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fMRI study of cannabidiol-induced changes in attention control in treatment-resistant epilepsy

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ABSTRACT

Patients with treatment-resistant epilepsy (TRE) frequently exhibit memory and attention deficits that contribute to their poor personal and societal outcomes. We studied the effects of adjunct treatment with pharmaceutical grade cannabidiol (CBD) oral solution (Epidiolex®; Greenwich Biosciences, Inc.) on attention control processes related to stimulus conflict resolution in patients with TRE. Twenty-two patients with TRE underwent 3 T magnetic resonance imaging (MRI) before receiving (PRE) and after achieving a stable dose of CBD (ON). Functional MRI (fMRI) data were collected while patients performed 2 runs of a flanker task (FT). Patients were instructed to indicate via button press the congruent (CON) and incongruent (INC) conditions. We performed t-tests to examine with FT attention control processes at PRE and ON visits and to compare the 2 visits using derived general linear model (GLM) data (INC – CON). We performed generalized psychophysiological interaction (gPPI) analyses to assess changes in condition-based functional connectivity on FT. Median time between fMRI visits was 10 weeks, and median CBD dose at follow-up was 25 mg/kg/d. From PRE to ON, participants experienced improvements in seizure frequency (SF) ($p = 0.0009$), seizure severity (Chalfont Seizure Severity Scale (CSSS); $p < 0.0001$), and mood (Total Mood Disturbance (TMD) score from Profile of Mood States (POMS); $p = 0.0026$). Repeated measures analysis of variance showed nonsignificant improvements in executive function from 34.6 (23.5)% to 41.9 (22.4)% CON accuracy and from 34.2 (25.7)% to 37.6 (24.4)% INC accuracy ($p = 0.199$). Change in CON accuracy was associated with change in INC accuracy ($r_S = 0.81$, $p = 0.0005$). Participants exhibited CBD-induced increases in fMRI activation in the right superior frontal gyrus (SFG) and right insula/middle frontal gyrus (MFG) and decrease in activation for both regions at ON relative to PRE (corrected $p = 0.05$). The subset of patients who improved in FT accuracy with CBD showed a negative association between change in right insula/MFG activation and change in accuracy for the INC condition ($r_S = -0.893$, $p = 0.0068$). The gPPI analysis revealed a CBD-induced decrease in condition-based functional connectivity differences for the right SFG seed region (corrected $p = 0.05$). Whole-brain regression analysis documented a negative association of change in right insula/MFG condition-based connectivity with change in INC accuracy (corrected $p = 0.005$). Our results suggest that CBD modulates attention control processing in patients with TRE by reducing right SFG and right insula/MFG activation related to stimulus conflict resolution and by dampening differences in condition-based functional connectivity of the right SFG. Our study is the first to provide insight into how CBD affects the neural substrates involved in attention processing and how modulation of the activity and functional connectivity related to attentional control processes in the right insula/MFG may be working to improve cognitive performance in TRE.

Abbreviations: ANOVA, analysis of variance; ASD, antiseizure drug; CBD, cannabidiol; CSSS, Chalfont Seizure Severity Scale; CON, congruent events (from FT); fMRI, functional magnetic resonance imaging; FT, flanker task; GLM, general linear model; gPPI, generalized psychophysiological interaction; INC, incongruent events (from FT); MFG, middle frontal gyrus; POMS, Profile of Mood States; PWE, persons with epilepsy; QOL, quality of life; rs-FC, resting state functional connectivity; SF, seizure frequency; SFG, superior frontal gyrus; TMD, total mood disturbance (from POMS); TRE, treatment-resistant epilepsy; UAB, University of Alabama at Birmingham.

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1. Introduction

Epilepsy affects approximately 70 million people worldwide, with about 1/3 carrying the diagnosis of treatment-resistant epilepsy (TRE) [1–4]. Persons with epilepsy (PWE), particularly those with TRE, have poorer health-related quality of life (QOL) than individuals without epilepsy [5]. Additionally, up to half of PWE suffer from cognitive impairment in one or more domains including memory, learning, attention, and executive functioning [6–11]. The presence of cognitive deficits in PWE contributes to poor QOL and has a major impact on their ability to participate in professional and societal activities [2,12]. Thus, there are ongoing efforts to develop strategies to ameliorate the negative impact of epilepsy on cognition.

In epilepsy, poor seizure control has been associated with worsening of cognitive functions [13,14]. Cannabidiol (CBD) is one of the more than 100 cannabinoids derived from the *Cannabis sativa* plant [15] and has received increasing attention for the treatment of TRE [16]. Highly-purified CBD (approved as Epidiolex® in U.S.; Greenwich Biosciences, Inc.) has demonstrated efficacy, with an acceptable safety profile, in randomized clinical trials (RCTs) for the treatment of Dravet [17] and Lennox–Gastaut [18,19] syndromes. Open-label expanded access CBD programs have also demonstrated improvements in seizure frequency (SF) and severity [20–22]. Much less is known about the effects of CBD on cognitive functions in epilepsy. The rapidly growing interest in CBD as an antiseizure drug (ASD), fueled by the recent United States (US) Food and Drug Administration (FDA) approval of Epidiolex® and its US Drug Enforcement Administration (DEA) rescheduling to schedule V [23,24], has prompted the need to evaluate its brain and behavior effects in PWE [25].

Successful cognition is often dependent on our ability to focus attention on relevant information while inhibiting distracting information. Attentional control is an important aspect of executive function that is necessary to allocate attention to relevant information and resolve the conflict between relevant and irrelevant information. Individuals with TRE have been shown to perform worse on attention and executive function tasks compared with healthy individuals and those with well-controlled epilepsy [14,26,27]. The effect of CBD on attention and executive functions in TRE and on corresponding brain changes has not been extensively explored [25].

The primary aim of this study was to assess how adjunct treatment with Epidiolex® affects executive functioning and attentional control processes in patients with TRE. The flanker task (FT) [28] probes attentional control processes through requiring the participant to attend to a centrally-located stimulus while inhibiting attention to flanking stimuli that may or may not be identical (i.e., congruent (CON) or incongruent (INC) stimuli). The interaction between a frontoparietal network with sensory and motor regions is thought to mediate attentional control processes [29,30]. Functional magnetic resonance imaging (fMRI) has the ability to noninvasively query CBD-induced brain changes in attentional control processing in TRE [25]. Studies in healthy volunteers have shown that CBD decreased fMRI activity during motor response inhibition (Go/No-Go task) without affecting task performance [31] and during salience processing (visual oddball task) [32] indicating decreased use of resources in order to complete the same task. Furthermore, single dose of CBD in patients with psychosis induced multiregion decreases in fMRI signals that were not observed in healthy controls and were less pronounced than deactivations in placebo-treated patients with psychosis indicating partial resolution/improvement of brain abnormalities associated with psychosis in response to CBD [33]. As well, CBD has been shown to have anxiolytic effects and act upon emotion regulation circuits, which may improve mood [34–36]. Therefore, based on these previously described studies, we hypothesized that CBD would improve performance on the FT independent of improved seizure control and decrease fMRI activity related to attentional control. Our secondary hypotheses are that CBD would also improve SF, seizure severity, and mood state.

2. Material and methods

2.1. Participants

Patients with TRE were prospectively recruited as part of the University of Alabama at Birmingham (UAB) CBD Program, an open-label compassionate-use study of Epidiolex®. This study was approved by the UAB Institutional Review Board, and all participants and/or their legal representative provided written informed consent before study participation. Details of specific inclusion and exclusion criteria for the UAB CBD Program have been described elsewhere [22,37] and are available at www.uab.edu/cbd. Briefly, participants were required to fulfill the following primary inclusion criteria: State of Alabama residency; video-electroencephalography confirmed diagnosis of TRE; unable to achieve seizure freedom after 4 trials of different ASDs including at least 1 trial of 2 concomitant ASDs; average of at least 4 seizures per month over the previous 3 months; more than 1 year of age; if applicable, have stable ketogenic diet ratio; detailed documentation of seizures in the form of a seizure diary for 3 months prior to study enrollment. Exclusion criteria included a history of substance addiction or abuse; history of allergies to CBD, marijuana products, or sesame; use of any CBD-based or medical marijuana product within 30 days of enrollment; hemoglobin <10 g/dl; white blood cell count <2000; hematocrit <30%; initiation of felbamate treatment within 12 months prior to study enrollment; and/or aspartate aminotransferase or alanine aminotransferase liver enzyme elevation at least 5 times the upper limit of normal levels. Antiseizure drug dose(s) were also required to be stable for at least a month prior to enrollment. An independent screening committee evaluated and approved patients for study participation before scheduling an initial visit.

Patients who were able to participate in the neuroimaging portion of the study were prospectively approached; participation required 2 scanning sessions at 3 T, one at baseline before receiving Epidiolex® (PRE) and another after achieving either the target dose of 25 mg/kg/d or a stable midrange CBD dose for at least 2 weeks (ON); all approached subjects agreed to participate. Median time between fMRI visits was 10 weeks. Female participants were administered urine pregnancy tests prior to fMRI and were negative. Out of the 77 children and 62 adults enrolled in the UAB CBD Program [22], we recruited 2 children and 25 adults of which 22 completed both scanning sessions. Five participants were withdrawn: 1 adult reported metal implant on the day of fMRI that could not be validated as magnetic resonance imaging (MRI)-safe; 1 child and 2 adults were noncompliant and withdrew from the program, and 1 adult became deceased.

2.2. Assessments

The following assessments were performed at PRE and ON visits. The Chalfont Seizure Severity Scale (CSSS) allows for the quantification of the severity of seizures and includes different aspects of seizures such as loss of awareness and incontinence that cause patients distress [38]. The Profile of Mood States (POMS) assesses fluctuations in mood states and provides a Total Mood Disturbance (TMD) score based on the mood scale scores for tension/anxiety, depression/dejection, anger/hostility, confusion/bewilderment, fatigue/inertia, and vigor/activity, with higher TMD scores reflecting greater mood severity [39]. Seizure frequency was verified at each visit via review of the submitted seizure calendar and was measured per 1 month with all seizures/seizure types included in the measure [22].

2.3. fMRI flanker task

To assess attention-related processing, fMRI data were collected while patients performed 2 runs of a modified FT using images of fish instead of arrows to indicate directionality to assess attention-related activation (Fig. 1), similar to the stimuli used on the Attention Network

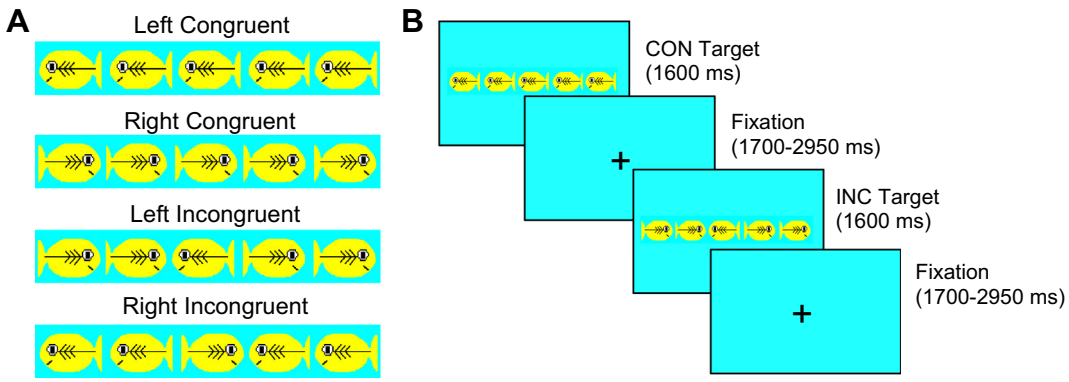

Fig. 1. Schematic of the modified flanker task. (A) Participants were instructed to indicate via button press what direction the center fish is swimming in (left or right) for congruent (CON) and incongruent (INC) target stimuli. (B) A target stimulus is presented for 1600 ms followed by a fixation screen for a variable amount of time, both during which responses are recorded. The exact stimulus presented for each target type (i.e., left or right congruent for CON target and left or right incongruent for INC target) was randomly selected for each run at each visit.

Test version for children [40]. The task was programmed and presented during the MRI session using DirectRT (Version 2008; Empirisoft, www.empirisoft.com). Each task run consisted of 36 CON target stimuli (row of 5 fish in the center of screen all swimming the same direction) and 36 INC target stimuli (center fish is swimming in direction opposite the adjacent fish). Each stimulus was presented for 1600 ms, followed by a fixation cross (jittered between 1700 and 2950 ms) in a fast event-related design. Participants were instructed to respond via button press which direction the center fish was swimming for each stimulus. Response selection and response times were recorded during both stimulus presentation and the following fixation cross to allow for more accurate behavioral assessment. Participants performed a practice task on the same day as the scanning session prior to the fMRI to ensure understanding of task instructions and ability to perform the task. The order in which CON and INC targets were presented was similar for both visits. However, in order to minimize practice effects, we employed random selection of the actual presented stimuli (i.e., left or right congruent for CON target and left or right incongruent for INC target; Fig. 1), while keeping the total number of presentations of each stimulus type balanced. For example, one participant may have "left congruent" presented as a CON target on the first trial at PRE and "right congruent" presented as the first trial at ON while another participant may have "right congruent" presented as a CON target on the first trial at PRE and "left congruent" presented as the first trial at ON.

2.4. Neuroimaging

Brain imaging was performed at UAB using a 3 T head-only Siemens Magnetom Allegra scanner, or after a scanner upgrade, a 3 T whole-body Siemens Prisma scanner. T1-weighted 3D high-resolution anatomical scans were acquired using a magnetization-prepared rapid acquisition with gradient echo (MP-RAGE) sequence with similar parameters on the Allegra (repetition time/echo time (TR/TE): 2300/2.17 ms, field of view (FOV): 25.6 × 25.6 × 19.2 cm, matrix: 256 × 256, flip angle: 9°, 1 mm isotropic) and on the Prisma (TR/TE: 2300/3.37 ms, FOV: 25.6 × 25.6 × 19.2 cm, matrix: 256 × 256, flip angle: 9°, 1 mm isotropic, 192 sagittal slices). At both scanners, T2*-weighted gradient echo-planar imaging pulse functional scans, while the participant performed 2 runs of the FT, were also acquired with the following parameters: TR/TE: 2000/23.0 ms, FOV: 25.6 × 25.6 × 15.2 cm, flip angle: 84°, 4 mm isotropic, 38 axial slices, and 161 volumes per scan.

2.5. Statistical analysis

Statistical analyses were performed using SAS (Statistical Analysis Software version 9.3, Cary, NC), with $p < 0.05$ considered significant. The sign test for paired data was performed to assess PRE- to ON-CBD changes in SF, CSSS scores, and TMD scores, with $p < 0.05$ considered significant. Repeated measures analysis of variance (ANOVA) examined FT behavioral data for the effects of condition (CON vs. INC), visit (PRE vs. ON), and their interaction for each measure, with $p < 0.05$ considered significant. Spearman correlations were performed to evaluate the relationships between ON–PRE changes in task performance (accuracy and response times for each condition) and changes in clinical and assessment scores (SF, CSSS, TMD), with $p < 0.0071$ considered significant after Bonferroni correction for multiple comparisons. Data are reported as median and interquartile range (IQR), mean and standard deviation (SD), and frequency and percentage, as appropriate.

2.6. fMRI data analysis

The Analysis of Functional NeuroImages (AFNI) software was used to analyze the task fMRI data [41]. Anatomical and fMRI scans were aligned. Slice-timing and coregistration of fMRI volumes to correct for head motion [42] were performed, followed by resampling to 4 mm voxels, normalization to Talairach space, and spatial smoothing using a 6 mm Gaussian kernel. Single-subject statistical analyses were performed to model the CON and INC events separately with a gamma variate hemodynamic response function, as well as contrasting INC versus CON events to determine fMRI activation related to attentional control (3dDeconvolve in AFNI). The single-subject general linear model (GLM) also accounted for MRI signal drift, and the six parameters of head motion were modeled as regressors of no interest. Additional correction for motion was performed by censoring volumes in which more than 3% of the total number of voxels are detected as outliers in signal from one volume to the next. Group statistical analyses were then performed, with 3dttest++ used to conduct one-sample t-tests to examine INC–CON differences at PRE and ON visits, as well as a paired t-test to assess CBD-induced changes in neural processing of attentional control. These analyses accounted for change in SF and scanner type. To determine statistical thresholding parameters, the spatial autocorrelation function in the 3dFWHx program was used to estimate noise smoothness and then fit to a mixed model, which was then used to generate noise random fields, estimate the probability of false-positive clusters, and determine the cluster threshold for different voxelwise thresholds using the 3dClustSim program [43]. Cluster thresholds for voxelwise $p = 0.02$ were calculated using 10,000 Monte Carlo simulations to achieve activation clusters significant at corrected $p < 0.05$ (voxelwise $p = 0.02$, cluster volume threshold of 1536 mm^3, and faces of voxels must touch). We further characterized the task-related fMRI activity by extracting the mean beta-weight values from regions showing significant ON vs. PRE differences and graphing them for each task condition at each time point. To examine brain activity specific to improved performance, we included only participants who showed improvements for both task conditions and performed Spearman correlations to evaluate the relationships between ON–PRE changes in task

Table 1
Demographic and clinical variables for the 22 patients with epilepsy who completed MRI visits before starting CBD treatment (PRE) and while on CBD (ON).

	PRE	ON
Age, years	30.5 (24.0)	–
Sex, female	14 (63.6)	–
Age of epilepsy onset, years	13.0 (22.0)	–
Epilepsy duration, years	18.0 (17.0)	–
Weeks between MRI visits[a]	–	10.0 (4.0)
Stable CBD dose, mg/kg/d	–	25.0 (5.0)
Monthly seizure frequency	12.5 (38.0)*	5.0 (14.0)*
Chalfont Seizure Severity Scale, total score	67.0 (52.0)#	11.5 (30.0)#
Profile of Mood States, Total Mood Disturbance score[b]	45.0 (49.0)*	25.5 (32.5)*

Data reported as median (interquartile range) except for sex, which is reported as frequency (percentage).
[a] One participant did not start CBD until 3 weeks after the PRE-CBD scan.
[b] Two participants did not complete the Profile of Mood States assessment at both visits.
* $p < 0.01$.
# $p < 0.001$.

performance (accuracy and response times for each condition) and changes in mean beta-weight values for each region showing significant differences, with $p < 0.025$ considered significant after Bonferroni correction for multiple comparisons.

2.7. Task-based connectivity analysis

Cannabidiol-induced neuroplasticity may be associated with dynamic changes in attentional control processing. We conducted a generalized psychophysiological interaction (gPPI) analysis using the gPPI toolbox for Statistical Parametric Mapping (SPM) software [44] to assess the context-specific changes in the relationship between a seed region to activity in other cortical regions by including a term specifying an interaction effect between the time series of the seed region and the time series of the task in each first-level GLM [45]. For each participant, the first principal component of the blood oxygen level-dependent (BOLD) time series from each scan was extracted from each seed region and entered into gPPI analysis. In order to reduce the influence of non-neural signals on estimates of task-dependent connectivity, signal from cerebrospinal fluid and white matter were also included as nuisance variables in the gPPI model [46]. For each participant, gPPI estimates quantifying the level of condition-dependent connectivity from each seed region to the rest of the cortex for each visit were then extracted from the gPPI model into connectivity maps. Those maps were then used in paired t-tests analysis in order to compare connectivity between ON and PRE visits, with differences significant at false discovery rate (FDR)-corrected $p < 0.05$ (voxelwise $p = 0.001$). The gPPI effects are interpreted as changes in interregional connectivity, and these changes are driven by psychological states related to the task being performed [44,47]. To examine neural underpinnings of improved attention processing in patients with TRE, we conducted exploratory whole-brain regression analysis of gPPI estimates for each seed region with accuracy on the INC and CON task conditions to determine ON–PRE differences in task-based connectivity related to performance, with differences significant at FDR-corrected $p < 0.05$ (voxelwise $p = 0.003$).

3. Results

3.1. Changes in clinical, assessment, and task performance data

Demographic and clinical data are summarized in Table 1. Participants underwent fMRI before receiving Epidiolex® (PRE) and after achieving a stable dose for at least 2 weeks (15 ($n = 4$), 20 ($n = 5$), or 25 ($n = 13$) mg/kg/d; ON), with a median time of 10 weeks between visits. Target dose was 25 mg/kg/d, but was adjusted based on a combination of seizure response and adverse events reported by the participants. From PRE to ON, median (IQR) for percent reduction was 71.2 (50.0) % for SF, 80.5 (45.7) % for CSSS total score, and 41.3 (62.0) % for POMS TMD score. Results of the signed-rank test for paired data show significant reductions in SF ($p = 0.0009$), CSSS total score ($p < 0.0001$), and TMD score ($p = 0.0026$) reflecting improvements in seizure control, seizure severity, and mood with CBD.

Because of technical glitch, behavioral data during FT fMRI were not recorded for 8 patients. For the remaining 14 patients, mean (SD) was 34.6 (23.5)% at PRE and 41.9 (22.4)% at ON for CON accuracy, and 34.2 (25.7)% at PRE and 37.6 (24.4)% at ON for INC accuracy (Fig. 2). Mean (SD) response times for CON were 884 (206) ms at PRE and 1161 (455) ms at ON, and for INC, 974 (282) ms at PRE and 1167 (460) ms at ON. There were no significant condition-by-visit interactions for accuracy ($p = 0.635$) or response times ($p = 0.625$). There was also no significant effect of condition for accuracy ($p = 0.777$) or response times ($p = 0.714$). There was a nonsignificant increase in accuracy ($p = 0.199$) and a significant slowing of response times ($p = 0.0178$) from PRE to ON visits. Change in CON accuracy was significantly associated with change in INC accuracy ($r_S = 0.81$, $p = 0.0005$). Change in either CON or INC accuracy was not associated with corresponding changes in response times or changes in SF, CSSS score, TMD score, or response times (all $p > 0.05$).

3.2. Flanker task fMRI and gPPI analysis

At the PRE visit, participants exhibited increased activation to INC relative to CON in the right precuneus; at the ON visit, participants exhibited decreased activation to INC relative CON in the right superior frontal gyrus (SFG) (Fig. 3A, Table 2). Paired t-test showed decreased attention-related activation in the right SFG and right insula extending into the middle frontal gyrus (MFG) at ON relative to PRE visits (Fig. 3B, Table 2). For participants who exhibited performance improvement for both

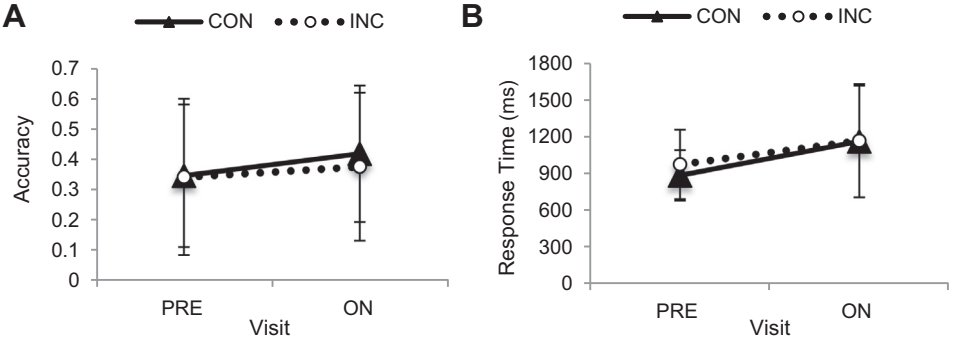

Fig. 2. Changes in accuracy for the congruent (CON) and incongruent (INC) conditions and corresponding response times on the modified flanker task. Repeated measures ANOVA shows (A) a nonsignificant increase in accuracy ($p = 0.199$) and (B) a significant increase in response time ($p = 0.0178$) from PRE to ON visits. There were no significant effects of condition (CON vs. INC) or interaction effects. Graphs show mean (SD) for each measure at each visit.

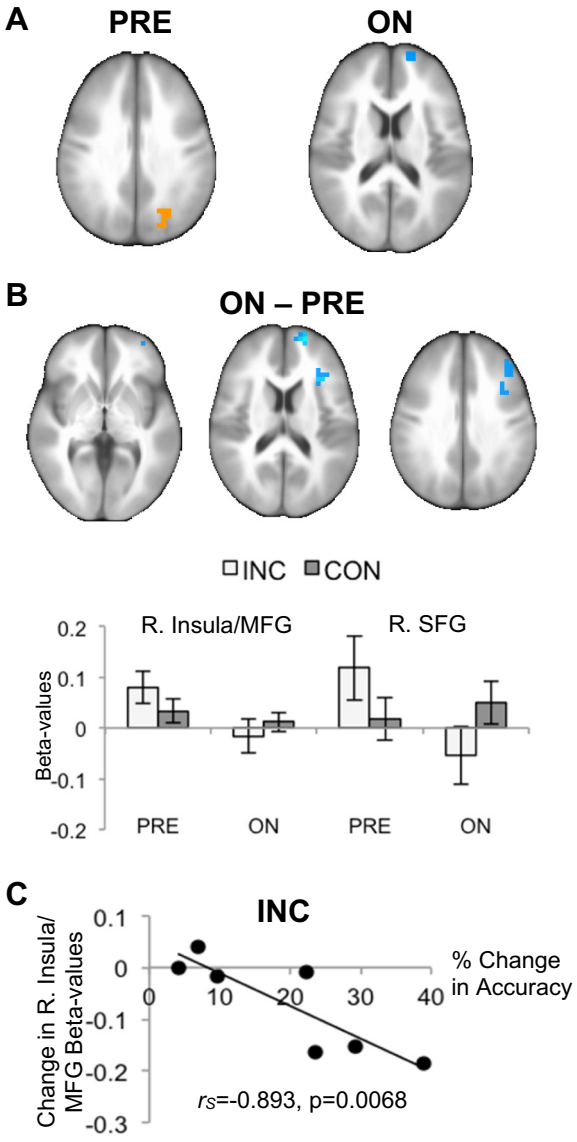

Fig. 3. Differential attention-related fMRI response during Flanker task. (A) Before starting CBD treatment (PRE) participants exhibited increased fMRI activation for incongruent (INC) relative to congruent (CON) conditions in the right precuneus (cluster in orange). While on CBD (ON), participants exhibited decreased activation for INC vs. CON in the right superior frontal gyrus (R. SFG; cluster in blue). (B) Paired t-test controlling for scanner type and change in seizure frequency showed decreased attention-related activation (INC > CON) in the right insula extending to the middle frontal gyrus (R. Insula/MFG) and in the R. SFG at ON versus PRE visits (clusters in blue). The graph of the beta-weight values showed that the difference was due to the inverse patterns of INC > CON activation at the PRE visit and INC < CON activation at the ON visit in these regions. (C) For patients who exhibited performance improvement for both conditions, INC accuracy improved with decreased INC-related activation of the R. Insula/MFG.

conditions, change in INC accuracy was significantly associated with change in right insula/MFG activation ($r_S = -0.893$, $p = 0.0068$) but not with change in right SFG activation ($r_S = 0.071$, $p = 0.88$) for the INC condition (Fig. 3C). Change in CON accuracy was not associated with changes in right insula/MFG activation ($r_S = -0.324$, $p = 0.48$) or right SFG activation ($r_S = 0.090$, $p = 0.85$) for the CON condition.

Using the right SFG and right insula/MFG seed regions in gPPI analysis revealed a significant change in task-based functional connectivity between the right SFG seed and a large bilateral midline cluster in the supplementary motor area and superior frontal area (1227 mm^3; centroid at $x = -1$, $y = +19$, $z = +47$), which was reduced at the ON visit compared with the PRE visit (Fig. 4A). Regression analysis showed that reduction in task-based functional connectivity of the right insula/MFG seed to left hemisphere brain regions encompassing Heschl's gyrus, postcentral gyrus, and insula (663 mm^3; centroid at $x = -47$, $y = -20$, $z = +15$) was related to increased INC accuracy at ON versus PRE visits (Fig. 4B).

Table 2
Location and extent of brain regions in which participants showed differences in flanker task fMRI activation at baseline (PRE) and after achieving a stable dose of CBD for at least 2 weeks (ON), and when comparing incongruent (INC) versus congruent (CON) task activations between the two visits.

	Brain regions	Peak voxel (x, y, z)	Peak t-value	Cluster extent (mm^3)
PRE				
INC > CON	R. precuneus/BA7	22, −69, 40	3.18	1664
ON				
INC < CON	R. superior frontal gyrus/BA10	14, 59, 24	−3.60	1600
ON–PRE				
INC–CON	R. superior frontal gyrus/BA10	18, 59, 20	−4.57	2176
	R. insula/middle frontal gyrus	30, 23, 16	−3.50	2688

4. Discussion

We hypothesized that CBD would improve performance on fMRI FT independent of improved seizure control and exert effects on executive functioning and attentional control processes in patients with TRE. In secondary analyses, participants experienced significant reductions in SF and CSSS in response to CBD, which were previously described in the entire cohort [22]. Our data also indicated significant improvements in mood state consistent with CBD's anxiolytic effects [34–36], slowing of response times on the INC FT trials, and a nonsignificant improvement in accuracy with CBD. As we hypothesized, changes in task accuracy for either condition were not explained by the significant improvements in seizure control (SF or CSSS) nor were they associated with changes in mood state or response times. Instead, change in CON accuracy was only significantly associated with change in INC accuracy, suggesting that participants who improved on one condition of the task also had a corresponding improvement in the other condition. Similarly, daily treatment with CBD in regular cannabis users did not significantly improve accuracy on an attentional set-shifting task, although it did reduce response times and decrease depressive and psychotic-like symptoms [48]. These authors also found increasing CBD concentrations in

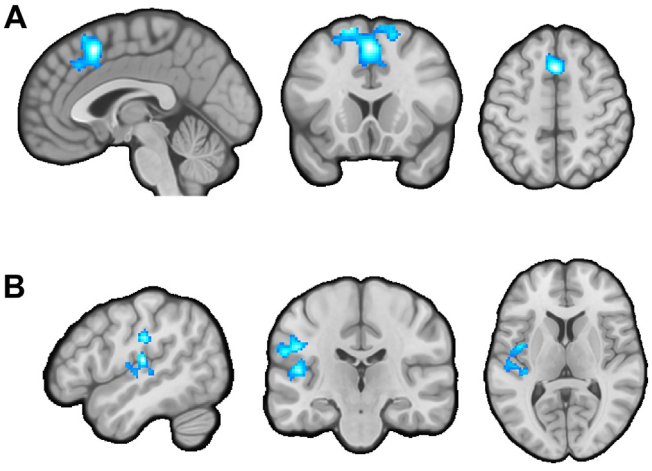

Fig. 4. Results of gPPI analysis. (A) Task-related connectivity (i.e., difference in connectivity between CON and INC conditions) between the right superior frontal gyrus seed region and a bilateral cluster in the supplementary motor area and superior frontal area was decreased at ON relative to PRE visits (in blue). (B) Whole brain regression analysis showed that increased accuracy on the INC condition was associated with the decreased difference in right insula/middle frontal gyrus seed task-based connectivity to left hemisphere brain regions (Heschel's gyrus, postcentral gyrus and insula) at ON relative to PRE visit.

plasma to be associated with better attentional control, but this cannot be tested in our study since plasma levels of CBD were not routinely measured at the time of the fMRI.

Interestingly, we did not observe an effect of task condition (INC vs. CON) on accuracy or response times whereas FT performance typically shows decreased accuracy and/or slower response times for INC compared with CON conditions [40,49–52]. It should also be noted that compared with the over 50% mean accuracy achieved in these previously published studies utilizing FT, our patients with epilepsy performed poorly overall with only 4 of the 14 achieving over 50% accuracy for either task condition at each time point. These results suggest a pronounced impairment in executive functioning in the majority of our sample, which is expected in TRE [7,27,53,54]. This suggests that the CON and INC conditions may not be differentially processed by participants despite the INC condition having stimulus conflict typically requiring greater attentional control.

With respect to the FT fMRI, we hypothesized that CBD would decrease fMRI activity related to attentional control given that studies in healthy volunteers and patients with psychosis showed decreased fMRI activity in other visual discrimination and response inhibition tasks (e.g., Go/No-Go, visual learning, and visual oddball task) [31–33]. At the baseline PRE visit, there was an increased right precuneus activation for INC relative to CON conditions, likely due to greater utilization of this region for visual processing of the stimulus conflict. Previous fMRI studies utilizing other versions of the FT have also observed an increased precuneus activation when resolving stimulus conflict [50,55] and during stimulus-driven reorienting [49]. Furthermore, in a meta-analysis of neuroimaging studies of tasks more generally involving interference resolution, the precuneus was one of the regions found to have overlap among all studies combined [56]. At the ON visit, there was a decreased right SFG activation for INC relative to CON conditions. The SFG has also been shown to play a role in resolving stimulus conflict [55], interference resolution [57], and response anticipation [58] during FT. Having decreased SFG activation for INC relative to CON conditions at the ON visit suggests that CBD may be modulating attention control processing such that the two conditions are being similarly processed and is consistent with our behavioral data results.

Direct examination of differences between attention control processing at ON versus PRE visits revealed a similar decrease in right SFG activation. Additionally, there was a decrease in right insula/MFG activation for ON versus PRE visits, which is further illustrated by the graph in Fig. 2B. Not only were the right insula and dorsolateral prefrontal cortex (DLPFC), which lies within the MFG, found to have overlap among all the interference resolution studies in meta-analysis, but also, these were the two regions specifically shared among the studies utilizing FT [56]. Both insula and DLPFC are thought to be involved in attention and response selection processes, with the right DLPFC more specifically involved in resolving interference from the INC flankers [56]. Similar to the SFG, the MFG is also involved in response anticipation as well as in conflict resolution on FT [58]. Additionally, the right MFG is consistently activated in tasks of sustained attention and thought to reflect top-down processing in order to maintain attention and performance (see [59,60]). However, in the presence of a distractor, increased right MFG activity has also been associated with decreasing performance on a sustained attention task [61]. Consistent with our data, decreased activation of the right insula/MFG region was associated with increased accuracy during the INC condition in patients with TRE who showed improved performance.

Comparison of task-based functional connectivity at ON versus PRE visits showed that with CBD, the difference in task condition-dependent connectivity of the right SFG with the supplementary motor area and superior frontal area is diminished. In healthy individuals, CBD was shown to acutely reduce prefrontal cortex to hippocampus functional connectivity related to salience processing [62]. Our results in TRE are consistent with this premise of reduced prefrontal functional connectivity with CBD. It also provides additional support for a CBD-facilitated shift in the processing of conflict stimuli in the INC condition to be more similar to the processing in the CON condition, since the condition-dependent connectivity differences seen at the baseline PRE visit are significantly reduced at the ON visit. Finally, exploratory regression analysis showed that improved INC accuracy was associated with CBD-induced decrease in condition-dependent connectivity of the right insula/MFG. Considering that we also showed that INC accuracy improvement was associated with greater decrease in right insula/MFG activity, these results are internally consistent.

There are study limitations that must be considered. We enrolled a mixture of different types of epilepsy. Though not examined to date, CBD may have different effects on attention and executive functioning in different types of epilepsy. Thus, a more homogeneous cohort of patients should be considered in future studies to tease apart these effects. Our study had a relatively small sample size, and behavioral data were not recorded for all patients, which likely limited our ability to detect significant changes in task accuracy and would be better resolved with a larger study. There is also the possibility of practice effects, which should be considered in the interpretation of our results. A 24-hour practice effect on the FT has been shown as faster response times [63], although we did not observe this over our study's average course of 10 weeks and in fact, observed a slowing of response times. Also, as described in our methods, random selection of the actual presented stimuli was employed for CON and INC targets in order to try and minimize practice effects. Further, we were unable to take into account the effects of ASDs on cognition. However, it should be noted that ASDs were kept relatively constant for the duration of trial with the exception of CBD. Other limitations included lack of a patient control group that did not receive CBD or follow-up fMRI to examine more prolonged effects of CBD treatment. These were beyond the scope of the current study and are important considerations for future research.

5. Conclusions

We show nonsignificant improvements in performance and significant CBD-induced changes in attention-related fMRI activation in executive control regions. The associations between increasing INC accuracy and both decreasing activation and decreasing condition-dependent connectivity of right insula/MFG suggests CBD-induced modulation of attention control processing in patients with TRE by dampening differences in both condition-based fMRI activation and functional connectivity. Our study is the first to provide insight into how CBD affects the neural substrates involved in attention processing and how modulation of the activity and functional connectivity of the right insula/MFG may be working to improve cognitive performance in patients with TRE.

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Declaration of interests

JBA is a consultant for LivaNova, Inc. and has served as a guest editor for Clinical Therapeutics. JPS has received funding from NIH, NSF, Shor Foundation for Epilepsy Research, EFA, Department of Defense, UCB Biosciences, NeuroPace Inc., FDA, AES, SAGE Therapeutics Inc., Greenwich Biosciences Inc., Serina Therapeutics Inc., and Eisai, Inc., is on the Consulting/Advisory Boards for SAGE Therapeutics Inc., Greenwich Biosciences Inc., NeuroPace, Inc., Upsher-Smith Laboratories, Inc., Medical

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