

Chronic cannabidiol treatment improves social and object recognition in double transgenic APP_{swe}/PS1ΔE9 mice

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Abstract

Rationale Patients suffering from Alzheimer's disease (AD) exhibit a decline in cognitive abilities including an inability to recognise familiar faces. Hallmark pathological changes in AD include the aggregation of amyloid- β (A β), tau protein hyperphosphorylation as well as pronounced neurodegeneration, neuroinflammation, neurotoxicity and oxidative damage. **Objectives** The non-psychoactive phytocannabinoid cannabidiol (CBD) exerts neuroprotective, anti-oxidant and anti-inflammatory effects and promotes neurogenesis. CBD also reverses A β -induced spatial memory deficits in rodents. **Materials and methods** Thus we determined the therapeutic-like effects of chronic CBD treatment (20 mg/kg, daily intraperitoneal injections for 3 weeks) on the APP_{swe}/PS1 Δ E9 (APPxPS1) transgenic mouse model for AD in a number of cognitive tests, including the social preference test, the novel object recognition task and the fear conditioning paradigm. We also analysed the impact of CBD on anxiety behaviours in the elevated plus maze.

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Results Vehicle-treated APPxPS1 mice demonstrated impairments in social recognition and novel object recognition compared to wild type-like mice. Chronic CBD treatment reversed these cognitive deficits in APPxPS1 mice without affecting anxiety-related behaviours.

Conclusions This is the first study to investigate the effect of chronic CBD treatment on cognition in an AD transgenic mouse model. Our findings suggest that CBD may have therapeutic potential for specific cognitive impairments associated with AD.

Keywords Alzheimer's disease · Novel therapeutic · Cannabidiol · Transgenic APP_{swe}/PS1 Δ E9 mice · Cognition · Behaviour · Social recognition memory · Object recognition memory

Introduction

Alzheimer's disease (AD) is the most prominent form of dementia. Patients suffering from AD demonstrate a decline in general cognitive ability including mild to severe memory loss, social withdrawal, an inability to recognise familiar faces and increased incidences of wandering (Chung and Cummings 2000; Reisberg et al. 1982). AD is characterised by two pathological hallmarks: (1) the aggregation of amyloid- β (A β) protein forming plaque deposits and (2) tau protein hyperphosphorylation, resulting in neurofibrillary tangle formation (Gotz and Ittner 2008). Neurodegeneration, neuroinflammation, neurotoxicity and oxidative damage are also prominent in post-mortem brain tissue of AD patients (Barger and Basile 2001; Koppel and Davies 2008; Marchalant et al. 2008; Pomara et al. 1992; Pratico and Sung 2004; Williams et al. 2006).

Existing treatments for AD such as acetylcholinesterase inhibitors and *N*-methyl-*D*-aspartate (NMDA) receptor antagonists provide short-term relief for cognitive and functional decline but are generally ineffective against disease

progression (Benito et al. 2007; Marchalant et al. 2008; Micale et al. 2007). Using compounds which target different aspects of AD pathology simultaneously (i.e. multimodal drug approach) may provide increased therapeutic benefits for patients compared to the more traditional interventions (Farlow et al. 2010; Tariot et al. 2004). Targeting the endocannabinoid (EC) system might be such an approach. The EC system has recently emerged as a possible therapeutic target for patients with AD, as it appears to be a neuroprotective system that responds to neurotoxic insult, including A β deposition [for review, see (Karl et al. 2012b)]. In particular, the phytocannabinoid cannabidiol (CBD), a non-psychoactive component of *cannabis sativa*, promises potential for the multimodal treatment of AD due to its neuroprotective, anti-inflammatory and anti-oxidant properties (Booz 2011; Iuvone et al. 2009; Krishnan et al. 2009; Scuderi et al. 2011; Zuardi 2008). Based on these properties, CBD may be able to counter many pathological symptoms of AD, and indeed a number of in vitro studies have shown that CBD treatment attenuates A β -induced neurotoxicity and cell death (Iuvone et al. 2004), tau protein-induced hyperphosphorylation (Esposito et al. 2006) and promotes hippocampal and adult neurogenesis (Esposito et al. 2011; Wolf et al. 2010).

Importantly, only a few studies have investigated the therapeutic potential of CBD in vivo and only in pharmacological rodent models for AD (i.e. intraventricular injection of A β). Treatment with CBD reduced A β -induced neuroinflammation (Esposito et al. 2007, 2011), rescued spatial memory deficits in the Morris water maze (MWM) and promoted microglial migration, a cellular mechanism that may enable the removal of A β deposits (Martin-Moreno et al. 2011).

Thus the multimodal nature of CBD suggests that CBD might be effective in treating a range of AD-relevant behaviours and brain pathologies. In the current study, we investigated for the first time the effectiveness of chronic CBD treatment to diminish behavioural deficits of an established transgenic mouse model of familial AD. Familial AD (early onset, autosomal dominant) is caused by mutations in one of three genes: amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2) (Gotz and Ittner 2008). The double transgenic APP_{swe}/PS1 Δ E9 (APPxPS1) mouse model we utilised exhibits accelerated amyloid pathology due to the co-expression of APP and PS1 mutant genes (Borchelt et al. 1997; Jankowsky et al. 2004a, b; Machova et al. 2010). We have previously reported that male APPxPS1 mice demonstrated a social recognition deficit and increased anxiety-like behaviour by the age of 6–7 months (Cheng et al. 2013), while other studies report impairment in object recognition in female APPxPS1 mice (Donkin et al. 2010; Jardanhazi-Kurutz et al. 2010). In the present study, we hypothesised that chronic treatment with CBD will reverse the behavioural and cognitive deficits of male APPxPS1 transgenic mice. To investigate this, we placed APPxPS1

transgenic males on a 3-week CBD treatment schedule before assessing them in a battery of behavioural and cognitive tests: the social preference test (SPT), the elevated plus maze (EPM), the novel object recognition task (NORT) and the fear conditioning paradigm (FC).

Materials and methods

Animals

Double transgenic mice expressing chimeric mouse/human APP (Mo/HuAPP695swe/Swedish mutations K595N/M596L) and mutant human PS1 (PS1/ Δ E9) mice (APPxPS1) were obtained from Jackson Laboratory (Bar Harbor, USA; stock no. 004462, line 85) and maintained as double hemizygotes on C57BL/6JxC3H/HeJ background as described previously (Borchelt et al. 1997; Jankowsky et al. 2001, 2004a, b). Male transgenic mice (APPxPS1; $N=23$) and their non-transgenic littermates [wild type-like (WT); $N=22$] were bred and group-housed in independently ventilated cages (Type Mouse Version 1: Airlaw, Smithfield, Australia) at Animal BioResources (Moss Vale, Australia). Test mice were transported to the Neuroscience Research Australia (NeuRA) at around 10 weeks of age, where they were group-housed in polysulfone cages (1144B: Tecniplast, Rydalmere, Australia) with corn cob bedding (Bed-O'Cobs: Able Scientific, Perth, Australia), a red transparent, polycarbonate igloo (certified mouse igloo from Bio-Serv, Frenchtown, NJ, USA) and some tissues for nesting. Mice were kept under a 12:12-h light/dark schedule [light phase between 0830 and 2030 hours and white light (illumination 124 lx)–dark phase, red light (illumination <2 lx)]. Environmental temperature was automatically regulated at 21 ± 1 °C and relative humidity was 40–60 %. Food (Rat and Mouse Maintenance Pellets: Gordon's Specialty Stockfeeds, Yanderra, Australia) and water were provided ad libitum, except where specified. Adult male A/JArc mice from the Animal Resources Centre (Canning Vale, Australia) were used as standard opponents in the social preference test. Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee in accordance with the Australian code of practice for the care and use of animals for scientific purposes.

Drug preparation and administration

Powdered cannabidiol (CAS: 13956-29-1 THC Pharma GmbH, Frankfurt/Main, Germany) was dissolved in equal amounts of Tween 80 (Sigma-Aldrich Co., St Louis, USA) and 100 % ethanol to the appropriate concentration and diluted with 0.9 % sodium chloride as published previously (Long et al. 2010, 2012). Ethanol and Tween 80 comprised 10 % of

the total volume. A vehicle (control) treatment group was set up similarly without the addition of CBD. Vehicle and CBD (20 mg/kg body weight) were administered by intraperitoneal (i.p.) injection (injection volume of 10 ml/kg body weight). The dose was chosen based on a previous study in a pharmacologically induced A β mouse model (Martin-Moreno et al. 2011).

Mice were assigned to either vehicle or CBD groups and treated daily for 3 weeks prior to the start of the behavioural assessment, beginning at 24 weeks of age (± 1 week). This treatment design was selected as the only other study evaluating the therapeutic potential of chronic CBD treatment for AD also used a 3-week treatment design and found that CBD could reverse A β -induced spatial memory deficits in a pharmacological model of AD (Martin-Moreno et al. 2011). Furthermore, therapeutic-like effects of CBD in mouse models of schizophrenia have been found after 3 weeks of CBD treatment (Long et al. 2010, 2012). Body weight of animals was monitored weekly. Treatment continued until the end of behavioural testing when mice were 32 weeks of age (± 2 weeks). The total duration of treatment was 8 weeks. Injections occurred post-testing in the afternoon (between 1400 and 1600 hours) to avoid acute effects of CBD administration modifying the behavioural performance of APPxPS1 and WT mice (Deiana et al. 2012) (for treatment details see Table 1).

Behavioural test battery

Mice were tested in a battery of tests sensitive to detect behavioural and cognitive deficits in APPxPS1 mice (Cheng et al. 2013; Karl et al. 2012a; Logge et al. 2012) (for test details see Table 1). All tests were conducted early in the light phase between 0930 and 1400 hours except for the elevated plus maze, which was conducted late in the dark phase of the light cycle (0500–0830 hours). An inter-test interval of at least 48 h was used to minimise the effect of repeated testing.

Equipment and apparatus were cleaned between trials using 70 % ethanol except where specified.

Social preference test (SPT) The SPT was used to assess sociability and social recognition memory (Moy et al. 2004) and performed as described in an earlier study assessing baseline behaviours of APPxPS1 males (Cheng et al. 2013). Test animals were isolated for 1 h prior to the start of testing. During the habituation trial, mice were allowed to explore a three-chamber apparatus, consisting of a centre chamber (length 9 cm; width 18 cm; depth 20 cm) and two outer chambers (16 cm \times 18 cm \times 20 cm), freely for 5 min. For the sociability test, an unfamiliar standard opponent (male A/J mouse) was placed in one of two animal enclosures (i.e. opponent chamber) in a quasi-randomised fashion (mouse enclosures allowed nose contact between mice but prevented fighting). The test mouse was returned to the apparatus and allowed to explore all three chambers and the animal enclosures for 10 min. Finally, test animals were observed in a 10-min social recognition test. For this, a second, unfamiliar standard opponent was placed in the previously empty chamber so that the test mouse had the choice to explore either the familiar mouse (from the previous trial) or the novel, unfamiliar mouse. The inter-trial interval (ITI) was 5 min. The chambers and enclosures were cleaned with 30 % ethanol in between trials and fresh corn cob bedding was added to the chambers prior to each test trial. ANY-MazeTM (Stoelting, Wood Dale, USA) tracking software was used to determine the time spent in the different chambers, number of entries and distance travelled by the test mice in each trial. Animals were excluded as non-performers if they climbed on top of the enclosures for more than 30 s during testing. A total of seven animals (N) were excluded (3 WT-VEH, 2 APPxPS1-VEH, 1 WT-CBD, 1 APPxPS1-CBD) leaving $N=8-11$ animals per test condition.

Table 1 Treatment schedule and test order of APPxPS1 and WT mice

Treatment	Vehicle		CBD	
	WT ($N=11$)	APPxPS1 ($N=11$)	WT ($N=11$)	APPxPS1 ($N=12$)
Age at start of treatment	25 \pm 1	25 \pm 1	24 \pm 1	25 \pm 1
Mice received daily i.p. injections of 20 mg/kg CBD for 3 weeks prior to behavioural testing				
Social preference test	28 \pm 1	28 \pm 1	27 \pm 1	28 \pm 1
EPM	29 \pm 1	29 \pm 1	28 \pm 1	29 \pm 1
NORT	30 \pm 1	30 \pm 1	29 \pm 1	30 \pm 1
Fear conditioning	32 \pm 2	33 \pm 3	32 \pm 2	33 \pm 2

Age [weeks] of APPxPS1 mice and their WT counterparts at the start of treatment, throughout behavioural tests and at the end of treatment (total duration of CBD treatment was 8 weeks)

APPxPS1 male APP_{Swe}/PS1 Δ E9 transgenic mice, CBD cannabidiol, WT wild type-like mice, EPM elevated plus maze, NORT novel object recognition task

Elevated plus maze (EPM) The EPM assesses the natural conflict between the tendency of mice to explore a novel environment and avoidance of a brightly lit, elevated open area (Montgomery 1955; Montgomery and Monkman 1955) and was employed to investigate potential anxiolytic-like effects of chronic CBD treatment. Indeed, CBD may exert acute anxiolytic effects that are detectable by the EPM (Guimaraes et al. 1990; Onaivi et al. 1990). The '+' apparatus consisted of two alternate open arms (35 cm×6 cm; without sidewalls; highly illuminated 70 lx) and two alternate enclosed arms (35 cm×6 cm; height of enclosing walls 28 cm; dimly illuminated 10 lx) connected by a central platform (6 cm×6 cm) and was elevated 70 cm above the floor. Mice were placed at the centre of the '+' of the grey PVC plus maze [for further details of apparatus see (Karl et al. 2008)] facing an enclosed arm and were allowed to explore the maze for 5 min. The time spent and distance travelled in the open and enclosed arms were recorded using ANY-Maze™ tracking software.

Novel object recognition test (NORT) Object recognition memory in the NORT is demonstrated by the animals' ability to distinguish between familiar and unfamiliar objects [as rodents have an innate preference towards novelty (Dere et al. 2007)]. The NORT was conducted over 3 days [as described previously (Logge et al. 2012)]: two trials (10 min per trial) were conducted per day with a 1 h ITI. On day 1, mice were habituated to the empty arena during both trials. On day 2, mice were habituated to the empty arena during trial 1 and to two identical objects during trial 2. On the test day, mice were exposed to two identical objects in the sample trial (trial 1; objects distinct from day 2) and a familiar and a novel object in the test trial (trial 2). The objects and their locations were counterbalanced across genotypes. Time spent *nosying* and *rearing* on the objects were recorded using ANY-Maze™ tracking software. The percentage of exploration time (time spent *nosying*+*rearing* objects) for the novel object (% novel exploration) was calculated using [(novel object exploration time/novel+familiar object exploration time)×100] and indicated short-term object recognition memory. Animals were excluded if they showed low levels of activity (i.e. <15 s of total object exploration time).

Fear conditioning (FC) Fear conditioning assesses hippocampus- and amygdala-dependent associative learning whereby a previously neutral stimulus elicits a fear response after it has been paired with an aversive stimulus. On conditioning day, mice were placed into the test chamber (model H10-11R-TC, Coulbourn Instruments, USA) for 2 min. Then, an 80 dB conditioned stimulus (CS) was presented for 30 s with a co-terminating 0.4 mA, 2 s foot shock (unconditioned stimulus; US) twice with an inter-pairing interval of 2 min. The test concluded 2 min later. The next day (context test), mice were returned to the apparatus for 7 min. On day 3 (cue

test), animals were placed in an altered context for 9 min. After 2 min (pre-CS/baseline), the CS was presented continuously for 5 min. The test concluded after another 2 min with the absence of the CS [for more details see (Chesworth et al. 2012; Duffy et al. 2010)]. Time spent *freezing* was measured for all three experimental days using ANY-Maze™ software.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to analyse behavioural parameters for main effects of 'genotype' and 'treatment' in all tests. Repeated measures (RM) two-way ANOVA was used to evaluate effects of 'chamber' (SPT) and '1 min block' (FC) as published previously (Logge et al. 2012). Performance in the SPT was also assessed using one-sample *t* tests to clarify whether the percentage of time spent in the opponent/novel chamber was above chance (50 %). Differences were regarded as significant if $p < 0.05$. Data are shown as means±standard error of means (SEM). *F* values and degrees of freedom are presented for ANOVA, and significant genotype and treatment effects are shown in figures and tables as '*' ($p < 0.05$) and '#' ($p < 0.05$), respectively, whereas *t* test results for social novelty preference are presented by '+' ($p < 0.05$), ++ ($p < 0.01$), +++ ($p < 0.001$). Analyses were conducted using SPSS 20.0 for Windows.

Results

Cognition

Sociability and social recognition All mice across treatments demonstrated a preference for sociability in the three-chamber social preference test. RM ANOVA revealed a significant effect of 'chamber' for time spent in both chambers [$F(1,41)=74.2, p < 0.001$], where all mice spent significantly more time in a chamber with an opponent over an empty chamber. *T* test for percentage of time spent with the novel mouse confirmed that all mice demonstrated significant levels of sociability above chance [WT-VEH, $t(10)=7.2, p < 0.001$; APPxPS1-VEH, $t(10)=5.4, p < 0.001$; WT-CBD, $t(10)=2.4, p < 0.05$; APPxPS1-CBD, $t(11)=5.8, p < 0.001$] (Fig. 1a).

In the social preference test, RM ANOVA revealed a significant effect of 'chamber', with test mice spending more time with the novel mouse than the familiar mouse [chamber $F(1,34)=26.7, p < 0.001$]. Importantly, an effect of 'genotype' for time spent in the chamber with the novel mouse was only found for vehicle-treated, but not CBD-treated, mice with APPxPS1-VEH mice showing reduced time spent with the novel mouse compared to WT-VEH mice [$F(1,34)=6.6, p < 0.05$] (Fig. 1b). Indeed, one-sample *t* test revealed that all groups, except APPxPS1-VEH animals, spent a significantly

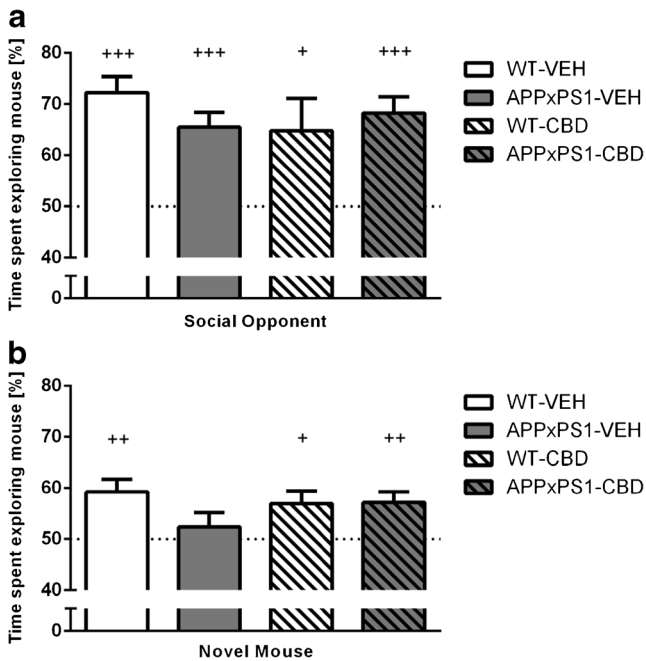


Fig. 1 Sociability and social recognition in the social preference test. **a** Percentage of time spent [%] in test chambers containing an unfamiliar mouse (i.e. opponent) compared to the total time spent with both the opponent and the empty mouse enclosure (WT-VEH, $N=11$; APPxPS1-VEH, $N=11$; WT-CBD, $N=11$; APPxPS1-CBD, $N=12$); **b** Percentage of time spent [%] in test chamber containing an unfamiliar (i.e. novel) mouse compared to the total time spent with both the novel and the familiar mouse (WT-VEH, $N=8$; APPxPS1-VEH, $N=9$; WT-CBD, $N=10$; APPxPS1-CBD, $N=11$). Data for non-transgenic WT control and double transgenic APPxPS1 male mice in vehicle and CBD groups are shown as means+SEM. One-sample t test results for novelty preference are presented by $^{+++}$ ($^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$). APPxPS1 male APP_{Swe}/PS1 Δ E9 transgenic mice, CBD cannabidiol, WT wild type-like mice

greater percentage of time with the novel mouse than the familiar mouse [WT-VEH, $t(7)=3.7$, $p<0.01$; APPxPS1-VEH, $t(8)=0.8$, $p=0.4$; WT-CBD, $t(9)=2.8$, $p<0.05$; APPxPS1-CBD, $t(10)=3.4$, $p<0.01$].

Novel object recognition Two-way ANOVA revealed no overall effects of ‘genotype’ or ‘treatment’ (all $p>0.05$), but an interaction between ‘genotype’ and ‘treatment’ was found for novel object recognition [$F(1,35)=4.7$, $p<0.05$]. To investigate this interaction, data were split by ‘treatment’ which revealed a deficit in vehicle-treated APPxPS1 mice in novel object recognition compared to WT-VEH mice [$F(1, 19)=4.5$, $p<0.05$; Fig. 2]. No genotype differences were found between CBD-treated mice ($p>0.05$). Furthermore, an effect of ‘treatment’ was found between APPxPS1-VEH and APPxPS1-CBD groups [$F(1,17)=4.7$, $p<0.05$], giving evidence that CBD treatment restored object recognition in APPxPS1 transgenic mice.

Fear conditioning All mice responded to the electric foot shocks delivered during the conditioning phase as determined

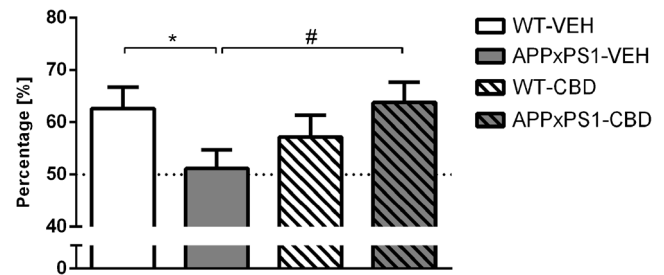


Fig. 2 Novel object recognition task. Time spent nosing towards the novel object over the familiar object expressed as a percentage of total time spent interacting with both objects [%]. Data for non-transgenic WT control and double transgenic APPxPS1 male mice in vehicle, and CBD groups are shown as means+SEM (WT-VEH, $N=11$; APPxPS1-VEH, $N=11$; WT-CBD, $N=11$; APPxPS1-CBD, $N=12$). $^{*}p<0.05$, significant ‘genotype’ effects; $^{\#}p<0.05$, significant ‘treatment’ effects. APPxPS1 male APP_{Swe}/PS1 Δ E9 transgenic mice, CBD cannabidiol, WT wild type-like mice

by their vocalisation. Two-way ANOVA found that all mice demonstrated similar amounts of *freezing* behaviour at baseline and during the context test (all $p>0.05$) (Table 2). Further, all mice responded with an increase in *freezing* post-CS onset [RM ANOVA $F(1,41)=13.7$, $p<0.001$] regardless of ‘genotype’ or ‘treatment’ (Table 2).

Anxiety

EPM testing found no effects of ‘genotype’ or ‘treatment’ on anxiety-like measures such as percentage of time spent in the open arms, percentage of distance travelled in the open arms and percentage of open arms entries (all $p>0.05$) (Table 3).

Discussion

Our study is the first to investigate the treatment effects of CBD in an established transgenic mouse model of AD. Daily treatment with CBD for 3 weeks rescued social recognition memory and improved object recognition deficits of male APPxPS1 transgenic mice. These effects were specific for recognition memory as CBD had no impact on fear-associated memory or anxiety measures.

The SPT measures both sociability and social recognition in the APPxPS1 transgenic mouse model. Sociability was intact in APPxPS1 mice whereas AD transgenic mice exhibited a clear social recognition memory deficit. This failure to distinguish between novel and familiar social opponents confirms our previous report for untreated male APPxPS1 mice of a similar age (Cheng et al. 2013). Importantly, animals of that study were also tested for their olfactory ability and no differences between transgenic and control mice were detected. The current study shows that chronic CBD treatment reversed the social recognition deficit observed in APPxPS1 mice, which regained a WT-like preference for the novel opponent post-treatment. AD mice have previously

Table 2 Fear-associated memory

	Vehicle		CBD	
	WT	APPxPS1	WT	APPxPS1
Baseline (first 2 min)				
Conditioning <i>freezing</i> [s]	9.7±3.7	4.3±1.4	4.7±1.5	7.7±2.2
Context <i>freezing</i> [s]	26.1±5.5	23.3±6.5	23.9±7.2	35.9±8.0
Cue <i>freezing</i> [s]	20.9±3.2	21.5±4.8	21.8±3.8	34.3±6.2
Context				
Total time spent <i>freezing</i> [s]	87.9±17.4	92.4±22.3	89.8±19.8	117.7±25.0
Cue				
Time spent <i>freezing</i> 1 min prior to cue onset [s]	13.1±1.7	16.3±3.7	15.2±2.4	21.7±3.3
Time spent <i>freezing</i> 1 min post-cue onset [s]	21.5±3.8	20.8±4.9	23.4±4.3	26.4±3.9

Time spent freezing [s] during baseline, context test and the cue test is shown for non-transgenic WT control and double transgenic APPxPS1 male mice treated with either vehicle or CBD. Data are presented as mean±SEM (WT-VEH, $N=11$; APPxPS1-VEH, $N=11$; WT-CBD, $N=11$; APPxPS1-CBD $N=12$)

APPxPS1 APP_{Swe}/PS1ΔE9 transgenic mice, CBD cannabidiol, WT wild type-like mice

been reported to develop impaired social recognition memory, including Thy1-hAPP(Lond/Swe+) transgenic mice (Faizi et al. 2012) and APPxPS1 mice maintained on pure C57BL/6J background (Filali et al. 2011), while AD patients have difficulties recognising familiar faces (Reisberg et al. 1982). Thus CBD's ability to rescue social recognition memory is worthy of further investigation as this therapeutic-like potential might have relevance in clinical settings.

Male APPxPS1 mice also demonstrated deficits in object recognition. Again, CBD treatment improved object recognition memory of APPxPS1 mice, as transgenic mice showed a wild type-like preference for a novel object post-CBD administration. Importantly, male APPxPS1 mice have not been assessed for object recognition memory previously, but impaired object recognition has been found in female APPxPS1 mice (Donkin et al. 2010; Jardanhazi-Kurutz et al. 2010). Furthermore, in support of our findings, male APPxPS1 mice bred on the C57BL/6J background also displayed deficits in object recognition (Yoshiike et al. 2008) as did other double

transgenic AD mouse models including the APP(swe)/PS1(L166P) and APP(NLh)/PS1(P264L) lines (Duszczyk et al. 2012; Webster et al. 2013). Impairments in object recognition have been linked to dysregulation of the glutamatergic system (Nilsson et al. 2007) as well as hippocampal and parahippocampal dysfunction (Fernandez et al. 2007). Interestingly, Hallak and colleagues found that CBD interacts with the glutamatergic system (Hallak et al. 2011); the phytocannabinoid augmented the effects of an *N*-methyl-*D*-aspartate (NMDA) receptor antagonist in a human study. CBD was also shown to be protective against glutamate neurotoxicity (Hampson et al. 1998). Finally, memantine, another NMDA receptor antagonist, improved object recognition in another transgenic AD mouse model (Scholtzova et al. 2008) suggesting that CBD may improve recognition memory via the glutamatergic pathway. It is interesting to note here that facial recognition is not linked to deficits in NMDA receptor activity (Rammsayer 2001), demonstrating the potential multimodality of CBD.

Table 3 Anxiety-like behaviours

	Vehicle		CBD	
	WT	APPxPS1	WT	APPxPS1
Time spent on open arms [%]	5.7±2.7	4.1±1.0	4.7±2.3	7.2±2.1
Time spent on open arms [s]	11.0±5.2	7.9±1.9	8.1±3.3	12.3±3.5
Entries into open arms [%]	15.5±3.5	16.4±2.0	15.5±4.0	20.2±2.5
Distance travelled on open arms [%]	4.1±1.8	3.7±1.0	3.5±1.3	5.7±3.1
Total distance travelled [m]	7.1±0.6	8.3±0.6	7.2±0.5	8.0±0.6

Anxiety-like behaviours in the EPM of non-transgenic WT control and double transgenic APPxPS1 male mice treated with either vehicle or CBD. Data are presented as mean±SEM (WT-VEH, $N=11$; APPxPS1-VEH, $N=11$; WT-CBD, $N=11$; APPxPS1-CBD, $N=12$)

APPxPS1 APP_{Swe}/PS1ΔE9 transgenic mice, CBD cannabidiol, EPM elevated plus maze, WT wild type-like mice

APPxPS1 transgenic mice did not develop an impairment in fear-associated memory which is in line with what we previously observed in male APPxPS1 mice (Cheng et al. 2013). Furthermore, CBD did not impact on this behavioural domain in WT or APPxPS1 transgenic mice. One other study found an effect of subchronic CBD treatment (i.e. 2-week treatment with 10 mg/kg CBD) on *freezing* behaviour in the conditioned emotional response paradigm (ElBatsh et al. 2012). CBD increased the *freezing* response of drug-treated Lister-hooded rats 24 h post-conditioning. This phenomenon could be related to an impact of CBD on anxiety (potentially anxiogenic but no baseline *freezing* was evaluated in those rats) or cognitive behaviours (potentially improving contextual fear conditioning). Importantly, ElBatsh and co-workers tested rats, not mice, and applied 10-foot shock-context pairings during the conditioning phase, which presents a more stressful test protocol than the one utilised in our study. In this context, it is important to mention that chronic CBD treatment did not modify the anxiety response of APPxPS1 mice in the elevated plus maze in the current study, and we also could not find an effect of genotype in this paradigm. Previously, we reported increased anxiety in male APPxPS1 mice when we employed a light-phase EPM test (Cheng et al. 2013). Thus, the differences between the earlier study and the current investigation are likely due to the circadian rhythm (animals were tested in the EPM during the dark phase in the current study) and the necessary daily injection procedure for all mice tested. Importantly, the earlier study did not reveal any differences in anxiety-like behaviour in the light-dark test (Cheng et al. 2013), suggesting that the anxiety phenotype of APPxPS1 mice is not only circadian rhythm but also task-dependent. Finally, previous studies reporting an anxiety phenotype in APPxPS1 mice did not specify test conditions (e.g. illumination, test time) thereby making a direct comparison of study outcomes impossible (Lalonde et al. 2004; Reiserer et al. 2007). CBD has previously been found to induce anxiolytic-like properties (Campos and Guimaraes 2008; Campos et al. 2013; Guimaraes et al. 1990; Long et al. 2010, 2012; Moreira et al. 2006; Onaivi et al. 1990). However, these effects were predominantly evident after acute treatment regimes (Campos and Guimaraes 2008; Campos et al. 2013; Guimaraes et al. 1990; Long et al. 2010; Moreira et al. 2006; Onaivi et al. 1990) whereas our behavioural testing commenced only after 3-week CBD treatment. Therefore, it is not that surprising that we could not detect an anxiolytic-like effect of CBD treatment on EPM behaviours. Our results are in line with Campos and colleagues who recently showed that subchronic (i.e. 2-week) CBD treatment (30 mg/kg) failed to produce anxiolytic-like effects in the EPM or the novelty suppressed feeding paradigm in control mice (although CBD reversed the anxiogenic effects of 14 days of unpredictable stress) (Campos et al. 2013).

In this study, we demonstrate for the first time a beneficial effect of chronic CBD treatment on recognition memory in a transgenic mouse model for AD. These findings are novel and

expand on earlier work describing the effectiveness of CBD to rescue A β -induced spatial memory deficits in wild type-like mice (Martin-Moreno et al. 2011). The same study described a profound effect of CBD on A β -induced neuroinflammation (i.e. reduction of interleukin-6, a biomarker for inflammation) and attenuation of microglial activation (Martin-Moreno et al. 2011). Furthermore, other studies reported similar anti-inflammatory effects of CBD in rats and mice (Esposito et al. 2007, 2011). Importantly, APPxPS1 mice reportedly show elevated levels of neuroinflammation (i.e. increased nitric oxide species and TNF- α) in the hippocampus (Kalifa et al. 2011) and neuroinflammatory processes are thought to be linked to neurodegeneration and cognitive impairment in humans (Johnson et al. 2013). Thus CBD might have improved the cognitive performance of APPxPS1 mice via the inhibition of neuroinflammation in transgenic mice. CBD's anti-inflammatory properties may also be strengthened indirectly by the phytocannabinoid's interaction with the endocannabinoid system (Thomas et al. 2007). Further, a previous study reported that APPxPS1 mice demonstrate impaired neurogenesis from the age of 10 months (Hamilton and Holscher 2012), which may contribute to the impaired cognitive abilities of transgenic mice compared to WT mice. Importantly, CBD has been shown to promote neurogenesis (Campos et al. 2013; Wolf et al. 2010) suggesting that the phytocannabinoid may also affect the cognitive performance of APPxPS1 mice via increasing neurogenesis in these mice. Other currently discussed mechanisms of action for CBD include the enhancement of endocannabinoid-mediated actions, by inhibiting the inactivation of the endocannabinoid anandamide, and the modulation of intracellular Ca²⁺ concentration (for more details see Campos et al. 2012; Hill et al. 2012; Izzo et al. 2009). Future studies are needed to clarify the mechanisms behind CBD's therapeutic-like effects in general and on AD-relevant pathological brain processes in particular.

In conclusion, our study provides the first evidence of CBD's therapeutic-like potential in a transgenic mouse model for AD. Chronic CBD treatment reversed deficits in social recognition and object recognition, without affecting fear-associated memory or anxiety behaviour. Thus CBD may have therapeutic potential as a treatment for AD patients.

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