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Endocannabinoid signaling as a synaptic circuit breaker in neurological disease

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***Cannabis sativa* is one of the oldest herbal plants in the history of medicine. It was used in various therapeutic applications from pain to epilepsy, but its psychotropic effect has reduced its usage in recent medical practice. However, renewed interest has been fueled by major discoveries revealing that cannabis-derived compounds act through a signaling pathway in the human body. Here we review recent advances showing that endocannabinoid signaling is a key regulator of synaptic communication throughout the central nervous system. Its underlying molecular architecture is highly conserved in synapses from the spinal cord to the neocortex, and as a negative feed-back signal, it provides protection against excess presynaptic activity. The endocannabinoid signaling machinery operates on demand in a synapse-specific manner; therefore, its modulation offers new therapeutic opportunities for the selective control of deleterious neuronal activity in several neurological disorders.**

Molecular architecture of synaptic endocannabinoid signaling

The core concept of neuronal communication involves the synaptic junction as the major site where chemical neurotransmitters convey information from presynaptic neurons to their postsynaptic partners. The molecular and morphological organization and the physiological operation of synaptic transmission follows a common scheme, with a predominantly anterograde flow of information throughout the central nervous system. Perturbations in elements of this scheme may lead to robust pathological consequences in the nervous system. Breakthrough discoveries in the last decade uncovered that endocannabinoid signaling is a principal regulator of synaptic communication; its molecular and anatomical organization is a common feature of most synapses, and perturbation of synaptic endocannabinoid signaling may contribute to several neurological diseases.

The notion that endocannabinoid signaling may have a general role in the regulation of synaptic transmission has been around for a long time. In 1990, Herkenham *et al.*[1] found that the high abundance of cannabinoid binding sites was comparable with the density of receptors for the two major neurotransmitters, glutamate and γ-aminobutyric acid (GABA). In fact, CB_1, the first cannabinoid receptor, is the most abundant G protein–coupled receptor in the brain[2–4]. Its central importance is supported by the observation that most behavioral effects of cannabinoid administration disappear after deletion of the gene encoding CB_1 (refs. 5–7). Even the subjective 'high' experience and the psychotropic effects induced by *Cannabis* smoking in humans can be alleviated by the selective blockade of CB_1 receptors[8].

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The CB_1 receptor is so abundant because it is found at nearly all types of central nervous system synapses, but, surprisingly, this receptor seems to consistently reside on the presynaptic side of the synapse[9]. The presynaptic localization was first shown on cortical GABAergic axon terminals[10], where immunogold labeling revealed an astonishing ~450 receptors within a single hippocampal GABAergic axon terminal[11]. Numerous examples of glutamatergic, often long-range projecting cells, including neocortical[12], hippocampal[13,14], hypothalamic[15] or cerebellar neurons[13], also bear presynaptic CB_1 receptors, and recent evidence suggests that even subcortical ascending pathways, such as cholinergic[16], noradrenergic[17] or serotonergic[18] axons, express CB_1.

What is the endogenous ligand for these receptors and where does it come from? CB_1 receptors are engaged by hydrophobic ligands, which may explain why this fascinating messenger system remained hidden from investigators for so long. Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive compound of the hemp plant, as well as *N*-arachidonoyl ethanolamide (anandamide)[19] and 2-arachidonoylglycerol (2-AG)[20,21], two endogenous ligands of CB_1 in the brain, are highly lipophilic. The hydrophobic nature of endocannabinoid molecules ensures that they don't need to be packed into conventional synaptic vesicles, but can rather be stored within cell membranes in their precursor forms and then synthesized and released upon relevant physiological stimuli[22]. Converging evidence from diverse experimental paradigms in several tissues suggests that endocannabinoids may not be primarily involved in basal and tonic intra- or intercellular communication (**Fig. 1a**). Instead, their main *modus operandi* is on-demand intercellular signaling[22]. This means that only precisely timed and positioned physiological stimuli evoke endocannabinoid biosynthesis and release from a selected subdomain of the cell surface (**Fig. 1b**,**c**).

Although anandamide was the first endogenous compound to be identified as an endocannabinoid[19], accumulating evidence suggests that 2-AG may be a more suitable candidate as an endogenous ligand of CB_1 receptors[23], at least in central synapses. Most importantly, a

b

c

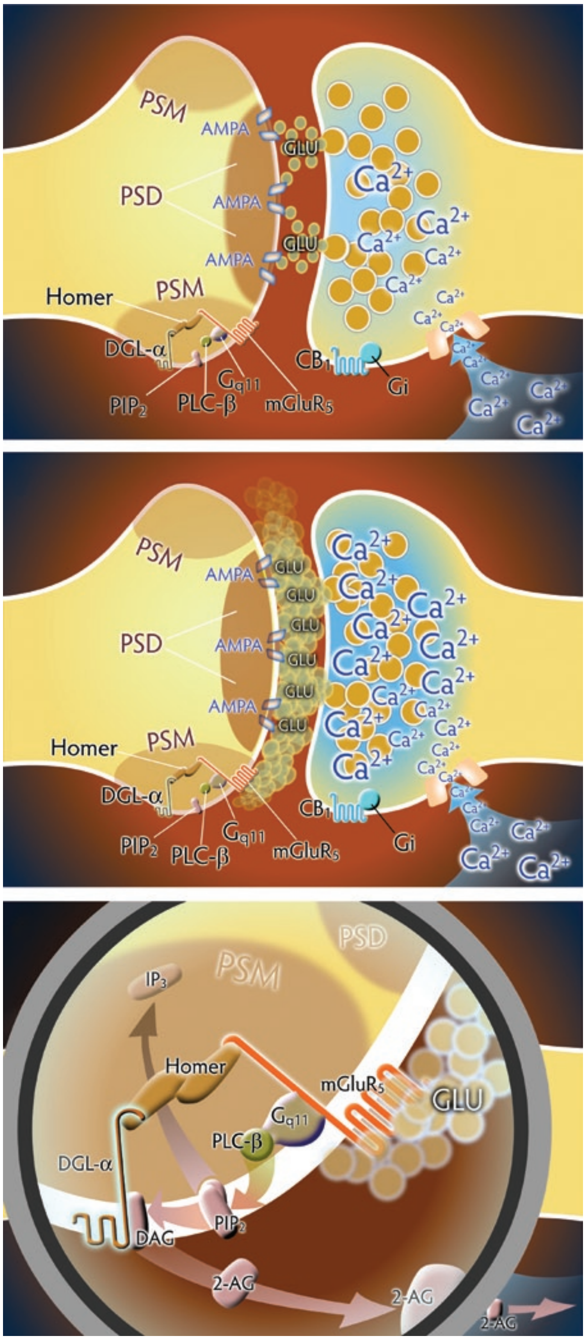

Figure 1 Activation of the perisynaptic signaling machinery (PSM) evokes retrograde endocannabinoid signaling. (**a**–**c**) Schematic diagrams illustrating the proposed physiological role of endocannabinoid-mediated retrograde synaptic signaling at glutamatergic synapses. (**a**) Basal synaptic activity involves gating of ionotropic glutamate receptors—predominantly α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), but also *N*-methyl-D-aspartate (NMDA), located in the postsynaptic density (PSD)—by synaptically released glutamate (GLU), which is triggered by Ca^{2+} influx through voltage-gated calcium channels. (**b**) In the case of excess presynaptic activity (depicted by highly elevated Ca^{2+} concentration in the axon terminal), increased release results in a spillover of glutamate from the synaptic cleft, where it will activate mGluRs associated with the PSM. (**c**) Signal transduction in the PSM begins with $mGluR_5$ activation, which triggers enzymatic activity of phospholipase C-β (PLC-β) via G_{q11} signaling. PLC-β cleaves the phosphatidyl inositol bisphosphate (PIP_2) pool into the signal transduction molecules inositol trisphosphate (IP_3) and diacylglycerol (DAG), the latter then further hydrolyzed by DGL-α to produce 2-AG. Notably, the molecular elements of the PSM are held together by the scaffolding protein Homer, as both $mGluR_5$ and DGL-α contain a Homer-binding motif in their C termini. Several well known features of synaptic transmission and plasticity (for example, NMDA receptors and other Homer-binding partners) are not indicated for reasons of clarity.

electrophysiological studies uncovered robust effects on synaptic neurotransmission by regulating 2-AG metabolism[24–27] but did not reveal significant changes upon pharmacologically modulating anandamide levels[26,28]. Furthermore, if 2-AG degradation was inhibited, the regional pattern of endogenous 2-AG accumulation overlapped with CB_1 receptors' distribution, and the increased 2-AG levels triggered CB_1-mediated signaling throughout the brain[29]. In contrast, elevation of anandamide levels by pharmacological inhibition of its degrading enzyme fatty acid amide hydrolase (FAAH) did not influence the activity of CB_1 receptors[29]. Moreover, CB_1 receptors share an evolutionary history with a recently identified major biosynthetic enzyme of 2-AG called diacylglycerol lipase-α (DGL-α)[30] but not with enzymes responsible for anandamide metabolism[31]. In addition, these two proteins and monoacylglycerol lipase (MGL)[32], the degrading enzyme of 2-AG, are all positioned in close proximity (albeit on opposite sides) at synapses in several brain areas[12,14,33–35]. Conversely, anandamide elimination takes place at a considerable distance from presynaptic CB_1 receptors, because FAAH does not show a preferential distribution at synapses and is instead predominantly located on intracellular membranes in postsynaptic cells[33]. Finally, an exciting possibility for the cross-talk of anandamide and 2-AG signaling was recently proposed on the basis of findings at striatal synapses, where anandamide acted as an inhibitor of 2-AG biosynthesis instead of competing for CB_1 receptors[36]. Though these findings all converge on 2-AG as being the primary candidate for a *synaptic* endocannabinoid, it is necessary to emphasize that anandamide may still turn out to be a *bona fide* ligand of CB_1 receptors under as yet unexplored conditions or in certain signaling processes at some selected parts of the body or even in the nervous system. In addition, anandamide may also influence physiological and pathophysiological processes via activation of several other molecular targets[37].

If 2-AG is a key endocannabinoid molecule at central synapses, then information on the upstream physiological events triggering its biosynthesis and release is crucial to understanding the functional significance of synaptic endocannabinoid signaling. Notably, the precursor molecule of 2-AG is diacylglycerol, a ubiquitously distributed signal transduction molecule[38]. Thus 2-AG biosynthesis may terminate signaling initiated by diacylglycerol (for example, the protein kinase C pathway), though spatial segregation of certain signaling machineries may circumvent this possibility. Indeed, not every upstream signaling molecule that triggers the diacylglycerol signal can also evoke 2-AG release. The most striking examples are the type 1 metabotropic glutamate receptors (mGluRs), namely $mGluR_1$ and $mGluR_5$ (ref. 39). Both mGluRs are primarily postsynaptic molecules distributed within similar synapse types[40]. The rationale for this colocalization is unknown, but, remarkably, activation of only one type of mGluR induces retrograde endocannabinoid signaling. At most synapses, $mGluR_5$ activation initiates 2-AG release[12,34,36,39,41,42], but, occasionally, $mGluR_1$ activates the signaling[25,43,44]. The reason for this selectivity is intriguing, given that both type 1 mGluRs are G_{q11}-coupled receptors, and their activation is known to be followed by the phospholipase C-β–mediated cleavage of the phosphatidyl inositol bisphosphate pool into inositol trisphosphate and diacylglycerol. The paradox that either $mGluR_5$ or $mGluR_1$ can predominantly initiate 2-AG release can be resolved by hypothesizing that certain specific signal transduction molecules are assembled together into distinct signaling machineries within the same postsynaptic structure. Quantitative neuroanatomical observations showed that both type 1 mGluRs and DGL-α have a striking overlapping distribution at central glutamatergic synapses[14,34,40,45]. None of these proteins was found intrasynaptically; instead, both were concentrated perisynaptically within a ~100-nm-wide annulus around the postsynaptic density (PSD). Remarkably, biochemical evidence suggests both type 1 mGluRs and DGL-α have a Homer-binding motif and are cross-linked via this key synaptic scaffolding protein[46,47]. This indicates that excitatory synapses consist of functionally distinct domains. Adjacent to the PSD, which contains most of the neurotransmitter receptors involved in basal synaptic neurotransmission (**Fig. 1a**), there is a perisynaptic signaling machinery (PSM) (**Fig. 1c**), which is designed to detect the spillover of glutamate from the synaptic cleft by means of perisynaptic type 1 mGluRs and translate this signal into a retrograde endocannabinoid (2-AG) message via activation of DGL-α (**Fig. 1b**,**c**). It is noteworthy that the above molecular machinery for the negative feedback pathway is conserved at glutamatergic synapses, as accumulating evidence indicates that this is so in the spinal cord (R. Nyilas and I.K., unpublished data), midbrain[35], striatum[34], hippocampus[14,45] and in the prefrontal[12] and somatosensory cortices (B. Dudok, T.F.F. and I.K., unpublished data). Because Homer is an important core protein of this perisynaptic signaling machinery, we must also emphasize that dysregulation of Homer signaling was shown to disrupt type 1 mGluR–mediated inhibition of glutamatergic excitatory postsynaptic currents[48], a phenomenon also known to be dependent on retrograde endocannabinoid signaling[34,43,49].

The above scenario suggests that 2-AG is synthesized by perisynaptic DGL-α enzymes located on the postsynaptic neuron and then activates presynaptic CB_1 receptors. How and where does the retrograde 2-AG signal terminate? A recent functional proteomic approach uncovered that 85% of the brain 2-AG content is eliminated by the serine hydrolase MGL[32,50]. In accordance with this data, high amounts of MGL were found in glutamatergic and selected GABAergic axon terminals[33], where it is situated in an ideal position to regulate the time course of retrograde endocannabinoid signaling. Taken together, the presynaptic localization of MGL and its predominant role in 2-AG degradation also implies that the vast majority of 2-AG molecules found in the brain may function as retrograde synaptic signals.

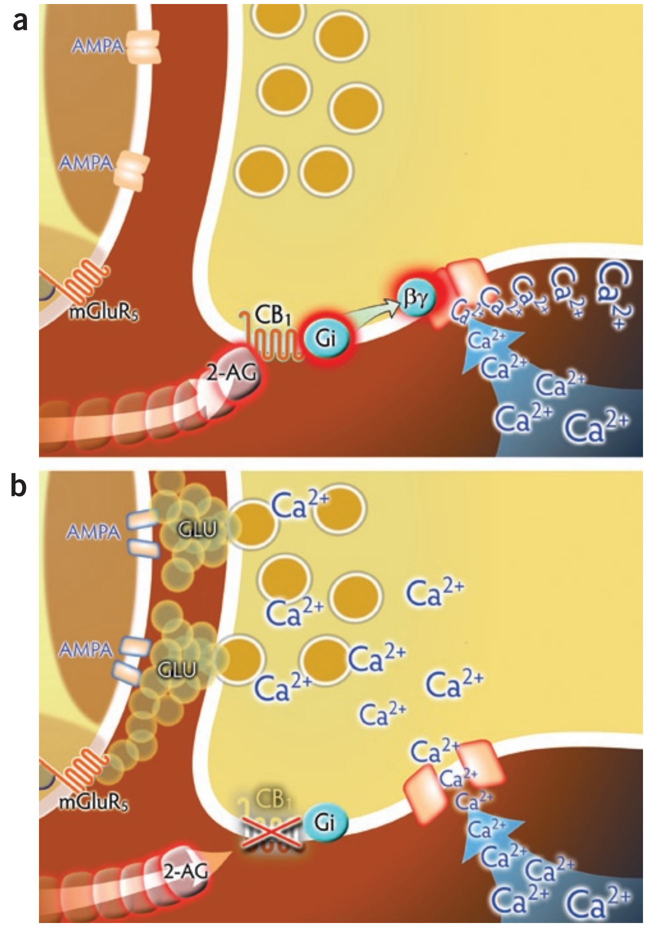

Figure 2 Operation of the perisynaptic signaling machinery as a synaptic circuit breaker. (**a**) Postsynaptically released 2-AG travels retrogradely through the synaptic cleft to engage presynaptic CB_1 cannabinoid receptors. Upon arrival and binding, a short-term suppression of neurotransmitter release will be induced by the βγ subunit of G_i, inhibiting voltage-gated calcium channels. (**b**) A loss of CB_1 receptors from glutamatergic axon terminals and the consequent impairment in both short-term and long-term control of glutamate release probably results in runaway excitation and a decreased seizure threshold, as observed both in humans and in animal models[68,69]. The entire process is depicted in **Supplementary Video 1** online.

Operation and malfunctioning of a synaptic circuit breaker

Presynaptic CB_1 receptors have an unusually clear effect on axon terminal activity. Irrespective of the chemical nature of a given bouton (for example, glutamatergic or GABAergic), its regional localization in the nervous system or the type of CB_1 ligand applied (for example, exogenous or endogenous cannabinoid), activation of presynaptic CB_1 receptors always results in the attenuation of neurotransmitter release[9]. Although the direction of the effect is always the same, its magnitude varies. However, when the readout of the physiological experiment was highly specific (for example, in paired recordings, when the measured synaptic currents originated from a single presynaptic neuron), the activation of CB_1 receptors could almost entirely block neurotransmitter release from both glutamatergic and GABAergic boutons[51,52]. Whether such robust veto of synaptic neurotransmission also occurs *in vivo* is not known; nevertheless, the same effect can also be achieved by the synaptic release of 2-AG from postsynaptic neurons[51,52], indicating that the perisynaptic signaling machinery has the intrinsic capacity to synthesize and release enough endocannabinoid molecules to behave as a synaptic circuit breaker (**Figs. 1** and **2**).

Although the general outcome of retrograde endocannabinoid signaling is always a decrease in synaptic transmission, the time course of expression of this phenomenon divides endocannabinoid-mediated synaptic plasticity into two types with potentially distinct physiological and pathophysiological implications[53]. Short-term synaptic depression has a rapid onset (<1s), but it is a transient event lasting seconds or sometimes minutes[53]. In contrast, the endocannabinoid-mediated form of long-term synaptic depression requires a longer induction paradigm, but it is sustained for at least several hours[53]. Notably, although CB_1 receptor activation is a necessary condition for both types[53], the underlying downstream signal transduction cascades are different (**Figs. 2** and **3**). Rapid but transient attenuation of neurotransmitter release is probably a membrane-delimited process requiring $G_{\beta\gamma}$-mediated inhibition of voltage-gated Ca^{2+} channels (VGCCs)[54,55]. In contrast, engagement of CB_1 receptors may also initiate downregulation of the adenylyl cyclase–protein kinase A (AC-PKA) pathway through $G\alpha_i$-coupling, resulting in long-term depression of synaptic transmission via the active zone protein Rab3-interacting molecule-1α (RIM-1α) (for example, at GABAergic synapses[56]) or via permanent inhibition of PQ-type Ca^{2+} channels (for example, at glutamatergic terminals[57]). An intriguing question to be answered in the future is how a given axon terminal and its presynaptic CB_1 receptors determine which signaling pathway and type of plasticity should be triggered. Are there two distinct macromolecular signaling complexes both linked to CB_1 receptors on a single axon terminal? In the case of hippocampal GABAergic boutons, high-resolution immunogold labeling showed two distribution peaks of CB_1 receptors[11], which may indeed reflect two functionally distinct populations. One population is positioned close to the presynaptic active zone and may react to local, postsynaptically released 2-AG and then directly act on VGCCs[11]. Another population is found on the preterminal segments, where it may be activated by 2-AG derived from heterosynaptic sources (as has been shown for hippocampal GABAergic terminals[58]) and may regulate the AC-PKA pathway[56]. An alternative possibility is that the same CB_1 receptor protein can initiate the two distinct signal transduction pathways by the two effector limbs of G protein activation (the α_i and $\beta\gamma$ limbs, **Figs. 2** and **3**), and a coincident signal—for example, activation of presynaptic NMDA receptors[59] or activation of the serine-threonine phosphatase, calcineurin, after repetitive firing of the presynaptic neuron[60] (**Fig. 3b**)—determines whether the long-term pathway can continue after the rapid decay of the short-term effect.

The step-by-step delineation of the crucial role of the endocannabinoid system in synaptic physiology and in short- and long-term synaptic plasticity evoked a clear paradigm shift in neurological research seeking to exploit this fascinating messenger system for therapeutic purposes. Evidence is rapidly accumulating for how synaptic endocannabinoid (or, more precisely, 2-AG) signaling is affected in certain neurological disorders, as well as for how pharmacological regulation of synaptic 2-AG levels or CB_1 activity may be therapeutically beneficial. The recent development of several new pharmacological and genetic tools targeting the endocannabinoid system, together with an increasing number of human studies, all contributed significantly to the change in this field.

If CB_1 receptors are key presynaptic regulators of synaptic transmission, then one of their most prominent applications may be the control of excess presynaptic activity. Increased abundance of glutamate is a feature of traumatic insults causing neuronal damage, for example, during cerebrovascular ischemia or epileptic seizures. Indeed, excitotoxicity-related neuronal damage and epilepsy are among the most intensively researched areas of the cannabinoid field[61]. A large body of literature shows that various forms of neuronal insults (for example, closed-head injury or convulsants) induce the release of endocannabinoids, including 2-AG[62,63], and, in several experimental models, CB_1 receptor agonists alleviate excitotoxicity and are neuroprotective[61,62,64–66]. In contrast, CB_1 antagonists reduce seizure threshold, further deteriorate malignant excitotoxic processes and increase neuronal death[65,66]. The underlying cellular and molecular processes of the involvement of synaptic endocannabinoid signaling in the brain's own protective system began to unfold after development of mouse models in which CB_1 receptors were deleted exclusively from selected cell types[67,68]. Although forebrain GABAergic axon terminals carry three to ten times more CB_1 receptors than their glutamatergic counterparts[13,34], selective inactivation of CB_1 receptors on these inhibitory axons surprisingly does not change the susceptibility of mice to convulsants[68]. Conversely, when CB_1 was deleted exclusively in principal forebrain neurons (which are glutamatergic; **Fig. 2b**), these mice expressed a severely reduced seizure threshold and showed (if they survived) augmented neuronal death[67,68].

These findings indicate that promoting endocannabinoid signaling at glutamatergic synapses may have a beneficial effect in epilepsy

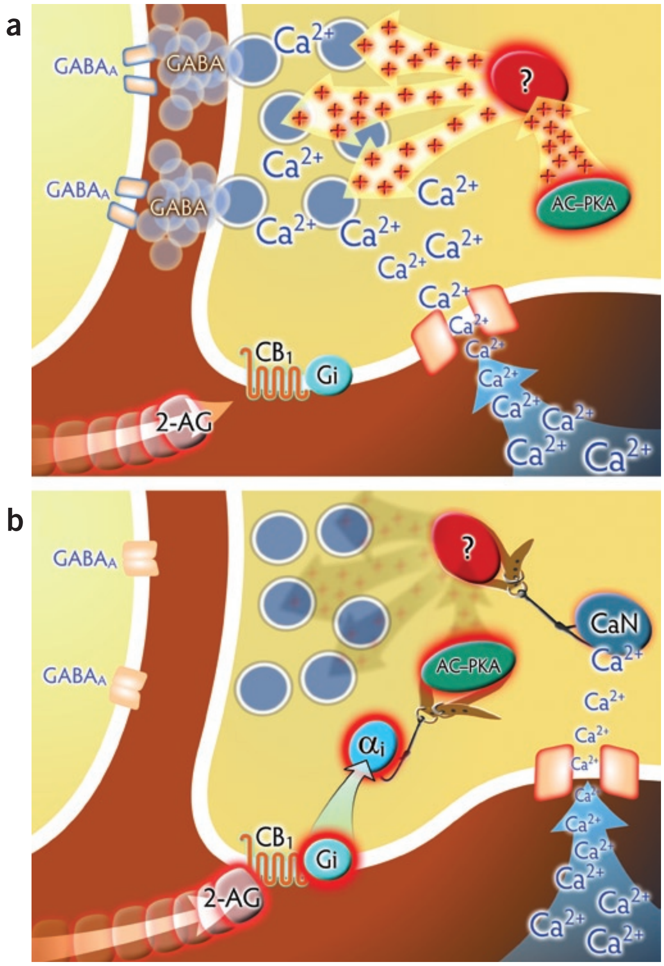

Figure 3 Molecular mechanism of endocannabinoid-mediated long-term depression. (**a**) Before 2-AG (synthesized by the postsynaptic cell) reaches the presynaptic terminal, transmitter release is mediated by Ca^{2+} influx and by active zone proteins (for example, by RIM-1α (ref. 58)) that are activated by PKA-mediated phosphorylation. (**b**) Upon CB_1 receptor activation, the AC-PKA pathway will be inhibited through $G\alpha_i$-coupling, which may result in long-term depression of synaptic transmission (for example, at GABAergic synapses in the basolateral amygdala or hippocampus[56], illustrated here, or at prefronto-accumbens glutamatergic synapses[57]). In addition, presynaptic cell firing ensures activation of the serine/threonine phosphatase calcineurin (CaN) by elevating intracellular Ca^{2+} levels. CaN then inhibits the activity of as yet unidentified active zone proteins (depicted by '?'; this protein may be RIM-1α at GABAergic synapses).

treatment, whereas a compound with an antagonistic profile—for example, the recently approved (in the EU) antiobesity drug rimonabant—may hold risks in individuals with a history of convulsions (irrespective of the underlying causes). In addition, these findings obtained in animal models also pose the question of whether impairment of endocannabinoid signaling contributes to increased network excitability in humans with epilepsy. In individuals with intractable temporal lobe epilepsy, the expression of CB_1 receptor mRNA is robustly downregulated together with DGL-α (ref. 69). Moreover, the majority of glutamatergic axon terminals in the dentate gyrus, which is a key subregion in epileptogenesis owing to its recurrent disynaptic excitatory circuitry, lost their CB_1 receptors[69] (**Fig. 2b**). In contrast, but in parallel with the animal models, GABAergic axon terminals were not affected[69]. Thus, it seems that the neuroprotective machinery involving synaptic endocannabinoid signaling may be impaired in people with epilepsy (**Fig. 2b**), which may further aggravate the progression of epileptic activity and neuronal damage by reducing seizure threshold.

It seems that not only presynaptic CB_1 receptors but also the entire perisynaptic signaling machinery responsible for retrograde 2-AG signaling should remain intact to enable protection against excess presynaptic activity. Perisynaptically positioned $mGluR_5$ receptors monitor the amount of glutamate spillover and initiate the entire retrograde signaling process. Notably, these $mGluR_5$ receptors were shown to be profoundly impaired after status epilepticus and kindling, whereas $mGluR_1$ receptors remained unaffected, further indicating that they may have a separate signaling function[70]. Moreover, epileptic seizures also reduced the level of the long isoform Homers[70], which cross-link $mGluR_5$ and DGL-α at glutamatergic synapses[47], and retrograde synaptic signaling did not operate properly in pilocarpine-treated epileptic rats[70]. Activation of $mGluR_5$ stimulates G_{q11}-mediated signaling, and forebrain principal cell–specific deletion of both Gα types markedly diminishes seizure thresholds, with several mice developing spontaneous epileptic seizures and dying at a younger age[63]. Excitotoxicity-induced 2-AG release was missing in these double-knockout mice, providing important evidence that excess neuronal activity evokes retrograde 2-AG signaling[63]. Finally, PLC-β1–deficient mice also develop severe epilepsy[71], indicating that this component of the perisynaptic signaling machinery is also crucial for the proper functioning of the synaptic circuit breaker in controlling network excitability.

Emerging evidence also points to the central role of endocannabinoid signaling in other neurological diseases, especially those in which neuronal damage is prominent. For example, 2-AG abundance is increased tenfold after closed head injury[62] and markedly reduces the size of brain edema via CB_1 receptor activation[62]. In accordance with these results, CB_1 activation reduced infarct volume and diminished neuronal cell loss by ~50% after both focal and global cerebral ischemia[64]. In the animal model for multiple sclerosis, experimental autoimmune encephalomyelitis, cannabinoid administration is neuroprotective through the activation of CB_1 receptors on neurons, probably by reducing the consequences of the immune attack–evoked excitotoxicity[72,73], which would otherwise further stimulate inflammation. Interestingly, in this fight, their partners are the CB_2 receptors on autoreactive T cells, which, when activated by endocannabinoids, suppress T cell proliferation and cytokine production[73]. Microglial cells also join the battle; they produce 2-AG upon stimulation of P2X7 receptors by ATP spilled from damaged cells[74]. Unfortunately, encephalitogenic T cells may also fight back with interferon-γ, which results in a decrease in 2-AG and disrupted endocannabinoid-mediated neuroprotection[75].

Synaptic endocannabinoid signaling in neurological disease

Several lines of recent evidence suggest that endocannabinoids are involved in remodeling of neuronal activity patterns by long-term synaptic plasticity, and these processes play a part in various brain disorders. The first discovery that long-term depression of central synapses is also mediated by retrograde endocannabinoid signaling was made in the dorsal striatum[76] and ventral striatum[42] (nucleus accumbens). The molecular machinery of the endocannabinoid system is indeed present at corticostriatal glutamatergic synapses[34]. From the medical point of view, synaptic endocannabinoid signaling may have a prominent pathophysiological role in both striatal areas. Movement disorders, especially Parkinson's disease, have been shown to be regulated by synaptic endocannabinoid signaling in the dorsal striatum[77], whereas research in the nucleus accumbens primarily explores the role of the endocannabinoid pathway in drug addiction[78]. Although at first glance these neurological diseases may have entirely different etiologies, it seems that at least the contribution of endocannabinoid-mediated long-term synaptic plasticity follows a similar logic.

Dendritic spine heads in the dorsal striatum are equipped with the perisynaptic signaling machinery to release 2-AG upon $mGluR_5$ activation[34], which can initiate both short- and long-term synaptic depression[34,77]. Recent data suggest that endocannabinoid-LTD can be elicited in synapses formed by cortical neurons on striatal medium spiny neurons projecting to the lateral globus pallidus (indirect pathway)[77]. It is noteworthy that both D_2 dopamine– and CB_1 receptor–deficient mice show characteristic movement impairments resembling symptoms of Parkinson's disease[5,79]. Thus, the synergestic activation of type 1 mGluRs coincidently with D_2 receptors may be required to achieve the most efficient synaptic depression[77,80]. This indicates that impaired dopaminergic innervation may have an impact on the operation of endocannabinoid signaling, and, indeed, in a mouse model of Parkinson's disease, the authors were unable to evoke endocannabinoid-LTD[77]. However, application of the D_2 receptor agonist quinpirole, together with inhibition of endocannabinoid degradation (both 2-AG and anandamide), was able to rescue endocannabinoid-LTD *in vitro* and compensate for the profound motor deficits due to the dopamine depletion used in the model[77]. It is tempting to speculate that, although synaptic endocannabinoid signaling follows a similar scheme throughout the central nervous system, variations on this common theme may have their specific physiological significance in certain brain areas, cells or synapse types. The pivotal role of D_2 receptors in the striatum (another example from the hypothalamus is described below) suggests that regulation of the initiation of endocannabinoid signaling may be a particularly good target to refine the operation of the endocannabinoid pathway according to specialized physiological requirements.

Addiction and related long-term reorganization of the brain's reward circuitry is also known to require an intact endocannabiniod system[78]. Genetic deletion or pharmacological blockade of CB_1 receptors either eliminates or at least robustly diminishes the addictive properties of most drugs of abuse, including nicotine, morphine, heroine, ethanol, cocaine or Δ^9-THC itself (for review, see ref. 78). The underlying neurobiological substrates are complex; endocannabinoid signaling is thought to contribute to the motivational aspects of drug-seeking behavior and also to be responsible for the relapse phenomenon induced by environmental stimuli and drug re-exposure at the level of the nucleus accumbens. In addition, it mediates the primary rewarding effects of several drugs at the level of the midbrain ventral tegmental area (VTA). Despite the multiple sites of action, substantial modification of synaptic efficacy is the main underlying mechanism in both brain areas. Although variations may turn out to be important at certain synapses, for example, in the regulation of 2-AG release, the molecular and anatomical organization of the endocannabinoid system and its mode of action seems to be remarkably conserved[34,35]. Drugs of abuse themselves regulate synaptic endocannabinoid signaling both in the nucleus accumbens and in the VTA. A single administration of either cocaine or Δ^9-THC eliminates both a homosynaptic form of endocannabinoid-mediated long-term depression at prefronto-accumbens synapses[81,82] and a heterosynaptic form of long-term depression induced by activation of hippocampal glutamatergic afferents but expressed at neighboring GABAergic synapses[81]. This latter cross-talk between distinct types of synapses was also shown to be present in the VTA, where repeated *in vivo* administration of cocaine together with excitatory afferent stimulation triggered long-term depression of GABAergic synapses on dopaminergic neurons[83]. Notably, the type 1 mGluR–PLC-β–DGL-α–CB_1 receptor pathway mediated this phenomenon, whereas D_2 receptors contributed to the detection of coincident administration of cocaine, a dopamine uptake inhibitor[83]. This heterosynaptic phenomenon may free dopaminergic neurons from GABAergic inhibition, shifting them into burst firing mode, and thereby may have a particularly key role in the primary rewarding effects of drugs of abuse.

Appreciation of 'natural rewards' in the brain is also an endocannabinoid-dependent process. The strong orexigenic (appetite stimulatory) effect of *Cannabis* is exploited in several countries as an effective treatment for anorexia. The underlying neurobiological basis seems to be the regulation of synaptic plasticity; for example, similar to the process in the VTA, perifornical lateral hypothalamic neurons also need to escape from their GABAergic inhibition, which they achieve by initiating retrograde synaptic endocannabinoid signaling[84]. Leptin[85], an important anorexigenic hormone, downregulates endocannabinoid release by reducing the depolarization-induced calcium increase required for PLC-β and DGL-α activity in the postsynaptic hypothalamic neurons[84]. Leptin-deficient mice have elevated hypothalamic endocannabinoid abundance[85] and show six times longer, but still transient, short-term synaptic depression, also indicating that the abundance of available endocannabinoids is an important determinant factor in the efficacy of retrograde inhibition of neurotransmitter release[84]. Whereas leptin acts against endocannabinoid signaling in the hypothalamus, glucocorticoids have been shown to support such signaling in the hypothalamic paraventricular nucleus. In this structure, glucocorticoids evoke an endocannabinoid-mediated depression of excitatory inputs on parvocellular neurosecretory neurons[86], which may be a key step in negative feedback control of glucocorticoid action on the hypothalamic-pituitary-adrenal axis[86].

Despite the fact that *Cannabis* is an ancient analgesic, and the molecular machinery for retrograde endocannabinoid signaling is present along the nociceptive signal transmission pathway (R. Nyilas and I.K., unpublished data), surprisingly little is known about the role of endocannabinoids in the regulation of synaptic plasticity in this system. Neurons of the descending analgesic pathway in the midbrain periaqueductal gray can also break free from their GABAergic inhibition by the $mGluR_5$-CB_1 pathway, and this process may underlie the well known analgesic effect of cannabinoids[41], especially the nonopioid form of stress-induced analgesia[87]. However, we must also emphasize that several aspects of cannabinoid-mediated analgesia have been shown to occur at the periphery, both at CB_1[88] and partially also at CB_2 receptors[89], and modulation of endocannabinoid signaling by drugs unable to cross the blood-brain barrier may thus have central importance in antinoceptive treatments.

Harvest *Cannabis* or exploit our own endocannabinoids?

Synaptic endocannabinoid signaling may have a pivotal role in a plethora of neurological diseases, either as a contributing factor to the etiology of a disease or as an alternative solution to circumvent other impaired signaling pathways. How can this system be harnessed to treat neurological disorders?

Rimonabant, the first marketed CB_1 receptor antagonist, is already in use in the EU under the name of Acomplia as an antiobesity drug, and a recent European cohort study also revealed its potency in cardiovascular disease linked to the metabolic syndrome[90]. However, the US Food and Drug Administration held back its approval owing to concerns over potential side effects, especially increased risk of depression and suicidal behavior. Therefore, it is of pivotal importance that serotonin, the major neurotransmitter implicated in the pathophysiology of depression, can also initiate retrograde endocannabinoid signaling via activation of the G_{q11}-coupled $5\text{-}HT_2$ receptors, which results in profound suppression of excitatory synapses via engagement of presynaptic CB_1 receptors[91]. This implies that rimonabant may counteract serotonergic signaling and suggests that it may also interfere with some of the beneficial effects of selective serotonin reuptake blockers in mood disorders.

Another promising approach to treating neurological disorders may be the potentiation of endocannabinoid signaling. This is a very promising option, because it seems that the synaptic endocannabinoid machinery is only activated at certain synapses in a manner restricted both when and where it is required for homeostatic operations of synapses and neuronal networks. Thus, a blockade of endocannabinoid degradation may be a fairly selective approach. At least three different families of chemical compounds have been identified as first-generation inhibitors of MGL[87,92,93]. Although not yet efficacious or selective, these molecules can serve as a good proof of principle for the approach itself, because their peripheral and central antinociceptive and anti-inflammatory effects have already been shown[87,94]. An even more selective approach could be the delineation of cell type–specific physiological signals contributing to the stimulation of endocannabinoid release, which, in a cocktail with MGL inhibitors, could further promote endocannabinoid signaling at the required synapses.

Finally, we must emphasize that 2-AG may only be the tip of the iceberg, as other chemically related endogenous molecules are also rapidly emerging, and their widespread, but patterned, distribution in the brain suggests that these molecules may be involved in various previously undescribed signaling pathways[22]. Anandamide is already well known, and a selective inhibitor of its degrading enzyme, FAAH, has shown its potential as an anxiolytic, antidepressant and antinociceptive compound[95–97]. Such new classes of lipid signaling molecules and the complex enzymatic networks regulating their life cycle may represent further research targets to be explored.

Note: Supplementary information is available on the Nature Medicine website.

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1. Herkenham, M. *et al.* Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. USA* **87**, 1932–1936 (1990).
2. Devane, W.A., Dysarz, F.A., III, Johnson, M.R., Melvin, L.S. & Howlett, A.C. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **34**, 605–613 (1988).
3. Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. & Bonner, T.I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561–564 (1990).
4. Piomelli, D. The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* **4**, 873–884 (2003).
5. Zimmer, A., Zimmer, A.M., Hohmann, A.G., Herkenham, M. & Bonner, T.I. Increased mortality, hypoactivity and hypoalgesia in cannabinoid CB_1 receptor knockout mice. *Proc. Natl. Acad. Sci. USA* **96**, 5780–5785 (1999).
6. Ledent, C. *et al.* Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. *Science* **283**, 401–404 (1999).
7. Monory, K. *et al.* Genetic dissection of behavioural and autonomic effects of Δ^9-tetrahydrocannabinol in mice. *PLoS Biol.* **5**, e269 (2007).
8. Huestis, M.A. *et al.* Blockade of effects of smoked marijuana by the CB_1-selective cannabinoid receptor antagonist SR141716. *Arch. Gen. Psychiatry* **58**, 322–328

(2001).
9. Freund, T.F., Katona, I. & Piomelli, D. Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* **83**, 1017–1066 (2003).
10. Katona, I. *et al.* Presynaptically located CB_1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* **19**, 4544–4558 (1999).
11. Nyiri, G., Cserep, C., Szabadits, E., Mackie, K. & Freund, T.F. CB_1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. *Neuroscience* **136**, 811–822 (2005).
12. Lafourcade, M. *et al.* Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. *PLoS ONE* **2**, e709 (2007).
13. Kawamura, Y. *et al.* The CB_1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J. Neurosci.* **26**, 2991–3001 (2006).
14. Katona, I. *et al.* Molecular composition of the endocannabinoid system at glutamatergic synapses. *J. Neurosci.* **26**, 5628–5637 (2006).
15. Wittmann, G. *et al.* Distribution of type 1 cannabinoid receptor (CB_1)-immunoreactive axons in the mouse hypothalamus. *J. Comp. Neurol.* **503**, 270–279 (2007).
16. Degroot, A. *et al.* CB_1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. *Mol. Pharmacol.* **70**, 1236–1245 (2006).
17. Oropeza, V.C., Mackie, K. & Van Bockstaele, E.J. Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. *Brain Res.* **1127**, 36–44 (2007).
18. Balazsa, T., Biro, J., Gullai, N., Ledent, C. & Sperlagh, B. CB_1-cannabinoid receptors are involved in the modulation of non-synaptic [^{3}H]serotonin release from the rat hippocampus. *Neurochem. Int.* **52**, 95–102 (2008).
19. Devane, W.A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
20. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83–90 (1995).
21. Sugiura, T. *et al.* 2-arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215**, 89–97 (1995).
22. Piomelli, D., Astarita, G. & Rapaka, R. A neuroscientist's guide to lipidomics. *Nat. Rev. Neurosci.* **8**, 743–754 (2007).
23. Sugiura, T., Kishimoto, S., Oka, S. & Gokoh, M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog. Lipid Res.* **45**, 405–446 (2006).
24. Makara, J.K. *et al.* Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. *Nat. Neurosci.* **8**, 1139–1141 (2005).
25. Melis, M. *et al.* Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol–mediated suppression of excitation in dopamine neurons. *J. Neurosci.* **24**, 10707–10715 (2004).
26. Hashimotodani, Y., Ohno-Shosaku, T. & Kano, M. Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus. *J. Neurosci.* **27**, 1211–1219 (2007).
27. Hashimotodani, Y., Ohno-Shosaku, T., Maejima, T., Fukami, K. & Kano, M. Pharmacological evidence for the involvement of diacylglycerol lipase in depolarization-induced endocanabinoid release. *Neuropharmacology* **54**, 58–67 (2008).
28. Kim, J. & Alger, B.E. Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nat. Neurosci.* **7**, 697–698 (2004).
29. Palomaki, V.A., Lehtonen, M., Savinainen, J.R. & Laitinen, J.T. Visualization of 2-arachidonoylglycerol accumulation and cannabinoid CB_1 receptor activity in rat brain cryosections by functional autoradiography. *J. Neurochem.* **101**, 972–981 (2007).
30. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163**, 463–468 (2003).
31. McPartland, J.M., Norris, R.W. & Kilpatrick, C.W. Coevolution between cannabinoid receptors and endocannabinoid ligands. *Gene* **397**, 126–135 (2007).
32. Dinh, T.P. *et al.* Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. USA* **99**, 10819–10824 (2002).
33. Gulyas, A.I. *et al.* Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.* **20**, 441–458 (2004).
34. Uchigashima, M. *et al.* Subcellular arrangement of molecules for 2-arachidonoyl-glycerol–mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J. Neurosci.* **27**, 3663–3676 (2007).
35. Matyas, F. *et al.* Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology* **54**, 95–107 (2008).
36. Maccarrone, M. *et al.* Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. *Nat. Neurosci.* **11**, 152–159 (2008).
37. Pertwee, R.G. Pharmacological actions of cannabinoids. *Handb. Exp. Pharmacol.* **168**, 1–51 (2005).
38. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–778 (1997).
39. Jung, K.M. *et al.* Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. *Mol. Pharmacol.* **68**, 1196–1202 (2005).
40. Lujan, R., Nusser, Z., Roberts, J.D., Shigemoto, R. & Somogyi, P. Perisynaptic location of metabotropic glutamate receptors $mGluR_1$ and $mGluR_5$ on dendrites and dendritic spines in the rat hippocampus. *Eur. J. Neurosci.* **8**, 1488–1500 (1996).
41. Drew, G.M., Mitchell, V.A. & Vaughan, C.W. Glutamate spillover modulates GABAergic synaptic transmission in the rat midbrain periaqueductal grey via metabotropic glutamate receptors and endocannabinoid signaling. *J. Neurosci.* **28**, 808–815 (2008).
42. Robbe, D., Kopf, M., Remaury, A., Bockaert, J. & Manzoni, O.J. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc. Natl. Acad. Sci. USA* **99**, 8384–8388 (2002).
43. Maejima, T., Hashimoto, K., Yoshida, T., Aiba, A. & Kano, M. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron* **31**, 463–475 (2001).
44. Azad, S.C. *et al.* Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. *J. Neurosci.* **24**, 9953–9961 (2004).
45. Yoshida, T. *et al.* Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB_1 receptor. *J. Neurosci.* **26**, 4740–4751 (2006).
46. Brakeman, P.R. *et al.* Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* **386**, 284–288 (1997).
47. Jung, K.M. *et al.* A key role for diacylglycerol lipase-α in metabotropic glutamate receptor–dependent endocannabinoid mobilization. *Mol. Pharmacol.* **72**, 612–621 (2007).
48. Kammermeier, P.J. & Worley, P.F. Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. *Proc. Natl. Acad. Sci. USA* **104**, 6055–6060 (2007).
49. Straiker, A. & Mackie, K. Metabotropic suppression of excitation in murine autaptic hippocampal neurons. *J. Physiol. (Lond.)* **578**, 773–785 (2007).
50. Blankman, J.L., Simon, G.M. & Cravatt, B.F. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356 (2007).
51. Ohno-Shosaku, T., Maejima, T. & Kano, M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* **29**, 729–738 (2001).
52. Straiker, A. & Mackie, K. Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. *J. Physiol. (Lond.)* **569**, 501–517 (2005).
53. Chevaleyre, V., Takahashi, K.A. & Castillo, P.E. Endocannabinoid-mediated synaptic plasticity in the CNS. *Annu. Rev. Neurosci.* **29**, 37–76 (2006).
54. Wilson, R.I., Kunos, G. & Nicoll, R.A. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron* **31**, 453–462 (2001).
55. Brown, S.P., Safo, P.K. & Regehr, W.G. Endocannabinoids inhibit transmission at granule cell to Purkinje cell synapses by modulating three types of presynaptic calcium channels. *J. Neurosci.* **24**, 5623–5631 (2004).
56. Chevaleyre, V., Heifets, B.D., Kaeser, P.S., Sudhof, T.C. & Castillo, P.E. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1α. *Neuron* **54**, 801–812 (2007).
57. Mato, S., Lafourcade, M., Robbe, D., Bakiri, Y. & Manzoni, O.J. Role of the cyclic-AMP/PKA cascade and of P/Q-type Ca^{++} channels in endocannabinoid-mediated long-term depression in the nucleus accumbens. *Neuropharmacology* **54**, 87–94 (2008).
58. Chevaleyre, V. & Castillo, P.E. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* **38**, 461–472 (2003).
59. Sjostrom, P.J., Turrigiano, G.G. & Nelson, S.B. Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. *Neuron* **39**, 641–654 (2003).
60. Heifets, B.D., Chevaleyre, V. & Castillo, P.E. Interneuron activity controls endocannabinoid-mediated presynaptic plasticity through calcineurin. *Proc. Natl. Acad. Sci. USA* **105**, 10250–10255 (2008).
61. Lutz, B. On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures. *Biochem. Pharmacol.* **68**, 1691–1698 (2004).
62. Panikashvili, D. *et al.* An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* **413**, 527–531 (2001).
63. Wettschureck, N. *et al.* Forebrain-specific inactivation of Gq/G11 family G proteins results in age-dependent epilepsy and impaired endocannabinoid formation. *Mol. Cell. Biol.* **26**, 5888–5894 (2006).
64. Nagayama, T. *et al.* Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J. Neurosci.* **19**, 2987–2995 (1999).
65. Wallace, M.J., Martin, B.R. & DeLorenzo, R.J. Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. *Eur. J. Pharmacol.* **452**, 295–301 (2002).
66. Wallace, M.J., Blair, R.E., Falenski, K.W., Martin, B.R. & DeLorenzo, R.J. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. *J. Pharmacol. Exp. Ther.* **307**, 129–137 (2003).
67. Marsicano, G. *et al.* CB_1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* **302**, 84–88 (2003).
68. Monory, K. *et al.* The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* **51**, 455–466 (2006).
69. Ludanyi, A. *et al.* Downregulation of the CB_1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. *J. Neurosci.* **28**, 2976–2990 (2008).
70. Kirschstein, T. *et al.* Loss of metabotropic glutamate receptor–dependent long-term depression via downregulation of $mGluR_5$ after status epilepticus. *J. Neurosci.* **27**, 7696–7704 (2007).
71. Kim, D. *et al.* Phospholipase C isozymes selectively couple to specific neurotransmitter receptors. *Nature* **389**, 290–293 (1997).

72. Baker, D. *et al.* Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* **404**, 84–87 (2000).
73. Maresz, K. *et al.* Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB_1 on neurons and CB2 on autoreactive T cells. *Nat. Med.* **13**, 492–497 (2007).
74. Witting, A., Walter, L., Wacker, J., Moller, T. & Stella, N. P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. *Proc. Natl. Acad. Sci. USA* **101**, 3214–3219 (2004).
75. Witting, A. *et al.* Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. *Proc. Natl. Acad. Sci. USA* **103**, 6362–6367 (2006).
76. Gerdeman, G.L., Ronesi, J. & Lovinger, D.M. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat. Neurosci.* **5**, 446–451 (2002).
77. Kreitzer, A.C. & Malenka, R.C. Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. *Nature* **445**, 643–647 (2007).
78. Maldonado, R., Valverde, O. & Berrendero, F. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci.* **29**, 225–232 (2006).
79. Baik, J.H. *et al.* Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. *Nature* **377**, 424–428 (1995).
80. Yin, H.H. & Lovinger, D.M. Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. *Proc. Natl. Acad. Sci. USA* **103**, 8251–8256 (2006).
81. Mato, S. *et al.* A single *in-vivo* exposure to Δ^9-THC blocks endocannabinoid-mediated synaptic plasticity. *Nat. Neurosci.* **7**, 585–586 (2004).
82. Fourgeaud, L. *et al.* A single *in vivo* exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J. Neurosci.* **24**, 6939–6945 (2004).
83. Pan, B., Hillard, C.J. & Liu, Q.S. Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. *J. Neurosci.* **28**, 1385–1397 (2008).
84. Jo, Y.H., Chen, Y.J., Chua, S.C., Jr, Talmage, D.A. & Role, L.W. Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. *Neuron* **48**, 1055–1066 (2005).
85. Di Marzo, V. *et al.* Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410**, 822–825 (2001).
86. Di, S., Malcher-Lopes, R., Halmos, K.C. & Tasker, J.G. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J. Neurosci.* **23**, 4850–4857 (2003).
87. Hohmann, A.G. *et al.* An endocannabinoid mechanism for stress-induced analgesia. *Nature* **435**, 1108–1112 (2005).
88. Agarwal, N. *et al.* Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat. Neurosci.* **10**, 870–879 (2007).
89. Guindon, J. & Hohmann, A.G. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *Br. J. Pharmacol.* **153**, 319–334 (2008).
90. Van Gaal, L., Pi-Sunyer, X., Despres, J.P., McCarthy, C. & Scheen, A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the rimonabant in obesity (RIO) program. *Diabetes Care* **31**, S229–S240 (2008).
91. Best, A.R. & Regehr, W.G. Serotonin evokes endocannabinoid release and retrogradely suppresses excitatory synapses. *J. Neurosci.* **28**, 6508–6515 (2008).
92. Saario, S.M., Savinainen, J.R., Laitinen, J.T., Jarvinen, T. & Niemi, R. Monoglyceride lipase–like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. *Biochem. Pharmacol.* **67**, 1381–1387 (2004).
93. Nomura, D.K. *et al.* Activation of the endocannabinoid system by organophosphorus nerve agents. *Nat. Chem. Biol.* **4**, 373–378 (2008).
94. Comelli, F., Giagnoni, G., Bettoni, I., Colleoni, M. & Costa, B. The inhibition of monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive effect in a murine model of acute inflammation. *Br. J. Pharmacol.* **152**, 787–794 (2007).
95. Gobbi, G. *et al.* Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc. Natl. Acad. Sci. USA* **102**, 18620–18625 (2005).
96. Kathuria, S. *et al.* Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* **9**, 76–81 (2003).
97. Jayamanne, A. *et al.* Actions of the FAAH inhibitor URB597 in neuropathic and inflammatory chronic pain models. *Br. J. Pharmacol.* **147**, 281–288 (2006).