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

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## **Abstract**

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- $\beta$  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- $\beta$  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 endocannabinoid system in AD before commenting on the potential future of CBD for AD therapy (including safety aspects).

## **Disciplines**

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***The Therapeutic Potential of the Phytocannabinoid Cannabidiol (CBD) for Alzheimer's Disease***

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***Short title:*** CBD therapy in Alzheimer's disease

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## **Abstract**

Alzheimer's disease (AD) is the most common neurodegenerative disorder, characterised by progressive loss of cognition. Over 35 million people are currently diagnosed with AD worldwide. Unfortunately, current therapies are limited to very modest symptomatic relief. Brains of AD patients are characterised by deposition of amyloid- $\beta$  ( $A\beta$ ) and hyperphosphorylated forms of tau protein. AD brains also exhibit neurodegeneration and show high levels of oxidative stress and inflammation. The phytocannabinoid cannabidiol (CBD) possesses neuroprotective, anti-oxidant, and anti-inflammatory properties and reduces  $A\beta$  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 give 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 CBD's therapeutic potential. We will also discuss the role of the endocannabinoid system in AD before commenting on the potential future of CBD for AD therapy (including safety aspects).

**Keywords:** Alzheimer's disease, dementia, cannabinoids, cannabidiol, endocannabinoid system, cannabinoid receptor, therapy, cognition, learning and memory, mouse model

## Abbreviations

2-AG: 2-arachidonylglycerol (endocannabinoid)

2-LG: 2-linoleoylglycerol

5-HT: 5-hydroxytryptamine / serotonin

5-HT<sub>1A</sub>: serotonergic 5-hydroxytryptamine 1A receptor

A<sub>2A</sub>: adenosine 2A receptor

A $\beta$ : beta-amyloid protein; majority of A $\beta$  is 40 residues in length (A $\beta$ <sub>40</sub>), whereas approximately 10% form the 42 residue-length variant (A $\beta$ <sub>42</sub>)

ABHD6: Serine hydrolase alpha/beta-hydrolase domain-containing 6

ACEA: arachidonyl-20-chloroethylamide (CB<sub>1</sub> agonist)

AICD: amyloid precursor protein intracellular domain

AEA: anandamide (also called *N*-arachidonylethanolamine)

AMPA: 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid

APP: amyloid precursor protein

ATP: adenosine triphosphate

BDNF: brain-derived neurotrophic factor

Ca<sup>2+</sup>: calcium ions

CB<sub>1</sub>: cannabinoid receptor 1

CB<sub>2</sub>: cannabinoid receptor 2

CBD: cannabidiol (phytocannabinoid)

CGS-21680: A<sub>2A</sub> receptor agonist

CNS: central nervous system

COX-2: cyclooxygenase 2

CP 55,940: synthetic cannabinoid, full agonist of CB<sub>1</sub> and CB<sub>2</sub> (>40 more potent than THC)

CSF: cerebrospinal fluid

DAGL- $\alpha/\beta$ : diacylglycerol lipases  $\alpha/\beta$

Dronabinol: pharmaceutical formulation of THC

DNA: deoxyribonucleic acid

$^{18}\text{F}$ FDG:  $^{18}\text{F}$ -fluoro-deoxyglucose

FAAH: fatty acid amide hydrolase

GABA:  $\gamma$ -aminobutyric acid

GFAP: glial fibrillary acidic protein

GPR18 / GPR55 / GPR118: G-protein coupled receptors 18, 55, and 119

GSK3- $\beta$ : glycogen synthase kinase 3  $\beta$

GW9662: peroxisome proliferator-activated receptor  $\gamma$  antagonist

HU-308: synthetic cannabinoid; selective CB<sub>2</sub> agonist

Iba1: ionised calcium binding adaptor molecule 1 (i.e. a microglia/macrophage-specific calcium-binding protein)

INF- $\gamma$ : interferon  $\gamma$

i.p.: intraperitoneal

i.c.v.: intracerebroventricular

IL: interleukin

JWH-133: synthetic cannabinoid selective for CB<sub>2</sub>

MDA7: 1-((3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl) carbonyl) piperidine - a novel highly selective CB<sub>2</sub> agonist

MAGL: monoacylglycerol lipase

Nabilone: synthetic cannabinoid mimicking the effects of THC

NAPE-PLD: *N*-acyl phosphatidylethanolamine-selective phospholipase D

NMDA: N-methyl-D-aspartate

NF- $\kappa$ B: transcription factor nuclear factor  $\kappa$ B

NFT: neurofibrillarly tangle

NO: nitric oxide

iNOS: inducible nitric oxide synthase

p-GSK3 $\beta$ : phosphorylated glycogen synthase kinase 3  $\beta$

p38 MAP kinase: p38 mitogen-associated protein kinase

PEA: palmitoylethanolamide

PET: positron emission tomography

PND: Postnatal day

PPAR $\gamma$ : peroxisome proliferator-activated receptor  $\gamma$

PS1: presenilin 1

PS2: presenilin 2

ROS: reactive oxygen species

Sativex®: 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), developed/produced by GW Pharmaceuticals Ltd (Cambridge, UK) in a 1:1 proportion

SAPK/JNK: stress-activated protein kinase/c-Jun NH(2)-terminal kinase

SR141716: CB<sub>1</sub> antagonist

SR144528: CB<sub>2</sub> antagonist

SOD1/2: superoxide dismutase 1/2

THC:  $\Delta^9$ -tetrahydrocannabinol (phytocannabinoid)

TNF- $\alpha$ : tumor necrosis factor-alpha

HU-210: synthetic cannabinoid selective for CB1 (100-800 times more potent than THC)

VR1: vanilloid receptor type 1; also known as the transient receptor potential cation channel subfamily

V member 1 (TRPV1)

VR2: vanilloid receptor type 2

WIN 55,212-2: synthetic cannabinoid with mixed CB<sub>1</sub>/CB<sub>2</sub> agonism (similar to THC)

ZM241,385: A<sub>2A</sub> receptor antagonist

## **Main Text**

### **1. 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 people globally are living with dementia (World Alzheimer Report 2015; (Ferri et al., 2005)). The most prominent form of dementia is Alzheimer's disease (AD), which is predicted to affect 1 in 85 people globally by the year 2050. AD is categorised by three progressive clinical stages: mild, moderate and severe (Zandi et al., 2002). The mild stage is characterised 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 recognise familiar people 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).

#### ***1.1 Biology of Alzheimer's disease***

AD is characterised 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 is believed to result from a complex interaction of various environmental risk factors and multiple susceptibility genes (Kamboh, 2004). A great deal of information has been gained from the analysis of genetic risk factors. *APOE* genotype is by far the most robust predictor of AD risk with the  $\epsilon 4$  allele affording increased risk and the  $\epsilon 2$  allele

affording protection as compared to the most common  $\epsilon 3$  allele (Corder et al., 1993). Genome-wide association studies (GWAS) 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 homeostasis (e.g. *GAB2*) (Belbin et al., 2011).

Familial AD is autosomal dominant and accounts for <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 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  $\gamma$ -secretase, one of the enzymes responsible for the cleavage of APP into  $\beta$ -amyloid peptides ( $A\beta$ ).  $A\beta$  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 anti-microbial 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  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases via two pathways, a non-amyloidogenic and an amyloidogenic pathway. The non-amyloidogenic pathway accounts for the majority of APP processing in the healthy brain whereby APP is cleaved by  $\alpha$ -secretase to generate: 1) a soluble N-terminal fragment ( $sAPP\alpha$ ), which has neuroprotective properties; 2) a C-terminal fragment ( $CTF\alpha$ ), that is retained in the membrane and processed further by  $\gamma$ -secretase to yield an N-terminal fragment ( $p3$ ); and, 3) a membrane-bound C-terminal fragment, the APP intracellular domain (*AICD*), which regulates gene transcription. A minority of APP is processed by the amyloidogenic pathway, leading to the generation of  $A\beta$ : first,  $\beta$ -secretase cleaves APP resulting in soluble APP and a cell-membrane bound fragment; second,  $\gamma$ -secretase cleaves this fragment further, producing  $A\beta$  and *AICD*. Importantly, the majority of  $A\beta$  produced are 40 residues in length ( $A\beta_{40}$ ), whereas approximately 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 $\beta$ <sub>42</sub>. The excessive production of A $\beta$ <sub>42</sub> increases its aggregation in extracellular deposits that form amyloid or senile plaques, one of the two distinct types of lesions observed post-mortem in brains of AD patients.

The second pathological hallmark of AD is the intracellular accumulation and hyperphosphorylation of microtubule-associated protein tau (MAPT), which leads to the formation of neurofibrillary tangles (NFT). Tau is predominantly found in axons of neurons, where it promotes the assembly of microtubules from tubulin, stabilises them, and supports microtubule-dependent axonal transport of organelles and biomolecules. In the healthy brain, 2-3 amino acid residues on tau are phosphorylated, whereas in AD, tau proteins are hyperphosphorylated (average of 9 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 A $\beta$  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 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 hypothesised that deposits of A $\beta$  are responsible for causing and exacerbating tau hyperphosphorylation and the generation of NFTs, as A $\beta$  depositions have been found prior to 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 demonstrate 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 regarding the role of A $\beta$  in AD suggests that resting microglia become activated in response to the presence of A $\beta$  and cluster at sites of amyloid deposition in the brain. This initiates neuroinflammatory processes and the release of neurotoxic factors (e.g. pro-inflammatory 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 one side, 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 side, 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 recognised that microglia exist in a spectrum of functional phenotypes that may reflect helpful (e.g. A $\beta$  clearance) or harmful (e.g. over-production of pro-inflammatory 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)

## ***1.2 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. Based on the complex pathology of AD, a preventative, multimodal drug approach targeting a combination of pathological AD events early in disease development appears 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 anti-oxidant properties and have immunosuppressive effects. More recently, cannabinoids have also been found to possess properties that may reduce A $\beta$  and tau pathology. 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 CBD's pharmacological properties and its potential role in AD therapy will be outlined in the following sections.

## **2. Cannabidiol (CBD)**

The phytocannabinoid CBD was first isolated from *cannabis sativa* in 1940 (Adams, 1940) and its structure was elucidated in the 60's (Mechoulam and Gaoni, 1965). CBD has very low toxicity. The LD<sub>50</sub> after intravenous administration to the rhesus monkey is 212 mg/kg whereas the oral LD<sub>50</sub> could not be established, probably due to 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 due to 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 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, oxidations to carboxylic acids,  $\beta$ -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)].

CBD pharmacological properties range from anti-convulsive, anti-anxiety, and anti-psychotic (Zuardi et al., 1991, Zuardi et al., 1995, Leweke et al., 2000, Schneider et al., 2002) to anti-nausea, anti-inflammatory, and anti-rheumatoid arthritic [outlined more comprehensively in (Pertwee, 2008, Russo, 2011)]. Importantly, CBD is a multi-target drug that can interact with many signaling systems including the endocannabinoid system (outlined in more detail in section 2.2).

### ***2.1 Brief introduction to the endocannabinoid system***

The endocannabinoid system (ECS) is an intercellular signalling system comprised of G-protein coupled cannabinoid receptors 1 (CB<sub>1</sub>) and 2 (CB<sub>2</sub>) as well as more recently discovered receptors (e.g. *N*-arachidonyl glycine receptor or G-protein coupled receptor 18: GPR18); endogenous ligands, the best characterised ones being the arachidonic acid derivatives *N*-arachidonylethanolamine (also called anandamide: AEA) and 2-arachidonoylglycerol (2-AG) and their homologues; and metabolic enzymes (for overview see Table 1). The enzymes responsible for the biosynthesis of AEA require complete characterisation but a *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) has been implicated in the process as has diacylglycerol lipase- $\alpha$  (DAGL- $\alpha$ ) for 2-AG. Metabolism of anandamide and 2-AG require 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, *Cannabis sativa*, including  $\Delta^9$ -tetrahydrocannabinol (THC), the main psychoactive and cognition-impairing component, CBD, as well as synthetic cannabinoids (i.e. cannabimimetics such as CP 55,940 and WIN 55,212-2) (Tanasescu and Constantinescu, 2010) (for overview see Table 1).

CB<sub>1</sub> 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<sub>1</sub> in learning and memory. CB<sub>1</sub> has also been implicated in cannabinoid-mediated modulation of immune functions (Cabral et al., 2008). CB<sub>2</sub> 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<sub>2</sub> is also densely expressed on activated microglia cells, which suggests a possible role in mediating neuroinflammatory responses in the CNS. Stimulation of CB<sub>2</sub> 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 (neonataly until 32 months of age) of cannabinoid receptors in the rat brain using the full CB<sub>1</sub> and CB<sub>2</sub> agonist CP 55,904 revealed that cannabinoid receptor binding capacity increases progressively from birth to postnatal day (PND) 60 in whole brain preparations whereas no further changes in binding are detected in adulthood and throughout the normal aging process (Belue et al., 1995). Interestingly, and in line with emerging evidence suggesting that A $\beta$  depositions in AD brain are the result of impaired clearance, is the fact that activation of CB<sub>1</sub>/CB<sub>2</sub> by 2-AG as well as the suppression of the endocannabinoid-degrading enzyme MGL (but not FAAH or ABHD6) elevates A $\beta$  clearance across the blood-brain barrier (Bachmeier et al., 2013).

## ***2.2 CBD pharmacology***

### CBD 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<sub>1</sub> and CB<sub>2</sub> receptors compared to 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<sub>1</sub> as well as CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptors, and that CBD has no agonistic activity of cannabinoid receptors even at high concentrations (Petitet et al., 1998). These findings were in line with earlier reports showing that CBD can reverse behavioural effects induced by THC (Karniol et al., 1974, Zuardi et al., 1981) although the study by Petitet and coworkers found no blockade of CP 55,940-induced hypothermia in mice by CBD. Interestingly, another study found that CBD antagonises (or inversely agonises) 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<sub>1</sub> or CB<sub>2</sub> receptors (Pertwee et al., 2002). In a follow-up study, CBD displayed inverse agonism at human CB<sub>2</sub> receptors and was a high potency antagonist (non-competitive) of cannabinoid receptor agonists in mouse brains as well as in membranes from CHO cells transfected with human CB<sub>2</sub> (Thomas et al., 2007). CBD induced inverse agonism at CB<sub>2</sub> receptors at concentrations well below those at which it displaces CP 55,940. This characteristic of CBD may contribute to its anti-inflammatory properties, as there is evidence that CB<sub>2</sub> 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 on GPR18 (McHugh et al., 2010) receptors and activates the putative abnormal CBD receptor (Pertwee,

2005, 2008). Bisogno and coworkers discovered that CBD also interacts with vanilloid receptor type 1 (VR1), the receptor for capsaicin (Bisogno et al., 2001), 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 (VR1) (Bisogno et al., 2001). Finally, there is limited evidence that suggests CBD might also activate VR2 (Izzo et al., 2009).

CBD also impacts brain endocannabinoid 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 due to 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).

#### CBD effects on other neurotransmitter systems and brain processes

The role of CBD in brain circuits other than the endocannabinoid system has also been evaluated. In 1998, CBD was found to protect against neurotoxicity mediated by glutamate receptors, i.e. 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 exhibit a modest agonistic affinity of human 5-HT<sub>1a</sub> 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<sub>1A</sub> receptor (Russo et al., 2005). Furthermore, CBD enhanced adenosine receptor A<sub>2A</sub> signalling via inhibition of cellular uptake of an adenosine transporter (Carrier et al., 2006). This effect may be at least partially responsible for CBDs ability to decrease inflammation and to be neuroprotective. Although not relevant for the *in vivo* effects (as CBD doses required were not biologically relevant), CBD was reported to possess allosteric modulator properties for  $\mu$ - and  $\delta$ -opioid

receptors at very high concentrations (i.e. dissociation rate induced by naloxone and naltrindole at receptors was accelerated by 100  $\mu\text{M}$  CBD) (Kathmann et al., 2006). This is an interesting finding considering that  $\delta$ -opioid receptors can form a complex with  $\beta$ - and  $\gamma$ -secretases thereby promoting the processing of APP to A $\beta$  (Teng et al., 2010). Finally, there is also some experimental evidence to support CBD activity in other pathways such as the dopamine and GABA 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<sub>1</sub> knockout mice suggesting that the effect of CBD on neurogenesis is mediated by an indirect activation of CB<sub>1</sub> receptors, possibly via inhibition of AEA metabolism/uptake (as discussed earlier). Supporting this finding is a recent *in vitro* study showing that CBD increases proliferation of hippocampal progenitor cells in culture, which can be prevented by antagonists for both CB<sub>1</sub> and CB<sub>2</sub> 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, sections 3.1 and 3.2) where the therapeutic properties of CBD in preclinical models for the disease are discussed (for full overview of pharmacological actions of CBD see Table 2).

### **3. CBD: A new treatment option for Alzheimer's disease – a preclinical perspective**

As discussed in section 2.2, the molecular mechanisms in which CBD exerts its various effects are still under debate with evidence suggesting that its actions are not confined to the receptors of the endocannabinoid system. Importantly for this review, a number of studies provide evidence that CBD exerts various properties including neuroprotection, anti-inflammatory and anti-oxidant effects, and is able to 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* (section 3.1) and *in vivo* models (section 3.2) relevant to AD.

### ***3.1 Effects of CBD on in vitro Alzheimer's disease models***

#### Tau pathology

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

Direct modulatory effects of CBD on APP processing have only recently been evaluated in *in vitro* studies. Transfected human neuroblastoma SHSY5YAPP<sup>+</sup> cells exhibited significantly elevated full-length APP expression compared to control neuronal cells (Scuderi et al., 2014). CBD counteracted this elevation in a dose-dependent manner by inducing ubiquitination of APP without having any effect on

control cells. The CBD effect on SHSY5YAPP<sup>+</sup> cells was paralleled by a progressive reduction of A $\beta$  peptide expression in cell lysates and consequentially less apoptotic events (i.e. number of apoptotic cell bodies and % neuron survival) in these cells (Scuderi et al., 2014). Importantly, the peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) antagonist GW9662 blocked these effects of CBD whereas the involvement of  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretases were ruled out. PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily and include three isoforms (i.e.  $\alpha$ ,  $\beta/\delta$ , and  $\gamma$ ). PPARs have been linked to inflammation, cell proliferation, and differentiation. Importantly, PPAR $\gamma$  receptors are expressed at low levels under physiological conditions but rise in response to some pathological situations including AD (Kitamura et al., 1999). In line with this, PPAR $\gamma$  activation has been found to reduce APP expression (d'Abramo et al., 2005) and dramatically enhance clearance of A $\beta$  *in vitro* (Camacho et al., 2004). Thus, CBD may exert a beneficial effect on the amyloidogenic pathway through a mechanism involving PPAR $\gamma$ . This presents a novel promising avenue to counteract the progression of AD and would be in line with suggestions that regulating PPAR $\gamma$  activity may be therapeutically effective for AD pathophysiology. Related to the impact CBD has on the amyloidogenic pathway, other synthetic cannabinoids (i.e. the CB<sub>2</sub> agonist JWH-015) have been found to increase the phagocytosis of A $\beta$  by mouse microglial cells (Ehrhart et al., 2005) and promote the removal of A $\beta$  from human frozen tissue sections at low doses (Tolon et al., 2009). Importantly, CB<sub>2</sub> expression is upregulated in glial cells under chronic neuroinflammatory conditions such as AD.

#### A $\beta$ -induced toxicity

CBD dose-dependently alleviated various other effects of A $\beta$ -induced toxicity in a cultured rat pheochromocytoma PC12 cell model (Iuvone et al., 2004). CBD administration prior to A $\beta$  treatment improved cell survival, reduced lipid peroxidation and production of reactive oxygen species (ROS). ROS have been found to play a role in A $\beta$ -induced cell damage and death (Brera et al., 2000) The phytocannabinoid also increased caspase 3 protein levels (used as a hallmark of apoptosis), DNA fragmentation and intracellular calcium levels, which were elevated in A $\beta$ -treated cells without CBD

treatment. Importantly, caspases are essential mediators of many of the pathways involved in executing the apoptotic programme following A $\beta$  accumulation (Nicholson and Thornberry, 1997). Furthermore, A $\beta$ -induced DNA fragmentation, a hallmark of apoptosis, has previously been found in AD models (Gschwind and Huber, 1995), and a rise in calcium levels has been suggested to be responsible for at least some of the toxic effects of A $\beta$  (Mattson, 2002). Finally, an A $\beta$ -induced decrease in the pro-caspase 3/total caspase 3 ratio was counteracted by CBD, suggesting that CBD could exert a protective role at 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<sub>1</sub> receptors, as SR141716A treatment (a CB<sub>1</sub> receptor antagonist) did not modulate the CBD properties observed. The authors concluded that CBD exerts a combination of neuroprotective, anti-oxidant, and anti-apoptotic effects against A $\beta$ -induced toxicity.

In line with the above findings, stimulation of PC12 cells with A $\beta$  caused 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 pro-inflammatory conditions typical for AD (Cardenas et al., 2005). CBD inhibited these consequences of A $\beta$  toxicity dose-dependently. This neuroprotective effect of CBD was mediated by suppressing the A $\beta$ -induced increase in phosphorylation of p38 mitogen-associated protein kinase (p38 MAP kinase) and activation of nuclear factor- $\kappa$ B activation (NF- $\kappa$ B) (Esposito et al., 2006b). The transcription factor NF- $\kappa$ B is stimulated by stress-responsive protein kinases (e.g. p38 MAP kinase) and regulates the expression of genes involved in cell differentiation, proliferation and apoptosis as well as oxidative and inflammatory responses. Furthermore, NF- $\kappa$ B activation is required to induce iNOS protein transcription in post-mortem AD brains (Haas et al., 2002).

A more recent study was not able to confirm the neuroprotective effects of CBD: CBD protected rat PC12 peripheral neuronal cells and human SH-SY5Y cells against oxidative stress and lipid

peroxidation induced by tert-butyl hydroperoxide but failed against A $\beta$ <sub>1-40</sub> fibril and aggregate-evoked neurotoxicity (Harvey et al., 2012). This unexpected finding was probably due to the fact that the previous studies incubated cells with non-fibrillar A $\beta$ , whereas Harvey *et al.* utilised A $\beta$  in its preformed (fibrillar) state when incubating the neuronal cell lines. Thus the neuroprotective efficacy of CBD appears dependent on A $\beta$  fibril formation occurring during cell exposure, which implies either a direct influence on fibril formation or interference with A $\beta$  fibril uptake or processing. CBD was neuroprotective against oxidative stress generated from tert-butyl hydroperoxide but not from A $\beta$  exposure as there are differences in the neurotoxicity profile caused by these two stressors. It is likely that A $\beta$  activates additional pathways in evoking cell death that are not surmountable via antioxidant capacity alone whereas tert-butyl hydroperoxide will directly attack membrane lipids (Harvey et al., 2012). Interestingly, AEA effectively protected neuronal cells against A $\beta$  fibril and aggregate-evoked neurotoxicity via a pathway unrelated to CB<sub>1</sub> or CB<sub>2</sub> receptor activation, although both neuronal cell lines expressed CB<sub>1</sub> receptors (low expression of CB<sub>2</sub> only) (Harvey et al., 2012).

The same team went on to analyse the potential inhibition of native A $\beta$  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 $\beta$ <sub>42</sub> directly 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 $\beta$ <sub>42</sub>, after which TNF- $\alpha$  and nitrite production were evaluated following CBD treatment (Janefjord et al., 2014). A $\beta$ <sub>42</sub> evoked a concentration-dependent loss of cell viability in SHSY5Y cells but negligible TNF- $\alpha$  and nitrite production in BV-2 cells compared to LPS or albumin. CBD protected against A $\beta$ -induced cell viability loss directly as well as against LPS-activated BV-2 conditioned media viability loss. CBD also altered the morphology of A $\beta$  fibrils and aggregates to some extent (but to a lesser degree than other cannabinoids). However, there was no clear correlation between changed morphology and neuroprotective actions. In line with the previous study outlining the role of endocannabinoids in A $\beta$ -relevant neuroprotection (Harvey et al., 2012), of all the other

cannabinoids used, only the endocannabinoid 2-AG was found to provide significant and direct neuroprotection against A $\beta$ <sub>42</sub> (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 A $\beta$  pretreatment). This may point towards a cellular proliferative or mitochondrial stabilising effect of CBD that could potentially offset the loss of viability induced by A $\beta$ <sub>42</sub>.

### 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 defense of the CNS against pathological events. When membrane receptors of microglia are activated [e.g. by adenosine triphosphate (ATP), which is released by dying cells], these glial cells migrate towards the site of injury and release e.g. pro-inflammatory 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 and colleagues (2003) discovered that microglia express both CB<sub>1</sub> and CB<sub>2</sub> 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 via antagonism at CB<sub>2</sub> and ‘abnormal CBD’ receptors [for the latter see (Jarai et al., 1999)]. In line with this, another study found that several synthetic cannabinoids blocked A $\beta$ -induced activation of cultured microglia, which was assessed by microglial cell activity, morphology, and TNF- $\alpha$  release (Ramirez et al., 2005). This effect was evident for HU-210 but also for cannabinoids with CB<sub>2</sub> selectivity (i.e. JWH-133) and no anti-oxidant properties (i.e. WIN 55,212-2).

Interestingly, JWH-133 was as effective as the mixed CB<sub>1</sub>/CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptors whereas those properties could be blocked by CB<sub>2</sub> 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<sub>2A</sub> receptor (i.e. CGS-21680) mimicked the actions of CBD. More importantly, an A<sub>2A</sub> 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 which could be stopped by CB<sub>1</sub> and CB<sub>2</sub> receptor antagonism (i.e. SR141716 and SR144528 respectively).

### Acetylcholine

It is important to realise that A $\beta$  not only induces neurodegeneration but also has 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 (ACh), 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 ACh-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 A $\beta$  *in vitro* (and does so more effectively than currently approved AD interventions such as donepezil and tacrine) (Eubanks et al., 2006).

### Glutamate

A $\beta$  can also cause long-term disruptions to glutamatergic neurotransmission (Schliebs and Arendt, 2011). Under normal physiological conditions, glutamate activates NMDA receptors, allowing calcium ions (Ca<sup>2+</sup>) to flow into the post-synaptic neuron. This triggers a signalling cascade that produces synaptic plasticity including long-term potentiation (LTP), 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<sup>2+</sup> influx. This prolonged Ca<sup>2+</sup> 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 remember that CBD has been shown to reduce glutamate-, NMDA-, AMPA-, and kainate-induced toxicity in rat cortical neurones (Hampson et al., 1998). However, the direct effect of CBD on the glutamatergic system in an AD-relevant context has not been investigated yet.

### ***3.2 Effects of CBD 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 utilising cannabis plant extract either high in CBD or THC revealed 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 given concomitantly with THC-rich extracts did not block the memory-impairing

(and catalepsy-inducing) effects of THC. It has been suggested that a >10-fold higher dose of CBD over THC is necessary to effectively antagonise THC-mediated behavioural deficits [(Fadda et al., 2004) – see also (Zuardi and Karniol, 1983)] (for further details on CBD-THC combination treatments/effects see section 5.1).

#### Mouse models developing AD-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 had therapeutic effects on hepatic encephalopathy [i.e normalising increased TNF- $\alpha$  receptor 1 gene and decreased brain-derived neurotrophic factor (BDNF) 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<sub>2A</sub> receptors. A follow-up study by the same research team also discovered an involvement of 5-HT<sub>1A</sub> receptor activation in the beneficial properties of CBD treatment in this model (Magen et al., 2010). However, CBD-induced BDNF expression changes were not mediated by either receptor suggesting the involvement of yet to be discovered pathways.

Iron content in brain appears 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 anti-oxidant 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 and coworkers found that the amnesic effects of A $\beta$ <sub>25-35</sub> and A $\beta$ <sub>42</sub>

(measured in mice using the step-through passive avoidance test) could be blocked by co-treatment with the CB<sub>1</sub> antagonist SR141716A (Mazzola et al., 2003). Another study using pharmacological rodent models for AD evaluated changes to the endocannabinoid system after rats had been exposed to cortical A $\beta$ <sub>42</sub> administration. A $\beta$ <sub>42</sub> enhanced hippocampal 2-AG (but not AEA) levels concomitant with the appearance of markers of neuronal damage, increased CB<sub>2</sub> (but not CB<sub>1</sub>) protein expression, and induced cognitive deficits in the passive avoidance task. Inhibition of endocannabinoid cellular reuptake reversed hippocampal damage and loss of memory retention (but only when given early after A $\beta$ <sub>42</sub> administration) (van der Stelt et al., 2006). These data suggested for the first time that early modifications to the endocannabinoid system might protect against A $\beta$  neurotoxicity and its consequences. Indeed, detrimental effects of bilateral injection of A $\beta$ <sub>40</sub> fibrils into the hippocampal CA1 area of rats on spatial memory and neuroinflammation (e.g. microglia and astrocyte activation, IL-1 $\beta$  expression and A $\beta$  clearance) were reversed by subchronic treatment with MDA7, a selective CB<sub>2</sub> receptor agonist. Furthermore, A $\beta$ <sub>40</sub> injections were accompanied by increased hippocampal CB<sub>2</sub> expression (Wu et al., 2013).

*CBD treatment:* Most relevant to this review are studies testing CBD in pharmacological models for AD. When mice were inoculated with A $\beta$ <sub>42</sub> in the hippocampus and co-treated with CBD (i.p. route), CBD dose-dependently suppressed A $\beta$ -induced increases in glial fibrillary acidic protein (GFAP) mRNA and protein expression (i.e. a marker of activated astrocytes) and reduced A $\beta$ -induced iNOS and IL-1 $\beta$  protein expression, and the related NO and IL-1 $\beta$  release (Esposito et al., 2007). IL-1 $\beta$  is involved in events related to neurodegeneration including synthesis and processing of APP and astrocyte activation, which is followed by iNOS over-expression and excessive production of NO. Thus, CBD was effective in counteracting aspects of the neuroinflammatory response to A $\beta$  challenge and a CB<sub>2</sub>-related mechanism was put forward by the authors (Esposito et al., 2007). Interestingly, CB<sub>2</sub> overexpression has been detected in an A $\beta$ -induced rat model of reactive gliosis (van der Stelt et al.,

2006), and CB<sub>2</sub> 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 co-workers is another study that determined the effects of chronic CBD treatment on these domains in mice injected i.v.c. with fibrillar A $\beta$ . CBD treatment reversed the compromising effects of A $\beta$  on Morris water maze learning (no data were available for the memory consolidation and retention of these mice). CBD did not alter the A $\beta$ -induced increase in TNF- $\alpha$  mRNA expression but decreased levels of IL-6 (Martin-Moreno et al., 2011).

#### Transgenic mouse models for Alzheimer's disease

*The endocannabinoid system in AD transgenic mice:* A limited number of studies paid close attention to the expression profile of the cannabinoid receptors CB<sub>1</sub> and CB<sub>2</sub> in the context of AD. CB<sub>1</sub> immunoreactivity was reduced in hippocampal regions (i.e. CA1 and CA2/3) of APP $\times$ PS1 mice, an established transgenic mouse model for familial AD (Kalifa et al., 2011). This CB<sub>1</sub> phenotype was associated with astroglial proliferation and elevated expression of the iNOS and TNF- $\alpha$  suggesting that lower CB<sub>1</sub> expression levels in AD transgenic mice may diminish anti-inflammatory processes thereby exacerbating AD-associated pathology. Another study found no changes to CB<sub>2</sub> protein expression in APP transgenic mice (Martin-Moreno et al., 2012). Finally, a triple AD transgenic mouse model (i.e. harboring PS1<sub>M146V</sub>, APP<sub>Swe</sub>, and Tau<sub>P301L</sub> transgenes), exhibited increased CB<sub>1</sub> expression in prefrontal cortex, dorsal hippocampus, and basolateral amygdala complex, whereas expression levels were lower compared to 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<sub>1</sub> knockout mice to study the impact of CB<sub>1</sub> deficiency on AD pathology (Stumm et al., 2013). Most double mutant mice died before the onset of AD pathology but surviving mice exhibited 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<sub>1</sub> on APP processing. Compared to APP23 transgenic and CB<sub>1</sub> knockout mice, double mutant APP23/CB<sub>1</sub> mice showed even more impaired 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 co-workers 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, prior to plaque pathology and cognitive deficits). The CB<sub>2</sub> 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 <sup>18</sup>F-fluoro-deoxyglucose (<sup>18</sup>FDG) uptake using PET), which is reduced in AD patients and correlated with cognitive deficits, was reduced in AD transgenic mice. Finally, JWH-133 intervention fully reversed this phenotype. Looking at neuroinflammation in this model system, JWH-133 normalised the density of ionised 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 COX-2 protein levels and TNF- $\alpha$  mRNA expression (both increased in AD patients and AD transgenic mouse models). Furthermore, the synthetic cannabinoids were able to reduce the enhanced levels of A $\beta$ <sub>40</sub> and of the more amyloidogenic A $\beta$ <sub>42</sub> in APP mice, probably by enhancement of A $\beta$  clearance through blood-brain or CSF-barrier (Martin-Moreno et al., 2012). Finally, WIN 55,212-2 (but not JWH-133) prevented reductions to p-GSK3- $\beta$  in AD transgenic mice.

A recent study confirmed most of these findings when treating APPxPS1 mice with JWH-133 at the pre-symptomatic stage (Aso et al., 2013). JWH-133: i) improved cognitive performance in the novel object recognition test and the active avoidance task; ii) decreased microglial reactivity and reduced expression of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, TNF $\alpha$ , and IFN $\gamma$ ; iii) reduced the expression of

active p38 and stress-activated protein kinase/c-Jun NH(2)-terminal kinase (SAPK/JNK); iv) increased the expression of inactive GSK3 $\beta$  and lowered tau hyperphosphorylation; v) enhanced the expression of superoxide dismutase 1 (SOD1) and SOD2 around plaques; but, vi) did not alter A $\beta$  production.

Inconsistencies between these two studies (e.g. effects of JWH-133 on A $\beta$  pathology and tau hyperphosphorylation-relevant pathways, i.e. GSK3- $\beta$ ) were attributed to methodological differences in regards to mouse model and treatment design chosen as well as 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:* Two studies so far 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 section 5.1). In a first experiment, Sativex® [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] was administered i.p., daily for a month to parkin-null, human tau overexpressing (PK<sup>-/-</sup>/Tau<sup>VLW</sup>) mice, which presents a model of complex frontotemporal dementia, parkinsonism, and lower motor neuron disease. Sativex® treatment resulted in less abnormal stress-related behaviours (e.g. overgrooming and stereotypies; 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 A $\beta$  plaques in cortex and hippocampus and increased autophagy. The mechanism behind the effects of Sativex® has not been evaluated yet.

In a second study, botanical extracts high in THC (CBD content less than 0.5%) or high in CBD (THC content less than 2.5%) as well as a combination thereof (CBD plus THC) were used to treat APPxPS1 transgenic mice at the early symptomatic phase (Aso et al., 2015). All three approaches preserved object recognition memory of AD transgenic mice but THC had detrimental effects on cognition in control mice. CBD+THC also reduced fear-associated learning impairments of APPxPS1 mice, decreased soluble A $\beta$ <sub>42</sub> (but not A $\beta$ <sub>40</sub>) levels in the cortex and changed plaque composition (but 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 APPxPS1 mice [mRNA expression levels; for details see (Aso et al., 2015)]. Interestingly, the redox protein thioredoxin 2 and the signaling 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 signaling 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 signaling pathway prevents A $\beta$ -induced neurotoxicity *in vitro*.

*Pure CBD treatment:* Our team has expanded on the aforementioned studies evaluating the potential role of CB<sub>2</sub> and CBD+THC combinations in AD therapy by focusing on CBD effects in AD transgenic mouse models. We have run 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<sub>swe</sub>/PS1 $\Delta$ E9 (APPxPS1) chronically with CBD (daily i.p. injections) after the development of cognitive deficits and A $\beta$  pathology. CBD rescued social recognition memory and reversed object recognition deficits of male APPxPS1 transgenic mice. These effects were specific for recognition memory as CBD had no

impact on fear-associated memory or anxiety 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 a 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). So CBD may improve recognition memory via 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 deficit in male APPxPS1 mice (Cheng et al., 2014b). The beneficial effect of CBD on social recognition memory was not associated with a direct effect on A $\beta$  levels. Insoluble and soluble levels of A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> were no different between vehicle and CBD-treated APPxPS1 mice in cortex and hippocampus. Levels of oxidation were not significantly altered in APPxPS1 mice in comparison to their age-matched WT 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 APPxPS1 and control mice are not evident anymore (normally observed at 3- 5 months of age). Although not significant, the data obtained based on the administration of one CBD dose only (i.e. 20 mg/kg) suggested that CBD might have a beneficial effect on cytokine levels, in particular TNF- $\alpha$ , which would be in line with earlier findings discussed above [e.g. (Martin-Moreno et al., 2011)].

The study also revealed 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 A $\beta$  and tau pathology) and dietary phytosterols can either interfere with critical functional processes in AD or decrease amyloidogenic processing.

#### **4. The endocannabinoid system and cannabinoid therapy in Alzheimer's disease patients**

To date there have been no clinical trials evaluating the therapeutic potential of CBD for AD. This is probably due to the limited number of preclinical research studies investigating the effects of CBD in AD thus far. However, two clinical trials testing CBD have been carried out, which have some relevance for AD. In 2009, an interventional study explored the value of CBD in treating cognitive dysfunction in schizophrenia (ClinicalTrials.gov identifier NCT00588731). The study was based on a six-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 (ClinicalTrials.gov identifier NCT01502046) started in 2011 and was a double blind, randomised, cross over, placebo-controlled phase 2 clinical trial to assess the neuroprotective properties of CBD, THC, and Sativex in Huntington's disease patients. Unfortunately, it was not possible to obtain any information from the [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) website or from the lead investigators about the effects of CBD effects on cognition or neuroprotection in humans. Nevertheless, a few studies evaluated the role of the endocannabinoid system in AD patients and the effectiveness of cannabinoid treatment other than CBD in AD therapy and those findings will be outlined in the following.

##### ***4.1 The endocannabinoid system in Alzheimer's disease***

Westlake and colleagues 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, Alzheimer's disease patients, and patients who died with other forms of cortical pathology (Westlake et al., 1994). In AD brains, compared to normal brains, [ $^3$ H]CP 55,940 binding was reduced in various

regions of the hippocampus and the caudate nucleus. Less significant reductions were detected in the substantia nigra and the globus pallidus. Other neocortical and basal ganglia structures were not different from control levels. Levels of mRNA expression did not differ between AD and control brains, but there were regionally discrete losses of the intensely expressing cells in the hippocampus. It is important to note that the reductions in binding did not correlate with or localise to areas showing histopathology (i.e. overall tissue quality or stainings for neuritic plaques and NFTs). Furthermore, reduced [<sup>3</sup>H]CP 55,940 binding was associated with increasing age and with other forms of cortical pathology. Thus, reductions in CB<sub>1</sub>/CB<sub>2</sub> receptor expression appeared related to generalised aging and/or disease process and were not selectively associated with AD.

These findings could not be replicated by follow up investigations. For example, Ramirez and coworkers detected CB<sub>1</sub> and CB<sub>2</sub> receptor expression together with markers of microglia activation in senile plaques in AD patients and that CB<sub>1</sub>-positive neurons were greatly reduced in areas of microglia activation (no change to CB<sub>2</sub> expression) (Ramirez et al., 2005). In line with this, immunoblotting for CB<sub>1</sub> receptors in postmortem cortical brain tissues (Brodmann area 10) from a cohort of neuropathologically confirmed AD patients and age-matched controls revealed reduced CB<sub>1</sub> expression in AD brains, which was consistent with the loss of pyramidal cortical neurons in which these receptors are substantially expressed. A correlation between reduced CB<sub>1</sub> expression and hypophagia was found, supporting the idea of a potential use of receptor agonists or cannabis sativa-derived cannabinoids in the management of AD-associated eating disorders (Solas et al., 2013).

Another study analysed the expression of not only CB<sub>1</sub> and CB<sub>2</sub> receptors and also of FAAH in hippocampus and entorhinal cortex sections from postmortem brains of AD patients using immunohistochemistry (Benito et al., 2003). FAAH expression was increased in neuritic plaque-associated astrocytes whereas CB<sub>2</sub> receptors were abundantly and selectively overexpressed in activated microglia. Supporting this finding is another study reporting elevated levels of CB<sub>2</sub> receptors in postmortem cortical brain tissues of AD patients. The elevated expression did not correlate with

cognitive status but two relevant AD markers, i.e. A $\beta$ <sub>42</sub> levels and and senile plaque manifestation (Solas et al., 2013). It can be postulated that CB<sub>2</sub> receptors might be modulators of the inflammatory response associated with neurodegenerative processes and therefore presents a possible target for new therapeutic approaches. Importantly, the expression of CB<sub>1</sub> receptors was not affected by AD. In line with Benito et al (2003) is a more recent study applying semi-quantitative (immunoblotting) and quantitative (radioligand binding) assessments to confirm that CB<sub>1</sub> receptor levels were unchanged in AD in several brain regions (i.e. frontal cortex, anterior cingulate gyrus, hippocampus, and caudate nucleus) (Lee et al., 2010). Finally, comparative protein profiling and quantitative morphometry showed that overall CB<sub>1</sub> protein levels in the hippocampi of AD patients remained unchanged relative to age-matched controls, and that CB<sub>1</sub>-positive presynapses engulfed A $\beta$ -containing senile plaques (Mulder et al., 2011). Lee and coworkers commented on the limitations of their study design regarding neuroanatomical resolution (i.e. no subregion analyses were carried out), which did not allow the detection of subtle CB<sub>1</sub> expression changes in specific cytoarchitectural or neuroanatomical domains. Furthermore, the functional status of CB<sub>1</sub> receptors was not considered, which might be important, as Ramirez and coworkers found elevated nitration of both CB<sub>1</sub> and CB<sub>2</sub> protein in AD brains (Ramirez et al., 2005). It is interesting that a correlation was found between frontal cortical CB<sub>1</sub> immunoreactivity and cognitive scores (i.e. MMSE and CAMCOG) assessed within a year before death in the AD patient group suggesting that CB<sub>1</sub> 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<sub>1</sub> expression and the cognitive status (Mini Mental State Examination score) of AD patients (Solas et al., 2013) (for overview on changes to the endocannabinoid system in AD brain see Table 3).

The endocannabinoid system seems to be further involved in human AD pathology. In a case-control study, the circulating levels of plasma endocannabinoids (eCBs) were analysed and the relationship between eCBs and TNF- $\alpha$  was explored in elderly control subjects and AD patients. In comparison

with controls, there were no significant differences in measured AEA or 2-AG concentrations in plasma samples from patients with AD. Furthermore, endocannabinoid levels in the CSF were not correlated to 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- $\alpha$ , although the levels of TNF- $\alpha$  were very low (Koppel et al., 2009). Further longitudinal studies will be required to conclusively assess the impact of progressive AD pathology on circulating endocannabinoid levels.

Another study found increased hippocampal protein concentrations for the 2-AG synthesizing enzyme DAGL- $\alpha/\beta$  in brain tissue of patients with definite Alzheimer's (Braak stage VI). In particular, DAGL- $\beta$  expression was found in microglia accumulating near senile plaques and apposing CB<sub>1</sub>-positive presynapses. Furthermore, microglia, expressing 2-AG-degrading enzymes (i.e. ABHD6 and MGL) 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 MGL expression (although at levels significantly lower than in neurons lacking NFT pathology). Finally, it was revealed that MGL recruitment to biological membranes was impaired in AD brains suggesting that disease progression slows the termination of 2-AG signalling.

To conclude, the 'endocannabinoid phenotype' of AD brains appears to be complex and findings appear at times contradictory and highly dependent on the methodologies applied (e.g. type of polyclonal antibody, issue of cellular resolution in autoradiography studies, selection of mixed CB<sub>1</sub>/CB<sub>2</sub> *versus* selective receptor agonists). However, summarising the diverse findings conservatively suggests that alterations in the localisation, expression, and function of cannabinoid receptors occur in AD and may play a role in its pathophysiology, thereby providing a target for therapeutic interventions.

#### ***4.2 Effects of cannabinoids other than CBD on Alzheimer's disease patients***

Volicer's team was the first to evaluate the therapeutic effectiveness of cannabinoids in AD (Volicer et al., 1997). Using a placebo-controlled crossover design, with each treatment period lasting six weeks, 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 increased body weight and showed 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).

A second study measured the effect of dronabinol on nocturnal motor activity, as nighttime agitation occurs frequently in patients with dementia. Six late-stage AD patients were treated daily for two 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 nighttime 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 nighttime 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 delivered disappointing results for this patient (Passmore, 2008). No blinded or placebo studies have been conducted to date.

## **5. The future of CBD 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 have to be expected. CBD has been

described as being non-toxic and non-cataleptic, 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 1,500 mg/day (orally) or 30 mg i.v. 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 fertilisation 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 missing 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 has an inhibiting effect on CYP isozymes, primarily CYP2C and CYP3A classes of isozymes. Thus, CBD could potentially impact on antiepileptic medication, as e.g. valproate and clobazam are metabolised via 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 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 regard 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% due to significant first-pass metabolism in the liver (Mechoulam et al., 2002, Devinsky et al., 2014). Smoking and intravenous administration of cannabinoids both 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 via vaporisation, which is regarded as an effective mode of delivery since 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 specialised equipment and patient cooperation with the administration method (Devinsky et al., 2014). The combined CBD+THC product Sativex® is administered as a oromucosal spray and appears to be safe and effective (Wade et al., 2010). The bioavailability achieved by oralmucosal delivery appears to be similar to the oral route but less variable. Finally, transdermal approaches have also been investigated, but due to 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).

### ***5.1 CBD+THC combination treatment strategy***

It would be beyond the focus of this review to discuss 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 via CBD co-treatment). However, the nature of the interactive relationship between THC and CBD appears 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 change blood pressure, which may have serious consequences in people with heart disease (Jones, 2002). Furthermore, THC impacts on the risk to develop psychosis, although this effect is predominantly seen after long-term adolescent THC/cannabis abuse and mostly in people with a genetic predisposition for psychosis (Arnold et al., 2012). Long-term use of THC can also cause the development of cannabis 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 users (Bolla et al., 2002). In this context it is important to realise 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)].

## **6. 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 1 in 85 people 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 >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 characterisation of CBD *in vivo*, using established rodent models, is required to understand the full consequences of long-term CBD treatment and to analyse potential side effects of CBD in an aging organism. Once those data are available, the translation of this preclinical work could be realized very quickly as CBD is readily available and appears 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 concerning medical applications of cannabis 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 been considered as a treatment option for e.g. Parkinson's disease and schizophrenia as well.

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## Tables

Component	Description	References
<i>Endocannabinoids</i>		
2-arachidonoylglycerol (2-AG)	Full agonist at CB <sub>1</sub> with neuromodulatory effects	(Stella et al., 1997)
<i>N</i> -arachidonylethanolamine (AEA or anandamide)	Agonistic properties at both CB <sub>1</sub> and CB <sub>2</sub> with effects on appetite, learning and memory, and the generation of anandamide motivation and pleasure	(Devane et al., 1992) (original paper identifying anandamide)  (Paradisi et al., 2006) (distinct effects on CB <sub>1</sub> and CB <sub>2</sub> receptors; affects appetite)  (Mallet and Beninger, 1996) (affects working memory in rats)  (Mahler et al., 2007) (affects motivation and

***Exocannabinoids***

Phytocannabinoids >100 constituents of *Cannabis* (Fellermeier et al., *sativa* including the main 2001) psychoactive component  $\Delta^9$ -tetrahydrocannabinol (THC) and the non-psychoactive constituent cannabidiol (CBD); multitude of effects have been described including on pain sensitivity, mood, appetite, and cognition

Cannabinimimetics Synthetic cannabinoids that (Lambert and mimic actions of Fowler, 2005) phytocannabinoids such as CP 55,940 or WIN 55,212-2 (for effect range see *Phytocannabinoids*)

***Homologues of endocannabinoids***

2-linoleoyl glycerol (2-LG) Natural ligand for CB<sub>1</sub>; (Ben-Shabat et al., 1998) potentiates activity of other endocannabinoids including 2-AG

Palmitoylethanolamide PPAR- $\gamma$  is the main target of (Lo Verme et al.,

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(PEA)	PEA, but it also has affinity to cannabinoid-like G-coupled receptors GPR55 and GPR119, but no affinity for CB <sub>1</sub> /CB <sub>2</sub> ; it can enhance effects of AEA probably through TRPV1	2005) (PPAR-alpha is main target of PEA) (Godlewski et al., 2009) (affinity for GPR55 and GPR119)
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***Synthesising / Metabolic enzymes***

N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD)	Synthesis of AEA and PEA	(Okamoto et al., 2004)
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Diacylglycerol lipase (DAGL) $\alpha$ and $\beta$	Synthesis of 2-AG	(Bisogno et al., 2003)
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Monoacylglycerol lipase (MGL)	Degradation of 2-AG	(Dinh et al., 2002, Makara et al., 2005)
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Serine hydrolase alpha/beta-hydrolase domain-containing	Degradation of 2-AG	(Marrs et al., 2010)
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6 (ABHD6)

Fatty acid amide hydrolase (FAAH)	Degradation of AEA and 2-AG	(Cravatt et al., 2001)
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***Main receptors for cannabinoids***

Cannabinoid receptor 1	Involved in the majority of	(D'Souza, 2007,
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(CB <sub>1</sub> )	CNS effects of cannabinoids including psychosis and in immune functions	Cabral et al., 2008)
Cannabinoid receptor 2 (CB <sub>2</sub> )	Involved in immune function and neuroinflammatory responses in CNS	(Walter et al., 2003, Ramirez et al., 2005, Pacher and Mechoulam, 2011)
<i>N</i> -arachidonyl glycine receptor or G-protein coupled receptor 18 (GPR18)	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)

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**Table 1:** Components of the endocannabinoid system (ECS)

<b>Pharmacological target</b>	<b>Effect</b>	<b>References</b>
<i>In vitro studies</i>		
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 A $\beta$	(Iuvone et al., 2004)
Inhibition of phosphorylated p38 MAP kinase; activation of nuclear factor-kB	Inhibits nitrite production and iNOS protein expression in PC12 cells exposed to A $\beta$	(Esposito et al., 2006b)
Rescue of Wnt/ $\beta$ -catenin pathway	Rescues A $\beta$ -induced toxicity and inhibits tau protein hyperphosphorylation in PC12 cells exposed to A $\beta$	(Esposito et al., 2006a)
NMDA, AMPA and kainate receptors	Reduction of glutamate-induced toxicity in primary cortical neurons	(Hampson et al., 1998)
Activation of PPAR- $\gamma$	Induced ubiquitination of APP and decreased A $\beta$ production in APP-expressing human neuroblastoma cells	(Scuderi et al., 2014)
<i>In vivo studies</i>		
Glial pathways	Reduction in interleukin-1 $\beta$ , iNOS expression and subsequent NO release in A $\beta$ -injected mice	(Esposito et al., 2007)

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Microglia	Induced microglial migration, (Martin-Moreno suppression of interleukin-6 and et al., 2011) prevented spatial memory deficits in A $\beta$ -injected mice
Indirect activation of CB <sub>1</sub> receptor	Increased adult neurogenesis in (Wolf et al., CB <sub>1</sub> receptor deficient mice with no 2010) effect on cognition in WT mice
Activation of PPAR- $\gamma$	Induced hippocampal neurogenesis (Esposito et al., and reduced reactive gliosis in A $\beta$ - 2011) injected rats
Inverse CB <sub>2</sub> receptor agonism	Antagonises CB <sub>2</sub> receptor agonists (Thomas et al., in WT mice 2007)

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**Table 2:** Pharmacological targets of CBD

<b>Component of ECS</b>	<b>AD-relevant effects</b>	<b>References</b>
CB <sub>1</sub> receptors	No differences were reported for receptor expression, distribution or availability in cortex or hippocampus of AD patients	(Benito et al., 2003, Lee et al., 2010, Mulder et al., 2011, Ahmad et al., 2014)
	CB <sub>1</sub> receptor expression in AD was comparable to normal ageing	(Westlake et al., 1994)
	CB <sub>1</sub> receptors reported to be reduced in cortical areas and neurons away from senile plaques	(Ramirez et al., 2005, Solas et al., 2013)
	CB <sub>1</sub> 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 <sub>2</sub> receptors	CB <sub>2</sub> receptor expression reported to correlate with A $\beta$ <sub>42</sub> levels and plaque deposition but not cognitive changes	(Solas et al., 2013)
	CB <sub>2</sub> receptor expression reported to be abundantly and selectively overexpressed in microglia	(Benito et al., 2003)

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	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 homogenates	(Mulder et al., 2011, Pascual et al., 2014)
	Decreased FAAH activity in frontal cortex of AD patients, which is mimicked by the addition of A $\beta$ <sub>40</sub> peptide to control brain samples	(Pascual et al., 2014)
AEA	No differences reported for AEA plasma concentration in AD patients compared to control subjects	(Koppel et al., 2009)
	Lower AEA concentration in AD brain (midfrontal and temporal cortices) compared to control and inversely correlated with A $\beta$ <sub>42</sub> peptide and severity of cognitive deficits	(Jung et al., 2012)
	Increased degradation of AEA in AD frontal cortex compared to controls	(Pascual et al., 2014)

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2-AG	No differences reported for 2-AG plasma concentration in AD patients compared to control subjects	(Koppel et al., 2009)
	Altered 2-AG signalling during late stages of AD due to combination of impaired MAGL recruitment and increased DAGL concentration	(Mulder et al., 2011)

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**Table 3:** The endocannabinoid system and Alzheimer’s disease