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Cannabinoids, eating behaviour, and energy homeostasis

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Q1 **Soon after the discovery of cannabis by western societies, its psychotropic effects overshadowed its medical benefits. However, investigation into the molecular action of the main constituents of cannabis has led to the discovery of an intercellular signalling system, called the endocannabinoid system (ECS). The ECS comprises a set of molecular components, including enzymes, signalling lipids and G-protein coupled receptors, which has an outstanding role in modulating eating behaviour and energy homeostasis. Interestingly, evidence has shown that the ECS is present at the central and peripheral nervous system, modulating the function of the hypothalamus, the brain reward system and the brainstem, and coordinating the crosstalk between these brain structures and peripheral organs. Indeed, the ECS is present and functional in metabolically relevant peripheral tissues, directly modulating their physiology. In the context of a global obesity pandemic, these discoveries are highly suggestive in order to design novel pharmaceutical tools to fight obesity and related morbidities. In fact, a cannabinoid-based first generation of drugs was developed and marketed. Their failure, due to central side-effects, is leading to a second generation of these drugs unable to cross the blood–brain barrier, as well as other ECS-focused strategies that are still in the pipeline. In the next few years we will hopefully know whether such an important player in energy homeostasis can be successfully targeted without significantly affecting other vital processes related to mood and sense of well-being. Copyright © 2013 John Wiley & Sons, Ltd.**

Q2 **Keywords:** endocannabinoids; phytocannabinoids; obesity; energy balance; feeding

Cannabis and society

Q3 Cannabis is currently an illicit substance in most western countries. Its trafficking, possession, selling, and consumption are generally penalized with different legal consequences depending on the country. The fight against cannabis use is mainly because of its psychotropic effects; indeed its use has been argued to lead to dependence and psychotic disorders, especially in young people.[1]

However, cannabis also has a 'good' side, i.e. its medicinal properties. References exist to the ancient use of cannabis for the alleviation of several medical conditions such pain, nausea and appetite loss as early as 2700 years BC (reviewed in Amar[2]). These benefits were also recognized after western societies discovered this herb, though a series of historical events led to cannabis being excluded from the United States pharmacopeia and later from that of Great Britain and most other European countries.

Paradoxically, alcohol and tobacco, both of which have well-documented harmful effects linked to an increased incidence of diseases like hepatic cirrhosis and lung cancer, remain legal while their medicinal effects are scarce or null. It is thus difficult to understand the different legal status of cannabis when compared to these other substances of abuse. The difficulty and cost of changing social paradigms and the actions of lobbies in favour of alcohol and tobacco are probably among the causes underlying this different status. Indeed, increasing evidence for the beneficial effects of medical cannabis and a deeper understanding of related cellular and molecular processes involved have clearly decreased the perceived risk-benefit ratio of cannabis and cannabinoid compounds in the last 20 years.

The endogenous cannabinoid system

The discovery of an endogenous cannabinoid signalling system in animals and humans has represented a milestone, boosting research into cannabinoids and cannabinoid-based therapeutic approaches. Since the seminal work by Devane *et al*., identifying the main receptor involved in cannabinoid effects,[3] the molecular components of this system have been cloned, anatomically localized and their physiology has been substantially elucidated. Briefly, the endocannabinoid system (ECS) comprises a set of molecular components including the endogenous cannabinoids (mainly 2-arachydonoil-glycerol (2-AG) and anandamide, both polyunsaturated fatty acid derivatives), the G-protein-coupled cannabinoid receptors (CB1 and CB2), and several enzymes involved in the synthesis and degradation of endocannabinoids.[4,5]

The ECS works as a local intercellular modulatory mechanism. When a given stimulus (e.g. membrane depolarization) reaches a cell provided with the enzymes to synthesize endocannabinoids, these compounds are generated from lipid precursors

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in the cytoplasmic membrane and then released into the extracellular space where they target, in an autocrine or paracrine way, cannabinoid receptors. Activation of these receptors, generally coupled to G inhibitory protein, leads to modulation of intracellular processes that eventually change the way cells respond to other stimuli. Examples of these intracellular events being modulated include decreased adenylyl cyclase activity, leading to lower cAMP production, and modulation of ion channel activity, such as activation of G-protein-coupled inwardly-rectifying potassium channels and inhibition of L-, N- and P/Q-type voltage-gated calcium channels. Also, different MAPK pathways are activated, such as ERK1/2, p38MAPK and JNK.[6]

The cannabinoid CB1 and CB2 receptors are differentially expressed in tissues and display different sensitivity to the endocannabinoids anandamide and 2-AG. CB1 receptors are found predominantly at nerve terminals, and are thus responsible for the psychotropic effects of exogenous cannabinoids, such as Δ^9-THC from cannabis, whereas CB2 receptors are mainly expressed on immune cells. Pharmacologically, both anandamide and 2-AG have slightly greater CB1 than CB2 sensitivity, but 2-AG exhibits higher CB1 and CB2 efficacy than anandamide, the latter thus behaving as a partial agonist at both receptor types. Indeed, anandamide has additional targets, such as TRPV1.[7]

Δ^9-THC, the main constituent of cannabis, is also a partial agonist at CB1 and CB2 receptors, whereas cannabinol (another cannabis constituent) also behaves as a partial agonist, at least at CB1 receptors.[7] Cannabidiol, another main component of cannabis, has however very low affinity for cannabinoid receptors.[8]

Synthetic cannabinoids are also available and are of great value in the pharmacological dissection of biological processes mediated through cannabinoid receptors. For instance, HU-210, CP5940 and R-(+)-WIN55212 are mixed CB1 and CB2 agonists with higher affinities than Δ^9-THC and endocannabinoids. Additionally, selective CB1 and CB2 agonists and antagonists have been developed, the best known of which is rimonabant, a CB1 antagonist/inverse agonist that was even marketed for complicated obesity. For a detailed and exhaustive description of these and other cannabinoid compounds (both exogenous and endogenous) as well as their pharmacology, the reader can refer to the following reviews.[7,9]

The endocannabinoid system, food intake, and energy homeostasis

The ECS is widely expressed throughout the body, participating in a wide range of biological functions, including nociception, regulation of motor activity, cognitive and emotional processes, immune function and energy balance. Because of its modulatory nature, the ECS has been suggested to be critically involved in the maintenance of homeostasis.[10] One such key homeostatic function is energy homeostasis. Living creatures need energy to maintain their physical integrity. Since life appeared on Earth, several mechanisms have had to be implemented in order to guarantee a constant energy supply to living organisms. Thus, evolution imposed in animals certain behaviours, among which is the eating behaviour. However, given the limited availability of edible energy, other mechanisms were developed to assure an optimized utilization of energy contained in food and ways to store the surplus within the body. To deal with these behavioural and metabolic challenges, a complex set of physiological processes operates in both the central nervous system (CNS) and the peripheral tissues, acting in a coordinated fashion. As explained below, the ECS plays an outstanding role in these processes (Figure 1). In fact, cannabis users are very familiar with the hyperphagic effects of cannabinoids, a phenomenon that has long been recognized and is colloquially known as 'the munchies', i.e. the overconsumption of foods, especially those of high palatability, after smoking marijuana. Research efforts have found that this behaviour is promoted by Δ^9-THC [11] through cannabinoid CB1 receptors.[12] Moreover, the effect of Δ^9-THC on feeding was found to be mimicked by exogenous administration of the endogenous cannabinoids anandamide and 2-AG, thus supporting the involvement of these lipids in the natural processes controlling feeding.[13,14] In agreement with these studies, other authors have found synthetic agonists and antagonists to promote and reduce, respectively, feeding behaviour. More information on one of these synthetic antagonists, rimonabant, can be found in the next section. Interestingly, natural compounds other than Δ^9-THC, found in Δ^9-THC standardized botanical drug

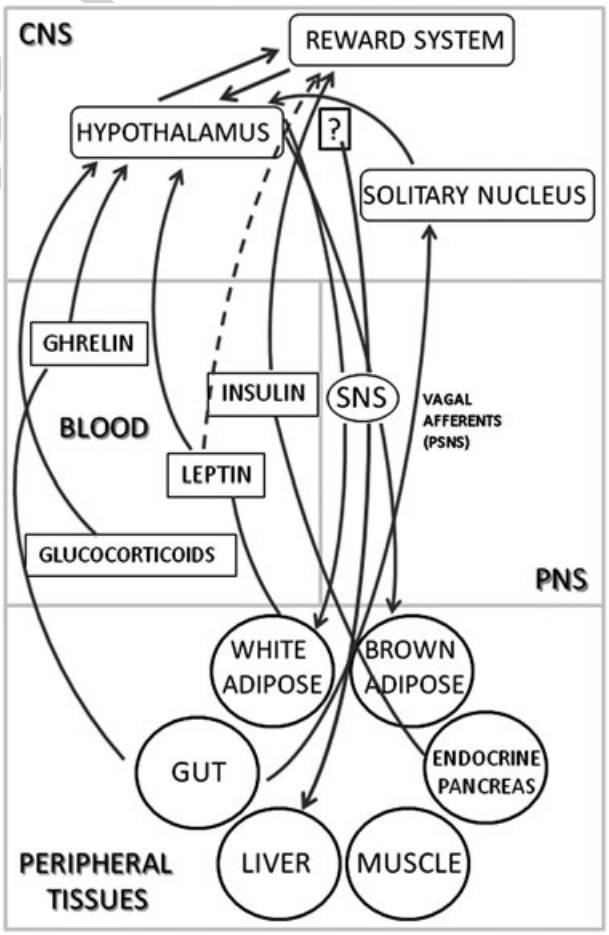

Figure 1. Schematic diagram depicting connections between CNS structures and peripheral tissues, mediated by humoral factors or PNS signalling, related to modulation of food intake or energy balance by the endocannabinoid system. Solid arrows represent connections in which the endocannabinoid system has a well-established role. Dashed arrows represent connections in which indirect findings or preliminary data suggest the endocannabinoid system plays a role. For purposes of clarity, connections between peripheral organs have been omitted. Abbreviations: CNS: Central Nervous System; PNS: Peripheral Nervous System; PSNS: Parasympathetic nervous system; SNS: Sympathetic nervous system; CCK: CholeCystoKinin; GLP-1: Glucagon-Like Peptide 1; PYY: Peptide YY

substance containing a typical array of non- Δ^9-THC phytocannabinoids, have been found to modulate feeding, thus supporting the therapeutic potential of non- Δ^9-THC phytocannabinoids for the treatment of energy balance disorders.[15,16] Moreover, Δ^9-THCV, a natural cannabinoid antagonist, has been found to exert hypophagic actions [17] and cannabidiol has been reported to decrease food intake or to counteract the orexigenic effects of cannabinoid agonists.[18,19] The reader can refer to excellent reviews in the field to have a full picture on animal feeding studies.[20,21]

Although peripheral mechanisms have also been involved in feeding,[22] the brain emerges as the major organ orchestrating food intake. Specifically, a key brain structure involved is the hypothalamus. In fact, the hypothalamus may be considered the main biological structure regulating food intake and energy balance by integrating both central and peripheral signals (reviewed in Morton[23]). Interestingly, the ECS is expressed in this brain structure and it has an important role in modulating the hypothalamic control of energy intake (reviewed in Bermudez-Silva et al.[24]; figure 1). Recent findings also suggest that hypothalamic CB1 controls energy expenditure through modulation of β(3)-adrenergic receptor and uncoupling protein-1 in the brown adipose tissue (Cardinal et al., 2012). Indeed, the hypothalamus, through sympathetic activation, inhibits anandamide synthesis in adipose tissue, decreasing lipogenesis.[25] In this sense, it is interesting to note the increasing evidence for the important role of the peripheral nervous system (both sympathetic efferents and parasympathetic vagal afferents) in mediating, through the engagement of the ECS, the crosstalk between the CNS and peripheral organs ([22,26,25,27]; Quarta et al., 2010; Cardinal et al., 2012; [28]). Furthermore, the hypothalamus receives input from peripheral hormones like leptin, ghrelin, glucocorticoids, and satiety hormones (CCK, GLP-1 and PYY) that inform it about the peripheral energy status, and there is clear evidence of ECS involvement in the crosstalk between the hypothalamus and peripheral organs through these hormones. The relationship between leptin and the ECS in the hypothalamus is well known, with leptin inhibiting local endocannabinoid production and thus decreasing food intake ([29]; Cardinal et al., 2012). On the other hand, compelling evidence suggests that ghrelin and endocannabinoids act synergistically at the hypothalamus to promote food intake (reviewed in Bermudez-Silva et al.[24]). Glucocorticoids, which have a known orexigenic action, inhibit hypothalamic neurons by suppressing excitatory glutamatergic synaptic input, and this is mediated through retrograde release of endocannabinoids and CB1activation.[30] Unfortunately, little is known about satiety hormones and the ECS, though several studies suggest a connection.[26,31–33]

Another key brain region involved in eating behaviour is the reward system. It comprises several synaptically interconected neural structures that modulate eating behaviour by processing and integrating the reinforcement properties of natural rewards such as food, i.e. food intake behaviour is promoted by inducing pleasant sensations and mitigating negative feelings of starvation, thus increasing the probability of that behaviour occurring.[34] However, the reward system is a double-edged sword, stimulation of which by non-natural reinforcers, like cannabinoids or other drugs of abuse, can lead to loss of control over behaviour, with sometimes dramatic consequences, such as those derived from risky behaviour after acute intoxication and drug dependence development. Cannabis abuse, which could be viewed in our current society as a 'perceived freedom behaviour', can be considered from a biological perspective to be a collateral consequence of this molecular system modulating 'biologically-imposed behaviours', like feeding. Indeed, the reward system is involved in hedonic homeostasis, what could explain the mood-related side effects of drugs blocking CB1 receptors (see below). As can be inferred, the ECS has an important role in modulating the reward system physiology. There is a vast literature dealing with behavioural and pharmacological studies on the regulation of brain reward by the ECS (reviewed in Vlachou and Panagis[35]). As an example of pioneering findings linking the reward system and food intake through the ECS, fasting was found to raise endocannabinoid levels in the Nucleus Accumbens (a brain region involved in the reward system) [14] and direct administration of the endocannabinoid 2-AG in this area increased food intake in a CB1-dependent manner.[14] Furthermore, dopamine release (considered a crucial mediator of the rewarding effects of food and drugs of abuse) in the Nucleus Accumbens was inhibited by pharmacological systemic blocking of CB1 receptors in animals exposed to novel highly palatable food.[36] Moreover, there is recent evidence that the reward system is directly modulated by insulin, through local endocannabinoids.[37] Several previous studies have suggested a peripheral-mediated modulation of the reward system through the hypothalamus ([38]; Leinninger et al., 2009).

In addition to the hypothalamus and the reward system, the brainstem, and specifically, the nucleus of the tractus solitarius (NTS), is another brain structure involved in feeding. It receives, among others, sensory inputs from the nodose ganglion that in turn originate in the gut in response to ingested food. Likewise, the NTS projects to, among other regions, the hypothalamus, thus forming a neural circuit that contributes to the autonomic regulation of food intake. Interestingly, CB1 receptors are expressed at the NTS and the nodose ganglion [26,39] and they participate in the modulation of food intake by this circuit.[22,28]

In addition to the role of the ECS in modulating CNS processes involved in food intake and energy homeostasis and its role in the crosstalk between the CNS and peripheral organs through the peripheral nervous system and hormones (Figure 1), metabolically-relevant peripheral tissues, i.e. the liver, adipose, muscle and endocrine pancreas, self-contain an ECS that modulates the normal physiology of these organs.

CB1 receptors in the adipose tissue have been found to promote lipogenesis by several mechanisms, including facilitation of adipocyte differentiation and increased expression of adipogenic enzymes. Specifically, pharmacological activation of CB1 receptors with WIN55212-2 was found to increase lipoprotein lipase activity in primary adipocytes while rimonabant prevented this effect.[40] Rimonabant induced the expression of adiponectin, involved in fatty acid breakdown, in adipose tissue,[41] anandamide and 2-AG levels were found to be increased just before adipocyte differentiation, together with changes in the expression of enzymes involved in synthesis and degradation of these endocannabinoids,[42] and adipogenic genes were induced after stimulation of CB1 receptors with HU-210.[43] Indeed, CB1 receptor antagonism by rimonabant and CB1 silencing by siRNA technology led to the transdifferentiation of white adipose tissue to thermogenic brown adipose tissue [44]. In this sense, it is important to note that the ECS in brown adipose tissue is an important player in energy homeostasis. In fact, increased energy expenditure has been found to be more important than reduced food intake in the body-weight-reducing effect of rimonabant,[45] and chronic administration of rimonabant increased brown adipose tissue temperature

and uncoupling protein 1 expression in this tissue.[27] Of note, rimonabant has been found to activate brown adipose tissue in diet-induced obese mice.[46]

In the liver, CB1 receptor activation also induces lipogenesis, which seems relevant in liver steatosis during obesity. Thus, pharmacological CB1 activation in hepatocytes induced the expression of lipogenic enzymes, in turn increasing de novo fatty acid synthesis, and a high-fat diet was found to increase hepatic levels of the endocannabinoid anandamide, CB1 density, and basal rates of fatty acid synthesis, with the latter being reduced by CB1 blockade.[47] Moreover, liver-specific CB1 knockout mice, though developing obesity at the same level as wild type mice when fed a high-fat diet, were found to have less steatosis, hyperglycemia, dyslipidemia, and insulin and leptin resistance.[48]

CB1 receptors have also been found in muscle [49] and pharmacological activation of CB1 receptors in isolated soleus decreased both basal and insulin-stimulated glucose transport activity.[50] Rimonabant directly improved glucose transport activity in a dose-dependent manner in this tissue.[51,50] Responsiveness of skeletal muscle toward insulin seems to be mediated through modulation of PI3-kinase/PKB and Raf-MEK1/2-ERK1/2 signalling pathways.[52]

Finally, the endocrine pancreas contains a full ECS which has been suggested to be involved in glucose-stimulated insulin secretion and beta-cell mass expansion. CB1 and CB2 receptors as well the ECS machinery have been found in islets.[53,54] Indeed, CB1 and CB2 expression showed species-specific differences (reviewed in Bermudez-Silva et al.;Juan-Pico et al[55,56]). Specific activation of CB1 receptors in human islets increased insulin, glucagon and somatostatin secretion, whereas CB2 activation decreased insulin secretion.[53] These effects seem to be mediated through modulation of glucose-induced intracellular calcium transients.[57,58] Furthermore, rimonabant was found to decrease insulin hypersecretion in islets from diabetic rats [59] and chronic treatment with rimonabant had a protective role against the development of hyperinsulinaemia, β-cell dysfunction and islet disorganization in diabetic rats.[60] Moreover, at least part of the benefits of CB1 antagonism in islets seems to be mediated through enhanced insulin receptor signalling leading to β-cell mass expansion.[54]

Taken together, apart from modulating lipid metabolism, the ECS modulates glucose metabolism by promoting pancreatic hormonal secretion that in turn favours glucose uptake by tissues such as the liver and white adipose tissue, leading to fatty acid synthesis and energy storage.

Overall, we can say that the hypothalamus, the reward system and the brainstem are major integrator centres for food intake and energy homeostasis that receive inputs from both vagal afferents and hormonal signals and that, in turn, coordinate the function of peripheral metabolically-relevant tissue by output signalling through sympathetic efferents. All these nervous structures are provided with the ECS; indeed peripheral organs contain their own ECS which modulates their physiology. From a physiological point of view, signalling through CB1 receptors has a general anabolic effect by (1) promoting food intake through modulation of both pleasure and motivation to eat, (2) re-directing lipid metabolism to pro-lipogenic and anti-lipolytic processes, and (3) re-directing glucose metabolism to hepatic and adipose lipogenic actions. These coordinated actions are compatible with a role of CB1 signalling as a molecular thrifty pathway. Consequently, blocking this pathway in people from western societies, where energy storage within the body is a problem rather than an advantage, is expected to be beneficial to tackle obesity and related morbidities, as will be explained in the following section.

The obesity pandemic and the development of endocannabinoid-based therapies

Obesity is currently a worldwide pandemic with a growing incidence and prevalence. In 2008, more than 1.4 billion adults, aged 20 and older, were overweight and of these, over 500 million were obese.[61] At least 2.8 million adults die each year as a result of being overweight or obese.[61] The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Paradoxically, overweight and obesity are largely preventable by a healthier choice of foods and regular physical activity. However, these recommendations currently have very poor adherence among the population, suggesting that supportive environments and communities are fundamental. In this sense, it seems clear that society and the food industry should play a more prominent role than that played so far. Likewise, there is a lack of effective pharmacological treatments to treat obesity, with the development of safe and successful anti-obesity drugs seeming a kind of utopia.

Apart from the above-mentioned findings regarding the outstanding role of the ECS in food intake and energy balance, increasing evidence points to a dysregulated ECS, including high endocannabinoid levels, in tissues from obese people (reviewed in Silvestri and Di Marzo, 2013). This suggests excessive Q8 endocannabinoid signalling (overactive endocannabinoid tone) that seems to be involved not only in the pathophysiology of obesity but also in its pathogenesis (Silvestri and Di Marzo, 2013). Thus, blocking the cannabinoid CB1 receptor would indeed help to control this overactivity of the ECS.

Consequently, it is not surprising that the pharmaceutical industry has focused on this molecular system to develop novel therapeutic approaches to fight obesity and related co-morbidities. From the seminal pre-clinical studies by Ravinet Trillou et al. [62] to Q9 phase III clinical trial results (see below), it has become clear that blockade of cannabinoid CB1 receptors is effective in decreasing body weight gain as well as ameliorating the metabolic profile in people with obesity, dyslipidemia and type 2 diabetes (reviewed in Bermudez-Silva et al.[63]). Rimonabant efficacy has been assessed in phase III clinical trials, called the RIO (Rimonabant In Obesity) studies. Rimonabant was administered as an adjuvant to a hypocaloric diet in overweight or obese patients,[64,65] in overweight patients with dyslipidaemia,[66] and in overweight or obese patients with type 2 diabetes.[67] These studies found that rimonabant significantly reduced body weight and waist circumference while improving cardiometabolic risk factors.

A CB1 antagonist/inverse agonist (rimonabant; Acomplia®) was marketed in the EU and other countries (the US Food and Drug administration never approved the drug due to safety concerns) as a co-adjuvant to diet and exercise for treating obese patients and overweight persons with a BMI $> 27\ kg/m^2$ and associated risk factors such as dyslipidaemia or type 2 diabetes. However, the European Medicine Agency (EMEA) had first to modify the indications for rimonabant, excluding patients with a history of psychiatric disorders and, finally, removed the drug from the market due to an unexpected increase in the risk-benefit ratio (reviewed in Bermudez-Silva et al.[63]). In parallel, drug-makers announced they would stop further clinical research on this type of cannabinoid-based drugs, like the CB1 inverse agonist

taranabant that was also reported to significantly decrease body weight in obese subjects,[68] thus ending the clinical development of this first generation of CB1 antagonists/inverse agonists.

However, pre-clinical studies as well as recent clinical findings strongly suggest that crossing the blood–brain-barrier is not indispensable for the metabolic improvement of CB1 antagonists/inverse agonists.[69–72] Indeed, avoiding these compounds reaching the CNS would prevent side effects such as mood disorders and depression, probably arising as a consequence of CB1 blockade preventing the hedonic processing at the reward system of natural reinforcers like food, sex, and social interactions. Thus, direct pharmacological actions on peripheral metabolically-relevant tissues could be enough to obtain metabolic benefits. Alternatively, these positive results of non-brain penetrable CB1 antagonists/inverse agonists could also be related to the reduced tightening of the hypothalamic blood–brain-barrier and/or the modulation of brain structures through vagal afferents, thus specifically blocking CB1 receptors in the hypothalamus but not in the reward system. In this line, interesting new findings showed that haemopressin, the first peptide ligand to be described for the CB1 cannabinoid receptor and which behaves as an inverse agonist in vivo crossing the blood–brain barrier, can inhibit appetite without altering reward system signalling.[73] This kind of compound could lead to novel therapeutic approaches, though more investigation is still needed.

Another research line suggests that inverse agonism could be the pharmacological event leading to the reported central side effects of CB1 antagonists/inverse agonists, such as those exerted by rimonabant. Accordingly, research efforts are also being devoted to the development of a neutral CB1 antagonist, i.e. not decreasing the CB1 receptor activity below the basal level, independently of its ability to cross the blood–brain-barrier (reviewed in Silvestri and Di Marzo[74]). Following this rationale, both research centres and the pharmaceutical industry are currently working on a second generation of CB1 antagonists unable to reach the CNS or to induce inverse agonism.

In addition, novel therapeutic strategies are being developed; for instance, those devoted to normalizing the endocannabinoid tone. These include pharmacological inhibition of diacylglycerol lipase (the enzyme involved in 2-AG synthesis)[75,76] and dietary intake of n-3 PUFAs (in order to decrease the availability of endocannabinoid precursors),[77,78] both of which seem to be more promising.

Furthermore, other constituents of cannabis could have anti-obesity properties. As described above, other natural compounds present in cannabis extracts, such as Δ^9-THCV and cannabidiol, have the potential to counteract the orexigenic effects of cannabinoid agonists.[17–19] Interestingly, recent evidence suggests that the cannabidiol effect is at least mediated through the cannabinoid CB2 receptor.[79] In agreement with this hypothesis, CB2 receptors have been found in the hypothalamus and their overexpression in animal models has been linked to decreased fasting-induced food intake and a lean phenotype.[80] Indeed, cannabidiol has been reported to have potent anti-inflammatory and anti-oxidant properties, which could be of great interest in obesity-related disorders like type 2 diabetes. Definitively, mechanisms of action of cannabidiol, Δ^9-THCV and non-Δ^9-THC phytocannabinoid cocktails, such as those used in Farrimond's studies,[15,16] as well as the putative therapeutic value of strategies aimed at mimicking their biological actions, merit more investigation.

The eventual success of these new approaches is largely a mystery. On the one hand, the ECS is a potent energy balance modulator. There is increasing knowledge about its mechanisms of action and previous clinical experience can eventually guide further clinical development of endocannabinoid-based therapy for obesity and metabolically-related diseases. On the other hand, the widespread presence of the ECS in the CNS and peripheral organs, known to work in a broad range of biological processes, the 'promiscuity' of endocannabinoids, binding to several types of receptors and thus increasing the probability of an off-target effect, and the shared molecular pathways (with other signalling lipids) involved in the synthesis and/or degradation of endocannabinoids, remain important challenges.

Conclusion

In summary, the ECS has a key role in modulating food intake and energy homeostasis, and the underlying mechanisms are being increasingly elucidated. Given the big challenge posed by obesity and related disorders, great expectation has been placed on drugs targeting this system. The first generation of CB1 blockers has failed, but the initial deception has turned into hopes resulting from new progress in the field. The second generation of CB1 blockers is in the pipeline, together with other ECS-based strategies. It is expected that in the next few years we will know whether an ECS-based therapy for obesity and/or related diseases is feasible or whether it will just increase the history of failures seen for the treatment of obesity.

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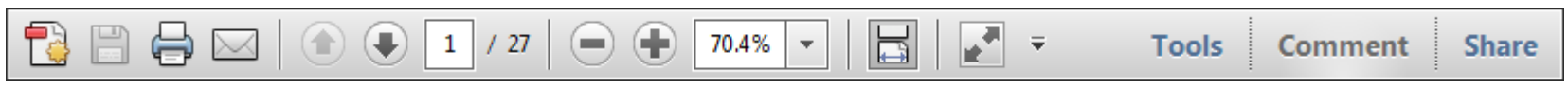

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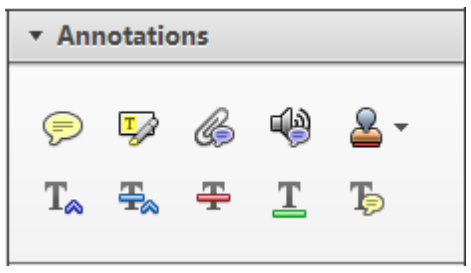

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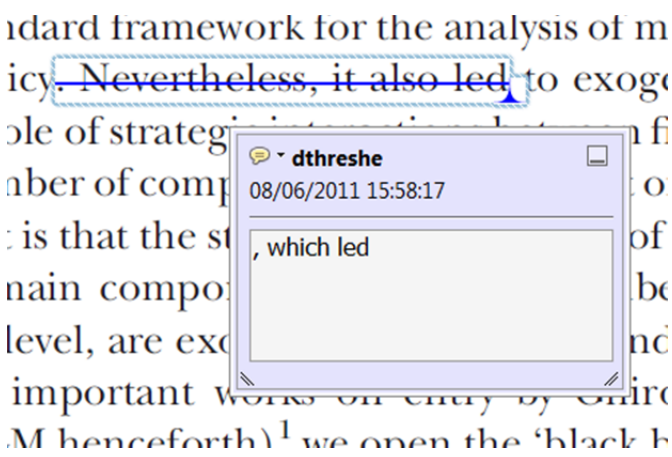

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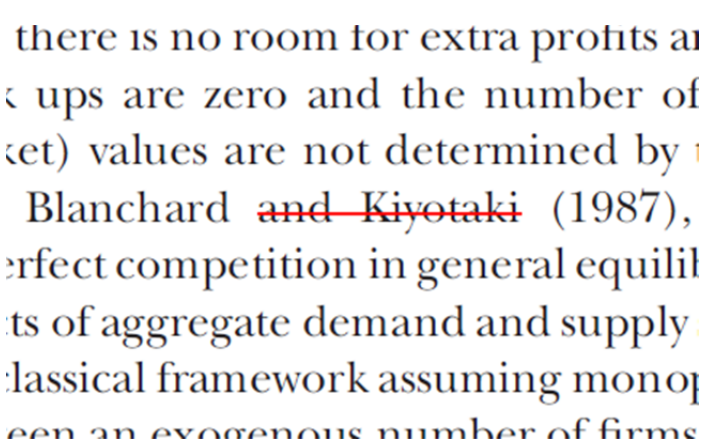

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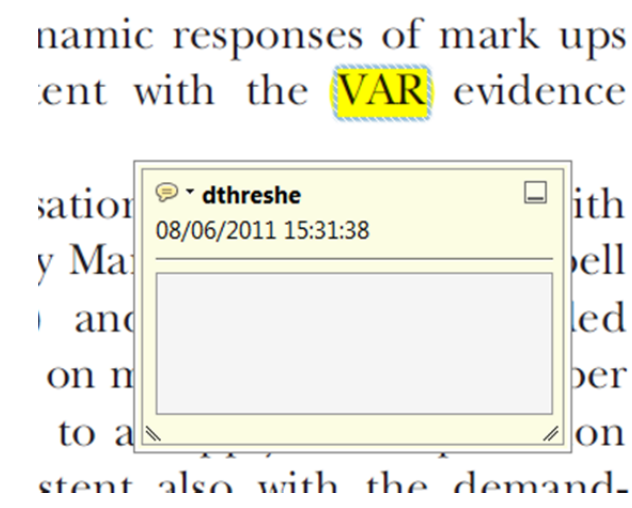

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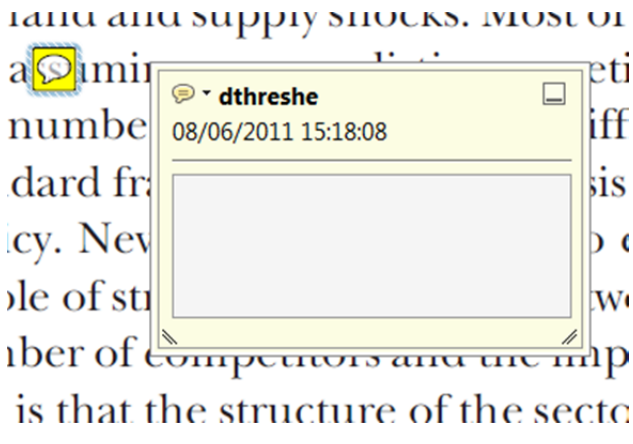

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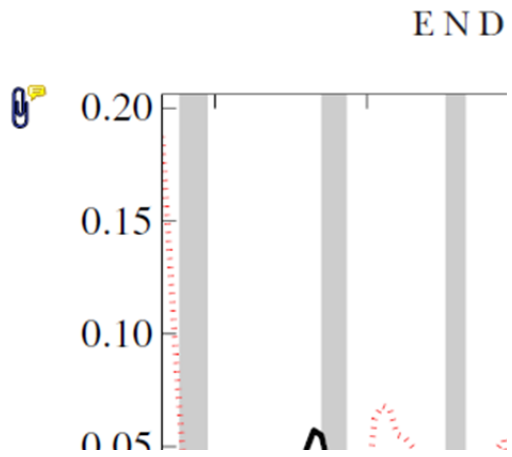
- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

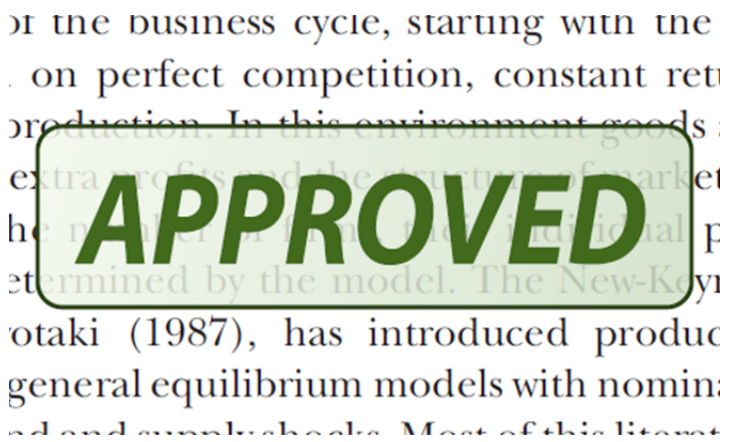


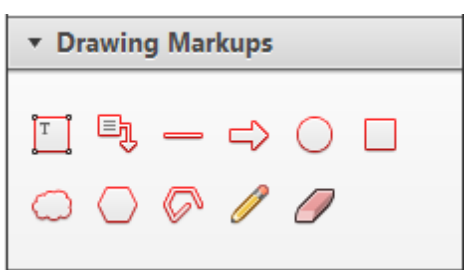
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).



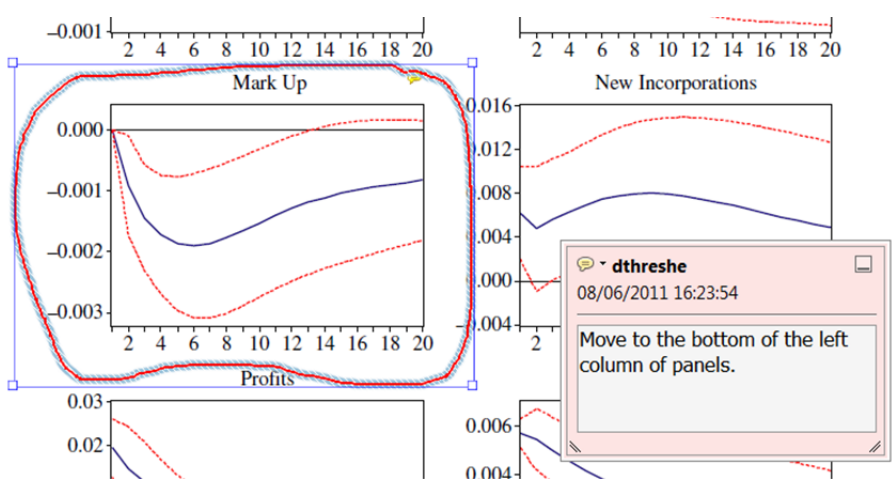


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: