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Combination treatment with medium dose THC and CBD had no therapeutic effect in a transgenic mouse model for Alzheimer's disease but affected other domains including anxiety-related behaviours and object recognition memory

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease that effects memory and behaviour. The phytocannabinoid cannabidiol (CBD) has been found to reverse impairments of recognition as well as spatial memory deficits of AD transgenic mice but had only limited effects on disease-relevant brain pathologies. Recent evidence suggests that combining CBD with other cannabinoids including delta-9-tetrahydrocannabinol (THC) may lead to improved therapeutic outcomes. Thus, this study evaluated the chronic effects of combined treatment with 3 mg/kg THC and 20 mg/kg CBD on 14.5-month-old *APP$_{Swe}$/PS1ΔE9* (*APP/PS1*) transgenic females and control littermates. Mice were treated with THCxCBD or vehicle (VEH) daily via intraperitoneal injections for 3 weeks before behavioural testing commenced. AD-relevant behavioural domains were analysed utilising Elevated Plus Maze (EPM), open field (OF), novel object recognition test (NORT), social interaction (SI), Y-maze (YM), and prepulse inhibition test (PPI). *APP/PS1* females showed an anxiety-like phenotype and object recognition deficits that remained unchanged by cannabinoid treatment. Interestingly, some effects of THCxCBD appeared genotype-dependent with cannabinoid treatment causing an anxiogenic EPM response in *APP/PS1* mice but having an anxiolytic-like effect in WT females. Moreover, THCxCBD administration disrupted the novel object preference of control females. Noteworthy, THCxCBD significantly decreased different fat depots and bodyweight of all mice across genotype. No other differences between genotypes or treatment groups were detected. In conclusion, the particular cannabinoid combination strategy utilised had no prominent therapeutic-like effect in 14.5-month-old *APP/PS1* females.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and the most common form of dementia. AD symptoms include memory loss, impaired learning, spatial disorientation and social withdrawal (Alzheimer's Association, 2023). These symptoms are associated with post-mortem brain pathologies like senile plaques due to amyloid-β-aggregation and neurofibrillary tangles (NFTs) due to tau hyperphosphorylation (Götz and Ittner, 2008; Reitz and Mayeux, 2014). Moreover, neuroinflammation including microglial activation and cerebral atrophy play a role in AD pathogenesis (Chen and Mobley, 2019). Two forms of AD can be distinguished: sporadic (or late onset) and familial (or early onset) AD. Familial AD is caused by mutations in the *amyloid precursor protein* (*APP*) gene, the *presenilin 1* (*PS1*) gene or the *presenilin 2* (*PS2*) gene (Götz and Ittner, 2008; Reitz and Mayeux, 2014). In the current study a mouse model for familial AD was utilised, i.e. double transgenic *APP$_{Swe}$/PS1ΔE9* (*APP/PS1*) mice. These AD transgenic mice show amyloid depositions from 6 months of age onwards (Borchelt et al., 1997; Jankowsky et al., 2004a) and display recognition memory deficits, increased anxiety and spatial memory impairments (Cheng et al., 2013; Cheng et al., 2014b).

Current treatment options for AD include acetylcholinesterase

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inhibitors and the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine (Therapeutic Guidelines, 2023). However, more effective treatment options need to be evaluated as these drugs do not alter the progression of the disease and have side effects (e.g. gastrointestinal issues, headache, confusion) (National Insitute on Aging (NIA), 2023). One promising new approach for AD therapy is targeting the endocannabinoid system by means of phytocannabinoid treatment (Karl et al., 2012). Phytocannabinoids are constituents of the *Cannabis sativa* plant with cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) being two of the major phytocannabinoids. CBD is a non-toxic, antipsychotic, anti-inflammatory and neuroprotective component of *Cannabis sativa* and promises therapeutic potential for AD (Booz, 2011; Iuvone et al., 2009). Indeed, CBD reduced tau hyperphosphorylation in PC12 cells (Esposito et al., 2006) and Aβ production by inducing APP ubiquitination in SHSY5Y^{APP+} cells (Scuderi et al., 2014). Importantly, our previous in vivo work also found that chronic CBD treatment can reverse social and object recognition impairments as well as spatial memory deficits of *APP/PS1* mice (Cheng et al., 2014a; Coles et al., 2020; Watt et al., 2020). However, purified CBD had only limited effects on reversing or preventing the development of AD-relevant brain pathologies (Watt et al., 2020). Thus, CBD treatment alone may not be sufficient to achieve clinically relevant changes in AD mice (Aso et al., 2015).

THC is the major psychoactive cannabinoid extracted from the *Cannabis sativa* plant (Ng et al., 2023) and the synthetic THC form dronabinol can for example impair the cognitive performance of 2-month-old C75BL/6 J mice (Bilkei-Gorzo et al., 2017). However, low dose THC treatment (0.75 mg/kg) has been found to reverse cognitive deficits in 6-month-old *APP/PS1* male mice (Aso et al., 2015) and chronic treatment with 3 mg/kg THC restored cognitive function in 12- and 18-month-old C57BL/6 J males (Bilkei-Gorzo et al., 2017). Thus, the current project aims to evaluate if a cannabinoid combination treatment using therapeutically relevant doses of CBD (20 mg/kg) and THC (3 mg/kg) may be effective in achieving clinically relevant changes in female 14.5-month-old double transgenic *APP/PS1* mice. Animals were tested for disease-relevant behavioural parameters including locomotion, anxiety, social behaviours, sensorimotor gating, as well as learning and memory.

2. Materials and methods

2.1. Animals

Test mice were female double transgenic *APP$_{Swe}$/PS1ΔE9* (*APP/PS1*) mice and wild type-like control littermates (WT). The *APP/PS1* mice expressed the chimeric mouse/human AβPP (*Mo/HuAPP695$_{swe}$/Swedish* mutations *K595N/M596I*) and the mutant human *PS1* (*PS1/ΔE9*) gene. The mouse model is generated as hemizygotes on the congenic C57BL/6JxC3H/HeJ background (Borchelt et al., 1997; Jankowsky et al., 2004a; Jankowsky et al., 2004b). All mice were bred and grouped-housed in individually ventilated cages (Type Mouse Version 1: Airlaw, Smithfield, Australia) at Australian BioResources (ABR: Moss Vale, Australia). Mice were transported to the Western Sydney University's animal facility (School of Medicine, Campbelltown, NSW, Australia) at approximately 10 weeks of age and were grouped housed in filter top cages (1284 L: Tecniplast, Rydalmere, Australia) equipped with corn cob bedding (1/8" Bed-o'Cobs, Tecniplast Australia), crinkle paper (Crink-l'Nest, Tecniplast, Australia), and tissues (Kleenex®, Facial Tissue, Kimberley-Clark, Australia) for nesting. A 12:12 h light:dark cycle (light phase: 0900–2100 h with white light at an illumination of 124 lx; dark phase: 2100–0900 h with red light at an illumination of less than 2 lx), a temperature of 23 ± 1 °C and relative humidity of 40–60 % were set as laboratory conditions. Food (Rat & Mouse Pellets: Gordon's Specialty Stockfeeds Pty Ltd., NSW, Australia) and water were provided ad libitum. For the social interaction test, female adult A/JArc mice from the Animal Resources Centre (ARC: Canning Vale, Australia) were used as social interaction partners. All research and animal care procedures were approved by the Western Sydney University Animal Care and Ethics Committee (ACEC: A14345) and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Drug treatment

The purified cannabinoid combination (THCxCBD) was prepared similar to what has been published previously (Coles et al., 2020; Long et al., 2013). Firstly, THC resin (Dronabinol, THC Pharm GmbH, Frankfurt/Main, Germany) was dissolved in 100 % ethanol to a concentration of 100 mg/ml to create a stock solution. Secondly, powdered cannabidiol (CAS: 13956–29-1; THC Pharm GmbH) in a dosage of 20 mg/kg bodyweight CBD and the THC stock solution in a dosage of 3 mg/kg bodyweight were dissolved and mixed, into a siliconized (Sigmacote®, Sigma-Aldrich Co., St Louis, United States) falcon tube, in equal parts of 100 % ethanol and Tween80 (Sigma-Aldrich Co., St Louis, United States) and diluted in 0.9 % sodium chloride to the concentration to a final ratio of 1:1:18 (ethanol:Tween80:sodium chloride). The vehicle (VEH) was prepared in a similar way, without the siliconization of the falcon tube and without the addition of powdered cannabidiol and THC stock solution (VEH: 1:1:18 ethanol:Tween80:sodium chloride). 10 ml/kg bodyweight of either THCxCBD or VEH were administered daily via intraperitoneal (i.p.) injections over a five-week period (WT-VEH $n = 13$; *APP/PS1*-VEH $n = 13$; WT-THCxCBD $n = 12$; *APP/PS1*-THCxCBD $n = 10$). Behavioural testing of the 48 test mice commences after the initial three weeks of treatment. At all times, drug administration was carried out in the afternoon (1400-1600 h) after behavioural tests had been completed to avoid any interference of the injections with the performance of the mice in the different tests (Cheng et al., 2014a; Coles et al., 2020; Watt et al., 2020). Bodyweight (BW) was monitored weekly. 3 mg/kg BW THC dose was chosen to use the beneficial low dose effects of THC detected in previous studies (Aso et al., 2016a; Aso et al., 2016b; Aso et al., 2015; Bilkei-Gorzo et al., 2017) and to avoid the detrimental effects caused by higher THC doses (e.g. hypolocomotion, anxiogenic effects at 5 or 10 mg/kg (Long et al., 2010a)). 20 mg/kg CBD was selected because of its therapeutic properties (Cheng et al., 2014a) and to counterbalance any potentially negative consequences of chronic THC administration (Calabrese and Rubio-Casillas, 2018).

2.3. Behavioural test battery

The mice were tested for different behavioural tests (Table 1) approximately at the age of 14.5 months with an intertest interval of at least 48 h. All tests were conducted during 0900–1400 h (i.e. the first 5 h of the light phase) to reduce the influence of the circadian rhythm on the performance of the experimental mice. Mice were habituated to the test

Table 1
Age across the experimental period: Age [weeks] of all mice for non-transgenic control (WT) and double transgenic *AβPPSwe/PS1ΔE9* (*APP/PS1*) female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD). EPM: Elevated Plus Maze; OF: Open field; NORT: Novel object recognition test; SI: Social interaction; YM: Y-maze; PPI: Prepulse inhibition.

Treatment	VEH		THCxCBD	
Genotype	WT	*APP/PS1*	WT	*APP/PS1*
Start of treatment	59 ± 0.1	60 ± 1.1	58 ± 0.2	62 ± 1.4
EPM	62 ± 0.1	63 ± 1.1	61 ± 0.2	65 ± 1.4
OF	62 ± 0.1	63 ± 1.1	62 ± 0.1	65 ± 1.4
NORT	62 ± 0.2	64 ± 1.1	62 ± 0.2	65 ± 1.3
SI	62 ± 0.2	64 ± 1.1	62 ± 0.2	65 ± 1.4
YM	63 ± 0.1	64 ± 1.1	63 ± 0.1	66 ± 1.4
PPI	63 ± 0.1	65 ± 1.1	63 ± 0.2	67 ± 1.4
Perfusion	64 ± 0.1	65 ± 1.1	63 ± 0.2	67 ± 1.4

room for 30 min prior to any testing (apart from the PPI test: mice were habituated to another room and only moved in the PPI room at time of testing) and all apparatuses were cleaned with 80 % ethanol and were allowed to dry between animals.

2.3.1. Elevated Plus Maze

The EPM is a "+"-shaped apparatus consisting of two opposing open and two opposing enclosed arms connected by a central platform (Elevated Plus Maze, San Diego Instruments, San Diego, USA). The mice were put on the central platform of the EPM facing towards an enclosed arm and were allowed to explore the EPM for 5 min while the AnyMaze™ (Stoeting, Wood Dale, USA) tracking software recorded the following parameters: distance travelled and time spent on the first and second outer half of the open arms as well as both enclosed arms. Mice have an innate conflict between the tendency to explore a new environment on one hand and to avoid an elevated, bright open area on the other hand (Montgomery and Monkman, 1955). Furthermore, anxiolytic drugs increase and anxiogenic drugs decrease the time spent on open arms (Komada et al., 2008; Rodgers and Dalvi, 1997). Therefore, time spent on open arms and the percentage distance travelled on open arms provide measures of anxiety-related behaviour. Moreover, to assess the tendency to explore the environment in the EPM, *rearing* (i.e. the mouse standing on both hind paws in a vertical upright position; (Seibenhener and Wooten, 2015)) and *head dipping* (i.e. the mouse dips its head below the height of the open arm) behaviours were analysed. Finally, *stretch attend* postures (i.e. forward elongation of head and shoulders followed by retraction to original position; (Espejo, 1997)) were assessed and used as a parameter for risk assessment behaviour (Espejo, 1997; Karl et al., 2008; Walf and Frye, 2007).

2.3.2. Open field (OF)

To conduct the open field test, mice were put into an empty arena made of grey Perspex (36 cm × 36 cm × 24 cm) for 10 min and the AnyMaze™ tracking software recorded the performance of the mice. The open arena was divided into a central (22 cm × 22 cm) and peripheral zone (7 cm from all walls) to not only record the total distance travelled, but also the time spent in centre and the percentage distance travelled in centre. Other behaviours like *rearing* [i.e. the mouse standing on both hind paws in a vertical upright position; (Seibenhener and Wooten, 2015)] and *freezing* (i.e. complete behavioural immobility except for respiration; (Crawley, 1999)) were scored manually. Analysing the combination of these parameters provides insights into anxiety-related behaviours (i.e. time spent in centre, percentage distance travelled in centre, and *freezing*) and exploration (i.e. *rearing*) (Crawley, 1999; Denenberg, 1969; Seibenhener and Wooten, 2015).

2.3.3. Novel object recognition test (NORT)

To evaluate the object recognition memory, the NORT uses the mice's ability to distinguish between familiar and unfamiliar objects and the mice's innate preference for novelty (Dere et al., 2007). The NORT was conducted over two consecutive days. On day one, the mice were habituated to the empty arena (see 2.3.2 for details). Two 10-min habituation trials were performed with an ITI (inter-trail interval) of two hours. During the 10 min, the mice were allowed to explore the NORT arena freely. The first of these habituation trials was utilised as the open field test (see 2.3.2 for details). On the second day, one training and one test trial were conducted. For the training trial, two identical objects were placed in two opposing quadrants of the NORT arena, and the mice were allowed to freely investigate the two identical objects. After an ITI (inter-trail interval) of 15 min, one of the identical objects was replaced by an unfamiliar object and the mice were allowed to freely investigate the two objects (one familiar object and one unfamiliar, novel object). Investigation of the objects (i.e. *sniffing*, *rearing* and *climbing* the object) was scored manually and calculated as (here based on the example *sniffing*)

$$\frac{\text{time } \textit{sniffing} \text{ novel object}}{\text{time } \textit{sniffing} \text{ both (familiar + novel) objects}} \times 100$$

to analyse object recognition memory. Based on previous literature, seven mice (two *APP/PS1*-VEH, three WT-THCxCBD and two *APP/PS1*-THCxCBD) were excluded from NORT because they did not show a minimum of 10 s of exploration of either or both objects during training (Cheng et al., 2014b; Coles et al., 2020; Watt et al., 2020). In fact, two vehicle-treated *APP/PS1* mice did not explore either object in the training trial at all (zero time spent *sniffing*).

2.3.4. Social interaction (SI)

To asses social behaviour in mice the social interaction test against a standard opponent mouse (i.e. female A/JArc mouse) was performed (Kudryavtseva et al., 2018). The female A/JArc mouse and the female experimental mouse were put into opposite corners of the empty Perspex arena (same as in OF and NORT so no habituation trial required) and were allowed to interact with each other freely for 10 min. During the test, the total frequency and time spent on *active social interaction* was measured, thereby scoring following active social behaviours: *sniffing*, *anogenital sniffing*, *rearing*, and *following* of the test mouse. One *APP/PS1*-THCxCBD mouse had to be excluded from the social interaction test as the mouse had to be euthanised for animal welfare reasons.

2.3.5. Y-maze (YM)

To evaluate short-term memory, the YM with spontaneous alternation can be used (Kraeuter et al., 2019). The Y-maze consists of three arms (30 cm × 9 cm × 17 cm, material: grey Perspex) at a 120° angle from each other with internal clues on the walls (dots, vertical stripes and chequered) that are all connected through a triangular central platform (9 cm side length). The mice were put in the centre of the Y-maze facing towards one of the arms and were allowed to explore the maze freely for 8 min in line with previously published studies (Long et al., 2010b). The sequence of arm entries between the arms (e.g. ABCBAB…) was scored manually, the number of correct triplets (i.e. an alternation between the arms ABC, CBA, ACB, …) were counted and the percentage of correct spontaneous alternation was calculated as

$$\text{spontaneuos alternation } [\%] = \frac{\text{number of correct triples}}{(\text{number of arm entries}) - 2} \times 100.$$

2.3.6. Prepulse inhibition (PPI)

As described previously, a startle response is an unconditioned response to a sudden stimulus (i.e. acoustic stimulus). The mechanism that a non-startling prepulse suppresses the startle response is called prepulse inhibition (PPI). The PPI model is one option to evaluate sensorimotor gating (i.e. the ability of the brain to filter redundant stimuli) (Ioannidou et al., 2018; Karl et al., 2003). Test mice were habituated to the animal enclosures and startle chambers (SR-LAB, San Diego Instruments, San Diego, USA) for 10 min on two consecutive days (continuous background noise of 70 dB was displayed in the chambers). On the third day, the PPI test (approximately 30 min) was conducted and included the following session parts: acclimatization phase (i.e. 5 min to 70 dB continuous background noise) followed by 97 trials presented in a pseudorandom order: 5 × 70 dB trials, 5 × 100 dB trials, 3 sets of 5 × 120 dB trials (presented at the beginning, in the middle and at the end of the test session to check for startle habituation), as well as 4 sets of 6 trials with 74, 82 or 86 dB prepulses followed by a 120 dB startle pulse with variable interstimulus intervals (ISI) of 32, 64, 128 or 254 ms). The intertrial interval (ITI) across test session differed randomly between 10 and 20 s. The acoustic startle response (ASR) was calculated as the mean amplitude to all startle trails and percentage PPI (%PPI) was calculated as

$$\%\text{PPI} = \frac{\text{mean startle response (120 dB)} - \text{PPI response}}{\text{mean startle response (120 dB)}} \times 100.$$

%PPI was averaged across interstimulus intervals (ISI) to produce a mean %PPI for each prepulse intensity (74, 82 or 86 dB) in line with previous publications (Karl et al., 2011).

2.4. Statistical analysis

Two-way ANOVA was used to discover main effects of "genotype", "treatment" and interactions between the two experimental between factors. In addition, three-way repeated measures (RM) ANOVA was used to determine effects of the within-subject factors "startle intensity" (PPI), "startle block" (PPI), "prepulse intensity" (PPI) and "time" (bodyweight). If interactions were evident, data were split by the corresponding factors and were further analysed with the corresponding follow up statistical analysis. Finally, one-sample *t*-test was performed in the NORT test to determine if "percentage time spent *sniffing* the novel object" or "percentage time spent *sniffing*, *rearing* and *climbing* the novel object" was greater than chance level (i.e. 50 %). Data are presented as mean ± standard error of the mean (SEM) and individual data points are added to figures where appropriate to increase clarity of results. Differences were assessed as statistically significant if $p < 0.05$. F-values and degrees of freedom are presented for ANOVAs, significant "genotype" effects are indicated by "*" ($^{*}p < 0.05$ and $^{**}p < 0.01$), "treatment" effects by "#" ($^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$), "repeated measure" effects by "^" ($^{\wedge\wedge}p < 0.001$) and significant interactions by "×" ($^{\times}p < 0.05$, $^{\times\times}p < 0.01$ and $^{\times\times\times}p < 0.001$). Significant *t*-test results against chance level (i.e. 50 %, NORT) are indicated by "+" ($^{+}p < 0.05$). Analyses were conducted using GraphPad Prism 9 and IBM SPSS Statistics 27 for Windows. All statistical details of non-significant findings have been added to Supplementary Table 1.

3. Results

3.1. Elevated Plus Maze (EPM)

3.1.1. Locomotion

APP/PS1 transgenic mice demonstrated WT-like locomotion and chronic treatment with THCxCBD had no significant effect on total distance travelled. No significant interaction effect between experimental factors was evident either (all *p*'s > 0.05; Fig. 1A and Supplementary Table 1). Analysing exploratory behaviour (i.e. frequency of *rearing* and *head dipping*) did not reveal any significant differences between mice regardless of genotype or treatment and no interaction effect was significant either (all *p*'s > 0.05; Table 2).

3.1.2. Anxiety

Two-way ANOVA showed that *APP/PS1* mice spent a similar amount of time on the open arms of the EPM as their WT littermates. Treatment with cannabinoids had no significant effect on time spent on open arms and no interaction between the main effects were evident (all *p*'s > 0.05; Fig. 1B). Analysing time spent on the second half of the open arms (i.e. further away from the center area; e.g. outer arm) revealed no main effects of "genotype" or "treatment" (both *p*'s > 0.05). However, a significant interaction between "genotype" and "treatment" for time spent on the outer open arm was evident ([$F(1,44) = 6.3$, $p = 0.02$]; splitting the data by "genotype" revealed a significant "treatment" effect in *APP/PS1* mice [$F(1,20) = 5.5$, $p = 0.03$] as cannabinoid treatment reduced the time spent compared to vehicle-treated *APP/PS1* mice (Fig. 1C – not significant in WT mice). Split by "treatment", a "genotype" effect was evident in the THCxCBD-treated mice with *APP/PS1* mice spending a reduced time on the outer open arms [$F(1,21) = 4.4$, $p = 0.049$] compared to WT females (Fig. 1C - not significant in vehicle-treated mice). When analysing the percentage distance travelled on open arms, two-way ANOVA revealed a significant main effect of "genotype" [$F(1,44) = 4.9$, $p = 0.03$] as *APP/PS1* mice exhibited reduced open arm locomotion compared to WT females (Fig. 1D). Chronic treatment with THCxCBD had no effect on this test parameter ($p > 0.05$). A trend interaction between "genotype" and "treatment" was evident ([$F(1,44) = 4.0$, $p = 0.0525$]; Fig. 1D). Following the explorative nature of this study, data were split by "genotype", revealing a "treatment" effect for *APP/PS1* transgenic mice [$F(1,20) = 4.8$, $p = 0.04$] but not their WT littermates ($p > 0.05$) with THCxCBD treatment reducing open arm locomotion in AD transgenic mice. Moreover, splitting the data by "treatment" revealed a "genotype" effect for the cannabinoid-treated mice [$F(1,21) = 6.8$, $p = 0.02$] with *APP/PS1* mice displaying reduced open arm locomotion in comparison to WT females (no significant effect of genotype under vehicle treatment conditions, $p > 0.05$). In addition, *APP/PS1* females exhibited reduced percentage of outer open arm locomotion compared to WT females ("genotype": [$F(1,44) = 4.2$, $p = 0.047$]; Fig. 1E). A significant interaction between "genotype" and "treatment" was also detected [$F(1,44) = 8.4$, $p = 0.006$]. Splitting the data by "genotype", THCxCBD-treated *APP/PS1* mice showed a reduction in outer open arm locomotion compared to the vehicle condition [$F(1,20) = 6.7$, $p = 0.02$] whereas cannabinoid-treated WT mice displayed an increase in this behaviour [$F(1,24) = 5.5$, $p = 0.03$] compared to VEH-WT mice (Fig. 1E). Split by "treatment", a "genotype" effect was evident under cannabinoid treatment conditions [$F(1,21) = 6.8$, $p = 0.02$] with *APP/PS1* showing a reduced percentage distance travelled on the outer open arms. This genotype difference was not detected in the vehicle treatment group ($p > 0.05$). THCxCBD treatment had no overall effect on outer open arm locomotion ($p > 0.05$; Fig. 1E). Finally, no significant main effects or interactions were detected for the frequency of *stretch attend postures* (all *p*'s > 0.05; Table 2).

3.2. Open field (OF)

3.2.1. Locomotion

Two-way ANOVA did not reveal any effects between experimental groups for total distance travelled in the OF test (no "genotype" or "treatment" effects and no interaction either; all *p*'s > 0.05; Fig. 2A). Furthermore, analysing explorative behaviours (i.e. frequency and duration of *rearing*) did not reveal any significant differences between experimental groups either (all *p*'s > 0.05; Table 2).

3.2.2. Anxiety

A significant main effect of "genotype" for time spent in the OF centre was evident [$F(1,44) = 8.1$, $p = 0.007$] as *APP/PS1* female mice spent less time in this area compared to control females (Fig. 2B). Cannabinoid treatment had no effect on OF centre time and also did not modulate the genotype difference detected (i.e. no main effect of "treatment" and no interaction with "genotype", both *p*'s > 0.05). Similarly, percentage distance travelled in the OF centre was decreased in AD transgenic females across treatment conditions [$F(1,44) = 10.9$, $p = 0.002$] and was not affected by treatment (i.e. no main 'treatment' effect and no interaction with "genotype": all *p*'s > 0.05; Fig. 2C). In line with these findings, time spent *freezing* was increased in *APP/PS1* mice regardless of their treatment ([$F(1,44) = 4.8$, $p = 0.03$], no main effect of "treatment" and no interaction were observed: all *p*'s > 0.05; Fig. 2D).

3.3. Novel object recognition test (NORT) - object recognition memory:

One-sample *t*-test revealed that vehicle-treated AD transgenic mice showed a deficit in recognizing the novel object as they were not *sniffing* the novel object significantly longer than by chance (i.e. 50 %) [$t(9) = 1.6$, $p = 0.1$] whereas the corresponding WT females had a significant preference for *sniffing* the novel object ([$t(12) = 2.4$, $p = 0.03$]; Fig. 3A). Mice treated with the purified cannabinoids did not show a preference for the novel object regardless of genotype [*APP/PS1*-THCxCBD: $t(7) = 1.3$, $p = 0.2$; WT-THCxCBD: $t(9) = 1.4$, $p = 0.2$]. Performing two-way ANOVAs to look for differences across experimental groups did not reveal any significant main effects or interaction for percentage time spent *sniffing* the novel object (all *p*'s > 0.05). Time spent exploring the novel object (e.g. percentage time spent *sniffing*, *rearing*, and *climbing* the

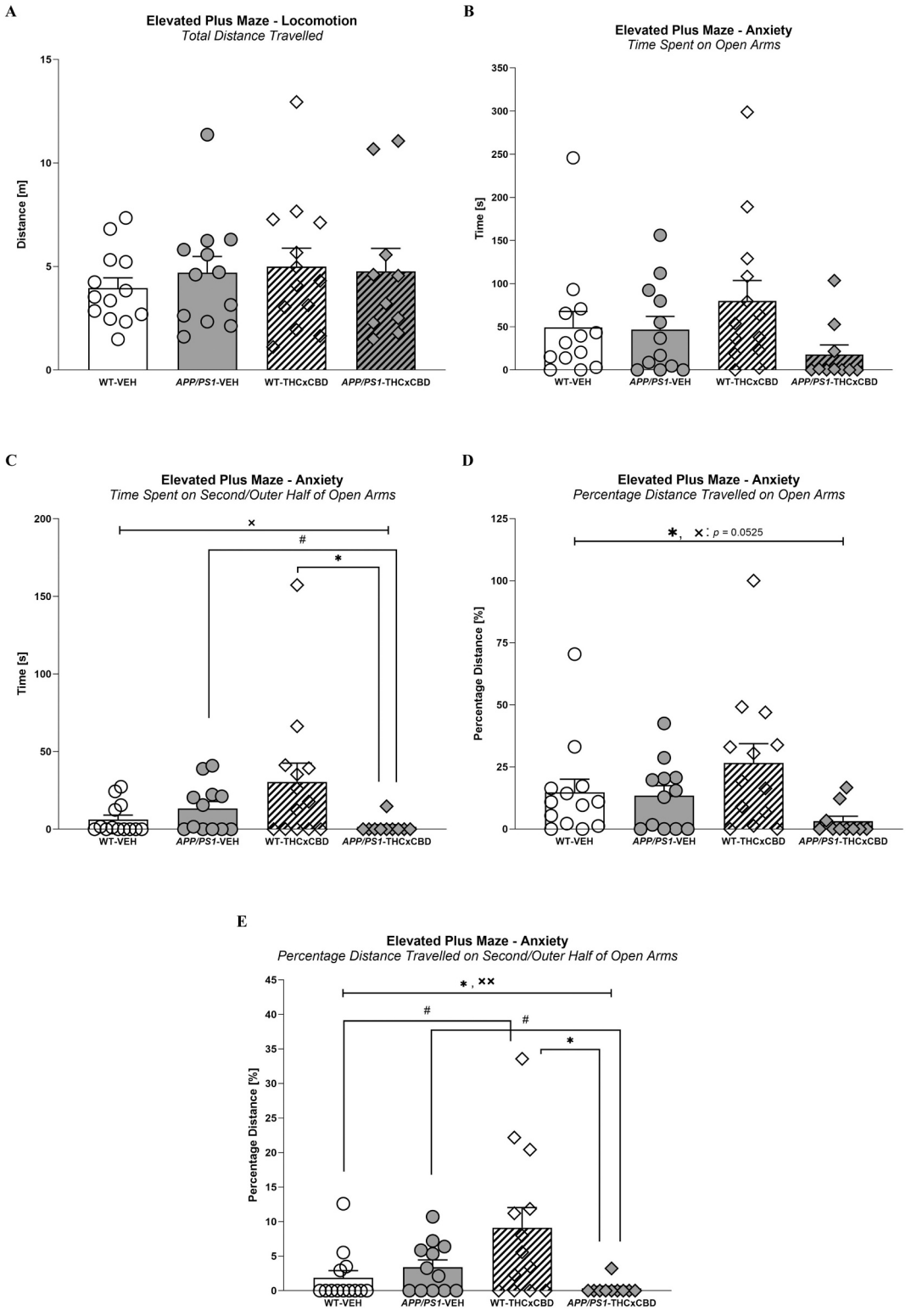

Fig. 1. A-E. Elevated Plus Maze (EPM) - Locomotion and anxiety-related behaviours: A) Total distance travelled [m], B) time spent on open arms [s], C) time spent on second/outer half of open arms [s], D) percentage distance travelled on open arms [%] and E) percentage distance travelled on second/outer half of open arms [%]. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9* (*APP/PS1*) female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM and individual data points are indicated. Significant "genotype" effects are indicated by "*" ($^{*}p < 0.05$), significant "treatment" effects are indicated by "$^{\#}$" ($^{\#}p < 0.05$), significant "genotype" by "treatment" interactions are indicated by "$^{\times}$" ($^{\times}p < 0.05$ and $^{\times\times}p < 0.01$) or the exact trend interaction has been indicated by $^{\times}p = 0.0525$; Fig. 1D).

Table 2
Exploration and anxiety-related behaviours in the Elevated Plus Maze (EPM) and the open field (OF): Frequencies [n] of explorative behaviours (*rearing, head dipping*) and risk assessment (*stretch attend posture*) in the EPM and frequencies [n] and time spent [s] *rearing* in the OF. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM.

Treatment	VEH		THCxCBD	
Genotype	WT	*APP/PS1*	WT	*APP/PS1*
EPM				
Frequency of *rearing* [n]	14.5 ± 2.8	11.2 ± 1.9	14.5 ± 2.5	14.1 ± 3.0
Frequency of *head dipping* [n]	23.7 ± 3.0	26.2 ± 5.6	20.4 ± 2.5	15.4 ± 3.3
Frequency of *stretch attend posture* [n]	10.6 ± 2.0	11.3 ± 1.7	11.1 ± 1.5	14.5 ± 2.2
OF				
Frequency of *rearing* [n]	42.8 ± 5.3	33.6 ± 8.1	30.8 ± 7.0	22.4 ± 5.4
Time spent *rearing* [s]	58.9 ± 7.5	50.9 ± 9.0	45.3 ± 10.3	34.5 ± 9.8

novel object) revealed similar results [one-sample *t*-test for WT-VEH: $t(12) = 3.5$, $p = 0.004$; all other p's > 0.05; no differences between experimental groups; Fig. 3B].

3.4. Social interaction (SI)

Two-way ANOVA did not reveal any significant main effects or interaction thereof for the various individual social behaviours (frequency and duration of *sniffing*, *anogenital sniffing*, *rearing*, and *following*; all p's > 0.05; Table 3 – frequency data not shown). No differences were detected for total *active social interaction* time either ($p > 0.05$; Table 3).

3.5. Y-maze (YM) - short-term memory

APP/PS1 transgenic mice and WT mice demonstrated a similar level of spontaneous alternation during Y-Maze testing. Cannabinoid treatment did not affect spontaneous alternation and no interaction effect was revealed either (all p's > 0.05; Fig. 4).

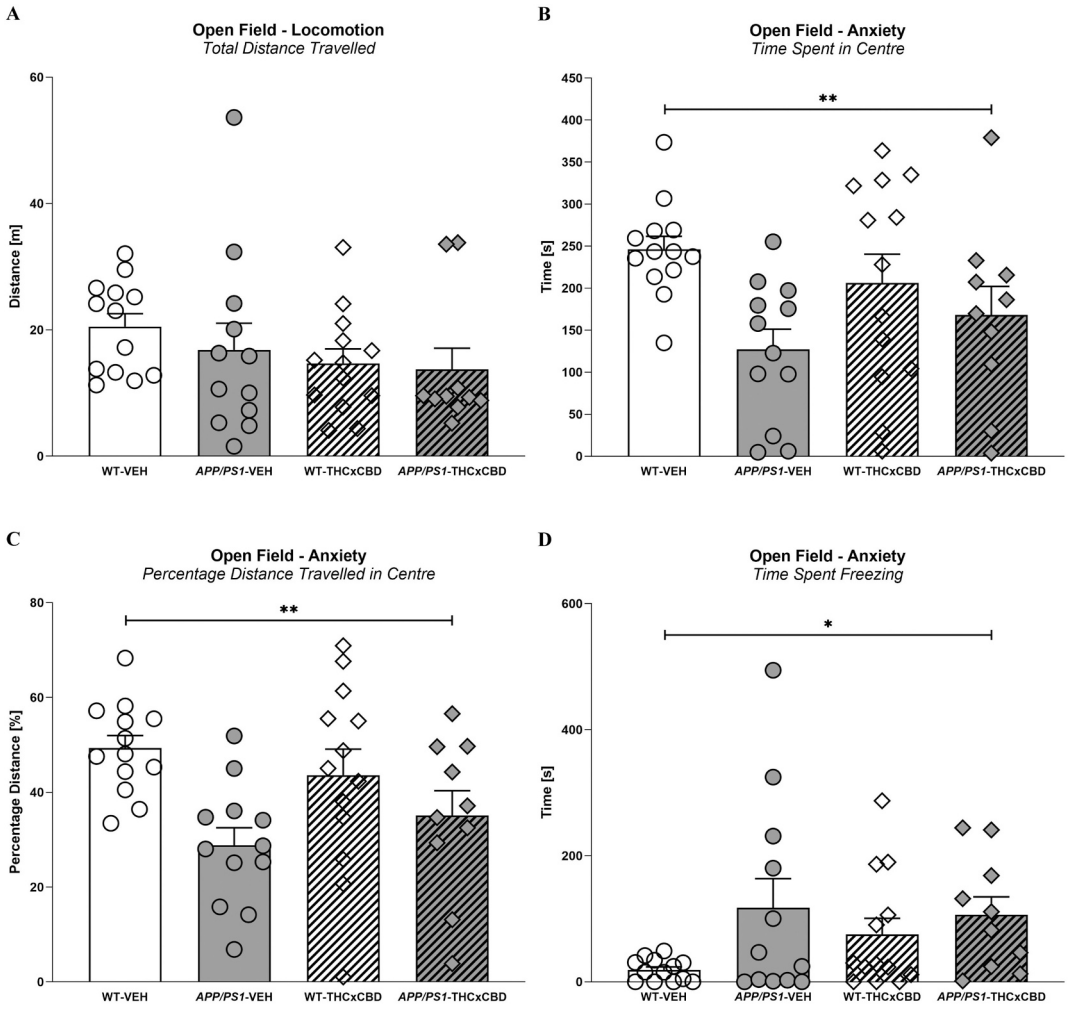

Fig. 2. A-D. Open field (OF) - Locomotion and anxiety-related behaviours: A) Total distance travelled [m], B) time spent in OF centre [s], C) percentage distance travelled in OF centre [%] and D) time spent *freezing* [s]. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM and individual data points are indicated. Significant "genotype" main effects across treatment conditions are indicated by "*" (*$p < 0.05$ and **$p < 0.01$).

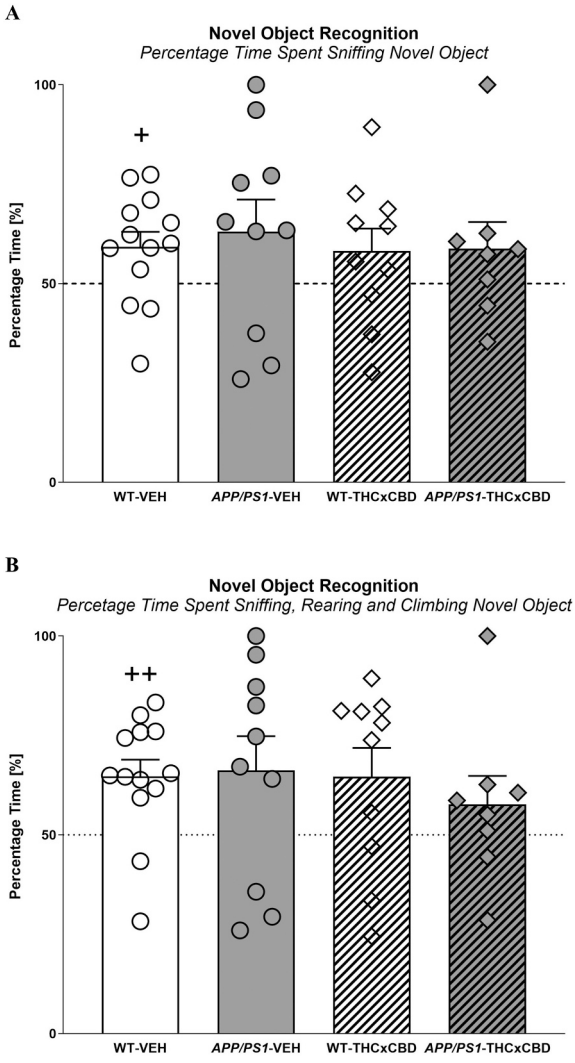

Fig. 3. A-B. Novel object recognition test (NORT) - object recognition memory: A) Time spent *sniffing* and B) combined time spent *sniffing*, *rearing,* and *climbing* the novel object as a percentage of total *sniffing* (or total *sniffing*, *rearing,* and *climbing*) both objects [%]. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM and individual data points are indicated. Significant single sample *t*-test results against chance level (i.e. 50 %, dotted line) are indicated by "$^{+}$" ($^{+}p < 0.05$ and $^{++}p < 0.01$).

3.6. Prepulse inhibition (PPI): acoustic startle response and sensorimotor gating

3.6.1. Acoustic startle response (ASR)

All mice showed increasing startle responses to increasing startle pulse intensities [three-way RM ANOVA for "startle intensity": $F(2,88) = 61.5$, $p < 0.0001$; Fig. 5A]. No main effects for "genotype" or "treatment" were evident across startle pulse intensities (all p's > 0.05). However, three-way RM ANOVA revealed an interaction between "startle intensity" and "genotype" [$F(2,88) = 3.2$, $p = 0.046$; Fig. 5A]. Splitting the data by "startle intensity" showed a trend effect of "genotype" [$F(1,44) = 3.5$, $p = 0.07$] at 120 dB with *APP/PS1* mice showing a higher ASR compared to WT littermates. No other significant effects were detected (all p's > 0.05). Analysing ASR habituation, all mice displayed decreasing ASR across the three 120 dB startle pulse blocks [three-way RM ANOVA for "startle block": $F(2,88) = 11.7$, $p < 0.0001$; Fig. 5B] indicating that all groups habituated to the 120 dB startle stimulus. No treatment effect or interactions were detected (all p's > 0.05).

Table 3
Social interaction (SI): Time spent [s] on *active social interaction* and on individual social behaviours (*sniffing*, *anogenital sniffing*, *rearing,* and *following)* in the SI test. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM.

Treatment	VEH		THCxCBD	
Genotype	WT	*APP/PS1*	WT	*APP/PS1*
Active social interaction [s]	119.1 ± 13.4	112 ± 13.6	120.8 ± 10.3	91.6 ± 9.4
Sniffing [s]	89.6 ± 10.0	85.1 ± 7.2	89.4 ± 8.4	72.0 ± 6.1
Anogenital sniffing [s]	13.0 ± 1.9	12.7 ± 3.7	15.3 ± 2.6	10.3 ± 1.9
Rearing [s]	15.6 ± 3.3	13.5 ± 5.0	15.6 ± 2.2	7.5 ± 2.5
Following [s]	1.0 ± 0.5	0.7 ± 0.4	0.5 ± 0.3	1.8 ± 1.0

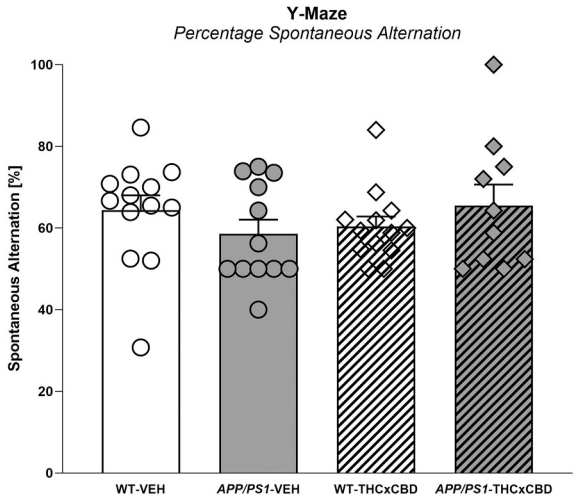

Fig. 4. Y-maze (YM) - short-term memory: Percentage spontaneous alternation [%] shown. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM and individual data points are indicated.

3.6.2. Prepulse inhibition (PPI)

Three-way RM ANOVA revealed that increasing prepulse intensities led to a significant increase in %PPI [three-way RM ANOVA for "prepulse intensity": $F(2,88) = 89.2$, $p < 0.0001$; Fig. 5C]. *APP/PS1* mice showed the same level of %PPI as non-transgenic mice, THCxCBD treatment had no effect on %PPI and no interaction between factors were evident either (all p's > 0.05). Finally, analysing %PPI averaged across all three prepulse intensities did not detect any differences between the experimental groups either (all p's > 0.05; Fig. 5D).

3.7. Bodyweight and fat weight analysis

3.7.1. Bodyweight

Three-way RM ANOVA detected a significant effect of "time" [$F(5,220) = 69.9$, $p < 0.0001$], a significant main effect of "treatment" [$F(1,44) = 11.7$, $p = 0.001$] as well as an interaction of both factors [three-way RM ANOVA for "time x treatment": $F(5,220) = 5.5$, $p < 0.0001$] on

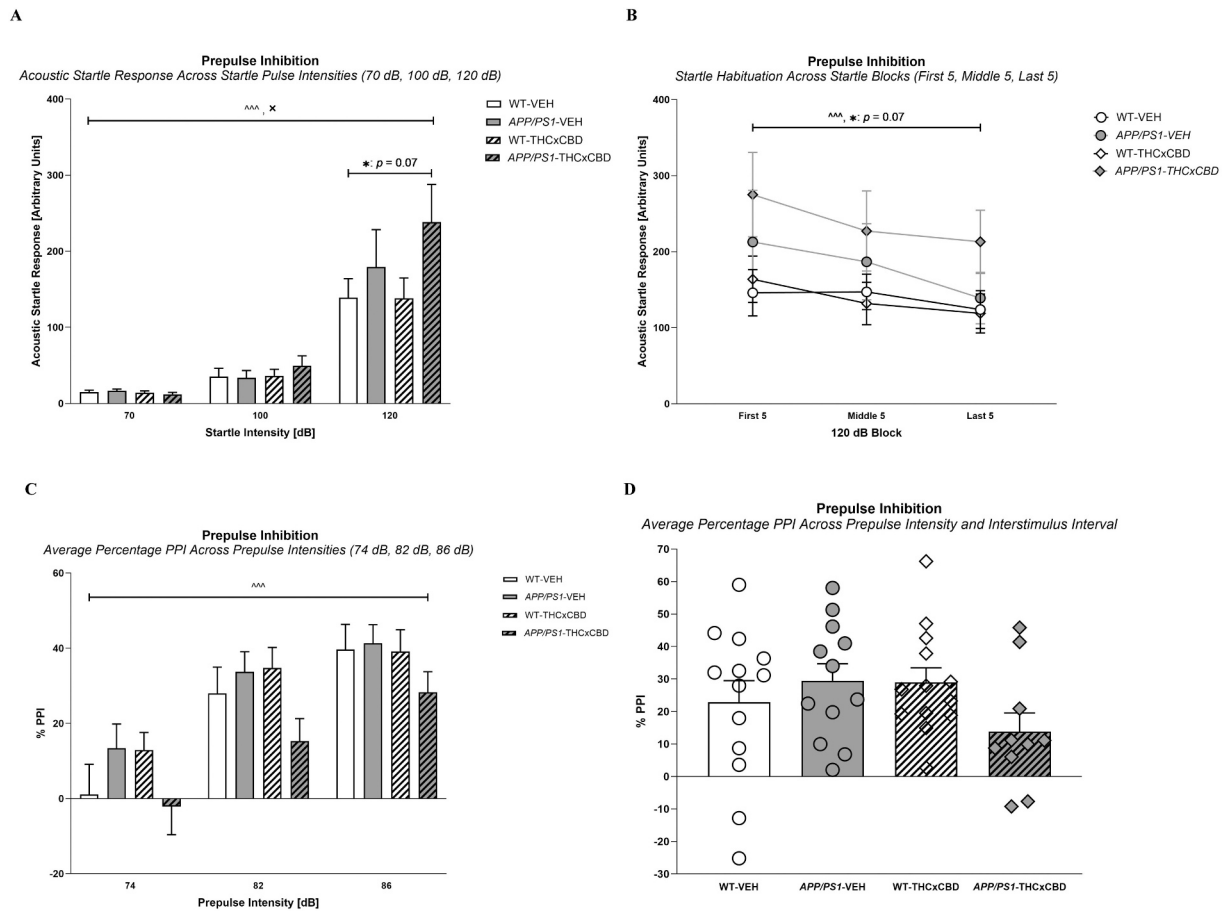

Fig. 5. A-D. Acoustic startle response (ASR) and sensorimotor gating (i.e. prepulse inhibition: PPI): A) ASR [arbitrary units] for three startle pulse intensities (70/100/120 dB), B) habituation to the ASR [arbitrary units] across the first, middle, and last block of five 120 dB startle pulse presentations, C) percentage PPI [%PPI] for three prepulse intensities (74/82/86 dB - averaged across inter-stimulus intervals), and D) percentage PPI [%PPI] averaged across prepulse intensities and inter-stimulus intervals. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM and individual data points are indicated where appropriate (D). Significant repeated measure effects across experimental conditions are indicated by "^" ($^{\wedge\wedge\wedge}p < 0.001$), significant "startle intensity" by "genotype" interactions are indicated by "×" ($^{\times}p < 0.05$) and an exact trend "genotype" main effect across treatment conditions has been indicated by $^{*}p = 0.07$; Fig. 5A and B).

bodyweight development with both treatment groups showing weight loss over the time and THCxCBD-treated mice generally losing weight quicker compared to VEH-treated mice (Fig. 6). Splitting the data by "time" confirmed main "treatment" effects across genotype in weeks 1–5. Importantly, prior to any treatment (i.e. week 0), no significant effect of "treatment" was detected [two-way ANOVA for "treatment" for week 0: $F(1,44) = 3.6$, $p = 0.06$; Fig. 6]. "Genotype" had no main effect on bodyweight development ($p > 0.05$ for main effect and all interactions with genotype as a factor).

3.7.2. Inguinal and retroperitoneal fat

Two-way ANOVA revealed significant "treatment" effects for the weight of inguinal fat as a percentage of total bodyweight [$F(1,44) = 18.9$, $p < 0.0001$], percentage of retroperitoneal fat [$F(1,44) = 16.0$, $p = 0.0002$], as well as the sum of both fat depots (i.e. total fat) [$F(1,44) = 19.3$, $p < 0.0001$] (Table 4). Cannabinoid-treated mice had less fat compared to vehicle-treated mice and this treatment effect was not influenced by "genotype" (all interactions: p's > 0.05) and no overall genotype differences were detected either (all p's > 0.05).

4. Discussion

This study presents the first evaluation of the therapeutic-like effects of a cannabinoid combination treatment with 3 mg/kg THC and 20 mg/kg CBD on female 14.5-month-old *APP/PS1* mice. AD transgenic mice displayed increased OF anxiety compared to control females regardless of treatment conditions whereas EPM anxiety was only elevated in *APP/PS1* females when they had been treated with cannabinoids. Interestingly, the effects of cannabinoid treatment on EPM anxiety were genotype-specific as WT females displayed a moderate anxiolytic-like EPM phenotype post treatment (no effects of treatment on OF anxiety). *APP/PS1* females also tended to startle more to a 120 dB startle stimulus compared to control littermates with cannabinoid treatment having no effect on this phenotype. Most importantly, vehicle-treated AD transgenic females exhibited a deficient object recognition memory. THCxCBD had no therapeutic effect on this deficit and rather impaired object recognition memory in WT females. Locomotion, exploration, spontaneous alternation, social interaction, and sensorimotor gating were neither affected by genotype nor treatment. Finally, cannabinoid treatment resulted in a stronger reduction of bodyweight across the experimental test period and also lowered the weight of inguinal and retroperitoneal fat deposits in both genotypes compared to vehicle-treated mice.

Female vehicle-treated *APP/PS1* mice displayed increased OF anxiety and also showed a trend for an elevated ASR, which can be associated with increased fear and anxiety (Brown et al., 1951; Walker and Davis, 1997). The anxiety phenotype of *APP/PS1* females appears to be task- as well as age-dependent. Indeed, previous studies in 10–12

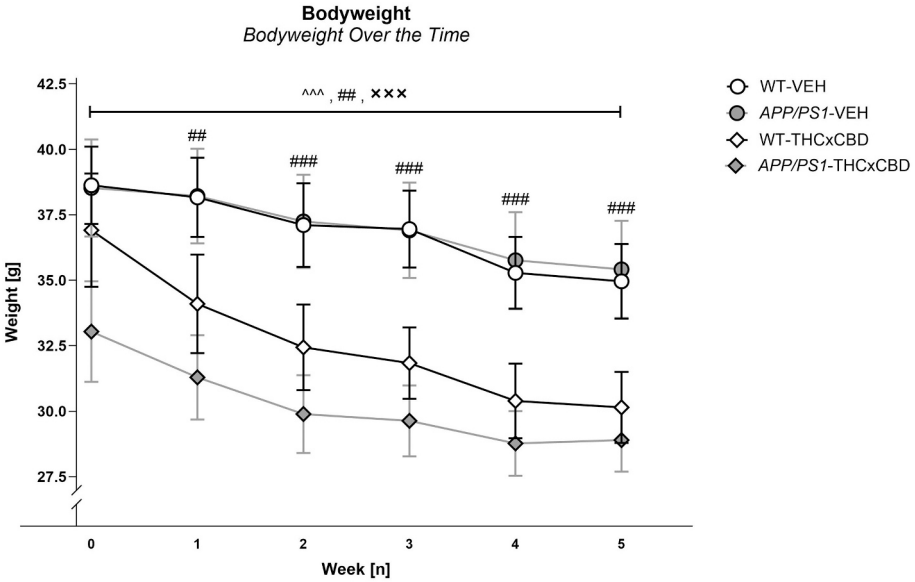

Fig. 6. Bodyweight development across time: Bodyweight [g] per week is shown. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM. Significant repeated measure effects across experimental factors are indicated by "^" ($^{\wedge\wedge\wedge}p < 0.001$), significant "treatment" effects are indicated by "#" ($^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ – including once data were split by 'time') and a significant "time" by "treatment" interaction is indicated by "×" ($^{\times\times\times}p < 0.001$).

Table 4
Inguinal, retroperitoneal, and total fat deposit weights: Inguinal, retroperitoneal, and total fat weights as a percentage of total bodyweight [%] are shown. Total fat is defined as the sum of inguinal and retroperitoneal fat. Data for non-transgenic control (WT) and double transgenic *AβPP$_{Swe}$/PS1ΔE9 (APP/PS1)* female mice treated with either vehicle (VEH) or combined purified cannabinoids (i.e. 3 mg/kg BW delta-9-tetrahydrocannabinol and 20 mg/kg BW cannabidiol: THCxCBD) are presented as mean ± SEM. Significant "treatment" effects across genotypes are indicated by "#" ($^{\#\#\#}p < 0.001$).

Treatment	VEH		THCxCBD	
Genotype	WT	*APP/PS1*	WT	*APP/PS1*
Inguinal Fat as a percentage of total BW [%]$^{\#\#\#}$	4.5 ± 0.4	4.9 ± 0.7	2.9 ± 0.4	2.4 ± 0.3
Retroperitoneal Fat as a percentage of total BW [%]$^{\#\#\#}$	2.0 ± 0.2	1.7 ± 0.2	1.1 ± 0.2	0.9 ± 0.1
Total Fat as a percentage of total BW [%]$^{\#\#\#}$	6.5 ± 0.7	6.6 ± 0.9	4.0 ± 0.5	3.2 ± 0.4

months old females revealed an anxiogenic-like phenotype of *APP/PS1* mice the light-dark (LD) apparatus (Coles et al., 2020) and the EPM (Chesworth et al., 2022) whereas 9-month-old females exhibited an anxiolytic-like phenotype in the LD test but not in the EPM (Cheng et al., 2014b). It is also important to note that although EPM, LD and OF are all spatio-temporal anxiety tests, they do not reliably reproduce each other (reviewed in (Mohammad et al., 2016)).

Chronic THCxCBD treatment increased anxiety in *APP/PS1* females but triggered an opposite although more subtle response in control females in the EPM. Previous work from our laboratory found no effect of chronic CBD treatment (20 mg/kg and 5 mg/kg) on the anxiety phenotype of *APP/PS1* females (Chesworth et al., 2022; Coles et al., 2020) and males (Cheng et al., 2014c). However, particular CBD doses triggered anxiolytic-like effects in male C57BL/6JArc mice (Long et al., 2010b). Looking at THC effects on anxiety, 3-week-treatment with 10 mg/kg but not 3 mg/kg THC increased OF and LD anxiety in C57BL/6JArc males (Long et al., 2010b) and lower doses of THC mediate anxiolytic-like responses (Berrendero and Maldonado, 2002) in line with the established biphasic effect of cannabinoids on anxiety (Viveros et al., 2005).

This genotype-specific cannabinoid effects reported here has also been found in other studies, for example for acute CBD treatment effects in wildtype-like and transgenic (Kv1.3−/− mice) females (Huffstetler et al., 2023).This could be related to the genotype-specific expression profile of *cannabinoid receptor 1* (*Cnr1*) (also evident for *cannabinoid receptor 2*) in *APP/PS1* mice. Higher mRNA levels of *Cnr1* have been reported in the prefrontal cortex, the hypothalamus and the olfactory bulb of AD transgenic females (Vidal-Palencia et al., 2022). Elevated *Cnr1* expression combined with the beforementioned biphasic effect of cannabinoids on anxiety (Viveros et al., 2005) may cause female *APP/PS1* mice to change from anxiolytic- to anxiogenic-like responses already at lower THC doses than control females. Considering the potentially interactive effects of 3 mg/kg THC and 20 mg/kg CBD, previous research found that high CBD low THC combinations often show anxiolytic-like properties (Liu et al., 2022) whereas e.g. an even 1:1 ratio of CBD and THC can induce anxiety according to information published on the UK Electronic Medicines Compendium website (Jazz Pharmaceuticals and Electronic Medicines Compendium, January 2025) or have no effect on this domain (Aso et al., 2015).

The detected object recognition memory deficits in *APP/PS1* females are in line with previous studies (Coles et al., 2020; Watt et al., 2020). Interestingly, *APP/PS1* females appear to exhibit these cognitive impairments later than male AD transgenic mice (Cheng et al., 2013; Cheng et al., 2014b). Interestingly, *APP/PS1* females develop glucose and insulin intolerance at a later stage of life than males (Li et al., 2016), these intolerances can be associated with synaptic failure, memory decline and Alzheimer's disease (reviewed in (De Felice et al., 2014)). It should be noted here that some mice (including two vehicle-treated *APP/PS1* females were excluded from the analysis as their exploration times of the two objects during training were below 10 s (in line with our previous publications (Cheng et al., 2014b; Coles et al., 2020; Watt et al., 2020)). This affects the statistical power of the data analysed but was necessary to not include 'false positives' as mice not exploring objects during the training would not be able to show intact object recognition memory during the following test trial.

THCxCBD treatment did not reverse the deficit in AD females and actually caused detrimental effects on recognition memory in wildtype-

like females. This is an important finding as both cannabinoids in isolation have been found to rescue cognitive deficits. Chronic treatment with 20 mg/kg and 5 mg/kg (but not 50 mg/kg) of purified CBD restored object recognition deficits in 7-month-old male and 12-month-old female AD transgenic mice, respectively (Cheng et al., 2014a; Coles et al., 2020; Watt et al., 2020) and 3 mg/kg of THC administered via subcutaneous minipumps improved cognitive function (novel object location recognition test) in 12- and 18-month-old mice (Bilkei-Gorzo et al., 2017). Furthermore, combination studies utilising a ratio of 1:1 (THC + CBD, 0.75 mg/kg each) in 6- and 12-month-old *APP/PS1* males found beneficial effects of THCxCBD on object recognition memory in the V-maze test (Aso et al., 2016b; Aso et al., 2015). However, a combination of 10 mg/kg THC and 20 mg/kg CBD caused object recognition impairments in female C57BL/6 sublines (Kasten et al., 2019). Future research will need to clarify further at which dose and ratio THCxCBD combination treatments may have clinical relevance for AD therapy.

In the current study, locomotion, exploration, spontaneous alternation, social interaction, and sensorimotor gating were not affected by genotype or treatment. Previous studies have reported task-dependent phenotypes with similar findings in 7–10 months old male and female *APP/PS1* mice in the EPM but not the LD test (Cheng et al., 2013; Cheng et al., 2014a, 2014b; Cheng et al., 2014c) or the OF (Hulshof et al., 2022). Spontaneous alternation was also not impaired in female *APP/PS1* mice, in line with another study in which 18-month-old but not 6- or 12-month-old male *APP/PS1* mice displayed spontaneous alternation impairment (Chaney et al., 2018). Interestingly, male *APP/PS1* mice exhibit spontaneous alternation deficits at a younger age (i.e. 7 and 12 months of age) when only external cues are available (Kim et al., 2016; Kim et al., 2014). The absence of any phenotype differences in social and sensorimotor gating reported here are in line with our previous baseline work in 9–11-month-old *APP/PS1* females (Cheng et al., 2014b) but in opposition to the PPI testing of 12-month-old *APP/PS1* transgenic females (Coles et al., 2020).

THCxCBD treatment did not modify locomotion, exploration, social interaction or sensorimotor gating. The particular dose regime has not been tested previously, however, our previous work utilising purified CBD in *APP/PS1* females found a similar inactivity on these domains (Coles et al., 2020). Furthermore, neither purified CBD (1, 5, 10 or 50 mg/kg) nor THC (0.3, 1, 3 or 10 mg/kg) had an effect on spontaneous alternation in 3-month-old C57BL/6JArc male mice (Long et al., 2010b). The locomotor depressant effects of THC reported elsewhere (Long et al., 2010a; Long et al., 2010b) were not evident in the current when THC was combined with 20 mg/kg CBD, which could be related to the antagonistic effect of CBD on the cannabinoid CB_1 receptor through negative allosteric modulation (Laprairie et al., 2015). Interestingly, cannabinoid effects on sensorimotor gating and social interaction appear highly dose-dependent with chronic CBD enhancing or lowering PPI in C57BL/6JArc mice ((Long et al., 2010b; Schleicher et al., 2019); chronic THC at various doses with no effect) and THC affecting social domains in opposing ways ((Bilkei-Gorzo et al., 2017; Long et al., 2010b); chronic CBD at various doses with no effect). Interestingly, acute administration of CBD reversed THC-induced decrement of social interaction in male rats (Malone et al., 2009), possibly explaining the lack of combined effect for social interaction in the current study.

Cannabinoid-treated females lost more weight compared to vehicle-treated mice across the experimental period and also exhibited lower inguinal and retroperitoneal fat with no genotype differences being evident. Another study on *APP/PS1* mice actually detected reduced bodyweight and lean tissue mass compared to age-matched WT mice at 12- and 18-months of age but these mice had not been chronically treated (Lin et al., 2019). Looking at a comprehensive review on the effects of CBD and THC on obesity, both an increase and a decrease in bodyweight and adipose tissue across species (primarily mice, rats and humans) have been described (Fearby et al., 2022) so further research is needed to clarify relationship between cannabinoid treatment and bodyweight and fat tissue development.

In summary, this study revealed that 14.5-month-old *APP/PS1* females displayed an anxiety-like phenotype and object recognition deficits. Combined cannabinoid treatment increased anxiety-like behaviour in *APP/PS1* mice task-specifically, had negative effects on object recognition memory in WT mice, and led to a significant reduction of bodyweight and inguinal and retroperitoneal fat deposits in all mice. Thus, the treatment design chosen did not improve symptoms associated with Alzheimer's disease (Alzheimer's Association, 2023) in female 14.5-month-old *APP/PS1* mice. In the future, different dosages of THC and CBD combinations (possibly with an even lower THC dosage) should be considered to further understand the potential value of cannabinoid combination treatment for Alzheimer's disease treatment.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2025.174101.

CRediT authorship contribution statement

Beate Aumer: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Rossana Rosa Porto:** Writing – review & editing, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Madilyn Coles:** Writing – review & editing, Supervision, Software, Methodology, Conceptualization. **Nina Ulmer:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Georgia Watt:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Heike Kielstein:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Tim Karl:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Data availability

Data will be made available on request.

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