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Programming and reprogramming neural cells by (endo-) cannabinoids: from physiological rules to emerging therapies

Mauro Maccarrone^{*,‡,+}, Manuel Guzman[§], Ken Mackie[¶], Patrick Doherty[#], and Tibor Harkany^{&,\$,+}

^{*}School of Medicine and Center of Integrated Research, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, I-00128 Rome, Italy

[‡]European Center for Brain Research/Santa Lucia Foundation, Via del Fosso di Fiorano 65, I-00143 Rome, Italy

[§]Department of Biochemistry and Molecular Biology I and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, Complutense University, E-28040 Madrid, Spain

[¶]Department of Psychological & Brain Sciences, Indiana University, 702 N Walnut Grove Ave, Bloomington, IN 47405-2204, USA

[#]Wolfson Centre for Age-Related Diseases, King's College London SE1 1UL, United Kingdom,

[&]Division of Molecular Neuroscience, Department of Medical Biochemistry & Biophysics, Scheeles väg 1:A1, Karolinska Institutet, SE-17177 Stockholm Sweden

^{\$}Department of Molecular Neurosciences, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, A-1090 Vienna, Austria

Abstract

Of the many signaling lipids, endocannabinoids are being increasingly recognized as having an important involvement in neuronal and glial development. Recent experimental evidence suggests that during neuronal differentiation, endocannabinoid signaling undergoes dynamic reorganization that results in a fundamental role-switch from the prenatal determination of cell fate to the homeostatic regulation of synaptic neurotransmission and bioenergetics in the mature nervous system. These studies also offer novel insights into neuropsychiatric disease mechanisms, and contribute to the public debate about the benefits and risks of cannabis use during pregnancy and in adolescence.

Keywords

adolescence; chemotaxis; drug abuse; localization; marijuana; migration; neural progenitor; synaptogenesis

⁺Address for correspondence: **Tibor Harkany** (at the Karolinska Institutet); Tel: +46 8 524 87656; Fax: +46 8 341 960, Tibor.Harkany@ki.se or Tibor.Harkany@meduniwien.ac.at or **Mauro Maccarrone** (at Campus Bio-Medico, University of Rome); Tel: +39 06 225 419169; Fax: +39 06 225 41456; m.maccarrone@unicampus.it.

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The brain's capacity for information processing relies on the number, molecular and functional diversity of neurons, their topographically precise connectivity, and their metabolic and signaling interactions with glial cells. The design logic of the developing nervous system encompasses a kaleidoscope of interacting signaling pathways individually orchestrated along unique temporal and spatial scales. During brain development, neural progenitor proliferation and asymmetric division, and the positioning and molecular diversification of neuronal and glial progenies are modulated by both cell autonomous and cell-cell interactions of morphogenetic signals vital to building complex tissues. Amongst these structurally and functionally diverse signaling domains, bioactive signal lipids (e.g., phospholipids, sphingolipids, glycolipids, prostanoids) are widely recognized as critical for neuronal and glial differentiation, as well as for synaptic plasticity¹⁻³.

Recent work has highlighted similar, yet unexpectedly diverse roles for the endocannabinoid family of small signaling lipids, *N*-acyl-amines and 2-acyl-glycerols typically containing an arachidonoyl moiety^{4,5}, in the developing nervous system. Indeed, contemporary evidence outlines a continuum of action by endocannabinoids, encompassing early stages of embryo development and implantation⁶, nervous system development⁷, bioenergetics⁸ and intercellular communication^{9,10} in the adult, including adult neurogenesis^{11,12}. More specifically for the nervous system, endocannabinoid signaling appears as a multimodal communication cassette involved in the patterning of the very neuronal connections whose synaptic efficacy, once mature, is often modulated by endocannabinoids⁷ (Figure 1a). The widespread nature of endocannabinoid modulation of both excitatory and inhibitory synaptic neurotransmission in the postnatal brain⁹ and spinal cord¹³ (Figure 1b) suggests that developmental rules might place these small signaling lipids into a critical arch of the molecular machinery controlling synaptic neurotransmission.

The family of endocannabinoids and their structural analogues¹⁴ potentially includes hundreds of bioactive molecules. This review will focus on 2-arachidonoylglycerol (2-AG), the most abundant mammalian endocannabinoid affecting synaptic neurotransmission^{15,16}, and to a lesser extent on anandamide (AEA)¹⁷, a mixed endovanilloid/endocannabinoid ligand. 2-AG, AEA and related lipids can stimulate CB₁ and CB₂ cannabinoid receptors (CB₁R/CB₂Rs) and the nuclear fatty acid receptors peroxisome proliferator-activated receptor α and γ (PPAR α and PPAR γ)¹⁸. 2-AG generally has higher efficacy at CB₁R/CB₂Rs, whereas AEA is a low efficacy agonist that can act as a partial agonist (or even antagonist) in tissues with low receptor reserve or at receptors inefficiently coupled to downstream effectors. AEA and 2-AG can stimulate PPARs at high concentrations, though related molecules such as *N*-oleoylethanolamine do so more potently. Moreover, AEA can engage TRPV1 and G protein-coupled receptor 55 (GPR55)¹⁸.

Although gaps persist in our knowledge of endocannabinoid action during formation of the subcortical forebrain, midbrain and spinal cord, the studies highlighted herein define multiple steps of cortical and cerebellar development modulated by endocannabinoids elucidated through the use of an impressive array of genetic and cellular tools¹⁹⁻²⁴, evolutionary deductions^{25,26}, as well as experimental models in invertebrates²⁷, non-mammalian vertebrates^{28,29}, rodents³⁰⁻³⁴ and human fetal tissues³⁵⁻³⁸. The evidence for endocannabinoid involvement in fundamental developmental processes comes through the

combination of sophisticated mouse genetics^{21,39}, neurophysiology⁴⁰, and the study of gene polymorphisms for CB₁R (*CNR1*), CB₂R (*CNR2*), α/β -hydrolase domain-containing 12 serine hydrolase (*ABHD12*) and fatty-acid amide hydrolase (*FAAH*) in humans with diseases that are thought to have a developmental origin, such as schizophrenia, bipolar disorder, drug addiction, metabolic disorders and neurodegeneration⁴¹⁻⁴⁵. The latter observations support causality between dysregulated endocannabinoid signaling and neuropsychiatric illnesses. Thus, the interdisciplinary nature of this review provides a translational framework bridging (at least) two key areas of neuroscience: neurophysiology and psychiatric/addiction research.

Endocannabinoid signaling at CB₂R/CB₁Rs in neural progenitors and postmitotic neurons^{11,24,46} is recognized as the primary molecular substrate for phytocannabinoids⁴⁷, most prominently for psychoactive Δ^9 -tetrahydrocannabinol (THC). In this context, and after discussing the multifarious roles of endocannabinoid signaling in the prenatal and perinatal brain, we address endocannabinoid contributions to regulating adult neurogenesis as cannabis use might impact the continued production of new neurons in adolescents. Next, we examine the molecular mechanisms and health benefits of exploiting the endocannabinoid system as a druggable target in pathologies arising from the loss of cell proliferation control, particularly gliomas. Lastly, we will highlight differential sensitivities of precisely orchestrated physiological processes *versus* disease pathomechanisms to help place the ongoing public debate on the use of THC during pregnancy and in adolescents into a more realistic context.

Molecular logic of endocannabinoid action

Much of what we know about the molecular organization of endocannabinoid signaling during neuronal development (schematically depicted in Figure 1a) comes from the comparative analysis of successive developmental stages in rodents (Figure 2a-d)^{11,22,32,33,46,48}. Arrangements at mature synapses are such that 2-AG synthesis is postsynaptic^{15,16,49} with *sn*-1-diacylglycerol lipase α (DAGL α) enriched in the “*perisynaptic annulus*” (that is, 50-100 nm from the postsynaptic density⁵⁰), allowing the fast coupling of postsynaptic metabotropic receptor activation to Ca²⁺/phospholipase C β -dependent 2-AG synthesis⁵¹ (Figure 1b). Diffusing retrogradely across the synapse, endocannabinoids activate presynaptic CB₁Rs, typically signaling *via* G_i proteins to inhibit synaptic neurotransmission (Figure 1b). 2-AG is then largely inactivated by presynaptic monoacylglycerol lipase (MAGL)⁵², with a possible contribution from α/β -hydrolase domain-containing 6 (ABHD6) and ABHD12 serine hydrolases^{45,53} partitioned to postsynaptic sites (Figure 1b). The situation with AEA is more complex. AEA can be produced postsynaptically to inhibit synaptic transmission in a retrograde fashion like 2-AG⁵⁴. Alternatively, *N*-arachidonylethanolamine-selective phospholipase D (NAPE-PLD) may produce AEA presynaptically, which then acts at postsynaptic TRPV1^{55,56} and is inactivated by postsynaptic FAAH⁵⁷. Notably, and in accord with the tripartite synapse hypothesis, perisynaptic astrocytes often contain MAGL (Figure 1b), thus forming a barrier that may limit 2-AG spread beyond its intended site of action (20-100 μ m in a temperature-sensitive manner)^{58,59}.

At least *four premises* should be considered to appreciate the unique modes of endocannabinoid signaling during brain development:

- i) In differentiating neural tissues, endocannabinoids can adopt a primarily *autocrine/cell-autonomous* mechanism of action^{19,48,60}, in contrast to retrograde signaling by endocannabinoids at adult synapses, which is a *paracrine* mechanism⁹. If receptor location remains constant, this implies positional differences in the localization of enzymes to influence 2-AG bioavailability. An example is DAGL α , which switches from an axonal to postsynaptic localization^{48,51} upon differentiation of the presynapse and commencement of synaptic neurotransmission⁶¹. For cell-autonomous endocannabinoid signals to drive neural progenitor proliferation, including pathological situations during tumorigenesis (*see below*), the intracellular transport and partitioning of DAGL α are proximal to the localization of CB₁Rs/CB₂Rs⁴⁶, poised for receptor activation by lateral ligand diffusion in the plasmalemma (Figure 1 and 3a). DAGL α , and to a lesser extent NAPE-PLD, are found in lipid rafts⁶², membrane specializations allowing CB₁R accumulation and focused signal transduction by recruitment of effector kinases⁶³. Following neuronal polarization and during directional axonal growth, 2-AG is protected from ligand degradation⁴⁸ by a mechanism that increases MAGL protein turnover⁶⁴ in motile neurite domains, particularly the growth cone (Figure 3a). High levels of axonal MAGL serves to prevent the premature, ectopic activation of CB₁Rs (and perhaps CB₂Rs⁶⁵) during axonal transport. Thus, endocannabinoid “hot spots” can be predicted to exist for focal signaling events.
- ii) Neural progenitors, in contrast to differentiated neurons, commonly co-express CB₁Rs and CB₂Rs. Pharmacological and histochemical evidence converge on predominant CB₂R expression in the subventricular zone of the cerebral cortex¹¹ (Figure 2a). Based on the simultaneous expression of CB₂R and DAGL α/β in neural progenitors, it is likely that autocrine endocannabinoid signaling supports asymmetric cell division, cell-cycle exit and long-range migration of the ensuing progenies^{11,46,60}. Upon commitment to a neuronal fate, a CB₂R-to-CB₁R switch occurs¹¹: CB₁R levels become up-regulated at the expense of CB₂Rs (Figure 2b,d). Although direct experimental evidence for causality is as yet lacking, experimental data from various vertebrate species^{28,29,66,67} suggest that early CB₁R expression might promote neuronal polarization and the commencement of long-range cell migration. The down-regulation of DAGL α/β expression that occurs following neuronal specification can be viewed as a pivotal step to increase the reliance of postmitotic neurons on extracellular 2-AG produced by, e.g., pyramidal cells in the cortical plate⁵¹, acting as positional cues (Figure 2a,c).
- iii) As neurogenesis precedes gliogenesis⁶⁸, the spatial spread of endocannabinoids in the fetal brain is less restricted than in adult brain. Thus, physiologically relevant ligand concentrations could affect, in bulk, cohorts of migrating neurons or axons during pathfinding⁶⁴ (Figure 2a,c). This suggests wider roles for endocannabinoids in cell-cell interactions than previously thought.

Nevertheless, it remains to be established whether these signaling lipids preferentially modulate contact guidance or indeed are even extracellularly released.

- iv) Enzymatic inactivation is a rate-limiting step for endocannabinoid signaling in the developing nervous system^{48,69}. Significant levels of FAAH and MAGL have been demonstrated in cultured neural progenitors^{19,20} and radial glia^{48,70,71}, which generate postmitotic cortical neurons^{72,73}, as well as act as cellular scaffolds for radial migration of neurons⁷⁴. Since radial glia produce a checkerboard-like patterned map converting neural progenitor distribution along the ventricular wall into a three-dimensional neuronal protomap in the cortical plate⁷³, this cell type is ideally positioned to limit endocannabinoid diffusion (Figure 2a). Furthermore, thalamocortical axons accumulate MAGL^{33,48}, producing corridors for CB₁R-containing corticothalamic axons during the corticothalamo-thalamocortical handshake. This MAGL localization can both limit axonal spread in the prospective internal capsule (Figure 3b), as well as delineate migratory routes for CB₁R-expressing cortical interneurons^{66,75}. Overall, the molecular architecture of endocannabinoid signaling networks can modulate cell proliferation, as well as the migration, polarization and synaptogenesis of post-mitotic neurons in the cerebral cortex.

Cannabinoid receptors in fetal brain

Although the role of CB₁R, CB₂R, TRPV1 and GPR55 has been examined in prenatal and/or postnatal neurodevelopment, including cell survival and tumorigenic transformation⁷⁶⁻⁸², a role for endocannabinoid interactions with PPARs remains to be established. Given the predominance of CB₁R mRNA and protein expression in the fetal nervous system of rodents^{46,83} and humans⁸⁴, we focus on signal transduction, heteromerization and upstream regulation of CB₁R levels and activity.

CB₁Rs primarily couple to G_{i/o} proteins, and commonly signal by inhibition of adenylyl cyclase and of certain voltage-dependent Ca²⁺ channels, and activation of some K⁺ channels (for review see ref.¹⁸). In stem and glial cells and neurons, CB₁Rs also activate the phosphatidylinositol-3-kinase (PI3K)-Akt/PKB-mammalian target of rapamycin (mTOR) cascade along with several mitogen-activated protein kinase pathways (e.g., ERK, p38, and JNK^{38,85}). Although these signaling events have recently been reviewed in detail^{70,86}, important concepts are that PI3K and Akt recruitment contributes to neuronal polarization by promoting neurite outgrowth⁸⁷, mTOR signaling regulates a proneural transcriptional cascade of Pax6 and Tbr2 for expansion of the cortical progenitor pool in mice^{32,46,88}, while JNK1 links CB₁R activity to cytoskeletal instability to determine the morphological phenotypes of neurons³⁸ (Figure 3a).

Additional modes of CB₁R (and likely CB₂R) signaling enrich its complexity and cell stage-specific impact. These include sequestration of G proteins⁸⁹, shifting the balance in the landscape of signaling by co-existent GPCRs, or heteromerization with other GPCRs⁹⁰ inducing G protein switching and differential pharmacology⁹¹. From a developmental standpoint, CB₁R interplay with neurotrophin signaling appears of particular significance.

Firstly, CB₁Rs can trans-activate certain receptor tyrosine kinases (Trks)⁹², particularly TrkB in the absence of brain-derived neurotrophic factor (BDNF)⁹³ in a mechanism involving Src kinase activation, as well as signaling *via* β -arrestins⁹⁴. Secondly, BDNF can sensitize CB₁Rs, and induce signal transduction (ERK and Akt phosphorylation) at otherwise sub-threshold levels of exogenously-applied agonists⁹⁵. Thirdly, 2-AG signaling at CB₁Rs can act as an essential effector of neurotrophin signaling^{69,96}. This concept (Figure 3a) was introduced when fibroblast growth factor receptor (FGFR) activation by neural cell adhesion molecules was shown to couple to neurite outgrowth in a CB₁R-dependent manner⁹⁶. Mechanistically, FGFR activation induces phospholipase C γ (PLC γ) activation, which generates arachidonoyl-containing diacylglycerol (DAG) for conversion to 2-AG by DAGL α/β and cell-autonomous action on CB₁Rs in motile growth cones. More recently, a similar mechanism was described for nerve growth factor/TrkA signaling⁶⁹ *via* the PI3K pathway leading to coincidentally increased DAGL/MAGL/CB₁R expression. Notably, increased 2-AG content and focal signaling in growth cones was maintained by the coordinated, NGF-dependent expression of E3 ubiquitin ligases, including breast cancer-associated protein 1 (BRCA1)^{69,87}, to mediate MAGL degradation (Figure 3a). As such, both DAGL and CB₁R inhibition abolishes neurotrophin-induced neurite outgrowth^{69,96,97} in cerebellar and subcortical neurons, supporting the hypothesis that neurotrophin signaling uses endocannabinoids to regulate neurite extension. Likewise, netrin signaling at its receptor, deleted in colorectal cancer (DCC), is modulated by CB₁Rs and CB₂Rs^{31,65}, impacting netrin-induced growth cone steering decisions and directional axonal growth in the developing visual system. Pathophysiological implications of these interactions are considerable, ranging from disrupted neuronal migration⁹³, faulty growth cone turning decisions^{21,31,65}, and incomplete hemispheric segregation of visual pathways³¹.

TRPV1 channels⁹⁸ may be located on the plasma membrane or on intracellular membranous compartments such as the endoplasmic reticulum⁹⁹. In both cases, TRPV1 activation by AEA will increase cytoplasmic Ca²⁺, thus stimulating diverse Ca²⁺-dependent signaling pathways. Activation of plasma membrane TRPV1 will also tend to depolarize the cell, which may not only be important for differentiation but also to adjust responsiveness to e.g., neurotrophins and netrins signaling through Ca²⁺-dependent cAMP production. Moreover, and countering the generally pro-survival actions of AEA through CB₁R, TRPV1 activation by AEA and related lipids can lead to cell death *via* the ATF3-dependent endoplasmic reticulum (ER) stress pathway⁷⁶. As such, the existence of a signaling axis between AEA-activated TRPV1 controlling 2-AG biosynthesis and biological activity at CB₁Rs in the fetal brain, like in adult striatum¹⁰⁰, remains to be determined. Therefore, future research might be increasingly directed towards advancing knowledge on the differential involvement of multi-receptor mechanisms by determining if the (dys-)balance of receptor activities pertains to unwanted changes in neuro- and gliogenesis and neuronal differentiation, impinging upon the wiring diagram of neuronal networks.

Endocannabinoids and neuronal development

The bioavailability of 2-AG and AEA diverges strikingly as the embryo develops^{101,102}. AEA predominates in the blastocyst and early embryonic stages, and is required for embryo implantation and the maintenance of pregnancy⁶. In contrast, 2-AG levels gradually increase

as tissue differentiation progresses, including in the nervous system, where DAGL α and DAGL β expression in developing axonal tracts during the mid- and late-gestational periods exceeds those in later stages of development^{48,51} (Figure 2a). A series of elegant experiments from Ismael Galve-Roperh's laboratory combined mouse genetics and *in vitro* models to show that endocannabinoids control the size of the neural progenitor pool in the developing forebrain and are particularly important to adopt neuronal vs. astroglial cell fate in the hippocampus and cerebral cortex^{19,20,88,103}. These studies revealed that neural progenitors express CB₂Rs, along with CB₁Rs, and that CB₂R activation by non-psychoactive cannabinoids and endocannabinoids and downstream PI3K-Akt-mTORC1 signaling modulates the expansion of the neural progenitor pool *in vitro* and *in vivo*^{103,104}. Although significant gaps in present knowledge exist as to the integration of endocannabinoid signals in neurogenic niches at the receptor level, as well as the specific contributions of 2-AG, AEA and other endocannabinoids, the consensus view is that neurogenic fate decision coincides with a CB₂R-to-CB₁R switch in postmitotic neurons¹¹, explaining the predominance of CB₁R expression (Figure 2b) and signaling during neuronal differentiation^{22,28,33,60,93} in all vertebrates studied^{28,29,46}, including humans³⁶ (Figure 2d).

The event of receptor switching parallels the down-regulation of DAGL expression in postmitotic neurons⁶⁰, allowing the cessation of proliferation-promoting autocrine 2-AG signaling (Figure 2a). Instead, reliance of the differentiating progeny on *paracrine* (target-derived) positional endocannabinoid signals increases. Such a mechanism is likely relevant for radially-migrating pyramidal cells to leave the cortical progenitor niche towards the prospective cortical plate along a vertically-decreasing 2-AG gradient. This also implies that CB₁Rs transduce repulsive signals to maintain the directionality of neuronal migration. A similar mechanism might operate in some, if not all, GABA interneurons⁶⁶ migrating tangentially in the superficial and deep migratory streams from subcortical ganglionic eminences towards endocannabinoid-rich cortical domains⁴⁸ (Figures 2a,c). We propose that GABA interneurons use 2-AG signals to avoid the DAGL α/β -rich cortical plate (note that pyramidal cells populate the cortical plate days earlier than interneurons¹⁰⁵), and migrate in endocannabinoid-sparse cortical “corridors” instead. This hypothesis also takes into account the likely presence of focal endocannabinoid gradients (*see below*) to control the direction of cell migration⁹³, and integrates radial glia as a cell contingent terminating long-range migration by endocannabinoid inactivation⁴⁶.

Besides its likely contribution to shaping the path of migrating GABA interneurons, DAGL expression in pyramidal cells can also subserve the growth of corticofugal axons. This hypothesis is compatible with the axonal localization of CB₁Rs, suggesting that autocrine and paracrine modes of action can promote neurite elongation⁴⁸, fasciculation and directionality^{29,31} (Figure 3b). Recent experimental – particularly *in vitro* – evidence suggests that CB₁Rs, CB₂Rs⁶⁵, TRPV1 and GPR55 couple to neurite growth by likely modulating lateralized Ca²⁺ signaling¹⁰⁶ in axonal growth cones, representing a direct link to cytoskeletal reorganization upon exogenous ligand exposure in various cell systems^{81,82,107-111}. Here, we focus on CB₁R-mediated axonal growth whose physiological significance is supported by genetic and pharmacological analyses (Table 1, Figure 4). Initially, CB₁Rs, even though signaling competent¹¹², were thought to be at “atypical

locations” in fetal brains (that is, in the white matter instead of synaptic sites)^{101,112}. In subsequent studies, the selective exposure of CB₁Rs on the axonal surface was demonstrated^{23,113}, and their activation shown to induce repulsive growth cone turning and eventual collapse, at least *in vitro*^{21,31}. The molecular mechanism of CB₁R-mediated cytoskeletal instability in growth cones is thought to involve Rho family GTPases²¹, Ras and PI3K-Akt- β -catenin signaling^{96,114} (Figure 3a). In single cell systems^{21,31}, CB₁R-mediated repulsive growth cone turning involves neurite extension, while growth cone collapse is followed by neurite retraction, growth cone reinstatement and renewed extension/motility along alternative paths. The highly dynamic steering behaviors of advancing growth cones in the presence of repulsive cues might be translated to the complex three-dimensional structure of the developing cerebrum where precisely-configured 2-AG sources are organized in corridor-like patterns such that repeated cycles of repulsion-alternative pathfinding responses, resulting in net neurite elongation, propel CB₁R⁺ axons along their growth trajectory. These considerations, together with CB₁R expression being restricted to developing neurons destined to the cerebral cortex^{46,66}, suggest that endocannabinoid signaling is particularly critical for cortical development and that the targeting of long-range axons leaving the cerebral cortex (termed corticofugal axons) might switch from endocannabinoid-dependent to non-endocannabinoid-mediated forms of axon guidance once passing the pallio-subpallial boundary. The fact that thalamocortical axons express MAGL (Figure 3b) raises the possibility that during the process of the “thalamocortical-corticothalamic handshake”, when axons along opposite paths use *in-trans* signaling to maintain directional motility in the internal capsule, MAGL-mediated 2-AG inactivation can *i*) change axonal growth rate, *ii*) modulate directionality and *iii*) determine the size of axon fascicles by adjusting the number of axons navigating together. Although data using JZL184, a MAGL inhibitor, in cell culture systems highlights a role for MAGL in growth rate control⁴⁸, *in vivo* support for this hypothesis is not yet available. Moreover, and even though glypican (a heparan sulfate proteoglycan) and neurocan (a chondroitin sulfate proteoglycan), extracellular matrix proteins, have been proposed to be downstream targets of CB₁Rs during axonal growth³⁸, mechanistic insights in endocannabinoid-mediated axon fasciculation remain elusive. Nevertheless, a key observation is that once synaptic wiring occurs, DAGL⁵¹ and MAGL⁴⁸ become redistributed in neurons (Figure 3c) with DAGL exclusively present in the somatodendritic domain of neurons, while MAGL assumes a presynaptic localization. Although we as yet do not know the molecular filters excluding these enzymes from particular subcellular compartments, this molecular reorganization is clearly critical for the retrograde control of synaptic neurotransmission to becoming operational in the juvenile brain¹¹⁵⁻¹¹⁷.

Endocannabinoid contributions to gliogenesis

Even though the majority of studies have focused on the role of endocannabinoids in neuronal differentiation, essential data suggest that these signal lipids are also significant in regulating astro- and oligodendroglial differentiation^{19,118-122}. Indeed, CB₁R activation promotes astroglial differentiation of mouse neural progenitors in culture^{19,123,124} and in the postnatal and adult mouse hippocampus *in vivo*¹²⁵. Furthermore, CB₁R stimulation enhances the survival of rat astrocytes in culture and in the adult rat hippocampus *in vivo* by a likely focal signaling mechanism (that is, cultured astroglia

express CB₁R, CB₂R, MAGL and FAAH raising the possibility of both autocrine and paracrine signaling^{19,20}) and reliant on the PI3K-Akt pathway (Figure 3a).

The enzymes responsible for 2-AG synthesis and degradation were found in oligodendrocytes^{119,120}, which produce high levels of 2-AG. Since oligodendrocytes also express CB₁R and CB₂R, and respond to MAGL inhibition (by JZL184) by rapid morphological differentiation, particularly the formation of highly-ramified processes¹¹⁹, it is likely that autocrine 2-AG signaling dominates in driving oligodendroglial maturation. Moreover, agonist activation of both CB₁Rs and CB₂Rs enhances the survival of oligodendrocyte progenitors in culture. These effects are mediated by the CB₁R/CB₂R-mediated activation of the ERK¹²⁰ and PI3K-Akt signaling pathways^{119,126} *in vitro*. These data were corroborated by studies showing that CB₁R/CB₂R activation also promotes oligodendrogenesis in the subventricular zone of the postnatal rodent brain *in vivo*¹¹⁸, particularly upon hypoxic-ischemic damage in neonatal¹²⁷ and adult animals, and viral encephalitis-induced disease¹²⁸. Although the above observations seem compelling, some investigators have been unable to reproduce these findings, at least when attempting to differentiate mouse neural progenitors to generate astroglia or oligodendroglia *in vitro*^{129,130}. Nonetheless, the recent finding that hemopressin, a putative *endogenous* CB₁R inverse agonist, might increase oligodendroglial differentiation of neural progenitors isolated from the subventricular zone of neonatal mice¹³¹ suggests that spatially-restricted, cell stage-specific endocannabinoid signals could differentially affect the proliferative capacity of neural progenitors and typify the cellular identity of ensuing progenies.

Neurogenesis precedes gliogenesis during brain development⁶⁸. Therefore, we propose that endocannabinoid signaling can be relevant to gliogenesis and differentiation in both autocrine and paracrine contexts. While the former might operate in glial progenitors to expand available cell pools in gliogenic niches of the brain, the latter, with DAGL-containing axons serving as the cellular sources of 2-AG, can promote the recruitment of astroglia and oligodendrocytes to specific neuronal foci for metabolic purposes or to myelinate long-range axons to increase their conductance velocity.

Adverse effects of cannabis during pregnancy

Developmental neurobiology commonly probes the relevance of any signaling system to brain development by a combination of genetic and pharmacological tools. The >400 bioactive components in *Cannabis* spp.⁴⁷ make the use of pharmacological approaches particularly significant in relation to endocannabinoid signaling. Here, we summarize data from genetic (knock-out) models, antagonism of CB₁Rs, and modulating endocannabinoid metabolism.

Genetic models

The lack of lethality or an otherwise robust nervous system phenotype in constitutive CB₁R knock-out mice¹³² prompted initial skepticism towards a mandatory neurodevelopmental role for endocannabinoids. The first wave of conditional knock-out models^{21,32,46} and detailed morphological analyses^{21,32,69} have begun to change this view (Table 1, Figure 4), and confirmed a particularly prevalent capacity of compensation and adaptation at the level

of cannabinoid receptors and metabolic enzymes. These findings also suggested that pharmacological challenges might similarly provoke unwanted developmental complications.

CB₁R modulation

Accordingly, a bolus injection of SR414716, a CB₁R antagonist, into the lateral ventricle of mouse fetuses (embryonic day 14)⁴⁶ recapitulated the arrest of neuroblast migration²⁴ from the subventricular zone, ipsilateral cortical delamination and growth defects of corticofugal axons seen in mice lacking CB₁R in pyramidal cells⁴⁶ (Figure 4). Moreover, perinatal injections of AM251, another CB₁R antagonist, altered the wiring diagram of the barrel cortex by mistargeted axons, thus modifying the whisker map and compromising somatosensory information processing³⁰. Likewise, injection of pregnant dams with WIN55,212-2, a potent and efficacious CB₁R/CB₂R-mixed agonist, derailed Tbr1 and Tbr2 expression¹³³, augmented both radial and tangential neuronal migration in the cerebral cortex with a particular increase in the number of Cajal-Retzius cells in the cortical marginal zone¹³³, which can underpin deranged¹³⁴ glutamate neurotransmission and impaired postnatal learning and memory encoding^{135,136}.

Manipulating endocannabinoid metabolism

Chronic MAGL inhibition by JZL184, producing multi-fold increases in embryonic 2-AG levels, modifies development of the cortical circuitry by a functional antagonist mode of action, with long-lasting consequences of CB₁R (and perhaps CB₂R) availability and signal competence^{69,137}. Although *in vivo* confirmation is lacking, based on the dependence of neuronal differentiation on precisely timed 2-AG signaling and the axonal mis-targeting phenotype in DAGL^{-/-} brains *in vivo*⁶⁴, we predict that pharmacological inhibition of DAGLs will be detrimental to nervous system development.

Endocannabinoid signaling constitutes a wide-ranging neural-cell communication system with many molecular nodes allowing for alternative metabolic checkpoints to tightly control endocannabinoid concentrations (“homeostatic balance”) and to allow for broad interactions with co-existent signaling cassettes (see interactions with neurotrophins and netrins above). Thus, one may postulate that instead of provoking a gross neurodevelopmental phenotype (“direct hit”), pharmacological or genetic manipulation of select molecular constituents in endocannabinoid signaling networks will induce subtle changes in many neurons and synapses, only manifesting as disease upon a secondary stressor (“double hit”). This concept is compatible with recent studies showing that pre- or perinatal administration (from embryonic day 10 to postnatal day 7) of URB597, a FAAH inhibitor¹³⁸ or GPR55 deletion¹³⁹ does not markedly affect neurogenesis or axonal development (Table 1). Yet both strategies induced long-lasting behavioral deficits, particularly indices of depression-like symptoms and memory impairment enduring into the adulthood of affected offspring. The lack of a clear anatomical phenotype in these models might be explained by *i*) moderate GPR55 mRNA expression (relative to CB₁R mRNA) in the brain¹³⁹ and *ii*) the 1,000-fold lower levels of AEA than 2-AG in the developing brain^{48,102}, reducing the impact of these molecular targets and likely triggering functional substitutions by other cannabinoid-sensing receptors and 2-AG, respectively. Nevertheless, the behavioral deficits observed are in

agreement with the outcome of longitudinal human studies in children presenting compulsive and depressive behaviors and deficits in recall and visual memory when prenatally exposed to cannabis^{140,141}.

Cannabis exposure during pregnancy

Research in relation to brain development has solely focused on the analysis of the impact of THC^{34,38,142,143}, its major psychoactive component, so far. Pioneering studies from the Hurd laboratory have demonstrated increased heroin seeking in offspring prenatally exposed to THC^{143,144}, trans-generational imprinting *via* epigenetic mechanisms through coincidentally enhanced transcriptional repression and reduced transcriptional activation³⁴, as well as misplacement of CB₁R⁺ hippocampal interneurons in neonatal offspring⁹³. The neuronal basis for THC-induced drug-seeking behaviors in the offspring might be due to the altered neuropeptide (dynorphin, enkephalin) expression in mesocorticolimbic reward circuits¹⁴⁵, and the erroneous synaptic wiring of glutamatergic cortical neurons³⁸. Modifications to glutamatergic axons were shown to originate during neuronal development and be due to the CB₁R-dependent degradation of SCG10/brain-specific stathmin-2, a determinant of microtubule elongation by sequestering tubulin dimers in a ternary T2S complex¹⁴⁶. The loss of SCG10/brain-specific stathmin-2 thus compromises cytoskeletal instability in a molecular mechanism involving the phosphorylation and activation of JNKs, particularly JNK1, which phosphorylate SCG10/stathmin-2 destining it to proteasomal degradation¹⁴⁷ (Figure 3a). Since reduced cytoskeletal instability leads to erroneous neuronal morphologies, particularly neurite outgrowth, the THC-induced loss of SCG10 compromised corticofugal development. THC-induced wiring deficits appeared permanent since adult offspring showed synaptic rearrangements, particularly CB₁R localization, and reduced long-term plasticity in the hippocampus³⁸.

Pharmacological manipulation of the endocannabinoid system is also detrimental in neonates and during adolescence. In a provocative series of experiments, Esther Fride and her colleagues suggested that a single injection of SR141716 in new-born mice compromises suckling behaviors so severely that it leads to the death of the offspring¹⁴⁸. Likewise, prolonged THC administration in neonates induced learning impairments enduring into adulthood¹⁴⁹. For the adolescent period (that is, postnatal days 28–45 in mice), THC administration impairs social behaviors and cognition, with concomitant alterations in neurochemical indices of both glutamatergic and GABAergic neurotransmission^{150,151} (e.g., vesicular transporters, postsynaptic and SNARE complex components). Although most of these data only allow circumstantial conclusions based on cross-correlations, it is tempting to speculate that pharmacological manipulation of endocannabinoid signaling during critical periods of synaptogenesis and/or postnatal pruning might precipitate or predispose to neuropsychiatric disease-like phenotypes. Our hypothesis is in accord with clinicopathological data showing signaling-competent CB₁Rs in major axonal trajectories in human fetal brains¹¹², disrupted endocannabinoid signaling (particularly CB₁R levels) and molecular differentiation of CB₁R⁺ GABA interneurons in schizophrenics^{152,153}, and deficient endocannabinoid signaling in rodent models of autism (neuroligin knock-outs⁴⁰) and deletion of the fragile X gene¹⁵⁴.

Endocannabinoids in restorative neuroscience

We live in an exciting time for restorative neuroscience, where key findings in developmental biology are being translated into the adult context to combat devastating human neurological conditions. In this section we focus on emerging evidence that endocannabinoids regulate the proliferation of neural stem cells in the adult brain with the hope that persistent neurogenesis might be harnessed for brain repair¹⁵⁵. In the adult rodent brain, neural stem cell niches exist in the hippocampus and the subventricular zone of the lateral ventricle and serve to generate new neurons that can integrate into mature synaptic circuits to facilitate specialized forms of learning and memory¹⁵⁶. In the hippocampus, the generation and differentiation of new neurons is a relatively local event within the dentate gyrus¹⁵⁶. In the subventricular zone, neural stem cells generally differentiate into migratory neuroblasts that travel a considerable distance along the rostral migratory stream (RMS) to populate the olfactory bulb (OB) with new neurons¹⁵⁷. In humans, a robust RMS is a transient structure that is only obvious for a few months after birth¹⁵⁸. Nonetheless, proliferative neural stem cells can still be identified in the subventricular zone of aged stroke patients, with new neurons appearing in the adjacent striatum throughout life¹⁵⁹. Interestingly, subventricular zone neuroblasts can be recruited to sites of brain injury where they might provide trophic support to help preserve function¹⁶⁰ with recent evidence suggesting that neural stem cells and progenitors resident in the subventricular zone might also provide “local” trophic support to neurons residing in the proximal striatum¹⁶¹.

Epidermal growth factor (EGF) and FGF receptor signaling play central roles in driving adult neurogenesis¹⁶², with their respective ligands operating alongside inhibitory factors like growth differentiation factor 11 (GDF11)^{163,164} to maintain the balance between neural stem cell quiescence and proliferation¹⁶⁵. This leaves the cells poised to respond in a positive or negative manner to other cues, some of which appear to be derived from local microglia^{166,167}. Genetic deletion of cannabinoid receptors, or relatively short term treatment with CB₁R- and CB₂R-selective antagonists, suppresses neural stem cell proliferation in the hippocampus and subventricular zone^{12,104} (Table 2). In contrast, activation of cannabinoid receptors with synthetic agonists and/or by increasing the level of endocannabinoids by genetic deletion or pharmacological inhibition of FAAH, stimulates neural stem cell proliferation in both niches^{11,12,20,104}. Unexpectedly, stimulating endocannabinoid signaling has more dramatic effects in older animals, suggesting that the natural age-related decline in neurogenesis might in part reflect a “run-down” in endocannabinoid tone. In this context, whereas treatment of 6 week-old mice with CB₂R agonists increases subventricular neural progenitor proliferation by 20-30%, the same treatments result in 300-400% increase in 6 month-old mice, with an associated 3-fold increase in the number of new neurons appearing in the olfactory bulb two weeks later¹¹.

2-AG appears to be the main endocannabinoid driving adult neurogenesis as there is a 50% reduction in neural stem cell proliferation in the hippocampus and subventricular zone of young adult mice when DAGL α and/or DAGL β are knocked-out¹⁵. Even greater effects (70-80% loss) are seen in the subventricular zone following acute pharmacological inhibition of these enzymes¹¹ (Table 2). Neural stem cells express both DAGLs as well as CB₁R and CB₂R¹¹, suggesting an autocrine signaling pathway. However, it is also possible

that 2-AG exerts paracrine effects on neighboring cells, with this type of “short-range” signaling recently shown to be capable of regulating axonal guidance⁶⁴.

In addition to promoting stem cell proliferation, DAGL-dependent activation of both the CB₁R and CB₂R promotes the migration of neuroblasts along the RMS towards the OB, again *via* an autocrine signaling mechanism²⁴. As such, endocannabinoid tone stabilizes the leading process on migratory neuroblasts, perhaps allowing them to better read and/or respond to spatial guidance cues within the RMS. The question remains as to how endocannabinoid tone arises and is maintained in neural stem cells and migratory neuroblasts. In this context, there are many candidates as a wide range of growth factors implicated in neurogenesis, including FGF-2, have the potential to directly and focally stimulate DAGL-dependent endocannabinoid signaling⁹⁶. Interestingly, recent progress in understanding the key downstream effectors that mediate endocannabinoid signaling has implicated mammalian target of rapamycin complex 1 (mTORC1) rather than PI3K or ERK¹⁰³ as downstream signal effector; with biochemical and transcriptional experiments suggesting that PI3K activity is largely regulated by the EGF receptor, while the EGF and FGF receptors synergistically regulate the ERK pathway¹⁶⁸.

In summary, adult neurogenesis can be considered as a form of structural or cellular plasticity operating within the developed brain; it is highly dynamic with correlative changes seen upon injury, in depression and in various neurodegenerative and inflammatory disease states¹⁶⁹. A considerable body of evidence now supports a key role for endocannabinoid signaling in the regulation of neural stem cell proliferation, and the lineage commitment and migration of their progeny. Stimulation of neurogenesis in the adult brain is beneficial in, e.g., depression¹⁷⁰, but substantial benefits in the aged, injured and/or diseased brain remain to be demonstrated. Nonetheless, FAAH inhibitors might be useful drugs for this purpose given the dramatic effects they have on neurogenesis in older mice¹²² and the fact that they have been developed for use in man, albeit for other conditions¹⁷¹.

Cannabinoid receptors in glioma therapy

It is widely believed that the optimal strategy for cancer treatment would involve targeted therapies capable of providing the most efficacious and selective treatment for each individual tumor patient (‘personalized therapy’). *Glioblastoma multiforme* (grade IV astrocytoma) is one of the most malignant forms of human cancer with very short survival times following diagnosis. The recent finding that a small molecule selectively affecting membrane turnover in human glioma cells was efficacious in a preclinical glioma model¹⁷² reinforces the concept that the maintenance of lipid mass in dividing tumor cells is critical for their continued growth. Moreover, it is known that cannabinoids inhibit tumor growth in animal models of glioma through a mechanism involving inhibition of PI3K-Akt-mTORC1 signaling that induces apoptosis of tumor cells without affecting their non-transformed counterparts¹⁷³ (Figure 5). Additional mechanisms such as inhibition of angiogenesis¹⁷⁴ and invasion^{175,176} can contribute to the CB₁R/CB₂R-induced impairment of glioma growth in mouse models. Moreover, in mice bearing gliomas, cannabinoids potentiate the anti-tumor efficacy of temozolomide, the benchmark agent for the clinical management of gliomas, in a synergistic manner and without overt toxicity¹⁷⁷. In a pilot phase I clinical study,

intracranial THC administration to 9 patients with actively-growing recurrent *glioblastoma multiforme* was safe and could be achieved without significant unwanted effects¹⁷⁸. Importantly, radiological monitoring and analyses of tumor specimens supported the anti-tumor action of cannabinoids in those cancer patients^{173,178}. Notably, endocannabinoid-sensing receptors (especially CB₂R and TRPV1) are up-regulated in human glioma tissue as compared with normal brain tissue, and their expression positively correlates with tumor grading^{76,179}. The differential receptor expression patterns thus allow potential endocannabinoid-drug targets to induce glioma cell death. These findings provide ‘proof-of-concept’ that endocannabinoid-based medicines can improve the clinical efficacy of classical cytotoxic drugs in glioma patients. In line with this, a phase I-II trial for a combination therapy with cannabinoids and temozolomide is currently ongoing (<http://clinicaltrials.gov/show/NCT01812603>).

The mechanism of action appears to involve ER stress, which is a common theme in (endo-)cannabinoid-triggered glioma cell death (Figure 5). ER stress is activated in response to ER-damaging stimuli and aims to lessen the protein load on the ER by coordinating a temporal shutdown of protein translation and a complex program of gene transcription to increase the ER's protein-folding capacity. If this transcriptional program fails to re-establish ER homeostasis, persistent ER stress will induce cell death. Cannabinoid-induced apoptotic death of glioma cells was shown to stimulate the CB₁R/CB₂R-dependent *de novo* synthesis of ceramide. This sphingolipid accumulates in the ER, thereby inducing ER stress through the phosphorylation/inhibition of eukaryotic initiation factor 2 α (eIF2 α) and the downstream activation of ER stress-related transcription factors such as p8, ATF-4 and CHOP¹⁸⁰. In parallel, fatty-acyl ethanolamides (like AEA) activate TRPV1 receptors located on the ER of glioma cells, inducing Ca²⁺ release into the cytoplasm; likely in convergence with ceramide accumulation to deplete ER Ca²⁺ stores and evoke the phosphorylation/inhibition of eIF2 α and the up-regulation of transcription factors such as ATF-4 and ATF-3⁷⁶ (Figure 5). These two branches of (endo)cannabinoid-mediated ER stress trigger the execution of glioma cell death. In the former branch, cannabinoid receptor-mediated ER stress up-regulates the expression of the pseudokinase tribbles-homologue-3 (TRIB-3), which interacts with and inhibits the pro-survival kinase Akt, thereby leading to the inhibition of mTORC1 and the subsequent stimulation of autophagy-mediated apoptotic cell death¹⁸¹. The molecular targets downstream of ATF-3 in the TRPV1-mediated ER stress response remain elusive. Incidentally, (endo)cannabinoids can engage additional ER stress-related executioners to trigger neuronal death. As such, AEA induces apoptosis of human neuroblastoma cells through a CB₁R-dependent pathway *via* p38 and Erk1/2 and BiP/GRP78, a ER stress sensor that triggers p53 and PUMA¹⁸².

The widely-reported induction of glioma cell death by cannabinoid receptor stimulation is in striking contrast with their well-known proliferative/pro-survival activity exerted on neural progenitors, neurons and glial cells. In fact, cannabinoid receptors regulate signal transduction pathways distinctly in tumor and non-tumor cells (Figure 5). The molecular basis of this ‘yin-yang’ behavior is incompletely understood. Nevertheless, the possibility that it relies on different patterns of CB₁R/CB₂R expression and/or pre-coupling to effectors seems unlikely for a number of reasons: glioma cell lines resistant to cannabinoid-induced

apoptosis express similar amounts of CB₁R and/or CB₂R compared with cannabinoid-sensitive lines, and pharmacological studies using cannabinoid receptor subtype-selective antagonists support that either CB₁R or CB₂R can signal apoptosis in cannabinoid-sensitive glioma cells^{183,184}. In addition, the short-term (minute-range) coupling of CB₁R/CB₂R to key cell signaling pathways such as Erk and Akt is similar in glioma cell lines that are sensitive or resistant to cannabinoid-induced apoptosis^{183,185}, as well as in primary astroglia¹²⁵ or oligodendroglia¹²¹. Altogether, this evidence supports that mechanisms other than changes in the expression/intrinsic functionality of cannabinoid receptors might account for the differences in cannabinoid sensitivity of glioma cells. Indeed, a series of studies indicates that this ‘yin-yang’ behavior could result from the differential capacity of tumor or non-tumor glial cells to induce long-term *de novo* ceramide synthesis in the ER and, in turn, trigger an ER stress response^{174,181,185}, which determines whether the mitogenic PI3K-Akt-mTORC1 pathway becomes stimulated (in non-tumor cells; Figure 5a) or inhibited (in tumor cells; Figure 5b) upon cannabinoid receptor engagement^{173,182}.

Conclusions

Here, we reviewed available experimental and translational evidence¹⁸⁶ implicating endocannabinoid signaling in brain development, from neural stem cell survival and proliferation, cell fate decisions and the motility and differentiation of ensuing neuronal and glial progenies. We highlighted multiple niches at which developmental endocannabinoid signaling, encompassing the fetal-to-young adult periods, is of significance: recreational cannabis use during pregnancy and in adolescence impacting structural and functional brain plasticity¹⁸⁶, adult neurogenesis in which endocannabinoids might be exploited to promote the colonization of areas of neuronal loss, and the molecular pathogenesis of gliomas where ceramide-induced ER stress is identified as an attractive therapeutic target. Despite great progress over the past decades, information is limited as to the involvement of endocannabinoids in the maintenance of neuron-glia interplay, particularly the recruitment of astroglia for metabolic control and oligodendroglia for ensheathing of axons. These areas of research are warranted since functional CB₁Rs are likely expressed by (adult) astroglia¹⁸⁷, neuron-derived endocannabinoids can induce Ca²⁺ signaling and glutamate release from astrocytes¹⁸⁸, and the assumed presence of CB₁Rs on mitochondria⁸. Even though the latter observation is as yet controversial¹⁸⁹, future studies aimed to define sequential endocannabinoid signal recruitment along distinct timescales to foci of neuronal and glial differentiation will undoubtedly provide fundamental insights in the contribution of signal lipids to shaping the nervous system. These studies will facilitate the molecular analysis of neuropsychiatric diseases with presumed developmental origins and in which modifications to endocannabinoid signaling have been observed (autism⁴⁰, schizophrenia^{152,190,191}, bipolar disorder^{41,192} and depression¹⁵²). Attempts to disentangle complex genetic traits and epigenetic mechanisms must rely, at least in large part, on modern molecular and cellular tools, to reconcile existing controversies in cannabinoid pharmacology. Thus, a sea-change in our understanding of endocannabinoid actions in subpallial, midbrain, and spinal networks are expected, and will facilitate the exploitation of axonal responsiveness and glial scaffolding for therapeutic purposes. Ultimately, further analysis using combinatorial platforms is warranted to define the relative significance of endocannabinoid signals amongst coexistent morphogenetic pathways, and their sensitivities

to metabolic pressures. Thus, we await the emergence of consensus views on alleviating the adverse impact of maternal drug abuse, obesity, diabetes and cardiovascular diseases¹⁹³, commonly associated with altered circulating endocannabinoid levels¹⁹⁴ and increased risk of neuropsychiatric and metabolic illnesses in the offspring¹⁹⁵, on future generations.

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Glossary

Fasciculation	A process where developing axons follow a primary pioneer axon, thus maintaining strict directionality towards a distal target
Ceramides	Molecules that consist of sphingosine plus a fatty acyl chain and are one of the structurally simplest forms of sphingolipids
Corticothalamo-thalamocortical handshake	The point at which corticothalamic and thalamocortical axons with opposite growth trajectories cross one another
Cytoskeletal instability	The dynamic reorganization of the cytoskeleton by elongation and shortening of the "+" and "-" ends of microtubules
Endocannabinoids	Endogenous compounds that bind to CB ₁ and/or CB ₂ cannabinoid receptors with high affinity, and evoke a 9-tetrahydrocannabinol-like behavioral tetrad
Functional antagonist	A drug that is pharmacologically classified as an agonist but induces a phenotype similar to that provoked by an antagonist
Morphogenetic signals	Gradients of molecules that determine the position of specialized cellular subtypes and instruct their communication and functional role during histogenesis
Neuroblast	A postmitotic cell in an undifferentiated form migrating towards its final position where it becomes a neuron
Psychoactive component	The molecular component of a plant extract that is responsible for provoking acute changes in perception or behaviour
Sn-1-diacylglycerol lipase	Two isoforms, which evolved evolutionarily through gene duplication ¹⁹⁶ , are chiefly responsible for 2-AG synthesis

Sphingolipids	Molecules that contain the organic aliphatic amino alcohol sphingosine or a structurally similar molecule as backbone
Subventricular zone	A part of the cerebral cortex what lines the dorsolateral surface of the lateral ventricle and is rich in progenitor cells and retains neurogenic capacity throughout life
Tripartite synapse	A characteristic doctrine of synapse anatomy, which recognizes the presynaptic terminal, postsynaptic specialization and astroglial end-feet that isolates the synaptic cleft as a specialized entity

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Online summary

- Endocannabinoid signaling, primarily via an autocrine mode of action and by engaging CB₁ and CB₂ cannabinoid receptors, modulates cell fate decisions of neural stem cells.
- Neuronal commitment coincides with a robust enhancement of CB₁ cannabinoid receptor expression, and the use of endocannabinoids as extracellular cues for neuronal migration and directional axonal growth.
- Growth factor signaling via receptor tyrosine kinases commonly uses endocannabinoid effectors to promote neurite outgrowth. In turn, cannabinoid receptors can trans-activate receptor tyrosine kinases.
- The endocannabinoid system is the molecular target of phytocannabinoids, particularly ⁹-tetrahydrocannabinol. Maternal cannabis consumption can adversely affect nervous system development.
- Cannabinoid receptor expression in adult neural progenitors and gliomas could be exploited for therapeutic benefit.

Fig. 1. Molecular architecture of the endocannabinoid system during synaptogenesis and at mature synapses

Neuronal and glial components of developing and mature synapses are shown. It is important to note that the molecular architecture shown here is typical. There are likely to be differences in neurotransmitter system-specific and developmentally-regulated enzyme and/or receptor expression and function at different types of synapse and at different stages of development. Most prominently, monoacylglycerol lipase (MAGL) is excluded from motile growth cones until synaptogenesis commences^{48,69}. Strike-through symbols indicate catalytic enzyme activity throughout.

(a) In developing synapse, AEA (green circles) and 2-AG (brown circles) orchestrate signaling by binding to their target receptors: CB₁R, CB₂R, GPR55, and TRPV1. Their availability is determined by biosynthesis enzymes (NAPE-PLD and DAGLα/β) and degrading enzymes (FAAH and MAGL). Note that information on alternative enzymes (e.g., ABHD6 and ABHD12) is at present not available. Unlike other endocannabinoid-binding receptors, CB₁Rs are preferentially recruited to and signal within cholesterol-enriched membrane microdomains termed lipid rafts⁶².

(b) At the mature synapse, the availability of AEA and 2-AG is determined by ABHD6 and ABHD12 in addition to FAAH and MAGL, and also by transmembrane (EMT) and

intracellular (AIT) transport mechanisms (e.g., fatty acid binding proteins¹⁹⁷, heat shock protein 70¹⁹⁸, and FAAH-like AEA transporter¹⁹⁹), and storage organelles (adiposomes or lipid droplets)²⁰⁰. There is compelling evidence that key receptor and enzyme components of the endocannabinoid system partition distinctly, both intracellularly and amongst pre- and post-synaptic neurons, microglia and astrocytes. CB₂Rs are expressed mainly upon brain injury²⁰¹. *Abbreviations*: ABHD6/12, α/β -hydrolase domain-containing 6/12 hydrolases; AIT, AEA intracellular transporter; CB₁R/CB₂R, G protein-coupled type-1 and type-2 cannabinoid receptors; DAGL α/β , *sn*-1-diacylglycerol lipase α/β ; EMT, putative endocannabinoid transmembrane transporter; ER, endoplasmic reticulum; FAAH, fatty-acid amide hydrolase; GPR55, G protein-coupled receptor 55; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidylethanolamine-specific phospholipase D; TRPV1, transient receptor potential vanilloid 1 channel.

Fig. 2. Molecular architecture of endocannabinoid signaling during corticogenesis, with emphasis on neurogenesis and neuronal migration

(a) During mid- or late-gestation in rodents, 2-AG-rich cortical microdomains are thought to repulse post-mitotic neurons that express CB₁R⁺, including radially migrating pyramidal cells and tangentially migrating GABA interneurons, in the cerebral cortex. DAGL expression in the fetal ventricular proliferative zone (1) and in the cortical plate (2) can produce physiologically-relevant extracellular 2-AG concentrations (pink shading). This molecular arrangement, producing “corridors” sparse in 2-AG (white areas, 3), could explain some features of spatially segregated radial (pyramidal cell) and tangential (interneuron) neuronal migration. Radial glia (green, 4), acting as scaffolds for migrating neurons, can synthesize and subsequently degrade endocannabinoids, thus promoting the

endocannabinoid-mediated radial detachment of neurons for final positioning (5).

Abbreviations: CP, cortical plate; dms/sms, deep/superficial migratory streams; IZ, intermediate zone; MZ; marginal zone; SVZ, subventricular zone; VZ, ventricular zone.

(b) At E14.5, CB₁R mRNA is predominantly expressed by neurons in the cortical plate (cp) and hippocampal primordium (hc). *Arrows* denote CB₁R mRNA expression in likely interneurons that leave the ganglionic eminence (ge) and migrate towards the cerebral cortex⁶⁶. *Abbreviations:* iz, intermediate zone; lv, lateral ventricle; mz; marginal zone; spt, septum.

(c) In mid-gestational mouse brain, DAGL α is found expressed at high levels by pyramidal cells and targeted towards their axons. Immunohistochemistry was performed in GAD67-GFP knock-in mice, marking some GABAergic interneurons²⁰² migrating in the superficial (sms) and deep migratory streams (dms).

(d) CB₁R mRNA expression concentrates in the cortical plate and proliferative germinal layers (ne)^{36,46} in the human fetal brain (second trimester). Data in **(b)** were modified from refs.⁴⁶, while images in **(c)** and **(d)** are courtesy of Dr. Erik Keimpema and Dr. Yasmin L. Hurd, respectively. Scale bar = 110 μ m (b), 30 μ m (c), 200 μ m (d).

Fig. 3. Design logic of endocannabinoid signaling during neurite outgrowth and synaptogenesis
(a) Signal transduction mechanisms implicated in the CB₁R-mediated control of cortical neuron specification and morphological differentiation. Activation of tyrosine kinase receptors (particularly the fibroblast growth factor receptor (FGFR) and the high-affinity nerve growth factor (NGF) receptor, TrkA) and their activity-dependent phosphorylation are thought to induce 2-AG production *via* sequential activation of phospholipase Cγ (producing diacylglycerol; DAG) and *sn*-1-diacylglycerol lipase α (DAGLα). The dashed arrow in the plasma membrane indicates lateral 2-AG diffusion that can activate CB₁Rs in an autocrine

fashion. Signaling *via* G proteins recruited to CB₁Rs upon agonist binding regulates neuronal morphology by e.g., the phosphorylation of C-Jun N-terminal kinases (e.g., JNK1)³⁸, which trigger the rapid degradation of SCG10/stathmin-2 and alter to cytoskeletal instability. Alternatively, CB₁R activation can modulate the activity of Rho-family GTPases, particularly RhoA, to induce growth cone repulsion and collapse^{21,31}. Neurotrophin (*blue circles*) and 2-AG signaling can coincidentally activate PI3K/Akt signaling. This, in turn, influences the activity of the transcriptional regulators Pax6 and CREB and their control of neural progenitor proliferation and fate decisions (for review see ref.⁸⁶). Cytoplasmic BRCA1 is referred to as one of the candidate E3 ubiquitin ligases controlling MAGL degradation^{69,87}.

(b) Spatial segregation of molecular determinants of 2-AG signaling during the corticothalamic-thalamocortical axonal “handshake”. Left panel: corticofugal axons are CB₁R⁺ (*red circles*) whereas thalamocortical axons are CB₁R-but MAGL⁺ (*green circles*)⁴⁸. Scale bar = 400 μ m. Right panel: corticofugal axons harbor DAGLs (ref.⁵¹) and can use paracrine 2-AG signaling for fasciculation (“1”). In turn, autocrine 2-AG signaling in corticofugal axons might be sufficient to promote their elongation (“2”). This molecular layout is compatible with MAGL⁺ thalamocortical axons limiting the spatial spread of 2-AG (“3”; dashed line indicates 2-AG inactivation), thus controlling the distribution of corticofugal fascicles and confining their growth trajectories to a subpallial corridor (“4”). Accordingly, pharmacological inhibition of MAGL activity during corticogenesis disrupts the formation of the corticofugal projection system²⁰³.

(c) The subcellular switch of DAGL and MAGL during neuronal polarization and synaptogenesis. In immature neurons, DAGL α is localized to the primary neurite (quiescent axon) including the growth cone for autocrine signaling. However, DAGL α is excluded from more proximal parts of the axon and redistributed to the somatodendritic axis of neurons once synapses formed. In contrast, MAGL becomes enriched in the presynapse, where it likely assumes a role as “stop” signal to limit 2-AG-mediated neurite elongation⁴⁸. The precisely timed molecular reconfiguration of 2-AG signaling supports a continuum of endocannabinoid actions during neuronal differentiation, leading up to the retrograde control of synaptic neurotransmission (*inset*). Here, DAGL α is selectively enriched in the perisynaptic annulus of dendritic spines (pink shading) apposing glutamatergic afferents^{49,204}.

Abbreviations: ctx, cerebral cortex; cfa, corticofugal axon; f, fimbria; hc, hippocampus; lv, lateral ventricle; tca, thalamocortical axon; th, thalamus.

Fig. 4. Defective development of the corticofugal system following genetic manipulation of CB₁Rs

Conditional deletion of CB₁Rs from cortical pyramidal cells results in errant corticofugal axon fasciculation (cfa; *arrows*) in mice²⁰⁵. Note that enlarged axon fascicles were also seen in extracortical areas, such as the striatum (cpu), where local CB₁R expression was unaffected. *Abbreviation*: G5, golga 5, a coiled-coil membrane protein that likely plays a role in vesicle tethering and docking; L1-NCAM, L1 neural cell adhesion molecule. *Scale bars* = 25 μm. Data were modified from ref.⁴⁶.

a Neural progenitor cell **b Glioma cell** **Fig. 5. Dysregulation of cannabinoid receptor signaling in glioma cells**

(a) In neural progenitors, agonist binding to cannabinoid receptors couples to the PI3K/Akt/mTORC1 pathway *via* G_i proteins. Trans-activated (by phosphorylation) receptor tyrosine kinases (Trks) might produce signal amplification by also using mTORC1 as a molecular effector. Akt can elicit cell growth and survival effects (thus being mitogenic) either by inhibiting glycogen synthase kinase 3 β (GSK-3 β) and activating β -catenin²⁰⁶ or by activating mTORC1, which leads to p27 inhibition¹⁰³ and Pax6 phosphorylation⁸⁷ and expressional up-regulation⁸⁸.

(b) In glioma cells, cannabinoids trigger ER stress by engaging (at least) two mechanisms: cannabinoid receptors stimulate *de novo* synthesis of ceramide in the ER *via* G_i -dependent and perhaps also G_i -independent mechanisms^{173,180}; and TRPV1 receptors on the ER, which mediate Ca^{2+} release from this organelle to the cytoplasm and, conceivably, deplete ER Ca^{2+} stores⁷⁶. Ceramide accumulation and Ca^{2+} depletion in the ER converge at the phosphorylation (that is, inhibition) of eIF2 α and the induction of ATF-4, which, in turn,

triggers cell death by two signaling cascades: up-regulation of TRIB-3 expression, leading to the inhibition of the AKT-mTORC1 axis¹⁸¹; and up-regulation of ATF-3 expression⁷⁶.

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Table 1

Pharmacological and/or genetic models used to dissect the contribution of developmentally-regulated endocannabinoid signaling to cell diversity and intercellular communication. Existing studies^{20,21,32,33,38,46,69,88,93,133-135,139,143,203} focused on forebrain development, particularly corticogenesis. Prenatal treatments with CB₁R ligands (SR141716, AM251 [antagonists] and WIN55,212-2 [agonist]), FAAH (URB597) or MAGL (JZL184) inhibitors were listed.

Model/approach	Phenotype	Reference
CB ₁ R ^{-/-} (global)	decreased neurogenesis migratory neuron misrouting axonal growth and guidance errors	Keimpema <i>et al.</i> , 2013 Wu <i>et al.</i> , 2013 Mulder <i>et al.</i> , 2008
CB ₁ R ^{-/-} (interneuron)	migratory neuron misrouting altered synapse distribution	Berghuis <i>et al.</i> , 2007
CB ₁ R ^{-/-} (pyramidal cell)	premature cell-cycle exit decreased neural progenitor proliferation corticofugal axon fasciculation errors deficient fine motor functions (postnatal)	Diaz-Alonso <i>et al.</i> , 2014 Mulder <i>et al.</i> , 2008 Diaz-Alonso <i>et al.</i> , 2012
FAAH ^{-/-}	augmented neurogenesis increased neural progenitor proliferation increased radial migration in cortex	Mulder <i>et al.</i> , 2008 Aguado <i>et al.</i> , 2005
DAGLα ^{-/-}	altered synapse distribution	Keimpema <i>et al.</i> , 2013
MAGL ^{-/-}	no developmental phenotype	Alpar <i>et al.</i> , 2014
GPR55 ^{-/-}	no developmental phenotype impaired movement coordination (postnatal)	Wu <i>et al.</i> , 2010 Wu <i>et al.</i> , 2014
⁹ -THC	interneuron misplacement axon fasciculation error impaired synaptic plasticity (postnatal) altered drug-seeking behavior (postnatal)	Berghuis <i>et al.</i> , 2005 Tortoriello <i>et al.</i> , 2014 Tortoriello <i>et al.</i> , 2014 Spagno <i>et al.</i> , 2007
SR141716A	axon fasciculation error	Mulder <i>et al.</i> , 2008
AM251	axon fasciculation error	Tortoriello <i>et al.</i> , 2014
URB597	no developmental phenotype impaired learning and memory (postnatal)	Wu <i>et al.</i> , 2014
WIN55,212-2	disrupted neuronal migration impaired synaptic neurotransmission and learning deficits (postnatal)	Saez <i>et al.</i> , 2014 Antonelli <i>et al.</i> , 2004 Mereu <i>et al.</i> , 2003
JZL184	axonal growth and guidance defects (prenatal)	Keimpema <i>et al.</i> , 2013 Alpar <i>et al.</i> , 2014

Table 2

The effect of pharmacological or genetic manipulation of endocannabinoid signaling on neurogenesis in the postnatal rodent brain. The impact of endocannabinoids on neurogenesis in the dentate gyrus (DG) or olfactory bulb (OB) was tested by selectively inhibiting CB₁Rs and CB₂Rs, by DAGL inhibitors, or by the deletion of genes. Conversely, endocannabinoid signaling can be mimicked or enhanced by CB₁R or CB₂R agonists, or by inhibition or knockout of FAAH (which results in multifold increases in brain AEA levels²⁰⁷). The wide range of tools^{11,15,19,20,104,208} that has been used to dissect that DAGL-dependent endocannabinoid signaling regulates neural stem cell (NSC) proliferation in both the DG of the hippocampus and the subventricular zone/rostral migratory stream in rodents with particular impact on neuroblast migration²⁴ and the appearance of new neurons in the OB.

Phenotype	Treatment	Response	Reference
<i>NSC proliferation, DG</i>	CB ₁ R antagonists	<i>decrease</i>	Jin <i>et al.</i> , 2004
	CB ₁ R ^{-/-}	<i>decrease</i>	Jin <i>et al.</i> , 2004
	DAGL ^{-/-}	<i>decrease</i>	Gao <i>et al.</i> , 2010
	CB ₁ R agonists	<i>increase</i>	Jin <i>et al.</i> , 2004
	CB ₂ R agonists	<i>increase</i>	Palazuelos <i>et al.</i> , 2006
	FAAH inhibitors	<i>increase</i>	Aguado <i>et al.</i> , 2005
	FAAH ^{-/-}	<i>increase</i>	Aguado <i>et al.</i> , 2005
<i>NSC proliferation, SVZ</i>	CB ₂ R antagonists	<i>decrease</i>	Goncalves <i>et al.</i> , 2008
	DAGL inhibitors	<i>decrease</i>	Goncalves <i>et al.</i> , 2008
	CB ₂ R agonists	<i>increase</i>	Goncalves <i>et al.</i> , 2008
	FAAH inhibitors	<i>increase</i>	Goncalves <i>et al.</i> , 2008
<i>Neuroblast migration</i>	CB ₁ R or CB ₂ R antagonists	<i>decrease</i>	Oudin <i>et al.</i> , 2011
	DAGL inhibitors	<i>decrease</i>	Oudin <i>et al.</i> , 2011
<i>New neurons in adult olfactory bulb</i>	CB ₂ R antagonists	<i>decrease</i>	Goncalves <i>et al.</i> , 2008
	CB ₂ R agonists	<i>increase</i>	Goncalves <i>et al.</i> , 2008
	FAAH inhibitors	<i>increase</i>	Goncalves <i>et al.</i> , 2008