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Care and Feeding of the Endocannabinoid System: A Systematic Review of Potential Clinical Interventions that Upregulate the Endocannabinoid System

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Abstract

Background: The "classic" endocannabinoid (eCB) system includes the cannabinoid receptors CB_1 and CB_2, the eCB ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and their metabolic enzymes. An emerging literature documents the "eCB deficiency syndrome" as an etiology in migraine, fibromyalgia, irritable bowel syndrome, psychological disorders, and other conditions. We performed a systematic review of clinical interventions that enhance the eCB system—ways to upregulate cannabinoid receptors, increase ligand synthesis, or inhibit ligand degradation.

Methodology/Principal Findings: We searched PubMed for clinical trials, observational studies, and preclinical research. Data synthesis was qualitative. Exclusion criteria limited the results to 184 *in vitro* studies, 102 *in vivo* animal studies, and 36 human studies. Evidence indicates that several classes of pharmaceuticals upregulate the eCB system, including analgesics (acetaminophen, non-steroidal anti-inflammatory drugs, opioids, glucocorticoids), antidepressants, antipsychotics, anxiolytics, and anticonvulsants. Clinical interventions characterized as "complementary and alternative medicine" also upregulate the eCB system: massage and manipulation, acupuncture, dietary supplements, and herbal medicines. Lifestyle modification (diet, weight control, exercise, and the use of psychoactive substances—alcohol, tobacco, coffee, cannabis) also modulate the eCB system.

Conclusions/Significance: Few clinical trials have assessed interventions that upregulate the eCB system. Many preclinical studies point to other potential approaches; human trials are needed to explore these promising interventions.

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Introduction

The endocannabinoid (eCB) system consists of receptors, endogenous ligands, and ligand metabolic enzymes. Metaphorically the eCB system represents a microcosm of psychoneuroimmunology or mind-body medicine. Cannabinoid receptor 1 (CB_1) is the most abundant G protein-coupled receptor expressed in the brain, with particularly dense expression in (rank order): the substantia nigra, globus pallidus, hippocampus, cerebral cortex, putamen, caudate, cerebellum, and amygdala [1]. CB_1 is also expressed in non-neuronal cells, such as adipocytes and hepatocytes, and in musculoskeletal tissues. Cannabinoid receptor 2 (CB_2) is principally associated with cells governing immune function, although it may also be expressed in the central nervous [2,3].

The quintessential eCB ligands are *N*-arachidonylethanolamide (anandamide, AEA) and *sn*-2-arachidonoylglycerol (2-AG). AEA and 2-AG are released upon demand from cell membrane-embedded phospholipid precursors. The primary biosynthetic enzyme of AEA is *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD). 2-AG is biosynthesized by two isoforms of diacylglycerol lipase, DAGLα and DAGLβ. AEA and 2-AG work in a homeostatic fashion, thus they are broken down after they activate CB_1 or CB_2. AEA is catabolized primarily by fatty acid amide hydrolase 1 (FAAH1), and 2-AG is catabolized by monoacylglycerol lipase (MAGL), and, to a lesser extent, α,β-hydrolase-6 (ABHD-6), cyclooxygenase 2 (COX2), and FAAH1.

This "classic eCB system" has expanded with the discovery of secondary receptors, ligands, and ligand metabolic enzymes [4]. For example, AEA, 2-AG, *N*-arachidonoyl glycine (NAGly) and the phytocannabinoids Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) may also serve, to different extents, as ligands at GPR55, GPR18, GPR119, and several transient receptor potential ion channels (*e.g.*, TRPV1, TRPV2, TRPA1, TRPM8). The effects of AEA and 2-AG can be enhanced by "entourage compounds" that inhibit their hydrolysis via substrate competition,

and thereby prolong their action. Entourage compounds include *N*-palmitylethanolamide (PEA), *N*-oleoylethanolamide (SEA), and *cis*-9-octadecenoamide (OEA, oleamide).

The eCB system's salient homeostatic roles have been summarized as, "relax, eat, sleep, forget, and protect" [5]. It modulates embryological development, neural plasticity, neuroprotection, immunity and inflammation, apoptosis and carcinogenesis, pain and emotional memory, and most importantly from the viewpoint of recent drug development: hunger, feeding, and metabolism. Obese individuals seem to display an increased eCB tone, driving CB_1 activation in a chronic, feed-forward dysfunction (reviewed by [6]). An antagonist or inverse agonist of CB_1 called rimonabant (*aka*, SR141716 in preclinical studies) was approved for the treatment of obesity. It was subsequently withdrawn from the market due to adverse effects [7].

Other diseases are associated with suboptimal functioning of the eCB system. Russo [8] proposed that migraine, fibromyalgia, irritable bowel syndrome, and related conditions represent CEDS, "clinical endocannabinoid deficiency syndromes." Fride [9] speculated that a dysfunctional eCB system in infants contributes to "failure to thrive" syndrome. Hill and Gorzalka [10] hypothesized that deficient eCB signaling could be involved in the pathogenesis of depressive illnesses. In human studies, eCB system deficiencies have been implicated in uncompensated schizophrenia [11], migraine [12], multiple sclerosis [13], Huntington's [14,15], uncompensated Parkinson's [16], irritable bowel syndrome [17], uncompensated anorexia [18], and chronic motion sickness [19].

Correcting CEDS may be accomplished via at least three molecular mechanisms: 1. augmenting eCB ligand biosynthesis; 2. decreasing eCB ligand degradation; 3. augmenting or decreasing receptor density or function. Clinical interventions for CEDS are largely unknown; this provided a rationale for reviewing potential clinical approaches. The paucity of human clinical trials led us to include preclinical studies in a systematic review. A systematic review uses an objective, transparent approach for research synthesis, with the aim of minimizing bias. Systematic reviews usually analyze human clinical trials, but the methodology can be applied to preclinical studies [20,21]. We previously conducted a systematic review of *in vitro* CB_1 ligand binding affinity and receptor distribution [22]. The review has alerted others to interspecies differences in preclinical studies, and other methodological issues (*e.g.*, [23]).

Potential clinical interventions (intervention groups) include pharmaceutical drugs, such as analgesics (acetaminophen, nonsteroidal anti-inflammatory drugs, opiates, glucocorticoids), antidepressants, antipsychotics, anxiolytic agents, and anticonvulsants. We also investigated therapeutic approaches classified as "complementary and alternative medicine" (CAM). The National Center for Complementary and Alternative Medicine (NCCAM) defines CAM as "a group of diverse medical and healthcare systems, practices, and products, that are not currently part of conventional medicine" (http://nccam.nih.gov/health/whatiscam/). The NCCAM categorizes CAM practices into three broad groups: "natural products" (dietary supplements and herbal remedies), "mind and body medicine" (meditation, yoga, and acupuncture), and "body-based practices" (massage, spinal manipulation). For the purposes of this review, we add "lifestyle modifications," including diet, weight control, exercise, and commonly-used psychoactive substances—alcohol, tobacco, coffee, and cannabis.

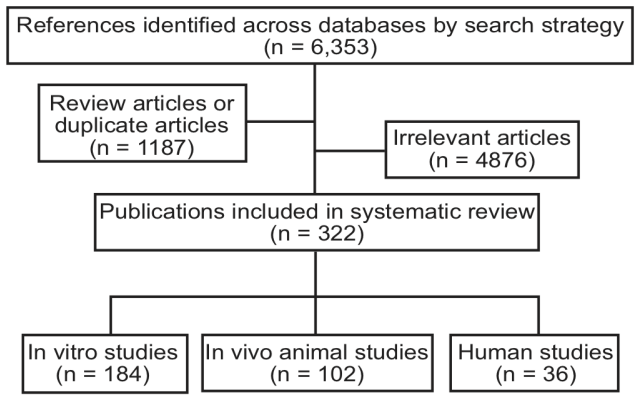

Figure 1. Selection process for study inclusion.
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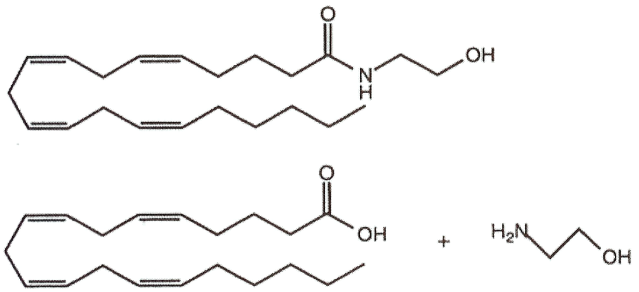

Figure 2. Anandamide (top figure) is metabolized into arachidonic acid and ethanolamine (bottom figures).
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Methods

Data Sources and Search Parameters

This review followed the guidelines proposed by PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [24], see Checklist S1. PubMed (www.ncbi.nlm.nih.gov/pubmed/) was searched through March 2013 using three MeSH keywords: endocannabinoids, cannabinoids, cannabinoid receptors. Each keyword was entered in a boolean combination with each of the intervention groups listed in the previous paragraph. Titles and abstracts of identified articles in all languages were screened for inclusion and exclusion criteria. We included randomized clinical trials, observational studies, and preclinical research on model organisms and *in vitro* studies. We excluded redundant articles that used identical methods and reported parallel results, or review articles that presented duplicate information.

Because this review focuses upon clinical interventions affecting the eCB system, we deemed as irrelevant (and excluded) articles that described the reverse scenario, such as eCB ligands modulating opioid receptors, THC enhancing tobacco or alcohol abuse, etc. Retrieved articles were scanned for supporting citations, and antecedent sources were retrieved and screened for inclusion and exclusion criteria. In addition, we checked reference lists of relevant narrative reviews.

Data Selection, Abstraction, and Synthesis

All three authors selected studies for inclusion and exclusion; the first author abstracted all data, the second and third authors arbitrated uncertainties and disagreements. We undertook a qualitative synthesis across studies because there was substantial heterogeneity with respect to research methodologies amongst the

identified articles—ranging from randomized clinical trials, observational studies, and preclinical research on model organisms and *in vitro* studies. The substantial heterogeneity amongst these methodologies precluded a single metric of quality assessment.

Many studies utilized *in vitro* measures of receptor density and signal transduction, as differences in means before- and after-interventions. Briefly: assays for CB_1/CB_2 receptor density include autoradiography with tritiated ligands (usually [^{3}H]CP55,940 or [^{3}H]SR141716), Western blot or immunostaining with antibodies to CB_1/CB_2 proteins, and Northern blot with radio-labeled or fluorescent riboprobes for CB_1/CB_2 mRNA. Signal transduction studies measure cannabinoid-stimulated inhibition of adenylyl cyclase, cannabinoid-stimulated [^{35}S]GTPγS binding, or electrophysiological assays of *ex vivo* brain slices. Electrophysiological studies include depolarization-induced suppression of excitation (DSE, via glutamatergic synapses), depolarization-induced suppression of inhibition (DSI, via GABAergic synapses), long-term depression of excitatory synaptic transmission (LTDE, via glutamatergic synapses), or long-term depression of inhibitory synaptic transmission (LTDI, via GABAergic synapsis).

Publication bias was addressed by asking investigators to contribute unpublished studies. Clinical interventions (intervention groups) with five or more studies are provided with an interpretive summary at the end of the section (*e.g.*, the sections on NSAIDs, glucocorticoids, opiates, etc.).

Results and Discussion

The search algorithm identified 6,353 potentially relevant articles. The majority of these were irrelevant. For example, combining the three MeSH keywords with "alcohol" generated 2450 hits, many of which concerned the relationship between alcohol and cannabis in motor vehicle accidents or suicides. Only 322 articles met the predefined selection criteria for relevance. See Figure 1 for a flowchart of articles included in this review. Few randomized clinical trials have been conducted on our topic; most of the articles concerned preclinical research. Fewer studies measured the effects of clinical interventions on CB_1 expression in humans. This is because the measurement of CB_1 expression requires positron emission tomography (PET) or brain biopsies. Although cannabinoid radioligands for PET scans are available, few PET studies on clinical interventions have been completed. Ethical issues circumscribe brain biopsies in living humans. A few studies measured postmortem CB_1 expression.

The use of PubMed as a stand-alone search engine may have generated bias regarding CAM practices. McPartand and Pruitt [25] used PubMed to compile a review of clinical trials regarding the CAM herbal medicine *Serenoa repens*; PubMed yielded only 33% of articles that they subsequently obtained by screening retrieved articles for supporting citations. Expanding our search by screening retrieved articles for supporting citations improved the yield, as it did in the *Serenoa* review.

The quality of *in vitro* studies such as [^{3}H]CP55,940 binding at CB_1 was generally high, for example, PMSF was used when appropriate. Methods used in two electrophysiology studies were controversial, and the studies were removed after urging by our manuscript reviewers. The quality of some rodent models of behavior was also questionable. Rather than judge their translational validity—a contentious issue [26]—we have named the specific behavioral assays in each study, allowing the reader to pass judgment.

Pharmaceutical drugs

Non-steroidal anti-inflammatory agents (NSAIDs). NSAIDs inhibit two cyclooxygenase (COX) enzymes, COX1 and COX2, and thereby block the conversion of arachidonic acid (AA) into inflammatory prostaglandins. Ibuprofen, ketorolac, and flurbiprofen also block the hydrolysis of AEA into arachidonic acid and ethanolamine [27]. See Figure 2. A binding site for some NSAIDs on FAAH has also been identified [28]. NSAID inhibition of COX2 blocks the metabolism of AEA and 2-AG into prostaglandin ethanolamides (PG-EAs) and prostaglandin glycerol esters (PG-GEs), respectively [29]. PG-EAs and PG-GEs *increase* the frequency of miniature inhibitory postsynaptic currents (mIPSCs) in primary cultured mouse hippocampal neurons, an effect opposite to that of their parent molecules [30].

Prostaglandin E_2 glycerol ester (PGE_2-GE), a COX2 metabolite of 2-AG, induced mechanical allodynia and thermal hyperalgesia in rat paws [31]. $PGF_2\alpha$-EA, a COX2 metabolite of AEA, was found in the spinal cord of mice with carrageenan-induced knee inflammation. $PGF_2\alpha$-EA contributed to pain perception and dorsal horn nociceptive neuron hyperactivity, thus providing a rationale for the combined use of COX2 and FAAH1 inhibitors against inflammatory pain [32].

Electrophysiology studies of rat hippocampal cells showed that meloxicam and nimesulide prolonged and increased DSI; that is to say, the COX2 inhibitors potentiated synaptic 2-AG release and CB_1 signaling [33]. Consistent with this, intrathecally applied indomethacin enhanced eCB-mediated antinociception in mice that was blocked by the CB_1 antagonist AM251 [34]. Intrathecally applied flurbiprofen produced a similar eCB-dependent antinociception in the rat formalin test [35].

Combining NSAIDs with cannabinoids (either eCBs or exogenous cannabinoids) produces additive or synergistic effects. A sub-effective dose of WIN55,212-2 became fully antinociceptive following administration of indomethacin in rats [36]. A local injection of ibuprofen plus AEA in the rat formalin test produced synergistic antinociceptive effects involving both CB_1 and CB_2 [37]. The FAAH inhibitor URB937, when coadministered to mice with indomethacin, produced a synergistic reduction in pain-related behaviors [38]. Furthermore, URB937 reduced the number and severity of gastric lesions produced by indomethacin. One contrary study showed that THC's decrease in intraocular pressure was partially blocked by indomethacin in rabbits [39].

In a small human study, the administration of indomethacin antagonized marijuana effects [40]. Yet a high dose of ibuprofen may cause sedation, possibly a cannabimimetic effect [41]. Clinical anecdotes of NSAIDs eliciting cannabimimetic effects have been reported; the individuals are usually familiar with the effects of cannabis, and usually females [42].

In summary, preclinical studies indicate that some NSAIDs inhibit FAAH and enhance the activity of eCBs, phytocannabinoids, and synthetic cannabinoids. Combinational effects may be particularly relevant at peripheral sites, such as the peripheral terminals of nociceptors.

Acetaminophen. Acetaminophen (paracetamol), following deacetylation to its metabolite *p*-aminophenol, is conjugated with AA to form *N*-arachidonoylphenolamine (NAP, *aka* AM404). It is likely that deacetylation takes place mainly in the liver, and conjugation occurs in the central nervous system. NAP blocks the breakdown of AEA by FAAH, inhibits COX1 and COX2, and acts as a TRPV1 agonist [43]. The analgesic activity of acetaminophen in rats is blocked by CB_1 or CB_2 antagonists [44,45]. Analgesic activity is also lost in $CB_1^{-/-}$ knockout mice [46]. A sub-effective dose of the synthetic cannabinoid WIN55,212-2 became effective following intracisternal adminis-

tration of acetaminophen in rats [36]. A sub-effective dose of AEA in mice became anxiolytic in the Vogel conflict test and the social interaction test when co-administered with acetaminophen; the effect was blocked by the CB_1 antagonist AM251 [47].

Small amounts of acetaminophen are also metabolized via the cytochrome P-450 pathway into *N*-acetyl-*p*-benzoquinone imine (NAPQI). Intrathecal administration of NAPQI activates TRPA1 and imparts antinociception in the mouse hot-plate test, and a similar action is found for Δ^9-tetrahydrocannabiorcol. These effects are lost in Trpa1(−/−) mice [48].

In summary, preclinical studies indicate that acetaminophen enhances the activity of eCBs and synthetic cannabinoids in rodents. Why acetaminophen fails to elicit cannabimimetic effects in humans is unknown. Acetaminophen-cannabinoid drug interactions may be species-specific; Gould *et al.* [49] demonstrated strain-specific differences in mice. They suggested that other indirect actions of acetaminophen, including 5-HT receptor agonism, may outweigh any CB_1 mediated effects in some mouse strains.

Glucocorticoids. The distribution of glucocorticoid receptors (GRs) and CB_1 overlap substantially in the central nervous system and other tissues, as do GRs and CB_2 in immune cells. Dual activation of GRs and CBs may participate in glucocorticoid-mediated anti-inflammatory activity, immune suppression, insulin resistance, and acute psychoactive effects. In a rat model of spinal nerve injury (sciatic nerve constriction with suture loops), the GR receptor agonist dexamethasone increased CB_1 density after spinal nerve injury, which suggests that CB_1 is a downstream target for GR actions [50]. Glucocorticoid administration also induced CB_1 expression in bone in mice [51] and rats [52].

The *acute* administration of glucocorticoids may shift AA metabolism toward eCB synthesis in parts of the brain. Electrophysiological studies of rat hypothalamic slices demonstrated that adding dexamethasone or corticosterone to slice baths caused a rapid suppression of synaptic activity, characterized as glucocorticoid-induced, eCB-mediated suppression of synaptic excitation (GSE). GSE was blocked by CB_1 antagonists, indicating that eCB release mediated GSE [53]. A follow-up study demonstrated that GSE correlated with increased levels of AEA and 2-AG [54]. The same group found no changes in AEA and 2-AG after exposure of cerebellar slices to dexamethasone. In hypothalamic slices, GSE could be blocked by leptin, suggesting that GSE is a nutritional state-sensitive mechanism [55]. Dexamethasone enhanced eCB-mediated GSE by inhibiting COX2 in dorsal raphe serotonin neurons [56].

Corticosterone administration increased AEA levels in several rat limbic structures (amygdala, hippocampus, hypothalamus), but not the prefrontal cortex. 2-AG levels were only elevated in the hypothalamus [57]. The same group conducted an *ex vivo* study of the rat medial prefrontal cortex (mPFC). Bath application of corticosterone to mPFC slices suppressed GABA release onto principal neurons in the prelimbic region, which was prevented by application of the CB_1 antagonist AM251 [58]. This indicates local recruitment of eCB signaling, probably through 2-AG. A previous study of rats receiving a single dose of corticosterone detected no change in 2-AG and a reduction of AEA in hippocampal homogenates [59]. Corticosterone increased hippocampal levels of 2-AG in rats; the impairment of contextual fear memory by corticosterone was blocked by the CB_1 antagonist AM251 [60].

Chronic exposure to glucocorticoids downregulates the eCB system. Chronic corticosterone administration decreased CB_1 densities in rat hippocampus [59] and mouse hippocampus and amygdala [61]. Chronic corticosterone administration in male rats led to visceral hyperalgesia in response to colorectal distension, accompanied by increased AEA, decreased CB_1 expression, and increased TRPV1 expression in dorsal root ganglia. Co-treatment with the corticoid receptor antagonist RU-486 prevented these changes [62].

In summary, preclinical rodent studies indicate that *acute* glucocorticoid administration enhances the activity of eCBs. The clinical phenomenon of acute "corticosteroid mania" may have a cannabimimetic component. *Chronic* exposure to glucocorticoids downregulates the eCB system, a scenario consistent with chronic stress, which we review below.

Opiates. Naloxone, a μ-opioid receptor antagonist, inhibited THC-induced Fos immunoreactivity in several regions of the rat central nervous system, including the ventral tegmental area, hypothalamus, caudate-putamen, and periaqueductal grey. Conversely, naloxone and THC had an additive effect on Fos immunoreactivity in the amygdala, stria terminalis, insular cortex, and paraventricular nucleus of the thalamus [63].

Short-term co-administration of morphine with THC caused an upregulation of CB_1 protein in the spinal column of rats, far greater than THC or morphine given alone [64]. A rodent study of chronic but voluntary intake of opiates (rats self-administering heroin) enhanced [^{3}H]CP55,940 binding in the amygdala and ventral tegmental area, plus a marked increase in cannabinoid-stimulated [^{35}S]GTPγS binding in the nucleus accumbens, caudate putamen, and amygdala [65]. Superperfusion of *ex vivo* rat nucleus accumbens slices with 4-aminopyridine and NMDA released glutamate and GABA, respectively, and either morphine or the CB_1 agonist HU210 predictably inhibited these responses. Combining HU210 and morphine caused a synergistic inhibition of GABA release, but a non-additive response in glutamate release [66].

Chronic morphine exposure in rats caused a reduction in hippocampal and cerebellar CB_1 density measured with [^{3}H]CP55,940, and a strong reduction in CP55,940-stimulated [^{35}S]GTPγS binding; 2-AG contents were also reduced [67]. Another rat study showed that chronic morphine exposure caused variable, regionally-specific modulations in [^{3}H]CP55,940 binding and CB_1 mRNA levels; CB_1 upregulated in some regions and dowregulated in other regions [68]. In human CB_1-transfected HEK293 cells, morphine induced a desensitization of the μ-opioid receptor and heterologous desensitization of CB_1, demonstrated by a reduction in WIN55212-2-induced $[Ca^{2+}]_i$ release [69]. μ-opioid receptor knockout mice showed a dramatic reduction in WIN55212-2-stimulated [^{35}S]GTPγS binding [70]. In human SH-SY5Y neuroblastoma cells, sequential activation of CB_1 and δ-opioid receptor produced synergistic elevations of intracellular Ca^{2+}, a response that each receptor alone did not trigger in an efficacious way [71].

In behavioral studies, heroin reinstated "drug-seeking" behavior for WIN55,212-2 in rats [72]. Morphine did the same for THC in monkeys [73]. The rewarding effects of THC, measured by conditioned place-preference, were reversed by naloxone in rats [74]. In rats trained to discriminate THC, morphine administration markedly potentiated the THC discriminative stimulus [75]. Morphine or codeine potentiated THC-induced antinociception and analgesia in mice and rats [76–79]; inactive doses of the drugs in combination produce potent, synergistic analgesia [80]. Synergistic analgesia was confirmed in an isobolographic analysis [64]. Historically this is the first isobolographic analysis of a cannabinoid since the days Walter Siegfried Loewe, who invented the isobologram to test drug combinations for synergy [81]. Loewe demonstrated synergy generated by cannabis extracts combined

Table 1. Effects of PUFA supplementation upon eCB levels.

Supplemented PUFA	assay; result compared to unsupplemented controls[1]	reference
DHA+AA	*in vivo* piglets, whole brain homogenates; ↑ AEA, ≈2-AG	[137]
AA	*in vivo* mice, whole brain homogenates; ↑ AEA	[137]
DHA	*in vivo* mice, whole brain homogenates; ↓ 2-AG	[325]
AA	*in vivo* mice, whole brain homogenates; ↑ 2-AG	[325]
DHA	*in vitro* mouse 3T3-F442A adipocytes; ↓ 2-AG, ↓ AEA	[326]
AA	*in vitro* mouse 3T3-F442A adipocytes; ↑ 2-AG	[326]
DHA+EPA	*in vivo* rats, whole brain homogenates; ≈AEA, ≈2-AG	[327]
or AA	*in vivo* rats, jejunum homogenates; ↑ AEA, ↑ 2-AG	
DHA+EPA	*in vivo* Zucker rats, visceral adipose tissue; ↓ ↓ 2-AG, ↓ AEA	[142]
DHA+EPA	*in vivo* Zucker rats, whole brain homogenates; ↓ 2-AG, ≈AEA	[143]
DHA+EPA	*in vivo* rats; serum: ↓ ↓ AEA, ↓ 2-AG; brain: ↓ AEA, ≈2-AG	[133]
DHA+EPA	*in vivo* obese humans; serum: ↓ 2-AG, ≈AEA	[144]
DHA+EPA	*in vivo* mice; liver: ↓ AEA, ≈2-AG; brain: ↓ AEA	[131]

[1] ↑, increase; ↓, decrease; ≈, no change;
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with other drugs [82,83], as well as synergy generated amongst the individual components within cannabis itself [84,85].

Normal men subjected to a thermal pain stimulus did not experience analgesia from a low dose of nabilone (a synthetic THC analogue), or a low dose of morphine. But co-administration of the drugs produced an analgesic effect [86].

Endorphins (endogenous opioids) enhance the effects of cannabinoids: Administering a low dose of THC to rats produced an anxiolytic response in the light-dark box test, which was abolished by beta-funaltrexamine, a μ-opioid receptor antagonist [87]. In rats trained to discriminate THC, microinjection of β-endorphin into the ventral tegmental area potentiated the THC discriminative stimulus [75]. Enkephalins (endogenous opioids) also enhance the effects of THC: the inhibition of encephalin-degrading enzymes augmented THC-induced antinociception in mice, an effect blocked by either rimonabant or naloxone [88]. Naltrexone, a μ- and κ-opioid receptor antagonist, significantly increased many of the "positive" subjective effects of oral THC [89] and smoked cannabis [90] in marijuana smokers. These results suggest that endogenous opioids contribute to the effects of cannabis.

In summary, preclinical studies and clinical trials indicate that *acute* opiate administration enhances the activity of eCBs, phytocannabinoids, and synthetic cannabinoids. Acute opiates may also upregulate CB_1 expression. *Chronic* opiate administration, however, may have a deleterious effect on the eCB system.

Antidepressant drugs. Serotonin selective uptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) are the most commonly prescribed antidepressant drugs. Treatment with fluoxetine, the archetypal SSRI, potentiated THC-induced hypothermia in rats [91], but did not change THC-induced behavioral effects—freezing behavior, social interaction or exploration, and preference for outer or inner zones [92]. Fluoxetine increased CB_1 binding density in the prefrontal cortex, without altering AEA or 2-AG levels in rat brains [93]. Chronic fluoxetine also increased WIN55212-2-stimulated [^{35}S]GTPγS binding in the rat prefrontal cortex [94]. Conversely, treatment with citalopram reduced HU210-stimulated [^{35}S]GTPγS binding in the rat hypothalamus and hippocampus [95].

Treatment with fluoxetine prevented synaptic defects in mice induced by chronic unpredictable stress (the CUS protocol included inversion of day/night light cycle, 45° tilted cage, cage rotation, tube restraint, predator sounds, strobe lights, food and water deprivation, cold environment, and wet bedding), and CUS preserved eCB- and WIN55,212-2-stimulated CB_1 signaling [96]. In the hands of Mato *et al.* [97], fluoxetine in rats enhanced the inhibition of adenylyl cyclase by WIN55212-2, but did not alter WIN55212-2-stimulated [^{35}S]GTPγS binding or CB_1 density measured with [^{3}H]CP55,940. They proposed that fluoxetine enhanced WIN55212-2 signaling through Gαi2 and Gαi3 subunits and not through Gαo subunits.

Treatment with the TCA desipramine increased CB_1 binding density in the hippocampus and hypothalamus, without significantly altering AEA or 2-AG levels in rat brains [98]. The CUS protocol altered CB_1 density in rat brains, and these changes were attenuated by concurrent treatment with imipramine [99]. Desipramine-induced weight gain was reduced by cotreatment with SR141716A, suggesting an eCB pathway [100].

Treatment with the MAOI tranylcypromine increased CB_1 binding density in the prefrontal cortex and hippocampus, and increased 2-AG but decreased AEA levels in the prefrontal cortex [93]. Repeated electroconvulsive shock treatment (EST) for depression produced complex and regionally specific effects. Generally EST downregulated CB_1 binding density and AEA levels in the cortex, but enhanced cannabinoid-stimulated [^{35}S]GTPγS binding in the amygdala [101].

In summary, the effects of antidepressant drugs or treatments upon the eCB system are not definitive, but likely result in CB_1 upregulation, at least in some brain regions. Preclinical studies suggest agonist trafficking may be responsible for variable responses.

Antipsychotic drugs. First-generation antipsychotic drugs, such as haloperidol and chlorpromazine (thorazine), are dopamine D_2 receptor inverse agonist. Second-generation "atypical" antipsychotics (*e.g.*, risperidone, olanzapine, clozapine, and aripiprazole) antagonize D_2 and 5-HT_{2A}, and also target other neuroreceptors. Acute administration of chlorpromazine enhanced the hypothermic response to THC [102]. Subchronic administration of haloperidol increased CB_1 density in rat brains, indicated by

Table 2. Effects of short- and long-term caloric restriction upon the brain eCB system in animal studies.

species, exercise	measure	reference
rats administered leptin	leptin (appetite-reducing hormone) decreases hypothalamic AEA and 2-AG levels	[6]
rats fasted	fasting for 24 h increased AEA and 2-AG in limbic forebrain and 2-AG in hypothalamus;	[328]
mice fasted	time-dependent effects: short-term fasting (24 h) increased hypothalamic 2-AG; long-term fasting (12 d) decreased hypothalamic 2-AG	[329]
goldfish fasted	food restriction decreased CB_1 mRNA in the forebrain and increased AEA levels in the telencephalon, two effects reversed by refeeding	[330,331]
rats after gastric bypass	weight loss after Roux-en-Y gastric bypass surgery decreased AEA and with no change in 2-AG levels in skeletal muscle	[332]
Zucker obese rats fasted	fasting decreased CB_1 mRNA in brainstem but not in hypothalamic nuclei	[333]

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increased binding of [^{3}H]CP55,940 in the substantia nigra>globus pallidus>striatum. Subchronic haloperidol also potentiated CP55,940-stimulated [^{35}S]GTPγS binding in the substantia nigra [103]. Sundram *et al.* [104] confirmed haloperidol's effects on [^{3}H]CP55,940 binding, and obtained similar results with chlorpromazine and olanzapine. In monkeys trained to discriminate THC, haloperidol sensitized the THC discriminative stimulus [105]. Risperidone increased [^{3}H]CP55,940 binding in rat brain without altering CB_1 mRNA levels [106]. Four weeks of aripiprazole upregulated CB_1 in rat frontal cortex [107]. Clozapine decreased [^{3}H]CP55,940 binding in rat brain [104], and attenuated THC-induced disruption of spatial working memory in the rat radial maze task [108].

Several researchers have proposed that CB_1 upregulation during antipsychotic drug treatment may explain appetite enhancement, weight gain, and CB_1 supersensitivity. D'Souza *et al.* [109] conducted a double-blind study on the effects of adding haloperidol to THC. Compared to THC alone, the combination of drugs significantly worsened verbal recall, distractibility, and vigilance scores. The drug combination did not affect other testing parameters, such as euphoric effects and motor outcomes. Another double-blind study showed that haloperidol reversed THC-induced increases in the Positive and Negative Syndrome Scale (used for measuring symptom severity in schizophrenia), but did not affect the THC-induced "high" in healthy male volunteers [110]. A double-blind study in healthy male volunteers showed that olanzapine reduced the effects of THC as measured on the positive and negative syndrome scale, and the visual analogue scale for psychedelic effects, but the reduction fell short of statistical significance, $p = 0.67$ [111].

In summary, antipsychotic drugs likely upregulate CB_1 expression in parts of the rodent brain. In human clinical studies, antipsychotic drugs do not affect THC-induced "high" or "euphoria," but dampen dysphoria and worsen verbal recall and distractibility.

Anxiolytics, sedatives, and anesthetics. Diazepam is used for treating anxiety, insomnia, muscle spasms, and seizure disorders. Combining diazepam with WIN55212-2 produced a supra-additive anxiolytic effect in the rat elevated plus maze test; combining diazepam with the FAAH inhibitor URB597 also led to a supra-additive effect; coadministration of diazepam with the CB_1 antagonist AM251 attenuated diazepam's anxiolytic effect [112]. These findings might be explained by the observation that both chronic and, particularly, acute administration of diazepam to mice is accompanied by strong elevation of brain eCB levels [113].

The anxiolytic and sedative effects of alprazolam were also attenuated by AM251 in mouse behavioral assays (light-dark box test, neurological severity score, and step-down inhibitory avoidance test) [114]. Surprisingly, however, the administration of alprazolam reduced WIN55212-2-stimulated [^{35}S]GTPγS binding in mouse amygdala and hippocampus [114]. $CB_1^{-/-}$ knockout mice showed impaired anxiolytic responses to both buspirone and bromazepam in light/dark box, elevated plus maze, and social interaction tests [115]. N-arachidonoyl-serotonin (AA-5-HT), a dual FAAH/TRPV1 blocker, imparted anxiolytic effects in the mouse elevated plus maze assay [116].

A sub-effective dose of THC given to mice caused catelpsy in the horizontal bar test after sub-effective doses of either flurazepam or baclofen were added [117]. The beta-adrenergic blocking agent propranolol causes mild sedation, but pretreatment with propranolol blocked cannabis-induced cardiovascular effects and learning impairment in a small clinical trial [118].

General anesthesia (midazolam, sufentanil, isoflurane, and sufentanil) resulted in decreased serum AEA in patients stressed by the anticipation of cardiac surgery [119]. The dissociative anesthetic phencyclidine (PCP) impairs rotarod performance and open-field behavior in rats, effects shared by THC; combining the two caused supra-additive results [120]. Low-grade Mexican marijuana was adultered with PCP and marketed as "superweed" in the 1970s [121]. Nitrious oxide and THC both increase pain threshold in the tail-flick and hot-plate test, and their combination caused supra-additive effects [122].

Anticonvulsants. Combining diazepam with WIN55212-2 produced a supra-additive anticonvulsant effect in rats; combining diazepam with the FAAH inhibitor URB597 also led to a synergistic effect; coadministration of diazepam with the CB_1 receptor antagonist AM251 attenuated the anticonvulsant effect of diazepam [123]. Chronic administration of valproate in rats increased CB_1 binding of the PET scan tracer [^{18}F]MK-9470; this was not seen with levetiracetam [124]. Tiagabine, an anticonvulsant GABA reuptake inhibitor, augmented THC-induced catalepsy [117] but not antinociception or hypothermia [125]. In a human study, tiagabine augmented THC discrimination and enhanced THC effects in other outcomes [126].

Pregabalin is a Ca^{2+} channel antagonist used for treating epilepsy and neuropathic pain. Isobolographic analysis demonstrated that combining WIN 55,212-2 with pregabalin exerted synergistic antinociceptive effects in the mouse hot-plate test [127]. Vagus nerve stimulation (VNS) is used as an add-on treatment to patients with drug-resistant epilepsy. Implantation of a vagus nerve stimulator in rats significantly decreased AEA and 2-AG in mesenteric adipose tissue, but increased PEA [128]. Chemical VNS by administration of the peptide hormone cholecystokinin 8

to fasted rats decreased expression of CB_1 in vagal afferent neurons [129].

Complementary and alternative medicine

Dietary supplements: PUFAs. Polyunsaturated fatty acids (PUFAs) play fundamental roles in many cellular and multicellular processes, including inflammation, immunity, and neurotransmission. They must be obtained through diet, and a proper balance between omega-6 (ω-6) PUFAs and ω-3 PUFAs is essential. The typical Western diet contains a surfeit of ω-6s and a deficiency of ω-3s [130].

Arachidonic acid (AA) is the archetypical ω-6, with 20 carbons and four double bonds (20:4ω-6). Some of its metabolites cause chronic diseases seen in Western populations: prostaglandins cause pain and swelling, and leukotrienes cause bronchoconstriction and asthma. The inflammatory metabolites of AA are countered by dietary ω-3s. The two best-known ω-3s are eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3).

eCBs are derived from AA (see Figure 2). Several preclinical studies showed that dietary supplementation with AA increased serum levels of AEA and 2-AG, summarized in Table 1. Although we clearly need AA to biosynthesize eCBs, *excessive* levels of AA, administered chronically, may lead to excessive levels of eCBs. This in turn may lead to desensitized and downregulated CB_1 and CB_2 receptors. Linoleic acid, an 18:2ω-6 PUFA, is converted into AA, and it elevated 2-AG and AEA levels and induces obesity in mice [131].

Dietary supplementation with ω-3s predictably increased the concentration of EPA and/or DHA in tissues, cells, and plasma, and decreased the relative concentration of AA in tissues, cells, and plasma [132,133]. ω-3 supplementation also decreased AEA and 2-AG in tissues, cells, and plasma (Table 1). However, the effects of ω-3 supplementation are nuanced and complex:

Piscitelli *et al.* [134] fed mice a high-fat diet (cholesterol and saturated fatty acids) with little AA. This diet caused a *decrease* in AEA and 2-AG in the liver. Supplementing that diet with DHA and EPA *increased* AEA and 2-AG in the liver. In contrast, the high-fat diet increased AEA and 2-AG in muscle tissue, and supplementation with krill oil *decreased* AEA and 2-AG. Similar trends were seen in heart, kidneys and white adipose tissue.

Adequate levels of dietary ω-3s are required for proper eCB signaling. Mice supplemented with ω-3s, compared to mice on a control diet, expressed greater levels of CB_1 and CB_2 mRNA. Mice supplemented with ω-3s also expressed greater levels of eCB synthetic enzymes—NAPE-PLD, DAGLα, and DAGβ [132]. Supplementation with ω-3s also modulated the concentrations of "entourage compounds" such as PEA and OEA [133,134].

In apparent contrast with the above findings, Lafourcade *et al.* [135] showed that ω-3 *deficiency* abolished eCB-mediated neuronal functions. They reasoned that lifelong ω-3 deficiency causes chronically elevated eCB levels within brain synapses, which leads to CB_1 desensitization. They tested a rodent model of depression-like behavior (the forced-swim test), and ω-3-deficient mice performed like $CB_1^{-/-}$ knockout mice. The administration of WIN55212-2 did not change their behavior, whereas in ω-3-rich mice, WIN55212-2 imparted typical cannabimimetic effects. Larrieu *et al.* [136] demonstrated depressive-like symptoms in ω-3-deficient mice compared to mice fed an ω-3 enriched diet. They used the forced-swim test as well as the more valid open-field and social-investigation tests. Mice deficient in ω-3 showed impairment in the CB_1 signaling pathway—ERK1/2 phosphorylation in the hippocampus was reduced after treatment with WIN55212-2, and the antianxiety effects of WIN55212-2 were absent in ω-3-deficient mice.

ω-3 PUFAs may impact the eCB system via a second mechanism: eCB biosynthetic enzymes readily accept ω-3s as substrates. An ω-3-rich diet markedly elevated the *N*-acyl-ethanolamide metabolite of DHA, called DHEA, the *N*-acyl-ethanolamide metabolite of EPA, called EPEA, and the *sn*-2-glycerol-ester metabolite of EPA, called 2-EPG [133,137]. FAAH catabolized DHEA [138,139]. DHEA and EPEA act as eCBs: DHEA and EPEA showed high binding affinity for CB_1 ($K_i = 124$ and 55 nM respectively) and acted as partial agonists [139]. Their affinity nearly equals that of AEA—a meta-analysis of affinity studies using the same binding assay (mouse brain, [^{3}H]CP55940 displacement, presence of PMSF) produced a modal K_i value of 61 nM for AEA [22]. DHEA, *aka* synaptamide, stimulates neurite growth and synaptogenesis in developing hippocampal neurons [140].

In natural fish oil, DHA and EPA are esterified in triacylglycerides (TAG), whereas in many fish oil capsules, DHA and EPA are esterified in EE (ethyl-ester) or TAG (rTAG). Krill oil contains DHA and EPA esterified in phospholipids, primarily phosphatidylcholines, which may improve their bioavailability; furthermore krill oil contains less AA than fish oil [141]. Batetta *et al.* [142] supplemented the diet of obese Zucker rats with fish oil or krill oil, which contained nearly identical amounts of EPA and DHA. The visceral adipose tissue of krill oil-supplemented rats contained less AEA and 2-AG than fish oil-supplemented rats. In the liver only AEA levels were significantly less. The effects of these dietary sources of DHA and EPA on brain eCB levels were much less pronounced, with krill oil producing only a small decrease of 2-AG levels [143]. The same research group reported similar results in an obese cohort mostly composed by women: krill oil but not fish oil significantly decreased serum 2-AG levels; no significant changes were seen in normo-weight subjects [144]. In a yet unpublished study, one of us observed that in obese men, dietary krill oil reduced plasma AEA levels and concomitantly counteracted hypertriglyceridemia (Di Marzo, unpublished data).

In summary, dietary ω-3s seem to act as homeostatic regulators of the eCB system. In obese rodents fed a high-AA diet, ω-3s significantly decrease eCBs, especially 2-AG, particularly in tissues that become dysregulated, such as adipose and liver tissues. Plasma eCB levels are reduced by krill oil also in obese humans. Little change in eCB levels are seen in normo-weight individuals not fed a high ω-6 diet, and dietary ω-3s are required for proper eCB signaling.

Dietary supplements: Probiotics. "Probiotics" are endosymbiotic microorganisms that confer a health benefit upon their human hosts. Probiotics occur in fermented foods, such as yogurt and kimchi. The best known organisms are *Lactobacillus acidophilus* and *Bifidobacterium* species. "Prebiotics" such as oligofructose are carbohydrates that serve as substrates for probiotic organisms. Human intestinal epithelial cells incubated with *L. acidophilus* produced more CB_2 mRNA [145]. Feeding *L. acidophilus* to mice and rats increased the expression of CB_2 mRNA in colonic epithelial cells. Lastly, mice fed *L. acidophilus* showed less pain behavior following colonic distension with butyrate than control mice, an effect reversed by the CB_2 antagonist AM630 [145].

Probiotics and prebiotics also modulate CB_1 expression. Acute probiotic treatment with *Enterococcus faecium* upregulated CB_1 mRNA in *Solea solea* [146]. Pathologically obese *ob/ob* mice expressed elevated levels of colon CB_1 mRNA [147]. When fed prebiotics such as oligofructose, they expressed less CB_1 mRNA, produced less AEA (due to increased FAAH mRNA expression in adipose tissue), and gained less fat mass.

Other dietary considerations. A natural phosphate derivative of vitamin E, α-tocopheryl phosphate (α-TP), is a common

Table 3. Effects of exercise upon the eCB system in rodent studies.

species, exercise	measure	reference
rats, forced swimming for 1 h/d×6 months	decreased CB_1 antibody expression in adipocytes	[334]
rats, voluntary wheelrunning, 24 h	running reversed chronic stress-induced deficits in GABAergic synapses to CB_1 stimulation by eCBs and HU210	[208]
mice, voluntary wheel running, 42 d	running rescued the sensitivity of striatal GABA synapses to CB_1 stimulation downregulated by EAE induction	[335]
mice, voluntary wheel running for 15 d	sensitivity of striatal GABA synapses to CB_1 stimulation increased	[336]
rats, forced treadmill running for 40 d	reduced CB_1 expression in the striatum and hippocampus	[337]
rats, voluntary wheel running for 8 d	increased CB_1 expression in the hippocampus, increased CB_1-mediated GTPγS binding, and increased AEA content in hippocampus	[338]
mice, voluntary wheel running for 10 d	increased CB_1 expression in the hippocampus	[321]
rats, forced treadmill running for 40 d	no change in gene expression of CB_1, CB_2, or FAAH in liver	[339]

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constituent in plant and animal tissues. Although α-TP does not bind to CB_1, it modulates synaptic transmission in rodent hippocampus slices, an effect blocked by the CB_1 antagonist AM251 [148].

Human breast milk contains small amounts of AEA and high levels of 2-AG, but the biological significance of this is not known [149]. The oral administration of AEA (300 mg/kg), OEA (200 mg/kg) and especially 2-AG (400 mg/kg) in rats produces calming properties [150]. Mouse breast milk also contains eCBs, and when newborn mice are fed the CB_1 antagonist SR141716A, they stop suckling and die [151].

Pesticides such as chlorpyrifos and diazinon alter normal eCB system function [152,153]. We hypothesize that eating organic foods lacking pesticide residues may promote eCB homeostasis. Piperonyl butoxide, which is a synergist added to insecticides such as pyrethrum, is an efficacious but low-potency antagonist of CB_1 [154]. Phthalates are plasticizers added to water bottles, tin cans, food packaging, and even the enteric coating of pharmaceutical pills. Phthalates may act as endocrine disruptors and carcinogens. They also block CB_1 as allosteric antagonists [155].

Herbal remedies. Some plants besides *Cannabis* produce vaguely cannabimimetic effects. Copal incense, extracted from *Protium* species (same plant family as *Boswellia*) contains a pentacyclic triterpene with high affinity for CB_1 and CB_2 [156]. Absinthe contains thujone, a constituent of wormwood, *Artemisia absinthium*. Thujone has weak affinity for CB_1 [157]. Pristimerin, an alkaloid found in khat, *Catha edulis*, acts as a potent inhibitor of MAGL ($IC_{50} = 93$ nM) and causes an elevation of 2-AG levels in rat cortical neurons [158]. Salvinorin A in *Salvia divinorum* produces CB_1-mediated effects in the gastrointestinal tract of rodents. Salvinorin A primarily acts as a kappa-opioid receptor agonist and is inactive as a ligand for CB_1 and CB_2 [159]; it may interact with a putative CB_1-kappa-opioid receptor heterodimer [160].

Flavonoids such as biochanin A (from red clover, *Trifolium pratense*), genistein (from soybean, *Glycine max*), and kaempferol (from tea, *Camelia sinensis*, and many other plants) exert modest inhibition of FAAH in the low micromolar range [161]. Cyanidin and delphinidin, two anthocyanidins found in a wide range of plants, have micromolar affinities for CB_1 [162]. Epigallocatechin-3-O-gallate, the most abundant catechin in tea, also has micromolar affinities for CB_1 [163].

Yangonin, a kavalactone extracted from kava, *Piper methysticum*, exhibits affinity for CB_1 with a $K_i = 0.72$ μM [164]. Curcumin, extracted from curry powders, elevates eCB levels and brain nerve growth factor (NGF) in a brain region-specific fashion, and pretreatment with CB_1 antagonist AM4113 blocks this effect [165]. A study suggested that curcumin and resveratrol could bind to CB_1, but the study was retracted [166].

Compounds with phytocannabinoid-like moieties have been extracted from legumes [167,168], *Helichrysum* [169], *Rhododendron* sp. [170], liverworts [171,172], and fungi [173–175]. Falcarinol is a skin irritant found in several plants that causes contact dermatitis. It covalently binds with the CB_1 receptor, causing potent inverse agonistic and pro-inflammatory effects in human skin [176].

Higher plants (angiosperms and gymnosperms) produce PUFAs with acyl tails limited to 18 carbons in length [177]. Hence reports of PUFAs in plants with longer acyl tails, such as AA, AEA, and 2-AG are controversial. Di Tomaso *et al.* [178] detected AEA in chocolate and cocoa powder derived from *Theobroma cacao*. A subsequent study showed very little, if any, AEA in cocoa powder [149]. Nakane *et al.*[179] reportedly extracted sciadonic acid (20:3ω-6) from seeds of a pine tree, *Sciadopitys verticillata*. This analog of 2-AG exhibited cannabimimetic activity in NG108-15 cells expressing CB_1.

Unlike higher plants, non-vascular plants such as club mosses, mosses, and algae express Δ^6-elongase enzymes, so they are capable of producing PUFAs with longer acyl tails [177]. Semiplenamide A, an AEA-like PUFA isolated from a blue-green alga, *Lyngbya semiplena*, has micromolar affinity for CB_1 and also blocks the AEA transporter, thereby inhibiting AEA breakdown [180]. Grenadamide, a PUFA in *Lyngbya majuscula*, has micromolar affinities for CB_1 [181]. Soderstrom *et al.* [182] extracted but did not identify an eCB-like compound from *L. majuscula*. Soderstrom also extracted a dozen eCB-like PUFAs from unidentified green algae (Chlorophyta), the brown alga *Laminaria angustata*, and the sponge *Mycale micracanthoxea*.

Some plant ligands bind to CB_2 and modulate the immune system, but have no affinity for CB_1 and do not elicit psychoactivity. Alkamides from *Echinacea* species bind to CB_2 with nanomolar affinity, and act as CB_2 agonists with immunomodulatory effects [183]. Several constituents from *E. purpurea* root and herb produce synergistic, pleiotropic effects—they bind to CB_2 as well as inhibit AEA uptake [184]. Other constituents from *Echinacea purpurea* act as weak CB_1 antagonists [185].

The principal terpenoid in black pepper, (E)-β-caryophyllene (BCP), binds to CB_2 with nanomolar affinity and acts as an agonist. Its anti-inflammatory effects are reduced in CB_2 knockout

Table 4. Effects of chronic or subchronic ethanol upon eCB levels.

species, assay	result compared to controls[1]	reference
human neuroblastoma cell line	↑ [^{3}H]AEA	[340]
rat cerebellar granule neurons	↑ [^{3}H]2-AG	[341]
rat, oral administration	↑ AEA limbic forebrain, ↓ AEA+2-AG midbrain	[258]
rat cerebellar granule neurons	↑ [^{3}H]AEA via ↓ AEA transport and ≈FAAH	[342]
mouse, ethanol vapor inhalation	↑ AEA cortex via ↓ FAAH	[252]

[1] ↑, increase; ↓, decrease; ≈, no change; assay; result compared to unsupplemented controls.
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mice [186]. The protective effects of BCP on colitis in mice are reversed by the CB_2 antagonist AM630 [187]. The protective effects of BCP on cisplatin-induced nephrotoxicity in mice are absent in CB_2 knockout mice [188]. Lastly, the antinociceptive effect of BCP in mice is prevented by pretreatment with AM630 [189].

Rutamarin in *Ruta graveolens* has micromolar affinity for CB_2 [190]. An unidentified constituent in noni fruit, *Morinda citrifolia*, shows weak affinity for CB_2 [191]. The aromatic resin extracted from mastic, *Pistacia lentiscus*, contains an essential oil (EO) rich with monoterpenoids and sesquiterpenoids. Rats fed mastic EO showed higher plasma levels of DHA, EPA, PEA, and OEA than control rats, with no change in AEA or 2-AG [192].

Shellfish are not herbal remedies, but they have been used medicinally. AEA and/or 2-AG have been isolated from the mussel *Mytilus galloprovincialis*, the clam *Tapes dicussatus*, the oyster *Crassosterea* sp. [193], the sea urchin *Paracentrotus lividus* [194], and the sea squirt *Ciona intestinalis* [195].

Mind and body medicine: chronic stress. Chronic or repeated stress results in a chronic elevation of endogenous corticosterone via the hypothalamic-pituitary-adrenocortical (HPA) axis. Chronic stress (repeated restraint) reduced AEA levels throughout the corticolimbic stress circuit in rodents [99,196,197]. In contrast, 2-AG levels decrease or increase, depending upon the nature of the stressor: Hill *et al.* [198] found reduced 2-AG content within rat hippocampus following the CUS protocol. But in the hypothalamus and midbrain, 2-AG increased in the same testing paradigm [99]. Elevations in 2-AG appear after chronic restraint stress within the amygdala [196,199], hypothalamus [200], and medial prefrontal cortex [58].

CB_1 expression decreased in rat hippocampus following the CUS protocol [198], whereas CB_1 expression increased in the prefrontal cortex in the same testing paradigm [99]. The same paradigm decreased hippocampal CB_1 expression in male rats, but increased CB_1 expression in female rats [201]. Social isolation stress decreased CB_1 density in the supraoptic nucleus of rats [202]. Immobilization/acoustic stress increased CB_1 mRNA and protein expression in the prefrontal cortex of mice [203]. A chronic mild stress protocol (subjecting rats to cage soiling with water, group housing in a confined space, water and/or food deprivation, intermittent lighting, reversal of light/dark cycle, cage tilting to 45°, exposure to loud white noise and strobe lights) increased CB_1 mRNA in the prefrontal cortex and decreased CB_1 in the midbrain [204].

Adult rats exposed to chronic restraint stress increased CB_1 binding of [^{3}H]CP55,940 in the prefrontal cortex (PFC) with a decrease in the hippocampus. A 40-day recovery period resulted in normalization of CB_1 in the PFC, and a pronounced upregulation of CB_1 density in the hippocampus, possibly indicative of a rebound effect. Adolescent rats did not show any change in hippocampal CB_1 density, but exhibited an upregulation in both the PFC and amygdala. They also exhibited a rebound in the hippocampus after 40 days [205].

Chronic water avoidance stress in male rats increased serum corticosterone levels and visceral hyperalgesia in response to colorectal distension, accompanied by increased AEA, decreased CB_1 expression, and increased TRPV1 expression in the dorsal root ganglia [62]. Co-treatment with the corticoid receptor antagonist RU-486 prevented these changes [206]. Seven daily sessions of social defeat stress in mice decreased AEA levels in the hypothalamus and hippocampus, but not in the striatum or the frontal cortex; 2-AG levels increased after the last, but not the first, session in the hypothalamus, hippocampus, and frontal cortex [207]. Fear expression after the sessions was prolonged in mice receiving rimonabant and in $CB_1^{-/-}$ knockouts. Conditional knockouts lacking CB_1 in two defined neuronal subpopulations—glutamatergic neurons and GABAergic neurons—indicated that the former CB_1 subpopulation was responsible for the fear responses.

Electrophysiological studies confirm the effects of chronic stress upon the eCB system: Chronic social defeat stress in mice (exposure to aggression) impaired GABAergic synapse sensitivity to eCBs (probably 2-AG) mobilized by group I metabotropic glutamate receptor stimulation [208]. The CUS protocol attenuated eCB-mediated DSE, LTD, and depression of field excitatory postsynaptic potentials [96]. Chronic restraint stress attenuated eCB-mediated DSI in rat hippocampus [209]. These chronic stressors also desensitized CB_1 to exogenous cannabinoids: they reduced electrophysiological responses to HU210 in mouse striatum [208], and to WIN55,212-2 in mouse striatum [96]. Chronic immobilization stress in rats impaired retrograde eCB signaling at GABAergic synapses, and a functional downregulation of CB_1 in the paraventricular nucleus of the hypothalamus [210].

Acute restraint challenge in rats induces corticosterone release in the paraventricular nucleus of the hypothalamus (PVN). This is inhibited by dexamethasone, a response blocked by the CB_1 antagonist AM251—suggesting that fast feedback requires local release of eCBs. Indeed, PVN content of 2-AG is elevated by the restraint challenge [200].

Acute footshock stress increased 2-AG and AEA levels in the periaqueductal gray and contributed to stress-induced analgesia (SIA) in male rats. SIA enhancement by a MAGL inhibitor and not by a FAAH inhibitor indicated that 2-AG was the primary eCB responsible for SIA [211]. SIA was modulated via CB_1 receptors in the basolateral nucleus of the amygdala (BLA); microinjection of SR141716A into the BLA suppressed SIA [212]. Glucocorticoid enhancement of memory consolidation in the acute footshock stress is dependent upon CB_1 activation in male rats; WIN55,212-2 infused into the amygdala enhances memory in an inhibitory avoidance apparatus, and AM251 impairs the

response [213]. Acute handling stress in male newts increased serum cortisol levels and induced behavioral changes (less sexual behavior); the latter was blocked by a cannabinoid antagonist, AM281, indicating dependence upon CB_1 activation [214].

Acute restraint stress in *male* rats increases hippocampal content of 2-AG and enhanced eCB-dependent modulation of GABA release measured by whole-cell voltage clamp of inhibitory post-synaptic currents (IPSCs) in hippocampal CA1 cells [215]. Responses in *female* rats are much more complex, because eCB levels fluctuate across the estrous cycle [216]. The eCB system has been implicated in cycle-dependent changes in pressure pain thresholds in human females [217].

In summary, chronic stress impairs the eCB system, via decreased levels of AEA and 2-AG. Changes in CB_1 expression are more labile. Stress management may reverse the effects of chronic stress on eCB signaling, although few studies exploring this possibility have been performed to date. Clinical anecdotes suggests that stress-reduction techniques, such as meditation, yoga, and deep breathing exercises impart mild cannabimimetic effects [218].

Rossi *et al.* [208] found that mice given access to a running wheel recovered their chronic stress-induced synaptic defects. Accordingly, social play in rats increased CB_1 phosphorylation (a marker of CB_1 activation) in the amygdala and enhanced AEA levels in the amygdala and nucleus accumbens [219]. The effects of exercise on the eCB system are elaborated below. Grooming behavior, which is a stress-reduction behavior in rodents, increased in response to SR141716A administration [220].

Mind and body medicine: acupuncture. Acupuncture reduced stress-related behavior (from maternal separation in rats) and normalized HPA-induced corticosterone release [221]. Electroacupunture (EA) reduced thermal hyperalgesia and mechanical allodynia induced by an injection of complete Freund's adjuvant into rat paws. EA increased AEA levels in skin tissue. The antinociceptive effects of EA were attenuated by the CB_2 antagonist AM630, but not by the CB_1 antagonist AM251 [222]. Moreover, EA upregulated the expression of CB_2 receptors in skin tissues [223]. It appears likely that CB_2 activation in the skin stimulates the release of β-endorphin, which then acts on peripheral μ-opioid receptors to inhibit nociception [224].

However, CB_1 may play a role in the *central* effects of EA: rats treated with EA showed reduced GABA levels in the ventrolateral periaqueductal gray, an effect reversed by CB_1 blockade with AM251 [225]. Enhanced activation of epsilon protein kinase C in rat brain by EA was reversed by CB_1 blockade with AM251 and not by CB_2 blockade with AM630 [226].

Mind and body medicine: body-based practices. Massage and osteopathic manipulation of asymptomatic participants increased serum AEA 168% over pretreatment levels; mean OEA levels decreased 27%, and no changes occurred in 2-AG. Participants receiving sham manipulation showed no changes [218]. Osteopathic manipulation of participants with low back pain increased serum PEA 1.6-fold over pretreatment levels, with no change in AEA. Participants receiving sham manipulation showed no changes [227].

Lifestyle modifications

Diet and weight change. Dozens of animal studies and human cohort studies have shown that diets rich in fats and sugars alter levels of AEA, 2-AG, their metabolic enzymes, and CB_1. The reverse causality is also true—many studies show that CB_1 agonists stimulate the consumption of fat and sugar. The rewarding properties of palatable foods are attenuated by CB_1 blockade and in $CB_1^{-/-}$ knockouts. Stimulation of feeding behavior by CB_1 agonists occurs across the phylogenetic scale, from humans to *Hydra*, although there is no molecular evidence for CB_1 orthologs in invertebrates other than the boneless chordates *Ciona intestinalis* and *Branchiostoma floridae*. Reviews on this topic are available [7,228,229], which we do not intend to duplicate here.

Upregulation of the eCB system in obese humans seems to be driven by excessive production of eCBs in several peripheral tissues such as visceral adipose tissue, liver, pancreas, and skeletal muscle. Differences arise between central (intra-abdominal) adipocytes versus peripheral (subcutaneous) adipocytes, with additional variations due to gender, age, and genetic polymorphisms in metabolic enzymes. Visceral adiposity particularly correlates with elevated levels of 2-AG in blood plasma [230]. Increases in circulating eCBs likely reflect spillover from adipose tissues and liver parenchyma, where CB_1 activation promotes *de novo* lipogenesis and reduces insulin sensitivity, respectively. In mice with diet-induced obesity, CB_1 mRNA and protein levels increased in the hippocampus, compared to lean controls [231]. Furthermore, hippocampal slices from obese mice showed increased CB_1 functionality, with no sign of CB_1 desensitization. We find it surprising that sustained elevations of eCB ligands do not result in CB_1 downregulation. This may be due to the fact that such elevations are not as dramatic as those caused, for example, by chronic MAGL inhibition. The lack of downregulation may contribute to the hedonic aspects of overeating, and influence cognitive processes.

Weight loss by caloric restriction or fasting predictably modulates the eCB system. Animal studies have demonstrated the complexities arising in adipose tissue versus the central nervous system (Table 2). In human studies, weight loss from caloric restriction has produced conflicting results. Engeli *et al.* [232] measured CB_1 and FAAH gene expression, and serum AEA and 2-AG, in obese postmenopausal women. They reported no changes after 5% weight loss from caloric restriction. Bennetzen *et al.* [233] analyzed a younger population of obese men and women; a 10–12% weight loss resulted in elevated 2-AG levels in gluteal adipose tissues, with no change in AEA levels. Weight loss increased CB_1 mRNA in abdominal adipose tissues but decreased CB_1 mRNA in gluteal adipose tissues.

In centrally obese men, decreased plasma AEA and 2-AG levels accompanied a weight loss intervention consisting of both caloric restriction and exercise. Only 2-AG levels correlated with decreased visceral adipose tissue, plasma triglycerides and insulin resistance, and improved HDL-cholesterol levels [234]. However, the influence of caloric restriction and exercise separately was not analyzed in this study. You *et al.* [235] measured CB_1 and FAAH mRNA in subcutaneous abdominal and gluteal adipose tissue in overweight or obese postmenopausal women. Caloric restriction resulted in 11% weight loss, which led to a reduction in gluteal CB_1 and FAAH gene expression but no significant changes in abdominal adipose tissue. You and associates also tested the effects of exercise, see below. A 12-week hospital-based weight loss program (moderate caloric restriction along with counseling by dieticians and physical activity teachers) resulted in a mean weight loss of 9.5% and a significant reduction in salivary AEA levels, while salivary 2-AG, OEA and PEA did not significantly change [236].

In summary, increased food intake, adiposity, and elevated levels of AEA and 2-AG apparently spiral in a feed-forward mechanism. Weight loss from caloric restriction breaks the cycle, possibly by reducing CB_1 expression and reducing eCB levels.

Exercise. Rodent studies have shown that exercise modulates the eCB system (Table 3). The results of these studies show a critical difference between short-term, voluntary exercises (*e.g.*,

Table 5. Partial agonism of THC at CB_1, based on assays of cannabinoid-stimulated signal transduction.

full agonist, species and substrate	assay; maximal stimulation by Δ^9-THC compared to the full agonist	reference
WIN55,212-2 rat cerebellar membranes	[^{35}S]GTPγS binding; 20%	[284]
WIN55,212-2 mouse brain membranes	[^{35}S]GTPγS binding; 25%	[343]
CP55,940 rat cerebellar membranes	[^{35}S]GTPγS binding; 54%	[344]
WIN55,212-2 rat hippocampal neurons	patch-clamp Ca++ currents and excitatory postsynaptic currents; 41% and 55%	[299]
HU-210, WIN55,212-2 transfected human CB_1	[^{35}S]GTPγS binding; 56% at Gai;, 89% at Gao;	[312]
WIN55,212-2 transfected human CB_1	inwardly rectifying potassium (GIRK) current amplitude, 35%	[296]

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wheel running) and long-term, coerced exercise (forced swimming, treadmills). Although both types of exercise regimens increased eCB ligand concentrations, only long-term-forced exercise led to sustained elevations of eCBs, and predictable CB_1 downregulation.

In humans, serum AEA levels doubled over baseline in male subjects after ≥30 min running, and increased significantly in male subjects after biking. Serum 2-AG levels did not significantly increase [237]. Heyman *et al.* [238] reported similar findings in male cyclists—serum AEA levels increased significantly during exercise, whereas 2-AG concentrations remained stable. AEA levels increased incrementally at 55% maximum work output, at 75% W_{max}, and during a 15 min recovery period. Beta-endorphin levels exhibited a different trajectory—they did not increase until the 75% W_{max} stage, and dropped significantly during the recovery period.

Feuerecker *et al.* [239] measured the effects of physical exercise in aerobically-trained male subjects. Strenuous hiking at high altitudes (up to 3196 m) significantly increased serum AEA levels over baseline. Strenuous hiking at low altitudes also increased AEA level, but to a lesser extent. In a small cadre of overweight or obese middle-aged women, 20 weeks of moderate-intensity aerobic exercise (CRM) or vigorous-intensity aerobic exercise (CRV) did not change CB_1 or FAAH gene expression [235]. However, combining data from the two groups (CRM+CRV) showed a decrease in FAAH mRNA in abdominal adipose tissue, compared to a control group that participated solely in caloric restriction. The CRM and CRV groups showed a slight increase in CB_1 mRNA expression in gluteal adipose tissue over baseline, whereas the control group that only participated in caloric restriction showed a significant decrease in CB_1 mRNA.

Raichlen *et al.*, [240] measured circulating eCBs in humans and dogs (cursorial mammals) and ferrets (a non-cursorial mammal) before and after treadmill exercise to test the hypothesis that neurobiological rewards are linked to high-intensity exercise in cursorial mammals. The authors showed that humans and dogs share significantly increased exercise-induced eCB signaling following high-intensity endurance running, whereas eCB signaling did not significantly increase following low-intensity walking, nor did it increase in the non-cursorial ferrets following exercise at any intensity. The same research group showed that serum AEA levels in male and female runners significantly increased after 30 minutes of moderately intense treadmill running (70–80% age-adjusted maximum heart rate), and not after very high or very low intensity exercises [240,241].

In summary, medium- to high-intensity voluntary exercise in cursorial mammals, including humans, increases eCB signaling, via increased serum AEA levels (but not 2-AG), and possibly increased CB_1 expression. "Runner's high" may be an eCB-induced reward for exercise.

Alcohol. *Acute* administration of a high dose of ethanol in rats decreased AEA levels in brain, serum, and adipose tissue; PEA also decreased in the brain. AEA decrease was associated with inhibition of AEA release and no change in NAPE-PLD or FAAH hydrolysis [242]. However, exposing *ex vivo* murine hippocampal neuron cultures to lower doses of ethanol increased AEA and 2-AG release [243]. This increase led to reduced presynaptic glutamate release in neuron cultures, which was blocked by SR141716A. There was no change in CB_1 density.

Electrophysiological studies of anesthetized rats showed that alcohol enhanced eCB signaling in mesolimbic circuits [244]. This effect was blocked by SR141716A, and increased by the FAAH inhibitor URB597—indicating AEA involvement. Another study by the same group showed parallel responses in rat amygdala. The downregulation of amygdala CB_1 with chronic WIN55212-2 blunted the response to alcohol [245].

Ex vivo exposure of rat striatal slices showed ethanol shifts synaptic plasticity from LTP to eCB-mediated LTDI. Ethanol-enhanced LTDI was blocked by the CB_1 antagonist AM251 [246]. The same group showed that ethanol modulated eCB-mediated striatal plasticity in a synapse-specific manner. Ethanol prevented CB_1-dependent long-lasting disinhibition (DLL) in the dorsolateral striatum [247]. Furthermore, the study showed that LTDI by an exogenous cannabinoid, WIN55,212-2, was actually prevented by ethanol.

Chronic ethanol treatment decreased CB_1 density and decreased cannabinoid-stimulated [^{35}S]GTPγS activation in various animal models [248–251]. One study of chronic ethanol did not alter CB_1 binding of [^{3}H]CP55,940 or CB_1 mRNA levels in rat brain homogenates [68]. Short-term chronic exposure (72 hours) of ethanol vapor in mice increased CB_1 density in the cortex, hippocampus, striatum and cerebellum, with downregulation of CB_1 receptor-stimulated [^{35}S]GTPγS binding [252]. The effects of chronic ethanol treatment upon eCB levels in various *in vitro* and animal models are shown in Table 4.

Vinod *et al.* [251] compared alcohol-preferring (aP) and alcohol-non preferring (NaP) rats, a pair of rat lines selectively bred for opposite alcohol preference. CB_1 receptor density, CB_1 receptor-stimulated [^{35}S]GTPγS coupling, and levels of AEA and 2-AG were higher in the brains of alcohol-naive aP compared to NaP

rats. Ethanol consumption in aP rats decreased CB_1 receptor-stimulated [^{35}S]GTPγS binding after 10 days, and moreso after 60 days. 2-AG levels elevated after 10 days, and both 2-AG and AEA levels increased after 60 days; FAAH levels decreased with no change in MAGL. Ethanol withdrawl upregulated [^{35}S]GTPγS binding.

A rat model of binge drinking—serial cycles of ethanol intoxication and withdrawal—increased CB_1 mRNA in the prefrontal cortex [253]. Another study of serial cycles in rats showed a transient decrease in hippocampal CB_1 mRNA and protein levels (two days after cessation of cycles), followed by a long term up-regulation in CB_1 mRNA and protein, 40 days after cessation of cycles. Serial cycles increased 2-AG in the hippocampus, two days and 40 days after cessation of cycles; AEA increased only at 40 days [254].

An electrophysiological study of intermittent ethanol consumption in rats showed depression of CB_1-dependent long-lasting disinhibition (DLL) in excised slices of the dorsolateral striatum [255]. Furthermore, the study showed that LTDI by an exogenous cannabinoid, WIN55,212-2, was prevented by intermittent ethanol consumption.

A human clinical trial assigned 55 adults to one of three groups—drinking either 250 ml of red wine, grape juice, or plain water. Within 10 minutes, the consumption of a moderate amount of alcohol reduces plasma AEA and 2-AG concentrations, whereas an equal volume of grape juice did not affect plasma eCBs. Interestingly, plain water reduced 2-AG concentrations without affecting AEA [256].

Alcoholics who died of natural causes or motor vehicle accidents expressed decreased CB_1 densities in the ventral striatum, decreased CP55,940-stimulated [^{35}S]GTPγS binding, and decreased FAAH activity, compared to controls [257]. Alcoholics who died of suicide in the same study had increased CB_1 densities, increased CB_1 receptor-stimulated G(i/o) protein activation, and decreased FAAH activity, compared to controls. Lehtonen *et al.* (2010) measured eCB levels in post-mortem brains of Cloninger type 1 and type 2 alcoholics. Type 1 alcoholics had lower levels of AEA than controls in the nucleus accumbens (NAcc), anterior cingulate cortex, and frontal cortex. PEA, OEA, and 2-AG were unchanged. They also showed dopaminergic deficiencies in the NAcc, suggesting a compensatory mechanism one direction or the other. Type 2 alcoholics produced slightly higher eCB levels than controls, but not significantly.

In summary, acute ethanol may enhance endogenous eCB release and eCB signaling, although it varies by brain area and synapse, and this complexity requires further testing. Two studies suggest ethanol dampens the effects of the eCB system. Chronic ethanol consumption and binge drinking likely desensitize or downregulate CB_1 and impair eCB signaling, except perhaps in areas involved in reward and motivation to self-administer this substance of abuse [258].

Nicotine. In a human randomized controlled trial, nicotine augmented THC-induced "high" and heart rate [259]. In rodent behavioral studies, *acute* nicotine augmented THC discrimination and THC-induced hypothermia, antinociception, locomotor inactivity, anxiolysis, and place aversion [260–264]. Nicotine-potentiated THC discrimination was blocked by rimonabant and URB-597 (a FAAH inhibitor), suggesting nicotine potentiation is mediated by the release of AEA acting at CB_1 [263]. CB_2 is also involved—the CB_2–selective agonist JWH133 induced antinociception in the mouse formalin test, and this effect was potentiated by nicotine [265]. Acute nicotine elicited marked increases in AEA in the amygdala, hypothalamus, and prefrontal cortex but decreased levels in the hippocampus; variations in 2-AG were less pronounced [266].

In a contrary study, intracelebellar microinfusion of nicotine attenuated THC-induced ataxia in mice. Microinfusion of synthetic subtype agonists indicated the involvement of $\alpha_4\beta_2$ but not α_7 nicotinic receptor subtypes [267]. Buczynski *et al.* [268] compared volitional self-administration (SA) versus forced nicotine exposure (FA) in the ventral tegmental area using *in vivo* microdialysis. SA but not FA increased AEA; both SA and FA increased 2-AG; these subtle changes were not seen in corresponding bulk brain tissue analysis of eCBs. Acute nicotine enhanced THC-induced c-Fos expression in various brain regions [264].

Chronic nicotine increased AEA levels in the limbic forebrain and increased AEA and 2-AG contents in rat brainstem, but decreased AEA and/or 2-AG contents in the hippocampus, the striatum and the cerebral cortex [258]. Chronic nicotine increased CB_1 density in the prelimbic prefrontal cortex, ventral tegmental area, and the hippocampus [269]. Seven days of nicotine exposure increased brain CB_1 densities in adolescent male rats and sensitized them to the locomotor-decreasing effects of THC and CP55,940 [270]. These changes were not seen in adult male rats. Chronic nicotine inhibited the development of tolerance to antinociceptive and hypothermic effects of THC [264].

Other plant products that exert cholinergic effects, such as calamus, *Acorus calamus*, have been admixed with cannabis to decrease cannabis-induced memory deficits, and "calm and center the effects of marijuana" [42]. Consistent with this, the synthetic cholinergic agent rivastigmine reversed memory deficits in rats induced by the synthetic cannabinoid WIN55,212-2 [271].

Caffeine. Co-administering caffeine and cannabis has a long history. Bell [272] claimed that oral administration of hashish with coffee increased the effects of cannabis, and at the same time diminished its duration. He proposed a pharmacokinetic mechanism—coffee promoted more rapid absorption of hashish.

Caffeine and theophylline are antagonists of adenosine receptors. Adenosine receptors are tonically activated by adenosine, their endogenous ligand. Rodent studies indicate that A_1-subtype adenosine receptors tonically inhibit CB_1 activity [273]. Thus the antagonism of A_1 receptors by caffeine and theophylline enhances eCB system function (*e.g.*, activation of CB_1 by 2-AG). Caffeine potentiated CB_1-mediated activity stimulated by THC and WIN-55,212 in hippocampus slices [273]. Consistent with this, the simultaneous application of WIN-55,212 plus an A_1 agonist produced less than additive stimulation of [^{35}S]GTPγS binding in mouse cerebellar membranes [274].

In whole animals, however, caffeine's effects are biphasic and vary by dosage and acute versus chronic administration. In humans, the acute administration of caffeine decreases headache pain, but exposure to chronic high doses, $\geq$300 mg/day, may exacerbate chronic pain [275]. In rabbits, an acute dose of caffeine antagonized THC-induced changes in cortico-hippocampal electroencephalogram recordings [276]. In mice, chronic caffeine at high doses potentiated CB_1-dependent stimulation by eCBs and HU210 at striatal GABAergic, but not glutamatergic, synapses [277]. A single dose or a subacute dose (one day of caffeine in water) rescued the sensitivity of GABAergic synapses to HU210 in mice exposed to chronic stress.

Chronic caffeine at moderate doses increased THC's effects on short-term memory in mice [278]. Surprisingly, CB_1 density decreased in the caffeinated mice, measured by [^{3}H]SR141716A binding. Cortical and hippocampal tissues also showed a decrease in WIN55,212-2-stimulated [^{35}S]GTPγS binding, but this attenuation was not seen in THC-stimulated [^{35}S]GTPγS binding. This

highlights the fact that caffeine-induced changes observed *in vitro* do not necessarily reflect the effects of caffeine upon integrated brain circuitry *in vivo*. Lastly, acute antagonism of A_1 with DPCPX did not modulate the effects of THC on short-term memory [278], which further supports our hypothesis that chronic and acute blockade of A_1 receptors have different functional consequences.

Cannabis. *Cannabis* and cannabis products are complex polypharmaceuticals, consisting of THC, cannabidiol (CBD), dozens of minor cannabinoids, as well as terpenoids, flavonoids, and other compounds. Fundamentally, THC mimics AEA and 2-AG by acting as an agonist at CB_1 and CB_2 [279]. But rather than simply substituting for AEA and 2-AG, McPartland and Guy [280] proposed that *Cannabis* and its many constituents work, in part, by "kick-starting" the eCB system. The acute administration of THC increased CB_1 density in rodent brains [281,282]. Acute upregulation of CB_1 mRNA continued for up to 14 days in some rat brain regions [283]. Acute THC also increased the sensitivity of CB_1 to cannabinoids, measured by WIN-55,212-2-stimulated [^{35}S]GTPγS binding in rat brains [284]. Lastly, acute THC stimulated AEA biosynthesis [285].

Chronic, high dosing of THC causes a predictable desensitization and downregulation of CB_1 and CB_2, accompanied by drug tolerance. Chronic THC decreased CB_1 density in rodent brains, and dampened cannabinoid-stimulated [^{35}S]GTPγS [282,284,286,287]. CB_1 in different regions of the brain downregulate and desensitize at unequal rates and magnitudes, with greatest decreases in the hippocampus and little or no change in the nucleus accumbens and basolateral amygdala. Chronic THC elicited few changes in AEA or 2-AG levels in rat brains, except for a significant augmentation of AEA levels in the limbic forebrain [288].

Similar results have been reported in two human studies. Villares [289] collected postmortem brain tissues from known cannabis smokers; [^{3}H]SR141716A binding and CB_1 mRNA was downregulated in several brain regions, compared to non-smoking control autopsies. Hirvonen *et al.* [290] employed PET scan imaging in living subjects. The degree of CB_1 downregulation correlated with years of chronic cannabis smoking. CB_1 densities returned to normal after four weeks of abstinence. Variable downregulation in different brain regions may explain why frequent users of cannabis develop tolerance to some effects of THC, such as anxiogenesis and cognitive impairment, but not to its euphoric effects [291]. Downregulation is partially epigenetic—the CB_1 promoter region in chronic marijuana smokers is hypermethylated, reducing CB_1 mRNA expression levels [292].

THC acts as a partial agonist of CB_1, compared to synthetic cannabinoids which act as full agonists (Table 5). Partial agonism likely explains why exposure to THC caused half as much CB_1 desensitization as the full agonist WIN55,212-2 in rat hippocampal neurons [293]. In a study of rat CB_1 transfected into AtT20 cells, THC caused less downregulation and internalization than WIN55,212-2 or CP-55,940 [294]. In agreement, drug tolerance studies utilizing the behavioral "tetrad" test show that chronic THC caused less tolerance than the full agonist CP-55,940 in mice [295]. In a study of human CB_1 transfected into *Xenopus* oocytes, the desensitization rate of THC was half that of WIN55,212-2 [296]. However, one [^{35}S]GTPγS autoradiography study of rat brains suggested that chronic THC and WIN55,212-2 caused equal desensitization [297]. Another study indicated that THC acts as a full agonist at mouse GABAergic synapses, with efficacy equal to WIN55,212-2, albeit at fairly high concentrations [298].

If THC is a partial agonist, then THC might functionally antagonize the effects of a full agonist when the two drugs are added together. THC antagonized the effects of WIN55,212-2 in rat brain sections [284,299], and mouse autaptic hippocampal neurons [300].

The capacity of THC to antagonize a full agonist depends, in part, upon ligand affinity—its ability to occupy and hold the CB_1 binding site. A meta-analysis of affinity studies calculated a mean $K_i = 42.6$ nM for THC in rat membranes—much less affinity than that of WIN-55,940, with a $K_d = 2.4$ nM [22]. This indicates that high concentrations of THC relative to WIN-55,940 are required to antagonize the full agonist. There are species differences—in human membranes, CB_1 affinity of THC ($K_i = 25.1$ nM) is much closer to that of WIN-55,940 ($K_d = 16.7$).

2-AG acts as a full agonist at rodent and human CB_1 and CB_2 [296,301–303]. The emetogenic effects of exogenously-administered 2-AG were blocked by THC [304]. THC dampened or occluded eCB-mediated retrograde signaling of CB_1, presumable mediated by 2-AG [300,305,306]. Roloff and Thayer [307] demonstrated another complexity in the relationship between THC and 2-AG: neuron firing rate in response to stimulus in rat hippocampal neurons. At low firing rates, THC mimicked 2-AG and behaved like an agonist; at high firing rates, THC antagonized endogenous 2-AG signaling.

AEA is a partial agonist like THC, with an efficacy somewhat greater than THC in mouse brain [308] and transfected human CB_1 [296]. Consistent with partial agonism, exogenously-administered AEA caused little tolerance in rodents [309,310]. Agonist trafficking adds further complexity—THC and AEA preferentially activate different G-protein subtypes [311]. At transfected human CB_1, AEA acted as a full agonist via Gαi subunits, and a partial agonist via Gαo subunits, with agonist efficacy much greater than THC at Gαi, and slightly greater than THC at Gαo [312].

AEA and THC can antagonize each other; this in part is due to cross-tolerance [313,314]. Falenski *et al.* [287] demonstrated that subchronic administration of THC in FAAH$^{-/-}$ knockout mice caused greater tolerance to THC than did subchronic administration of THC in wildtype mice. Thus elevated levels of AEA in FAAH$^{-/-}$ knockouts produced additive effects with THC. Vann *et al.* [315] trained rats to discriminate THC; trained rats injected with PMSF, which inhibits FAAH, showed 2.7-fold greater discrimination than rats injected with vehicle. In other words, inhibiting AEA degradation led to an increase in the potency of THC. Further, THC was more potent at producing antinociception, decreasing spontaneous activity, and increasing ring immobility when co-administered with PMSF as compared to vehicle.

In summary, the effects of THC upon the eCB system oscillate between potentiation and suppression, depending on acute versus chronic dosage. The dividing line between "acute" and "chronic" is a gray zone, and likely differs amongst individuals. Suplita *et al.* [316] summarized the situation: they studied "stress antinociception," where rodents become less responsive to painful stimuli following exposure to an environmental stressor. Stress antinociception is mediated, in part, by the coordinated release of 2-AG and AEA. Acute administration of THC potentiated eCB-mediated stress antinociception. The converse was also true: animals exposed acutely to foot shock, which elicits eCB-mediated stress antinociception, became sensitized to the effects of THC. Chronic administration of THC predictably dampened stress antinociception. The converse was *not* true: chronic exposure to foot shock (3 min/day for 15 days) failed to dampen antinociception induced by either WIN-55,212-2 or by further footshocks.

The potential synergy between THC and the eCB system is analogous to the potential synergy between AEA and 2-AG: Rodent studies that combined FAAH and MAGL inhibitors indicated that AEA and 2-AG may activate CB_1 receptors in different parts of the central nervous system. Each causes unique

behavioral effects, and when both are enhanced, new effects emerge. Long and colleagues [317] showed that AEA and 2-AG independently dampen pain sensation, but together their effects are dramatically enhanced.

Cannabis is more than THC [318,319]. Adding CBD to THC in mice enhanced CB_1 expression in hippocampus and hypothalamus [320]. CBD increased hippocampal cell survival and neurogenesis, whereas THC had the opposite effect; the CBD response was absent in $CB_1^{-/-}$ knockout mice [321]. CBD inhibited the cellular uptake of AEA and its breakdown by FAAH [322,323]. A separate systematic review regarding the effects of CBD on THC is currently underway (McPartland, unpublished). Several other non-THC cannabinoids interact with enzymes of the eCB system. For example, cannabidivarin and cannabidiolic acid are moderately potent inhibitors of DAGLα, and cannabigerol and cannabichromene are relatively potent inhibitors of anandamide cellular uptake [323]. Interestingly, cannabis extracts ("botanical drug substances," BDS) enriched in cannabinoids, such as THC-acid BDS and CBD-BDS, were more potent than the corresponding pure compounds at inhibiting MAGL and AEA cellular uptake [323].

Conclusions

Many randomized controlled trials identified in this systematic review have been conducted on lifestyle modifications (*e.g.*, exercise, maintenance of ideal body weight) and CAM interventions (*e.g.*, dietary supplements, stress modification, acupuncture, massage and manipulation). In our opinion these are sensible methods of enhancing the eCB system.

Preclinical studies identified useful prescription drugs, such as SSRIs, anxiolytics, antipsychotics, and anticonvulsants. However, these drugs are generally administered in a chronic fashion, and this comes with a caveat: generating chronic elevations in AEA and 2-AG may be counterproductive. Faced with constant activation by agonists, CB_1 and CB_2 desensitize and downregulate. A desensitized receptor drives less receptor-mediated signal transduction, and develops cross-tolerance to all agonists—eCBs and phytocannabinoids alike. A downregulated receptor is not functional—either it does not bind ligand or has internalized away from the cell membrane.

The difference between acute and chronic augmentation has been demonstrated in rodent studies: acute blockade of MAGL with JZL184 elevated 2-AG levels and provided analgesia [324]. In the face of chronic blockade with JZL184 this analgesia was lost, because sustained elevation of 2-AG caused CB_1 desensitization. This led to a loss in eCB-dependent synaptic plasticity, cross-tolerance to other cannabinoids, and physical dependence.

Other drugs identified in preclinical studies have side effect profiles too severe to warrant their use for upregulating the eCB system (*e.g.*, corticosteroids, opioids, nicotine). Preclinical studies suggest a number of over-the-counter medications, such as analgesics, seem to be acting through eCB-mediated mechanisms. Clinical trials are warranted, although over-the-counter medications lack patent protection, so expensive clinical trials seem unlikely.

Supporting Information

Checklist S1 Online supporting material. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.
(DOC)

Author Contributions

Conceived and designed the experiments: JM GWG VD. Performed the experiments: JM GWG VD. Analyzed the data: JM GWG VD. Contributed reagents/materials/analysis tools: JM GWG VD. Wrote the paper: JM GW VD.

References

1. Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77: 299–318.
2. Onaivi ES (2011) Commentary: functional neuronal CB2 cannabinoid receptors in the CNS. Current Neuropharmacology 9: 205–208.
3. Atwood BK, Mackie K (2010) CB2: a cannabinoid receptor with an identity crisis. British Journal of Pharmacology 160: 467–479.
4. De Petrocellis L, Di Marzo V (2010) Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. Journal of Neuroimmune Pharmacology 5: 103–121.
5. Di Marzo V (1998) 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: biochemistry and possible physiopathological relevance. Biochimica et Biophysica Acta - Lipids and Lipid Metabolism 1392: 153–175.
6. Di Marzo V, Piscitelli F, Mechoulam R (2011) Cannabinoids and endocannabinoids in metabolic disorders with focus on diabetes. Handbook of Experimental Pharmacology: 75–104.
7. Bermudez-Silva FJ, Viveros MP, McPartland JM, de Fonseca FR (2010) The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? Pharmacology Biochemistry and Behavior 95: 375–382.
8. Russo EB (2004) Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? Neuro Endocrinol Lett 25: 31–39.
9. Fride E (2004) The endocannabinoid-CB receptor system: Importance for development and in pediatric disease. Neuroendocrinology Letters 25: 24–30.
10. Hill MN, Gorzalka BB (2005) Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol 16: 333–352.
11. Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, et al. (2004) Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology 29: 2108–2114.
12. Sarchielli P, Pini LA, Coppola F, Rossi C, Baldi A, et al. (2007) Endocannabinoids in chronic migraine: CSF findings suggest a system failure. Neuropsychopharmacology 32: 1384–1390.
13. Di Filippo M, Pini LA, Pelliccioli GP, Calabresi P, Sarchielli P (2008) Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis. Journal of Neurology Neurosurgery and Psychiatry 79: 1224–1229.
14. Allen KL, Waldvogel HJ, Glass M, Faull RLM (2009) Cannabinoid (CB1), $GABA_A$ and $GABA_B$ receptor subunit changes in the globus pallidus in Huntington's disease. Journal of Chemical Neuroanatomy 37: 266–281.
15. Van Laere K, Casteels C, Dhollander I, Goffin K, Grachev I, et al. (2010) Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. Journal of Nuclear Medicine 51: 1413–1417.
16. Pisani V, Moschella V, Bari M, Fezza F, Galati S, et al. (2010) Dynamic changes of anandamide in the cerebrospinal fluid of Parkinson's disease patients. Movement Disorders 25: 920–924.
17. Wong BS, Camilleri M, Eckert D, Carlson P, Ryks M, et al. (2012) Randomized pharmacodynamic and pharmacogenetic trial of dronabinol effects on colon transit in irritable bowel syndrome-diarrhea. Neurogastroenterology and Motility 24.
18. Gerard N, Pieters G, Goffin K, Bormans G, Van Laere K (2011) Brain type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. Biological Psychiatry 70: 777–784.
19. Chouker A, Kaufmann I, Kreth S, Hauer D, Feuerecker M, et al. (2010) Motion sickness, stress and the endocannabinoid system. PLoS ONE 5.
20. Sandercock P, Roberts I (2002.) Systematic reviews of animal experiments. Lancet 360: 586.
21. Macleod MR, O'Collins T, Howells DW, Donnan GA (2004) Pooling of animal experimental data reveals influence of study design and publication bias. Stroke 35: 1203–1208.
22. McPartland JM, Glass M, Pertwee RG (2007) Meta-analysis of cannabinoid ligand binding affinity and cannabinoid receptor distribution: interspecies differences. British Journal of Pharmacology 152 583–589
23. van der Worp HB, Macleod MR (2011) Preclinical studies of human disease: time to take methodological quality seriously. Journal of Molecular and Cellular Cardiology 51: 449–450.

24. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 6: e1000100.
25. McPartland JM, Pruitt PL (2000) Benign prostatic hyperplasia treated with saw palmetto: a literature search and an experimental case study. J Am Osteopath Assoc 100: 89–96.
26. Hornberg JR (2013) Measuring behaviour in rodents: towards translational neuropsychiatric research. Behavioural Brain Research 236: 295–306.
27. Fowler CJ, Janson U, Johnson RM, Wahlstrom G, Stenstrom A, et al. (1999) Inhibition of anandamide hydrolysis by the enantiomers of ibuprofen, ketorolac, and flurbiprofen. Archives of Biochemistry and Biophysics 362: 191–196.
28. Bertolacci L, Romeo E, Veronesi M, Magotti P, Albani C, et al. (2013) A binding site for nonsteroidal anti-inflammatory drugs in fatty acid amide hydrolase. Journal of the American Chemical Society 135: 22–25.
29. Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, et al. (2002) Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem 277: 44877–44885.
30. Sang N, Zhang J, Chen C (2006) PGE_2 glycerol ester, a COX-2 oxidative metabolite of 2-arachidonoyl glycerol, modulates inhibitory synaptic transmission in mouse hippocampal neurons. Journal of Physiology-London 572: 735–745.
31. Hu SSJ, Bradshaw HB, Chen JSC, Tan B, Walker JM (2008) Prostaglandin E-2 glycerol ester, an endogenous COX-2 metabolite of 2-arachidonoylglycerol, induces hyperalgesia and modulates NF kappa B activity. British Journal of Pharmacology 153: 1538–1549.
32. Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, et al. (2012) Discovery of prostamide F-2 alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. Plos One 7.
33. Kim J, Alger BE (2004) Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. Nature Neuroscience 7: 697–698.
34. Gühring H, Hamza M, Sergejeva M, Ates M, Kotalla CE, et al. (2002) A role for endocannabinoids in indomethacin-induced spinal antinociception. European Journal of Pharmacology 454: 153–163.
35. Ates M, Hamza M, Seidel K, Kotalla CE, Ledent C, et al. (2003) Intrathecally applied flurbiprofen produces an endocannabinoid-dependent antinociception in the rat formalin test. European Journal of Neuroscience 17: 597–604.
36. Ahn DK, Choi HS, Yeo SP, Woo YW, Lee MK, et al. (2007) Blockade of central cyclooxygenase (COX) pathways enhances the cannabinoid-induced antinociceptive effects on inflammatory temporomandibular joint (TMJ) nociception. Pain 132: 23–32.
37. Guindon J, Hohmann AG (2009) The endocannabinoid system and pain. CNS & Neurological Disorders-Drug Targets 8: 403–421.
38. Sasso O, Bertorelli R, Bandiera T, Scarpelli R, Colombano G, et al. (2012) Peripheral FAAH inhibition causes profound antinociception and protects against indomethacin-induced gastric lesions. Pharmacological Research 65: 553–563.
39. Green K, Kearse EC, McIntyre OL (2001) Interaction between Delta-9-tetrahydrocannabinol and indomethacin. Ophthalmic Research 33: 217–220.
40. Perez Reyes M, Burstein SH, White WR, Mcdonald SA, Hicks RE (1991) Antagonism of marihuana effects by indomethacin in humans. Life Sciences 48: 507–515.
41. Easley RB, Altemeier WA 3rd (2000) Central nervous system manifestations of an ibuprofen overdose reversed by naloxone. Pediatr Emerg Care 16: 39–41.
42. McPartland JM, Blanchon DJ, Musty RE (2008) Cannabimimetic effects modulated by cholinergic compounds. Addiction Biology 13: 411–415.
43. Högestätt ED, Jonsson BAG, Ermund A, Andersson DA, Bjork H, et al. (2005) Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. Journal of Biological Chemistry 280: 31405–31412.
44. Dania M, Guindon J, Lambert C, Beaulieu P (2007) The local antinociceptive effects of paracetamol in neuropathic pain are mediated by cannabinoid receptors. European Journal of Pharmacology 573: 214–215.
45. Ottani A, Leone S, Sandrini M, Ferrari A, Bertolini A (2006) The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors. European Journal of Pharmacology 531: 280–281.
46. Mallet C, Daulhac L, Bonnefont J, Ledent C, Etienne M, et al. (2008) Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia. Pain 139: 190–200.
47. Umathe SN, Manna SSS, Utturwar KS, Jain NS (2009) Endocannabinoids mediate anxiolytic-like effect of acetaminophen via CB1 receptors. Progress in Neuro-Psychopharmacology & Biological Psychiatry 33: 1191–1199.
48. Andersson DA, Gentry C, Alenmyr L, Killander D, Lewis SE, et al. (2011) TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid D^9-tetrahydrocannabiorcol. Nature Communications 2: 551.
49. Gould GG, Seillier A, Weiss G, Giuffrida A, Burke TF, et al. (2012) Acetaminophen differentially enhances social behavior and cortical cannabinoid levels in inbred mice. Progress in Neuro-Psychopharmacology & Biological Psychiatry 38: 260–269.
50. Wang S, Lim G, Mao J, Sung B, Yang L (2007) Central glucocorticoid receptors regulate the upregulation of spinal cannabinoid-1 receptors after peripheral nerve injury in rats. Pain 131: 96–105.
51. Wu RW, Lin TP, Ko JY, Yeh DW, Chen MW, et al. (2011) Cannabinoid receptor 1 regulates ERK and GSK-3 beta-dependent glucocorticoid inhibition of osteoblast differentiation in murine MC3T3-E1 cells. Bone 49: 1255–1263.
52. Ko JY, Wu RW, Kuo SJ, Chen MW, Yeh DW, et al. (2012) Cannabinoid receptor 1 mediates glucocorticoid-induced bone loss in rats by perturbing bone mineral acquisition and marrow adipogenesis. Arthritis and Rheumatism 64: 1204–1214.
53. Di S, Malcher-Lopes R, Halmos KC, Tasker JG (2003) Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. Journal of Neuroscience 23: 4850–4857.
54. Di S, Malcher-Lopes R, Marcheselli VL, Bazan NG, Tasker JG (2005) Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. Endocrinology 146: 4292–4301.
55. Malcher-Lopes R, Di S, Marcheselli VS, Weng FJ, Stuart CT, et al. (2006) Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. Journal of Neuroscience 26: 6643–6650.
56. Wang J, Shen RY, Haj-Dahmane S (2012) Endocannabinoids mediate the glucocorticoid-induced inhibition of excitatory synaptic transmission to dorsal raphe serotonin neurons. Journal of Physiology-London 590: 5795–5808.
57. Hill MN, Karatsoreos IN, Hillard CJ, McEwen BS (2010) Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo. Psychoneuroendocrinology 35: 1333–1338.
58. Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, et al. (2011) Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. Journal of Neuroscience 31: 10506–10515.
59. Hill MN, Carrier EJ, Ho WSV, Shi L, Patel S, et al. (2008) Prolonged glucocorticoid treatment decreases cannabinoid CB_1 receptor density in the hippocampus. Hippocampus 18: 221–226.
60. Atsak P, Hauer D, Campolongo P, Schelling G, McGaugh JL, et al. (2012) Glucocorticoids interact with the hippocampal endocannabinoid system in impairing retrieval of contextual fear memory. Proceedings of the National Academy of Sciences of the United States of America 109: 3504–3509.
61. Bowles NP, Hill MN, Bhagat SM, Karatsoreos IN, Hillard CJ, et al. (2012) Chronic, noninvasive glucocorticoid administration suppresses limbic endocannabinoid signaling in mice. Neuroscience 204: 83–89.
62. Hong S, Fan J, Kemmerer ES, Evans S, Li Y, et al. (2009) Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. Gut 58: 202–210.
63. Allen KV, McGregor IS, Hunt GE, Singh ME, Mallet PE (2003) Regional differences in naloxone modulation of Δ^9-THC induced Fos expression in rat brain. Neuropharmacology 44: 264–274.
64. Cichewicz DL, Haller VL, Welch SP (2001) Changes in opioid and cannabinoid receptor protein following short-term combination treatment with Δ^9- tetrahydrocannabinol and morphine. Journal of Pharmacology and Experimental Therapeutics 297: 121–127.
65. Fattore L, Vigano D, Fadda P, Rubino T, Fratta W, et al. (2007) Bidirectional regulation of mu-opioid and CB1-cannabinoid receptor in rats self-administering heroin or WIN 55,212-2. European Journal of Neuroscience 25: 2191–2200.
66. Schoffelmeer ANM, Hogenboom F, Wardeh G, De Vries TJ (2006) Interactions between CB1 cannabinoid and mu opioid receptors mediating inhibition of neurotransmitter release in rat nucleus accumbens core. Neuropharmacology 51: 773–781.
67. Viganò D, Valenti M, Cascio MG, Di Marzo V, Parolaro D, et al. (2004) Changes in endocannabinoid levels in a rat model of behavioural sensitization to morphine. European Journal of Neuroscience 20: 1849–1857.
68. Gonzalez S, Fernández-Ruiz J, Sparpaglione V, Parolaro D, Ramos JA (2002) Chronic exposure to morphine, cocaine or ethanol in rats produced different effects in brain cannabinoid CB1 receptor binding and mRNA levels. Drug and Alcohol Dependence 66: 77–84.
69. Chu J, Zheng H, Zhang YH, Loh HH, Law PY (2010) Agonist-dependent mu-opioid receptor signaling can lead to heterologous desensitization. Cellular Signalling 22: 684–696.
70. Berrendero F, Mendizabal V, Murtra P, Kieffer BL, Maldonado R (2003) Cannabinoid receptor and WIN 55 212-2-stimulated [35S]-GTPgammaS binding in the brain of mu-, delta- and kappa-opioid receptor knockout mice. Eur J Neurosci 18: 2197–2202.
71. Marini P, Moriello AS, Cristino L, Palmery M, De Petrocellis L, et al. (2009) Cannabinoid CB1 receptor elevation of intracellular calcium in neuroblastoma SH-SY5Y cells: interactions with muscarinic and delta-opioid receptors. Biochimica Et Biophysica Acta-Molecular Cell Research 1793: 1289–1303.
72. Spano MS, Fattore L, Cossu G, Deiana S, Fadda P, et al. (2004) CB1 receptor agonist and heroin, but not cocaine, reinstate cannabinoid-seeking behaviour in the rat. British Journal of Pharmacology 143: 343–350.
73. Justinova Z, Munzar P, Panlilio LV, Yasar S, Redhi GH, et al. (2008) Blockade of THC-seeking behavior and relapse in monkeys by the cannabinoid CB1-receptor antagonist rimonabant. Neuropsychopharmacology 33: 2870–2877.
74. Braida D, Iosuè S, Pegorini S, Sala M (2004) Δ^9-Tetrahydrocannabinol-induced conditioned place preference and intracerebroventricular self-administration in rats. European Journal of Pharmacology 506: 63–69.

75. Solinas M, Zangen A, Thiriet N, Goldberg SR (2004) beta-Endorphin elevations in the ventral tegmental area regulate the discriminative effects of delta-9-tetrahydrocannabinol. European Journal of Neuroscience 19: 3183–3192.
76. Welch SP, Stevens DL (1992) Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. Journal of Pharmacology and Experimental Therapeutics 262: 10–18.
77. Pugh G, Smith PB, Dombrowski DS, Welch SP (1996) The role of endogenous opioids in enhancing the antinociception produced by the combination of delta-9-tetrahydrocannabinol and morphine in the spinal cord. Journal of Pharmacology and Experimental Therapeutics 279: 608–616.
78. Cichewicz DL, Martin ZL, Smith FL, Welch SP (1999) Enhancement of μ opioid antinociception by oral Δ^9-tetrahydrocannabinol: dose-response analysis and receptor identification. Journal of Pharmacology and Experimental Therapeutics 289: 859–867.
79. Cox ML, Haller VL, Welch SP (2007) Synergy between Δ^9-tetrahydrocannabinol and morphine in the arthritic rat. European Journal of Pharmacology 567: 125–130.
80. Reche I, Fuentes JA, Ruiz-Gayo M (1996) Potentiation of Δ^9-tetrahydrocannabinol-induced analgesia by morphine in mice: involvement of μ- and κ-opioid receptors. European Journal of Pharmacology 318: 11–16.
81. Loewe WS (1928) Die quantitative Problem der Pharmakologie. Ergebnisse der Physiologie 27: 47–187.
82. Loewe WS (1940) Synergism of cannabis and butyl-bromathyl-barbituric acid. Journal of the American Pharmaceutical Association (Scientific edition) 29: 162–163.
83. Loewe WS (1944) Studies on the pharmacology of marihuana. In: Committee L, editor. The Marihuana Problem in the City of New York. Lancaster, PA: Jaques Cattell Press. pp. 149–212.
84. Loewe WS, Modell W (1941) The action of chemical components of cannabis extracts. Journal of Pharmacology and Experimental Therapeutics 72: 72.
85. Loewe WS (1945) Marihuana activity of cannabinol. Science 102: 615–616.
86. Roberts JD, Gennings C, Shih M (2006) Synergistic affective analgesic interaction between delta-9-tetrahydrocannabinol and morphine. European Journal of Pharmacology 530: 54–58.
87. Berrendero F, Maldonado R (2002) Involvement of the opioid system in the anxiolytic-like effects induced by Delta(9)-tetrahydrocannabinol. Psychopharmacology 163: 111–117.
88. Reche I, Ruiz-Gayo M, Fuentes JA (1998) Inhibition of opioid-degrading enzymes potentiates Δ^9-tetrahydrocannabinol-induced antinociception in mice. Neuropharmacology 37: 215–222.
89. Haney M, Bisaga A, Foltin RW (2003) Interaction between naltrexone and oral THC in heavy marijuana smokers. Psychopharmacology 166: 77–85.
90. Cooper ZD, Haney M (2010) Opioid antagonism enhances marijuana's effects in heavy marijuana smokers. Psychopharmacology (Berl) 211: 141–148.
91. Malone DT, Taylor DA (1998) Modulation of Δ^9-tetrahydrocannabinol-induced hypothermia by fluoxetine in the rat. British Journal of Pharmacology 124: 1419–1424.
92. Goddard M, Smith PF, Ashton JC (2010) Behavioural effects of co-administration of delta 9-tetrahydrocannabinol with fluoxetine in rats. Pharmacology 86: 125–128.
93. Hill MN, Ho WSV, Hillard CJ, Gorzalka BB (2008) Differential effects of the antidepressants tranylcypromine and fluoxetine on limbic cannabinoid receptor binding and endocannabinoid contents. Journal of Neural Transmission 115: 1673–1679.
94. Rodriguez-Gaztelumendi A, Rojo ML, Pazos A, Diaz A (2009) Altered CB1 receptor-signaling in prefrontal cortex from an animal model of depression is reversed by chronic fluoxetine. Journal of Neurochemistry 108: 1423–1433.
95. Hesketh SA, Brennan AK, Jessop DS, Finn DP (2008) Effects of chronic treatment with citalopram on cannabinoid and opioid receptor-mediated G-protein coupling in discrete rat brain regions. Psychopharmacology 198: 29–36.
96. Wang W, Sun DL, Pan B, Roberts CJ, Sun XL, et al. (2010) Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. Neuropsychopharmacology 35: 2249–2261.
97. Mato S, Vidal R, Castro E, Diaz A, Pazos A, et al. (2010) Long-term fluoxetine treatment modulates cannabinoid type 1 receptor-mediated inhibition of adenylyl cyclase in the rat prefrontal cortex through 5-hydroxytryptamine$_{1A}$ receptor-dependent mechanisms. Molecular Pharmacology 77: 424–434.
98. Hill MN, Ho WSV, Sinopoli KJ, Viau V, Hillard CJ, et al. (2006) Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology 31: 2591–2599.
99. Hill MN, Carrier EJ, McLaughlin RJ, Morrish AC, Meier SE, et al. (2008) Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. Journal of Neurochemistry 106: 2322–2336.
100. Gobshtis N, Ben-Shabat S, Fride E (2007) Antidepressant-induced undesirable weight gain: prevention with rimonabant without interference with behavioral effectiveness. European Journal of Pharmacology 554: 155–163.
101. Hill MN, Barr AM, Ho WSV, Carrier EJ, Gorzalka BB, et al. (2007) Electroconvulsive shock treatment differentially modulates cortical and subcortical endocannabinoid activity. Journal of Neurochemistry 103: 47–56.
102. Gray GA, Hedley D, Pertwee RG (1987) Enhancement of the hypothermic response of mice to delta-9-tetrahydrocannabinol by subhypothermic doses of chlorpromazine and phentolamine. Neuropharmacology 23: 229–235.
103. Andersson M, Terasmaa A, Fuxe K, Stromberg I (2005) Subchronic haloperidol increases CB(1) receptor binding and G protein coupling in discrete regions of the basal ganglia. J Neurosci Res 82: 264–272.
104. Sundram S, Copolov D, Dean B (2005) Clozapine decreases [3H] CP 55940 binding to the cannabinoid 1 receptor in the rat nucleus accumbens. Naunyn Schmiedebergs Arch Pharmacol 371: 428–433.
105. Schulze DR, Carroll FI, McMahon LR (2012) Interactions between dopamine transporter and cannabinoid receptor ligands in rhesus monkeys. Psychopharmacology 222: 425–438.
106. Secher A, Husum H, Holst B, Egerod KL, Mellerup E (2010) Risperidone treatment increases CB1 receptor binding in rat brain. Neuroendocrinology 91: 155–168.
107. Cheng MC, Liao DL, Hsiung CA, Chen CY, Liao YC, et al. (2008) Chronic treatment with aripiprazole induces differential gene expression in the rat frontal cortex. International Journal of Neuropsychopharmacology 11: 207–216.
108. Rodrigues LCD, Conti CL, Nakamura-Palacios EM (2011) Clozapine and SCH 23390 prevent the spatial working memory disruption induced by Delta(9)-THC administration into the medial prefrontal cortex. Brain Research 1382: 230–237.
109. D'Souza DC, Braley G, Blaise R, Vendetti M, Oliver S, et al. (2008) Effects of haloperidol on the behavioral, subjective, cognitive, motor, and neuroendocrine effects of delta-9-tetrahydrocannabinol in humans. Psychopharmacology 198: 587–603.
110. Liem-Moolenaar M, te Beek ET, de Kam ML, Franson KL, Kahn RS, et al. (2010) Central nervous system effects of haloperidol on THC in healthy male volunteers. Journal of Psychopharmacology 24: 1697–1708.
111. Kleinloog D, Liem-Moolenaar M, Jacobs G, Klaassen E, de Kam M, et al. (2012) Does olanzapine inhibit the psychomimetic effects of Δ^9-tetrahydrocannabinol? Journal of Psychopharmacology 26: 1307–1316.
112. Naderi N, Haghparast A, Saber-Tehrani A, Rezaii N, Alizadeh AM, et al. (2008) Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice. Pharmacol Biochem Behav 89: 64–75.
113. Micale V, Cristino L, Tamburella A, Petrosino S, Leggio GM, et al. (2009) Altered responses of dopamine D3 receptor null mice to excitotoxic or anxiogenic stimuli: possible involvement of the endocannabinoid and endovanilloid systems. Neurobiology of Disease 36: 70–80.
114. Garcia-Gutierrez MS, Manzanares J (2010) The cannabinoid CB1 receptor is involved in the anxiolytic, sedative and amnesic actions of benzodiazepines. Journal of Psychopharmacology 24: 757–765.
115. Urigüen L, Perez-Rial S, Ledent C, Palomo T, Manzanares J (2004) Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology 46: 966–973.
116. Micale V, Cristino L, Tamburella A, Petrosino S, Leggio GM, et al. (2009) Anxiolytic effects in mice of a dual blocker of fatty acid amide hydrolase and transient receptor potential vanilloid type-1 channels. Neuropsychopharmacology 34: 593–606.
117. Pertwee RG, Greentree SG, Swift PA (1988) Drugs that stimulate or facilitate central GABAergic transmission interact synergistically with delta-9-tetrahydrocannabinol-induced to produce marked catalepsy in mice. Neuropharmacology 27: 1265–1270.
118. Sulkowski A, Vachon L, Rich ES (1977) Propranolol effects on acute marihuana intoxication in man. Psychopharmacology (Berl) 23: 47–53.
119. Weis F, Beiras-Fernandez A, Hauer D, Hornuss C, Sodian R, et al. (2010) Effect of anaesthesia and cardiopulmonary bypass on blood endocannabinoid concentrations during cardiac surgery. British Journal of Anaesthesia 105: 139–144.
120. Pryor GT, Husain S, Larsen FF, McKenzie CE, Carr JD, et al. (1977) Interactions between delta9-tetrahydrocannabinol and phencyclidine hydrochloride in rats. Pharmacology Biochemistry and Behavior 6: 123–136.
121. McPartland JM, Pruitt PL (1997) Medical marijuana and its use by the immunocompromised. Altern Ther Health Med 3: 39–45.
122. Novelli GP, Peduto VA, Bertol E, Mari F, Pieraccioli E (1983) Analgesic interaction between nitrous oxide and delta-9-tetrahydrocannabinol in the rat. British Journal of Anaesthesia 55: 997–1000.
123. Naderi N, Ahari FA, Shafaghi B, Najarkolaei AH, Motamedi F (2008) Evaluation of interactions between cannabinoid compounds and diazepam in electroshock-induced seizure model in mice. Journal of Neural Transmission 115: 1501–1511.
124. Goffin K, Bormans G, Casteels C, Bosier B, Lambert DM, et al. (2008) An in vivo [18F]MK-9470 microPET study of type 1 cannabinoid receptor binding in Wistar rats after chronic administration of valproate and levetiracetam. Neuropharmacology 54: 1103–1106.
125. Pertwee RG, Browne SE, Ross TM, Stretton CD (1991) An investigation of the involvement of GABA in certain pharmacological effects of delta-9-tetrahydrocannabinol. Pharmacology Biochemistry and Behavior 40: 581–585.
126. Lile JA, Kelly TH, Hays LR (2012) Separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9-THC in humans discriminating Δ^9-THC. Drug and Alcohol Dependence 122: 61–69.

127. Luszczki JJ, Florek-Luszczki M (2012) Synergistic interaction of pregabalin with the synthetic cannabinoid WIN 55,212-2 mesylate in the hot-plate test in mice: an isobolographic analysis. Pharmacological Reports 64: 723–732.
128. Banni S, Carta G, Murru E, Cordeddu L, Giordano E, et al. (2012) Vagus nerve stimulation reduces body weight and fat mass in rats. Plos One 7.
129. Burdyga G, Varro A, Dimaline R, Thompson DG, Dockray GJ (2010) Expression of cannabinoid CB1 receptors by vagal afferent neurons: kinetics and role in influencing neurochemical phenotype. American Journal of Physiology-Gastrointestinal and Liver Physiology 299: G63–G69.
130. Riediger ND, Othman RA, Suh M, Moghadasian MH (2009) A systemic review of the roles of n-3 fatty acids in health and disease. Journal of the American Dietetic Association 109: 668–679.
131. Alvheim AR, Malde MK, Osei-Hyiaman D, Lin YH, Pawlosky RJ, et al. (2012) Dietary linoleic acid elevates endogenous 2-AG and anandamide and induces obesity. Obesity 20: 1984–1994.
132. Hutchins-Wiese HL, Li Y, Hannon K, Watkins BA (2012) Hind limb suspension and long-chain omega-3 PUFA increase mRNA endocannabinoid system levels in skeletal muscle. Journal of Nutritional Biochemistry 23: 986–993.
133. Wood JT, Williams JS, Pandarinathan L, Janero DR, Lammi-Keefe CJ, et al. (2010) Dietary docosahexaenoic acid supplementation alters select physiological endocannabinoid-system metabolites in brain and plasma. Journal of Lipid Research 51: 1416–1423.
134. Piscitelli F, Carta G, Bisogno T, Murru E, Cordeddu L, et al. (2011) Effect of dietary krill oil supplementation on the endocannabinoidome of metabolically relevant tissues from high-fat-fed mice. Nutrition & Metabolism 8.
135. Lafourcade M, Larrieu T, Mato S, Duffaud A, Sepers M, et al. (2011) Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Nature Neuroscience 14: 345–350.
136. Larrieu T, Madore C, Joffre C, Laye S (2012) Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice. Journal of Physiology and Biochemistry 68: 671–681.
137. Berger A, Crozier G, Bisogno T, Cavaliere P, Innis S, et al. (2001) Anandamide and diet: Inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding N-acylethanolamines in piglets (vol 98, pg 6402, 2001). Proceedings of the National Academy of Sciences of the United States of America 98: 7647–7647.
138. Bisogno T, Delton Vandenbroucke I, Milone A, La Garde M, Di Marzo V (1999) Biosynthesis and inactivation of N-arachidonoylethanolamine (anandamide) and N-docosahexaenoylethanolamine in bovine retina. Archives of Biochemistry and Biophysics 370: 300–307.
139. Brown I, Cascio MG, Wahle KWJ, Smoum R, Mechoulam R, et al. (2010) Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines. Carcinogenesis 31: 1584–1591.
140. Kim HY, Spector AA (2013) Synaptamide, endocannabinoid-like derivative of docosahexaenoic acid with cannabinoid-independent function. Prostaglandins Leukotrienes and Essential Fatty Acids 88: 121–125.
141. Schuchardt JP, Schneider I, Meyer H, Neubronner J, von Schacky C, et al. (2011) Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations–a comparative bioavailability study of fish oil vs. krill oil. Lipids Health Dis 10: 145.
142. Batetta B, Griinari M, Carta G, Murru E, Ligresti A, et al. (2009) Endocannabinoids may mediate the ability of (n-3) fatty acids to reduce ectopic fat and inflammatory mediators in obese Zucker rats. Journal of Nutrition 139: 1495–1501.
143. Di Marzo V, Griinari M, Carta G, Murru E, Ligresti A, et al. (2010) Dietary krill oil increases docosahexaenoic acid and reduces 2-arachidonoylglycerol but not N-acylethanolamine levels in the brain of obese Zucker rats. International Dairy Journal 20: 231–235.
144. Banni S, Carta G, Murru E, Cordeddu L, Giordano E, et al. (2011) Krill oil significantly decreases 2-arachidonoylglycerol plasma levels in obese subjects. Nutr Metab (Lond) 8: 7.
145. Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, et al. (2007) Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med 13: 35–37.
146. Palermo FA, Mosconi G, Avella MA, Carnevali O, Verdenelli MC, et al. (2011) Modulation of cortisol levels, endocannabinoid receptor 1A, proopiomelanocortin and thyroid hormone receptor alpha mRNA expressions by probiotics during sole (Solea solea) larval development. General and Comparative Endocrinology 171: 293–300.
147. Muccioli GG, Naslain D, Backhed F, Reigstad CS, Lambert DM, et al. (2010) The endocannabinoid system links gut microbiota to adipogenesis. Molecular Systems Biology 6.
148. Crouzin N, de Jesus Ferreira MC, Cohen-Solal C, M'Kadmi C, Bernad N, et al. (2011) alpha-tocopherol and alpha-tocopheryl phosphate interact with the cannabinoid system in the rodent hippocampus. Free Radic Biol Med 51: 1643–1655.
149. Di Marzo V, Sepe N, De Petrocellis L, Berger A, Crozier G, et al. (1998) Trick or treat from food endocannabinoids? Nature 396: 636–637.
150. Crozier Willi G, Berger A, Di Marzo V, Bisogno T, De Petrocellis L, et al. (2001) Lipids in neural function: modulation of behavior by oral administration of endocannabinoids found in foods. Nestle Nutr Workshop Ser Clin Perform Programme: 169–184; discussion 185–167.
151. Fride E, Ginzburg Y, Breuer A, Bisogno T, Di Marzo V, et al. (2001) Critical role of the endogenous cannabinoid system in mouse pup suckling and growth. Eur J Pharmacol 419: 207–214.
152. Carr RL, Borazjani A, Ross MK (2011) Effect of developmental chlorpyrifos exposure, on endocannabinoid metabolizing enzymes, in the brain of juvenile rats. Toxicological Sciences 122: 112–120.
153. Quistad CB, Sparks SE, Casida JE (2001) Fatty acid amide hydrolase inhibition by neurotoxic organophosphorus pesticides. Toxicology and Applied Pharmacology 173: 48–55.
154. Dhopeshwarkar AS, Jain S, Liao CY, Ghose SK, Bisset KM, et al. (2011) The actions of benzophenanthridine alkaloids, piperonyl butoxide and (S)-methoprene at the G-protein coupled cannabinoid CB1 receptor in vitro. European Journal of Pharmacology 654: 26–32.
155. Bisset KM, Dhopeshwarkar AS, Liao CY, Nicholson RA (2011) The G protein-coupled cannabinoid-1 (CB_1) receptor of mammalian brain: inhibition by phthalate esters in vitro. Neurochemistry International 59: 706–713.
156. Simao da Silva KAB, Paszcuk AF, Passos GF, Silva ES, Bento AF, et al. (2011) Activation of cannabinoid receptors by the pentacyclic triterpene alpha,beta-amyrin inhibits inflammatory and neuropathic persistent pain in mice. Pain 152: 1872–1887.
157. Meschler JP, Howlett AC (1999) Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. Pharmacology Biochemistry and Behavior 62: 473–480.
158. King AR, Dotsey EY, Lodola A, Jung KM, Ghomian A, et al. (2009) Discovery of potent and reversible monoacylglycerol lipase inhibitors. Chem Biol 16: 1045–1052.
159. Capasso R, Borrelli F, Cascio MG, Aviello G, Huben K, et al. (2008) Inhibitory effect of salvinorin A, from Salvia divinorum, on ileitis-induced hypermotility: cross-talk between kappa-opioid and cannabinoid CB1 receptors. British Journal of Pharmacology 155: 681–689.
160. Fichna J, Dicay M, Lewellyn K, Janecka A, Zjawiony JK, et al. (2012) Salvinorin A has antiinflammatory and antinociceptive effects in experimental models of colitis in mice mediated by KOR and CB1 receptors. Inflammatory Bowel Diseases 18: 1137–1145.
161. Thors L, Burston JJ, Alter BJ, McKinney MK, Cravatt BF, et al. (2010) Biochanin A, a naturally occurring inhibitor of fatty acid amide hydrolase. British Journal of Pharmacology 160: 549–560.
162. Korte G, Dreiseitel A, Schreier P, Oehme A, Locher S, et al. (2009) An examination of anthocyanins' and anthocyanidins' affinity for cannabinoid receptors. Journal of Medicinal Food 12: 1407–1410.
163. Korte G, Dreiseitel A, Schreier P, Oehme A, Locher S, et al. (2010) Tea catechins' affinity for human cannabinoid receptors. Phytomedicine 17: 19–22.
164. Ligresti A, Villano R, Allara M, Ujvary I, Di Marzo V (2012) Kavalactones and the endocannabinoid system: the plant-derived yangonin is a novel CB1 receptor ligand. Pharmacological Research 66: 163–169.
165. Hassanzadeh P, Hassanzadeh A (2012) The CB1 receptor-mediated endocannabinoid signaling and NGF: the novel targets of curcumin. Neurochemical Research 37: 1112–1120.
166. Prather PL, Seely KA, Levi MS (2009) The dietary polyphenols trans-resveratrol and curcumin selectively bind human CB1 cannabinoid receptors with nanomolar affinities and function as antagonists/inverse agonists (vol 330, pg 31, 2009). Journal of Pharmacology and Experimental Therapeutics 331: 1147–1147.
167. Botta B, Gacs-Baitz E, Vinciguerra V, Delle Monache G (2003) Three isoflavanones with cannabinoid-like moieties from Desmodium canum. Phytochemistry 64: 599–602.
168. Muhammad I, Li XC, Dunbar DC, ElSohly MA, Khan IA (2001) Antimalarial (+)-trans-hexahydrodibenzopyran derivatives from Machaerium multiflorum. Journal of Natural Products 64: 1322–1325.
169. Bohlmann F, Hoffmann E (1979) Cannabigerol-ähnliche verbendungen aus *Helichrysum umbraculigerum*. Phytochemistry 18: 1371–1374.
170. Iwata N, Kitanaka S (2011) New cannabinoid-like chromane and chromene derivatives from Rhododendron anthopogonoides. Chemical & Pharmaceutical Bulletin 59: 1409–1412.
171. Harinantenaina L, Takahara Y, Nishizawa T, Kohchi C, Soma GI, et al. (2006) Chemical constituents of malagasy liverworts, Part V: Prenyl bibenzyls and clerodane diterpenoids with nitric oxide inhibitory activity from Radula appressa and Thysananthus spathulistipus. Chemical & Pharmaceutical Bulletin 54: 1046–1049.
172. Toyota M, Shimamura T, Ishii H, Renner M, Braggins J, et al. (2002) New bibenzyl cannabinoid from the New Zealand liverwort Radula marginata. Chemical & Pharmaceutical Bulletin 50: 1390–1392.
173. Gao JT, Leon F, Radwan MM, Dale OR, Husni AS, et al. (2011) Benzyl derivatives with in vitro binding affinity for human opioid and cannabinoid receptors from the fungus Eurotium repens. Journal of Natural Products 74: 1636–1639.
174. Lin H, Ji-Kai L (2001) Two novel phenylacetoxylated p-terphenyls from Thelephora ganbajun Zang. Z Naturforsch C 56: 983–987.
175. Quaghebeur K, Coosemans J, Toppet S, Compernolle F (1994) Cannabiorcin and 8-chlorocannabiorcichromenic acid as fungal antagonists from Cylindrocarpon olidum. Phytochemistry 37: 159–161.

176. Leonti M, Casu L, Raduner S, Cottiglia F, Floris C, et al. (2010) Falcarinol is a covalent cannabinoid CB1 receptor antagonist and induces pro-allergic effects in skin. Biochemical Pharmacology 79: 1815–1826.
177. Zank TK, Zahringer U, Lerchl J, Heinz E (2000) Cloning and functional expression of the first plant fatty acid elongase specific for Delta(6)-polyunsaturated fatty acids. Biochemical Society Transactions 28: 654–658.
178. di Tomaso E, Beltramo M, Piomelli D (1996) Brain cannabinoids in chocolate. Nature 382: 677–678.
179. Nakane S, Tanaka T, Satouchi K, Kobayashi Y, Waku K, et al. (2000) Occurrence of a novel cannabimimetic molecule 2- sciadonoylglycerol (2-eicosa-5′, 11′, 14′-trienoylglycerol) in the umbrella pine Sciadopitys verticillata seeds. Biological & Pharmaceutical Bulletin 23: 758–761.
180. Gutierrez M, Pereira AR, Debonsi HM, Ligresti A, Di Marzo V, et al. (2011) Cannabinomimetic lipid from a marine cyanobacterium. Journal of Natural Products 74: 2313–2317.
181. Sitachitta N, Gerwick WH (1998) Grenadadiene and grenadamide, cyclopropyl-containing fatty acid metabolites from the marine cyanobacterium Lyngbya majuscula. J Nat Prod 61: 681–684.
182. Soderstrom K, Murray TF, Yoo HD, Ketchum S, Milligan K, et al. (1997) Discovery of novel cannabinoid receptor ligands from diverse marine organisms. Advances in Experimental Medicine and Biology 433: 73–77.
183. Raduner S, Majewska A, Chen JZ, Xie XQ, Hamon J, et al. (2006) Alkylamides from Echinacea are a new class of cannabinomimetics - cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. Journal of Biological Chemistry 281: 14192–14206.
184. Chicca A, Raduner S, Pellati F, Strompen T, Altmann KH, et al. (2009) Synergistic immunomopharmacological effects of N-alkylamides in Echinacea purpurea herbal extracts. International Immunopharmacology 9: 850–858.
185. Hohmann J, Redei D, Forgo P, Szabo P, Freund TF, et al. (2011) Alkamides and a neolignan from Echinacea purpurea roots and the interaction of alkamides with G-protein-coupled cannabinoid receptors. Phytochemistry 72: 1848–1853.
186. Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, et al. (2008) Beta-caryophyllene is a dietary cannabinoid. Proceedings of the National Academy of Sciences of the United States of America 105: 9099–9104.
187. Bento AF, Marcon R, Dutra RC, Claudino RF, Cola M, et al. (2011) Beta-caryophyllene inhibits dextran sulfate sodium-Induced colitis in mice through CB2 receptor activation and PPAR gamma pathway. American Journal of Pathology 178: 1153–1166.
188. Horvath B, Mukhopadhyay P, Kechrid M, Patel V, Tanchian G, et al. (2012) β-Caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner. Free Radical Biology and Medicine 52: 1325–1333.
189. Katsuyama S, Mizoguchi H, Kuwahata H, Komatsu T, Nagaoka K, et al. (2013) Involvement of peripheral cannabinoid and opioid receptors in β-caryophyllene-induced antinociception. European Journal of Pain 17: 664–675.
190. Rollinger JM, Schuster D, Danzl B, Schwaiger S, Markt P, et al. (2009) In silico target fishing for rationalized ligand discovery exemplified on constituents of Ruta graveolens. Planta Med 75: 195–204.
191. Palu AK, Kim AH, West BJ, Deng SX, Jensen J, et al. (2008) The effects of Morinda citrifolia L. (noni) on the immune system: its molecular mechanisms of action. Journal of Ethnopharmacology 115: 502–506.
192. Quartu M, Serra MP, Boi M, Pillolla G, Melis T, et al. (2012) Effect of acute administration of Pistacia lentiscus L. essential oil on rat cerebral cortex following transient bilateral common carotid artery occlusion. Lipids in Health and Disease 11.
193. Sepe N, De Petrocellis L, Montanaro F, Cimino G, Di Marzo V (1998) Bioactive long chain N-acylethanolamines in five species of edible bivalve molluscs - Possible implications for mollusc physiology and seafood industry. Biochimica et Biophysica Acta Lipids and Lipid Metabolism 1389: 101–111.
194. Bisogno T, Ventriglia M, Milone A, Mosca M, Cimino G, et al. (1997) Occurrence and metabolism of anandamide and related acyl-ethanolamides in ovaries of the sea urchin Paracentrotus lividus. Biochimica et Biophysica Acta Lipids and Lipid Metabolism 1345: 338–348.
195. Matias I, McPartland JM, Di Marzo V (2005) Occurrence and possible biological role of the endocannabinoid system in the sea squirt Ciona intestinalis. Journal of Neurochemistry 93: 1141–1156.
196. Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TTY, et al. (2010) Endogenous cannabinoid signaling is essential for stress adaptation. Proceedings of the National Academy of Sciences of the United States of America 107: 9406–9411.
197. Patel S, Roelke CT, Rademacher DJ, Hillard CJ (2005) Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. European Journal of Neuroscience 21: 1057–1069.
198. Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, et al. (2005) Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology 30: 508–515.
199. Patel S, Kingsley PJ, Mackie K, Marnett LJ, Winder DG (2009) Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at iInhibitory synapses in basolateral amygdala. Neuropsychopharmacology 34: 2699–2709.
200. Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP (2010) Fast feedback inhibition of the HPA axis by glucocorticoids Is mediated by endocannabinoid signaling. Endocrinology 151: 4811–4819.
201. Reich CG, Taylor ME, McCarthy MM (2009) Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. Behavioural Brain Research 203: 264–269.
202. Sciolino NR, Bortolato M, Eisenstein SA, Fu J, Oveisi F, et al. (2010) Social isolation and chronic handling alter endocannabinoid signaling and behavioral reactivity to context in adult rats. Neuroscience 168: 371–386.
203. Zoppi S, Nievas BGP, Madrigal JLM, Manzanares J, Leza JC, et al. (2011) Regulatory role of cannabinoid receptor 1 in stress-induced excitotoxicity and neuroinflammation. Neuropsychopharmacology 36: 805–818.
204. Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, et al. (2007) Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biological Psychiatry 62: 1103–1110.
205. Lee TTY, Hill MN (2013) Age of stress exposure modulates the immediate and sustained effects of repeated stress on corticolimbic cannabinoid CB1 receptor binding in male rats. Neuroscience 249: 106–114.
206. Hong SS, Zheng G, Wu XY, Snider NT, Owyang C, et al. (2011) Corticosterone mediates reciprocal changes in CB 1 and TRPV1 receptors in primary sensory neurons in the chronically stressed rat. Gastroenterology 140: 627-U371.
207. Dubreucq S, Matias I, Cardinal P, Haring M, Lutz B, et al. (2012) Genetic dissection of the role of cannabinoid type-1 receptors in the emotional consequences of repeated social stress in mice. Neuropsychopharmacology 37: 1885–1900.
208. Rossi S, De Chiara V, Musella A, Kusayanagi H, Mataluni G, et al. (2008) Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. Journal of Neuroscience 28: 7284–7292.
209. Hu W, Zhang MY, Czeh B, Zhang WQ, Flugge G (2011) Chronic restraint stress impairs endocannabinoid mediated suppression of GABAergic signaling in the hippocampus of adult male rats. Brain Research Bulletin 85: 374–379.
210. Wamsteeker JI, Kuzmiski JB, Bains JS (2010) Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. Journal of Neuroscience 30: 11188–11196.
211. Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, et al. (2005) An endocannabinoid mechanism for stress-induced analgesia. Nature 435: 1108–1112.
212. Connell K, Bolton N, Olsen D, Piomelli D, Hohmann AG (2006) Role of the basolateral nucleus of the amygdala in endocannabinoid-mediated stress-induced analgesia. Neuroscience Letters 397: 180–184.
213. Campolongo P, Roozendaal B, Trezza V, Hauer D, Schelling G, et al. (2009) Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proceedings of the National Academy of Sciences of the United States of America 106: 4888–4893.
214. Coddington E, Lewis C, Rose JD, Moore FL (2007) Endocannabinoids mediate the effects of acute stress and corticosterone on sex behavior. Endocrinology 148: 493–500.
215. Wang MN, Hill MN, Zhang LH, Gorzalka BB, Hillard CJ, et al. (2012) Acute restraint stress enhances hippocampal endocannabinoid function via glucocorticoid receptor activation. Journal of Psychopharmacology 26: 56–70.
216. Bradshaw HB, Rimmerman N, Krey JF, Walker JM (2006) Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 291: R349–R358.
217. Dunnett AJ, Roy D, Stewart A, McPartland JM (2007) The diagnosis of fibromyalgia in women may be influenced by menstrual cycle phase. Journal of Bodywork and Movement Therapies 11: 99–105.
218. McPartland JM, Giuffrida A, King J, Skinner E, Scotter J, et al. (2005) Cannabimimetic effects of osteopathic manipulative treatment. Journal of the American Osteopathic Association 105: 283–291.
219. Trezza V, Damsteegt R, Manduca A, Petrosino S, Van Kerkhof LWM, et al. (2012) Endocannabinoids in amygdala and nucleus accumbens mediate social play reward in adolescent rats. Journal of Neuroscience 32: 14899–14908.
220. Navarro M, Hernández E, Muñoz RM, del Arco I, Villanúa MA, et al. (1997) Acute administration of the CB1 cannabinoid receptor antagonist SR141716A induces anxiety-like responses in the rat. Neuroreport 8: 491–496.
221. Park HJ, Park HJ, Chae Y, Kim JW, Lee H, et al. (2011) Effect of acupuncture on hypothalamic-pituitary-adrenal system in maternal separation rats. Cell Mol Neurobiol 31: 1123–1127.
222. Chen L, Zhang J, Li F, Qiu Y, Wang L, et al. (2009) Endogenous anandamide and cannabinoid receptor-2 contribute to electroacupuncture analgesia in rats. Journal of Pain 10: 732–739.
223. Zhang J, Chen L, Su TF, Cao FY, Meng XF, et al. (2010) Electroacupuncture increases CB2 receptor expression on keratinocytes and infiltrating inflammatory cells in inflamed skin tissues of rats. Journal of Pain 11: 1250–1258.
224. Su TF, Zhang LH, Peng M, Wu CH, Pan W, et al. (2011) Cannabinoid CB2 receptors contribute to upregulation of beta-endorphin in inflamed skin tissues by electroacupuncture. Molecular Pain 7.
225. Fu LW, Longhurst JC (2009) Electroacupuncture modulates vlPAG release of GABA through presynaptic cannabinoid CB1 receptors. J Appl Physiol 106: 1800–1809.
226. Wang QA, Li XY, Chen YK, Wang F, Yang QZ, et al. (2011) Activation of epsilon protein kinase C-mediated anti-apoptosis is involved in rapid tolerance

induced by electroacupuncture pretreatment through cannabinoid receptor type 1. Stroke 42: 389–396.
227. Darmani NA, Izzo AA, Degenhardt B, Valenti M, Scaglione G, et al. (2005) Involvement of the cannabimimetic compound, N-palmitoyl-ethanolamine, in inflammatory and neuropathic conditions: Review of the available pre-clinical data, and first human studies. Neuropharmacology 48: 1154–1163.
228. Di Marzo V (2011) Endocannabinoids: an appetite for fat. Proc Natl Acad Sci U S A 108: 12567–12568.
229. Di Marzo V, Matias I (2005) Endocannabinoid control of food intake and energy balance. Nature Neuroscience 8: 585–589.
230. Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, et al. (2007) Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. International Journal of Obesity 31: 692–699.
231. Massa F, Mancini G, Schmidt H, Steindel F, Mackie K, et al. (2010) Alterations in the hippocampal endocannabinoid system in diet-Induced obese mice. Journal of Neuroscience 30: 6273–6281.
232. Engeli S, Böhnke J, Feldpausch M, Gorzelniak K, Janke J, et al. (2005) Activation of the peripheral endocannabinoid system in human obesity. Diabetes 54: 2838–2843.
233. Bennetzen MF, Wellner N, Ahmed SS, Ahmed SM, Diep TA, et al. (2011) Investigations of the human endocannabinoid system in two subcutaneous adipose tissue depots in lean subjects and in obese subjects before and after weight loss. International Journal of Obesity 35: 1377–1384.
234. Di Marzo V, Cote M, Matias I, Lemieux I, Arsenault BJ, et al. (2009) Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. Diabetologia 52: 213–217.
235. You TJ, Disanzo BL, Wang XW, Yang RZ, Gong DW (2011) Adipose tissue endocannabinoid system gene expression: depot differences and effects of diet and exercise. Lipids in Health and Disease 10.
236. Matias I, Gatta-Cherifi B, Tabarin A, Clark S, Leste-Lasserre T, et al. (2012) Endocannabinoids measurement in human saliva as potential biomarker of obesity. Plos One 7.
237. Sparling PB, Giuffrida A, Piomelli D, Rosskopf L, Dietrich A (2003) Exercise activates the endocannabinoid system. Neuroreport 14: 2209–2211.
238. Heyman E, Gamelin FX, Goekint M, Piscitelli F, Roelands B, et al. (2012) Intense exercise increases circulating endocannabinoid and BDNF levels in humans - possible implications for reward and depression. Psychoneuroendocrinology 37: 844–851.
239. Feuerecker M, Hauer D, Toth R, Demetz F, Holzl J, et al. (2012) Effects of exercise stress on the endocannabinoid system in humans under field conditions. European Journal of Applied Physiology 112: 2777–2781.
240. Raichlen DA, Foster AD, Gerdeman GL, Seillier A, Giuffrida A (2012) Wired to run: exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the 'runner's high'. Journal of Experimental Biology 215: 1331–1336.
241. Raichlen DA, Foster AD, Seillier A, Giuffrida A, Gerdeman GL (2013) Exercise-induced endocannabinoid signaling is modulated by intensity. European Journal of Applied Physiology 113: 869–875.
242. Ferrer B, Bermudez-Silva FJ, Bilbao A, Alvarez-Jaimes L, Sanchez-Vera I, et al. (2007) Regulation of brain anandamide by acute administration of ethanol. Biochemical Journal 404: 97–104.
243. Basavarajappa BS, Ninan I, Arancio O (2008) Acute ethanol suppresses glutamatergic neurotransmission through endocannabinoids in hippocampal neurons. Journal of Neurochemistry 107: 1001–1013.
244. Perra S, Pillolla G, Melis M, Muntoni AL, Gessa GL, et al. (2005) Involvement of the endogenous cannabinoid system in the effects of alcohol in the mesolimbic reward circuit: electrophysiological evidence in vivo. Psychopharmacology 183: 368–377.
245. Perra S, Pillolla G, Luchicchi A, Pistis M (2008) Alcohol inhibits spontaneous activity of basolateral amygdala projection neurons in the rat: involvement of the endocannabinoid system. Alcoholism-Clinical and Experimental Research 32: 443–449.
246. Yin HH, Park BS, Adermark L, Lovinger DM (2007) Ethanol reverses the direction of long-term synaptic plasticity in the dorsomedial striatum. Eur J Neurosci 25: 3226–3232.
247. Clarke RBC, Adermark L (2010) Acute ethanol treatment prevents endocannabinoid-mediated long-lasting disinhibition of striatal output. Neuropharmacology 58: 799–805.
248. Basavarajappa BS, Cooper TB, Hungund BL (1998) Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. Brain Res 793: 212–218.
249. Basavarajappa BS, Hungund BL (1999) Down-regulation of cannabinoid receptor agonist-stimulated [35S]GTP gamma S binding in synaptic plasma membrane from chronic ethanol exposed mouse. Brain Res 815: 89–97.
250. Ortiz S, Oliva JM, Perez-Rial S, Palomo T, Manzanares J (2004) Chronic ethanol consumption regulates cannabinoid CB1 receptor gene expression in selected regions of rat brain. Alcohol Alcohol 39: 88–92.
251. Vinod KY, Maccioni P, Garcia-Gutierrez MS, Femenia T, Xie S, et al. (2012) Innate difference in the endocannabinoid signaling and its modulation by alcohol consumption in alcohol-preferring sP rats. Addiction Biology 17: 62–75.
252. Vinod KY, Yalamanchili R, Xie S, Cooper TB, Hungund BL (2006) Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid system. Neurochemistry International 49: 619–625.
253. Rimondini R, Arlinde C, Sommer W, Heilig M (2002) Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. Faseb Journal 16: 27–35.
254. Mitrirattanakul S, Lopez-Valdes HE, Liang J, Matsuka Y, Mackie K, et al. (2007) Bidirectional alterations of hippocampal cannabinoid 1 receptors and their endogenous ligands in a rat model of alcohol withdrawal and dependence. Alcohol Clin Exp Res 31: 855–867.
255. Adermark L, Jonsson S, Ericson M, Soderpalm B (2011) Intermittent ethanol consumption depresses endocannabinoid-signaling in the dorsolateral striatum of rat. Neuropharmacology 61: 1160–1165.
256. Feuerecker M, Hauer D, Gresset T, Lassas S, Kaufmann I, et al. (2012) Effect of an acute consumption of a moderate amount of ethanol on plasma endocannabinoid levels in humans. Alcohol and Alcoholism 47: 226–232.
257. Vinod KY, Kassir SA, Hungund BL, Cooper TB, Mann JJ, et al. (2010) Selective alterations of the CB1 receptors and the fatty acid amide hydrolase in the ventral striatum of alcoholics and suicides. Journal of Psychiatric Research 44: 591–597.
258. Gonzalez S, Cascio MG, Fernández-Ruiz J, Fezza F, Di Marzo V, et al. (2002) Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. Brain Research 954: 73–81.
259. Penetar DM, Kouri EM, Gross MM, McCarthy EM, Rhee CK, et al. (2005) Transdermal nicotine alters some of marihuana's effects in male and female volunteers. Drug Alcohol Depend 79: 211–223.
260. Balerio GN, Aso E, Maldonado R (2006) Role of the cannabinoid system in the effects induced by nicotine on anxiety-like behaviour in mice. Psychopharmacology (Berl) 184: 504–513.
261. Le Foll B, Wiggins M, Goldberg SR (2006) Nicotine pre-exposure does not potentiate the locomotor or rewarding effects of Delta-9-tetrahydrocannabinol in rats. Behav Pharmacol 17: 195–199.
262. Pryor GT, Larsen FF, Husain S, Braude MC (1978) Interactions of delta9-tetrahydrocannabinol with d-amphetamine, cocaine, and nicotine in rats. Pharmacol Biochem Behav 8: 295–318.
263. Solinas M, Scherma M, Tanda G, Wertheim CE, Fratta W, et al. (2007) Nicotinic facilitation of delta-9-tetrahydrocannabinol discrimination involves endogenous anandamide. Journal of Pharmacology and Experimental Therapeutics 321: 1127–1134.
264. Valjent E, Mitchell JM, Besson M-J, Caboche J, Maldonado R (2002) Behavioural and biochemical evidence for interactions between Δ^9-tetrahydrocannabinol and nicotine. British Journal of Pharmacology 135: 564–578.
265. Jafari MR, Golmohammadi S, Ghiasvand F, Zarrindast MR, Djahanguiri B (2007) Influence of nicotinic receptor modulators on CB2 cannabinoid receptor agonist (JWH133)-induced antinociception in mice. Behav Pharmacol 18: 691–697.
266. Cippitelli A, Astarita G, Duranti A, Caprioli G, Ubaldi M, et al. (2011) Endocannabinoid regulation of acute and protracted nicotine withdrawal: effect ofFAAH inhibition. PLoS ONE 6.
267. Smith AD, Dar MS (2006) Mouse cerebellar nicotinic-cholinergic receptor modulation of Δ^9-THC ataxia: role of the $\alpha_4\beta_2$ subtype. Brain Research 1115: 16–25.
268. Buczynski MW, Polis IY, Parsons LH (2013) The volitional nature of nicotine exposure alters anandamide and oleoylethanolamide levels in the ventral tegmental area. Neuropsychopharmacology 38: 574–584.
269. Marco EM, Llorente R, Moreno E, Biscaia JM, Guaza C, et al. (2006) Adolescent exposure to nicotine modifies acute functional responses to cannabinoid agonists in rats. Behav Brain Res 172: 46–53.
270. Werling LL, Reed SC, Wade D, Izenwasser S (2009) Chronic nicotine alters cannabinoid-mediated locomotor activity and receptor density in periadolescent but not adult male rats. International Journal of Developmental Neuroscience 27: 263–269.
271. Robinson L, Goonawardena AV, Pertwee R, Hampson RE, Platt B, et al. (2010) WIN55,212-2 induced deficits in spatial learning are mediated by cholinergic hypofunction. Behavioural Brain Research 208: 584–592.
272. Bell J (1857) On the haschisch or Cannabis indica. Boston Medical and Surgical Journal 56: 209–216.
273. Hoffman AF, Laaris N, Kawamura M, Masino SA, Lupica CR (2010) Control of cannabinoid CB1 receptor function on glutamate axon terminals by endogenous adenosine acting at A_1 receptors. Journal of Neuroscience 30: 545–555.
274. Selley DE, Cassidy MP, Martin BR, Sim-Selley LJ (2004) Long-term administration of Delta9-tetrahydrocannabinol desensitizes CB1-, adenosine A1-, and GABAB-mediated inhibition of adenylyl cyclase in mouse cerebellum. Mol Pharmacol 66: 1275–1284.
275. McPartland JM, Mitchell JA (1997) Caffeine and chronic back pain. Archives of Physical Medicine and Rehabilitation 78: 61–63.
276. Consroe P, Jones B, Laird H, 2nd (1976) EEG and behavioral effects of delta9-tetrahydrocannabinol in combination with stimulant drugs in rabbits. Psychopharmacology (Berl) 50: 47–52.
277. Rossi S, De Chiara V, Musella A, Mataluni G, Sacchetti L, et al. (2009) Caffeine drinking potentiates cannabinoid transmission in the striatum: Interaction with stress effects. Neuropharmacology 56: 590–597.

278. Sousa VC, Assaife-Lopes N, Ribeiro JA, Pratt JA, Brett RR, et al. (2011) Regulation of hippocampal cannabinoid CB1 receptor actions by adenosine A_1 receptors and chronic caffeine administration: Implications for the effects of Δ^9-tetrahydrocannabinol on spatial memory. Neuropsychopharmacology 36: 472–487.
279. Mechoulam R, Fride E, Di Marzo V (1998) Endocannabinoids. Eur J Pharmacol 359: 1–18.
280. McPartland JM, Guy GW (2004) The evolution of Cannabis and coevolution with the cannabinoid receptor—a hypothesis. In: Guy GW, Robson R, Strong K, Whittle B, editors. The Medicinal Use of Cannabis. London: Royal Society of Pharmacists. pp. 71–102.
281. Cichewicz DL, Welch SP (2001) Opioid and cannabinoid receptor protein levels following chronic treatment. FASEB Journal 15: A228–A228.
282. Romero J, Garcia L, Fernandez-Ruiz JJ, Cebeira M, Ramos JA (1995) Changes in rat brain cannabinoid binding sites after acute or chronic exposure to their endogenous agonist, anandamide, or to delta-9-tetrahydrocannabinol. Pharmacology Biochemistry and Behavior 51: 731–737.
283. Zhuang S, Kittler J, Grigorenko EV, Kirby MT, Sim LJ, et al. (1998) Effects of long-term exposure to delta9-THC on expression of cannabinoid receptor (CB1) mRNA in different rat brain regions. Brain Res Mol Brain Res 62: 141–149.
284. Sim LJ, Hampson RE, Deadwyler SA, Childers SR (1996) Effects of chronic treatment with Δ^9-tetrahydrocannabinol on cannabinoid-stimulated [^{35}S]GTPγS autoradiography in rat brain. Journal of Neuroscience 16: 8057–8066.
285. Burstein SH, Hunter SA (1995) Stimulation of anandamide biosynthesis in N-18TG2 neuroblastoma cells by delta-9-tetrahydrocannabinol (THC). Biochemical Pharmacology 49: p855–858.
286. Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Vogt LJ, et al. (1999) Chronic Δ^9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. Journal of Neurochemistry 73: 2447–2459.
287. Falenski KW, Thorpe AJ, Schlosburg JE, Cravatt BF, Abdullah RA, et al. (2010) FAAH$^{-/-}$ mice display differential tolerance, dependence, and cannabinoid receptor adaptation after Δ^9-tetrahydrocannabinol and anandamide administration. Neuropsychopharmacology 35: 1775–1787.
288. Di Marzo V, Berrendero F, Bisogno T, Gonzalez S, Cavaliere P, et al. (2000) Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of D^9-tetrahydrocannabinol-tolerant rats. Journal of Neurochemistry 74: 1627–1635.
289. Villares J (2007) Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. Neuroscience 145: 323–334.
290. Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, et al. (2012) Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. Molecular Psychiatry 17: 642–649.
291. D'Souza DC, Ranganathan M, Braley G, Gueorguieva R, Zimolo Z, et al. (2008) Blunted Psychotomimetic and Amnestic Effects of Delta-9-Tetrahydrocannabinol in Frequent Users of Cannabis. Neuropsychopharmacology.
292. Rotter A, Bayerlein K, Hansbauer M, Weiland J, Sperling W, et al. (2013) CB1 and CB2 receptor expression and promoter methylation in patients with cannabis dependence. European Addiction Research 19: 13–20.
293. Lundberg DJ, Daniel AR, Thayer SA (2005) Δ^9-Tetrahydrocannabinol-induced desensitization of cannabinoid-mediated inhibition of synaptic transmission between hippocampal neurons in culture. Neuropharmacology 49: 1170–1177.
294. Hsieh C, Brown S, Derleth C, Mackie K (1999) Internalization and recycling of the CB1 cannabinoid receptor. J Neurochem 73: 493–501.
295. Fan F, Compton DR, Ward S, Melvin L, Martin BR (1994) Development of cross-tolerance between delta-9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. Journal of Pharmacology and Experimental Therapeutics 271: 1383–1390.
296. Luk T, Jin WZ, Zvonok A, Lu D, Lin XZ, et al. (2004) Identification of a potent and highly efficacious, yet slowly desensitizing CB1 cannabinoid receptor agonist. British Journal of Pharmacology 142: 495–500.
297. Sim-Selley LJ, Martin BR (2002) Effect of chronic administration of *R*-(+)-[2,3-dihydro-5- methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4- benzoxazinyl]-(1-naphthalenyl)methanone mesylate (WIN55,212-2) or Δ^9-tetrahydrocannabinol on cannabinoid receptor adaptation in mice. Journal of Pharmacology and Experimental Therapeutics 303: 36–44.
298. Laaris N, Good CH, Lupica CR (2010) Δ^9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. Neuropharmacology 59: 121–127.
299. Shen M, Thayer SA (1999) Δ^9-Tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture. Molecular Pharmacology 55: 8–13.
300. Straiker A, Mackie K (2005) Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. Journal of Physiology-London 569: 501–517.
301. Gonsiorek W, Lunn C, Fan XD, Narula S, Lundell D, et al. (2000) Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. Molecular Pharmacology 57: 1045–1050.
302. Savinainen JR, Jarvinen T, Laine K, Laitinen JT (2001) Despite substantial degradation, 2-arachidonoylglycerol is a potent full efficacy agonist mediating CB_1 receptor-dependent G-protein activation in rat cerebellar membranes. British Journal of Pharmacology 134: 664–672.
303. Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature 388: 773–778.
304. Darmani NA (2002) The potent emetogenic effects of the endocannabinoid, 2-AG (2- arachidonoylglycerol) are blocked by Δ^9-tetrahydrocannabinol and other cannnabinoids. Journal of Pharmacology and Experimental Therapeutics 300: 34–42.
305. Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE, et al. (2004) A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci 7: 585–586.
306. Kelley BG, Thayer SA (2004) Δ^9-Tetrahydrocannabinol antagonizes endocannabinoid modulation of synaptic transmission between hippocampal neurons in culture. Neuropharmacology 46: 709–715.
307. Roloff AM, Thayer SA (2009) Modulation of excitatory synaptic transmission by Δ^9-tetrahydrocannabinol switches from agonist to antagonist depending on firing rate. Molecular Pharmacology 75: 892–900.
308. Burkey TH, Quock RM, Consroe P, Ehlert FJ, Hosohata Y, et al. (1997) Relative efficacies of cannabinoid CB1 receptor agonists in the mouse brain. Eur J Pharmacol 336: 295–298.
309. Aceto MD, Scates SM, Razdan RK, Martin BR (1998) Anandamide, an endogenous cannabinoid, has a very low physical dependence potential. Journal of Pharmacology and Experimental Therapeutics 287: 598–605.
310. Fride E (1995) Anandamides: tolerance and cross-tolerance to delta-9-tetrahydrocannabinol. Brain Research 697: 83–90.
311. Bonhaus DW, Chang LK, Kwan J, Martin GR (1998) Dual activation and inhibition of adenylyl cyclase by cannabinoid receptor agonists: evidence for agonist-specific trafficking of intracellular responses. J Pharmacol Exp Ther 287: 884–888.
312. Glass M, Northup JK (1999) Agonist selective regulation of G proteins by cannabinoid CB(1) and CB(2) receptors. Mol Pharmacol 56: 1362–1369.
313. Fride E, Barg J, Levy R, Saya D, Heldman E, et al. (1995) Low doses of anandamides inhibit pharmacological effects of delta-9-tetrahydrocannabinol. Journal of Pharmacology and Experimental Therapeutics 272: 699–707.
314. Pertwee RG, Stevenson LA, Griffin G (1993) Cross-tolerance between delta-9-tetrahydrocannabinol and the cannabimimetic agents, CP 55,940, WIN 55,212-2 and anandamide. British Journal of Pharmacology 110: 1483–1490.
315. Vann RE, Walentiny DM, Burston JJ, Tobey KM, Gamage TF, et al. (2012) Enhancement of the behavioral effects of endogenous and exogenous cannabinoid agonists by phenylmethyl sulfonyl fluoride. Neuropharmacology 62: 1019–1027.
316. Suplita RL, Eisenstein SA, Neely MH, Moise AM, Hohmann AG (2008) Cross-sensitization and cross-tolerance between exogenous cannabinoid antinociception and endocannabinoid-mediated stress-induced analgesia. Neuropharmacology 54: 161–171.
317. Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, et al. (2009) Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. Proceedings of the National Academy of Sciences of the United States of America 106: 20270–20275.
318. McPartland JM, Pruitt PL (1999) Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. Alternative Therapies in Health and Medicine 5: 57–62.
319. McPartland JM, Russo EB (2001) Cannabis and cannabis extracts: Greater than the sum of their parts? Journal of Cannabis Therapeutics 1: 103–132.
320. Hayakawa K, Mishima K, Hazekawa M, Sano K, Irie K, et al. (2008) Cannabidiol potentiates pharmacological effects of Δ^9-tetrahydrocannabinol via CB1 receptor-dependent mechanism. Brain Research 1188: 157–164.
321. Wolf SA, Bick-Sander A, Fabel K, Leal-Galicia P, Tauber S, et al. (2010) Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. Cell Communication and Signaling 8.
322. Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, et al. (2001) Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. British Journal of Pharmacology 134: 845–852.
323. De Petrocellis L, Ligresti A, Moriello AS, Allarà M, Bisogno T, et al. (2011) Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. British Journal of Pharmacology 163: 1479–1494.
324. Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, et al. (2010) Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. Nature Neuroscience 13: 1113–1121.
325. Watanabe S, Doshi M, Hamazaki T (2003) n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. Prostaglandins Leukot Essent Fatty Acids 69: 51–59.
326. Matias I, Carta G, Murru E, Petrosino S, Banni S, et al. (2008) Effect of polyunsaturated fatty acids on endocannabinoid and N-acyl-ethanolamine levels in mouse adipocytes. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1781: 52–60.
327. Artmann A, Petersen G, Hellgren LI, Boberg J, Skonberg C, et al. (2008) Influence of dietary fatty acids on endocannabinoid and N-acylethanolamine

levels in rat brain, liver and small intestine. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1781: 200–212.
328. Kirkham TC, Williams CM, Fezza F, Di Marzo V (2002) Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. British Journal of Pharmacology 136: 550–557.
329. Hanus L, Avraham Y, Ben-Shushan D, Zolotarev O, Berry EM, et al. (2003) Short-term fasting and prolonged semistarvation have opposite effects on 2-AG levels in mouse brain. Brain Research 983: 144–151.
330. Cottone E, Guastalla A, Pomatto V, Campantico E, Di Marzo V, et al. (2009) Goldfish CB1 mRNA expression is affected by fasting and anandamide administration. Neuroreport 20: 595–599.
331. Valenti M, Cottone E, Martinez R, De Pedro N, Rubio M, et al. (2005) The endocannabinoid system in the brain of Carassius auratus and its possible role in the control of food intake. Journal of Neurochemistry 95: 662–672.
332. Guijarro A, Osei-Hyiaman D, Harvey-White J, Kunos G, Suzuki S, et al. (2008) Sustained weight loss after roux-en-y gastric bypass is characterized by down regulation of endocannabinoids and mitochondrial function. Annals of Surgery 247: 779–790.
333. Jelsing J, Larsen PJ, Vrang N (2009) The effect of leptin receptor deficiency and fasting on cannabinoid receptor 1 mRNA expression in the rat hypothalamus, brainstem and nodose ganglion. Neuroscience Letters 463: 125–129.
334. Yan ZC, Liu DY, Zhang LL, Shen CY, Ma QL, et al. (2007) Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-delta. Biochemical and Biophysical Research Communications 354: 427–433.
335. Rossi S, Furlan R, De Chiara V, Musella A, Lo Giudice T, et al. (2009) Exercise attenuates the clinical, synaptic and dendritic abnormalities of experimental autoimmune encephalomyelitis. Neurobiology of Disease 36: 51–59.
336. De Chiara V, Errico F, Musella A, Rossi S, Mataluni G, et al. (2010) Voluntary exercise and sucrose consumption enhance cannabinoid CB1 receptor sensitivity in the striatum. Neuropsychopharmacology 35: 374–387.
337. da Silva SG, Araujo BHS, Cossa AC, Scorza FA, Cavalheiro EA, et al. (2010) Physical exercise in adolescence changes CB1 cannabinoid receptor expression in the rat brain. Neurochemistry International 57: 492–496.
338. Hill MN, Titterness AK, Morrish AC, Carrier EJ, Lee TTY, et al. (2010) Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. Hippocampus 20: 513–523.
339. Yasari S, Prud'homme D, Tesson F, Jankowski M, Gutkowska J, et al. (2012) Effects of exercise training on molecular markers of lipogenesis and lipid partitioning in fructose-induced liver fat accumulation. J Nutr Metab 2012: 181687.
340. Basavarajappa BS, Hungund BL (1999) Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor N-arachidonoyl-phosphatidylethanolamine in SK-N-SH cells. Journal of Neurochemistry 72: 522–528.
341. Basavarajappa BS, Saito M, Cooper TB, Hungund BL (2000) Stimulation of cannabinoid receptor agonist 2-arachidonylglycerol by chronic ethanol and its modulation by specific neuromodulators in cerebellar granule neurons. Biochimica Et Biophysica Acta-Molecular Basis of Disease 1535: 78–86.
342. Basavarajappa BS, Saito M, Cooper TB, Hungund BL (2003) Chronic ethanol inhibits the anandamide transport and increases extracellular anandamide levels in cerebellar granule neurons. European Journal of Pharmacology 466: 73–83.
343. Burkey TH, Quock RM, Consroe P, Roeske WR, Yamamura HI (1997) delta 9-Tetrahydrocannabinol is a partial agonist of cannabinoid receptors in mouse brain. Eur J Pharmacol 323: R3–4.
344. Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq M-C (1998) Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of Δ^9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. Life Sciences 63: PL1–6.