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Cannabinol (CBN) alleviates age-related cognitive decline by improving synaptic and mitochondrial health

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ABSTRACT

Age-related cognitive decline and neurodegenerative diseases, such as Alzheimer's disease, represent major global health challenges, particularly with an aging population. Mitochondrial dysfunction appears to play a central role in the pathophysiology of these conditions by driving redox dysregulation and impairing cellular energy metabolism. Despite extensive research, effective therapeutic options remain limited. Cannabinol (CBN), a cannabinoid previously identified as a potent inhibitor of oxytosis/ferroptosis through mitochondrial modulation, has demonstrated promising neuroprotective effects. In cell culture, CBN targets mitochondria, preserving mitochondrial membrane potential, enhancing antioxidant defenses and regulating bioenergetic processes. However, the *in vivo* therapeutic potential of CBN, particularly in aging models, has not been thoroughly explored. To address this gap, this study investigated the effects of CBN on age-associated cognitive decline and metabolic dysfunction using the SAMP8 mouse model of accelerated aging. Our results show that CBN significantly improves spatial learning and memory, with more pronounced cognitive benefits observed in female mice. These cognitive improvements are accompanied by sex-specific changes in metabolic parameters, such as enhanced oxygen consumption and energy expenditure. Mechanistically, CBN modulates key regulators of mitochondrial dynamics, including mitofusin 2 (MFN2) and dynamin-related protein 1 (DRP1), while upregulating markers of mitochondrial biogenesis including mitochondrial transcription factor A (TFAM) and translocase of outer mitochondrial membrane 20 (TOM20). Additionally, CBN upregulates key synaptic proteins involved in vesicle trafficking and postsynaptic signaling suggesting that it enhances synaptic function and neurotransmission, further reinforcing its neuroprotective effects. This study provides *in vivo* evidence supporting CBN's potential to mitigate age-related cognitive and metabolic dysfunction, with notable sex-specific effects, highlighting its promise for neurodegenerative diseases and cognitive decline.

1. Introduction

Age-related cognitive decline and neurodegenerative disorders represent a major global health concern, exacerbated by the rapidly increasing aging population [1]. Cognitive impairment in older adults has been linked to mitochondrial dysfunction, oxidative stress and impaired energy metabolism, all of which appear to play a role in the pathophysiology of neurodegenerative diseases, including Alzheimer's disease (AD) [2,3]. This growing understanding has broadened the focus of research towards identifying therapeutic agents capable of mitigating these pathological mechanisms. Despite significant research on mitochondrial dysfunction and its role in cognitive decline, therapeutic interventions remain largely ineffective [4,5]. Current pharmacological approaches primarily target symptom management rather than addressing the underlying metabolic and mitochondrial deficits associated with neurodegeneration [6]. This highlights the urgent need for novel neuroprotective strategies that can modulate mitochondrial homeostasis, redox balance, and synaptic function. Cannabis-derived compounds, including non-psychoactive cannabinoids, have gained attention as potential neuroprotective agents [7]. Among these, cannabinol (CBN) has emerged as a promising candidate due to its reported antioxidant and anti-inflammatory properties, coupled with its ability to modulate mitochondrial function [8]. Notably, mitochondria are vital for neuronal health, playing a key role in energy production, calcium homeostasis, and the regulation of oxytosis/ferroptosis [9,10]. The delicate balance of mitochondrial fusion and fission dynamics is critical

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for maintaining mitochondrial homeostasis, with disruptions in these processes implicated in cognitive decline and neurodegeneration [11]. A previous study from our laboratory demonstrated that CBN protects cultured neuronal cells from oxytosis/ferroptosis, a regulated form of cell death associated with neurodegenerative diseases [8,12]. CBN was found to exert its neuroprotective effects by directly targeting mitochondria independent of traditional cannabinoid receptors, preserving mitochondrial membrane potential, enhancing antioxidant defenses and regulating bioenergetic processes. These findings provided a strong rationale for investigating CBN's therapeutic potential *in vivo*, particularly in mouse models of age-related cognitive decline. While the SAMP8 model exhibits age-related cognitive impairments, it does not develop classical hallmark AD pathologies such as amyloid plaques or tau tangles. However, with age, the brains of SAMP8 mice do exhibit some of the pathophysiological hallmarks of AD including abnormal amyloid-β (Aβ) accumulation in the vasculature, hyperphosphorylation of tau [13,14], increased oxidative stress, mitochondrial dysfunction and synaptic deficits making it a valuable model for studying aging-related cognitive decline. To further expand on these findings, we investigated the effects of CBN on cognitive performance, metabolic parameters, and mitochondrial function in SAMP8 mice, providing a robust framework for potential neurodegenerative interventions.

2. Material and methods

2.1. Study design

In this study, we investigated the effects of cannabinol (CBN) on male and female aging-accelerated SAMP8 mice. The study included three groups: 9-month-old SAMP8 mice serving as the younger control group, 13-month-old SAMP8 mice as the aged control group, and 13-month-old SAMP8 mice treated with CBN (provided by FloraWorks, 99.9 % purity). The CBN-treated mice received either 200 ppm or 400 ppm of CBN in their diet for 4 months, beginning at 9 months of age. The diets used were LabDiet 5015 re-pelleted as the vehicle control, LabDiet 5015/200PPM for the 200 ppm (200 mg CBN/kg diet) CBN group, and LabDiet 5015/400PPM for the 400 ppm (400 mg CBN/kg diet) CBN group. All diets were prepared by TestDiet (Richmond, IN). The major constituents of LabDiet 5015 include ground wheat, dehulled soybean meal, ground corn, wheat germ, brewers dried yeast, porcine animal fat, condensed whey, and various vitamins and minerals. Importantly, both the control and CBN-supplemented diets share all of the same basic ingredients, ensuring that any observed effects are attributable to CBN rather than dietary components. The 9-month and 13-month untreated groups indicated the progression of age-related cognitive decline and metabolic changes in the SAMP8 model. The CBN-treated groups at 13 months enabled us to evaluate how CBN intervention might mitigate these age-associated changes. The 13-month timepoint in SAMP8 mice represents a critical transitional stage with moderate pathology that parallels early cognitive decline in humans. While there is no agreed-upon direct equivalence between mouse and human ages, however 13 months in SAMP8 mice, due to their unique phenotype, corresponds to late middle age or early elderly in humans, a period when cognitive decline and neurodegenerative processes become clinically significant. This age was therefore selected to model a stage when pathology is pronounced but not end-stage, allowing us to test interventions at a window that is relevant to early-to-moderate human disease [15–17]. By comparing the 13-month CBN-treated groups (at 200 ppm and 400 ppm) with their age-matched untreated controls, we could directly assess the impact of CBN on cognitive function, metabolic parameters, and mitochondrial dynamics in aged SAMP8 mice. These concentrations in the diet correspond to ~10 and 20 mg/kg/day for the mice which is equivalent to ~57 and 114 mg/day for a 70 kg human. Both male and female mice were included to investigate any sex-specific effects. This experimental setup provided a comprehensive view of the potential therapeutic effects of CBN in age-related cognitive decline and metabolic dysfunction. The study was initiated with a cohort of 80 SAMP8 mice, equally distributed between males and females (n = 40 per sex). As expected in longitudinal aging studies, some attrition occurred over the course of the experiment. Although the final analysis included a total of 68 mice, figure legends may report slightly different sample sizes, reflecting variations due to specific assay requirements or exclusions. The attrition rate was within the expected range for aging studies using SAMP8 mice, known for their accelerated aging phenotype. Despite the attrition, the remaining sample sizes were sufficient to maintain statistical power, allowing for a robust evaluation of CBN effects while considering both age and sex-related variables.

2.2. Detection of cannabinol (CBN) in plasma via targeted LC-MS/MS

Targeted metabolomics analysis of cannabinol (CBN) in the mouse plasma was conducted by the Salk Institute Mass Spectrometry Core. CBN was extracted with a 1:4 ratio of plasma to hexane:ethyl acetate solution (9:1). Plasma samples were vortexed and centrifuged (10,000×*g*, 4 °C, 10 min). The organic layer was transferred to a new tube and dried under nitrogen gas. Samples were reconstituted in methanol prior to injection. Samples were analyzed on a Dionex Ultimate 3000 LC system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) coupled to a TSQ Quantiva mass spectrometer (Thermo Fisher Scientific) fitted with a Waters BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters Corporation, Milford, MA, USA). LC solvents consisted of solution A, 0.1 % formic acid in water and solution B, 0.1 % formic acid in acetonitrile. Total run time was 30 min with a flow rate of 0.4 mL/min. The gradient was as follows: 20 % B from 0 to 1 min, 20–80 % B from 1 to 15 min, 80–100 % B from 15 to 21 min, 100 % B from 21 to 25 min, 100-20 % B from 25 to 25.1 min, and 20 % B from 25.1 to 30 min. The sample injection volume was 20 μL, column oven temperature was set to 30 °C, and the autosampler at 4 °C. MS analysis was performed using electrospray ionization in positive mode, spray voltages of 3500 V, ion transfer tube temperature of 342 °C, and vaporizer temperature of 358 °C. Multiple reaction monitoring (MRM) was performed with a CBN precursor mass of 311.17 and quantifier product ion of 222.94, as well as the qualifier product ions' 207.9, 195.12, 202.99, 213.33. A CBN chemical standard was used to optimize instrument parameters for CBN detection and enabled additional confirmation of identification via retention time match of 15.3 min. Peak areas were measured using the software Skyline 24.1 [18]. CBN concentrations in plasma were quantified using a 10-point external calibration curve (9.7–5000 ng/mL) processed identically to study samples. The calibration curve (Peak Area = 3986.6 × Concentration + 18,251; $R^2 = 0.9998$) was used to calculate sample concentrations by solving for concentration. A volume correction factor was applied to account for reconstitution (final concentration = calculated concentration × [100 μL/75 μL]. Controls with peak areas below the lowest calibration standard were reported as not detected (ND).

2.3. Study approval

All experiments were performed in accordance with the U.S. Public Health Service Guide for Care and Use of Laboratory Animals and protocols approved by the IACUC at the Salk Institute. Mice were housed in an environmentally controlled vivarium with lights on from 06:00–18:00h, temperature at 20–23 °C, lighting levels at 300 lux (less than 50 lux in the cages) and humidity at 40–60 %. Cage cleaning was not performed on the same day as any of the behavioral testing. Food and water were available ad libitum.

2.4. Behavioral assays

Elevated Plus Maze (EPM): The EPM test was conducted following established procedures as previously described [19]. The maze consists of four arms arranged in a plus-shape, two of which are enclosed with

15.25 cm high walls, and the other two are open. The point at which the open and closed arms cross is called the center zone and is considered to be a decision zone, neither open nor closed. Each arm measures 30 cm in length and 5 cm in width and the arms are elevated above the floor by 50 cm. A video-tracking system (AnyMaze) was connected to a video camera directly above the plus maze and utilized to automatically record the exploratory activity of the mice into open or closed arms, as well as the time spent in each. Mice underwent a 30 min acclimatization period in the testing room prior to testing. The mice were observed for 5 min during which behavioral data were recorded. Disinhibition, a measure of impairment in executive function, was assessed by comparing the time spent in the open arms versus the closed arms.

Barnes Maze: The Barnes maze is a circular platform (90 cm in diameter) with 20 evenly spaced holes (5 cm in diameter) around its perimeter, one of which leads to a dark hide box while the others are false openings, too small for entry. The platform was lit by a bright light (approx. 100 lux). Cues are placed on the walls adjacent to the maze with of the compass points North, East, South and West having a different shape to aid with spatial navigation. The primary measure is the latency to locate the hide box. In the training phase, a hide box was placed under one hole. Each animal was placed in the center of the maze within an opaque cylinder for 30 s to induce spatial disorientation. After the cylinder was removed, the animal was allowed to explore the maze for a total of 3 min or until it located the hide box, with incorrect entries, velocity and distance traveled being recorded via a camera connected to a laptop using Anymaze video-tracking software. If the animal failed to find the box within 3 min, it was gently guided to the correct location. Once inside, the animal remained in the box for 20 s before being returned to its home cage. 24h after an initial habituation session of 3 min, training sessions were repeated three times daily over a 4-day period, with the hide box location remaining consistent for each animal but randomized across animals to control for unintended bias to one area of the platform. After a 2-day break, 90sec trial was conducted to assess memory retention, no hide box available. After the retention trial, mice were given a 3 min "refresher" trial with the hide box in the same location as during the training trials. 24h after the retention trial, the hide box was moved to a position 180° opposite to the initial training location ("reversal" trial) and the animal trained to find the new location for a maximum of 3 min per trial. The reversal trial session was repeated 3 times on 2 separate days.

2.5. Metabolic chamber analysis

All mice were housed under controlled environmental conditions with a 12-h light/dark cycle and had ad libitum access to food and water. Physiological parameters, including oxygen consumption (VO2), carbon dioxide production (VCO2), energy expenditure (EE), respiratory exchange ratio (RER), total activity (XYTOT), and ambulatory activity (XYAMB), were measured using an automated metabolic system. Measurements were recorded at approximately 26-min intervals to ensure accurate data collection. Data were processed using Python (version 3.x) with pandas, numpy, and scipy libraries. Descriptive statistics (mean and standard error of the mean) were computed for each parameter. Effect sizes (Cohen's d) were calculated to assess the practical significance of differences between CBN treatments and control. Body mass data were analyzed to control for potential confounding effects on the physiological measurements. This methodology aimed to provide a detailed assessment of CBN's effects on metabolic and activity parameters, accounting for potential differences between male and female mice.

2.6. Tissue preparation

Mice were anesthetized and their blood collected by cardiac puncture. After perfusing with PBS, their brains were removed and dissected to collect the hippocampus which was then processed for Western blotting.

2.7. Western blotting

Hippocampal tissue samples were homogenized in RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 % NP-40, 0.1 % SDS, 0.5 % sodium deoxycholate) supplemented with protease and phosphatase inhibitors. Homogenates were sonicated twice for 10 s each and centrifuged at 100,000×*g* for 60 min at 4 °C. Protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of protein (20–25 μg per sample) were resolved on 4–20 % Criterion precast gels (Bio-Rad, Hercules, CA, USA) under denaturing conditions and transferred onto PVDF membranes using a semi-dry transfer system. Membranes were blocked in 5 % non-fat skim milk in TBS-T buffer (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 0.1 % Tween-20) for 1 h at room temperature and incubated overnight at 4 °C with primary antibodies diluted in 5 % BSA in TBS-T (0.05 % Tween-20). The primary antibodies used in this study were anti-OPA1 (Cat# 80471, 1:3000), anti-SOD2 (Cat# 13141, 1:3000), anti-MFN2 (Cat# 9482, 1:3000), anti-TOM20 (Cat# 42406, 1:3000), anti-DRP1 (Cat# 8570, 1:3000), anti-VDAC (Cat# 4661, 1:3000), anti-MCU (Cat# 14997, 1:3000), anti-SNAP25 (A195, Cat# 4117, 1:3000) and anti-GAPDH (14C10 Rabbit mAb, Cat# 2118, 1:3000) from Cell Signaling Technology (Danvers, MA). Antibodies against total OXPHOS (Cat# ab110413, 1:3000) and TFAM (Cat# ab131607, 1:3000) were obtained from Abcam (Cambridge, MA). Anti-PSD95 (Cat# MA1-046, 1:1000) and anti-synaptophysin SP11 (Cat# MA5-16402, 1:1000) were purchased from Invitrogen. Horseradish peroxidase-conjugated secondary antibodies (Cat# 1706515 and 1706516) were obtained from Bio-Rad (Hercules, CA). Following three washes in TBS-T, membranes were incubated with HRP-conjugated secondary antibodies (1:5000 in 5 % skim milk in TBS-T) for 1 h at room temperature. Protein bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Pierce), and densitometry analysis was performed using ImageJ (NIH) with normalization to GAPDH.

2.8. Statistical analysis

Data are presented as the mean ± SEM with individual data points overlaid on bar graphs. Statistical analyses were performed using one-way ANOVA with Tukey's multiple comparisons post hoc test or Student's *t*-test where applicable. For behavioral data, Western blot results, and metabolic chamber experiments, p values less than 0.05 were considered statistically significant, with significance levels indicated as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.001$ (***). For metabolic chamber experiments, physiological parameters (VO_2, VCO_2, EE, RER, XYTOT, and XYAMB) were processed using Python (version 3.x) with pandas, numpy, and scipy libraries. Descriptive statistics (mean ± SEM) were computed, and one-way ANOVA was used to compare groups, with effect sizes (Cohen's d) calculated where applicable. Body mass was included as a covariate to adjust for potential confounding effects.

3. Results

3.1. Targeted LC-MS/MS analysis of cannabinol (CBN) in plasma

Targeted metabolomics analysis of plasma samples using LC-MS/MS confirmed the presence of cannabinol (CBN) in the plasma of mice following consumption of diets containing 200 ppm or 400 ppm CBN. In the control group, no detectable levels of CBN were observed (Suppl Fig. 1), confirming the absence of endogenous CBN or cross-contamination in untreated samples. In the 200 ppm CBN group, the average plasma CBN concentration was 8.62 ng/mL (corresponding to an average peak area of 86,155.67) (Suppl Fig. 2). In the 400 ppm CBN group, the average plasma concentration increased to 15.49 ng/mL

(average peak area: 282,014.13) (Suppl Fig. 3). These findings demonstrate that dietary CBN is effectively absorbed into the systemic circulation in a dose-dependent manner. The observed increase in peak area and corresponding concentrations with higher CBN doses supports the conclusion that plasma CBN levels correlate with dietary intake, reinforcing the reliability of LC-MS/MS quantification for CBN detection in biological matrices.

3.2. Behavioral Analysis of Male and Female SAMP8 mice: effects of age and CBN treatment

To evaluate the impact of cannabinol (CBN) on cognitive performance (learning, memory and executive function), we assessed male and female SAMP8 mice at two ages (9 months and 13 months) and at 13 months following treatment with CBN mixed into their food at two concentrations (200 ppm and 400 ppm). Behavioral outcomes were measured using the Barnes maze for learning and spatial memory and the Elevated Plus Maze (EPM) for disinhibition as a measure of impairment in executive function (Fig. 1A).

3.3. Barnes Maze (latency to find the target hole)

In male SAMP8 mice, no significant change in the latency to find the target hole was observed in the 13-month-old group compared to the 9-month-old group, and CBN had no effect either (Fig. 1B). In contrast, female SAMP8 mice exhibited a significant increase in latency with age, reflecting a decline in spatial learning efficiency in the 13-month-old group compared to their younger counterparts (Fig. 1E). CBN treatment significantly improved performance in the older females. However, while the 400 ppm dose appeared to show further improvement, the difference between the doses was not statistically significant (Fig. 1E). These findings highlight that while male SAMP8 mice maintained consistent spatial learning performance across age groups regardless of CBN treatment, females benefited more markedly from CBN, suggesting a sex-dependent response to CBN treatment.

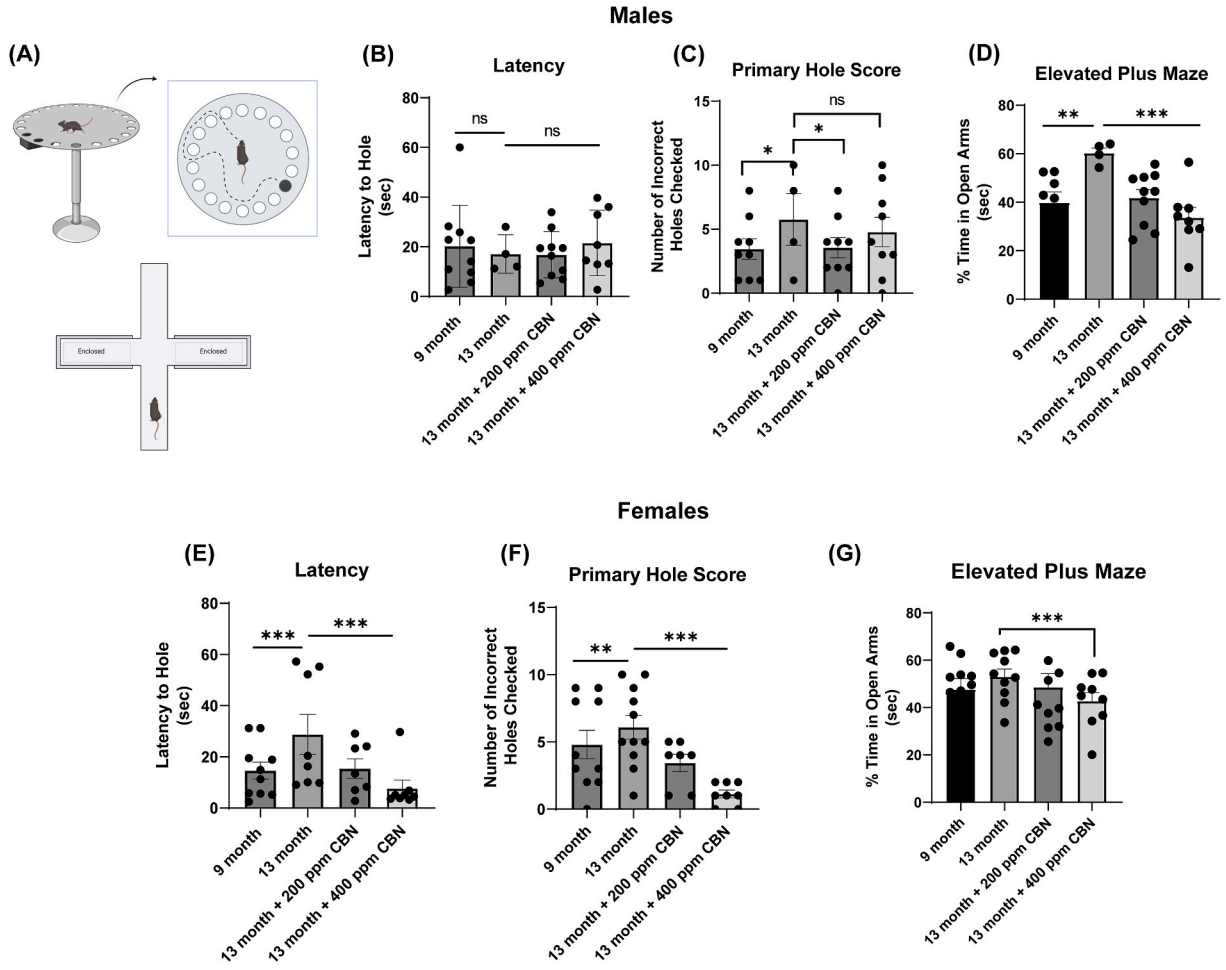

Fig. 1. Behavioral Analysis of Male and Female SAMP8 Mice in Response to CBN Treatment
(A) Schematic of the Barnes maze setup for assessing spatial memory and learning and the Elevated Plus Maze (EPM) protocol for evaluating disinhibition. (B, E) Latency to locate the target hole in the Barnes maze. In males (B), no significant effects of time or treatment were observed. In females (E), latency increased with age, indicating reduced spatial learning efficiency. CBN treatment, particularly at 400 ppm, significantly improved performance. (C, F) Primary hole score in the Barnes maze. In males (C), CBN treatment at 200 ppm mitigated the age-related increase in primary hole score in 13-month-old mice, but no significant effect was observed at 400 ppm. In females (F), the primary hole score increased with age but was significantly reduced by CBN treatment, with the greatest improvement at 400 ppm. (D, G) Time spent in the open arms of the EPM, a measure of disinhibition. In males (D), CBN treatment significantly reduced time spent in the open arms at 13 months, with a stronger effect observed at 400 ppm. In females (G), a significant reduction in time spent in the open arms was only observed in 13-month-old mice treated with CBN 400 ppm, while no significant effect was observed with CBN 200 ppm or between 9-month-old and 13-month-old females. Number of mice per group: 9-month control (males: n = 10, females: n = 10), 13-month control (males: n = 4, females: n = 10), CBN 200 ppm (males: n = 10, females: n = 7), CBN 400 ppm (males: n = 8, females: n = 8). Statistical tests: One-way ANOVA with Tukey's multiple comparisons post hoc test. * indicates significance levels: $^{**}p < 0.01$, $^{***}p < 0.001$, ns = not significant. Bar graphs represent mean ± SEM with individual data points superimposed to show variability across biological replicates.

3.4. Barnes Maze (primary hole score)

Male SAMP8 mice demonstrated an age-related increase in the number of incorrect holes checked (primary hole score) in the 13-month-old group compared to the 9-month-old group. This age-related decline in performance was mitigated by CBN treatment at 200 ppm but not at 400 ppm (Fig. 1C). Female SAMP8 mice also exhibited significant age-related differences in primary hole score, with older females making more errors than younger ones (Fig. 1F). Interestingly, CBN treatment at both concentrations (200 ppm and 400 ppm) significantly reduced the primary hole score in 13-month-old females, with the 400 ppm group showing the most pronounced improvement (Fig. 2C). These results suggest that CBN enhances spatial learning accuracy in aged mice, particularly in females, highlighting its potential as a cognitive enhancer.

3.5. Elevated Plus Maze (disinhibition)

The EPM results indicate that male SAMP8 mice displayed an increase in disinhibited behavior with age, as 13-month-old males spent more time in the open arms compared to 9-month-old males (Fig. 1D). Both the 200 ppm and 400 ppm doses of CBN significantly reduced the time spent in the open arms among older males, with the higher dose showing a more pronounced effect. In female SAMP8 mice, a significant difference was observed only between the 13-month-old and 13-month-old females treated with CBN 400 ppm. CBN treatment at 400 ppm demonstrated a significant decrease in disinhibited behavior compared to untreated older females (Fig. 1G). There was no difference in disinhibition between 9-month-old and 13-month-old female mice. These findings suggest that CBN effectively modulates disinhibition, but the specific effects appear to vary based on sex and age.

3.6. Cannabinol (CBN)-Induced metabolic modulation in SAMP8 mice

To further investigate the effects of CBN on SAMP8 mice, we conducted metabolic chamber analyses following the behavioral assessments on the 13 month old mice. Understanding how CBN influences metabolic parameters is important, as metabolic health can significantly impact cognitive function and overall behavior. The metabolic parameters measured included oxygen consumption (VO2), carbon dioxide production (VCO2), energy expenditure (EE), respiratory exchange ratio (RER), ambulatory activity (XYAMB), and total activity (XYTOT).

3.7. Oxygen consumption (VO2)

In male SAMP8 mice, CBN treatment led to a significant reduction in oxygen consumption compared to controls (Fig. 2A) (Control: 119.33 ± 0.36 mL/kg/hr; CBN 200: 111.64 ± 0.33 mL/kg/hr; CBN 400: 111.46 ± 0.33 mL/kg/hr) in 13-month-old mice; $p < 0.05$). In females, no significant differences in VO2 were observed between the old control and CBN-treated groups (Fig. 3A), indicating a sex-specific metabolic response to CBN.

3.8. Carbon dioxide production (VCO2)

Old male mice exhibited a significant decrease in VCO2 with CBN treatment (Control: 112.25 ± 0.40 mL/kg/hr; CBN 200: 103.18 ± 0.37 mL/kg/hr; CBN 400: 103.51 ± 0.35 mL/kg/hr; $p < 0.05$), similar to the observed reductions in VO2 and indicating an overall decline in metabolic activity (Fig. 2B). In contrast, female mice showed a significant increase in VCO2 with CBN treatment (Control: 69.47 ± 0.79; CBN 200: 76.05 ± 0.65; CBN 400: 77.94 ± 0.63, $p < 0.05$) suggesting enhanced metabolic activity or a compensatory response (Fig. 3B).

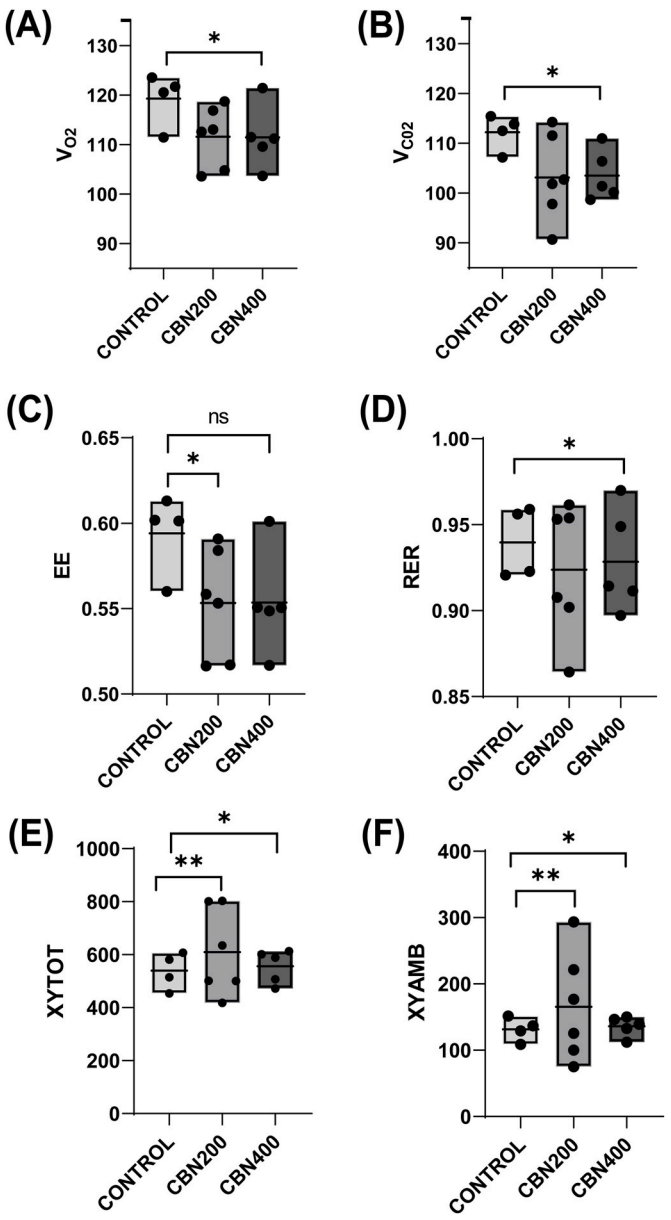

Fig. 2. Metabolic Effects of CBN Treatment in Male SAMP8 Mice
(A) Oxygen consumption (VO2) was significantly reduced with CBN treatment, indicating decreased metabolic demand. (B) Carbon dioxide production (VCO2) decreased with CBN treatment, supporting reduced metabolic activity. (C) Energy expenditure (EE) was lower in CBN-treated males, consistent with decreased metabolic activity. (D) Respiratory exchange ratio (RER) suggested a shift towards fat oxidation following CBN treatment. (E) Total activity levels peaked at 200 ppm and slightly decreased at 400 ppm, indicating a biphasic dose response. (F) Ambulatory activity increased with CBN treatment. Number of mice per group: 13-month control (n = 4), CBN 200 ppm (n = 6), CBN 400 ppm (n = 6). Statistical tests: One-way ANOVA with body mass as a covariant to adjust for potential confounding effects. *$p < 0.05$, *ns* = not significant. Bar graphs represent mean ± SEM with individual data points superimposed to show variability across biological replicates.

3.9. Energy expenditure (EE)

CBN treatment led to a slight but not significant decrease in energy expenditure in old male SAMP8 mice, (Control: 0.594 ± 0.002 kcal/h; CBN 200: 0.553 ± 0.002 kcal/h; CBN 400: 0.553 ± 0.002 kcal/h) consistent with the reduced VO2 and VCO2 measurements (Fig. 2C). In female mice, however, CBN treatment resulted in a significant increase

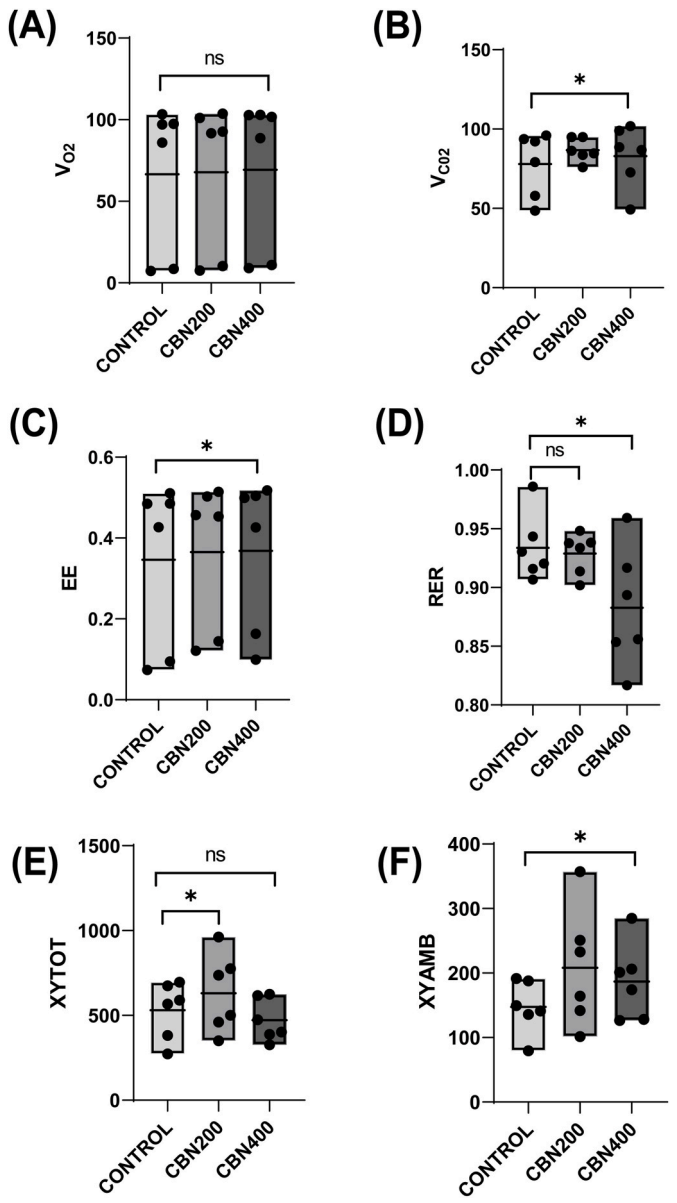

Fig. 3. Metabolic Effects of CBN Treatment in Female SAMP8 Mice
(A) Oxygen consumption (VO2) remained unchanged between control and CBN-treated groups. (B) Carbon dioxide production (VCO2) increased with CBN treatment, suggesting enhanced metabolic activity. (C) Energy expenditure (EE) was elevated in CBN-treated females, reflecting an adaptive metabolic response. (D) Respiratory exchange ratio (RER) was reduced with CBN 400 treatment (E) Total activity decreased slightly at 400 ppm, potentially indicating a biphasic effect. (F) Ambulatory activity increased with both CBN doses. Number of mice per group: 13-month control (n = 6), CBN 200 ppm (n = 6), CBN 400 ppm (n = 6). Statistical tests: One-way ANOVA with body mass as a covariate to adjust for potential confounding effects. *$p < 0.05$, *ns* = not significant. Bar graphs represent mean ± SEM with individual data points superimposed to show variability across biological replicates.

in energy expenditure (Fig. 3C), (Control: 0.23 ± 0.00; CBN 200: 0.25 ± 0.00; CBN 400: 0.26 ± 0.00; $p < 0.05$), indicating a potential adaptive response.

3.10. Respiratory exchange ratio (RER)

Old male mice showed a slight reduction in RER following CBN treatment (Control: 0.940 ± 0.020; CBN 200: 0.924 ± 0.036; CBN 400: 0.928 ± 0.030, $p < 0.05$) suggesting a shift towards greater fat oxidation rather than carbohydrate utilization [20] (Fig. 2D). In female mice, a reduction in RER was noted in the CBN 400 group only (Control: 0.934 ± 0.028; CBN 200: 0.932 ± 0.016; CBN 400: 0.883 ± 0.052, $p < 0.05$) (Fig. 2D).

3.11. Total activity (XYTOT)

In male mice, total activity was highest in the CBN 200 ppm group (Control: 539.24 ± 9.46 counts/hr; CBN 200: 608.53 ± 9.79 counts/hr; CBN 400: 556.74 ± 7.47 counts/hr; $p < 0.05$), indicating a stimulatory effect of this lower dose. However, total activity decreased slightly at the higher 400 ppm dose as compared to 200 ppm (Fig. 2E) suggesting a plateau or mild reduction in activity. In female mice, total activity also decreased at the higher CBN dose (Fig. 3E) (Control: 486.76 ± 6.70; CBN 200: 500.17 ± 8.88; CBN 400: 424.96 ± 6.10; $p < 0.05$)

3.12. Ambulatory activity (XYAMB)

Both male and female SAMP8 mice exhibited increased ambulatory activity with CBN treatment, particularly with the 200 ppm dose. In males, the 200 ppm group demonstrated the highest activity levels (Fig. 2F) (Control: 131.59 ± 3.50 counts/hr; CBN 200: 164.89 ± 4.63 counts/hr; CBN 400: 136.29 ± 2.83 counts/hr; $p < 0.05$), suggesting a dose-dependent enhancement of physical activity. This increase in activity suggests that CBN may positively influence overall vitality. Female mice also showed increased activity with CBN treatment (Fig. 3F), (Control: 143.54 ± 2.90; CBN 200: 159.36 ± 4.37; CBN 400: 156.93 ± 3.10; $p < 0.05$).

3.13. Impact of CBN treatment on mitochondrial dynamics and function

To elucidate the molecular mechanisms underlying the observed behavioral and metabolic changes, we conducted Western blot analyses of key proteins involved in mitochondrial dynamics and function using the hippocampi of the mice. Mitochondrial dysfunction is a hallmark of aging and neurodegenerative diseases, and modulation of mitochondrial dynamics has been proposed as a potential therapeutic strategy [21,22]. Moreover, we have shown that CBN can modulate mitochondrial dynamics and function in cell culture [8]. During aging, levels of the mitochondrial fusion proteins MFN2 and OPA1 decline, compromising mitochondrial quality control, reducing ATP synthesis efficiency, and contributing to metabolic dysfunction and neurodegeneration [23]. These proteins are essential for maintaining mitochondrial integrity and function [24–26]. In our study, CBN treatment at both 200 ppm and 400 ppm significantly prevented the loss of MFN2 and OPA1 levels in the hippocampi of aging mice (Fig. 4A–A-C). This effect was particularly marked in male mice ($p < 0.001$), in accordance with the hypothesis that enhanced mitochondrial fusion can contribute to improved mitochondrial function. The regulation of mitochondrial fission, mediated by Dynamin-related protein 1 (DRP1), showed sex-specific variations in response to aging and CBN treatment. In female mice, DRP1 levels significantly decreased at 13 months (Fig. 4A and D) compared to 9 months ($p < 0.01$), suggesting an age-related reduction in mitochondrial fission. In contrast, male mice did not exhibit any statistically significant changes in DRP1 levels with aging. However, CBN at 200 ppm significantly increased DRP1 levels (Fig. 4A and D), suggesting that CBN may modulate mitochondrial fission specifically in males in a dose-dependent manner. Notably, no significant effect of CBN treatment on DRP1 levels was observed in females. These findings suggest that CBN's effect on mitochondrial dynamics might differ between sexes, with potential effects on mitochondrial fusion in both males and females, and fission in males. The Voltage-dependent anion channel (VDAC), a critical component of the mitochondrial outer membrane that regulates metabolic cross-talk between mitochondria and the cytosol [27], was examined next. VDAC levels were decreased in 13-month-old mice compared to 9-month-old controls (Fig. 4A and E). However, CBN

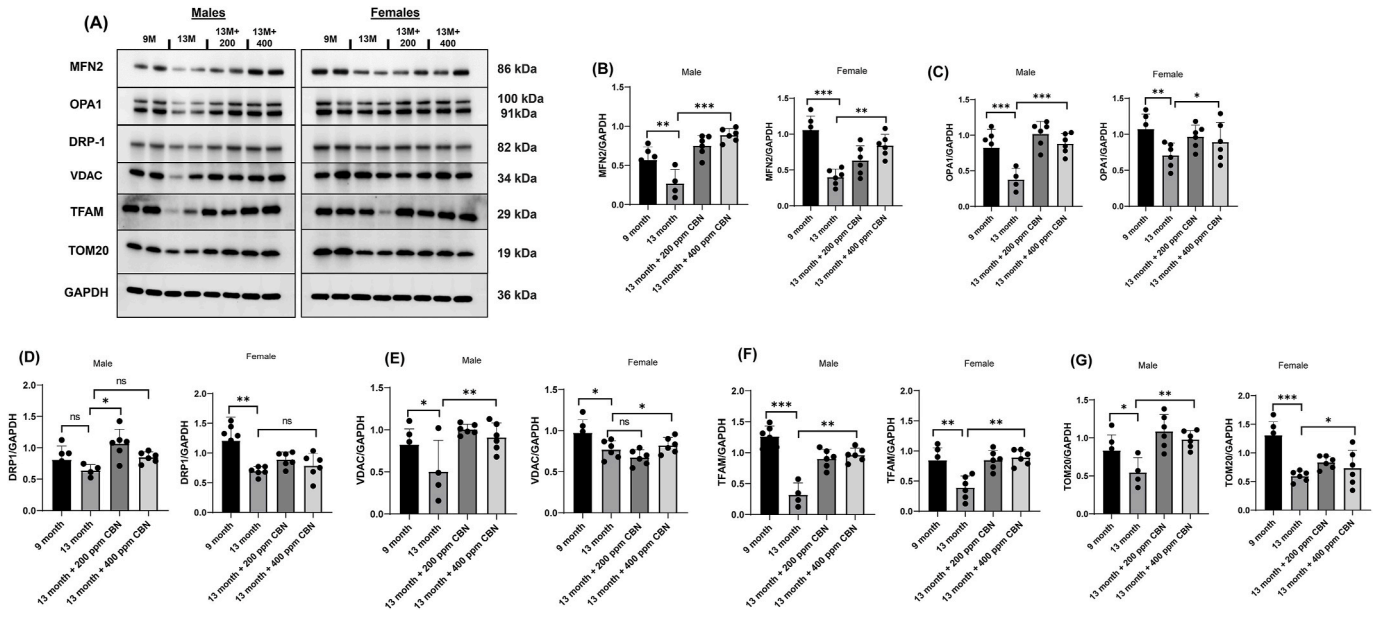

Fig. 4. Effects of CBN on Mitochondrial Dynamics in SAMP8 Mice

(A) Western blot analyses revealed changes in mitochondrial fusion and fission proteins. (B, C) MFN2 and OPA1 levels, indicative of mitochondrial fusion, were significantly maintained by CBN treatment, with more pronounced effects in males. (D) DRP1, a fission protein, increased with CBN treatment in 13 month old males but remained unchanged in females. (E) VDAC levels, which decline with age, were preserved by CBN treatment in both males and females. (F, G) TFAM and TOM20 levels decreased with age and this was prevented by treatment with CBN. In both sexes. Number of mice per group (Male & Female): 9-month control (n = 6&6), 13-month control (n = 4&6), CBN 200 ppm (n = 6&6), CBN 400 ppm (n = 6&6). Statistical tests: One-way ANOVA with Tukey's multiple comparison post hoc test. * indicates significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *ns* = not significant. Bar graphs represent mean ± SEM with individual data points superimposed to show variability across biological replicates.

treatment led to an increase in VDAC levels in both males and females, with a more pronounced effect in males ($p < 0.001$). In females treated with CBN 200, the increase was not statistically significant (Fig. 4A and E). Building on the findings of the prevention of age-related changes in markers of mitochondrial dynamics by CBN, we further examined its effects on markers of mitochondrial biogenesis, a process that typically declines with age and contributes to reduced cellular energy production [28]. Our results show that both mitochondrial transcription factor A (TFAM) and translocase of the outer mitochondrial membrane 20 (TOM20) were reduced in 13 month old mice as compared to 9 month mice with more notable effects in males for TFAM and in females for TOM20 (Fgure5A, F&G). CBN treatment effectively prevented the loss of TFAM (Fig. 4A and F) and (TOM20) (Fig. 4A and G) in both males and females. The maintenance of these proteins with CBN treatment suggests that CBN plays a role in promoting mitochondrial biogenesis and enhancing the mitochondrial protein import machinery.

3.14. CBN Modulates Oxidative Phosphorylation and stress response

The efficiency of oxidative phosphorylation (OXPHOS) complexes, which are essential for ATP production and overall energy metabolism, often declines with age [28,29]. Our analysis demonstrated significant age-related reductions in markers of complexes I and II in both male and female mice between 9 and 13 months ($p < 0.05$) (Fig. 5A and C). The loss of the complex I marker was prevented by CBN in both males and females ($p < 0.05$). In contrast, complex III did not show significant changes between 9 and 13 months in males, suggesting a lack of significant age-related alterations in this complex (Fig. 5A and C). However, in 13-month-old females, complex III expression was reduced, and this reduction was prevented by CBN treatment, suggesting a sex-specific response to aging and treatment (Fig. 5A and C) [30]. Importantly, complex V (ATP synthase) also displayed a significant age-related decline in both males and females and this was also prevented by CBN treatment in both male and female mice ($p < 0.01$) (Fig. 5A and C). In parallel with the improvements observed in ETC complex protein levels, we further assessed CBN's role in mitigating mitochondrial oxidative stress, which is closely linked to mitochondrial efficiency and cellular energy homeostasis. Superoxide dismutase 2 (SOD2), a key mitochondrial antioxidant enzyme, is essential for maintaining mitochondrial integrity and reducing oxidative damage [31]. Our findings revealed that while in male mice SOD2 levels did not show statistically significant age-related changes between 9 and 13 months, CBN treatment significantly increased SOD2 expression ($p < 0.01$) (Fig. 5A and B), suggesting a positive response to treatment despite stable baseline levels. In contrast, female mice exhibited a significant age-related decline in SOD2 levels ($p < 0.01$), suggesting increased oxidative stress with aging. Notably, CBN administration effectively prevented this decline in females ($p < 0.05$) (Fig. 5A and B), further highlighting CBN's potential to sustain mitochondrial antioxidant defenses. Altogether, these results highlight CBN's ability to modulate multiple aspects of mitochondrial function.

3.15. CBN's effects on synaptic and cellular protein dynamics in SAMP8 mice

Age-related changes in synaptic proteins and cellular dynamics also exhibited sex-specific responses to CBN treatment. In male mice, while synaptophysin, a critical presynaptic vesicle protein [32], showed no significant age-related changes between 9 and 13 months, CBN treatment significantly increased its levels in males ($p < 0.05$) (Fig. 6A and B), suggesting enhanced synaptic function. In contrast, female mice exhibited a significant reduction in synaptophysin levels at 13 months compared to 9 months ($p < 0.01$), suggesting an age-associated decline in synaptic function. CBN effectively maintained synaptophysin levels in aged females (Fig. 6A and B), demonstrating its potential to counteract age-related synaptic changes and support synaptic integrity. Further supporting the ability of CBN to prevent age-related changes in synaptic function, postsynaptic density protein 95 (PSD-95), a key scaffolding protein for postsynaptic signaling [33], exhibited a significant decline in males ($p < 0.001$), which was robustly prevented by CBN treatment (p

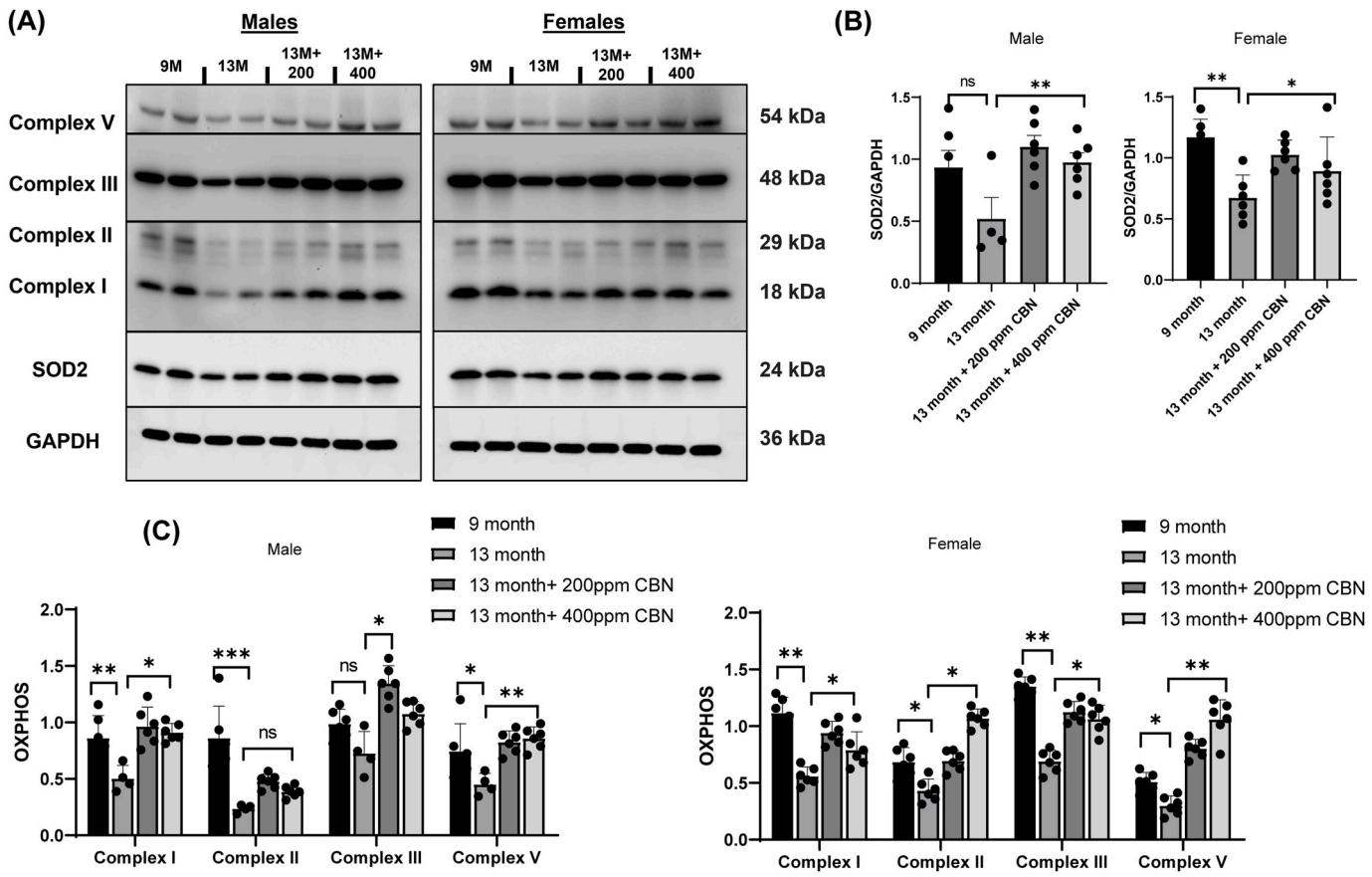

Fig. 5. CBN Modulates Oxidative Phosphorylation in SAMP8 Mice

(A) Western blot analysis showed age-related declines in oxidative phosphorylation (OXPHOS) complex proteins and SOD2. (B) SOD2 expression was elevated with CBN treatment, indicating reduced oxidative stress and improved mitochondrial function (C) Complex I and V levels decreased with age and these decreases were prevented by CBN treatment in both sexes. Number of mice per group (Male & Female): 9-month control (n = 6&6), 13-month control (n = 4&6), CBN 200 ppm (n = 6&6), CBN 400 ppm (n = 6&6). Statistical tests: One-way ANOVA with Tukey's multiple comparison post hoc test. * indicates significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *ns* = not significant. Bar graphs represent mean ± SEM with individual data points superimposed to show variability across biological replicates.

< 0.001) (Fig. 6A and C). Female mice also showed a significant age-dependent decrease in PSD-95 levels which was significantly prevented by 200 ppm CBN treatment ($p < 0.05$) while the response was less pronounced at 400 ppm. SNAP-25, a key protein involved in neurotransmitter release and synaptic vesicle dynamics [34], was significantly decreased in males with age and increased with CBN treatment ($p < 0.05$) (Fig. 6A and E). This increase also suggests enhanced synaptic function, as SNAP-25 is essential for the exocytotic release of neurotransmitters at chemical synapses [35,36]. In females, SNAP-25 levels were significantly different between 9 and 13 months ($p < 0.001$), with CBN treatment maintaining SNAP-25 levels in aged females ($p < 0.05$). MCU, the mitochondrial calcium uniporter, regulates mitochondrial calcium uptake, which is crucial for energy production and cell survival. Dysregulated MCU function can lead to mitochondrial dysfunction, characterized by impaired energy production and increased oxidative stress [37,38]. In both males and females, MCU levels were significantly decreased with age (Fig. 6A and D) and were effectively maintained by treatment with CBN ($p < 0.001$) (Fig. 6A and D). Together, these results further support CBN's role in promoting synaptic health through both mitochondrial protein regulation and synaptic vesicle dynamics, with distinct responses observed between males and females.

4. Discussion

In this study, we investigated the therapeutic effects of cannabinol (CBN) in ameliorating age-related cognitive decline, metabolic dysfunction, and mitochondrial impairment in the senescence-accelerated SAMP8 mice. These mice exhibit a progressive, age-associated decline in brain function similar to human aging [39], and our previous studies have shown that SAMP8 mice develop early deterioration in learning and memory, increased oxidative stress, inflammation, and vascular impairment [19,40]. We have also demonstrated that fisetin, a neuroprotective flavonol, effectively mitigated these age-related issues in SAMP8 mice [19]. Moreover, we have shown recently that the AD drug candidates CMS121 and J147 reduce cognitive decline and metabolic markers of aging in SAMP8 mice, further supporting the use of this model for studying age-related brain function [40]. Our findings described here suggest that CBN has the potential to positively impact several aspects of aging in a sex-dependent manner, including cognitive function, metabolic efficiency, and mitochondrial health. These results are in accordance with existing literature on the neuroprotective and metabolic benefits of cannabinoids [41,42], while providing additional new insights into how CBN can modulate sex-dependent biological processes in aging.

CBN significantly improved spatial learning and memory in aged SAMP8 mice, with the effects most pronounced in females. These findings are consistent with previous studies that suggest that cannabinoids can enhance cognitive function by mitigating oxidative stress and inflammation, key drivers of neurodegeneration [43,44]. While the exact mechanisms underlying the observed sex-specific effects of CBN remain unclear, potential factors could include differences in neurobiological responses to cannabinoids or variations in age-associated physiological and molecular changes between males and females

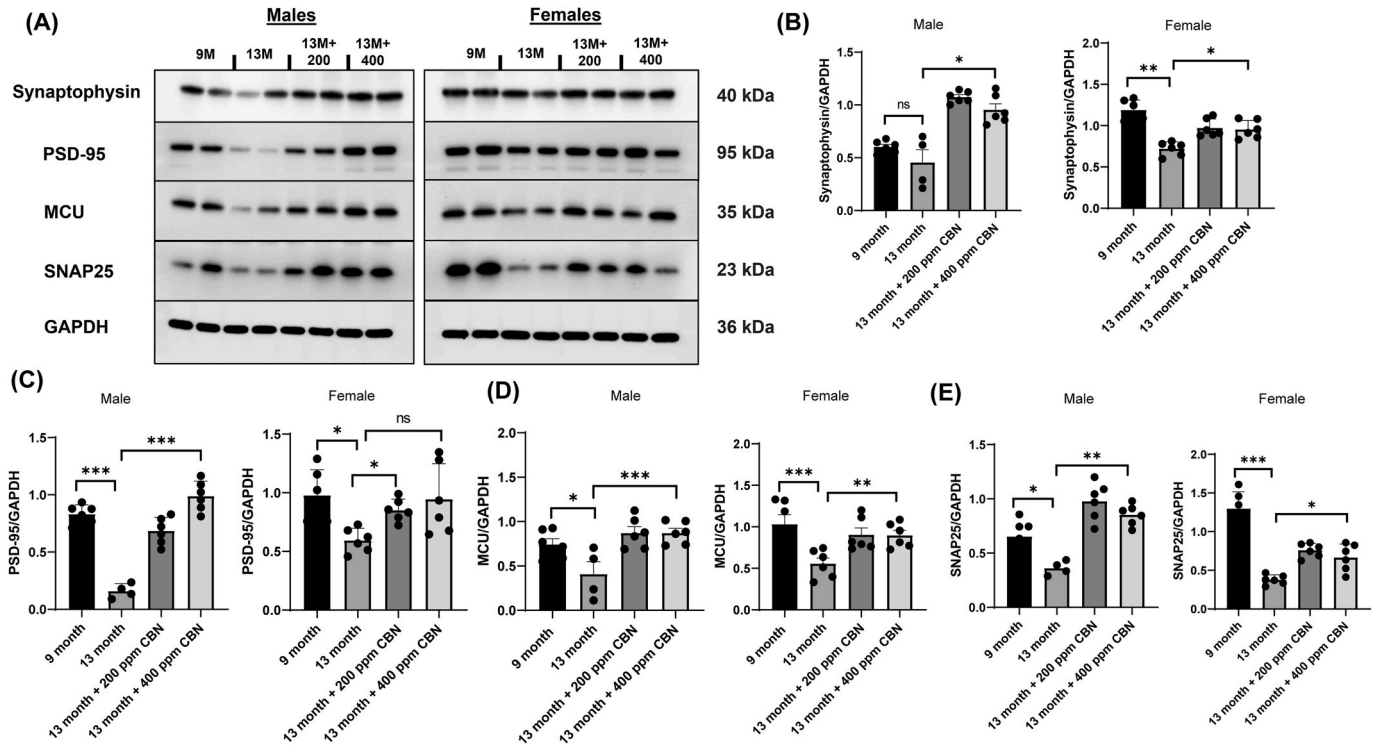

Fig. 6. Synaptic Protein Expression in Male and Female SAMP8 Mice Treated with CBN

(A) Representative Western blots showing levels of synaptophysin, PSD-95, MCU, and SNAP-25 in male and female SAMP8 mice treated with CBN. **(B)** In male mice, synaptophysin levels did not exhibit significant age-related changes between 9 and 13 months; however, CBN treatment significantly increased synaptophysin levels. In female mice, synaptophysin levels were significantly reduced at 13 months compared to 9 months which was prevented by CBN treatment. **(C)** Male mice showed significant age-related declines in PSD-95 levels, which were robustly prevented by CBN treatment. In female mice, PSD-95 levels also decreased with age and CBN treatment (200 ppm) prevented this loss, although the response was less pronounced with 400 ppm. **(D)** Mitochondrial calcium uptake protein (MCU levels) decreased with age and CBN treatment significantly prevented this loss in both sexes, with a more pronounced effect in males. **(E)** Age-related declines in SNAP-25, a protein critical for neurotransmitter release and synaptic vesicle dynamics, were prevented in both sexes by CBN treatment. The 200 ppm dose was most effective in both sexes. Number of mice per group (Male & Female): 9-month control (n = 6&6), 13-month control (n = 4&6), CBN 200 ppm (n = 6&6), CBN 400 ppm (n = 6&6). Statistical tests: One-way ANOVA with Tukey's multiple comparison post hoc test. * indicates significance levels: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, *ns* = not significant. Bar graphs represent mean ± SEM with individual data points superimposed to show variability across biological replicates.

[45–47].

Moreover, CBN's potential to reduce disinhibited behaviors was most evident in males, as demonstrated by highly significant reductions in the EPM. Specifically, CBN treatment reduced open-arm time (Fig. 1D), suggesting improved executive function by mitigating age-related disinhibition, consistent with behavioral deficits previously reported in SAMP8 mice [19]. This conclusion is supported by evidence that cannabinoids enhance prefrontal cortex-mediated executive function, reducing impulsive behavior in rodent models [48]. Alternatively, some cannabinoids (e.g., CBD) reduce anxiety in mice, which could increase open-arm time in the EPM due to anxiolytic effects, highlighting varied behavioral outcomes of cannabinoid treatment [49].These behavioral effects indicate that CBN not only supports learning and memory but also promotes executive function, which is vital in addressing age-related neurodegenerative conditions. In females, while significant improvements were not observed in the EPM because there were no significant changes with age in this task, behavioral enhancements were noted in the Barnes maze which measures spatial learning and memory, indicating that CBN can influence multiple aspects of cognitive function.

The metabolic effects of CBN provide further evidence of its therapeutic versatility. In males, reduced oxygen consumption (VO2) and carbon dioxide production (VCO2) suggest a shift toward metabolic efficiency, which has been shown to alleviate oxidative stress and enhance mitochondrial function in aging [2]. Conversely, the increase in VCO2 and energy expenditure (EE) in females potentially reflects an adaptive metabolic response, potentially supporting the energy demands of improved learning and memory. These sex-specific metabolic responses highlight the role of cannabinoids in modulating energy homeostasis differently between sexes, possibly involving broader physiological changes beyond the brain. In males, reduced VO2 (Fig. 2A) could enhance mitochondrial efficiency in the brain [50], although given CBN's influence on physical activity, it could also indicate systemic effects, suggesting that multiple potential mechanisms likely underlie these whole-body metabolic changes [51]. These opposite metabolic effects can both be beneficial. Reduced VO2 and VCO2 in males enhances metabolic efficiency and mitochondrial function, reducing oxidative stress and improving overall health and longevity [50]. Increased VCO2 and EE in females may support cognitive performance by promoting synaptic plasticity, which is crucial for brain health [52]. Overall, these sex-specific metabolic responses highlight the role of cannabinoids in modulating energy homeostasis differently between sexes [53–55]. Additionally, the increased ambulatory and total activity observed in CBN-treated mice supports the idea that improved metabolic efficiency can translate into enhanced physical vitality and cognitive engagement. Similar findings have been reported in cannabis studies, where improved mitochondrial and metabolic functions were linked to better behavioral outcomes in aging models [56].

Mitochondrial dysfunction is a hallmark of aging and neurodegenerative diseases, characterized by disrupted fusion and fission dynamics, impaired biogenesis, and oxidative damage [3,57]. In our earlier study, CBN treatment was shown to preserve key proteins related to mitochondrial fusion and biogenesis in cell culture models of oxytosis/ferroptosis [8]. In the current study, we observed that CBN

similarly prevented the age-related decline of critical mitochondrial fusion (MFN2, OPA1) and biogenesis (TFAM, TOM20) related proteins in the hippocampi of both male and female aged mice. These results highlight the potential of CBN to maintain mitochondrial integrity during aging, aligning with evidence that enhancing mitochondrial fusion and biogenesis supports cognitive function and neuronal survival [11,58]. In males, the increased expression of DRP1 suggests a compensatory enhancement of mitochondrial fission, balancing the fusion-fission dynamic to maintain mitochondrial integrity. While this process may prevent the accumulation of damaged mitochondria, it is also possible that it represents a stress response to aging-related mitochondrial deficits, similar to compensatory mechanisms observed in other neurodegenerative models [59,60]. The upregulation of VDAC further highlights CBN's role in improving mitochondrial function. Our findings that CBN prevented age-related declines in VDAC align with evidence suggesting that preservation of mitochondrial functions is critical for treating neurodegeneration [61]. CBN's enhancement of oxidative phosphorylation (OXPHOS) complexes, particularly complex V, further supports its potential for restoring ATP synthesis capabilities. In addition, the maintenance or enhancement of superoxide dismutase 2 (SOD2) levels in the aged mice further illustrates CBN's potential to mitigate age-related oxidative damage. These findings align with studies emphasizing the importance of sustaining mitochondrial antioxidant defenses to counteract the detrimental effects of aging on cellular energy homeostasis [62]. Interestingly, the sex-specific restoration of SOD2 levels, with females exhibiting a more pronounced prevention of age-related declines, further suggests that CBN targets mitochondrial stress pathways in a sex-dependent manner. This differential response warrants further investigation, as understanding the mechanisms underlying the regulation of sex-specific antioxidant defenses could inform more tailored therapeutic approaches.

Synaptic dysfunction is a critical feature of age-related cognitive decline. CBN treatment elevated synaptophysin and PSD-95 levels in aged SAMP8 mice, providing strong evidence for its potential to preserve synaptic integrity. Synaptophysin, a presynaptic protein essential for neurotransmitter vesicle trafficking, and PSD-95, a postsynaptic scaffolding protein crucial for synaptic signaling, are integral to synaptic function and plasticity [63]. These results align with studies indicating cannabinoids such as anandamide and 2-arachidonoylglycerol (2-AG), enhance synaptic plasticity and neurotransmitter release, mechanisms central to learning and memory [64]. The increases in both synaptophysin and PSD-95 likely contribute to the improved cognitive performance observed in the different tasks. In males, the increase in PSD-95, which is vital for postsynaptic function and plasticity, may contribute to their improved performance [65]. In contrast, the significant increase in synaptophysin levels in females correlates with their improved performance in spatial learning tasks. Synaptophysin's role in synaptic vesicle trafficking suggests that its upregulation enhances synaptic activity in regions critical for spatial memory, such as the hippocampus [66]. While PSD-95 upregulation in males and synaptophysin upregulation in females correlate with their respective cognitive enhancements, it remains unclear whether these changes directly drive the behavioral improvements or if they reflect broader neuroplastic adaptations. CBN treatment also increased the levels of mitochondrial calcium uniporter (MCU) in both male and female mice, indicating enhanced mitochondrial calcium uptake, a process associated with improved mitochondrial function and synaptic health. [67]. Additionally, SNAP-25, a key protein involved in neurotransmitter release and synaptic vesicle dynamics, showed an age-related decline that was mitigated by CBN treatment in both sexes. In males, SNAP-25 levels were significantly upregulated, suggesting enhanced synaptic vesicle exocytosis essential for efficient neurotransmission, while in females, CBN partially restored SNAP-25 levels that had markedly decreased between 9 and 13 months. These findings highlight CBN's dual role in promoting synaptic integrity and mitochondrial efficiency, consistent with evidence linking mitochondrial dysfunction and impaired synaptic dynamics to aging and neurodegeneration [35,68]. Overall, CBN shows promise as a therapeutic approach to enhancing synaptic function and mitochondrial dynamics, offering a potential strategy for mitigating age-related cognitive decline.

Limitations of the study: Although this study demonstrates the therapeutic potential of CBN for age-related cognitive decline, it has several limitations. We examined whole hippocampal homogenates to evaluate changes in mitochondrial and synaptic proteins, providing a comprehensive overview of the effects of CBN on learning and memory. This approach reflects the collective contributions of neurons, astrocytes, microglia, and oligodendrocytes to these processes. However, it does not identify the specific effects of CBN on individual cell types or map the spatial distribution of proteins across hippocampal subregions. Techniques such as single-cell analyses, electron microscopy or histology could provide these finer mechanistic details. Additionally, while hippocampal changes are relevant to the cognitive findings, the metabolic cage data represents whole body physiology, indicating that peripheral tissues (e.g., liver, adipose) and other brain regions (e.g., cortex, hypothalamus) likely contribute to the observed effects. Future research examining cell-type-specific mRNA and protein expression and responses across multiple tissues would provide a more complete picture of CBN's therapeutic mechanisms.

5. Conclusion and future directions

This study elucidates the neuroprotective effects of cannabinol (CBN) in aging-accelerated SAMP8 mice, a model of age-related cognitive decline. CBN demonstrated its ability to enhance cognitive function, improve mitochondrial dynamics, and modulate metabolism. Specifically, it improved spatial learning and memory in females and executive function in males, with distinct metabolic responses in both sexes. These findings place CBN as a promising agent for mitigating age-related cognitive decline and associated metabolic disruptions, particularly in preserving mitochondrial function during aging. The sex-specific differences in response to CBN treatment suggest that CBN's molecular effects may be gender-dependent, which warrants further investigation into the underlying mechanisms. Future studies should focus on identifying the molecular pathways responsible for CBN's sex-specific effects and further exploring its long-term impact on neurodegenerative processes. Additionally, examining the potential synergistic effects of CBN with other neuroprotective compounds may provide additional insights into therapeutic strategies.

CRediT authorship contribution statement

Nawab John Dar: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Antonio Currais:** Writing – review & editing, Validation. **Taketo Taguchi:** Methodology. **Nick Andrews:** Validation, Methodology. **Pamela Maher:** Writing – review & editing, Visualization, Validation, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2025.103692.

Data availability

Data will be made available on request.

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Abbreviations:

AD: Alzheimer's Disease
ANOVA: Analysis of Variance
BCA: Bicinchoninic Acid
CBN: Cannabinol
DRP1: Dynamin-Related Protein 1
EE: Energy Expenditure
EPM: Elevated Plus Maze
GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase
IACUC: Institutional Animal Care and Use Committee
MCU: Mitochondrial Calcium Uniporter
MFN1: Mitofusin-1
MFN2: Mitofusin-2
OPA1: Optic Atrophy 1
OXPHOS: Oxidative Phosphorylation
PBS: Phosphate-Buffered Saline
PSD-95: Postsynaptic Density Protein 95
RER: Respiratory Exchange Ratio
RIPA: Radioimmunoprecipitation Assay
SAMP8: Senescence-Accelerated Mouse Prone 8
SDS: Sodium Dodecyl Sulfate
SNAP-25: Synaptosomal-Associated Protein 25
SOD2: Superoxide Dismutase 2
TBS-T: Tris-Buffered Saline with Tween
TFAM: Mitochondrial Transcription Factor A
TOM20: Translocase of Outer Mitochondrial Membrane 20
VDAC: Voltage-Dependent Anion Channel
VCO2: Carbon Dioxide Production
VO2: Oxygen Consumption
XYAMB: Ambulatory Activity
XYTOT: Total Activity