Distribution of the Endocannabinoid System in the Central Nervous System

Sherry Shu-Jung Hu and Ken Mackie

Contents

1 Introduction 61
1.1 Overview 61
1.2 Cells Expressing Components of the ECS 62
1.3 Subcellular Localization of CB_1 Cannabinoid Receptors 63
2 Retina 64
2.1 Receptors 64
2.2 Synthetic Enzymes 64
2.3 Degradative Enzymes 64
3 Cerebral Cortex 65
3.1 Neocortex 65
3.1.1 Receptors 65
3.1.2 Synthetic Enzymes 66
3.1.3 Degradative Enzymes 66
3.2 Olfactory Areas (Olfactory Bulb, Piriform Cortex, Associated Regions) 68
3.2.1 Receptors 68
3.2.2 Synthetic Enzymes 68
3.2.3 Degradative Enzymes 68
3.3 Hippocampal Formation 69
3.3.1 Receptors 69
3.3.2 Synthetic Enzymes 71
3.3.3 Degradative Enzymes 71

S.S.-J. Hu
Department of Psychology, National Cheng Kung University, Tainan 70101, Taiwan
e-mail: shujunghu@gmail.com

K. Mackie (✉)
Department of Psychological and Brain Sciences, Indiana University, 47405 Bloomington, IN, USA

Gill Center for Biomolecular Science, Indiana University, 47405 Bloomington, IN, USA
e-mail: kmackie@indiana.edu

© Springer International Publishing Switzerland 2015

R.G. Pertwee (ed.), *Endocannabinoids*, Handbook of Experimental Pharmacology 231, DOI 10.1007/978-3-319-20825-1_3

3.4 Cortical Subplate (Other Amygdala Nuclei) 73
3.4.1 Receptors 73
3.4.2 Synthetic Enzymes 73
3.4.3 Degradative Enzymes 73
4 Subcortical Nuclei (Striatum, Basal Ganglia) 74
4.1 Striatum (Dorsal, Caudate) 74
4.1.1 Receptors 74
4.1.2 Synthetic Enzymes 74
4.1.3 Degradative Enzymes 75
4.2 Striatum (Ventral, Accumbens) 75
4.2.1 Receptors 75
4.2.2 Synthetic Enzymes 75
4.2.3 Degradative Enzymes 75
4.3 Striatum Medial (Lateral Septum, Septohippocampal, etc.) 76
4.3.1 Receptors 76
4.3.2 Synthetic Enzymes 76
4.3.3 Degradative Enzymes 76
4.4 Striatum Caudal (Striatum-like Amygdala Nuclei, Central Amygdala, Bed Nucleus, Medial Amygdala, Etc.) 76
4.4.1 Receptors 76
5 Cerebellum and Associated Nuclei 77
5.1 Cerebellar Cortex 77
5.1.1 Receptors 77
5.1.2 Synthetic Enzymes 77
5.1.3 Degradative Enzymes 78
5.2 Deep Cerebellar Nuclei (Fastigial, Interpos, Dentate Nucleus) 78
5.2.1 Receptors 78
5.2.2 Synthetic Enzymes 78
5.2.3 Degradative Enzymes 78
6 Brainstem 79
6.1 Diencephalon 79
6.1.1 Thalamus (All Nuclei, Including Reticular Thalamic Nucleus, Habenula) .. 79
6.1.2 Hypothalamus (All Nuclei) 80
6.1.3 Mesencephalon (Colliculi, VTA, PAG, SN, Raphe) 80
6.2 Hindbrain 82
6.2.1 Medulla (Area Postrema, Cochlear Nuclei, Nucleus of the Solitary Tract, Trigeminal Nuclei, Various Other Cranial Nerve Nuclei) 82
7 Spinal Cord (Dorsal, Ventral, Dorsal Root Ganglion) 83
7.1 Receptors 83
7.2 Synthetic Enzymes 84
7.3 Degradative Enzymes 85
8 Summary, Concluding Thoughts, and Future Directions 85
References 85

Abstract

The endocannabinoid system consists of endogenous cannabinoids (endocannabinoids), the enzymes that synthesize and degrade endocannabinoids, and the receptors that transduce the effects of endocannabinoids. Much of what we know about the function of endocannabinoids comes from studies that combine localization of endocannabinoid system components with physiological

or behavioral approaches. This review will focus on the localization of the best-known components of the endocannabinoid system for which the strongest anatomical evidence exists.

Keywords
CB_1 cannabinoid receptor • Immunocytochemistry • In situ hybridization

Abbreviations

2-AG	2-Arachidonoylglycerol
ABHD4/6/12	α/β-hydrolase domain 4/6/12
AEA or anandamide	*N*-arachidonoylethanolamine
BLA	Basolateral amygdala
CB_1 and CB_2	Cannabinoid receptor 1 and 2
CRIP1a	Cannabinoid receptor interacting protein 1a
DAGLα/β	Diacylglycerol lipase α/β
DRG	Dorsal root ganglion
FAAH	Fatty acid amide hydrolase
GDE1	Glycerophosphodiesterase 1
M1	Muscarinic cholinergic receptor
MAGL	Monoacylglycerol lipase
mGluR5	Metabotropic glutamate receptor 5
MSNs	Striatal medium spiny neurons
NAAA	*N*-acylethanolamine-hydrolyzing acid amidase
NAPE-PLD	*N*-acyl phosphatidylethanolamine phospholipase D
PAG	Periaqueductal gray
SN	Substantia nigra
TH	Tyrosine hydroxylase
VTA	Ventral tegmental area

1 Introduction

1.1 Overview

Studies of the distribution of the protein components of the endocannabinoid system (ECS) are motivated by the notion that we can gain important insights into the function of the ECS by understanding the location of its component enzymes and receptors. This review has been written with that concept in mind, with an emphasis on studies that integrate function and location. Because of space limitations, this review will focus on components of the ECS in the CNS with the highest quality localization data. Thus, the emphasis will be on the cannabinoid

CB_1 receptors and on the enzymes *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) (Di Marzo et al. 1994; Okamoto et al. 2004), fatty acid amide hydrolase (FAAH) (Cravatt et al. 2001), diacylglycerol lipase (DAGL) (Bisogno et al. 2003; Gao et al. 2010), monoacylglycerol lipase (MAGL) (Dinh et al. 2002, 2004), and α/ß-hydrolase domain 6 (ABHD6) (Blankman et al. 2007; Marrs et al. 2010). The interesting topic of neuronal expression of CB_2 receptors is complicated by the high inducibility of CB_2 in pathological conditions, the low levels of CB_2 compared to CB_1, the presence of CB_2 in microglia and endothelial cells, and nonspecific antibodies. Nonetheless, CB_2 may be present on a limited population of neurons with functional consequences, for example: (Viscomi et al. 2009; den Boon et al. 2012; Zhang et al. 2014). The interested reader can refer to the literature for additional details and consideration (Marsicano and Kuner 2008; Atwood and Mackie 2010). We will also focus on ECS component localization in the mature brain in this review. The ECS and its components are subject to dynamic regulation in the developing CNS, a topic that has been well covered in recent reviews (Harkany et al. 2008; Maccarrone et al. 2014).

A variety of techniques are available to determine protein localization in tissues. These include autoradiography (labeled ligands or GTPγS), in situ hybridization, and antibody-based techniques. Each of these techniques gives complementary information, which taken together, can enhance our understanding of ECS function—autoradiography requires high affinity binding of a probe to the protein, *in situ* hybridization detects mRNA (often useful for identifying cell types synthesizing a protein), and antibody-based techniques detect a (protein) epitope resembling the epitope that antibody was raised against. As antibody-based techniques are most commonly used for ECS localization, it is important to be aware of some of their caveats. The issue of spurious results from antibody studies has received considerable attention in the neuroscience community (Saper 2005; Rhodes and Trimmer 2006; Michel et al. 2009; Manning et al. 2012), though numerous examples of poor practice continue to be published. "Best practices" have been discussed and implemented by several journals (Saper 2005; Rhodes and Trimmer 2006; Manning et al. 2012) and are summarized in Table 1. As much as is possible, studies that have adhered to those practices will be emphasized in this review.

1.2 Cells Expressing Components of the ECS

While the ECS in neurons has received the most attention due to the prominent effects of endogenous and exogenous cannabinoids on neuronal function, it is important to appreciate that glial cells are a major component of the ECS, sometimes acting independently of neurons and sometimes in concert. Strong evidence supports the presence of CB_1 receptors in some astrocytes and microglia (Rodriguez et al. 2001; Stella 2010; Bosier et al. 2013), and these cells as well as oligodendrocytes are prodigious synthesizers and degraders of endocannabinoids (Walter et al. 2002; Stella 2009); however, their complement of enzymes vary somewhat from those in neurons (Marrs et al. 2010). In addition, the CNS

Table 1 Antibody controls for immunocytochemistry

Approach	Strength
Block with immunizing protein	Weak (block with immunizing protein is a necessary, but not sufficient condition to establish antibody specificity)
Lack of staining with preimmune serum	Weak (immune response can induce the expression of many serum proteins that can interact with extraneous epitopes)
Lack of staining in knockout (KO)	Strong (requires knockout; need to understand how the KO was made, articularly if the exon which the antibody was raised against remains; knockout studies should be conducted in parallel (identical tissue processing, incubation times, etc.) with experiments performed with tissue that expresses the genetically deleted protein
Identical staining with antibody directed against independent epitopes	Strong (for alternatively spliced proteins need to ascertain that the appropriate exons are expressed)
Lack of staining in knockdown	Strong (need independent verification of knockdown; stronger if knockdown from limited population of cells so "controls" are adjacent)
Detection of protein in transfected cells	Medium (demonstrates antibody can detect protein, but not necessarily in tissue; stronger if conducted in a mixed population of cells (expressing and non-expressing) and target protein is epitope-tagged to allow its unequivocal detection; difficult to apply if target protein is present in the cell line used)
Detection of appropriate band on western blot	Can be helpful correlation (however, the conformation of protein in fixed tissue and denatured gel is quite different; many examples where antibody won't detect a specific band in Western blots and works for immunocytochemistry and vice versa)
Correlation with *in situ* hybridization	Useful at the level of cell populations (assumes mRNA is translated to protein)
Correlation with function	Helpful (e.g., CB_1-mediated responses and CB_1 receptors detected; however can lead to circular reasoning)

vasculature also participates in endocannabinoid signaling (Gebremedhin et al. 1999; Schley et al. 2009; Zhang et al. 2009; Dowie et al. 2014).

1.3 Subcellular Localization of CB_1 Cannabinoid Receptors

Due to their prominent effects on presynaptic calcium channels and synaptic transmission, it is not surprising that high levels of CB_1 receptors are found on some presynaptic terminals and preterminal axon segments (Katona et al. 1999; Nyiri et al. 2005a). The ability of endocannabinoids to suppress spiking in low threshold spiking cortical interneurons and some pyramidal cells suggests that CB_1 receptors are also located on neuron somata (Marinelli et al. 2009). Within neurons,

CB_1 receptors are sometimes associated with specialized structures (e.g., multilamellar bodies (Katona et al. 1999)) that may be involved in their trafficking. A recent though not uncontroversial finding (Benard et al. 2012; Hebert-Chatelain et al. 2014a, b; vs. Morozov et al. 2013) is that CB_1 receptors are associated with some mitochondria, including mitochondria found in astrocytes, where they contribute to energy balance and may play a role in synaptic plasticity.

2 Retina

2.1 Receptors

Using the immunocytochemical approach, CB_1 receptors are found in subsets of amacrine cells and horizontal cells and densely expressed in the inner plexiform layer (Straiker et al. 1999a; Yazulla et al. 1999). CB_1 are also present in rod and cone photoreceptor terminals in a wide range of vertebrate retinas, including human (Straiker et al. 1999a, b; Hu et al. 2010), as well as in rod bipolar cells in rat retina (Yazulla et al. 1999).

2.2 Synthetic Enzymes

While the presence of synthetic enzymes for *N*-arachidonoylethanolamine (anandamide or AEA) has not yet been examined in retina, two isoforms of the major synthetic enzyme for 2-arachidonoylglycerol (2-AG), diacylglycerol lipase-α and -β (DAGLα/β) (Bisogno et al. 2003; Gao et al. 2010), were both found in the mouse retina. DAGLα appears in the two synaptic layers, the outer plexiform layer and inner plexiform layer, whereas DAGLβ immunoreactivity is limited to retinal blood vessels. Furthermore, DAGLα is present in postsynaptic type 1 OFF cone bipolar cells juxtaposed to CB_1-containing cone photoreceptor terminals (Hu et al. 2010). These findings suggest that retrograde 2-AG signaling exists at type 1 OFF bipolar cell-cone photoreceptor synapses, consisting of presynaptic CB_1 receptors and postsynaptic DAGLα.

2.3 Degradative Enzymes

Both degradative enzymes for anandamide and related *N*-acylethanolamines (NAEs), fatty acid amide hydrolase (FAAH) (Cravatt et al. 2001) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) (Guo et al. 2005; Tsuboi et al. 2005), are present in retina. FAAH immunoreactivity was first detected in large ganglion cells, in large dopaminergic amacrine cells, in the dendrites of star-burst amacrine cells, and in the somata of horizontal cells of the rat retina (Yazulla et al. 1999). In the mouse retina, FAAH is widely expressed in the inner segments, outer nuclear layer, ganglion cell layer, and in the axon terminals of photoreceptors in the outer plexiform layer, and also co-localizes with CB_1 in subpopulation of amacrine cells

in the inner nuclear layer and in cells in the ganglion cell layer (Hu et al. 2010). The most notable difference between mouse and rat FAAH staining is the absence of staining in horizontal cells of the mouse, possibly a function of species difference. Finally, NAAA immunoreactivity is limited to retinal pigment epithelium in the mouse retina (Hu et al. 2010).

Among five candidate degradative enzymes for 2-AG, monoacylglycerol lipase (MAGL) and α/β-hydrolase domain 6 (ABHD6) (Blankman et al. 2007) were found in the mouse retina. Similar to DAGLα, MAGL staining was found in the outer and inner plexiform layers and additionally in the ganglion cell layer (Hu et al. 2010). While MAGL is not co-localized with DAGLα in the inner plexiform layer, it is distal to DAGLα in the outer plexiform layer. MAGL is present in photoreceptor terminals, including the rod spherules and cone pedicles (Hu et al. 2010), where it is well positioned to break down 2-AG after retrograde release onto both rod and cone terminals.

On the other hand, ABHD6 is widely distributed in the inner plexiform layer, inner nuclear layer, and ganglion cell layer. ABHD6 is localized to the calbindin- and GAD67-positive amacrine cells in the inner nuclear layer and to the dendrites of ganglion or displaced amacrine cells in the proximal inner plexiform layer (Hu et al. 2010). The postsynaptic staining of ABHD6 suggests its potential role in the breakdown of extrasynaptic 2-AG that has diffused beyond its intended target synapses.

3 Cerebral Cortex

3.1 Neocortex

3.1.1 Receptors

CB_1 receptors are densely expressed in all regions of the cortex, with high levels found in cingulate gyrus, frontal cortex, secondary somatosensory, and motor cortex, reviewed in (Mackie 2005). An immunocytochemical study found a heterogeneous distribution of CB_1-immunoreactive axons across neocortex in macaque monkeys and humans. Most neocortical association regions, such as the prefrontal and cingulate cortex, contain a higher density of CB_1-immunoreactive axons compared to the primary motor and somatosensory cortices (Eggan and Lewis 2007). Furthermore, in many cortical regions, CB_1-immunoreactivity displays a distinctly laminar pattern of expression, corresponding to the cytoarchitectonic boundaries. Although the distribution of CB_1 immunoreactivity across monkey neocortical regions is broadly similar to that observed in the rat using immunocytochemical (Egertova and Elphick 2000; Hajos et al. 2000; Katona et al. 2001; Bodor et al. 2005) and autoradiographic (Herkenham et al. 1991) approaches, there are several species differences in the laminar distribution of CB_1-immunoreactive axons. For example, CB_1-immunoreactive axons were reported to be most densely expressed in layers 2–3 and 6 and least densely expressed in layer 4 of the rat frontal and cingulate cortex (Egertova and Elphick 2000). In contrast, the highest density of CB_1-immunoreactive axons is localized in layer 4 of the same regions in monkey

(Eggan and Lewis 2007). In the primary somatosensory cortex, CB_1-immunoreactive axons are most densely localized in layer 5A in rat (Bodor et al. 2005), whereas a relatively similar density of CB_1-immunoreactive axons exists in layers 2–3 and 5–6, and a sparse axonal labeling presents in layer 4 of monkey (Eggan and Lewis 2007). Despite these differences, the laminar distribution of CB_1-immunoreactivity is quite similar within primates. For example, both autoradiographic (Glass et al. 1997) and immunocytochemical (Eggan and Lewis 2007) methods yield similar laminar distribution of CB_1 receptors in human and monkey neocortex, respectively.

In forebrain, high levels of CB_1 receptors have been primarily found on large cholecystokinin (CKK)-containing basket interneurons, with lesser levels found in non-CCK-expressing neurons (Marsicano and Lutz 1999). For example, CB_1 immunoreactivity is absent in nonadapting multipolar interneurons, such as the parvalbumin or bi-tufted adapting somatostatin-expressing interneurons (Tsou et al. 1999; Bodor et al. 2005). Despite original studies suggesting lack of expression of CB_1 immunoreactivity in principal glutamatergic neurons (Tsou et al. 1998a; Freund et al. 2003), more sensitive in situ hybridization studies revealed low but detectable levels of CB_1 mRNA in the great majority of glutamatergic neurons in many cortical regions including neocortex (Monory et al. 2006). Moreover, a single-cell real-time polymerase chain reaction (qPCR) study revealed that at least 50% of neocortical glutamatergic pyramidal neurons contain CB_1 mRNA (Hill et al. 2007), which is consistent with functional data showing that the CB_1 receptor agonist WIN-55212-2 decreased the intracortical electrical stimulation-evoked excitatory postsynaptic currents (EPSCs) in a CB_1 antagonist-dependent fashion (Hill et al. 2007). In summary, CB_1 receptors are densely expressed in multiple cortical regions. While CB_1 expression is highest on CCK-positive interneurons, functionally important CB_1 receptors are present on multiple neuron populations.

3.1.2 Synthetic Enzymes

Studies examining the protein and mRNA distribution of the anandamide synthesizing enzymes, NAPE-PLD, in neocortex have found it to be widespread, but at relatively low levels (Egertova et al. 2008). On the other hand, in situ hybridization revealed that moderate levels of DAGLα and DAGLβ mRNA are expressed in mouse cerebral cortex (Yoshida et al. 2006). DAGLα is typically dendritic, as shown for a cultured cortical neuron in Fig. 1.

3.1.3 Degradative Enzymes

FAAH-immunoreactive neuronal somata and dendrites are present throughout the transitional and neocortical regions of the mouse cerebral cortex and are often surrounded by CB_1-immunoreactive fibers. Except for layer 1, FAAH immunostaining is evident in all cortical layers, especially in the large cells in layer 5 (Egertova et al. 2003).

In situ hybridization revealed that high levels of MAGL mRNA exist throughout the rat brain cortex, especially in layers 4, deep 5, and 6 (Dinh et al. 2002).

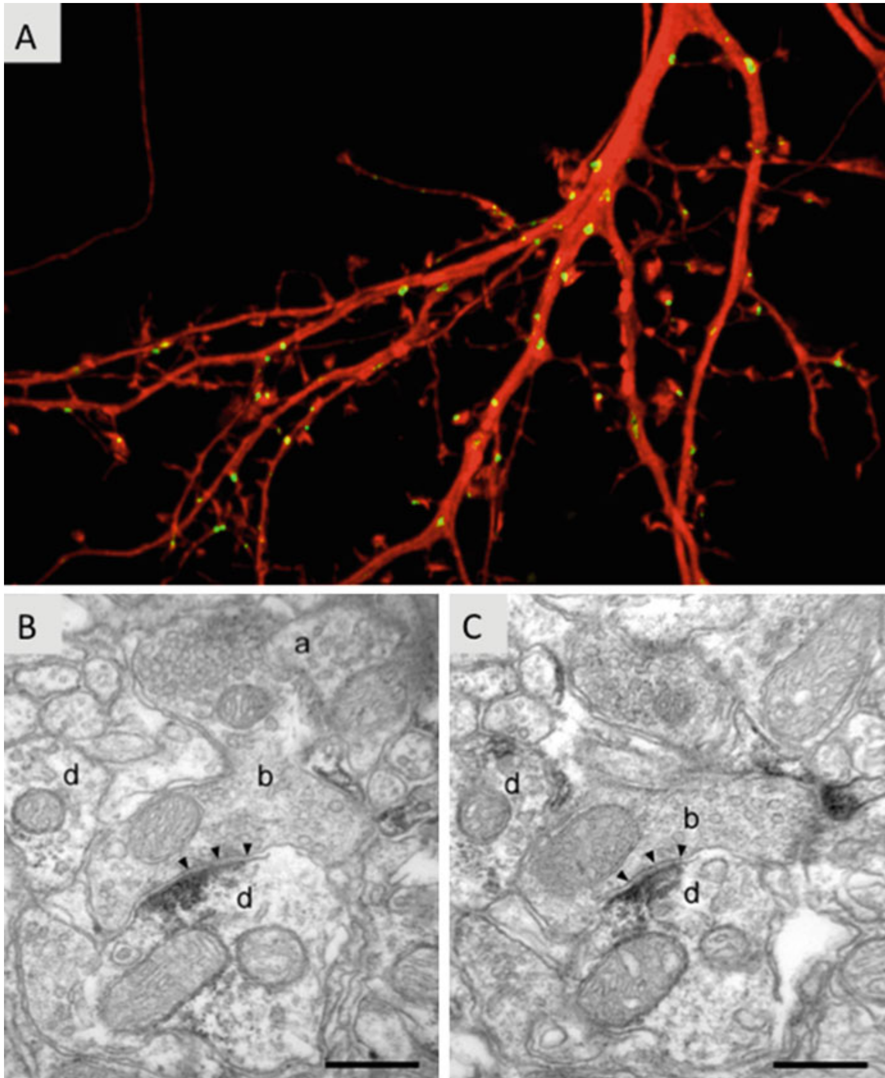

Fig. 1 Postsynaptic expression of diacylglycerol lipase alpha (DAGLα). (**A**) Dual immunofluorescent staining for DAGLα (*green*) and MAP2 (*red*). DAGLα as detected by a C-terminal antibody in a cultured neuron was found in close proximity to many dendritic spines. (**B** and **C**) Two consecutive ultrathin sections from mouse spinal cord demonstrate that the electron-dense reaction product representing DAGLα immunoreactivity (*arrowheads*) is present in the dendrite (*d*) close to the asymmetric postsynaptic density across from an excitatory terminal (*b*). Scale bar = 200 nm. Original figures provided by Barna Dudok and Istvan Katona (**A**) and Rita Nyilas and Istvan Katona (**B**, **C**)

In the mouse prefrontal cortex, ABHD6 immunoreactivity is predominantly localized to the postsynaptic dendritic spines, which is juxtaposed to the CB_1-positive presynaptic terminals (Marrs et al. 2010). Moreover, selective inhibition of ABHD6 allowed the induction of CB_1-mediated long-term depression by the subthreshold stimulation, suggesting this enzyme is a *bona fide* member of the endocannabinoid signaling system (Marrs et al. 2010).

3.2 Olfactory Areas (Olfactory Bulb, Piriform Cortex, Associated Regions)

3.2.1 Receptors

In the olfactory bulb, CB_1 receptors are highly expressed in the inner granular cell layer, followed by the inner plexiform layer, while less are expressed in the external plexiform layer, the glomerular layer, and the accessory olfactory bulb (Herkenham et al. 1991; Tsou et al. 1998a; Egertova and Elphick 2000). However, a detailed examination revealed that CB_1 receptor immunoreactivity is abundant in the periglomerular processes of GAD65-positive interneurons and the inner granular cell layer (A. Straiker, personal communication). Furthermore, CB_1 receptors are expressed uniformly by most neurons in the anterior olfactory nucleus and the anterior commissure, which connect the olfactory bulbs (Herkenham et al. 1991; Matsuda et al. 1993; Glass et al. 1997; Tsou et al. 1998a; Egertova and Elphick 2000). Moreover, most neurons in the piriform cortex contain CB_1 mRNA (Marsicano and Lutz 1999). Finally, CB_1 receptor immunoreactivity is present on dendritic processes in the olfactory epithelium of *Xenopus laevis* tadpoles, where it mediates cannabinoid modulation of odor-induced spike-associated currents in individual olfactory receptor neurons (Czesnik et al. 2007).

3.2.2 Synthetic Enzymes

NAPE-PLD mRNA expression has been detected in several olfactory areas. For example, NAPE-PLD mRNA is present in granule and periglomerular cells in the olfactory bulb and in neuronal cell body layers in the olfactory tubercle and the piriform cortex (Egertova et al. 2008). Interestingly, the immunostaining of NAPE-PLD is very intense in glomeruli of the accessory olfactory bulb and in the vomeronasal axons projecting into the accessory olfactory bulb (Egertova et al. 2008). Moderate expression of DAGLα and DAGLβ mRNA was found in mouse olfactory bulb by in situ hybridization (Yoshida et al. 2006).

3.2.3 Degradative Enzymes

Despite the overall low levels of CB_1 receptor expression in the olfactory bulb, FAAH immunoreactivity is intense, especially in fibers of the olfactory nerve and in the olfactory glomeruli, as well as in the somata and dendrites of mitral cells (Egertova et al. 2003). Moreover, FAAH-immunoreactive neuronal somata are evident in the majority of cortical olfactory regions receiving direct input from the olfactory bulb, including the anterior olfactory nucleus, the piriform cortex,

the tenia tecta, and the indusium griseum (Egertova et al. 2003). Importantly, in all of these regions, FAAH-immunoreactive postsynaptic neuronal somata are surrounded by a complementary network of CB_1-immunoreactive fibers, which supports the hypothesis that anandamide and other acyl amides may function as transynaptic signaling molecules (Egertova et al. 2000).

3.3 Hippocampal Formation

3.3.1 Receptors

The hippocampus is highly involved in cognitive functions such as the spatial and declarative learning and memory, thereby drawing much attention as a site of action of endogenous and exogenous cannabinoids due to their effects on memory. Early autoradiographic studies found very high levels of CB_1 receptors in all subfields of the hippocampus as well as the dentate gyrus (Herkenham et al. 1991; Jansen et al. 1992). In situ hybridization studies revealed that most CB_1 receptor expression arose from a restricted subset of interneurons (Matsuda et al. 1990, 1993; Mailleux and Vanderhaeghen 1992). Immunocytochemical studies showed high levels of CB_1 receptors on large CKK-positive basket and Schaffer collateral-associated interneurons in the hippocampal pyramidal cell layer, as well as the molecular layer and at the base of the granule cell layer in the dentate gyrus (Katona et al. 1999; Marsicano and Lutz 1999; Tsou et al. 1999; Egertova and Elphick 2000) (e.g., Fig. 2a), while few or no CB_1 receptors were found on parvalbumin-positive interneurons. Agonist activation of CB_1 receptors on these interneurons was found to decrease power in theta, gamma, and ripple oscillations in the hippocampus (Robbe et al. 2006). Actions of cannabinoids such as these appear to be widespread in cortical and subcortical circuits (Sales-Carbonell et al. 2013) and may contribute to the effects of cannabinoids on memory (Chen et al. 2003; Freund et al. 2003; Klausberger et al. 2005; Robbe et al. 2006).

Building on earlier studies that detected CB_1 mRNA, but not protein in CA1 and CA3 pyramidal neurons, studies in which CB_1 receptors were selectively deleted from either GABAergic or glutamatergic neurons allowed the conclusive anatomical identification of low levels of CB_1 protein in glutamatergic hippocampal pyramidal neurons (Marsicano et al. 2003; Lutz 2004). Indeed, several groups independently identified CB_1 protein in glutamatergic pyramidal neurons in the CA1 and CA3 regions (Degroot et al. 2006; Katona et al. 2006) (examples of CB_1 expression in hippocampal inhibitory and excitatory terminals are shown in Fig. 2A–D). However, among glutamatergic neurons, the highest CB_1 levels are present in dentate gyrus mossy cells (Kawamura et al. 2006; Monory et al. 2006). Interestingly, many CB_1-positive hilar mossy cells also contain dopamine D_2 receptors, suggesting that this region might be involved in the interactions of these two neuromodulatory systems (Degroot et al. 2006). Moreover, CB_1 immunoreactivity was also identified in the majority of hippocampal cholinergic nerve terminals, where presynaptic CB_1 receptors control acetylcholine release in vitro (Gifford and Ashby 1996) and in vivo (Degroot et al. 2006). On the other hand, CB_1

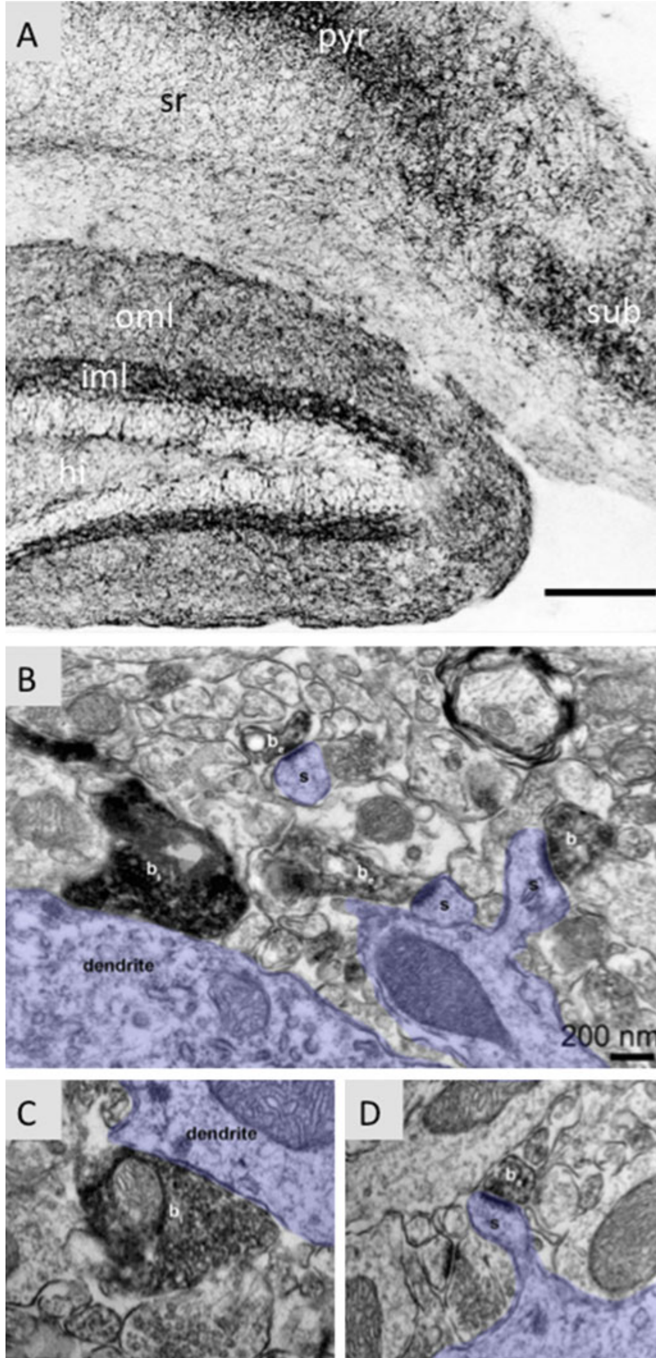

Fig. 2 CB_1 expression in rodent brain is primarily presynaptic. (**A**) CB_1 receptors in mouse hippocampal formation were detected by an antibody directed to its C-terminus. A dense meshwork of CB_1-expressing axons is evident. Levels are particularly high in the inner molecular layer

receptors appeared to be absent from granule cells of the dentate gyrus. Finally, low but detectable levels of CB_1 receptors were found in a subset of progenitor cells in the subgranular zone of the dentate gyrus, which regulate proliferation, survival, and differentiation of these adult progenitor cells (Aguado et al. 2005; Galve-Roperh et al. 2007).

3.3.2 Synthetic Enzymes

NAPE-PLD mRNA, as revealed by in situ hybridization, is most intensely expressed in the dentate gyrus granule cell layer, followed by the pyramidal cell layer of the hippocampus throughout all three fields (CA1-CA3) (Cristino et al. 2008; Egertova et al. 2008; Nyilas et al. 2008). Moreover, NAPE-PLD immunoreactivity was detected in granule cell axons (Egertova et al. 2008; Nyilas et al. 2008) as well as in many neurons of the hilus region in mouse dentate gyrus (Cristino et al. 2008; Nyilas et al. 2008). Therefore, in contrast to 2-AG's prominent role as a retrograde signaling messenger (Kano et al. 2009), anandamide and related NAEs generated by NAPE-PLD in axons may act as anterograde synaptic signaling molecules to regulate the activity of postsynaptic neurons (Egertova et al. 2008).

High levels of DAGLα mRNA and protein have been found in postsynaptic dendritic spines of the majority of hippocampal pyramidal neurons, including the spine head, neck, or both (Katona et al. 2006; Yoshida et al. 2006). A similar pattern for DAGLα was found in the postmortem human hippocampus, with highest levels in strata radiatum and oriens of the cornu ammonis and in the inner third of stratum moleculare of the dentate gyrus (Ludanyi et al. 2011). The juxtaposition of DAGL-α-immunoreactive postsynaptic dendritic spines to CB_1-expressing presynaptic terminals at excitatory glutamatergic synapses highlights the prominence of 2-AG as a retrograde messenger at many synapses (Katona et al. 2006).

3.3.3 Degradative Enzymes

FAAH is highly expressed in the somata and proximal dendrites of the pyramidal cells, which are densely innervated by CB_1-positive axon terminals (Egertova et al. 1998, 2003; Tsou et al. 1998b; Gulyas et al. 2004). However, it is important to note that FAAH is more frequently present on the membrane surface of intracellular organelles (e.g., mitochondria, smooth endoplasmic reticulum) than on somatic or dendritic plasma membranes (Gulyas et al. 2004). In addition, FAAH

Fig. 2 (continued) of the dentate gyrus and the pyramidal neuron layer: *hi* hilus, *iml* inner molecular layer, *oml* outer molecular layer, *pyr* pyramidal cell layer, *sr* stratum radiatum, *sub* subiculum. (**B–D**) Expression of CB_1 receptors at the ultrastructural level in the mouse hippocampus assessed using a C-terminal CB_1 antibody. (**B**) Electron-dense reaction product is present in both inhibitory (b_i) and excitatory (b_e) boutons. Note that the excitatory boutons synapse onto spines (*s*) while the inhibitory boutons form synapses on a dendritic shaft. Dendritic structures are pseudocolored blue for ease of identification. (**C**) Higher magnification image showing a CB_1-positive inhibitory terminal synapsing onto the shaft of a dendrite. (**D**) Higher magnification image showing a CB_1-positive excitatory bouton forming an asymmetric synapse onto a dendritic spine. Scale bar = 200 nm in **B**. Original figures provided by Jim Wager-Miller (**A**) and Chris Henstridge and Istvan Katona (**B–D**)

immunostaining is also evident in the somata of mouse dentate gyrus granule cells (Egertova et al. 2003), which contrasts to the absence of FAAH immunoreactivity in granule cells of the rat dentate gyrus (Tsou et al. 1998b; Gulyas et al. 2004). An in situ hybridization study showed that MAGL mRNA is abundantly expressed in the CA3 field of rat hippocampus (Dinh et al. 2002). Additional immunocytochemical studies revealed that MAGL protein is particularly prominent in axon terminals of granule cells, CA3 pyramidal cells, and some interneurons of rat hippocampus (Gulyas et al. 2004), as well as in axon terminals of glutamatergic neurons in both rodent and human hippocampus (Yoshida et al. 2006; Ludanyi et al. 2011) (Fig. 3A, B).

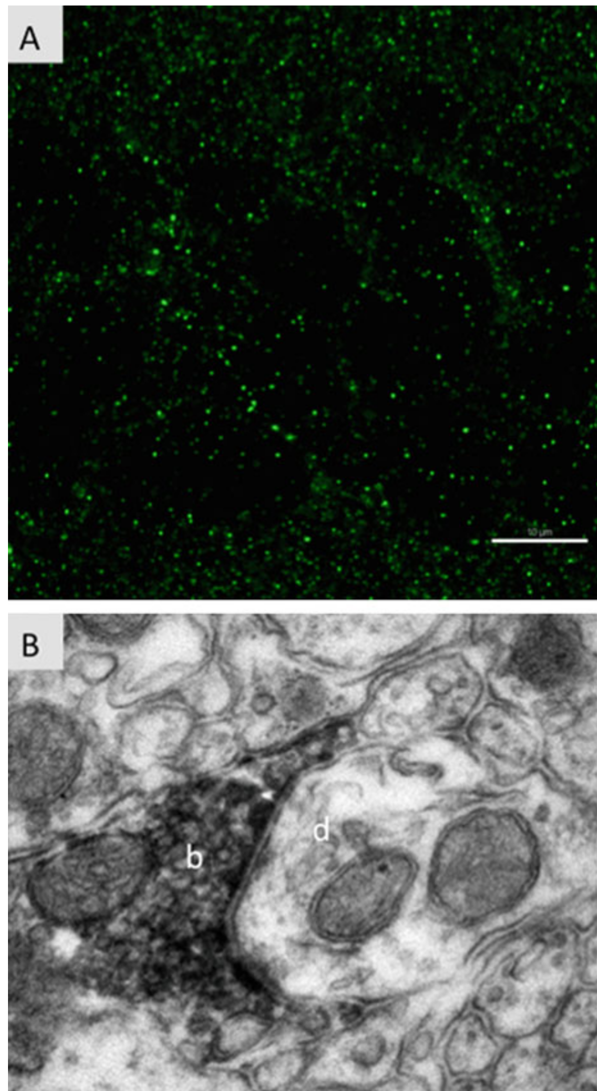

Fig. 3 Presynaptic localization of monoacylglycerol lipase (MAGL) detected with an antibody recognizing residues positioned in the middle of mouse MAGL. (**A**) Punctate expression of MAGL immunoreactivity in the mouse hippocampus pyramidal cell layer. Scale bar = 10 μm. (**B**) Electron-dense reaction product representing MAGL immunoreactivity is present in an inhibitory terminal (*b*) synapsing onto a dendritic shaft (*d*). Original figures provided by Stephen Woodhams and Istvan Katona

3.4 Cortical Subplate (Other Amygdala Nuclei)

3.4.1 Receptors

The amygdala is divided into a cortical component (e.g., the basolateral, lateral, and basomedial nuclei) and a striatal component (e.g., the central and medial nuclei) (Swanson and Petrovich 1998). This subdivision corresponds to the different structural organization and neurochemical properties of the two components. For example, while most principal neurons in the cortical subdivision of the amygdala are glutamatergic, the great majority of neurons in the striatal subdivision are GABAergic. Therefore, similar to cortex, high levels of CB_1 receptors are primarily expressed in the CCK-positive GABAergic basket cells (Marsicano and Lutz 1999; Katona et al. 2001). For example, in the basal (but not lateral) nucleus of the basolateral amygdala (BLA), high levels of CB_1 receptors are localized to presynaptic CCK-positive GABAergic terminals at invaginating synapses (Yoshida et al. 2011). On the other hand, as in cortex, low but functionally significant levels of CB_1 receptor are present in glutamatergic neurons in the cortical part of amygdala (Monory et al. 2006; Yoshida et al. 2011). Consistent with the above anatomical data, functional studies suggest that CB_1 receptors and endocannabinoids facilitated extinction of fear conditioning *via* inhibiting GABA release in the BLA (Marsicano et al. 2002). Finally, in contrast to earlier studies finding very weak CB_1 immunoreactivity within the central amygdala (Katona et al. 2001; Kamprath et al. 2011), a recent study using a highly sensitive CB_1 receptor antibody showed the presence of functional CB_1 receptors in the central amygdala (Ramikie et al. 2014).

3.4.2 Synthetic Enzymes

In the amygdaloid complex, NAPE-PLD mRNA is expressed in neurons of the cortical and medial amygdaloid nuclei, while less staining has been found in the basal and lateral nuclei (Egertova et al. 2008). At invaginating synapses, a unique type of perisomatic synapses in the basal nucleus of the BLA, DAGLα is recruited to somatic membrane of postsynaptic pyramidal neurons, juxtaposed to the CB_1-, MAGL-, and CCK-containing presynaptic terminals (Yoshida et al. 2011). In the central amygdala, DAGLα is localized to postsynaptic dendritic spine heads and dendritic shafts, juxtaposed to the CB_1-containing presynaptic terminals at glutamatergic synapses (Ramikie et al. 2014).

3.4.3 Degradative Enzymes

FAAH-immunoreactive neuronal somata are also present throughout the basolateral complex of the amygdala, which includes the lateral, basolateral (BLA), and basomedial nuclei. In all of these nuclei, the FAAH-immunoreactive somata are surrounded by CB_1-immunoreactive fibers (Egertova et al. 2003).

As mentioned above (Sect. 3.4.2), MAGL is co-expressed with CB_1 receptors in the presynaptic CCK-positive terminals at invaginating synapses in the basal nucleus of the BLA. Together with the postsynaptic DAGLα expression, this constitutes a molecular convergence for 2-AG-mediated retrograde signaling.

This contrasts with the flat perisomatic synapses made by parvalbumin-positive interneurons (Yoshida et al. 2011) where no such arrangement is present.

4 Subcortical Nuclei (Striatum, Basal Ganglia)

4.1 Striatum (Dorsal, Caudate)

4.1.1 Receptors

The subcortical nuclei with the highest level of CB_1 receptor expression are the basal ganglia. In situ hybridization studies showed that many striatal medium spiny neurons (MSNs) express CB_1 receptors, while adult pallidal and nigral neurons contain little or no CB_1 mRNA (Matsuda et al. 1993; Julian et al. 2003). Due to its axonal terminal localization, the high levels of pallidal and nigral CB_1 receptor binding and protein observed in autoradiographic and immunocytochemical studies mostly arose from GABAergic neurons projecting from the caudate putamen (Tsou et al. 1998a; Egertova and Elphick 2000). The staining for CB_1 in axons is denser in the globus pallidus than in the caudate putamen, while both show a gradient in the staining intensity increasing from medial to lateral (Tsou et al. 1998a; Egertova and Elphick 2000). Moreover, CB_1 receptors are present on both the striatonigral and striatopallidal projection pathways; thus, they are well positioned to modulate both the direct and indirect striatal output pathways (Hohmann and Herkenham 2000). In addition, a study utilizing a high-sensitivity CB_1 receptor antibody showed CB_1 protein to be intensely expressed on GABAergic axon terminals of striatal MSNs and parvalbumin-positive interneurons (Uchigashima et al. 2007). Finally, CB_1 protein was found on excitatory corticostriatal afferents (Gerdeman and Lovinger 2001; Rodriguez et al. 2001; Uchigashima et al. 2007), GABAergic aspiny interneurons (Hohmann and Herkenham 2000) and neurons in the subthalamic nucleus (Matsuda et al. 1993), with important functional implications (Kreitzer and Malenka 2007).

4.1.2 Synthetic Enzymes

The immunostaining of NAPE-PLD is present in the caudate putamen (Egertova et al. 2008). On the other hand, because 2-AG is synthesized by DAGL after membrane depolarization and G_q-coupled receptor activation, an immunocytochemical study was carried out to examine the subcellular distribution of DAGLα, metabotropic glutamate receptor 5 (mGluR5), and muscarinic cholinergic receptor 1 (M1) in mouse striatum (Uchigashima et al. 2007). Even though all three proteins were present on somatodendritic membranes of MSNs, only DAGLα and mGluR5 were present in spines and the perisynaptic region, while M1 receptors were absent in these domains (Uchigashima et al. 2007). This subcellular arrangement may account for the differential involvement of mGluR5 and M1 in endocannabinoid-mediated depolarization-induced suppression of inhibition and depolarization-induced suppression of excitation (Uchigashima et al. 2007).

4.1.3 Degradative Enzymes

In the mouse caudate putamen, FAAH-immunoreactive oligodendrocytes are present in fiber tracts (white matter) (Egertova et al. 2003). FAAH protein is also localized to the myelin sheath surrounding the unstained axons of large neurons (Egertova et al. 2003). This is consistent with the findings that FAAH mRNA is present in white matter of the rat brain (Thomas et al. 1997). However, the functional significance of FAAH expression in these non-neuronal cells is not yet understood. Moreover, although FAAH and CB_1 receptors are anatomically associated in many brain regions (Egertova et al. 1998), FAAH-expressing neurons are present in some brain areas such as thalamus and midbrain that express few or no CB_1 receptors (Egertova et al. 2003). Conversely, there is a population of striatal GABAergic MSNs where CB_1 receptors are present in their axonal terminals projecting to globus pallidus, entopeduncular nucleus, and substantia nigra, but FAAH-expressing neurons are absent (Egertova et al. 2003). In these situations, FAAH's biological role may be to degrade related acyl amides other than anandamide, for example, oleoylethanolamine and palmitoylethanolamine (Melis et al. 2008).

4.2 Striatum (Ventral, Accumbens)

4.2.1 Receptors

CB_1 receptors are expressed at low to moderate levels in the nucleus accumbens, with a pattern reminiscent of the striatum. CB_1 receptor protein is also localized on the terminals of the prefrontal glutamatergic efferents projecting to the nucleus accumbens as well as on the GABAergic axon terminals of accumbens MSNs and parvalbumin-positive interneurons (Robbe et al. 2001; Uchigashima et al. 2007). However, CB_1 receptors seem to be absent in the dopaminergic terminals projecting from the ventral tegmental area (VTA) to the accumbens. Therefore, cannabinoid stimulation of dopamine release in nucleus accumbens is likely mediated by inhibition of GABA release (either from within the nucleus accumbens or in the VTA) (Tanda et al. 1997; Szabo et al. 1999, 2002).

4.2.2 Synthetic Enzymes

DAGLα immunostaining, similar to that of mGluR5, is intense in spines and the perisynaptic region of the somatodendritic surface of striatal MSNs (Uchigashima et al. 2007).

4.2.3 Degradative Enzymes

FAAH immunoreactivity is absent in the nucleus accumbens (Egertova et al. 2003). However, moderate amounts of MAGL mRNA were found in the nucleus accumbens shell, islands of Calleja, and pontine nuclei by in situ hybridization (Dinh et al. 2002).

4.3 Striatum Medial (Lateral Septum, Septohippocampal, etc.)

4.3.1 Receptors

Moderate levels of CB_1 protein and mRNA are present in the basal forebrain including the medial and lateral septum and the nucleus of the diagonal band (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Marsicano and Lutz 1999). In situ hybridization studies showed the presence of CB_1 mRNA in cholinergic territories, especially the medial septum in mice. An immunocytochemical study revealed that a dense network of CB_1-positive fibers is present in the tenia tecta, ventral pallidum, and substantia innominate, whereas a fine network of CB_1-positive fibers is localized to the medial septum, diagonal bands, and nucleus basalis (Harkany et al. 2003). In the same study, no CB_1 immunoreactivity was detected in the cell bodies of basal forebrain cholinergic cells; instead these cells contain high levels of FAAH (Harkany et al. 2003). This is consistent with CB_1 receptors being synthesized and rapidly transported to axon terminals. Indeed, a later study showed that CB_1 protein is expressed in at least one-third of cholinergic neuron somata when axonal protein transport was blocked by cholchicine (Nyiri et al. 2005b). In rat medial septum, at least two types of cholinergic cells were identified, one with large somata, expressing CB_1 and $GABA_B$ receptors and projecting to the hippocampus, whereas the other had smaller somata and lacked these two receptors (Nyiri et al. 2005b). Therefore, endocannabinoid signaling is implicated in the function of a population of septohippocampal cholinergic neurons, including cognition as well as generation of hippocampal theta rhythms.

4.3.2 Synthetic Enzymes

The immunostaining of NAPE-PLD is localized to the lateral nucleus of the septum (Egertova et al. 2008).

4.3.3 Degradative Enzymes

Neuronal FAAH-immunoreactivity has been detected in the lateral septum and the triangular septal nucleus (Egertova et al. 2003), as well as in the basal forebrain cholinergic neurons (Harkany et al. 2003).

4.4 Striatum Caudal (Striatum-like Amygdala Nuclei, Central Amygdala, Bed Nucleus, Medial Amygdala, Etc.)

4.4.1 Receptors

In contrast to the cortical component of the amygdala, the striatal component of amygdala (e.g., central and medial nuclei) displays much lower levels of CB_1 receptors. CB_1 mRNA in the striatal amygdala was revealed by sensitive in situ hybridization with the absence of signal in the same region of CB_1 knockout mice (Marsicano and Lutz 1999).

5 Cerebellum and Associated Nuclei

5.1 Cerebellar Cortex

5.1.1 Receptors

The patterns of CB_1 receptor expression in the cerebellum are striking. While autoradiographic and immunocytochemical studies showed intense labeling of CB_1 protein in the molecular layer, in situ hybridization studies yielded robust CB_1 mRNA in the granule cell layer (Matsuda et al. 1990; Herkenham et al. 1991; Glass et al. 1997; Tsou et al. 1998a; Egertova and Elphick 2000). Purkinje neurons are devoid of CB_1 protein labeling, while the axon terminals of basket cells surrounding the Purkinje cell axon initial segment display extremely strong CB_1 labeling. Putting this together, CB_1 receptors are mainly expressed in the axon terminals of the climbing fibers, parallel fibers, and (some) basket cells, suggesting a prominent presynaptic localization of CB_1 receptors, mediating modulatory effects of (endo)cannabinoids at glutamatergic and GABAergic inputs onto Purkinje neurons. Consistent with this pattern of expression, several elegant electrophysiological studies demonstrated a role for endocannabinoid inhibition of glutamatergic and GABAergic neurotransmission onto Purkinje neurons (Kreitzer and Regehr 2001; Maejima et al. 2001; Diana et al. 2002; Brenowitz and Regehr 2003). However, additional electrophysiological studies supported the functional somatic expression of CB_1 receptors (Kreitzer et al. 2002).

5.1.2 Synthetic Enzymes

NAPE-PLD mRNA has been found in the granule cell layer and Purkinje cells, but not in the molecular layer or white matter (Egertova et al. 2008). However, NAPE-PLD immunoreactivity is localized in the pre- and post-synaptic areas of the Purkinje neurons and in the somata of the basket cells in the molecular layer (Cristino et al. 2008; Suarez et al. 2008).

DAGLα immunoreactivity is highly concentrated at the base of the spine neck of cerebellar Purkinje cells. In contrast to its distribution in hippocampal pyramidal cells, DAGLα is excluded from the main body of spine neck and head (Yoshida et al. 2006). However, there are no DAGLα-immunoreactive neurons in the granular layer and in any subdivisions of the inferior olive, suggesting that DAGLα is not present in parallel and climbing fibers (Suarez et al. 2008).

High levels of DAGLβ mRNA have been found in the mouse cerebellar granular layer by in situ hybridization (Yoshida et al. 2006). However, DAGLβ immunostaining in the cerebellar cortex is less intense than that of DAGLα. DAGLβ protein is localized to cell bodies of Purkinje neurons and in the molecular layer. In contrast to DAGLα, DAGLβ-containing neuropil of the molecular layer probably represents parallel and climbing fibers from the granular cells and inferior olive neurons, respectively (Suarez et al. 2008).

5.1.3 Degradative Enzymes

FAAH immunoreactivity is present in the somata and dendrites of the Purkinje cells, which are innervated by CB_1-positive axon terminals (Egertova et al. 1998, 2003; Tsou et al. 1998b; Gulyas et al. 2004). Weak FAAH immunostaining is also evident in the somata of granule cells (Egertova et al. 2003), which is consistent with the detection of FAAH mRNA in rat cerebellar granule cells (Thomas et al. 1997).

Both Northern blot and in situ hybridization analyses revealed that MAGL mRNA is present in rat cerebellum (Dinh et al. 2002), whereas MAGL immunoreactivity is localized to the axon terminals in the molecular layer but absent in the FAAH-positive Purkinje cell dendrites in rat cerebellum (Gulyas et al. 2004). Interestingly, a recent immunocytochemical study found that MAGL is heterogeneously expressed in mouse cerebellum, with highest levels in parallel fiber terminals, weak levels in Bergman glia, and complete absence in other synaptic terminals (Tanimura et al. 2012). Even though the expression of MAGL is limited to a subset of nerve terminals and astrocytes in the cerebellum, MAGL still regulates 2-AG retrograde signaling broadly at parallel fiber or climbing fiber to Purkinje cell synapses (Zhong et al. 2011; Tanimura et al. 2012).

5.2 Deep Cerebellar Nuclei (Fastigial, Interpos, Dentate Nucleus)

5.2.1 Receptors

All cerebellar nuclei (medial, lateral, and interposed nuclei) contain very weak CB_1-immunoreactivity throughout the neuropil. However, intense CB_1 immunostaining was found in the neuropil of the dorsal part of the principal nucleus of the inferior olive (Suarez et al. 2008).

5.2.2 Synthetic Enzymes

Most cerebellar nuclei showed intense neuropil NAPE-PLD immunoreactivity and a number of moderately NAPE-PLD-labeled neurons. On the other hand, both the posterior parvicellular part of the interposed cerebellar and lateral cerebellar nuclei have considerably fewer NAPE-PLD-labelled neurons and a less intense neuropil immunoreactivity (Suarez et al. 2008).

DAGLα immunoreactivity is absent in cell bodies of cerebellar and vestibular nuclei and in other regions with mossy fiber projections in the granular layer such as the pontine nuclei or the spinal cord (Suarez et al. 2008). In contrast, DAGLβ immunoreactivity is associated mainly with cell bodies embedded in a network of fibers. As with NAPE-PLD immunostaining, the posterior parvicellular parts of the interposed cerebellar and lateral cerebellar nuclei contain fewer DAGLβ-positive neurons when compared with the remaining cerebellar nuclei (Suarez et al. 2008).

5.2.3 Degradative Enzymes

The strong FAAH immunoreactivity observed in all cerebellar nuclei is related mainly to the presence of a dense meshwork of fibers, consisting of FAAH-positive

punctate labeling that contain immunoreactive somata (Egertova et al. 2003; Suarez et al. 2008). These cerebellar nuclei are devoid of CB_1 immunoreactivity and receive synaptic input mainly from Purkinje cell axons. In white matter surrounding the cerebellar nuclei, FAAH-immunoreactive oligodendrocytes are conspicuous (Egertova et al. 2003).

Cerebellar and functionally related vestibular nuclei have numerous MAGL-immunoreactive neurons, showing a perikaryal and dendritic Golgi-like labeling, similar to that of DAGLβ (Suarez et al. 2008).

6 Brainstem

6.1 Diencephalon

6.1.1 Thalamus (All Nuclei, Including Reticular Thalamic Nucleus, Habenula)

Receptors

CB_1 receptor expression is very low in most areas of the thalamus, with the exception of strong labeling in the lateral habenular nucleus, the anterior dorsal thalamic nucleus, and the reticular thalamic nucleus (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992; Tsou et al. 1998a; Marsicano and Lutz 1999). Since CB_1 mRNA is quite abundant in the lateral habenular nucleus, which has extremely diverse projections, it is likely that (endo)cannabinoids, acting through CB_1 receptors, significantly affect the diverse functions of the lateral habenula (Herkenham and Nauta 1977; Herkenham et al. 1991).

Synthetic Enzymes

NAPE-PLD mRNA is found in several thalamic nuclei such as lateral posterior nuclei and the medial geniculate nucleus, albeit at quite low intensity in mice (Egertova et al. 2008; Nyilas et al. 2008). In contrast, the highest levels of NAPE-PLD activity, protein and mRNA, were identified by enzyme assay, western blotting, and qPCR in rat thalamus, among nine different brain regions examined (Morishita et al. 2005). In addition, in situ hybridization revealed that moderate levels of DAGLα mRNA are expressed in mouse thalamus (Yoshida et al. 2006).

Degradative Enzymes

FAAH immunoreactivity has been detected in neuronal somata in the majority of thalamic nuclei, including the anterodorsal, the anteroventral, the anteromedial, the ventroanterior, the paratenial, the mediodorsal, the reticulothalamic, the ventrolateral, the ventroposterior, the ventromedial, the posterior, the lateral geniculate, and the medial geniculate (Egertova et al. 2000, 2003). Interestingly, FAAH protein is much more abundant in these nuclei than the cannabinoid CB_1 receptor, suggesting its role may be to degrade acyl amides other than anandamide in these regions.

An in situ hybridization study revealed that MAGL mRNA is abundantly expressed in the anterior thalamus, particularly in the anterodorsal nucleus, whereas it is sparse in other thalamic nuclei (Dinh et al. 2002).

6.1.2 Hypothalamus (All Nuclei)

Receptors

High levels of CB_1 immunoreactivity have been found in the arcuate, paraventricular, ventromedial, dorsomedial nuclei, and the external zone of the median eminence (Wittmann et al. 2007), as well as in the infundibular stem and lateral hypothalamic area (Tsou et al. 1998a). Further analysis revealed that CB_1 immunoreactivity is detectable in the preterminals of approximately equal numbers of symmetric and asymmetric synapses, suggesting the occurrence of retrograde signaling by endocannabinoids in both excitatory and inhibitory hypothalamic neuronal networks (Wittmann et al. 2007). An in situ hybridization study suggests that CB_1 mRNA is primarily present on glutamatergic neurons in the hypothalamus (Marsicano and Lutz 1999). Despite the relatively low levels of CB_1 receptors in the hypothalamus, functional GTPγS assays revealed these receptors are more efficiently coupled to G proteins than in many other regions (Breivogel and Childers 1998). Finally, a recent study showed that mice with viral-mediated knockdown of the CB_1 receptor gene (~60 % decrease of the mRNA level) in the hypothalamus, while maintained on a normocaloric standard diet, displayed decreased body weight gain over time, subsequent to increased energy expenditure and elevated β_3-adrenergic receptor expression in brown adipose tissues (Cardinal et al. 2012). This result suggests that hypothalamic CB_1 receptor signaling plays an important role in energy expenditure under basal conditions, contributing to the antiobesity effect of CB_1 receptor antagonism.

Synthetic Enzymes

The staining of NAPE-PLD mRNA is evident in cells of the ventromedial nucleus (Egertova et al. 2008).

6.1.3 Mesencephalon (Colliculi, VTA, PAG, SN, Raphe)

Receptors

A. Substantia Nigra

Both autoradiographic and immunocytochemical studies showed extremely high levels of CB_1 receptor protein in the substantia nigra (SN) pars reticulata (Herkenham et al. 1991; Egertova and Elphick 2000). In contrast, in situ hybridization studies showed very low amounts of CB_1 mRNA in the SN (Matsuda et al. 1993), suggesting the high levels of CB_1 protein are restricted to incoming axonal projections from other brain regions. For example, CB_1 receptor protein is restricted to the GABAergic axonal terminals from the putamen MSNs and the glutamatergic terminals from the subthalamic nucleus, which may be involved in

the control of locomotility by CB_1 activation in the SN (Mailleux and Vanderhaeghen 1992; Sanudo-Pena and Walker 1997; Sanudo-Pena et al. 1999a). On the other hand, very low levels of CB_1 receptors exist in sparse intrinsic nigral neurons, which may exert a direct control on dopaminergic transmission (Matsuda et al. 1993; Julian et al. 2003). Finally, in rat striatal nerve terminals, a low but significant percentage of CB_1–immunoreactivity is co-localized with tyrosine hydroxylase (TH), a marker for both noradrenergic and dopaminergic terminals (Kofalvi et al. 2005).

B. Ventral Tegmental Area

Both cannabinoids and endocannabinoids modulate the primary rewarding effects of many abused drugs, including exogenous cannabinoids, *via* regulation of drug-induced increases in dopaminergic neural activity in the VTA (Maldonado et al. 2006). Therefore, it is of interest to elucidate the expression and function of CB_1 receptors in the VTA. A sensitive CB_1 polyclonal antibody (Fukudome et al. 2004) revealed a dense neuropil labeling of CB_1 receptors in the VTA (Matyas et al. 2008). The CB_1 immunoreactivity is restricted to presynaptic axon terminals of symmetric synapses, which may belong to local intrinsic GABAergic neurons (Matyas et al. 2008). Moreover, CB_1-immunoreactivity is co-localized with vesicular glutamate transporter in presynaptic terminals near dopamine neuron dendrites in the VAT, indicating the presence of CB_1 receptors in glutamatergic terminals (Kortleven et al. 2011). Interestingly, co-localization of CB_1 receptor and TH has been revealed in several brain areas including VTA, thereby pointing to a possible direct influence of CB_1 receptor activation on dopaminergic neurons (Wenger et al. 2003). However, further studies are required to clarify this possibility.

C. Periaqueductal Gray

Low to moderate levels of CB_1 receptors have been found in the midbrain periaqueductal gray (PAG), where the ECS is involved in the control of pain sensation, including stress-induced analgesia (Walker et al. 1999; Hohmann et al. 2005; Gregg et al. 2012). In contrast to opiate receptors on GABAergic aqueductal neurons, CB_1 receptors are preferentially, but not exclusively, localized in the dorsal portion of the PAG (Tsou et al. 1998a; Azad et al. 2001). In addition, moderate levels of CB_1 mRNA and protein have been found in the reticular formation and raphe nucleus, the latter being the main neuronal source of serotonin in the brain (Glass et al. 1997; Haring et al. 2007), which might have functional implications in emotion/mood modulation.

Synthetic Enzymes

A. Ventral Tegmental Area

Moderate to high levels of DAGLα are expressed in most neurons of the VTA (Matyas et al. 2008). High-resolution electron microscopy further revealed that DAGLα is accumulated in postsynaptic plasma membrane of glutamatergic and GABAergic synapses, of both TH-positive and negative dendrites. The finding that

DAGLα is present in postsynaptic dendrites juxtaposed to presynaptic CB_1 receptors suggests that 2-AG-CB_1-mediated retrograde synaptic signaling may modulate the drug-reward circuitry at multiple types of synapses in the VTA.

B. Periaqueductal Gray
DAGLα protein is co-localized with mGluR5 within the same dendritic spine heads at postsynaptic excitatory synapses in rat dorsolateral PAG, which is involved in 2-AG-mediated stress-induced analgesia (Gregg et al. 2012).

Degradative Enzymes

A. Substantia Nigra
FAAH-immunoreactive neurons are not evident in the pars reticulata of the SN, which contains a very high concentration of CB_1 immunoreactivity in both mouse and rat (Egertova et al. 2000, 2003).

B. Other Nuclei
FAAH-immunoreactive neurons are present in the superior and inferior colliculus, the rhabdoid nucleus, and several mesencephalic raphe nuclei and are also intensely stained in the mesencephalic trigeminal nucleus (Egertova et al. 2003). FAAH-immunoreactive oligodendrocytes associated with fiber tracts are abundant in the midbrain. For example, FAAH immunostaining was found to be localized in the myelin sheath surrounding the unstained axons of the mesencephalic trigeminal tract (Egertova et al. 2003).

6.2 Hindbrain

6.2.1 Medulla (Area Postrema, Cochlear Nuclei, Nucleus of the Solitary Tract, Trigeminal Nuclei, Various Other Cranial Nerve Nuclei)

Receptors

Expression of CB_1 receptors, in contrast to the opioid receptors, in the medullary respiratory control centers is relatively low (Herkenham et al. 1991; Glass et al. 1997). This likely explains the low mortality caused by cannabinoid intoxication in humans and animals. However, relatively high levels of CB_1 receptors are present in the medullary nuclei associated with emesis and vagal control of gut motility, which may underlie the inhibition of emesis and gastrointestinal motility by cannabinoids (Krowicki et al. 1999; Van Sickle et al. 2001, 2003). For example, high to moderate levels of CB_1 receptors were found in the area postrema, the dorsal motor nucleus of the vagus, as well as the medial subnucleus and the subnucleus gelatinosus of the nucleus of the solitary tract (Van Sickle et al. 2001, 2003; Mackie 2005; Storr and Sharkey 2007).

In mouse dorsal cochlear nucleus, CB_1 receptors are highly expressed in glutamatergic terminals of the parallel fibers, at intermediate levels in glycinergic terminals, and completely absent in the auditory nerve inputs innervating to the same DCN principle neurons—fusiform and cartwheel cells (Zhao et al. 2009). Therefore, CB_1 receptors are well positioned to mediate short- and long-term plasticities exhibited at parallel fiber synapses, but not at auditory nerve inputs (Zhao et al. 2011; Zhao and Tzounopoulos 2011).

Synthetic Enzymes

Both DAGLα and DAGLβ proteins were identified in the somata of fusiform and cartwheel cells of mouse DCN, while they were present only in the dendritic spines of cartwheel cells (Zhao et al. 2009). These findings suggest that the synthesis of 2-AG is more distant from parallel fiber synapses in fusiform than cartwheel cells.

7 Spinal Cord (Dorsal, Ventral, Dorsal Root Ganglion)

7.1 Receptors

Intrathecal application of cannabinoids has been found to suppress pain in various pain models (Smith and Martin 1992; Welch et al. 1995), which is consistent with their suppression of noxious stimulus-evoked neuronal firing (Hohmann and Herkenham 1998) and Fos protein expression in the spinal dorsal horn (Hohmann et al. 1999b). Moreover, cannabinoids inhibited glutamate release from afferents in lamina I of dorsal horn in a CB_1 receptor-dependent fashion (Jennings et al. 2001; Morisset and Urban 2001). Compatible with these functional studies, moderate levels of CB_1 receptors were found in the superficial layers of the dorsal horn, the dorsolateral funiculus, and lamina X, all regions of the spinal cord associated with analgesia (Farquhar-Smith et al. 2000; Nyilas et al. 2009).

However, only a small percentage of CB_1 receptors are localized at central terminals of primary afferent C fibers, with many more present on large, myelinated Aβ and Aδ fibers, as well as postsynaptic interneurons (Hohmann and Herkenham 1998, 1999; Hohmann et al. 1999a; Farquhar-Smith et al. 2000). A dorsal rhizotomy produced time-dependent 50 % losses in cannabinoid binding densities in the dorsal horn since rhizotomy destroyed the terminals of both small- and large-diameter fibers (Hohmann et al. 1999a). However, a quantitative autoradiographic study showed a modest (16 %) decrease in cannabinoid binding sites in the superficial dorsal horn by neonatal capsaicin-mediated destruction of sensory C fibers (Hohmann and Herkenham 1998). Moreover, another study showed little decrease in CB_1 receptor immunoreactivity following dorsal rhizotomy or hemisection of the spinal cord, suggesting CB_1 receptors are primarily expressed on interneurons (Farquhar-Smith et al. 2000). Similarly, CB_1 receptors are only minimally co-localized with markers for C primary afferents both in the superficial dorsal horn and dorsal root ganglion (DRG) (Farquhar-Smith et al. 2000; Bridges et al. 2003). The above data suggest that the majority of CB_1 receptors are not

localized at the presynaptic terminals of nociceptive primary afferents, but rather may exist on postsynaptic interneurons (Farquhar-Smith et al. 2000; Salio et al. 2002), a CB_1 distribution which differs from the strong presynaptic axon terminal localization of CB_1 receptors in most other brain regions (Katona et al. 1999; Nyiri et al. 2005a). Indeed, research showed that CB_1 receptors localized at dorsal horn inhibitory postsynaptic interneurons mediate C-fiber-induced pain sensitization. However, it is important to emphasize the unexpected pro-nociceptive role of endocannabinoids is specific for C-fiber-mediated activity-dependent hyperalgesia, in contrast to the anti-nociceptive effect of these endogenous lipid molecules in models of inflammatory and neuropathic pain (Pernia-Andrade et al. 2009). Moreover, a conditional nociceptor-specific loss of CB_1 was found to reduce spinal CB_1-specific ligand binding by approximately 20 % only and did not substantially decrease CB_1-immunoreactivity in spinal laminae I and II (Agarwal et al. 2007). However, this conditional knockout of CB_1 in peripheral nociceptive neurons led to a substantial reduction of analgesia produced by local and systemic delivery of cannabinoids, suggesting that low levels of CB_1 expression did not necessarily mean lack of functional significance (Agarwal et al. 2007). Therefore, it is likely that the interplay between cannabinoid actions on peripheral primary afferents, interneurons, and descending pathways collectively contributes to the analgesic effects of CB_1 receptor activation in the spinal cord.

Interestingly, despite the earlier reports of minimal localization of CB_1 receptors in nociceptive DRG neurons (Hohmann and Herkenham 1999; Bridges et al. 2003), more recent studies suggest a much broader (40–80 %) distribution of CB_1 receptors in nociceptive neurons of DRG and trigeminal ganglia (Mitrirattanakul et al. 2006; Agarwal et al. 2007). While the possible reasons for these discrepancies have been well discussed elsewhere (Marsicano and Kuner 2008), it is also important to note that the expression and peripheral transport of CB_1 receptors in the DRG can be upregulated by peripheral inflammation (Amaya et al. 2006). Finally, some immunocytochemical evidence suggests CB_1 receptors are also present in the ventral horn (Tsou et al. 1998a; Sanudo-Pena et al. 1999b), a spinal cord area associated with movement.

7.2 Synthetic Enzymes

DAGLα mRNA is widely expressed in spinal dorsal horn neurons (Nyilas et al. 2009). Similar to CB_1 receptors, high levels DAGLα protein have been found to be localized at the superficial dorsal horn. High-resolution electron microscopy demonstrated a postsynaptic localization of DAGLα at nociceptive synapses, which is juxtaposed to the presynaptic CB_1-containing excitatory primary afferents (Nyilas et al. 2009) (Fig. 1B, C). Interestingly, postsynaptic DAGLα is co-localized with mGluR5, whose activation induces 2-AG biosynthesis (Nyilas et al. 2009).

7.3 Degradative Enzymes

FAAH has been found in the cell bodies of ventral horn neurons (Tsou et al. 1998b). The presence of FAAH and CB_1 receptors in circuits involved in spinal reflexes may underlie the antispastic effects of (endo)cannabinoids.

8 Summary, Concluding Thoughts, and Future Directions

As this brief review has shown, the components of the endocannabinoid system (ECS) are widespread throughout the CNS. Endocannabinoids have a major role as retrograde transmitters in many brain regions, although often with region-specific specialization as discussed above. Thus, in most brain regions, the highest levels of CB_1 receptors are found presynaptically, while endocannabinoid synthesizing enzymes are present postsynaptically. Interestingly, some endocannabinoid degrading enzymes are found presynaptically (e.g., MAGL) and others postsynaptically (e.g., ABHD6 and FAAH), suggesting specialized roles of each in endocannabinoid degradation (Gulyas et al. 2004; Blankman et al. 2007), and introducing an extra layer of complexity in endocannabinoid metabolism. A variation on the presynaptic localization of CB_1 is the somatic expression of CB_1, most commonly in some cortical and cerebellar neurons, where endocannabinoid signaling is cell autonomous. Future studies are needed to better define the distribution of some less-well studied (putative) ECS components, including α/β-hydrolase domain 4 (ABHD4) (Simon and Cravatt 2006), glycerophosphodiesterase 1 (GDE1) (Simon and Cravatt 2006, 2010), ABHD6 (Blankman et al. 2007; Marrs et al. 2010), α/β-hydrolase domain 12 (ABHD12) (Blankman et al. 2007), cannabinoid receptor interacting protein 1a (CRIP1a) (Niehaus et al. 2007), and DAGLβ (Gao et al. 2010).

References

Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, Brenner GJ, Rubino T, Michalski CW, Marsicano G, Monory K, Mackie K, Marian C, Batkai S, Parolaro D, Fischer MJ, Reeh P, Kunos G, Kress M, Lutz B, Woolf CJ, Kuner R (2007) Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. Nat Neurosci 10:870–879

Aguado T, Monory K, Palazuelos J, Stella N, Cravatt B, Lutz B, Marsicano G, Kokaia Z, Guzman M, Galve-Roperh I (2005) The endocannabinoid system drives neural progenitor proliferation. FASEB J 19:1704–1706

Amaya F, Shimosato G, Kawasaki Y, Hashimoto S, Tanaka Y, Ji RR, Tanaka M (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

Azad SC, Marsicano G, Eberlein I, Putzke J, Zieglgansberger W, Spanagel R, Lutz B (2001) Differential role of the nitric oxide pathway on delta(9)-THC-induced central nervous system effects in the mouse. Eur J Neurosci 13:561–568

Benard G et al (2012) Mitochondrial CB(1) receptors regulate neuronal energy metabolism. Nat Neurosci 15:558–564

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello-A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di Marzo V, Doherty P (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163:463–468

Blankman JL, Simon GM, Cravatt BF (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol 14:1347–1356

Bodor AL, Katona I, Nyiri G, Mackie K, Ledent C, Hajos N, Freund TF (2005) Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. J Neurosci 25:6845–6856

Bosier B, Bellocchio L, Metna-Laurent M, Soria-Gomez E, Matias I, Hebert-Chatelain E, Cannich A, Maitre M, Leste-Lasserre T, Cardinal P, Mendizabal-Zubiaga J, Canduela MJ, Reguero L, Hermans E, Grandes P, Cota D, Marsicano G (2013) Astroglial CB1 cannabinoid receptors regulate leptin signaling in mouse brain astrocytes. Mol Metab 2:393–404

Breivogel CS, Childers SR (1998) The functional neuroanatomy of brain cannabinoid receptors. Neurobiol Dis 5:417–431

Brenowitz SD, Regehr WG (2003) Calcium dependence of retrograde inhibition by endocannabinoids at synapses onto Purkinje cells. J Neurosci 23:6373–6384

Bridges D, Rice AS, Egertova M, Elphick MR, Winter J, Michael GJ (2003) Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. Neuroscience 119:803–812

Cardinal P, Bellocchio L, Clark S, Cannich A, Klugmann M, Lutz B, Marsicano G, Cota D (2012) Hypothalamic CB1 cannabinoid receptors regulate energy balance in mice. Endocrinology 153:4136–4143

Chen K, Ratzliff A, Hilgenberg L, Gulyas A, Freund TF, Smith M, Dinh TP, Piomelli D, Mackie K, Soltesz I (2003) Long-term plasticity of endocannabinoid signaling induced by developmental febrile seizures. Neuron 39:599–611

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci USA 98:9371–9376

Cristino L, Starowicz K, De Petrocellis L, Morishita J, Ueda N, Guglielmotti V, Di Marzo V (2008) Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. Neuroscience 151:955–968

Czesnik D, Schild D, Kuduz J, Manzini I (2007) Cannabinoid action in the olfactory epithelium. Proc Natl Acad Sci USA 104:2967–2972

Degroot A, Kofalvi A, Wade MR, Davis RJ, Rodrigues RJ, Rebola N, Cunha RA, Nomikos GG (2006) CB1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. Mol Pharmacol 70:1236–1245

den Boon FS, Chameau P, Schaafsma-Zhao Q, van Aken W, Bari M, Oddi S, Kruse CG, Maccarrone M, Wadman WJ, Werkman TR (2012) Excitability of prefrontal cortical pyramidal neurons is modulated by activation of intracellular type-2 cannabinoid receptors. Proc Natl Acad Sci USA 109:3534–3539

Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D (1994) Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372:686–691

Diana MA, Levenes C, Mackie K, Marty A (2002) Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. J Neurosci 22:200–208

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002) Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci USA 99:10819–10824

Dinh TP, Kathuria S, Piomelli D (2004) RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. Mol Pharmacol 66:1260–1264

Dowie MJ, Grimsey NL, Hoffman T, Faull RL, Glass M (2014) Cannabinoid receptor CB2 is expressed on vascular cells, but not astroglial cells in the post-mortem human Huntington's disease brain. J Chem Neuroanat 59–60:62–71

Egertova M, Elphick MR (2000) Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. J Comp Neurol 422:159–171

Egertova M, Cravatt BF, Elphick MR (2000) Fatty acid amide hydrolase expression in rat choroid plexus: possible role in regulation of the sleep-inducing action of oleamide. Neurosci Lett 282:13–16

Egertova M, Cravatt BF, Elphick MR (2003) Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119:481–496

Egertova M, Giang DK, Cravatt BF, Elphick MR (1998) A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc Biol Sci 265:2081–2085

Egertova M, Simon GM, Cravatt BF, Elphick MR (2008) Localization of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression in mouse brain: A new perspective on N-acylethanolamines as neural signaling molecules. J Comp Neurol 506:604–615

Eggan SM, Lewis DA (2007) Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. Cereb Cortex 17:175–191

Farquhar-Smith WP, Egertova M, Bradbury EJ, McMahon SB, Rice AS, Elphick MR (2000) Cannabinoid CB(1) receptor expression in rat spinal cord. Mol Cell Neurosci 15:510–521

Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 83:1017–1066

Fukudome Y, Ohno-Shosaku T, Matsui M, Omori Y, Fukaya M, Tsubokawa H, Taketo MM, Watanabe M, Manabe T, Kano M (2004) Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling. Eur J Neurosci 19:2682–2692

Galve-Roperh I, Aguado T, Palazuelos J, Guzman M (2007) The endocannabinoid system and neurogenesis in health and disease. Neuroscientist 13:109–114

Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, Shen R, Zhang MY, Strassle BW, Lu P, Mark L, Piesla MJ, Deng K, Kouranova EV, Ring RH, Whiteside GT, Bates B, Walsh FS, Williams G, Pangalos MN, Samad TA, Doherty P (2010) Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J Neurosci 30:2017–2024

Gebremedhin D, Lange AR, Campbell WB, Hillard CJ, Harder DR (1999) Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. Am J Physiol 276:H2085–2093

Gerdeman G, Lovinger DM (2001) CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85:468–471

Gifford AN, Ashby CR Jr (1996) Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212–2, and is potentiated by the cannabinoid antagonist, SR 141716A. J Pharmacol Exp Ther 277:1431–1436

Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77:299–318

Gregg LC, Jung KM, Spradley JM, Nyilas R, Suplita RL 2nd, Zimmer A, Watanabe M, Mackie K, Katona I, Piomelli D, Hohmann AG (2012) Activation of type 5 metabotropic glutamate receptors and diacylglycerol lipase-alpha initiates 2-arachidonoylglycerol formation and endocannabinoid-mediated analgesia. J Neurosci 32:9457–9468

Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF (2004) Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci 20:441–458

Guo Y, Wang H, Okamoto Y, Ueda N, Kingsley PJ, Marnett LJ, Schmid HH, Das SK, Dey SK (2005) N-acylphosphatidylethanolamine-hydrolyzing phospholipase D is an important determinant of uterine anandamide levels during implantation. J Biol Chem 280:23429–23432

Hajos N, Katona I, Naiem SS, MacKie K, Ledent C, Mody I, Freund TF (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci 12:3239–3249

Haring M, Marsicano G, Lutz B, Monory K (2007) Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience 146:1212–1219

Harkany T, Mackie K, Doherty P (2008) Wiring and firing neuronal networks: endocannabinoids take center stage. Curr Opin Neurobiol 18:338–345

Harkany T, Hartig W, Berghuis P, Dobszay MB, Zilberter Y, Edwards RH, Mackie K, Ernfors P (2003) Complementary distribution of type 1 cannabinoid receptors and vesicular glutamate transporter 3 in basal forebrain suggests input-specific retrograde signalling by cholinergic neurons. Eur J Neurosci 18:1979–1992

Hebert-Chatelain E, Reguero L, Puente N, Lutz B, Chaouloff F, Rossignol R, Piazza PV, Benard G, Grandes P, Marsicano G (2014a) Studying mitochondrial CB1 receptors: yes we can. Mol Metab 3:339

Hebert-Chatelain E, Reguero L, Puente N, Lutz B, Chaouloff F, Rossignol R, Piazza PV, Benard G, Grandes P, Marsicano G (2014b) Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB1 receptor. Mol Metab 3:495–504

Herkenham M, Nauta WJ (1977) Afferent connections of the habenular nuclei in the rat. A horseradish peroxidase study, with a note on the fiber-of-passage problem. J Comp Neurol 173:123–146

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563–583

Hill EL, Gallopin T, Ferezou I, Cauli B, Rossier J, Schweitzer P, Lambolez B (2007) Functional CB1 receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons. J Neurophysiol 97:2580–2589

Hohmann AG, Herkenham M (1998) Regulation of cannabinoid and mu opioid receptors in rat lumbar spinal cord following neonatal capsaicin treatment. Neurosci Lett 252:13–16

Hohmann AG, Herkenham M (1999) Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 90:923–931

Hohmann AG, Herkenham M (2000) Localization of cannabinoid CB(1) receptor mRNA in neuronal subpopulations of rat striatum: a double-label in situ hybridization study. Synapse 37:71–80

Hohmann AG, Briley EM, Herkenham M (1999a) Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. Brain Res 822:17–25

Hohmann AG, Tsou K, Walker JM (1999b) Intrathecal cannabinoid administration suppresses noxious stimulus-evoked Fos protein-like immunoreactivity in rat spinal cord: comparison with morphine. Zhongguo Yao Li Xue Bao 20:1132–1136

Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D (2005) An endocannabinoid mechanism for stress-induced analgesia. Nature 435:1108–1112

Hu SS, Arnold A, Hutchens JM, Radicke J, Cravatt BF, Wager-Miller J, Mackie K, Straiker A (2010) Architecture of cannabinoid signaling in mouse retina. J Comp Neurol 518:3848–3866

Jansen EM, Haycock DA, Ward SJ, Seybold VS (1992) Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. Brain Res 575:93–102

Jennings EA, Vaughan CW, Christie MJ (2001) Cannabinoid actions on rat superficial medullary dorsal horn neurons in vitro. J Physiol 534:805–812

Julian MD, Martin AB, Cuellar B, Rodriguez De Fonseca F, Navarro M, Moratalla R, Garcia-Segura LM (2003) Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. Neuroscience 119:309–318

Kamprath K, Romo-Parra H, Haring M, Gaburro S, Doengi M, Lutz B, Pape HC (2011) Short-term adaptation of conditioned fear responses through endocannabinoid signaling in the central amygdala. Neuropsychopharmacology 36:652–663

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M (2009) Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89:309–380

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 19:4544–4558

Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, Freund TF (2001) Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci 21:9506–9518

Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci 26:5628–5637

Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, Ohno-Shosaku T, Kano M (2006) The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. J Neurosci 26:2991–3001

Klausberger T, Marton LF, O'Neill J, Huck JH, Dalezios Y, Fuentealba P, Suen WY, Papp E, Kaneko T, Watanabe M, Csicsvari J, Somogyi P (2005) Complementary roles of cholecystokinin- and parvalbumin-expressing GABAergic neurons in hippocampal network oscillations. J Neurosci 25:9782–9793

Kofalvi A, Rodrigues RJ, Ledent C, Mackie K, Vizi ES, Cunha RA, Sperlagh B (2005) Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: a combined immunochemical and pharmacological analysis. J Neurosci 25:2874–2884

Kortleven C, Fasano C, Thibault D, Lacaille JC, Trudeau LE (2011) The endocannabinoid 2-arachidonoylglycerol inhibits long-term potentiation of glutamatergic synapses onto ventral tegmental area dopamine neurons in mice. Eur J Neurosci 33:1751–1760

Kreitzer AC, Regehr WG (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29:717–727

Kreitzer AC, Malenka RC (2007) Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models. Nature 445:643–647

Kreitzer AC, Carter AG, Regehr WG (2002) Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. Neuron 34:787–796

Krowicki ZK, Moerschbaecher JM, Winsauer PJ, Digavalli SV, Hornby PJ (1999) Delta9-tetrahydrocannabinol inhibits gastric motility in the rat through cannabinoid CB1 receptors. Eur J Pharmacol 371:187–196

Ludanyi A, Hu SS, Yamazaki M, Tanimura A, Piomelli D, Watanabe M, Kano M, Sakimura K, Magloczky Z, Mackie K, Freund TF, Katona I (2011) Complementary synaptic distribution of enzymes responsible for synthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in the human hippocampus. Neuroscience 174:50–63

Lutz B (2004) On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures. Biochem Pharmacol 68:1691–1698

Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T (2014) Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci 15:786–801

Mackie K (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol 168:299–325

Maejima T, Ohno-Shosaku T, Kano M (2001) Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. Neurosci Res 40:205–210

Mailleux P, Vanderhaeghen JJ (1992) Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. Neuroscience 48:655–668

Maldonado R, Valverde O, Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 29:225–232

Manning CF, Bundros AM, Trimmer JS (2012) Benefits and pitfalls of secondary antibodies: why choosing the right secondary is of primary importance. PLoS One 7, e38313

Marinelli S, Pacioni S, Cannich A, Marsicano G, Bacci A (2009) Self-modulation of neocortical pyramidal neurons by endocannabinoids. Nat Neurosci 12:1488–1490

Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, Bodor AL, Muccioli GG, Hu SS, Woodruff G, Fung S, Lafourcade M, Alexander JP, Long JZ, Li W, Xu C, Moller T, Mackie K, Manzoni OJ, Cravatt BF, Stella N (2010) The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci 13:951–957

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

Marsicano G, Kuner R (2008) Cannabinoids and the brain. Springer, Boston, MA

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530–534

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van der Stelt M, Lopez-Rodriguez ML, Casanova E, Schutz G, Zieglgansberger W, Di Marzo V, Behl C, Lutz B (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science 302:84–88

Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327:535–550

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

Matyas F, Urban GM, Watanabe M, Mackie K, Zimmer A, Freund TF, Katona I (2008) Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. Neuropharmacology 54:95–107

Melis M, Pillolla G, Luchicchi A, Muntoni AL, Yasar S, Goldberg SR, Pistis M (2008) Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. J Neurosci 28:13985–13994

Michel MC, Wieland T, Tsujimoto G (2009) How reliable are G-protein-coupled receptor antibodies? Naunyn Schmiedebergs Arch Pharmacol 379:385–388

Mitrirattanakul S, Ramakul N, Guerrero AV, Matsuka Y, Ono T, Iwase H, Mackie K, Faull KF, Spigelman I (2006) Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain. Pain 126:102–114

Monory K, Massa F, Egertova M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M, Long J, Rubenstein JL, Goebbels S, Nave KA, During M, Klugmann M, Wolfel B, Dodt HU, Zieglgansberger W, Wotjak CT, Mackie K, Elphick MR, Marsicano G, Lutz B (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron 51:455–466

Morishita J, Okamoto Y, Tsuboi K, Ueno M, Sakamoto H, Maekawa N, Ueda N (2005) Regional distribution and age-dependent expression of N-acylphosphatidylethanolamine-hydrolyzing phospholipase D in rat brain. J Neurochem 94:753–762

Morisset V, Urban L (2001) Cannabinoid-induced presynaptic inhibition of glutamatergic EPSCs in substantia gelatinosa neurons of the rat spinal cord. J Neurophysiol 86:40–48

Morozov YM, Dominguez MH, Varela L, Shanabrough M, Koch M, Horvath TL, Rakic P (2013) Antibodies to cannabinoid type 1 receptor co- react with stomatin-like protein 2 in mouse brain mitochondria. Eur J Neurosci 38:2341–2348

Niehaus JL, Liu Y, Wallis KT, Egertova M, Bhartur SG, Mukhopadhyay S, Shi S, He H, Selley DE, Howlett AC, Elphick MR, Lewis DL (2007) CB1 cannabinoid receptor activity is modulated by the cannabinoid receptor interacting protein CRIP 1a. Mol Pharmacol 72:1557–1566

Nyilas R, Gregg LC, Mackie K, Watanabe M, Zimmer A, Hohmann AG, Katona I (2009) Molecular architecture of endocannabinoid signaling at nociceptive synapses mediating analgesia. Eur J Neurosci 29:1964–1978

Nyilas R, Dudok B, Urban GM, Mackie K, Watanabe M, Cravatt BF, Freund TF, Katona I (2008) Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. J Neurosci 28:1058–1063

Nyiri G, Cserep C, Szabadits E, Mackie K, Freund TF (2005a) CB1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. Neuroscience 136:811–822

Nyiri G, Szabadits E, Cserep C, Mackie K, Shigemoto R, Freund TF (2005b) GABAB and CB1 cannabinoid receptor expression identifies two types of septal cholinergic neurons. Eur J Neurosci 21:3034–3042

Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem 279:5298–5305

Pernia-Andrade AJ, Kato A, Witschi R, Nyilas R, Katona I, Freund TF, Watanabe M, Filitz J, Koppert W, Schuttler J, Ji G, Neugebauer V, Marsicano G, Lutz B, Vanegas H, Zeilhofer HU (2009) Spinal endocannabinoids and CB1 receptors mediate C-fiber-induced heterosynaptic pain sensitization. Science 325:760–764

Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, Watanabe M, Katona I, Patel S (2014) Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. Neuron 81:1111–1125

Rhodes KJ, Trimmer JS (2006) Antibodies as valuable neuroscience research tools versus reagents of mass distraction. J Neurosci 26:8017–8020

Robbe D, Alonso G, Duchamp F, Bockaert J, Manzoni OJ (2001) Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J Neurosci 21:109–116

Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL, Buzsaki G (2006) Cannabinoids reveal importance of spike timing coordination in hippocampal function. Nat Neurosci 9:1526–1533

Rodriguez JJ, Mackie K, Pickel VM (2001) Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. J Neurosci 21:823–833

Sales-Carbonell C, Rueda-Orozco PE, Soria-Gomez E, Buzsaki G, Marsicano G, Robbe D (2013) Striatal GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony. Proc Natl Acad Sci USA 110:719–724

Salio C, Fischer J, Franzoni MF, Conrath M (2002) Pre- and postsynaptic localizations of the CB1 cannabinoid receptor in the dorsal horn of the rat spinal cord. Neuroscience 110:755–764

Sanudo-Pena MC, Walker JM (1997) Role of the subthalamic nucleus in cannabinoid actions in the substantia nigra of the rat. J Neurophysiol 77:1635–1638

Sanudo-Pena MC, Tsou K, Walker JM (1999a) Motor actions of cannabinoids in the basal ganglia output nuclei. Life Sci 65:703–713

Sanudo-Pena MC, Strangman NM, Mackie K, Walker JM, Tsou K (1999b) CB1 receptor localization in rat spinal cord and roots, dorsal root ganglion, and peripheral nerve. Zhongguo Yao Li Xue Bao 20:1115–1120

Saper CB (2005) An open letter to our readers on the use of antibodies. J Comp Neurol 493:477–478

Schley M, Stander S, Kerner J, Vajkoczy P, Schupfer G, Dusch M, Schmelz M, Konrad C (2009) Predominant CB2 receptor expression in endothelial cells of glioblastoma in humans. Brain Res Bull 79:333–337

Simon GM, Cravatt BF (2006) Endocannabinoid biosynthesis proceeding through Glycerophospho-N-Acyl ethanolamine and a role for alpha/beta hydrolase 4 in this pathway. J Biol Chem 281:26465–72

Simon GM, Cravatt BF (2010) Characterization of mice lacking candidate N-acyl ethanolamine biosynthetic enzymes provides evidence for multiple pathways that contribute to endocannabinoid production in vivo. Mol Biosyst 6:1411–1418

Smith PB, Martin BR (1992) Spinal mechanisms of delta 9-tetrahydrocannabinol-induced analgesia. Brain Res 578:8–12

Stella N (2009) Endocannabinoid signaling in microglial cells. Neuropharmacology 56(Suppl 1):244–253

Stella N (2010) Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia 58:1017–1030

Storr MA, Sharkey KA (2007) The endocannabinoid system and gut-brain signalling. Curr Opin Pharmacol 7:575–582

Straiker A, Stella N, Piomelli D, Mackie K, Karten HJ, Maguire G (1999a) Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc Natl Acad Sci USA 96:14565–14570

Straiker AJ, Maguire G, Mackie K, Lindsey J (1999b) Localization of cannabinoid CB1 receptors in the human anterior eye and retina. Invest Ophthalmol Vis Sci 40:2442–2448

Suarez J, Bermudez-Silva FJ, Mackie K, Ledent C, Zimmer A, Cravatt BF, de Fonseca FR (2008) Immunohistochemical description of the endogenous cannabinoid system in the rat cerebellum and functionally related nuclei. J Comp Neurol 509:400–421

Swanson LW, Petrovich GD (1998) What is the amygdala? Trends Neurosci 21:323–331

Szabo B, Muller T, Koch H (1999) Effects of cannabinoids on dopamine release in the corpus striatum and the nucleus accumbens in vitro. J Neurochem 73:1084–1089

Szabo B, Siemes S, Wallmichrath I (2002) Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15:2057–2061

Tanda G, Pontieri FE, Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science 276:2048–2050

Tanimura A, Uchigashima M, Yamazaki M, Uesaka N, Mikuni T, Abe M, Hashimoto K, Watanabe M, Sakimura K, Kano M (2012) Synapse type-independent degradation of the endocannabinoid 2-arachidonoylglycerol after retrograde synaptic suppression. Proc Natl Acad Sci USA 109:12195–12200

Thomas EA, Cravatt BF, Danielson PE, Gilula NB, Sutcliffe JG (1997) Fatty acid amide hydrolase, the degradative enzyme for anandamide and oleamide, has selective distribution in neurons within the rat central nervous system. J Neurosci Res 50:1047–1052

Tsou K, Mackie K, Sanudo-Pena MC, Walker JM (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience 93:969–975

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998a) Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83:393–411

Tsou K, Nogueron MI, Muthian S, Sanudo-Pena MC, Hillard CJ, Deutsch DG, Walker JM (1998b) Fatty acid amide hydrolase is located preferentially in large neurons in the rat central nervous system as revealed by immunohistochemistry. Neurosci Lett 254:137–140

Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N (2005) Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem 280:11082–11092

Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M (2007) Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. J Neurosci 27:3663–3676

Van Sickle MD, Oland LD, Mackie K, Davison JS, Sharkey KA (2003) Delta9-tetrahydrocannabinol selectively acts on CB1 receptors in specific regions of dorsal vagal complex to inhibit emesis in ferrets. Am J Physiol Gastrointest Liver Physiol 285:G566–576

Van Sickle MD, Oland LD, Ho W, Hillard CJ, Mackie K, Davison JS, Sharkey KA (2001) Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. Gastroenterology 121:767–774

Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, Molinari M, Maccarrone M (2009) Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. J Neurosci 29:4564–4570

Walker JM, Huang SM, Strangman NM, Tsou K, Sanudo-Pena MC (1999) Pain modulation by release of the endogenous cannabinoid anandamide. Proc Natl Acad Sci USA 96:12198–12203

Walter L, Franklin A, Witting A, Moller T, Stella N (2002) Astrocytes in culture produce anandamide and other acylethanolamides. J Biol Chem 277:20869–20876

Welch SP, Thomas C, Patrick GS (1995) Modulation of cannabinoid-induced antinociception after intracerebroventricular versus intrathecal administration to mice: possible mechanisms for interaction with morphine. J Pharmacol Exp Ther 272:310–321

Wenger T, Moldrich G, Furst S (2003) Neuromorphological background of cannabis addiction. Brain Res Bull 61:125–128

Wittmann G, Deli L, Kallo I, Hrabovszky E, Watanabe M, Liposits Z, Fekete C (2007) Distribution of type 1 cannabinoid receptor (CB1)-immunoreactive axons in the mouse hypothalamus. J Comp Neurol 503:270–279

Yazulla S, Studholme KM, McIntosh HH, Deutsch DG (1999) Immunocytochemical localization of cannabinoid CB1 receptor and fatty acid amide hydrolase in rat retina. J Comp Neurol 415:80–90

Yoshida T, Fukaya M, Uchigashima M, Miura E, Kamiya H, Kano M, Watanabe M (2006) Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. J Neurosci 26:4740–4751

Yoshida T, Uchigashima M, Yamasaki M, Katona I, Yamazaki M, Sakimura K, Kano M, Yoshioka M, Watanabe M (2011) Unique inhibitory synapse with particularly rich endocannabinoid signaling machinery on pyramidal neurons in basal amygdaloid nucleus. Proc Natl Acad Sci USA 108:3059–3064

Zhang HY, Gao M, Liu QR, Bi GH, Li X, Yang HJ, Gardner EL, Wu J, Xi ZX (2014) Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. Proc Natl Acad Sci USA 111:E5007–5015

Zhang M, Adler MW, Abood ME, Ganea D, Jallo J, Tuma RF (2009) CB2 receptor activation attenuates microcirculatory dysfunction during cerebral ischemic/reperfusion injury. Microvasc Res 78:86–94

Zhao Y, Tzounopoulos T (2011) Physiological activation of cholinergic inputs controls associative synaptic plasticity via modulation of endocannabinoid signaling. J Neurosci 31:3158–3168

Zhao Y, Rubio ME, Tzounopoulos T (2009) Distinct functional and anatomical architecture of the endocannabinoid system in the auditory brainstem. J Neurophysiol 101:2434–2446

Zhao Y, Rubio M, Tzounopoulos T (2011) Mechanisms underlying input-specific expression of endocannabinoid-mediated synaptic plasticity in the dorsal cochlear nucleus. Hear Res 279:67–73

Zhong P, Pan B, Gao XP, Blankman JL, Cravatt BF, Liu QS (2011) Genetic deletion of monoacylglycerol lipase alters endocannabinoid-mediated retrograde synaptic depression in the cerebellum. J Physiol 589:4847–4855