In an increasingly ageing population, the incidence of neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease and Huntington’s disease are rising. While the aetiologies of these disorders are different, a number of common mechanisms that underlie their neurodegenerative components have been elucidated; namely neuroinflammation, excitotoxicity, mitochondrial dysfunction and reduced trophic support. Current therapies focus on treatment of the symptoms and attempt to delay the progression of these diseases but there is currently no cure. Modulation of the endogenous cannabinoid system is emerging as a potentially viable option in the treatment of neurodegeneration. Endocannabinoid signalling has been found to be altered in many neurodegenerative disorders. To this end, pharmacological manipulation of the endogenous cannabinoid system, as well as application of phytocannabinoids and synthetic cannabinoids have been investigated. Signalling from the CB1 and CB2 receptors are known to be involved in the regulation of Ca\(^{2+}\) homeostasis, mitochondrial function, trophic support and inflammatory status, respectively, while other receptors gated by cannabinoids such as PPAR\(\gamma\), are gaining interest in their anti-inflammatory properties. Through multiple lines of evidence, this evolutionarily conserved neurosignalling system has shown neuroprotective capabilities and is therefore a potential target for neurodegenerative disorders. This review details the mechanisms of neurodegeneration and highlights the beneficial effects of cannabinoid treatment.
economic burden (Brookmeyer et al., 2007; Zuccato et al., 2010; Taylor et al., 2013). Age remains the highest risk factor for these diseases and with a degree of neurodegeneration also occurring during normal ageing the threat to the quality of life and health of the global population is ever present (Marchant et al., 2009). Although neurodegenerative diseases are a heterogeneous group of disorders, current research has identified a number of common underlying mechanisms namely protein misfolding, neuroinflammation, excitotoxicity and oxidative stress. These triggers are known to contribute to the progression of symptoms, functional alteration and microanatomical deficits found in neurodegenerative states.

Inflammation within the CNS is centred around the activation of the resident immune cells, the microglia (Akiyama et al., 2000; Taylor et al., 2013). Maintained in a quiescent state and associated with the production of neurotrophic and anti-inflammatory factors, microglia become activated by the recognition of highly conserved structural motifs on either pathogens (pathogen associated molecular patterns; PAMPs) or from damaged or stressed cells (damage associated molecular patterns; DAMPs) (Arroyo et al., 2011). The binding of PAMPs or DAMPs to pattern-recognition receptors, such as the Toll-like receptors (TLR) or receptors for advanced glycation end-products (RAGE), cause the migration of microglia followed by the synthesis and release of proinflammatory cytokines and reactive oxygen species (ROS) (Van et al., 1996; Arroyo et al., 2011). Oxidative stress is a cytotoxic condition brought on by the increased intracellular production or accumulation of ROS and reactive nitrogen species (RNS) (Taylor et al., 2013). ROS are normal products of the mitochondrial respiratory chain but activated microglia generate excessive amounts as a result of intracellular peroxidases, oxidative processes and NADPH oxidase activity (Block and Hong, 2007). Regulation of ROS and RNS is vital to cell survival as their increased production leads to the damage of proteins, lipids, carbohydrates and nucleic acids resulting in significant disruption of cellular function (Mehta et al., 2013). Furthermore, oxidative stress can lead to the activation of the mitochondrial permeability transition pore causing the collapse of the trans-membrane electrochemical gradient and the release of proapoptotic factors like cytochrome c, procaspases and caspase activated DNase (Emerit et al., 2004). Excitotoxicity is the pathological process of damaging and killing neuronal cells as a result of excessive stimulation of ionotropic receptors by glutamate and similar substances (Mehta et al., 2013). This process leads to impairment of intracellular Ca\(^{2+}\) buffering, generation of ROS and RNS, activation of the mitochondrial permeability transition pore and secondary excitotoxicity (Dong et al., 2009). In an attempt to reduce the intracellular Ca\(^{2+}\) load, neurons expend considerable energy using ion pumps on the endoplasmic reticulum, plasma membrane and mitochondria, reducing ATP levels and causing excitotoxic lesions (Beal, 2000). Activation of the proapoptotic cascade is associated with a number of insults such as generation of ROS/RNS, mitochondrial dysfunction, excitotoxicity and trophic factor withdrawal. This process depends upon initiator and effector caspases which cause DNA cleavage, proteolytic cascades and mitochondrial permeability resulting in the release of proapoptotic factors such as cytochrome c and DIABLO (Bredesen et al., 2006). A dynamic interplay between these neurodegenerative pro-

cesses has been reported in AD, PD and HD and is the focus of many prospective therapeutic agents (Bredesen et al., 2006; Lin and Beal, 2006). Decreased neurogenesis and neurotrophic support has also emerged as a common characteristic in neurodegenerative states often presenting early in disease progression (Simuni and Sethi, 2008). Genes which have been identified as problematic in neurodegenerative disorders such as those for α-synuclein, presenilin 1, tau and huntingtin are also involved in brain plasticity and their aberrant aggregation is detrimental to adult neurogenesis (Winner et al., 2011).

### The endogenous cannabinoid (eCB) system

The eCB system is composed of the endocannabinoid signalling molecules, 2-arachidonoyl glycerol (2AG) and anandamide (AEA) and their G-protein coupled cannabinoid CB\(_1\) and CB\(_2\) receptors (Piomelli, 2003: receptor nomenclature follows Alexander et al., 2013). Endocannabinoid signalling molecules are synthesized in the post-synaptic terminal as a result of depolarization and work in a retrograde fashion on presynaptic CB receptors. The primary pathway through which AEA is synthesized involves the Ca\(^{2+}\)-dependent cleavage of its membrane precursor N-arachidonoyl phosphatidylethanolamine by phospholipase D (Di Marzo et al., 1994). In most cases, 2AG is synthesized by the hydrolysis of two sn-1 diacylglycerol lipase isoforms, diacylglycerol lipase-α (DGLα) and diacylglycerol lipase-β (DGLβ) (Bisogno et al., 2003). The CB\(_1\) receptor is highly expressed in the CNS at the terminals of central and peripheral neurons where they regulate neurotransmitter release and psychoactivity (Sanchez and Garcia-Merino, 2012). CB\(_2\) receptor expression is associated with the peripheral immune system, neurons within the brainstem and microglia during neuroinflammation (Van Sickle et al., 2005; Nunez et al., 2008). CB\(_1\) and CB\(_2\) receptors have also been associated with postnatal oligodendrogenesis. CB\(_1\) activation increases the number of glial precursors in the subventricular zone of postnatal rats while CB\(_2\) activation increases polysialylated neural cell adhesion molecule expression which is necessary for the migration of oligodendrocyte precursors (Arevalo-Martin et al., 2007). CB receptors act via the G\(_\alpha\) or G\(_\beta\) protein to stimulate the MAPK pathway and inhibit adenylylate cyclase, attenuating the conversion of ATP to cyclic AMP (Howlett et al., 2002). CB receptor activation is also tightly linked to ion channel regulation through inhibition of voltage-dependent Ca\(^{2+}\) channels and activation of K\(^+\) channels (Mackie et al., 1993; Deadwyler et al., 1995; Hampson et al., 2000). The TRPV1 receptor is also activated by the endocannabinoid AEA and has been linked to its anti-inflammatory actions (Zygmunt et al., 1999). Degradation of endocannabinoids is carried out by two enzymes: fatty acid amide hydrolase (FAAH) and monooacylglycerol lipase (MGL) which act upon AEA and 2AG respectively (Cravatt et al., 1996; Ben-Shabat et al., 1998). A number of exogenous ligands to CB receptors are also known such as the phytocannabinoids derived from the *Cannabis sativa* plant as well as synthetic CB\(_1\)/CB\(_2\) agonists and antagonists. Manipulation of the eCB system has also been carried out by the inhibition of
endocannabinoid biosynthesis, membrane transport and degradation (Bisogno et al., 2005). The eCB system has been identified as a possible therapeutic target against neurodegeneration as a number of alterations in the eCB system have been noted in AD, PD and HD, as discussed below (Figure 1).

**Alzheimer’s disease**

AD is a progressive age-related neurodegenerative disorder that affects over 26 million people worldwide (Brookmeyer et al., 2007). It is estimated that 10% of people over 65 and 25% of people over 80 years of age are afflicted by this debilitating disease, and that number is set to rise to 1 in every 85 people by 2050 (Hebert et al., 2003; Brookmeyer et al., 2007). AD is defined by the progressive deterioration of cognition and memory and is the most common form of dementia among the elderly (Minati et al., 2009). The characteristic hallmarks of AD include the formation of neuritic plaques, containing aggregated forms of the amyloid-β (Aβ) peptide and dystrophic neurites, and neurofibrillary tangles caused by the hyperphosphorylation of the microtubule associated protein, tau, resulting in severe neurodegeneration.

Over the past two decades, neuroinflammation has emerged as an integral process in the pathogenesis of AD. Post-mortem analysis of the brains of AD patients has revealed an increase in the amount of activated microglia and astrocytes as well as a significantly higher levels of proinflammatory cytokines, IL-1, IL-6 and TNF-α and ROS (Akiyama et al., 2000; Rojo et al., 2008). Furthermore, clinical studies have identified a positive correlation between TNF-α levels and cognitive decline (Holmes et al., 2009) and numerous trials have shown that anti-inflammatory drugs delay the onset or slow the progression of AD (Arroyo et al., 2011). Fibrillated Aβ can be recognized by immune cells and phagocytosed. However, once the peptides oligomerize, aggregate and form neuritic plaques this is not possible, leading to the chronic activation of the immune system (Salminen et al., 2009). Activation of TLR, nucleotide-binding oligomerization domain-like receptors and RAGE by Aβ can stimulate phagocytosis but also results in reduced antioxidant defence and the release of proinflammatory cytokines and proapoptotic mediators (Salminen et al., 2009; Heneka et al., 2010). The pathophysiological relevance of neuroinflammation to neurodegeneration in AD has been well established through multiple lines of evidence. Direct evidence of neurotoxicity has
been shown as a result of the release of IL-1, IL-6 and TNF-α (Allan and Rothwell, 2001). Colocalization of the inflammatory response to areas most affected by AD pathology and the absence of such a response in areas less affected implies a strong relationship between the two (Akiyama et al., 2000).

The dysregulation of intracellular Ca\(^{2+}\) concentration and excessive activation of NMDA receptors are characteristic of AD (Sonkusare et al., 2005). Accumulation of glutamate as a result of Ββ-mediated reduction in astrocytic uptake, as well as direct activation of NMDA receptors, leads to excessive NMDA activity and excitotoxicity (Sonkusare et al., 2005; Texido et al., 2011). Ββ has been shown to increase voltage-dependant Ca\(^{2+}\) channel activity (MacManus, 2000) and to form Ca\(^{2+}\) permeable pores in membrane bilayers (Alarcon et al., 2006). Ββ-induced excitotoxicity has long been associated with the neurodegenerative process as rises in intracellular Ca\(^{2+}\) concentration have been shown to activate a number of apoptotic pathways including the activation of caspase-3, calpain and lysosomal cathepsins (Hajnoczky et al., 2003; Harvey et al., 2012). Activated microglia, which can be seen in excess around neuritic plaques, are a major source of ROS production and oxidative stress in the CNS. ROS can further perpetuate the inflammatory response by activating proinflammatory pathways (Taylor et al., 2013).

Several components of the eCB system are altered in AD. In the post-mortem brains of patients with AD, CB\(_2\) receptor expression was significantly increased in areas containing microglia associated with the neuritic plaques, such as the entorhinal cortex and parahippocampus (Benito et al., 2003; Solas et al., 2013). This increase in CB\(_2\) expression is thought to be an attempt to counteract the chronic inflammation found in AD as CB\(_2\) receptor activation reduces microglial activation and cytokine production (Ramirez et al., 2005; Koppel and Davies, 2010). CB\(_1\) receptor expression in the AD brain remains a contentious issue with reports of both intact and increased expression levels (Lee et al., 2010; Solas et al., 2013). However, Farkas et al. (2012) have recently reported an initial rise, followed by a steady decline in CB\(_1\) receptor expression in the prefrontal cortex of AD patients. When patients were grouped depending on the progression of AD, at the earliest stages of disease progression (Braak stages I-II) CB\(_1\) receptor density was at its highest when compared to age-matched controls and those CB\(_1\) receptor levels were found to decline with the progression of AD while remaining above age-matched control levels (Farkas et al., 2012).

Furthermore, pharmacological investigation has shown that the CB\(_1\) receptor becomes functionally impaired by nitrosylation in the AD brain, affecting the G protein coupling and downstream signaling (Ramirez et al., 2005). Lipidomic analysis of post-mortem brain tissue from AD patients has revealed significantly reduced levels of AEA and its precursors in the midfrontal and temporal cortex when compared to age-matched controls (Jung et al., 2012). Interestingly, increased degradation of AEA may also occur as a consequence of the up-regulation of the metabolizing enzyme, FAAH, on plaque-associated astrocytes that has been noted in the AD brain (Benito et al., 2003). Inhibition of MGL in an in vivo model of AD has recently been shown to suppress the production and accumulation of Ββ via reduced expression of θ-site amyloid precursor protein cleaving enzyme 1, a key enzyme in the synthesis of Ββ (Chen et al., 2012). 2AG signalling in AD patients (Braak stage VI) is functionally impaired with increased expression of DGLα and DGLβ as well as the hydrolyzing enzyme MGL although membrane-associated 2AG hydrolysis by MGL was decreased (Mulder et al., 2011).

**Parkinson’s disease**

PD is the second most common neurodegenerative disease affecting 1% of people over 60 and 4% of people over 80 years of age (de Lau and Breteler, 2006). PD is characterized by the progressive loss of dopaminergic neurons primarily in the substantia nigra (SN) affecting the circuits of the basal ganglia resulting in bradykinesia, rigidity and tremors (Bartels and Leenders, 2009). In a rat model of PD, symptomatology followed an approximate 50% reduction of dopaminergic neurons in the SN combined with an 80% loss of dopamine levels in the striatum (Deumens et al., 2002). In degenerating neurons, Lewy bodies form containing neurofilaments with aggregated α-synuclein (Wakabayashi et al., 2007). The disease has been associated with genetic mutations, inflammation, exogenous toxins and oxidative stress (Bartels and Leenders, 2009).

The link between PD and dopamine loss has been affirmed by PET studies showing a presynaptic dopamine deficit in PD patients and post mortem biochemical analysis revealing decreased levels of dopamine metabolites in the affected areas (Bartels and Leenders, 2009). Intracellular degradation of dopamine generates high levels of ROS, promotes H\(^{+}\) leakage from the mitochondria and reduces levels of glutathione, a key antioxidant enzyme (Hald and Lotharius, 2005). This intrinsic increase in ROS and concomitant decrease in antioxidant enzymes may be the reason for the high levels of oxidative stress found in PD patients. Furthermore, ROS have been shown to induce excitotoxicity through the activation of NMDA receptors and induction of proinflammatory cascades (Barnham et al., 2004). Indeed, PET scans and post-mortem analysis have reported an increased number of activated microglia in the PD brain (McGeer et al., 1988; Gerhard et al., 2006). In line with this, post mortem analysis has also revealed an increased amount of proinflammatory cytokines, namely IL1-β, IL-2, IL-4, IL-6 and TNF-α (Taylor et al., 2013).

The eCB system has been shown to modulate GABAergic and glutamatergic transmission in the basal ganglia (Kofalvi et al., 2005) which affects motor function (Fernández-Ruiz, 2009) and has therefore gained interest as a possible therapeutic target for motor disorders. A recent study has shown a decrease in the availability of CB\(_2\) receptors in the SN of PD patients when compared with healthy controls (Van Laere et al., 2012). However, a marked increase in CB\(_1\) receptors was found in the nigrostriatal, mesolimbic and mesocortical dopaminergic projection areas of the same patients. It is important to note that no difference in CB\(_1\) availability was found between patients that had developed levodopa-induced dyskinesias and those without such symptoms (Van Laere et al., 2012). AEA levels in the cerebrospinal fluid of untreated PD patients were found to be more than double that found in age-matched controls. Interestingly, AEA levels returned to control levels in patients receiving chronic dopa-
mine replacement therapy (Pisani et al., 2010). Furthermore, a sevenfold increase in 2AG levels was found in the globus pallidus of the reserpine-treated animal model of PD and this has been linked to suppression of locomotion (Di Marzo et al., 2000). A decrease in endocannabinoid degradation has also been noted in an animal model of PD with reduced levels of FAAH and AEA membrane transporter found in the striatum (Gubellini et al., 2002). This increase in endocannabinoid tone and CB1 receptor activity in the brain of PD patients has been proposed to be an attempt to normalize striatal function following dopamine depletion as enhanced CB1 receptor signalling reduces glutamate release and activates the pool of G-proteins usually activated by the dopamine D2 receptor (Meschler and Howlett, 2001; Brotchie, 2003).

Huntington’s disease

HD is a progressive neurodegenerative disease that affects 4–10 people per 100,000. The average age of onset is 40 years and it is fatal within 15–20 years (Ross and Tabrizi, 2011). The disease is inherited in an autosomal dominant fashion and is caused by an expanded cytosine, adenine, guanine repeat in the huntingtin gene. Expansion of this gene results in an elongated glutamine repeat at the NH2 terminus of the huntingtin protein (HTT) (Macdonald, 1993). The exact functions of HTT are not fully known although it is believed to play a role in vesicular transport and regulation of gene transcription (Cattaneo et al., 2005; Sadri-Vakili and Cha, 2006). Mutation of HTT can result in intracellular toxic protein aggregation through the formation of abnormal conformations, typically β-sheet structures, protein modifications and the disruption of cellular processes such as protein degradation and metabolic pathways (Ross and Tabrizi, 2011). The resulting clinical features of this are atrophy of the cerebral cortex, severe striatal neuronal loss and up to a 95% reduction of GABAergic medium spiny projection neurons (Halliday et al., 1998; Vonsattel, 2008). The pathological processes implicated in HD are the loss of trophic factors, specifically brain-derived neurotrophic factor (BDNF), excitotoxicity, oxidative stress and inflammation resulting in progressive neurodegeneration. Symptoms associated with HD include progressive motor dysfunction, cognitive decline and psychiatric disturbance (Ross and Tabrizi, 2011).

A number of studies have reported the dependency of medium spiny neurons on BDNF which is depleted by approximately 35% in animal models of HD (Baquet et al., 2004; Zuccato and Cattaneo, 2007). Reduced BDNF mRNA expression has also been reported in the post mortem analysis of brain tissue from HD patients (Zuccato et al., 2008). Decreased levels of BDNF have been closely linked to the HD phenotype since BDNF partial knock-out mice showed very similar phenotypes to HD models, namely progressive brain damage and hindlimb claspng as well as reduced striatal volumes (Baquet et al., 2004). Indeed, BDNF replacement is believed to be a possible therapeutic for HD and has been shown to decrease excitotoxicity and attenuate motor dysfunction and cell loss in animal models of HD (Kells et al., 2004; Kells et al., 2008). This may prove beneficial as mounting evidence implicates excitotoxicity in the pathophysiology of HD. Hassel et al. (2008) have reported a 43% decrease in glutamate uptake in HD patients and defective activity of the glutamate transporter, GLT1. The subsequent accumulation of extracellular glutamate could well be the cause of excessive NMDA activity and excitotoxicity. Mutant HTT has also been found to bind directly to mitochondria, disrupting metabolic activity and up-regulating the proapoptotic factors Bcl2-associated X protein and p53-up-regulated modulator of apoptosis (Bae et al., 2005). Neuroinflammatory processes are also gaining interest in the investigation of HD. PET imaging, in vitro studies and post-mortem analysis have reported an increase in microglial activation in HD which correlates with neurodegeneration and the severity of the condition (Ross and Tabrizi, 2011).

A clear parallel has been made between the graded progression of HD and decreasing CB1 receptor density, particularly in the caudate nucleus, putamen and the globus pallidus (Glass et al., 2000). Recently, it has been reported that CB1 receptor down-regulation is specific to certain striatal subpopulation such as medium spiny neurons and neuropeptide Y/neuronal nitric oxide synthase-expressing interneurons (Horne et al., 2013). Much work has been carried out in analysing the components of the eCB system in R6/2 transgenic mice, a common model of HD. A loss of CB1 receptor density was found presymptomatically (Denovan-Wright and Robertson, 2000) as a result of mutant HTT-associated impairment of CB1 receptor gene expression (Blazquez et al., 2011). Genetic ablation of CB1 receptors aggravated HD symptoms in mice while pharmacological activation by Δ9-tetrahydrocannabinol (THC) attenuated symptomatology indicating that impairment of CB1 receptor function may be a primary pathogenic feature of HD (Blazquez et al., 2011). CB1 receptor expression, however, was found to increase in the striatal microglia of these transgenic mice and HD patients and this may confer neuroprotection as genetic ablation of CB1 receptors in transgenic HD mice results in increased microglial activation, aggravation of disease symptomatology and decreased life span (Palazuelos et al., 2009). In the striatum, a reduction in AEA, 2AG and their respective biosynthetic enzymes N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D and diacylglycerol lipase activity was found (Bisogno et al., 2008; Bari et al., 2013). In the cortex, a reduction in 2AG levels was accompanied by an increase in AEA levels while their respective hydrolytic enzymes MGL, was decreased, and FAAH increased (Bisogno et al., 2008; Bari et al., 2013). These data clearly indicate the alteration of multiple components of the eCB system in the progression of HD.

Ageing

Ageing is a time-dependent and progressive deterioration of biological function that leads to death. The typical characteristics of ageing include a decrease in physiological capacity, reduced adaptive capabilities to changes in environment and an increased vulnerability to disease and death (Farooqui and Farooqui, 2009). Indeed, normal ageing presents many of the same pathophysiologic mechanisms found in neurodegenerative diseases and is believed to further aggravate disease progression. Many theories have been put forward to explain...
the degenerating nature of age such as Ca$^{2+}$ dyshomeostasis, oxidative stress and mitochondrial dysfunction but a consensus is yet to be reached.

The atrophy of the human brain with age is believed to be as a result of neurodegeneration and the loss of myelinated axons (Peters, 2002). Increased Ca$^{2+}$ influx has been reported in the CA1 hippocampal region of aged rats, mediated by increased voltage-operated Ca$^{2+}$ channels (Landfield and Pittler, 1984; Thibault and Landfield, 1996). Furthermore, intracellular Ca$^{2+}$ regulation is altered in the aged brain. Efflux of Ca$^{2+}$ through plasma membrane pumps as well as its uptake to mitochondrial sinks is affected by ageing (Michaelis et al., 1996; Toescu, 2005) resulting in impairments in intracellular Ca$^{2+}$ homeostasis. Oxidative stress is also prominent in the aged brain. Membrane lipid peroxidation coupled with oxidative damage of proteins and DNA is reported to increase with age (Sohal and Weindruch, 1996). Prolonged oxidative damage of mitochondrial DNA and lipids increases ROS generation resulting in further oxidative damage and vulnerability towards apoptosis (Paradies et al., 2011). Chronic activation of microglia and alterations in their morphologic and immunophenotypic nature have also been reported. Normal ageing is believed to prime microglia for an exaggerated response, preferentially releasing proinflammatory cytokines. Increased basal levels of IL-6 and enhanced LPS-induced levels of IL-6 and IL-1β have been reported in the aged brain (Nakanishi and Wu, 2009).

Conflicting reports have emerged on the state of the eCB system as a result of ageing. Decreased CB$_1$ receptor density has been reported in the cerebellum and cerebral cortex of aged rats, while reduced CB$_1$ mRNA levels were found in the hippocampus and brainstem (Berrendero et al., 1998). Conversely, Wang et al. (2003) have shown that there is no change in endocannabinoid tone or CB$_1$ receptor density in the hippocampus limbic forebrain, amygdala or cerebellum of aged mice. However, decreased coupling of CB$_1$ receptor to G-proteins was reported in the limbic forebrain.

**The eCB system as a therapeutic target**

The use of cannabinoids as a therapeutic remains a controversial issue. However, some success has been gained with the use of cannabinoid-based drugs to regulate appetite, sleep, pain and some psychotic tendencies. Dronabinol, derived from the phytocannabinoid THC, is beneficial in reducing anorexia, increasing body weight and improving behaviour in elderly AD patients (Volcier et al., 1997). Dronabinol has more recently been assessed in a pilot study with AD patients where it improved nocturnal motor activity and reduced agitation and aggression, without undesired side effects (Walther et al., 2006). In animal models of PD, THC attenuates motor inhibition and the loss of tyrosine hydroxylase-positive (dopamine producing) neurons. Furthermore, preclinical studies have investigated the anti-inflammatory and antioxidant capabilities of the phytocannabinoid cannabidiol (CBD), combined with THC, in the form of the cannabinoid-based medicine Sativex, which is already used as a therapeutic agent for multiple sclerosis. Sativex has been shown to successfully treat neuropathic pain and spasticity in multiple sclerosis patients (Nurmikko et al., 2007; Notcutt et al., 2012). Maresz et al. (2007) have demonstrated that CB$_1$ and CB$_2$ receptors are required for mediation of the immune system in animal models of multiple sclerosis. This combination is now emerging as a viable therapeutic option for PD and HD (Valdeolivas et al., 2012; Fernandez-Ruiz et al., 2013). The eCB system is believed to be a promising therapeutic target for delaying disease progression and ameliorating Parkinsonian symptoms (Garcia et al., 2011).

**Cannabinoids and neuroinflammation**

Chronic neuroinflammation has been identified as a key mediator of neurodegeneration in AD, PD and HD. Various models of inflammation have reported the beneficial effects of cannabinoid action on reducing the inflammatory burden (Figure 2). The CB$_2$ selective agonist, JWH015 a synthetic cannabinoid, has been shown to reduce interferon-γ-induced up-regulation of CD40 in cultured mouse microglial cell through interfering with the JAK/STAT pathway. Furthermore, this intervention suppressed the production of proinflammatory cytokines and promoted the phagocytosis of Aβ (Ehrhart et al., 2005). Mobilization of intracellular Ca$^{2+}$ in response to ATP is a key mediator of microglial activation and inducer of the inflammatory response. CBD, along with the synthetic cannabinoids WIN 55212-2, a mixed CB$_1$/CB$_2$ receptor agonist and JWH-133, a CB$_2$ receptor selective agonist, were all shown to decrease the ATP-induced rise in intracellular Ca$^{2+}$ concentration in the N13 microglial cell line (Martin-Moreno et al., 2011). The effects of WIN 55212-2 and JWH-133 were fully reversed by the selective CB$_2$ antagonist, SR144528 (100 nM) indicating a CB$_2$ receptor-mediated effect. This antagonism was not seen in CBD-treated cells suggesting that CB$_2$-independent mechanisms may also be beneficial. Furthermore, the Aβ-induced rise in the proinflammatory cytokine IL-6 was reduced almost sixfold by 20 mg kg$^{-1}$ CBD or 0.5 mg kg$^{-1}$ WIN 55212-2 in vivo (Martin-Moreno et al., 2011). Further in vivo studies using transgenic APP 2576 mice have reported that oral administration of JWH-133 (0.2 mg kg$^{-1}$ day$^{-1}$ for 4 months) decreased microglial activation, reduced COX-2 and TNF-α mRNA and reduced cortical levels of Aβ, with no impact on cognitive performance (Martin-Moreno et al., 2012). A number of studies have identified the PPARγ as a key mediator of the cannabinoid anti-inflammatory effect. The PPAR family are a group of nuclear hormone receptors known to be involved in gene expression, lipid and glucose metabolism and the inflammatory response. In cultured rat astrocytes, reactive gliosis was induced by treatment with 1 mg mL$^{-1}$ Aβ for 24 h and this was significantly reduced by CBD in a concentration-dependant manner. The beneficial effects of CBD were blunted by PPARγ antagonism by GW9662, suggesting the involvement of PPARγ in the anti-inflammatory effects of CBD (Esposito et al., 2011). Hippocampal fractions isolated from adult rats injected with Aβ (10 μg mL$^{-1}$) to the CA1 region and treated with CBD (10 mg kg$^{-1}$) intraperitoneally for 15 days replicated the results found in vitro. Fakhfouri et al. (2012) have further elucidated the relationship between cannabinoids and PPARγ in vivo and have
identified that Aβ, when administered intrahippocampally to adult rats, increased PPARγ transcriptional activity and protein expression is observed which was further increased as a result of i.c.v. administration of WIN 55212-2. The beneficial effects caused by WIN 55212-2 were partially halted by the antagonism of PPARγ by i.c.v. administration of GW9662.

A common model for inflammation in the brain is the infusion of lipopolysaccharide into the fourth ventricle of young rats. Marchalant et al. (2007) have shown that daily i.p. injections of WIN 55212-2 (0.5 mg kg\(^{-1}\)) successfully reduced microglial activation in this model. However, when the dosing regimen was raised to 1 mg kg\(^{-1}\) day\(^{-1}\), microglial activation was potentiated by WIN 55212-2. Normal aging has also been shown to cause neuroinflammation and in this context cannabinoids have also been shown to confer neuroprotection. In rats aged 23 months, WIN 55212-2 injections of 2 mg kg\(^{-1}\) i.p. for 4 weeks reduced the number of activated microglia in the hippocampus and dentate gyrus (Marchalant et al., 2009). Interestingly, when incubated with the CB₁ receptor antagonists SR141716A and SR144528, WIN 55212-2 had no effect. The same treatment was found to decrease the mRNA levels of the proinflammatory cytokine IL-6 as well as the anti-inflammatory cytokine IL1-RA. Protein levels of TNF-α and IL-1β were decreased while an increase in IL1-RA was seen (Marchalant et al., 2009). It is now clear that at multiple steps throughout the inflammatory process, cannabinoids can help to reduce the inflammatory burden during neurodegeneration.

Cannabinoids, excitotoxicity and mitochondrial dysfunction

The excitotoxic increase of intracellular Ca\(^{2+}\) concentration in neurodegenerative disorders can lead to the activation of apoptotic and proinflammatory pathways, as well as disrupting metabolic processes leading to cell death.
Endocannabinoids are most commonly synthesized in a Ca\textsuperscript{2+}-dependent fashion as a result of depolarization and are believed to help reduce excitotoxic damage. Indeed, AEA levels increase rapidly in the hippocampi of mice after administration of the excitotoxin kainic acid (KA) (30 mg kg\textsuperscript{-1}) and genetic ablation of the CB\textsubscript{1} receptor lowered the threshold for KA-induced seizures with more than 75% of CB\textsubscript{1}-null mice dying within 1 h of KA injection. The neuroprotective capabilities of CB\textsubscript{1} are suggested to act primarily on principal glutamatergic neurons. Furthermore, the intracellular events involved in this neuroprotection have been attributed to the CB\textsubscript{1}-mediated activation of ERKs and the subsequent expression of the immediate early genes c-fos and zif268 (Marsicano et al., 2003). Cannabinoid action, via CB\textsubscript{1} receptors in particular, regulates intracellular Ca\textsuperscript{2+} levels through a number of mechanisms (Figure 2). Exposure of murine cortical cultures to 20 \mu M NMDA for 24 h results in 70% cell death and WIN 55212-2 has been shown to decrease cell death though the inhibition of nitric oxide signalling and PKA (Kim et al., 2006a). This CB\textsubscript{1} receptor-mediated regulation of PKA has long been associated with neuroprotection against excitotoxicity (Kim et al., 2005). Another route for Ca\textsuperscript{2+} influx is through TNF-\alpha mediated surface delivery of Ca\textsuperscript{2+} permeable AMPA receptors which contribute to in vitro excitotoxicity. WIN 55212-2 inhibits this TNF-\alpha-induced increase in surface AMPA receptors and reduces excitotoxic damage in rat hippocampal cultures (Zhao et al., 2010). TNF-\alpha also increased PKA activity (Zhang et al., 2002) which in turn can phosphorylate AMPA receptors at Ser\textsuperscript{412} and traffic them to the plasma membrane (Oh et al., 2006). It is therefore believed that the inhibition of PKA by CB\textsubscript{1} receptor stimulation is beneficial in reducing excitotoxic damage by interfering with AMPA trafficking. Furthermore, the CB\textsubscript{1} receptor agonists, WIN 55212-2 and AEA, inhibited glutamate release from rat hippocampal synaptosomes which would reduce NMDA activation and the resulting Ca\textsuperscript{2+} influx (Wang, 2003). As well as reducing the influx of Ca\textsuperscript{2+}, cannabinoid action regulates intracellular Ca\textsuperscript{2+} homeostasis. WIN 55212-2 reduced the NMDA-mediated release of Ca\textsuperscript{2+} from intracellular stores in cultured rat hippocampal cells thereby increasing cell viability. This involved the CB\textsubscript{1}-mediated reduction in cAMP-dependent PKA phosphorylation of ryanodine receptors (Zhuang et al., 2005). Furthermore, in high-excitability conditions CBD (1 \mu M) increased the levels of Ca\textsuperscript{2+} uptake by mitochondria in cultured rat hippocampal neurons (Ryan et al., 2009). Intense elevation of intracellular Ca\textsuperscript{2+} is known to induce proapoptotic cascades. Activation of cytosolic calpains by Ca\textsuperscript{2+} results in permeabilization of the lysosome and the release of proapoptotic proteins such as the caspase and cathepsin family (Yamashima and Oikawa, 2009). Noonan et al. (2010) have shown in vitro that increasing endocannabinoid tone through inhibiting FAAH degradation of 2AG prevented the A\beta-induced increase in calpain activation, permeabilization of the lysosome and the resulting neurodegeneration.

Mitochondrial dysfunction has also been addressed by cannabinoid research (Figure 2). Oxygen-glucose deprivation/reoxygenation of neuronal-glial cultures causes mitochondrial depolarization and oxidative stress. In rat neuronal-glial cultures, the cannabinoid trans-caryophyllene (1 \mu M) has been shown to increase neuronal viability through a reduction of mitochondrial depolarization and oxidative stress, and by increasing the expression of BDNF. This study has identified CB\textsubscript{2} receptor activation as a mechanism for enhancing the phosphorylation of AMP-activated protein kinase and cAMP responsive element-binding protein and increasing expression of the CREB target protein, BDNF (Choi et al., 2013). In an in vitro model of PD, 1-methyl-4-phenylpyridinium iodide, paraquat and lactacystin were used to inhibit mitochondrial function, generate free radicals and inhibit the ubiquitin proteasome respectively. These treatments resulted in cell death brought on by ROS generation, caspase-3 activation and cytotoxicity. THC (10 \mu M) was shown to reduce these effects in human neuroblastoma cells (SH-SY5Y) while increasing cell viability. This result was not reproduced by the CB\textsubscript{1} receptor agonist WIN 55212-2 (1 \mu M) but was blocked by inhibition of PPAR\textgamma, the activity of which was increased by THC treatment (Carroll et al., 2012).

**Cannabinoids and adult neurogenesis**

Adult neurogenesis is the process by which new neurons are generated and integrated into the developed brain. Regulation of neurogenesis is strictly controlled through a number of different factors such as adrenal and sex hormones, neurotransmitter systems, trophic factors and inflammatory cytokines. The formation of new neurons and neuronal connections may prove vital to sustaining neuronal function in neurodegenerative disorders where neurogenesis is impaired such as AD and HD (Molero et al., 2009; Crews et al., 2010). The eCB system has been closely linked to the process of adult neurogenesis. DGL\textalpha and DGL\beta synthesize the endocannabinoid 2AG, and DGL\alpha and DGL\beta null mice have an 80 and 50% reduction in 2AG respectively. These transgenic mice were shown to have impaired neurogenesis, believed to be as a result of the loss of 2AG-mediated transient suppression of GABAergic transmission at inhibitory synapses (Gao et al., 2010). Furthermore, mice lacking CB\textsubscript{1} receptors displayed an almost 50% reduction in neurogenesis in the dentate gyrus and subventricular zone when compared to wild type. In line with this, the mixed CB\textsubscript{1}/CB\textsubscript{2} receptor agonist WIN 55212-2 enhanced BrdU incorporation into murine neuronal culture in a CB\textsubscript{2} receptor-mediated fashion (Kim et al., 2006b). CB\textsubscript{1} receptor-mediated stimulation of adult neurogenesis has been shown to act through its opposition of the antineurogenic effect of nitric oxide (Kim et al., 2006b; Marchalant et al., 2009). Neuronal precursor cell proliferation and the number of migrating neurons have been shown to increase in neurogenic regions in response to seizure, ischaemia and excitotoxic and mechanical lesions indicating a possible contributing factor in the repair of lesioned circuits (Gould and Tanapat, 1997; Arvidsson et al., 2001; Parent et al., 2002; Lie et al., 2004). KA-induced neural progenitor proliferation is reduced in CB\textsubscript{1} receptor deficient mice as well as in wild-type mice administered with the selective CB\textsubscript{1} receptor antagonist SR141716A. This effect was attributed to the CB\textsubscript{1}-dependent expression of basic fibroblast growth factor and epidermal growth factor (Aguado et al., 2007). BDNF is vital for the survival of new neurons and is significantly reduced in neurodegenerative conditions such as HD (Zuccato and Cattaneo, 2007). De March et al. (2008) have shown that 2 weeks post-excitotoxic lesion in rats, tran-
sient up-regulation of BDNF coincides with higher binding activity and protein expression of CB₁ receptor. This is believed to be an attempt to rescue the striatal neuronal population. In a reciprocal fashion, BDNF (10 ng mL⁻¹) was shown in vitro to increase neuronal sensitivity to the endocannabinoids 2AG and noladin ether as measured by the phosphorylation of Akt (Maison et al., 2009). Indeed, CB₁ receptor activation has been implicated in neural precursor proliferation and neurogenesis while CB₁ and CB₂ receptor activation is involved in neural progenitor cell proliferation, both of which are vital to the generation and survival of new neurons (Palazuelos et al., 2006; Aguado et al., 2007).

Summary

Neurodegenerative diseases are a heterogeneous group of age-related disorders. While AD, PD and HD have a variety of different genetic and environmental causes, the principal factor involved is the progressive and severe loss of neurons. It is widely accepted that neuroinflammation, excitotoxicity and oxidative stress are key mediators of neurodegeneration, and impaired neurogenesis as well as reduced trophic support leave neuronal systems unable to cope. The eCB system is emerging as a key regulator of many neuronal systems that are relevant to neurodegenerative disorders. Activation of CB₁ receptors regulates many neuronal functions such as Ca²⁺ homeostasis and metabolic activity while the CB₂ receptor is mainly involved in regulating the inflammatory response.

Here, we have put forward the mechanisms of neurodegeneration in the three most prevalent neurodegenerative disorders, AD, PD and HD, as well as showing the vulnerability of the brain as a result of age. We have summarized evidence of the beneficial role of modulating the cannabinoid system to reduce the burden of neurodegeneration. Pharmacological modulation of the eCB system (Figure 3) has been shown to reduce chronic activation of the neuroinflammatory response, aid in Ca²⁺ homeostasis, reduce oxidative stress, mitochondrial dysfunction and the resulting proapoptotic cascade, while promoting neurotrophic support.

**Figure 3**

Chemical structures of the common CB receptor agonists.
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Conflict of interest

There are no conflicts of interest associated with this paper.

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