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## The thrifty lipids: Endocannabinoids and the neural control of energy conservation

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### Abstract

The “thrifty gene hypothesis” posits that evolution preferentially selects physiological mechanisms that optimize energy storage to increase survival under alternating conditions of abundance and scarcity of food. Recent experiments suggest that endocannabinoids – a class of lipid-derived mediators that activate cannabinoid receptors in many cells of the body – are key agents of energy conservation. The new evidence indicates that these compounds increase energy intake and decrease energy expenditure by controlling the activity of peripheral and central neural pathways involved in the sensing and hedonic processing of sweet and fatty foods, as well as in the storage of their energy content for future use.

### Keywords

2-arachidonoylglycerol; cannabinoid receptors; dietary fat

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“During the first 99 percent or more of man’s life on earth, while he existed as a hunter and gatherer, it was often feast or famine”. [1]

### Introduction

The endocannabinoid system plays a critical role in monitoring energy needs and maintaining metabolic balance in mammals [2]. This signaling complex is found in most mammalian organs and tissues, and comprises a set of lipid-derived messengers (i.e., endocannabinoids) [3], as well as proteins that control their formation and deactivation [4–12] and cell-surface receptors that transduce their actions (CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors) [13, 14]. One of the best-understood endocannabinoids, the fatty acyl ester 2-arachidonoyl-*sn*-glycerol (2-AG), is produced in the brain and spinal cord through the consecutive activation of two receptor-operated lipases, phospholipase C-β (PLC-β) and diacylglycerol lipase-α (DGL-α). After release into the extracellular space, 2-AG diffuses to presynaptic nerve terminals, where it reduces neurotransmitter release by recruiting G<sub>i/o</sub>

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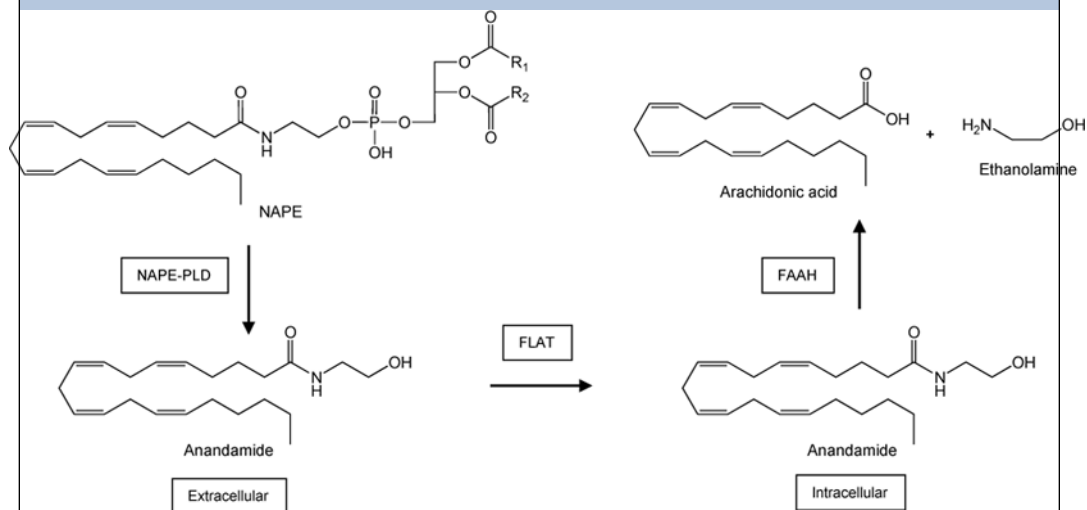
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protein-coupled CB<sub>1</sub> receptors (CB<sub>1</sub>Rs), to be finally eliminated through enzyme-mediated hydrolysis [6, 8, 9, 15] (see Figure 1). Less is known on the workings of another important endocannabinoid, anandamide (arachidonylethanolamide), though it is generally assumed that, like 2-AG, this compound acts in the brain as a local modulator of synaptic activity [3] (see Box 1).

### Box 1

#### Anandamide formation and deactivation

Anandamide biosynthesis occurs through enzyme-mediated hydrolysis of the membrane phospholipid precursor, *N*-arachidonoyl phosphatidylethanolamine (NAPE) (Figure I), but the identification and neuroanatomical localization of the enzymes involved in this reaction are still incomplete [4, 7]. After release into the extracellular space, anandamide acts as a partial agonist at presynaptic CB<sub>1</sub>Rs, and is subsequently deactivated by cellular uptake and intracellular hydrolysis. Anandamide hydrolysis is catalyzed by fatty acid amide hydrolase-1 (FAAH-1) and FAAH-2, two serine hydrolases found on intracellular membranes [12]. The transport of anandamide from the extracellular space to intracellular FAAH is facilitated by the chaperon action of a splicing variant of FAAH-1, called FAAH-like anandamide transporter (FLAT) [10]. In addition to 2-AG and anandamide, other endogenous molecules that bind CB<sub>1</sub>Rs have been identified, such as noladin ether [3, 73]. Their physiological significance remains, however, largely unknown.



**Figure I.** Schematic illustrating the main enzymatic steps involved in the formation and hydrolysis of anandamide.

The central nervous system (CNS) has long been recognized as a site of endocannabinoid control of feeding behavior [16, 17]. There is substantial evidence, however, that endocannabinoid signaling in peripheral tissues also plays an important role in energy homeostasis [18–21]. In the present review, we highlight recent findings suggesting that an overarching function of the endocannabinoids, and particularly of 2-AG, both inside and outside the CNS might be to regulate the sensing and seeking of sweet and fatty foods, when these are available, and promote the storage of their energy content to allow for its utilization during periods of scarcity. According to this view, endocannabinoid signaling is a key molecular instantiation of the “thrifty gene hypothesis”, which states that evolution

selects physiological mechanisms that maximize energy storage to increase survival under alternating conditions of feast and famine [1].

## Peripheral endocannabinoids and food taste

### Endocannabinoids in the tongue control neural responses to sweets

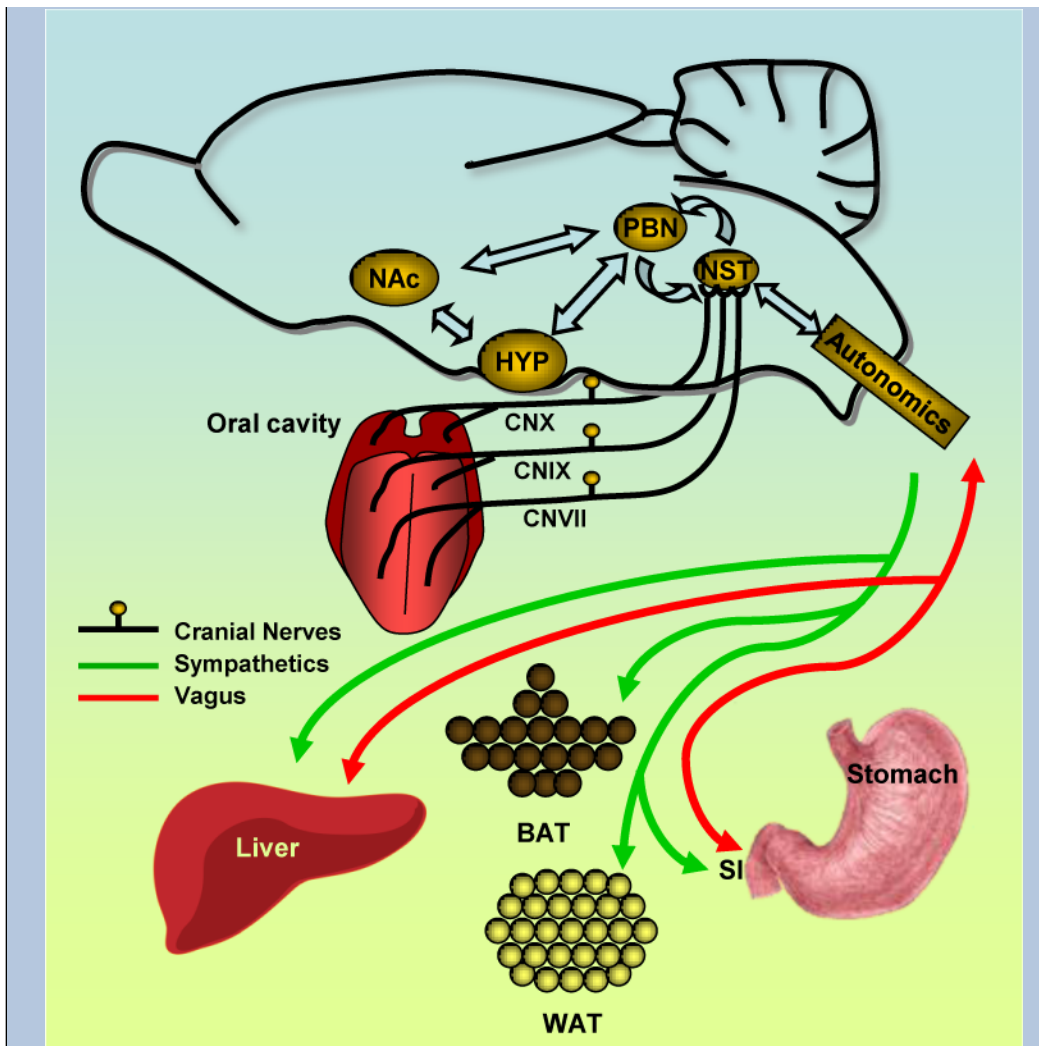
Food is first sensed in the taste buds, clusters of cells located on protrusions of the tongue called *papillae*. The *chorda tympani* branch of the facial nerve (VII) innervates taste buds from the anterior two-thirds of the tongue, while the lingual branch of the glossopharyngeal nerve (IX) innervates taste buds from the posterior one-third of the tongue. Both the VII and IX nerves enter the brainstem to terminate in the nucleus of the solitary tract (NST) [22] (see Box 2). Recent work has shown that CB<sub>1</sub>Rs are present in taste buds and that their pharmacological activation enhances neural responses to sweet foods [23].

#### Box 2

##### Endocannabinoid mechanisms throughout the mammalian body regulate the seeking, sensing, and utilization of energy-dense foods

The mammalian brain reciprocally communicates with peripheral organs and tissues via autonomic signals to control food intake and energy homeostasis (Figure I). Emerging evidence places the endocannabinoid system at the driver's seat of this regulatory action. CB<sub>1</sub>Rs are present on the tongue, where their activation increases neural responses to sweet substances [23]. Fat taste is increasingly recognized as a basic taste quality [26, 27] and a host of taste receptors located on the tongue have been identified as critical for maintaining fat intake [26, 28, 29]. Oral exposure to dietary fats mobilizes endocannabinoids in the rat proximal small intestine through efferent vagal signaling, and their local blockade with a CB<sub>1</sub> receptor antagonist curbs fat intake, suggesting that endocannabinoids in the gut play a major role in driving the intake of fatty meals [19].

CB<sub>1</sub> receptors in the PBN are thought to gate the gustatory neurotransmission associated with palatable foods. Their activation increases the consumption of such foods, but fails to affect the intake of a standard diet [38]. Neural signals from the hindbrain are transmitted throughout the forebrain to, but not limited to, the NAc and hypothalamus. Pharmacological activation of CB<sub>1</sub>Rs in these regions increases food intake [42, 44, 45, 47–50], while CB<sub>1</sub>R activation in the NAc shell enhances positive affective reactions to sweets [42]. Furthermore, endocannabinoid levels increase in the hippocampus of diet-induced obese mice, which may promote hedonic eating [74]. In addition to their role in food intake, forebrain endocannabinoids regulate energy homeostasis by modifying activity of the sympathetic nervous system [58, 59], which communicates with the periphery to control thermogenesis in BAT. Enhanced endocannabinoid activity in the hypothalamus may conserve energy, at least in part, by reducing BAT thermogenesis. Furthermore, peripheral endocannabinoid mechanisms are critical for the maintenance of lipid metabolism and energy utilization. Endocannabinoids in liver may regulate lipogenesis [61]. CB<sub>1</sub>R activation promotes adipogenesis [63–66] and reduces fatty-acid oxidation in liver and skeletal muscle [68, 69].



**Figure I.**

Schematic representing key central and peripheral organs involved in food intake and energy balance. Gustatory neural signals, including those likely associated with fat or sweet taste, are transmitted from the tongue and oral cavity to the brainstem along the facial (CNVII), glossopharyngeal (CNIX), and vagus (CNX) nerves [22]. These afferent sensory signals terminate in the nucleus of the solitary tract (NST). Neural signals are subsequently transmitted rostrally in rats to the parabrachial nucleus (PBN). Neurons in the NST and PBN respond to and integrate gustatory information derived from the oral cavity, with satiation/satiety-related neural signals transmitted from the gut by the afferent vagus nerve (red arrows). The hindbrain communicates sensory information from food to areas throughout the forebrain, including the nucleus accumbens (NAc) and the hypothalamus (HYP). Importantly, the brain communicates with peripheral organs and tissues, including brown adipose tissue (BAT), liver, white adipose tissue (WAT), and small intestine (SI), via the autonomic nervous system—which comprises vagal afferent and efferents (red arrows), and sympathetics (green arrows)—to maintain food intake and energy balance.

In the mouse tongue, CB<sub>1</sub>R<sub>s</sub> are found in cells of the *fungiform* papillae, present at the front of the tongue, and the *circumvallate* papillae, located toward the back of the tongue [23]. In

70% of those cells, CB<sub>1</sub>Rs co-localize with type 1 taste receptor 3, a putative sweet receptor [24, 25] and, when activated by exogenously administered endocannabinoids, increase the neural activity elicited in the chorda tympani by sweeteners – but not by bitter, umami, salty, or sour substances [23]. This effect is not only observed *in vivo*, but also *in vitro* after application of CB<sub>1</sub>R agonists to isolated taste cells [23], which is suggestive that local endocannabinoid signaling in the tongue might enhance neural responses to sweet nutrients.

### Endocannabinoids in the gut promote dietary fat intake

The existence of a “fat taste” is now generally accepted [26, 27] and receptors located on the tongue have been identified as being critical for initiating and maintaining dietary fat intake [26, 28, 29]. In the brain, dopamine and opioid pathways have been implicated in the “wanting” and “liking”, respectively, of fatty and sweet meals [30]. Experiments in rodents have shown that tasting a fatty meal (without ingesting it) is sufficient to elicit dopamine outflow in the ventral striatum [31], a brain region that controls the hedonic evaluation of rewarding stimuli [32, 33]. Likewise, tasting a fatty meal promotes its further intake once this has begun [34] and elicits conditioned place preference — a test used to assess preference for an environment that has been associated with a rewarding experience [35]. Collectively, these data support the theory that orosensory positive feedback mechanisms play a key role in the rewarding properties of dietary fat [34].

New investigations suggest that endocannabinoid signals in the gut might contribute to the attraction that many mammals display for fatty foods [19, 36]. These experiments utilized a rat sham-feeding model in which the orosensory effects of fat can be separated from its post-ingestive influences [34]. The results show that sham-feeding rats with a high-fat liquid meal causes the two primary endocannabinoids, 2-AG and anandamide, to accumulate in the jejunal portion of the small intestine [19]. This effect is due to a coordinated increase in the production and decrease in the degradation of the two substances. Importantly, surgical transection of the vagus nerve blocks the effect of fat sham feeding on endocannabinoid mobilization, implying that the gustatory signals elicited by this nutrient are transmitted from the brainstem to the intestine through the vagus nerve (see Box 2). Furthermore, sham feeding rats with a nutritionally complete diet produces a biochemical response similar to that elicited by fat, whereas sham feeding meals containing either carbohydrate or protein exerts no such effect [19].

Fat sham feeding modifies endocannabinoid levels only in the proximal small intestine, not in other peripheral tissues or the brain. This raises the question of whether such a restricted change in endocannabinoid signaling might be able to influence feeding behavior. To address this question, rats were fitted with duodenal catheters and a low concentration of the CB<sub>1</sub>R inverse agonist, rimonabant, was infused into their intestine just prior to sham feeding them with a high-fat meal. Local blockade of CB<sub>1</sub>Rs in the small intestine was found to markedly reduce fat sham-feeding [19]. Moreover, a similar effect was observed after systemic administration of a CB<sub>1</sub>R neutral antagonist that was restricted access to the CNS [19]. These findings indicate that endocannabinoid signals in the gut may be a critical component of the positive orosensory feedback mechanism that drives fat intake. The results further suggest that therapeutic strategies aimed at restraining small-intestinal endocannabinoid activity – for example, peripherally restricted CB<sub>1</sub>R antagonists – might help reduce the overeating of fatty foods and control overweight and obesity (see Box 3).

**Box 3****Peripherally restricted CB<sub>1</sub>R antagonists for the treatment of obesity**

The CB<sub>1</sub>R inverse agonist, rimonabant (SR141716A), has been invaluable in probing the functions served by the endocannabinoid system in the control of energy balance [75]. In both humans and experimental animals, the drug produces a temporary decrease in food intake that is followed by a more prolonged reduction in body weight [76]. In obese human subjects, rimonabant reduces waist circumference and improves metabolic parameters [77] but, due to its ability to cross the blood-brain barrier, also exerts psychiatric side effects (e.g., increased risk of depression and anxiety), which have ultimately prevented its clinical use. To avoid these central interferences, CB<sub>1</sub>R antagonists and inverse agonists have been developed that are restricted to the periphery of the body. These compounds reduce food intake and body-weight gain and improve the metabolic profile in rodents [62, 78], presumably by interacting with CB<sub>1</sub>R in the digestive tract [79], liver [61], pancreas [80], white adipose tissue [81], and skeletal muscle [82]. The metabolic effects of peripherally restricted CB<sub>1</sub>R antagonists/inverse agonists are particularly important, because they underscore the critical functions served by peripheral endocannabinoids in the control of systemic metabolism.

**Central endocannabinoid signaling and food intake**

Experiments with genetically modified mice have shown that central CB<sub>1</sub>R can exert opposite effects on food intake, depending on whether they are localized to presynaptic terminals of excitatory or inhibitory neurons [37]. Peripheral administration of low doses of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the psychoactive component of *Cannabis*, stimulates food intake in wild-type mice, but fails to do so in mice that selectively lack CB<sub>1</sub>R on nerve terminals of excitatory glutamate-releasing neurons [37]. Conversely, high doses of  $\Delta^9$ -THC decrease food intake in wild-type mice, but not in mice lacking CB<sub>1</sub>R in inhibitory GABA-releasing neurons. Administration of the CB<sub>1</sub>R inverse agonist, AM251, into the ventral striatum of wild-type mice completely blocked the hypophagic actions of high-dose  $\Delta^9$ -THC, but failed to block the hyperphagic effects of low-dose  $\Delta^9$ -THC [37]. A plausible interpretation of these findings is that low levels of CB<sub>1</sub>R activation stimulate feeding through inhibition of glutamatergic transmission (see Figure 1) in neural circuits that likely exclude the ventral striatum, whereas high levels of CB<sub>1</sub>R activation reduce feeding through inhibition of GABA-ergic transmission within the ventral striatum. The opposing functions of CB<sub>1</sub>R at glutamatergic and GABA-ergic synapses underscore the complexity of the neurobiological control of food intake and energy balance, and the critical need for further investigations to delineate the roles that brain endocannabinoid mechanisms play in controlling these processes.

**Brainstem**

A recent report suggests that endocannabinoids in the hindbrain regulate gustatory neurotransmission initiated by palatable foods [38]. Taste-related neural signals, including those associated with fatty and sweet foods, are transmitted from the oral cavity first to the NST and then to the pontine parabrachial nucleus (PBN) in the caudal brainstem [22] (see Box 2). Neurons in the NST and PBN integrate taste information derived from the oral cavity with satiation- and satiety-related signals transmitted from the gut by the afferent vagus nerve. This information is processed, and appropriate motor commands are sent to modulate meal size and inter-meal interval [39, 40].

CB<sub>1</sub>R<sub>s</sub> are present in the PBN, where local administration of 2-AG increases intake of palatable foods, but not standard chow [38]. This effect is blocked by the CB<sub>1</sub>R inverse agonist, AM251. Furthermore, indirect activation of CB<sub>1</sub>R<sub>s</sub> in the PBN with microinfusions of an inhibitor of anandamide degradation enhances intake of a palatable fat-rich diet [41]. This work suggests that endocannabinoid signaling in the PBN may modulate the intake of foods with hedonically positive sensory properties and might gate neurotransmission inherent to fat and sweet taste.

### Ventral striatum

Sensory information about food is processed in forebrain areas that include the shell of the nucleus accumbens (NAc), which is specifically involved in the affective evaluation of rewarding sensory stimuli [32]. In the NAc, endocannabinoids may regulate the motivation to consume palatable nutrients. This possibility is supported by studies that utilized the taste reactivity test [42], which evaluates the hedonic impact (“liking”) of sensory stimuli [43]. Microinfusions of anandamide into select “hot spots” of the NAc shell was shown to increase “liking” responses to a sucrose solution in rats, and this effect was prevented by CB<sub>1</sub>R blockade [42]. Furthermore, administration of anandamide into the NAc increased the intake of a palatable sweet solution, but not one containing the aversive bitter substance, quinine [44].

While these results identify the NAc shell as a brain site where pharmacological CB<sub>1</sub>R activation modulates the pleasurable properties of food, they leave open the question of whether intrinsic endocannabinoid activity contributes to “liking” responses under physiological conditions. A partial answer to this question comes from studies which show that fasting increases endocannabinoid levels in the limbic forebrain (including NAc) of rats [45], and that infusion of inhibitors of endocannabinoid degradation in NAc stimulates feeding [46]. These data offer a possible scenario in which fasting stimulates endocannabinoid production in NAc [45] to enhance both the hedonic properties of palatable foods and the motivation to consume them [42].

### Hypothalamus

2-AG levels increase in the rat hypothalamus after a 24-h fast [45] and infusions of CB<sub>1</sub>R agonists into various hypothalamic nuclei stimulate food intake [47–50]. Leptin, an adipose-derived circulating hormone that reduces energy intake and promotes energy utilization [51], interferes with these signaling events. Systemic administration of leptin lowers endocannabinoid content in the mouse hypothalamus [52], suggesting that this hormone accelerates energy consumption at least in part by modulating hypothalamic endocannabinoid activity. Consistent with this idea, endocannabinoid levels in the hypothalamus are abnormally elevated in genetically obese rodents, including Zucker rats, leptin-deficient (*ob/ob*) mice, and leptin-receptor deficient (*db/db*) mice [52].

The mechanisms involved in the regulation of endocannabinoid signaling by leptin are not entirely clear. However, electrophysiological studies in rat hypothalamic slices have provided important information about this process. The studies have shown that glucocorticoids stimulate, via a non-genomic mechanism, endocannabinoid biosynthesis and the ensuing suppression of excitatory neurotransmission in perifornical neurosecretory neurons located in the paraventricular nucleus of the hypothalamus [53, 54]. Importantly, endocannabinoid signaling is blocked by leptin, which is consistent with an integrated role for endocannabinoids with hormones involved in energy homeostasis [53]. Further supporting this idea, leptin treatment reduces endocannabinoid-mediated depolarization-induced suppression of inhibition in the perifornical region of the lateral hypothalamus (LH) in rat brain slices [55]. In this region, endocannabinoids may be released from neurons that



secrete melanin-concentrating hormone (MCH) to inhibit GABAergic transmission and ultimately disinhibit MCH release. Thus, under fasting conditions, when concentrations of circulating leptin are low, endocannabinoid levels in the hypothalamus may rise unopposed by leptin, ultimately enhancing the release of MCH and promoting an overall anabolic tone [55]. The complexity of the circuits controlling these processes is emphasized, however, by recent studies that show a reduction in CB<sub>1</sub>R signaling at GABA-ergic synapses in the dorsomedial hypothalamus under fasting conditions [56], a time in which circulating leptin levels are low.

## Central and peripheral integration in the control of energy balance

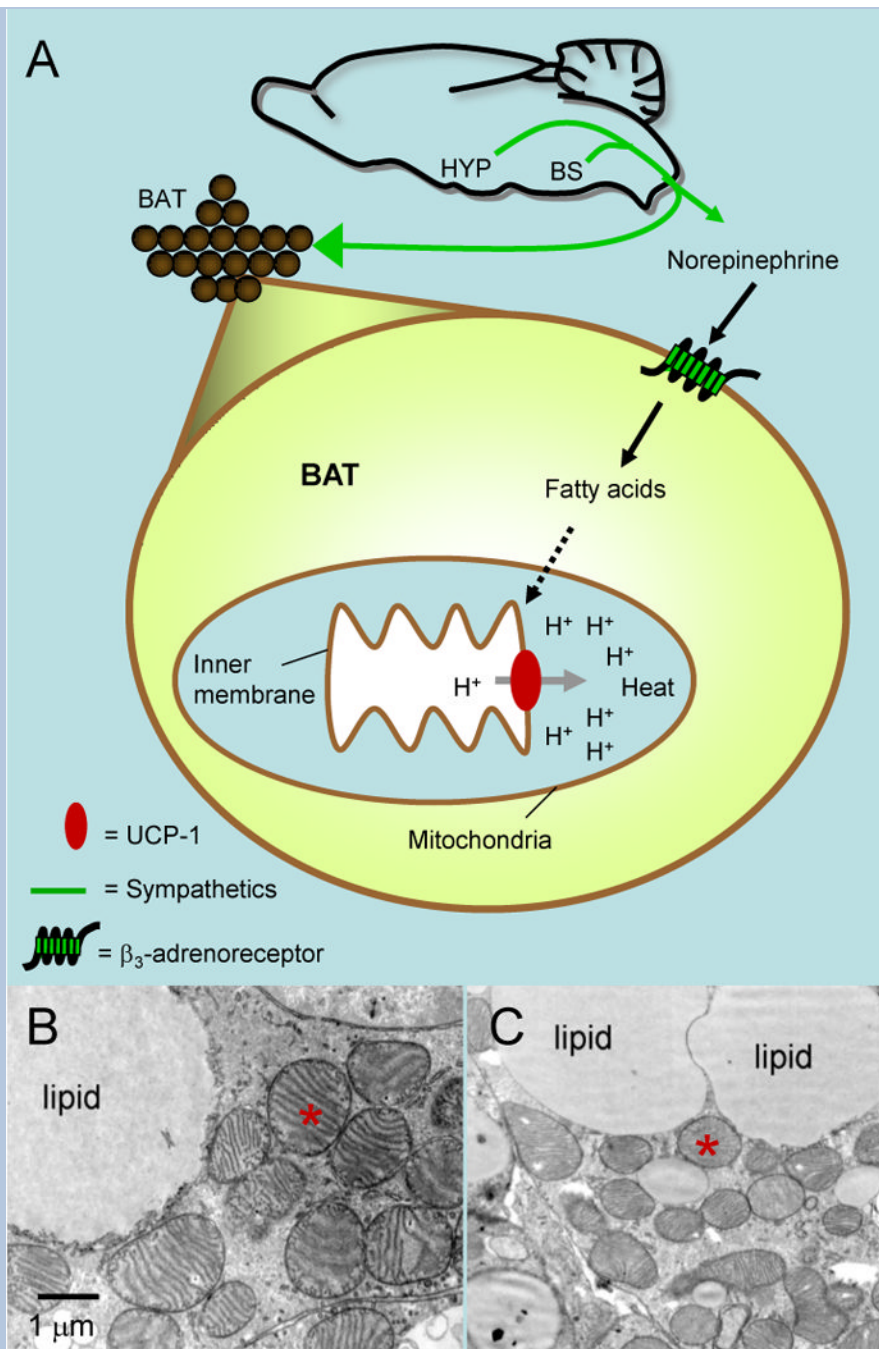
The brain reciprocally communicates with peripheral organs and tissues to maintain energy balance (see Box 2). Several recent studies suggest that central endocannabinoid mechanisms contribute to energy homeostasis by toning down processes controlled by the sympathetic nervous system, such as thermogenesis in brown adipose tissue (BAT; see Box 4) [57–60], which activates energy expenditure.

### Box 4

#### Brown adipose tissue (BAT) generates heat

BAT is responsible for non-shivering thermogenesis — a heat-generating mechanism that helps mammals to maintain metabolic balance in the face of fluctuating energy requirements and uncertain energy availability [83]. Though initially uncertain, the role of BAT in human thermogenesis is now convincingly documented [84–86]. Thermogenesis in BAT is controlled by the sympathetic nervous system, which extensively innervates this tissue, but is ultimately governed by neural centers located in the hypothalamus (HYP) and brainstem (BS) [87] (see Figure IA). Norepinephrine released from sympathetic nerve terminals activates G<sub>s</sub> protein-coupled β<sub>3</sub>-adrenoreceptors on the surface of brown adipocytes, stimulating lipolysis and the delivery of fatty acids to mitochondria, and activating uncoupling protein-1 (UCP-1). UCP-1 disconnects the mitochondrial respiratory chain from adenosine-5'-triphosphate synthesis during fatty-acid oxidation, which leads to a leakage of protons (H<sup>+</sup>) across the inner mitochondrial membrane, and the release of energy in the form of heat [83].

Recent findings from a variety of laboratories have suggested a major role for endocannabinoid mechanisms in controlling sympathetic activity that drives BAT thermogenesis [57–60]. For example, mutant mice that overexpress MGL in the forebrain, when compared to wild-type, display reduced levels of 2-AG in this brain region, a lean phenotype that is resistant to diet-induced obesity [59]. Moreover, these mice have increased levels of sympathetically-mediated BAT thermogenesis and mitochondrial density in the forebrain compared to wildtype mice (Figures IB and IC) [59].



**Figure I. Endocannabinoid mechanisms are involved in controlling sympathetic activity that drives BAT thermogenesis**

(A) Schematic diagram of the rat brain (upper panel) illustrating neural centers in the HYP and BS that control thermogenesis in BAT. Lower panel: Norepinephrine released from sympathetic nerve terminals results in the release of heat from brown adipocytes. (B–C) Electron microscopy revealed increased mitochondrial density in BAT from MGL-transgenic mice (left panel) compared to wild-type mice (right panel). Images adapted from [59]; red asterisk represents mitochondria.

### Forebrain 2-AG controls energy dissipation

Mice lacking CB<sub>1</sub>Rs in the forebrain and sympathetic ganglia are resistant to diet-induced obesity [58]. When exposed to a high-fat diet, these mice display an abnormally low energy efficiency (body weight gain per unit of energy intake) compared to wild-type littermates. This phenotypic change was attributed to an enhanced thermogenic activity in BAT. Indeed, mice lacking CB<sub>1</sub>Rs in forebrain and sympathetic ganglia showed increased transcription of BAT genes involved in thermogenesis, including uncoupling protein-1 (UCP-1), improved thermogenic response and O<sub>2</sub> consumption following cold exposure, and enhanced uptake of 2-deoxy-2-[<sup>18</sup>F]-fluoro-D-glucose, as measured by positron emission tomography (PET) [58]. Enhanced sympathetic outflow mediates at least some of these effects. Uptake of the norepinephrine analog PET tracer, <sup>11</sup>C-meta-hydroxyephedrine, by BAT was heightened in mutant mice during cold exposure, which is suggestive of an accelerated norepinephrine turnover. An economical interpretation of these results is that endocannabinoid signaling in the forebrain, and possibly sympathetic ganglia, controls energy balance by regulating thermogenesis in BAT.

To test this hypothesis further, subsequent studies utilized mice that overexpressed the presynaptic 2-AG-hydrolyzing enzyme monoacylglycerol lipase (MGL) (see Fig. 1) under the control of the Ca<sup>2+</sup>-calmodulin kinase II- $\alpha$  promoter [59]. 2-AG hydrolysis is increased and 2-AG levels are concomitantly reduced throughout the forebrain – but not in cerebellum, sympathetic ganglia, or peripheral tissues – of these mice. Moreover, levels of anandamide and CB<sub>1</sub>R expression are normal in these mutants. When compared to wild-type controls, MGL-overexpressing mice displayed a complex series of phenotypic changes that included leanness, increased feeding, reduced motor activity, and marked resistance to diet-induced obesity and its metabolic consequences (e.g., high serum triglyceride, insulin and leptin levels; increased liver steatosis) [59]. This phenotypic profile may be explained by the finding that, compared to wild-type controls, MGL-overexpressing mice utilized a greater amount of energy during motor activity, showing increased thermogenic responses to pharmacological activation of  $\beta_3$ -adrenergic receptors, as well as displaying elevated mitochondrial density in BAT (see Box 4). Importantly, treatment with an irreversible MGL inhibitor normalized thermogenesis in transgenic mice [59]. As this effect is blocked by the CB<sub>1</sub>R inverse agonist, rimonabant, it can be reasonably concluded that increased thermogenesis in MGL-overexpressing mice is a consequence of deficient 2-AG signaling at forebrain CB<sub>1</sub>Rs. Although it is possible that CB<sub>1</sub>Rs in peripheral sympathetic fibers control BAT-mediated thermogenesis - as evidenced by enhanced thermogenesis in mice lacking CB<sub>1</sub>Rs both in forebrain and sympathetic ganglia [58] - 2-AG appears to regulate BAT-mediated thermogenesis primarily in the forebrain, since MGL overexpression in transgenic mice is strictly confined to this region [59].

### Endocannabinoids and the non-neuronal control of lipid storage and utilization

Endocannabinoid activity may regulate lipogenesis in the liver. Administration of CB<sub>1</sub>R agonists in mice increases expression of liver proteins that support lipogenesis: steroid regulatory element-binding protein 1c (SREBP-1c), acetyl-CoA-carboxylase-1, and fatty acid synthase [61]. Further suggesting a facilitating role for endocannabinoids in liver lipogenesis, it was shown that the beneficial effects of peripheral CB<sub>1</sub>R blockade on liver triglyceride content and diet-induced insulin resistance are retained in transgenic animals that express CB<sub>1</sub>Rs exclusively in the liver, but not in mice that lack CB<sub>1</sub>R in all organs and tissues [62].

CB<sub>1</sub>R activation promotes adipogenesis. Mutant mice globally lacking CB<sub>1</sub>R exhibit reduced fat mass and increased lean mass [63]. CB<sub>1</sub>Rs are present in adipose cells and their activation *in vitro* increases lipoprotein lipase activity [63], whereas CB<sub>1</sub>R blockade decreases adipocyte proliferation in mouse preadipocytes [64]. In these cells, endocannabinoids increase expression of a variety of genes associated with adipocyte proliferation, including peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), an early marker of adipocyte differentiation, and cause buildup of triglyceride-rich lipid droplets [65]. CB<sub>1</sub>R activation in rat adipocytes also promotes adipocyte differentiation, expression of PPAR- $\gamma$  [66], and insulin-stimulated glucose uptake [67].

One mechanism through which the endocannabinoid system may regulate energy utilization in liver and skeletal muscle is by reducing fatty acid  $\beta$ -oxidation. Systemic administration of  $\Delta^9$ -THC reduces the activity of liver and adipocyte AMP-dependent kinase (AMPK), which promotes fatty acid oxidation and glucose uptake [68]. In contrast, the CB<sub>1</sub>R inverse agonist AM251 increases expression of the AMPK isoform, AMPK $\alpha$ 1 in myotubes obtained from human muscle [69]. This effect may contribute to the beneficial metabolic properties of CB<sub>1</sub>R antagonists. Peripheral endocannabinoids might also participate in the transport of lipids through the bloodstream. Systemic administration of a non-selective irreversible inhibitor of endocannabinoid degradation in mice causes hypertriglyceridemia by impairing apolipoprotein-E-mediated clearance of triglyceride-rich lipoproteins [70]. Conversely, blockade of CB<sub>1</sub>Rs lowers circulating levels of triglycerides and non-esterified fatty acids, as well as triglyceride content in skeletal muscle of diet-induced obese rats [71]. Furthermore, incubation of liver and intestinal cell lines with endocannabinoids reduces the expression of apolipoprotein A-1 (apo A-1) [72], the major protein component of high-density lipoproteins (HDL).

## Summary

The evidence reviewed here suggests that an overarching function of the endocannabinoid system in mammals might be to modulate the activity of central and peripheral neural pathways involved in the intake of sweet and fatty foods, as well as the storage of the energy content of these nutrients for future utilization (see Box 5 for *Outstanding questions*). In the tongue, endocannabinoids acting at CB<sub>1</sub>Rs may strengthen the gustatory response to sweet substances and possibly help enhance their intake [23]. The taste of dietary fat, like sweet, is inherently palatable to most mammals; however, only oral exposure to fat, not sugar or protein, enhances endocannabinoid mobilization in the proximal small intestine [19]. Local pharmacological blockade of this signaling event attenuates dietary fat intake, suggesting that the endocannabinoid system in the gut is a critical constituent of the positive feedback mechanism that drives fat consumption.

### Box 5

#### Outstanding questions

- What receptors in the tongue sense fatty and sweet foods, and how do they initiate endocannabinoid signaling? Potential candidates include CD36 for fat [88] and type 1 taste receptor 3 for sugar [24, 25]. Although both proteins are found in lingual sensory epithelium [89, 90], their role in activating endocannabinoid signaling has not been investigated.
- How do endocannabinoids in the tongue and small intestine communicate with the brain to influence behavior? In the gut, endocannabinoids may promote fat intake by activating CB<sub>1</sub> receptors on vagal fibers and enteric neurons [91], thereby modifying the action or generation of neurohumoral factors that affect

satiation (meal size) and satiety (inter-meal interval), such as ghrelin [92] or cholecystokinin (CCK) [93].

- Do small-intestinal endocannabinoids regulate the absorption and processing of dietary fat, in addition to its intake? Such a role would be compatible with the anatomical localization of fat-induced endocannabinoid mobilization [19], but has not been examined yet.
- How does forebrain 2-AG control thermogenesis in BAT? One possibility is that 2-AG may work by suppressing the activity of neural circuits that drive sympathetic outflow and thermogenesis. Such circuits might include those in the paraventricular nucleus of the hypothalamus that utilize the neuropeptide cocaine- and amphetamine- related transcript (CART) [59].
- Injections of CB<sub>1</sub>R antagonists/inverse agonists into select brain regions have consistently failed to affect food intake [38, 45, 46, 49, 50]. By contrast, injections of CB<sub>1</sub>R antagonists/inverse agonists into cerebral ventricles have been shown to produce anorexia in most studies [94–96], albeit not all (eg. [18]). How can these contrasting findings be reconciled? It is reasonable to assume that the concomitant blockade of CB<sub>1</sub>Rs in multiple brain sites is necessary to affect food intake, but this possibility has not been investigated yet.
- What is the role for circulating endocannabinoids in controlling food intake and energy balance? Studies have reported that plasma endocannabinoid levels in humans correlate with increases in various markers of obesity –including body mass index, waist circumference and visceral fat mass [97–99]. Plasma endocannabinoid concentrations are relatively low (less than 10 nM) [97–100], and hence, likely represent spillover from sites of production in peripheral organs. The possibility cannot be excluded, however, that circulating endocannabinoids participate in signaling events that control food intake and energy homeostasis (for example, through selective accumulation in target tissues).

Endocannabinoid mechanisms in the brain are known to be involved in the control of feeding behavior [37, 38, 45, 46, 48, 49, 52], but the results of experiments with genetically modified mice suggest that they also regulate energy balance independently of food intake [58, 59]. Overexpression of the 2-AG-hydrolyzing enzyme, MGL, in mouse forebrain neurons greatly reduces 2-AG content and produces a lean, obesity-resistant phenotype [59]. Forebrain-selective deletion of CB<sub>1</sub>Rs causes phenotypic changes that largely (albeit not completely) overlap with those seen in MGL-overexpressing mice [58]. These complementary findings strongly indicate that forebrain 2-AG signaling at CB<sub>1</sub>Rs promotes energy conservation. This function is synergistic with those served by the endocannabinoids in peripheral tissues, such as liver and adipose, in which these compounds stimulate both lipogenesis and adipogenesis.

In conclusion, the endocannabinoid system may act throughout the body as an agent of energy conservation: a messenger of thrift selected by evolution to maximize chances of survival in natural environments where feast is inevitably followed by famine. While adaptive in the wild, this signaling mechanism can become maladaptive in environments where food is plentiful and the energy necessary to find it is minimal. In such conditions, this pro-survival mechanism might turn into an agent of obesity, diabetes and cardiovascular disease.

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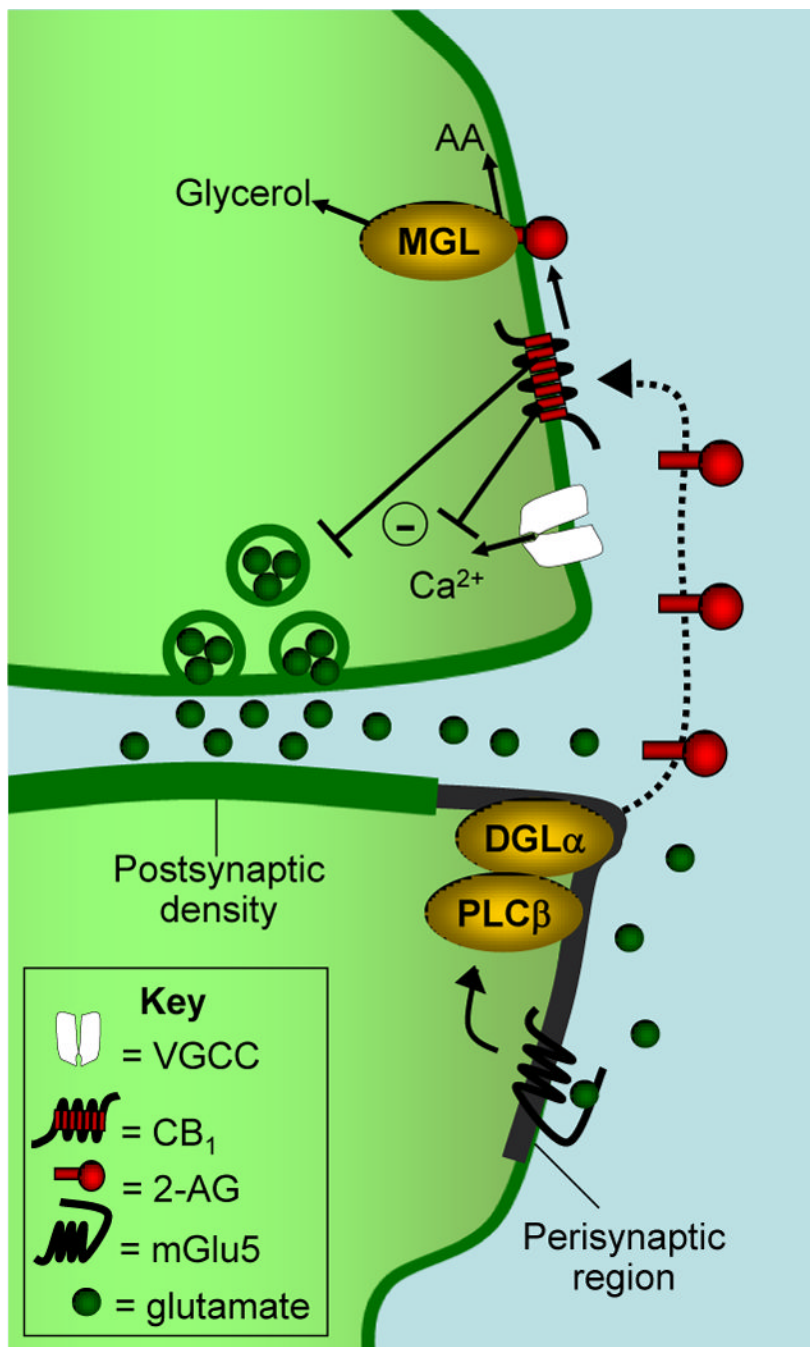
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**Figure 1.** Receptor-dependent production of 2-arachidonoyl-*sn*-glycerol (2-AG) and retrograde signaling at excitatory synapses. The biosynthesis of 2-AG at excitatory synapses may be initiated following spillover of glutamate into the perisynaptic region. Glutamate signaling at type-5 metabotropic glutamate receptors (mGlu5-Rs) stimulates phospholipase C-β (PLC-β) activity generating 1,2-arachidonoylglycerol, which is cleaved by diacylglycerol lipase-α (DGL-α) to produce 2-AG [6, 8, 15]. This endocannabinoid diffuses to the nerve ending where it binds to presynaptic CB<sub>1</sub> cannabinoid receptors, reducing both calcium influx at voltage gated calcium channels (VGCC) and vesicular release of glutamate (inhibition

denoted by  $\rightarrow$ ). 2-AG is rapidly degraded by monoacylglycerol lipase (MGL) and other hydrolases [9] into arachidonic acid (AA) and glycerol.