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Effects of Chronic, Low-Dose Cannabinoids, Cannabidiol, Delta-9-Tetrahydrocannabinol and a Combination of Both, on Amyloid Pathology in the 5xFAD Mouse Model of Alzheimer's Disease

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Abstract

Background: There is an urgent need for novel therapies to treat Alzheimer's disease. Among others, the use of cannabinoids such as delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) has been proposed as a putative approach based on their anti-inflammatory effects.

Methods: The present work was designed to explore the effects of chronic (28 days) treatment with low doses of cannabinoids: CBD (0.273 mg/kg), THC (0.205 mg/kg) or a combination of both (CBD:THC; 0.273 mg/kg:0.205 mg/kg) in the 5xFAD mouse model of AD.

Results: Our data revealed that THC-treated 5xFAD mice (but not other treatment groups) exhibited anxiogenic and depressant-like behavior. A significant improvement in spatial memory was observed only in the CBD:THC-treated group. Interestingly, all cannabinoid-treated groups showed significantly increased cortical levels of the insoluble form of beta amyloid 1-42. These effects were not accompanied by changes in molecular parameters of inflammation at the mRNA or protein level.

Conclusions: These data reveal differential effects of chronic, low-dose cannabinoids and point to a role of these cannabinoids in the processing of amyloid peptides in the brains of 5xFAD mice.

Keywords: Alzheimer; CBD; THC; behavior; amyloid

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative condition that leads to progressive cognitive impairment in the elderly and accounts for the majority of cases of dementia worldwide.[1] The main neuropathological features of this disease include the loss of neurons in the hippocampus and cortex, deposition of amyloid peptides and hyperphosphorylated tau, and development of a neuroinflammatory environment that is thought to aggravate the loss of neurons.[2,3]

Clinically, AD is characterized by initial short-term memory loss, progressing to mild cognitive impairment and to dementia within years from the disease diagnosis. These features are accompanied by other comorbidities such as sleep alterations, mood disorders, and visual and hearing impairments that eventually compromise basic functions and lead to death.[1,4]

Considering epidemiologic data, the search for novel therapies for AD is utterly urgent.[4] In humans, cannabinoids have been administered in several small clinical trials, mostly for treatment of behavioral symptoms of AD such as agitation and aggression.[5–9] Among the cannabinoids, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have been proposed as putative therapeutics for AD. THC is the most abundant cannabinoid present in the *Cannabis sativa* plant and is responsible for the psychoactivity of plant derivatives consumed worldwide.[10] This compound binds both CB_1 and CB_2

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receptors[11] and exhibits several properties potentially relevant to AD, such as reduction in inflammation,[12] induction of hippocampal neurogenesis,[13] and enhancement of amyloid beta (Aβ) removal.[14]

Moreover, THC was found to decrease Aβ plaques and to induce neuroprotection in the 5xFAD mouse model of AD through a mechanism mediated by cyclooxygenase-2, providing a link between neuroinflammation and THC in the context of AD.[15] However, at high doses, THC also triggers anxiety and memory impairment, which could be a limiting factor for its use as a drug in AD.[6,16]

CBD, on the other hand, has been proposed as an attractive compound for treatment of several Central Nervous System (CNS) diseases, mainly because of its lack of psychoactive effects.[17] The clinical use of CBD has been hampered by the complexity of its pharmacological properties that allow its interaction with a variety of receptors, such as CB_1 and CB_2, and several other GPCRs, transient receptor potential vanilloid-1 (TRPV1), several other ion channel receptors, peroxisome proliferator-activated receptor gamma (PPARγ), and fatty acid amide hydrolase (FAAH).[10,18]

CBD has been also shown to have potent antioxidant and anti-inflammatory properties and to induce neuroprotection *in vitro*[19] and *in vivo*.[20] In the context of AD, CBD has been shown to have beneficial effects in neuronal cell lines,[21,22] in glial cells,[23] and in animal models of this disease.[20,24,25]

The present studies have been designed to explore the potential anti-inflammatory and neuroprotective effects of cannabinoids, CBD alone, THC alone, or a combination of both (CBD:THC), in the 5xFAD mouse model of AD.

Materials and Methods

Mice

Eight-month-old, male hemizygous mice ($N = 16$ per treatment group) coexpressing five familial AD mutations (5xFAD) and their wild-type (WT) littermates were used in these experiments.[26] Mice were housed at the animal facility of Universidad Francisco de Vitoria (authorization No. 281150000013). Experimental protocols met the European and Spanish regulations for protection of experimental animals (2010/63/EU and RD 1201/2005 and 53/2013) and were authorized by the local ethics committee (PROEX149/18).

Drugs, dosing, and treatments

High-purity (>97%) synthesized CBD and THC were purchased from Purisys, LLC (Athens, GA). Cannabinoids or vehicle stock solutions were prepared with ethanol (supplemented with 0.05% alpha tocopherol) and stored at −20°C and protected from light. To date, several small clinical trials have investigated the use of isolated cannabinoids as well as medical cannabis extracts for treatment of symptoms of dementia.[27–36] A dose analysis revealed a safe and potentially efficacious THC oral dose of 0.75–12 mg daily in this elderly population.

To use translationally relevant chronic doses in the 5xFAD mice, we converted between the human equivalent doses (HEDs) and mouse doses according to the FDA Guidance for Industry on "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers." We selected doses that were on the lower end of clinically tested human doses for subjects with dementia, considering that all trials used oral dosing with associated low bioavailability and variable absorption, while 5xFAD mice received chronic i.p. administration.

The ratio between THC and CBD in this study was selected based on a screen of MediCane's proprietary full-spectrum extracts on zebrafish neurological models (data not shown). Injectable solutions were prepared fresh each day from the ethanolic stock solutions using a ratio of 1:1:18 (ethanol:Cremophor®:saline) and were continuously stirred until injection. Mice were treated for 28 consecutive days.

The 5xFAD mice were divided into four groups (VEH, CBD, THC, and CBD + THC) with their WT (VEH) counterparts as controls. Each mouse received a daily i.p. injection containing vehicle, CBD (0.273 mg/kg), THC (0.205 mg/kg), or CBD:THC (0.273:0.205 mg/kg).

Behavioral tests

Elevated plus maze. The elevated plus maze (EPM) test was performed as described.[37,38] Mice were analyzed for 5 min, allowing them to move freely in the platform. All tests were video recorded and analyzed by an independent researcher. Time spent in open arms was measured and expressed as % of time.

Rotarod. The Rotarod (RR) test (Panlab, Barcelona, Spain) was employed to quantify motor coordination in mice. Each mouse was tested for three consecutive trials, allowing 15 min of recovery between tests. The speed of the rolling bar was initially set at 4 rpm and then it was increased up to 40 rpm over 10 min. The time that the mouse stayed on the rolling bar was the latency time and only the third trial was represented.

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Open-field test. Mice were placed in a flat circular arena and allowed to move freely for 10 min.[39] An independent researcher carried out the experiment and analyzed the images recorded with the SMART Video Tracking System, v.3 (Panlab). Parameters analyzed included total distance, time spent, and distance covered in the central and peripheral sections.

Tail suspension test. The tail suspension (TS) test provides a notion of the "depressive-like" state of a mouse.[40] Mice were tested for 6 min and images were recorded for analysis. Immobility time was quantified for each mouse.

Barnes maze. The Barnes maze (BM) test was used to get an estimation of memory impairment in mice.[41] During the training sessions, individual mice were tested twice a day for 4 consecutive days. The mouse was placed in a cylindrical, black start chamber in the middle of the platform. After 10 sec, the chamber was removed to let the animal move freely for 3 min. All trials were recorded for the analysis. On the fifth day, a short-term memory retention test was performed (one trial).

An independent researcher carried out the experiment and analyzed images recorded with the SMART Video Tracking System, v.3 (Panlab). Latency time to the target hole was analyzed during the training sessions (days 1–4) and in the memory trial (day 5).

Molecular determinations

Quantification of Aβ 1–42 by enzyme-linked immunosorbent assay. To determine Aβ 1–42 levels in brain tissue, the hippocampus was homogenized in 10 volumes of ice-cold guanidine buffer (5.0 M guanidine·HCl/50 mM Tris·Cl, pH 8.0) containing the protease inhibitor cocktail (Roche).

Soluble and insoluble amyloid fractions were separated according to the following procedure: hippocampi were homogenized for a sequential extraction in 10 volumes of ice-cold TBS extraction buffer (140 mM NaCl, 3 mM KCl, 25 mM Tris [pH 7.4], and 5 mM EDTA) and the protease inhibitor cocktail (Roche) for the soluble fraction and guanidine buffer (5.0 M guanidine-HCl and 50 mM Tris, pH 8.0) containing the protease inhibitor cocktail (Roche) for the insoluble fraction.

Protein concentrations were determined in both fractions (Micro BCA™ protein assay kit; Thermo Scientific). Human enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen) were used according to manufacturer's specifications. Optical signals at 450 nm were read on a Sunrise microplate reader (Tecan), and sample concentrations were determined by comparison with the respective standard curves.

Protein quantification by western blot. Cortices were homogenized in lysis buffer (MLB: 25 mM HEPES, pH 7.5, 150 mM NaCl, 1% Igepal CA-630, 10 mM $MgCl_2$, 1 mM EDTA, 10% glycerol, 1 mM Na_3VO_4, 25 mM NaF, and protease inhibitor cocktail; Roche). Then, samples were centrifuged at 12,000 *g* for 20 min at four degrees and protein concentrations were quantified (BCA Protein Assay Kit; Thermo Scientific).

Tissue lysates (50 μg/lane) were loaded into sodium dodecyl sulfate (SDS)–polyacrylamide gels, and the proteins were transferred onto nitrocellulose membranes (Bio-Rad). After blocking in TTBS, they were incubated overnight at 4°C, as appropriate, with anti-GFAP (1:500; Bio-Rad), anti-Iba1 (1:1000; FUJIFILM Wako), anti-GAPDH (1:7000; Abcam), anti-PSD95 (1:1000; Abcam), and antisynaptophysin (1:1000; Abcam).

Membranes were incubated with corresponding horseradish peroxidase-conjugated mouse secondary antibody (1:10,000) or anti-rabbit (1:5000) and developed using a chemiluminescent reagent (GE Healthcare). Developed signals were recorded on the ChemiDoc Imaging System (Bio-Rad) for densitometric analysis (ImageJ).

Quantification of messenger RNA levels by real time-quantitative polymerase chain reaction. RNA from the cortex and hippocampus was isolated using the TriPure isolation reagent (Roche). Then, a Transcriptor First Strand complementary DNA (cDNA) Synthesis Kit (Roche) was used according to manufacturer's specifications, and different relative gene expression levels were measured using the CFX Connect™ Real-Time System (Bio-Rad), Quantimix Easy Probes Kit (Biotools), and the following mouse probes conjugated with the FAM fluorophore PrimePCR™ Probe Assay (Bio-Rad): *Rps18* (qMmuCEP0053856), *Cnr2* (custom), *Cnr1* (qMmuCEP0038879), *Tnf* (qMmuCEP0028054), *Il6* (qMmuCEP0054186), and *Il1b* (qMmuCEP0054181).

RNA expression was calculated using the comparative Ct method normalized to the 18S ribosomal subunit. Data were expressed relative to a calibrator using the $2^{-(\Delta\Delta Ct)} \pm s$ formula.

Statistical analysis

The statistical analysis was performed with GraphPad 9.0. Normality was analyzed with the D'Agostino and Pearson or Shapiro–Wilk tests; normal data were checked for outliers with ROUT in GraphPad (10%),

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while non-normal data were assessed with the 1.5xSD approach. In the case of normal data, a two-tailed Student's *t*-test, one-way analysis of variance (ANOVA) (followed by Tukey's *post hoc* test), or two-way ANOVA (followed by Sidak's *post hoc* test) was used; for non-normally distributed data, the Mann–Whitney U test or Kruskal–Wallis test was used.

A *p*-value <0.05 was considered statistically significant.

Results

Behavioral data

Spatial memory. As expected, 5xFAD mice exhibited impaired spatial memory (Fig. 1A) [genotype

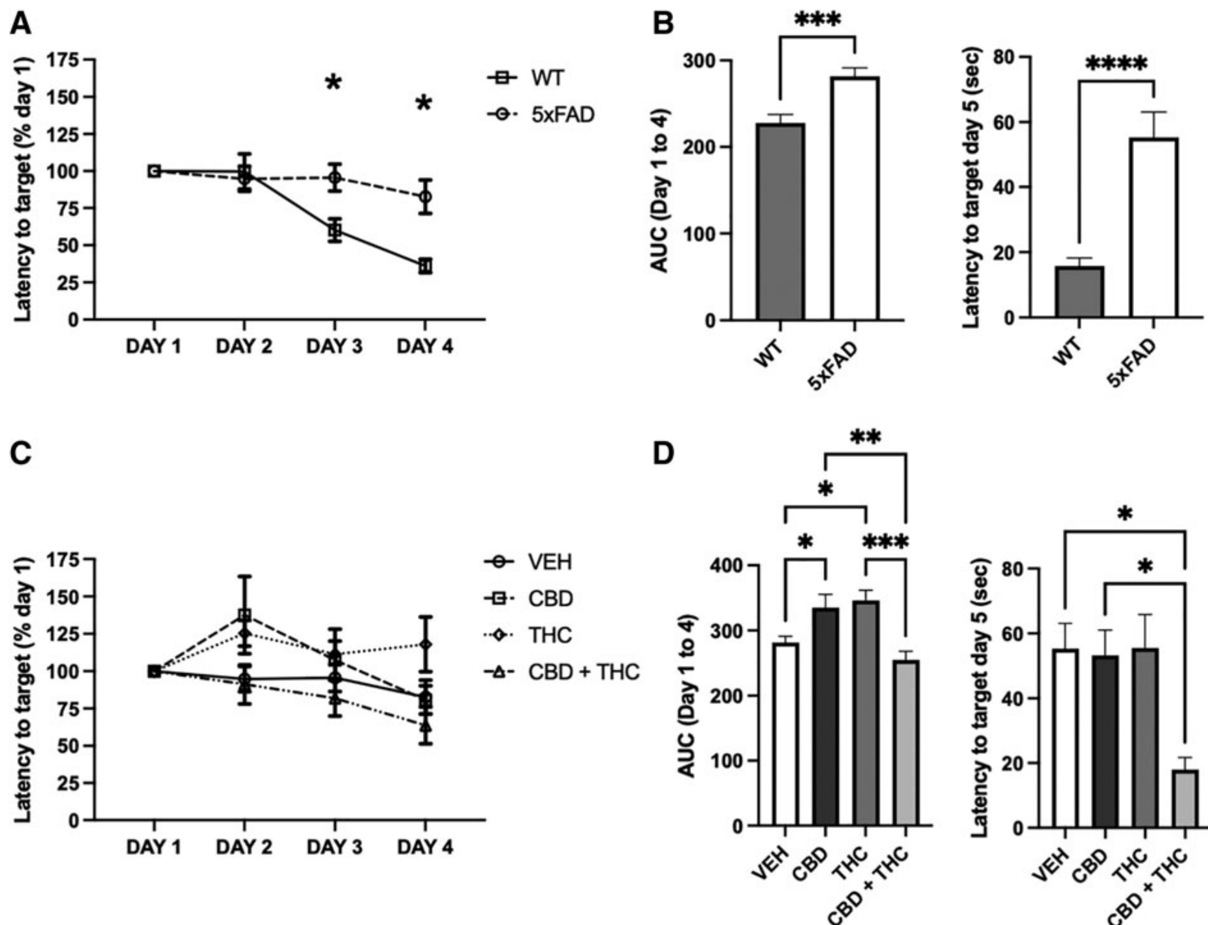

FIG. 1. Spatial memory was not modified by treatment with CBD, THC, or CBD:THC on days 1–4, but was significantly improved by treatment with CBD:THC on day 5. **(A)** The analysis of the latency to target confirmed a significant deficit in 5xFAD versus WT mice (**p* < 0.05; two-way ANOVA, followed by Sidak's test for multiple comparisons); **(B)** area under the curve analysis confirmed a significant worsening in spatial memory in 5xFAD mice compared with WT mice on days 1–4 (****p* = 0.0007; two-tailed unpaired *t* test) as well as on day 5 (*****p* < 0.0001; Mann–Whitney test); **(C)** latency to target showed no effects due to the treatment with cannabinoids; **(D)** analysis of the area under the curve showed that CBD- and THC-treated mice exhibited worse performance on days 1–4 compared with VEH-treated mice (**p* < 0.05, ***p* < 0.01, and ****p* < 0.001; one-way ANOVA, followed by Tukey's test for multiple comparisons); on day 5, CBD:THC-treated mice showed significant improvement in memory (**p* < 0.05; Kruskal–Wallis test, followed by Dunn's test for multiple comparisons). *N* = 43 (WT mice) and *N* = 16–24 (5xFAD mice). ANOVA, analysis of variance; CBD, cannabidiol; THC, delta-9-tetrahydrocannabinol; WT, wild-type.

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$F(1, 65) = 6.909$, $p = 0.0107$; time $F(2.239, 132.1) = 12.85$, $p < 0.0001$; and interaction $F(3, 177) = 5.678$, $p = 0.0010$] in the BM test. Sidak's *post hoc* test revealed a significant effect on days 3 ($p = 0.0181$) and 4 ($p = 0.0029$). The AUC analysis confirmed this effect on days 1–4 ($p = 0.0007$, Fig. 1B, left). Difference in the latency to target was also significant on day 5 ($p < 0.0001$, Fig. 1B, right).

However, 5xFAD mice treated with the different cannabinoid combinations revealed differential results (Fig. 1C) [genotype $F(3, 73) = 3.288$; $p = 0.0273$; time $F(2.351, 154.4) = 3.852$; $p = 0.0177$; and interaction $F(9, 197) = 1.439$; $p = 0.1734$]. While CBD alone and THC alone worsened spatial memory on days 1–4 (Tukey's *post hoc* test: $p = 0.0420$ and $p = 0.0151$, respectively), the CBD:THC combination of both did not ($p = 0.5523$; Fig. 1C, D).

Interestingly, the latency to target on day 5 revealed a significant improvement in CBD:THC-treated mice over vehicle-treated mice (Tukey's *post hoc* test: $p = 0.0319$) (Fig. 1D).

Anxiety. To measure anxiety-like behavior, we quantified the time spent in the open arms of the EPM. We found that 5xFAD mice showed decreased levels of anxiety compared with their WT counterparts ($p = 0.0064$; Fig. 2A). In addition, THC-treated 5xFAD mice showed increased anxiety [decreased time in open arms; $F(3, 50) = 2.623$, $p = 0.0607$; Tukey's *post hoc* test: $p = 0.048$] (Fig. 2B). No changes were observed in mice exposed to CBD or CBD:THC (Fig. 2B).

Depression. The TS test was used as an indicator of the depressive state in mice. Our data showed no changes due to the amyloid pathology ($p = 0.9652$) (Fig. 2C). A significant increase in depressive-like behavior (increased immobility time) was observed only in THC-treated 5xFAD mice compared with vehicle-treated 5xFAD mice [$F(3, 72) = 2.991$, $p = 0.0365$; Tukey's *post hoc* test: $p = 0.0284$] (Fig. 2D).

Motor coordination. Analysis of the performance in the RR test showed that motor coordination was enhanced in 5xFAD mice ($p < 0.0001$; Fig. 2E). No

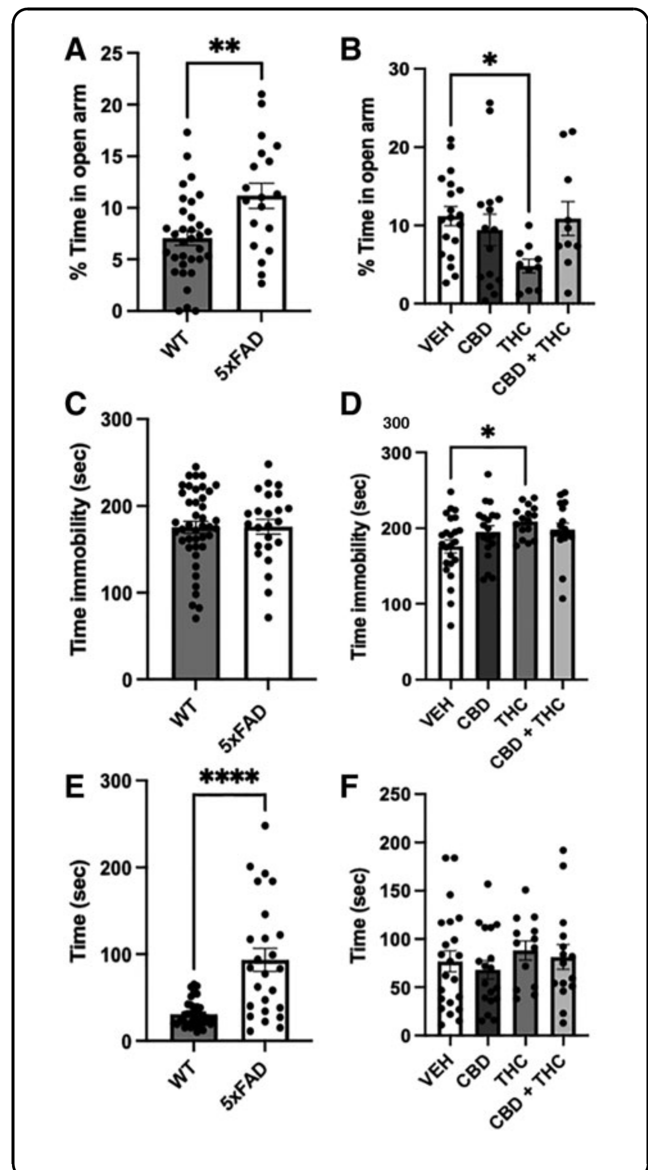

FIG. 2. Anxiety, depression, and locomotor coordination were measured using EPM **(A, B)**, TS **(C, D)**, and RR **(E, F)** tests, respectively. **(A, B)** The 5xFAD mice exhibited decreased anxious state compared with WT mice (**$p < 0.01$; Mann–Whitney test) and treatment with THC exacerbated anxiety in 5xFAD mice (*$p < 0.05$; one-way ANOVA, followed by Tukey's test for multiple comparisons). **(C, D)** No differences were found between WT and 5xFAD mice, and a significant increase in time of immobility was triggered by the exposure to THC (*$p < 0.05$; one-way ANOVA, followed by Tukey's test for multiple comparisons). **(E, F)** Locomotor coordination was enhanced in 5xFAD mice compared with WT mice (****$p < 0.0001$; two-tailed unpaired t test) and no differences were found to be associated with treatment with the different cannabinoids. $N = 43$ (WT mice) and $N = 16$–24 (5xFAD mice). EPM, elevated plus maze; RR, rotarod; TS, tail suspension.

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changes associated with cannabinoid treatments were evident (Kruskal–Wallis test; $p = 0.5004$; Fig. 2F).

Locomotor activity. The open-field test allows for determination of locomotor activity. Our data indicate that 5xFAD mice exhibited decreased locomotor activity (distance covered in the periphery and central part; $p < 0.0001$ and $p = 0.0192$, respectively) (Fig. 3C, D) that was not modified by treatment with any of the cannabinoids tested [periphery: $F(3, 70) = 0.2195$, $p = 0.8826$; center: $F(3, 70) = 1.316$, $p = 0.2762$] (Fig. 3).

Expression levels of cannabinoid receptors and markers of inflammation were unaltered by exposure to cannabinoids

We next analyzed the expression levels of cannabinoid *Cnr1* and *Cnr2* receptors and of several markers of neuroinflammation in the hippocampus (Fig. 4) and cortex (Fig. 5). No changes were observed in expression levels of cannabinoid CB_1 receptors due to the pathology or treatment with the different cannabinoids (Figs. 4A, 5A).

Regarding CB_2, a significant increase was detected in samples from 5xFAD mice compared with WT mice ($p < 0.0001$), but no changes were found after exposure to CBD, THC, or CBD:THC (Figs. 4B, 5B). As expected, all inflammatory markers studied (TNFα, IL1β, and IL6) were increased as a consequence of the increased amyloid production in 5xFAD mice compared with WT mice, both in the hippocampus and cortex ($p = 0.0002$ and $p = 0.0001$ for *Tnf*; $p = 0.001$ and $p < 0.0001$ for *Il1b*; and $p < 0.0001$ and $p < 0.0001$ for *Il6*) (Figs. 4C–E, 5C–E).

Exposure to CBD, THC, or the combination did not induce any changes in any of the parameters analyzed.

Protein levels of markers of gliosis and neuronal damage were not modified after treatment with cannabinoids

We employed western blot to quantify hippocampal and cortical levels of several proteins that are representative of the gliotic process and neurodegeneration and characteristic of the amyloid pathology. Protein levels

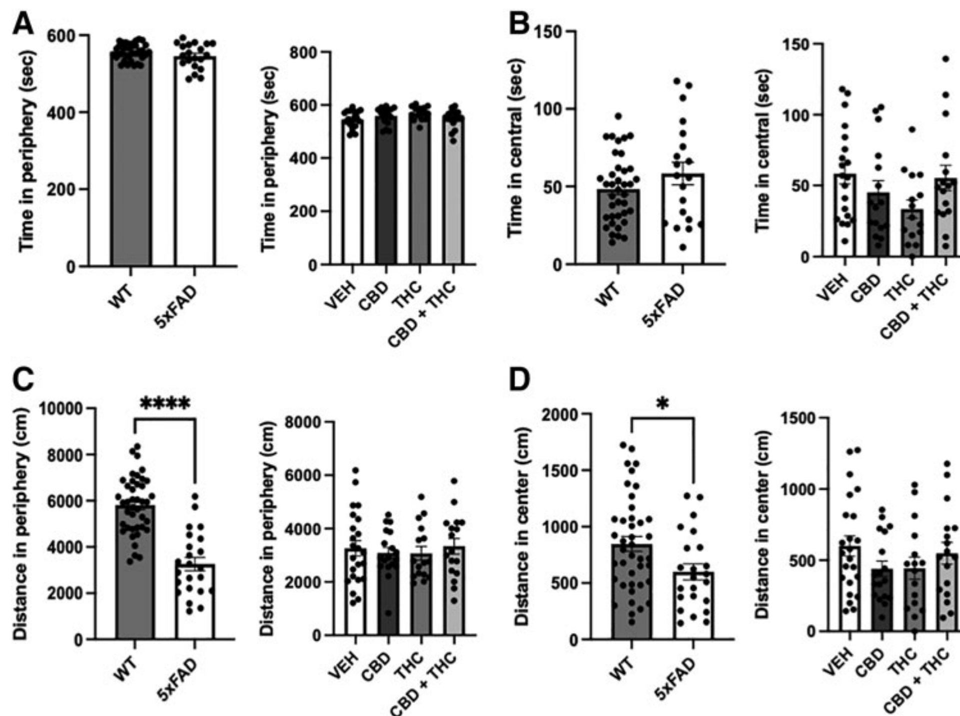

FIG. 3. The analysis of locomotor activity by the open-field test revealed no changes in time spent in peripheral or central areas of the arena **(A, B)** and showed a significant decrease in the distance covered **(C, D)** by 5xFAD mice compared with WT mice both in the periphery (****$p < 0.0001$; two-tailed unpaired *t* test) and in the center (*$p < 0.05$; two-tailed unpaired *t* test) of the field. Treatment with the different cannabinoids tested did not lead to any significant changes. $N = 43$ (WT mice) and $N = 16–24$ (5xFAD mice).

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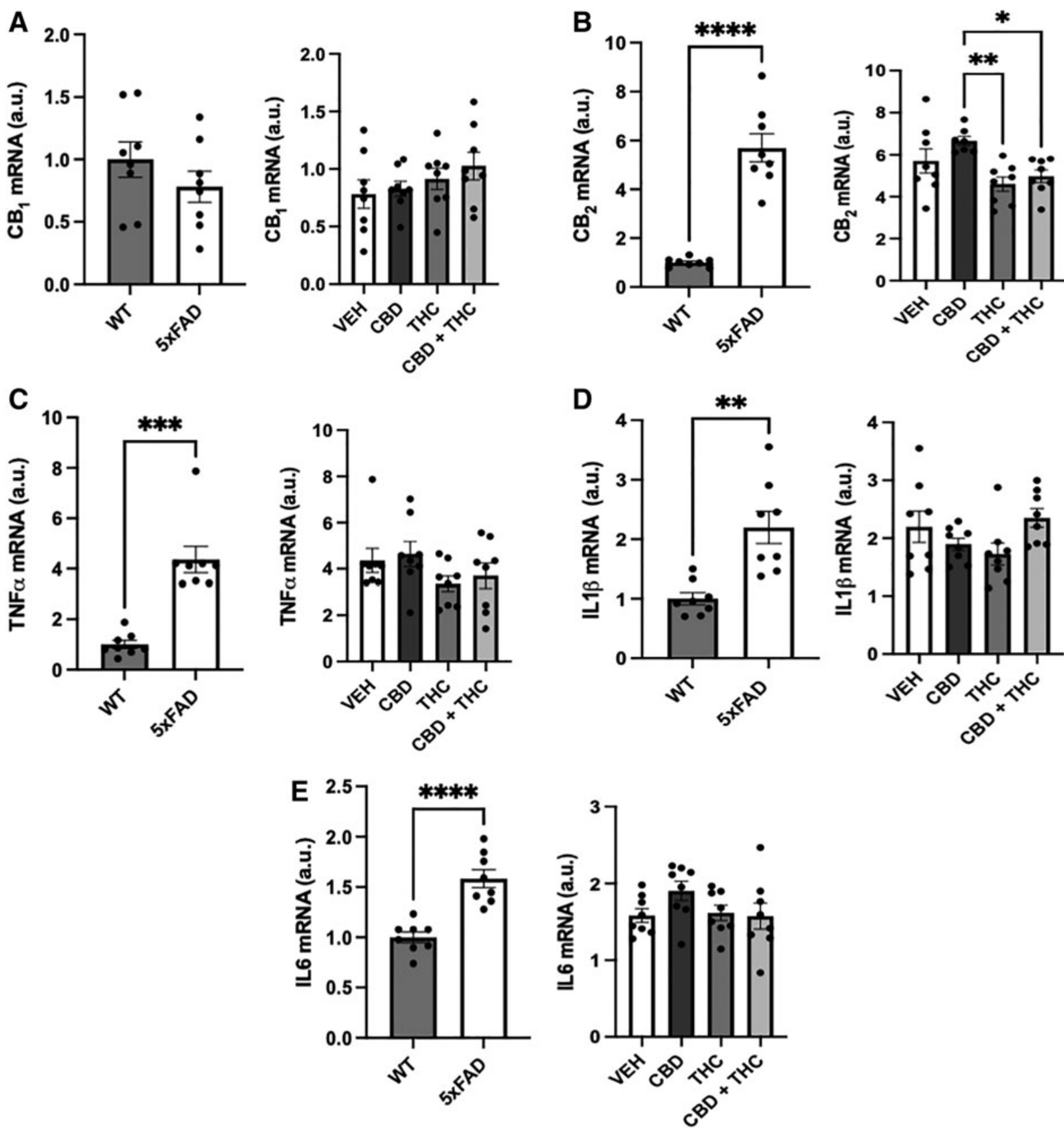

FIG. 4. Hippocampal mRNA levels of cannabinoid CB_1 and CB_2 receptors and several markers of neuroinflammation. Quantification of the expression levels of *Cnr1* (CB1 receptors) **(A)** showed no differences linked to the amyloid pathology or to exposure to the different cannabinoids tested. A significant increase in *Cnr2* (CB2 receptors) expression levels **(B)** was evident in 5xFAD versus WT mice, which was not modified after treatment with CBD, THC, or CBD:THC. Analysis of mRNA levels of *Tnf*, *Il1b*, and *Il6* **(C–E)** confirmed amyloid-induced changes that did not vary due to exposure to the cannabinoids tested (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$; two-tailed unpaired t test). $N = 8$ mice per group. mRNA, messenger RNA.

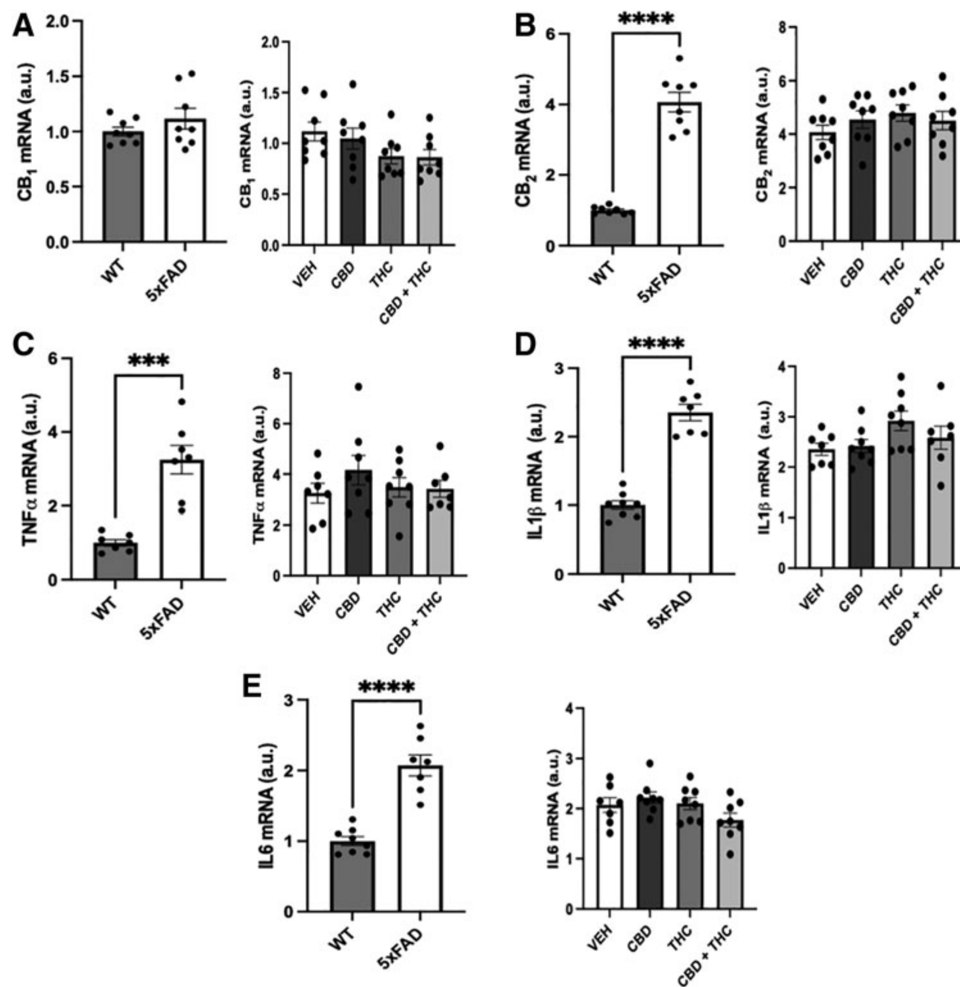

FIG. 5. Cortical mRNA levels of cannabinoid CB_1 and CB_2 receptors and several markers of neuroinflammation. Amyloid pathology led to significant increases in the expression levels of *Cnr2*, *Tnf*, *Il1b*, and *Il6* **(B–E)**, but not of *Cnr1* **(A)**. Treatment with CBD, THC, or CBD:THC did not induce any significant changes (***$p < 0.001$ and ****$p < 0.0001$; two-tailed unpaired *t* test). $N = 8$ mice per group.

of Iba1, GFAP, synaptophysin, and PSD95 were determined. Our data confirmed the expected astrogliosis and microgliosis that are characteristic of 5xFAD mice, with significant increases in GFAP in the hippocampus ($p = 0.0027$) and cortex ($p = 0.0098$) and Iba1 ($p = 0.041$ and $p = 0.0003$, respectively) proteins (Figs. 6A, 6B, 7A, 7B). Treatment with the different cannabinoids, however, did not modify protein levels of the gliosis markers.

Quantification of markers of synaptic integrity revealed a decrease in the presynaptic marker, synaptophysin, in the cortex due to the AD-like pathology ($p = 0.0278$; Fig. 6C), but not of PSD95 (Figs. 6D, 7D). No changes in these markers were observed after exposure to cannabinoids (Figs. 6C, 6D, 7C, 7D).

Aβ 1–42 levels

Finally, we quantified the hippocampal levels of the main pathological form of the amyloid peptide (Aβ 1–42) by ELISA. We found that while soluble amyloid levels remained unaltered after treatment with cannabinoids [$F(3, 28) = 0.9667$, $p = 0.4222$] (Fig. 8A), those of insoluble amyloid were significantly modified [$F(3, 28) = 10084$, $p < 0.0001$] (Fig. 8B).

Thus, exposure to CBD, THC, or the combination of both induced significant increases in respect to vehicle-

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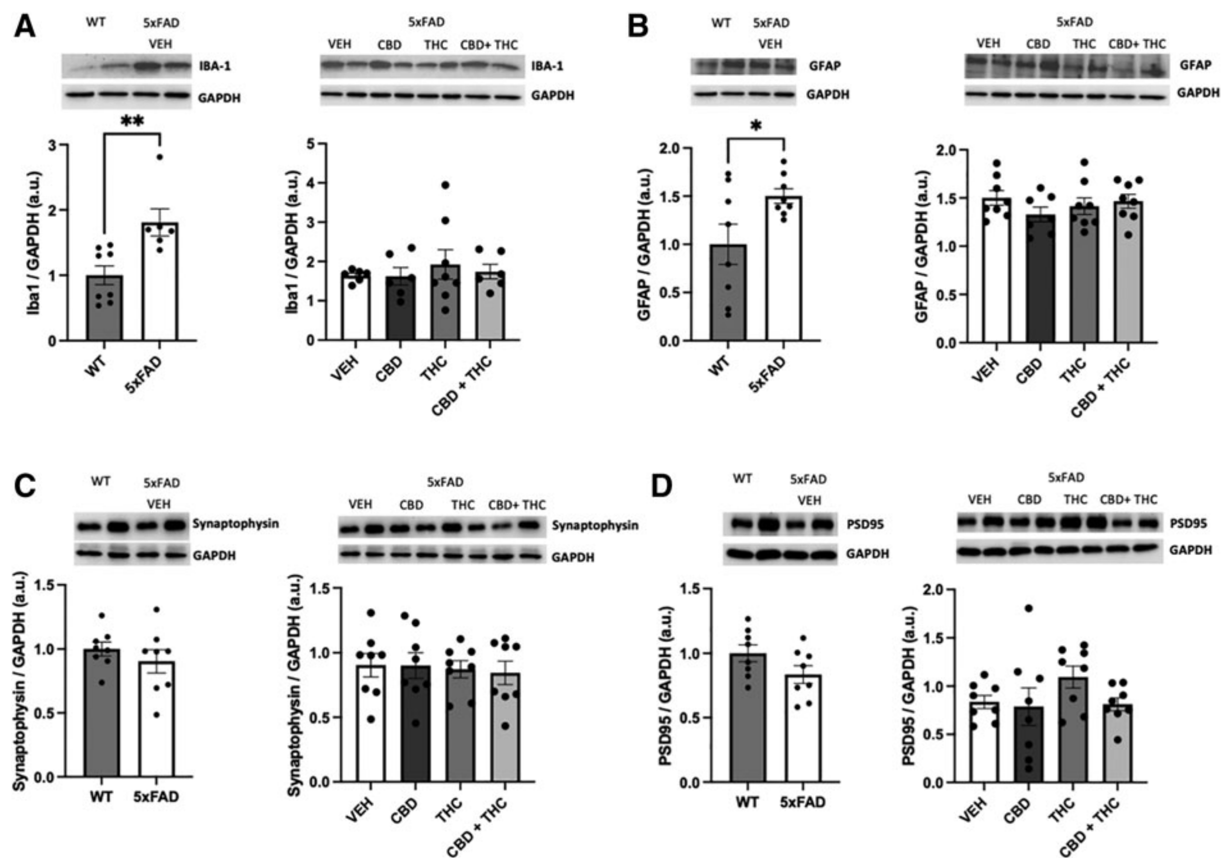

FIG. 6. Hippocampal protein levels indicative of gliosis and neuronal integrity. The 5xFAD mice exhibited increased microgliosis **(A)** and astrogliosis **(B)** and no changes in the levels of the presynaptic marker, synaptophysin **(C)**, and the postsynaptic marker, PSD95 **(D)**. No changes were found after treatment with the cannabinoids tested (* $p < 0.05$; ** $p < 0.01$; two-tailed unpaired *t* test). $N = 8$ mice per group.

treated 5xFAD mice. Furthermore, this increase was maximal in CBD:THC-treated mice (Tukey's *post hoc* test: $p < 0.0001$) followed by THC-treated mice and, in lower levels, CBD-treated mice (Fig. 8B).

Discussion

In this study, we report the effects of the treatment with CBD, THC, or a mixture of both on behavioral and molecular parameters in the 5xFAD mouse model of AD. The main findings of this study are that (1) THC enhanced anxiety and depression (EPM and TS tests), (2) CBD and THC showed different effects when administered alone than in combination (BM test), and (3) all treatments with these cannabinoids led to an increase in the insoluble form of Aβ.

Importantly, these effects were not accompanied by significant changes in molecular parameters of inflammation at the messenger RNA (mRNA) or protein level. We also confirmed previously published observations on 5xFAD mice, such as increased motor coordination of these mice over controls at 6 months of age,[42] decreased locomotion,[43] and induction of proinflammatory markers as a consequence of exacerbated amyloid production.[26]

THC-induced amnesic effects are known to be mediated by hippocampal CB_1 receptors,[44] expressed in GABAergic interneurons, and involve postsynaptic NMDA glutamatergic receptors.[45] Interestingly, low doses (1 and 3 mg/kg) of THC induce improvements in cognitive performance in aged mice.[46,47] Furthermore, the coadministration of CBD prevented this

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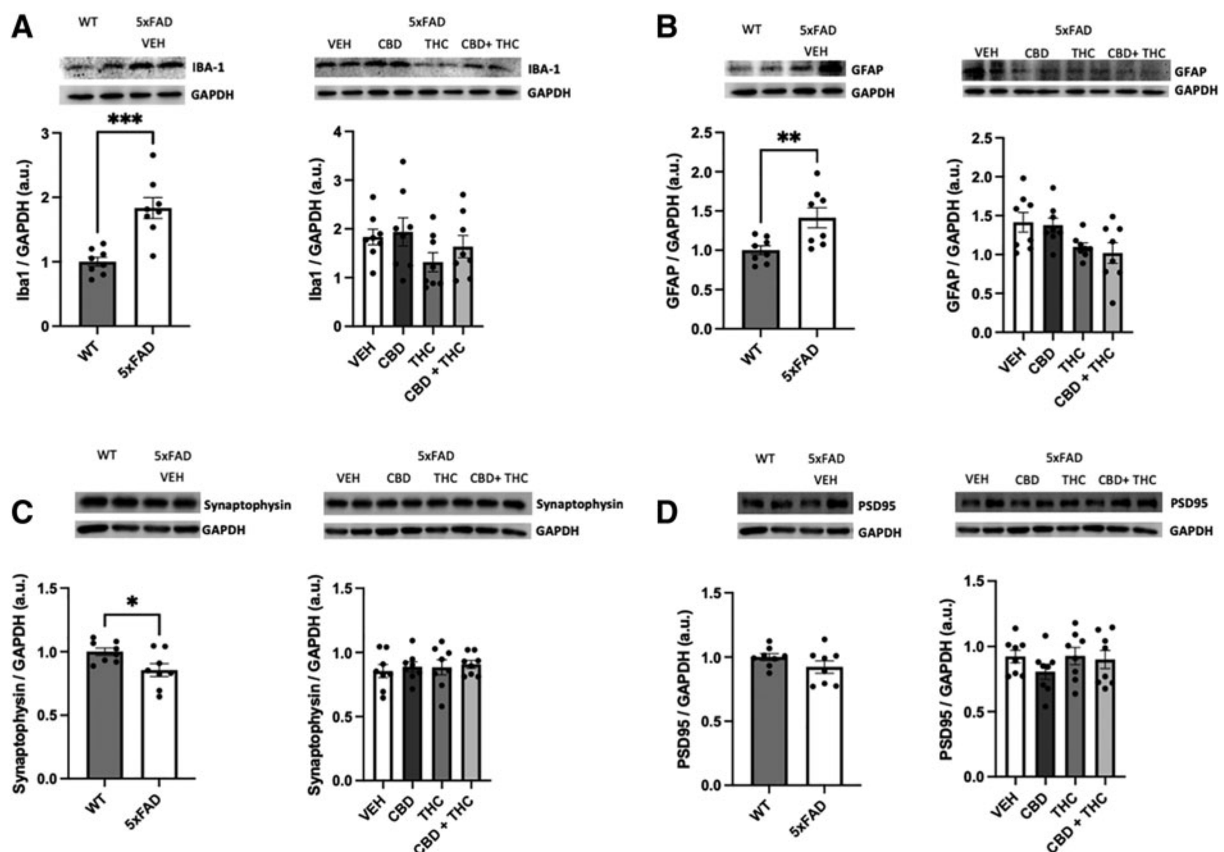

FIG. 7. Cortical protein levels indicative of gliosis and neuronal integrity. The 5xFAD mice exhibited increased microgliosis **(A)** and astrogliosis **(B)**, together with decreased levels of the presynaptic marker, synaptophysin **(C)**, and no changes in the postsynaptic marker, PSD95 **(D)**. No changes were found after treatment with the cannabinoids tested (*$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$; two-tailed unpaired t test). $N = 8$ mice per group.

beneficial effect.[47] In our study, CBD also impaired learning working memory in 5xFAD mice (Fig. 1).

This observation is in contrast with previously published data reporting a beneficial effect of this cannabinoid in both *in vitro* and *in vivo* models of AD (reviewed by Watt and Karl[48]). We do not have an explanation for this observation, other than the dose employed and the age of mice (8 months old) differing from previously published literature. As has been recently highlighted,[49] dosage, ratios, route of administration, and age of mice may be crucial determinants to explain the disparity in data after administration of cannabinoids in the context of age-associated behavioral and molecular effects.

Our data also suggest the existence of an antagonizing effect between CBD and THC, leading to significantly different consequences when used in combination in the context of amyloid-induced memory impairment.[10] This is in agreement with recently published reports demonstrating that CBD is capable of dampening the psychoactive effects of THC, including memory impairment.[49]

After its description by Ben-Shabat et al.,[50] different mechanisms have been suggested to explain how nonactive cannabinoids may modulate the activity of, for instance, THC. Examples of these interactions between phytocannabinoids and endocannabinoids have been obtained in pre-clinical models of cancer and pain, but no conclusive data have been reported yet. It has been speculated, for instance, cannabinoids may regulate the interplay of other cannabinoids with CB_1 and/or CB_2, alter the metabolism of

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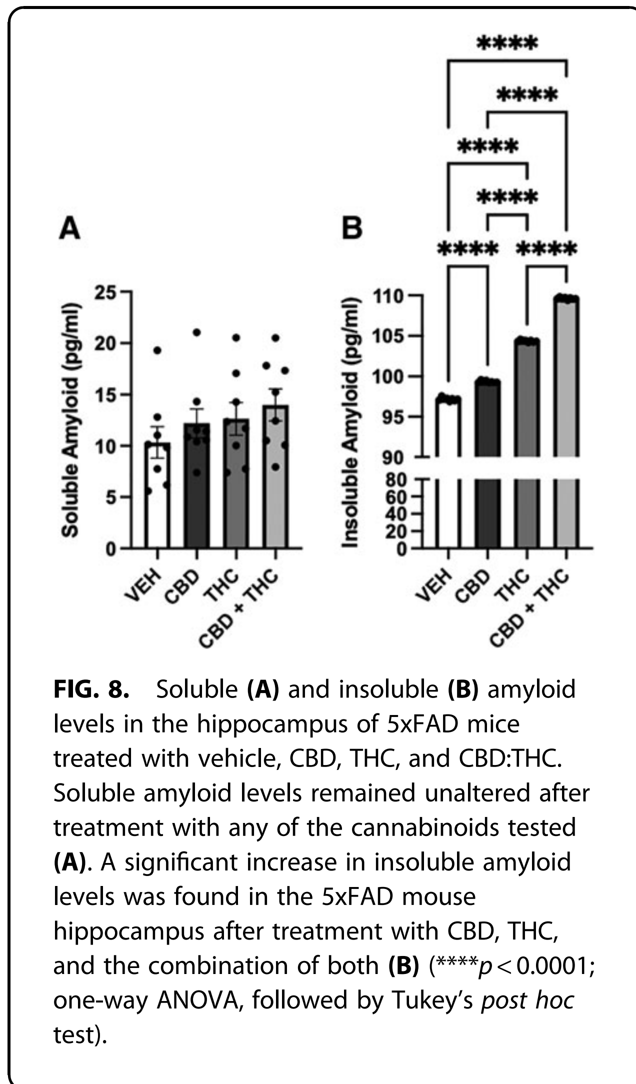

FIG. 8. Soluble **(A)** and insoluble **(B)** amyloid levels in the hippocampus of 5xFAD mice treated with vehicle, CBD, THC, and CBD:THC. Soluble amyloid levels remained unaltered after treatment with any of the cannabinoids tested **(A)**. A significant increase in insoluble amyloid levels was found in the 5xFAD mouse hippocampus after treatment with CBD, THC, and the combination of both **(B)** (****$p < 0.0001$; one-way ANOVA, followed by Tukey's *post hoc* test).

endocannabinoids, or enhance the interaction with additional receptors such as TRPV1 or GPR55.[51]

The molecular basis for these effects is not clear. We have measured inflammatory parameters both in the hippocampus and cortex because, as previously shown by Negrón-Oyarzo et al.,[52] the interplay between these two brain structures is critical for spatial memory consolidation. We found no significant differences in neuroinflammation-related parameters in the hippocampus or cortex after exposure to any of the cannabinoids tested, thus ruling out a possible effect on neuroinflammatory conditions as a putative explanation for the observed changes in memory.

Aso et al. addressed this question in the AβPP/PS1 mouse model of AD.[53,54] These authors chronically treated 6- and 12-month-old mice with low doses (0.75 mg/kg) of plant-extracted THC, CBD, and a combination of both and found a cannabinoid-induced improvement in memory impairment. Interestingly, mice exposed to the CBD:THC mixture also exhibited improvement in a complex cognitive task, such as the active avoidance test.[54]

Discrepancies between our present data and those reported by these authors may be due to the drugs used (plant-extracted vs. synthetic drugs), doses administered (0.75 mg/kg vs. 0.273 and 0.205 mg/kg for CBD and THC, respectively), and time of testing (5 weeks of treatment vs. 28 days).

Our data also revealed that 5xFAD mice exhibited decreased anxiety compared with their WT littermates, but that treatment of 5xFAD mice with THC alone led to increased anxiety, as revealed by the EPM test. These data parallel those recently reported by Forner et al.,[55] who found a significant increase in the time spent in the open arm of the EPM by 5xFAD mice and confirm previous observations linking THC with the development of an anxious state in mice (recently reviewed by Iglesias et al.[56]).

We also observed significant increases in the insoluble form of Aβ in CBD-, THC-, and CBD:THC-treated mice, being maximal in this last group. These data are in partial agreement with those of Aso et al.,[53] who found a facilitative effect of the CBD:THC mixture on Aβ deposition. On the contrary, these authors found no changes in plaque composition in CBD- and THC-treated mice.

In vitro[57,58] and molecular modeling[59,60] studies have shown that THC is capable of modifying Aβ fibrillation, although the biological relevance of these interactions is not clear. It is now established that the complexity of the diverse amyloid species may be a critical contributing factor to AD pathology.[61] Increasing toxicity is significantly associated with soluble low-molecular-mass oligomers, which are significantly more neurotoxic and interfere with key neuronal functions such as long-term potentiation.[62] Soluble oligomeric species show a high surface-to-volume ratio and hydrophobic-like properties and are prone to bind to membranes, which lead to pore formation and membrane permeability.[3,59]

As well as exhibiting structural differences in the monomeric state, oligomers may initiate aberrant cellular processes through specific and non-specific interactions with receptors, mitochondria, synaptic vesicles, and membranes, leading to aberrant signaling or cellular dysfunction.[3] In this line of reasoning, the increase

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in insoluble amyloid levels observed herein might be considered as a protective effect, directed to prevent the spread of damage linked to small-sized oligomers.

In addition to THC and CBD, other plant cannabinoids have been recently tested on their putative effects on amyloid-induced damage. A study by Patil et al. has demonstrated that cannabidiorcol (a phytocannabinoid present in trace amounts in *C. sativa*) and cannabinol inhibited enzymes related to AD, including acetylcholinesterase and butyrylcholinesterase.[63]

In addition, delta-8-tetrahydrocannabinol (an isomer of THC) has been recently shown to inhibit endoplasmic reticulum stress, leading to enhancement in cell viability in an *in vitro* model of amyloid-induced toxicity.[64] This phytocannabinoid also prevented neuronal apoptosis by inhibiting Bax and increasing Bcl-2 protein levels.

Finally, the *in vivo* and *in vitro* protective effects of the acidic variants of CBD and THC, cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA), respectively, have been studied by Kim et al.[65] These authors found that both CBDA and THCA decreased Aβ and p-tau levels and enhanced the neuroprotective effect of the brain-derived neurotrophic factor by normalizing intracellular calcium levels in neurons.

Altogether, these novel data highlight the potential of different cannabinoids as effective modulators of amyloid-induced alterations.

Authors' Contributions

M.A.A. and S.R.M.E. were involved in methodology and investigation; A.M.M.R. and M.T.G. were involved in methodology, visualization, and investigation; N.R. was involved in conceptualization, resources, and writing; N.T.Z. was involved in conceptualization and resources; and J.R. was involved in visualization, supervision, and writing.

Author Disclosure Statement

The authors declare no conflicts of interest.

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Abbreviations Used

Aβ = amyloid beta
AD = Alzheimer's disease
ANOVA = analysis of variance
BM = Barnes maze
CBD = cannabidiol
CBDA = cannabidiolic acid
ELISA = enzyme-linked immunosorbent assay
EPM = elevated plus maze
mRNA = messenger RNA
RR = rotarod
THC = delta-9-tetrahydrocannabinol
THCA = tetrahydrocannabinolic acid
TRPV1 = transient receptor potential vanilloid-1
TS = tail suspension
WT = wild-type

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