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REVIEW

The endocannabinoid system as a target for the treatment of neurodegenerative disease

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The *Cannabis sativa* plant has been exploited for medicinal, agricultural and spiritual purposes in diverse cultures over thousands of years. Cannabis has been used recreationally for its psychotropic properties, while effects such as stimulation of appetite, analgesia and anti-emesis have lead to the medicinal application of cannabis. Indeed, reports of medicinal efficacy of cannabis can been traced back as far as 2700 BC, and even at that time reports also suggested a neuroprotective effect of the cultivar. The discovery of the psychoactive component of cannabis resin, Δ^9-tetrahydrocannabinol (Δ^9-THC) occurred long before the serendipitous identification of a G-protein coupled receptor at which Δ^9-THC is active in the brain. The subsequent finding of endogenous cannabinoid compounds, the synthesis of which is directed by neuronal excitability and which in turn served to regulate that excitability, further widened the range of potential drug targets through which the endocannabinoid system can be manipulated. As a result of this, alterations in the endocannabinoid system have been extensively investigated in a range of neurodegenerative disorders. In this review we examine the evidence implicating the endocannabinoid system in the cause, symptomatology or treatment of neurodegenerative disease. We examine data from human patients and compare and contrast this with evidence from animal models of these diseases. On the basis of this evidence we discuss the likely efficacy of endocannabinoid-based therapies in each disease context.
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Abbreviations: 2-AG, 2-arachidonoylglycerol; 3-NP, 3-nitropropionic acid; 6-OHDA, 6-hydroxy dopamine; Δ^9-THC, Δ^9-tetrahydrocannabinol; AD, Alzheimer's disease; AEA, anandamide/arachidonoylethanolamide; ALS, amyotrophic lateral sclerosis; AMT, anandamide membrane transporter; BAP, β-amyloid peptide; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CNS, central nervous system; CREAE, chronic relapsing EAE; CSF, cerebrospinal fluid; DAGL, diacylglycerol lipase; EAAT2, glutamate transporter/excitatory amino acid transporter 2; EAE, experimental autoimmune encephalomyelitis; FAAH, fatty acid amide hydrolyse; GABA, γ-amino butyric acid; HD, Huntington's disease; hSOD1, human superoxide dismutase 1; LID, levodopa-induced dyskinesia; MAGL, monoacylglycerol lipase; MS, multiple sclerosis; NAPE-PLD, N-acyl phosphatidylethanolamine phospholipase D; PEA, palmitoylethanolamide; PD, Parkinson's disease; PPAR, peroxisome proliferator-activated receptor; TMEV, Theiler's murine encephalomyelitis virus; TNF, tumour necrosis factor; TRPV1, transient receptor potential (vanilloid) receptor 1; WT, wildtype

The psychoactive component of cannabis resin, Δ^9-tetrahydrocannabinol (Δ^9-THC), was first isolated in 1964 (Gaoni and Mechoulam, 1964), and at least 70 other structurally related 'phytocannabinoid' compounds have since been isolated (Elsohly and Slade, 2005). The development of synthetic cannabimimetic drugs has aided in the pharmacological characterization of an endogenous system which responds to cannabis. However, it was the serendipitous identification of a G-protein coupled cannabinoid receptor at which cannabinoid compounds are active in the brain (Matsuda *et al*., 1990), which heralded an explosion in endocannabinoid research. In this review, we examine the reports of changes to endocannabinoid levels in human patients and compare and contrast this with evidence from animal models. We also

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consider the reported changes to other elements of the endocannabinoid system, with a view to understanding their relationship to endocannabinoid levels and disease symptomatology. Based upon these examinations, and upon the reported effects of manipulation of the cannabinoid system in each disease context, we discuss the likely efficacy of endocannabinoid-based therapies.

The endocannabinoid system

The endocannabinoid system comprises the cannabinoid receptors CB_1, CB_2 and possibly others; the fatty acid derivatives which act as their endogenous ligands; and the proteins responsible for the synthesis, reuptake and degradation of these endogenous ligands.

Cannabinoid receptors

Various studies have mapped the localization of cannabinoid receptors in tissues and at a subcellular level, and these have been critical to our understanding of the effects of cannabinoids in disease. CB_1 receptors are expressed in both the central nervous system and periphery. They are the most abundant GPCR in the brain, with high expression levels in the basal ganglia nuclei and moderately high expression in the hippocampus, cerebellum and neocortex (Herkenham *et al*., 1990; Glass *et al*., 1997). At the subcellular level, CB_1 has been localized to pre-synaptic terminals, and is found at significantly higher levels on GABAergic than glutamatergic neurons in various brain regions (Katona *et al*., 1999; Katona *et al*., 2001; Puighermanal *et al*., 2009).

CB_1 is expressed at much lower levels peripherally than in the brain. Nonetheless, CB_1 expression has been detected in a variety of circulating immune cells (Bouaboula *et al*., 1993), and in resident microglia in rat brain (Sinha *et al*., 1998). Activation of microglial CB_1 has been shown to inhibit the release of nitric oxide, suggesting that CB_1 may be anti-inflammatory (Waksman *et al*., 1999). There is evidence that changes in CB_1 expression may occur with activation in immune cell lines; however, reports are conflicting as to whether CB_1 is up- or down-regulated in these activated cells (reviewed in Klein, 2005). Astrocytic expression of CB_1 has been demonstrated in cultured cells (Bouaboula *et al*., 1995; Sheng *et al*., 2005), and after injury (Garcia-Ovejero *et al*., 2009); however, there has been some controversy over whether normal astrocytes *in situ* express CB_1. Several studies have found no overlap between astrocytic and CB_1 immunostaining (McDonald and Mascagni, 2001; Marchalant *et al*., 2007; Marchalant *et al*., 2008), therefore there was a call for more definitive studies to confirm that the CB_1-positive perivascular glia reported by several other groups were indeed astrocytes (Moldrich and Wenger, 2000; Rodriguez *et al*., 2001; Salio *et al*., 2002). A recent investigation has gone some way towards achieving this, by demonstrating that the response to cannabinoids by astrocytes in hippocampal slices was blocked by a CB_1-specific antagonist (Navarrete and Araque, 2008).

The myriad peripheral and immune activities of cannabinoid compounds, despite the low levels of CB_1 expression in cognate immune organs (reviewed in Klein *et al*., 2003), prompted the search for a peripheral receptor for cannabinoids, CB_2 (Munro *et al*., 1993). CB_2 shows only 44% overall identity to CB_1 (Munro *et al*., 1993). CB_2 is found in particular abundance in peripheral organs with immune function, including macrophages, spleen, tonsils, thymus and leucocytes, as well as the lung and testes (Munro *et al*., 1993; Galiegue *et al*., 1995; Brown *et al*., 2002). Early studies suggested that CB_2 was absent from the brain (Griffin *et al*., 1999; Brown *et al*., 2002). However, a number of studies have now shown CB_2 expression in diseased brain cells, including astrocytomas (Sanchez *et al*., 2001; Ellert-Miklaszewska *et al*., 2007), microglia and astrocytes in Alzheimer's disease (Benito *et al*., 2003; Esposito *et al*., 2007b), and T cells, microglia and astrocytes in multiple sclerosis (Benito *et al*., 2007).

These studies and others provide strong evidence that CB_2 is up-regulated in response to inflammatory cues or immune cell activation. It should be noted, however, that a specific marker to distinguish macrophages and microglia remains to be identified, making it difficult to determine whether CB_2 up-regulation is attributable to expression by activated resident microglia or by peripheral macrophages invading the brain. More recently, CB_2 expression has also been reported to occur in normal brain; in neural progenitors (Palazuelos *et al*., 2006); select neuronal subsets in the brainstem (Van Sickle *et al*., 2005), cerebellar granule layer (Skaper *et al*., 1996), and dorsal root of the spinal cord (Beltramo *et al*., 2006); and in microglial populations (Klegeris *et al*., 2003; Nunez *et al*., 2004; Ashton *et al*., 2006), but not normal astrocytes (Ashton *et al*., 2006; Benito *et al*., 2007; Racz *et al*., 2008; Garcia-Ovejero *et al*., 2009). Several reports originating from one research group, of extensive CB_2 immunoreactivity in various neuronal subtypes throughout the brain, have been somewhat controversial and warrant further investigation (Gong *et al*., 2006; Onaivi *et al*., 2006).

The possible existence of other cannabinoid-responsive receptors has been brought to light by the inability of specific CB_1 or CB_2 antagonists to abolish the effects or binding of certain cannabinoids, and by the persistence of cannabinoid effects in CB_1 and/or CB_2 knockout animals. One such receptor is the abnormal-cannabidiol receptor, which is activated by abnormal cannabidiol, AEA, 2-AG and WIN55,212-2, and antagonized by cannabidiol, the cannabidiol analogue O-1918 and the endogenous compound N-arachidonoyl L-serine (Jarai *et al*., 1999; Begg *et al*., 2003; McHugh *et al*., 2008; Kreutz *et al*., 2009). The orphan receptor designated GPR55 has also received considerable attention as a possible cannabinoid receptor. Endo-, phyto- and synthetic cannabinoids have been shown to bind and activate GPR55 (Ryberg *et al*., 2007; Lauckner *et al*., 2008), although subsequent studies dispute a number of these findings (Henstridge *et al*., 2009; Kapur *et al*., 2009). Indeed, those studies reported that an array of cannabinoids failed to activate GPR55, with the exception of AM251 and SR141716A, which are CB_1 antagonists, and lysophosphatidylinositol, which is not a cannabinoid (Oka *et al*., 2007; Henstridge *et al*., 2009; Kapur *et al*., 2009). Two of the studies suggested that CP55,940, a cannabinoid receptor agonist, acts as an antagonist or partial agonist at GPR55 (Henstridge *et al*., 2009; Kapur *et al*., 2009). The adoption of either the abnormal-cannabidiol receptor or GPR55 into the cannabinoid receptor family remains an issue of debate. However, a possible role for non-CB_1/CB_2 receptors in the effects of cannabinoids in neurodegenerative disorders should not be overlooked.

Endocannabinoids

Endocannabinoids were first discovered in brain, but are also present in the periphery in both humans and animals. They are produced by cultured neurons (Di Marzo *et al*., 1994), microglia (Walter *et al*., 2003) and astrocytes (Walter *et al*., 2002), as well as by isolated macrophages (Wagner *et al*., 1997; Di Marzo *et al*., 1999) To date, five endogenous cannabinoids or 'endocannabinoids' have been identified; arachidonoyl ethanolamide (anandamide, AEA) (Devane *et al*., 1992), 2-arachidonoyl glycerol (2-AG) (Mechoulam *et al*., 1995), O-arachidonoyl ethanolamine (virodhamine) (Porter *et al*., 2002), N-arachidonyldopamine (NADA) (Huang *et al*., 2002) and 2-arachidonoyl glyceryl ether (noladin ether) (Hanus *et al*., 2001). While publications subsequent to Hanus *et al*. also report noladin ether to occur in brain, several other groups have failed to corroborate this finding (Oka *et al*., 2003; Richardson *et al*., 2007), leaving doubt as to whether it is a *bona fide* endocannabinoid.

In addition to these compounds, the AEA analogue palmitoylethanolamide (PEA) is receiving increasing interest as a potential endocannabinoid. PEA shows no affinity for CB_1, and reports are conflicting as to whether it activates CB_2 (Facci *et al*., 1995; Ryberg *et al*., 2007) or not (Showalter *et al*., 1996; Lambert *et al*., 1999; Sugiura *et al*., 2000). However, reports of specific mechanisms regulating the synthesis (Stella and Piomelli, 2001) and metabolism (Tsuboi *et al*., 2005) of PEA support the notion that this endogenous fatty acid may act as more than just an 'entourage' molecule for the effects of 'true' endocannabinoids (for review, see Mackie and Stella, 2006). There has also been significant interest in oleamide as an endocannabinoid. It has shown efficacy both *in vivo* at the abnormal-cannabidiol receptor (Hoi and Hiley, 2006; Sudhahar *et al*., 2009) and more controversially *in vitro* at CB_1 (for commentary see Fowler, 2004; Leggett *et al*., 2004). The investigation of oleamide as an endocannabinoid may be complicated by its use in, and organic solvent-mediated leaching from, disposable laboratory plastic ware (McDonald *et al*., 2008).

As some of these potential endocannabinoids remain under debate, and because the majority of studies of endocannabinoids levels in neurodegenerative disease have quantified only AEA and 2-AG, these two endocannabinoids will thus form the focus of our review.

AEA has been found both in the brain and periphery (Felder *et al*., 1996). AEA is an agonist for CB_1 (Devane *et al*., 1992; Mackie *et al*., 1993; Showalter *et al*., 1996), CB_2 (Felder *et al*., 1995; Showalter *et al*., 1996), and the vanilloid TRPV1 receptor (Zygmunt *et al*., 1999). In the brain, AEA levels are high in the hippocampus, thalamus, striatum and brainstem and lower, but still detectable, in the cerebral cortex and cerebellum (Felder *et al*., 1996; Bisogno *et al*., 1999). This pattern of distribution shows poor correlation with that described for CB_1 (Herkenham *et al*., 1990; Bisogno *et al*., 1999). AEA is also found, albeit at far lower levels than in brain, in human blood (serum and plasma) and CSF (Felder *et al*., 1996).

Like AEA, 2-AG is found in both the brain and periphery, although in the brain it is found at concentrations approximately 150 times that of AEA (Bisogno *et al*., 1999). 2-AG is found at high levels in the brainstem, hippocampus, striatum and medulla in rats, showing a correlation with AEA but not CB_1 localization (Bisogno *et al*., 1999). 2-AG is an agonist at CB_1 and also CB_2, where its potency is greater than that of AEA; while this has been taken to suggest that 2-AG may be the endogenous ligand for CB_2, this finding could equally be due to greater stability of 2-AG than AEA (Mechoulam *et al*., 1995; Gonsiorek *et al*., 2000; Sugiura *et al*., 2000).

Endocannabinoid synthesis and inactivation

Various synthetic and degradative enzymes have been identified which dynamically regulate the levels of endogenous cannabinoids under normal and diseased conditions, and which may be key targets for therapeutics. Both AEA and 2-AG are produced by cleavage of plasma membrane phospholipids. They are synthesized 'on demand' in an activity-dependent fashion, whereby calcium acts as a biosensor of membrane depolarization to induce synthesis as required (Di Marzo *et al*., 1994; Stella *et al*., 1997). AEA is synthesized from its arachidonic acid and phosphatidylethanolamine precursors by the sequential actions of two intracellular enzymes; an N-acyltransferase and a phospholipase (NAPE-PLD)(Di Marzo *et al*., 1994; Cadas *et al*., 1997). 2-AG is formed by the hydrolysis of membrane-derived diacylglycerol by sn1 diacylglycerol lipase (DAGL) found in the membranes of neuronal dendritic spines (Bisogno *et al*., 2003; Katona *et al*., 2006). DAGL expression may also be induced in reactive astrocytes (Garcia-Ovejero *et al*., 2009). How these highly lipophilic endocannabinoids are released from the membrane into synaptic and extra-synaptic spaces remains poorly understood.

Inactivation of endocannabinoids occurs rapidly *in vivo* (Di Marzo *et al*., 1994; Maccarrone *et al*., 1998). The fatty acid amide hydrolase (FAAH) enzyme is found intracellularly on the membranes of organelles in mainly post-synaptic neurons, showing complementary expression to CB_1 (Egertova *et al*., 1998; Gulyas *et al*., 2004). Reactive astrocytes show increased expression of FAAH (Benito *et al*., 2005; Benito *et al*., 2007; Nunez *et al*., 2008). FAAH is responsible for the degradation of AEA predominantly, although 2-AG can also act as a substrate (Di Marzo *et al*., 1998; Goparaju *et al*., 1998). The inactivation of 2-AG occurs preferentially through hydrolysis by the pre-synaptically localized monoacylglycerol lipase (MAGL) enzyme (Dinh *et al*., 2002). The membrane transporter for AEA (AMT) is yet to be cloned and its existence is a topic of hot debate. On one side of the controversy, the saturable, temperature-dependent and specific nature of AEA uptake argues against passive diffusion through the membrane (Di Marzo *et al*., 1994). In addition, specific inhibitors of uptake but not hydrolysis bolster the claims for the AMT's existence. Many (Vandevoorde and

Fowler, 2005; Alexander and Cravatt, 2006; Kaczocha *et al*., 2006), but not all (Ortar *et al*., 2003; Fegley *et al*., 2004), of these uptake inhibitors have subsequently been shown to inhibit FAAH, which would reduce the concentration gradient for diffusion. Recently, intracellular transporters were identified which facilitate the delivery of AEA to FAAH through the hydrophilic cytosol (Kaczocha *et al*., 2009). This finding may help reconcile the data supporting both passive membrane diffusion and carrier-mediated kinetics for AEA uptake; however, while this model abrogates the need for an AMT, it does not yet rule out the possibility of its existence.

Endocannabinoid function

Our growing understanding of the roles of the endogenous cannabinoids has suggested two main pathways by which cannabinoids may impact upon neurodegenerative processes; neuromodulation and immunomodulation. The neuromodulatory action of endocannabinoids has been well characterized in several elegant studies, and CB_1 and CB_2 signal transduction have been detailed in various reviews (Felder *et al*., 1995; McAllister and Glass, 2002; Dalton *et al*., 2009; Scotter *et al*., 2009). Briefly, it has been shown that endocannabinoids synthesized by depolarized post-synaptic dendrites, particularly 2-AG (Kim and Alger, 2004), can act as retrograde ligands at CB_1 located at pre-synaptic terminals to inhibit the release of excitatory or inhibitory neurotransmitter from the pre-synaptic neuron (Maejima *et al*., 2001; Wilson and Nicoll, 2001).

Aside from this crucial regulatory role in the activity of neurons, endocannabinoids also play a key role in peripheral and brain immune function. As mentioned, CB_2 is expressed on various circulating and resident immune cells, particularly when these cells are activated, and its agonism is typically associated with a dampening of their pro-inflammatory activities. This includes the inhibition of release of inflammatory mediators, including nitric oxide, interleukin-2 and TNF-α, inhibition of the activation of the cell-mediated immune processes, and inhibition of proliferation and chemotaxis (Ehrhart *et al*., 2005; Coopman *et al*., 2007; Maresz *et al*., 2007; Romero-Sandoval *et al*., 2009; and reviewed in Walter and Stella, 2004a).

Endocannabinoids in neurodegenerative disease

There has been anecdotal and preliminary scientific evidence of cannabis affording symptomatic relief in diverse neurodegenerative disorders. These include multiple sclerosis, Huntington's, Parkinson's and Alzheimer's diseases, and amyotrophic lateral sclerosis. This evidence implied that hypofunction or dysregulation of the endocannabinoid system may be responsible for some of the symptomatology of these diseases. Given also the abundance of CB_1 in areas associated with movement and executive thought, interest soon developed in measuring endocannabinoids levels in patients with degenerative movement disorders.

Huntington's disease

Huntington's disease (HD) is a hereditary disorder with an incidence of approximately 1 in 15 000 and is characterized by a triad of symptoms; disturbances in movement, mood and cognition (for reviews, see Rosenblatt and Leroi, 2000; and Ross and Margolis, 2001). In 1993 the *huntingtin* gene, mutation of which is responsible for the disease, was mapped, and its misfolding was identified as a requisite pathological event in the degeneration of neurons in the striatum, cortex and other diffuse brain regions affected by HD (The Huntington's Disease Collaborative Research Group, 1993). However, despite this finding and much subsequent research, both the wildtype function of the huntingtin protein and the pathophysiological pathways activated by the mutant protein remain ill-defined.

Endocannabinoid changes system in human HD

One of the first detectable signs of cellular dysfunction in human HD brains is the loss of CB_1 from GABAergic efferent terminals and somata. In HD patients with early symptoms but without gross neuropathology (Grade zero (Vonsattel *et al*., 1985)), there is a significant decrease in CB_1 density in the internal and external globus pallidus and substantia nigra (Glass *et al*., 2000). While CB_1 loss in the external segment of the globus pallidus is likely due to the degeneration of the terminals of the GABA/enkephalin efferents lost first in HD (Reiner *et al*., 1988), in the internal segment significant CB_1 loss is seen prior to changes in co-localized receptors or GABA/substance P neuronal pathology (Glass *et al*., 2000; Allen *et al*., 2009). Contrary to findings with CB_1, CB_2 has recently been demonstrated to be up-regulated in post-mortem HD striatum (Palazuelos *et al*., 2009), consistent with marked gliosis in this region.

Recently, it was reported that lymphocyte preparations from HD patients contained levels of AEA that were sixfold greater than those of control patient lymphocytes (Battista *et al*., 2007). This was attributed to an inhibition of function of FAAH in AEA metabolism. While the relationship between the peripheral and central endocannabinoid systems remains unclear, in the same study a corresponding reduction in FAAH activity was also detected in the cerebral cortex of post-mortem HD brains.

Endocannabinoid changes in animal models of HD

Various animal models have been developed which reproduce the selective degeneration of medium spiny neurons of the striatum in HD. Intrastriatal administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) to rats produces an interneuron-sparing striatal lesion and HD-like motor abnormalities (Beal *et al*., 1993). An examination of endocannabinoids levels in this model revealed decreases in AEA and 2-AG in the striatum, but not the cerebral cortex, and increases in the region corresponding to the substantia nigra (Lastres-Becker *et al*., 2001b). These changes are difficult to interpret however, given the acute ablation of medium spiny neurons which likely produce these endocannabinoids (van der Stelt *et al*., 2003). Transgenic mouse models of HD, carrying the pathologically expanded huntingtin gene with 115 (R6/1) or 150 (R6/2) repeats, also recapitulate the motor phenotype of HD (Mangiarini *et al*., 1996). Unlike lesion models, the progression of disease in these animals occurs in the absence of overt cell death (Mangiarini *et al*., 1996), which may enable the analysis of endocannabinoid changes associated with early cellular dysfunction. Loss of CB_1 is recapitulated by various transgenic rodent models, despite their lack of overt cell death (Denovan-Wright and Robertson, 2000; Lastres-Becker *et al*., 2002a; McCaw *et al*., 2004), suggesting that CB_1 loss may represent an early marker of, or exacerbating factor in, neuronal dysfunction.

Interestingly, these CB_1 changes occur considerably earlier than alterations in endocannabinoid levels in R6/2 mice; with receptor changes occurring pre-symptomatically while endocannabinoid alterations (as described below) were concurrent with distinct motor symptomatology (Denovan-Wright and Robertson, 2000; McCaw *et al*., 2004; Bisogno *et al*., 2008). This indicates that changes to cannabinoid receptor levels may be a part of or induced by HD pathology, rather than occurring secondarily to changes in endocannabinoid levels.

Profiles of endocannabinoid levels in pre-symptomatic R6/1 mice (12 weeks gestational age) showed decreased AEA in the hippocampus and increased 2-AG in the cerebral cortex. Interestingly no changes were detected for either endocannabinoid in the striatum (Dowie *et al*., 2009a), the brain region in which dysfunction occurs early in both this model and human sufferers (Vonsattel *et al*., 1985; Mangiarini *et al*., 1996). Endocannabinoid quantification in pre-symptomatic R6/2 mice (4.5 weeks gestational age) suggested a decrease, rather than an increase, in 2-AG levels in the cerebral cortex, while AEA levels were unchanged in all areas examined (Bisogno *et al*., 2008). Analysis of motor symptomatic R6/2 mice (10 weeks gestational age) in the same study showed 2-AG levels to be decreased in the cerebral cortex, striatum, and also trending towards a decrease in the hippocampus. AEA levels were also decreased in the striatum and hippocampus, but increased in the cerebral cortex (Bisogno *et al*., 2008). This latter finding, of increased AEA in the cerebral cortex, could be the result of inhibition of FAAH, as was reported for human HD cerebral cortex (Battista *et al*., 2007).

Recently, CB_2 alterations have also been detected in the human and transgenic animal HD brain. Palazuelos *et al*. (2009) detected increased CB_2 protein in the striatum in both human HD patients and R6/2 mice compared with controls. In mouse models the increase in CB_2 occurred concurrently with increased immunoreactivity for the microglial (and macrophage) markers CD68 and CD11b, with which CB_2 was shown to co-localize (Palazuelos *et al*., 2009). Surprisingly, the greatest increase in CB_2 immunoreactivity in R6/2 mice (~3.5-fold) was seen at a pre-symptomatic time-point, suggesting that increases in CB_2-positive microglia occurred early in the disease. This is in accordance with human HD studies which have found that striatal microglia are activated in preclinical and low grade patients (Sapp *et al*., 2001; Tai *et al*., 2007). The intrinsic activation of CB_2 in HD may reduce pro-inflammatory microglial cascades to some extent; however, this mechanism is clearly insufficient to prevent the inevitable progression of neuronal death.

Cannabinoid agents as therapeutics in HD

The question of whether CB_1 activation may be therapeutic in HD has been explored in various rodent lesion models; however, reports are conflicting as to whether CB_1 agonism is neuroprotective, exacerbatory, or is more useful in the treatment of HD symptoms. It has been found that administration of the endocannabinoid uptake inhibitors UM404 or UCM707 attenuated both the neurotransmitter deficits and increase in ambulation ('hyperkinesia') associated with 3-NP lesion (Lastres-Becker *et al*., 2002b; de Lago *et al*., 2006). However, further investigation suggested that the target responsible for much of the anti-hyperkinetic effect may have been the vanilloid TRPV1 receptor, with CB_1 playing only a minor role (Lastres-Becker *et al*., 2003b).

A neuroprotective role for CB_1 in HD has also been tested. Lastres-Becker *et al*. (2004) found that the number of rats which developed a significant lesion due to 3-NP administration was reduced in a cohort treated daily with Δ^9-THC. However, the same group had previously reported an exacerbation of malonate lesion with either Δ^9-THC or SR141716A treatment, despite their opposing actions at CB_1 (Lastres-Becker *et al*., 2003a), and later the endocannabinoid uptake inhibitor UCM707 also failed to reduce lesion volume in the malonate model (de Lago *et al*., 2006). In contrast, in quinolinic acid-lesioned rats, pre- and then co-administration of WIN55,212-2 with the excitotoxin reduced the induction of glutamate release and reduced the lesion volume (Pintor *et al*., 2006). Similarly, following lesion with kainic acid, wildtype but not CB_1-knockout mice showed an injury-induced recruitment of brain-derived neurotrophic factor which decreased neuronal damage and gliosis (Marsicano *et al*., 2003; Khaspekov *et al*., 2004). The ability of the neuromodulatory cannabinoid system to attenuate excitotoxic damage, as is inflicted by the aforementioned toxins, is widely accepted. However, the contribution of excitotoxicity to the pathophysiology of HD is unknown, and as such care must be taken in extrapolating the efficacy of cannabinoids in lesion models to transgenic or human HD.

In the R6/1 transgenic mouse model of HD, exposure to enriched environments caused an up-regulation of CB_1 and also delayed the onset of symptoms (van Dellen *et al*., 2000; Glass *et al*., 2004). Environmental enrichment was also found to almost completely rescue the deficit in brain-derived neurotrophic factor associated with mutant huntingtin expression (Spires *et al*., 2004). However, despite the efficacy of CB_1 signalling in attenuating molecular and behavioural HD, the early and dramatic loss of these receptors may preclude their use as therapeutics (Dowie *et al*., 2009b).

In contrast, CB_2 on microglia in the R6/2 striatum has been shown to increase prior to symptom-onset (Palazuelos *et al*., 2009). Because CB_2 knockout leads to enhanced gliosis and an exacerbation of motor symptoms, the reported CB_2 up-regulation may be a protective compensatory mechanism (Palazuelos *et al*., 2009). The selective CB_2 agonist HU308 has been shown to reduce neuronal loss, potentially through suppression of glial activation, in the quinolinic acid, 3-NP and malonate lesion models (Sagredo *et al*., 2007; Palazuelos *et al*., 2009; Sagredo *et al*., 2009); however, neuroprotection via CB_2 manipulation is yet to be confirmed in transgenic HD models. Indeed the CB_1/CB2 agonist HU210 failed to modify disease in the R6/1 transgenic model of HD (Dowie *et al*., 2009b). Interestingly, cannabidiol has been found to almost completely reverse the neuronal changes induced by 3-NP administration to rats, suggesting that anti-oxidant effects, abnormal-cannabinoid receptor antagonism, or as yet unidentified cannabinoid receptors may be protective in this model of HD (Sagredo *et al*., 2007). A 6-week clinical trial of cannabidiol in humans failed to ameliorate symptoms (Consroe *et al*., 1991), but further studies need to be performed to evaluate the effectiveness of cannabinoid treatment in patients with HD.

Alzheimer's disease

The disruptive effects of Δ^9-THC on memory are well documented and have recently been more fully characterized at the molecular level (Puighermanal *et al*., 2009). Alzheimer's disease (AD), a disease with major impact on memory systems, has therefore been investigated for evidence of dysfunction of the endocannabinoid system resulting from, or contributing to, disease pathophysiology. AD is the most common neurodegenerative disorder, with a prevalence of approximately 10% in humans over 80 years old (Ferri *et al*., 2005). There is both genetic and idiopathic aetiology for the disease, which is characterized by gross atrophy of cholinergic neurons projecting to the cerebral cortex and hippocampus, and also of glutamatergic neurons of those regions (Whitehouse *et al*., 1982; Greenamyre *et al*., 1985; Wenk, 2003). Neurodegeneration appears to follow the extracellular deposition of β-amyloid protein in 'plaques' and/or the formation of intracellular 'tangles' of hyperphosphorylated tau protein (see Minati *et al*. 2009 for a recent review).

There is much debate regarding which, if either, of these proteins is central to the neurodegenerative process (reviewed in Mudher and Lovestone, 2002). While anti-β-amyloid vaccination of humans (Hock *et al*., 2003; Nicoll *et al*., 2003; Gilman *et al*., 2005) and transgenic animals (Schenk *et al*., 1999; Dodart *et al*., 2002) has led to various degrees of cognitive or histological improvement, a Phase III trial currently underway may further implicate or absolve β-amyloid plaques in the impairment of cognitive function. Already there is much compelling evidence for β-amyloid cascade/neuroinflammation hypothesis, which proposes that misfolded β-amyloid is both injurious to neurons and also invokes a microglial response which, while perhaps evolved to phagocytically clear plaques, is itself neurotoxic (Haga *et al*., 1989; Itagaki *et al*., 1989; Hardy and Higgins, 1992; Streit *et al*., 2005; Hickman *et al*., 2008). The finding that CB_2 is expressed on the microglia clustered around β-amyloid plaques therefore suggests that endocannabinoids may have the ability to modulate the effector cells of AD (Benito *et al*., 2003).

Endocannabinoid system changes in human AD

Studies have found CB_1 expression on neurons to be reduced (Westlake *et al*., 1994; Ramirez *et al*., 2005) or unchanged (Benito *et al*., 2003) in human AD brain, Remaining CB_1 protein was shown to be excessively nitrated and to have decreased efficacy of G-protein coupling (Ramirez *et al*., 2005). In contrast, CB_2 expression is dramatically up-regulated, particularly in the microglial cells surrounding β-amyloid plaques in human AD brain (Benito *et al*., 2003; Ramirez *et al*., 2005).

Endocannabinoid levels *per se* have not been assayed in post-mortem human AD brain, however, enhanced enzymatic activity of both DAGL and MAGL, the synthetic and catabolic enzymes for 2-AG, respectively, has been detected in the hippocampus in human AD (Farooqui *et al*., 1988). In addition, FAAH protein expression and activity are reported to be elevated in activated astrocytes adjacent to the plaques (Benito *et al*., 2003). These data have been suggested to imply that local production and turnover of 2-AG may also be elevated in the disease. Interestingly, quantification of AEA and 2-AG from plasma of Alzheimer's disease patients did not detect any significant differences from age-matched controls (Koppel *et al*., 2009).

Endocannabinoid system changes in animal models of AD

Animal models of AD provide support to the hypothesis that 2-AG levels may be elevated in the hippocampus in human AD patients. Rats injected unilaterally in the frontal cortex with fragments of β-amyloid peptide (BAP rats) develop AD-like molecular pathology with significant neuronal and neuritic degeneration in the distantly located hippocampus, and impaired learning and memory (Kowall *et al*., 1991; van der Stelt *et al*., 2006). In agreement with the increased activity of DAGL and MAGL in human AD (six- to eightfold) (Farooqui *et al*., 1988), mRNA expression of these enzymes was elevated by 1.2- and 1.6-fold, respectively, in BAP rats (van der Stelt *et al*., 2006). 2-AG levels were rapidly and dramatically elevated in the ipsilateral and, to a lesser degree, the contralateral hippocampus in these animals. Increases in AEA levels were of a greater magnitude in the contralateral than ipsilateral hippocampus (van der Stelt *et al*., 2006). *In vitro*, C6 glioma cells exposed to β-amyloid produced fourfold less AEA and 1.5-fold more 2-AG than control cells, suggesting that elevated 2-AG levels at least are a conserved feature of these AD models (Esposito *et al*., 2007a). Consistent with human studies, CB_1 has been found to be reduced in BAP rats and C6 glioma cells challenged with β-amyloid (Esposito *et al*., 2007a).

Cannabinoid agents as therapeutics in AD

Synthetic Δ^9-THC (dronabinol) has been shown to alleviate behavioural disturbances and weight loss, and night-time agitation symptoms in human studies of Alzheimer's and severe dementia respectively (Volicer *et al*., 1997; Walther *et al*., 2006). As yet however, cannabinoid neuroprotection studies have only been conducted in animals. In BAP rats, the AMT inhibitor VDM11 was able to attenuate hippocampal neuron damage through the elevation of AEA levels alone, while in BAP mice neuronal rescue was associated with elevations in both AEA and 2-AG levels (van der Stelt *et al.*, 2006). Pharmacological dissection suggests that these endocannabinoids may mediate neuroprotection through activation of CB_1, and inhibit the inflammatory microglial response through activation of CB_2 (Ramirez *et al.*, 2005). CB_2 agonists have been shown to inhibit TNF-α and nitric oxide production by microglia/macrophages, as well as stimulating their phagocytosis of β-amyloid peptide (Ehrhart *et al.*, 2005; Tolon *et al.*, 2009). In a study by Esposito *et al.* a CB_2 antagonist was able to attenuate markers of astrogliosis (Esposito *et al.*, 2007a). Interestingly, the same group showed similar anti-inflammatory effects in another *in vivo* AD model following administration of cannabidiol, which does not bind to CB_2 (Esposito *et al.*, 2007b).

The unifying hypothesis encompassing most of these studies is that pathologic changes in endocannabinoid levels and CB_2 expression are induced by the inflammatory environment which occurs in AD. Activation of CB_2 by up-regulated endocannabinoids goes some way towards halting microglial activation; however, this innate compensation is insufficient to prevent the subsequent inflammatory damage to neurons, which may also suffer from a loss of protection due to the down-regulation of CB_1. On the basis of the pre-clinical efficacy already demonstrated, cannabinoid stimulators may have therapeutic benefit by augmenting the brain's innate response.

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory CNS disease which in contrast to the other neurodegenerative disorders reviewed here, presents most frequently in early adulthood (Liguori *et al.*, 2000). The CNS infiltration of autoreactive T cells, with specificity for myelin or other CNS proteins, is followed by their clonal expansion. Other immune mediators are recruited, including B cells and microglia, and together with T cells differentiated into 'cytotoxic' effectors, cause demyelination of neurons of the brain and spinal cord (Friese and Fugger, 2005). This is particularly catastrophic for motor and sensory function, and symptoms of MS include spasticity, hyperreflexia, pain and sensory disturbance (Noseworthy *et al.*, 2000). Focal inflammatory lesions or 'plaques' in the white matter are characterized by clusters of immune cells causing axonal demyelination and destruction, death of oligodendrocytes and bystander neurons, and a sclerotic astroglial 'scar' (Frohman *et al.*, 2006). Aetiology of the disease is enigmatic; however, correlations have been found between MS and birth/residence in non-equatorial countries, viral infection and mutations in several genes with immune function (Marrie, 2004; Compston and Coles, 2008). The 1-year prevalence for MS is approximately 0.9 per 1000 (Hirtz *et al.*, 2007). The majority of MS cases (~85%) are initially of relapsing-remitting nature, where occasional 'attacks' are followed by periods of remission where no progression of disease occurs and indeed damage sustained during the attack may be partially or completely repaired (Lublin and Reingold, 1996). The other subtypes of MS; primary or secondary progressive (subsequent to relapsing-remitting disease), and progressive relapsing, are characterized by progressive decline in motor function with infrequent or no remission respectively (Lublin and Reingold, 1996).

Endocannabinoid system changes in human MS

Both CB_1- and CB_2-expressing cells are reported to collect around plaques in human MS (Benito *et al.*, 2007). CB_1-expressing oligodendrocytes, their precursors, and macrophages, have been identified clustered around active lesions, and CB_1-positive perivascular T cells are also evident in MS. CB_2 expression has been localized to plaque-associated microglia and macrophages, nearby astrocytes, and perivascular T cells. FAAH was detected in plaque-associated astrocytes (Benito *et al.*, 2007). In the same study neuronal CB_1 expression was noted to appear more intense in areas of demyelination, although the authors suggested increased CB_1 epitope availability rather than *bona fide* up-regulation.

Interestingly, an up-regulation of CB_1 and CB_2 levels has also been detected in blood sampled from primary progressive MS patients, suggesting that peripheral immune regulation of the endocannabinoid system paralleled that occurring in the human brain (Jean-Gilles *et al.*, 2009).

Endocannabinoid levels in human MS patients have been investigated in several studies. The first of these reported a significant increase in AEA, but not 2-AG levels, in the CSF of relapsing-remitting MS patients experiencing current relapse, with a strong correlation between AEA levels and the number of inflammatory lesions visible on imaging (Centonze *et al.*, 2007). Another recent study also found elevated AEA levels compared with controls in MS patients across the clinical spectrum, this time in the plasma, again suggesting that the peripheral endocannabinoid system may be subject to similar processes to those occurring centrally (Jean-Gilles *et al.*, 2009). In contrast, Di Filippo *et al.* (2008) reported a deficit in all endocannabinoids tested (AEA, 2-AG, PEA and OEA) in the CSF of MS patients compared with controls. Interestingly, despite this underlying hypofunctionality, the authors suggest that the inducible nature of the endocannabinoid system was retained. Compared with patients in remission, endocannabinoid levels were higher (though still below those of controls) in the subset of patients currently in relapse, and higher again in patients with active lesions (Di Filippo *et al.*, 2008). This is in keeping with an earlier report which demonstrated that AEA was released from both silent human MS lesions, and to a greater degree from active lesions (Eljaschewitsch *et al.*, 2006). All three of these studies suggest a relationship between disease state and the regulation of endocannabinoid levels, and demonstrate induction of the endocannabinoid system by inflammatory cues or neuronal activity. FAAH, which is expressed by macrophages (Di Marzo *et al.*, 1999; Di Marzo *et al.* 1998), platelets (Maccarrone *et al.*, 2000) and mast cells (Maccarrone *et al.*, 2000) in blood, showed decreased expression in secondary progressive MS and trended towards a decrease in relapsing-remitting and primary progressive MS patient blood (Jean-Gilles *et al.*, 2009). Decreased expression of the inactivating enzyme FAAH may underpin the elevations in AEA detected in human blood and CSF described earlier.

Endocannabinoid changes in animal models of MS

In the experimental autoimmune encephalomyelitis (EAE) model, reductions in the order of 25–65% in AEA and 2-AG have been detected in motor (striatum, midbrain) and other brain areas (brainstem, hippocampus and cerebral cortex); and in the midbrain, diencephalon and limbic forebrain respectively (Cabranes *et al.*, 2005). Another group reported no change in brain levels of either endocannabinoid in the same model (Witting *et al.*, 2006). EAE is induced in rats by inoculation with immunogenic myelin components, causing significant inflammation and infiltration of mononuclear cells into the spinal cord, while infiltrated lesions in the brain, as seen in human MS, are rare (Berrendero *et al.*, 2001; Cabranes *et al.*, 2005). It has been suggested that these differences from the pattern of human MS, and the acute nature of EAE, make it a poor model of MS, in which the majority of patients show chronic relapse/remission (Sriram and Steiner, 2005). Indeed, this model may not appropriately recapitulate the acute attack phase either, as the reported reductions in endocannabinoid levels in EAE rats are in conflict with the relapse-induced elevations in endocannabinoids seen in human MS patients.

In the chronic relapsing experimental allergic encephalomyelitis (CREAE) model, induced by sensitizing specific strains of mice, with foreign CNS myelin, AEA and 2-AG were increased in the brain (~19% and ~70% respectively) and spinal cord (~200% and ~70% respectively) of spastic mice compared with non-spastic and control mice (Baker *et al.*, 2001). CREAE mice, like EAE rats, also show a lack of infiltrating lesions of the brain, despite abundant lesions in the spinal cord (Baker *et al.*, 1990). However, findings from the CREAE model are more in keeping with the trends in human multiple sclerosis patients, and together these studies suggest that 'on-demand' production of endocannabinoids may occur in response to relapse/lesion.

In the EAE model the changes in endocannabinoid levels and receptor expression conflict somewhat with those described in the human brain and periphery. A reduction in CB_1 expression in the cerebral cortex and striatum has been detected in EAE rats (Berrendero *et al.*, 2001; Centonze *et al.*, 2007). Interestingly, given the decrease in endocannabinoid levels described above for EAE models, an increase in both the AEA biosynthetic (NAPE-PLD) and inactivation (FAAH) enzymes has been identified in both EAE mouse striatum and in the lymphocytes of humans with current relapse of MS (Centonze *et al.*, 2007). Also of interest, CREAE mice, which more closely recapitulated the endocannabinoid changes seen in the acute phases of human MS than did EAE rats, showed a pattern of CB_1 reduction that was remarkably similar to that in EAE rats. Despite these conflicting patterns one may conclude that: i) that components of the endocannabinoid system are altered in multiple sclerosis and models thereof; and ii) that those components may be altered independently of one another.

Cannabinoid agents as therapeutics in MS

Sativex, an oromucosal spray delivering Δ^9-THC and cannabidiol, is licensed for use in multiple sclerosis in several countries, following demonstrations of its efficacy as a treatment for symptoms of neuropathic pain and disturbed sleep (Rog *et al.*, 2005; Rog *et al.*, 2007; and reviewed in Wade *et al.*, 2006). It is generally well-tolerated even with long-term use. The large majority of adverse events reported in trials have been mild to moderate and the use of Sativex has not been correlated with any decline in cognitive measures in MS patients (Rog *et al.*, 2007; Aragona *et al.*, 2009; reviewed in Wade *et al.*, 2006; Smith, 2007). Sativex may be useful not only in the control of pain, but also spasticity, as several trials have found. A 2007 study of 189 patients found nearly twice the reduction in spasticity in patients taking Sativex compared with placebo over a 6-week period (Collin *et al.*, 2007). A more recent 6-week Sativex study failed to detect any clinical improvement, alterations to NAPE-PLD or FAAH enzyme activity, or receptor expression; however, significant findings may have been limited by the small scale of the study (20 patients) (Centonze *et al.*, 2009).

Similar findings have emerged from trials delivering synthetic or plant-derived Δ^9-THC (dronabinol or nabilone respectively). Meta-analysis of three such trials found these cannabinoids to significantly reduce neuropathic and spasticity-induced pain (Karst *et al.*, 2003; Wade *et al.*, 2003; Svendsen *et al.*, 2004; Iskedjian *et al.*, 2007; and see Wissel *et al.*, 2006). Those studies, and a large 15-week clinical trial for oral Δ^9-THC, found no measurable improvement in motor function or spasticity itself, although Δ^9-THC-treated patients perceived improvement (Zajicek *et al.*, 2003). A 12-month follow-up to this large trial showed that perceived improvement now correlated with a measurable reduction in spasticity in patients treated with Δ^9-THC, and to a lesser degree, those treated with cannabis extract (Zajicek *et al.*, 2005).

Cannabinoids and endocannabinoid modulation have also elicited a therapeutic response in the EAE, CREAE and Theiler's murine encephalomyelitis virus (TMEV) models of MS. In the EAE model, the endocannabinoid/endovanilloid agonist arvanil and the AMT inhibitors AM404 and OMDM2 reduced neurological decline, while VDM11 and UCM707, also AMT inhibitors, did not (Cabranes *et al.*, 2005; de Lago *et al.*, 2006). While AM404 and arvanil are agonists at the vanilloid TRPV1 receptor, the protective OMDM2 has virtually no efficacy at TRPV1 (Ortar *et al.*, 2003) and indeed the TRPV1-selective agonist capsaicin was unable to ameliorate the neurological deficit in these mice, indicating that protection more likely occurred via cannabinoid than vanilloid receptors (Cabranes *et al.*, 2005). In the CREAE model, limb spasticity was attenuated by each of the endocannabinoids AEA, 2-AG or PEA, or exogenous cannabinoids or cannabinoid modifiers WIN, AM404, AM374, VDM11, OMDM1, OMDM2 or UCM707 (Baker *et al.*, 2001; de Lago *et al.*, 2006; de Lago *et al.* 2004). It appears that both CB_1 and CB_2 receptors may have a role in this improved symptomatic profile. While the CB_1-selective antagonist SR141716A more efficiently antagonized the improvement in spasticity and tremor, the CB_2-selective ligand JWH-133 was also able to reduce spasticity, at a dose which was sub-effective via CB_1 (Baker *et al.*, 2000).

In the much-studied TMEV mice, endocannabinoid augmentation has been shown to attenuate inflammatory events such as the differentiation of myelin-specific T cells, the production of pro-inflammatory mediators (TNF-α, nitric oxide, interleukin-1β and interleukin-6) and the activation and infiltration of microglia, leading to a slowed progression of the experimental disease (Croxford and Miller, 2003; Ortega-Gutierrez *et al*., 2005). As Ortega-Gutierrez *et al*. (2005) found these effects to be only partially blocked by a combination of selective CB_1 and CB_2 antagonists, additional, non-CB_1/CB_2 receptors may also be involved. A recent study suggests inhibition of the TASK1 channel by AEA may play an important role in the regulation of T cells in EAE (Bittner *et al*., 2009). Several studies have also implicated the PPAR-γ receptor as contributing to the therapeutic profile of some cannabinoid agonists (Mestre *et al*., 2009; Loria *et al*., 2010).

Most of these studies have shown cannabinoids, via CB_2, to control the runaway inflammatory cascade which initiates neuronal damage in MS. However, as suggested by Benito *et al*. (2007), CB_1 may also help to limit excitotoxic damage to neurons, by suppressing both the neuronal release of glutamate and the neuronal depolarization response to glutamate. Maresz *et al*. (2007) attributed the neuroprotective properties of Δ^9-THC in multiple sclerosis to the activation of both CB_2 receptors expressed by T cells and CB_1 receptors expressed by neurons (Maresz *et al*., 2007).

In addition to potentially preventing inflammatory and excitotoxic damage in MS, cannabinoids may also have a role in promoting repair of the axonal myelin sheath. Several studies have indicated that cannabinoids, via CB_1 or CB_2 (or both), may regulate myelination in the developing brain (Arevalo-Martin *et al*., 2007), the normal adult brain (Kittler *et al*., 2000) and the inflamed brain in the TMEV model of MS (Arevalo-Martin *et al*., 2003). The increased remyelination seen in the TMEV model may reflect the ability of cannabinoids to reduce inflammatory mediators which retard remyelination processes. Alternatively, the cannabinoids may have a *bona fide* stimulatory effect upon myelination, by enhancing the survival (Molina-Holgado *et al*., 2002), migration and differentiation towards an oligodendrocyte fate (Arevalo-Martin *et al*., 2007) of oligodendrocyte progenitor cells in the inflamed brain. If these exciting findings of cannabinoid-mediated attenuation of inflammation, stimulation of remyelination, and behavioural and symptomatic recovery translate from model systems to humans, cannabinoids may be promising therapeutics in MS.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative cause of adult death, after Alzheimer's disease and Parkinson's disease (Nicholson *et al*., 2000). ALS results in the degeneration of motor neurons in the cortex, brainstem and spinal cord (Brown, 1997; Nicholson *et al*., 2000). Most causes of ALS are presently unknown and several mechanisms of insult to motor neurons have been suggested (Ludolph *et al*., 2000; Robberecht, 2000; Cleveland and Rothstein, 2001). Two of the primary theories underlying motor neuron vulnerability are susceptibility to excitotoxicity and oxidative damage, including neuroinflammation (Ludolph *et al*., 2000; Robberecht, 2000).

Further insights into the aetiology of sporadic ALS, responsible for 90% of the diagnosed cases of the disease, have come from studies of regulation of glutamate activity. Plasma, CSF and post-mortem brain tissue from patients with ALS showed significant increases in glutamate levels (Plaitakis and Caroscio, 1987; Rothstein *et al*., 1990). Thus, glutamate may be the (or one of the) neurotoxic agent(s) reported in serum from ALS patients (Wolfgram and Myers, 1973). Rothstein *et al*. (Rothstein *et al*. 1992) demonstrated impaired glutamate uptake in vesicular preparations from post-mortem spinal cord and motor cortex of ALS patients. They subsequently confirmed a loss of function of EAAT2 expressed exclusively by glia, in the motor cortex and spinal cord of 60–70% of sporadic ALS patients (Rothstein *et al*., 1995). Importantly, compounds that act on the endocannabinoid system have the potential to reduce excitotoxic and oxidative cell damage as well as neuroinflammation (Hampson *et al*., 1998; Carter and Rosen, 2001; Walter and Stella, 2004b).

Endocannabinoid system changes in human ALS

To date there have been few studies on the endocannabinoid system in human ALS. Changes to the endocannabinoid system in ALS may reflect the neuroinflammatory component of disease pathogenesis. In the spinal cord of human ALS patients, areas of motor neuron damage were marked by an increased cohort of CB_2-positive microglia/macrophages (Yiangou *et al*., 2006b).

Endocannabinoid changes in animal models of ALS

Mutations in Cu/Zn superoxide dismutase (hSOD1) are the primary cause of up to 20% of familial ALS cases (Rosen *et al*., 1993). Over 100 different base-pair substitutions have been documented in human patients to date. It should be noted that familial ALS constitutes only 10% of ALS cases, nonetheless, to date hSOD1 mutations are one of the few aetiologies established for the disease. Until recently, no animal model accurately reflected the pathology of ALS. Now, transgenic mice expressing human SOD1 mutations have been generated (hSOD1G93A, hSOD1G85R, hSOD1G37R) (Gurney *et al*., 1994; Wong *et al*., 1995; Bruijn *et al*., 1997). The three mutant mouse strains have slightly different pathologies; however, they exhibit pathologic and cytologic motor neuron degeneration similar to patients with familial as well as sporadic ALS. Transgenic mice over-expressing wild-type (WT) hSOD1 and mice lacking hSOD1 do not show these disease signs (Bruijn *et al*., 1998). The hSOD1G93A mice are the strain predominantly used for preclinical testing of compounds for treating ALS; the disease in these animals follows a consistent onset, progression and outcome that closely mimics human ALS (Gurney *et al*., 1996; Klivenyi *et al*., 1999; Zhu *et al*., 2002).

Recent studies have described the involvement of the endocannabinoid system in the progression of disease in ALS mice and the benefits associated with the administration of cannabinoid agonists (Raman *et al*., 2004; Witting *et al*., 2004; Weydt *et al*., 2005; Bilsland *et al*., 2006; Kim *et al*., 2006; Yiangou *et al*., 2006a; Shoemaker *et al*., 2007). AEA and 2-AG accumulate in the lumbar spinal cord of ALS mice during disease progression and are presumed to be part of an endogenous defence mechanism (Witting *et al*., 2004; Bilsland *et al*., 2006).

Unlike in human tissue, increased CB_2 immunoreactivity was not detected in G93ASOD1 mice; however, there was a pre-symptomatic decrease, followed by a symptomatic increase, in CB_1 mRNA and protein compared with wildtype animals (Zhao *et al*., 2008). Up-regulation of CB_1 may underpin the findings of a recent study which demonstrated heightened control of both inhibitory and excitatory transmission by striatal CB_1 in symptomatic G93ASOD1 mice (Rossi *et al*., 2009).

Cannabinoid agents as therapeutics in ALS

Support for a protective role of the endocannabinoid system comes from *in vivo* studies in ALS mice. Interestingly, treatment with Δ^9-THC was effective if administered either before or after onset of signs in the ALS mouse model (Raman *et al*., 2004). Administration at the onset of tremors delayed motor impairment by 6% and prolonged survival by 5% in Δ^9-THC treated mice when compared with vehicle controls. This delay, while modest, is similar to that seen with ceftriaxone (Rothstein *et al*., 2005), which is in a clinical trial for ALS.

Bilsland *et al*. (2006) found a significant delay in disease progression when another cannabinoid agonist WIN55,212-2 was administered to ALS mice beginning after symptom onset (at 90 days of age); however, survival was not extended. Importantly, functional motor unit survival was enhanced in the cannabinoid-treated animals at 120 days, a relatively late stage in disease; these functional studies were supported by evaluating motor neuron numbers in the treated animals (Bilsland *et al*., 2006). FAAH knockout mice, which have increased AEA levels due to the lack of its hydrolytic enzyme, crossed with ALS mice also showed improvement in motor neuron survival, supporting the theory that endocannabinoids are neuroprotective in ALS (Bilsland *et al*., 2006).

Deletion of CB_1 in ALS mice (in the ABH strain background), while not altering motor neuron survival, extended lifespan by 15 days, a 13% increase in survival (Bilsland *et al*., 2006). It will be important to define the role of CB_2, and the relationship between CB_1 and CB_2, in modifying disease progression in a single standard background.

Microglia from ALS mice possess increased cytotoxic potential (Weydt *et al*., 2004). CB_2 activation blocks β-amyloid-induced microglial activation (Ramirez *et al*., 2005). Conversely, with other stimuli, CB_2 activation can increase microglial migration and proliferation (Walter *et al*., 2003; Carrier *et al*., 2004). One study using a selective CB_2 agonist, AM1241, in ALS mice showed slowing of disease progression when administered after disease onset (Kim *et al*., 2006). A second study using a different dosing paradigm found that a dose of $3\ mg\cdot kg^{-1}$ AM1241 produced a 56% increase in survival interval (an 11% increase in lifespan) (Shoemaker *et al*., 2007). A recent study in human ALS patients demonstrated increased CB_2 immunostaining in activated microglia from spinal cord (Yiangou *et al*., 2006a). These results suggest that CB_2-mediated processes may modify disease progression in ALS.

An important consideration for treatment is that ALS is a chronic disease therefore long-term toxicity of treatment drugs becomes an important issue. Δ^9-THC is well tolerated and already in clinical usage for nausea associated with cancer chemotherapy and appetite stimulation with the AIDS wasting syndrome. In a pilot study of the safety and tolerability of Δ^9-THC in ALS patients, symptomatic benefits were seen in insomnia, appetite and spasticity (Gelinas *et al*., 2002). Other endocannabinoid compounds may have a similar tolerability profile without the psychotropic side effects found with Δ^9-THC. If they are effective in a pre-clinical model of ALS, they could be evaluated for its effectiveness in the human disease. Cannabinoids may prove to be novel therapeutic targets for the treatment of ALS.

Parkinson's disease

Parkinson's disease (PD) is characterized by muscle rigidity, tremor and a slowing of physical movement (bradykinesia). The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, due to death of dopaminergic neurons of the substantia nigra. Secondary symptoms may include high level cognitive dysfunction and subtle language problems. The neuromodulatory effects of the endocannabinoid system are of particular importance to the dopaminergic system, which in turn exerts reciprocal regulation upon the endocannabinoid system. For example, CB_1 and D1/D2-like receptors are co-localized in striatal neurons (Hohmann and Herkenham, 2000; Hermann *et al*., 2002) and exhibit complex signalling interactions (Glass and Felder, 1997; Meschler and Howlett, 2001; Kearn *et al*., 2005). Endocannabinoids and FAAH inhibitors influence the firing activity of dopaminergic neurons through PPARα (Melis *et al*., 2008) and TRPV1 receptors (Marinelli *et al*., 2003). Cannabinoid CB_1-mediated effects on dopamine release are complex; AEA has been demonstrated to reduce dopamine release in striatal slice cultures (Cadogan *et al*., 1997), and to increase dopamine release in the nucleus accumbens *in vivo* (Cheer *et al*., 2004; Solinas *et al*., 2006). Likewise, activation of dopamine D2 receptors has been demonstrated to increase AEA levels in the basal ganglia (Giuffrida *et al*., 1999; Ferrer *et al*., 2003).

Endocannabinoid changes in human PD

In Parkinsonian tissue the level of CB_1 mRNA has been shown to be decreased in the caudate nucleus, anterior dorsal putamen and external segment of the globus pallidus (Hurley *et al*., 2003). In contrast to this, an increase in CB_1 binding in the caudate nucleus and the putamen has been observed by others (Lastres-Becker *et al*., 2001a). These studies are complicated to interpret as all patients have undergone drug treatment, and the effects of drug treatment on the cannabinoid system are not clear. To date endocannabinoids levels have only been investigated in one study. Pisani *et al*. showed AEA levels in the CSF of PD patients (either untreated or undergoing drug-washout) were more than twice that of controls (Pisani *et al*., 2005).

Endocannabinoid changes in animal models of PD

Conflicting data exist around the alterations in both cannabinoid receptors and endocannabinoids in models of Parkinson's disease. Increased CB_1 binding and CB_1 mRNA levels have been reported in the brain of MPTP-treated primates (Lastres-Becker *et al*., 2001a) and in rats with 6-OHDA lesions (Romero *et al*., 2000), however, others have seen no alteration in receptor levels (Zeng *et al*., 1999). Decreased (Giuffrida *et al*., 1999) and increased AEA (Gubellini *et al*., 2002) have both been observed following 6-OHDA lesion, while dopamine depletion has been shown to elevate 2-AG levels in rat globus pallidus following reserpine treatment (Di Marzo *et al*., 2000). In MPTP non-human primate models, both AEA and 2AG have been demonstrated to be elevated (van der Stelt *et al*., 2005). A recent study has suggested that in models of PD, indirect-pathway endocannabinoid-mediated long-term depression is absent but is rescued by a D2 receptor agonist or inhibitors of endocannabinoid degradation, consistent with the suggestion of decreased endocannabinoid tone in 6-OHDA-treated rats (Kreitzer and Malenka, 2007). Interestingly, administration of levodopa, a dopamine precursor that is the mainstay treatment for PD, does not elevate AEA in the basal ganglia of 6-OHDA-treated rats (Giuffrida *et al*., 1999), but does lead to up-regulation of striatal CB_1 receptors (Zeng *et al*., 1999) in the dopamine-depleted striatum. Collectively, these observations indicate that degeneration of nigro-striatal projections dramatically affects endocannabinoid transmission although the precise nature of these changes may depend on the model utilized and the timeframe of the observations.

Cannabinoid agents as therapeutics in PD

Studies on the potential therapeutic utility of cannabinoid agonists and antagonists in PD have also produced conflicting results. In several studies of MPTP-treated primates, rimonabant and other cannabinoid antagonists failed to alleviate the motor deficits of parkinsonism (Meschler and Howlett, 2001; Mesnage *et al*., 2004; Cao *et al*., 2007), although Cao *et al*. (2007) found increased responses to suboptimal doses of levodopa in the presence of the antagonist. However, in contrast to these studies, motor activity was improved with rimonabant following both MPTP treatment in a primate (van der Stelt *et al*., 2005) and in a recent 6-OHDA rodent lesion study (Kelsey *et al*., 2009). This recent study has also found improved responses to low (but not high) levodopa concentrations in the presence of sub-optimal concentrations of rimonabant (Kelsey *et al*., 2009). Levodopa-induced dyskinesias (LID), a disabling motor complication resulting from long-term use of levodopa, are however, alleviated by activation of CB_1 (Ferrer *et al*., 2003; Morgese *et al*., 2009), and recent data suggest that this is through CB_1-mediated alterations in dopamine and glutamate outputs (Morgese *et al*., 2009). FAAH inhibitors failed to produce an anti-dyskinetic effect when administered alone in levodopa-treated 6-OHDA-lesioned rats (Morgese *et al*., 2007), suggesting that AEA elevation is not sufficient to attenuate LID. Interpretation of this finding, however, is complicated by the finding that URB597 in combination with the TRPV1 antagonist capsazepine produced a significant anti-dyskinetic effect, suggesting that the beneficial actions of CB_1 stimulation on LID may be counteracted by TRPV1 agonism (Morgese *et al*., 2007). Furthermore, Lee *et al*. (2006) showed that URB597 alone or stimulation of TRPV1 receptors by capsaicin can attenuate levodopa-induced hyperactivity in reserpine-treated rats. Whether these discrepancies are due to the different animal models and/or the type of behavioural measure used remains to be clarified.

As for the animal studies, drug trials in humans have produced conflicting results. In a randomized, double-blind, placebo-controlled, crossover trial (n = 7), the cannabinoid receptor agonist nabilone significantly reduced LID in PD (Sieradzan *et al*., 2001). In contrast, in a seventeen patient double-blind, cross-over study, cannabis, while well tolerated, had no effect on LID (Carroll *et al*., 2004).

Conclusion

An overarching paradigm in the diseases summarized in this review is that hypofunction or dysregulation of the endocannabinoid system may be responsible for some of the symptomatology of these diseases. In Huntington's disease, Alzheimer's disease, as well as in ALS, pathologic changes in endocannabinoid levels and CB_2 expression are induced by the inflammatory environment. Activation of CB_2 by up-regulated endocannabinoids goes some way towards halting microglial activation; however, this innate compensation is insufficient to prevent the subsequent inflammatory damage to neurons, which may also suffer from the loss of protection conferred by the down-regulated CB_1 in HD and AD. In multiple sclerosis, cannabinoids have shown promise in animal models. Furthermore, Sativex, an oromucosal spray delivering Δ^9-THC and cannabidiol, is licensed for use in multiple sclerosis in several countries, following demonstrations of its efficacy as a treatment for symptoms of neuropathic pain and disturbed sleep. If the exciting findings of cannabinoid-mediated attenuation of inflammation, stimulation of remyelination, and behavioural and symptomatic recovery translate from model systems to humans, cannabinoids may be promising therapeutics in MS.

However, there are conflicting data emerging from animal models of all of the diseases, which highlight the need for better models. In addition, improved technologies for studying human disease as it progresses (rather than just *post-mortem*) will be critical for evaluating the therapeutic potential of any new treatments, including cannabinoids. While alterations in the endocannabinoid system in a range of diseases has led to speculation that the endocannabinoid system is intricately involved in the pathology of these disorders, the extensive cell loss and inflammatory environment that characterizes these diseases makes it difficult to ascertain whether the alterations are simply the result of the pathological process. Whether integral to the disease, or a symptom of it, the studies described in this review highlight the potential role that endocannabinoids may play in either protecting cells from the disease process, or treating the symptoms of the disease. CB_1 activation has been shown to be effective in limiting cell death following excitotoxic lesions, while CB_2 is involved in dampening inflammatory immune cell response to disease. These two targets may therefore work together to provide both neuroprotection to acute injury and immune suppression during more chronic responses. Modulation of endocannabinoid levels through targeting synthesis or degradation enzymes also holds promise for providing more temporally and regionally appropriate enhancement of cannabinoid activity. Cannabinoid agonists in human trials to date have been well tolerated and safe, but clearly psychoactivity following CB_1 activation is often an unacceptable consequence, particularly for long-term drug treatment; it is hoped that modulation of endocannabinoid levels may provide a more suitable alternative.

Conflict of interest

The authors declare no conflict of interest.

References

Alexander JP, Cravatt BF (2006). The putative endocannabinoid transport blocker LY2183240 is a potent inhibitor of FAAH and several other brain serine hydrolases. *J Am Chem Soc* **128**: 9699–9704.

Allen KL, Waldvogel HJ, Glass M, Faull RL (2009). Cannabinoid (CB(1)), GABA(A) and GABA(B) receptor subunit changes in the globus pallidus in Huntington's disease. *J Chem Neuroanat* **37**: 266–281.

Aragona M, Onesti E, Tomassini V, Conte A, Gupta S, Gilio F *et al*. (2009). Psychopathological and cognitive effects of therapeutic cannabinoids in multiple sclerosis: a double-blind, placebo controlled, crossover study. *Clin Neuropharmacol* **32**: 41–47.

Arevalo-Martin A, Garcia-Ovejero D, Rubio-Araiz A, Gomez O, Molina-Holgado F, Molina-Holgado E (2007). Cannabinoids modulate Olig2 and polysialylated neural cell adhesion molecule expression in the subventricular zone of post-natal rats through cannabinoid receptor 1 and cannabinoid receptor 2. *Eur J Neurosci* **26**: 1548–1559.

Arevalo-Martin A, Vela JM, Molina-Holgado E, Borrell J, Guaza C (2003). Therapeutic action of cannabinoids in a murine model of multiple sclerosis. *J Neurosci* **23**: 2511–2516.

Ashton JC, Friberg D, Darlington CL, Smith PF (2006). Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. *Neurosci Lett* **396**: 113–116.

Baker D, O'Neill JK, Gschmeissner SE, Wilcox CE, Butter C, Turk JL (1990). Induction of chronic relapsing experimental allergic encephalomyelitis in Biozzi mice. *J Neuroimmunol* **28**: 261–270.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman JW *et al*. (2000). Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* **404**: 84–87.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Makriyannis A *et al*. (2001). Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J* **15**: 300–302.

Battista N, Bari M, Tarditi A, Mariotti C, Bachoud-Levi AC, Zuccato C *et al*. (2007). Severe deficiency of the fatty acid amide hydrolase (FAAH) activity segregates with the Huntington's disease mutation in peripheral lymphocytes. *Neurobiol Dis* **27**: 108–116.

Beal MF, Brouillet E, Jenkins B, Henshaw R, Rosen B, Hyman BT (1993). Age-dependent striatal excitotoxic lesions produced by the endogenous mitochondrial inhibitor malonate. *J Neurochem* **61**: 1147–1150.

Begg M, Mo FM, Offertaler L, Batkai S, Pacher P, Razdan RK *et al*. (2003). G protein-coupled endothelial receptor for atypical cannabinoid ligands modulates a Ca2+-dependent K+ current. *J Biol Chem* **278**: 46188–46194.

Beltramo M, Bernardini N, Bertorelli R, Campanella M, Nicolussi E, Fredduzzi S *et al*. (2006). CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *Eur J Neurosci* **23**: 1530–1538.

Benito C, Kim WK, Chavarria I, Hillard CJ, Mackie K, Tolon RM *et al*. (2005). A glial endogenous cannabinoid system is upregulated in the brains of macaques with simian immunodeficiency virus-induced encephalitis. *J Neurosci* **25**: 2530–2536.

Benito C, Nunez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ *et al*. (2003). Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci* **23**: 11136–11141.

Benito C, Romero JP, Tolon RM, Clemente D, Docagne F, Hillard CJ *et al*. (2007). Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis. *J Neurosci* **27**: 2396–2402.

Berrendero F, Sanchez A, Cabranes A, Puerta C, Ramos JA, Garcia-Merino A *et al*. (2001). Changes in cannabinoid CB(1) receptors in striatal and cortical regions of rats with experimental allergic encephalomyelitis, an animal model of multiple sclerosis. *Synapse* **41**: 195–202.

Bilsland LG, Dick JR, Pryce G, Petrosino S, Di Marzo V, Baker D *et al*. (2006). Increasing cannabinoid levels by pharmacological and genetic manipulation delay disease progression in SOD1 mice. *Faseb J* **29**: 29.

Bisogno T, Berrendero F, Ambrosino G, Cebeira M, Ramos JA, Fernandez-Ruiz JJ *et al*. (1999). Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem Biophys Res Commun* **256**: 377–380.

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A *et al*. (2003). Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol* **163**: 463–468.

Bisogno T, Martire A, Petrosino S, Popoli P, Di Marzo V (2008). Symptom-related changes of endocannabinoid and palmitoylethanolamide levels in brain areas of R6/2 mice, a transgenic model of Huntington's disease. *Neurochem Int* **52**: 307–313.

Bittner S, Meuth SG, Gobel K, Melzer N, Herrmann AM, Simon OJ *et al*. (2009). TASK1 modulates inflammation and neurodegeneration in autoimmune inflammation of the central nervous system. *Brain* **132**: 2501–2516.

Bouaboula M, Bourrié B, Rinaldi-Carmona M, Shire D, Fur GL, Casellas P (1995). Stimulation of cannabinoid receptor CB1 induces Krox-24 expression in human astrocytoma cells. *J Biol Chem* **270**: 13973–13980.

Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpech B, Shire D *et al*. (1993). Cannabinoid-receptor expression in human leukocytes. *Eur J Biochem* **214**: 173–180.

Brown RH, Jr (1997). Amyotrophic lateral sclerosis. Insights from genetics. *Arch Neurol* **54**: 1246–1250.

Brown SM, Wager-Miller J, Mackie K (2002). Cloning and molecular characterization of the rat CB2 cannabinoid receptor. *Biochim Biophys Acta* **1576**: 255–264.

Bruijn L, Becher M, Lee M, Anderson K, Jenkins N, Copeland N *et al*. (1997). ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* **18**: 327–338.

Bruijn L, Houseweart M, Kato S, Anderson K, Anderson S, Ohama E *et al*. (1998). Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**: 1851–1854.

Cabranes A, Venderova K, de Lago E, Fezza F, Sanchez A, Mestre L *et al*. (2005). Decreased endocannabinoid levels in the brain and beneficial effects of agents activating cannabinoid and/or vanilloid receptors in a rat model of multiple sclerosis. *Neurobiol Dis* **20**: 207–217.

Cadas H, di Tomaso E, Piomelli D (1997). Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J Neurosci* **17**: 1226–1242.

Cadogan AK, Alexander SP, Boyd EA, Kendall DA (1997). Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. *J Neurochem* **69**: 1131–1137.

Cao X, Liang L, Hadcock JR, Iredale PA, Griffith DA, Menniti FS *et al*. (2007). Blockade of cannabinoid type 1 receptors augments the antiparkinsonian action of levodopa without affecting dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated rhesus monkeys. *J Pharmacol Exp Ther* **323**: 318–326.

Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K *et al*. (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Mol Pharmacol* **65**: 999–1007.

Carroll CB, Bain PG, Teare L, Liu X, Joint C, Wroath C *et al*. (2004). Cannabis for dyskinesia in Parkinson disease: a randomized double-blind crossover study. *Neurology* **63**: 1245–1250.

Carter GT, Rosen BS (2001). Marijuana in the management of amyotrophic lateral sclerosis. *Am J Hosp Palliat Care* **18**: 264–270.

Centonze D, Bari M, Rossi S, Prosperetti C, Furlan R, Fezza F *et al*. (2007). The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* **130**: 2543–2553.

Centonze D, Mori F, Koch G, Buttari F, Codeca C, Rossi S *et al*. (2009). Lack of effect of cannabis-based treatment on clinical and laboratory measures in multiple sclerosis. *Neurol Sci* **30**: 531–534.

Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM (2004). Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J Neurosci* **24**: 4393–4400.

Cleveland DW, Rothstein JD (2001). From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. *Nat Rev Neurosci* **2**: 806–819.

Collin C, Davies P, Mutiboko IK, Ratcliffe S (2007). Randomized controlled trial of cannabis-based medicine in spasticity caused by multiple sclerosis. *Eur J Neurol* **14**: 290–296.

Compston A, Coles A (2008). Multiple sclerosis. *Lancet* **372**: 1502–1517.

Consroe P, Laguna J, Allender J, Snider S, Stern L, Sandyk R *et al*. (1991). Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacol Biochem Behav* **40**: 701–708.

Coopman K, Smith LD, Wright KL, Ward SG (2007). Temporal variation in CB2R levels following T lymphocyte activation: evidence that cannabinoids modulate CXCL12-induced chemotaxis. *Int Immunopharmacol* **7**: 360–371.

Croxford JL, Miller SD (2003). Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212. *J Clin Invest* **111**: 1231–1240.

Dalton GD, Bass CE, Van Horn C, Howlett AC (2009). Signal Transduction via Cannabinoid Receptors. *CNS & Neurol Disord Drug Targets* **8**: 422–431.

van Dellen A, Blakemore C, Deacon R, York D, Hannan AJ (2000). Delaying the onset of Huntington's in mice. *Nature* **404**: 721–722.

Denovan-Wright EM, Robertson HA (2000). Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. *Neuroscience* **98**: 705–713.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al*. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**: 1946–1949.

Di Filippo M, Pini LA, Pelliccioli GP, Calabresi P, Sarchielli P (2008). Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **79**: 1224–1229.

Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Orlando P, Wagner JA *et al*. (1999). Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. *Eur J Biochem* **264**: 258–267.

Di Marzo V, Bisogno T, Sugiura T, Melck D, De Petrocellis L (1998). The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide. *Biochem J* **331**: 15–19.

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC *et al*. (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**: 686–691.

Di Marzo V, Hill MP, Bisogno T, Crossman AR, Brotchie JM (2000). Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *Faseb J* **14**: 1432–1438.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL *et al*. (2002). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A* **99**: 10819–10824.

Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C *et al*. (2002). Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. *Nat Neurosci* **5**: 452–457.

Dowie MJ, Bradshaw HB, Howard ML, Nicholson LF, Faull RL, Hannan AJ *et al*. (2009a). Altered CB1 receptor and endocannabinoid levels precede motor symptom onset in a transgenic mouse model of Huntington's disease. *Neuroscience* **163**: 456–465.

Dowie MJ, Bradshaw HB, Howard ML, Nicholson LF, Faull RL, Hannan AJ *et al*. (2009b). Molecular impacts of chronic cannabinoid treatment in Huntington's disease transgenic mice. *19th Annual Symposium of the International Cannabinoid Research Society*; St. Charles, Illinois.

Egertova M, Giang DK, Cravatt BF, Elphick MR (1998). A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. *Proc Biol Sci* **265**: 2081–2085.

Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y *et al*. (2005). Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* **2**: 29.

Eljaschewitsch E, Witting A, Mawrin C, Lee T, Schmidt PM, Wolf S *et al*. (2006). The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron* **49**: 67–79.

Ellert-Miklaszewska A, Grajkowska W, Gabrusiewicz K, Kaminska B, Konarska L (2007). Distinctive pattern of cannabinoid receptor type II (CB2) expression in adult and pediatric brain tumors. *Brain Res* **1137**: 161–169.

Elsohly MA, Slade D (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci* **78**: 539–548.

Esposito G, Iuvone T, Savani C, Scuderi C, De Filippis D, Papa M *et al*. (2007a). Opposing control of cannabinoid receptor stimulation on amyloid-beta-induced reactive gliosis: in vitro and in vivo evidence. *J Pharmacol Exp Ther* **322**: 1144–1152.

Esposito G, Scuderi C, Savani C, Steardo L, Jr, De Filippis D, Cottone P *et al*. (2007b). Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *Br J Pharmacol* **151**: 1272–1279.

Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A (1995). Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci U S A* **92**: 3376–3380.

Farooqui AA, Liss L, Horrocks LA (1988). Stimulation of lipolytic enzymes in Alzheimer's disease. *Ann Neurol* **23**: 306–308.

Fegley D, Kathuria S, Mercier R, Li C, Goutopoulos A, Makriyannis A *et al*. (2004). Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. *Proc Natl Acad Sci U S A* **101**: 8756–8761.

Felder C, Joyce K, Briley E, Mansouri J, Mackie K, Blond O *et al*. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol Pharmacol* **48**: 443–450.

Felder CC, Nielsen A, Briley EM, Palkovits M, Priller J, Axelrod J *et al*. (1996). Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. *FEBS Lett* **393**: 231–235.

Ferrer B, Asbrock N, Kathuria S, Piomelli D, Giuffrida A (2003). Effects of levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. *Eur J Neurosci* **18**: 1607–1614.

Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M *et al*. (2005). Global prevalence of dementia: a Delphi consensus study. *Lancet* **366**: 2112–2117.

Fowler CJ (2004). Oleamide: a member of the endocannabinoid family? *Br J Pharmacol* **141**: 195–196.

Friese MA, Fugger L (2005). Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? *Brain* **128**: 1747–1763.

Frohman EM, Racke MK, Raine CS (2006). Multiple sclerosis – the plaque and its pathogenesis. *N Engl J Med* **354**: 942–955.

Galiegue S, Mary S, Marchand J, Dussossoy D, Carriere D, Carayon P *et al*. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem* **232**: 54–61.

Gaoni Y, Mechoulam R (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* **86**: 1646–1647.

Garcia-Ovejero D, Arevalo-Martin A, Petrosino S, Docagne F, Hagen C, Bisogno T *et al*. (2009). The endocannabinoid system is modulated in response to spinal cord injury in rats. *Neurobiol Dis* **33**: 57–71.

Gelinas D, Miller R, Abood M (2002). A pilot study of safety and tolerability of Delta 9-THC (Marinol) treatment for ALS. *Amyotrophic Later Scler & Motor Neuron Disord* **3**: 23.

Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC *et al*. (2005). Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology* **64**: 1553–1562.

Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D (1999). Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat Neurosci* **2**: 358–363.

Glass M, Felder CC (1997). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci* **17**: 5327–5333.

Glass M, Dragunow M, Faull RL (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**: 299–318.

Glass M, Dragunow M, Faull RL (2000). The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* **97**: 505–519.

Glass M, van Dellen A, Blakemore C, Hannan AJ, Faull RL (2004). Delayed onset of Huntington's disease in mice in an enriched environment correlates with delayed loss of cannabinoid CB1 receptors. *Neuroscience* **123**: 207–212.

Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A *et al*. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res* **1071**: 10–23.

Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW (2000). Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol Pharmacol* **57**: 1045–1050.

Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S (1998). Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett* **422**: 69–73.

Greenamyre JT, Penney JB, Young AB, D'Amato CJ, Hicks SP, Shoulson I (1985). Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. *Science* **227**: 1496–1499.

Griffin G, Wray EJ, Tao Q, McAllister SD, Rorrer WK, Aung MM *et al*. (1999). Evaluation of the cannabinoid CB2 receptor-selective antagonist, SR144528: further evidence for cannabinoid CB2 receptor absence in the rat central nervous system. *Eur J Pharmacol* **377**: 117–125.

Gubellini P, Picconi B, Bari M, Battista N, Calabresi P, Centonze D *et al*. (2002). Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. *J Neurosci* **22**: 6900–6907.

Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F *et al*. (2004). Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci* **20**: 441–458.

Gurney ME, Cutting FB, Zhai P, Doble A, Taylor CP, Andrus PK *et al*. (1996). Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Ann Neurol* **39**: 147–157.

Gurney ME, Pu H, Chiu A, Canto MD, Polchow C, Alexander D *et al*. (1994). Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* **264**: 1772–1775.

Haga S, Akai K, Ishii T (1989). Demonstration of microglial cells in and around senile (neuritic) plaques in the Alzheimer brain. An immunohistochemical study using a novel monoclonal antibody. *Acta Neuropathol* **77**: 569–575.

Hampson A, Grimaldi M, Axelrod J, Wink D (1998). Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U S A* **95**: 8268–8273.

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE *et al*. (2001). 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci U S A* **98**: 3662–3665.

Hardy JA, Higgins GA (1992). Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**: 184–185.

Henstridge CM, Balenga NA, Ford LA, Ross RA, Waldhoer M, Irving AJ (2009). The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca2+ signaling and NFAT activation. *FASEB J* **23**: 183–193.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR *et al*. (1990). Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A* **87**: 1932–1936.

Hermann H, Marsicano G, Lutz B (2002). Coexpression of the cannabinoid receptor type 1 with dopamine and serotonin receptors in distinct neuronal subpopulations of the adult mouse forebrain. *Neuroscience* **109**: 451–460.

Hickman SE, Allison EK, El Khoury J (2008). Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J Neurosci* **28**: 8354–8360.

Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R (2007). How common are the 'common' neurologic disorders? *Neurology* **68**: 326–337.

Hock C, Konietzko U, Streffer JR, Tracy J, Signorell A, Muller-Tillmanns B *et al*. (2003). Antibodies against beta-amyloid slow cognitive decline in Alzheimer's disease. *Neuron* **38**: 547–554.

Hohmann AG, Herkenham M (2000). Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a double-label in situ hybridization study. *Synapse* **37**: 71–80.

Hoi PM, Hiley CR (2006). Vasorelaxant effects of oleamide in rat small mesenteric artery indicate action at a novel cannabinoid receptor. *Br J Pharmacol* **147**: 560–568.

Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F *et al*. (2002). An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci U S A* **99**: 8400–8405.

Hurley MJ, Mash DC, Jenner P (2003). Expression of cannabinoid CB1 receptor mRNA in basal ganglia of normal and parkinsonian human brain. *J Neural Transm* **110**: 1279–1288.

Iskedjian M, Bereza B, Gordon A, Piwko C, Einarson TR (2007). Meta-analysis of cannabis based treatments for neuropathic and multiple sclerosis-related pain. *Curr Med Res Opin* **23**: 17–24.

Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D (1989). Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J Neuroimmunol* **24**: 173–182.

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

Jean-Gilles L, Feng S, Tench CR, Chapman V, Kendall DA, Barrett DA *et al*. (2009). Plasma endocannabinoid levels in multiple sclerosis. *J Neurol Sci*.

Kaczocha M, Glaser ST, Deutsch DG (2009). Identification of intracellular carriers for the endocannabinoid anandamide. *Proc Natl Acad Sci U S A* **106**: 6375–6380.

Kaczocha M, Hermann A, Glaser ST, Bojesen IN, Deutsch DG (2006). Anandamide uptake is consistent with rate-limited diffusion and is regulated by the degree of its hydrolysis by fatty acid amide hydrolase. *J Biol Chem* **281**: 9066–9075.

Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS *et al*. (2009). Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem* **284**: 29817–29827.

Karst M, Salim K, Burstein S, Conrad I, Hoy L, Schneider U (2003). Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain: a randomized controlled trial. *JAMA* **290**: 1757–1762.

Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N *et al*. (2001). Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci* **21**: 9506–9518.

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K *et al*. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* **19**: 4544–4558.

Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D *et al*. (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* **26**: 5628–5637.

Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M (2005). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol Pharmacol* **67**: 1697–1704.

Kelsey JE, Harris O, Cassin J (2009). The CB(1) antagonist rimonabant is adjunctively therapeutic as well as monotherapeutic in an animal model of Parkinson's disease. *Behav Brain Res* **203**: 304–307.

Khaspekov LG, Brenz Verca MS, Frumkina LE, Hermann H, Marsicano G, Lutz B (2004). Involvement of brain-derived neurotrophic factor in cannabinoid receptor-dependent protection against excitotoxicity. *Eur J Neurosci* **19**: 1691–1699.

Kim J, Alger BE (2004). Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nat Neurosci* **7**: 697–698.

Kim K, Moore DH, Makriyannis A, Abood ME (2006). AM1241, a cannabinoid CB2 receptor selective compound, delays disease progression in a mouse model of amyotrophic lateral sclerosis. *Eur J Pharmacol* **542**: 100–105.

Kittler JT, Grigorenko EV, Clayton C, Zhuang SY, Bundey SC, Trower MM *et al*. (2000). Large-scale analysis of gene expression changes during acute and chronic exposure to [Delta]9-THC in rats. *Physiol Genomics* **3**: 175–185.

Klegeris A, Bissonnette CJ, McGeer PL (2003). Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. *Br J Pharmacol* **139**: 775–786.

Klein TW (2005). Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* **5**: 400–411.

Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L *et al*. (2003). The cannabinoid system and immune modulation. *J Leukoc Biol* **74**: 486–496.

Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA *et al*. (1999). Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med* **5**: 347–350.

Koppel J, Bradshaw H, Goldberg T, Khalili H, Marambaud P, Walker M *et al*. (2009). Endocannabinoids in Alzheimer's disease and their impact on normative cognitive performance: a case-control and cohort study. *Lipids Health Dis* **8**: 2.

Kowall NW, Beal MF, Busciglio J, Duffy LK, Yankner BA (1991). An in vivo model for the neurodegenerative effects of beta amyloid and protection by substance P. *Proc Natl Acad Sci U S A* **88**: 7247–7251.

Kreitzer AC, Malenka RC (2007). Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* **445**: 643–647.

Kreutz S, Koch M, Bottger C, Ghadban C, Korf HW, Dehghani F (2009). 2-Arachidonoylglycerol elicits neuroprotective effects on excitotoxically lesioned dentate gyrus granule cells via abnormal-cannabidiol-sensitive receptors on microglial cells. *Glia* **57**: 286–294.

de Lago E, Fernandez-Ruiz J, Ortega-Gutierrez S, Cabranes A, Pryce G, Baker D *et al*. (2006). UCM707, an inhibitor of the anandamide uptake, behaves as a symptom control agent in models of Huntington's disease and multiple sclerosis, but fails to delay/arrest the progression of different motor-related disorders. *Eur Neuropsychopharmacol* **16**: 7–18.

de Lago E, Ligresti A, Ortar G, Morera E, Cabranes A, Pryce G *et al*. (2004). In vivo pharmacological actions of two novel inhibitors of anandamide cellular uptake. *Eur J Pharmacol* **484**: 249–257.

Lambert DM, DiPaolo FG, Sonveaux P, Kanyonyo M, Govaerts SJ, Hermans E *et al*. (1999). Analogues and homologues of N-palmitoylethanolamide, a putative endogenous CB(2) cannabinoid, as potential ligands for the cannabinoid receptors. *Biochim Biophys Acta* **1440**: 266–274.

Lastres-Becker I, Berrendero F, Lucas JJ, Martin-Aparicio E, Yamamoto A, Ramos JA *et al*. (2002a). Loss of mRNA levels, binding and activation of GTP-binding proteins for cannabinoid CB1 receptors in the basal ganglia of a transgenic model of Huntington's disease. *Brain Res* **929**: 236–242.

Lastres-Becker I, Bizat N, Boyer F, Hantraye P, Brouillet E, Fernandez-Ruiz J (2003a). Effects of cannabinoids in the rat model of Huntington's disease generated by an intrastriatal injection of malonate. *Neuroreport* **14**: 813–816.

Lastres-Becker I, Bizat N, Boyer F, Hantraye P, Fernandez-Ruiz J, Brouillet E (2004). Potential involvement of cannabinoid receptors in 3-nitropropionic acid toxicity in vivo. *Neuroreport* **15**: 2375–2379.

Lastres-Becker I, Cebeira M, de Ceballos ML, Zeng BY, Jenner P, Ramos JA *et al*. (2001a). Increased cannabinoid CB1 receptor binding and activation of GTP-binding proteins in the basal ganglia of patients with Parkinson's syndrome and of MPTP-treated marmosets. *Eur J Neurosci* **14**: 1827–1832.

Lastres-Becker I, de Miguel R, De Petrocellis L, Makriyannis A, Di Marzo V, Fernandez-Ruiz J (2003b). Compounds acting at the endocannabinoid and/or endovanilloid systems reduce hyperkinesia in a rat model of Huntington's disease. *J Neurochem* **84**: 1097–1109.

Lastres-Becker I, Fezza F, Cebeira M, Bisogno T, Ramos JA, Milone A *et al*. (2001b). Changes in endocannabinoid transmission in the basal ganglia in a rat model of Huntington's disease. *Neuroreport* **12**: 2125–2129.

Lastres-Becker I, Hansen HH, Berrendero F, De Miguel R, Perez-Rosado A, Manzanares J *et al*. (2002b). Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. *Synapse* **44**: 23–35.

Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci U S A* **105**: 2699–2704.

Lee J, Di Marzo V, Brotchie JM (2006). A role for vanilloid receptor 1 (TRPV1) and endocannabinnoid signalling in the regulation of spontaneous and L-DOPA induced locomotion in normal and reserpine-treated rats. *Neuropharmacology* **51**: 557–565.

Leggett JD, Aspley S, Beckett SR, D'Antona AM, Kendall DA (2004). Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. *Br J Pharmacol* **141**: 253–262.

Liguori M, Marrosu MG, Pugliatti M, Giuliani F, De Robertis F, Cocco E *et al*. (2000). Age at onset in multiple sclerosis. *Neurol Sci* **21**: S825–S829.

Loria F, Petrosino S, Hernangomez M, Mestre L, Spagnolo A, Correa F *et al*. (2010). An endocannabinoid tone limits excitotoxicity in vitro and in a model of multiple sclerosis. *Neurobiol Dis* **37**: 166–176.

Lublin FD, Reingold SC (1996). Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology* **46**: 907–911.

Ludolph AC, Meyer T, Riepe MW (2000). The role of excitotoxicity in ALS – what is the evidence? *J Neurol* **247**: 17–16.

McAllister SD, Glass M (2002). CB(1) and CB(2) receptor-mediated signalling: a focus on endocannabinoids. *Prostaglandins Leukot Essent Fatty Acids* **66**: 161–171.

Maccarrone M, van der Stelt M, Rossi A, Veldink GA, Vliegenthart JF, Agro AF (1998). Anandamide hydrolysis by human cells in culture and brain. *J Biol Chem* **273**: 32332–32339.

Maccarrone M, Fiorucci L, Erba F, Bari M, Finazzi-Agro A, Ascoli F (2000). Human mast cells take up and hydrolyze anandamide under the control of 5-lipoxygenase and do not express cannabinoid receptors. *FEBS Lett* **468**: 176–180.

McCaw EA, Hu H, Gomez GT, Hebb AL, Kelly ME, Denovan-Wright EM (2004). Structure, expression and regulation of the cannabinoid receptor gene (CB1) in Huntington's disease transgenic mice. *Eur J Biochem* **271**: 4909–4920.

McDonald AJ, Mascagni F (2001). Localization of the CB1 type cannabinoid receptor in the rat basolateral amygdala: high concentrations in a subpopulation of cholecystokinin-containing interneurons. *Neuroscience* **107**: 641–652.

McDonald GR, Hudson AL, Dunn SMJ, You H, Baker GB, Whittal RM *et al*. (2008). Bioactive Contaminants Leach from Disposable Laboratory Plasticware. *Science* **322**: 917.

McHugh D, Tanner C, Mechoulam R, Pertwee RG, Ross RA (2008). Inhibition of human neutrophil chemotaxis by endogenous cannabinoids and phytocannabinoids: evidence for a site distinct from CB1 and CB2. *Mol Pharmacol* **73**: 441–450.

Mackie K, Stella N (2006). Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J* **8**: E298–E306.

Mackie K, Devane WA, Hille B (1993). Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol Pharmacol* **44**: 498–503.

Maejima T, Ohno-Shosaku T, Kano M (2001). Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. *Neurosci Res* **40**: 205–210.

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C *et al*. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87**: 493–506.

Marchalant Y, Cerbai F, Brothers HM, Wenk GL (2008). Cannabinoid receptor stimulation is anti-inflammatory and improves memory in old rats. *Neurobiol Aging* **29**: 1894–1901.

Marchalant Y, Rosi S, Wenk GL (2007). Anti-inflammatory property of the cannabinoid agonist WIN-55212-2 in a rodent model of chronic brain inflammation. *Neuroscience* **144**: 1516–1522.

Maresz K, Pryce G, Ponomarev ED, Marsicano G, Croxford JL, Shriver LP *et al*. (2007). Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med* **13**: 492–497.

Marinelli S, Di Marzo V, Berretta N, Matias I, Maccarrone M, Bernardi G *et al*. (2003). Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat substantia nigra by endogenous stimulation of vanilloid receptors. *J Neurosci* **23**: 3136–3144.

Marrie RA (2004). Environmental risk factors in multiple sclerosis aetiology. *Lancet Neurol* **3**: 709–718.

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A *et al*. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* **302**: 84–88.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**: 561–564.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR *et al*. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**: 83–90.

Melis M, Pillolla G, Luchicchi A, Muntoni AL, Yasar S, Goldberg SR *et al*. (2008). Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. *J Neurosci* **28**: 13985–13994.

Meschler JP, Howlett AC (2001). Signal transduction interactions between CB1 cannabinoid and dopamine receptors in the rat and monkey striatum. *Neuropharmacology* **40**: 918–926.

Mesnage V, Houeto JL, Bonnet AM, Clavier I, Arnulf I, Cattelin F *et al*. (2004). Neurokinin B, neurotensin, and cannabinoid receptor antagonists and Parkinson disease. *Clinical Neuropharmacology* **27**: 108–110.

Mestre L, Docagne F, Correa F, Loria F, Hernangomez M, Borrell J *et al*. (2009). A cannabinoid agonist interferes with the progression of a chronic model of multiple sclerosis by downregulating adhesion molecules. *Mol Cell Neurosci* **40**: 258–266.

Minati L, Edginton T, Bruzzone MG, Giaccone G (2009). Current concepts in Alzheimer's disease: a multidisciplinary review. *Am J Alzheimers Dis Other Demen* **24**: 95–121.

Moldrich G, Wenger T (2000). Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study. *Peptides* **21**: 1735–1742.

Molina-Holgado E, Vela JM, Arevalo-Martin A, Almazan G, Molina-Holgado F, Borrell J *et al*. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *J Neurosci* **22**: 9742–9753.

Morgese MG, Cassano T, Cuomo V, Giuffrida A (2007). Anti-dyskinetic effects of cannabinoids in a rat model of Parkinson's disease: role of CB(1) and TRPV1 receptors. *Exp Neurol* **208**: 110–119.

Morgese MG, Cassano T, Gaetani S, Macheda T, Laconca L, Dipasquale P *et al*. (2009). Neurochemical changes in the striatum of dyskinetic rats after administration of the cannabinoid agonist WIN55,212-2. *Neurochem Int* **54**: 56–64.

Mudher A, Lovestone S (2002). Alzheimer's disease-do tauists and baptists finally shake hands? *Trends Neurosci* **25**: 22–26.

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**: 61–65.

Navarrete M, Araque A (2008). Endocannabinoids mediate neuron-astrocyte communication. *Neuron* **57**: 883–893.

Nicholson SJ, Witherden AS, Hafezparast M, Martin JE, Fisher EM (2000). Mice, the motor system, and human motor neuron pathology. *Mamm Genome* **11**: 1041–1052.

Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO (2003). Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* **9**: 448–452.

Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG (2000). Multiple sclerosis. *N Engl J Med* **343**: 938–952.

Nunez E, Benito C, Pazos MR, Barbachano A, Fajardo O, Gonzalez S *et al*. (2004). Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. *Synapse* **53**: 208–213.

Nunez E, Benito C, Tolon RM, Hillard CJ, Griffin WS, Romero J (2008). Glial expression of cannabinoid CB(2) receptors and fatty acid amide hydrolase are beta amyloid-linked events in Down's syndrome. *Neuroscience* **151**: 104–110.

Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T (2007). Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem Biophys Res Commun* **362**: 928–934.

Oka S, Tsuchie A, Tokumura A, Muramatsu M, Suhara Y, Takayama H *et al*. (2003). Ether-linked analogue of 2-arachidonoylglycerol (noladin ether) was not detected in the brains of various mammalian species. *J Neurochem* **85**: 1374–1381.

Onaivi ES, Ishiguro H, Gong JP, Patel S, Perchuk A, Meozzi PA *et al*. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann N Y Acad Sci* **1074**: 514–536.

Ortar G, Ligresti A, De Petrocellis L, Morera E, Di Marzo V (2003). Novel selective and metabolically stable inhibitors of anandamide cellular uptake. *Biochem Pharmacol* **65**: 1473–1481.

Ortega-Gutierrez S, Molina-Holgado E, Arevalo-Martin A, Correa F, Viso A, Lopez-Rodriguez ML *et al*. (2005). Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis. *FASEB J* **19**: 1338–1340.

Palazuelos J, Aguado T, Egia A, Mechoulam R, Guzman M, Galve-Roperh I (2006). Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. *FASEB J* **20**: 2405–2407.

Palazuelos J, Aguado T, Pazos MR, Julien B, Carrasco C, Resel E *et al*. (2009). Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain* **132**: 3152–3164.

Pintor A, Tebano MT, Martire A, Grieco R, Galluzzo M, Scattoni ML *et al*. (2006). The cannabinoid receptor agonist WIN 55,212-2 attenuates the effects induced by quinolinic acid in the rat striatum. *Neuropharmacology* **51**: 1004–1012.

Pisani A, Fezza F, Galati S, Battista N, Napolitano S, Finazzi-Agrò A *et al*. (2005). High endogenous cannabinoid levels in the cerebrospinal fluid of untreated Parkinson's disease patients. *Ann Neurol* **57**: 777–779.

Plaitakis A, Caroscio J (1987). Abnormal glutamate metabolism in amyotrophic lateral sclerosis. *Ann Neurol* **22**: 575–579.

Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J *et al*. (2002). Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* **301**: 1020–1024.

Puighermanal E, Marsicano G, Busquets-Garcia A, Lutz B, Maldonado R, Ozaita A (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci* **12**: 1152–1158.

Racz I, Nadal X, Alferink J, Banos JE, Rehnelt J, Martin M *et al*. (2008). Crucial role of CB(2) cannabinoid receptor in the regulation of central immune responses during neuropathic pain. *J Neurosci* **28**: 12125–12135.

Raman C, McAllister SD, Rizvi G, Patel SG, Moore DH, Abood ME (2004). Amyotrophic lateral sclerosis: delayed disease progression in mice by treatment with a cannabinoid. *Amyotroph Lateral Scler Other Motor Neuron Disord* **5**: 33–39.

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

Reiner A, Albin RL, Anderson KD, D'Amato CJ, Penney JB, Young AB (1988). Differential loss of striatal projection neurons in Huntington disease. *Proc Natl Acad Sci U S A* **85**: 5733–5737.

Richardson D, Ortori CA, Chapman V, Kendall DA, Barrett DA (2007). Quantitative profiling of endocannabinoids and related compounds in rat brain using liquid chromatography-tandem electrospray ionization mass spectrometry. *Anal Biochem* **360**: 216–226.

Robberecht W (2000). Oxidative stress in amyotrophic lateral sclerosis. *J Neurol* **247**).

Rodriguez JJ, Mackie K, Pickel VM (2001). Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. *J Neurosci* **21**: 823–833.

Rog DJ, Nurmikko TJ, Friede T, Young CA (2005). Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology* **65**: 812–819.

Rog DJ, Nurmikko TJ, Young CA (2007). Oromucosal delta9-tetrahydrocannabinol/cannabidiol for neuropathic pain associated with multiple sclerosis: an uncontrolled, open-label, 2-year extension trial. *Clin Ther* **29**: 2068–2079.

Romero J, Berrendero F, Perez-Rosado A, Manzanares J, Rojo A, Fernandez-Ruiz JJ *et al*. (2000). Unilateral 6-hydroxydopamine lesions of nigrostriatal dopaminergic neurons increased CB1 receptor mRNA levels in the caudate-putamen. *Life Sci* **66**: 485–494.

Romero-Sandoval EA, Horvath R, Landry RP, DeLeo JA (2009). Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation. *Mol Pain* **5**: 25.

Rosen D, Siddique T, Patterson D, Figlewicz D, Sapp P, Hentati A *et al*. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**: 59–62.

Rosenblatt A, Leroi I (2000). Neuropsychiatry of Huntington's disease and other basal ganglia disorders. *Psychosomatics* **41**: 24–30.

Ross CA, Margolis RL (2001). Huntington's disease. *Clin Neurosci Res* **1**: 142–152.

Rossi S, De Chiara V, Musella A, Cozzolino M, Bernardi G, Maccarrone M *et al*. (2009). Abnormal sensitivity of cannabinoid CB1 receptors in the striatum of mice with experimental amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* **19**: 1–8.

Rothstein J, Kammen MV, Levey A, Martin L, Kuncl R (1995). Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* **38**: 73–84.

Rothstein J, Martin L, Kuncl R (1992). Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N Engl J Med* **326**: 1464–1468.

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE *et al*. (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* **433**: 73–77.

Rothstein J, Tsai G, Kuncl R, Clawson L, Cornblath D, Drachman D *et al*. (1990). Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann Neurol* **28**: 18–25.

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

Sagredo O, Gonzalez S, Aroyo I, Pazos MR, Benito C, Lastres-Becker I *et al*. (2009). Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. *Glia* **57**: 1154–1167.

Sagredo O, Ramos JA, Decio A, Mechoulam R, Fernandez-Ruiz J (2007). Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *Eur J Neurosci* **26**: 843–851.

Salio C, Doly S, Fischer J, Franzoni MF, Conrath M (2002). Neuronal and astrocytic localization of the cannabinoid receptor-1 in the dorsal horn of the rat spinal cord. *Neurosci Lett* **329**: 13–16.

Sanchez C, de Ceballos ML, Gomez del Pulgar T, Rueda D, Corbacho C, Velasco G *et al*. (2001). Inhibition of glioma growth in vivo by selective activation of the CB(2) cannabinoid receptor. *Cancer Res* **61**: 5784–5789.

Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K *et al*. (2001). Early and progressive accumulation of reactive microglia in the Huntington disease brain. *J Neuropathol Exp Neurol* **60**: 161–172.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T *et al*. (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**: 173–177.

Scotter E, Graham S, Glass M (2009). Cannabinoid receptor signal transduction pathways. In: Reggio PH (ed.). *The Cannabinoid Receptors*. Humana Press: New York, pp. 153–172.

Sheng WS, Hu S, Min X, Cabral GA, Lokensgard JR, Peterson PK (2005). Synthetic cannabinoid WIN55,212-2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes. *Glia* **49**: 211–219.

Shoemaker JL, Seely KA, Reed RL, Crow JP, Prather PL (2007). The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. *J Neurochem* **101**: 87–98.

Showalter VM, Compton DR, Martin BR, Abood ME (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther* **278**: 989–999.

Sieradzan KA, Fox SH, Hill M, Dick JP, Crossman AR, Brotchie JM (2001). Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology* **57**: 2108–2111.

Sinha D, Bonner TI, Bhat NR, Matsuda LA (1998). Expression of the CB1 cannabinoid receptor in macrophage-like cells from brain tissue: immunochemical characterization by fusion protein antibodies. *J Neuroimmunol* **82**: 13–21.

Skaper SD, Buriani A, Dal Toso R, Petrelli L, Romanello S, Facci L *et al*. (1996). The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc Natl Acad Sci U S A* **93**: 3984–3989.

Smith PF (2007). Symptomatic treatment of multiple sclerosis using cannabinoids: recent advances. *Expert Rev Neurother* **7**: 1157–1163.

Solinas M, Justinova Z, Goldberg SR, Tanda G (2006). Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J Neurochem* **98**: 408–419.

Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C *et al*. (2004). Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. *J Neurosci* **24**: 2270–2276.

Sriram S, Steiner I (2005). Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. *Ann Neurol* **58**: 939–945.

Stella N, Piomelli D (2001). Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur J Pharmacol* **425**: 189–196.

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

van der Stelt M, Hansen HH, Veldhuis WB, Bar PR, Nicolay K, Veldink GA *et al*. (2003). Biosynthesis of endocannabinoids and their modes of action in neurodegenerative diseases. *Neurotox Res* **5**: 183–200.

van der Stelt M, Fox SH, Hill M, Crossman AR, Petrosino S, Di Marzo V *et al*. (2005). A role for endocannabinoids in the generation of parkinsonism and levodopa-induced dyskinesia in MPTP-lesioned non-human primate models of Parkinson's disease. *Faseb J* **19**: 1140–1142.

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

Streit WJ, Conde JR, Fendrick SE, Flanary BE, Mariani CL (2005). Role of microglia in the central nervous system's immune response. *Neurol Res* **27**: 685–691.

Sudhahar V, Shaw S, Imig JD (2009). Mechanisms involved in oleamide-induced vasorelaxation in rat mesenteric resistance arteries. *Eur J Pharmacol* **607**: 143–150.

Sugiura T, Kondo S, Kishimoto S, Miyashita T, Nakane S, Kodaka T *et al*. (2000). Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. *J Biol Chem* **275**: 605–612.

Svendsen KB, Jensen TS, Bach FW (2004). Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double blind placebo controlled crossover trial. *BMJ* **329**: 253.

Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ *et al*. (2007). Microglial activation in presymptomatic Huntington's disease gene carriers. *Brain* **130**: 1759–1766.

The Huntington's Disease Collaborative Research Group (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**: 971–983.

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

Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N (2005). Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J Biol Chem* **280**: 11082–11092.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al*. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–332.

Vandevoorde S, Fowler CJ (2005). Inhibition of fatty acid amide hydrolase and monoacylglycerol lipase by the anandamide uptake inhibitor VDM11: evidence that VDM11 acts as an FAAH substrate. *Br J Pharmacol* **145**: 885–893.

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

Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP, Jr (1985). Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol* **44**: 559–577.

Wade DT, Makela PM, House H, Bateman C, Robson P (2006). Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. *Mult Scler* **12**: 639–645.

Wade DT, Robson P, House H, Makela P, Aram J (2003). A preliminary controlled study to determine whether whole-plant cannabis extracts can improve intractable neurogenic symptoms. *Clin Rehabil* **17**: 21–29.

Wagner JA, Varga K, Ellis EF, Rzigalinski BA, Martin BR, Kunos G (1997). Activation of peripheral CB1 cannabinoid receptors in haemorrhagic shock. *Nature* **390**: 518–521.

Waksman Y, Olson JM, Carlisle SJ, Cabral GA (1999). The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J Pharmacol Exp Ther* **288**: 1357–1366.

Walter L, Franklin A, Witting A, Moller T, Stella N (2002). Astrocytes in culture produce anandamide and other acylethanolamides. *J Biol Chem* **277**: 20869–20876.

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

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

Walter L, Stella N (2004b). Cannabinoids and neuroinflammation. *Br J Pharmacol* **141**: 775–785. Epub 2004 Feb 2002.

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

Wenk GL (2003). Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry* **64**: 7–10.

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

Weydt P, Hong S, Witting A, Moller T, Stella N, Kliot M (2005). Cannabinol delays symptom onset in SOD1 (G93A) transgenic mice without affecting survival. *Amyotroph Lateral Scler Other Motor Neuron Disord* **6**: 182–184.

Weydt P, Yuen EC, Ransom BR, Moller T (2004). Increased cytotoxic potential of microglia from ALS-transgenic mice. *Glia* **48**: 179–182.

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**: 1237–1239.

Wilson RI, Nicoll RA (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**: 588–592.

Wissel J, Haydn T, Muller J, Brenneis C, Berger T, Poewe W *et al*. (2006). Low dose treatment with the synthetic cannabinoid Nabilone significantly reduces spasticity-related pain: a double-blind placebo-controlled cross-over trial. *J Neurol* **253**: 1337–1341.

Witting A, Chen L, Cudaback E, Straiker A, Walter L, Rickman B *et al*. (2006). Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. *Proc Natl Acad Sci U S A* **103**: 6362–6367.

Witting A, Weydt P, Hong S, Kliot M, Moller T, Stella N (2004). Endocannabinoids accumulate in spinal cord of SOD1 G93A transgenic mice. *J Neurochem* **89**: 1555–1557.

Wolfgram F, Myers L (1973). Amyotrophic lateral sclerosis: effect of serum on anterior horn cells in tissue culture. *Science* **179**: 579–580.

Wong P, Pardo C, Borchelt D, Lee M, Copeland N, Jenkins N *et al*. (1995). An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* **14**: 1105–1116.

Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C *et al*. (2006a). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol* **6**: 12.

Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C *et al*. (2006b). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol* **6**: 12.

Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A *et al*. (2003). Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. *Lancet* **362**: 1517–1526.

Zajicek JP, Sanders HP, Wright DE, Vickery PJ, Ingram WM, Reilly SM *et al*. (2005). Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *J Neurol Neurosurg Psychiatry* **76**: 1664–1669.

Zeng BY, Dass B, Owen A, Rose S, Cannizzaro C, Tel BC *et al*. (1999). Chronic L-DOPA treatment increases striatal cannabinoid CB1 receptor mRNA expression in 6-hydroxydopamine-lesioned rats. *Neurosci Lett* **276**: 71–74.

Zhao P, Ignacio S, Beattie EC, Abood ME (2008). Altered presymptomatic AMPA and cannabinoid receptor trafficking in motor neurons of ALS model mice: implications for excitotoxicity. *Eur J Neurosci* **27**: 572–579.

Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M *et al*. (2002). Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* **417**: 74–78.

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V *et al*. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400**: 452–457.