

## Cannabinoids and the expanded endocannabinoid system in neurological disorders

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**Abstract** | Anecdotal evidence that cannabis preparations have medical benefits together with the discovery of the psychotropic plant cannabinoid  $\Delta^9$ -tetrahydrocannabinol (THC) initiated efforts to develop cannabinoid-based therapeutics. These efforts have been marked by disappointment, especially in relation to the unwanted central effects that result from activation of cannabinoid receptor 1 (CB1), which have limited the therapeutic use of drugs that activate or inactivate this receptor. The discovery of CB2 and of endogenous cannabinoid receptor ligands (endocannabinoids) raised new possibilities for safe targeting of this endocannabinoid system. However, clinical success has been limited, complicated by the discovery of an expanded endocannabinoid system — known as the endocannabinoidome — that includes several mediators that are biochemically related to the endocannabinoids, and their receptors and metabolic enzymes. The approvals of nabiximols, a mixture of THC and the non-psychotropic cannabinoid cannabidiol, for the treatment of spasticity and neuropathic pain in multiple sclerosis, and of purified botanical cannabidiol for the treatment of otherwise untreatable forms of paediatric epilepsy, have brought the therapeutic use of cannabinoids and endocannabinoids in neurological diseases into the limelight. In this Review, we provide an overview of the endocannabinoid system and the endocannabinoidome before discussing their involvement in and clinical relevance to a variety of neurological disorders, including Parkinson disease, Alzheimer disease, Huntington disease, multiple sclerosis, amyotrophic lateral sclerosis, traumatic brain injury, stroke, epilepsy and glioblastoma.

*Cannabis sativa* is a common plant that has been used for several purposes for millennia. Desiccated flowers of some cannabis plant varieties that contain psychotropic compounds were used by so-called healers in early civilizations<sup>1</sup>. Anecdotal evidence and, more recently, medical case reports suggest that the plant has therapeutic effects<sup>1</sup>. In the late 20th century, the first cannabis-derived compound was approved for clinical use<sup>2</sup>, and subsequently the first was approved for a neurological disorder<sup>3,4</sup>.

The typical natural products that derive from cannabis plant flowers (cannabinoids) — such as  $\Delta^9$ -tetrahydrocannabinol (THC) and the non-euphoric cannabidiol (CBD)<sup>5,6</sup> — were characterized in the 1960s, leading to major breakthroughs in our understanding of the plant's effects. Insights into the mechanism of action of THC, which is the psychotropic component of marijuana, led to identification of the cannabinoid receptors in the 1990s<sup>7,8</sup> and, consequently, of endogenous ligands of these receptors, which became known as

endocannabinoids<sup>9–12</sup>. Subsequently, it became clear that cannabinoid receptors and endocannabinoids are pleiotropic signalling molecules involved in re-establishing homeostasis after pathological insults, suggesting therapeutic opportunities for multiple pathologies, including neurological disorders<sup>13–16</sup>. Studies in animal models soon showed that this signalling system is altered in neurological diseases, motivating efforts to translate these findings into treatments<sup>17</sup>. The approval of nabiximols — a combination of THC and CBD — for the treatment of pain and/or spasticity in multiple sclerosis (MS) in 2005 (REF.<sup>3</sup>) was a milestone in cannabis research.

In this Review, we first provide an overview of cannabinoids and the extended endocannabinoid system (the endocannabinoidome). We then consider the molecular and cellular bases of endocannabinoidome function and malfunction in the brain, and discuss preclinical and clinical studies of cannabinoids and endocannabinoidome-based drugs as potential therapies in neurological disorders.

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## Key points

- Cannabinoid receptors 1 and 2 (CB1 and CB2), the two endocannabinoids anandamide and 2-arachidonoylglycerol, and endocannabinoid anabolic and catabolic enzymes form the endocannabinoid system.
- Endocannabinoid signalling is involved in regulation of cell, tissue, organ and organism homeostasis, brain development, neurotransmitter release and synaptic plasticity, and cytokine release from microglia, and hence is implicated in multiple neurological disorders.
- Endocannabinoid signalling is altered in most neurological disorders; enhancers or inhibitors of endocannabinoid signalling can have therapeutic effects in preclinical models, depending on disease characteristics and the roles of CB1 and CB2.
- Endocannabinoids can activate different receptors and their biosynthetic and catabolic pathways are often shared with other mediators. Consequently, the system is considered to be part of an expanded signalling system, the endocannabinoidome.
- The endocannabinoidome hinders therapeutic targeting of endocannabinoid anabolic or catabolic enzymes but inhibitors of endocannabinoid inactivation and allosteric modulators of CB1 and CB2 are being actively investigated in neurological disorders.
- The existence of the endocannabinoidome explains in part why some non-euphoric cannabinoids, which affect several endocannabinoidome proteins, are useful for the treatment of neurological disorders, such as multiple sclerosis and epilepsy.

### Cannabinoid signalling *Cannabis and cannabinoids*

Following increased recreational use of marijuana in the 1960s, anecdotal reports indicated its benefits in conditions such as MS, epilepsy and Tourette syndrome. Consequently, major efforts were made to identify the chemicals responsible for the euphoric, perception-altering and potential medicinal effects of marijuana and other preparations of cannabis flowers<sup>18–21</sup>. These efforts culminated in identification of cannabiol (later shown to be a processing product of THC), CBD and THC<sup>5,6,22</sup>. In animals, only THC (and to a lesser degree cannabiol) produce similar effects to those of marijuana, such as catalepsy, hypolocomotion, analgesia and hypothermia in mice and static ataxia in dogs<sup>23,24</sup>. Consequently, THC was considered to be the major psychotropic component of recreational cannabis preparations. Indeed, >100 unique cannabinoids have now been identified and almost all are non-psychotropic<sup>25</sup>, although many are present in recreational cannabis preparations.

Although THC mediates the euphoric effects of cannabis preparations<sup>26</sup>, there is no reason to believe that it also mediates the apparent medicinal effects of cannabis, and CBD is also considered clinically interesting for its therapeutic potential in several disorders. Specific central and peripheral targets of THC and CBD have been identified<sup>7,8,25</sup> — THC is relatively specific for cannabinoid receptors, and CBD modulates the activity of several proteins. The psychoactivity of THC narrows its therapeutic window and limits its applications, but CBD is more amenable to clinical development, even for paediatric populations<sup>27,28</sup>.

### The endocannabinoid system

Use of a synthetic, radiolabelled THC analogue led to the initial identification of high-affinity binding sites for THC in the brain<sup>29</sup>, later identified as the cannabinoid receptor 1 (CB1), a G protein-coupled receptor (GPCR) that is expressed most abundantly in the brain. Cannabinoid receptor 2 (CB2), which is also a GPCR,

was later identified by homology cloning and found to be highly expressed in the immune system<sup>7,8</sup>. These fundamental breakthroughs led to identification of endogenous CB1 and CB2 ligands. The lipids anandamide (the ethanolamide of arachidonic acid) and 2-arachidonoylglycerol (2-AG) (FIG. 1) were identified in brain and intestinal samples and shown to activate CB1 and CB2 with high affinity and efficacy<sup>9–11</sup>. Consequently, these lipids were named endocannabinoids<sup>12</sup>.

Subsequently, enzymes involved in endocannabinoid biosynthesis and inactivation were identified<sup>30–33</sup> (FIG. 1). *N*-acylphosphatidylethanolamine (NAPE)-specific phospholipase D-like hydrolase (NAPE-PLD) catalyses the synthesis of anandamide and other *N*-acylethanolamines<sup>33</sup>, and fatty acid amide hydrolase (FAAH) catalyses the hydrolysis of anandamide (and other *N*-acylethanolamines and fatty acid primary amides)<sup>31</sup>. Diacylglycerol lipase  $\alpha$  (DAGL $\alpha$ ) and DAGL $\beta$  catalyse the biosynthesis of 2-AG and other monoacylglycerols<sup>30</sup> and monoacylglycerol lipase (MAGL) catalyses the hydrolysis of 2-AG (and that of other monoacylglycerols)<sup>32</sup>. This system of endogenous signals, receptors and metabolic enzymes became known as the endocannabinoid system (TABLE 1).

Alterations in the endocannabinoid system are found in experimental models of, and patients with, most neurological diseases<sup>34–36</sup> and genetic manipulation of the system in mouse models alters susceptibility to neurodegenerative disorders<sup>37–41</sup>. These findings suggest that targeting components of the endocannabinoid system is a possible therapeutic strategy<sup>42</sup>.

### The endocannabinoidome

The endocannabinoid system is complicated by promiscuity of mediators, overlap with other pathways and alternative metabolic processes, so modulation of its components affects a wider endocannabinoid-related network known as the endocannabinoidome (TABLE 1). This complex system poses a challenge for the development of selective endocannabinoid-based drugs but also offers new opportunities for the exploitation of non-THC cannabinoids, which often modulate several endocannabinoidome proteins (FIG. 1). The main elements of and mechanisms involved in the endocannabinoidome are outlined below.

**Endocannabinoid degradation.** Direct activation of CB1 — claimed to be the most abundant GPCR in the mammalian brain — is accompanied by CNS-related adverse effects that can be serious<sup>16</sup>. Inhibition of FAAH (which increases levels of anandamide and therefore increases CB1 activation) does not normally have such effects but does increase levels of other endogenous FAAH substrates that activate other receptors, including peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ), orphan GPCR 119 (GPR119), orphan GPCR 55 (GPR55) and the transient receptor potential cation channel subfamily V member 1 (TRPV1)<sup>17</sup>. These receptors often have roles opposite to those of cannabinoid receptors<sup>43–46</sup>. Similarly, substrates of MAGL include monoacylglycerols other than 2-AG<sup>47</sup> that also target receptors other than CB1 and CB2, including TRPV1 and GPR119 (REFS<sup>45,48</sup>). FAAH and MAGL inhibitors have been proposed as

safer alternatives to direct CB1 agonists, but their effects are occasionally unpredictable because they indirectly activate non-cannabinoid receptors.

Inhibition of their enzymatic hydrolysis makes anandamide and 2-AG available for other enzymatic reactions that produce mediators with different receptors. Anandamide and 2-AG can be metabolized via oxidation by cyclooxygenase 2 (REF. 49) and the end products (prostaglandin ethanolamides and prostaglandin glycerol esters) act at receptors other than cannabinoid and prostanoid receptors<sup>50,51</sup>. Furthermore, 2-AG can be phosphorylated to the corresponding lysophosphatidic acid, which acts at its own receptors<sup>52</sup>. Anandamide and 2-AG can also be inactivated by other hydrolases<sup>47,53</sup> (FIG. 1), but these enzymes also metabolize other lipids, so targeting them would create other problems.

Finally, 2-AG is a more effective CB1 agonist than anandamide, so increasing its levels by inhibition of MAGL can cause desensitization of CB1. Consequently, chronic administration of MAGL inhibitors can produce effects that are opposite to those of CB1 activation or cause tolerance<sup>54,55</sup>. Conversely, 2-AG is a precursor of arachidonic acid and pro-inflammatory prostanoids, so beneficial effects of MAGL inhibitors, particularly those seen in experimental models of Alzheimer disease (AD) and Parkinson disease (PD)<sup>56,57</sup>, might be mediated by inhibition of prostanoid receptor signalling.

**Endocannabinoid biosynthesis.** Redundancy and promiscuity are hallmarks of endocannabinoid biosynthesis as well as degradation. Anandamide and 2-AG can be produced by several pathways and enzymes that are also involved in the biosynthesis of other *N*-acylethanolamines and monoacylglycerols<sup>17</sup> (FIG. 1). Therefore, inhibition of the two main enzymes involved in endocannabinoid synthesis might not always selectively or effectively reduce tissue levels of the two endocannabinoids and could affect levels of other mediators.

**Promiscuity of endocannabinoid targets.** To complicate things further, endocannabinoids act on other targets; for example, anandamide activates TRPV1 and PPAR $\gamma$  and inhibits Ca<sub>v3.2</sub> Ca<sup>2+</sup> channels and transient receptor potential cation channel subfamily M member 8 (TRPM8) channels, whereas 2-AG activates TRPV1 channels and GABA<sub>A</sub> receptors<sup>17</sup>. Consequently, even if anandamide and 2-AG were hydrolysed selectively or were not precursors of other bioactive molecules, their inhibition could indirectly modulate the activity of receptors other than CB1 and CB2.

**Endocannabinoid-like mediators.** The complexity of endocannabinoid-related molecules extends to other long-chain *N*-acyl-amides, including *N*-acyl-aurines<sup>58</sup>, *N*-acyl-serotonins<sup>59</sup>, *N*-acyl-dopamines<sup>60</sup>, fatty acid primary amides<sup>31</sup> and a plethora of *N*-acyl-amino acids. Each of these mediators has its own molecular targets and metabolic enzymes (FIG. 1) and interacts with these promiscuously. These receptors and enzymes are often shared with the endocannabinoids, justifying the name of endocannabinoidome for this complex signalling system<sup>61</sup> (FIG. 1).

**Allosteric modulators of CB1 and CB2.** Positive and negative allosteric modulators of CB1 and CB2 are emerging as possible solutions to the complexity of the endocannabinoidome. By modulating endocannabinoid signalling at these receptors, they preserve the site-selectivity and time-selectivity of endocannabinoid action but, unlike FAAH and MAGL inhibitors, do not interfere with other mediators<sup>16</sup>. Allosteric modulators of CB1 and CB2 that have been identified<sup>62,63</sup> include endogenous molecules, such as the haemopressins and related peptides, which are hydrolytic products of  $\alpha$ -haemoglobin<sup>64</sup>, and some previously discovered lipids, such as lipoxin A4 and pregnenolone<sup>65,66</sup>.

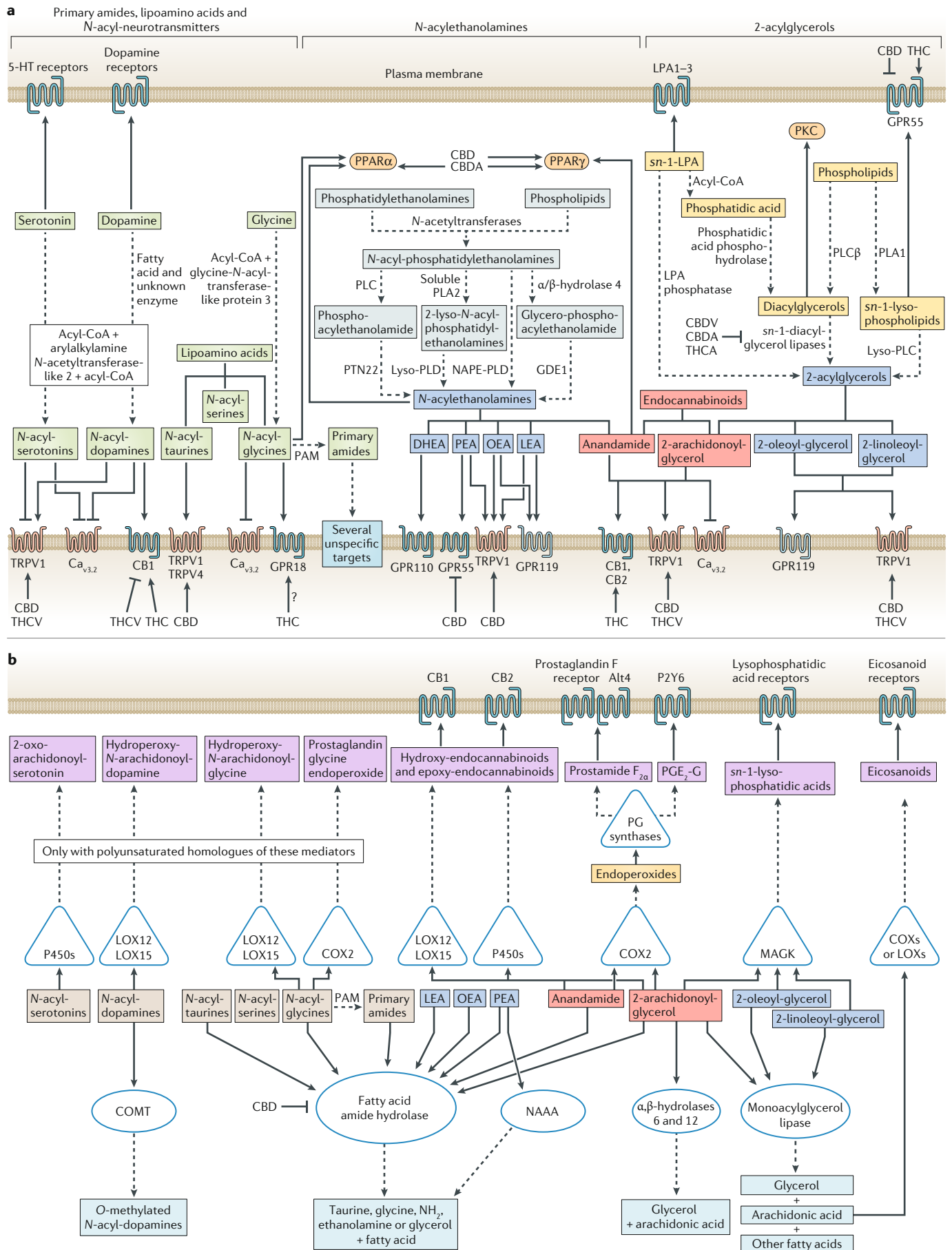
## Physiological roles

### CB1 receptors

Advanced microscopy techniques, such as electron microscopy and super-resolution microscopy, have refined knowledge of the anatomical distribution of CB1 receptors in the brain<sup>67</sup> and have revealed some molecular mechanisms behind the major effects of THC and synthetic CB1 agonists on mood, perception, cognition and locomotion in humans and animals<sup>68</sup>. A major breakthrough was the discovery that CB1 is mostly located presynaptically in excitatory and inhibitory neurons<sup>69,70</sup>. Other important findings include the presence of DAGL $\alpha$  in postsynaptic membranes and of MAGL in axon terminals, and that presynaptic CB1 can inhibit voltage-gated Ca<sup>2+</sup> channels and vesicular release of GABA or glutamate<sup>69</sup>. Together, these findings indicate that endocannabinoids, particularly 2-AG, are inhibitory retrograde neuromodulators<sup>71</sup> (FIG. 2).

This hypothesis has subsequently been confirmed in almost all brain regions investigated. Depending on whether CB1 is expressed in glutamatergic or GABAergic afferents, retrograde activation of the receptor underlies short-term and long-term forms of synaptic plasticity, including depolarization-induced and metabotropic receptor-mediated suppression of excitatory and inhibitory neurotransmission, long-term depression of excitation or inhibition, and long-term potentiation<sup>72</sup>. These effects, often through modulation of multi-synaptic circuitries, are thought to underlie most CB1-mediated effects of endocannabinoids. In neurological disorders<sup>42,72</sup>, the timing of CB1 activation and the distribution of the receptor between inhibitory and excitatory terminals might be altered, thereby leading to profound alterations of CB1 function.

CB1 receptors are not only expressed presynaptically or only in neurons (FIG. 2). Postsynaptic CB1 receptors mediate slow self-inhibition of neocortical interneurons<sup>73</sup> and change expression of precursors of appetite-controlling peptides in the arcuate nucleus of the hypothalamus<sup>74,75</sup>. Some evidence suggests that a small proportion of postsynaptic CB1 is located in the external membrane of mitochondria<sup>76</sup>, where it inhibits electron transport and the respiratory chain, thereby affecting brain metabolism and memory formation<sup>77</sup>. In astrocytes, CB1 is involved in the regulation of synaptic plasticity in the hippocampus and in leptin signalling in the hypothalamus<sup>78,79</sup>. Activation of CB1 also stimulates proliferation of adult progenitor stem cells and their





**Fig. 1 | The expanded endocannabinoid system. a** | The endocannabinoids anandamide and 2-arachidonoylglycerol (red boxes) are often accompanied by their congeners, the *N*-acylethanolamines and the 2-acylglycerols (dark blue boxes). These congeners share biosynthetic pathways and enzymes with the endocannabinoids (pale blue for *N*-acylethanolamines and yellow for 2-acylglycerols) and modulate targets other than cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), such as transient receptor potential cation channel subfamily V member 1 (TRPV1), peroxisome proliferator-activated nuclear receptor- $\alpha$  (PPAR $\alpha$ ) and PPAR $\gamma$ , T-type  $\text{Ca}^{2+}$  ( $\text{Ca}_{v3.2}$ ) channels, and orphan G protein-coupled receptors such as GPR18, GPR55, GPR110 and GPR119. The biosynthetic precursors of 2-acylglycerols also have their own targets, such as protein kinase C (PKC), GPR55 and lysophosphatidic acid receptors 1–3 (LPA1–3). Other long-chain fatty acid amides, such as primary amides, lipoamino acids and some *N*-acyl-neurotransmitters have also been identified as elements of the expanded endocannabinoid system with promiscuous targets, whereas no receptor for *N*-acyl-serines has been identified. Distinct biosynthetic pathways exist for different lipoamino acids and *N*-acyl-neurotransmitters (pale green boxes). Intracellular targets are shown as orange rounded boxes. Plant cannabinoids modulate several targets of the expanded endocannabinoid system or endocannabinoidome. **b** | The endocannabinoids, their congeners and the various long-chain fatty acid amides often share inactivating enzymes, although these enzymes have different substrate selectivity. Fatty acid amide hydrolase breaks down long-chain *N*-acylethanolamines, *N*-acyltaurines and *N*-acylglycines; fatty acid amide hydrolase 2 (so far found only in human tissues) has a preference for oleoylethanolamide (OEA) and linoleoylethanolamide (LEA); *N*-acylethanolamine acid amidohydrolase (NAHA) recognizes saturated *N*-acylethanolamines, such as palmitoylethanolamide (PEA); monoacylglycerol lipase is specific for long-chain 2-acylglycerols, especially those that are unsaturated; and  $\alpha$ , $\beta$ -hydrolases 6 and 12 also recognize long-chain 2-acylglycerols and have non-endocannabinoidome ester substrates. In addition, some oxidizing enzymes of the arachidonate cascade, such as cyclooxygenase 2 (COX2), and various lipoxygenases (LOX) recognize the polyunsaturated fatty acid-containing endocannabinoid congeners. Several metabolic products of these congeners have their own receptors, whereas the LOX and cytochrome P450 oxygenase (P450) derivatives of endocannabinoids can still activate CB1 and CB2 receptors. Solid arrows denote modulation or interaction with protein targets, dashed arrows denote metabolic transformation. 5-HT, 5-hydroxytryptamine; Alt4, splicing variant 4 of the FP receptor; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarin; COMT, catechol O-methyltransferase; DHEA, *N*-docosahexaenoyl-ethanolamine; GDE1, glycerophosphodiester phosphodiesterase 1; lyso-PLD, lysophospholipase D; MAGK, monoacylglycerol kinase; NAPE-PLD, *N*-acyl-phosphatidylethanolamine-specific phospholipase D; PAM, peptidyl-glycine  $\alpha$ -amidating monooxygenase; P2Y6, P2Y purinoceptor 6; PG, prostaglandin; PLA, phospholipase A; PLC, phospholipase C; PTN22, tyrosine-protein phosphatase non-receptor type 22; THC,  $\Delta^9$ -tetrahydrocannabinol; THCA,  $\Delta^9$ -tetrahydrocannabinolic acid; THCV,  $\Delta^9$ -tetrahydrocannabivarin. Adapted from REF.<sup>340</sup>, Springer Nature Limited.

differentiation into neurons or astrocytes<sup>80</sup>, a role that could be relevant to neurodegenerative disorders.

### CB2 receptors

Evidence from studies in the context of neurological disease indicates that the major role of CB2 is immune modulation. Studies of human brain samples indicate that CB2 is strongly and selectively expressed in microglia in diseases such as AD, MS and amyotrophic lateral sclerosis (ALS)<sup>37</sup>. Another study has indicated that CB2 reduces pro-inflammatory cytokine release from activated microglia in AD<sup>81</sup> (FIG. 2).

As for CB1, CB2 activation also stimulates adult neurogenesis<sup>82</sup>, and some evidence indicates a role for the receptor in regulating blood–brain barrier (BBB) permeability<sup>83</sup>. Some studies have suggested that CB2 is expressed at very low levels in healthy neurons and that their activation has the opposite effects to CB1 activation<sup>84,85</sup>. However, the strength of these studies is uncertain because some relied on pharmacological or immunological tools that were later found to have low selectivity<sup>86,87</sup>.

In addition, the mechanism by which CB2 alters neuronal function is still undefined. One study has suggested that activation of postsynaptic CB2 reduces neuronal excitability in the CA3 and CA2 regions of the hippocampus through functional coupling with the sodium-bicarbonate transporter<sup>88</sup>. Developing CB2 agonists as safe drugs for neurological disorders might be difficult if it is confirmed that they alter mood and cognition.

### Other endocannabinoidome receptors

The most studied of the receptors involved in the wider endocannabinoidome are TRPV1, PPAR $\gamma$  and PPAR $\alpha$ , although some work has addressed the role of two orphan GPCRs, GPR55 and GPR18.

TRPV1 was thought not to have a function in the brain until it was found in GABAergic and glutamatergic terminals and neuronal somata in the hippocampus and cerebellum<sup>89,90</sup>. Demonstration that TRPV1 in these neurons generates  $\text{Ca}^{2+}$  influx and depolarization as it does in spinal or sensory neurons has been difficult, but its role in short-term and long-term synaptic plasticity is well established and has implications in the regulation of mood, fear, memory, food intake, visual development and locomotion<sup>91</sup>. TRPV1 is thought to increase excitability of central neurons, as suggested by studies in epilepsy models<sup>92,93</sup>. However, TRPV1 also mediates long-term depression through upregulation of AMPA receptor reuptake<sup>94</sup> (FIG. 2). Conversely, TRPV1 increases glutamatergic neurotransmission via microvesicle release from microglia, particularly in neuroinflammatory conditions<sup>95</sup>, although its activation inhibits release of inflammatory cytokines from activated microglia<sup>96</sup>.

PPAR $\alpha$  and PPAR $\gamma$  are expressed in neurons, astrocytes and microglia in the brain, where they have anti-inflammatory and neuroprotective effects during acute and chronic neuroinflammatory insults, such as brain trauma, ischaemia, AD and MS<sup>97</sup>. Experiments in mice without active forms of these receptors have provided insight into their physiological functions. For example, both isoforms have been associated with ethanol consumption<sup>98</sup>, whereas PPAR $\alpha$  activation by some *N*-acylethanolamines or *N*-oleoyl-glycine<sup>46,99</sup> reduces nicotine preference. Additionally, strong evidence suggests that PPAR $\alpha$  reduces food intake<sup>100</sup>, whereas PPAR $\gamma$  is involved in neuronal differentiation<sup>101</sup>.

The role of GPR55 as an endocannabinoid receptor is controversial, but evidence suggests that its activation stimulates excitatory hippocampal neurons<sup>102</sup>. On this basis, GPR55 activation by endocannabinoidome mediators, such as anandamide, 2-AG and palmitoylethanolamide, might be detrimental in epilepsy or conditions characterized by glutamate excitotoxicity<sup>103</sup>. Little information is available on the role of GPR18 in brain physiology. However, expression in microglia suggests that this receptor has a function in neuroinflammation<sup>104,105</sup>.

### The endocannabinoidome and gut microbiota

The endocannabinoid system has a major role in regulating myenteric neuron activity, vagal and sympathetic nerve function, and the release of gastrointestinal neuropeptides (ghrelin and cholecystokinin-8), which in turn modulate endocannabinoid levels<sup>106</sup>. Another aspect of

the gut–brain axis that is becoming better appreciated is the effects of dysbiosis on endocannabinoid signalling, and the role of endocannabinoid signalling in dysbiosis<sup>107</sup>. CB1 has been implicated in dysbiosis-induced increases in intestinal permeability, the ensuing systemic inflammation, and modulation of the microbiota composition in a way that favours dysmetabolism<sup>108–110</sup>. Conversely, evidence suggests that CB2 activation partly

mediates the analgesic effects of probiotics against visceral pain<sup>111</sup>.

Endocannabinoidome receptors, including TRPV1, GPR119 and PPAR $\alpha$ , reduce intestinal permeability, and altered levels of their endocannabinoidome ligands could mediate the negative effects of dysbiosis and the beneficial effects of the commensal microorganism *Akkermansia muciniphila* on increased intestinal

**Table 1 | Components of the endocannabinoid system and the endocannabinoidome and their role**

Component type	Component	Role
<b>Endocannabinoid system</b>		
Receptors	Cannabinoid receptor 1 (CB1)	Receptor for THC and endocannabinoids
	Cannabinoid receptor 2 (CB2)	Receptor for THC and endocannabinoids
Enzymes	Fatty acid amide hydrolase (FAAH)	Hydrolysis of anandamide (and other <i>N</i> -acylethanolamines), fatty acid primary amides, <i>N</i> -acyltaurines, <i>N</i> -acylglycines and possibly 2-AG
	<i>N</i> -acylphosphatidylethanolamine-specific phospholipase D-like hydrolase (NAPE-PLD)	Biosynthesis of anandamide and other <i>N</i> -acylethanolamines
	Monoacylglycerol lipase (MAGL)	Hydrolysis of 2-AG and other monoacylglycerols
	Diacylglycerol lipase $\alpha$ and $\beta$ (DAGL $\alpha$ and DAGL $\beta$ )	Biosynthesis of 2-AG and other monoacylglycerols from diacylglycerols
<b>Endocannabinoidome</b>		
Receptors	Peroxisome proliferator-activated receptor- $\alpha$ (PPAR $\alpha$ )	Activated by palmitoylethanolamide, oleoylethanolamide and <i>N</i> -oleoyl-glycine
	Orphan GPCR 119 (GPR119)	Activated by some endocannabinoid congeners
	Orphan GPCR 55 (GPR55)	Activated by palmitoylethanolamide
	Transient receptor potential cation channel subfamily V member 1 (TRPV1) channel	Activated by anandamide, 2-AG and some of their congeners
	Peroxisome proliferator-activated receptor- $\gamma$ (PPAR $\gamma$ )	Activated by anandamide at micromolar concentrations and by some oxidation products of 2-AG
	Ca <sub>v3.2</sub> (T-type) Ca <sup>2+</sup> channel	Inhibited by anandamide and several unsaturated long-chain fatty acid amides
	Transient receptor potential cation channel subfamily M member 8 (TRPM8) channels	Inhibited by anandamide and <i>N</i> -arachidonoyl-dopamine
	GABA <sub>A</sub> receptors	Activated by 2-AG
Endocannabinoid congener mediators	<i>N</i> -acylethanolamines (e.g. palmitoylethanolamide, oleoylethanolamide, docosahexaenylethanolamide)	Agonists of PPAR $\alpha$ and/or TRPV1 and/or GPR55 and/or GPR119; docosahexaenylethanolamide activates GPR110
	2-Acylglycerols	Some are agonists for TRPV1 and/or GPR119
Other long-chain fatty acid amide-derived mediators	Primary fatty acid amides (e.g. oleamide)	Oleamide is a sleep-inducing factor with multiple targets
	<i>N</i> -acyl-amino acids (e.g. <i>N</i> -acylglycines, <i>N</i> -acylserines, <i>N</i> -acyltaurines)	Some <i>N</i> -acylglycines activate GPR18 and/or PPAR $\alpha$ ; some <i>N</i> -acyl-taurines activate TRPV1 and TRPV4
	<i>N</i> -acyl-neurotransmitters (e.g. <i>N</i> -acyl-serotonins, <i>N</i> -acyl-dopamines)	Unsaturated <i>N</i> -acyl-serotonins are TRPV1 antagonists and FAAH inhibitors; <i>N</i> -arachidonoyl-dopamine is a dual CB1 and TRPV1 agonist
	Endocannabinoid oxidation products (e.g. 12-lipoxygenase, 15-lipoxygenase and cytochrome P450 oxygenase products, prostamides and prostaglandin glycerol esters)	12-Hydroxy-anandamide, 15-hydroxy-anandamide and 5,6-epoxy-anandamide activate cannabinoid receptors; prostamide F <sub>2a</sub> activates a heterodimer of the prostaglandin F receptor and a splice variant of the same receptor; prostaglandin E <sub>2</sub> glycerol ester activates the P2Y6 purinergic receptor
Enzymes (those most specifically belonging to the endocannabinoidome)	Glycerophosphodiester phosphodiesterase 1 (GDE1) and $\alpha/\beta$ -hydrolase 4 (ABHD4)	Alternative to NAPE-PLD in the biosynthesis of <i>N</i> -acylethanolamine
	Ca <sup>2+</sup> -dependent and Ca <sup>2+</sup> -independent <i>N</i> -acyltransferases (including phospholipase A2 group IVE and phospholipase A/acyltransferase 1)	Produce <i>N</i> -acylphosphatidylethanolamines for <i>N</i> -acylethanolamine biosynthesis
	Peptidyl-glycine $\alpha$ -amidating monooxygenase (PAM) and glycine <i>N</i> -acyltransferase-like protein 3 (GLYATL3)	Act in sequence in the biosynthesis of <i>N</i> -acylglycines and primary fatty acid amides

2-AG, 2-arachidonoyl-glycerol; GPCR, G protein-coupled receptor; THC,  $\Delta^9$ -tetrahydrocannabinol.

permeability and the ensuing systemic inflammation<sup>112</sup>. Given the ever-increasing evidence that alterations in the gut microbiota are a cause of comorbidity in chronic neuroinflammatory conditions and are related to nutritional and metabolic issues<sup>113</sup>, the importance of the endocannabinoidome–gut microbiome axis in neurology deserves further investigation.

### Involvement in neurological disorders

Endocannabinoidome signalling is altered in experimental models of neurological disorders and in plasma and post-mortem brain samples from humans with these disorders (Supplementary Table 1). Such alterations are often difficult to interpret owing to the number of endocannabinoidome mediators involved and the multi-faceted nature of the changes. Studies in animal models suggest that in neurological disorders, endocannabinoids may no longer be tightly regulated, pro-homeostatic mediators but become dysregulated and contribute to disease in different ways depending on the location and timing of their production and on the stage of the disease<sup>17</sup>. Consequently, cannabinoid receptor antagonists and agonists can produce beneficial effects; for example, CB2 agonists and antagonists can both be beneficial in animal models of MS<sup>15</sup>. The realization that enhancers and blockers of endocannabinoid signalling could be used in treatment of the same disorder is both challenging and exciting for drug developers. Exploitation of this opportunity requires development of clinically relevant animal models to investigate the ‘yin and yang’ of the system. Ways in which the endocannabinoidome is affected in various neurological conditions in experimental models and humans are outlined below, ordered according to the amount of preclinical evidence available for each disorder.

### Parkinson disease

**CB1 and CB2 receptors.** Animal models of PD are generated by either reproducing degeneration of dopaminergic neurons in the substantia nigra with neurotoxins or by manipulating genes that encode PD-associated proteins, such as parkin or  $\alpha$ -synuclein. Biphasic dysregulation of CB1 (hypoactivity in pre-symptomatic and early PD and hyperactivity at later stages) occurs in different models, including  $\alpha$ -synuclein and parkin knockout animals<sup>114</sup>, 6-hydroxydopamine (6-OHDA)-treated rats, and a monkey model of treatment-induced dyskinesia<sup>115,116</sup>. PET and MRI have shown that CB1 levels are increased in patients with PD<sup>117,118</sup>, and imaging in rats and patients has revealed CB2 upregulation<sup>118,119</sup>.

Whether alterations in CB1 levels in PD are protective or maladaptive is unclear. In marmosets and rats with toxin-induced lesions (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment in marmosets and 6-OHDA treatment in rats), CB1 agonists ameliorated levodopa-induced dyskinesia<sup>120,121</sup>. However, CB1 antagonists were also beneficial in MPTP-lesioned marmosets that had been treated with levodopa, in 6-OHDA-treated rats with severe nigral lesions and in MPTP-treated rhesus monkeys<sup>122–124</sup>. The outcome of modulation might depend on the severity of the lesion, which might cause preferential localization of CB1 to

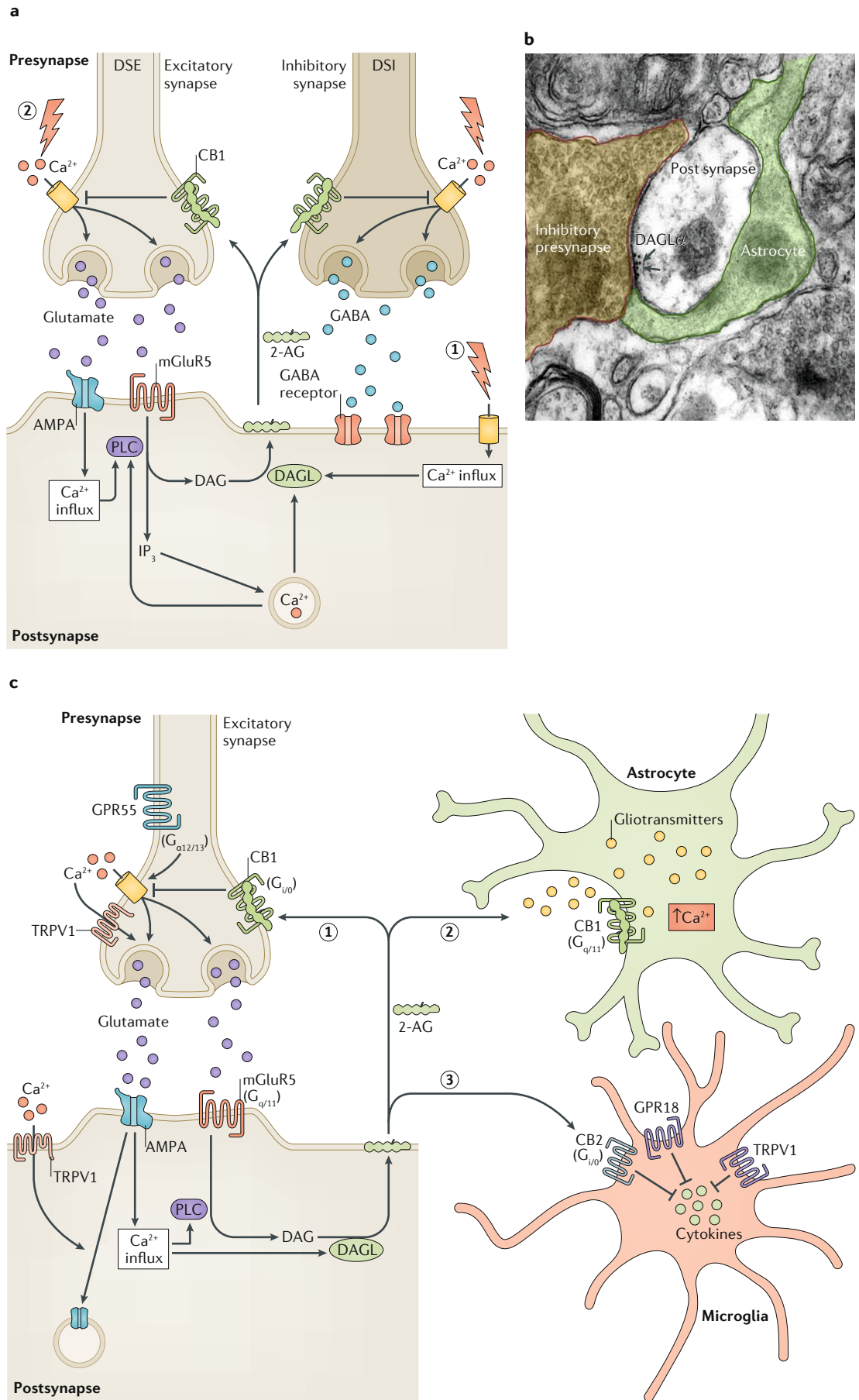
glutamatergic or GABAergic terminals (FIG. 3), although this mechanism is speculative.

CB2 receptor modulation has produced more predictable results. Activation of CB2 reduced dopamine depletion in 6-OHDA-treated rats<sup>125</sup> and counteracted MPTP-induced neurotoxic and neuroinflammatory events in mice<sup>83,126</sup>. Moreover, CB2 is upregulated in lipopolysaccharide-treated rats, and activation of these receptors reduced expression of inflammatory markers<sup>119</sup>.

**Endocannabinoids.** In most studies, endocannabinoid levels are increased in PD. Abnormal endocannabinoid levels in the cerebrospinal fluid (CSF) of untreated patients with PD and in 6-OHDA-treated and reserpine-treated rats were reversed by levodopa treatment<sup>122,127–130</sup>, suggesting that the changes are related to disease symptoms. However, these observations are not easy to interpret, and inhibition of endocannabinoid metabolic enzymes has provided more insight. In MPTP-treated mice, MAGL inhibition led to CB2-mediated neuroprotection<sup>131</sup>. Blockade of FAAH in the same model improved motor behaviour via CB1 and/or CB2 activation but had no neuroprotective effects<sup>39</sup>. In 6-OHDA-treated rats, FAAH blockade reduced dyskinesia only when administered with a TRPV1 antagonist<sup>120</sup>, suggesting that levodopa-induced dyskinesia is worsened by activation of TRPV1 by FAAH substrates. The neuroprotective effects of FAAH inhibitors could result from increased tissue levels of non-endocannabinoid *N*-acylethanolamines, such as palmitoylethanolamide, administration of which reduced MPTP-induced neurotoxicity and neuroinflammation in mice, in part via PPAR $\alpha$  activation<sup>132</sup>. In 6-OHDA-treated rats, the PPAR $\alpha$  agonist oleoylethanolamide (OEA) had TRPV1-mediated antidyskinetic effects<sup>133</sup>. The fact that TRPV1 antagonism and activation can have similar effects on levodopa-induced dyskinesia could be explained by the fact that TRPV1 agonists immediately desensitize the channel, an effect also seen in models of epilepsy (see Seizures and epilepsy below).

**Effects of phytocannabinoids.** In studies in 6-OHDA-treated and lipopolysaccharide-treated rats, THC, CBD and  $\Delta^9$ -tetrahydrocannabinavarin (THCV) had anti-parkinsonian effects<sup>125,134,135</sup>. The investigators suggested that these effects were due to the antioxidant properties of these phytocannabinoids and, in the case of THCV, to CB2 activation and CB1 antagonism.

**Clinical studies.** In an exploratory, double-blind trial of CBD in patients with PD, the highest dose tested (300 mg daily) improved quality of life<sup>136</sup>. In another pilot study, both THC and nabilone, a synthetic analogue of THC, reduced levodopa-induced dyskinesia in PD<sup>137</sup>. Finally, ultramicrosized palmitoylethanolamide produced beneficial effects as an adjuvant therapy in patients with advanced PD<sup>138</sup>. Palmitoylethanolamide is the only endocannabinoidome mediator for which clinical results are available, and its efficacy in several types of neuropathic pain has warranted its marketing as a ‘special food with medical purposes’<sup>139</sup>.





# Fig. 2 | Neurophysiological roles of the expanded endocannabinoid system.

**a** | The role of endocannabinoid retrograde signalling in short-term plasticity, known as depolarization-induced suppression of excitation (DSE) or depolarization-induced suppression of inhibition (DSI). Two events can induce production of the endocannabinoid 2-arachidonoyl glycerol (2-AG). Postsynaptic step depolarization or an action potential induces  $\text{Ca}^{2+}$  influx via voltage-gated  $\text{Ca}^{2+}$  channels (stimulus 1) that is amplified by metabotropic receptor-induced intracellular  $\text{Ca}^{2+}$  release. Alternatively, brief tetanic stimulation of excitatory afferents (stimulus 2) leads to glutamate release, which stimulates the metabotropic glutamate receptor (mGluR5), thereby initiating 2-AG biosynthesis. AMPA receptor activation can contribute to this effect. Other  $\text{G}_{q/11}$ -coupled receptors can also activate phospholipase C (PLC) and diacylglycerol lipase (DAGL), which are required for synthesis of 2-AG, and induce inositol-trisphosphate ( $\text{IP}_3$ ) production that causes intracellular calcium mobilization. 2-AG activates the presynaptic cannabinoid receptor 1 (CB1), leading to depression of neurotransmitter release. **b** | A transmission electron micrograph (L.C., unpublished observations) of the tripartite synapse that shows DAGLa immunogold labelling (arrows) at the postsynaptic membrane of an active zone receiving a symmetrical synapse from a putative inhibitory neuron (orange) and enveloped by astroglial processes (green). **c** | The physiological role of the endocannabinoidome in modulating synaptic plasticity at the tripartite synapse. In addition to conventional intercellular signalling (step 1), postsynaptic transient receptor potential cation channel subfamily V member 1 (TRPV1) can reduce excitatory synaptic transmission by increasing AMPA receptor reuptake and mediating TRPV1 long-term depression. Activation of mGluR5 instead produces CB1-mediated retrograde long-term depression. Conversely, presynaptic activation of TRPV1 and  $\text{G}_{\alpha_{12/13}}$ -coupled receptor GPR55 contribute to presynaptic  $\text{Ca}^{2+}$  influx, thereby facilitating synaptic transmission. 2-AG also amplifies  $\text{Ca}^{2+}$  influx via CB1 in astrocytes (step 2), thereby promoting release of gliotransmitters (for example, glutamate) into the synaptic cleft and amplification of 2-AG signalling. By binding CB2 and/or GPR18 and/or TRPV1 on microglia (step 3), endocannabinoids and related mediators modulate the release of cytokines, which might participate in synaptic activity and pruning. DAG, sn-1 acyl-2-arachidonoyl-glycerol; GPR, G protein-coupled receptor. Parts **a** and **c** (left) adapted with permission from REF.<sup>341</sup>, Elsevier.

## Alzheimer disease

**CB1 and CB2 receptors.** Several experimental models of AD that mimic accumulation of amyloid- $\beta$  (A $\beta$ ) peptides, hyper-phosphorylation of tau or genetic dysfunctions have been widely employed to search for new treatments. Studies of CB1 in these models have produced varying results. CB1 levels were unaltered in Tg2576 transgenic mice, which overexpress a mutant form of amyloid precursor protein (APP), and in APP/PS1 mice, which express the same mutant APP and mutant presenilin 1 (REF.<sup>140</sup>). However, CB1 localization and signalling were altered in presymptomatic Tg2576 mice<sup>141</sup>.

CB1 and/or CB2 agonists ameliorated memory and/or cognitive impairments in Tg2576 mice, APP/PS1 mice<sup>142</sup> and rodents that had received intracerebral injections of A $\beta$ <sup>143,144</sup>. Conversely, CB1 antagonism protected against A $\beta$ -induced memory impairment in mice<sup>13</sup>, suggesting that activation of CB1 by endocannabinoids inhibits neurotoxicity but worsens its long-term consequences (such as reduced acetylcholine signalling) that lead to cognitive impairment. CB1-related findings in the brains of patients with AD have also been variable. Downregulation, upregulation and no alteration of CB1 have all been reported<sup>145–148</sup>.

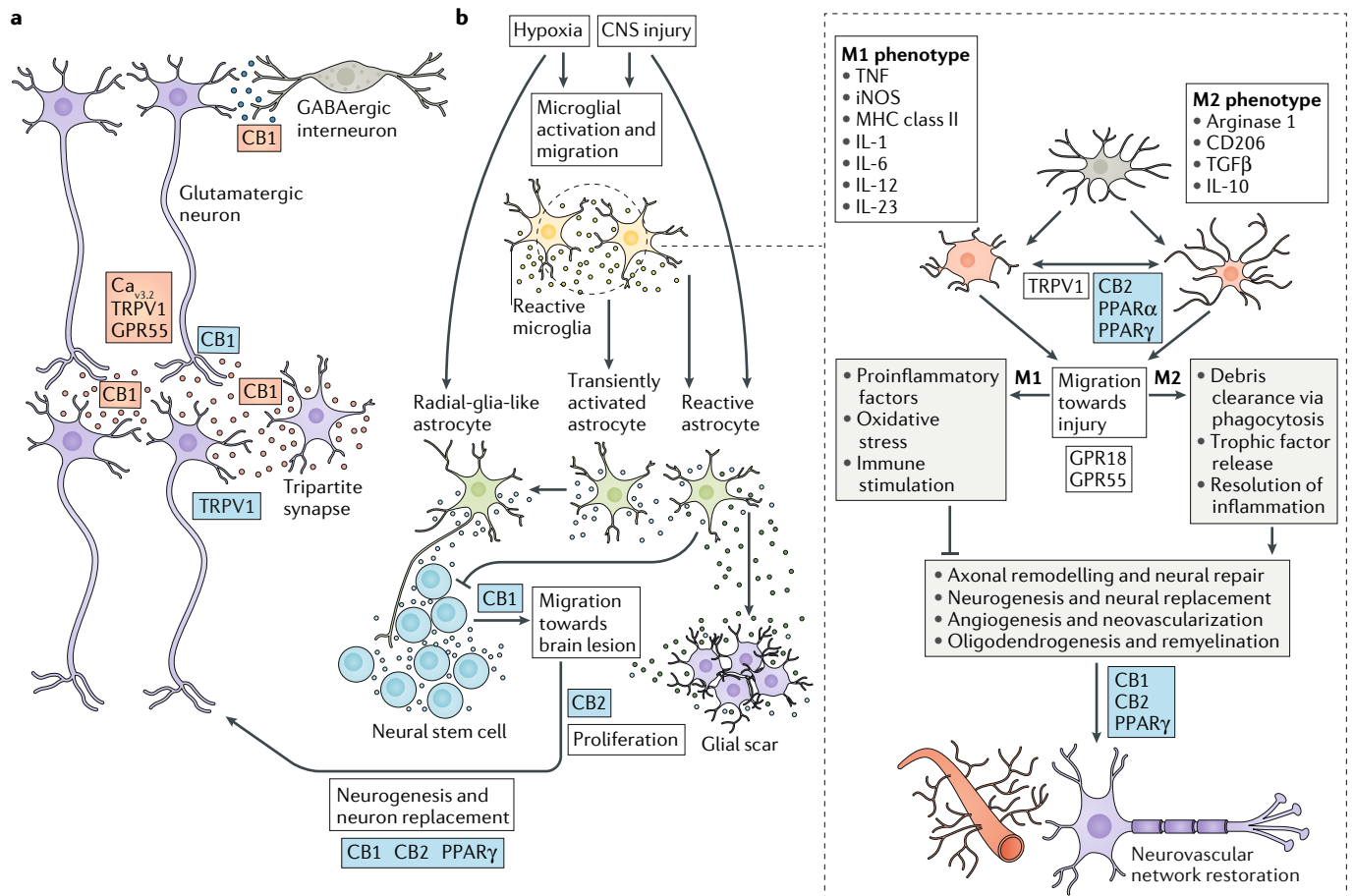
Studies of CB2 in AD consistently indicate its upregulation. Marked increases in CB2 levels have been found in microglia in APP/PS1 mice and in mice that have received intracerebral injection of A $\beta$ <sup>142,149</sup>, suggesting that CB2 protects against AD-associated inflammation. In various in vitro and in vivo AD models, CB2 activation reduced levels of neurotoxic

factors and pro-inflammatory mediators produced by reactive astrocytes and microglial cells<sup>143,150–152</sup>, stimulated microglial proliferation and migration<sup>153</sup>, and decreased A $\beta$  levels. Accordingly, CB2 receptor knock-out in amyloidogenic J20 mice (another AD model) led to increased levels of A $\beta$ <sup>154</sup>. In humans with AD, CB2 is upregulated in neuritic clear plaque-associated astrocytes and microglia, whereas CB1 expression is unchanged<sup>143,155</sup>.

**Endocannabinoids.** In 5 $\times$ FAD mice, which co-express five common AD-associated mutations, levels of anandamide and 2-AG were unchanged<sup>156</sup>. In mice with A $\beta$ -induced neurotoxicity and cognitive impairment, hippocampal levels of 2-AG were increased in the early stages of disease and levels of anandamide were decreased in later stages<sup>157</sup>. These findings are in partial agreement with those in human AD<sup>34,38</sup>. In one post-mortem study of patients with AD, levels of anandamide were reduced in the midfrontal and temporal cortex and inversely correlated with A $\beta$  accumulation<sup>34</sup>. This observation agrees with a previous finding that FAAH expression and activity was increased in neuritic plaque-associated astrocytes and microglia from post-mortem brains from patients with AD<sup>155</sup>. In another post-mortem study, 2-AG-mediated signalling was increased in the hippocampus of patients with AD, and DAGLa levels were increased near amyloid plaques<sup>38</sup>. Increased plasma levels of 2-AG have also been observed in patients with AD, particularly those with ischaemic heart disease or cerebral leukoaraiosis<sup>35</sup>.

In 5 $\times$ FAD mice, pharmacological elevation of 2-AG levels with an MAGL inhibitor prevented neuroinflammation, decreased neurodegeneration and improved memory<sup>158</sup>, but these effects were independent of CB2 (REF.<sup>159</sup>). MAGL inhibition also reduced microglia-mediated neuroinflammation in APdE9 mice, another genetic model of AD<sup>160</sup>. Genetic inactivation of MAGL produced similar effects in APP/PS1 mice by reducing prostaglandin production<sup>57</sup>.

Genetic ablation of FAAH in 5 $\times$ FAD mice reduced A $\beta$  levels, neuritic plaques and gliosis independent of CB1 (REF.<sup>156</sup>) but worsened the neuroinflammatory effects of A $\beta$  in astrocytes in vitro via a mechanism that involved PPAR $\alpha$ , PPAR $\gamma$  and TRPV1, but not CB1 or CB2 (REF.<sup>44</sup>). The neuroprotective effects of FAAH inhibition might be mediated via other substrates of the enzyme, such as palmitoylethanolamide, administration of which reduced toxicity and reversed memory deficits in a PPAR $\alpha$ -dependent manner in A $\beta$ -treated rats<sup>161</sup> and counteracted astroglyosis and improved neuronal viability in a triple transgenic model of AD<sup>162</sup>. Inhibition of a putative endocannabinoid membrane transporter that facilitates endocannabinoid reuptake by cells from the extracellular medium had beneficial and deleterious effects on memory deficits in mice with A $\beta$ -induced neurotoxicity and cognitive impairment; whether the effects were beneficial or exacerbating depended on the timing of administration<sup>157</sup>, highlighting the time dependence and site dependence of endocannabinoid signalling in the aetiopathology of AD (FIG. 3).



**Fig. 3 | Endocannabinoid receptors in acute or degenerative neurological disorders.** Activation of receptors in red boxes is deleterious, activation of receptors in blue boxes is protective, and activation of receptors in uncoloured boxes can be deleterious or protective depending on the context, or has unclear effects. **a** | Retrograde endocannabinoid activation of presynaptic cannabinoid receptor 1 (CB1) in some glutamatergic neurons is protective in acute and chronic neurodegenerative disorders. However, in chronic conditions, CB1 signalling can lose spatial selectivity, spread over other CB1 populations, such as those in other neurons, or in GABAergic terminals and astrocytes in tripartite synapses; in the latter two cases, CB1 signalling contributes to excitotoxicity. Likewise, endocannabinoid interactions with presynaptic T-type Ca<sup>2+</sup> channels (Ca<sub>v3.2</sub>) and G protein-coupled receptor 55 (GPR55) in neurons, and with transient receptor potential cation channel subfamily V member 1 (TRPV1) in neurons or inflammatory microglia can counteract or contribute to neuronal excitability and glutamate excitotoxicity, whereas postsynaptic TRPV1 reduces glutamate excitotoxicity by inhibiting AMPA receptor signalling. **b** | Hypoxic conditions or neuronal injury activate microglia, which can have pro-inflammatory (M1) or protective (M2) phenotypes. Activation of CB2 and peroxisome proliferator-activated receptor-γ (PPARγ) and PPARα promote the M2 phenotype. GPR18 and GPR55 regulate microglial migration, but whether they are anti-inflammatory or pro-inflammatory is not clear. The role of TRPV1 in regulating the M1–M2 balance is controversial and might depend on context. Reactive M2 microglia produce cytokines (green dots) that stimulate formation of reactive astrocytes, which in turn modulate neural stem cell migration and differentiation by releasing positive and negative trophic factors (light blue and dark green dots) and participate in gliosis. CB1 and CB2 activation stimulate neural stem cell migration and proliferation, respectively, and, together with PPARγ, induce adult neurogenesis. iNOS, inducible nitric oxide synthase; MHC, major histocompatibility complex; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor. Part **b**, left, adapted from REF.<sup>342</sup>, Springer Nature Limited. Part **b**, right, adapted from REF.<sup>343</sup>, Springer Nature Limited.

**Effects of phytocannabinoids.** Experiments in vitro and in vivo models of Aβ-induced neurotoxicity have shown that CBD can protect against Aβ-induced insults, as it reduces oxidative stress, tau phosphorylation and expression of inducible nitric oxide synthase via the WNT-β-catenin pathway, which mediates several of the neurotoxic effects of Aβ<sup>163</sup>. Moreover, CBD ameliorated cognitive impairments and prevented development of a social recognition deficit in APP/PS1 mice<sup>164</sup>. Finally, CBD and THC together preserved memory function and

reduced astrogliosis and inflammation in APP/PS1 mice, and the combination was more effective than either cannabinoid alone<sup>165</sup>.

**Clinical studies.** Clinical tests of cannabinoids in patients with AD are limited. THC and nabilone have been tested in controlled clinical trials for the treatment of some consequences and comorbidities of AD, such as anxiety, agitation and depression<sup>166–169</sup>. THC was ineffective against neuropsychiatric symptoms, although it showed

some beneficial effects on balance and gait and was well tolerated, thus warranting further studies with higher dosages. Nabilone reduced the severity of agitation.

### Huntington disease

**CB1 and CB2 receptors.** Huntington disease (HD) is an inherited disorder that causes death of dopaminergic neurons in the globus pallidus, leading to progressive locomotor impairment and mood and/or mental impairments. Experimental models of HD reproduce either the neurodegeneration, via injection of neurotoxins into the globus pallidus, or its cause — expansion of CAG triplet repeats in the gene that encodes the huntingtin protein. Substantial loss of CB1 has been seen in different animal models with different CAG repeat lengths (R6/1, R6/2 and HD94 mice), suggesting that reduced endocannabinoid signalling is associated with HD severity and progression<sup>170–172</sup>. Similar changes have been seen in post-mortem samples from patients with HD<sup>173</sup>. The use of conditional CB1 knockout mice and the designer receptor exclusively activated by designer drug (DREADD) pharmacogenetic technique showed that CB1 exerts its neuroprotective effects at glutamatergic synapses<sup>174,175</sup>. This mechanism is expected given that endocannabinoids mediate retrograde inhibition of glutamate excitotoxicity at excitatory terminals (see the discussion above on the physiological roles of the endocannabinoidome). Accordingly, in animal models of striatal damage, activation of CB1 on corticostriatal projections by inhibition of glutamatergic transmission selectively protect medium spiny neuron populations that are damaged<sup>176</sup>. In addition, THC attenuated striatal degeneration in R6/2 mice independent of CB1, and genetic deficiency of CB1 worsened disease signs in N171-82Q transgenic mice (which express an N-terminal fragment of huntingtin with 82 glutamine repeats) and after 3-nitropropionic intoxication<sup>177</sup>.

As in other neurodegenerative disorders, CB2 expression is increased in post-mortem brains from patients with HD and in experimental models, including R6/2 mice<sup>178,179</sup> and malonate-lesioned rats<sup>180</sup>. Genetic ablation of CB2 exacerbates disease in R6/2 mice, BACHD mice (which express full-length, human, mutant huntingtin)<sup>178,179</sup> and malonate-lesioned rats<sup>180</sup>. An agonist of CB1 and CB2 receptors prevented motor impairment and the loss of medium spiny neurons in R6/1 mice<sup>181</sup>.

**Endocannabinoids.** Reduced striatal levels of anandamide and 2-AG have been observed in 3-nitropropionic-lesioned rats and R6/2 mice<sup>182,183</sup>. In R6/1 mice, 2-AG levels were increased and anandamide levels were decreased<sup>172</sup>. In humans with HD, FAAH activity is decreased and, consequently, anandamide levels are increased in lymphocytes<sup>184</sup>.

Pharmacological modulation of endocannabinoid metabolism has been shown to protect neurons in models of HD. Inhibitors of endocannabinoid cellular reuptake had anti-hyperkinetic effects in the 3-nitropropionic model, although mostly via activation of TRPV1 (REF<sup>185</sup>). A DAGL inhibitor ameliorated (and an MAGL inhibitor exacerbated) malonate-induced damage of striatal neurons by reducing cyclooxygenase 2-mediated

oxidation of 2-AG to form the pro-inflammatory prostaglandin E<sub>2</sub> glycerol ester<sup>50</sup>.

**Effects of phytocannabinoids.** Studies in the 3-nitropropionic model of HD have shown that THC<sup>134</sup>, CBD<sup>186</sup> and cannabigerol<sup>187</sup> can protect striatal neurons. Similar effects were seen with a nabiximols-like combination of THC and CBD in malonate-treated and 3-nitropropionic-treated rats; the effects were mediated by CB1 and/or CB2 in the malonate model and independent of CB1 and CB2 in the 3-nitropropionic model<sup>188</sup>.

**Clinical studies.** In one trial in 26 patients with HD, nabiximols was well tolerated but did not improve disease<sup>189</sup>, although in a subsequent study of seven patients with early-onset HD, it reduced dystonia<sup>190</sup>. CBD has been tested in 15 patients with HD, but no therapeutic effect was seen, even with a high dose (700 mg daily)<sup>191</sup>. Nabilone has also been tested for the treatment of motor symptoms in patients with HD, with contrasting results<sup>190,192,193</sup>.

### Multiple sclerosis

**CB1 and CB2 receptors.** Complex alterations in CB1 and CB2 expression occur in patients with MS and in experimental models. These models include experimental autoimmune encephalomyelitis (EAE) and Theiler murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), which recreate the brain and spinal cord demyelination that occurs in MS, and chronic relapsing EAE (CREAE), which also reproduces the relapsing–remitting MS phenotype<sup>194–198</sup>. Several lines of evidence suggest that activation of CB1 and CB2 has beneficial effects. In CREAE mice, CB1 agonists ameliorated tremor and spasticity, whereas antagonists worsened them<sup>199,200</sup>. In TMEV-IDD mice, CB1 and CB2 agonists improved clinical scores via immunomodulatory and anti-inflammatory mechanisms<sup>201,202</sup>. In lymphocytes isolated from EAE mice or patients with MS, CB2 activation suppressed immune responses<sup>203,204</sup>. Finally, genetic ablation of CB1 or CB2 caused more severe clinical manifestations in various models<sup>203,205</sup>.

**Endocannabinoids.** In relapsing CREAE mice, anandamide and/or 2-AG levels were increased in the brain or spinal cord, but only anandamide levels were increased in EAE mice and only 2-AG levels in TMEV-IDD mice<sup>194,197,200,206</sup>. In two studies in patients with MS, blood levels of endocannabinoids were increased and CSF levels were decreased, although in other studies, anandamide levels were increased in the CSF as well as in peripheral lymphocytes and the brain<sup>204,206,207</sup>. These findings suggest that modulation of endocannabinoid signalling is an adaptive response in MS to counteract symptoms and progression. Accordingly, inhibitors of the putative endocannabinoid transporter<sup>194,199,200,208,209</sup>, FAAH<sup>200,210</sup>, MAGL<sup>210,211</sup> and the monoacylglycerol lipase ABHD6 (REF<sup>212</sup>), all of which increase endocannabinoid levels, had beneficial effects in various models. In addition, CNS levels of palmitoylethanolamide were increased in models of MS<sup>197,200</sup>, but decreased in the CSF

of patients with MS<sup>207</sup>, and administration of exogenous palmitoylethanolamide transiently ameliorated spasticity in CREAE mice<sup>200</sup> and reduced motor disability and inflammation in TMEV-IDD mice<sup>197</sup>. Similarly, early administration of palmitoylethanolamide with CBD ameliorated EAE in mice<sup>213</sup>. Endocannabinoids and palmitoylethanolamide also act via TRPV1 channel activation and desensitization. Interestingly, in patients with MS, the presence of the G allele of the SNP rs222747 of *TRPV1*, which causes increased expression and function of TRPV1, is associated with lower CSF levels of TNF, indicating anti-inflammatory effects of this channel on microglia<sup>96</sup>. This observation suggests that TRPV1 could be a target for the treatment of MS.

**Effects of phytocannabinoids.** CBD ameliorates EAE in mice<sup>214</sup>. The underlying mechanism includes activation of PI3K–AKT–mTOR signalling, reduction of pro-inflammatory mediators, and PPAR $\gamma$  activation<sup>215</sup>. In TMEV-IDD mice, CBD had immunoregulatory effects via adenosine 2A receptor activation and downregulation of vascular cell adhesion molecule-1 (REF.<sup>216</sup>). In CREAE mice, CBD potentiated the anti-spasticity effects of THC (see Clinical studies below)<sup>217</sup>.

**Clinical studies.** Nabiximols is approved for the treatment of neuropathic pain and treatment-resistant spasticity in patients with MS in several countries<sup>4</sup>, although not yet by the FDA. Clinical practice has confirmed that nabiximols is useful for MS spasticity<sup>215</sup> as an add-on therapy with other anti-spastic agents<sup>218</sup>. Neurophysiological studies have revealed that nabiximols has beneficial effects on cortical and spinal excitability, metaplastic effects on the motor cortex (but not on upper motor neurons) and — relevant to its analgesic effects — improves sensory responses and laser-evoked potentials<sup>219–222</sup>. In discontinuation studies in an Italian population of patients with MS, ~40% of patients were resistant to the anti-spastic action of the drug<sup>223,224</sup>.

Some evidence is emerging that nabiximols has immunomodulatory effects in MS, raising the possibility that it could be used to alter disease progression<sup>225</sup>. This possibility is supported by studies in rodents that have demonstrated benefits of THC via CB2 activation and of CBD via multi-target anti-inflammatory effects (see CB1 and CB2 receptors above). Ultramicronized palmitoylethanolamide has also been tested in patients with MS. The treatment reduced circulating levels of pro-inflammatory cytokines and reduced the adverse effects of interferon- $\beta$ 1a treatment for relapsing–remitting MS<sup>226</sup>.

#### Amyotrophic lateral sclerosis

**CB1 and CB2 receptors.** ALS is an incurable neurodegenerative disorder of motor neurons. The cause is usually unknown, so experimental models are limited. In SOD1 mice — a controversial model of ALS in which mice overexpress superoxide dismutase 1 (SOD1)<sup>227</sup> — CB1 expression was downregulated<sup>228</sup> or unchanged<sup>229</sup>, and genetic deletion of the receptor extended lifespan with no effect on disease onset<sup>41</sup>, so CB1 activation is unlikely to be beneficial. CB2 was upregulated in the

spinal cord of SOD1 mice<sup>227,230</sup> and in activated microglia in the spine of TAR-DNA binding protein 43 (TDP43) mutant mice<sup>231</sup>, another ALS model developed on the basis that mutant TDP43 aggregates in the brain and spinal cord of patients with familial ALS. CB2 is also upregulated in post-mortem primary motor cortex and spinal cord samples from patients with ALS<sup>232</sup>. Furthermore, a selective CB2 agonist slowed disease progression in SOD1 mice<sup>230</sup>, and these findings together suggest that CB2 has a protective role in ALS.

**Endocannabinoids.** In SOD1 mice, anandamide and 2-AG concentrations are increased in the lumbar spinal cord<sup>41,233</sup>. Genetic knockout of FAAH in SOD1 mice prevented development of symptoms without prolonging survival<sup>41</sup>, and administration of an MAGL inhibitor delayed disease onset, slowed progression and increased survival<sup>229</sup>, suggesting that the increases in anandamide and, in particular, 2-AG are neuroprotective. In TDP43 mutant mice, endocannabinoid levels are unchanged<sup>231</sup>.

**Phytocannabinoids and clinical studies.** Some evidence suggests that phytocannabinoids and multi-target endocannabinoidome mediators might be useful in ALS. In human gingiva-derived mesenchymal stromal cells, CBD modulated expression of genes associated with ALS<sup>234</sup>, and nabiximols-like combinations of THC and CBD slightly delayed disease progression in SOD1 mice<sup>227</sup>.

In *Xenopus* oocytes transplanted with muscle membranes from selected patients with ALS, palmitoylethanolamide reduced desensitization of acetylcholine-evoked currents after repetitive neurotransmitter application<sup>235</sup>. Given that ALS involves defects in the expression of acetylcholine receptors in skeletal muscle even in the absence of motor neuron anomalies, this observation suggests that palmitoylethanolamide could be beneficial in this disease. Accordingly, in patients with ALS, palmitoylethanolamide slowed reductions in forced vital capacity over time — suggesting that it can improve pulmonary function in this disease — and improved the clinical condition of one patient<sup>235,236</sup>.

#### Traumatic brain injury

**CB1 and CB2 receptors.** Traumatic brain injury (TBI) is the most common cause of epilepsy in people aged >35 years. Experimental models of TBI involve subjecting animals to head impacts to mimic mild, moderate or severe brain injury. In a porcine model of TBI, CB1 was over-expressed after injury<sup>237</sup>. Use of selective and unselective CB2 agonists and CB1 antagonists in mice and studies of CB1 receptor knockout mice<sup>238–240</sup> have suggested that targeting these receptors could have therapeutic potential.

**Endocannabinoids.** In a mouse model of TBI, levels of 2-AG in the brain hemisphere ipsilateral to injury were increased between 1 h and 24 h after injury<sup>241</sup>. In the same model, administration of 2-AG protected the BBB, reduced inflammation and oedema and improved clinical recovery via CB1-mediated mechanisms<sup>238,241,242</sup>. In another mouse model, levels of anandamide, but not 2-AG, were increased in the ipsilateral hemisphere 3 days after TBI<sup>243</sup>. Inhibition of endocannabinoid degradation



by blocking FAAH, MAGL or ABHD6 reduced neurodegeneration and inflammation, protected BBB integrity and improved motor impairments, memory deficits and anxiety behaviour in different TBI models<sup>243–246</sup>.

In addition to classic endocannabinoids, several endocannabinoidome mediators seem to be involved in TBI. In mouse models, palmitoylethanolamide and *N*-arachidonoyl-L-serine had beneficial effects in TBI, including a reduction in oedema, gliosis and behavioural deficits and induction of neurogenesis<sup>247,248</sup>. In addition, *N*-oleoyl-glycine was increased in the insular cortex of mice with mild injury, and reduced nicotine reward and withdrawal effects, possibly explaining previously reported reductions in nicotine dependence in smokers after mild TBI<sup>99</sup>. Finally, TRPV1 antagonism attenuated BBB disruption after TBI in a cortical impact injury model in mice<sup>249</sup>.

**Clinical studies.** In several studies in experimental TBI, dexamabinol (also known as HU-211) — an enantiomer of the ultra-potent synthetic CB1 and CB2 ligand HU-210 that is inactive at cannabinoid receptors — exhibited potent neuroprotective activity, probably by inhibiting the NMDA receptor<sup>250</sup>. On this basis, the drug was tested in a phase III randomized, placebo-controlled clinical trial, but the results were negative<sup>251</sup>. Despite the good safety profile of this compound, studies with higher doses were never conducted.

### Stroke and neonatal ischaemia

**CB1 and CB2 receptors.** Activation of CB1 protects against acute stroke through various mechanisms, including attenuation of BBB disruption, reductions in brain oedema and infarcted tissue volume, and induction of hypothermia, effects that are all usually reversed by CB1 antagonists<sup>252–254</sup>. Stroke severity is increased in CB1 knockout mice<sup>255</sup>, although one study has suggested that CB1 antagonists could be protective in transient or permanent cerebral artery occlusion<sup>256</sup>.

In mice with middle cerebral artery occlusion, CB2 activation reduced infarct volume and improved neurological outcome and cerebral microcirculatory function<sup>257,258</sup>. Intriguingly, double knockout of CB1 and CB2 in mice improved recovery after stroke, suggesting that unidentified compensatory mechanisms are activated<sup>259</sup>. Indeed, palmitoylethanolamide and other *N*-acylethanolamines protected against transient focal cerebral ischaemia in rats and against the effects of middle cerebral artery occlusion in mice via mechanisms that did not require activation of CB1, CB2 or TRPV1, whereas OEA reduced infarct volume via PPARα in the latter model, and improved spatial cognitive deficits through enhancement of hippocampal neurogenesis in mice with transient focal cerebral ischaemia<sup>260–262</sup>.

**Endocannabinoids.** Several studies in rodent models of ischaemia after stroke have shown that brain levels of *N*-acylethanolamines are elevated. This increase seems to be even greater after reperfusion<sup>256,263,264</sup>. Levels of 2-AG in the brain are unaffected<sup>256</sup>. In agreement with these murine studies, palmitoylethanolamide levels are elevated in the blood of patients with acute stroke<sup>265</sup>.

**Effects of phytocannabinoids.** The neuroprotective effects of CBD after ischaemic stroke have been widely investigated. In the middle cerebral artery occlusion mouse model, CBD reduced infarct size, increased cerebral blood flow, improved motor behaviour and increased survival by acting at 5-HT<sub>1A</sub> receptors<sup>266</sup>. CBD also reduced ischaemic injury by upregulating the Na<sup>+</sup>–Ca<sup>2+</sup> exchanger NCX2 and NCX3 proteins<sup>267</sup> and protected against hypoxic–ischaemic damage in newborn rats and piglets<sup>268–271</sup>. After hypoxia–ischaemia in newborn pigs, CBD reduced brain oedema and seizures<sup>268</sup> and brain damage was reversed after 72 h from treatment<sup>270</sup>. In the same model, CBD treatment reduced infarct volume and improved functional parameters<sup>271</sup>. In the forebrain from newborn mice that were deprived of oxygen and glucose, CBD had a neuroprotective effect that was partly mediated by adenosine 2A receptors<sup>269</sup>. Finally, CBD reduced brain damage and improved long-term functional recovery in a rat model of perinatal arterial ischaemic stroke<sup>272</sup>.

**Clinical studies.** Nabiximols is currently being tested as an add-on therapy for post-stroke spasticity<sup>273</sup>. Previously, palmitoylethanolamide with luteolin was tested in patients with stroke during rehabilitation and improved cognitive impairments, spasticity, pain and independence in daily living activities<sup>274</sup>.

### Seizures and epilepsy

**CB1 and CB2 receptors.** Most preclinical models of acute seizure involve treatments that induce strong neuronal depolarization, such as kainic acid, pentylenetetrazole or electric shock (as in the maximal electroshock model, which produces tonic–clonic (grand mal) generalized seizures). Treatment with pilocarpine can induce true status epilepticus in rodents. In various models of temporal lobe epilepsy, an agonist of CB1 and CB2 had anti-epileptogenic effects<sup>93,275,276</sup> and CB1 and CB2 blockade had pro-epileptogenic effects<sup>277,278</sup>. However, the CB1 antagonist SR141716A can have anti-epileptogenic effects, particularly in trauma-induced or febrile seizures<sup>279–281</sup>. In the pentylenetetrazole and maximal electroshock models, activation of CB1 protected against acute seizures<sup>282–285</sup>; PPARγ activation had a synergistic effect in the pentylenetetrazole model<sup>284</sup>.

**Endocannabinoids.** Hippocampal concentrations of endocannabinoids are transiently increased after kainic acid injection in mice<sup>286</sup>. Levels of endocannabinoids, palmitoylethanolamide and OEA were altered in a brain-region-specific manner 1 h after kainic acid-induced seizures<sup>287</sup>. Although endocannabinoid levels in the hippocampus were unchanged in a rat model of fever-induced convulsions<sup>288</sup>, 2-AG was upregulated in a model of acute epilepsy induced by pilocarpine<sup>277</sup>. These data support the hypothesis that anandamide and 2-AG are released after neuronal hyperexcitability to counteract glutamate excitotoxicity during seizures<sup>286</sup>.

In several studies in preclinical models of acute seizure, pharmacological elevation of endocannabinoid levels had anti-convulsant effects. FAAH inhibitors protected against seizures induced by pentylenetetrazole

or kainic acid<sup>289,290</sup>. Inhibitors of anandamide reuptake or hydrolysis had mixed effects on pentylenetetrazole-induced seizures — a CB1-dependent anti-convulsant effect was seen at lower doses of the inhibitors and a TRPV1-mediated pro-convulsant effect was seen at higher doses. This observation suggests that extracellular accumulation of 2-AG or anandamide has anti-convulsive effects via the CB1 receptor, whereas intracellular anandamide accumulation is pro-convulsive via TRPV1 activation<sup>291,292</sup>. In the pentylenetetrazole model, inhibition of ABHD6 had an anticonvulsive effect via a GABA<sub>A</sub> receptor-mediated mechanism independent of CB1 and CB2 (REF.<sup>293</sup>), in agreement with the hypothesis that 2-AG directly activates GABA<sub>A</sub> receptors. In models of epileptogenesis caused by kindled seizures, MAGL inhibitors had anticonvulsive and protective effects<sup>294</sup>, but high doses and long-term use of these inhibitors in pilocarpine-induced temporal lobe epilepsy in mice had epileptogenic effects<sup>295</sup>, possibly owing to CB1 desensitization.

In the pentylenetetrazole model, TRPV1 activation by *N*-oleoyl-dopamine was pro-convulsant<sup>296</sup>. By contrast, palmitoylethanolamide had anti-epileptic effects, but these were partially reversed by CB1 and CB2 antagonists<sup>297</sup>; these findings are in line with the idea that palmitoylethanolamide also acts by increasing the effects of endocannabinoids at their receptors<sup>139</sup>.

**Effects of phytocannabinoids.** CBD had anti-convulsant effects in the pilocarpine model of temporal lobe epilepsy, the penicillin model of partial seizure and the pentylenetetrazole model<sup>298,299</sup>, possibly via GPR55 antagonism and TRPV1 desensitization<sup>5</sup>. It also rescued morphological anomalies in interneurons induced by epilepsy<sup>300</sup>. Cannabidiol had anticonvulsant effects in several mouse and rat models of seizures, including the maximal electroshock model and audiogenic and pentylenetetrazole-induced seizures, but was inactive in the pilocarpine model<sup>301</sup>.

**Clinical studies.** In 2018, the FDA approved CBD for the treatment of seizures in Dravet syndrome and Lennox–Gastaut syndrome on the basis of two successful double-blind, placebo-controlled phase III trials<sup>28,302</sup> and previous open-label studies<sup>27</sup>. In patients with Dravet syndrome, CBD (20 mg/kg daily) decreased the median frequency of convulsive seizures from 12.4 per month to 5.9 per month, compared with a decrease from 14.9 per month to 14.1 per month with placebo<sup>28</sup>. A 50% reduction in frequency of convulsive seizures was seen in 43% of patients who received CBD and 27% who received placebo<sup>28</sup>. In patients with Lennox–Gastaut syndrome, the efficacy of CBD was similar: the median reduction in drop seizure frequency was 41.9% in patients who received 20 mg/kg daily, 37.2% in patients who received 10 mg/kg daily, and 17.2% in patients who received placebo. In both studies, some patients discontinued treatment owing to adverse events (including diarrhoea, decreased appetite and somnolence), but these were deemed less serious than with other anti-convulsant treatments<sup>302</sup>. In a prospective, open-label study published in 2018, CBD as an add-on treatment reduced the

frequency and severity of seizures and reduced adverse events in 72 children and 60 adults with treatment-resistant epilepsy<sup>303</sup>. Finally, in an expanded access programme, CBD has been tested for the treatment of seizures in patients with other rare disorders, including CDKL5 deficiency disorder, Aicardi syndrome, Dup15q syndrome, Doose syndrome, febrile infection-related epilepsy syndrome and other treatment-resistant paediatric epilepsies. Reported efficacies and safety profiles were similar to those in the studies discussed above<sup>303–305</sup>. Several other clinical studies of CBD are ongoing and its interactions with other anti-convulsants are being investigated<sup>306</sup>.

## Glioblastoma

**CB1 and CB2 receptors.** Glioblastoma is a rare, incurable brain tumour with an average survival time of <2 years from diagnosis. Studies of CB1 expression in glioblastoma have produced conflicting results<sup>307,308</sup>. By contrast, CB2 receptors are consistently upregulated in the brains of patients with glioblastoma and in human glioblastoma cells, and their expression positively correlates with tumour grade<sup>309</sup>. CB1 and CB2 agonists decreased tumour size and increased survival by reducing angiogenesis in xenograft models with human glioma cells<sup>310–313</sup>. Moreover, CB2 activation induced differentiation and inhibited gliomagenesis of glioma-derived stem-like cells, which express all elements of the endocannabinoid system<sup>314</sup>.

**Endocannabinoids.** Elevated or reduced brain levels of anandamide and elevated levels of 2-AG have been reported in patients with glioma<sup>308</sup>. In various implantable or grafted tumour models, anandamide suppressed proliferation, adhesion, migration and invasion of temozolomide-resistant human U251 glioblastoma cells<sup>315</sup>. A cocktail of anandamide, OEA and palmitoylethanolamide that is released by adult neural progenitor cells caused apoptosis of high-grade glioblastoma cells via activation of TRPV1 (REF.<sup>316</sup>). As further evidence for a protective role of TRPV1, the 5′-untranslated regions of human *TRPV1* generate a stable transcript that encodes TRPV1v3, a variant of the channel that is very highly expressed in human glioblastoma tissue and stem-like cells and is associated with longer survival of patients<sup>317</sup>.

**Effects of phytocannabinoids.** CBD inhibits glioma cell proliferation and migration in vitro; these effects are independent of CB1 but at least partly mediated by CB2 (REF.<sup>318</sup>). THC had concentration-dependent effects on xenografts of temozolomide-resistant human glioblastoma T98G cells in mice — low doses stimulated proliferation and high doses inhibited proliferation<sup>319</sup>. Evidence suggests that a functional dimeric complex between GPR55 and CB2 could be responsible for these effects. CBD potentiated the anti-proliferative effect of THC, and administration of the two cannabinoids with temozolomide or radiation greatly increased glioma cell death<sup>320,321</sup>. Finally, CBD increased uptake of chemotherapeutic drugs and caused cytotoxicity in human glioma cells by activating TRPV2 (REF.<sup>322</sup>), and promoted differentiation while reducing proliferation of

glioma-derived stem-like cells by upregulating acute myeloid leukaemia 1, a driver of tumour initiation that promotes TRPV2 expression<sup>323</sup>.

**Clinical studies.** A phase II clinical trial of nabiximols in glioblastoma has produced promising, although yet unpublished, results<sup>324</sup>. According to the manufacturer's press release<sup>324</sup>, "the study showed that patients with documented recurrent glioblastoma treated with THC:CBD had an 83% 1 year survival rate compared with 53% for patients in the placebo cohort ( $P=0.042$ ). Median survival for the THC:CBD group was greater than 550 days compared with 369 days in the placebo group". A new clinical trial of the non-psychotropic, synthetic cannabinoid dexanabinol in glioblastoma has also been completed but the results are yet to be disclosed<sup>325</sup>.

### Summary

The actions of endocannabinoids, endocannabinoid-like mediators and phytocannabinoids in several neurological disorders are multi-faceted, but some common threads can be identified (FIG. 3). These compounds often counteract infiltration of peripheral immune cells to the CNS, an aetiopathological factor in most neurodegenerative diseases<sup>326</sup>. They also commonly shift the phenotypes of microglia and infiltrating macrophages from pro-inflammatory to anti-inflammatory<sup>327</sup>, an effect often mediated by CB2, TRPV1 or PPAR $\gamma$ . When effective, CB1 agonists often reduce excitotoxicity. Studies of CB2 agonists and inhibitors of endocannabinoid inactivation are still at the preclinical stages, but major advances have been made in the clinical development of multi-target drugs that act within and beyond the endocannabinoidome (Supplementary Table 2). In addition to the clinical studies mentioned above, several clinical trials of nabiximols and CBD are ongoing or recruiting.

### Conclusions

Major efforts have been made to develop endocannabinoidome-targeted drugs. THC and its synthetic analogue nabilone were ineffective in clinical trials against the primary symptoms of AD, PD and MS, although THC has proved effective in Tourette syndrome<sup>328</sup>. Clinical results with nabiximols, CBD and palmitoylethanolamide are more promising, possibly owing to their multi-target nature that means they address the redundancy and promiscuity in the expanded endocannabinoid system. Notably, anecdotal and observational evidence on the use of cannabis preparations (including marijuana) in neurological disorders is increasing, but we have chosen not to discuss in this Review any controversial case reports or clinical studies with non-standardized preparations.

Controversy and, often unjustified, societal and regulatory barriers hinder development of cannabinoid-based treatments. A common misconception is that cannabinoids are all psychoactive, and pharmacologists have not been able to convey to the media and the layman that of >100 cannabinoids present in cannabis flowers, only THC is responsible for the central effects of marijuana. In addition, the fact that several varieties of cannabis plants exist with different

compositions of cannabinoids is neglected. CBD is still a controlled substance in the USA, even though it has been administered to hundreds of patients with no euphoric effects and a relatively safe profile. This anomaly makes clear that, despite considerable scientific evidence, talks about legalization, and the many industrial and medical uses of the plant, stigma around cannabis still hinders the conclusive assessment of the therapeutic potential of the plant's most abundant components. Further education is needed to reduce the negative impact of these factors on research. Emerging data on other non-euphoric cannabinoids (for example, cannabidivarin and  $\Delta^9$ -tetrahydrocannabivarin) that are being tested in epilepsy<sup>329</sup>, some rare forms of autism<sup>330</sup> and PD and metabolic disorders<sup>135</sup> might help with this education.

Research into targeting the expanded endocannabinoid system is in its infancy. The involvement of allosteric modulation in endocannabinoid signalling and the promiscuity of endocannabinoid-like mediators suggest that targeting non-orthosteric binding sites of CB1 and CB2 and/or development of multi-target compounds could be the best approach to developing neuroprotective drugs. Endogenous lipids, such as palmitoylethanolamide<sup>331</sup>, that act simultaneously at GPCRs, ion channels and PPARs<sup>139</sup> can be taken as templates for the development of synthetic multi-target drugs that deal with the multi-factorial aetiology of most neurological disorders. For example, preclinical studies indicate that the neuroprotective actions of palmitoylethanolamide involve modulation of at least three cell types<sup>139,264,332,333</sup>. Deeper knowledge of the allosteric sites on CB1 and CB2 (REF.<sup>62</sup>) is needed to enable their exploitation in the clinic.

In addition, more research is needed on the role of the gut microbiota in neuroinflammation and of endocannabinoidome signalling in the regulation of the gut microbiome<sup>107,109,112,113,334</sup>. Modulation of the gut-brain axis by targeting gut endocannabinoidome receptors could offer new therapeutic opportunities. For example, evidence suggests that OEA and/or palmitoylethanolamide not only have central therapeutic effects but also reduce 'leaky gut'-associated systemic inflammation and modulate gut microbiota composition<sup>335</sup>. Conversely, the intestinal flora produces neurotransmitters, such as serotonin and GABA<sup>110,336,337</sup>, and endocannabinoid-like molecules that act at the same receptors as the endogenous signalling molecules<sup>338</sup>. These mediators could affect the brain directly by diffusing through the BBB or indirectly via myenteric and vagal fibres. Levels of these molecules have not yet been measured in most neurological disorders associated with dysbiosis<sup>339</sup>.

Given that most studies of endocannabinoidome targeting have been preclinical, more neurologically relevant animal models are needed to reduce the translation gap. In addition, further placebo-controlled and double-blind trials of endocannabinoidome-targeted therapies are needed to clarify whether the dream of developing new neurotherapies from cannabinoids and their endogenous counterparts can be fully realized.

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The authors contributed equally to all aspects of the article.

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