The therapeutic potential of the phytocannabinoid cannabidiol for Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterized by progressive loss of cognition. Over 35 million individuals currently have AD worldwide. Unfortunately, current therapies are limited to very modest symptomatic relief. The brains of AD patients are characterized by the deposition of amyloid-β and hyperphosphorylated forms of tau protein. AD brains also show neurodegeneration and high levels of oxidative stress and inflammation. The phytocannabinoid cannabidiol (CBD) possesses neuroprotective, antioxidant and anti-inflammatory properties and reduces amyloid-β production and tau hyperphosphorylation *in vitro*. CBD has also been shown to be effective *in vivo* making the phytocannabinoid an interesting candidate for novel therapeutic interventions in AD, especially as it lacks psychoactive or cognition-impairing properties. CBD treatment would be in line with preventative, multimodal drug strategies targeting a combination of pathological symptoms, which might be ideal for AD therapy. Thus, this review will present a brief introduction to AD biology and current treatment options before outlining comprehensively CBD biology and pharmacology, followed by in-vitro and in-vivo evidence for the therapeutic potential of CBD. We will also discuss the role of the endocannabinioid system in AD before commenting on the potential future of CBD for AD therapy (including safety aspects). *Behavioural Pharmacology* 28:142–160 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

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Alzheimer's disease

As the world's population ages and life expectancy increases, many individuals are faced with an increased risk of developing dementia. Dementia is the severe loss of cognitive abilities that is not part of the normal ageing process and currently, over 46 million individuals worldwide are living with dementia (Ferri *et al.*, 2005; Prince *et al.*, 2015). The most prominent form of dementia is Alzheimer's disease (AD), which is predicted to affect one in 85 individuals globally by the year 2050. AD is categorized by three progressive clinical stages: mild, moderate and severe (Zandi *et al.*, 2002). The mild stage is characterized by short-term memory loss, subtle difficulties in learning and communication as well as spatial disorientation. During the moderate stage, memory decline (e.g. noticeable lapses in short-term memory and loss of reading and writing ability) begins to affect everyday tasks, resulting in increased frustration and loss of emotional control. In the severe stage, AD patients face a universal disruption of cognitive abilities including severely impaired learning and speech, inability to recognize familiar individuals and loss of control over bodily functions. Eventually, individuals are left in a weakened physical state where they are prone to other illnesses (e.g. infections).

Biology of Alzheimer's disease

AD is characterized as either sporadic (late onset) or familial (early onset, autosomal dominant). Sporadic AD is the most common and least understood form of AD, accounting for up to 95% of reported AD cases (Gotz and Ittner, 2008). The age of onset for sporadic AD is usually around 65 years. The cause of sporadic AD remains to be elucidated, but it is believed to result from a complex interaction of various environmental risk factors and multiple susceptibility genes (Kamboh, 2004). Considerable information has been obtained from the analysis of genetic risk factors. The *APOE* genotype is by far the most robust predictor of AD risk, with the ε4 allele leading to an increased risk and the ε2 allele conferring protection compared with the most common ε3 allele (Corder *et al.*, 1993). Genome-wide association studies have confirmed the importance of *APOE* in AD risk and also identified several additional genetic risk factors, many of which are, like *APOE*, related to lipid homoeostasis (e.g. *GAB2*) (Belbin *et al.*, 2011).

Familial AD is autosomal dominant and accounts for less than 10% of cases, with an earlier age of onset than the sporadic form, and often occurring at 40–50 years of age. Familial AD is linked to mutations in the amyloid precursor protein gene (*APP*) or in genes encoding presenilin

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1 (*PS1*) and presenilin 2 (*PS2*). Mutations in *PS1* or *PS2* cause the most common and aggressive forms of familial AD and are responsible for the activity of γ-secretase, one of the enzymes responsible for the cleavage of APP into β-amyloid peptides (Aβ). Aβ peptides are found in human brains and have an important damaging pathological function in AD. However, they are also involved in several other processes, including the regulation of cholesterol transport, antioxidant and antimicrobial properties (Baruch-Suchodolsky and Fischer, 2009; Soscia *et al.*, 2010; Umeda *et al.*, 2010). They possess high turnover rates and are associated with synaptic vesicle release, implying a role in neurotransmission (Marchesi, 2011).

APP is cleaved and processed by α-, β- and γ-secretases via two pathways: a nonamyloidogenic and an amyloidogenic pathway. The nonamyloidogenic pathway accounts for the majority of APP processing in the healthy brain, whereby APP is cleaved by α-secretase to generate: (a) a soluble N-terminal fragment (sAPPα), which has neuroprotective properties; (b) a C-terminal fragment (CTFα), which is retained in the membrane and processed further by γ-secretase to yield an N-terminal fragment (p3); and (c) a membrane-bound C-terminal fragment, the APP intracellular domain, which regulates gene transcription.

The first pathological hallmark of AD is the deposition of Aβ. A minority of APP is processed by the amyloidogenic pathway, leading to the generation of Aβ: first, β-secretase cleaves APP, resulting in soluble APP and a cell-membrane bound fragment, and second, γ-secretase cleaves this fragment further, producing Aβ and APP intracellular domain. Importantly, the majority of Aβ produced are 40 residues in length ($A\beta_{40}$), whereas ~10% form the 42 residue-length variant ($A\beta_{42}$). $A\beta_{42}$ is the longer, more hydrophobic isoform that is more prone to fibril formation and therefore found predominantly in cerebral plaques. Mutations in *APP* or the genes for the APP-processing enzymes presenilin 1 or presenilin 2 appear to influence the overproduction of $A\beta_{42}$. The excessive production of $A\beta_{42}$ increases its aggregation in extracellular deposits that form amyloid or senile plaques, one of the two distinct types of lesions observed post mortem in the brains of AD patients.

The second pathological hallmark of AD is the intracellular accumulation and hyperphosphorylation of the microtubule-associated protein tau, which leads to the formation of neurofibrillary tangles (NFTs). Tau is predominantly found in axons of neurons, where it promotes the assembly of microtubules from tubulin, stabilizes them and supports microtubule-dependent axonal transport of organelles and biomolecules. In the healthy brain, two to three amino acid residues on tau are phosphorylated, whereas in AD, tau proteins are hyperphosphorylated (average of nine phosphates per molecule), leading to lowered tau affinity for microtubules, increased tau resistance to calcium-activated neutral proteases and finally the aggregation and formation of NFTs (Lie *et al.*, 2005). However, so far, no tau mutations have been linked to AD (Andorfer *et al.*, 2003; Wolfe, 2009; Armstrong, 2013).

The presence of elevated Aβ in the brain is strongly correlated with cognitive decline in patients diagnosed with early dementia (Naslund *et al.*, 2000) and this has been confirmed using transgenic AD mouse models that routinely express mutant forms of APP and PS1 (Hsiao *et al.*, 1996; Holcomb *et al.*, 1998). However, other preclinical data argue against a direct correlation of amyloid plaque load with cognitive abilities in AD mouse models, suggesting that amyloid plaques might not be the cause of AD, but rather a consequence of pathologic changes in brain metabolism (Stumm *et al.*, 2013). It has been hypothesized that deposits of Aβ are responsible for causing and exacerbating tau hyperphosphorylation and the generation of NFTs as Aβ depositions have been found before any signs of tau pathology (Gotz *et al.*, 2001). Accumulation of tau and the associated NFTs induce cognitive deficits, which correlate with neurodegeneration. Transgenic mouse models expressing tau mutations show cognitive deficits and AD-relevant pathology (Barten *et al.*, 2012). These processes eventually lead to neuronal death and ultimately dementia (amyloid cascade hypothesis). In humans, extensive tau pathology is generally associated with later stages of AD, but changes in tau biology could potentially also occur much earlier (Kuret *et al.*, 2005). Some researchers argue that tau pathology correlates best with AD progression (Gotz *et al.*, 2008).

An additional hypothesis on the role of Aβ in AD suggests that resting microglia become activated in response to the presence of Aβ and cluster at sites of amyloid deposition in the brain. This initiates neuroinflammatory processes and the release of neurotoxic factors (e.g. proinflammatory cytokines and reactive oxygen and nitrogen species) (Streit, 2004), resulting in the manifestation of several characteristic AD pathologies such as neurodegeneration, neuroinflammation, neurotoxicity and oxidative damage (amyloid cascade-neuroinflammation hypothesis). On the one hand, neuroinflammation could be a beneficial event, inducing an immune response to start the phagocytosis of amyloid species in an attempt to limit the development of the disease. On the other hand, prevailing evidence suggests that neuroinflammation is a driving force in the acceleration of AD development as it triggers the production of proinflammatory chemokines, cytokines and neurotoxins by the activated microglia and astrocytes in the brain. It should also be recognized that microglia exist in a spectrum of functional phenotypes that may reflect helpful (e.g. Aβ clearance) or harmful (e.g. overproduction of proinflammatory cytokines and free radicals) roles. Recently, approaches to selectively upregulate the helpful functions of microglia have received increased attention (Perry *et al.*, 2010; McGeer and McGeer, 2015).

Treatment of Alzheimer's disease

Given the looming burden of AD, pharmacological regimens that could delay or even prevent the onset of AD would offer tremendous public health benefits. On the basis of the complex pathology of AD, a preventative, multimodal drug approach targeting a combination of pathological AD events early in disease development appears to be ideal. Unfortunately, current AD treatments only provide limited relief for cognitive and functional decline in the early stages of the disease and are ineffective against disease progression (Zandi and Breitner, 2001; Benito *et al.*, 2007; Marchalant *et al.*, 2008; Karl *et al.*, 2012). Furthermore, these treatment options cause a range of adverse side effects (e.g. nausea, vomiting, abdominal pain, headache, depression and dizziness) (Benito *et al.*, 2007; Micale *et al.*, 2007; Marchalant *et al.*, 2008). Therefore, it is necessary to explore new therapeutic avenues. Importantly, cannabinoids show anti-inflammatory, neuroprotective and antioxidant properties and have immunosuppressive effects. More recently, cannabinoids have also been found to possess properties that may reduce Aβ and tau pathology (Karl *et al.*, 2012). Thus, cannabinoid-related intervention strategies may have therapeutic properties in AD. The phytocannabinoid cannabidiol (CBD) is of particular interest as it lacks the psychoactive and cognition-impairing properties of other cannabinoids. What is currently known about the pharmacological properties of CBD and its potential role in AD therapy will be outlined in the following sections.

Cannabidiol

The phytocannabinoid CBD was first isolated from *Cannabis sativa* in 1940 (Adams, 1940) and its structure was elucidated in the 1960s (Mechoulam and Gaoni, 1965). CBD has very low toxicity. The LD_{50} after intravenous administration to the rhesus monkey is 212 mg/kg, whereas the oral LD_{50} could not be established, probably because of the fact that CBD is barely absorbed systemically after oral administration (i.e. oral bioavailability ranges between 13 and 19%) (Mechoulam *et al.*, 2002). If injected, CBD is rapidly distributed and easily passes the blood–brain barrier because of its lipophilicity, which in turn provides CBD a prolonged elimination (Grotenhermen, 2003). A high volume of distribution (~32 l/kg) has been estimated, with rapid distribution not only in the brain but also in adipose tissue and other organs (Devinsky *et al.*, 2014). Preferential distribution to fat raises the possibility of accumulation of CBD depots in chronic administration schemes, especially in patients with high adiposity. The metabolism of CBD shows biotransformation routes typically observed for phytocannabinoids [but species differences must be considered when assessing preclinical research data; see Bergamaschi *et al.* (2011)]. CBD undergoes multiple hydroxylations, and oxidations to carboxylic acids, β-oxidation, conjugation and epoxidation (Harvey *et al.*, 1991). Eventually, CBD is preferentially excreted in urine, both in the free state and as its glucuronide, with a half-life of 9 h [reviewed in Iuvone *et al.* (2009)].

The pharmacological properties of CBD range from anticonvulsive, antianxiety and antipsychotic (Zuardi *et al.*, 1991, 1995; Leweke *et al.*, 2000; Schneider *et al.*, 2002) to antinausea, anti-inflammatory and anti-rheumatoid arthritic [outlined more comprehensively in Pertwee (2008); Russo (2011)]. Importantly, CBD is a multitarget drug that can interact with many signalling systems including the endocannabinoid system (ECS; outlined in more detail in the Cannabidiol pharmacology section).

Brief introduction to the endocannabinoid system

The ECS is an intercellular signalling system comprised of (a) G-protein-coupled (GPR) cannabinoid receptors 1 (CB_1) and 2 (CB_2) as well as more recently discovered receptors (e.g. *N*-arachidonylglycine receptor or G-protein-coupled receptor 18: GPR18), (b) endogenous ligands, the best characterized ones being the arachidonic acid derivatives *N*-arachidonoylethanolamine (AEA, also called anandamide) and 2-arachidonoylglycerol (2-AG) and their homologues, and (c) metabolic enzymes (for an overview, see Table 1). The enzymes responsible for the biosynthesis of AEA require complete characterization, but an *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) has been implicated in the process as has diacylglycerol lipase-α (DAGL-α) for 2-AG. The metabolism of anandamide and 2-AG requires fatty amide acid hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Piomelli, 2003; Howlett *et al.*, 2011). The ECS is involved in a variety of physiological processes including appetite, pain sensation, mood and cognition. There are two main groups of cannabinoids that interact with the receptors of the ECS, namely, the endogenous ligands (i.e. 2-AG and AEA) and exogenous cannabinoids. Exogenous cannabinoids include various phytocannabinoids derived from the marijuana plant, *C. sativa*, including Δ^9-tetrahydrocannabinol (THC), the main psychoactive and cognition-impairing component, and CBD, as well as synthetic cannabinoids (i.e. cannabimimetics such as CP 55,940 and WIN 55,212-2) (Tanasescu and Constantinescu, 2010) (for an overview, see Table 1).

CB_1 receptors are highly expressed throughout the brain by many different classes of neurons and also at lower levels by glial cells and many peripheral cell types (Pertwee, 2008). These receptors are found in abundance in the basal ganglia, cerebellum and more importantly the hippocampus, parahippocampal and entorhinal cortices, suggesting an involvement of CB_1 in learning and memory. CB_1 has also been implicated in cannabinoid-mediated modulation of immune functions (Cabral *et al.*, 2008). CB_2 receptors are predominantly found on a variety of immune cells including B lymphocytes, natural killer cells, monocytes/macrophages and T cells, suggesting a role in immunomodulation. Importantly, CB_2 is also

Table 1 Components of the endocannabinoid system

Component	Description	References
Endocannabinoids		
2-Arachidonoylglycerol (2-AG)	Full agonist at CB_1 with neuromodulatory effects	Stella *et al.* (1997)
N-arachidonoylethanolamine (AEA or anandamide)	Agonistic properties at both CB_1 and CB_2 with effects on appetite, learning and memory, and the generation of motivation and pleasure	Devane *et al.* (1992) Paradisi *et al.* (2006) Mallet and Beninger (1996) Mahler *et al.* (2007)
Exocannabinoids		
Phytocannabinoids	>100 constituents of *Cannabis sativa* including the main psychoactive component Δ^9-tetrahydrocannabinol (THC) and the nonpsychoactive constituent cannabidiol (CBD); a multitude of effects have been described including on pain sensitivity, mood, appetite and cognition	Fellermeier *et al.* (2001)
Cannabimimetics	Synthetic cannabinoids that mimic actions of phytocannabinoids such as CP 55,940 or WIN 55,212-2 (for effect range, see Phytocannabinoids)	Lambert and Fowler (2005)
Homologues of endocannabinoids		
2-Linoleoyl glycerol (2-LG)	Natural ligand for CB_1; potentiates activity of other endocannabinoids including 2-AG	Ben-Shabat *et al.* (1998)
Palmitoylethanolamide (PEA)	PPARγ is the main target of PEA, but it also has affinity to cannabinoid-like G-coupled receptors GPR55 and GPR119, but no affinity for CB_1/CB_2; it can enhance the effects of AEA probably through TRPV1	Lo Verme *et al.* (2005) Godlewski *et al.* (2009)
Synthesizing/metabolic enzymes		
N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD)	Synthesis of AEA and PEA	Okamoto *et al.* (2004)
Diacylglycerol lipase α and β (DAGL-α/β)	Synthesis of 2-AG	Bisogno *et al.* (2003)
Monoacylglycerol lipase (MAGL)	Degradation of 2-AG	Dinh *et al.* (2002); Makara *et al.* (2005)
Serine hydrolase α/β-hydrolase domain-containing 6 (ABHD6)	Degradation of 2-AG	Marrs *et al.* (2010)
Fatty acid amide hydrolase (FAAH)	Degradation of 2-AG and AEA	Cravatt *et al.* (2001)
Main receptors for cannabinoids		
Cannabinoid receptor 1 (CB_1)	Involved in the majority of CNS effects of cannabinoids including psychosis and in immune functions	D'Souza (2007); Cabral *et al.* (2008)
Cannabinoid receptor 2 (CB_2)	Involved in immune function and neuroinflammatory responses in CNS	Walter *et al.* (2003); Ramirez *et al.* (2005); Pacher and Mechoulam (2011)
N-arachidonyl glycine receptor or GRP18	Abnormal cannabinoid receptor, activation by AEA and phytocannabinoids (e.g. THC and CBD)	McHugh *et al.* (2010); McHugh (2012)
GPR55	Potential cannabinoid receptor, activated by both endocannabinoids and phytocannabinoids such as THC and CBD	Brown (2007); McHugh *et al.* (2010); Henstridge (2012)
GPR119	Potential cannabinoid receptor, implicated in regulation of food intake and body weight; activation by AEA	Brown (2007); McHugh *et al.* (2010)

CNS, central nervous system; GPR, G-protein-coupled receptor.

densely expressed on activated microglia cells, which suggests a possible role in mediating neuroinflammatory responses in the central nervous system. Stimulation of CB_2 receptors in microglia not only drives the proliferation and migration of microglia but can also block their differentiation into a neurotoxic phenotype (Stella, 2010).

Radioligand binding studies into the developmental pattern (neonatally until 32 months of age) of cannabinoid receptors in the rat brain, using the full CB_1 and CB_2 agonist CP 55,904, showed that cannabinoid receptor-binding capacity increases progressively from birth to postnatal day 60 in whole-brain preparations, whereas no further changes in binding are detected in adulthood and throughout the normal ageing process (Belue *et al.*, 1995). Interestingly, and in line with emerging evidence suggesting that Aβ depositions in AD brain are the result of impaired clearance, is the fact that activation of CB_1/CB_2 by 2-AG as well as the suppression of the endocannabinoid (eCB)-degrading enzyme MAGL (but not FAAH or α/β-hydrolase domain containing 6: ABHD6) elevates Aβ clearance across the blood–brain barrier (Bachmeier *et al.*, 2013).

Cannabidiol pharmacology

Cannabidiol effects on the endocannabinoid system

The pharmacokinetic plasma pattern of CBD resembles that of THC, as does its metabolic pattern (Grotenhermen, 2003). CBD has been found to have very low displacement activities at CB_1 and CB_2 receptors compared with other cannabinoids such as THC and WIN 55,212-2 (Thomas *et al.*, 1998). Another study in the same year confirmed that CBD has a very low affinity (micromolar range) for CB_1 as well as CB_2 receptors. More importantly, that work suggested that CBD develops antagonistic-like properties against the synthetic cannabinoid, CP 55,940, which is a full and highly potent agonist of CB_1 and CB_2 receptors, and that CBD has no agonistic activity at cannabinoid receptors even at high concentrations (Petitet *et al.*, 1998). These findings were in line with earlier reports showing that CBD can reverse the behavioural effects induced by THC (Karniol *et al.*, 1974; Zuardi *et al.*, 1981), although the study

by Petitet *et al.* (1998) found no CBD-induced blockade of CP 55,940-induced hypothermia in mice. Interestingly, another study found that CBD antagonizes (or inversely agonizes) not only CP 55,940 but also WIN 55,212-2, but because CBD produced this antagonism at concentrations well below those at which it binds to cannabinoid receptors, the authors concluded that CBD acts at prejunctional sites that are unlikely to be CB_1 or CB_2 receptors (Pertwee *et al.*, 2002). In a follow-up study, CBD showed inverse agonism at human CB_2 receptors and was a high potency, non-competitive antagonist of cannabinoid receptor agonists in mouse brains as well as in membranes from CHO cells transfected with human CB_2 (Thomas *et al.*, 2007). CBD induced inverse agonism at CB_2 receptors at concentrations well below those at which it displaces CP 55,940. This characteristic of CBD may contribute towards its anti-inflammatory properties as there is evidence that CB_2 inverse agonism can inhibit immune cell migration (Lunn *et al.*, 2006). In line with this, CBD is a potent inhibitor of evoked migration both of murine microglial cells and macrophages and of human neutrophils [reviewed in Pertwee (2008)]. In addition, CBD appears to be an antagonist on GPR55 (Ryberg *et al.*, 2007) and GPR18 (McHugh *et al.*, 2010) receptors, and activates the putative abnormal CBD receptor (Pertwee, 2005, 2008). Bisogno *et al.* (2001) discovered that CBD also interacts with vanilloid receptor type 1 (VR1), the receptor for capsaicin as it stimulated VR1 with a maximal effect similar in efficacy to that of capsaicin, suggesting that VR1 may mediate some of the pharmacological effects of CBD (Bisogno *et al.*, 2001). Finally, there is limited evidence that suggests CBD might also activate the vanilloid receptor type 2 (Izzo *et al.*, 2009).

CBD also impacts brain eCB levels directly. An initial study reported interactions between CBD and proteins that inactivate AEA (Bisogno *et al.*, 2001). CBD inhibited AEA uptake and, to a lesser extent, AEA hydrolysis. These findings suggested that increased levels of endogenous AEA because of CBD-induced inhibition of AEA uptake and degradation (Watanabe *et al.*, 1996) might mediate some of the pharmacological effects of CBD. Supporting this idea is another study, which reported that CBD blunts the expression and the activity of FAAH, the enzyme required for the degradation of both AEA and 2-AG (De Filippis *et al.*, 2008; Leweke *et al.*, 2012).

Cannabidiol effects on other neurotransmitter systems and brain processes

The role of CBD in brain circuits other than the ECS has also been evaluated. In 1998, CBD was found to protect against neurotoxicity mediated by glutamate receptors, that is, *N*-methyl-D-aspartate (NMDA) receptors, 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid (AMPA) receptors and kainate receptors (Hampson *et al.*, 1998). CBD has also been reported to show a modest agonistic affinity of human serotonergic 5-HT_{1a} receptors (Russo *et al.*, 2005), where it inhibits 5-HT re-uptake and reduces overall 5-HT neurotransmission [reviewed in Pertwee (2004)]. In line with the earlier statement that CBD has species-dependent properties, CBD discriminated between human and rat orthologues of the 5-HT_{1A} receptor (Russo *et al.*, 2005). Furthermore, CBD enhanced adenosine receptor A_{2A} signalling by inhibition of cellular update of an adenosine transporter (Carrier *et al.*, 2006). This effect may at least partially be responsible for CBD's ability to decrease inflammation and to be neuroprotective. Although not relevant for the in-vivo effects (as the CBD doses required were not biologically relevant), CBD was reported to possess allosteric modulator properties for μ-opioid and δ-opioid receptors at very high concentrations (i.e. the dissociation rate induced by naloxone and naltrindole at receptors was accelerated by 100 μm CBD) (Kathmann *et al.*, 2006). This is an interesting finding considering that δ-opioid receptors can form a complex with β-secretases and γ-secretases, thereby promoting the processing of APP to Aβ (Teng *et al.*, 2010). Finally, there is also some experimental evidence to support CBD activity in other pathways such as the dopamine and γ-aminobutyric acid neurotransmitter systems [reviewed in Pertwee (2004)].

It has also been shown that CBD can increase adult hippocampal neurogenesis (Wolf *et al.*, 2010). Interestingly, this effect is absent in CB_1 knockout mice, suggesting that the effect of CBD on neurogenesis is mediated by an indirect activation of CB_1 receptors, possibly by inhibition of AEA metabolism/uptake (as discussed earlier). Supporting this finding is a recent in-vitro study showing that CBD increases the proliferation of hippocampal progenitor cells in culture, which can be prevented by antagonists for both CB_1 and CB_2 receptors or overexpression of FAAH (Campos *et al.*, 2011). Further, AD-specific pharmacological actions of CBD have been reported. These will be outlined in more detail in the following sections (in particular, the sections on the effects of CBD in in-vitro and in-vivo AD models), where the therapeutic properties of CBD in preclinical models for the disease are discussed (for a full overview of pharmacological actions of CBD, see Table 2).

Cannabidiol: a new treatment option for Alzheimer's disease – a preclinical perspective

As discussed earlier, the molecular mechanisms by which CBD exerts its various effects are still under debate, with evidence suggesting that its actions are not confined to the receptors of the ECS. Importantly, for this review, a number of studies provide evidence that CBD has various properties including neuroprotection, anti-inflammatory and antioxidant effects, and can modulate the function of the immune system [as reviewed in Campbell and Gowran (2007); Pertwee (2008); Izzo *et al.* (2009); Scuderi *et al.* (2009); Booz (2011)]. This evidence will be outlined in the following sections in the context of in-vitro and in-vivo models relevant to AD.

Table 2 Pharmacological targets of CBD

Pharmacological target	Effect	References
In-vitro studies		
Inhibition of caspase 3 (involved in the signalling pathway for CBD)	Increased cell survival, decreased ROS production and lipid peroxidation in PC12 cells exposed to Aβ	Iuvone *et al.* (2004)
Inhibition of phosphorylated p38 MAP kinase; activation of nuclear factor-κβ (NF-κβ)	Inhibits nitrite production and iNOS protein expression in PC12 cells exposed to Aβ	Esposito *et al.* (2006b)
Rescue of Wnt/β-catenin pathway	Rescues Aβ-induced toxicity and inhibits tau protein hyperphosphorylation in PC12 cells exposed to Aβ	Esposito *et al.* (2006a)
NMDA, AMPA and kainate receptors	Reduction of glutamate-induced toxicity in primary cortical neurons	Hampson *et al.* (1998)
Activation of PPARγ	Induced ubiquitination of APP and decreased Aβ production in APP-expressing human neuroblastoma cells	Scuderi *et al.* (2014)
In-vivo studies		
Glial pathways	Reduction in interleukin-1β, iNOS expression and subsequent NO release in Aβ-injected mice	Esposito *et al.* (2007)
Microglia	Induced microglial migration, suppression of interleukin-6 and prevented spatial memory deficits in Aβ-injected mice	Martin-Moreno *et al.* (2011)
Indirect activation of CB_1 receptor	Increased adult neurogenesis in CB_1 receptor deficient mice with no effect on cognition in WT mice	Wolf *et al.* (2010)
Activation of PPARγ	Induced hippocampal neurogenesis and reduced reactive gliosis in Aβ-injected rats	Esposito *et al.* (2011)
Inverse CB_2 receptor agonism	Antagonizes CB_2 receptor agonists in WT mice	Thomas *et al.* (2007)

AMPA, 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid; APP, amyloid precursor protein; CBD, cannabidiol; iNOS, inducible nitric oxide synthase; MAP, mitogen-associated protein; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; PPARγ, peroxisome proliferator-activated receptor-γ; WT, wild type-like.

Effects of cannabidiol on in-vitro Alzheimer's disease models

Tau pathology

CBD was reported to suppress the hyperphosphorylation of tau protein in Aβ-stimulated PC12 neuronal cells in a dose-dependent manner. The CBD-induced suppression was associated with a reduction in phosphorylated glycogen synthase kinase 3-β (p-GSK3-β), the active form of GSK3-β, a multifunctional phosphorylating serine/threonine kinase (Esposito *et al.*, 2006a). Importantly, active p-GSK3-β is also known as tau protein kinase and is responsible for tau protein hyperphosphorylation and NFT formation in the brains of patients with AD (Sperber *et al.*, 1995). GSK3-β activation also induces Aβ overproduction because of its impact on APP processing (Phiel *et al.*, 2003). Furthermore, Aβ peptide induces GSK3-β phosphorylation in hippocampal and cortical neurons, thereby disrupting the Wnt signalling function (Garrido *et al.*, 2002). Aβ-induced Wnt pathway disruptions are pivotal events in the neuronal apoptosis characteristic of AD, involving p-GSK3-β upregulation and β-catenin degradation (De Ferrari and Inestrosa, 2000). In line with this, β-catenin levels are decreased in the brains of AD patients (Satoh and Kuroda, 2000). These data suggest that CBD inhibits tau hyperphosporylation by disrupting phosphorylation of GSK3-β and thereby rescues at least some aspects of the Wnt signalling pathway. Importantly, pharmacological interventions that rescue Wnt activity have been proposed as novel therapeutics for AD treatment in the past (Esposito *et al.*, 2006a).

Amyloid-β pathology

The direct modulatory effects of CBD on APP processing have only recently been evaluated in in-vitro studies. Transfected human neuroblastoma SHSY5YAPP$^+$ cells showed significantly elevated full-length APP expression compared with control neuronal cells (Scuderi *et al.*, 2014). CBD counteracted this elevation in a dose-dependent manner by inducing ubiquitination of APP without exerting any effect on control cells. The CBD effect on SHSY5YAPP$^+$ cells was paralleled by a progressive reduction of Aβ peptide expression in cell lysates and consequentially fewer apoptopic events [i.e. number of apoptopic cell bodies and neuron survival (%)] in these cells (Scuderi *et al.*, 2014). Importantly, the peroxisome proliferator-activated receptor-γ (PPARγ) antagonist GW9662 blocked these effects of CBD, whereas the involvement of α-, β- and γ-secretases was ruled out. PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily and include three isoforms (i.e. α, β/δ and γ). PPARs have been linked to inflammation, cell proliferation and differentiation. Importantly, PPARγ receptors are expressed at low levels under physiological conditions, but increase in response to some pathological conditions including AD (Kitamura *et al.*, 1999). In line with this, PPARγ activation has been found to reduce APP expression (D'Abramo *et al.*, 2005) and considerably enhance clearance of Aβ *in vitro* (Camacho *et al.*, 2004). Thus, CBD may exert a beneficial effect on the amyloidogenic pathway through a mechanism involving PPARγ. This presents a novel promising avenue to counteract the progression of AD and would be in line with suggestions that the regulation of PPARγ activity may be therapeutically effective for AD pathophysiology. Related to the impact that CBD has on the amyloidogenic pathway, other synthetic cannabinoids (i.e. the CB_2 agonist JWH-015) have been found to increase the phagocytosis of Aβ by mouse microglial cells (Ehrhart *et al.*, 2005) and promote the removal of Aβ from human tissue sections at low doses (Tolon *et al.*, 2009). Importantly, CB_2 expression is

upregulated in glial cells under chronic neuroinflammatory conditions such as AD.

Amyloid-β-induced toxicity

CBD dose-dependently alleviated various other effects of Aβ-induced toxicity in a cultured rat pheocromocytoma PC12 cell model (Iuvone *et al.*, 2004). CBD administration before Aβ treatment improved cell survival, and reduced lipid peroxidation and the production of reactive oxygen species. Reactive oxygen species have been found to play a role in Aβ-induced cell damage and death (Brera *et al.*, 2000). The phytocannabinoid also decreased caspase 3 protein levels (used as a hallmark of apoptosis), DNA fragmentation and intracellular calcium levels, which were elevated in Aβ-treated cells without CBD treatment. Importantly, caspases are essential mediators of many of the pathways involved in executing the apoptopic programme following Aβ accumulation (Nicholson and Thornberry, 1997). Furthermore, Aβ-induced DNA fragmentation, a hallmark of apoptosis, has been found previously in AD models (Gschwind and Huber, 1995), and an increase in calcium levels has been suggested to be responsible for at least some of the toxic effects of Aβ (Mattson, 2002). Finally, an Aβ-induced decrease in the procaspase 3/total caspase 3 ratio was counteracted by CBD, suggesting that CBD could play a protective role in the execution phase of apoptosis as activation of procaspase 3 to caspase 3 normally serves as the convergence point of different apoptotic signalling pathways (Iuvone *et al.*, 2004). The effects of CBD appeared to be independent of CB_1 receptors as SR141716A treatment (a CB_1 receptor antagonist) did not modulate the CBD properties observed. The authors concluded that CBD exerts a combination of neuroprotective, antioxidant and anti-apoptotic effects against Aβ-induced toxicity.

In line with the above findings, stimulation of PC12 cells with Aβ led to a significant increase in nitrite production and inducible nitric oxide synthase (iNOS) protein expression. iNOS and its enzymatic product nitric oxide (NO) are among the most important neurotoxic effectors in AD. NO is predominantly released by activated glial cells and may disrupt neurons, thereby sustaining the proinflammatory conditions typical for AD (Cardenas *et al.*, 2005). CBD inhibited these effects of Aβ toxicity dose dependently. This neuroprotective effect of CBD was mediated by suppressing the Aβ-induced increase in phosphorylation of p38 mitogen-associated protein kinase and activation of nuclear factor-κβ activation (NF-κβ) (Esposito *et al.*, 2006b). The transcription factor NF-κβ is stimulated by stress-responsive protein kinases (e.g. p38 mitogen-associated protein kinase) and regulates the expression of genes involved in cell differentiation, proliferation and apoptosis as well as oxidative and inflammatory responses. Furthermore, NF-κβ activation is required to induce iNOS protein transcription in post-mortem AD brains (Haas *et al.*, 2002).

A more recent study could not confirm the neuroprotective effects of CBD: CBD protected rat PC12 peripheral neuronal cells and human SHSY5Y cells against oxidative stress and lipid peroxidation induced by tert-butyl hydroperoxide, but failed against $A\beta_{40}$ fibril and aggregate-induced neurotoxicity (Harvey *et al.*, 2012). This unexpected finding was probably due to the fact that the previous studies incubated cells with nonfibrillar Aβ, whereas Harvey *et al.* (2012) utilized Aβ in its preformed (fibrillar) state when incubating the neuronal cell lines. Thus, the neuroprotective efficacy of CBD appears to be dependent on Aβ fibril formation occurring during cell exposure, which implies either a direct influence on fibril formation or interference with Aβ fibril uptake or processing. CBD was neuroprotective against oxidative stress generated from tert-butyl hydroperoxide, but not from Aβ exposure, as there are differences in the neurotoxicity profile caused by these two stressors. It is likely that Aβ activates additional pathways in inducing cell death that are not surmountable by antioxidant capacity alone, whereas tert-butyl hydroperoxide directly attacks membrane lipids (Harvey *et al.*, 2012). Interestingly, AEA effectively protected neuronal cells against Aβ fibril and aggregate-induced neurotoxicity through a pathway unrelated to CB_1 or CB_2 receptor activation, although both neuronal cell lines expressed CB_1 receptors (low expression of CB_2 only) (Harvey *et al.*, 2012).

The same team went on to analyse the potential inhibition of native Aβ fibrils and oligomer formation by CBD (and other cannabinoids, e.g. THC, JWH-015 and 2-AG). Human neuroblastoma SHSY5Y cells were exposed to $A\beta_{42}$ and cell viability was measured in the presence of CBD. SHSY5Y cells were also exposed to microglia-conditioned media (BV-2 cells) activated with lipopolysaccharide (LPS), albumin or $A\beta_{42}$, after which the production of tumour necrosis factor (TNF-α) and nitrite was evaluated following CBD treatment (Janefjord *et al.*, 2014). $A\beta_{42}$ induced a concentration-dependent loss of cell viability in SHSY5Y cells, but negligible TNF-α and nitrite production in BV-2 cells compared with LPS or albumin. CBD protected against Aβ-induced cell viability loss directly as well as against LPS-activated BV-2 conditioned media viability loss. CBD also altered the morphology of Aβ fibrils and aggregates to some extent (but to a lesser degree than other cannabinoids). However, there was no clear correlation between altered morphology and neuroprotective actions. In line with the previous study outlining the role of eCBs in Aβ-relevant neuroprotection (Harvey *et al.*, 2012), of all the other cannabinoids used, only 2-AG was found to provide significant and direct neuroprotection against $A\beta_{42}$ (Janefjord *et al.*, 2014). The authors raised another interesting point: there was a trend for cell viability levels to be improved in the CBD control group (i.e. without Aβ pretreatment). This may point towards a cellular proliferative or mitochondrial stabilizing effect of CBD that could potentially offset the loss of viability induced by $A\beta_{42}$.

Microglial function

A consistent pathology of AD is glial activation. Microglia are the resident macrophages of the brain and play a major role in the active immune defence of the central nervous system against pathological events. When membrane receptors of microglia are activated (e.g. by ATP, which is released by dying cells), these glial cells migrate towards the site of injury and release, for example, proinflammatory cytokines and NO. In AD, microglia overstimulation may be responsible for the inflammatory conditions typically found in patient brains and may result in neurodegeneration. Thus, pharmacological manipulation of microglia activity may have therapeutic potential for neurodegenerative diseases in general and for AD in particular. However, microglia can have helpful and harmful phenotypes. Thus, it is very likely that a balanced immune-modulation is required for AD therapy as immune activation of microglia can clear plaques, whereas chronic neuroinflammation can cause neuronal death/dysfunction (Krause and Muller, 2010).

Walter *et al.* (2003) discovered that microglia express both CB_1 and CB_2 receptors. Furthermore, stimulation of microglia with ATP *in vitro* increased the production of 2-AG and triggered glial cell migration (Walter *et al.*, 2003). Importantly, CBD prevented the 2-AG-induced migration of microglial cells possibly by antagonism at CB_2 and 'abnormal CBD' receptors [for the latter, see Jarai *et al.* (1999)]. In line with this, another study found that several synthetic cannabinoids blocked Aβ-induced activation of cultured microglia, which was assessed by microglial cell activity, morphology and TNF-α release (Ramirez *et al.*, 2005). This effect was evident for HU-210, but also for cannabinoids with CB_2 selectivity (i.e. JWH-133) and no antioxidant properties (i.e. WIN 55,212-2). Interestingly, JWH-133 was as effective as the mixed CB_1/CB_2 agonist WIN 55,212-2 in the inhibition of microglia activation (Ramirez *et al.*, 2005). A more recent study confirmed and expanded on these findings as not only WIN 55,212-2 and JWH-133 but also CBD dose dependently decreased the ATP-induced increase in intracellular calcium in cultured N13 microglial cells and in rat primary microglia. The properties of CBD in the N13 cell model were independent of its actions on CB_1 and CB_2 receptors, whereas these properties could be blocked by CB_2 antagonism in the primary microglia model (Martin-Moreno *et al.*, 2011). The research team also investigated a potential involvement of adenosine receptors and found that an agonist of the adenosine A_{2A} receptor (i.e. CGS-21680) mimicked the actions of CBD. More importantly, an A_{2A} antagonist (i.e. ZM241,385) suppressed the effects of CBD in both the N13 and the primary microglial cell models (Martin-Moreno *et al.*, 2011). Finally, CBD promoted primary microglial migration, a phenomenon that could be stopped by CB_1 and CB_2 receptor antagonists (i.e. SR141716 and SR144528, respectively).

Acetylcholine

It is important to realize that Aβ not only induces neurodegeneration but also exerts downstream effects including the severe disruption of several neurotransmitter systems. For example, cholinergic neurons are lost in brain areas relevant for memory processing (i.e. amygdala, hippocampus and frontal cortex) and this deterioration is accompanied by a decrease in acetylcholine, which plays a crucial role in cortical development and activity and the modulation of cognition, learning and memory (Schliebs and Arendt, 2011). So far, no research has been carried out to determine the effects of CBD on the cholinergic system and the acetylcholine-related changes in AD. However, it is interesting to note that the phytocannabinoid THC was found to completely inhibit the enzyme acetylcholinesterase and its aggregating effect on Aβ *in vitro* (and does so more effectively than currently approved AD interventions such as donepezil and tacrine) (Eubanks *et al.*, 2006).

Glutamate

Aβ can also cause long-term disruptions to glutamatergic neurotransmission (Schliebs and Arendt, 2011). Under normal physiological conditions, glutamate activates NMDA receptors, enabling calcium ions (Ca^{2+}) to flow into the postsynaptic neuron. This triggers a signalling cascade that produces synaptic plasticity including long-term potentiation, thereby facilitating higher order processes such as learning and memory (Parsons *et al.*, 2013). In AD, NMDA receptors are overstimulated by the presence of excess glutamate, leading to sustained Ca^{2+} influx. This prolonged Ca^{2+} overload increases the production of NO, inhibiting mitochondrial activity and depleting intracellular ATP levels (Takeuchi, 2010). The loss of energy results in impaired dendritic and axonal transport, and neuronal function, generating an excitotoxic state and eventually neurodegeneration. In this context, it is important to keep in mind that CBD has been shown to reduce glutamate-induced, NMDA-induced, AMPA-induced and kainate-induced toxicity in rat cortical neurons (Hampson *et al.*, 1998). However, the direct effect of CBD on the glutamatergic system in an AD-relevant context has not been investigated as yet.

Effects of cannabidiol on in-vivo Alzheimer's disease models

There is a body of literature available evaluating the therapeutic-like effects of THC and synthetic cannabinoids on AD-relevant behaviours and brain pathology [e.g. Ramirez *et al.* (2005); Aso *et al.* (2013)]. The available in-vivo evidence for the therapeutic properties of CBD in AD is much sparser. Pure CBD was found to be inactive in cognitive domains of healthy rhesus monkeys and mice [e.g. Lichtman *et al.* (1995); Winsauer *et al.* (1999); Long *et al.* (2010)], although there is also limited evidence that CBD (i) facilitates extinction of a contextual fear memory in rats (Bitencourt *et al.*, 2008) and (ii) blocks

reconsolidation of aversive memories in rodents (Stern *et al.*, 2012). Furthermore, work utilizing *Cannabis* spp. plant extract high in either CBD or THC showed that acute treatment with CBD-rich extracts did not impact the working memory of healthy rats in a delayed-matching-to-place task in the water maze, whereas THC-rich extracts impaired cognitive performance (Fadda *et al.*, 2004). Interestingly, CBD-rich extracts administered concomitantly with THC-rich extracts did not block the memory-impairing (and catalepsy-inducing) effects of THC. It has been suggested that a greater, 10-fold higher dose of CBD over THC is necessary to effectively antagonize THC-mediated behavioural deficits [Fadda *et al.*, 2004 – see also (Zuardi and Karniol (1983)] (for further details on CBD–THC combination treatments/effects, see below).

Mouse models developing Alzheimer's disease-relevant phenotypes

The following in-vivo findings were not obtained in AD models, but may be relevant to some of the AD-related pathological and behavioural characteristics. In a mouse model for chronic liver disease, CBD exerted therapeutic effects on hepatic encephalopathy (i.e. normalizing increased TNF-α receptor 1 gene and decreased brain-derived neurotrophic factor expression) and improved cognitive functioning, which is impaired in mouse models for chronic liver disease (Magen *et al.*, 2009). The majority of CBD effects were blocked by pharmacological antagonism at A_{2A} receptors. A follow-up study by the same research team also discovered an involvement of $5\text{-}HT_{1A}$ receptor activation in the beneficial properties of CBD treatment in this model (Magen *et al.*, 2010). However, CBD-induced brain-derived neurotrophic factor expression changes were not mediated by either receptor, suggesting the involvement of yet to be discovered pathways.

Iron content in the brain appears to be positively correlated with poorer cognitive performance of AD patients (Ding *et al.*, 2009). Furthermore, iron-induced memory impairments are associated with increased oxidative stress markers in the brain (De Lima *et al.*, 2005). Thus, iron-induced cognitive deficits might be linked to oxidative damage and the antioxidant properties of CBD might be beneficial in this context. Indeed, high-dose acute CBD as well as subchronic CBD treatment recovered the object recognition memory performance of iron-treated rats without affecting cognition of control rats (Fagherazzi *et al.*, 2011).

Pharmacological rodent models for Alzheimer's disease

Manipulations to the endocannabinoid system: AD-relevant experimental strategies were implemented for the first time when Mazzola *et al.* (2003) found that the amnesic effects of $A\beta_{25–35}$ and $A\beta_{42}$ (measured in mice using the step-through passive avoidance test) could be blocked by cotreatment with the CB_1 antagonist SR141716A. Another study using pharmacological rodent models for AD evaluated changes to the ECS after rats had been exposed to cortical $A\beta_{42}$ administration. $A\beta_{42}$ enhanced hippocampal 2-AG (but not AEA) levels concomitant with the appearance of markers of neuronal damage, increased CB_2 (but not CB_1) protein expression and induced cognitive deficits in the passive avoidance task. Inhibition of eCB cellular reuptake reversed hippocampal damage and loss of memory retention (but only when administered early after the administration of $A\beta_{42}$) (Van der Stelt *et al.*, 2006). These data suggested for the first time that early modifications to the ECS might protect against Aβ neurotoxicity and its consequences. Indeed, the detrimental effects of a bilateral injection of $A\beta_{40}$ fibrils into the hippocampal CA1 area of rats on spatial memory and neuroinflammation [e.g. microglia and astrocyte activation, interleukin (IL)-1β expression and Aβ clearance] were reversed by subchronic treatment with MDA7, a selective CB_2 receptor agonist. Furthermore, $A\beta_{40}$ injections were accompanied by increased hippocampal CB_2 expression (Wu *et al.*, 2013).

Cannabidiol treatment: Most relevant to this review are studies testing CBD in pharmacological models for AD. When mice were inoculated with $A\beta_{42}$ in the hippocampus and cotreated with CBD (by the intraperitoneal route), CBD dose dependently suppressed Aβ-induced increases in glial fibrillary acidic protein (GFAP) mRNA and protein expression (i.e. a marker of activated astrocytes) and reduced Aβ-induced iNOS and IL-1β protein expression, and the related NO and IL-1β release (Esposito *et al.*, 2007). IL-1β is involved in events related to neurodegeneration including synthesis and processing of APP and astrocyte activation, which is followed by iNOS overexpression and excessive production of NO. Thus, CBD was effective in counteracting aspects of the neuroinflammatory response to Aβ challenge and a CB_2-related mechanism was put forward by the authors (Esposito *et al.*, 2007). Interestingly, CB_2 overexpression has been detected in an Aβ-induced rat model of reactive gliosis (Van der Stelt *et al.*, 2006) and CB_2 affects reactive gliosis at neuroinflammatory sites, thereby playing a role in the progression of brain damage (Walter and Stella, 2004).

Confirming the cognition-rescuing and anti-inflammatory effects of CBD reported by Esposito and colleagues is another study that determined the effects of chronic CBD treatment on these domains in mice injected intracerebroventricularly with fibrillar Aβ. CBD treatment reversed the compromising effects of Aβ on Morris water maze learning (no data were available for the memory consolidation and retention of these mice). CBD did not alter the Aβ-induced increase in TNF-α mRNA expression, but decreased levels of IL-6 (Martin-Moreno *et al.*, 2011).

Transgenic mouse models for Alzheimer's disease

The endocannabinoid system in Alzheimer's disease transgenic mice: A limited number of studies have focused attention on the expression profile of the cannabinoid receptors CB_1 and CB_2 in the context of AD. CB_1 immunoreactivity was reduced in hippocampal regions (i.e. CA1 and CA2/3) of *APP/PS1* mice, an established transgenic mouse model for familial AD (Kalifa *et al.*, 2011). This CB_1 phenotype was associated with astroglial proliferation and elevated expression of the iNOS and TNF-α, suggesting that lower CB_1 expression levels in AD transgenic mice may decrease anti-inflammatory processes, thereby exacerbating AD-associated pathology. Another study found no changes to CB_2 protein expression in APP transgenic mice (Martin-Moreno *et al.*, 2012). Finally, a triple AD transgenic mouse model (i.e. harbouring $PS1_{M146V}$, APP_{Swe} and Tau_{P301L} transgenes) showed increased CB_1 expression in the prefrontal cortex, dorsal hippocampus and basolateral amygdala complex, whereas expression levels were lower compared with control mice in the ventral hippocampus from 6 months of age onwards (Bedse *et al.*, 2014).

Expanding on these earlier findings, APP23 transgenic mice were crossed with CB_1 knockout mice to study the impact of CB_1 deficiency on AD pathology (Stumm *et al.*, 2013). Most double-mutant mice died before the onset of AD pathology, but surviving mice showed reduced levels of APP and its fragments, which were accompanied by a reduced plaque load and less inflammation. These findings point to a regulatory role of CB_1 in APP processing. Compared with *APP23* transgenic and CB_1 knockout mice, double-mutant $APP23/CB_1$ mice showed even more learning and memory deficits in the Morris water maze.

Manipulations to the endocannabinoid system: Moving on from these expression studies using AD transgenic mice, a small number of research teams have evaluated the effects of cannabinoids other than CBD on AD-relevant behaviours and brain pathology of established transgenic mouse models for the disease. Martin-Moreno and colleagues studied the effects of prolonged oral administration of WIN 55,212-2 or JWH-133 on cognition and inflammation in *APP* transgenic mice (i.e. for 4 months starting at 7 months before onset of plaque pathology and cognitive deficits). The CB_2 agonist JWH-133 (but not WIN 55,212-2) prevented the development of object recognition memory impairments (Martin-Moreno *et al.*, 2012). Furthermore, glucose uptake in the brain (as measured by fluorine-18 fluorodeoxyglucose uptake using PET), which is reduced in AD patients and correlated with cognitive deficits, was reduced in AD transgenic mice. JWH-133 intervention fully reversed this phenotype. Looking at neuroinflammation in this model system, JWH-133 normalized the density of ionized calcium-binding adaptor molecule 1 (Iba1)-positive microglia (increased in AD transgenic mice) and both JWH-133 and WIN 55,212-2 reduced the enhancement of cyclooxygenase 2 (COX-2) protein levels and TNF-α mRNA expression (both increased in AD patients and AD transgenic mouse models). Furthermore, the synthetic cannabinoids could reduce the enhanced levels of $A\beta_{40}$ and of the more amyloidogenic $A\beta_{42}$ in *APP* mice, probably by enhancement of Aβ clearance through the blood–brain or CSF barrier (Martin-Moreno *et al.*, 2012). Finally, WIN 55,212-2 (but not JWH-133) reversed the reduced levels of inactive GSK3-β (i.e. pSer9-GSK3-β) in AD transgenic mice without having an effect in wild type-like mice. Thus, WIN 55,212-2 normalized the pathological pSer9-GSK3-β activity in these mice. The total protein levels of GSK3-β remained largely unchanged (Martin-Moreno *et al.*, 2012).

A recent study confirmed most of these findings when treating *APP/PS1* mice with JWH-133 at the presymptomatic stage (Aso *et al.*, 2013). JWH-133 (a) improved cognitive performance in the novel object recognition test and the active avoidance task; (b) decreased microglial reactivity and reduced the expression of proinflammatory cytokines IL-1β, IL-6, TNF-α and IFNγ; (c) reduced the expression of active p38 and stress-activated protein kinase/c-Jun NH(2)-terminal kinase (SAPK/JNK); (d) increased the expression of inactive GSK3-β and lowered tau hyperphosphorylation; and (e) enhanced the expression of superoxide dismutase 1 (SOD1) and SOD2 around plaques, but (f) did not alter Aβ production.

Inconsistencies between these two studies (e.g. effects of JWH-133 on Aβ pathology and tau hyperphosphorylation-relevant pathways, i.e. GSK3-β) were attributed to methodological differences in terms of mouse model and treatment design chosen as well as the specifics of biochemical analyses (Aso *et al.*, 2013). In this context, it is important to note that other cannabinoids have been found to lack any therapeutic-like properties in AD transgenic mouse models [e.g. HU-210 in *APP23/PS1* transgenic mice: Chen *et al.* (2010)].

CBD + THC combination treatment: so far, two studies have explored the potential of CBD + THC combination compounds for AD therapy using AD transgenic mice (for further information on the potential and issues around combination treatments using THC and CBD, see below). In a first experiment, Sativex [a mixture of a THC botanical extract (containing 67.1% THC, 0.3% CBD, 0.9% cannabigerol, 0.9% cannabichromene and 1.9% other phytocannabinoids) and a CBD botanical extract (containing 64.8% CBD, 2.3% THC, 1.1% cannabigerol, 3.0% cannabichromene and 1.5% other phytocannabinoids) in a 1 : 1 proportion; developed/produced by GW Pharmaceuticals Ltd (Cambridge, UK)] was administered intraperitoneally, daily for a month to parkin-null, human tau overexpressing ($PK^{-/-}/Tau^{VLW}$) mice, which presents a model of complex frontotemporal dementia, parkinsonism and lower motor neuron disease. Sativex treatment resulted in fewer abnormal stress-related behaviours (e.g.

overgrooming and stereotypes; cognition was not assessed, except spontaneous alternation version of the Y maze). Furthermore, the treatment reduced the metabolism of dopamine (but not the level of dopamine itself) as well as gliosis (e.g. Iba1 levels), neuroinflammation (e.g. GFAP levels) and iNOS levels in the cerebral cortex (Casarejos *et al.*, 2013). Most relevant to AD is the finding that Sativex decreased the concentration of phosphorylated tau and Aβ plaques in the cortex and hippocampus and increased autophagy. The mechanism behind the effects of Sativex has not been evaluated as yet.

In a second study, botanical extracts high in THC (CBD content < 0.5%) or high in CBD (THC content < 2.5%) as well as a combination thereof (CBD + THC) were used to treat *APP/PS1* transgenic mice in the early symptomatic phase (Aso *et al.*, 2015). All three approaches preserved object recognition memory of AD transgenic mice, but THC exerted detrimental effects on cognition in control mice. CBD + THC also reduced fear-associated learning impairments of *APP/PS1* mice, decreased soluble $A\beta_{42}$ (but not $A\beta_{40}$) levels in the cortex and altered plaque composition (but the total amyloid burden was unchanged). All cannabinoids reduced astrogliosis (decreased GFAP staining), but only CBD + THC reduced microgliosis (i.e. decreased Iba1 staining). CBD + THC was also most effective in reducing inflammation and modifying neuroinflammatory responses in *APP/PS1* mice [mRNA expression levels; for details, see Aso *et al.* (2015)]. Interestingly, the redox protein thioredoxin 2 and the signalling protein Wnt16 were identified as significant substrates for the CBD + THC-induced effects in AD transgenic mice. Thioredoxin 2 is a key component of the mitochondrial antioxidant system that is responsible for the clearance of reactive intermediates and repairs proteins with oxidative damage. The Wnt gene family encodes secreted signalling proteins, which have been implicated in several developmental processes, including axon guidance during development and in response to traumatic injury. Moreover, activation of the Wnt signalling pathway prevents Aβ-induced neurotoxicity *in vitro*.

Pure cannabidiol treatment: Our team has expanded on the aforementioned studies evaluating the potential role of CB_2 and CBD + THC combinations in AD therapy by focusing on CBD effects in AD transgenic mouse models. We have carried out two studies exposing AD transgenic mice and control littermates to chronic CBD (or vehicle) treatment, both to test for remedial as well as preventative properties of CBD for AD therapy. In the remedial arm of this study, we treated double transgenic *APP_{swe}/PS1ΔE9* (*APP/PS1*) chronically with CBD (daily intraperitoneal injections) after the development of cognitive deficits and Aβ pathology. CBD rescued social recognition memory and reversed object recognition deficits of male *APP/PS1* transgenic mice. These effects were specific for recognition memory as CBD had no impact on fear-associated memory or anxiety behaviours (Cheng *et al.*, 2014a). Impairments in object recognition have been linked to dysregulation of the glutamatergic system (Nilsson *et al.*, 2007) and CBD has been found to augment the effects of an NMDA receptor antagonist in humans (Hallak *et al.*, 2011). Furthermore, CBD protects against glutamate neurotoxicity (Hampson *et al.*, 1998) and memantine, another NMDA receptor antagonist, improved object recognition in another transgenic AD mouse model (Scholtzova *et al.*, 2008). Therefore, CBD may improve recognition memory through the glutamatergic pathway.

For the preventative research strategy, we treated *APPxPS1* mice with CBD or vehicle using a daily voluntary oral administration scheme for 8 months beginning at 2.5 months of age when AD-like pathophysiology is still sparse. Long-term oral CBD treatment prevented the development of social recognition deficits in male *APP/PS1* mice (Cheng *et al.*, 2014b). The beneficial effect of CBD on social recognition memory was not associated with a direct effect on Aβ levels. Insoluble and soluble levels of $A\beta_{40}$ and $A\beta_{42}$ were not different between vehicle-treated and CBD-treated *APP/PS1* mice in the cortex and hippocampus. Levels of oxidation were not significantly altered in *APP/PS1* mice in comparison with their age-matched wild-type littermates, nor did we detect changes in the level of lipid oxidation in the cortex of CBD-treated animals, despite its known antioxidant properties. It is possible that brains were collected at an age (i.e. around 10 months of age) where nucleic acid oxidation differences between *APP/PS1* and control mice are no longer evident (normally observed at 3–5 months of age). Although not significant, the data obtained on the basis of the administration of only one CBD dose (i.e. 20 mg/kg) suggested that CBD might exert a beneficial effect on cytokine levels, in particular, TNF-α, which would be in line with the earlier findings discussed above [e.g. Martin-Moreno *et al.* (2011)].

The study also showed a complex relationship between CBD treatment, AD genotype and brain levels of cholesterol and phytosterols. These findings will be followed up in future work. This is important as disturbances in brain cholesterol metabolism are associated with the major pathological features of AD (including Aβ and tau pathology) and dietary phytosterols can either interfere with critical functional processes in AD or decrease amyloidogenic processing.

The endocannabinoid system and cannabinoid therapy in patients with Alzheimer's disease

To date, there have been no clinical trials evaluating the therapeutic potential of CBD for AD. This is probably because of the limited number of preclinical research studies investigating the effects of CBD in AD thus far. However, two clinical trials testing CBD have been conducted, which have some relevance for AD. In 2009,

an interventional study explored the value of CBD in treating cognitive dysfunction in schizophrenia (*http://www.ClinicalTrials.gov* identifier NCT00588731). The study was based on a 6-week, randomized, placebo-controlled, fixed-dose trial comparing CBD with placebo added to a stable dose of antipsychotic medications in patients diagnosed with schizophrenia. The second interventional study (*http://www.ClinicalTrials.gov* identifier NCT01502046) started in 2011 and was a double-blind, randomized, crossover, placebo-controlled phase 2 clinical trial to assess the neuroprotective properties of CBD, THC and Sativex in patients with Huntington's disease. Unfortunately, it was not possible to obtain any information from the *http://www.ClinicalTrials.gov* website or from the lead investigators on the effects of CBD effects on cognition or neuroprotection in humans. Nevertheless, a few studies evaluated the role of the ECS in AD patients and the effectiveness of cannabinoid treatment other than CBD in AD therapy and these findings will be outlined in the following sections.

The endocannabinoid system in Alzheimer's disease

Westlake *et al.* (1994) carried out autoradiographic studies using [^{3}H]CP 55,940 binding (i.e. synthetic CB_1 and CB_2 receptor agonist) in fresh-frozen brain sections from normal aged humans, AD patients and patients who died with other forms of cortical pathology. In AD brains, compared with normal brains, [^{3}H]CP 55,940 binding was reduced in various regions of the hippocampus and the caudate nucleus. Fewer significant reductions were detected in the substantia nigra and the globus pallidus. Other neocortical and basal ganglia structures were not different from control levels. The levels of mRNA expression did not differ between AD and control brains, but there were regionally discrete losses of CB_1/CB_2 mRNA in cells that had high expression levels of these endocannabinoid receptors in the hippocampus. It is important to note that the reductions in binding did not correlate with or localize to areas showing histopathology (i.e. overall tissue quality or stainings for neuritic plaques and NFTs). Furthermore, reduced [^{3}H]CP 55,940 binding was associated with increasing age and with other forms of cortical pathology. Thus, reductions in CB_1/CB_2 receptor expression appeared to be related to generalized ageing and/or disease process and were not selectively associated with AD.

These findings could not be replicated by follow-up investigations. For example, Ramirez *et al.* (2005) detected CB_1 and CB_2 receptor expression together with markers of microglia activation in senile plaques in AD patients and that CB_1-positive neurons were considerably reduced in areas of microglia activation (no change in CB_2 expression). In line with this, immunoblotting for CB_1 receptors in post-mortem cortical brain tissues (Brodmann area 10) from a cohort of neuropathologically confirmed AD patients and age-matched controls showed reduced CB_1 expression in AD brains, which was consistent with the loss of pyramidal cortical neurons in which these receptors are highly expressed. A correlation between reduced CB_1 expression and hypophagia was found, supporting the idea of a potential use of receptor agonists or *C. sativa*-derived cannabinoids in the management of AD-associated eating disorders (Solas *et al.*, 2013).

Another study analysed the expression of not only CB_1 and CB_2 receptors and also of FAAH in hippocampus and entorhinal cortex sections from post-mortem brains of AD patients using immunohistochemistry (Benito *et al.*, 2003). FAAH expression was increased in neuritic plaque-associated astrocytes, whereas CB_2 receptors were abundantly and selectively overexpressed in activated microglia. Supporting this finding is another study reporting elevated levels of CB_2 receptors in post-mortem cortical brain tissues of AD patients. The elevated expression did not correlate with cognitive status, but two relevant AD markers, that is, $A\beta_{42}$ levels and senile plaque manifestation (Solas *et al.*, 2013). It can be postulated that CB_2 receptors might be modulators of the inflammatory response associated with neurodegenerative processes and therefore present a possible target for new therapeutic approaches. Importantly, the expression of CB_1 receptors was not affected by AD. In line with Benito *et al.* (2003) is a more recent study applying semiquantitative (immunoblotting) and quantitative (radioligand binding) assessments to confirm that CB_1 receptor levels were unchanged in AD in several brain regions (i.e. the frontal cortex, anterior cingulate gyrus, hippocampus and caudate nucleus) (Lee *et al.*, 2010). Finally, comparative protein profiling and quantitative morphometry showed that overall CB_1 protein levels in the hippocampi of AD patients remained unchanged relative to age-matched controls and that CB_1-positive presynapses engulfed Aβ-containing senile plaques (Mulder *et al.*, 2011). Lee *et al.* (2010) commented on the limitations of their study design on neuroanatomical resolution (i.e. no subregion analyses were carried out), which did not enable the detection of subtle CB_1 expression changes in specific cytoarchitectural or neuroanatomical domains. Furthermore, the functional status of CB_1 receptors was not considered, which might be important, as Ramirez *et al.* (2005) found elevated nitration of both CB_1 and CB_2 protein in AD brains. It is interesting that a correlation was found between frontal cortical CB_1 immunoreactivity and cognitive scores (i.e. MMSE and CAMCOG) assessed within a year before death in the AD patient group, suggesting that CB_1 receptors are intact in AD and may play a role in preserving cognitive function (Lee *et al.*, 2010). However, a more recent study using immunoblotting could not replicate this finding when analysing correlations between cortical CB_1 expression and the cognitive status (Mini Mental State Examination score) of AD patients

(Solas *et al.*, 2013) (for an overview on changes to the ECS in AD brain, see Table 3).

The ECS seems to be further involved in human AD pathology. In a case–control study, the circulating levels of plasma eCBs were analysed and the relationship between eCBs and TNF-α was explored in elderly control participants and AD patients. In comparison with the controls, there were no significant differences in measured AEA or 2-AG concentrations in plasma samples from patients with AD. Furthermore, eCB levels in the CSF were not correlated with cognitive performance in healthy controls at risk for AD. In pooled plasma samples, an inverse correlation was observed between plasma levels of 2-AG (but not AEA) and TNF-α, although the levels of TNF-α were very low (Koppel *et al.*, 2009). Further longitudinal studies will be required to conclusively assess the impact of progressive AD pathology on circulating eCB levels.

In other studies increased hippocampal protein concentrations for the 2-AG synthesizing enzyme DAGL-α/β was found in brain tissue of patients with definite AD (Braak stage VI). In particular, DAGL-β expression was found in microglia accumulating near senile plaques and apposing CB_1-positive presynapses. Furthermore, microglia, expressing 2-AG-degrading enzymes (i.e. ABHD6 and MAGL) began to surround senile plaques in brain tissue of patients with probable AD (Braak stage III) (Mulder *et al.*, 2011). Interestingly, ABHD6 expression ceased in NFT-bearing pyramidal cells, whereas pyramidal cells containing hyperphosphorylated tau retained MAGL expression (although at levels significantly lower than those in neurons lacking NFT pathology). Finally, it was shown that MAGL recruitment to biological membranes was impaired in AD brains, suggesting that disease progression slows the termination of 2-AG signalling.

To conclude, the 'eCB phenotype' of AD brains appears to be complex and findings appear contradictory at times and highly dependent on the methodologies applied (e.g. type of polyclonal antibody, issue of cellular resolution in autoradiograpy studies, selection of mixed CB_1/CB_2 vs. selective receptor agonists). However, summarizing the diverse findings conservatively suggests that alterations in the localization, expression and function of cannabinoid receptors occur in AD and may play a role in its physiopathology, thereby providing a target for therapeutic interventions.

Effects of cannabinoids other than cannabidiol on Alzheimer's disease patients

Volicer *et al.* (1997) were the first to evaluate the therapeutic effectiveness of cannabinoids in AD. Using a placebo-controlled crossover design, with each treatment period lasting 6 weeks, the effects of dronabinol (i.e. a pharmaceutical formulation of THC) on patients with a diagnosis of probable AD who refused food were determined. AD patients on dronabinol treatment showed an increase in body weight and decreased severity of disturbed behaviour. This effect persisted during the placebo period in patients who received dronabinol first. Adverse reactions observed more commonly during the dronabinol treatment than during placebo periods included euphoria, somnolence and tiredness, but did not require discontinuation of therapy (Volicer *et al.*, 1997).

Table 3 The endocannabinoid system and Alzheimer's disease

Component of ECS	AD-relevant effects	References
CB_1 receptors	No differences were reported for receptor expression, distribution or availability in the cortex or the hippocampus of AD patients	Benito *et al.* (2003); Lee *et al.* (2010); Mulder *et al.* (2011); Ahmad *et al.* (2014)
	CB_1 receptor expression in AD was comparable with normal ageing	Westlake *et al.* (1994)
	CB_1 receptors reported to be reduced in cortical areas and neurons away from senile plaques	Ramirez *et al.* (2005); Solas *et al.* (2013)
	CB_1 receptor expression was reported to not correlate with any AD biomarkers or cognitive deficits	Solas *et al.* (2013)
	Nitrosylated in AD brain allowing the potential for impaired coupling of receptors	Ramirez *et al.* (2005)
CB_2 receptors	CB_2 receptor expression reported to correlate with $A\beta_{42}$ levels and plaque deposition, but not cognitive changes	Solas *et al.* (2013)
	CB_2 receptor expression reported to be abundantly and selectively overexpressed in AD brains	Benito *et al.* (2003)
	Nitrosylated in AD brain, allowing the potential for impaired coupling of receptors	Ramirez *et al.* (2005)
FAAH	Significantly increased FAAH concentration in neuritic plaque-associated glia and in peripheral blood mononuclear cells of AD patients	Benito *et al.* (2003); D'Addario *et al.* (2012)
	No changes in FAAH protein content in hippocampal of AD patients	Mulder *et al.* (2011); Pascual *et al.* (2014)
	Decreased FAAH activity in the frontal cortex of AD patients, which is mimicked by the addition of $A\beta_{40}$ peptide to control brain samples	Pascual *et al.* (2014)
AEA	No differences reported for AEA plasma concentration in AD patients compared with control participants	Koppel *et al.* (2009)
	Lower AEA concentration in AD brain (midfrontal and temporal cortices) compared with controls and inversely correlated with $A\beta_{42}$ peptide and severity of cognitive deficits	Jung *et al.* (2012)
	Increased degradation of AEA in AD frontal cortex compared with the controls	Pascual *et al.* (2014)
2-AG	No differences reported for 2-AG plasma concentration in AD patients compared with control participants	Koppel *et al.* (2009)
	Altered 2-AG signalling during late stages of AD because of a combination of impaired MAGL recruitment and increased DAGL concentration	Mulder *et al.* (2011)

AD, Alzheimer's disease; AEA, *N*-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol; DAGL, diacylglycerol lipase; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase.

A second study measured the effect of dronabinol on nocturnal motor activity as night-time agitation occurs frequently in patients with dementia. Six late-stage AD patients were treated daily for 2 weeks. Dronabinol led to a reduction in nocturnal motor activity and the patients also showed improved Neuropsychiatric Inventory scores including for agitation, aberrant motor and night-time behaviours. No side effects were observed for dronabinol treatment (Walther *et al.*, 2006). These authors followed up on their initial findings with a first randomized, controlled crossover trial of dronabinol for night-time agitation in two AD patients using actigraphy as the objective measure. Administration of dronabinol led to reduced night-time activity and strengthened circadian rhythms (Walther *et al.*, 2011).

Finally, another case study investigated the effects of nabilone, a synthetic cannabinoid supposedly mimicking the effects of THC, in the context of AD. Nabilone reduced the severity of agitation and resistiveness of an AD patient during evening personal care, with no emergent side effects. Previous treatment attempts using donepezil and memantine had yielded disappointing results for this patient (Passmore, 2008). No blinded or placebo studies have been carried out to date.

The future of cannabidiol in Alzheimer's disease therapy

To consider CBD as a novel therapeutic option for AD naturally requires an assessment of how well humans tolerate CBD and what potential side effects might be expected. CBD has been described as being nontoxic and noncataleptic, with no impact on food intake or physiological parameters, such as heart rate, blood pressure and body temperature, and no role in psychomotor or psychological functions. Chronic CBD use and high doses of up to 1500 mg/day (orally) or 30 mg intravenously are well tolerated in humans. However, some studies report that CBD can inhibit hepatic drug metabolism, be immunosuppressive, induce lymphocyte apoptosis *in vitro* and decrease fertilization capacity and the activity of p-glycoprotein and other drug transporters [reviewed in depth in Bergamaschi *et al.* (2011)]. Importantly, long-term safety studies are lacking to this date and the effect of CBD in particular in the elderly has not been assessed at all. Drug–drug interactions have not been evaluated in any detail either. However, it is known that CBD exerts an inhibiting effect on CYP isozymes, primarily CYP2C and CYP3A classes of isozymes. Thus, CBD could potentially impact on antiepileptic medication, as for example, valproate and clobazam are metabolized by these isozymes. In conclusion, further studies are needed to clarify these potential in-vitro and in-vivo side effects before CBD can be trialled clinically [for a detailed overview of CBD safety *in vitro* and *in vivo*, both for rodents and humans, see Bergamaschi *et al.* (2011): Tables 1 and 2; for side effects of CBD *in vitro* and *in vivo*, for rodents, monkeys and humans, see Bergamaschi *et al.* (2011): Tables 3 and 4].

It will also be necessary to work out the best possible administration route for CBD to achieve clinically effective plasma and brain concentration routes. Some initial studies have been completed in this respect using rodent models (Deiana *et al.*, 2012), but recent focus has shifted to assessing different delivery methods for cannabinoids in humans. CBD has been delivered orally in an oil-based capsule in some human trials, but because of the low water solubility of CBD, oral cannabinoid administration can result in slow and erratic absorption. Furthermore, and as discussed earlier, CBD is barely absorbed after oral administration (and absorption rates can be highly variable): bioavailability from oral delivery has been estimated to be as low as 6% because of significant first-pass metabolism in the liver (Mechoulam *et al.*, 2002; Devinsky *et al.*, 2014). Both smoking and intravenous administration of cannabinoids produce reliable and similar pharmacokinetic profiles. However, smoking carries toxic risks and loss of active drug by combustion. An alternate method is intrapulmonary administration of cannabinoids through vaporization, which is considered an effective mode of delivery as it results in fast onset of action and high systemic bioavailability and avoids risks associated with smoking and the formation of pyrolytic toxic compounds as a result of combustion (Solowij *et al.*, 2014). However, this delivery method is limited by the need for specialized equipment and patient cooperation with the administration method (Devinsky *et al.*, 2014). The combined CBD + THC product Sativex is administered as an oromucosal spray and appears to be safe and effective (Wade *et al.*, 2010). The bioavailability achieved by oral mucosal delivery appears to be similar to the oral route, but less variable. Finally, transdermal approaches have also been investigated, but because of CBD's high lipophilicity, special ethosomal delivery systems would be required to prevent drug accumulation in the skin, which are impractical and costly (Lodzki *et al.*, 2003; Devinsky *et al.*, 2014).

CBD + THC combination treatment strategy

It would be beyond the focus of this review to discuss in detail the potential of combined CBD + THC treatment for AD therapy, although recent evidence suggests that such a combination therapy might provide the 'best' AD pathology-counteracting properties of cannabinoids without the known detrimental effects of pure THC treatment (i.e. by blocking those effects through CBD cotreatment). However, the nature of the interactive relationship between THC and CBD appears to be very complex and the evidence provided in the literature to date is inconclusive, if not contradictory [i.e. CBD blocking and/or facilitating THC effects; e.g. Karniol *et al.* (1974); Varvel *et al.* (2006); Klein *et al.* (2011); comprehensively reviewed in McPartland *et al.* (2015)].

Reviewing this growing body of literature, it is important to pay close attention to the timing of CBD versus THC intake, the CBD : THC ratio and the route of administration. CBD can increase the potency of THC by pharmacokinetic interaction if CBD is administered before THC or a pharmacodynamic interaction may occur when both cannabinoids are taken together, mainly at a high-dose ratio of CBD : THC [reviewed in Bergamaschi *et al.* (2011)].

It should be emphasized here that THC alone increases heart rate and alters blood pressure, which may have serious consequences in patients with heart disease (Jones, 2002). Furthermore, THC impacts on the risk of developing psychosis, although this effect is predominantly observed after long-term adolescent THC/*Cannabis* spp. abuse and mostly in individuals with a genetic predisposition for psychosis (Arnold *et al.*, 2012). Long-term use of THC can also lead to the development of *Cannabis* spp. dependency, a growing problem in Western society. Finally, the negative effects of THC on cognitive abilities seem to be reversible after abstinence, except in heavy *Cannabis* spp. users (Bolla *et al.*, 2002). In this context, it is important to realize that there are no systematic data available determining the physiological and psychological effects of long-term THC treatment in the elderly population to date [reviewed in Grotenhermen (2007)].

Concluding remarks

AD is the most common form of dementia (i.e. around 70% of all dementia cases) and it is predicted that AD will affect one in 85 individuals globally by 2050. For example, over 300 000 Australians are currently affected by dementia at an estimated cost of $6.6 billion per annum, with the numbers expected to grow to more than 700 000 by 2050. Considering the looming burden of AD, treatments that could delay or even prevent the onset of AD would offer tremendous public health benefits. Unfortunately, current therapeutic options are limited to modest symptomatic relief, without preventing disease progression. The studies reviewed in this paper suggest that CBD could well provide symptomatic relief and/or prevent disease progression for AD patients. However, a more systematic and in-depth characterization of CBD *in vivo*, using established rodent models, is required to understand the full consequences of long-term CBD treatment and to analyse the potential side effects of CBD in an ageing organism. Once these data are available, the translation of this preclinical work could be realized very quickly as CBD is readily available and appears to be safe for human use. In fact, a number of countries (e.g. Canada and Germany) have already approved CBD-containing products for the treatment of pain and inflammation in multiple sclerosis patients.

Such research would be very timely as it also falls within existing and developing federal regulations on medical applications of *Cannabis* spp. and more importantly, extracts thereof, worldwide (e.g. Australia, Canada and Germany). Finally, understanding the pharmacology of CBD in more detail including its long-term effects in the elderly will be relevant beyond research into AD therapy as CBD has also been considered a treatment option for conditions such as Parkinson's disease and schizophrenia.

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Conflicts of interest

There are no conflicts of interest.

References

Adams R (1940). Marihuana. *Science* **92**:115–119.

Ahmad R, Goffin K, van den Stock J, de Winter FL, Cleeren E, Bormans G, *et al.* (2014). In vivo type 1 cannabinoid receptor availability in Alzheimer's disease. *Eur Neuropsychopharmacol* **24**:242–250.

Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, *et al.* (2003). Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem* **86**:582–590.

Armstrong RA (2013). What causes Alzheimer's disease? *Folia Neuropathol* **51**:169–188.

Arnold JC, Boucher AA, Karl T (2012). The yin and yang of Cannabis-induced psychosis: the actions of Δ(9)-tetrahydrocannabinol and cannabidiol in rodent models of schizophrenia. *Curr Pharm Des* **18**:5113–5130.

Aso E, Juvés S, Maldonado R, Ferrer I (2013). CB2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in AβPP/PS1 mice. *J Alzheimers Dis* **35**:847–858.

Aso E, Sánchez-Pla A, Vegas-Lozano E, Maldonado R, Ferrer I (2015). Cannabis-based medicine reduces multiple pathological processes in AβPP/PS1 mice. *J Alzheimers Dis* **43**:977–991.

Bachmeier C, Beaulieu-Abdelahad D, Mullan M, Paris D (2013). Role of the cannabinoid system in the transit of beta-amyloid across the blood–brain barrier. *Mol Cell Neurosci* **56**:255–262.

Barten DM, Fanara P, Andorfer C, Hoque N, Wong PY, Husted KH, *et al.* (2012). Hyperdynamic microtubules, cognitive deficits, and pathology are improved in tau transgenic mice with low doses of the microtubule-stabilizing agent BMS-241027. *J Neurosci* **32**:7137–7145.

Baruch-Suchodolsky R, Fischer B (2009). Abeta40, either soluble or aggregated, is a remarkably potent antioxidant in cell-free oxidative systems. *Biochemistry* **48**:4354–4370.

Bedse G, Romano A, Cianci S, Lavecchia AM, Lorenzo P, Elphick MR, *et al.* (2014). Altered expression of the CB1 cannabinoid receptor in the triple transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis* **40**: 701–712.

Belbin O, Carrasquillo MM, Crump M, Culley OJ, Hunter TA, Ma L, *et al.* (2011). Investigation of 15 of the top candidate genes for late-onset Alzheimer's disease. *Hum Genet* **129**:273–282.

Belue RC, Howlett AC, Westlake TM, Hutchings DE (1995). The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. *Neurotoxicol Teratol* **17**:25–30.

Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, Vogel Z, *et al.* (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* **353**:23–31.

Benito C, Núñez E, Tolón RM, Carrier EJ, Rábano A, Hillard CJ, Romero J (2003). Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively

overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci* **23**:11136–11141.
Benito C, Núñez E, Pazos MR, Tolón RM, Romero J (2007). The endocannabinoid system and Alzheimer's disease. *Mol Neurobiol* **36**:75–81.
Bergamaschi MM, Queiroz RH, Zuardi AW, Crippa JA (2011). Safety and side effects of cannabidiol, a *Cannabis sativa* constituent. *Curr Drug Saf* **6**:237–249.
Bisogno T, Hanus L, de Petrocellis L, Tchilibon S, Ponde DE, Brandi I, *et al.* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* **134**:845–852.
Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, *et al.* (2003). Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol* **163**:463–468.
Bitencourt RM, Pamplona FA, Takahashi RN (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur Neuropsychopharmacol* **18**:849–859.
Bolla KI, Brown K, Eldreth D, Tate K, Cadet JL (2002). Dose-related neurocognitive effects of marijuana use. *Neurology* **59**:1337–1343.
Booz GW (2011). Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. *Free Radic Biol Med* **51**:1054–1061.
Brera B, Serrano A, de Ceballos ML (2000). beta-amyloid peptides are cytotoxic to astrocytes in culture: a role for oxidative stress. *Neurobiol Dis* **7**:395–405.
Brown AJ (2007). Novel cannabinoid receptors. *Br J Pharmacol* **152**:567–575.
Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F (2008). CB2 receptors in the brain: role in central immune function. *Br J Pharmacol* **153**:240–251.
Camacho IE, Serneels L, Spittaels K, Merchiers P, Dominguez D, de Strooper B (2004). Peroxisome-proliferator-activated receptor gamma induces a clearance mechanism for the amyloid-beta peptide. *J Neurosci* **24**:10908–10917.
Campbell VA, Gowran A (2007). Alzheimer's disease; taking the edge off with cannabinoids? *Br J Pharmacol* **152**:655–662.
Campos AC, Palazuelos J, Aguado T, Aguiar DC, Guzmán M, GalveRoperh I, Guimaraes FS (2011). Hippocampal proliferative processes are needed for behavioral effects of cannabidiol. *Revista de Neurosciencias* **19**:81.
Cárdenas A, Moro MA, Hurtado O, Leza JC, Lizasoain I (2005). Dual role of nitric oxide in adult neurogenesis. *Brain Res Brain Res Rev* **50**:1–6.
Carrier EJ, Auchampach JA, Hillard CJ (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci USA* **103**:7895–7900.
Casarejos MJ, Perucho J, Gomez A, Muñoz MP, Fernandez-Estevez M, Sagredo O, *et al.* (2013). Natural cannabinoids improve dopamine neurotransmission and tau and amyloid pathology in a mouse model of tauopathy. *J Alzheimers Dis* **35**:525–539.
Chen B, Bromley-Brits K, He G, Cai F, Zhang X, Song W (2010). Effect of synthetic cannabinoid HU210 on memory deficits and neuropathology in Alzheimer's disease mouse model. *Curr Alzheimer Res* **7**:255–261.
Cheng D, Low JK, Logge W, Garner B, Karl T (2014a). Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice. *Psychopharmacology (Berl)* **231**:3009–3017.
Cheng D, Spiro AS, Jenner AM, Garner B, Karl T (2014b). Long-term cannabidiol treatment prevents the development of social recognition memory deficits in Alzheimer's disease transgenic mice. *J Alzheimers Dis* **42**:1383–1396.
Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, *et al.* (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**:921–923.
Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci USA* **98**:9371–9376.
D'Abramo C, Massone S, Zingg JM, Pizzuti A, Marambaud P, Dalla Piccola B, *et al.* (2005). Role of peroxisome proliferator-activated receptor gamma in amyloid precursor protein processing and amyloid beta-mediated cell death. *Biochem J* **391** (Pt 3):693–698.
De Ferrari GV, Inestrosa NC (2000). Wnt signaling function in Alzheimer's disease. *Brain Res Brain Res Rev* **33**:1–12.
De Filippis D, Iuvone T, d'amico A, Esposito G, Steardo L, Herman AG, *et al.* (2008). Effect of cannabidiol on sepsis-induced motility disturbances in mice: involvement of CB receptors and fatty acid amide hydrolase. *Neurogastroenterol Motil* **20**:919–927.
De Lima MN, Polydoro M, Laranja DC, Bonatto F, Bromberg E, Moreira JC, *et al.* (2005). Recognition memory impairment and brain oxidative stress induced by postnatal iron administration. *Eur J Neurosci* **21**:2521–2528.
Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, *et al.* (2012). Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berl)* **219**:859–873.
Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**:1946–1949.
Devinsky O, Cilio MR, Cross H, Fernandez-Ruiz J, French J, Hill C, *et al.* (2014). Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* **55**:791–802.
Ding B, Chen KM, Ling HW, Sun F, Li X, Wan T, *et al.* (2009). Correlation of iron in the hippocampus with MMSE in patients with Alzheimer's disease. *J Magn Reson Imaging* **29**:793–798.
Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, *et al.* (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci USA* **99**:10819–10824.
D'Addario C, di Francesco A, Arosio B, Gussago C, Dell'Osso B, Bari M, *et al.* (2012). Epigenetic regulation of fatty acid amide hydrolase in Alzheimer disease. *PLoS One* **7**:e39186.
D'Souza DC (2007). Cannabinoids and psychosis. *Int Rev Neurobiol* **78**: 289–326.
Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y, *et al.* (2005). Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* **2**:29.
Esposito G, de Filippis D, Carnuccio R, Izzo AA, Iuvone T (2006a). The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. *J Mol Med* **84**:253–258.
Esposito G, de Filippis D, Maiuri MC, de Stefano D, Carnuccio R, Iuvone T (2006b). Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in beta-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-kappaB involvement. *Neurosci Lett* **399**:91–95.
Esposito G, Scuderi C, Savani C, Steardo L Jr, de Filippis D, Cottone P, *et al.* (2007). Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *Br J Pharmacol* **151**:1272–1279.
Esposito G, Scuderi C, Valenza M, Togna GI, Latina V, de Filippis D, *et al.* (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One* **6**:e28668.
Eubanks LM, Rogers CJ, Beuscher AE 4th, Koob GF, Olson AJ, Dickerson TJ, Janda KD (2006). A molecular link between the active component of marijuana and Alzheimer's disease pathology. *Mol Pharm* **3**:773–777.
Fadda P, Robinson L, Fratta W, Pertwee RG, Riedel G (2004). Differential effects of THC- or CBD-rich Cannabis extracts on working memory in rats. *Neuropharmacology* **47**:1170–1179.
Fagherazzi EV, Garcia VA, Maurmann N, Bervanger T, Halmenschlager LH, Busato SB, *et al.* (2012). Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders. *Psychopharmacology (Berl)* **219**:1133–1140.
Fellermeier M, Eisenreich W, Bacher A, Zenk MH (2001). Biosynthesis of cannabinoids. Incorporation experiments with (13)C-labeled glucoses. *Eur J Biochem* **268**:1596–1604.
Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, *et al.* (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**: 2112–2117.
Garrido JL, Godoy JA, Alvarez A, Bronfman M, Inestrosa NC (2002). Protein kinase C inhibits amyloid beta peptide neurotoxicity by acting on members of the Wnt pathway. *FASEB J* **16**:1982–1984.
Godlewski G, Offertáler L, Wagner JA, Kunos G (2009). Receptors for acylethanolamides-GPR55 and GPR119. *Prostaglandins Other Lipid Mediat* **89**:105–111.
Götz J, Ittner LM (2008). Animal models of Alzheimer's disease and frontotemporal dementia. *Nat Rev Neurosci* **9**:532–544.
Götz J, Chen F, van Dorpe J, Nitsch RM (2001). Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. *Science* **293**:1491–1495.
Götz J, Ittner LM, Fändrich M, Schonrock N (2008). Is tau aggregation toxic or protective: a sensible question in the absence of sensitive methods? *J Alzheimers Dis* **14**:423–429.
Grotenhermen F (2003). Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin Pharmacokinet* **42**:327–360.
Grotenhermen F (2007). The toxicology of Cannabis and Cannabis prohibition. *Chem Biodivers* **4**:1744–1769.
Gschwind M, Huber G (1995). Apoptotic cell death induced by beta-amyloid 1–42 peptide is cell type dependent. *J Neurochem* **65**:292–300.

Haas J, Storch-Hagenlocher B, Biessmann A, Wildemann B (2002). Inducible nitric oxide synthase and argininosuccinate synthetase: co-induction in brain tissue of patients with Alzheimer's dementia and following stimulation with beta-amyloid 1-42 in vitro. *Neurosci Lett* **322**:121–125.

Hallak JE, Dursun SM, Bosi DC, de Macedo LR, Machado-de-Sousa JP, Abrão J, *et al.* (2011). The interplay of cannabinoid and NMDA glutamate receptor systems in humans: preliminary evidence of interactive effects of cannabidiol and ketamine in healthy human subjects. *Prog Neuropsychopharmacol Biol Psychiatry* **35**:198–202.

Hampson AJ, Grimaldi M, Axelrod J, Wink D (1998). Cannabidiol and (−)delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci USA* **95**:8268–8273.

Harvey DJ, Samara E, Mechoulam R (1991). Comparative metabolism of cannabidiol in dog, rat and man. *Pharmacol Biochem Behav* **40**:523–532.

Harvey BS, Ohlsson KS, Mååg JL, Musgrave IF, Smid SD (2012). Contrasting protective effects of cannabinoids against oxidative stress and amyloid-β evoked neurotoxicity in vitro. *Neurotoxicology* **33**:138–146.

Henstridge CM (2012). Off-target cannabinoid effects mediated by GPR55. *Pharmacology* **89**:179–187.

Holcomb L, Gordon MN, McGowan E, Yu X, Benkovic S, Jantzen P, *et al.* (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med* **4**:97–100.

Howlett AC, Reggio PH, Childers SR, Hampson RE, Ulloa NM, Deutsch DG (2011). Endocannabinoid tone versus constitutive activity of cannabinoid receptors. *Br J Pharmacol* **163**:1329–1343.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, *et al.* (1996). Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* **274**:99–102.

Iuvone T, Esposito G, Esposito R, Santamaria R, di Rosa M, Izzo AA (2004). Neuroprotective effect of cannabidiol, a non-psychoactive component from *Cannabis sativa*, on beta-amyloid-induced toxicity in PC12 cells. *J Neurochem* **89**:134–141.

Iuvone T, Esposito G, de Filippis D, Scuderi C, Steardo L (2009). Cannabidiol: a promising drug for neurodegenerative disorders? *CNS Neurosci Ther* **15**: 65–75.

Izzo AA, Borrelli F, Capasso R, di Marzo V, Mechoulam R (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* **30**:515–527.

Janefjord E, Mååg JL, Harvey BS, Smid SD (2014). Cannabinoid effects on β amyloid fibril and aggregate formation, neuronal and microglial-activated neurotoxicity in vitro. *Cell Mol Neurobiol* **34**:31–42.

Járai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR, *et al.* (1999). Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc Natl Acad Sci USA* **96**: 14136–14141.

Jones RT (2002). Cardiovascular system effects of marijuana. *J Clin Pharmacol* **42**:58S–63S.

Jung KM, Astarita G, Yasar S, Vasilevko V, Cribbs DH, Head E, *et al.* (2012). An amyloid β42-dependent deficit in anandamide mobilization is associated with cognitive dysfunction in Alzheimer's disease. *Neurobiol Aging* **33** :1522–1532.

Kalifa S, Polston EK, Allard JS, Manaye KF (2011). Distribution patterns of cannabinoid CB1 receptors in the hippocampus of APPswe/PS1ΔE9 double transgenic mice. *Brain Res* **1376**:94–100.

Kamboh MI (2004). Molecular genetics of late-onset Alzheimer's disease. *Ann Hum Genet* **68** (Pt 4):381–404.

Karl T, Cheng D, Garner B, Arnold JC (2012). The therapeutic potential of the endocannabinoid system for Alzheimer's disease. *Expert Opin Ther Targets* **16**:407–420.

Karniol IG, Shirakawa I, Kasinski N, Pfeferman A, Carlini EA (1974). Cannabidiol interferes with the effects of delta 9 – tetrahydrocannabinol in man. *Eur J Pharmacol* **28**:172–177.

Kathmann M, Flau K, Redmer A, Tränkle C, Schlicker E (2006). Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn Schmiedebergs Arch Pharmacol* **372**:354–361.

Kitamura Y, Shimohama S, Koike H, Ji Kakimura, Matsuoka Y, Nomura Y, *et al.* (1999). Increased expression of cyclooxygenases and peroxisome proliferator-activated receptor-gamma in Alzheimer's disease brains. *Biochem Biophys Res Commun* **254**:582–586.

Klein C, Karanges E, Spiro A, Wong A, Spencer J, Huynh T, *et al.* (2011). Cannabidiol potentiates Δ^9-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. *Psychopharmacology (Berl)* **218**:443–457.

Koppel J, Bradshaw H, Goldberg TE, Khalili H, Marambaud P, Walker MJ, *et al.* (2009). Endocannabinoids in Alzheimer's disease and their impact on normative cognitive performance: a case–control and cohort study. *Lipids Health Dis* **8**:2.

Krause DL, Muller N (2010). Neuroinflammation, microglia and implications for anti-inflammatory treatment in Alzheimer's disease. *Int J Alzheimers Dis* **2010**. doi: 10.4061/2010/732806.

Kuret J, Chirita CN, Congdon EE, Kannanayakal T, Li G, Necula M, *et al.* (2005). Pathways of tau fibrillization. *Biochim Biophys Acta* **1739**:167–178.

Lambert DM, Fowler CJ (2005). The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *J Med Chem* **48**: 5059–5087.

Lee JH, Agacinski G, Williams JH, Wilcock GK, Esiri MM, Francis PT, *et al.* (2010). Intact cannabinoid CB1 receptors in the Alzheimer's disease cortex. *Neurochem Int* **57**:985–989.

Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM (2000). Different effects of nabilone and cannabidiol on binocular depth inversion in Man. *Pharmacol Biochem Behav* **66**:175–181.

Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, *et al.* (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry* **2**:e94.

Lichtman AH, Dimen KR, Martin BR (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology (Berl)* **119**:282–290.

Lie DC, Colamarino SA, Song HJ, Désiré L, Mira H, Consiglio A, *et al.* (2005). Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**: 1370–1375.

Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, Piomelli D (2005). The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. *Mol Pharmacol* **67**:15–19.

Lodzki M, Godin B, Rakou L, Mechoulam R, Gallily R, Touitou E (2003). Cannabidiol-transdermal delivery and anti-inflammatory effect in a murine model. *J Control Release* **93**:377–387.

Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T (2010). A behavioural comparison of acute and chronic delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *Int J Neuropsychopharmacol* **13**: 861–876.

Lunn CA, Fine JS, Rojas-Triana A, Jackson JV, Fan X, Kung TT, *et al.* (2006). A novel cannabinoid peripheral cannabinoid receptor-selective inverse agonist blocks leukocyte recruitment in vivo. *J Pharmacol Exp Ther* **316**:780–788.

Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM (2009). Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. *J Hepatol* **51**:528–534.

Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM (2010). Cannabidiol ameliorates cognitive and motor impairments in bile-duct ligated mice via 5-HT1A receptor activation. *Br J Pharmacol* **159**:950–957.

Mahler SV, Smith KS, Berridge KC (2007). Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacology* **32**:2267–2278.

Makara JK, Mor M, Fegley D, Szabó SI, Kathuria S, Astarita G, *et al.* (2005). Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. *Nat Neurosci* **8**:1139–1141.

Mallet P, Beninger R (1996). The endogenous cannabinoid receptor agonist anandamide impairs memory in rats. *Behav Pharmacol* **7**:276–284.

Marchalant Y, Brothers HM, Wenk GL (2008). Inflammation and aging: can endocannabinoids help? *Biomed Pharmacother* **62**:212–217.

Marchesi VT (2011). Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: implications for early detection and therapy. *FASEB J* **25**:5–13.

Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, *et al.* (2010). The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* **13**:951–957.

Martín-Moreno AM, Reigada D, Ramírez BG, Mechoulam R, Innamorato N, Cuadrado A, de Ceballos ML (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Mol Pharmacol* **79**:964–973.

Martín-Moreno AM, Brera B, Spuch C, Carro E, García-García L, Delgado M, *et al.* (2012). Prolonged oral cannabinoid administration prevents neuroinflammation, lowers β-amyloid levels and improves cognitive performance in Tg APP 2576 mice. *J Neuroinflammation* **9**:8.

Mattson MP (2002). Oxidative stress, perturbed calcium homeostasis, and immune dysfunction in Alzheimer's disease. *J Neurovirol* **8**:539–550.

Mazzola C, Micale V, Drago F (2003). Amnesia induced by beta-amyloid fragments is counteracted by cannabinoid CB1 receptor blockade. *Eur J Pharmacol* **477**:219–225.

McGeer PL, McGeer EG (2015). Targeting microglia for the treatment of Alzheimer's disease. *Expert Opin Ther Targets* **19**:497–506.

McHugh D (2012). GPR18 in microglia: implications for the CNS and endocannabinoid system signalling. *Br J Pharmacol* **167**:1575–1582.

McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, Bradshaw HB (2010). *N*-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci* **11**:44.

McPartland JM, Duncan M, di Marzo V, Pertwee RG (2015). Are cannabidiol and Δ(9)-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *Br J Pharmacol* **172**:737–753.

Mechoulam R, Gaoni Y (1965). A total synthesis of Dl-delta-1-tetrahydrocannabinol, the active constituent of hashish. *J Am Chem Soc* **87**:3273–3275.

Mechoulam R, Parker LA, Gallily R (2002). Cannabidiol: an overview of some pharmacological aspects. *J Clin Pharmacol* **42**:11s–19s.

Micale V, Mazzola C, Drago F (2007). Endocannabinoids and neurodegenerative diseases. *Pharmacol Res* **56**:382–392.

Mulder J, Zilberter M, Pasquaré SJ, Alpár A, Schulte G, Ferreira SG, *et al.* (2011). Molecular reorganization of endocannabinoid signalling in Alzheimer's disease. *Brain* **134** (Pt 4):1041–1060.

Näslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD (2000). Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA* **283**:1571–1577.

Nicholson DW, Thornberry NA (1997). Caspases: killer proteases. *Trends Biochem Sci* **22**:299–306.

Nilsson M, Hansson S, Carlsson A, Carlsson ML (2007). Differential effects of the *N*-methyl-D-aspartate receptor antagonist MK-801 on different stages of object recognition memory in mice. *Neuroscience* **149**:123–130.

Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004). Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem* **279**:5298–5305.

Pacher P, Mechoulam R (2011). Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* **50**:193–211.

Paradisi A, Oddi S, Maccarrone M (2006). The endocannabinoid system in ageing: a new target for drug development. *Curr Drug Targets* **7**:1539–1552.

Parsons CG, Danysz W, Dekundy A, Pulte I (2013). Memantine and cholinesterase inhibitors: complementary mechanisms in the treatment of Alzheimer's disease. *Neurotox Res* **24**:358–369.

Pascual AC, Martín-Moreno AM, Giusto NM, de Ceballos ML, Pasquaré SJ (2014). Normal aging in rats and pathological aging in human Alzheimer's disease decrease FAAH activity: modulation by cannabinoid agonists. *Exp Gerontol* **60**:92–99.

Passmore MJ (2008). The cannabinoid receptor agonist nabilone for the treatment of dementia-related agitation. *Int J Geriatr Psychiatry* **23**:116–117.

Perry VH, Nicoll JA, Holmes C (2010). Microglia in neurodegenerative disease. *Nat Rev Neurol* **6**:193–201.

Pertwee RG (2004). The pharmacology and therapeutic potential of cannabidiol. In: Di Marzo V, editor. *Cannabinoids*. New York, NY: Kluwer Academic/Plenum Publishers. pp. 1–52.

Pertwee RG (2005). Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. *Life Sci* **76**:1307–1324.

Pertwee RG (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br J Pharmacol* **153**:199–215.

Pertwee RG, Ross RA, Craib SJ, Thomas A (2002). (−)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. *Eur J Pharmacol* **456**:99–106.

Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC (1998). Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci* **63**:PL1–PL6.

Phiel CJ, Wilson CA, Lee VM, Klein PS (2003). GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature* **423**:435–439.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* **4**:873–884.

Prince M, Wimo A, Guerchet M, Ali G-C, Wu Y-T, Prina M (2015). *World Alzheimer Report 2015. The Global Impact of Dementia*. London: Alzheimer's Disease International.

Ramírez BG, Blázquez C, Gómez del Pulgar T, Guzmán M, de Ceballos ML (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci* **25**:1904–1913.

Russo EB (2011). Taming THC: potential Cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br J Pharmacol* **163**:1344–1364.

Russo EB, Burnett A, Hall B, Parker KK (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res* **30**:1037–1043.

Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, *et al.* (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* **152**:1092–1101.

Satoh J, Kuroda Y (2000). Amyloid precursor protein beta-secretase (BACE) mRNA expression in human neural cell lines following induction of neuronal differentiation and exposure to cytokines and growth factors. *Neuropathology* **20**:289–296.

Schliebs R, Arendt T (2011). The cholinergic system in aging and neuronal degeneration. *Behav Brain Res* **221**:555–563.

Schneider U, Borsutzky M, Seifert J, Leweke FM, Huber TJ, Rollnik JD, Emrich HM (2002). Reduced binocular depth inversion in schizophrenic patients. *Schizophr Res* **53**:101–108.

Scholtzova H, Wadghiri YZ, Douadi M, Sigurdsson EM, Li YS, Quartermain D, *et al.* (2008). Memantine leads to behavioral improvement and amyloid reduction in Alzheimer's-disease-model transgenic mice shown as by micromagnetic resonance imaging. *J Neurosci Res* **86**:2784–2791.

Scuderi C, Filippis DD, Iuvone T, Blasio A, Steardo A, Esposito G (2009). Cannabidiol in medicine: a review of its therapeutic potential in CNS disorders. *Phytother Res* **23**:597–602.

Scuderi C, Steardo L, Esposito G (2014). Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5YAPP+ cells through PPARγ involvement. *Phytother Res* **28**:1007–1013.

Solas M, Francis PT, Franco R, Ramirez MJ (2013). CB2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients. *Neurobiol Aging* **34**:805–808.

Solowij N, Broyd SJ, van Hell HH, Hazekamp A (2014). A protocol for the delivery of cannabidiol (CBD) and combined CBD and 9-tetrahydrocannabinol (THC) by vaporisation. *BMC Pharmacol Toxicol* **15**:58.

Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, *et al.* (2010). The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS One* **5**:e9505.

Sperber BR, Leight S, Goedert M, Lee VM (1995). Glycogen synthase kinase-3 beta phosphorylates tau protein at multiple sites in intact cells. *Neurosci Lett* **197**:149–153.

Stella N (2010). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* **58**:1017–1030.

Stella N, Schweitzer P, Piomelli D (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**:773–778.

Stern CA, Gazarini L, Takahashi RN, Guimarães FS, Bertoglio LJ (2012). On disruption of fear memory by reconsolidation blockade: evidence from cannabidiol treatment. *Neuropsychopharmacology* **37**:2132–2142.

Streit WJ (2004). Microglia and Alzheimer's disease pathogenesis. *J Neurosci Res* **77**:1–8.

Stumm C, Hiebel C, Hanstein R, Purrio M, Nagel H, Conrad A, *et al.* (2013). Cannabinoid receptor 1 deficiency in a mouse model of Alzheimer's disease leads to enhanced cognitive impairment despite of a reduction in amyloid deposition. *Neurobiol Aging* **34**:2574–2584.

Takeuchi H (2010). Neurotoxicity by microglia: Mechanisms and potential therapeutic strategy. *Clin Exp Immunol* **1**:12–21.

Tanasescu R, Constantinescu CS (2010). Cannabinoids and the immune system: an overview. *Immunobiology* **215**:588–597.

Teng L, Zhao J, Wang F, Ma L, Pei G (2010). A GPCR/secretase complex regulates beta- and gamma-secretase specificity for Abeta production and contributes to AD pathogenesis. *Cell Res* **20**:138–153.

Thomas BF, Gilliam AF, Burch DF, Roche MJ, Seltzman HH (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J Pharmacol Exp Ther* **285**:285–292.

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br J Pharmacol* **150**:613–623.

Tolón RM, Núñez E, Pazos MR, Benito C, Castillo AI, Martínez-Orgado JA, Romero J (2009). The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. *Brain Res* **1283**:148–154.

Umeda T, Mori H, Zheng H, Tomiyama T (2010). Regulation of cholesterol efflux by amyloid beta secretion. *J Neurosci Res* **88**:1985–1994.

Van der Stelt M, Mazzola C, Esposito G, Matias I, Petrosino S, de Filippis D, *et al.* (2006). Endocannabinoids and beta-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol Life Sci* **63**:1410–1424.

Varvel SA, Wiley JL, Yang R, Bridgen DT, Long K, Lichtman AH, Martin BR (2006). Interactions between THC and cannabidiol in mouse models of cannabinoid activity. *Psychopharmacology (Berl)* **186**:226–234.

Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ (1997). Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. *Int J Geriatr Psychiatry* **12**:913–919.

Wade DT, Collin C, Stott C, Duncombe P (2010). Meta-analysis of the efficacy and safety of Sativex (nabiximols), on spasticity in people with multiple sclerosis. *Mult Scler* **16**:707–714.

Walter L, Stella N (2004). Cannabinoids and neuroinflammation. *Br J Pharmacol* **141**:775–785.

Walter L, Franklin A, Witting A, Wade C, Xie Y, Kunos G, *et al.* (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J Neurosci* **23**:1398–1405.

Walther S, Mahlberg R, Eichmann U, Kunz D (2006). Delta-9-tetrahydrocannabinol for nighttime agitation in severe dementia. *Psychopharmacology (Berl)* **185**:524–528.

Walther S, Schüpbach B, Seifritz E, Homan P, Strik W (2011). Randomized, controlled crossover trial of dronabinol, 2.5 mg, for agitation in 2 patients with dementia. *J Clin Psychopharmacol* **31**:256–258.

Watanabe K, Kayano Y, Matsunaga T, Yamamoto I, Yoshimura H (1996). Inhibition of anandamide amidase activity in mouse brain microsomes by cannabinoids. *Biol Pharm Bull* **19**:1109–1111.

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994). Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* **63**:637–652.

Winsauer PJ, Lambert P, Moerschbaecher JM (1999). Cannabinoid ligands and their effects on learning and performance in rhesus monkeys. *Behav Pharmacol* **10**:497–511.

Wolf SA, Bick-Sander A, Fabel K, Leal-Galicia P, Tauber S, Ramirez-Rodriguez G, *et al.* (2010). Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Commun Signal* **8**:12.

Wolfe MS (2009). Tau mutations in neurodegenerative diseases. *J Biol Chem* **284**:6021–6025.

Wu J, Bie B, Yang H, Xu JJ, Brown DL, Naguib M (2013). Activation of the CB2 receptor system reverses amyloid-induced memory deficiency. *Neurobiol Aging* **34**:791–804.

Zandi PP, Breitner JC (2001). Do NSAIDs prevent Alzheimer's disease? And, if so, why? The epidemiological evidence. *Neurobiol Aging* **22**: 811–817.

Zandi PP, Breitner JC, Anthony JC (2002). Is pharmacological prevention of Alzheimer's a realistic goal? *Expert Opin Pharmacother* **3**:365–380.

Zuardi AW, Karniol IG (1983). Effects on variable-interval performance in rats of delta 9-tetrahydrocannabinol and cannabidiol, separately and in combination. *Braz J Med Biol Res* **16**:141–146.

Zuardi AW, Finkelfarb E, Bueno OF, Musty RE, Karniol IG (1981). Characteristics of the stimulus produced by the mixture of cannabidiol with delta 9-tetra-hydrocannabinol. *Arch Int Pharmacodyn Ther* **249**:137–146.

Zuardi AW, Rodrigues JA, Cunha JM (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology (Berl)* **104**:260–264.

Zuardi AW, Morais SL, Guimarães FS, Mechoulam R (1995). Antipsychotic effect of cannabidiol. *J Clin Psychiatry* **56**:485–486.