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REVIEW

The dynamic nature of type 1 cannabinoid receptor (CB_1) gene transcription

RB Laprairie, MEM Kelly and EM Denovan-Wright

Department of Pharmacology, Dalhousie University, Halifax, NS, Canada

Correspondence
EM Denovan-Wright, Department of Pharmacology, Dalhousie University, 6E Sir Charles Tupper Medical Bldg., 5850 College St., Halifax, NS, Canada B3H 4R2.
E-mail: emdenova@dal.ca

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The type 1 cannabinoid receptor (CB_1) is an integral component of the endocannabinoid system that modulates several functions in the CNS and periphery. The majority of our knowledge of the endocannabinoid system involves ligand–receptor binding, mechanisms of signal transduction, and protein–protein interactions. In contrast, comparatively little is known about regulation of CB_1 gene expression. The levels and anatomical distribution of CB_1 mRNA and protein are developmental stage-specific and are dysregulated in several pathological conditions. Moreover, exposure to a variety of drugs, including cannabinoids themselves, alters CB_1 gene expression and mRNA levels. As such, alterations in CB_1 gene expression are likely to affect the optimal response to cannabinoid-based therapies, which are being developed to treat a growing number of conditions. Here, we will examine the regulation of CB_1 mRNA levels and the therapeutic potential inherent in manipulating expression of this gene.

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Abbreviations
2-AG, 2-arachidonyl glycerol; AEA, anandamide; AP-1, activator protein 1; CB_1, type 1 cannabinoid receptor; CB_2, type 2 cannabinoid receptor; *CNR1*, type 1 cannabinoid receptor gene; D_2Sh, type 2 dopamine receptor – short variant; DRG, dorsal root ganglion; eCBs, endocannabinoids; ECS, endocannabinoid system; mAEA, meth-anadamide; NFAT, nuclear factor of activated T cells; PPE, preproenkephalin; RAR, retinoic acid receptor; REST, repressive element 1 silencing transcription factor; STAT, signal transducers and activators of transcription; TH, tyrosine hydroxylase; THC, Δ^9-tetrahydrocannabinol; UTR, untranslated region

Introduction

In the past decade, evidence has accumulated indicating that the endocannabinoid system (ECS) plays a critical role in the regulation of numerous biological processes including embryonic development, metabolism and neurotransmission (Mechoulam and Hanu, 2001; Howlett *et al.*, 2002; Pertwee *et al.*, 2010). The ECS consists of endogenously synthesized endocannabinoids [eCBs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG)], their receptors (the type 1 and type 2 cannabinoid receptors) and their anabolic and catabolic enzymes (Figure 1; Matsuda *et al.*, 1990; Munro *et al.*, 1993; Di Marzo *et al.*, 1994; Cravatt *et al.*, 1996; Martin *et al.*, 1999). In addition to eCBs, phytocannabinoids and synthetic cannabinoids act as cannabinoid receptor ligands. The type 1 cannabinoid receptor (CB_1) mediates cannabinoid-dependent signal transduction in the CNS and periphery (Howlett *et al.*, 2002; Basavarajappa *et al.*, 2009), while the type 2 cannabinoid receptor (CB_2) is localized to, and highly inducible in, peripheral haemopoietic cells and glial cells in specific areas of the CNS during the inflammatory response (Basavarajappa *et al.*, 2009; Atwood *et al.*, 2012). To date, the majority of CB_1 research has focused on ligand–receptor binding, signal transduction and protein–protein interactions. In contrast, knowledge of CB_1 gene regulation is limited. CB_1 receptor abundance and the function of the ECS may change in response to altered CB_1 gene expression in different developmental or disease conditions or in response to drug exposure. While other reviews have explored the general factors that regulate CB_1 and CB_2 levels during disease pathogenesis (Miller and Devi, 2011), this review will focus on regulation of CB_1 mRNA expression during development and in several

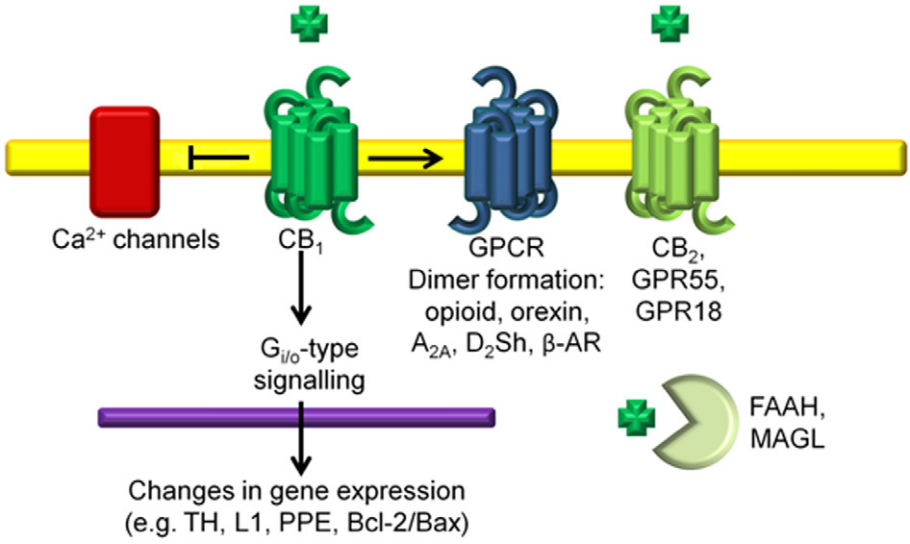


Figure 1

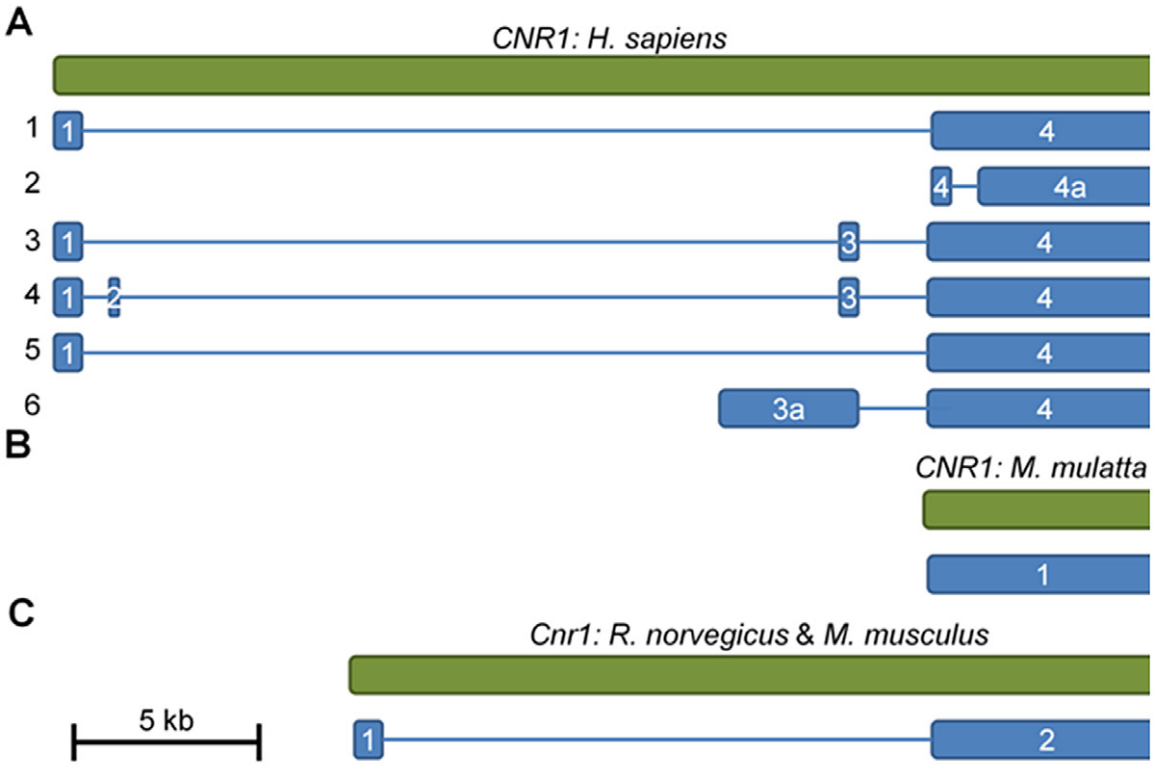
The ECS and CB_1. The GPCR CB_1 is activated by endocannabinoid ligands such as AEA and exogenous ligands such as THC. In the CNS, activation of CB_1, which is typically coupled to $G_{i/o}$-proteins, inhibits AC and causes changes in gene expression (e.g. TH, L1 adhesion molecule, PPE and the Bcl-2/Bax regulators of apoptosis). Activation of CB_1 also causes inhibition of L-, N- and P/Q-type Ca^{2+} channels. CB_1 can couple to several other GPCRs, which influences receptor trafficking and ligand affinity. Other components of the ECS include CB_2, the putative cannabinoid receptors, GPR55 and GPR18, and the catabolic enzymes of cannabinoids fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL).

pathological conditions where specific pharmacologically tractable regulators of CB_1 transcription have been elucidated. The data reviewed here demonstrate that CB_1 mRNA transcription is malleable and may be exploited for therapeutic benefit.

Architecture, splice variants and isoforms of the CNR1 gene

The human CB_1 gene (*CNR1*) spans 26.1 kb of chromosome 6 (6q14–q15). *CNR1* contains four exons (Figure 2A), and the protein coding region of CB_1 is contained entirely within exon 4 (Zhang *et al.*, 2004). Outside of the coding region, alternative splicing of CB_1 mRNA produces six 5′ untranslated region (5′-UTR) splice variants. The precise transcription start sites within exon 1 included in 5′ UTR variants 1, 3, 4 and 5 have not been defined; although it appears that multiple transcription start sites may exist within the first 60 bp of exon 1 (Shire *et al.*, 1996). Transcription of variant 6 begins within intron 2, and thus the 5′ most exon of variant 6 has been redefined as exon 3a. Transcription of variant 2 begins at the 5′ end of exon 4. Transcript variants 1, 3, 4, 5 and 6 encode full-length CB_1 that is 472 amino acids in length encoded without interruption by a single region in exon 4. Exon 4, however, can be differentially spliced to remove 102 nts separating the 5′ end of exon 4 and a new exon identified as exon 4a. This splicing occurs in transcript variant 2 that encodes the truncated, 439 amino acid and CB_{1b} protein (Figure 2D). In CB_{1a}, different intra-exon 4 splice sites result in the loss of 167 nts. Furthermore, two translation start sites are present at the 5′ end of exon 4. Translation from the first produces CB_1 and CB_{1b}. Translation from the second is thought to produce the amino-terminal variant CB_{1a}, also known as CB_{1short} (Ryberg *et al.*, 2005). The macaque monkey (*Macaca mulatta*) CB_1 gene is located on chromosome 4. Although the number of exons is not known, the protein coding region of the gene is contained entirely within one contiguous coding region (Figure 2B) (National Centre for Biotechnology Information (NCBI), 2011). The mouse and rat CB_1 genes are located on chromosomes 4 and 5, respectively; both genes contain 2 exons with the protein coding regions existing entirely within the second exon in both species (Figure 2C; Miller and Devi, 2011).

To date, CB_{1a} and CB_{1b} isoforms have only been identified in humans and higher primates (Ryberg *et al.*, 2005; Gustafsson *et al.*, 2008; Palermo *et al.*, 2009), and some evidence suggests CB_{1a} may be expressed in the rat (Shire *et al.*, 1996). Several authors have demonstrated that CB_1, CB_{1a} and CB_{1b} receptors signal *via* $G_{i/o}$-type G-proteins and that the amino-terminal variants CB_{1a} and CB_{1b} have reduced affinity for cannabinoid agonists and antagonists (Rinaldi-Carmona *et al.*, 1996; Ryberg *et al.*, 2005). However, Xiao *et al.* (2008) did not observe differences in the ligand affinity or localization of the three CB_1 protein isoforms. Moreover, the signalling properties of CB_1 receptor variants may be altered depending on the model system they are being studied in (Straiker *et al.*, 2012), which complicates our ability to understand receptor differences. In the majority of reports, steady-state CB_1 mRNA levels were measured *via* amplification of the 3′ end of the CB_1 coding region outside of the 5′ region in exon 4 involved in differential splicing. The cell-specific relative abundance of CB_1 versus CB_{1a} or CB_{1b} is, therefore, poorly characterized (Gustafsson *et al.*, 2008). Early research suggested that CB_{1a} mRNA accounted for approximately 20% of the CB_1 transcript population (Shire *et al.*, 1996), yet more recent evidence suggests that less than 5% of the total population of CB_1 transcripts obtained from human fetal and adult

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CB1     ACTGAGGTTA TGAAGTCGAT CCTAGATGGC CTTGCAGATA CCACCTTCCG      350
CB1b    ACTGAGGTTA TGAAGTCGAT CCTAGATGGC CTTGCAGATA CCACCTTCCG
CB1a    ACTGAGGTTA TGAAGTCGAT CCTAGATGGC CTTGCAGATA CCACCTTCCG

CB1     CACCATCACC ACTGACCTCC TGTACGTGGG CTCAAATGAC ATTCAGTACG      400
CB1b    CACCATCACC ACTGACCT-- ---------- ---------- ----------
CB1a    CACCATCACC ACTGACCTCC TGTACGTGGG CTCAAATGAC ATTCAGTACG
CB1     AAGACATCAA AGGTGACATG GCATCCAAAT TAGGGTACTT CCCACAGAAA      450
CB1b    ---------- ---------- ---------- ---------- ----------
CB1a    AAGACATCAA AG-------- ---------- ---------- ----------
CB1     TTCCCTTTAA CTTCCTTTAG GGGAAGTCCC TTCCAAGAGA AGATGACTGC      500
CB1b    ---------- ---------- GGGAAGTCCC TTCCAAGAGA AGATGACTGC
CB1a    ---------- ---------- ---------- ---------- ----------
CB1     GGGAGACAAC CCCCAGCTAG TCCCAGCAGA CCAGGTGAAC ATTACAGAAT      550
CB1b    GGGAGACAAC CCCCAGCTAG TCCCAGCAGA CCAGGTGAAC ATTACAGAAT
CB1a    ---------- ---------- ---------- ---------- ----------
CB1     TTTACAACAA GTCTCTCTCG TCCTTCAAGG AGAATGAGGA GAACATCCAG      600
CB1b    TTTACAACAA GTCTCTCTCG TCCTTCAAGG AGAATGAGGA GAACATCCAG
CB1a    ---------- ---------- ---------G AGAATGAGGA GAACATCCAG
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Figure 2

(A) The human CB_1 gene, *CNR1*, spans 26.1 kb on chromosome 6. Six splice variants of the 5′ UTR have been identified by sequencing cDNA ESTs. Splice variants are illustrated in blue and numbered on the left. Exons are numbered within the blue boxes. Each splice variant is aligned with respect to its nucleotide sequence in the CNR1 gene (at top). The scale bar represents 5 kb of nucleotides. (B) The non-human primate (*M. mulatta*) CB_1 gene is poorly characterized, yet it is known that the entire protein coding region is contained within 1 exon (NCBI, 2011). The protein isoforms CB1a and CB1b have been described in non-human primates (Gustafsson *et al.*, 2008). (C) The rat and mouse CB_1 genes span approximately 20 kb on chromosome 4 and contain two exons. The second exon contains the entire protein coding region. (D) Three CNR1 coding region variants for protein isoforms of CB_1 have been described in humans and non-human primates: the 472 amino acid, intron-less CB_1, the 439 amino acid CB_{1b} and the 411 amino acid CB_{1a}. In this figure, position 1 is 300 bp downstream of the 5′ end of exon 4 in *CNR1*. Translation of CB_1 and CB_{1b} begins at the same ATG codon located 309 bp downstream of the first nucleotide in exon 4. Translation of CB_{1a} begins 326 bp downstream of the first nucleotide in exon 4. Fifty-nine basepairs downstream of the CB_{1b} translation start site, CB_{1b} contains a 102 bp intron that is spliced from the pre-mRNA at an atypical intron–exon splice junction (s, CT/cc and ag/GG). Eighty-eight basepairs downstream of the CB_{1a} translation start site, CB_{1a} contains a 167 bp intron that is spliced from the pre-mRNA at a typical 5′ intron-exon boundary (AG/gt) and an atypical ag/GA 3′ splice junction. Downstream of the CB_{1a} intron–exon junction the coding sequences of the three CB_1 isoforms are identical.

brain tissue are CB_{1a} or CB_{1b} (Xiao *et al.*, 2008). Studies to define the relative abundance and distribution of the 5′ UTR variants 1–6 have measured the levels of expressed sequence tags. The 5′ UTR transcript variants 1 (5732 bp), 3 (5863 bp), 4 (5901 bp) and 5 (5776 bp) are most abundant in the brain, lymphocytes, testes and liver, relative to other tissues (NCBI, 2011). Transcript variant 2 (5387 bp mRNA) is expressed at highest levels in the brain and testes (NCBI, 2011). Transcript variant 6 (8974 bp mRNA) has only been isolated from brain tissue (NCBI, 2011). Regulation of the transcription of 5′ UTR variants and how 5′ UTR differences relate to CB_1 mRNA stability and translation to different CB_1 isoforms has not been characterized. The abundance and activity of the different amino-terminal CB_1 isoforms may be regulated by different physiological conditions, isoform-specific ligand–receptor affinity and the CB_1 isoform complement expressed in a given cell type (Ryberg *et al.*, 2005).

The 'when and where' of CB_1 mRNA expression

In mammals, steady-state levels of CB_1 mRNA vary in different tissues and during different developmental periods. In humans, CB_1 is detected in neocortical progenitor cells and in the subventricular zone during the early cortical plate stages of development (9 to 17 weeks gestation; Zurolo *et al.*, 2010). CB_1 mRNA is also abundant at 19 weeks gestation in humans in white matter, which is nearly devoid of CB_1 expression in adulthood. In the human visual cortex, CB_1 mRNA levels rise during early development and plateau approximately 1 year after birth (Romero *et al.*, 1997; Pinto *et al.*, 2010). Following the steady-state CB_1 mRNA plateau achieved 1 year after birth, CB_1 mRNA levels increase further in the visual cortex to reach a new steady-state level during adolescence, after which CB_1 mRNA abundance declines throughout adulthood (Pinto *et al.*, 2010). In the non-human primate, *M. mulatta*, high levels of CB_1 mRNA have been observed in the prefrontal cortex during neonatal development (Eggan *et al.*, 2010); CB_1 mRNA abundance increases in the prefrontal cortex until reaching a steady-state at postnatal day 5 (Eggan *et al.*, 2010). In the same manner as is observed in the human visual cortex, a higher steady-state level of CB_1 mRNA is observed in the *M. mulatta* prefrontal cortex during adolescence, and steady-state CB_1 mRNA levels decline in the prefrontal cortex following adolescence (Eggan *et al.*, 2010). In mice, CB_1 mRNA is detectable during embryonic development as early as four-cell and eight-cell/morula stages (Paria *et al.*, 1995), and can still be detected at embryonic day 12 in glutamatergic neurons of the cerebral cortex and hippocampus (Vitalis *et al.*, 2008). CB_1 mRNA is abundant in the adult mouse thalamus, amygdala, dorso-lateral prefrontal cortex, hypothalamus and pituitary (NCBI, 2011). Furthermore, CB_1 expression is enriched in the striatum, relative to other brain regions, within the adult mouse CNS (Fernandez-Ruiz *et al.*, 2004; McCaw *et al.*, 2004). It is within the striatum that a high steady-state level of CB_1 expression are dysregulated in Parkinson's and Huntington's diseases (Zeng *et al.*, 1999; Denovan-Wright and Robertson, 2000). The temporal and anatomical distribution of CB_1 expression during early development is similar in mice and rats (NCBI, 2011). Six to 8-week-old rats, which are sexually mature, have lower levels of CB_1 mRNA in the limbic/associative brain areas compared with adolescents (Heng *et al.*, 2011). Following periods of peak neurodevelopment associated with high CB_1 levels, CB_1 mRNA abundance declines in these brain regions (Heng *et al.*, 2011). Taken together, these data demonstrate that, in mammals, CB_1 mRNA levels peak during adolescence within the prefrontal cortex, limbic/associative areas and visual cortex and subsequently with age. Early development and adolescence represent critical developmental windows where the regulation of CB_1 expression changes in order for higher levels of expression to be achieved. It is likely that developmental stage-specific transcription factors or modifiers regulate the different steady states of CB_1 expression. A representative illustration of the temporal–spatial expression of CB_1 mRNA, in the CNS, based on data obtained from mouse, rat, monkey and human is presented in Figure 3.

High levels of CB_1 expression are related to the establishment of neuronal circuitry. During critical development periods, such as late gestation and early postnatal life, areas associated with neurogenesis and synapse formation, such as the subventricular zone and white matter, are transiently enriched for CB_1 and subsequently depleted of CB_1 expression in adulthood (Romero *et al.*, 1997). The activity or abundance of the factors that enabled high steady-state CB_1 levels during development and adolescence may decrease in concentration or activity as part of the aging process (Eggan *et al.*, 2010; Heng *et al.*, 2011). Greater expression and subsequent activation of CB_1 receptors facilitates higher expression of several genes required for brain development, including tyrosine hydroxylase (TH), preproenkephalin (PPE), the neural adhesion molecule L1 and Bcl-2/Bax genes involved in apoptotic regulation of development (reviewed in Fernandez-Ruiz *et al.*, 2004). Mice lacking CB_1 exhibit transcriptional dysregulation of PPE and substance P (Steiner *et al.*, 1999), altered dendritic morphology and lower synapse density in the prefrontal cortex (Fitzgerald *et al.*, 2012), impaired locomotor activity (Zimmer *et al.*, 1999) and increased anxiety (Hill *et al.*, 2011) compared to wild-type littermates. Thus, the developmental stage-specific expression of CB_1 facilitates the proper establishment of neuronal circuitry and the consequent normalization of behaviour (Fernandez-Ruiz *et al.*, 2004).

Expression of CB_1 is cell-specific within the CNS. Striatal medium spiny projection neurons and interneurons are enriched for CB_1 mRNA expression, relative to other cell populations, within the basal ganglia (Marsicano and Lutz, 1999; Fernandez-Ruiz *et al.*, 2004). Consistent with CNS anatomical distribution, CB_1 appears to be involved with aspects of motor coordination, mechanisms of reward and motivation, emotion and central endocrine regulation during adulthood (Fernandez-Ruiz *et al.*, 2004). CB_1 is co-localized, co-expressed and can dimerize with the pre-synaptic type 2 dopamine short (D_2Sh), μ-and δ-opioid, adenosine A_{2a}, and orexin, receptors (Navarro *et al.*, 2008; Pacheco *et al.*, 2009; Uriguen *et al.*, 2009; Bortolato *et al.*, 2010; Rozenfeld *et al.*, 2012). Thus, in addition to alterations in CB_1-mediated signalling, changing CB_1 mRNA and protein abundance could cause dysregulated signalling *via* other GPCRs, such as D_2Sh, opioid and orexin receptors; by affecting dimer formation,

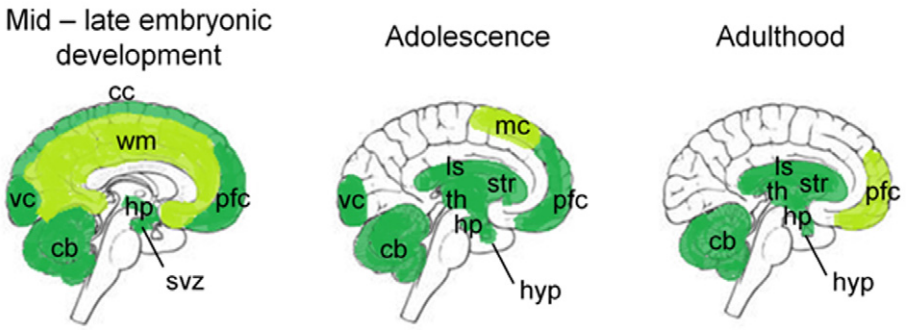

Figure 3

CB_1 mRNA abundance and distribution shift throughout development in the CNS. This simplified schematic illustrates the areas of the brain where CB_1 mRNA levels are moderate and high relative to other regions of the CNS during mid – late embryonic development, adolescence and adulthood. The images were created using data obtained in humans, non-human primates, mice and rats. cb, cerebellum; cc, cerebral cortex; hp, hippocampus; hyp, hypothalamus; ls, limbic system; mc, motor cortex; pfc, prefrontal cortex; svz, sub-ventricular zone; th, thalamus; str, striatum; vc, visual cortex; wm, white matter (Denovan-Wright and Robertson, 2000; Vitalis *et al.*, 2008; Eggan *et al.*, 2010; Gensat, 2010; Zurolo *et al.*, 2010; Heng *et al.*, 2011; NCBI, 2011).

receptor trafficking and localization, and signal transduction (Hudson *et al.*, 2010; reviewed in Smith *et al.*, 2010).

In non-neuronal tissue, CB_1 expression is associated with immune and endocrine homeostasis as well as reproductive system development and maturation. CB_1 mRNA is abundant in helper (CD4) and cytotoxic (CD8) T cells, hepatocytes, beta-islet cells and adipose tissue, during adulthood where these receptors regulate inflammatory and metabolic processes through autocrine and paracrine signalling mechanisms (Borner *et al.*, 2008; Mukhopadhyay *et al.*, 2010). CB_1 mRNA levels increase in primary cultured rat leydig cells (testosterone-producing testis cells) from postnatal day 14 onwards, spermatids from postnatal days 31 through 61 and sertoli cells from postnatal day 41 onwards (Cacciola *et al.*, 2008). Cells within the testes exhibit a biphasic pattern of CB_1 expression, in which CB_1 levels are elevated at 1 week post partum, decline to a minimum by 2 weeks post partum and rise again to highest levels as mice reach sexual maturity at 4 weeks post partum (Cacciola *et al.*, 2008). Certain fish species, for example the gilthead seabream (*Sparus aurata*) and the puffer fish (*Fugu rubripes*), are capable of undergoing sexual reversal, which is the process of shifting the reproductive organs from being functionally male to functionally female, or vice versa. Sexual reversal, therefore, represents a period of altered gene expression leading to changes in cellular phenotype within teleost reproductive organs. CB_1 mRNA expression increases in the testes of these fish during the process of sexual reversal (Cottone *et al.*, 2008). Increased CB_1 expression could contribute to sexual reversal by altering the complement of genes expressed and thus the phenotype of the testes (Cottone *et al.*, 2008). In the developing CNS, changes in CB_1 expression facilitate downstream changes in the expression of many other genes (Fernandez-Ruiz *et al.*, 2004). Therefore, up-regulation of CB_1 expression may also sufficiently alter the gene expression profile in reproductive tissue to produce changes in phenotype and facilitate developmental processes. In reproductive systems, as in the CNS, CB_1 expression appears to be coordinated in order to facilitate development and maturation during early development and adolescence.

Understanding changes in CB_1 mRNA expression that occur in diverse pathological conditions

CB_1 mRNA expression is induced by inflammation in non-neuronal tissue

Although CB_2 receptors are considered the major eCB receptor in the periphery, particularly as regulators of inflammation (Rajesh *et al.*, 2008; reviewed in Atwood and Mackie, 2010), CB_1 receptors also contribute to regulation of the inflammatory response. Pro-inflammatory molecules induce CB_1 and CB_2 mRNA expression in cells that mediate the inflammatory responses (Gutierrez *et al.*, 2006; Borner *et al.*, 2008). The involvement of CB_1 in the inflammatory response was first examined in rat dorsal root ganglia (DRG), where complete Freund's adjuvant increased CB_1 mRNA abundance in glial cells of the DRG 4 h post-treatment, relative to untreated controls (Amaya *et al.*, 2006). Freund's adjuvant produces an inflammatory response and activates such transcription factors as nuclear factor of activated T cells (NFAT) and NF-κB in glial cells (Amaya *et al.*, 2006; Borner *et al.*, 2007a). Activation of NFAT and NF-κB is dependent on the endogenous pro-inflammatory cytokines CD3/28 and IL-4 (Borner *et al.*, 2007a). CD3/28 and IL-4 induce CB_1 mRNA expression in human peripheral T cells and immortalized Jurkat cells (Borner *et al.*, 2007a; 2008). Borner *et al.* (2007a) examined CD3/28- or IL-4-mediated induction of CB_1 *via* a promoter–reporter plasmid in which chloramphenicol acetyl transferase activity was driven by a 3 kb fragment of the *CNR1* promoter. Short, double-stranded, decoy oligonucleotides containing the consensus sequences normally bound by NFAT or NF-κB were used to titrate NFAT or NF-κB enhancers of transcription away from their endogenous promoters (Borner *et al.*, 2007a). NFAT and NF-κB facilitate a CD3/28- or IL-4-dependent increase in CB_1 expression (Borner *et al.*, 2007a). Using the same techniques, it was found that activator protein 1 (AP-1) and the signal transducers and activators of transcription 5 and 6 (STAT5 and STAT6) are also recruited to the *CNR1* promoter to mediate increased mRNA expression

in Jurkat cells (Borner *et al.*, 2007a,b; 2008). Together, these data demonstrate that pro-inflammatory cues mediate an increase in CB_1 mRNA levels from an initial steady-state to a second, higher state through common mechanisms.

CB_1 mRNA expression is changed in various cancers

The ECS regulates cell fate and division during oncogenesis (Malfitano *et al.*, 2012). For example, in breast cancer, CB_2 agonism inhibits cell cycle progression *via* down-regulation of Cdc2 (Caffarel *et al.*, 2006), whereas in fibrosarcoma cells, CB_1 antagonism up-regulates the cell cycle inhibitor $p21^{WAF1}$ and down-regulates cyclins E and D (Malfitano *et al.*, 2012). Up- and down-regulation of CB_1 levels influences cell growth, just as cannabinoid treatment affects cell growth. Treatment of transformed cells with anti-neoplastic agents has been shown to both increase and decrease CB_1 mRNA expression depending on drug and cell line (Larrinaga *et al.*, 2010b; Proto *et al.*, 2011). CB_1 mRNA levels are reduced in the DLD-1 and SW620 cell culture models of colorectal cancer, relative to primary colorectal cell cultures (Proto *et al.*, 2011). Furthermore, the CB_1 promoter is highly methylated in human colorectal carcinoma cells, compared with healthy tissue (Wang *et al.*, 2008). Demethylation of the CB_1 promoter results in elevated CB_1 expression and reduced cell division (Wang *et al.*, 2008), suggesting that elevated CB_1 expression consequently alters the capacity for cell division in colorectal carcinoma cells. This may be the result of CB_1-mediated up-regulation of $p21^{WAF1}$ or down-regulation of Cdc2 and cyclins, or both (Caffarel *et al.*, 2006; Malfitano *et al.*, 2012). Treatment of DLD-1 and SW620 cells with 17β-estradiol or the synthetic cannabinoid meth-anandamide (mAEA) increases CB_1 mRNA levels (Proto *et al.*, 2011). Following treatment with 17β-estradiol or mAEA, the rate of division of these colorectal cancer cells is significantly reduced (Proto *et al.*, 2011). CB_1 mRNA levels are lower in primary adrenocarcinoma tumour cells than in healthy tissue (Larrinaga *et al.*, 2010b). In healthy tissue, CB_1 activation decreases cell division and proliferation (Larrinaga *et al.*, 2010a,b). The chemotherapeutic agent gemcitabine arrests adrenocarcinoma tumour growth (Larrinaga *et al.*, 2010a). Gemcitabine has also been reported to induce CB_1 mRNA expression *via* NF-κB, as demonstrated by chromatin immunoprecipitation of the *CNR1* promoter (Larrinaga *et al.*, 2010a). These data demonstrate that CB_1 mRNA expression can be manipulated pharmacologically, and that the level of CB_1 expression and activity negatively correlates with cell division. Consequently, pharmacological manipulation of CB_1 expression may represent a therapeutic option for the treatment of adrenocarcinomas (Larrinaga *et al.*, 2010a,b).

In contrast to observations of decreased CB_1 mRNA levels in adrenocarcinoma, CB_1 mRNA abundance is increased in biopsied human tissue taken from patients with prostate cancer or benign prostate hyperplasia relative to healthy tissue (reviewed in Gustafsson *et al.*, 2008). Similarly, CB_1 mRNA levels are increased in non-Hodgkin lymphoma tissues, relative to healthy tissues (Gustafsson *et al.*, 2008). Despite the increase in CB_1 mRNA levels, Gustafsson *et al.* (2008) found that the relative proportions of CB_{1a} and CB_{1b} mRNA levels were lower in biopsied lymphoma tissue compared with normal lymphocytes (Gustafsson *et al.*, 2008). If CB_1, CB_{1a} and CB_{1b} are not equally abundant, then this suggests that expression of each isoform is differentially regulated (Gustafsson *et al.*, 2008), which is likely the result of differences in mRNA processing and stability (Shire *et al.*, 1996; Ryberg *et al.*, 2005). Conversely, the hypothesis that each CB_1 isoform is regulated by different promoter elements and upstream differences in cell signalling (Gustafsson *et al.*, 2008) is unlikely because all three isoforms share a common, highly active promoter (Borner *et al.*, 2007a,b; 2008; reviewed in Miller and Devi, 2011). CB_1 levels are also elevated in alveolar rhabdosarcoma, which is caused by expression of a chimeric PAX3/7-FOX01 transcription factor (Marshall *et al.*, 2011). Up-regulation of CB_1 in alveolar rhabdosarcoma does not affect the rate or capacity of cells to divide but rather increases the metastatic ability of the cells (Marshall *et al.*, 2011). Consequently, elevated CB_1 expression in certain cancers, such as prostate cancer, lymphoma or rhabdosarcoma may impact metastasis, but not cell division.

CB_1 mRNA abundance fluctuates in obesity and diabetes

The hormone 17β-estradiol and mediators of the inflammatory response, such as CD3/28, increase CB_1 mRNA levels. It is not surprising, therefore, that obesity and diabetes – two pathologies associated with hormonal dysregulation and inflammation – are also associated with changes in CB_1 mRNA levels (Howlett *et al.*, 2002; Kempf *et al.*, 2007). Whether obesity correlates with higher or lower CB_1 mRNA levels remains controversial. CB_1 mRNA abundance has been measured in primary cultured adipocytes of lean and obese individuals. In one study, CB_1 mRNA was shown to be less abundant in primary cultured adipocytes derived from white adipose tissue of obese children compared with that from lean children (Karvela *et al.*, 2010). Similarly, CB_1 levels were lower in adipocytes derived from the visceral adipose tissue of obese adults compared with healthy adults (Kempf *et al.*, 2007). In another study, CB_1 mRNA was more abundant in adipocytes derived from the visceral adipose tissue of obese adults, relative to non-obese individuals (Sarzani *et al.*, 2009). Different cell culture conditions may account for the discrepancies in CB_1 mRNA levels reported by these groups. Karvela *et al.* (2010) and Kempf *et al.* (2007) cultured adipocytes in the presence of adiponectin, while Sarzani *et al.* (2009) measured CB_1 mRNA abundance in tissue samples without culturing the adipocytes. Therefore, the primary culturing and treatment of adipocytes may alter CB_1 expression. However, the functional consequence of altered CB_1 levels in adipocytes may be altered by cell survival and proliferation because CB_1 activation is often associated with pro-survival signalling (Kempf *et al.*, 2007; Sarzani *et al.*, 2009; Karvela *et al.*, 2010). In obese individuals, enhanced survival of adipocytes may exacerbate their condition (Sarzani *et al.*, 2009).

Diabetes is associated with a decreased production of, or response to, insulin. Insulin can penetrate the blood–brain barrier, act on insulin receptors and stimulate glucose uptake in the central nervous system (Bingham *et al.*, 2002). Streptozotocin-treated rats lack insulin-producing beta-islet cells and are used to model diabetes (Zhang *et al.*, 2007). CB_1 levels are increased in the striatum and hypothalamus of streptozotocin-treated rats compared to untreated controls (Diaz-Asensio *et al.*, 2008). In the rat pancreas, β-, α, and

δ-islet cells express CB_1 mRNA (Zhang *et al.*, 2007). Treatment of rats with glucose (20–50 mM) is associated with an increase in plasma insulin concentration and a decrease in CB_1 mRNA levels in the pancreas, white adipose tissue and DRG, relative to untreated rats (Zhang *et al.*, 2007). Thus, increased glucose, leading to increased plasma insulin is associated with CB_1 down-regulation. Therefore, insulin appears to inhibit CB_1 expression in the CNS. Conversely, up- or down-regulation of CB_1 may lead to altered insulin receptor expression (Zhang *et al.*, 2007), glucose uptake (Diaz-Asensio *et al.*, 2008) or both.

Susceptibility to schizophrenia is associated with changes in CB_1 mRNA expression

During adolescence, CB_1 mRNA and eCB levels peak in the dorso-lateral prefrontal cortex in mammals (Eggan *et al.*, 2010). Mice exposed to Δ^9-tetrahydrocannabinol (THC) during adolescence (postnatal day 40) are more likely to develop schizophrenia modelling behaviours, namely pre-attentional sensorimotor and executive function deficits, than mice exposed to THC later in adulthood (Heng *et al.*, 2011). Eggan *et al.* (2010) also found that CB_1 mRNA abundance peaks in the dorso-lateral prefrontal cortex of macaque monkeys 2 months after puberty. These data suggest that adolescence may be a period of hypersensitivity to cannabinoids (Caspi *et al.*, 2005). Polymorphisms that relate *cannabis* use to the *CNR1* gene have been described. One study reported that a TAG allele in the 5′ region of *CNR1* exon 3 and a polymorphic AAT repeat in the 3′ region of exon 4 were more prevalent among European, African American and Japanese substance abusers than individuals that did not have a history of substance abuse from the same region (Zhang *et al.*, 2004). Intriguingly, the presence of the 'TAG' allele was associated with less CB_1 mRNA compared to other alleles (Zhang *et al.*, 2004). Subsequent analyses support that the length of the polymorphic AAT repeat region may be correlated with substance abuse (Benyamina *et al.*, 2011). Therefore, a strong connection exists between the allelic variability of *CNR1* and adolescent exposure *cannabis* use.

CB_1 mRNA abundance differs in individuals with schizophrenia compared with healthy controls. CB_1 mRNA levels were higher in postmortem tissue from the dorso-lateral prefrontal cortex of individuals with schizophrenia compared with age-matched healthy subjects (Uriguen *et al.*, 2009). Immunohistochemical analyses of postmortem brains isolated from individuals with schizophrenia revealed that individuals treated with the atypical antipsychotics olanzapine or clozapine expressed significantly less CB_1 protein in the dorso-lateral prefrontal cortex than age-matched individuals with schizophrenia who did not receive atypical antipsychotics (Uriguen *et al.*, 2009). Olanzapine and clozapine treatment were not associated with a change in CB_1 mRNA levels (Uriguen *et al.*, 2009). Therefore, these atypical antipsychotics that are, among other activities, D_2 receptor antagonists, decrease CB_1 protein, but not mRNA, levels. The functional implication of this observation is that pharmacological manipulation of the dopaminergic system can impact the cannabinergic system and possibly vice versa (El Khoury *et al.*, 2012). Thus, therapeutics aimed at reducing cannabinergic or dopaminergic tone, such as antipsychotics, may impact both systems simultaneously. Conversely, certain compounds may increase the tone of both systems, which could have undesirable effects on cognition, behaviour and motor control if the ECS is over-activated and beneficial effects on cognition, behaviour and motor control in pathologies where CB_1 levels are reduced.

A striatal cell-specific decrease in CB_1 mRNA is observed in Parkinson's disease

CB_1 mRNA is highly expressed in the caudate and putamen, globus pallidus and substantia nigra of healthy individuals (Fernandez-Ruiz *et al.*, 2004). Hurley *et al.* (2003) examined CB_1 mRNA levels in postmortem tissue from normal controls and individuals with Parkinson's disease. CB_1 levels were reduced in the caudate nucleus, anterior dorsal putamen and external segment of the globus pallidus, relative to controls or other brain regions of Parkinson's patients (Hurley *et al.*, 2003). A key feature of Parkinson's disease is decreased dopamine levels. Parkinson's disease can be modelled by selective lesioning of the nigrostriatal pathway by 6-hydroxydopamine or administration of 6-hydroxydopamine to the medial forebrain bundle causes cell loss in the substantia nigra, which in turn depletes the striatum of dopamine (Zeng *et al.*, 1999). Consequently, CB_1 mRNA levels are reduced within the dopamine-depleted rat striatum (Zeng *et al.*, 1999). CB_1 mRNA expression can be increased in the striatum of 6-hydroxydopamine-lesioned rats by subsequent, chronic, treatment with L-DOPA, which increases dopaminergic signalling (Zeng *et al.*, 1999; Garcia-Arencibia *et al.*, 2009). Reserpine depletes catecholamines, including dopamine, in the CNS (Thrash *et al.*, 2009). Reserpine-treated rats have been used to model Parkinson's disease and depression (Silverdale *et al.*, 2001; Thrash *et al.*, 2009). Similarly to 6-hydroxydopamine-lesioned rats, CB_1 mRNA levels were reduced in the caudate and putamen of reserpine-treated rats relative to age-matched, control rats (Silverdale *et al.*, 2001). Overall, CB_1 mRNA levels appear to be regulated by dopamine in Parkinson's disease, which provides more evidence for a link between cannabinergic and dopaminergic signalling.

A cell-specific decrease in CB_1 mRNA is observed in Huntington's disease and contributes to disease pathogenesis

CB_1 mRNA levels are reduced in the caudate and putamen of human subjects with Huntington's disease and the striatum of all Huntington's disease mouse models tested to date relative to age-matched controls (Pazos *et al.*, 2008). Expression and nuclear localization of the amino-terminus of mutant huntingtin protein reduces transcription of CB_1 in striatal medium spiny projection neurons early in disease progression (Gafni and Ellerby, 2002; McCaw *et al.*, 2004). CB_1 expression is not altered in the presence of mutant huntingtin protein in the hippocampus, or prior to adulthood in mice (Denovan-Wright and Robertson, 2000; McCaw *et al.*, 2004). Also, CB_1 mRNA levels are lower in cultured neuronal cell models of Huntington's disease, which lack inter-cellular signalling, compared to cells that do not express mutant huntingtin (Blázquez *et al.*, 2011). Wild-type huntingtin binds to the repressive element 1 silencing transcription factor (REST), which can interact with repressor elements at the CB_1 pro-

moter (Blázquez *et al.*, 2011). Blázquez *et al.* (2011) utilized a CB_1 promoter–reporter and decoy oligonucleotide-based assay to demonstrate that REST inhibits transcription of CB_1 in cells expressing mutant huntingtin. Therefore, tissue-, cell- and developmental stage-specific factors that normally accommodate high-level CB_1 mRNA expression in the adult striatum are affected by the cell-autonomous overexpression of amino-terminal mutant huntingtin. In contrast, CB_1 mRNA expression does not appear to change in Alzheimer's disease, a neurodegenerative disease, like Huntington's and Parkinson's diseases, characterized by protein misfolding and aggregation (Kalifa *et al.*, 2011). While CB_1 protein levels (Kalifa *et al.*, 2011) and receptor binding (Westlake *et al.*, 1994) may be decreased, in the hippocampus, neocortex and basal ganglia during Alzheimer's disease progression (Westlake *et al.*, 1994; Lee *et al.*, 2010), mRNA abundance is unaffected in humans and mouse models.

Decreased CB_1 receptor function may contribute to progressive decline in Huntington's disease. Separate research groups bred two different mouse models of Huntington's disease with homozygous CB_1 knock-out mice ($CB_1^{-/-}$; Blázquez *et al.*, 2011; Mievis *et al.*, 2011). Both research groups found that mice over-expressing amino-terminal mutant huntingtin and having reduced CB_1 exhibited an earlier Huntington's disease symptom onset, a more rapid disease progression and a greater degree of medium spiny projection neuron degeneration than wild-type mice or mice over-expressing amino-terminal mutant huntingtin with a full complement of CB_1 (Blázquez *et al.*, 2011; Mievis *et al.*, 2011). Their findings suggest CB_1 normally performs a neuroprotective role in the striatum and loss of this receptor correlates with Huntington's disease pathogenesis. Thus, therapeutic strategies capable of elevating CB_1 mRNA abundance may restore or enhance the neuroprotective role of CB_1 where decreased expression of this receptor may contribute to disease pathology.

Pharmacological manipulation of CB_1 mRNA abundance

CB_1 mRNA levels can be modulated by methamphetamine and alcohols

Methamphetamine use is associated with region-specific changes in CB_1 mRNA levels in the CNS. Acute treatment of rats with methamphetamine is associated with increases in steady-state CB_1 mRNA levels in the prefrontal cortex, caudate and putamen, basolateral amygdala, CA1 hippocampal region and perirhinal cortex, relative to other brain regions and untreated controls (Bortolato *et al.*, 2010). These region-specific increases are detectable up to 3 weeks after a single post-acute treatment (Bortolato *et al.*, 2010). Acute methamphetamine use is associated with increased dopamine neurotransmission. These findings align with findings in schizophrenia and Parkinson's disease that suggest CB_1 levels are influenced by dopamine acting on pre- and post-synaptic receptors.

Ethanol use is also associated with region-specific changes in CB_1 mRNA levels in the CNS and in cell culture. In humans, CB_1 protein levels have been compared in the ventral striatum of individuals that were alcohol-dependent to age-matched, non-alcoholic individuals (Vinod *et al.*, 2005). CB_1 protein levels were lower in alcohol-dependent individuals relative to non-alcoholic controls (Vinod *et al.*, 2005). Barbier *et al.* (2008) found that mice exposed to ethanol *in utero* have significantly lower levels of CB_1 mRNA in the cortex, striatum and hippocampus from postnatal days 14 through 90 relative to age-matched controls. Consequently, exogenous ethanol exposure appears to alter CB_1 expression during early development and adulthood and may lead to chronic alterations in neurotransmission and gene expression that are normally facilitated by CB_1.

CB_1 mRNA levels can be modulated by estradiol and retinoic acid

Estradiol and retinoic acid alter CB_1 mRNA levels. 17β-estradiol and retinoic acid act upon their respective nuclear receptors to increase CB_1 mRNA levels in cell culture (Mukhopadhyay *et al.*, 2010; Proto *et al.*, 2011). Administration of 17β-estradiol increases CB_1 mRNA levels in DLD-1 and SW620 colon cancer cells (Nortarnicola *et al.*, 2008; Proto *et al.*, 2011). The effect of 17β-estradiol requires the oestrogen receptor, retinoic acid receptor (RAR)α and PPARγ to co-localize within the promoter region 1 kb from the human *CNR1* transcription start site (Proto *et al.*, 2011). Retinoic acid also increases CB_1 mRNA levels in mouse primary hepatic stellate cells (Mukhopadhyay *et al.*, 2010). This induction requires retinaldehyde dehydrogenase and RARγ (Mukhopadhyay *et al.*, 2010). RARγ interacts with a retinoic acid response element approximately 350 bp upstream of the mouse *Cnr1* transcription start site. Together, these data demonstrate 17β-estradiol and retinoic acid induce CB_1 transcription *via* oestrogen receptor, RAR- and PPAR-dependent mechanisms from a basal state to a higher steady state. Therefore, CB_1 levels can be manipulated by the activation or inhibition of well-known, pharmacologically tractable regulators of transcription.

CB_1 mRNA level is modulated by cannabinoids

Cannabinoids modulate steady-state CB_1 mRNA abundance. Chronic treatment with THC has been shown to decrease CB_1 mRNA levels in the CNS of rodents. Repeated exposure to THC, once daily for 14 days by i.p. injection, decreases CB_1 mRNA levels in the caudate and putamen of adult male rats (Corchero *et al.*, 1999). The extent of CB_1 mRNA decrease correlates to the number of repeated exposures. In another study, THC treatment increased CB_1 mRNA levels in the rat cerebellum and hippocampus over a 3 day period, while simultaneously decreasing CB_1 mRNA levels in the rat striatum (Zhuang *et al.*, 1998). Cannabinoids have also been shown to increase CB_1 mRNA levels in primary and immortalized cell culture systems. Treatment of primary mouse hepatic, stellate cells with 2-AG induces CB_1 mRNA, up to 30-fold relative to basal expression in untreated cells (Mukhopadhyay *et al.*, 2010). 2-AG-mediated CB_1 induction is RARγ- and CB_1 receptor-dependent in this model system (Mukhopadhyay *et al.*, 2010). AEA has been reported to increase CB_1 mRNA levels in DLD-1 and SW620 cells (Proto *et al.*, 2011). This effect was oestrogen receptor- and RARα-

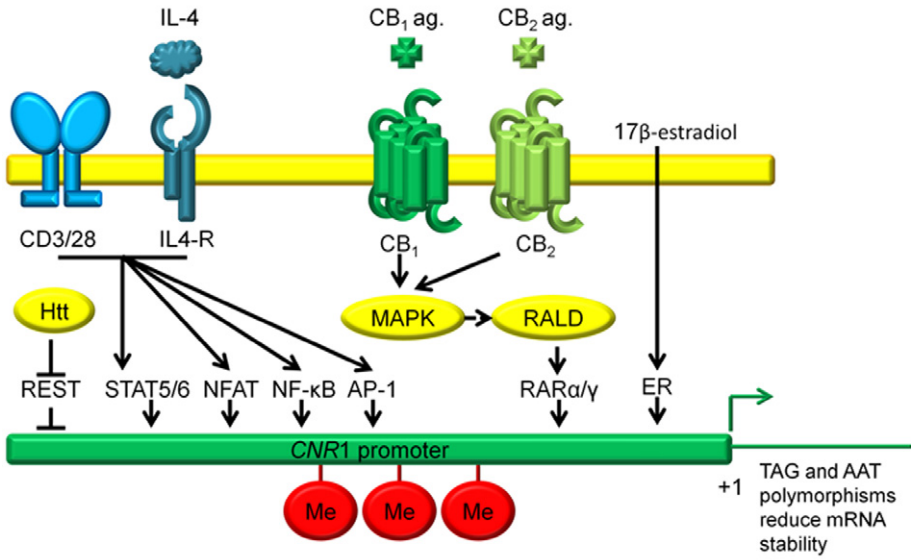

Figure 4

CB_1 mRNA expression is regulated by several co-activators of transcription. This schematic illustrates regulation of CB1 transcription at several functional transcription factor binding sites known to enhance CB_1 expression within 3 kb of the *CNR1* transcription start site at exon 1. STAT5, STAT6 (approximately –2769 bp, Borner *et al.*, 2007a,b), NFAT, NF-κB, AP-1 (within –2490 bp, Borner *et al.*, 2008), REST (approximately –898 bp, Blázquez *et al.*, 2011), RARα/γ (–350 bp, McCaw *et al.*, 2004; Mukhopadhyay *et al.*, 2010; Proto *et al.*, 2011), ERE (–1073 bp and –366 bp, Proto *et al.*, 2011), TAG and AAT polymorphisms 5′ of exon 1 and 3′ of exon 4, respectively (Zhang *et al.*, 2004), Me, DNA methylation leading to promoter repression (Miller and Devi, 2011).

dependent. Finally, THC, methanandamide and the CB_2-selective agonist JWH-015 induce CB_1 mRNA expression in Jurkat cells in a CB_2-dependent manner (Borner *et al.*, 2007b). Borner *et al.* (2007b) observed that CB_2 activation leads to phosphorylation of STAT5, transactivation of IL-4 and activation of STAT6, thereby inducing CB_1 promoter activity (Borner *et al.*, 2007b). Thus, in some systems, cannabinoid-dependent activation of CB_1 and CB_2 receptors stimulates the activity of specific transcription factors, such as the oestrogen receptor, RARα and STAT6, and augments steady-state CB_1 mRNA levels above basal levels. In other systems, cannabinoid exposure down-regulates CB_1 mRNA levels (Corchero *et al.*, 1999). Cannabinoid treatment therefore, as in various pathological conditions, is associated with malleable context-specific regulation of CB_1 expression. *In vivo*, repeated exposure to cannabinoid agonists is associated with tachyphylaxis (Corchero *et al.*, 1999), whereas in cell culture, single acute doses of cannabinoid agonists induce CB_1 mRNA expression (Borner *et al.*, 2007b; Mukhopadhyay *et al.*, 2010). From these observations, it is clear that the response of the CB_1 mRNA levels to cannabinoid treatment depends on the nature of treatment, chronic versus acute as well as the potency and efficacy of the ligand. For example, CB1 mRNA expression may be inducible in *in vivo* studies examining acute doses of cannabinoids, indirect cannabinoid agonism *via* fatty acid amide hydrolase inhibitors (Kim and Alger, 2010), or allosteric modulation of CB_1 receptor activity (Navarro *et al.*, 2009; Ahn *et al.*, 2012).

CB_1 protein levels are also increased following acute cannabinoid-dependent induction of CB_1 mRNA levels (Mukhopadhyay *et al.*, 2010; Proto *et al.*, 2011). This increase is modest (four- to fivefold) compared with the increased mRNA expression observed (29- to 30-fold, Mukhopadhyay *et al.*, 2010; Proto *et al.*, 2011) yet represents an increase in the pool of CB_1 receptors. In these studies, CB_1 protein abundance was quantified *via* western blot. Therefore, it is not known whether cannabinoid-mediated CB_1 induction affects the localization or functionality of CB_1 receptors.

Conclusions

Manipulation of CB_1 expression may have wide ranging affects on physiological processes such as embryogenesis (Paria *et al.*, 1995) and neural development (Fitzgerald *et al.*, 2012). Given that CB_1 gene expression is highest in many regions of the brain during early development and adolescence, and it is these areas of high expression where expression is often altered during disease progression, the effect of CB_1 therapeutics on CB_1 expression will depend on the existing level of CB_1 expression. CB_1 levels can be modulated pharmacologically by pro-inflammatory peptides, oestrogen, insulin, atypical antipsychotics, methamphetamine, ethanol, retinoic acid and, importantly, endogenous and exogenous cannabinoids (Figure 4). The observation that cannabinoids can induce CB_1 mRNA expression in cell culture suggests that cannabinoid ligands could regulate CB_1 levels. Although long-term treatment with THC *in vivo* is associated with tachyphylaxis (Corchero *et al.*, 1999), other cannabinoids have not been examined; the extent of tachyphylaxis may vary in

a ligand-specific manner (Hudson *et al.*, 2010), as well as with the duration of exposure (Zhuang *et al.*, 1998), half-life, efficacy, and potency of the cannabinoid. For instance, acute doses of less-potent agonists of CB_1 (e.g. AEA relative to THC; Pertwee *et al.*, 2010), or allosteric modulators of CB_1 (Ahn *et al.*, 2012) may induce CB_1 expression *in vivo*, whereas chronic treatment with more-potent agonists may cause receptor desensitization. If this is the case, then cannabinoid-dependent manipulation of CB_1 levels may represent a useful therapeutic strategy for diseases where reduced or elevated CB_1 levels correlate with disease progression. Overall, the affect of cannabinoid-based therapies may depend upon their modulation of CB_1 gene expression.

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Conflict of interest

None declared.

References

Ahn KH, Mahmoud MM, Kendall DA (2012). Allosteric modulator ORG27569 induces CB1 cannabinoid receptor high affinity agonist binding state, receptor internalization, and Gi protein-independent ERK1/2 kinase activation. J Biol Chem 287: 12070–12082.

Amaya F, Shimosato G, Kawasaki Y, Hashimoto S, Tanaka Y, Ji RR *et al.* (2006). Induction of CB1 cannabinoid receptor by inflammation in primary afferent neurons facilitates antihyperalgesic effect of peripheral CB1 agonist. Pain 124: 175–183.

Atwood BK, Mackie K (2010). CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol 160: 467–479.

Atwood BK, Straiker A, Mackie K (2012). CB2: therapeutic target-in-waiting. Prog Neuropsychopharmacol 38: 16–20.

Barbier E, Pierrefiche O, Vaudry D, Vaudry H, Daoust M, Naassila M (2008). Long-term alterations in vulnerability to addiction to drugs of abuse in brain gene expression after early life ethanol exposure. Neuropharmacology 55: 1199–2211.

Basavarajappa B, Nixon R, Arancio O (2009). Endocannabinoid system: emerging role from neurodevelopment to neurodegeneration. Mini Rev Med Chem 9: 448–462.

Benyamina A, Kebir O, Blecha L, Reynaud M, Krebs MO (2011). CNR1 gene polymorphisms in addictive disorders: a systematic review and a meta-analysis. Addict Biol 16: 12–16.

Bingham EM, Hopkins D, Smith D, Pernet A, Hallett W, Reed L *et al.* (2002). The role of insulin in human brain glucose metabolism: an ^{18}fluoro-deoxyglucose positron emission tomography study. Diabetes 51: 3384–3390.

Blázquez C, Chiarlone A, Sagredo O, Aguado T, Pazos MR, Resel E *et al.* (2011). Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. Brain 134: 119–136.

Borner C, Holt V, Kraus J (2007a). Activation of human T cells induces upregulation of cannabinoid type 1 transcription. Neuroimmunomodulation 14: 281–286.

Borner C, Holt V, Kraus J (2007b). Transcriptional regulation of the cannabinoid receptor type 1 gene in T cells by cannabinoids. J Leukoc Biol 81: 336–343.

Borner C, Bedini A, Holt V, Kraus J (2008). Analysis of promoter regions regulating basal and interleukin-4 inducible expression of the human CB1 receptor gene in T lymphocytes. Mol Pharmacol 73: 1013–1019.

Bortolato M, Frau R, Bini V, Luesu W, Loriga R, Collu M *et al.* (2010). Methamphetamine neurotoxicity increase brain expression and alters behavioural functions of CB1 cannabinoid receptors. J Psychiatr Res 44: 944–955.

Cacciola G, Chioccarelli T, Mackie K, Meccariello R, Ledent C, Fasano S *et al.* (2008). Expression of type-1 cannabinoid receptor during rat postnatal testicular development: possible involvement in adult leydig cell differentiation. Biol Reprod 79: 758–765.

Caffarel MM, Sarrio D, Palacios J, Guzman M, Sanchez C (2006). Delta-9- tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. Cancer Res 66: 6615–6621.

Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington HL *et al.* (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-o-methyltrasnferase gene: longitudinal evidence of a gene x environment interaction. Biol Psychiatry 57: 1117–1127.

Corchero J, Romero J, Berrendero F, Fernandez-Ruiz J, Ramos JA, Fuentes JA *et al.* (1999). Time-dependent differences of repeated administration with Delta9-tetrahydrocannabinol in proenkephalin and cannabinoid receptor gene expression and G-protein activation by mu-opiod and CB1-cannabinoid receptors in the caudate-putamen. Brain Res Mol Brain Res 67: 148–157.

Cottone E, Guastalla A, Mackie K, Franzoni MF (2008). Endocannabinoids affect the reproductive functions in teleosts and amphibians. Mol Cell Endocrinol 286: S41–S45.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384: 83–87.

Denovan-Wright EM, Robertson HA (2000). Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex, and hippocampus of transgenic Huntington's disease mice. Neuroscience 98: 705–713.

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: 686–691.

Diaz-Asensio C, Setien R, Echevarna E, Casis L, Casis E, Garrido A *et al.* (2008). Type 1 diabetes alters brain cannabinoid receptor expression and phosphorylation status in rats. Horm Metab Res 40: 454–458.

Eggan SM, Melchitsky DS, Sesack SR, Fish KN, Lewis DA (2010). Relationship of cannabinoid CB1 receptor and cholecystokinin immunoreactivity in monkey dorsolateral prefrontal cortex. Neuroscience 169: 1651–1661.

El Khoury MA, Gorgievski V, Moutsimilli L, Giros B, Tzavara ET (2012). Interactions between the cannabinoid and dopaminergic systems: evidence from animal studies. Prog Neuropsychopharmacol Biol Psychiatry 38: 56–60.

Fernandez-Ruiz J, Gomez M, Hernandez M, de Miguel R, Ramos JA (2004). Cannabinoids and gene expression during brain development. Neurotox Res 6: 389–401.

Fitzgerald ML, Chan J, Mackie K, Lupica CR, Pickel VM (2012). Altered dendritic distribution of dopamine D2 receptors and reduction in mitochondrial number in parvalbumin-containing interneurons in medial prefrontal cortex of cannabinoid-1 (CB1) receptor knockout mice. J Comp Neurol DOI: 10.1002/cne.23141. E-pub ahead of print.

Gafni J, Ellerby LM (2002). Calpain activation in Huntington's disease. J Neurosci 22: 4842–4849.

Garcia-Arencibia M, Garcia C, Kurz A, Rodriguez-Navarro JA, Gispert-Sachez S, Mena MA *et al.* (2009). Cannabinoid CB1 receptors are early downregulated followed by a further upregulation in the basal ganglia of mice with deletion of specific park genes. J Neural Transm Suppl 73: 269–275.

Gene Expression Nervous System Atlas (GENSAT) Project (2010). Gene name: cannabinoid receptor 1 (brain). NINDS Contracts N01NS02331 & HHSN271200723015 to The Rockefeller University (New York, NY). Available at: http://www.gensat.org/GeneProgressTracker.jsp?gensatGeneID=397.

Gustafsson K, Wang X, Severa D, Eriksson M, Kimby E, Merup M *et al.* (2008). Expression of cannabinoid receptors type 1 and type 2 in non-Hodgkin lymphoma: growth inhibition by receptor activation. Int J Cancer 123: 1025–1033.

Gutierrez T, Farthing JN, Zvonok AM, Makriyannis A, Hohmann AG (2006). Activation of peripheral CB1 and CB2 receptors suppresses the maintenance of inflammatory nociception: a comparative analysis. Br J Pharmacol 150: 153–163.

Heng L, Beverley JA, Steiner H, Tseng KY (2011). Differential developmental trajectories for CB1 cannabinoid receptor expression in limbic/associative and sensorimotor cortical areas. Synapse 65: 278–286.

Hill MN, Hillard CJ, McEwen BS (2011). Alterations in corticolimbic dendritic morphology and emotional behaviour in cannabinoid CB1 receptor-deficient mice parallel the effects of chronic stress. Cereb Cortex 21: 2056–2064.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al.* (2002). International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors. Pharmacol Rev 54: 161–202.

Hudson BD, Hebert TE, Kelly ME (2010). Physical and functional interaction between CB1 cannabinoid receptors and beta2-adrenoceptors. Br J Pharmacol 160: 627–642.

Hurley MJ, Mash DC, Jenner P (2003). Expression of cannabinoid CB1 receptor mRNA in basal ganglia of normal and parkinsonian human brain. J Neural Transm 110: 1279–1288.

Kalifa S, Polston EK, Allard JS, Manaye KF (2011). Distribution patterns of cannabinoid CB1 receptors in the hippocampus of APPswe/PS1ΔE9 double transgenic mice. Brain Res 1376: 94–100.

Karvela A, Rojas-Gil AP, Samkinidou E, Papadaki H, Pappa A, Georgiou G *et al.* (2010). Endocannabinoid (EC) receptor, CB1, and EC enzymes' expression in primary adipocyte cultures of lean and obese pre-pubertal children in relation to adiponectin and insulin. J Pediatr Endocrinol Metab 23: 1011–1024.

Kempf K, Hector J, Strate T, Schwartzloh B, Rose B, Herder C *et al.* (2007). Immune-mediated activation of the endocannabinoid system in visceral adipose tissue in obesity. Horm Metab Res 39: 596–600.

Kim J, Alger BE (2010). Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. Nat Neurosci 13: 592–600.

Larrinaga G, Sanz B, Perez I, Blanco L, Candenas ML, Pinto FM *et al.* (2010a). Cannabinoid CB1 receptor is downregulated in clear cell renal cell carcinoma. J Histochem Cytochem 58: 1129–1134.

Larrinaga G, Varona A, Perez I, Sanz B, Ugalde A, Candenas ML *et al.* (2010b). Expression of cannabinoid receptors in human kidney. Histol Histopathol 25: 1133–1138.

Lee JH, Agacinski G, Williams JH, Wilcock GK, Esiri MM, Francis PT *et al.* (2010). Intact cannabinoid CB1 receptors in Alzheimer's disease cortex. Neurochem Int 57: 985–989.

McCaw EA, Hu H, Gomez GT, Hebb AL, Kelly ME, Denovan-Wright EM (2004). Structure, expression, and regulation of the cannabinoid receptor gene (CB1) in Huntington's disease transgenic mice. Eur J Biochem 271: 4909–4920.

Malfitano AM, Laezza C, Galgani M, Matarese G, D'Alessandro A, Gazzerro P *et al.* (2012). The CB1 receptor antagonist rimonabant controls cell viability and ascitic tumour growth in mice. Pharmacol Res 65: 365–371.

Marshall AD, Lagutina I, Grosveld GC (2011). PAX3-FOX01 induces cannabinoid receptor 1 to enhance cell invasion and metastasis. Cancer Res 71: 7471–7480.

Marsicano G, Lutz B (1999). Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci 11: 4213–4225.

Martin BR, Mechoulam R, Razdan RK (1999). Discovery and characterization of endogenous cannabinoids. Life Sci 65: 573–595.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346: 561–564.

Mechoulam R, Hanu L (2001). The cannabinoids: an overview. Therapeutic implications in vomiting and nausea after cancer chemotherapy, in appetite promotion, in multiple sclerosis, and in neuroprotection. Pain Res Manag 6: 67–73.

Mievis S, Blum D, Ledent C (2011). Worsening of Huntington's disease phenotype in CB1 receptor knockout mice. Neurobiol Dis 42: 524–529.

Miller LK, Devi LA (2011). The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. Pharmacol Rev 63: 461–470.

Mukhopadhyay B, Liu J, Osei-Hyiaman D, Godlewski G, Mukhopadhyay P, Wang L *et al.* (2010). Transcriptional regulation of cannabinoid receptor-1 expression in the liver by retinoic acid acting via retinoic acid receptor-gamma. J Biol Chem 285: 19002–19011.

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. Nature 365: 61–65.

National Centre for Biotechnology Information (NCBI) (2011). EST Profile Hs.75110-CNR1: cannabinoid receptor 1 (brain). Database of Expressed Sequence Tags (dbEST) EST Profile Viewer. Available at: http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.75110.

Navarro G, Carriba P, Gandia J, Ciruela F, Casado V, Cortes A *et al*. (2008). Detections of heteromers formed by cannabinoid CB1, dopamine D2, and adenosine A2A G-protein- coupled receptors by combining bimolecular fluorescence complementation and bioluminescence energy transfer. ScientificWorldJournal 11: 1088–1097.

Navarro HA, Howard JL, Pollard GT, Carroll FI (2009). Positive allosteric modulation of the human cannabinoid (CB) receptor by RTI-371, a selective inhibitor of the dopamine transporter. Br J Pharmacol 156: 1178–1184.

Nortarnicola M, Messa C, Orlando A, Bifulco M, Laezza C, Gazzerro P *et al*. (2008). Estrogenic induction of cannabinoid CB1 receptor in human colon cancer cell lines. Scand J Gastroenterol 43: 66–72.

Pacheco DF, Klein A, Perez AC, Pacheco CM, de Francischi JN, Reis GM *et al*. (2009). Central antinociception induced by mu-opioid receptor agonist morphine, but not delta- or kappa-, is mediated by cannabinoid CB1 receptor. Br J Pharmacol 158: 225–231.

Palermo FA, Angelini M, Cottone E, Virgili M, Franzoni MF, Mosconi G *et al*. (2009). Involvement of endocannabinoid CB1 receptor in the modulation of stress responses related to xenoestrogen exposure. Ann N Y Acad Sci 1163: 504–507.

Paria BC, Das SK, Dey SK (1995). The preimplantation mouse embryo is a target for cannabinoind ligand-receptor signalling. Proc Natl Acad Sci U S A 92: 9460–9464.

Pazos MR, Sagredo O, Fernandez-Ruiz J (2008). The endocannabinoid system in Huntington's disease. Curr Pharm Des 14: 2317–2325.

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR *et al*. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev 62: 588–631.

Pinto JG, Hornby KR, Jones DG, Murphy KM (2010). Developmental changes in GABAergic mechanisms in human visual cortex across the lifespan. Front Cell Neurosci 4: 1–12.

Proto MC, Gazzerro P, Di Croce L, Santoro A, Malfitano AM, Pisanti S *et al*. (2011). Interaction of endocannabinoid system and steroid hormones in the control of colon cancer cell growth. J Cell Physiol 27: 250–258.

Rajesh M, Mukhopadhyay P, Haskó G, Huffman JW, Mackie K, Pacher P (2008). CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. Br J Pharmacol 153: 347–357.

Rinaldi-Carmona M, Calandra B, Shire D, Bouaboula M, Oustric D, Barth F *et al*. (1996). Characterization of two cloned human CB1 receptor isoforms. J Pharmacol Exp Ther 278: 871–878.

Romero J, Garcia-Palomero E, Berrendero F, Garcia-Gil J, Hernandez ML, Ramos JA *et al*. (1997). Atypical location of cannabinoid receptors in white matter areas during rat brain development. Synapse 26: 317–323.

Rozenfeld R, Bushlin I, Gomes I, Tzavaras N, Gupta A, Neves S *et al*. (2012). Receptor heteromerization expands the repertoire of cannabinoid signaling in rodent neurons. PLoS ONE 7: e29239.

Ryberg E, Vu HK, Larsson N, Groblewski T, Hjorth S, Elebring T *et al*. (2005). Identification and characterisation of a novel splice variant of the human CB1receptor. FEBS Lett 579: 259–264.

Sarzani R, Bordicchia M, Marcucci P, Bedetta S, Santini S, Giovagnoli A *et al*. (2009). Altered pattern of cannabinoid 1 receptor expression in adipose tissue of dysmetabolic and overweight patients. Metabolism 58: 361–367.

Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessègue B, Bonnin-Cabanne O *et al*. (1996). Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. Biochim Biophys Acta 1307: 132–136.

Silverdale MA, McGuire S, McInnes A, Crossman AR, Brotchie JM (2001). Striatal cannabinoid CB1 receptor mRNA expression is decreased in the reserpine-treated rat model of Parkinson's disease. Exp Neurol 169: 400–406.

Smith TH, Sim-Selley LJ, Selley DE (2010). Cannabinoid CB1 receptor-interacting proteins: novel targets for central nervous system drug discovery? Br J Pharmacol 160: 454–466.

Steiner H, Bonner TI, Zimmer AM, Kitai ST, Zimmer A (1999). Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. Proc Natl Acad Sci U S A 96: 5786–5790.

Straiker A, Wagner-Miller J, Hutchens J, Mackie K (2012). Differential signaling in human cannabinoid CB1 receptors and their splice variants in autaptic hippocampal neurons. Br J Pharmacol 165: 2660–2671.

Thrash B, Thiruchelvan K, Ahuja M, Suppiramaniam V, Dhanasekaran M (2009). Methampethamine-induced neurotoxicity: the road to Parkinson's disease. Pharmacol Rep 61: 966–977.

Uriguen L, Garcia-Fuster MJ, Callado LF, Morentin B, La Harpe R, Cassado V *et al*. (2009). Immunodensity and mRNA expression of A2Aadenosine, D2 dopamine, and CB1 cannabinoid receptors in post-mortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. Psychopharmacology (Berl) 206: 313–324.

Vinod KY, Arango V, Xie S, Kassir SA, Mann JJ, Cooper TB *et al*. (2005). Elevated levels of endocannabinoids and CB1 receptor-mediated G-protein signalling in the prefrontal cortex of alcoholic suicide victims. Biol Psychiatry 57: 480–486.

Vitalis T, Laine J, Simon A, Roland A, Leterrier C, Lenkei Z (2008). The type 1 cannabinoid receptor is highly expressed in embryonic cortical projection neurons and negatively regulates neurite growth in vitro. Eur J Neurosci 28: 1705–1718.

Wang D, Wang H, Ning W, Backlund MG, Dey SK, DuBois RN (2008). Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. Cancer Res 68: 6468–6476.

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994). Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. Neuroscience 63: 637–652.

Xiao JC, Jewell JP, Lin LS, Hagmann WK, Fong TM, Shen CP (2008). Similar in vitro pharmacology of human cannabinoid CB1 receptor variants expressed in CHO cells. Brain Res 1238: 36–43.

Zeng BY, Dass B, Owen A, Rose S, Cannizzaro C, Tel BC *et al*. (1999). Chronic L-DOPA treatment increases striatal cannabinoid CB1 receptor mRNA expression in 6-hydroxydopamine-lesioned rats. Neurosci Lett 276: 71–74.

Zhang F, Hong S, Stone V, Smith PJ (2007). Expression of cannabinoid CB1 receptors in models of diabetic neuropathy. J Pharmacol Exp Ther 323: 508–515.

Zhang P-W, Ishiguro H, Ohtsuki T, Hess J, Carillo F, Walther D *et al*. (2004). Human cannabinoid receptor 1: 5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. Mol Psychiatry 9: 916–931.

Zhuang S, Kittler J, Grigorenko EV, Kirby MT, Sim LJ, Hampson RE (1998). Effects of long- term exposure to delta9-THC on expression of cannabinoid receptor (CB1) mRNA in different rat brain regions. Brain Res Mol Brain Res 62: 141–149.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI (1999). Increased motality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A 96: 5780–5785.

Zurolo E, Iyer AM, Spliet WG, Van Rijen PC, Troost D, Gorter JA *et al*. (2010). CB1 and CB2 cannabinoid receptor expression during development and in epileptogenic development pathologies. Neuroscience 170: 28–41.