



**HAL**  
open science

## **Cannabis sativa and the endogenous cannabinoid system: therapeutic potential for appetite regulation**

Jonathan Andrew Farrimond, Marion Simone Mercier, Ben Whalley, Claire M Williams

► **To cite this version:**

Jonathan Andrew Farrimond, Marion Simone Mercier, Ben Whalley, Claire M Williams. Cannabis sativa and the endogenous cannabinoid system: therapeutic potential for appetite regulation. *Phytotherapy Research*, 2011, 10.1002/ptr.3375 . hal-00606643

**HAL Id: hal-00606643**

**<https://hal.science/hal-00606643>**

Submitted on 7 Jul 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Cannabis sativa and the endogenous cannabinoid system:  
therapeutic potential for appetite regulation**

Journal:	<i>Phytotherapy Research</i>
Manuscript ID:	PTR-10-1149.R2
Wiley - Manuscript type:	Review
Date Submitted by the Author:	06-Nov-2010
Complete List of Authors:	Farrimond, Jonathan; University of Reading, School of Pharmacy; University of Reading, School of Psychology & Clinical Language Sciences Mercier, Marion; University of Reading, School of Pharmacy; University of Reading, School of Psychology & Clinical Language Sciences Whalley, Ben; University of Reading, School of Pharmacy Williams, Claire; University of Reading, School of Psychology & Clinical Language Sciences
Keyword:	Feeding, Appetite, Phytocannabinoid, Endocannabinoid, Delta-9- tetrahydrocannabinol

SCHOLARONE™  
Manuscripts

view

1  
2  
3  
4  
5 1 ***Cannabis sativa* and the endogenous cannabinoid system:**  
6  
7  
8 2 **therapeutic potential for appetite regulation**  
9

10  
11 3 Short title: A therapeutic role for *C. sativa* in appetite regulation  
12

13  
14 4 **Jonathan A. Farrimond<sup>1,2</sup>, Marion S. Mercier<sup>1,2</sup>, Benjamin J. Whalley<sup>1</sup> and Claire**  
15  
16 **M. Williams<sup>2</sup>.**  
17

18  
19 6  
20 7 <sup>1</sup>School of Pharmacy and <sup>2</sup>School of Psychology and Clinical Language Sciences,  
21  
22 8 University of Reading.  
23

24  
25 9  
26  
27 10 Corresponding author: Jonathan Farrimond (j.a.farrimond@reading.ac.uk)  
28

29 11 School of Pharmacy,  
30

31 12 University of Reading,  
32

33 13 Whiteknights,  
34

35 14 Reading,  
36

37 15 Berkshire,  
38

39 16 RG6 6AP,  
40

41 17 U.K.  
42

43 18 Phone: +44 (0)118 378 8464  
44

45 19 Fax: +44 (0)118 378 4703  
46  
47  
48  
49  
50

51 22 This research was supported in part by the University of Reading Research Endowment  
52 23 Trust Fund (to JAF).  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 **Abstract**  
5

6 The herb *Cannabis sativa* (*C. sativa*) has been used in China and on the Indian  
7 subcontinent for thousands of years as a medicine. However, since it was brought to the  
8 U.K. and then the rest of the western world in the late 19<sup>th</sup> century, its use has been a  
9 source of controversy. Indeed, its psychotropic side effects are well reported but only  
10 relatively recently has scientific endeavour begun to find valuable uses for either the  
11 whole plant or its individual components. Here, we discuss evidence describing the  
12 endocannabinoid system, its endogenous and exogenous ligands and their varied effects  
13 on feeding cycles and meal patterns. Furthermore we also critically consider the  
14 mounting evidence which suggests non- $\Delta^9$ tetrahydrocannabinol phytocannabinoids play  
15 a vital role in *C. sativa*-induced feeding pattern changes. Indeed, given the wide range  
16 of phytocannabinoids present in *C. sativa* and their equally wide range of intra-, inter-  
17 and extra-cellular mechanisms of action, we demonstrate that non-  
18  $\Delta^9$ tetrahydrocannabinol phytocannabinoids retain an important and, as yet, untapped  
19 clinical potential.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

17 **Keywords:** Feeding, appetite, phytocannabinoid, endocannabinoid,  
18  $\Delta^9$ tetrahydrocannabinol

## 1 Introduction

2 For ~10,000 years, *Cannabis sativa* (*C. sativa*) has been used medicinally and  
3 recreationally for its diverse pharmacological actions and psychotropic properties.  
4 Typical actions include sedation, analgesia, hypothermia, catalepsy and euphoria which  
5 are thought to arise mainly from cannabinoid type 1 receptor (CB<sub>1</sub>R) activation  
6 (Childers *et al.*, 1998; Little *et al.*, 1988). These effects have typically been ascribed to  
7 the sixty (Howlett, 2002; Pertwee, 2008b) to eighty (Izzo *et al.*, 2009)  
8 phytocannabinoids (pCBs) present in *C. sativa*. In more recent times, *C. sativa* has been  
9 used less widely for medicinal purposes, due in part to the development of other  
10 therapeutic agents with better side effect profiles (e.g. Tramer *et al.*, 2001). Indeed,  
11 cannabinoid side-effects are known to include reduced motor coordination, tachycardia,  
12 transient memory loss, disruption to the sense of time, cognitive impairments, anxiety  
13 and a disruption of appetite regulation (Childers *et al.*, 1998; Dewey, 1986; Grant *et al.*,  
14 2005; Hollister, 1986; Kirkham *et al.*, 2001b; Wilkinson *et al.*, 2003) (for a recent  
15 review on the safety of *C. sativa*-based therapy see Wang *et al.*, 2008). However, the  
16 relatively recent identification of the CB<sub>1</sub>R and cannabinoid type 2 (CB<sub>2</sub>R) receptors,  
17 alongside the subsequent characterisation of the endocannabinoid (eCB) system (for a  
18 recent review see Maccarrone, 2009), has driven a resurgence of interest in the potential  
19 applications of cannabinoid-based therapy. Cannabinoid therapies have now been  
20 investigated for a wide range of neurological and neurodegenerative disorders (Glass,  
21 2001; Pryce *et al.*, 2003), both in terms of treatment (for a review see Consroe, 1998)  
22 and/or symptom management (Davis *et al.*, 2007). One notable example is in the  
23 treatment of multiple sclerosis (Pryce *et al.*, 2005) and its symptoms (eg. spasticity), for

1 which a cannabis-based compound (Sativex®) has recently been licensed in the U.K.,  
2 Spain and Canada by GW Pharmaceuticals.

3 'Cannabinoid' is a well-known term used to describe the compounds isolated  
4 from *C. Sativa*, the pCBs such as  $\Delta^9$ tetrahydrocannabinol ( $\Delta^9$ THC), cannabidiol (CBD)  
5 and cannabinol (CBN). In addition to these, the term now also encompasses both  
6 synthetic exogenous (e.g. SR141716A, a CB<sub>1</sub>R specific antagonist/inverse agonist) and  
7 endogenously produced ligands (the eCBs anandamide (AEA) and 2-arachidonyl  
8 glycerol (2-AG)) at CB<sub>1</sub>- and CB<sub>2</sub>Rs (for an overview of the cannabinoid receptor  
9 (CBR) ligands referred to in this review, see table 1). The development and  
10 characterisation of such pharmacological tools has enabled significant progress to be  
11 made into understanding the eCB system over the last 20 years (Onaivi *et al.*, 2002).  
12  $\Delta^9$ THC, the main psychoactive component in *C. sativa*, was first isolated and  
13 synthesised in 1964 by Gaoni *et al.* (Gaoni *et al.*, 1964a). However, it was not until  
14 1988 that the first cannabinoid specific G-protein coupled receptor (GPCR; CB<sub>1</sub>R) at  
15 which it acts was characterised (Devane *et al.*, 1988; Matsuda *et al.*, 1990), lending  
16 credence to the postulated existence of an eCB system (Howlett *et al.*, 1990) (for review  
17 see Pertwee, 2005). A series of studies (Herkenham *et al.*, 1991; Herkenham *et al.*,  
18 1990) identifying the widespread expression of this receptor in the brain further  
19 supported this discovery. These findings were rapidly followed by the isolation and  
20 structural determination of the first eCB ligand, AEA, in 1992 (Devane *et al.*, 1992), the  
21 identification and characterisation of CB<sub>2</sub>R expressed, on immune cells, in 1993 (Munro  
22 *et al.*, 1993), and the identification of a second eCB ligand, 2-AG, in 1995 (Mechoulam  
23 *et al.*, 1995b; Sugiura *et al.*, 1995) (See Fig.1). Since then, significant academic and  
24 commercial effort has been invested in characterising and exploiting the eCB system

1 (for example reviews see Basavarajappa, 2007a; Basavarajappa, 2007b; Bisogno *et al.*,  
2 2005; Freund *et al.*, 2003).

3 As previously outlined, it is now apparent that the eCB system is physiologically  
4 ubiquitous and linked to many pathophysiological states (de Fonseca, 2005), resulting in  
5 its identification as a therapeutic target, as well as the publication of numerous reviews  
6 regarding the potential benefits of cannabinoid based therapies (e.g. Agrawal *et al.*,  
7 2009; Baker *et al.*, 2003; Glass, 2001; Goutopoulos *et al.*, 2002; Makriyannis *et al.*,  
8 2005). This is particularly true in the field of body weight regulation and energy balance  
9 (Yates *et al.*, 2009), where significant advances have been made in the development of  
10 both anti-anorectic (e.g. in hepatitis C (Costiniuk *et al.*, 2008); in cancer (Walsh *et al.*,  
11 2003); in human immunodeficiency virus (HIV) (Woolridge *et al.*, 2005)) and anti-  
12 obesity (Van Gaal *et al.*, 2005) cannabinoid-based therapies. However, these advances  
13 must be made with care (Grant *et al.*, 2005), and it is clear that further long-term studies  
14 are warranted if the safety profiles of such therapies are to be fully understood (Wang *et*  
15 *al.*, 2008). Indeed, the withdrawal of Acomplia (Rimonabant: SR141716A; see EMA,  
16 2009 for details) from clinical use for the treatment of obesity due to serious cognitive  
17 side effects, combined with the cessation of Phase III clinical trials for a second putative  
18 anti-obesity drug, Taranabant (MK-0364; Clark, 2009), illustrates the difficulties of  
19 manipulating such a complex and ubiquitous system. Nonetheless, it is clear that the  
20 eCB system plays an important, if not yet fully understood, role in appetite, obesity and  
21 energy balance (for reviews see Cota *et al.*, 2003a; Kirkham, 2008). This review will  
22 describe the significant progress made, as well as the work still required, in order to  
23 fully understand the role played by the eCB system in feeding and appetite regulation.  
24 Importantly, by focusing on those studies that have examined pCB effects and how they

1 might interact with the eCB system to modulate appetite and feeding, this review  
2 provides insight into the potential use of these compounds as therapeutic tools in the  
3 treatment of eating- and weight-related disorders.

4

5

For Peer Review



## 1     **The endocannabinoid system in the central nervous system**

2     Although, CBRs are present in both the peripheral and central nervous systems (CNS),  
3     the majority of cannabinoid feeding research to date has focused primarily on centrally-  
4     mediated feeding mechanisms, a focus that is consequently reflected in this review. A  
5     short section outlining the biological components that comprise the eCB system within  
6     the CNS is presented hereafter (see also figures 2 and 3).

7             ECBs (AEA and 2-AG) are produced on demand by postsynaptic neurons (Di  
8     Marzo *et al.*, 2004) in the CNS in response to rises in intracellular  $[Ca^{2+}]$  (Cadas *et al.*,  
9     1997; Di Marzo *et al.*, 1994). Such increases typically occur following  $G_q$ -coupled  
10    receptor activation which stimulates production of inositol trisphosphate ( $IP_3$ ) by  
11    phospholipase C (PLC) leading to a release of  $Ca^{2+}$  from intracellular stores (for  
12    reviews see Berridge, 1995; Berridge *et al.*, 1988; Putney Jr, 1986). Alternatively,  
13    ionotropic glutamate receptor activation permits the influx of  $Ca^{2+}$  to increase eCB  
14    production (Freund *et al.*, 2003).

15            AEA synthesis from its precursors, arachidonic acid and N-arachidonoyl  
16    phosphatidyl-ethanolamine (NaPE) is an on demand effect. Phosphatidylethanolamine is  
17    first catalysed by  $Ca^{2+}$ -dependent N-acyltransferase (Cadas *et al.*, 1997) into NaPE  
18    (although  $Ca^{2+}$ -independent processes have also been proposed; Jin *et al.*, 2007); NaPE  
19    is then converted to the N-acylethanolamine, AEA, (Di Marzo *et al.*, 1994) by NaPE  
20    phospholipase-D (Okamoto *et al.*, 2004). However, it has been suggested that at least  
21    three other pathways for AEA synthesis may also exist (for reviews, see Liu *et al.*,  
22    2008; Okamoto *et al.*, 2009a; Okamoto *et al.*, 2009b). 2-AG is formed by the  
23    diacylglycerol (DAG) lipases  $\alpha$  and  $\beta$ -catalyzed hydrolysis of DAG (Di Marzo *et al.*,  
24    2007). After formation, eCB release into the extracellular space is thought to occur

1 immediately via passive diffusion or through a putative eCB transporter as no storage  
2 systems for eCBs have yet been identified and some eCB precursors are known to be  
3 membrane bound, suggesting that eCBs are produced at their release site (Di Marzo,  
4 2008; Pope *et al.*, 2010.).

5       Once released into the intercellular space, unlike classical neurotransmission,  
6 eCB signalling is retrograde such that eCBs released from a postsynaptic neuron diffuse  
7 across the synaptic cleft to bind with presynaptically located CB<sub>1</sub>Rs to exert effects (see  
8 figure 3; Ohno-Shosaku *et al.*, 2001; Piomelli *et al.*, 2000; Wilson *et al.*, 2001) that are  
9 now thought to be intimately linked to synaptic plasticity (see Alger, 2009). The  
10 activation of presynaptic CB<sub>1</sub>Rs by AEA and 2-AG induces a PLC- $\beta$ -mediated  
11 suppression of [Ca<sup>2+</sup>] entry and a protein kinase A (PKA)-mediated upregulation of [K<sup>+</sup>]  
12 efflux, thereby driving neuronal resting membrane potential to more hyperpolarized  
13 values and so reducing the further pre-synaptic release of neurotransmitter (for review  
14 see Mackie, 2006; Südhof *et al.*, 2008). Indeed, presynaptically it has been conclusively  
15 demonstrated that CB<sub>1</sub>R activation has a direct effect on membrane polarisation, for  
16 example, in cultured hippocampal neurones (Deadwyler *et al.*, 1995; Schweitzer, 2000)  
17 and in CA1 pyramidal cells (Kirby *et al.*, 2000).

18       ECB reuptake however remains the subject of much debate. Fatty acid amide  
19 hydrolase (FAAH; Cravatt *et al.*, 1996) and monoacylglycerol lipase (MAGL; Lambert  
20 *et al.*, 2005) are the two main enzymes involved in eCB degradation, and have been  
21 known to degrade AEA and 2-AG for some time. However, although a specific  
22 transmembrane transporter protein has yet to be identified, it seems that reuptake may  
23 be a rapid carrier-mediated transport process (e.g. Gerdeman *et al.*, 2002; Ronesi *et al.*,  
24 2004) since eCBs within the synaptic cleft are almost immediately inactivated (Di

1  
2  
3  
4 1 Marzo *et al.*, 2007). 2-AG is typically inactivated by MAGL (Di Marzo, 2008) but also  
5  
6 2 to some extent by FAAH (Blankman *et al.*, 2007), whereas, AEA is thought to be  
7  
8 3 degraded by FAAH alone (Vandevorde, 2008). Four mechanisms are currently  
9  
10 4 proposed to underlie eCB reuptake; a membrane-localised transmembrane carrier  
11  
12 5 protein, FAAH-induced passive diffusion through the lipid bilayer, the creation of  
13  
14 6 caveolae with AEA binding sites on the cell membrane which then internalise AEA  
15  
16 7 and/or 2-AG, and via intracellular sequestration into the cell within a lipid shell (for a  
17  
18 8 recent review see Yates *et al.*, 2009). Intracellular FAAH-mediated AEA hydrolysis  
19  
20 9 creates arachidonic acid and ethanolamine (Deutsch *et al.*, 2002 thoroughly review the  
21  
22 10 biochemistry of FAAH) whilst 2-AG undergoes primarily MAGL-mediated hydrolysis  
23  
24 11 into arachidonic acid and glycerol (Blankman *et al.*, 2007).

25  
26  
27  
28  
29  
30 12 While the mechanisms involved in AEA and 2-AG degradation remain poorly  
31  
32 13 understood, it is clear that FAAH and MAGL blockade could be of therapeutic value.  
33  
34 14 FAAH inhibition, for instance by URB597 (Piomelli *et al.*, 2006; see Vandevorde,  
35  
36 15 2008 for a review of FAAH and MAGL inhibitors), is expected to potentiate AEA  
37  
38 16 effects (and to some extent 2-AG). Such inhibition could, for instance, be expected to  
39  
40 17 have effects on energy homeostasis as AEA and 2-AG administration into the  
41  
42 18 hypothalamic nuclei has been conclusively demonstrated to induce hyperphagia in rats  
43  
44 19 (Jamshidi *et al.*, 2001; Kirkham *et al.*, 2001a; Kirkham *et al.*, 2002). Consequently,  
45  
46 20 increasing AEA concentrations in the synaptic cleft via FAAH inhibition could produce  
47  
48 21 similar effects. Although MAGL inhibition (e.g. by URB602; King *et al.*, 2007) which  
49  
50 22 could potentiate 2-AG effects is less well studied than FAAH inhibition, it too could be  
51  
52 23 of comparable therapeutic use. It is however important to consider that both FAAH and  
53  
54 24 MAGL inhibition could have secondary effects besides increasing AEA and 2-AG  
55  
56  
57  
58  
59  
60

1 levels respectively. For instance, some evidence suggests that FAAH inhibition can also  
2 decrease 2-AG levels (see Di Marzo *et al.*, 2008 for a review of these data), and MAGL  
3 inhibition, by the particularly potent and selective MAGL inhibitor JZL-184, has been  
4 found to induce behavioural effects similar to those produced by agonist-induced CB<sub>1</sub>R  
5 activation (Long *et al.*, 2009). The dynamic interplay between these systems must be  
6 taken into account when considering new therapeutic approaches if unwanted side-  
7 effects are to be minimised.

8         The cannabinoid receptors, CB<sub>1</sub> and CB<sub>2</sub>R, are members of the superfamily of  
9 GPCRs and are characterised by seven transmembrane helices (Turu *et al.*, 2009). CB<sub>1</sub>R  
10 was cloned in 1990 (Matsuda *et al.*, 1990), 26 years after the isolation of Δ<sup>9</sup>THC.  
11 CB<sub>2</sub>Rs were first identified and characterised via polymerised chain reaction  
12 experiments in 1993 (Munro *et al.*, 1993; Onaivi *et al.*, 2006), and were initially thought  
13 to be present only in **inflammatory/immune cells**; Basavarajappa, 2007b; Munro *et al.*,  
14 1993), although evidence now shows CB<sub>2</sub>R expression in the brain (Gong *et al.*, 2006;  
15 Onaivi *et al.*, 2006; Van Sickle *et al.*, 2005). Indeed, it is now known that both CB<sub>1</sub>- and  
16 CB<sub>2</sub>Rs are widely expressed in the brain (Gong *et al.*, 2006; Moldrich *et al.*, 2000)  
17 although it is postulated that the majority of the functional eCB effects in the CNS are  
18 CB<sub>1</sub>R mediated (Piomelli, 2003). This receptor is one of the most abundant GPCRs in  
19 the CNS (Herkenham *et al.*, 1991) and its distribution within the mammalian brain  
20 reflects the behavioural effects of cannabinoid administration. For instance, CB<sub>1</sub>R levels  
21 are particularly high in the cortex, hippocampus, cerebellum and basal ganglia,  
22 correlating with the well-known effects of cannabinoids on cognition, memory and  
23 motor control respectively. (Herkenham *et al.*, 1991; Tsou *et al.*, 1997; for a detailed  
24 review of CB<sub>1</sub>R localisation see Mackie, 2005). Although also present in the

1 hypothalamus, CB<sub>1</sub>R levels are lower in this brain region than in those mentioned above  
2 (Herkenham *et al.*, 1991; Tsou *et al.*, 1997) which is somewhat surprising considering  
3 the well-known hypothalamic involvement in feeding-related processes (Elmqvist *et al.*,  
4 1999). However, a study by Breivogel *et al.* found that hypothalamic CB<sub>1</sub>Rs are more  
5 strongly coupled to G-proteins than those in numerous other brain regions including the  
6 cortex and hippocampus (Breivogel *et al.*, 1997), supporting important functional  
7 effects of cannabinoids in this brain region, and suggesting that functional gain  
8 associated with downstream intracellular signalling cascades following CB<sub>1</sub>R activation  
9 may be of crucial importance.

10 While CB<sub>1</sub>- and CB<sub>2</sub>Rs are thought to mediate the majority of cannabinoid  
11 effects, both exo- and endo-genous cannabinoids also exert effects via other receptors.  
12 In line with this, it has been conclusively demonstrated that the non-psychotropic  
13 exogenous cannabinoids (excluding CBN and Δ<sup>9</sup>tetrahydrocannabivarin; Δ<sup>9</sup>THCV)  
14 exhibit almost no affinity for either CB<sub>1</sub> or CB<sub>2</sub>Rs (Petrosino *et al.*, 2009) and must  
15 therefore exert their known pharmacological effects via other routes. A number of  
16 alternative receptor sites are currently being investigated, including GPR55 (Brown *et*  
17 *al.*, 2001; Pertwee, 2007; Ryberg *et al.*, 2007), GPR119 (Overton *et al.*, 2006) and/or  
18 receptor-coupled or receptor independent ion channels (Oz, 2006) (e.g. transient  
19 receptor potential vanilloid 1; VR<sub>1</sub>). The former two of these will briefly be reviewed  
20 here since both GPR55 and GPR119 activation have been found to be involved in  
21 aspects of feeding (for reviews of CB<sub>1</sub>- and CB<sub>2</sub>R-independent cannabinoid effects see  
22 Brown, 2007; De Petrocellis *et al.*, 2009).

23 GPR55, first identified by Sawzdargo in human brain and spleen and in rat  
24 brain, spleen and intestine (Sawzdargo *et al.*, 1999; for a gastrointestinal tract review

1 see Sanger, 2007), is currently a putative third CBR. Upon activation, GPR55  
2 suppresses M-type  $K^+$  currents and stimulates phosphatidylinositol 4,5-bisphosphate  
3 (PIP<sub>2</sub>) -induced  $Ca^{2+}$  release from intracellular stores (Henstridge *et al.*, 2009; Lauckner  
4 *et al.*, 2008). While the status of GPR55 as a CBR has been a topic of fierce academic  
5 debate for some years (Petitet *et al.*, 2006), somewhat more compelling evidence has  
6 been presented recently suggesting that it is indeed a CBR (Baker *et al.*, 2006;  
7 Henstridge *et al.*, 2010; Kapur *et al.*, 2009; Lauckner *et al.*, 2005; Pertwee, 2007; Ross,  
8 2009; Ryberg *et al.*, 2007; Staton *et al.*, 2008). Both Baker (Baker *et al.*, 2006) and  
9 Ryberg (using radioligand binding assays in human embryonic kidney (HEK293) cells;  
10 Ryberg *et al.*, 2007) have shown that CP55,940 (a non-selective CBR agonist), but not  
11 WIN55212-2 (a CBR agonist), binds with strong affinity to GPR55; Baker also showed  
12 that AEA, 2-AG and  $\Delta^9$ THC bind to this receptor. Moreover, Ryberg presented further  
13 evidence in the same manuscript suggesting that GPR55 was  $G_{\alpha 13}$  coupled, and that the  
14 comparatively late detection of GPR55 as a possible CBR was because the affinity of  
15 CP55940 for GPR55 binding was 25 times lower than that for CB<sub>1</sub>R binding. However,  
16 in a similar study Lauckner and colleagues (Lauckner *et al.*, 2005) found that while  
17  $\Delta^9$ THC could activate GPR55 in dorsal root ganglia (DRG) cells, neither WIN55212-2  
18 nor CP55,940 bound to GPR55 in HEK293 and Chinese Hamster Ovary (CHO) cells.  
19 Furthermore the observed effects were blocked by application of SR141716A, a CB<sub>1</sub>R  
20 specific antagonist, (although not by SR144528, a CB<sub>2</sub>R specific antagonist), suggesting  
21 that these were CB<sub>1</sub>R rather than GPR55 mediated. The reasons behind the difference in  
22 CP55,940 binding between the studies by Baker, Ryberg and Lauckner are not  
23 immediately apparent, although Lauckner *et al.* suggest it may be due to a difference in  
24 GPR55 transfection between the cell types used. Despite such discrepancies, however,

1 evidence is mounting for GPR55's role in the eCB system, as demonstrated for instance  
2 by Kapur and colleagues, who observed AM251 (CB<sub>1</sub>R inverse agonist) and  
3 SR141716A (a CB<sub>1</sub>R specific antagonist) binding to GPR55 (Kapur *et al.*, 2009).  
4 Whilst it clearly remains an 'enigmatic' receptor (Ross, 2009), research is beginning to  
5 focus on possible GPR55 based therapies, with recent studies linking it to  
6 neuroinflammation (Pietr *et al.*, 2009), inflammatory and neuropathic pain (Staton *et al.*,  
7 2008) and anorexia (Ishiguro *et al.*, 2010).

8 Finally, although the identification of GPR119 in 2003 did not initially suggest  
9 links to the eCB system (Fredriksson *et al.*, 2003), later work by Overton and Soga  
10 showing N-oleoylethanolamide (OEA), an AEA analogue, induced GPR119 activation  
11 (Overton *et al.*, 2006; Soga *et al.*, 2005) suggested otherwise. Soga *et al.* and more  
12 recently Ning *et al.* observed that GPR119 agonism mediates glucose-dependent insulin  
13 production and release to some extent, as well as increasing intercellular cyclic  
14 adenosine monophosphate (cAMP) accumulation (Ning *et al.*, 2008; Soga *et al.*, 2005).  
15 However, despite findings that GPR119 agonism by OEA suppresses feeding in rats  
16 (Rodriguez de Fonseca *et al.*, 2001), this receptor is unlikely to be involved in centrally  
17 mediated eCB feeding effects as it has been found in the gut (Overton *et al.*, 2006;  
18 Overton *et al.*, 2008). It should nevertheless still be considered when interpreting *in vivo*  
19 feeding study results and could represent a useful target for anti-obesity therapies  
20 (Hughes, 2009).

21 Although, as previously mentioned, this review focuses primarily on central  
22 cannabinoid mediated feeding mechanisms, peripheral effects, such as those mediated  
23 by peripheral CBRs or GPR119 add considerable complexity when interpreting *in vivo*  
24 feeding data. This is particularly true in light of findings describing **peripheral** CB<sub>1</sub>R

1 expression e.g. in the vas deferens: Pertwee *et al.*, 1996; in the heart, blood vessels and  
2 bladder: Howlett, 2002; the liver and fatty tissue: Cota *et al.*, 2003b; Osei-Hyiaman *et*  
3 *al.*, 2005), and a potential modulatory role of these peripheral receptors on feeding  
4 behaviour (Gomez *et al.*, 2002). **Equally**, although it is thought that most of the  
5 functional eCB effects in the brain are CB<sub>1</sub>R-mediated (Piomelli, 2003), recent work  
6 has suggested some functional expression of CB<sub>2</sub>Rs in the brain (Onaivi *et al.*, 2006),  
7 particularly at synapses (Morgan *et al.*, 2009), and that these may also be involved in  
8 feeding behaviours (Onaivi *et al.*, 2008). **Furthermore, CBR activation in the gut (as**  
9 **recently reviewed by Izzo *et al.*, 2010) for example, in terms of gut motility, is also**  
10 **likely to have considerable effects on feeding patterns.** As such, it is often difficult,  
11 when interpreting *in vivo* experimental results, to exclusively assign cannabinoid effects  
12 to either **central or peripheral** sites of action, or to differentiate between CB<sub>1</sub>- and CB<sub>2</sub>-,  
13 or indeed non-CBR-mediated effects. Moreover, ligands which produce well-  
14 characterised effects at specific CBRs may also exert effects at other receptor sites,  
15 thereby creating unexpected and potentially unwanted secondary effects. This is well  
16 illustrated by SR141716A (Acomplia/Rimonabant), a well-known selective CB<sub>1</sub>R  
17 antagonist/inverse agonist which has recently been found by Kapur *et al.* to agonise  
18 GPR55, and which the authors suggest may have played a part in some of the ‘off-  
19 target’ effects that led to its withdrawal as an anti-obesity drug (Kapur *et al.*, 2009).

20       There is still much to clarify about how cannabinoids produce their endogenous  
21 effects, and how they might interact with each other *in vivo* (Ben Amar, 2006; Whalley  
22 *et al.*, 2004). Furthermore, adding to this complexity is the eCB system’s interaction  
23 with other systems, in particular the opioid system which itself has been linked to  
24 appetite regulation for some time (for an early review of opiates and appetite regulation



1 see Morley *et al.*, 1982). Indeed, it is now apparent that both the eCB and opioid  
2 systems modulate energy balance, that both systems can modulate food intake  
3 independently and that activation or suppression of both systems together can increase  
4 or reduce food intake to a greater extent than modulation of either system alone (Gallate  
5 *et al.*, 1999; Kirkham *et al.*, 2001c; Solinas *et al.*, 2005). Whilst detailed discussion of  
6 such interactions is beyond the scope of this review, they, along with the numerous  
7 other complexities outlined above, are important factors to consider when looking at, or  
8 indeed conducting and analysing, research on the eCB system and feeding.

9

## 1 Cannabinoid-mediated effects upon feeding

2 The last decade has seen considerable advances in our understanding of eCB-mediated  
3 control of feeding behaviours. Numerous anecdotal reports exist, and the ‘munchies’  
4 effect has become a well-accepted physiological response to *C. sativa* consumption, yet  
5 few quantitative reports from this period exist (for a complete review see Kirkham *et*  
6 *al.*, 2001b). While the ‘classical’ tetrad of cannabinoid effects was defined as decreased  
7 spontaneous motor activity, hypothermia, analgesia and catalepsy (Little *et al.*, 1988), it  
8 was only comparatively recently that  $\Delta^9$ THC was demonstrated to induce hyperphagia  
9 (Williams *et al.*, 1998), an effect clearly linked to CB<sub>1</sub>R rather than CB<sub>2</sub>R activation  
10 (Williams *et al.*, 2002b). Since CB<sub>2</sub>R have only recently been localised to the CNS  
11 (Morgan *et al.*, 2009), and have not been found expressed on feeding pathways, little  
12 research has considered the possible effects that CB<sub>2</sub>R stimulation may have on feeding.  
13 While a limited number of reports which consider CB<sub>2</sub>R effects on feeding do exist (e.g.  
14 Onaivi *et al.*, 2008), they have so far presented inconclusive evidence. Furthermore,  
15 possible opioid/cannabinoid and cannabinoid/serotonergic interactions have been  
16 studied, for example by Williams *et al.*, (2002b). Williams demonstrated a functional  
17 relationship between the cannabinoid and opioid systems in the control of appetite, and  
18 notably rejected any serotonergic interaction: upon administration of  $\Delta^9$ THC and either  
19 SR141716A, SR144528, naloxone or dexfenfluramine, Williams observed that while  
20 naloxone could block  $\Delta^9$ THC-mediated feeding increases, dexfenfluramine could not.  
21 Unfortunately, a detailed description of such interactions falls beyond the scope of this  
22 review, although a recent review of the effects of CB/opioid interactions on the  
23 behavioural satiety sequence can be found in Cota *et al.* (2006). Given the relatively  
24 recent success of treatment with  $\Delta^9$ THC-based CB<sub>1</sub>R partial agonists (e.g. dronabinol or

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 **nabilone**) and the remaining unmet clinical need in a range of disease states, further  
2 research into pCB-mediated feeding effects is on-going and is reviewed here alongside  
3 classical CB<sub>1</sub>R agonism and antagonism.

4

For Peer Review

1 *Endocannabinoid agonism and its effects on feeding*

2  $\Delta^9$ THC was first shown to exert hyperphagic effects in rats in 1998 (Williams *et al.*,  
3 1998).  $\Delta^9$ THC (0.063-2.0mg/kg) administered orally (per **ora**; p.o.) to pre-satiated (a  
4 situation in which eCB tone is reduced by the highly palatable prefeed process), adult,  
5 male, Lister hooded rats, at doses >0.5mg/kg significantly increased chow intake  
6 relative to control in the first hour following drug administration. **Furthermore**, no  
7 significant differences in food intake between control and any  $\Delta^9$ THC doses were seen  
8 over the twenty four hour period following  $\Delta^9$ THC administration. This early study  
9 importantly demonstrated that very low concentrations of orally administered  $\Delta^9$ THC  
10 could substantially change an animal's motivation to consume; particularly given that a  
11 considerable percentage of the administered drug would have undergone first pass  
12 metabolism as a result of oral administration. Intriguingly, a later study by Avraham  
13 (Avraham *et al.*, 2004) also showed that extremely low  $\Delta^8$ THC doses (a pCB similar to  
14  $\Delta^9$ THC but regarded as more stable; 0.001mg/kg; intraperitoneal; i.p.) increased feeding  
15 in mice. These studies suggest that subtle modulation of the eCB system can be  
16 achieved without the manifestation of the non-specific behavioural side effects  
17 traditionally associated with *C. sativa* administration (Howlett *et al.*, 2004). The drug  
18 administration routes used by Williams (1998; p.o.) and Avraham (i.p.) do, however,  
19 limit comparison of these results and prevent specific attribution of the effects to central  
20 or peripheral sites.

21 Exogenous AEA administration (0.5, 1.0, 5.0 and 10.0mg/kg; subcutaneous;  
22 s.c.) has also been observed to induce hyperphagia in pre-satiated rats (Williams *et al.*,  
23 1999). This study demonstrated that AEA administration significantly increased food  
24 intake relative to control in the first hour of testing, in a similar fashion to  $\Delta^9$ THC

1 (Williams *et al.*, 1998), but unlike  $\Delta^9$ THC also induced increases in total food intake. It  
2 was proposed that the observed changes in food intake pattern represented an AEA-  
3 induced modification in the *motivation* to feed, since AEA administration caused  
4 feeding to occur far sooner than under control conditions, suggesting a cannabinoid-  
5 mediated modulation of normal feeding pathways. Indeed, close inspection of these data  
6 shows that while vehicle-treated animals consumed only small amounts of food in the  
7 first hour of testing, they consumed ten-fold more during the third hour. In slightly later  
8 work, Williams *et al.* (2002a) used two groups of twelve, male, adult, Lister-hooded rats  
9 which received  $\Delta^9$ THC (0.5, 1.0 or 2.0mg/kg; s.c.) or AEA (0.0, 1.0, 5.0 or 10.0mg/kg;  
10 s.c.) and video recordings of post-administration activity to investigate feeding  
11 behaviours. It became clear that administration of both  $\Delta^9$ THC and AEA significantly  
12 decrease the latency to feeding onset, increase the duration of intake and the number of  
13 meals, but that only AEA increased total intake. Alongside the well-reported increases  
14 in short term chow intake associated with  $\Delta^9$ THC, these data suggest that  $\Delta^9$ THC and  
15 AEA administration induce their effects by subtly different, and yet to be elucidated  
16 pathways since two distinct changes to feeding behaviours were observed.

17 Like  $\Delta^9$ THC, extremely low doses of AEA (0.001mg/kg; i.p.) have also been  
18 demonstrated to induce significant increases in food intake in female mice in  
19 experiments which used a 40% diet restriction protocol (Hao *et al.*, 2000). It is  
20 interesting that in a situation when eCB tone would already be increased due to the diet  
21 restriction paradigm used, further slight increases in eCB tone caused by extremely low  
22 doses of AEA induced changes in feeding patterns. Furthermore, it is also interesting  
23 that when Hao and colleagues administered doses of 0.7 and 4.0mg/kg AEA (also i.p.)  
24 in the same set of experiments no significant feeding effects were observed, even

1 though, Williams (1999) demonstrated AEA effects at 1.0 and 5.0mg/kg (s.c.).  
2 However, as the authors note, it is particularly difficult to compare Williams *et al.*  
3 (1999) and Williams *et al.* (2002a) to Hao *et al.* (2000) since the experimental  
4 paradigms are almost complete opposites. Indeed, Williams used a prefeed paradigm  
5 followed by s.c. injections in male rats and recorded food intake over a period of six  
6 hours whereas Hao used a diet restricted paradigm followed by i.p. injections in female  
7 mice and recorded daily food intake over a period of one week.

8 AEA feeding effects were localised to CB<sub>1</sub>R sites by Williams using a pre-  
9 satiated paradigm in 1999. Rats received AEA (0.5, 1.0, 5.0 and 10.0mg/kg; s.c.) and, in  
10 a subsequent trial, AEA (1.0mg/kg; s.c.) plus SR141716A (0.1, 0.5 and 1.0mg/kg; s.c.;  
11 Williams *et al.*, 1999). This demonstrated that AEA significantly increased food intake  
12 relative to control (in a similar fashion to  $\Delta^9$ THC; Williams *et al.*, 1998) and that the  
13 effect could be blocked by SR141716A, thus confirming a CB<sub>1</sub>R-mediated mechanism  
14 of action for the observed feeding effects. In 2002 the hyperphagia induced by  $\Delta^9$ THC  
15 administration was also localised to CB<sub>1</sub>R sites (Williams *et al.*, 2002b). Williams first  
16 induced hyperphagia by administering  $\Delta^9$ THC (1.0mg/kg; s.c.) and in further trials  
17 attenuated this effect with the coadministration of SR141716A (0.1, 0.5 and 1.0mg/kg;  
18 s.c.) thereby demonstrating CB<sub>1</sub>R involvement in this effect. CB<sub>2</sub>R-mediation was then  
19 discounted as coadministration of  $\Delta^9$ THC (1.0mg/kg; s.c.) and SR144528 (0, 0.05, 0.1,  
20 0.5 and 1.0mg/kg; s.c.) failed to reduce the hyperphagia caused by  $\Delta^9$ THC  
21 administration.

22 Although Williams *et al.* (1999) localised AEA-mediated effects on feeding to  
23 CB<sub>1</sub>R, an effect which one can attribute to CNS receptors, evidence which links  
24 peripheral mechanisms to these effects also exists. Gomez and colleagues (Gomez *et al.*,

1  
2  
3  
4 2002) demonstrated that intracerebroventricular (i.c.v.) AEA administration (10.0µg/kg)  
5  
6 and WIN55,212 (10.0µg/kg) increased food intake, but that i.c.v. SR141716A  
7  
8 administration (0.1, 0.4, 2.0 or 10.0µg/kg) did not reduce feeding. In contrast, whilst i.p.  
9  
10 administration of AEA (0.1, 1.0 and 10.0mg/kg) and WIN55,212 (0.4, 2.0 and  
11  
12 10.0mg/kg) similarly increased food intake, i.p. administration of SR141716A (0.3, 1.0  
13  
14 and 3.0mg/kg) reduced feeding. Furthermore, the authors demonstrated that the changes  
15  
16 in feeding patterns they observed were unaffected by a CB<sub>2</sub>R antagonist (SR144528;  
17  
18 3.0mg/kg; i.p.), **principally** rejecting the hypothesis that these peripheral effects were in  
19  
20 fact CB<sub>2</sub>R- as opposed to CB<sub>1</sub>R-mediated. SR141716A's failure to reduce feeding after  
21  
22 i.c.v. but not i.p. administration led Gomez to suggest that CB<sub>1</sub>Rs must be present in the  
23  
24 periphery and that these peripheral CB<sub>1</sub>Rs can affect feeding. Gomez supports this  
25  
26 assertion by showing that intestinal AEA levels increase after food deprivation,  
27  
28 suggesting that intestinal endocannabinergic tone plays a role in energy balance.  
29  
30 However, the experimental paradigm used by Gomez did not account for the  
31  
32 concentrations of SR141716A, AEA and WIN55,212 that would be present in the CNS  
33  
34 following i.p. injection or for the considerably different dose ranges used. As such,  
35  
36 while this evidence is suggestive of a peripheral CB<sub>1</sub>R-mediated role in energy balance,  
37  
38 further experiments are needed to confirm this.  
39  
40  
41  
42  
43  
44  
45  
46

47 It has been well described that upon administration of an exogenous cannabinoid  
48  
49 agonist endocannabinergic tone will be increased and that alterations to feeding patterns  
50  
51 can then be observed. Furthermore, it has been demonstrated that naturally occurring  
52  
53 eCB concentrations fluctuate in various feeding states. Alongside evidence gathered  
54  
55 using exogenous cannabinoid administration to affect feeding patterns, this elegantly  
56  
57 demonstrates a link between how the natural eCB system modulates feeding patterns  
58  
59  
60

1 and how artificial alterations to its state modulate the same patterns. AEA and 2-AG  
2 levels in the rat limbic forebrain, hypothalamus and cerebellum were quantified in three  
3 feeding states; fasted, feeding and satiated (Kirkham *et al.*, 2002). Four groups of  
4 between eight and ten male rats were either: 1) given *ad libitum* access to food and  
5 sacrificed during a period of low spontaneous feeding; 2) fed with a wet mash for 15  
6 minutes and then sacrificed during feed consumption; 3) fed with a wet mash and  
7 sacrificed once they stopped eating or 4) given a 20% maintenance diet for 24hrs and  
8 sacrificed at the start of their red light cycle the following day. Brain tissue was then  
9 extracted and AEA and 2-AG levels were assessed in the limbic forebrain,  
10 hypothalamus and cerebellum. AEA and 2-AG levels were significantly elevated by  
11 food deprivation in the limbic forebrain while 2-AG concentration was significantly  
12 reduced in the hypothalamus during the feeding state but significantly increased during  
13 the deprived state. Since 2-AG levels were increased in the limbic forebrain and  
14 hypothalamus, and AEA levels were significantly increased in the limbic forebrain, it  
15 appears that elevated eCB levels in important reward-related brain areas during food  
16 deprivation play a role in motivating animals towards food. The reduction of 2-AG  
17 levels in the hypothalamus during feeding suggests that 2-AG is actively suppressed  
18 during feeding to facilitate satiation. Such data suggest subtly differing roles for AEA  
19 and 2-AG in feeding cycles.

20 Further experiments published in the same manuscript revealed that 2-AG (i.c.v.  
21 into the nucleus accumbens at 0.5 and 2.0 $\mu$ g) could significantly increase food intake  
22 over a one hour period while coadministration of SR141716A (0.5mg/kg; s.c. plus  
23 0.5 $\mu$ g 2-AG; i.c.v.) could almost fully attenuate this effect, thereby demonstrating CB<sub>1</sub>R  
24 mediation. The increases in food intake seen in Kirkham *et al.* (2002) are comparable to



1 the similar studies performed by the same group following  $\Delta^9$ THC administration  
2 (Williams *et al.*, 2002b; Williams *et al.*, 1998), however are considerably different from  
3 a feeding study which administered AEA (Williams *et al.*, 1999). Indeed, AEA  
4 (1.0mg/kg; s.c.) effects were observed over four hours and during the first hour  
5 significantly increased intake by a factor of approximately two, as well as significantly  
6 elevating food intake over the entire four hour period. In contrast, 2-AG (0.5 and  
7 1.0 $\mu$ g/kg; i.c.v.) significantly increased food intake by factors of nine and seven,  
8 respectively, over a one hour period. However, it is important to note that in this  
9 instance 2-AG administration was i.c.v. and AEA administration was s.c., and it is  
10 possible that AEA-mediated feeding increases would have been larger had  
11 administration been i.c.v. Given the strong anatomical connections between the nucleus  
12 accumbens shell and the hypothalamus (Stratford *et al.*, 1999) and the importance of the  
13 hypothalamus in the integration of feeding pathways (Maccarrone *et al.*, 2010) these  
14 data represent a demonstration that modulation of this pathway can have profound  
15 feeding effects.

16 As considerable differences in eCB levels in the limbic forebrain were seen  
17 during various feeding states in (Kirkham *et al.*, 2002) and because the limbic forebrain  
18 is traditionally associated with reward processing, possible interactions between the  
19 eCB and opioid systems were then considered by Williams and Kirkham (Williams *et*  
20 *al.*, 2002b). It was demonstrated that  $\Delta^9$ THC-induced hyperphagia could be attenuated  
21 by the opioid antagonist naloxone. This proves a link between opioid food reward  
22 processing and the eCB system. These experiments are also **exciting** in terms of putative  
23 anti-obesity treatments. Indeed, it has been reported that in obese humans, eCB levels  
24 are elevated by up to 52% (Engeli *et al.*, 2005); as such experiments which artificially

1 elevate eCB activity using food-restriction paradigms are particularly **valuable** since  
2 they more accurately model the human obese condition. Moreover, since in both the  
3 food deprived and obese states eCB levels are increased, this further supports the theory  
4 that eCB dysfunction plays an important part in human obesity. Further studies which  
5 directly compared AEA and 2-AG-induced feeding effects (both centrally and  
6 peripherally administered) would be extremely **useful**. It would be equally **compelling**  
7 for further work to consider fully the putative effects of peripheral cannabinoid  
8 receptors on feeding behaviours, for example in terms of gut motility.

9       Given the previously discussed alterations to eCB concentrations in the limbic  
10 forebrain, an area traditionally associated with reward processing and well documented  
11 opioid/cannabinoid interactions, it is clear that the changes to behavioural patterns  
12 observed during cannabinoid administration are due to changes in reward levels.  
13 Therefore, cannabinoid agonist effects on the consumption of different types of  
14 rewarding ingesta, such as sweet versus fatty foods, was addressed in a study by Koch  
15 (2001). Here,  $\Delta^9$ THC (0.0, 0.5, 1.0 and 2.5mg/kg; i.p.) was administered to rats  
16 receiving either control (chow), high fat (HF) or high fat plus sugar (HFS) diets and  
17 food intake was recorded over 24 hours post-injection. After the first hour, 0.5 and  
18 1.0mg/kg  $\Delta^9$ THC significantly increased food intake versus control within the HF diet  
19 group only, while doses of 2.5mg/kg had no significant effects on intake in any group.  
20 **However**, overall food intake during the first hour was significantly greater in the HF  
21 group than in either the control chow or HFS groups. During the second hour of testing,  
22 the 0.5 and 1.0mg/kg  $\Delta^9$ THC doses significantly increased intake versus control in all  
23 three groups with 1.0mg/kg causing the largest increases. This dose also induced a  
24 significantly greater intake increase in the HF (but not in the HFS) than in the control

1 chow group, although overall food intake across all doses was significantly greater in  
2 both the HF and HFS groups, relative to the control chow group. Finally in the fourth  
3 hour, unlike previous feeding studies employing  $\Delta^9$ THC which showed effects lasting  
4 fewer than four hours,  $\Delta^9$ THC doses of 1.0mg/kg still induced significant increases in  
5 consumption compared to vehicle in the HF and HFS diet groups.

6       The increases in intake seen at lower dose levels by Koch appear comparable  
7 (though reduced) to those previously reported (Williams *et al.*, 1998) and support a  
8 cannabinoid-mediated stimulation of intake (Williams *et al.*, 1999; Williams *et al.*,  
9 1998), although the non-significant effects of 2.5mg/kg  $\Delta^9$ THC remain unexplained,  
10 particularly as no changes to motor coordination or the appearance of tetrad effects were  
11 reported for this dose. **Therefore**, it is clear from these results that eCB system  
12 stimulation induces greater increases in the intake of highly fatty food-types (HF) than  
13 other, less calorific or more palatable, diets (HFS). Previous work by Arnone *et al.*  
14 (1997) and Simiand *et al.* (1998) (this work is fully discussed in ‘Endocannabinoid  
15 antagonists and their effects on feeding’) would suggest that the HFS intake should also  
16 have been increased by  $\Delta^9$ THC administration. Indeed,  $\Delta^9$ THC administration induced  
17 similar intake increases in the HFS and HF diet groups but only after the fourth and  
18 twenty-fourth hours of testing. These data could suggest that the eCB system is  
19 involved more strongly in increasing the motivation to consume high fat foods and that  
20 this effect manifests more quickly than eCB increases due to other ‘bland’ food types.  
21 From the perspective of evolution, an increased motivation to consume high fat foods is  
22 obvious and as such it is reasonable to suggest from this data that the eCB system is  
23 intimately involved in this process.

1 In a later study specifically designed to look at cannabinoid modulation of  
2 palatability, Higgs *et al.* (2003) tested the effects of various cannabinoid receptor  
3 ligands on consumption of a 10% sucrose solution in rats. In this test  $\Delta^9$ THC or AEA  
4 (0.5, 1.0 or 3.0mg/kg in both cases; i.p.) but not 2-AG (0.2, 0.5 and 2.0mg/kg; i.p.)  
5 significantly increased sucrose solution consumption. By analysing lick patterns using  
6 the mathematical model of ingestive control proposed by Davis *et al.* (1977), this effect  
7 was ascribed to a cannabinoid-induced increase in perceived palatability. The lack of  
8 significant 2-AG-induced effect is surprising, since both 2-AG and AEA are partial  
9 agonists at CB<sub>1</sub>R sites (although it has been suggested that 2-AG is the main ligand  
10 associated with CB<sub>2</sub>R sites; Sugiura *et al.*, 1999; Sugiura *et al.*, 2000), and because of  
11 the significant effects observed by Kirkham *et al.*, (2002). Higgs suggests that different  
12 speeds at which the exogenous and endogenous ligands act, coupled with the probable  
13 lack of 2-AG brain penetration (due to the route of administration), can explain the  
14 reduced effects of AEA compared to  $\Delta^9$ THC and could, to some extent, shed light on  
15 the limited effects of 2-AG. Administration of SR141716A alone was also tested (0.5,  
16 1.0 and 3.0mg/kg; i.p.) by Higgs, and found to elicit a dose-dependent decrease in  
17 consumption, thus linking the observed effects to modulation of endogenous CB<sub>1</sub>R  
18 activation. Yet, it could be argued that the dose-dependent decreases in consumption  
19 induced by SR141716A seen here were caused by a reduction in eCB tone of which 2-  
20 AG plays a part, and as such it remains impossible to discount any effects of 2-AG  
21 acting at CB<sub>2</sub>R sites on this process. This finding demonstrates that the eCB system  
22 must have an active effect upon appetite and feeding since CB<sub>1</sub>R blockade alone,  
23 without co-administration of a cannabinoid agonist (c.f. Gallate *et al.*, 1999; Williams *et*  
24 *al.*, 1999), significantly reduces consumption. Considering the works of Koch (2001),

1  
2  
3  
4  
5 1 Gallate *et al.* (1999) and Williams *et al.* (1999) it can be suggested that eCB system  
6  
7 2 stimulation upregulates the palatability of high calorie, low palatability foods and  
8  
9 3 conversely, reducing endocannabinergic tone diminishes perceived palatability. This  
10  
11 4 could explain why in Koch's work the HF diet intake was increased above HFS diet as  
12  
13 5 it was both palatable and calorie-rich.

14  
15  
16  
17 6 In a very recent study Yoshida and colleagues (2010) administered AEA and 2-  
18  
19 7 AG to both wild type and CB<sub>1</sub>R knock-out mice. They observed that AEA and 2-AG  
20  
21 8 preferentially increased taste responses to sweet rather than salty, sour, bitter or umami  
22  
23 9 (a Japanese term for a meaty or savoury taste) flavours in the chorda tympani nerve,  
24  
25 10 fungiform taste buds and during a short-term lick response test. Furthermore, they did  
26  
27 11 not observe this effect in their knock-out mice, hence linking this effect to CB<sub>1</sub>R. Given  
28  
29 12 their i.p. route of administration, the authors have suggested a peripheral effect,  
30  
31 13 alongside the well described central effect, of eCBs on taste. In comparison with the  
32  
33 14 work of Higgs *et al.* (2003) who found 2-AG to have little effect on palatability in a  
34  
35 15 progressive lick-based paradigm, and considering that both studies used i.p.  
36  
37 16 administration and observed peripheral taste responses, it is not immediately apparent  
38  
39 17 why different eCBs are identified as the principal modulators of taste.

40  
41  
42  
43  
44  
45  
46 18 In earlier taste reactivity studies, Gallate *et al.* (1999) presented rats with various  
47  
48 19 concentrations of 'beer' (<0.5%, 2.7% or 4.0% ethanol solutions) or an 8.6% sugar  
49  
50 20 solution, and their motivation to consume these substances was measured using a lick-  
51  
52 21 based progressive ratio paradigm. In order to assess CB<sub>1</sub>R involvement, animals were  
53  
54 22 treated with either vehicle, the CB<sub>1</sub>R agonist CP55,940 (10.0, 30.0 or 50.0µg/kg; i.p.),  
55  
56 23 CP55,940 (30.0µg/kg; i.p.) plus SR141716A (1.5mg/kg; i.p.), or naloxone (2.5mg/kg;  
57  
58 24 i.p.) plus CP55,940 (30.0µg/kg; i.p.). This drug regime therefore also examined

1 potential interactions between the cannabinoid and opioid systems. In agreement with  
2 previous research, CP55,940-treated animals exhibited a significant trend whereby  
3 increasing dose led to increases in both beer and sucrose solution consumption.  
4 SR141716A-induced CB<sub>1</sub>R blockade during CP55,940 treatment attenuated this effect,  
5 reducing beer solution intake to levels that did not differ from vehicle treatments.  
6 Furthermore, CP55,940 plus naloxone administration significantly reduced beer intake  
7 to approximately 50% of vehicle intake, confirming a strong interaction between CB<sub>1</sub>  
8 and opioid receptors in the control of alcohol consumption. This study demonstrated  
9 that CB<sub>1</sub>R activation could induce significant increases in palatable solution intake  
10 when compared to vehicle-treatments, and that this effect was attenuated by CB<sub>1</sub>R  
11 antagonists; further supporting the theory of CB<sub>1</sub>R involvement in perceived  
12 palatability.

13 In 2005 and 2007 Jarrett *et al.* (Jarrett *et al.*, 2005; Jarrett *et al.*, 2007)  
14 demonstrated that the eCB system mediated the perceived palatability of sweet and  
15 bitter tastes. In trials using adult, male Sprague-Dawley rats,  $\Delta^9$ THC (0.5mg/kg; i.p.),  
16  $\Delta^9$ THC (0.5mg/kg; i.p.) plus SR141716A (2.5mg/kg; i.p.) or AM251 (1.0mg/kg; i.p.), or  
17 AM251 (1.0mg/kg; i.p.) alone were administered via intraoral cannulae and the  
18 orofacial reactions to intraoral administration of either sucrose (2, 10 and 32%), or  
19 quinine (0.01 or 0.05%) solutions were recorded.  $\Delta^9$ THC increased the perceived  
20 palatability of the sucrose solution, an effect attenuated in animals treated with  $\Delta^9$ THC  
21 plus SR141716A (Jarrett *et al.*, 2005). In the later study,  $\Delta^9$ THC reduced aversiveness  
22 to 0.05% quinine solution, an effect that was blocked by coadministration of AM251  
23 (Jarrett *et al.*, 2007). The authors suggest that their results indicate that increases in  
24 CB<sub>1</sub>R responsiveness could be involved in a general upregulation of the ingesta

1 palatability. In further experiments during the same two studies, Jarrett and colleagues  
2 also demonstrated that SR141716A administration alone does not reduce perceived  
3 palatability (Jarrett *et al.*, 2005) but AM251 administration enhances aversiveness  
4 (Jarrett *et al.*, 2007). This may suggest that the increased palatability of sweet food can  
5 activate the eCB system and upregulate eCB tone. However, in the case of bitter tasting  
6 food, when eCB tone would not be increased by the low palatability of the food, CB<sub>1</sub>R  
7 antagonism can then reduce the perceived palatability of the food further.

8 Further supporting this palatability hypothesis, it has recently been demonstrated  
9 that, under a reinforced learning paradigm which included food type (normal lab chow,  
10 chocolate pellets or fat-enriched pellets) selection as an experimental variable, mice will  
11 select the sugar rich, highly-palatable food type or fatty foods over standard food after  
12  $\Delta^9$ THC (1.0mg/kg; i.p.) administration (Barbano *et al.*, 2009). Since, the chocolate-  
13 enhanced pellets used in this study had the same calorific content as the standard food  
14 pellets also employed while the fat enriched pellets contained approximately 66% more  
15 calories per gram but no added chocolate, it can be observed that  $\Delta^9$ THC administration  
16 increased the palatability of fatty foods to a level comparable to that of sweet foods.

17 The conclusion that the eCB system exerts a level of control over palatability  
18 reward is well supported. It has been shown that increased eCB tone increases the  
19 palatability of sweet liquids (Gallate *et al.*, 1999; Higgs *et al.*, 2003), bland fatty foods  
20 (Koch, 2001) or even aversive, bitter liquids (Jarrett *et al.*, 2005; Jarrett *et al.*, 2007).  
21 Indeed, eCB levels change naturally reflecting the current feeding state, and it is this  
22 basal tone that can be altered with the application of CB<sub>1</sub>R ligands. Furthermore, the  
23 meal pattern changes induced by stimulation of the eCB system are also well  
24 understood.  $\Delta^9$ THC administration has been demonstrated to reduce the latency to

1 feeding onset in periods of reduced endocannabinergic tone and has also been shown to  
2 increase meal sizes. If we consider appetitive behaviours as those that regulate the  
3 latencies to meal onset and consummatory behaviours as those that regulate the size of  
4 any given meal, what becomes clear from these data is that modulation of the eCB  
5 system can affect consummatory *and/or* appetitive behaviours and that these different  
6 behavioural changes can be manipulated individually. For example, in the work of  
7 Williams *et al.* (2002a), a distinct separation was seen between the AEA and  $\Delta^9$ THC  
8 groups in terms of feeding behaviour. In this instance, AEA administration produced  
9 effects comparable to  $\Delta^9$ THC: AEA significantly reduced the latency to feeding onset,  
10 whilst also increasing the number of eating bouts. AEA administration also increased  
11 meal duration and the duration of the longest bout versus control conditions. However,  
12 unlike  $\Delta^9$ THC, AEA induced significant increases in total intake over the test period.  
13 Given these data it can be clearly seen that the changes to feeding patterns (either in  
14 gross terms or in terms of palatability levels) are due to changes to reward processing.  
15 Indeed, the decreased latency to feeding onset and the increased size and length of  
16 meals thereafter coupled with increased lick responses can all be linked to increased  
17 reward.



1  
2  
3  
4  
5 1 *Endocannabinoid antagonism and its effect on feeding*

6  
7 2 Alongside the well documented hyperphagic effects of  $\Delta^9$ THC administration there is  
8  
9 3 comprehensive complimentary evidence which details the effects of reducing eCB tone  
10  
11 4 on feeding behaviours. Indeed the use of CB<sub>1</sub>R antagonist-based studies represents an  
12  
13 5 elegant way to study the effects of basal eCB tone on physiological feeding  
14  
15 6 mechanisms. This is particularly true as many CBR antagonists are highly selective. In  
16  
17 7 1997 Arnone (Arnone *et al.*, 1997) and colleagues performed a comprehensive set of  
18  
19 8 experiments which revealed the changes to feeding behaviours induced by CB<sub>1</sub>R  
20  
21 9 blockade by the administration of SR141716A (0.0, 0.1, 0.3, 1.0 or 3.0mg/kg: i.p.). In  
22  
23 10 experiments which studied either intake of sucrose pellets, intake of a 5% sucrose  
24  
25 11 solution or a 10% ethanol solution, Arnone demonstrated that eCB tone plays an  
26  
27 12 important role in the perceived appetitive values of both alcohol and sugar. Firstly,  
28  
29 13 Arnone and colleagues measured the weight of food consumed in 30min open field tests  
30  
31 14 where food-restricted male, Wistar rats (a situation in which one would expect eCB tone  
32  
33 15 to be increased by the food restriction (Kirkham *et al.*, 2002)) had access to either  
34  
35 16 sucrose pellets and standard lab chow or standard lab chow alone after SR141716A  
36  
37 17 administration. The quantity of sucrose pellets consumed by rats was reduced from  
38  
39 18 3.1±0.4g under control conditions to 1.0±0.4g after the 3.0mg/kg SR141716A dose in a  
40  
41 19 dose-dependent fashion; furthermore, the two highest doses of SR141716A induced  
42  
43 20 significant reductions when compared to control intakes. Standard chow intake was  
44  
45 21 unaffected. **However**, when Arnone repeatedly administered 3.0mg/kg SR141716A in  
46  
47 22 mice with a predisposition to alcohol consumption over four, six hour test periods in  
48  
49 23 one day, SR141716A induced a suppression of ethanol intake. Secondly, rats (male,  
50  
51 24 Sprague-Dawley) were given access to a 5% sucrose solution for a period of four hours  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 without access to food and water. As a control experiment another group of rats was  
2 given access to water for the same test period. Sucrose solution intake was significantly  
3 reduced by the two highest doses of SR141716A and a significant dose-dependent  
4 reduction in sucrose solution intake was apparent. Water intake was unaffected by  
5 SR141716A administration at any dose. Thirdly, Arnone tested the effect SR141716A  
6 had on ethanol consumption in male C57BL6 mice; once again administration of either  
7 1.0 or 3.0mg/kg SR141716A induced significant reductions in consumption versus  
8 control-treatment and a significant dose-dependent effect was apparent for the entire  
9 dose range. Water-only tests were used as a control, as per the 5% sucrose solution test,  
10 and no effect of SR141716A was apparent. These three experiments demonstrate that  
11 the eCB system plays an active role in the control of the appetitive value of sweet and  
12 alcoholic ingesta. Indeed, such experiments prove that the eCB system must have a  
13 constantly active basal tone as antagonists applied to an inactive system would induce  
14 no behavioural changes alone. Furthermore, given the selectivity of SR141716A we can  
15 be confident that this effect is localised to CB<sub>1</sub>R sites.

16       Reductions in eCB tone induced by the administration of SR141716A (0.0, 1.0  
17 and 3.0mg/kg; p.o.) were extended to marmosets in 1998 by Simiand (Simiand *et al.*,  
18 1998). Marmosets were habituated to a high-sugar mash (HSM; 33% sugar, 67% milk  
19 and cereal) and after habituation administered SR141716A twice a week; their food  
20 (either HSM or standard diet) intake was then observed for a period of six hours.  
21 Administration of the 3.0mg/kg dose induced significant reductions in HSM intake  
22 versus control-treatments after one, three and six hours while the 1.0mg/kg dose only  
23 reduced HSM intake versus control-treatments during hours one and three. **Notably**  
24 standard diet intake was unaffected by SR141716A administration with the exception of

1 a significant increase in intake seen during hour six. These data demonstrate that  
2 SR141716A can preferentially reduce intake of sweet, highly palatable food, versus  
3 bland food. This suggests that eCB tone is responsible for increasing the appetitive  
4 values of high-sugar foods under normal physiological conditions. Indeed, as previously  
5 discussed, such work has been supported by later experiments which showed  
6 SR141716A administration could reduce the reward associated with sucrose solutions  
7 (Higgs *et al.*, 2003). Since Arnone *et al.* (1997) and Simiand *et al.* (1998), together,  
8 used two strains of rats, one strain of mouse and one type of primate and observed  
9 similar results, it can be suggested that the effects of the eCB system are similar across  
10 species. However, the work of Arnone and Simiand did not consider a high fat diet  
11 alternative to the high-sugar foods they used, and they only antagonised the eCB system  
12 over a shorter period of time. It is **worthwhile comparing** the works of Arnone and  
13 Simiand to that of Williams and colleagues. Indeed, while Williams observed CB<sub>1</sub>R  
14 stimulation to increase feeding, and that this effect could be blocked by  
15 coadministration of SR141716A, Arnone and Simiand demonstrated a basal  
16 endocannabinergic tone which when blocked with SR141716A alone could reduce  
17 feeding. Furthermore, as discussed, the eCB system has been demonstrated to alter its  
18 activity level dependent on feeding state (e.g. deprived of food versus eating; Kirkham  
19 *et al.*, 2002). Taken together, these studies demonstrate that eCB tone is a powerful  
20 effector of perceived hunger and that feeding reduces endocannabinergic tone in the  
21 same way as blocking CB<sub>1</sub>R sites in the CNS.

22  $\Delta^9$ THC-induced effects on high fat versus high sugar foods were well described  
23 by Koch in 2001. However, only recently were antagonist studies used to facilitate the  
24 understanding of the role of CB<sub>1</sub>R alone in the selection and consumption of highly

1 fatty or sweet foods. In 2007, a study by Thornton-Jones *et al.* (2007) examined the  
2 effects of SR141716A-induced CB<sub>1</sub>R blockade on consumption of sweet (10% sucrose)  
3 or fatty (10% intralipid) solutions and analysed the lick-based response in a way similar  
4 to Higgs *et al.* (2003). **Appealingly**, this paradigm also compared the hypophagic  
5 SR141716A effects with those induced by behavioural manipulations designed to  
6 reduce either motivation to feed (pre-feeding) or food palatability (addition of quinine to  
7 the intralipid solution and reduction of sucrose concentration to 5%) which, combined  
8 with the lick-based microstructure analysis, allowed for more detailed interpretation of  
9 the results. Supporting previous findings, this study demonstrated that SR141716A  
10 treatment reduced both sucrose and intralipid solution intake, and microstructure  
11 analysis revealed that these reductions were due to decreased perceived palatability of  
12 ingesta. However, whilst these significant reductions were induced by both 1.0mg/kg  
13 and 3.0mg/kg SR141716A for the intralipid solution, only 3.0mg/kg SR141716A  
14 significantly reduced sucrose solution consumption. Furthermore, reduced lick duration  
15 of intralipid solution observed after drug administration led the authors to suggest that  
16 the SR141716A-induced reduction in consumption of this solution was a result of a  
17 decrease in motivation to feed, as well as an effect on perceived palatability. In contrast,  
18 reductions in sucrose solution intake were less pronounced than those seen for the  
19 intralipid solution, suggesting that eCBS may be more strongly implicated in  
20 consumption of highly calorific than sweet, palatable food-types. Taken alone, this work  
21 suggests that the eCB system can play its role in energy homeostasis via modulation of  
22 the motivation to consume fatty foods specifically; this long term effect may be due to  
23 changes in meal patterning over the shorter term, particularly with respect to the control  
24 of fat intake. Indeed, the work of Thornton-Jones further supports the findings of

1 Arnone *et al.* (1997), Simiand *et al.* (1998) and Koch (2001), as previously discussed,  
2 and strengthens the link between CB<sub>1</sub>R activation and the control of feeding.

3 Using a different experimental approach, a recent study by Mathes *et al.* (2008)  
4 also addressed these issues, and provided further support for eCB system involvement  
5 in the specific consumption of highly fatty and/or sweet foods. A ‘dessert protocol’ was  
6 employed, whereby rats were given *ad libitum* access to a wet mash diet made from  
7 standard laboratory chow in addition to simultaneous but time limited access to a  
8 ‘dessert’ diet high in fat and sugar. Vitaly, this methodology gave rats a choice between  
9 two different diets, allowing within-group analysis of diet selection. eCB system  
10 involvement in the consumption of these two different food-types was assessed by  
11 administration of either of the CB<sub>1</sub>R antagonists, SR141716A (1.0mg/kg; i.p.) or  
12 AM251 (0.3, 1.0 or 3.0mg/kg; i.p.) 30min prior to dessert access. Both compounds  
13 significantly reduced caloric intake over a 24 hour period, but this effect was due to a  
14 significant decrease in the consumption of the high fat and sugar diet only, with no  
15 effect on the consumption of the standard chow. When considered alongside Arnone *et*  
16 *al.* (1997), Koch (2001), Simiand *et al.* (1998) and Thornton-Jones *et al.* (2007), these  
17 findings suggest that CB<sub>1</sub>R-mediation of intake not only modulates intake of fatty food  
18 more than sugary food, but does so in a preferential fashion. Unfortunately, although  
19 Mathes *et al.*, in the earlier stages of this study, compared the consumption of the high  
20 fat and sugar diet with another diet high in sugar only, the effects of CB<sub>1</sub>R blockade on  
21 this sugar-only diet were not examined.

22 While the use of the previously discussed antagonists has been shown to reduce  
23 feeding in a number of models, there is considerable on-going research looking for  
24 novel compounds which perform similar actions. Such research is performed to further

1 our understanding of the eCB system and its ligands, whilst also searching for new anti-  
2 obesity drugs (for examples please see PSNCBAM-1, Horswill *et al.*, 2007; AM4113,  
3 Sink *et al.*, 2007; O-2050, Gardner *et al.*, 2006; MK55-96, Yan *et al.*, 2010; and MK-  
4 0364, Fong *et al.*, 2007). While, O-2050 was found to induce significant reductions in  
5 motor coordination, which may have been the cause for its effects on feeding  
6 behaviours, PSNCBAM-1, AM4113 and MK-5596 have been demonstrated to reduce  
7 short term intake and reduce body weight in animal models without apparent side  
8 effects. Further work using these novel agents would benefit from the inclusion of meal  
9 patterning analysis as this would allow the study of appetitive and/or consummatory  
10 behaviours, as well as effects on perceived palatability; such an analysis may shed more  
11 light on eCB mediation of feeding behaviours.

12

1  
2  
3  
4  
5 1 *Non- $\Delta^9$ THC phytocannabinoid effects on feeding*

6  
7 2 Very recently it has been suggested that non- $\Delta^9$ THC pCBs can also modulate rodent  
8  
9 3 feeding patterns (Farrimond *et al.*, 2010a; Farrimond *et al.*, 2010b) and induce different  
10  
11 4 changes than  $\Delta^9$ THC, without any apparent non-specific behavioural effects. Before  
12  
13 5 2009 there had been a limited number of studies which demonstrated non- $\Delta^9$ THC pCB-  
14  
15 6 mediated feeding effects. Unfortunately these studies were either unrepeated or  
16  
17 7 contradictory. Sofia and Knobloch (1976) reported that the CB<sub>1</sub>R agonist CBN reduced  
18  
19 8 intake in rats, an effect yet to be repeated, and that CBD also reduced feeding in rats.  
20  
21 9 However, Wiley *et al.*, (2005) reported that CBD administration had no effect on food  
22  
23 10 intake in mice, a result repeated by Riedel (2009) who observed only a small, non-  
24  
25 11 significant reduction in food intake and weight gain, also in mice. It should be noted  
26  
27 12 that the dose ranges used by Sofia, Knobloch and Wiley are considerably higher than  
28  
29 13 those used by Farrimond and colleagues and, given the well described bi-phasic dose  
30  
31 14 effects of CB<sub>1</sub>R activation (Glick *et al.*, 1972) on feeding, this may explain the  
32  
33 15 differences in observed effect patterns. Work in our laboratory has demonstrated that a  
34  
35 16 range of pCBs may have significant effects on feeding patterns. Thirty-six pre-satiated,  
36  
37 17 male, Lister-hooded rats received a purified, *C. sativa*-derived  $\Delta^9$ THC (hereafter  
38  
39 18 purified  $\Delta^9$ THC), a synthetic  $\Delta^9$ THC and a high- $\Delta^9$ THC standardised botanical drug  
40  
41 19 substance (BDS), similar in composition to that used in Sativex and containing a typical  
42  
43 20 array of non- $\Delta^9$ THC pCBs (high- $\Delta^9$ THC BDS; all treatments were dose matched for  
44  
45 21  $\Delta^9$ THC content; 0.00, 0.34, 0.67, 1.34 and 2.68mg/kg; p.o.; Farrimond *et al.*, 2010a).  
46  
47 22 Synthetic and purified  $\Delta^9$ THC administration induced classical  $\Delta^9$ THC hyperphagia in  
48  
49 23 this study: the latency to the first meal was reduced and both the first hour intake and  
50  
51 24 first meal duration were increased versus vehicle treatments. Intriguingly, the effects of  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 the high- $\Delta^9$ THC BDS administered showed significantly reduced hyperphagia in  
2 comparison. These results suggested that the combination of pCBs (and, potentially,  
3 non-cannabinoid components) in the high- $\Delta^9$ THC BDS attenuated the hyperphagic  
4 effects of  $\Delta^9$ THC. Given the constituents of the high- $\Delta^9$ THC BDS, ( $\Delta^9$ THC; 67.0%,  
5 Cannabigerol (CBG); 1.7%, Cannabichromene (CBC); 1.6%, CBN; 1.5%,  $\Delta^9$ THCV;  
6 0.9%,  $\Delta^9$ tetrahydrocannabinolic acid ( $\Delta^9$ THCA); 0.3%, CBD; 0.3% and Cannabitrinol  
7 (CBO); 0.2%) it is possible that the pCBs present could have affected feeding  
8 synergistically, or that at least one pCB modulated the  $\Delta^9$ THC-induced hyperphagia.  
9 Indeed, the high- $\Delta^9$ THC BDS used in Farrimond *et al.*, (2010a) contained known CB<sub>1</sub>R  
10 antagonists ( $\Delta^9$ THCV, Dennis *et al.*, 2008; Ma *et al.*, 2008; Thomas *et al.*, 2005) in  
11 addition to modulators of Ca<sup>2+</sup> homeostasis (CBD; Ryan *et al.*, 2009) and CBN (Rao *et*  
12 *al.*, 2006); which could modulate eCB levels. However, these results do not preclude the  
13 possibility that non-cannabinoid material also present in the high- $\Delta^9$ THC BDS also  
14 contributed to the observed behavioural effects.

15 Consistent with these results we have also demonstrated that non- $\Delta^9$ THC pCBs  
16 can affect feeding when co-administered with sub-effective concentrations of  $\Delta^9$ THC  
17 (Farrimond *et al.*, 2010b; please also see Riedel *et al.*, 2009 for non- $\Delta^9$ THC pCB-  
18 induced hypophagia fully discussed below). Ten, pre-satiated, adult, male, Lister-  
19 hooded rats received a low- $\Delta^9$ THC standardised BDS (low- $\Delta^9$ THC BDS; 0.0, 0.5, 1.0,  
20 2.0 and 4.0mg/kg; p.o.) containing only 6.9%  $\Delta^9$ THC, alongside 14.2% other pCBs.  
21 **Conversely**, at these concentrations, rather than having no effect on feeding (as it might  
22 be expected, given the use of a prefeed paradigm with an extremely low dose of  $\Delta^9$ THC  
23 and pCBs that have previously been shown to reduce  $\Delta^9$ THC-mediated hyperphagia),  
24 the low- $\Delta^9$ THC BDS significantly increased chow intake during the first hour, by



1 significantly reducing the latency to the first contact with food for the highest doses  
2 versus vehicle-treatments. These reductions in meal one latency mirror those previously  
3 demonstrated for considerably higher concentrations of pure  $\Delta^9$ THC (Farrimond *et al.*,  
4 2010a; Williams *et al.*, 1998). Furthermore, while the latency to the first meal was  
5 significantly reduced, the size and duration of this meal was unaffected. Therefore, we  
6 suggested that the ratio of non- $\Delta^9$ THC pCBs present in the low- $\Delta^9$ THC extract  
7 significantly increased appetitive (but not consummatory behaviours), since the  
8 concentrations of  $\Delta^9$ THC present were between two- and ten-fold lower than those  
9 previously demonstrated to induce hyperphagia (Farrimond *et al.*, 2010b).

10 When these two studies are considered together (Farrimond *et al.* 2010a and  
11 2010b), a number of suggestions can be made: firstly, since the observed effects of the  
12 non- $\Delta^9$ THC components of the extracts administered reduced hyperphagia when  
13 presented in one ratio and increased hyperphagia when presented in a different ratio, it  
14 is highly unlikely that the plant matter also present was responsible for these effects,  
15 given that its content did not change between the two tests. Secondly, it is possible that  
16 the pCBs present in both studies did not act in isolation such that more than one  
17 compound induced the observed effects. Thirdly, these effects could involve non-CBR  
18 dependent pathways since several of the non- $\Delta^9$ THC pCBs present are known to act via  
19 mechanisms only partially connected to CBR (see Izzo *et al.*, (2009) for review).  
20 Finally, these results give further credence to the already well supported therapeutic  
21 potential of non- $\Delta^9$ THC pCBs for the treatment of energy balance disorders. Indeed,  
22 non- $\Delta^9$ THC pCB-based treatment may prove more tolerable than  $\Delta^9$ THC treatment  
23 alone (e.g. Nabilone) due to the absence of psychotropic side effects associated with  
24 non- $\Delta^9$ THC pCBs.

1           Whilst a definitive mechanism underlying the findings reported by Farrimond *et*  
2 *al.*, (2010a and b) remains unknown, the naturally occurring pCB and CB<sub>1</sub>R neutral  
3 antagonist Δ<sup>9</sup>THCV, which was also present in the extracts employed in Farrimond *et*  
4 *al.*, has been considered alone and shown to exert hypophagic actions (Riedel *et al.*,  
5 2009). Indeed, given the difficulties associated with the use of the highly specific and  
6 potent CB<sub>1</sub>R antagonist/inverse agonist SR141716A in the clinic, the use of Δ<sup>9</sup>THCV,  
7 as a neutral antagonist, to reduce food intake may be of therapeutic value. Riedel *et al.*  
8 (2009) demonstrated that purified Δ<sup>9</sup>THCV (3.0, 10.0 and 30.0mg/kg; i.p.) significantly  
9 reduced chow intake over a period 12 hours and induced weight loss in male C57BL6  
10 mice. Subsequent treatment with extremely low Δ<sup>9</sup>THCV BDS doses (which contained  
11 between 0.1 and 0.3mg/kg Δ<sup>9</sup>THC) failed to induce changes to feeding patters; however  
12 the Δ<sup>9</sup>THC quantities presented were within the range previously observed to affect  
13 meal patterning in rats (Farrimond *et al.*, 2010). Unfortunately, meal microstructure was  
14 not analysed in this study. In a further experiment, Riedel hypothesised that if CBD  
15 (10.0mg/kg; i.p.) were to be co-administered with these very low Δ<sup>9</sup>THCV BDS doses,  
16 the effects of the present Δ<sup>9</sup>THC would be antagonised, and the hypophagic properties  
17 previously induced by purified Δ<sup>9</sup>THCV would therefore emerge. However, the  
18 evidence for *in vivo* antagonism of Δ<sup>9</sup>THC by CBD has so far only been demonstrated  
19 in a memory task (Fadda *et al.*, 2004), and Riedel observed no significant effects on  
20 feeding or weight change following CBD co-administration. The authors suggest that  
21 this lack of effect may also be attributed to other pCBs, present in his extract, or simply  
22 because the concentrations of Δ<sup>9</sup>THCV administered were too low. However, Riedel  
23 recorded all feeding data during the light phase of the rats' day during which time little  
24 feeding typically occurs. Furthermore, it is also possible that the experimental paradigm

1 used was not optimised for the observation of hypophagia. For instance, introducing  
2 food-deprivation prior to dosing could accentuate possible differences between control  
3 and conditions measurements, thereby rendering the experimental paradigm more  
4 sensitive to  $\Delta^9$ THCV-mediated reductions in feeding. While, Riedel has confirmed that  
5 purified  $\Delta^9$ THCV can reduce food intake in mice, further experiments which use more  
6 sensitive paradigms and analyse meal microstructure after  $\Delta^9$ THCV BDS administration  
7 may prove useful in fully understanding Riedel's data.

8         The work discussed throughout this manuscript demonstrates that some pCBs  
9 can alter appetitive and consummatory behaviours separately (e.g. Higgs *et al.*, 2003).  
10 As such if it were possible to use at least one non- $\Delta^9$ THC pCB to modulate  
11 consummatory behaviours (to increase or decrease the quantity consumed during a  
12 natural eating event) rather than inducing immediate short term post-administration  
13 hunger (the 'munchies', a colloquial term meaning increased hunger normally due to  
14 smoking cannabis recreationally) it may be possible to produce a *C. sativa*-based  
15 medicine which could control absolute intake quantity. Even though the mechanisms by  
16 which the eCB system modulates palatability and therefore diet selection remain  
17 unclear, direct effects on diet selection caused by cannabinoid administration have also  
18 been demonstrated. Given the presented data which suggests that increased eCB tone  
19 leads to increased selection of fatty foods over sweet foods, it is a possibility that non-  
20 psychotropic pCBs could also be used to modulate diet selection i.e. to reduce fatty food  
21 palatability in obesity. Indeed, putative anti-obesity treatments using pCBs are of great  
22 interest. Equally, while some pCBs have been shown to induce significant reductions in  
23 food intake and in some cases reduce body weight (e.g.  $\Delta^9$ THCV), little research is

- 1 currently considering possible mechanisms by which intake and body weight may be
- 2 increased, which would be valuable in the treatment of anorectic disorders.
- 3

For Peer Review

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 1 **Conclusions**

2 A considerable quantity of characterisation work has been performed on  $\Delta^9$ THC-  
3 mediated feeding effects, but the specific roles of other pCBs remain less well  
4 understood. Work in our laboratory and others suggests that non- $\Delta^9$ THC pCBs can have  
5 profound effects on feeding behaviours (Farrimond *et al.*, 2010a; Farrimond *et al.*,  
6 2010b; Riedel *et al.*, 2009; Sofia *et al.*, 1976; Wiley *et al.*, 2005). However, these are  
7 not yet well characterised. As such considerable further work is required to assess the  
8 effects of both purified single pCBs as well as combinations of numerous pCBs. With  
9 the recent UK approval of the  $\Delta^9$ THC CBD combination in Sativex, it can be clearly  
10 seen that the use of pCBs in the clinic is gaining public acceptance that regulatory  
11 bodies are willing to accept plant-derived medicines alongside their synthetic  
12 counterparts. Indeed, non- $\Delta^9$ THC pCBs may represent an untapped clinical potential for  
13 a number of feeding disorders. Such a need is particularly important given the non-  
14 specific behavioural side effects associated with the use of  $\Delta^9$ THC and its analogues in  
15 the clinic. Given the encouraging results demonstrating non- $\Delta^9$ THC pCB-mediated  
16 modulation of feeding, it is tempting to suggest that these compounds may provide at  
17 least the basis for novel therapies for the treatment of feeding disorders.

1  
2  
3  
4  
5 **1 Acknowledgements**  
6

7 2 The authors are grateful to the members of our research group who have kindly  
8  
9 3 commented on this manuscript, in particular to Drs A. Hill and G. Bucci. The authors  
10  
11 4 have no conflict of interest to declare.  
12  
13  
14  
15 5  
16  
17 6  
18  
19 7  
20  
21 8  
22  
23 9  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

1 **References**

- 2 Adams R, Baker B (1940). Structure of Cannabidiol. VII. A Method of Synthesis of a  
3 Tetrahydrocannabinol which Possesses Marijuana Activity1. *J Am Chem Soc* **62**(9):  
4 2405-2408.
- 5  
6 Agrawal A, Lynskey MT (2009). Candidate genes for cannabis use disorders: findings,  
7 challenges and directions. *Addiction* **104**(4): 518-532.
- 8  
9 Ahern G (2003). Activation of TRPV1 by the satiety factor oleoylethanolamide. *J Biol*  
10 *Chem* **278**(33): 30429.
- 11  
12 Alger BE (2009). *Endocannabinoid Signaling in Neural Plasticity*. edn, vol. 1. Springer  
13 Berlin Heidelberg.
- 14  
15 Armstrong HE, Galka A, Lin LS, Lanza Jr TJ, Jewell JP, Shah SK, *et al.* (2007).  
16 Substituted acyclic sulfonamides as human cannabinoid-1 receptor inverse agonists.  
17 *Bioorg Med Chem Lett* **17**(8): 2184-2187.
- 18  
19 Arnone M, Maruani J, Chaperon F, Thiébot MH, Poncelet M, Soubrié P, *et al.* (1997).  
20 Selective inhibition of sucrose and ethanol intake by SR141716, an antagonist of central  
21 cannabinoid (CB1) receptors. *Psychopharmacology* **132**(1): 104-106.
- 22  
23 Avraham Y, Ben-Shushan D, Breuer A, Zolotarev O, Okon A, Fink N, *et al.* (2004).  
24 Very low doses of [Delta]8-THC increase food consumption and alter neurotransmitter  
25 levels following weight loss. *Pharmacol Biochem Be* **77**(4): 675-684.
- 26  
27 Bachur N, Masek K, Melmon K, Udenfriend S (1965). Fatty acid amides of  
28 ethanolamine in mammalian tissues. *J Biol Chem* **240**(3): 1019.
- 29  
30 Baker D, Pryce G, Davies WL, Hiley CR (2006). In silico patent searching reveals a  
31 new cannabinoid receptor. *Trends Pharmacol Sci* **27**(1): 1-4.
- 32  
33 Baker D, Pryce G, Giovannoni G, Thompson A (2003). The therapeutic potential of  
34 cannabis. *Lancet Neurol* **2**: 291-296.
- 35  
36 Barbano M, Castane A, Martin-Garcia E, Maldonado R (2009). Delta-9-  
37 tetrahydrocannabinol enhances food reinforcement in a mouse operant conflict test.  
38 *Psychopharmacology* **205**(3): 475-487.
- 39

- 1  
2  
3  
4  
5 1 Basavarajappa BS (2007a). Critical Enzymes Involved in Endocannabinoid Metabolism.  
6 2 *Protein Peptide Lett* **14**(3): 237.  
7  
8 3  
9 4 Basavarajappa BS (2007b). Neuropharmacology of the Endocannabinoid Signalling  
10 5 System - Molecular Mechanisms, Biological Actions and Synaptic Plasticity. *Curr*  
11 6 *Neuropharmacol* **5**(2): 81-97.  
12  
13 7  
14 8 Ben-Zvi Z, Mechoulam R, Burstein S (1970). Identification through synthesis of an  
15 9 active .DELTA.1(6)-tetrahydrocannabinol metabolite. *J Am Chem Soc* **92**(11): 3468-  
16 10 3469.  
17  
18  
19 11  
20 12 Ben Amar M (2006). Cannabinoids in medicine: A review of their therapeutic potential.  
21 13 *J Ethnopharmacol* **105**(1-2): 1-25.  
22  
23 14  
24 15 Berridge M (1995). Capacitative calcium entry. *Biochem J* **312**(Pt 1): 1.  
25  
26 16  
27 17 (1988). Inositol trisphosphate and calcium signaling. *Cold Spring Harbor Symposia on*  
28 18 *Quantitative Biology*. Cold Spring Harbor Laboratory Press. p 927.  
29  
30  
31 19  
32 20 Bisogno T, Ligresti A, Di Marzo V (2005). The endocannabinoid signalling system:  
33 21 Biochemical aspects. *Pharmacol Biochem Be* **81**(2): 224-238.  
34  
35 22  
36 23 Blankman JL, Simon GM, Cravatt BF (2007). A comprehensive profile of brain  
37 24 enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol*  
38 25 **14**(12): 1347-1356.  
39  
40  
41 26  
42 27 Bortolato M, Mangieri R, Fu J, Kim J, Arguello O, Duranti A, *et al.* (2007).  
43 28 Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat  
44 29 model of chronic mild stress. *Biol Psychiat* **62**(10): 1103-1110.  
45  
46  
47 30  
48 31 Breivogel CS, Sim LJ, Childers SR (1997). Regional differences in cannabinoid  
49 32 receptor/G-protein coupling in rat brain. *J Pharmacol Exp Ther* **282**(3): 1632-1642.  
50  
51 33  
52 34 Brown A, J. , Wise A (2001). Identification of modulators of GPR55 activity:  
53 35 GlaxoSmithKline.  
54  
55 36  
56 37 Brown AJ (2007). Novel cannabinoid receptors. *Brit J Pharmacol* **152**(5): 567-575.  
57  
58 38  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 Burkey TH, Quock RM, Consroe P, Roeske WR, Yamamura HI (1997).  $\Delta^9$ -  
2 Tetrahydrocannabinol is a partial agonist of cannabinoid receptors in mouse brain. *Eur J*  
3 *Pharmacol* **323**(2-3): 3-4.
- 4  
5 Cadas H, di Tomaso E, Piomelli D (1997). Occurrence and biosynthesis of endogenous  
6 cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. *J*  
7 *Neurosci* **17**(4): 1226.
- 8  
9 Cahn RS (1933). Cannabis indica resin, part IV. The synthesis of some 2:2-  
10 dimethyldibenzopyrans, and confirmation of the structure of cannabinol. *J. Chem. Soc.:*  
11 1400–1405.
- 12  
13 Chan W, Magnus K, Watson H (1976). The structure of cannabitrinol. *Cell Mol Life Sci*  
14 **32**(3): 283-284.
- 15  
16 Childers SR, Breivogel CS (1998). Cannabis and endogenous cannabinoid systems.  
17 *Drug Alcohol Depen* **51**(1-2): 173-187.
- 18  
19 Clark RT (2009). *Annual Report Pursuant to Section 13 or 15(d) of the Securities*  
20 *Exchange Act of 1934 For the Fiscal Year Ended December 31, 2008*. Washington:  
21 Merck & Co., Inc.
- 22  
23 Comelli F, Giagnoni G, Bettoni I, Colleoni M, Costa B (2007). The inhibition of  
24 monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive  
25 effect in a murine model of acute inflammation. *Brit J Pharmacol* **152**(5): 787-794.
- 26  
27 Consroe P (1998). Brain cannabinoid systems as targets for the therapy of neurological  
28 disorders. *Neurobiol Dis* **5**(6 Pt B): 534-551.
- 29  
30 Costiniuk CT, Mills E, Cooper CL (2008). Evaluation of oral cannabinoid-containing  
31 medications for the management of interferon and ribavirin-induced anorexia, nausea  
32 and weight loss in patients treated for chronic hepatitis C virus. *Can J Gastroenterol*  
33 **22**(4).
- 34  
35 Cota D, Marsicano G, Lutz B, Vicennati V, Stalla GK, Pasquali R, *et al.* (2003a).  
36 Endogenous cannabinoid system as a modulator of food intake. *Int J Obes Relat Metab*  
37 *Disord* **27**(3): 289-301.
- 38  
39 Cota D, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, *et al.*  
40 (2003b). The endogenous cannabinoid system affects energy balance via central  
41 orexigenic drive and peripheral lipogenesis. *J Clin Invest* **112**(3): 423-431.

- 1  
2  
3  
4  
5 1  
6 2 Cota D, Tschop MH, Horvath TL, Levine AS (2006). Cannabinoids, opioids and eating  
7 3 behavior: the molecular face of hedonism? *Brain Res Brain Res Rev* **51**(1): 85-107.  
8  
9 4  
10 5 Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996).  
11 6 Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid  
12 7 amides. *Nature* **384**(6604): 83-87.  
13  
14 8  
15 9 Davis J, Levine M (1977). A model for the control of ingestion. *Psychol Rev* **84**(4):  
16 10 379-412.  
17  
18 11  
19 12 Davis M, Maida V, Daeninck P, Pergolizzi J (2007). The emerging role of cannabinoid  
20 13 neuromodulators in symptom management. *Supportive care cancer* **15**(1): 63-71.  
21  
22 14  
23 15 De Filippis Dea (2008). Effect of cannabidiol on sepsis-induced motility disturbances in  
24 16 mice: involvement of CB1 receptors and fatty acid amide hydrolase.  
25 17 *Neurogastroenterol Motil* **20**: 919-927.  
26  
27 18  
28 19 de Fonseca R (2005). The endocannabinoid system: physiology and pharmacology.  
29 20 *Alcohol Alcoholism* **40**(1): 2.  
30  
31 21  
32 22 De Petrocellis L, Di Marzo V (2009). Non-CB 1, Non-CB 2 Receptors for  
33 23 Endocannabinoids, Plant Cannabinoids, and Synthetic Cannabimimetics: Focus on G-  
34 24 protein-coupled Receptors and Transient Receptor Potential Channels. *J Neuroimmune*  
35 25 *Pharm*: 1-19.  
36  
37 26  
38 27 De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P, *et*  
39 28 *al.* (2008). Plant-derived cannabinoids modulate the activity of transient receptor  
40 29 potential channels of ankyrin type-1 (TRPA1) and melastatin type-8 (TRPM-8). *J*  
41 30 *Pharmacol Exp Ther*.  
42  
43 31  
44 32 Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S (1995). Cannabinoids  
45 33 modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-  
46 34 dependent process. *J Pharmacol Exp Ther* **273**(2): 734-743.  
47  
48 35  
49 36 Dennis I, Whalley BJ, Stephens GJ (2008). Effects of  $\Delta^9$ -tetrahydrocannabivarin on  
50 37 [35S] GTP $\gamma$ S binding in mouse brain cerebellum and piriform cortex membranes. *Brit J*  
51 38 *Pharmacol* **154**(6): 1349-1358.  
52  
53 39  
54 40 Deutsch D, Ueda N, Yamamoto S (2002). The fatty acid amide hydrolase (FAAH).  
55 41 *Prostag Leukotr Ess* **66**(2-3): 201-210.  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* **34**(5): 605-613.

5  
6 Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**(5090): 1946-1949.

9  
10 Dewey WL (1986). Cannabinoid pharmacology. *Pharmacol Rev* **38**(2): 151-178.

11  
12 Di Marzo V (2008). Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov* **7**(5): 438-455.

14  
15 Di Marzo V, Bifulco M, De Petrocellis L (2004). The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* **3**(9): 771-784.

17  
18 Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, *et al.* (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**(6507): 686-691.

21  
22 Di Marzo V, Maccarrone M (2008). FAAH and anandamide: is 2-AG really the odd one out? *Trends Pharmacol Sci* **29**(5): 229-233.

24  
25 Di Marzo V, Petrosino S (2007). Endocannabinoids and the regulation of their levels in health and disease. *Curr opin lipidol* **18**(2): 129.

27  
28 Elmquist J, Elias C, Saper C (1999). From Lesions to Leptin: Review Hypothalamic Control of Food Intake and Body Weight. *Neuron* **22**: 221-232.

30  
31 EMA (2009). Procedural steps taken and scientific information after the authorisation Acomplia. In: *EMEA/H/C/000666/A20/0012*, Agency EM (ed) Vol. 2010. Brussels: European Union.

34  
35 Engeli S, Böhnke J, Feldpausch M, Gorzelniak K, Janke J, Bátkai S, *et al.* (2005). Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* **54**(10): 2838.

38  
39 Evans AT, Formukong E, Evans FJ (1987). Activation of phospholipase A2 by cannabinoids: Lack of correlation with CNS effects. *FEBS Letters* **211**(2): 119-122.

- 1  
2  
3  
4  
5 1  
6 2 Fadda P, Robinson L, Fratta W, Pertwee R, Riedel G (2004). Differential effects of  
7 3 THC-or CBD-rich cannabis extracts on working memory in rats. *Neuropharmacology*  
8 4 **47**(8): 1170-1179.  
9  
10 5  
11 6 Farrimond J, Hill A, Whalley B, Williams C (2010a). Cannabis constituents modulate  
12 7 9-tetrahydrocannabinol-induced hyperphagia in rats. *Psychopharmacology* **210**(1): 97-  
13 8 106.  
14  
15 9  
16 10 Farrimond JA, Whalley BJ, Williams CM (2010b). A low  $\Delta^9$ tetrahydrocannabinol  
17 11 cannabis extract induces hyperphagia in rats. *Behav pharmacol in press*.  
18  
19 12  
20 13 Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, *et al.* (1995).  
21 14 Comparison of the pharmacology and signal transduction of the human cannabinoid  
22 15 CB1 and CB2 receptors. *Mol Pharmacol* **48**(3): 443-450.  
23  
24 16  
25 17 Fong TM, Guan X-M, Marsh DJ, Shen C-P, Stribling DS, Rosko KM, *et al.* (2007).  
26 18 Antiobesity Efficacy of a Novel Cannabinoid-1 Receptor Inverse Agonist, N-[(1S,2S)-  
27 19 3-(4-Chlorophenyl)-2-(3-cyanophenyl)-1-methylpropyl]-2-methyl-2-{[5-  
28 20 (trifluoromethyl)pyridin-2-yl]oxy}propanamide (MK-0364), in Rodents. *J Pharmacol*  
29 21 *Exp Ther* **321**(3): 1013-1022.  
30  
31 22  
32 23 Fredriksson R, Höglund PJ, Gloriam DEI, Lagerström MC, Schiöth HB (2003). Seven  
33 24 evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close  
34 25 relatives. *FEBS Letters* **554**(3): 381-388.  
35  
36 26  
37 27 Freund TF, Katona I, Piomelli D (2003). Role of Endogenous Cannabinoids in Synaptic  
38 28 Signaling. *Physiol. Rev.* **83**(3): 1017-1066.  
39  
40 29  
41 30 Gaetani S, Fu J, Cassano T, Dipasquale P, Romano A, Righetti L, *et al.* (2010). The Fat-  
42 31 Induced Satiety Factor Oleoylethanolamide Suppresses Feeding through Central  
43 32 Release of Oxytocin. *J Neurosci* **30**(24): 8096.  
44  
45 33  
46 34 Gallate JE, Saharov T, Mallet PE, McGregor IS (1999). Increased motivation for beer in  
47 35 rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol*  
48 36 **370**(3): 233-240.  
49  
50 37  
51 38 Gaoni Y, Mechoulam R (1966). Cannabichromene, a new active principle in hashish.  
52 39 *Chemical Communications (London)* **1966**(1): 20-21.  
53  
54  
55  
56  
57  
58  
59 40  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 Gaoni Y, Mechoulam R (1964a). Isolation, Structure, and Partial Synthesis of an Active  
2 Constituent of Hashish. *J Am Chem Soc* **86**(8): 1646-1647.
- 3  
4 (1964b). The structure and synthesis of cannabigerol, a new hashish constituent.  
5 *Chemistry Society*; London.
- 6  
7 Gardner A, Mallet PE (2006). Suppression of feeding, drinking, and locomotion by a  
8 putative cannabinoid receptor [ $\text{CB1}$ ]silent antagonist'. *Eur J Pharmacol* **530**(1-2): 103-106.
- 9  
10 Gatley S, Gifford A, Volkow N, Lan R, Makriyannis A (1996).  $^{123}\text{I}$ -labeled AM251: a  
11 radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors.  
12 *Eur J Pharmacol* **307**(3): 331-338.
- 13  
14 Gerdeman GL, Ronesi J, Lovinger DM (2002). Postsynaptic endocannabinoid release is  
15 critical to long-term depression in the striatum. *Nat Neurosci* **5**(5): 446-451.
- 16  
17 Gill E, Paton W, Pertwee R (1970). Preliminary experiments on the chemistry and  
18 pharmacology of cannabis. *Nature* **228**: 134-136.
- 19  
20 Glass M (2001). The role of Cannabinoids in neurodegenerative diseases. *Prog Neuro-*  
21 *Psychoph* **25**(4): 743-765.
- 22  
23 Glick SD, Milloy S (1972). Increased and decreased eating following THC  
24 administration. *Psychonom. Sci.* **29**(6).
- 25  
26 Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Del Arco I, *et al.* (2002). A  
27 Peripheral Mechanism for CB1 Cannabinoid Receptor-Dependent Modulation of  
28 Feeding. *J. Neurosci.* **22**(21): 9612-9617.
- 29  
30 Gong J, Onaivi E, Ishiguro H, Liu Q, Tagliaferro P, Brusco A, *et al.* (2006).  
31 Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. *Brain Res*  
32 **1071**: 10-23.
- 33  
34 Goutopoulos A, Makriyannis A (2002). From cannabis to cannabinergics: new  
35 therapeutic opportunities. *Pharmacology and Therapeutics* **95**: 103-117.
- 36  
37 Grant I, Cahn B (2005). Cannabis and endocannabinoid modulators: Therapeutic  
38 promises and challenges. *Clin Neurosci Res* **5**: 185-199.
- 39

- 1 Hao S, Avraham Y, Mechoulam R, Berry EM (2000). Low dose anandamide affects  
2 food intake, cognitive function, neurotransmitter and corticosterone levels in diet-  
3 restricted mice. *Eur J Pharmacol* **392**(3): 147-156.
- 4  
5 Henstridge C, Balenga N, Schröder R, Kargl J, Platzer W, Martini L, *et al.* (2010).  
6 GPR55 ligands promote receptor coupling to multiple signalling pathways. *Brit J*  
7 *Pharmacol* **9999**(9999).
- 8  
9 Henstridge CM, Balenga NAB, Ford LA, Ross RA, Waldhoer M, Irving AJ (2009). The  
10 GPR55 ligand L- $\alpha$ -lysophosphatidylinositol promotes RhoA-dependent Ca<sup>2+</sup>  
11 signaling and NFAT activation. *The FASEB Journal* **23**(1): 183.
- 12  
13 Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991).  
14 Characterization and localization of cannabinoid receptors in rat brain: a quantitative in  
15 vitro autoradiographic study. *J Neurosci* **11**(2): 563-583.
- 16  
17 Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, *et al.*  
18 (1990). Cannabinoid receptor localization in brain. *P Natl Acad Sci USA* **87**(5): 1932.
- 19  
20 Higgs S, Williams CM, Kirkham TC (2003). Cannabinoid influences on palatability:  
21 microstructural analysis of sucrose drinking after  $\Delta^9$ -tetrahydrocannabinol, anandamide,  
22 2-arachidonoyl glycerol and SR141716. *Psychopharmacology* **165**(4): 370-377.
- 23  
24 Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, *et al.* (2005).  
25 An endocannabinoid mechanism for stress-induced analgesia. *Nature* **435**(7045): 1108-  
26 1112.
- 27  
28 Holland ML, Allen JD, Arnold JC (2008). Interaction of plant cannabinoids with the  
29 multidrug transporter ABCC1 (MRP1). *Eur J Pharmacol* **591**(1-3): 128-131.
- 30  
31 Hollister LE (1986). Health aspects of cannabis. *Pharmacol Rev* **38**(1): 1-20.
- 32  
33 Horswill J, Bali U, Shaaban S, Keily J, Jeevaratnam P, Babbs A, *et al.* (2007).  
34 PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with  
35 hypophagic effects in rats. *Brit J Pharmacol* **152**(5): 805.
- 36  
37 Hosohata K, Quock RM, Hosohata Y, Burkey TH, Makriyannis A, Consroe P, *et al.*  
38 (1997). AM630 is a competitive cannabinoid receptor antagonist in the guinea pig brain.  
39 *Life Sci* **61**(9): PL115-118.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 Howlett AC (2002). The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* **68-69**: 619-631.
- 2
- 3
- 4 Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M (1990). The cannabinoid receptor: biochemical, anatomical and behavioral characterization. *Trends Neurosci* **13**(10): 420-423.
- 5
- 6
- 7
- 8 Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ (2004). Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* **47**(Supplement 1): 345-358.
- 9
- 10
- 11
- 12 Huffman J, Dai D, Martin B, Compton D (1994). Design, Synthesis and Pharmacology of Cannabimimetic Indoles. *Bioorg Med Chem Lett* **4**: 563-566.
- 13
- 14
- 15 Hughes TE (2009). Emerging therapies for metabolic diseases--the focus is on diabetes and obesity. *Curr Opin Chem Biol* **In Press, Corrected Proof**.
- 16
- 17
- 18 Ishiguro H, Onaivi ES, Horiuchi Y, Imai K, Komaki G, Ishikawa T, *et al.* (2010). Functional polymorphism in the GPR55 gene is associated with anorexia nervosa. *Synapse*: **Epub ahead of print**.
- 19
- 20
- 21
- 22 Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* **30**(10): 515-527.
- 23
- 24
- 25
- 26 Jamshidi N, Taylor DA (2001). Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Brit J Pharmacol* **134**(6): 1151-1154.
- 27
- 28
- 29 Jarrett MM, Limebeer CL, Parker LA (2005). Effect of [Delta]9-tetrahydrocannabinol on sucrose palatability as measured by the taste reactivity test. *Physio Behav* **86**(4): 475-479.
- 30
- 31
- 32
- 33 Jarrett MM, Scantlebury J, Parker LA (2007). Effect of [Delta]9-tetrahydrocannabinol on quinine palatability and AM251 on sucrose and quinine palatability using the taste reactivity test. *Physio Behav* **90**(2-3): 425-430.
- 34
- 35
- 36
- 37 Jin X, Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2007). Discovery and characterization of a Ca<sup>2+</sup>-independent phosphatidylethanolamine N-acyltransferase generating the anandamide precursor and its congeners. *J Biol Chem* **282**(6): 3614.
- 38
- 39
- 40

- 1 Kapur A, Zhao P, Sharir H, Bai Y, Caron M, Barak L, *et al.* (2009). Atypical  
2 responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem*  
3 **284**(43): 29817.
- 4  
5 King A, Duranti A, Tontini A, Rivara S, Rosengarth A, Clapper J, *et al.* (2007).  
6 URB602 inhibits monoacylglycerol lipase and selectively blocks 2-  
7 arachidonoylglycerol degradation in intact brain slices. *Chem Biol* **14**(12): 1357-1365.
- 8  
9 Kirby MT, Hampson RE, Deadwyler SA (2000). Cannabinoid receptor activation in  
10 CA1 pyramidal cells in adult rat hippocampus. *Brain Res* **863**(1-2): 120-131.
- 11  
12 Kirkham T, Williams C (2001a). Endocannabinoids: neuromodulators of food craving.  
13 *Food Cravings and Addiction. Leatherhead: Leatherhead Publishing*: 85–120.
- 14  
15 Kirkham TC (2008). Endocannabinoids and the Neurochemistry of Gluttony. *J*  
16 *Neuroendocrinol* **20**(9): 1099-1100.
- 17  
18 Kirkham TC, Williams CM (2001b). Endogenous cannabinoids and appetite. *Nutr Res*  
19 **14**(01): 65-86.
- 20  
21 Kirkham TC, Williams CM (2001c). Synergistic effects of opioid and cannabinoid  
22 antagonists on food intake. *Psychopharmacology* **153**(2): 267-270.
- 23  
24 Kirkham TC, Williams CM, Fezza F, Di Marzo V (2002). Endocannabinoid levels in rat  
25 limbic forebrain and hypothalamus in relation to fasting, feeding and satiation:  
26 stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol* **136**(4): 550-557.
- 27  
28 Koch JE (2001). [Delta]9-THC stimulates food intake in Lewis rats: effects on chow,  
29 high-fat and sweet high-fat diets. *Pharmacol Biochem Be* **68**(3): 539-543.
- 30  
31 Lambert D, Fowler C (2005). The endocannabinoid system: drug targets, lead  
32 compounds, and potential therapeutic applications. *J. Med. Chem* **48**(16): 5059-5087.
- 33  
34 Landsman RS, Burkey TH, Consroe P, Roeske WR, Yamamura HI (1997). SR141716A  
35 is an inverse agonist at the human cannabinoid CB1 receptor. *Eur J Pharmacol* **334**(1):  
36 R1-2.
- 37  
38 Lauckner JE, Hille B, Mackie K (2005). The cannabinoid agonist WIN55,212-2  
39 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proc*  
40 *Natl Acad Sci USA* **102**(52): 19144-19149.



- 1  
2  
3  
4  
5 1  
6 2 Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K (2008). GPR55 is a  
7 3 cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc*  
8 4 *Natl Acad Sci USA* **105**(7): 2699.  
9  
10 5  
11 6 Ligresti A, Moriello A, Starowicz K, Matias I, Pisanti S, De Petrocellis L, *et al.* (2006).  
12 7 Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on  
13 8 human breast carcinoma. *J Pharmacol Exp Ther* **318**(3): 1375.  
14  
15  
16 9  
17 10 Little P, Compton D, Johnson M, Melvin L, Martin B (1988). Pharmacology and  
18 11 stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther*  
19 12 **247**(3): 1046.  
20  
21 13  
22 14 Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, *et al.* (2008). Multiple  
23 15 pathways involved in the biosynthesis of anandamide. *Neuropharmacology* **54**(1): 1-7.  
24  
25  
26 16  
27 17 Long J, Li W, Booker L, Burston J, Kinsey S, Schlosburg J, *et al.* (2009). Selective  
28 18 blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects.  
29 19 *Nat chem biol* **5**(1): 37.  
30  
31 20  
32 21 Ma Y, Weston S, Whalley B, Stephens G (2007). Cannabinoid-induced modulation of  
33 22 cerebellar output occurs via effects on fast inhibitory synaptic transmission. *J.*  
34 23 *Neurosci.*  
35  
36  
37 24  
38 25 Ma YL, Weston SE, Whalley BJ, Stephens GJ (2008). The phytocannabinoid  $\Delta^9$ -  
39 26 tetrahydrocannabivarin modulates inhibitory neurotransmission in the cerebellum. *Brit J*  
40 27 *Pharmacol* **154**(1): 204-215.  
41  
42  
43 28  
44 29 Maccarrone M (2009). Endocannabinoids: Friends and foes of reproduction. *Prog Lipid*  
45 30 *Res* **48**(6): 344-354.  
46  
47 31  
48 32 Maccarrone M, Gasperi V, Catani M, Diep T, Dainese E, Hansen H, *et al.* (2010). The  
49 33 Endocannabinoid System and Its Relevance for Nutrition. *Ann Rev Nut* **30**.  
50  
51 34  
52 35 Mackie K (2005). Distribution of cannabinoid receptors in the central and peripheral  
53 36 nervous system. In: Pertwee R (ed) (eds). *Cannabinoids*, edn, Vol. 168. Berlin:  
54 37 Springer-Verlag. pp 299-325.  
55  
56  
57 38  
58 39 Mackie K (2006). Mechanisms of CB1 receptor signaling: endocannabinoid modulation  
59 40 of synaptic strength. *Int J Obes (Lond)* **30 Suppl 1**: S19-23.

- 1  
2  
3  
4  
5 1  
6 2 Makriyannis A, Mechoulam R, Piomelli D (2005). Therapeutic opportunities through  
7 3 modulation of the endocannabinoid system. *Neuropharmacology* **48**(8): 1068-1071.  
8  
9 4  
10 5 (2002). Agonists and silent antagonists in a series of cannabinoid sulfonamides.  
11 6 *International Cannabinoid Research Society Conference*; Pacific Grover, California,  
12 7 USA. ICRS.  
13  
14 8  
15 9 Mathes CM, Ferrara M, Rowland NE (2008). Cannabinoid-1 receptor antagonists  
16 10 reduce caloric intake by decreasing palatable diet selection in a novel dessert protocol in  
17 11 female rats. *Am J Physiol-Reg I* **295**(1): R67.  
18  
19 12  
20 13 Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a  
21 14 cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**(6284):  
22 15 561-564.  
23  
24 16  
25 17 Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, *et al.*  
26 18 (1995a). Identification of an endogenous 2-monoglyceride, present in canine gut, that  
27 19 binds to cannabinoid receptors. *Biochem Pharmacol* **50**(1): 83-90.  
28  
29 20  
30 21 Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, *et al.*  
31 22 (1995b). Identification of an endogenous 2-monoglyceride, present in canine gut, that  
32 23 binds to cannabinoid receptors. *Biochem Pharmacol* **50**(1): 83-90.  
33  
34 24  
35 25 Melvin LS, Johnson MR, Milne GM (1983). A cannabinoid derived analgetic (CP-  
36 26 55,940) Abstract. In: *186th National Meeting of the American Chemical Society*.  
37 27 Washington: American Chemical Society.  
38  
39 28  
40 29 Moldrich G, Wenger T (2000). Localization of the CB<sub>1</sub> cannabinoid receptor in the rat  
41 30 brain. An immunohistochemical study. *Peptides* **21**(11): 1735-1742.  
42  
43 31  
44 32 Morgan NH, Stanford IM, Woodhall GL (2009). Functional CB2 type cannabinoid  
45 33 receptors at CNS synapses. *Neuropharmacology* **57**(4): 356-368.  
46  
47 34  
48 35 Morley J, Levine A (1982). The role of the endogenous opiates as regulators of appetite.  
49 36 *Am J Clin Nut* **35**(4): 757.  
50  
51 37  
52 38 Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral  
53 39 receptor for cannabinoids. *Nature* **365**(6441): 61-65.  
54  
55  
56  
57  
58  
59  
60 40

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 Naidu P, Kinsey S, Guo T, Cravatt B, Lichtman A (2010). Regulation of Inflammatory  
2 Pain by Inhibition of Fatty Acid Amide Hydrolase. *J Pharmacol Exp Ther* **334**(1): 182.
- 3
- 4 Ning Y, Neill KO, Lan H, Pang L, Shan LX, Hawes BE, *et al.* (2008). Endogenous and  
5 synthetic agonists of GPR119 differ in signalling pathways and their effects on insulin  
6 secretion in MIN6c4 insulinoma cells. *Brit J Pharmacol* **155**(7): 1056-1065.
- 7
- 8 Ohno-Shosaku T, Maejima T, Kano M (2001). Endogenous cannabinoids mediate  
9 retrograde signals from depolarized postsynaptic neurons to presynaptic terminals.  
10 *Neuron* **29**(3): 729-738.
- 11
- 12 Okamoto K, Wang J, Morishita J, Ueda N (2009a). Biosynthetic Pathways of the  
13 Endocannabinoid Anandamide. In: Lambert DM (ed)<sup>(eds)</sup>. *Cannabinoids in Nature and*  
14 *Medicine*, edn. Zurich and Weinheim: VHCA and WILEY-VCH. p<sup>^</sup>pp 267-320.
- 15
- 16 Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004). Molecular characterization  
17 of a phospholipase D generating anandamide and its congeners. *J Biol Chem* **279**(7):  
18 5298-5305.
- 19
- 20 Okamoto Y, Tsuboi K, Ueda N (2009b). Chapter 1 Enzymatic Formation of  
21 Anandamide. In: Gerald L (ed)<sup>(eds)</sup>. *Vitamins & Hormones*, edn, Vol. Volume 81:  
22 Academic Press. p<sup>^</sup>pp 1-24.
- 23
- 24 Onaivi E, Carpio O, Ishiguro H, Schanz N, Uhl G, Benno R (2008). Behavioral effects  
25 of CB2 cannabinoid receptor activation and its influence on food and alcohol  
26 consumption. *Ann. NY Acad. Sci* **1139**: 426–433.
- 27
- 28 Onaivi E, Ishiguro H, Gong J, Patel S, Perchuk A, Meozzi P, *et al.* (2006). Discovery of  
29 the Presence and Functional Expression of Cannabinoid CB2 Receptors in Brain. *Ann.*  
30 *N.Y. Acad. Sci.*(1074): 514-536.
- 31
- 32 Onaivi E, Leonard C, Ishiguro H, Zhang P, Lin Z, Akinshola B, *et al.* (2002).  
33 Endocannabinoids and cannabinoid receptor genetics. *Progress in Neurobiol* **66**: 307-  
34 344.
- 35
- 36 Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, *et al.* (2005).  
37 Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and  
38 contributes to diet-induced obesity. *J Clin Invest* **115**(5): 1298-1305.
- 39

- 1  
2  
3  
4  
5 1 Overton HA, Babbs AJ, Doel SM, Fyfe MCT, Gardner LS, Griffin G, *et al.* (2006).  
6 2 Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in  
7 3 the discovery of small-molecule hypophagic agents. *Cell Metabol* **3**(3): 167-175.  
8  
9 4  
10 5 Overton HA, Fyfe MCT, Reynet C (2008). GPR119, a novel G protein-coupled receptor  
11 6 target for the treatment of type 2 diabetes and obesity. *Brit J Pharmacol* **153**(S1): S76-  
12 7 S81.  
13  
14 8  
15 9 Oz M (2006). Receptor-independent effects of endocannabinoids on ion channels. *Curr*  
16 10 *Pharm Des* **12**(2): 227-239.  
17  
18  
19 11  
20 12 Pacheco M, Childers S, Arnold R, Casiano F, Ward S (1991). Aminoalkylindoles:  
21 13 actions on specific G-protein-linked receptors. *J Pharmacol Exp Ther* **257**(1): 170.  
22  
23 14  
24 15 Pertwee R (2001). Cannabinoid receptor ligands. *Tocris Rev* **16**: 1-8.  
25  
26 16  
27 17 Pertwee R, Griffin G, Fernando S, Li X, Hill A, Makriyannis A (1995). AM630, a  
28 18 competitive cannabinoid receptor antagonist. *Life Sci* **56**(23-24): 1949-1955.  
29  
30 19  
31 20 Pertwee R, Joe-Adigwe G, Hawksworth G (1996). Further evidence for the presence of  
32 21 cannabinoid CB1 receptors in mouse vas deferens. *Eur J Pharmacol* **296**(2): 169-172.  
33  
34 22  
35 23 Pertwee RG (2008a). The diverse CB1 and CB2 receptor pharmacology of three plant  
36 24 cannabinoids:  $\Delta^9$ -tetrahydrocannabinol, cannabidiol and  $\Delta^9$ -tetrahydrocannabivarin. *Brit*  
37 25 *J Pharmacol* **153**(2): 199-215.  
38  
39 26  
40 27 Pertwee RG (2008b). The diverse CB<sub>1</sub> and CB<sub>2</sub> receptor pharmacology of three plant  
41 28 cannabinoids:  $\Delta^9$ -tetrahydrocannabinol, cannabidiol and  $\Delta^9$ -tetrahydrocannabivarin. *Brit*  
42 29 *J Pharmacol* **153**(2): 199-215.  
43  
44 30  
45 31 Pertwee RG (2007). GPR55: a new member of the cannabinoid receptor clan? *Brit J*  
46 32 *Pharmacol* **152**(7): 984-986.  
47  
48 33  
49 34 Pertwee RG (1999). Pharmacology of cannabinoid receptor ligands. *Curr Med Chem*  
50 35 **6**(8): 635-664.  
51  
52 36  
53 37 Pertwee RG (2005). The therapeutic potential of drugs that target cannabinoid receptors  
54 38 or modulate the tissue levels or actions of endocannabinoids. *Aaps J* **7**(3): E625-654.  
55  
56 39  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1 Petitet F, Donlan M, Michel A (2006). GPR55 as a new cannabinoid receptor: still a  
2 long way to prove it. *Chem Biol Drug Des* **67**(3): 252-253.
- 3
- 4 Petrosino S, Ligresti A, Di Marzo V (2009). Endocannabinoid chemical biology: a tool  
5 for the development of novel therapies. *Curr Opin Chem Bio* **13**(3): 309-320.
- 6
- 7 Pietr M, Kozela E, Levy R, Rimmerman N, Lin YH, Stella N, *et al.* (2009). Differential  
8 changes in GPR55 during microglial cell activation. *FEBS Letters* **583**(12): 2071-2076.
- 9
- 10 Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev*  
11 *Neurosci* **4**(11): 873-884.
- 12
- 13 Piomelli D, Giuffrida A, Calignano A, Rodriguez de Fonseca F (2000). The  
14 endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol Sci* **21**(6):  
15 218-224.
- 16
- 17 Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR, *et al.* (2006).  
18 Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). *CNS*  
19 *Drug Rev* **12**(1): 21-38.
- 20
- 21 Pope C, Mechoulam R, Parsons L (2010). Endocannabinoid signaling in neurotoxicity  
22 and neuroprotection. *Neurotox* **In Press, Corrected Proof**.
- 23
- 24 Pryce G, Ahmed Z, Hankey D, Jackson S, Croxford J, Pocock J, *et al.* (2003).  
25 Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain* **126**:  
26 2191-2202.
- 27
- 28 Pryce G, Baker D (2005). Emerging properties of cannabinoid medicines in  
29 management of multiple sclerosis. *Trends Neurosci* **28**(5): 272-276.
- 30
- 31 Putney Jr J (1986). A model for receptor-regulated calcium entry. *Cell Calcium* **7**(1): 1-  
32 12.
- 33
- 34 Rao GK, Kaminski NE (2006). Cannabinoid-mediated elevation of intracellular  
35 calcium: a structure-activity relationship. *J Pharmacol Exp Ther* **317**(2): 820-829.
- 36
- 37 Riedel G, Fadda P, McKillop-Smith S, Pertwee R, Platt B, Robinson L (2009).  
38 Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic  
39 properties in fasted and non-fasted mice. *Brit J Pharmacol* **156**(7): 1154-1166.

- 1  
2  
3  
4  
5 1  
6 2 Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, *et al.* (1994).  
7 3 SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS*  
8 4 *Lett* **350**(2-3): 240-244.  
9  
10 5  
11 6 Rinaldi-Carmona M, Barth F, Millan J, Derocq J, Casellas P, Congy C, *et al.* (1998). SR  
12 7 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J*  
13 8 *Pharmacol Exp Ther* **284**(2): 644.  
14  
15 9  
16 10 Rodriguez de Fonseca F, Navarro M, Gomez R, Escuredo L, Nava F, Fu J, *et al.* (2001).  
17 11 An anorexic lipid mediator regulated by feeding. *Nature* **414**(6860): 209-212.  
18  
19 12  
20 13 Ronesi J, Gerdeman GL, Lovinger DM (2004). Disruption of Endocannabinoid Release  
21 14 and Striatal Long-Term Depression by Postsynaptic Blockade of Endocannabinoid  
22 15 Membrane Transport. *J. Neurosci.* **24**(7): 1673-1679.  
23  
24 16  
25 17 Ross RA (2009). The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci* **30**(3):  
26 18 156-163.  
27  
28 19  
29 20 Ross RA, Brockie HC, Stevenson LA, Murphy VL, Templeton F, Makriyannis A, *et al.*  
30 21 (1999). Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors  
31 22 of L759633, L759656, and AM630. *Brit J Pharmacol* **126**(3): 665-672.  
32  
33 23  
34 24 Ryan D, Drysdale AJ, Lafourcade C, Pertwee RG, Platt B (2009). Cannabidiol Targets  
35 25 Mitochondria to Regulate Intracellular Ca<sup>2+</sup> Levels. *J. Neurosci.* **29**(7): 2053-2063.  
36  
37 26  
38 27 Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J, *et al.* (2007).  
39 28 The orphan receptor GPR55 is a novel cannabinoid receptor. *Brit J Pharmacol* **152**(7):  
40 29 1092-1101.  
41  
42 30  
43 31 Sanger GJ (2007). Endocannabinoids and the gastrointestinal tract: what are the key  
44 32 questions? *Brit J Pharmacol* **152**(5): 663-670.  
45  
46 33  
47 34 Sawzdargo M, Nguyen T, Lee DK, Lynch KR, Cheng R, Heng HH, *et al.* (1999).  
48 35 Identification and cloning of three novel human G protein-coupled receptor genes  
49 36 GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain*  
50 37 *Res Mol Brain Res* **64**(2): 193-198.  
51  
52 38  
53 39 Schweitzer P (2000). Cannabinoids decrease the K(+) M-current in hippocampal CA1  
54 40 neurons. *J Neurosci* **20**(1): 51-58.  
55  
56  
57  
58  
59  
60

- 1  
2  
3  
4  
5 1  
6 2 Showalter V, Compton D, Martin B, Abood M (1996). Evaluation of binding in a  
7 3 transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification  
8 4 of cannabinoid receptor subtype selective ligands. *J Pharmacol Exp Ther* **278**(3): 989.  
9  
10 5  
11 6 Simiand J, Keane M, Keane P, Soubrie P (1998). SR 141716, a CB1 cannabinoid  
12 7 receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav*  
13 8 *Pharmacol* **9**(2): 179.  
14  
15 9  
16 10 Sink K, McLaughlin P, Wood J, Brown C, Fan P, Vemuri V, *et al.* (2007). The novel  
17 11 cannabinoid CB1 receptor neutral antagonist AM4113 suppresses food intake and food-  
18 12 reinforced behavior but does not induce signs of nausea in rats. *Neuropsychopharmacol*  
19 13 **33**(4): 946-955.  
20  
21  
22 14  
23 15 Sofia RD, Knobloch LC (1976). Comparative effects of various naturally occurring  
24 16 cannabinoids on food, sucrose and water consumption by rats. *Pharmacol Biochem*  
25 17 *Be***4**(5): 591-599.  
26  
27  
28 18  
29 19 Soga T, Ohishi T, Matsui T, Saito T, Matsumoto M, Takasaki J, *et al.* (2005).  
30 20 Lysophosphatidylcholine enhances glucose-dependent insulin secretion via an orphan  
31 21 G-protein-coupled receptor. *Biochem Bioph Res Co* **326**(4): 744-751.  
32  
33  
34 22  
35 23 Solinas M, Goldberg S (2005). Motivational effects of cannabinoids and opioids on  
36 24 food reinforcement depend on simultaneous activation of cannabinoid and opioid  
37 25 systems. *Neuropsychopharmacol* **30**(11): 2035-2045.  
38  
39 26  
40 27 Staton PC, Hatcher JP, Walker DJ, Morrison AD, Shapland EM, Hughes JP, *et al.*  
41 28 (2008). The putative cannabinoid receptor GPR55 plays a role in mechanical  
42 29 hyperalgesia associated with inflammatory and neuropathic pain. *Pain* **139**(1): 225-236.  
43  
44  
45 30  
46 31 Stratford TR, Kelley AE (1999). Evidence of a Functional Relationship between the  
47 32 Nucleus Accumbens Shell and Lateral Hypothalamus Subserving the Control of  
48 33 Feeding Behavior. *J. Neurosci.* **19**(24): 11040-11048.  
49  
50 34  
51 35 Südhof TC, Starke K (2008). *Pharmacology of neurotransmitter release*. edn. Springer  
52 36 Verlag.  
53  
54  
55 37  
56 38 Sugiura T, Kodaka T, Nakane S, Miyashita T, Kondo S, Suhara Y, *et al.* (1999).  
57 39 Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. *J Biol*  
58 40 *Chem* **274**(5): 2794.  
59  
60 41

- 1  
2  
3  
4  
5 1 Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, *et al.* (1995). 2-  
6 Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain.  
7 *Biochem Biophys Res Commun* **215**(1): 89-97.  
8  
9 4  
10 5 Sugiura T, Waku K (2000). 2-Arachidonoylglycerol and the cannabinoid receptors.  
11 *Chem Phys Lipids* **108**(1-2): 89-106.  
12  
13 7  
14 8 Takeda S, Misawa K, Yamamoto I, Watanabe K (2008). Cannabidiolic Acid as a  
15 Selective Cyclooxygenase-2 Inhibitory Component in Cannabis. *Drug Metab Dispos*  
16 **36**(9): 1917-1921.  
17  
18  
19 11  
20 12 Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA, *et al.* (2005).  
21 Evidence that the plant cannabinoid  $\Delta^9$ -tetrahydrocannabivarin is a cannabinoid CB<sub>1</sub>  
22 and CB<sub>2</sub> receptor antagonist. *Brit J Pharmacol* **7**(146): 917-926.  
23  
24 15  
25 16 Thornton-Jones Z, Kennett G, Vickers S, Clifton P (2007). A comparison of the effects  
26 of the CB 1 receptor antagonist SR141716A, pre-feeding and changed palatability on  
27 the microstructure of ingestive behaviour. *Psychopharmacology* **193**(1): 1-9.  
28  
29 19  
30 20 Tramer M, Carroll D, Campbell F, Reynolds D, Moore R, McQuay H (2001).  
31 Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative  
32 systematic. *Brit Med J* **323**(7303): 16.  
33  
34 23  
35 24 Tsou K, Brown S, Sa udo-Pe a M, Mackie K, Walker J (1997). Immunohistochemical  
36 distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neurosci*  
37 **83**(2): 393-411.  
38  
39 27  
40 28 Turu G, Hunyady L (2009). Signal transduction of the CB1 cannabinoid receptor. *J Mol*  
41 *Endocrin.* **44**: 75-85  
42  
43 30  
44 31 Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rössner S (2005). Effects of the  
45 cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk  
46 factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet*  
47 **365**(9468): 1389-1397.  
48  
49 35  
50 36 Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, *et al.*  
51 (2005). Identification and functional characterization of brainstem cannabinoid CB2  
52 receptors. *Science* **310**(5746): 329-332.  
53  
54 39  
55 40 Vandevoorde S (2008). Overview of the chemical families of fatty acid amide hydrolase  
56 and monoacylglycerol lipase inhibitors. *Curr Top Med Chem* **8**(3): 247-267.  
57  
58  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 Walsh DM, Nelson KA, Mahmoud F (2003). Established and potential therapeutic applications of cannabinoids in oncology. *Supportive care cancer* **11**(3): 137-143.

4  
5 Wang T, Collet J, Shapiro S, Ware M (2008). Adverse effects of medical cannabinoids: a systematic review. *Can Med Assoc J* **178**(13): 1669.

8  
9 Werner N, Koch J (2003). Effects of the cannabinoid antagonists AM281 and AM630 on deprivation-induced intake in Lewis rats. *Brain Res* **967**(1-2): 290-292.

11  
12 Whalley BJ, Wilkinson JD, Williamson EM, Constanti A (2004). A novel component of cannabis extract potentiates excitatory synaptic transmission in rat olfactory cortex in vitro. *Neurosci Lett* **365**(1): 58-63.

15  
16 Wiley J, Burston J, Leggett D, Alekseeva O, Razdan R, Mahadevan A, *et al.* (2005). CB1 cannabinoid receptor-mediated modulation of food intake in mice. *Brit J Pharmacol* **145**(3): 293-300.

19  
20 Wilkinson JD, Whalley BJ, Baker D, Pryce G, Constanti A, Gibbons S, *et al.* (2003). Medicinal cannabis: is delta9-tetrahydrocannabinol necessary for all its effects? *J Pharm Pharmacol* **55**(12): 1687-1694.

23  
24 Williams CM, Kirkham TC (1999). Anandamide induces overeating: mediation by central cannabinoid CB<sub>1</sub> receptors. *Psychopharmacology* **143**(3): 315-317.

26  
27 Williams CM, Kirkham TC (2002a). Observational analysis of feeding induced by Delta9-THC and anandamide. *Physiol Behav* **76**(2): 241-250.

29  
30 Williams CM, Kirkham TC (2002b). Reversal of  $\Delta^9$ -THC hyperphagia by SR141716 and naloxone but not dexfenfluramine. *Pharmacol Biochem Be* **71**(1-2): 333-340.

32  
33 Williams CM, Rogers PJ, Kirkham TC (1998). Hyperphagia in pre-fed rats following oral  $\Delta^9$ -THC. *Physiol Behav* **65**(2): 343-346.

35  
36 Williamson EM, Evans FJ (2000). Cannabinoids in clinical practice. *Drugs* **60**(6): 1303.

37  
38 Wilson RI, Nicoll RA (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**(6828): 588-592.

- 1  
2  
3  
4 1 Woolridge E, Barton S, Samuel J, Osorio J, Dougherty A, Holdcroft A (2005). Cannabis  
5 2 use in HIV for pain and other medical symptoms. *J Pain Symptom manag* **29**(4): 358-  
6 3 367.  
7  
8  
9 4  
10 5 Yamauchi T, Shoyama Y, Aramaki H, Azuma T, Nishioka I (1967).  
11 6 Tetrahydrocannabinolic acid, a genuine substance of tetrahydrocannabinol. *Chem*  
12 7 *Pharm Bull* **15**(7): 1075.  
13  
14  
15 8  
16 9 Yan L, Huo P, Debenham JS, Madsen-Duggan CB, Lao J, Chen RZ, *et al.* (2010).  
17 10 Discovery of N-[(4R)-6-(4-Chlorophenyl)-7-(2,4-dichlorophenyl)-2,2-dimethyl-3,4-  
18 11 dihydro-2H-pyrano[2,3-b]pyridin-4-yl]-5-methyl-1H-pyrazole-3-carboxamide (MK-  
19 12 5596) as a Novel Cannabinoid-1 Receptor (CB1R) Inverse Agonist for the Treatment of  
20 13 Obesity. *J Med Chem.* **53**(10):4028-37.  
21  
22 14  
23 15 Yates ML, Barker EL (2009). Inactivation and Biotransformation of the Endogenous  
24 16 Cannabinoids Anandamide and 2-Arachidonoylglycerol. *Mol Pharmacol* **76**(1): 11.  
25  
26 17 **Yoshida R, Ohkuri T, Jyotaki M, Yasuo T, Horio N, Yasumatsu K, *et al.* (2010).**  
28 18 **Endocannabinoids selectively enhance sweet taste. *P Natl Acad Sci USA* **107**(2): 935-**  
29 19 **939.**  
30  
31 20 Zhu HJ, Wang JS, Markowitz JS, Donovan JL, Gibson BB, Gefroh HA, *et al.* (2006).  
32 21 Characterization of P-glycoprotein inhibition by major cannabinoids from marijuana. *J*  
33 22 *Pharmacol Exp Ther* **317**(2): 850-857.  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 **Figure 1;** the development of cannabinoid pharmacology 1964 – 2010.

2 **Figure 2;** eCB signalling pathways. Upon invasion of an action potential into the  
3 synaptic region of the presynapse, the release of neurotransmitter (in this case glutamate  
4 or acetylcholine) and the binding of neurotransmitter to postsynaptic receptor sites: the  
5 eCBs AEA and 2-AG are produced on demand from arachidonic acid by NaPE and  
6 DAG respectively and released into the synaptic cleft from the postsynapse (red). Upon  
7 neurotransmitter binding to both metabotropic Glutamate and Acetylcholine receptors G  
8 subunits disassociate and stimulate an increase in PLC- $\beta$  levels. This leads to an  
9 upregulation of intracellular IP<sub>3</sub> which gates calcium channels on the endoplasmic  
10 reticulum thus increasing intracellular Ca<sup>2+</sup>. Furthermore, the release of G<sub>ai/o</sub> subunits  
11 upregulates the production of cAMP and therefore allows further Ca<sup>2+</sup> entry into the cell  
12 via VGCCs. Equally upon activation of iGluR channels calcium is allowed into the cell.  
13 Once released into the extracellular space the endocannabinoids bind to CB<sub>1</sub>R sites on  
14 the presynapse (green) causing the presynaptic cell to move closer to its resting  
15 membrane potential principally by closing VGCCs and opening VGPCs thus reducing  
16 the available intracellular Ca<sup>2+</sup> required to facilitate further neurotransmitter release.

17 **Figure 3;** simplified diagram illustrating retrograde signalling by eCBs in the CNS.  
18 Following vesicular neurotransmitter release from the presynaptic cell (green),  
19 neurotransmitters bind to receptor proteins (black) on the postsynaptic cell (red)  
20 stimulating eCB production and release from the postsynaptic cell. eCBs then diffuse  
21 back across the synaptic cleft and bind to CBRs (blue) on the presynaptic cell. This  
22 predominately leads to a suppression of [Ca<sup>+</sup>] influx but also an increase in [K<sup>+</sup>] efflux,  
23 thus hyperpolarising the presynaptic cell and inhibiting further neurotransmitter release.

1 **Table 1;** endocannabinergic tone effector ligands. A brief overview of the CBR ligands  
2 discussed throughout this review, giving further background to their discovery and  
3 where appropriate, their non-feeding effects.

4 Abbreviations: 2-AG, 2-archidonlyglycerol;  $\Delta^8$ THC,  $\Delta^8$ tetrahydrocannabinol;  $\Delta^9$ THC,  
5  $\Delta^9$ tetrahydrocannabinol;  $\Delta^9$ THCA,  $\Delta^9$ tetrahydrocannabinolic acid;  $\Delta^9$ THCV,  
6  $\Delta^9$ tetrahydrocannbivarin; AEA, anandamide; CB<sub>1</sub>R, cannabinoid type 1 receptor; CB<sub>2</sub>R,  
7 cannabinoid type 2 receptor; CBR, cannabinoid receptor; CNS, central nervous system;  
8 FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; OEA, n-  
9 oleoylethanolamide; P-gp, p-glycoprotein; VR<sub>1</sub>, transient receptor potential vannilioid 1;  
10 TRPA<sub>1</sub>, transient receptor potential cation channel, subfamily A, member 1.

11

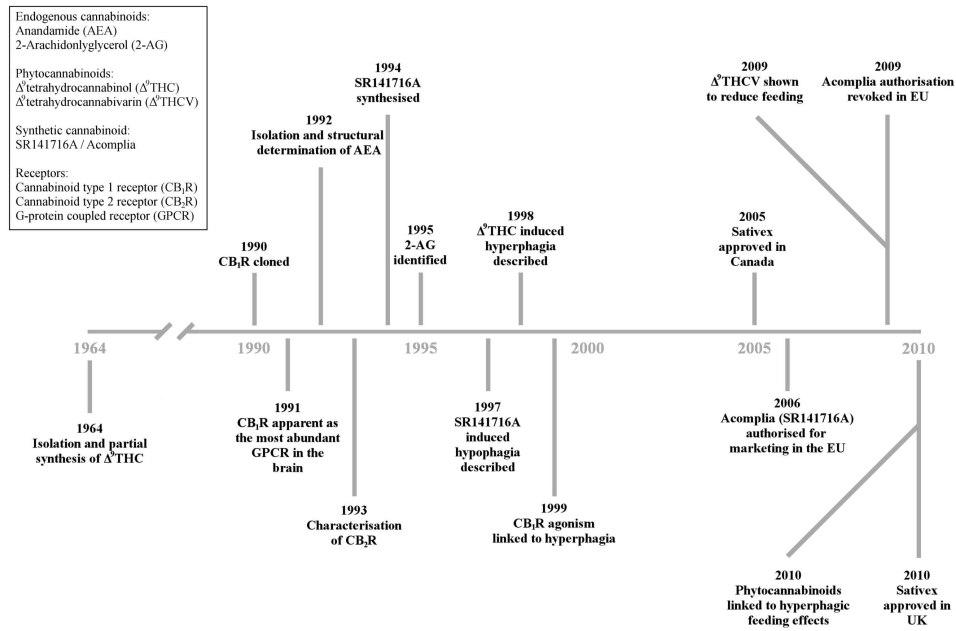


Fig 1

Figure 1; the development of cannabinoid pharmacology 1964 – 2010.  
117x78mm (600 x 600 DPI)

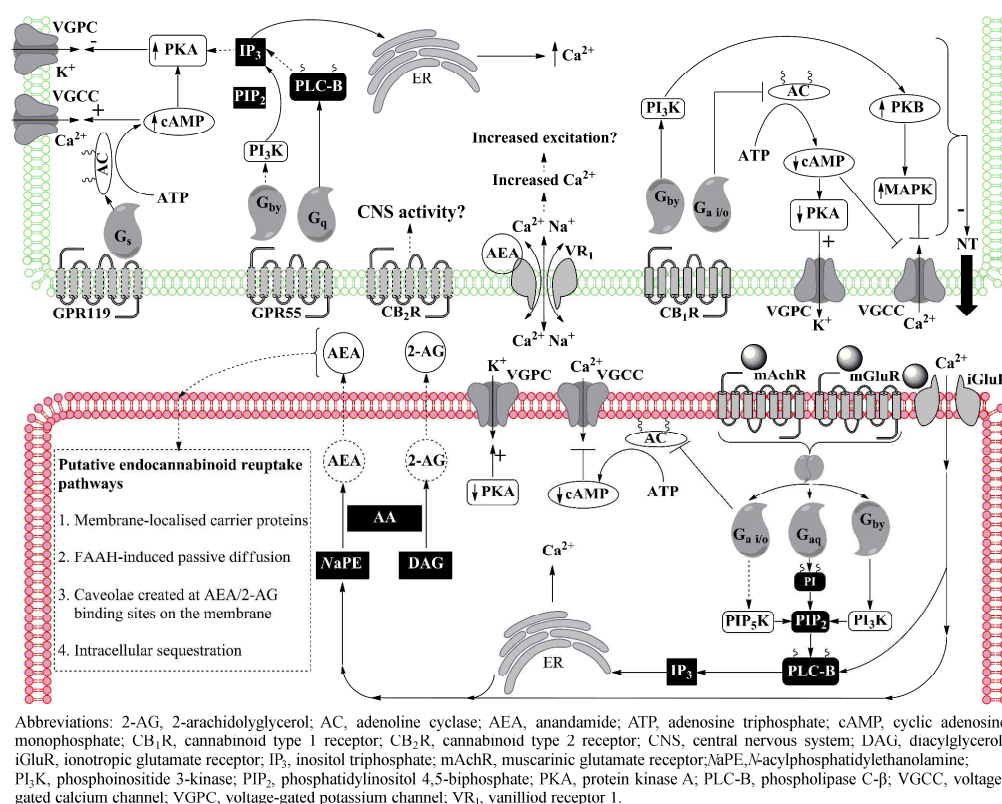
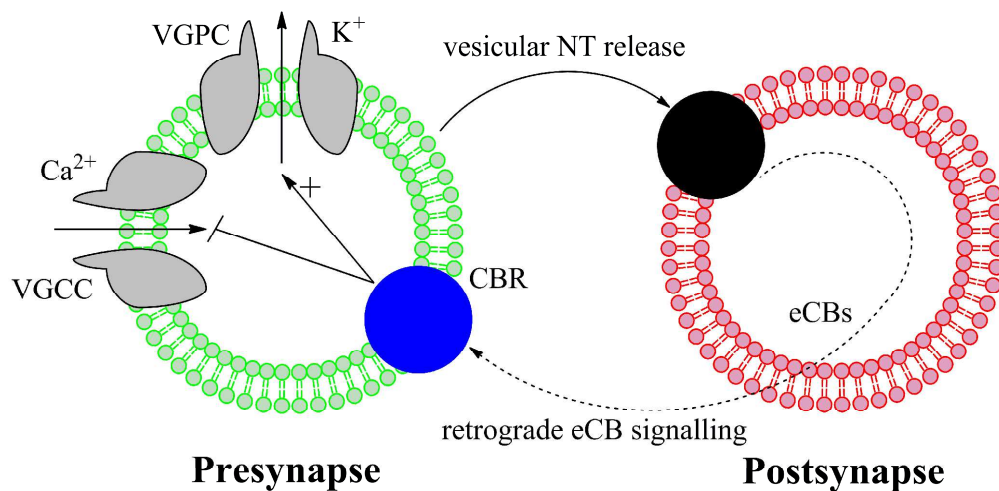


Fig 2

Figure 2; eCB signalling pathways. Upon invasion of an action potential into the synaptic region of the presynapse, the release of neurotransmitter (in this case glutamate or acetylcholine) and the binding of neurotransmitter to postsynaptic receptor sites: the eCBs AEA and 2-AG are produced on demand from arachidonic acid by NaPE and DAG respectively and released into the synaptic cleft from the postsynapse (red). Upon neurotransmitter binding to both metabotropic Glutamate and Acetylcholine receptors G subunits disassociate and stimulate an increase in PLC-β levels. This leads to an upregulation of intracellular IP<sub>3</sub> which gates calcium channels on the endoplasmic reticulum thus increasing intracellular Ca<sup>2+</sup>. Furthermore, the release of G<sub>ai/o</sub> subunits upregulates the production of cAMP and therefore allows further Ca<sup>2+</sup> entry into the cell via VGCCs. Equally upon activation of iGluR channels calcium is allowed into the cell. Once released into the extracellular space the endocannabinoids bind to CB<sub>1</sub>R sites on the presynapse (green) causing the presynaptic cell to move closer to its resting membrane potential principally by closing VGCCs and opening VGPCs thus reducing the available intracellular Ca<sup>2+</sup> required to facilitate further neurotransmitter release.

175x148mm (600 x 600 DPI)



Abbreviations: CBR, cannabinoid receptor; eCB, endocannabinoid; NT, neurotransmitter; VGCC, voltage-gated calcium channel; VGPC, voltage-gated potassium channel

**Fig 3**

Figure 3; simplified diagram illustrating retrograde signalling by eCBs in the CNS. Following vesicular neurotransmitter release from the presynaptic cell (green), neurotransmitters bind to receptor proteins (black) on the postsynaptic cell (red) stimulating eCB production and release from the postsynaptic cell. eCBs then diffuse back across the synaptic cleft and bind to CBRs (blue) on the presynaptic cell. This predominately leads to a suppression of  $[Ca^{2+}]$  influx but also an increase in  $[K^{+}]$  efflux, thus hyperpolarising the presynaptic cell and inhibiting further neurotransmitter release.

175x102mm (600 x 600 DPI)