THE MOLECULAR LOGIC OF ENDOCANNABINOID SIGNALLING

Daniele Piomelli

The endocannabinoids are a family of lipid messengers that engage the cell surface receptors that are targeted by Δ^9-tetrahydrocannabinol, the active principle in marijuana (*Cannabis*). They are made on demand through cleavage of membrane precursors and are involved in various short-range signalling processes. In the brain, they combine with CB_1 cannabinoid receptors on axon terminals to regulate ion channel activity and neurotransmitter release. Their ability to modulate synaptic efficacy has a wide range of functional consequences and provides unique therapeutic possibilities.

ARACHIDONIC ACID
Common name of 5,8,11,14-eicosatetraenoic acid, an essential (diet-derived) fatty acid that serves as precursor for eicosanoids and endocannabinoids.

Department of Pharmacology, University of California, Irvine 92697-4625, USA.
e-mail: piomelli@uci.edu
doi:10.1038/nrn1247

The *Cannabis* plant has been used in Europe since antiquity, mostly to make cordage and fabric, but first attracted the attention of European scientists when Napoleon's troops brought back from Egypt intriguing accounts of its psychotropic activity. In 1810, a member of Napoleon's *Commission des Sciences et des Arts* wrote[1]:

> "For the Egyptians, hemp is the plant par excellence, not for the uses they make of it in Europe and many other countries, but for its peculiar effects. The hemp cultivated in Egypt is indeed intoxicating and narcotic."

Before long, detailed descriptions of the plant's properties began to appear[2,3] and *Cannabis* extracts were introduced to the medical community. An 1848 commentary of the *British Pharmacopoeia* outlined quite accurately the psychotropic effects of *Cannabis* and pointed out its merit as an analgesic and antispasmodic[4]:

> "Numerous observers have described the Indian hemp as producing in the natives of the East, who familiarly use it instead of intoxicating spirits, sometimes a heavy, lazy state of agreeable reverie, from which the individual may be easily roused to discharge any simple duty – sometimes a cheerful, active state of inebriation causing him to dance, sing and laugh, provoking the venereal appetite, and increasing the desire for food – and sometimes a quarrelsome drunkenness, leading to acts of violence. During this condition pain is assuaged and spasm arrested. […] On the whole, it is a remedy which deserves a more extensive inquiry than any hitherto instituted."

The inquiry into the active chemical constituents of *Cannabis* turned out to be more time consuming than expected. Many other plant-derived compounds, such as morphine and atropine, had long been identified when the *Cannabis* plant finally yielded its active principle, the terpenoid derivative Δ^9-tetrahydrocannabinol (THC)[5,6] (FIG. 1).

The psychoactive properties of THC were recognized immediately, but the drug's unique chemical structure offered no hints as to its mechanism of action. To complicate matters further, the hydrophobic nature of THC delayed experimentation and indicated that the compound might act by influencing membrane fluidity, rather than by combining with a specific receptor. This impasse was resolved by the development of new classes of potent and selective THC analogue[7] (FIG. 1), which led eventually to the pharmacological identification of cannabinoid-sensitive sites in the brain[8].

The CB_1 cannabinoid receptor was molecularly cloned from rat brain in 1990 (REF. 9) and its immune-system counterpart, the CB_2 receptor, was identified by sequence homology three years later[10]. These discoveries not only established the mechanism of action of THC, thereby fuelling the development of subtype-selective agonists and antagonists (FIG. 1), but they also initiated a hunt for brain-derived cannabinoid ligands. Surprisingly, the first THC-like factor to be isolated was a lipid, rather than the peptide that had been expected on the basis of the precedent set by morphine and the enkephalins. It was identified as the amide of ARACHIDONIC ACID

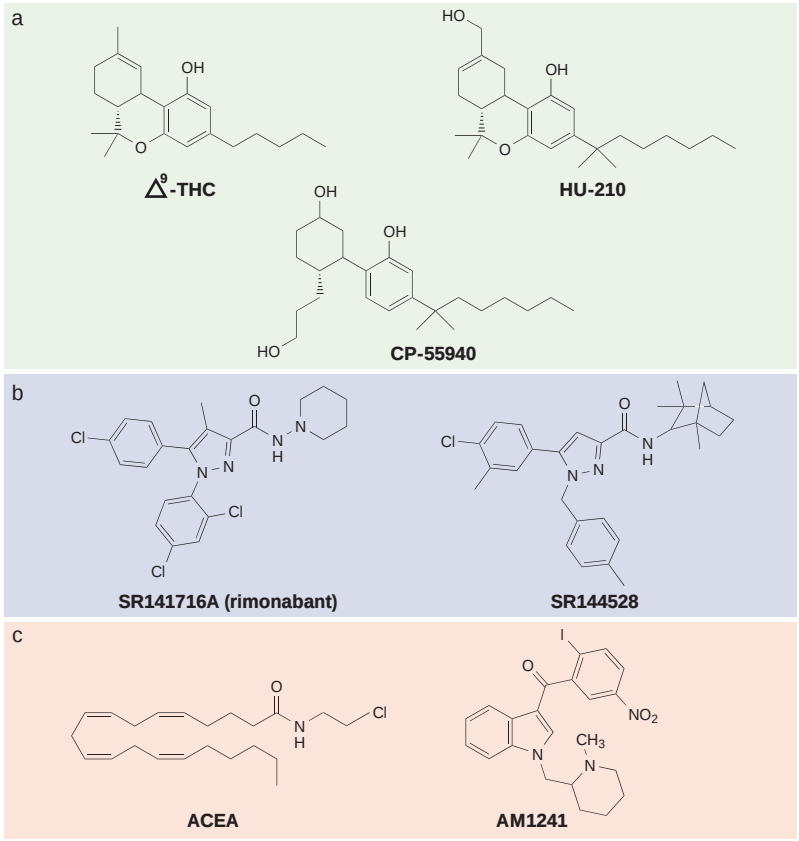

Figure 1 | **Chemical structures of plant-derived and synthetic compounds that bind to cannabinoid receptors. a** | Cannabinoid receptor agonists, which activate both CB_1 and CB_2 receptors. THC, tetrahydrocannabinol. **b** | Selective CB_1 antagonist (SR141716A, rimonabant) and CB_2 antagonist (SR144528). **c** | Selective CB_1 agonist (arachidonoyl-2′-chloroethanolamide, ACEA)[145] and CB_2 agonist (AM1241)[146].

with ethanolamine, and named anandamide after the Sanskrit word for bliss, *ananda*[11] (FIG. 2).

This small lipid molecule resembled no known neurotransmitter, but it did share structural features with the EICOSANOIDS, mediators of inflammation and pain with various functions in neural communication[12]. Though initially controversial[13], the signalling roles of anandamide were confirmed by the elucidation of the compound's unique metabolic pathways and the demonstration of its release in the live brain[14–16]. As the search for THC-like compounds continued, other bioactive lipids were extracted from animal tissues. These include 2-arachidonoylglycerol (2-AG)[17,18], noladin ether[19], virodhamine[20] and *N*-arachidonoyldopamine[21] (FIG. 2).

In this article, I review the synthesis, release and deactivation of the endogenous cannabinoids (also called endocannabinoids). I then outline the properties and distribution of brain CB_1 receptors. Last, I describe the function of the endocannabinoids as local modulators of synaptic activity and their contribution to memory, anxiety, movement and pain.

Synthesis

Anandamide. The membranes of plant cells contain a family of unusual lipids that consist of a long-chain FATTY ACID tethered to the head group of PHOSPHATIDYLETHANOLAMINE (PE) through an amide bond. When attacked by a PHOSPHOLIPASE D (PLD) enzyme, these membrane constituents generate a set of FATTY ACID ETHANOLAMIDES, which are used by plants as intercellular signalling molecules. They are released from cells in response to stress or infection, and stimulate the expression of genes engaged in systemic plant immunity[22]. This ancestral biochemical device is conserved in mammalian cells, which use the ethanolamide of arachidonic acid, anandamide, as a primary component of the endocannabinoid signalling system.

Anandamide formation in neurons is a two-step process, which parallels fatty acid ethanolamide production in plants[14,23,24] (FIG. 3). The first step is the stimulus-dependent cleavage of the phospholipid precursor *N*-arachidonoyl-PE. This reaction is mediated by an uncharacterized PLD and produces anandamide and phosphatidic acid, a metabolic intermediate that is used by cells in the synthesis of other glycerol-derived phospholipids. Genes encoding two PLD isoforms have been cloned in mammals[25], but it is not known whether either of these enzymes is responsible for anandamide synthesis.

The brain contains tiny quantities of *N*-arachidonoyl-PE (20–40 pmol g^{-1})[23,24] — probably too little to sustain anandamide release for an extended time. The cellular stores of this precursor are replenished by the enzyme *N*-acyltransferase (NAT), which catalyses the intermolecular passage of an arachidonic acid group from the *SN*-1 position of PHOSPHATIDYLCHOLINE to the head group of PE[14,23,24] (FIG. 3). In cultures of rat cortical neurons, two intracellular second messengers control NAT activity: Ca^{2+} and cyclic AMP. Ca^{2+} is required to engage NAT, which is inactive in its absence, whereas cAMP works through protein kinase A-dependent phosphorylation to enhance NAT activity[26]. Although catalysed by separate enzymes, the syntheses of anandamide and its parent lipid are thought to proceed in parallel because Ca^{2+}-stimulated anandamide production is generally accompanied by *de novo* formation of *N*-arachidonoyl-PE[23,24].

As expected of a Ca^{2+}-activated process, anandamide formation can be elicited by Ca^{2+} ionophores, which carry Ca^{2+} ions across cell membranes. For example, in cultures of rat striatal neurons labelled by incubation with [^{3}H]ethanolamine, the Ca^{2+} ionophore ionomycin stimulates accumulation of [^{3}H]anandamide[14]. A similar stimulation is produced by kainate (a glutamate receptor agonist), 4-aminopyridine (a K^+ channel blocker) or membrane-depolarizing concentrations of K^+, and can be prevented by chelating extracellular Ca^{2+} (REFS 14,26). The Ca^{2+} dependence of anandamide synthesis was also demonstrated using MICRODIALYSIS. Administration of a high-K^+ pulse in the rat striatum caused a reversible increase in interstitial anandamide concentrations, which was prevented by removal of Ca^{2+} from the perfusing solution[15].

Although neural activity induces anandamide release in a Ca^{2+}-dependent manner, Ca^{2+} entry into neurons is not the only determinant of anandamide generation: there is evidence that G-protein-coupled receptors can

EICOSANOIDS
A family of biologically active compounds produced through the enzymatic oxygenation of arachidonic acid. Examples are prostaglandins, leukotrienes and lipoxins.

FATTY ACID
An organic acid characterized by a non-branched carbon chain and an even number of carbon atoms. Examples of saturated fatty acids (without double bonds) are palmitic (16 carbons) and stearic (18 carbons). Examples of unsaturated and polyunsaturated fatty acids include oleic (18 carbons, one double bond) and arachidonic (20 carbons, 4 double bonds).

PHOSPHATIDYLETHANOLAMINE
An important class of membrane phospholipids comprising a glycerol skeleton linked to two fatty acid residues, phosphoric acid and ethanolamine.

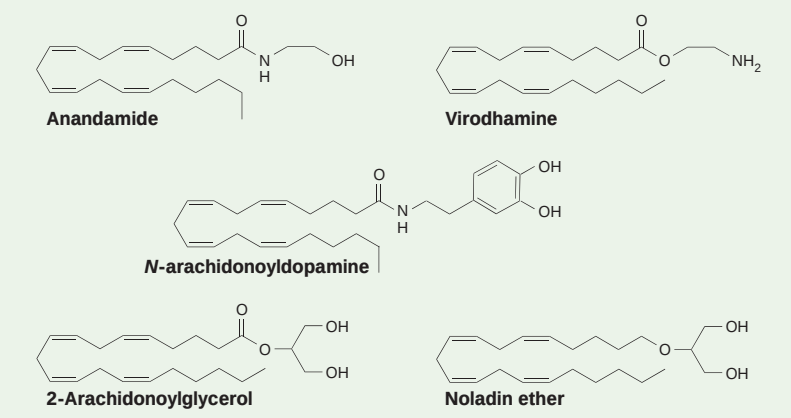

Figure 2 | **Chemical structures of endogenous compounds that bind to cannabinoid receptors.**

also trigger this process. For example, application of the dopamine D_2-receptor agonist quinpirole causes an eightfold increase in anandamide outflow in the rat striatum, which is prevented by the D_2-receptor antagonist raclopride[15]. This response is accompanied by an elevation in tissue anandamide content, indicating that it might be due to a net increase in anandamide formation rather than to extracellular release of preformed anandamide[27]. Muscarinic acetylcholine receptors and metabotropic glutamate receptors can also cause endocannabinoid release in hippocampal slices in a Ca^{2+}-independent manner, but the substance(s) involved have not been identified[28,29].

How does occupation of D_2 receptors initiate anandamide synthesis? Inhibition of cAMP formation, a hallmark of D_2-receptor signalling, is unlikely to be responsible for this effect because cAMP positively regulates NAT activity[26]. Alternatively, D_2 receptors could interact with the Rho family of small G proteins to stimulate PLD activity[30], or they might engage β–γ subunits of G proteins to activate phospholipase C_β (PLC_β)[31]. PLC_β catalyses the cleavage of phosphatidylinositol-4,5-bisphosphate to produce inositol-1,4,5-trisphosphate, which might then recruit the NAT/PLD pathway by mobilizing Ca^{2+} from internal stores.

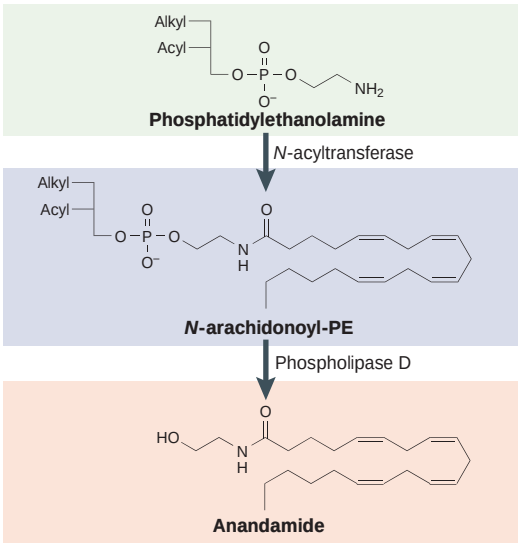

Figure 3 | **Mechanism of anandamide formation in neurons.** The sequence of reactions is thought to include: first, the synthesis of the anandamide precursor *N*-arachidonoyl-phosphatidylethanolamine (PE), catalysed by the enzyme *N*-acyltransferase; second, the cleavage of *N*-arachidonoyl-PE to yield anandamide, catalysed by phospholipase D.

2-Arachidonoylglycerol. Like other MONOACYLGLYCEROLS, 2-AG is at the crossroads of multiple routes of lipid metabolism, where it can serve interchangeably as an end-product for one pathway and precursor for another. These diverse metabolic roles can explain its high concentration in brain tissue (about 200-fold greater than anandamide's)[17,32], and imply that a significant fraction of brain 2-AG is engaged in housekeeping functions rather than in signalling.

The place occupied by 2-AG at central intersections of lipid metabolism also complicates efforts to define the biochemical pathway(s) responsible for its physiological synthesis. There is, however, enough information to indicate two possible routes (FIG. 4). The first begins with the phospholipase-mediated formation of 1,2-diacylglycerol (DAG). This product regulates protein kinase C activity — an important second messenger function — and is a substrate for two enzymes: DAG kinase[33], which attenuates DAG signalling by catalysing its phosphorylation to phosphatidic acid; and DAG lipase (DGL), which hydrolyses DAG to monoacylglycerol[34]. The fact that drug inhibitors of PLC and DGL block Ca^{2+}-dependent 2-AG accumulation in rat cortical neurons indicates primary involvement of this pathway in 2-AG formation[32].

An alternative pathway of 2-AG synthesis begins with the production, mediated by phospholipase A1 (PLA1)[35,36], of a 2-arachidonoyl-LYSOPHOSPHOLIPID, which might be hydrolysed to 2-AG by lyso-PLC activity (FIG. 4). Although there is no direct evidence for this mechanism in 2-AG formation, the high level of PLA1 expression in brain tissue[34,35] makes it an intriguing target for future investigation. In addition to the phospholipase-operated pathways outlined above, monoacylglycerols can be produced by hormone-sensitive lipase acting on triacylglycerols or by lipid phosphatases acting on lysophosphatidic acid. In general, however, these enzymes preferentially target lipids that are enriched in saturated or monounsaturated fatty acids, rather than the polyunsaturated species that would give rise to 2-AG.

Irrespective of its exact mechanism, neuronal 2-AG production can be initiated by an increase in the concentration of intracellular Ca^{2+}. In cultures of rat cortical neurons, the Ca^{2+} ionophore ionomycin and the glutamate receptor agonist NMDA (*N*-methyl-D-aspartate) stimulate 2-AG synthesis in a Ca^{2+}-dependent manner[32,37]. Likewise, in freshly dissected hippocampal slices, high-frequency stimulation of the SCHAFFER COLLATERALS produces a Ca^{2+}-dependent increase in tissue 2-AG content[32]. Importantly, this treatment has no effect on the concentrations of non-cannabinoid monoacylglycerols, such as 1(3)-palmitoylglycerol, which indicates

PHOSPHOLIPASE
(PL). A group of enzymes that catalyse the hydrolysis of phospholipids at their glycerol ester (PLA) or phosphodiester (PLC, PLD) bonds.

FATTY ACID ETHANOLAMIDE
A lipid-derived signalling molecule characterized by an ethanolamine residue linked to a long-chain fatty acid through an amide bond. Examples are anandamide (arachidonoylethanolamide), oleoylethanolamide and palmitoylethanolamide.

SN: STEREOSPECIFIC NUMBERING
Defines a convention on how to designate the stereochemistry of glycerol-based lipids. When the glycerol moiety is drawn with the secondary hydroxyl to the left, the carbons are numbered 1,2,3 from top to bottom.

PHOSPHATIDYLCHOLINE
A major class of membrane phospholipids comprised of a glycerol skeleton linked to two fatty acid residues, phosphoric acid and choline. In the mammalian brain, the *sn*-2 position of phosphatidylcholine most often contains an arachidonic acid residue, but a small pool of this fatty acid is also stored in the *sn*-1 position.

MICRODIALYSIS
A technique that allows the sampling of neurochemicals in the brain of live animals.

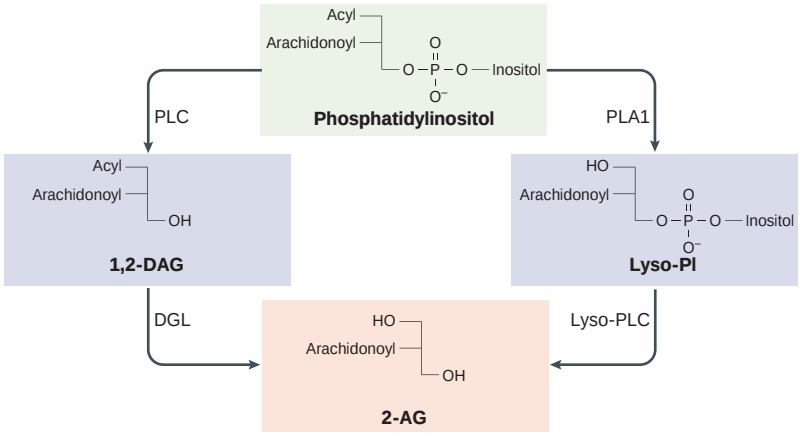

Figure 4 | **Pathways of 2-arachidonoylglycerol (2-AG) formation in neurons.** One possible sequence of reactions, shown on the left, includes the cleavage of phosphatidylinositol (PI) to yield 1,2-diacylglycerol (DAG), catalysed by a phospholipase such as phospholipase C (PLC), and the subsequent conversion of DAG to 2-AG, catalysed by diacylglycerol lipase (DGL). An alternative route, shown on the right, comprises the formation of a 2-arachidonoyl-lysophospholipid such as lyso-PI, catalysed by phospholipase A1 (PLA1), followed by the hydrolysis of the lysophospholipid to 2-AG, catalysed by lyso-PLC.

that 2-AG formation is not due to a generalized increase in the rate of lipid turnover[32]. Furthermore, high-frequency stimulation does not alter hippocampal anandamide concentrations, indicating that the syntheses of 2-AG and anandamide can be independently regulated[32,37]. In further support of this idea, activation of D_2 receptors — a potent stimulus for anandamide formation in the rat striatum — has no effect on striatal 2-AG concentrations[15,27].

Other putative endogenous ligands. Noladin ether is an ether-linked analogue of 2-AG that binds to and activates CB_1 receptors[19] (FIG. 2). Its pathway of formation has not been characterized, and its occurrence in the normal brain has been questioned[38]. Virodhamine, the ester of arachidonic acid and ethanolamine (FIG. 2), might act as an endogenous CB_1 antagonist[20]. Its presence in brain tissue has been documented[20], but is intriguing because this chemically unstable molecule is rapidly converted to anandamide in aqueous environments. The mechanism of its synthesis is unknown, and its deactivation might share anandamide's pathways of uptake and intracellular hydrolysis[20]. Finally, the endogenous vanilloid agonist, *N*-arachidonoyl-dopamine, also exhibits affinity for cannabinoid receptors *in vitro*[21] (FIG. 2).

Release from neurons

How are endocannabinoids released from cells and how do they reach their targets? Classical transmitters and neuropeptides can diffuse through the water-filled space that surrounds neurons, but hydrophobic compounds such as anandamide and 2-AG tend to remain associated with lipid membranes. One possibility is that endocannabinoids might not leave the cell where they are produced; rather, they could move sideways within the plasmalemma until they collide with membrane-embedded CB_1 receptors. This hypothesis is supported by the role of an intramembranous amino-acid residue (lysine-192) in the binding of anandamide to CB_1 (REF. 39), as well as by the finding that certain cannabinoid agonists can approach the receptor by lateral membrane diffusion[40]. Nevertheless, it does not account for two pieces of evidence. First, anandamide is found in incubation media of cells and in brain interstitial fluid, implying that it can overcome its tendency to partition in membranes[14–16]. Perhaps more importantly, physiological experiments have shown that an endocannabinoid substance does leave postsynaptic cells to activate CB_1 receptors on adjacent axon terminals[41–46]. This unidentified compound might travel as far as 20 μm from its cell of origin before being eliminated[41].

If endocannabinoids are released from neurons, what is the mechanism of their release? The fact that plasma membranes contain precursor molecules for both anandamide and 2-AG indicates that they could leave the cell as soon as they are formed. Extracellular lipid-binding proteins such as the lipocalins, which are expressed at high levels in the brain[47], might facilitate this step and help to deliver endocannabinoids to their cellular targets. Although this scenario awaits confirmation, it does mirror what happens in the bloodstream, where anandamide's movements are made possible by its reversible binding to serum albumin[48].

Deactivation

Two mechanisms cooperate in attenuating endocannabinoid signalling in the brain: carrier-mediated transport into cells and intracellular hydrolysis (FIG. 5).

Transport. Anandamide and 2-AG can diffuse passively through lipid membranes, but this process is accelerated by a rapid and selective carrier system that is present in both neurons and glial cells[49,50]. Although it is superficially similar to other transmitter systems, endocannabinoid transport is not driven by transmembrane Na^+ gradients, indicating that it might be mediated by a FACILITATED DIFFUSION mechanism[49,50]. In this respect, neural cells seem to internalize anandamide and 2-AG in a manner similar to fatty acids, eicosanoids and other biologically relevant lipids, by using energy-independent carriers. Several lipid-carrier proteins have been molecularly cloned[51], inspiring optimism that, despite current controversy (BOX 1), endocannabinoid transporter(s) will eventually be characterized.

Meanwhile, to gain insight into the role of transport in endocannabinoid inactivation, we can rely on an expanding series of pharmacological transport inhibitors. The prototype is AM404, which slows the elimination of both anandamide and 2-AG, magnifying their biological effects[49,52,53] (FIG. 6). This inhibitor has helped to unmask important roles of the endocannabinoid system in the regulation of neurotransmission and synaptic plasticity, but suffers from various limitations, including an affinity for VANILLOID RECEPTORS and susceptibility to enzymatic attack by fatty acid amide hydrolase (FAAH). These limitations have prompted an ongoing search for more selective and stable analogues[54,55] (FIG. 6).

MONOACYLGLYCEROL
A glycerol derivative in which one of the hydroxyl groups is linked to a fatty acid residue by an ester bond.

LYSOPHOSPHOLIPID
A phospholipid containing only one fatty acid chain. Examples include lysophosphatidic acid and lysophosphatidyl-ethanolamine.

SCHAFFER COLLATERALS
Axons of the CA3 pyramidal cells of the hippocampus that form synapses with the apical dendrites of CA1 neurons.

FACILITATED DIFFUSION
A common mechanism of transmembrane transfer that involves a protein carrier, but does not require expenditure of cellular energy.

VANILLOID RECEPTORS
Membrane receptor-channels permeable to monovalent cations. They are activated by noxious heat and capsaicin, the active constituent of hot chili peppers.

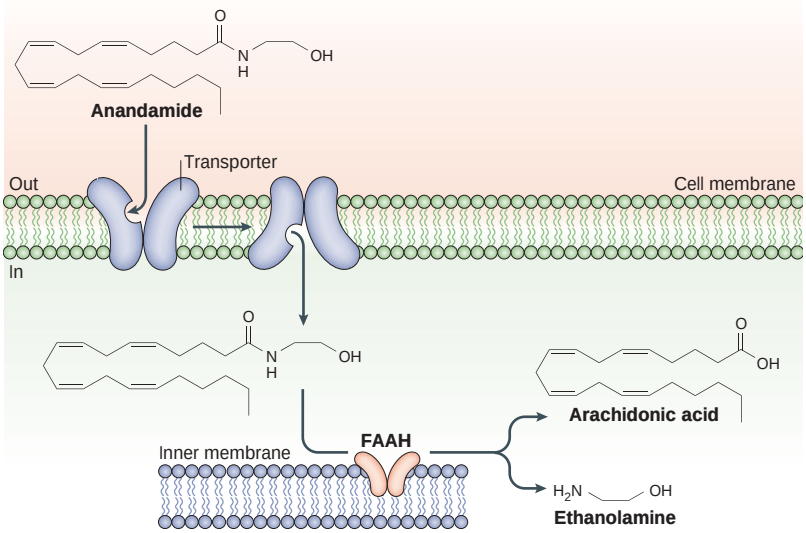

Figure 5 | **Mechanisms of endocannabinoid deactivation in neurons.** Anandamide and 2-arachidonoylglycerol (2-AG) can be internalized by neurons through a high-affinity transport mechanism, the 'endocannabinoid transporter'. Once inside cells, they can be hydrolysed by distinct serine hydrolases – anandamide by fatty acid amide hydrolase (FAAH) and 2-AG by monoglyceride lipase (MGL) (not shown) – to yield inactive breakdown products.

Fatty acid amide hydrolase. FAAH is an intracellular membrane-bound serine hydrolase that breaks down anandamide into arachidonic acid and ethanolamine[56–59] (FIG. 5). It has been molecularly cloned and its catalytic mechanism, which allows it to recognize a broad spectrum of amide and ester substrates, has been elucidated in detail[60]. Particularly notable is FAAH's ability to hydrolyse bioactive fatty amides, which do not bind to any of the known cannabinoid receptors: these include the satiety factor oleoylethanolamide[61] and the anti inflammatory/analgesic mediator palmitoylethanolamide[62,63]. FAAH tightly controls brain concentrations of these compounds[64,65], but the functional significance of this regulation is unknown.

FAAH is widely distributed in the rat brain, where it is expressed at high concentrations in cell bodies and dendrites of principal neurons[66,67]. In the hippocampus, neocortex and cerebellum, FAAH-positive cell bodies are juxtaposed to axon terminals that contain CB_1 receptors, indicating not only that FAAH participates in the inactivation of neurally generated anandamide, but also that this process occurs postsynaptically. This idea can now be tested in FAAH-deficient mice[64] or using selective FAAH inhibitors with long-lasting systemic actions[65] (FIG. 6).

Monoacylglycerol lipase. The pig brain contains two chromatographically distinct 2-AG-hydrolysing activities[68], one of which is probably due to the enzyme monoacylglycerol lipase (MGL). The rat brain isoform of this cytosolic serine hydrolase has been characterized both molecularly and morphologically[69]. It has a broad distribution in the central nervous system (CNS), which partially overlaps with that of FAAH; however, whereas FAAH is predominantly found in postsynaptic structures, MGL might be mostly associated with nerve endings[69]. In the hippocampal CA1 field, MGL-positive axon terminals surround cell bodies of pyramidal neurons containing FAAH. This localization could reflect a functional role of presynaptic MGL in terminating RETROGRADE SIGNALLING events mediated by 2-AG (discussed later in this article).

Box 1 | To transport or not to transport?

Anandamide uptake in neural cells exhibits three identifying features of carrier-mediated transport. First, saturation kinetics: plots of the initial rate of [^{3}H]anandamide accumulation against extracellular anandamide concentrations yield apparent Michaelis constants (K_M) of 0.32 μM in astrocytes and 1.2 μM in cortical neurons[49]. These values are very similar to those obtained with the transporters for serotonin (K_M = 0.3–0.5 μM), dopamine (K_M = 0.9–1.2 μM) and noradrenaline (K_M = 0.45 μM)[148]. Higher K_M values for anandamide transport were obtained, however, in cerebellar granule cells[50]. Second, substrate specificity: rat brain neurons and other cells in culture internalize [^{3}H]anandamide, but not closely related analogues[14,49,50,52]. Third, selective inhibition: [^{3}H]anandamide transport is blocked competitively by AM404 and other anandamide derivatives[49,54,55]. Importantly, some chiral analogues of anandamide inhibit transport in a stereospecific manner, which is indicative of a macromolecular recognition site[52].

Nevertheless, the finding that [^{3}H]anandamide uptake does not require cellular energy[49,50] has led to proposals that this process might be explained by passive membrane diffusion driven by intracellular fatty acid amide hydrolase (FAAH) activity[149]. This hypothesis is readily testable, as it assumes that anandamide uptake inhibitors act by blocking FAAH, and that disruption of FAAH by either genetic or pharmacological means should eliminate anandamide transport. Although it is clearly possible to inhibit anandamide transport independently of FAAH[54,55], a stringent test of this hypothesis – for example, an examination of anandamide uptake in FAAH-knockout mice – has not yet been reported. Irrespective of the outcome of such a test, definitive proof of the transporter's existence will come only from its molecular characterization.

CB_1 signalling

CB_1 is considered to be the most abundant G-protein-coupled receptor in the mammalian brain, and its presence in the neocortex, hippocampus, basal ganglia, cerebellum and brainstem accounts for most of the behavioural actions of cannabinoid drugs[70]. The four symptoms that are often used to define cannabinoid intoxication in the rodent – hypothermia, rigid immobility, analgesia and decreased motor activity[71] – are strikingly absent in mice in which the *cb1* gene has been deleted by targeted recombination[72,73].

Aside from its unusually high concentrations in the brain, CB_1 is a standard $G_{i/o}$-coupled receptor and can initiate signalling events typical of this class of transducing proteins. These include closure of Ca^{2+} channels, opening of K^+ channels, inhibition of adenylyl cyclase activity (with its consequent decrease in cytosolic cAMP concentrations) and stimulation of kinases that phosphorylate tyrosine, serine and threonine residues in proteins. Each of these mechanisms seems to have distinct functions in translating CB_1-receptor occupation into biological responses.

Cannabinoid agonists inhibit N- and P/Q-type voltage-activated Ca^{2+} channels[74–76]. This effect, which has been suggested to result from a direct interaction of $G_{i/o}$-protein β–γ subunits with the channels[77], might underlie CB_1-mediated depression of transmitter release at GABA (γ-aminobutyric acid) synapses in the CA1 field of the hippocampus[78] and at glutamatergic synapses in the dorsal striatum[79,80] (FIG. 7a). Importantly,

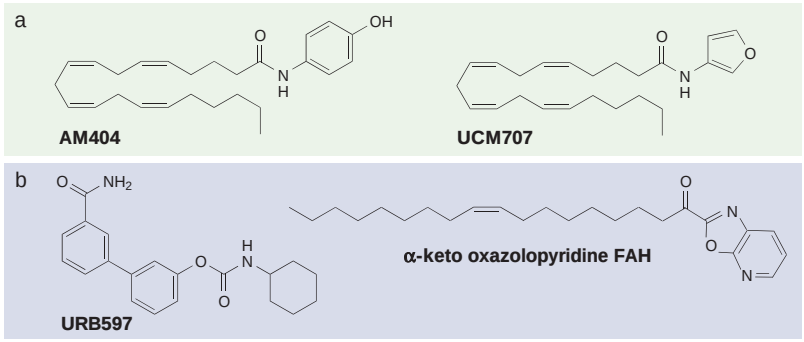

Figure 6 | **Chemical structures of endocannabinoid deactivation inhibitors.** **a** | Endocannabinoid transport inhibitors: AM404 (REF. 40) and UCM707 (REF. 54). **b** | Fatty acid amide hydrolase (FAAH) inhibitors: substituted carbamates (URB597)[65] and substituted α-keto oxazolopyridines[147].

however, endocannabinoid-mediated suppression of GABA release in hippocampal slices seems primarily to involve N-type Ca^{2+} channels[81].

Cannabinoid regulation of voltage-gated K^+ currents[82] is also implicated in presynaptic inhibition at GABA[83] and glutamate synapses. The latter include PARALLEL FIBRE–Purkinje cell synapses in the cerebellum, as well as synapses in the nucleus accumbens and lateral amygdala[84–86] (FIG. 7b). The sensitivity of these responses to PERTUSSIS TOXIN implies that they are mediated by $G_{i/o}$ proteins, but it is still unclear whether transduction is direct (β–γ subunit-mediated) or indirect (second messenger-mediated). Inhibition of cAMP formation does not seem to be involved[85,86].

On the other hand, cAMP can contribute to the regulation of neuronal gene expression by CB_1. This process, which is necessary to produce lasting changes in synaptic strength, depends on the recruitment of complex networks of intracellular protein kinases[87]. Two components of these networks, extracellular signal-regulated kinase (ERK) and focal adhesion kinase (FAK), become activated when hippocampal slices are treated with cannabinoid agonists[88,89]. This activation is mimicked by inhibitors of cAMP-dependent kinase and is lost when the slices are exposed to cell-permeant cAMP analogues, implying that it might result from a decrease in intracellular cAMP concentrations. The involvement of ERK and FAK in synaptic plasticity indicates that these protein kinases could participate in the changes in gene expression and the persistent neural adaptations that accompany cannabinoid administration[90].

CB_1 distribution

In the rodent and human cortices, CB_1 receptors are primarily found on axon terminals of cholecystokinin-8 (CCK-8)-positive GABA interneurons[91–95]. This expression pattern dominates the neocortex, hippocampal formation and amygdala, where nerve terminals that form excitatory synapses are ostensibly devoid of CB_1 immunoreactivity[91,94]. However, there is evidence that excitatory terminals in these regions do contain the receptor; for example, cannabinoid agonists reduce glutamatergic transmission in the amygdala of normal mice, but fail to do so in CB_1-deficient mutants[86]. In addition, low concentrations of CB_1 messenger RNA have been found in many neurons of the cortex that do not contain GABA[92].

CB_1 receptors are also expressed at very high levels throughout the basal ganglia. In the striatum they are localized to three distinct neuronal elements: glutamatergic terminals originating in the cortex[79,80], local-circuit GABA interneurons ('fast-spiking' interneurons that do not express CCK-8)[96] and axon terminals of GABA projection neurons ('medium spiny neurons')[97]. Medium spiny neurons project to striatal outflow nuclei, where CB_1 receptors are especially abundant; for example, in the globus pallidus they outnumber dopamine D_1 receptors by a factor of 45 (REF. 97).

In the cerebellum, CB_1 is present on excitatory terminals of climbing and parallel fibres (but not on their postsynaptic partners, the Purkinje neurons) as well as on GABA interneurons[70,93]. Smaller numbers of CB_1 receptors are also found in the thalamus (especially in the anterior dorsal nucleus and habenula), hypothalamus (ventromedial and anterior nuclei), midbrain (periaqueductal grey and superior colliculus), medulla (dorsal vagal complex and rostral ventromedial medulla) and spinal cord (dorsal horn)[70,93]. Last, CB_1 is expressed in peripheral sensory neurons[98], where it is localized in cells that express N52, a protein marker of mechanosensitive Aβ fibres[99].

Another brain receptor?

A few cannabinoid effects persist in CB_1-null mice, implying that this receptor might not act alone in mediating brain cannabinoid signalling[73,100]. Although cannabinoid agonists lose their ability to inhibit GABA and glutamate transmission in some brain regions of adult CB_1-knockout mice[86], they can still reduce excitatory transmission in the hippocampal CA1 field of these animals[101,102]. This discrepancy is reinforced by the finding that GABA and glutamate synapses in CA1 respond in different ways to cannabinoid drugs. For example, cannabinoid depression of excitatory currents is blocked by CAPSAZEPINE, whereas depression of inhibitory currents is not[103]. These results make a persuasive case for the existence of a hippocampal cannabinoid-sensitive site that is distinct from CB_1 (sometimes called 'CB_3'), but other evidence appears to contradict them; for example, in newborn CB_1-null mice, cannabinoid agonists affect neither GABA nor glutamate transmission[104]. Although this difference could be due to the developmental stage of the preparation used – adult[101] versus one-week-old mice[104] – more studies are needed to establish whether the CB_3 site is molecularly distinct from CB_1. A novel cannabinoid site has also been identified in the vascular endothelium[105], but seems to be different from CB_3 because it is not antagonized by capsazepine[105] or activated by the CB_1/CB_2 agonist Win-55212-2 (REF. 106).

A local message

Outside the brain, the endocannabinoids are produced on demand and act on cells located near their site of synthesis. For example, they are formed by circulating

RETROGRADE SIGNALLING
The backward movement of signalling molecules from postsynaptic to presynaptic structures, which underlies a variety of short- and long-term changes in synaptic efficacy.

PARALLEL FIBRES
Axons of cerebellar granule cells. Parallel fibres emerge from the molecular layer of the cerebellar cortex towards the periphery, where they extend branches perpendicular to the main axis of the Purkinje neurons and form *en passant* synapses with this cell type.

PERTUSSIS TOXIN
The causative agent of whooping cough, pertussis toxin causes the persistent activation of G_i proteins by catalysing the ADP-ribosylation of the α-subunit.

CAPSAZEPINE
A synthetic drug that acts as a competitive antagonist of capsaicin at vanilloid receptors.

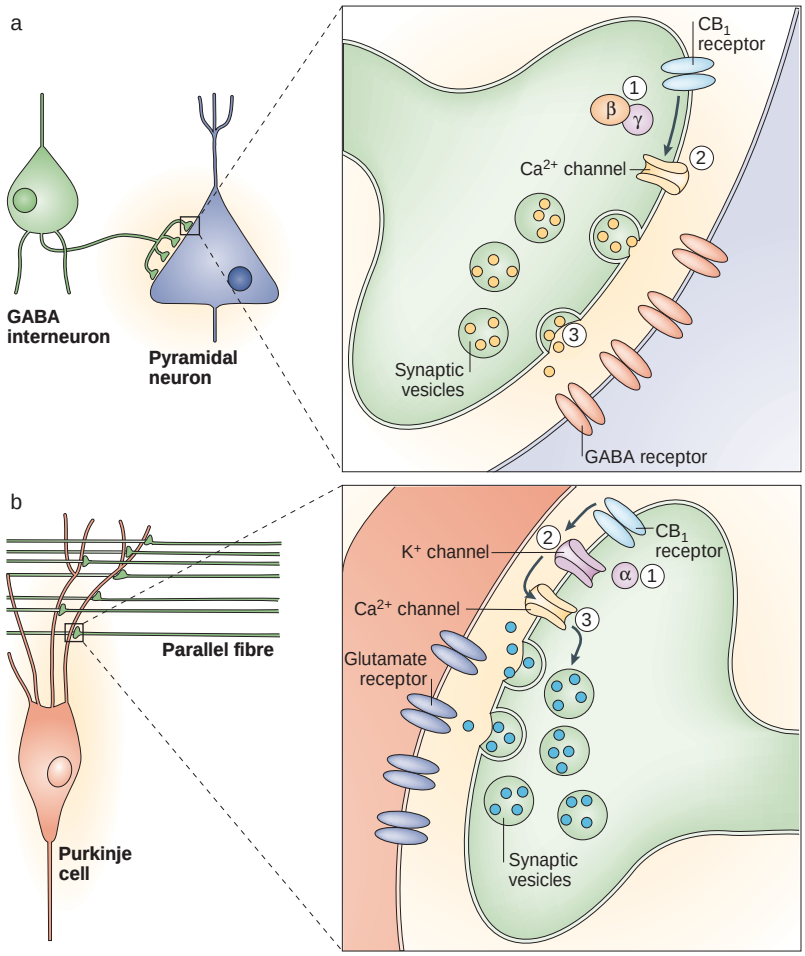

Figure 7 | **Regulation of presynaptic ion channel activities by CB_1 cannabinoid receptors.** **a** | At synapses between GABA (γ-aminobutyric acid) interneurons and pyramidal cells in the CA1 field of the hippocampus, activation of CB_1 receptors can initiate a series of intracellular events, which include (1) activation of G-protein β–γ subunits, (2) closure of voltage-gated Ca^{2+} channels and (3) inhibition of GABA release. **b** | At parallel fibre–Purkinje cell synapses in the cerebellum, CB_1 activation can (1) engage G-protein α-subunits that (2) cause the opening of K^+ channels; the resulting membrane hyperpolarization can (3) reduce Ca^{2+} entry and inhibit glutamate release. Mechanisms similar to those illustrated above are thought to underlie cannabinoid-mediated inhibition of neurotransmitter release in other brain regions.

leukocytes and platelets, and induce vascular relaxation by interacting with cannabinoid receptors on the surface of neighbouring endothelial and smooth muscle cells[107]. Similar PARACRINE actions are thought to occur in the CNS, where the endocannabinoids might mediate a localized signalling mechanism through which principal neurons modify the strength of incoming synaptic inputs.

Regulation of GABA transmission

Hippocampus: modulation of memory. When a pyramidal neuron in the CA1 field of the hippocampus is depolarized, the inhibitory GABA inputs received by that cell are transiently suppressed. This phenomenon, called depolarization-induced suppression of inhibition (DSI), is initiated postsynaptically by voltage-dependent influx of Ca^{2+} into the soma and dendrites of the neuron, but is expressed presynaptically through inhibition of transmitter release from axon terminals of GABA interneurons[108]. This indicates that a chemical messenger generated during depolarization of the pyramidal cell must travel backwards across the synapse to induce DSI (FIG. 8).

There is evidence that this retrograde signalling process involves an endocannabinoid substance, possibly 2-AG. First, CB_1 agonists mimic DSI, whereas CB_1 antagonists block it[41–43]. Second, DSI is absent in CB_1-deficient mice[81,109]. Third, the GABA interneurons that are implicated in DSI express high levels of CB_1 receptors, which are localized to their axon terminals[91]. Fourth, neural activity and Ca^{2+} entry stimulate the hippocampal synthesis of 2-AG, but have no effect on anandamide concentrations[32]. Nevertheless, we still don't know whether the endocannabinoid actually crosses back to the presynaptic nerve ending or is produced there by the action of another, unidentified retrograde signal (FIG. 8).

The fact that DSI is induced *in vitro*[43] by levels of neural activity that could also be encountered *in vivo* indicates that this process might have a role in normal brain function. Although this idea is still questioned[110], various results link DSI to the regulation of hippocampal GAMMA OSCILLATIONS[77]. These network oscillations are coordinated by CB_1-positive GABA interneurons and are influenced by cannabinoid agonists, raising the possibility that an endocannabinoid substance might modulate their expression[111] and be involved in the organization of hippocampal cell assemblies[112]. Another function of DSI might relate to synaptic plasticity. By weakening GABA-mediated inhibition, DSI could facilitate the induction of long-term potentiation in individual CA1 pyramidal neurons; this might contribute in turn to the formation of 'place fields' or to other forms of hippocampus-dependent learning[113]. Such a cognitive-enhancing action would not contradict the well-known amnesic effects of cannabinoid drugs[114] as the latter might result from a generalized, circuit-independent activation of CB_1 receptors in the hippocampus and other brain areas.

Outside the hippocampus, endocannabinoid-mediated DSI has been shown to occur at interneuron–principal cell synapses of the cerebellum[115–117] and probably will soon be discovered elsewhere.

Amygdala: modulation of emotions. CB_1-bearing interneurons are selectively localized to a subdivision of the amygdala called the basolateral complex[94,95], a key station in the neural circuitry that processes emotions and a primary site of cannabinoid analgesia[118]. This localization, and the fact that CB_1 inactivation causes anxiety-like and aggressive responses in rodents[119,120], indicate that the endocannabinoid system might influence affective states through changes in the amygdala's efferent activity. This idea is further supported by two findings: first, presentation of anxiogenic stimuli increases anandamide and 2-AG concentrations in the mouse amygdala[121]; second, FAAH inhibitors exhibit marked anxiolytic-like properties in rats[65].

Locally formed endocannabinoids could modify the amygdala's output in two complementary ways.

PARACRINE
A bioactive substance formed in the body by the action of primary messengers (hormones, neurotransmitters) on their receptors, which produces its effects by acting on cells near its sites of synthesis.

GAMMA OSCILLATIONS
Fast (20–80 Hz) synchronous oscillations of brain activity, which are thought to contribute to cognition and movement.

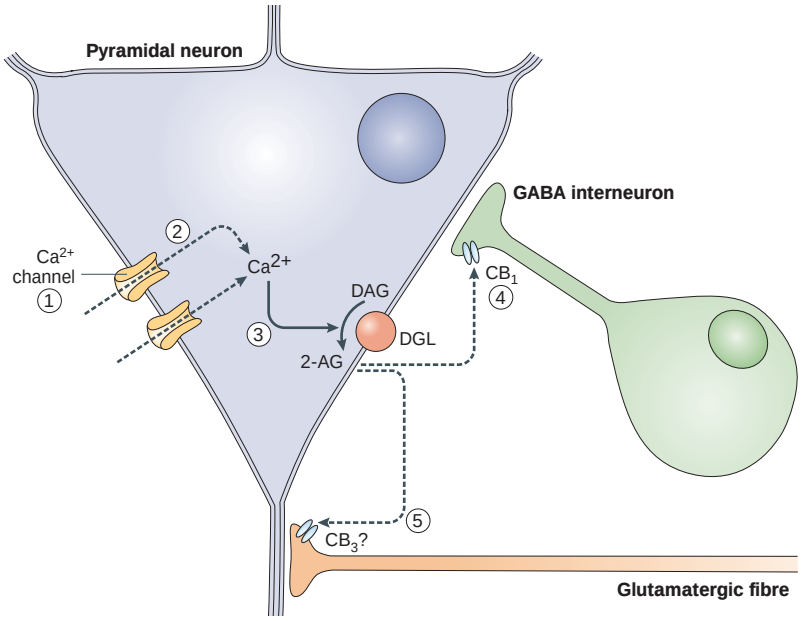

Figure 8 | **Endocannabinoid-mediated synaptic signalling.** In the CA1 field of the hippocampus, membrane depolarization (1) opens voltage-activated Ca^{2+} channels in pyramidal neurons, producing (2) an elevation of intracellular Ca^{2+} concentrations. Ca^{2+} can (3) stimulate the synthesis of 2-arachidonoylglycerol (2-AG) through the diacylglycerol lipase (DGL) pathway or the synthesis of anandamide through the phospholipase D pathway (not shown). The newly formed endocannabinoids might travel across the synapse to interact with (4) CB_1 receptors on axon terminals of GABA (γ-aminobutyric acid) interneurons, leading to depolarization-induced suppression of inhibition, or (5) 'CB_3' sites on glutamatergic terminals, leading to depolarization-induced suppression of excitation. Alternatively, the endocannabinoid might not actually cross back to the presynaptic nerve ending, but could be produced *in situ* by the action of another, unidentified retrograde signal.

They could depress glutamate release from axon terminals originating in the cortex and other brain regions[86]. In addition, by reducing GABA release from basolateral interneurons, they might disinhibit GABA cells in the adjacent intercalated nuclei and consequently decrease the activity of their postsynaptic targets, the pyramidal neurons in the central nucleus of the amygdala, which constitute the structure's primary efferent pathway[94].

Basal ganglia: modulation of motor activity. The terminal fields of striatal projection neurons contain the highest densities of CB_1 receptors in the brain. Here, local administration of cannabinoid agonists inhibits GABA release and profoundly affects motor behaviours[122]. Membrane depolarization and dopamine D_2-receptor activation stimulate striatal anandamide formation[15], indicating that this endocannabinoid might contribute to the regulation of basal ganglia function. In agreement with this hypothesis, the CB_1 antagonist rimonabant enhances the stimulation of movement that is induced in rats by dopamine agonists[123], whereas the endocannabinoid transport inhibitor AM404 attenuates this stimulation in a CB_1-dependent manner[53].

Anandamide might act at multiple sites in the basal ganglia, including GABA projection neurons, corticostriatal glutamatergic terminals and local-circuit interneurons[79,96,97]. Local-circuit interneurons are particularly notable because of their functional resemblance to CB_1-positive interneurons in the hippocampus, with which they share not only a GABA-containing phenotype, but also the ability to discharge high-frequency bursts of action potentials that can inhibit firing in large assemblies of projection cells[124]. Does locally released anandamide gain access to these interneurons? Or does it primarily act on medium spiny cells and their cortical afferents? We don't know yet. But these unanswered questions do not diminish the significance of striatal endocannabinoid signalling, which is further highlighted by the effectiveness of cannabinoid agonists in the symptomatic treatment of LEVODOPA-INDUCED DYSKINESIAS[125] and TOURETTE'S SYNDROME[126], two disorders with strong striatal underpinnings.

Hindbrain: central analgesia. Beside their actions in the amygdala, cannabinoid agonists can influence the central processing of pain by interacting with CB_1 receptors in the periaqueductal grey[127], rostral ventromedial medulla[128] and spinal trigeminal nucleus[129]. At each of these sites, CB_1 activation depresses GABA release through a presynaptic mechanism, without causing significant changes in somatic membrane conductances[129,130]. In the trigeminal nucleus, glycinergic transmission also is inhibited[129]. Painful stimuli elicit anandamide release in the rat periaqueductal grey[16], and systemic administration of CB_1 antagonists produces HYPERALGESIA in rats and mice[63,131,132]. So, noxious stimuli can engage a central analgesic circuit operated by the endocannabinoids, which, working in combination with a parallel mechanism in the periphery, could underlie the analgesic properties of cannabinoid drugs[133].

Regulation of glutamate transmission

Principal neurons in the hippocampus and cerebellum use endocannabinoids to carry out a signalling process that is analogous in mechanism, but opposite in sign, to DSI, called depolarization-induced suppression of excitation (DSE)[108]. Like DSI, DSE is induced by neuronal depolarization, it consists of a transient depression in neurotransmitter release, and it requires a retrograde endocannabinoid messenger. But unlike DSI, DSE targets glutamatergic rather than GABA axon terminals, and results therefore in reduced excitatory input to the affected cell[108] (FIG. 8).

Do DSI and DSE occur simultaneously in a single neuron and, if so, how are they coordinated? In cerebellar Purkinje cells, the two opposing phenomena can be elicited by similar stimulation protocols and so are likely to coexist[42]. Although they might be topographically segregated along the longitudinal axis of the neuron, the significance of their coexistence is not known. On the other hand, in the hippocampus, the induction of DSE requires longer periods of depolarization than does DSI, and its magnitude is smaller[104]. This could be explained by the lower sensitivity of glutamatergic terminals to endocannabinoid activation[104], which would indicate that a switch from DSI to DSE might occur when endocannabinoid concentrations at hippocampal synapses attain a certain threshold value. Again, the role of such a switch, if any, is undefined.

LEVODOPA-INDUCED DYSKINESIAS
Unwanted movements that appear after prolonged use of the anti-Parkinsonian drug levodopa, β-(3,4-dihydroxyphenyl)-L-alanine.

TOURETTE'S SYNDROME
A psychiatric disorder of unknown aetiology, characterized by the presence of compulsive vocal and motor tics.

HYPERALGESIA
A state of enhanced sensitivity to painful stimuli.

Box 2 | Addicted to food

Although it is well established that marijuana smokers eat more marshmallows than they should, exactly why this occurs is still quite mysterious. However, a few facts seem to be clear[150]. Cannabinoid agonists stimulate food intake in partially fed animals. This effect is probably due to the activation of CB_1 receptors and is accompanied by enhanced food palatability. Moreover, CB_1 antagonists such as rimonabant reduce food intake and body weight in animals. Last, feeding status and feeding-regulating hormones such as leptin can affect endocannabinoid synthesis in the hypothalamus[151], as well as in intestinal tissue[152]. The neural substrates of these actions have not been elucidated, but their therapeutic potential is under study. The cannabinoid agonist THC (dronabinol, Marinol) has been approved by the US Food and Drug Administration for the treatment of anorexia associated with AIDS, and the antagonist rimonabant is in advanced clinical development for the treatment of obesity. In a proof-of-concept trial (Phase II), which was recently completed, the drug produced a marked reduction of body weight in obese patients; after four months of treatment, patients taking rimonabant lost 4.5 kg, whereas those taking placebo lost only 1 kg. Larger trials (Phase III) are now underway in the United States and Europe to compare the effects of rimonabant and placebo on obesity, hyperlipidaemia and diabetes. Importantly, rimonabant is also under consideration as a treatment for alcohol and tobacco abuse, a reminder of the common neural substrates that underlie drug and food rewards[153].

Inhibition of glutamatergic neurotransmission by cannabinoid agonists has been documented in a variety of brain structures besides the hippocampus and cerebellum. These include the prefrontal cortex[134], amygdala[86], nucleus accumbens[85], striatum[79] and substantia nigra pars reticulata[135]. Whether such effects reflect the existence of regional DSE-like phenomena is an important question that remains to be addressed.

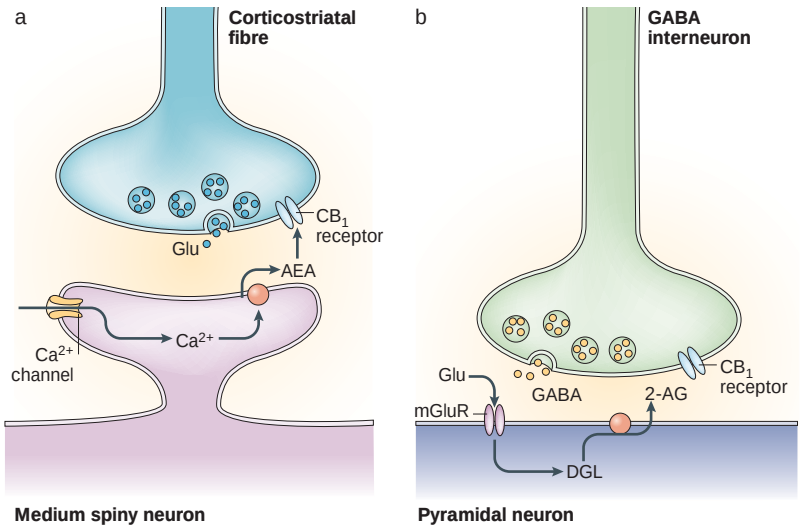

Figure 9 | **Roles of the endocannabinoids in long-term synaptic plasticity. a** | Repetitive activation of corticostriatal fibres causes a persistent reduction of glutamate release, called long-term depression (LTD), which might be mediated by anandamide. The elevated Ca^{2+} concentrations produced in postsynaptic spines of striatal medium spiny neurons after the stimulation could trigger anandamide (AEA) formation, which in turn might induce LTD by engaging CB_1 cannabinoid receptors on glutamatergic axon terminals. **b** | High-frequency stimulation of glutamatergic Schaffer collaterals in the hippocampus elicits a prolonged reduction of GABA (γ-aminobutyric acid) release that might be mediated by 2-arachidonoylglycerol (2-AG). This heterosynaptic form of plasticity, called inhibitory-LTD (I-LTD), is induced when glutamate activates metabotropic receptors (mGluR) on pyramidal neurons, eliciting 2-AG formation through the diacylglycerol lipase (DGL) pathway. 2-AG might then travel sideways to engage CB_1 receptors on contiguous terminals of GABA interneurons, producing I-LTD.

Other neurotransmitters

The ability of cannabinoid agonists to inhibit the release of neurotransmitters in the CNS is not restricted to glutamate and GABA[136]. A particularly convincing case has been made for acetylcholine, the release of which is reduced by cannabinoids both *in vitro* and *in vivo*, and is enhanced by inactivation of CB_1 receptors[136–138]. Acetylcholine release in the neocortex and hippocampus facilitates learning and memory, so disruption of this facilitatory process might contribute to the detrimental effects of cannabinoid drugs on cognition. Cannabinoids also reduce the release of the biogenic amines noradrenaline and serotonin[136], and the neuropeptide CCK-8 (REF. 139). Analogous, but as yet unknown, actions on peptide release in the hypothalamus might underlie the central involvement of the endocannabinoid system in the secretion of stress hormones and regulation of appetite (BOX 2).

Enduring inhibitions

Basal ganglia: a role in habit formation? High-frequency stimulation of cortical fibres that innervate the striatum leads to a form of persistent synaptic plasticity called long-term depression (LTD)[140]. Like its hippocampal counterpart, striatal LTD is induced when Ca^{2+} enters the somatodendritic compartment of projection neurons, and is expressed as a decrease in glutamate release from axon terminals of corticostriatal fibres[141]. These analogies with DSI are suggestive of an endocannabinoid-dependent process, an idea that has been confirmed experimentally[44]. Striatal LTD is absent in CB_1-deficient mice and is blocked by the CB_1 antagonist rimonabant; moreover, it is induced in a CB_1-dependent manner by anandamide or AM404 (REF. 44) (FIG. 9a).

A similar form of endocannabinoid-dependent LTD can be produced by low-frequency stimulation of cortical fibres that innervate the nucleus accumbens[45]. Despite differences in induction protocols *in vitro* — one is produced by high-frequency[44], the other by low-frequency, stimulation[45] — striatal and accumbal LTD could serve complementary functions. For example, they might both contribute to habit formation, a type of striatum-dependent learning that underlies the development of motor skills and is implicated in the pathogenesis of drug addiction[142]. Notably, cannabinoid drugs provoke in rats a relapse to drug-seeking behaviour after prolonged periods of abstinence, whereas CB_1 antagonists attenuate the relapse induced by drug-associated cues[143,144]. These findings have provided the rationale for current clinical trials of rimonabant as a treatment for alcohol and tobacco addiction (BOX 2).

Hippocampus: a role in cognition? In the hippocampal CA1 field, stimulation protocols that cause long-term potentiation at excitatory synapses onto pyramidal neurons simultaneously produce LTD at adjacent inhibitory synapses (I-LTD)[46]. Like striatal LTD, I-LTD might be endocannabinoid-mediated, but its molecular mechanism seems to be remarkably different. According to a current model, glutamate released from excitatory terminals activates metabotropic receptors on dendrites

of pyramidal neurons, which in turn stimulates 2-AG formation through the DGL pathway. The newly formed endocannabinoid can then depress GABA release by engaging CB_1 receptors on inhibitory nerve endings[46] (FIG. 9b). How this long-lasting disinhibitory process interacts with other forms of endocannabinoid-dependent plasticity and contributes to the overall effects of cannabinoids on hippocampus-dependent learning will surely be the object of future discussion and experiments.

Missing pieces

A decade of research in the biology of the endocannabinoid system has led to a series of exciting discoveries. We have learned that the brain contains multiple endocannabinoid lipids, and that neurons produce them using membrane constituents as starting material. We have also discovered that these lipids behave differently from traditional transmitters. Rather than being secreted from vesicle stores, they are released in a non-synaptic manner and combine with cannabinoid receptors located near their sites of synthesis.

Despite this progress, many crucial pieces of the endocannabinoid puzzle are still missing. For example, we need to map the neuronal circuits that produce anandamide and 2-AG, and this requires in turn the molecular characterization of the synthetic enzymes involved. We also need to understand how classical neurotransmitters and drugs of abuse interact with these circuits, and to explore the functional consequences of such interactions. Last, but not least, we must continue to develop selective pharmacological tools that target not only the different cannabinoid receptor subtypes, but also the mechanisms of endocannabinoid synthesis and deactivation. Although these tasks are far from trivial, what is already known about the endocannabinoid system indicates that they are well worth pursuing.

1. Rouyer, M. Sur les medicaments usuels des Egyptiens. *Bull. Pharmacie* **2**, 25 (1810).
2. O'Shaugnessy, W. B. On the *Cannabis indica* or Indian hemp. *Pharmacol. J. Trans.* **2**, 594 (1843).
3. Moreau, J. J. *Du Hachisch et de l'Aliénation Mentale* (Fortin, Masson & Co., Paris, 1845).
4. Christison, R. in *A Dispensatory, or Commentary on the Pharmacopoeias of Great Britain (and the United States)* 971–974 (Lea and Blanchard, Philadelphia, 1848).
5. Adams, R. Marihuana. *Harvey Lect.* **37**, 168 (1941).
6. Gaoni, Y. & Mechoulam, R. Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **86**, 1646–1647 (1964).
7. Melvin, L. S. & Johnson, M. R. Structure–activity relationships of tricyclic and nonclassical bicyclic cannabinoids. *NIDA Res. Monogr.* **79**, 31–47 (1987).
8. Devane, W. A., Dysarz, F. A., 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).
   This paper describes the original discovery of selective cannabinoid sites in the rat brain and outlines their pharmacological properties.
9. 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).
10. Munro, S., Thomas, K. L. & Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61–65 (1993).
    References 9 and 10 report on the molecular cloning and structural characterization of CB_1 and CB_2, the two cannabinoid receptors identified in mammalian tissues.
11. Devane, W. A. *et al.* Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949 (1992).
    This paper outlines the ground-breaking isolation of anandamide from pig brain and the ability of this lipid derivative to activate cannabinoid receptors.
12. Piomelli, D. & Greengard, P. Lipoxygenase metabolites of arachidonic acid in neuronal transmembrane signalling. *Trends Pharmacol. Sci.* **11**, 367–373 (1990).
13. Kempe, K., Hsu, F. F., Bohrer, A. & Turk, J. Isotope dilution mass spectrometric measurements indicate that arachidonylethanolamide, the proposed endogenous ligand of the cannabinoid receptor, accumulates in rat brain tissue post mortem but is contained at low levels in or is absent from fresh tissue. *J. Biol. Chem.* **271**, 17287–17295 (1996).
14. Di Marzo, V. *et al.* Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**, 686–691 (1994).
15. Giuffrida, A. *et al.* Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nature Neurosci.* **2**, 358–363 (1999).
16. Walker, J. M., Huang, S. M., Strangman, N. M., Tsou, K. & Sañudo-Peña, M. C. Pain modulation by release of the endogenous cannabinoid anandamide. *Proc. Natl Acad. Sci. USA* **96**, 12198–12203 (1999).
    By showing that anandamide is produced in and released from brain neurons under physiological conditions, references 14–16 established the role of this compound as an endogenous ligand for cannabinoid receptors.
17. Sugiura, T. *et al.* 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215**, 89–97 (1995).
18. 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).
19. Hanus, L. *et al.* 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB_1 receptor. *Proc. Natl Acad. Sci. USA* **98**, 3662–3665 (2001).
20. Porter, A. C. *et al.* Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J. Pharmacol. Exp. Ther.* **301**, 1020–1024 (2002).
21. Huang, S. M. *et al.* An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc. Natl Acad. Sci. USA* **99**, 8400–8405 (2002).
22. Chapman, K. D. Emerging physiological roles for *N*-acylphosphatidylethanolamine metabolism in plants: signal transduction and membrane protection. *Chem. Phys. Lipids* **108**, 221–229 (2000).
23. Sugiura, T. *et al.* Transacylase-mediated and phosphodiesterase-mediated synthesis of *N*-arachidonoylethanolamine, an endogenous cannabinoid-receptor ligand, in rat brain microsomes. Comparison with synthesis from free arachidonic acid and ethanolamine. *Eur. J. Biochem.* **240**, 53–62 (1996).
24. Cadas, H., di Tomaso, E. & Piomelli, D. Occurrence and biosynthesis of endogenous cannabinoid precursor, *N*-arachidonoyl phosphatidylethanolamine, in rat brain. *J. Neurosci.* **17**, 1226–1242 (1997).
25. Kodaki, T. & Yamashita, S. Cloning, expression, and characterization of a novel phospholipase D complementary DNA from rat brain. *J. Biol. Chem.* **272**, 11408–11413 (1997).
26. Cadas, H., Gaillet, S., Beltramo, M., Venance, L. & Piomelli, D. Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J. Neurosci.* **16**, 3934–3942 (1996).
27. Ferrer, B., Asbrock, N., Kathuria, S., Piomelli, D. & Giuffrida, A. Effects of levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. *Eur. J. Neurosci.* **18**, 1607–1614 (2003).
28. Varma, N., Carlson, G. C., Ledent, C. & Alger, B. E. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J. Neurosci.* **21**, 1–5 (2001).
29. Kim, J., Isokawa, M., Ledent, C. & Alger, B. E. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J. Neurosci.* **22**, 10182–10191 (2002).
30. Senogles, S. E. The D2s dopamine receptor stimulates phospholipase D activity: a novel signaling pathway for dopamine. *Mol. Pharmacol.* **58**, 455–462 (2000).
31. Hernández-López, S. *et al.* D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca^{2+} currents and excitability via a novel PLCβ1-IP3-calcineurin-signaling cascade. *J. Neurosci.* **20**, 8987–8995 (2000).
32. Stella, N., Schweitzer, P. & Piomelli, D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–778 (1997).
33. Kanoh, H., Yamada, K. & Sakane, F. Diacylglycerol kinases: emerging downstream regulators in cell signaling systems. *J. Biochem.* **131**, 629–633 (2002).
34. Farooqui, A. A., Rammohan, K. W. & Horrocks, L. A. Isolation, characterization, and regulation of diacylglycerol lipases from the bovine brain. *Ann. NY Acad. Sci.* **559**, 25–36 (1989).
35. Higgs, H. N. & Glomset, J. A. Identification of a phosphatidic acid-preferring phospholipase A1 from bovine brain and testis. *Proc. Natl Acad. Sci. USA* **91**, 9574–9578 (1994).
36. Pete, M. J., Ross, A. H. & Exton, J. H. Purification and properties of phospholipase A1 from bovine brain. *J. Biol. Chem.* **269**, 19494–19500 (1994).
37. Stella, N. & Piomelli, D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur. J. Pharmacol.* **425**, 189–196 (2001).
38. Oka, S. *et al.* Ether-linked analogue of 2-arachidonoylglycerol (noladin ether) was not detected in the brains of various mammalian species. *J. Neurochem.* **85**, 1374–1381 (2003).
39. Song, Z. H. & Bonner, T. I. A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN55212-2. *Mol. Pharmacol.* **49**, 891–896 (1996).
40. Xie, X. Q., Melvin, L. S. & Makriyannis, A. The conformational properties of the highly selective cannabinoid receptor ligand CP-55,940. *J. Biol. Chem.* **271**, 10640–10647 (1996).
41. Wilson, R. I. & Nicoll, R. A. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**, 588–592 (2001).
42. Kreitzer, A. C. & Regehr, W. G. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* **29**, 717–727 (2001).
43. Ohno-Shosaku, T., Maejima, T. & Kano, M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* **29**, 729–738 (2001).
    References 41–43 provided the first unequivocal demonstration that endocannabinoids regulate synaptic transmission in the brain.
44. Gerdeman, G. L., Ronesi, J. & Lovinger, D. M. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nature Neurosci.* **5**, 446–451 (2002).
45. 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).

46. Chevaleyre, V. & Castillo, P. E. Heterosynaptic LTD of hippocampal GABAergic synapses. A novel role of endocannabinoids in regulating excitability. *Neuron* **38**, 461–472 (2003).
47. Beuckmann, C. T. *et al.* Cellular localization of lipocalin-type prostaglandin D synthase (β-trace) in the central nervous system of the adult rat. *J. Comp. Neurol.* **428**, 62–78 (2000).
48. Bojensen, I. N. & Hansen, H. S. Binding of anandamide to bovine serum albumin. *J. Lipid Res.* **44**, 1790–1794 (2003).
49. Beltramo, M. *et al.* Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science* **277**, 1094–1097 (1997).
Together with reference 50, this paper identifies facilitated transport as the first step in anandamide deactivation and introduces the first anandamide transport inhibitor, AM404.
50. Hillard, C. J., Edgemond, W. S., Jarrahian, A. & Campbell, W. B. Accumulation of *N*-arachidonoylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J. Neurochem.* **69**, 631–638 (1997).
51. Abumrad, N., Coburn, C. & Ibrahimi, A. Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. *Biochim. Biophys. Acta* **1441**, 4–13 (1999).
52. Piomelli, D. *et al.* Structural determinants for recognition and translocation by the anandamide transporter. *Proc. Natl Acad. Sci. USA* **96**, 5802–5807 (1999).
53. Beltramo, M. *et al.* Reversal of dopamine D_2 receptor responses by an anandamide transport inhibitor. *J. Neurosci.* **20**, 3401–3407 (2000).
54. Lopez-Rodriguez, M. L. *et al.* Design, synthesis and biological evaluation of novel arachidonic acid derivatives as highly potent and selective endocannabinoid transporter inhibitors. *J. Med. Chem.* **44**, 4505–4508 (2001).
55. De Petrocellis, L., Bisogno, T., Davis, J. B., Pertwee, R. G. & Di Marzo, V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. *FEBS Lett.* **483**, 52–56 (2000).
56. Schmid, P. C., Zuzarte-Augustin, M. L. & Schmid, H. H. Properties of rat liver *N*-acylethanolamine amidohydrolase. *J. Biol. Chem.* **260**, 14145–14149 (1985).
Published long before the discovery of anandamide, this paper describes a membrane-associated enzyme activity that breaks down fatty acid ethanolamides. This enzyme, which also catalyses the hydrolysis of anandamide, is now called fatty acid amide hydrolase.
57. Hillard, C. J., Wilkison, D. M., Edgemond, W. S. & Campbell, W. B. Characterization of the kinetics and distribution of *N*-arachidonylethanolamine (anandamide) hydrolysis by rat brain. *Biochim. Biophys. Acta* **1257**, 249–256 (1995).
58. Ueda, N., Kurahashi, Y., Yamamoto, S. & Tokunaga, T. Partial purification and characterization of the porcine brain enzyme hydrolyzing and synthesizing anandamide. *J. Biol. Chem.* **270**, 23823–23827 (1995).
59. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
The first of an elegant series of papers (including references 60 and 64) that unveils the molecular properties of fatty acid amide hydrolase.
60. Bracey, M. H., Hanson, M. A., Masuda, K. R., Stevens, R. C. & Cravatt, B. F. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* **298**, 1793–1796 (2002).
61. Fu, J. *et al.* Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. *Nature* **425**, 90–93 (2003).
62. Mazzari, S., Canella, R., Petrelli, L., Marcolongo, G. & Leon, A. *N*-(2-hydroxyethyl)hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. *Eur. J. Pharmacol.* **300**, 227–236 (1996).
63. Calignano, A., La Rana, G., Giuffrida, A. & Piomelli, D. Control of pain initiation by endogenous cannabinoids. *Nature* **394**, 277–281 (1998).
64. Cravatt, B. F. *et al.* Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc. Natl Acad. Sci. USA* **98**, 9371–9376 (2001).
65. Kathuria, S. *et al.* Modulation of anxiety through blockade of anandamide hydrolysis. *Nature Med.* **9**, 76–81 (2003).
Selective and systemically active inhibitors of fatty acid amide hydrolase activity reveal a crucial role for anandamide in the regulation of emotion.
66. Tsou, K. *et al.* Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. *Neurosci. Lett.* **254**, 137–140 (1998).
67. Egertová, M., Cravatt, B. F. & Elphick, M. R. Comparative analysis of fatty acid amide hydrolase and CB_1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. *Neuroscience* **119**, 481–496 (2003).
68. Goparaju, S. K., Ueda, N., Taniguchi, K. & Yamamoto, S. Enzymes of porcine brain hydrolyzing 2-arachidonoylglycerol, an endogenous ligand of cannabinoid receptors. *Biochem. Pharmacol.* **57**, 417–423 (1999).
69. Dinh, T. P. *et al.* Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl Acad. Sci. USA* **99**, 10819–10824 (2002).
70. Herkenham, M. *et al.* Cannabinoid receptor localization in brain. *Proc. Natl Acad. Sci. USA* **87**, 1932–1936 (1990).
71. Adams, I. B. & Martin, B. R. Cannabis: pharmacology and toxicology in animals and humans. *Addiction* **91**, 1585–1614 (1996).
A comprehensive review of the pharmacology of *Cannabis* derivatives.
72. Ledent, C. *et al.* Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**, 401–404 (1999).
73. Zimmer, A., Zimmer, A. M., Hohmann, A. G., Herkenham, M. & Bonner, T. I. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc. Natl Acad. Sci. USA* **96**, 5780–5785 (1999).
74. Mackie, K. & Hille, B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc. Natl Acad. Sci. USA* **89**, 3825–3829 (1992).
75. Caulfield, M. P. & Brown, D. A. Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. *Br. J. Pharmacol.* **106**, 231–232 (1992).
76. Twitchell, W., Brown, S. & Mackie, K. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J. Neurophysiol.* **78**, 43–50 (1997).
77. Wilson, R. I. & Nicoll, R. A. Endocannabinoid signaling in the brain. *Science* **296**, 678–682 (2002).
78. Hoffman, A. F. & Lupica, C. R. Mechanisms of cannabinoid inhibition of $GABA_A$ synaptic transmission in the hippocampus. *J. Neurosci.* **20**, 2470–2479 (2000).
79. Gerdeman, G. & Lovinger, D. M. CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J. Neurophysiol.* **85**, 468–471 (2001).
80. Huang, C. C., Lo, S. W. & Hsu, K. S. Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. *J. Physiol.* **532**, 731–748 (2001).
81. Wilson, R. I., Kunos, G. & Nicoll, R. A. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron* **31**, 1–20 (2001).
82. Mu, J., Zhuang, S. Y., Kirby, M. T., Hampson, R. E. & Deadwyler, S. A. Cannabinoid receptors differentially modulate potassium A and D currents in hippocampal neurons in culture. *J. Pharmacol. Exp. Ther.* **291**, 893–902 (1999).
83. Kreitzer, A. C., Carter, A. G. & Regehr, W. G. Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. *Neuron* **34**, 787–796 (2002).
84. Daniel, H. & Crepel, F. Control of Ca^{2+} influx by cannabinoid and metabotropic glutamate receptors in rat cerebellar cortex requires K^+ channels. *J. Physiol.* **537**, 793–800 (2001).
85. Robbe, D., Alonso, G., Duchamp, F., Bockaert, J. & Manzoni, O. J. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J. Neurosci.* **21**, 109–116 (2001).
86. Azad, S. C. *et al.* Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. *Learn. Mem.* **10**, 116–128 (2003).
87. Adams, J. P. & Sweatt, J. D. Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annu. Rev. Pharmacol. Toxicol.* **42**, 135–163 (2002).
88. Derkinderen, P. *et al.* Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* **273**, 1719–1722 (1996).
89. Derkinderen, P. *et al.* Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *J. Neurosci.* **23**, 2371–2382 (2003).
90. Hoffman, A. F., Oz, M., Caulder, T. & Lupica, C. R. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *J. Neurosci.* **23**, 4815–4820 (2003).
91. Katona, I. *et al.* Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* **19**, 4544–4558 (1999).
92. Marsicano, G. & Lutz, B. Expression of the cannabinoid receptor CB_1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* **11**, 4213–4225 (1999).
93. Tsou, K., Brown, S., Sañudo-Peña, M. C., Mackie, K. & Walker, J. M. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**, 393–411 (1998).
94. Katona, I. *et al.* Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* **21**, 9506–9518 (2001).
95. McDonald, A. J. & Mascagni, F. Localization of the CB1 type cannabinoid receptor in the rat basolateral amygdala: high concentrations in a subpopulation of cholecystokinin-containing interneurons. *Neuroscience* **107**, 641–652 (2001).
96. Hohmann, A. G. & Herkenham, M. Localization of cannabinoid CB_1 receptor mRNA in neuronal subpopulations of rat striatum: a double-label *in situ* hybridization study. *Synapse* **37**, 71–80 (2000).
97. Herkenham, M., Lynn, A. B., de Costa, B. R. & Richfield, E. K. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* **547**, 267–274 (1991).
98. Hohmann, A. G. & Herkenham, M. Cannabinoid receptors undergo axonal flow in sensory nerves. *Neuroscience* **92**, 1171–1175 (1999).
99. Price, T. J., Helesic, G., Parghi, D., Hargreaves, K. M. & Flores, C. M. The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. *Neuroscience* **120**, 155–162 (2003).
100. Breivogel, C. S., Griffin, G., Di Marzo, V. & Martin, B. R. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol.* **60**, 155–163 (2001).
101. Hájos, N., Ledent, C. & Freund, T. F. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* **106**, 1–4 (2001).
Together with reference 100, this study provided the first indication that an additional brain cannabinoid receptor remains to be cloned.
102. Rouach, N. & Nicoll, R. A. Endocannabinoids contribute to short-term but not long-term mGluR-induced depression in the hippocampus. *Eur. J. Neurosci.* **18**, 1017–1020 (2003).
103. Hájos, N. & Freund, T. F. Pharmacological separation of cannabinoid sensitive receptors on hippocampal excitatory and inhibitory fibers. *Neuropharmacology* **43**, 503–510 (2002).
104. Ohno-Shosaku, T. *et al.* Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J. Neurosci.* **22**, 3864–3872 (2002).
105. Jarai, Z. *et al.* Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB1 or CB2 receptors. *Proc. Natl Acad. Sci. USA* **96**, 14136–14141 (1999).
106. Wagner, J. A., Varga, K., Jarai, Z. & Kunos, G. Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension* **33**, 429–434 (1999).
107. Bátkai, S. *et al.* Endocannabinoids acting at vascular CB_1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nature Med.* **7**, 827–832 (2001).
108. Alger, B. E. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog. Neurobiol.* **68**, 247–286 (2002).
109. Yoshida, T. *et al.* The cannabinoid CB1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar Purkinje cells. *J. Neurosci.* **22**, 1690–1697 (2002).
110. Hampson, R. E., Zhuang, S. Y., Weiner, J. L. & Deadwyler, S. A. Functional significance of cannabinoid-mediated, depolarization-induced suppression of inhibition (DSI) in the hippocampus. *J. Neurophysiol.* **90**, 55–64 (2003).
111. Hájos, N. *et al.* Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur. J. Neurosci.* **12**, 3239–3249 (2000).
112. Harris, K. D., Csicsvari, J., Hirase, H., Dragoi, G. & Buzsaki, G. Organization of cell assemblies in the hippocampus. *Nature* **424**, 552–556 (2003).
113. Carlson, G., Wang, Y. & Alger, B. E. Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nature Neurosci.* **5**, 723–724 (2002).
This paper reports that endocannabinoids can facilitate hippocampal long-term potentiation (LTP) at the single-cell level, although pharmacological administration of cannabinoid agonists inhibits LTP and impairs memory (reviewed in reference 114).
114. Hampson, R. E. & Deadwyler, S. A. Cannabinoids, hippocampal function and memory. *Life Sci.* **65**, 715–723 (1999).
115. Llano, I., Leresche, N. & Marty, A. Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* **6**, 565–574 (1991).

116. Kreitzer, A. C. & Regehr, W. G. Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *J. Neurosci.* **21**, 717–727 (2001).
117. Trettel, J. & Levine, E. S. Endocannabinoids mediate rapid retrograde signaling at interneuron right-arrow pyramidal neuron synapses of the neocortex. *J. Neurophysiol.* **89**, 2334–2338 (2003).
118. Martin, W. J. *et al.* Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. *Brain Res.* **822**, 237–242 (1999).
119. Navarro, M. *et al.* Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. *Neuroreport* **8**, 491–496 (1997).
120. Martin, M., Ledent, C., Parmentier, M., Maldonado, R. & Valverde, O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl.)* **159**, 379–387 (2002).
121. Marsicano, G. *et al.* The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530–534 (2002).
122. Romero, J. *et al.* The endogenous cannabinoid system and the basal ganglia: biochemical, pharmacological, and therapeutic aspects. *Pharmacol. Ther.* **95**, 137–152 (2002).
123. Gorriti, M. A., Rodríguez de Fonseca, F., Navarro, M. & Palomo, T. Chronic (–)-Δ^9-tetrahydrocannabinol treatment induces sensitization to the psychomotor effects of amphetamine in rats. *Eur. J. Pharmacol.* **365**, 133–142 (1999).
124. Koos, T. & Tepper, J. M. Inhibitory control of neostriatal projection neurons by GABAergic interneurons. *Nature Neurosci.* **2**, 467–472 (1999).
125. Sieradzan, K. A. *et al.* Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology* **57**, 2108–2111 (2001).
126. Muller-Vahl, K. R. *et al.* Δ^9-Tetrahydrocannabinol (THC) is effective in the treatment of tics in Tourette syndrome: a 6-week randomized trial. *J. Clin. Psychiatry* **64**, 459–465 (2003).
127. Lichtman, A. H., Cook, S. A. & Martin, B. R. Investigation of brain sites mediating cannabinoid-induced antinociception in rats: evidence supporting periaqueductal gray involvement. *J. Pharmacol. Exp. Ther.* **276**, 585–593 (1996).
128. Meng, I. D., Manning, B. H., Martin, W. J. & Fields, H. L. An analgesia circuit activated by cannabinoids. *Nature* **395**, 381–383 (1998).
129. Jennings, E. A., Vaughan, C. W. & Christie, M. J. Cannabinoid actions on rat superficial medullary dorsal horn neurons *in vitro*. *J. Physiol.* **534**, 805–812 (2001).
130. Vaughan, C. W., Connor, M., Bagley, E. E. & Christie, M. J. Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons *in vitro*. *Mol. Pharmacol.* **57**, 288–295 (1999).
131. Richardson, J. D., Aanonsen, L. & Hargreaves, K. M. SR 141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. *Eur. J. Pharmacol.* **319**, R3–R4 (1997).
132. Strangman, N. M., Patrick, S. L., Hohmann, A. G., Tsou, K. & Walker, J. M. Evidence for a role of endogenous cannabinoids in the modulation of acute and tonic pain sensitivity. *Brain Res.* **813**, 323–328 (1998).
133. Iversen, L. & Chapman, V. Cannabinoids: a real prospect for pain relief. *Curr. Opin. Pharmacol.* **2**, 50–55 (2002).
A recent review of the therapeutic potential of cannabinoid drugs as analgesic agents.
134. Auclair, N., Otani, S., Soubrie, P. & Crepel, F. Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex pyramidal neurons. *J. Neurophysiol.* **83**, 3287–3293 (2000).
135. Szabo, B., Wallmichrath, I., Mathonia, P. & Pfreundtner, C. Cannabinoids inhibit excitatory neurotransmission in the substantia nigra pars reticulata. *Neuroscience* **97**, 89–97 (2000).
136. Schlicker, E. & Kathmann, M. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* **22**, 565–572 (2001).
An exhaustive review of the effects of cannabinoids on the release of brain neurotransmitters.
137. Gifford, A. N. & Ashby, C. R. Jr. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J. Pharmacol. Exp. Ther.* **277**, 1431–1436 (1996).
138. Gessa, G. L., Casu, M. A., Carta, G. & Mascia, M. S. Cannabinoids decrease acetylcholine release in the medial-prefrontal cortex and hippocampus, reversal by SR 141716A. *Eur. J. Pharmacol.* **355**, 119–124 (1998).
139. Beinfeld, M. C. & Connolly, K. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci. Lett.* **301**, 69–71 (2001).
140. Calabresi, P., Maj, R., Pisani, A., Mercuri, N. B. & Bernardi, G. Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *J. Neurosci.* **12**, 4224–4233 (1992).
141. Choi, S. & Lovinger, D. M. Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. *Proc. Natl Acad. Sci. USA* **94**, 2665–2670 (1997).
142. Gerdeman, G. L., Partridge, J. G., Lupica, C. R. & Lovinger, D. M. It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci.* **26**, 184–192 (2003).
143. De Vries, T. J. *et al.* A cannabinoid mechanism in relapse to cocaine seeking. *Nature Med.* **7**, 1151–1154 (2001).
This article showed that the cannabinoid antagonist rimonabant prevents relapse to cocaine abuse in animals, revealing a key role for the endocannabinoid system in the regulation of reward.
144. Fattore, L., Spano, M. S., Cossu, G., Deiana, S. & Fratta, W. Cannabinoid mechanism in reinstatement of heroin-seeking after a long period of abstinence in rats. *Eur. J. Neurosci.* **17**, 1723–1726 (2003).
145. Hillard, C. J. *et al.* Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J. Pharmacol. Exp. Ther.* **289**, 1427–1433 (1999).
146. Ibrahim, M. M. *et al.* Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl Acad. Sci. USA* **100**, 10529–10533 (2003).
147. Boger, D. L. *et al.* Exceptionally potent inhibitors of fatty acid amide hydrolase: the enzyme responsible for degradation of endogenous oleamide and anandamide. *Proc. Natl Acad. Sci. USA* **97**, 5044–5049 (2000).
148. Masson, J., Sagne, C., Hamon, M. & Mestikawy, S. E. Neurotransmitter transporters in the central nervous system. *Pharmacol. Rev.* **51**, 439–464 (1999).
149. Glaser, S. T. *et al.* Evidence against the presence of an anandamide transporter. *Proc. Natl Acad. Sci. USA* **100**, 4269–4274 (2003).
150. Berry, E. M. & Mechoulam, R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol. Ther.* **95**, 185–190 (2002).
151. Di Marzo, V. *et al.* Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410**, 822–825 (2001).
152. Gomez, R. *et al.* A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J. Neurosci.* **22**, 9612–9617 (2002).
153. Koob, G. F. & Le Moal, M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology* **24**, 97–129 (2001).

Acknowledgements

Work in the author's laboratory was supported by the National Institute on Drug Abuse, and by the National Alliance for Research on Schizophrenia and Depression.

Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/

CB_1 | CB_2 | D_2 receptor | FAAH | MGL

FURTHER INFORMATION

International Cannabinoid Research Society: http://cannabinoidsociety.org/

International Association for Cannabis as Medicine: http://www.acmed.org/

National Institute on Drug Abuse: http://www.drugabuse.gov/ResearchReports/Marijuana/Marijuana6.html

Access to this interactive links box is free online.