

Endocannabinoid signalling and the deteriorating brain

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Abstract | Ageing is characterized by the progressive impairment of physiological functions and increased risk of developing debilitating disorders, including chronic inflammation and neurodegenerative diseases. These disorders have common molecular mechanisms that can be targeted therapeutically. In the wake of the approval of the first cannabinoid-based drug for the symptomatic treatment of multiple sclerosis, we examine how endocannabinoid (eCB) signalling controls — and is affected by — normal ageing and neuroinflammatory and neurodegenerative disorders. We propose a conceptual framework linking eCB signalling to the control of the cellular and molecular hallmarks of these processes, and categorize the key components of endocannabinoid signalling that may serve as targets for novel therapeutics.

After millennia of anecdotal use (both medicinal and recreational) of *Cannabis* plants, as described in Chinese, Indian and Arab pharmacopeias¹, centuries of their documented medicinal use throughout the world, and nearly five decades of research on the mechanism of action of their bioactive constituents (the phytocannabinoids), the medical use of cannabis extracts was approved in June 2010 by ten European countries². This first *Cannabis*-based medicine (known as nabiximols in the United States and marketed as Sativex (GW Pharmaceuticals) in more than 25 countries worldwide) was prepared by combining botanical extracts from two varieties of *Cannabis sativa* plants, one producing mainly Δ^9 -tetrahydrocannabinol (THC; the major psychotropic component in the flowers) and the other producing mainly cannabidiol (CBD; an abundant non-psychotropic phytocannabinoid), with a ratio of approximately 1:1. Thus, despite the medicinal uses of THC for the treatment of emesis and cachexia in patients with cancer undergoing chemotherapy, and for promoting appetite in patients with AIDS^{1,3}, cannabis extracts were finally given *bona fide* therapeutic status after a decade of clinical trials dedicated to the testing of a combination of THC and CBD on the neurodegenerative disease multiple sclerosis.

A wealth of results obtained from animal models and human studies over the past two decades greatly increased our understanding of the molecular mechanism by which THC, its endogenous ‘counterparts’ (the endocannabinoids, eCBs) and its synthetic analogues, modulate cannabinoid receptors. These studies also

provided scientific support for targeting the eCB signalling system to treat several devastating diseases, including neurodegenerative and chronic inflammatory diseases. This Review focuses on recent evidence that has added a new layer of complexity to the idea of targeting the eCB signalling system for therapeutic benefit, as it is now clear that the expression level of the different molecular components forming this signalling system (the receptors and enzymes producing and inactivating eCBs) change substantially both in the brain and peripheral tissues as a function of ageing. This suggests that the bioactivity of eCB-based therapeutics is likely to vary depending on the age of the patient, the disease type and the stage of disease at the time of treatment^{4,5}. Accordingly, such treatments may have to be tailored for different subsets of patients.

The endocannabinoid system

The first guanine-nucleotide-binding protein (G protein)-coupled receptor (GPCR) activated by THC, cannabinoid receptor 1 (CB1; encoded by gene *CNR1*), was discovered in the brain in 1988 (REF. 6) and cloned in 1990, (REF. 7) and the second GPCR, CB2, was identified in immune cells in 1993 (REF. 8). More than 70 phytocannabinoids are present in *Cannabis* plants (in varying amounts depending on strain or growing conditions), but only THC potently activates CB1 and CB2. The existence of these two receptors, of which one (CB1) is the most abundant GPCR in the brain, could only be explained by the presence of endogenous ligands; the eCBs. These were discovered in the early nineties, shortly after the

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discoveries of CB1 and CB2, as derivatives of the non-oxidative metabolism of the polyunsaturated fatty acid, arachidonic acid. *N*-arachidonoyl-ethanolamine (anandamide)⁹ and 2-arachidonoyl-glycerol (2-AG)^{10,11} are the two best-studied eCBs so far, and together with enzymes involved in their biosynthesis and inactivation¹², and the two cannabinoid receptors, they form the 'eCB system'. CB1 receptors are most abundant in brain regions such as the hippocampus, cortex, basal ganglia and cerebellum, and expressed in both excitatory (glutamatergic) and inhibitory (GABAergic) presynaptic terminals, although their postsynaptic localization has also been observed^{13,14}. In the healthy brain, CB2 receptors are barely detectable, although they seem to be present in some neuronal populations¹³. However, CB2 receptor expression can increase in both microglia and astrocytes under specific conditions of neuroinflammation (see below)¹³. Both CB1 and CB2 receptors couple to G protein type $G_{i/o}$, and less frequently to the $G_{q/11}$ type. It is through this coupling that they activate mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinase 1 (ERK1), ERK2, p38 MAPKs and JUN N-terminal kinases (JNKs), and inhibit adenylyl cyclase and cyclic AMP–protein kinase A (PKA) signalling. Activation of CB1 receptors also inhibits L-, N- and P/Q-type voltage-activated Ca^{2+} channels and stimulates inwardly rectifying K^+ channels, the result of which is to reduce neurotransmitter release¹⁵.

Anandamide is a high-affinity, low-efficacy CB1 agonist, with even lower efficacy at CB2 receptors, whereas 2-AG has lower affinity, but is a fully effective agonist at both CB1 and CB2 (REF. 16). The eCBs are produced from phospholipid precursors when intracellular Ca^{2+} is elevated, following either neuron depolarization or activation of metabotropic $G_{q/11}$ -coupled receptors. Anandamide, like other *N*-acyl-ethanolamines (NAEs), is produced from the hydrolysis of the corresponding *N*-acyl-phosphatidyl-ethanolamine (NAPE)¹⁷. This can occur in one step, when catalysed by the NAPE-selective phospholipase D (NAPE-PLD) enzyme¹⁸, or in two or three steps through alternative routes^{19,20}. 2-AG and other 2-acylglycerols are produced in one step from the hydrolysis of diacylglycerols (DAGs) by either of two diacylglycerol lipases (DAGLs), DAGLa or DAGL β (REF. 21), although in the adult brain it is the former of these two enzymes that accounts for nearly all of 2-AG acting as an eCB²². DAGs are produced in most cases from the hydrolysis of phosphoinositides by phospholipase C β (PLC β)²³. Although most eCB biosynthetic enzymes are sensitive to Ca^{2+} , it is thought that the rate controlling and Ca^{2+} -dependent steps in anandamide and 2-AG formation are undertaken by NAPE and DAG biosynthesis, respectively. A Ca^{2+} -dependent acyltransferase²⁴ catalyses the biosynthesis of NAPEs through the transfer of the corresponding acyl chains from the *sn*-1 position of phospholipids to the amine group of phosphatidyl-ethanolamine; however, its molecular identity has not yet been reported.

eCBs are taken up by cells through a mechanism that is not yet elucidated fully — but likely to involve membrane carriers, intracellular carrier proteins and/or

possibly endocytosis²⁵ — and are then inactivated by enzymatic hydrolysis. For anandamide, this reaction is catalysed uniquely by fatty acid amide hydrolase 1 (FAAH)²⁶, whereas for 2-AG most of this reaction seems to be catalysed by monoacylglycerol lipase (MAGL)²⁷, although ABHD6 (an abbreviation of α,β hydrolase 6)²⁸ and ABHD12, as well as FAAH²⁹, are also capable of hydrolysing this eCB. Both anandamide and 2-AG are also oxidized by the enzyme cyclooxygenase 2 (COX2; also known as prostaglandin G/H synthase 2)³⁰.

The postsynaptic and presynaptic distribution in the brain of DAGLa and MAGL, respectively, is ideal for enabling the retrograde inhibition of potentially excessive neurotransmitter release by 2-AG activation of presynaptic CB1 receptors³¹ (FIG. 1). Indeed, a flurry of landmark studies have demonstrated the pleiotropic nature of the eCB system, which is activated locally and on demand to regulate specific physiological functions, often following perturbations of homeostasis, to help restore a physiological steady state in mammalian cells, organs and organisms³².

How eCB signalling controls specific cellular functions, and how various cannabinoid agonists and antagonists modulate these functions is now much better understood, owing to the power of new pharmacological tools and genetic models capable of interrogating individual components of this system in selected cell populations. These approaches demonstrated that CB1 receptor signalling differs between glutamatergic and GABAergic neurons (see below), and also established that anandamide can activate non-cannabinoid receptors — the transient receptor potential cation channel subfamily V member 1 (TRPV1) channel and, possibly, the peroxisome proliferator-activated receptor- γ (PPAR γ)^{33,34} — which confer further functional flexibility to this mediator. Together, the wealth of studies using these powerful tools has provided a picture of the role and components of eCB signalling in the healthy brain. Here we review how the basic components of this signalling system are affected by, and in turn regulate, ageing-related processes, and outline the molecular commonalities between the deregulation of this signalling system in normal ageing and during neuroinflammation and neurodegeneration.

Endocannabinoid signalling in ageing

There are numerous lines of evidence from rodents and humans to indicate that eCB signalling undergoes age-dependent changes. In rodents, the CB1 mRNA levels and/or specific binding of CB1 agonists in the brain peak at the onset of puberty, stay at a relatively stable high level in the adult³⁵, and decline markedly in the cerebellum, cortex, and hippocampal and hypothalamic structures of older animals³⁶. The old-age-dependent decrease in receptor availability to the plasma membrane is likely to be due to post-transcriptional mechanisms, because changes in CB1 mRNA levels do not always reflect changes in the density of receptor binding and hence the amount of CB1 protein^{36,37}. Furthermore, the coupling of CB1 receptors to G proteins is also reduced in specific

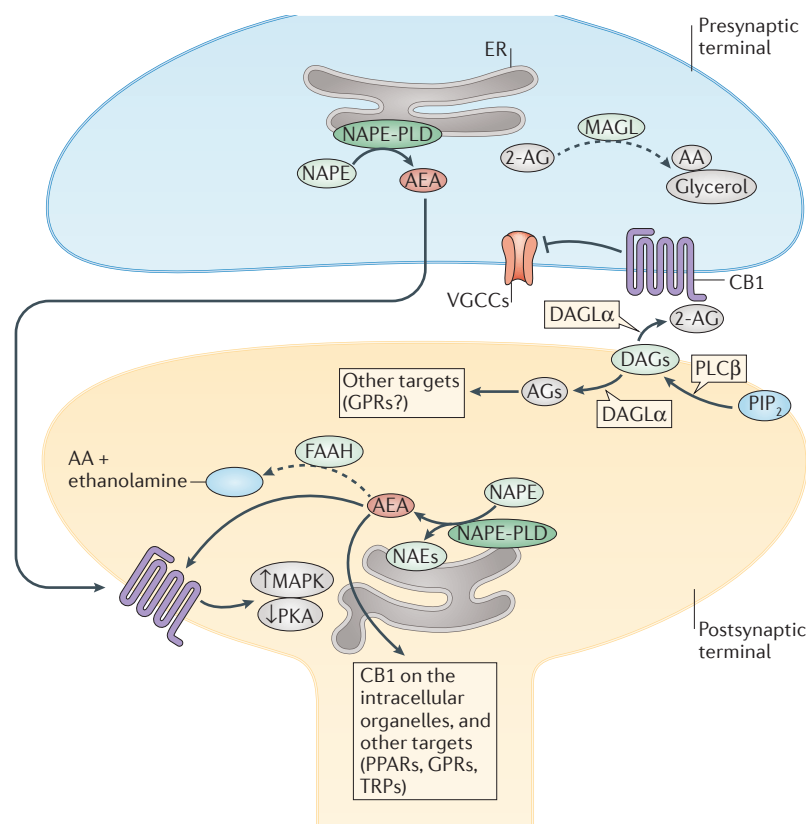


Figure 1 | Main biosynthetic and inactivating enzymes in endocannabinoid signalling. The subcellular distribution in neurons of enzymes regulating the levels of endocannabinoids (eCBs) is shown, including the proposed role of these lipid mediators in retrograde (mainly for 2-arachidonoyl-glycerol (2-AG)), anterograde and intracellular (for anandamide (AEA)) signalling. The biosynthesis of AEA occurs through the action, among others, of *N*-acylphosphatidylethanolamine (NAPE)-specific phospholipase D (NAPE-PLD), which is located in intracellular membranes both pre- and postsynaptically. AEA is degraded by fatty acid amide hydrolase 1 (FAAH), which is located postsynaptically. This distribution of the enzymes responsible for synthesis and degradation of AEA enables this and other *N*-acylethanolamines (NAEs) to function as anterograde signals acting at postsynaptic targets, or as intracellular mediators. 2-AG is biosynthesized by diacylglycerol lipase- α (DAGL α), which is located postsynaptically, and degraded by monoacylglycerol lipase (MAGL), which instead is presynaptic, thus accounting for the retrograde signalling action suggested for this endocannabinoid (see FIG. 2b). The complexity arising from the fact that many of these enzymes also regulate the levels of eCB-related mediators, with non-cannabinoid receptors as targets, is also depicted. For further complexity in eCB signalling see FIG. 4 and [Supplementary Information S1](#) (table). Solid arrows denote transformation into active metabolites or activation; dashed arrows denote transformation into metabolites inactive at cannabinoid receptors; blunt arrow denotes inhibition. AA, arachidonic acid; AGs, 2-acylglycerols; DAGs, diacylglycerols; ER, endoplasmic reticulum; GPRs, orphan G-protein-coupled receptors; MAPK, mitogen-activated protein kinases; PIP₂, phosphoinositide bisphosphate; PKA, protein kinase A; PLC β , phospholipase C β ; PPARs, peroxisome proliferator-activated receptors; TRPs, transient receptor potential channels; VGCCs, voltage-gated calcium channels.

brain areas in older animals³⁸. The levels of NAPE-PLD mRNA and protein increase during early postnatal development of rats³⁹. This is accompanied by increasing brain anandamide concentrations during the early postnatal period⁴⁰, with a peak at the onset of puberty⁴¹. Studies in humans also showed that CB1 receptor levels are much higher in younger compared to older individuals^{42,43}. The levels of CB1 mRNA

decrease by approximately 50% (REF. 44), and binding of the non-selective cannabinoid receptor agonist [³H] CP55,940 also decreases in cortical tissues of older individuals⁴⁵. The mRNA levels of eCB biosynthetic and metabolic enzymes also change during ageing in the human prefrontal cortex. NAPE-PLD levels increase continuously during ageing, whereas DAGL α peaks in young adulthood and declines in older individuals. FAAH mRNA shows only a minor increase with ageing. MAGL mRNA levels are highest during infancy and decrease thereafter, while ABHD6 levels increase steadily throughout life⁴⁴. Notably, we still do not know whether these changes correlate with corresponding changes in the levels of eCBs. Nevertheless, these data indicate that the age-related changes in the expression of the components of eCB signalling are similar in rodents and humans. These changes might be responsible for some of the age-specific alterations in behavioural paradigms measured both under basal conditions and following cannabinoid exposure. This could explain why, at a young age (2 months), animals lacking CB1 show better performance than wild-type littermate controls in several learning and memory tasks. Remarkably, when older individuals are tested, the outcome is the opposite. Specifically, at age 3–5 months, CB1-null mice no longer perform better, and in fact sometimes perform worse, than wild-type controls. When they are 12 months old, CB1-null mice perform substantially worse than wild-type animals in all cognitive tests used in these studies^{46–48}. Exposure of animals to doses of THC in the range that cause psychoactive effects has been shown to impair cognitive functions⁴⁹, similar to observations in humans after cannabis exposure⁵⁰. These cognitive deficits persist even after an extended period of abstinence⁵¹, and it has been suggested that cannabis use during early life may accelerate brain pathologies associated with ageing and psychiatric disorders^{52,53}. However, it is important to note that almost all studies on these THC effects, even in rodents, were performed with younger or adult (but not aged) individuals^{54,55} and it remains unclear how relevant these results are to older individuals.

Evidence obtained with new-generation genetic mouse models in which selective components of eCB signalling were knocked out globally or in specific cell populations indicate that disrupting this system affects fundamental aspects of the normal ageing process. For example, the age-dependent acceleration of cognitive decline in mice with global deletion of CB1 is accompanied by accelerated loss of hippocampal CA1 and CA3 neurons, and induction of reactive astrogliosis in this brain region, as well as an increased number of activated microglia and enhanced levels of pro-inflammatory cytokines⁵⁶. This phenotype is similar to that observed in mice with a deletion of CB1 selectively in GABAergic neurons; however, CB1 deletion in glutamatergic neurons does not induce any marked age-related cognitive deficits^{47,56}. These data indicate that absence of CB1 receptors in specific neuronal types accelerates the appearance of brain ageing indicators, including neuronal loss and chronic neuroinflammation. Importantly, this phenotype

is only observed in the brain and the skin of these animals, and not in other organs, suggesting a tissue-specific aspect of the involvement of eCB signalling in ageing⁵⁶. The expression profile and role of CB2 receptors in mouse brain remains to be established clearly, but it is interesting to note that mice lacking CB2 receptors show a phenotype that is also reminiscent of accelerated ageing, although outside of the brain. These mice suffer from severe osteoporosis^{57,58}, which is consistent with the reported function of this receptor in bone biology. Overall, these studies indicate that the disruption of cannabinoid receptors seems to enhance age-related decline in a number of tissues in which they have important physiological functions, and implicate eCB signalling in the control of the ageing process⁵⁹ (FIG. 2).

One of the hallmarks of ageing in rodents that is known to be influenced by eCB signalling is the depletion and eventual exhaustion of neural stem cell populations. Proliferation of progenitor cells in the rodent hippocampus and olfactory bulb is reduced by at least 50% in older animals (aged over 14 months) compared to younger individuals (aged 2–6 months)⁶⁰. This particular response to ageing probably involves both cell-extrinsic factors and alterations in the network of genes regulating proliferation, in particular those controlling eCB signalling^{61,62}. Indeed, pharmacological compounds that enhance CB1 and CB2 activity, including inhibitors of anandamide degradation by FAAH, stimulate adult hippocampal neurogenesis^{63–66}, whereas inhibition of eCB biosynthesis reduces it^{67,68}. These pharmacological findings are supported by genetic studies showing that the deletion of CB1 receptors or the 2-AG-producing enzyme DAGLA reduce the proliferation of neuronal progenitors to approximately 50% of that of wild-type controls^{67,69}. Conversely, mice with deletion of FAAH, which exhibit higher levels of anandamide in brain, show increased levels of adult neurogenesis⁷⁰. As these experiments were performed in 4-month-old adult mice, it will be important to establish whether impaired eCB stimulation of neurogenesis underlies at least part of the neuronal decline typical of ageing. Furthermore, CB1 receptors also have a crucial role in axonal guidance, distance migration of neurons and synapse formation during brain development⁷¹, whereas activation of both cannabinoid receptors is needed to stimulate myelin formation in the subcortical white matter⁷². Notably, eCB signalling is also likely to have a crucial role in insult-induced adult neuron replacement and remyelination. How this fundamental function of eCBs on progenitor cells is affected by ageing is not known.

Endocannabinoids and neuroinflammation

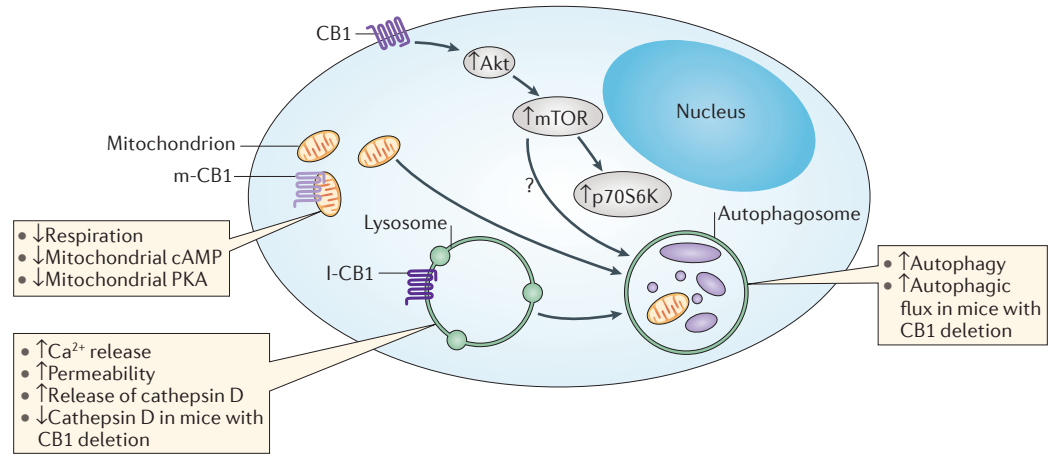
The expression level of both cannabinoid and eCB-related receptors in neurons and glial cells may change independently either as a function of normal ageing or earlier in life in response to a neurological disease⁷³ (FIG. 2). In neurological disease, chronic alterations in eCB signalling are often accompanied with chronic activation of the immune system and a number of immune cells that invade the brain parenchyma also express such receptors and thus contribute to the number of

cells that will respond to eCB and cannabinoids⁷⁴. This dynamic change in brain neuroinflammation in eCB signalling in neurons, glia and immune cells has been mapped in detail in mice undergoing experimental allergic encephalomyelitis (EAE), a model of multiple sclerosis. For example, resting microglial cells in the healthy adult brain express low levels of CB2, whereas induction of EAE induces a 200-fold upregulation in the expression of this receptor in activated microglia⁷⁵. As CB2 receptors regulate the cellular function of microglia and macrophages (both important mediators of EAE pathogenesis)⁷⁶, such a pronounced upregulation of these receptors in these cells might at first suggest that eCB signalling represents a critical molecular component controlling neuroinflammation and pathogenesis in EAE mouse models. However, an elegant study performed with a conditional genetic mouse model that enabled control of CB2 receptor expression in invading immune T cells in adult mice showed that it is this smaller population of cannabinoid receptors expressed by invading immune cells that has a key role in the development of the pathogenesis modeled by EAE⁷⁷. These findings provide an example of how low levels of cannabinoid receptors expressed by non-CNS cells may critically control disease initiation and progression (FIG. 3). It is unknown whether the ability to control neuroinflammation (and allied neurodegeneration through the action of cannabinoids on invading T cells) changes as a function of ageing. Furthermore, how ageing might predispose individuals affected by multiple sclerosis or other neuroinflammatory disorders to cannabinoid-based treatments also remains an open question.

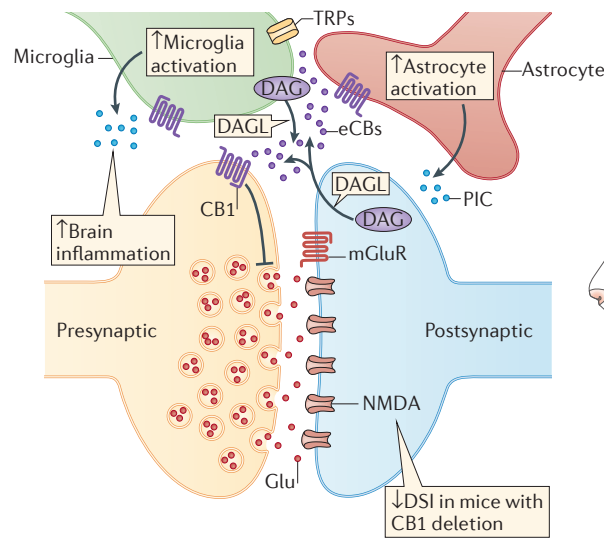
Variations in the levels of eCBs are also important to survey, as any change in the expression of cannabinoid receptors will alter their functionality, particularly if accompanied by a corresponding change in its endogenous agonists. Such changes in eCB levels have been detected in plasma from patients with multiple sclerosis⁷⁸, as well as in patients with disorders ranging from Parkinson's disease and Huntington's disease to amyotrophic lateral sclerosis, and/or in tissues from the corresponding animal models⁷⁹. These changes are likely to be influenced also by age-related alterations in the composition of the eCB signalling system. Therefore, to understand the possible role of this system in neuroinflammatory conditions, the field still needs to outline how cannabinoid receptor expression in neurons, astrocytes, microglia and invading immune cells, as well as eCB bioavailability, are affected by ageing. A better understanding of these processes will be essential if cannabinoid-based treatments are to be optimized for patients of different ages suffering from distinct disorders.

A key hallmark of ageing is found in the age-dependent deregulation of intercellular communication. This deregulation affects neuronal, endocrine and immune cell signalling⁸⁰, and the molecular components involved in this process are potential targets for the prevention of age-related disorders. One example of deregulated intercellular communication that has

a Cellular level



b Tissue level



c Organism level

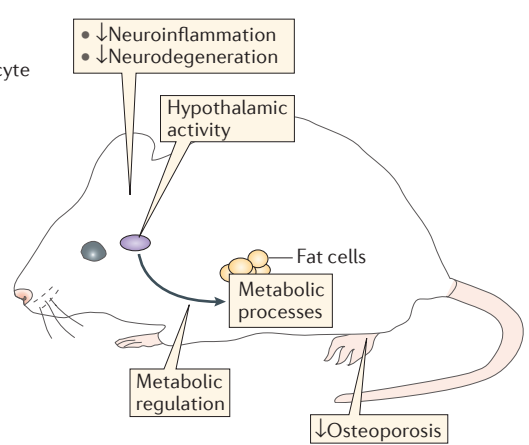


Figure 2 | Age-related changes at different system levels and their regulation by endocannabinoid signalling. Ageing is accompanied by changes in cellular processes, impairments in the integration of cellular activities, and deficits in physiological functions. **a** | At the cellular level, important hallmarks of ageing in the CNS are impairments in mitochondrial functions, disruption of proteostasis and autophagy, and alterations in signalling pathways involved in nutrient sensing, such as the mammalian target of rapamycin (mTOR) pathway. Endocannabinoids (eCBs) act as intracellular signalling molecules that modulate mitochondrial activity through cannabinoid type 1 (CB1) receptors located either in the plasma membrane or on lysosomes (l-CB1) and possibly mitochondria (m-CB1). In particular, activation of m-CB1 seems to reduce mitochondrial respiration, and to decrease mitochondrial cyclic AMP levels and protein kinase A (PKA) activity¹¹¹. CB1 receptors located on lysosomes enhance the intracellular release of Ca²⁺, increase the permeability of lysosomes and the release of cathepsin D. CB1 receptors on the cell surface also stimulate mTOR signalling, through an Akt-dependent mechanism, resulting in an enhanced activity of phosphoprotein 70 ribosomal protein S6 kinase (p70S6K). The mechanism by which cannabinoids stimulate autophagy is not entirely clear (indicated by a question mark), and is probably independent of mTOR. **b** | At the tissue level, disruption of intercellular communication is another hallmark of ageing. Endocannabinoids are best known as signalling molecules for short-range cell–cell communication. At synapses they provide a retrograde feedback system, in which activation of presynaptic CB1 receptors reduces neurotransmitter release probability. Endocannabinoids also modulate the activity of astrocytes and microglia. These cells may also be a source for brain endocannabinoids (eCBs). Increased numbers of activated microglia and astrocytes are typically found in the ageing brain and result in an increased production of pro-inflammatory cytokines (PICs). This process leads to a change towards a more pro-inflammatory milieu in the brain. **c** | At the organism level, eCBs modulate the activity of several systems that are important in ageing, such as metabolic processes or hypothalamic activity. Cannabinoids are generally protective against age-related pathologies, including neuroinflammation and neurodegeneration. They also protect against some age-related pathologies outside the CNS, such as osteoporosis⁵⁸. Akt, serine/threonine Akt; DAG, diacylglycerol; DAGLs, diacylglycerol lipases; DSI, depolarization-induced suppression of inhibitory neurotransmission; Glu, Glutamate; IC, intracellular; mGluR, metabotropic glutamate receptor; NMDA, N-methyl-D-aspartate; PKA, protein kinase A; TRP, transient receptor potential channel.

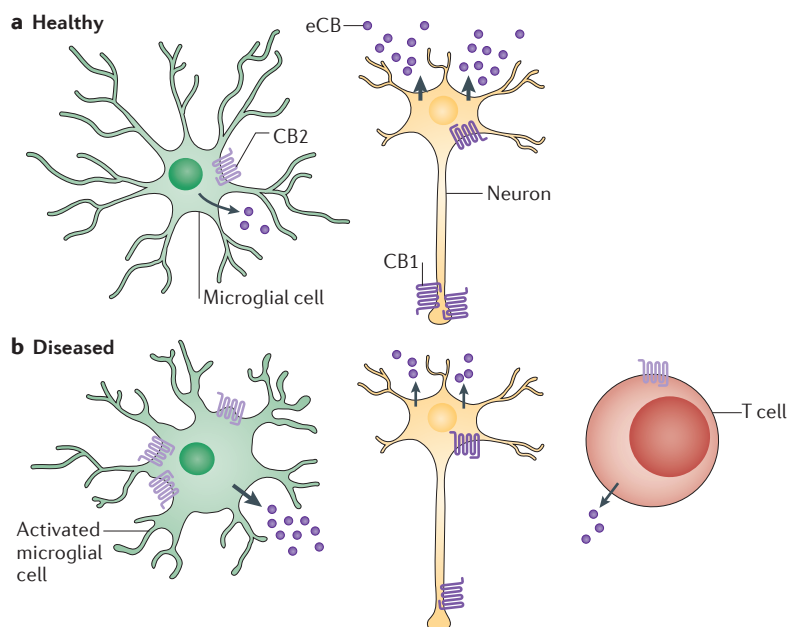


Figure 3 | Effect of neuroinflammation on endocannabinoid signalling. **a** | In healthy brain tissue, neurons express cannabinoid receptor 1 (CB1) receptors in the dendritic tree and also higher levels at axon terminals. Resting microglia express low levels of CB2 receptors⁷³. Endocannabinoid (eCB) production by neurons is high (indicated by the thick arrows), whereas eCB production in microglia is low (indicated by the thinner arrow). **b** | In diseased brain in which the immune system has been activated (for example, in multiple sclerosis), the cell-specific expression profile of cannabinoid receptors changes, resulting in lower levels of CB1 receptors in both the dendritic tree and axon terminal of neurons and higher expression of CB2 receptors in activated microglia. In addition, T cells expressing low levels of CB2 receptors invade the diseased brain area^{75,77}. eCB production by neurons decreases (indicated by the thinner arrows), while eCB production in microglia increases (thick arrow). This overall change in the cell-specific expression profile of cannabinoid receptors and eCB levels in brain as a function of disease and possibly of the ageing process suggests that the responses of humans to cannabinoid-based therapeutics is likely to differ depending on the age of the patient and disease phase.

been studied extensively occurs in monocytes and macrophages — major cellular components of the innate immune system — as ageing is often accompanied by accumulation of a low-grade pro-inflammatory milieu in certain tissues. This phenomenon has been termed ‘inflammaging’⁸¹. Specifically, older individuals have elevated levels of pro-inflammatory cytokines and nuclear factor- κ B (NF- κ B) signalling, and consequently, increased expression of inflammation-related proteins that are regulated by these molecules. This process is not unidirectional, as inflammaging also engages counter-adaptive processes, which include the activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in an increased release of anti-inflammatory cortisol. Thus, it is thought that the balance between these opposing processes is an important determinant of the health-related consequences of ageing. Although the function of eCBs in inflammaging has not been studied in detail, decades of research indicate that this signalling system is an important modulator of immune cell functions and inflammatory responses. For example, it is known that brain eCB signalling is activated by stress⁸² as well as by pro-inflammatory stimuli, and is involved

in the maintenance of HPA and immune homeostasis⁸³. As mentioned above, mice with CB1 deletion show all characteristics of an increased inflammaging condition in the brain, indicating that CB1 activation is important to counteract this process. These findings suggest that during ageing and neuroinflammation (or when both are present together) there is a disruption of brain tissue homeostasis that involves eCB signalling, and that this contributes to specific cell dysfunction. Furthermore, downregulation of CB1 receptors induced by chronic THC administration, or deletion of CB1, also cause cerebellar microglial activation in mice⁸⁴.

Another hallmark of ageing that is worth exploring is how age-related pathology that occurs in one tissue can adversely affect other tissues. Indeed, it was shown recently that a pro-inflammatory response that develops in the hypothalamus of older mice can exacerbate ageing-related changes in the physiology outside the brain, including changes in muscle endurance, dermal thickness, bone mass, tendon elasticity and cognitive performance in the whole animal⁸⁵. In line with this evidence, inhibiting the age-related induction of hypothalamic inhibitor of nuclear factor- κ B kinase subunit- β (IKK- β) or NF- κ B (which have both been shown to be crucial to hypothalamic inflammation in older mice) reduces the age-related cognitive decline of these animals and prolongs their lifespan⁸⁵. Interestingly, eCB signalling in the hypothalamus controls the HPA axis⁸² and modulates energy homeostasis in peripheral tissues. Activation of hypothalamic CB1 receptors induces orexigenic effects and inhibits energy expenditure. However, production of eCBs in the hypothalamus is inhibited by leptin⁸⁶, a hormone produced peripherally that has anorexigenic effects (increases energy expenditure and suppresses appetite) but also has pro-inflammatory actions⁸⁷. In adult mice with obesity induced by a high-fat diet, eCB signalling in the hypothalamus becomes deregulated following the development of leptin resistance in the arcuate nucleus, but not in other hypothalamic and extra-hypothalamic areas (see REF. 88 and references cited therein). This response is one likely cause of increased food intake and reduced energy expenditure, and contributes to further accumulation of adipose tissue and more leptin release. In turn, leptin promotes pro-inflammatory responses that might contribute to inducing gliosis and hence promote ageing and neuroinflammatory disorders, particularly multiple sclerosis^{87,89}. Thus, although the activation of eCB signalling in lean animals may counteract neuroinflammation (but less so in older animals), the opposite may be true during obesity (and possibly more so in older animals), indicating an overarching change in the functionality of eCB signalling associated with ageing and disease with different, and sometimes even opposing, effects.

eCB signalling, cell metabolism and disease

Many of the signalling events downstream of cannabinoid receptors are known to funnel through molecular integrators (‘hub’ molecules) within the intracellular signalling networks that control basic cell functions, including cell viability and, by extension, normal ageing.

One key downstream signalling event of CB1 (and leptin) receptors is the serine/threonine kinase mammalian target of rapamycin (mTOR), a molecular integrator that enables both cellular nutrient sensing and energy homeostasis (FIG. 2) through the ERK/MAPK–Akt pathway (FIG. 1). Specifically, mTOR is located in two multiprotein complexes, mTORC1 and mTORC2, which control anabolic metabolism through regulation of protein translation⁹⁰. The mTORC1 complex has a role in regulating synaptic plasticity and cognitive functions, including memory, through mechanisms that depend on *de novo* protein synthesis. Acute injections of THC induce rapid and transient stimulation of mTORC1 activity in the hippocampus, striatum, cerebellum, frontal cortex and amygdala^{91,92}, whereas repeated administration of THC leads to more sustained activation of mTORC1, lasting for several days after the cessation of treatment⁹². Accordingly, the amnesic effects of THC depends on mTOR signalling and can be abolished by the mTOR inhibitor rapamycin. Further supporting a functional link between these modalities, deregulated mTOR activity is associated with metabolic, neurological and psychiatric disorders, and overactivation of mTOR signalling through enhanced eCB activity contributes to cognitive impairment in fragile X syndrome⁹³. Deregulation of this system is likely to represent an important sign of ageing⁵⁹.

The regulation of mTORC1 by CB1 receptors is remarkably wide-ranging, as mTOR has also been implicated as a central regulator of autophagy, a cellular response thought to contribute to the ageing-associated loss of protein homeostasis or ‘proteostasis’. Proteostasis refers to cellular processes of stabilizing correctly folded functional proteins, or removing misfolded dysfunctional ones through proteasomal or lysosomal mechanisms. Fundamentally, autophagy (or specifically, macroautophagy) is a cytoprotective mechanism through which potentially harmful cytoplasmic components are sequestered in vesicles and delivered to lysosomes for degradation⁹⁴. This process can also provide nutrients under conditions of starvation. Evidence shows that autophagy is reduced in aged tissues and that rapamycin, and hence mTOR inhibition, increases lifespan mainly through induction of autophagy⁹⁵. Interestingly, autophagy measured in cancer cells is also induced by eCB signalling^{96–98}. However, the underlying mechanism of this latter response is unlikely to involve the above-mentioned CB1 activation of mTOR, as this mechanism inhibits (rather than stimulates) autophagy. Indeed, there is also evidence for inhibition of autophagy by eCB signalling, as CB1 antagonism and global deletion of CB1 were found to enhance autophagy flux in mice, although in an mTOR-independent manner⁹⁹. Mice with CB1 deletion show a significantly increased accumulation of lipofuscin¹⁰⁰, an age-related lipopigment that consists of highly crosslinked non-degradable lysosomal aggregates harmful to cells¹⁰¹. This phenomenon was related to a decreased expression of the lysosomal enzyme cathepsin D, which indicates that lysosomal degradation is impaired. Thus, in mice with CB1 deletion, the enhanced autophagy may be secondary to a reduced expression of cathepsin D, rather than inhibition of mTOR¹⁰⁰ (FIG. 2).

The depiction of the molecular mechanisms mediating the age-dependent deregulation of eCB signalling and regulation of autophagy reveals an overlapping signalling framework that encompasses several common molecular hubs, many of which have also been implicated in Huntington’s disease. This devastating autosomal-dominant neurodegenerative disease is caused by CAG expansions in exon 1 of the *IT15* gene (also known as *HTT*), which encodes the protein huntingtin¹⁰². Mutated huntingtin directly affects the expression of many genes and the activity of many enzymes that participate together in the development of this disease^{102,103}. A remarkably early response induced by mutant huntingtin is to inhibit the *Cnr1* promoter in neurons. This early downregulation of CB1 receptor expression can be detected in the neurons of human patients with Huntington’s disease, and genetic mouse and cellular models of Huntington’s disease^{49,104}. Recently, genetic manipulations (deletion or rescue) of CB1 receptor expression in select neuronal populations of Huntington’s disease genetic mouse models demonstrated that downregulation of this receptor has a key role in striatal neurodegeneration, including the loss of cortical–striatal connections^{105,106}. Thus, an additional conceptual link may be drawn that connects the age-dependent loss of functional CB1 receptors in select neuronal populations, which influence mTOR1 signalling, to the ability to promote autophagy in this neuronal population, with the two contributing together to the loss of functional synaptic contacts associated with Huntington’s disease.

An additional parallel may be found in studies showing that eCBs prevents amyloid- β -induced lysosomal destabilization in cortical neurons. Importantly, this activity is dependent on lysosomal CB1 receptors, but not on receptors located on the cell surface¹⁰⁷. Indeed, it has been suggested recently that eCB signalling may be involved not only in intercellular but in intracellular communication through its ability to regulate the functions of intracellular organelles. In some cells, a substantial proportion of CB1 receptors are localized on lysosomes^{108–110}, where they may be functionally active¹⁰⁹. Furthermore, CB1 receptors have been found recently on the external membrane of brain mitochondria¹¹¹. Although this finding is still somewhat controversial¹¹², these mitochondrial receptors seem to regulate neuronal energy metabolism directly, because incubation of isolated mitochondria with THC reduces the activity of the respiratory chain complex I, decreases the level of mitochondrial cAMP, and decreases mitochondrial PKA activity¹¹² (FIG. 1). The putative mitochondrial CB1 receptors were also shown to contribute to a form of eCB-mediated short-term feedback inhibition of synaptic activity, termed depolarization-induced suppression of inhibition, with potential impact on cognitive function¹¹¹. These findings are important in light of the fact that mitochondrial dysfunctions contribute to physiological ageing⁵⁹ as well as neurodegenerative disorders¹¹³.

In summary, the studies reviewed so far, together with evidence suggesting that changes in eCB levels might have a role in amyotrophic lateral sclerosis and Parkinson’s

disease⁷⁹, outline a molecular framework that connects extra- and intracellular eCB signalling to an age- and cell-metabolism-dependent deterioration of neural cells that might contribute to some neurodegenerative diseases.

The endocannabinoidome in neurodegeneration

Since the discovery of anandamide and 2-AG, it has been clear that these signalling lipids are accompanied in tissues by congeners; that is, other NAEs and 2-acylglycerols. These lipids are biologically active and share with the two eCBs their respective biosynthetic and inactivating enzymes (FIG. 1), but not their ability to activate CB1 and CB2 receptors. We now know that the brain concentrations of NAE and 2-acylglycerol congeners increase in animals treated with inhibitors of eCB-hydrolysing enzymes (FAAH or MAGL), as well as in mice lacking these enzymes^{114,115}. Remarkably, most congeners activate more than one receptor (Supplementary information S1 (table)) and many of these eCB-related receptors are expressed by different cell types of the CNS and have been shown to be involved in ageing and neuroinflammation and/or neurodegeneration. Thus, for example, when the activity of FAAH is blocked in animal models of Parkinson's or Huntington's disease^{116,117}, non-CB1-, non-CB2-mediated biological responses might be produced by NAE congeners, and this needs to be taken into consideration when interpreting the role of this enzyme in such pathological states.

NAEs and 2-acylglycerols are not the only examples of eCB-related mediators produced by cells. Amides of several fatty acids with amino acids (known as lipoaminoacids) or amine transmitters (such as the *N*-acyldopamines and *N*-acylserotonins)^{118–120}, have been isolated and their bioactivity investigated. Although our current knowledge of the biochemistry of these putative lipid mediators is still limited, it has been observed that their biosynthesis or degradation might be affected when FAAH is inhibited^{118,120}. The role of these long-chain fatty acid derivatives in the ageing brain or during neurodegenerative disorders is largely understudied, yet several molecular targets involved in excitotoxicity and neuroinflammation are known to be modulated by them (Supplementary information S1 (table)). Striking examples are T-type Ca²⁺ channels (particularly Ca_v3.2 and Ca_v3.3), which are involved in the establishment of associative long-term potentiation, and whose levels wane with ageing¹²¹. These channels are blocked by physiological concentrations of *N*-arachidonoyldopamine and *N*-arachidonoylserotonin, which exhibit nanomolar affinity in binding assays and are therefore among their strongest endogenous inhibitors¹²². Thus, if the levels of these eCB-like mediators were to be enhanced or reduced following pharmacological inhibition of FAAH, this could impact upon synaptic plasticity and potentially result in memory disturbances. These memory deficits could be more problematic if the levels of these mediators were shown to be altered during ageing.

To complicate things further, both anandamide and 2-AG can be oxidized efficiently by enzymes of the arachidonate cascade, in particular by COX2, and thus yield yet another class of bioactive lipids that act through yet

other sets of receptors^{30,123}. This reaction has now been demonstrated to occur also *in vivo*, especially under conditions in which COX2 is upregulated, and eventually leads to prostaglandin ethanolamides (or prostamides) and prostaglandin glycerol esters for anandamide and 2-AG, respectively. These metabolites produce biological effects via GPCRs that are pharmacologically different from both cannabinoid and prostaglandin receptors and are yet to be fully identified at the molecular level¹²⁴. It is reasonable to expect that the formation of these bioactive metabolites is enhanced following pharmacological inhibition of the hydrolytic catabolism of eCBs by FAAH and MAGL (or ABHD6)¹²³. One example was reported in relation to the neurotoxicity model of striatal neurons induced by malonate (which, if injected into the striatum of rats, produces effects that recapitulate some of the features of Huntington's disease). In this model, inhibitors of MAGL (which would increase 2-AG accumulation and thus would be expected to be neuroprotective) were found, surprisingly, to worsen malonate-induced neurotoxicity and neuroinflammation, whereas inhibition of 2-AG biosynthesis by blocking DAGLα produced beneficial effects¹²⁵. The mechanism underlying these unexpected results appears to involve a bioactive COX2 metabolite of 2-AG because treatment of striatal neurons with malonate in the presence MAGL inhibitors leads to the formation of prostaglandin E2 glycerol ester, which produces neurotoxic effects attenuated by a specific antagonist of prostaglandin E2 glycerol ester receptors¹²⁵. Another example comes from experimental models of Parkinson's disease, in which 2-AG, which has been known for decades to act as a biosynthetic precursor for arachidonic acid and eicosanoids in sensory neurons, may contribute to the neuroinflammatory response. Genetic or pharmacological inhibition of MAGL leads to reduced activity in the prostaglandin-mediated pro-inflammatory signalling cascades that underlie the neurodegeneration process measured in this model¹²⁶. A key control was to show that the amelioration of Parkinson's disease pathogenesis following MAGL inhibition was not due to indirect activation of cannabinoid receptors, but instead to reduction of prostaglandin biosynthesis via the 2-AG–arachidonate pathway¹²⁶. Additional evidence comes from animal models of Alzheimer's disease. In one study¹²⁷, inhibition of MAGL was shown to attenuate neuroinflammation and lower amyloid-β levels and plaques, effects that seem to involve 2-AG-derived prostaglandins. In another study, inhibiting MAGL prevented neuroinflammation, neurodegeneration and decreases in integrity of hippocampal synaptic structure and function, thereby improving long-term synaptic plasticity, spatial learning and memory¹²⁸. In both these studies, the effects were independent of CB1 and CB2 receptors. Thus, MAGL inhibitors might cause opposing actions on neuroinflammation depending on whether or not, in a given context and experimental set-up, 2-AG acts as a substrate for COX2 per se, thereby producing pro-inflammatory prostaglandin E2 glycerol ester, or only after its hydrolysis to arachidonic acid, thereby producing more conventional pro-inflammatory prostaglandins (FIG. 4).

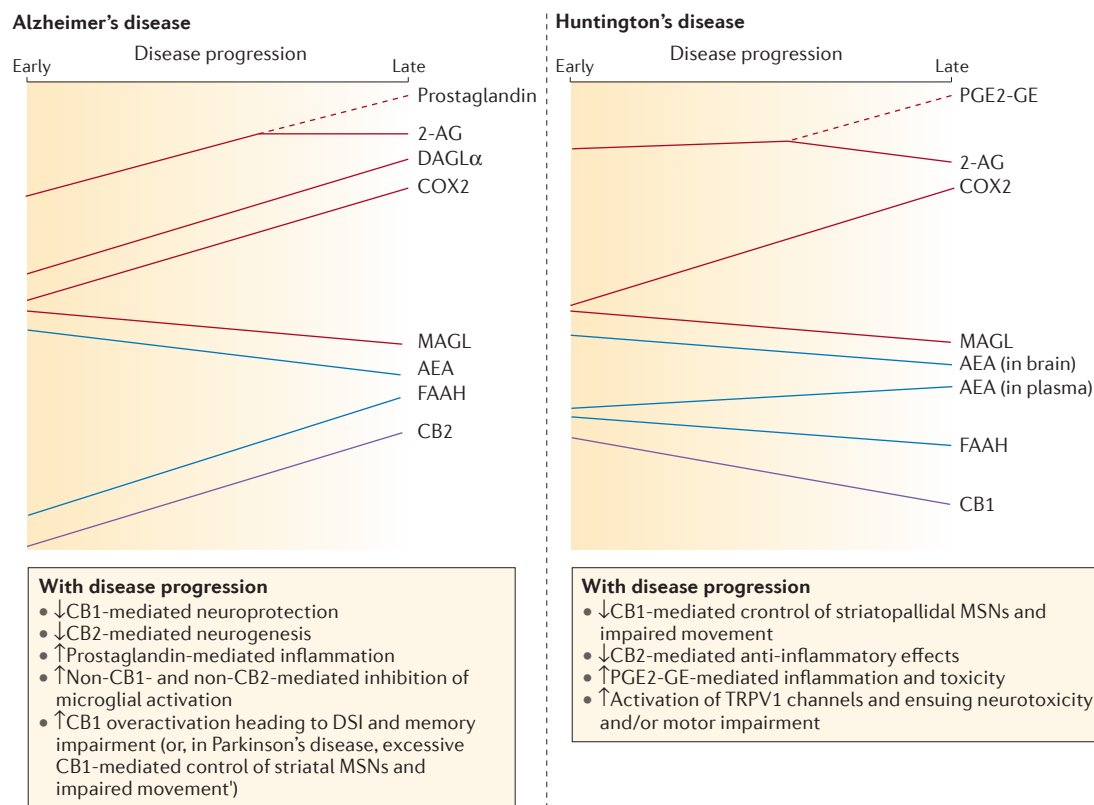


Figure 4 | The endocannabinoidome in neurodegenerative diseases. The complexity of the alterations of components of the endocannabinoid (eCB) system and of some elements of the 'endocannabinoidome', in terms of redundancy of metabolic pathways, involvement of non-cannabinoid type 1 (CB1), non-CB2 receptors and tissue- or time-dependent changes is summarized. Time-dependent changes of the brain levels of some of the eCB synthesizing and inactivating enzymes and eCBs, and of the expression of eCB molecular targets, in animal models of neurodegenerative diseases (and, when available, in post-mortem brains of patients with these disorders) are depicted with their potential consequences. Red lines indicate changes in 2-arachidonoyl-glycerol (2-AG) and its metabolic enzymes or products over time during the progression of either Huntington's or Alzheimer's disease. Time-dependent changes in anandamide (AEA) and fatty acid amide hydrolase 1 (FAAH) over the course of these diseases are shown in blue. Purple lines denote changes in CB1 and CB2 during Alzheimer's and Huntington's disease. Note that CB1 and CB2 receptor levels are unchanged in Alzheimer's disease and Huntington's disease, respectively (not shown). Dashed branches show when a mediator begins to be partially metabolized into another one. In this case, the levels of the mediator being transformed start being reduced and those of its product start being increased. Note how the levels of AEA and 2-AG may change in different or opposite ways in the two conditions. They can also produce opposite effects^{147,148}, depending on the production of cyclooxygenase 2 (COX2) metabolites (in the case of 2-AG)^{125–128} or because of activation of non-cannabinoid receptors (for example, transient receptor potential cation channel subfamily V member 1 (TRPV1) channels in the case of AEA)^{116,117}. Also within the same disorder, a given eCB may first increase and then decrease (as with 2-AG in Alzheimer's disease)¹⁴⁷, or change in opposite ways in different brain areas or blood (as with AEA in Huntington's disease)¹⁴⁹. Finally, some of the features of Alzheimer's disease, such as the formation of 2-AG-derived prostaglandins, or the participation of CB1 receptors in determining some of the symptoms¹⁴⁸, also occur in models of Parkinson's disease. DSI, depolarization-induced suppression of inhibitory neurotransmission; MSNs, medium-spiny neurons; PGE2-GE, prostaglandin E2 glycerol ester.

The multifunctionality of these bioactive lipids is still more complex than discussed above. At submicromolar concentrations, anandamide and 2-AG — even before their catabolism — may modulate the activity of additional non-cannabinoid receptors directly. Modulating these other targets may either reduce or enhance the potential therapeutic advantage of agents designed to pharmacologically manipulate eCB tissue levels. For example, activation of TRPV1 channels is perhaps the best-established non-CB1-, non-CB2-receptor-mediated action of anandamide³³. TRPV1 is expressed by both central neurons and glial cells (although less abundantly than

in peripheral sensory neurons), and is involved in exacerbating glutamate excitotoxicity and neuroinflammation¹²⁹; yet activation of this channel also contributes to changes in synaptic plasticity that can be beneficial to behavioural and cognitive deficits associated with neurodegenerative disorders^{129,130}. Accordingly, TRPV1 activation can both exacerbate and counteract some of the major symptoms in animal models of Parkinson's and Huntington's diseases^{116,117,131,132}. Another example is found in 2-AG that directly enhances GABA_A receptor activity¹³³, which may influence several aspects of ageing and neurodegeneration, including excitotoxicity, the development of

neuroinflammation and the establishment of synaptic plasticity. Finally, both anandamide and 2-AG may also activate PPAR γ , which is also emerging as a player in the control of neuronal activity and neuroinflammation by these signalling lipids^{34,134}.

When reviewing this evidence, it is possible to see that the discovery of the eCB system has in fact unravelled a new world of lipid mediators, enzymes and molecular targets, which could be seen as an 'endocannabinoidome' (Supplementary information S1 (table)). This concept is not just a matter of mere definitions, or of viewing things in a holistic manner, but may influence the future planning, design and eventual testing — in appropriate patient populations — of drugs that interfere with selected molecular components of this complex signalling system.

Concluding remarks

The wealth of studies on the action of eCBs and synthetic cannabinoids at CB1 and CB2 receptors in the context of neuroinflammation, neurodegeneration and ageing points to several molecular hubs that are likely to dictate the cellular response induced by these ligands. For example, as eCBs modulate a wide range of biological function through specific receptors, the enzymes involved in their inactivation provide a target through which one can indirectly regulate the activity of such receptors and, hence, the pathological consequences of their malfunctioning. A control mechanism over eCB levels and signalling has been reported for FAAH, MGL and ABHD6, and we now understand that these enzymatic hubs control the levels of several distinct signalling lipids that target a broad panel of receptors within a signalling framework. A recent example of this

complexity was provided with ABHD6 inhibitors that control seizure incidence in a mouse model of the juvenile form of Huntington's disease, an effect mediated by the action of 2-AG at GABA $_A$ rather than cannabinoid receptors¹³⁵. We also appreciate that this fine tuning of receptor activity by signalling lipids is in sharp contrast to the more sustained bioavailability of synthetic agonists, which is mainly determined by their pharmacokinetic profile typified by their accumulation in brain tissue. Thus, it will be important to bear in mind that control of cellular responses by synthetic compounds will not precisely mimic the more rapid and time- and space-specific activation and inactivation by endogenous signalling lipids. In particular, when considering the role of the eCB signalling system in controlling multiple modalities involved in ageing, neuroinflammation and neurodegeneration, a framework appears in which either one or several components of the endocannabinoidome may have to be targeted for therapy (TABLE 1). Specifically, the effects of manipulating eCB levels pharmacologically using inhibitors of FAAH and MAGL have been tested in experimental models of multiple sclerosis, and Alzheimer's, Parkinson's and Huntington's diseases, often yielding contradictory results, possibly owing to the intrinsic differences and heterogeneity of these model systems. Such inhibitors produce clear symptomatic relief (in terms of alleviation of spasticity) in chronic or relapsing EAE¹³⁶, and this might be one way by which CBD contributes to the anti-spasticity effects of Sativex in multiple sclerosis, as this phytocannabinoid is known to inhibit FAAH and can lead to increases in AEA levels in humans^{137,138}. Importantly, following induction of EAE, reduced neuroinflammation and neurodegeneration have also been

Table 1 | Possible endocannabinoid-system-based approaches for the treatment of neurodegenerative disorders.

eCB-system-based drugs	Rationale	Advantages	Disadvantages	Disease	Refs*
Inhibitors of eCB cellular reuptake and enzymatic hydrolysis (by FAAH, MAGL, ABHD6)	Mimic the beneficial neuromodulatory effects of CB1 activation and the anti-inflammatory effects of CB2 activation in a time- and tissue-selective manner	Activity-dependent control of therapeutic effect, and fewer side effects	<ul style="list-style-type: none"> • Indirect activation of non-CB1, non-CB2 receptors (with FAAH inhibitors) • Possible receptor desensitization at high dose (MAGL inhibitors) 	<ul style="list-style-type: none"> • Multiple sclerosis • Alzheimer's disease (depending on model and disease phase) • Parkinson's disease 	116, 136, 147, 150
Inhibitors of MAGL	Inhibition of 2-AG-derived inflammatory prostaglandins	Activity-dependent control of therapeutic effect, fewer side effects	Inhibition of beneficial effects of prostaglandins in other tissues or organs	<ul style="list-style-type: none"> • Alzheimer's disease • Parkinson's disease 	126–128
CB2 agonists	Reduction of inflammatory component of disease	Relative lack of psychotropic effects compared to CB1 agonists	<ul style="list-style-type: none"> • Possible receptor desensitization at high dose • May favour leukocyte infiltration due to chemotaxis 	<ul style="list-style-type: none"> • Huntington's disease • Amyotrophic lateral sclerosis • Multiple sclerosis 	145, 151–153
CB1 antagonists or inverse agonists	Reduction of the neurochemical imbalance due to prolonged alterations of eCB signalling	Already tested in humans	Potential psychiatric adverse events (anxiety, depression)	Parkinson's disease and Alzheimer's disease (depending on model and disease phase)	141, 142, 154

2-AG, 2-arachidonoyl-glycerol; ABHD6, α,β hydrolase 6; CB1, cannabinoid receptor 1; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase 1; MAGL, monoacylglycerol lipase. *Selected references.

shown in mice with FAAH deletion compared to wild-type mice^{139,140}. By contrast, studies testing the efficacy of FAAH inhibitors in animal models of Alzheimer's, Parkinson's and Huntington's diseases and of amyotrophic lateral sclerosis have been less successful. Possible explanations include: the potential harmful role played by eCB signalling in the Alzheimer's- and Parkinson's-disease-like pathogenesis (to the point that CB1 blockers also produce beneficial actions in these cases)^{141,142}; the possibility of inducing indirect activation of non-cannabinoid receptors (for example, TRPV1 channels) when inhibiting FAAH in Parkinson's and Huntington's diseases^{116,117}; the downregulated CB1 receptor expression and/or FAAH activity in Huntington's disease^{117,143,144}; the seemingly opposite roles of CB1 and CB2 receptors in determining survival in the superoxide dismutase (SOD1) mouse model of amyotrophic lateral sclerosis¹⁴⁵; and the different role of CB1 receptors when expressed on glutamatergic versus GABAergic terminals (neuroprotective only in the

former case), for example, in a model of Huntington's disease¹⁰⁶. Indeed, differential coupling and function between CB1 receptors expressed by either glutamatergic and GABAergic neurons has been described in detail¹⁴⁶. Thus, it is also possible that, owing to the time- and age-dependent nature of neurodegenerative disorders, and the plastic changes and roles of cannabinoid and eCB-related receptors in each of their phases (FIGS 3,4), similar therapeutic effects might be observed with drugs producing opposite actions on eCB actions or levels^{117,125}; or an inhibitor of eCB inactivation might produce both amelioration and worsening of the symptoms, in different phases of a given condition¹⁴⁷. These considerations provide a strong stepping stone to develop new therapies for both symptom alleviation and disease modification in neuroinflammatory and neurodegenerative disorders based on the pharmacological manipulation of eCB signalling, which may be a challenging but nevertheless fascinating task for our field of research to tackle in the future.

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Competing interests statement

The authors declare **competing interests**: see Web version for details.

FURTHER INFORMATION

Vincenzo Di Marzo's homepage: <http://www.icb.cnr.it/erg>
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