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Review

Endocannabinoids via CB_1 receptors act as neurogenic niche cues during cortical development

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During brain development, neurogenesis is precisely regulated by the concerted action of intrinsic factors and extracellular signalling systems that provide the necessary niche information to proliferating and differentiating cells. A number of recent studies have revealed a previously unknown role for the endocannabinoid (ECB) system in the control of embryonic neuronal development and maturation. Thus, the CB_1 cannabinoid receptor in concert with locally produced ECBs regulates neural progenitor (NP) proliferation, pyramidal specification and axonal navigation. In addition, subcellularly restricted ECB production acts as an axonal growth cone signal to regulate interneuron morphogenesis. These findings provide the rationale for understanding better the consequences of prenatal cannabinoid exposure, and emphasize a novel role of ECBs as neurogenic instructive cues involved in cortical development. In this review the implications of altered CB_1-receptor-mediated signalling in developmental disorders and particularly in epileptogenesis are briefly discussed.

Keywords: cortical progenitor; neurogenesis; endocannabinoid signalling

1. INTRODUCTION

The developing nervous system is characterized by highly active and dynamically regulated cellular processes involving cell generation and differentiation, migration to their final destination, neuronal maturation and establishment of appropriate neuronal connectivity [1,2]. The precise regulation of these processes is achieved by a complex network of intrinsic molecular determinants and intracellular signalling pathways that are in turn modulated by surrounding information from the neurogenic niche [3]. Numerous studies have begun to delineate some of these determinants and signalling pathways involved in neural cell fate decisions, such as the regulatory switch responsible for neuronal versus glial differentiation [4] and the specification of dorsal (pallial) versus ventral (subpallial) neurons [3]. Developmental neurobiology studies and advances in stem cell research have allowed the identification of some of the molecular mechanisms involved in the specification and differentiation of specific neuronal lineages with different neurotransmitter phenotypes (e.g. glutamatergic, GABAergic, dopaminergic, etc.) [5]. However, the precise extracellular signalling pathways that modulate the acquisition of the diversity of developing neuronal populations and guarantee their appropriate integration are still only partially understood. Exposure of the developing and maturing nervous system to marijuana-derived cannabinoids exerts a significant impact on behavioural aspects, particularly regarding the control of emotions and cognitive responses. The implications of cannabinoid exposure in human neuropsychiatric disorders (see other reviews in this special issue and [6,7]) have driven the investigation on the mechanism of action and neurobiological substrate underlying developmental action of cannabinoids. Endocannabinoids (ECBs) have recently been underscored as neurodevelopmental signalling cues that, by targeting the CB_1 cannabinoid receptor, exert a regulatory role on the molecular and cellular mechanisms involved in brain development. Here, we review the experimental evidence supporting the functional role of the ECB system during cortical development, as derived from genetic and pharmacological manipulation studies. The CB_1 receptor has emerged as a novel signalling platform that drives neuronal generation and specification, thereby modulating brain maturation and connectivity. We also discuss the potential implications of these findings in proper neuronal activity of the adult brain.

2. THE ENDOCANNABINOID SYSTEM IN THE DEVELOPING BRAIN

The expression pattern of the ECB system elements (including receptors and enzymes of synthesis and degradation) in the developing brain has been addressed, revealing the presence of diverse ECB-metabolizing

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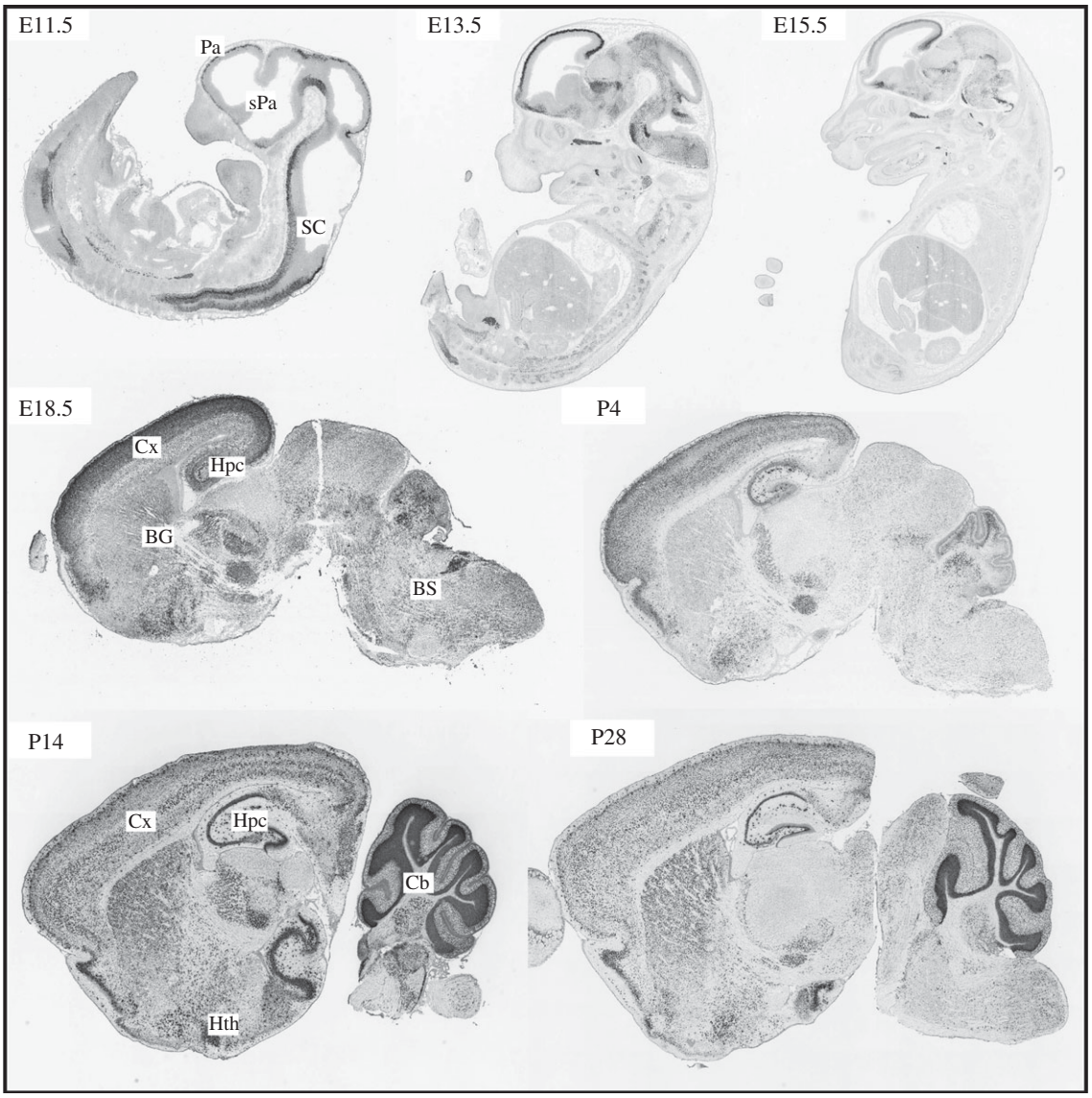

Figure 1. Expression pattern of the CB_1 cannabinoid receptor mRNA at different developmental stages. CB_1 mRNA *in situ* hybridization in the developing mouse nervous system is shown at the indicated stages. BG, basal ganglia; BS, brainstem; Cx, cortex; Hpc, hippocampus; Hth, hypothalamus; Pa, pallium; sPa, subpallium; SC, spinal cord. Published with permission of Allen Developing Mouse Brain Atlas, Seattle (WA), Allen Institute for Brain Science. Copyright ©2009. Available at: http://developingmouse.brain-map.org.

enzymes with restricted subcellular and spatio-temporal distribution. This complexity and the rapid rates of ECB synthesis/degradation reveal the existence of a dynamically regulated ECB tone during active neurogenesis. We will focus here on the expression pattern of the CB_1 receptor as the most important molecular target of the ECB tone [8]. The CB_1 receptor is expressed from very early stages of embryonic development, even before the appearance of the neural tube and neuroectoderm development. CB_1 is present in trophoblast stem cells and its deletion results in reduced cell proliferation and differentiation that is followed by aberrant placentation and compromised embryo implantation [9]. In addition to CB_1 receptor expression in the blastocyst stage, the other G-protein-coupled cannabinoid receptor, the CB_2 receptor, is also present in the inner cell mass, and has been proposed to be involved in embryonic stem-derived haematopoietic cell proliferation and lineage differentiation [10]. Cannabinoid administration during chick gastrulation results in alterations of neural tube formation and patterning, thus revealing the early sensitivity of the developing nervous system to cannabinoid signalling interference [11].

In mammals, CB_1 receptor expression during neural development is characterized by its abundant levels in white matter areas, with their levels progressively increasing from prenatal stages to adulthood in grey matter areas [12]. This atypical distribution of CB_1 receptor expression during development occurs while active neurogenesis and axonal migration occurs and prior to synaptic maturation and neuronal activity. Therefore, neurodevelopmental CB_1 receptor actions are likely to be independent of their regulatory role of neurotransmitter release and neuronal activity. The CB_1 mRNA expression pattern in the developing mouse brain is summarized in figure 1. CB_1 is present in the telecenphalon from E11.5 and its early expression is also observed in the developing spinal cord. During cortical development, CB_1 receptors are present in pioneer neurons that populate the marginal zone of the

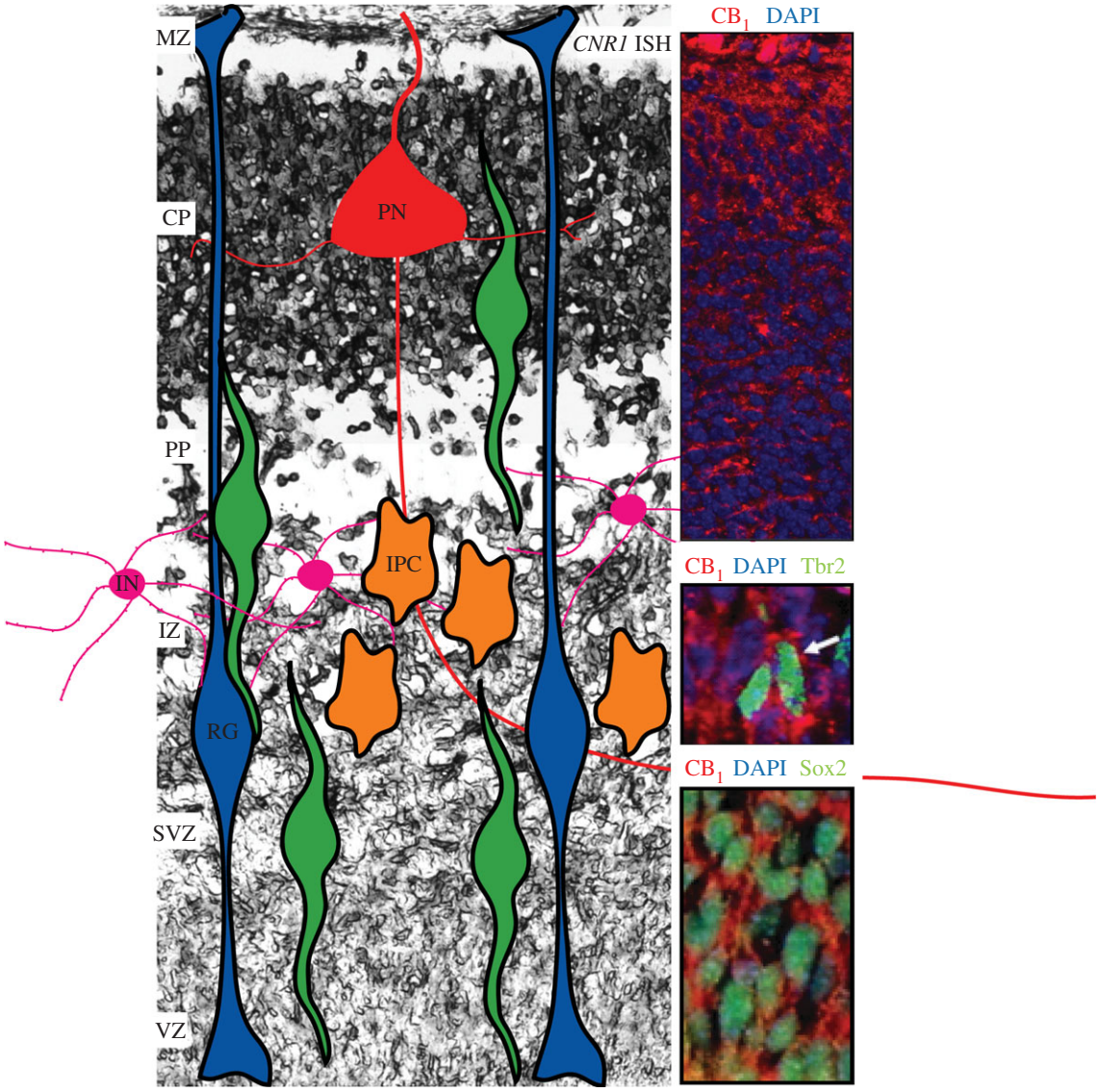

Figure 2. CB_1 cannabinoid receptor expression during cortical development. The CB_1 receptor is present in the developing cortex, showing increasing expression levels from undifferentiated to differentiated projection neurons (PNs). The CB_1 receptor is present in Cajal–Retzius cells of the marginal zone (MZ) and apical and basal progenitors in the ventricular and subventricular (VZ/SVZ) proliferative area. Representative immunofluorescence images showing the colocalization of the CB_1 receptor in radial glial (RG) progenitors and intermediate amplifying progenitor cells (IPCs) as identified with Sox2 and Tbr2 antibodies, respectively [13] (copyright National Academy of Sciences, USA 2009). Higher expression levels of the CB_1 receptor are evident in maturing neurons that have reached the CP, that correspond to locally generated PNs. CB_1 receptor is present in certain interneuron (IN) populations that reach the pallium upon tangential migration from the ganglionic eminences. Image background corresponds to a representative *in situ* hybridization of the *CNR1* mRNA at E.16.5 (by C. Hoffman and B. Lutz, Johannes Gutenberg University Mainz, Germany).

dorsal cortex (figure 2). In particular, CB_1 is present in Cajal–Retzius cells (E12.5) that are characterized by reelin expression [14,15]. Reelin is well known for its role as an instructive signalling cue that, among other actions, promotes radial migration of differentiating neurons. At embryonic day E13.5–E14.5, mouse developing cortex shows higher CB_1 receptor expression in the intermediate zone and developing cortical plate (figure 2), where postmitotic neuroblasts and differentiating neurons are located, and expresses early neuronal markers such as class III β-tubulin [13,14]. At these stages, the CB_1 receptor is also present in the subpial area of the ganglionic eminences and the primordium of the hippocampus [15]. Later, CB_1 receptors are heterogeneously distributed through cortical layers and the hippocampus, in both excitatory glutamatergic projection neurons, as identified by vGlut1 expression, and cholecystokinin (CCK)-expressing GABAergic interneurons colabelled with vGlut3 [16–18]. $CB_1^+CCK^+$ interneurons derived from the ganglionic eminences follow tangential migratory routes from the ventral telencephalon and reach the developing cortex, hippocampus and amygdala [15,19–21]. The regulatory role of the ECB system in development of excitatory and inhibitory neuronal lineages is also conserved in the adult brain, in which CB_1 receptors are functional in cortical excitatory projecting neurons and inhibitory GABAergic interneurons [8,22].

The expression and functionality of the ECB system has also been characterized in human brain development [23,24]. In human foetal brain, *in situ* hybridization and binding assays evidence a heterogeneous pattern of CB_1

receptor expression with preferential limbic expression and high levels throughout the cerebral cortex, hippocampus, caudate nucleus, putamen and cerebellum. CB_1 receptors are present at gestational week 9 in the subventricular zone (SVZ) and Cajal–Retzius cells of the marginal zone [25]. In the second trimester of gestation, intense labelling for CB_1 receptors is evident in the hippocampal CA region [24]. High densities of CB_1 receptors are detected during prenatal development in fibre-enriched areas that later in the adult brain are practically devoid of these receptors [23]. Overall, the early expression and functionality of the CB_1 receptor during nervous system development and its transient and atypical localization in prenatal stages suggest a specific role of the ECB system in human brain development, with potential implications in neuropsychiatric disorders [6,26].

3. THE CB_1 CANNABINOID RECEPTOR IN NEURAL STEM/PROGENITOR CELLS

Neural stem and progenitor cells of different embryonic brain areas express a functional ECB system. ECBs are actively produced in the neurogenic niche of the developing cortex and engage CB_1 receptors on NPs of the ventricular zone (VZ; figure 2), as identified by the expression of the neuroepithelial marker nestin and the transcription factor Sox2 [13,27]. Intermediate progenitor cells of the SVZ, characterized by the expression of the transcription factor Eomes/Tbr2, that contribute to the generation of pyramidal cells in all layers of the cerebral cortex [28], are also targeted by CB_1 receptors (Díaz-Alonso *et al.* 2012, unpublished results). CB_1 receptors are present in dividing cells identified by 5-bromo-2′-deoxyuridine labelling, the expression of endogenous cell cycle markers (Ki-67, phosphorylated-histone 3) and the phosphorylation of vimentin (a marker of radial progenitor cell division) [13,29,30]. These observations indicate that the CB_1 receptor present in both apical radial progenitors and basal intermediate progenitor cells, albeit at low expression levels when compared to differentiated neurons, exerts a regulatory role in progenitor cell fate. Whereas in the developing chick embryonic CB_1 receptor expression follows neuronal differentiation and, at least in the spinal cord, might be restricted to postmitotic neurons [31,32], its expression pattern in the nervous system of the zebrafish is suggestive of its involvement in neurogenesis [33].

Neurospheres (non-adherent *in vitro* culture of NP cells) from embryonic and postnatal development stages express CB_1 receptors and the anandamide (AEA)-degrading enzyme fatty acid amide hydrolase (FAAH), and elevations in their intracellular Ca^{2+} concentration increase ECB production [29]. In addition, the CB_{2-} receptor and diacylglycerol lipase (DAGL), the enzyme responsible for 2AG generation, are also functional in NP cultures [34,35]. AEA and 2-arachidonoylglycerol (2AG) can act therefore in an autocrine or paracrine manner on NPs or surrounding neighbour cells. DAGL expressed at embryonic stages is preferentially located in axon growth cones and is later redistributed to dendrites where it controls the 2AG retrograde neuromodulatory signalling role [36]. During corticogenesis, as well as in the developing retina, ECB production by *N*-acyl phosphatidylethanolamine–phospholipase D (NAPE-PLD; one of the enzymes responsible for AEA generation) and DAGL participate in axon guidance [37,38]. *In vitro* studies in neuroblastoma cells confirmed the positive action of 2AG production in neurite outgrowth and the existence of different mechanisms of action according to the metabolic origin of 2AG [39]. Unfortunately, the precise contribution of the two DAGL enzyme isoforms (α and β) in neural development remains to be clarified (see accompanying paper by Doherty *et al.*). In the adult, SVZ DAGLα is present in ependymal cells that are intimately related to neural stem cells, and mediates 2AG generation involved in the regulation of neurogenesis [40]. The analysis and characterization of the DAGL locus identified the minimal core promoter sequence and the involvement of the transcriptional regulator specificity protein Sp1 in DAGLα expression. High expression levels of DAGLα in the NSC line Cor-1 rapidly decrease through their differentiation into GABAergic neuronal cells [41], whereas in neuroblastoma cells retinoic acid-induced neuronal-like differentiation increases first DAGLα expression and later DAGLβ [39]. In the developing forebrain, monoacylglycerol lipase (MGL) expression is preferentially observed in the thalamus, thus restricting local 2AG levels and relieving non-permissive axonal growth of corticothalamic projections [42].

Although not discussed here in detail, the regulatory role of the CB_1 receptor in neuronal generation and maturation in the embryonic brain is preserved in the neurogenic niches of the adult brain. NPs in adult neurogenic brain areas also express the CB_1 receptor and produce ECB ligands [29,30,43]. CB_1 receptors are expressed in NP cells of the subgranular zone (SGZ) and SVZ, in which they drive progenitor proliferation and tune neural differentiation. These findings indicate that the role of ECBs as developmental signalling cues is conserved in the mature nervous system [44].

(a) *The CB_1 cannabinoid receptor drives neural progenitor cell proliferation*

CB_1 receptor activity in NPs regulates cell proliferation and survival. *In vitro*, the use of neurosphere cultures of embryonic cortical NPs derived from knockout mice has shown that inactivation of the CB_1 receptor, as well as of the CB_2 receptor, reduces cell proliferation and impairs self-renewal [29,34]. Accordingly, pharmacological regulation with selective CB_1 and CB_2 receptor agonists or antagonists exerts a positive or negative action, respectively, on NP cell division [29,30,34,40,45]. *In vivo*, CB_1 receptor loss of function induces alterations of cortical and hippocampal development [20,29] and, whereas CB_1-null mice have reduced cortical progenitor proliferation, in FAAH-deficient mice the opposite is observed [13,29]. Abnormal cortical development in CB_1-deficient mice is characterized by defective SVZ/VZ pyramidal progenitor proliferation and radial migration, deficits in axonal navigation and aberrant corticofugal projections [13]. The role of the ECB system in the regulation of pyramidal NP cell expansion during cortical development is also recapitulated in brain slices, in which pharmacological regulation of CB_1 receptors or genetic

manipulation of the ECB tone disrupts proper pyramidal neuron generation [13]. NP proliferation from other brain areas such as the cerebellum is also dependent on CB_1 receptor activation [45].

4. CANNABINOID SIGNALLING IN NEURAL PROGENITOR/STEM CELLS

CB_1 receptor signalling in neural cells has been extensively studied, but the existence of selective CB_1 receptor-mediated signalling mechanisms in progenitor cells remains to be investigated in detail. CB_1 receptor-evoked signal transduction pathways can be divided into two large categories: canonical signalling via the classical repertoire of heterotrimeric Gi protein partners, and crosstalk with other membrane receptor-dependent signalling events (in particular those elicited by neurotrophin/growth factor receptors). Current understanding of the signal transduction mechanisms regulated by CB_1 receptors in NPs is summarized in figure 3.

(a) *CB_1 cannabinoid receptor signalling mechanism and cell proliferation*

The CB_1 receptor-mediated proliferative and pro-survival actions have been attributed, at least in part, to the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt axis and extracellular signal-regulated protein kinase (ERK; figure 3) [48]. The CB_1 receptor, via canonical Gi-mediated inhibition of adenylyl cyclase, decreases cAMP concentration, and this in turn plays a prominent role by de-inhibiting the ERK pathway by protein kinase A [49,50]. In addition, G protein βγ subunits liberated upon CB_1 receptor activation stimulate the ERK pathway in a PI3K-dependent manner [51]. Therefore, both regulation of cAMP levels and PI3K signalling contribute to CB_1-mediated ERK activation. However, the mechanisms of CB_1 receptor-mediated ERK activation are multiple and interconnected, thus providing a rather complex scenario. It is likely that, at different time points, upon CB_1 receptor activation ERK activation may occur by different mechanisms [52]. According to this model, early ERK activation would be strongly dependent on cAMP levels, activation of members of the cytosolic tyrosine kinase Src family and transactivation of tyrosine kinase receptors. In cerebellar granular progenitor cells, CB_1 receptor coupling to the PI3K/Akt pathway is followed by the activation of the glycogen synthase kinase-3β/β-catenin pathway [45]. CB_1 receptor activation therefore increases β-catenin nuclear localization and the activation of lymphoid enhancer factor/T-cell factor transcription factors induces proliferation, thereby modulating cell cycle regulatory genes such as cyclin D1.

CB_1 signalling in neural cells may also involve the activation of mammalian target of rapamycin complex 1 (mTORC1), a serine/threonine protein kinase that regulates cell growth, proliferation and survival [53]. CB_1 receptor stimulation in hippocampal GABAergic neurons activates mTORC1 and downstream p70S6K in pyramidal neurons that, by controlling protein synthesis, is responsible for some amnesic effects of Δ^9-tetrahydrocannabinol administration [54,55]. Therefore, CB_1 receptor-induced mTORC1 and protein synthesis regulation can explain some long-term cannabinoid actions on neuronal plasticity and cognition. The role of CB_1 receptors in mTORC1 signalling during brain development remains unknown, although CB_2 receptors have recently been shown to be coupled to mTORC1 activation in NP cells both in the developing cortex and in the SGZ of the adult hippocampus [56]. At postnatal stages, mTORC1 signalling is known to be involved in oligodendrocyte differentiation and myelination [53,57], and the ECB system drives oligodendroglial differentiation and cell survival at least partially via mTORC1 regulation [58]. In contrast to neurons and progenitor cells, in which mTORC1 is activated by cannabinoid receptors [54], in transformed glioma cells cannabinoids, via tribbles homologue 3, inhibit the Akt/mTORC1 axis and can

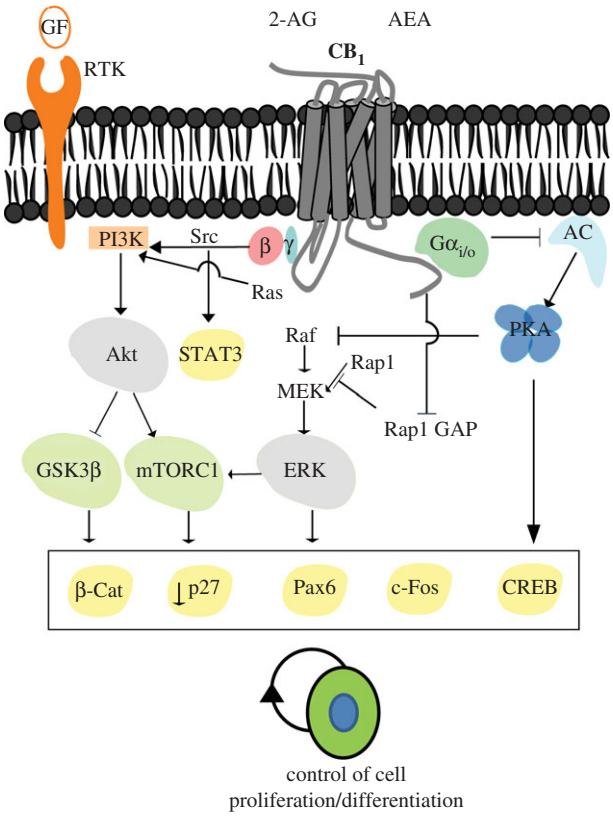

Figure 3. CB_1 cannabinoid receptor signalling and regulation of neural stem/progenitor cell proliferation. CB_1 receptors are coupled to Gi proteins, thereby mediating the inhibition of adenylyl cyclase (AC) and protein kinase A (PKA). CB_1 receptor coupling to Gi signalling is also associated with activation of the extracellular signal-regulated kinase (ERK) pathway via different mechanisms (see text for details). Direct activation of the PI3K/Akt and ERK pathways by CB_1 receptors may converge, thus synergizing with their activation by other receptors such as growth factor receptors with tyrosine kinase activity (RTK). CB_1 receptor-induced activation of RTKs can occur by promoting the processing of membrane-bound growth factor inactive precursors to yield active growth factors, or by activating intracellular Src family protein kinases. In some circumstances, CB_1 activity can antagonize RTK-mediated ERK signalling (see [46,47] for further details). Activation of the CB_1 receptor ultimately controls different transcriptional regulators, including CREB, STAT-3, PAX-6 and β-catenin. The CB_1 receptor may also regulate mammalian target of rapamycin complex 1 (mTORC1) in NPs as it occurs in differentiated neurons.

switch on an autophagy programme that results in cell death by apoptosis [59,60].

How this diversity of intracellular CB_1 receptor signalling mechanisms in neural cells is regulated remains poorly understood. CB_1 receptors may form homo- or heterodimers with other G-protein-coupled receptors [61,62] and this may shift intracellular signalling coupling. Importantly, although CB_2 receptors share some of the CB_1 receptor signalling effectors (inhibition of cAMP production, ERK and PI3K/Akt activation), their opposite pattern of expression during NP cell differentiation (i.e. NPs are $CB_1^{low}CB_2^{+}$, while differentiated neurons are $CB_1^{+}CB_2^{neg}$) may lead to different ratios of homo- and heterodimers that can promote alternative cell fate decisions according to the major signalling pathway engaged.

(b) *CB_1 cannabinoid receptor crosstalk with other extracellular signalling pathways*

CB_1 receptors have been shown to crosstalk with growth factor and neurotrophin signalling events at different levels (figure 3). CB_1 receptor activation is associated to changes in growth factor expression, and can regulate tyrosine kinase growth factor receptors by direct transactivation mechanisms. In the adult nervous system, CB_1 receptor expression is involved in the regulation of the levels of the neurotrophin brain-derived neurotrophic factor (BDNF), and thus CB_1-deficient mice have reduced hippocampal BDNF levels under basal circumstances, which could explain some of the neuronal plasticity and emotional alterations shown in those animals [63–65]. Transactivation of growth factor receptors with tyrosine kinase activity (EGFR, Trk B and others) has been shown to be involved in some CB_1 receptor-mediated neurodevelopmental actions [52]. CB_1 receptor-induced transactivation can be mediated by growth factor or cytokine (e.g. TNFα) expression or their processing and shedding from inactive membrane-bound precursors [66,67]. Moreover, transactivation can occur via cytosolic tyrosine kinases of the Src family and this mechanism may influence interneuron migration [20]. Growth factor levels are also regulated by cannabinoid signalling under different neurodegenerative paradigms, such as hippocampal and striatal excitotoxicity, in which BDNF, fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) are tuned by CB_1 receptors [68–70]. Reciprocally, FGF receptors promote axonal growth and guidance via DAGL activation and 2AG generation [71].

CB_1 receptor activation can also lead to the regulation of small G proteins and subsequent control of cytoskeleton and microtubule dynamics, which may be responsible for cannabinoid actions on neuritogenesis and synaptogenesis. Activation of CB_1 receptors can induce either neurite outgrowth or retraction [72–76]. CB_1 receptors are enriched in the axonal growth cones of GABAergic interneurons at late gestation and, when activated, they induce a chemorepulsive collapse of axonal growth cones by activating RhoA [37,73]. CB_1 receptor-induced neurite outgrowth in neuroblastoma Neuro2A cells occurs via Rap1, Src and the signal transducer and activator of transcription 3 (STAT 3) [74,76]. CB_1 receptor activation and IL6 receptor signalling exert a synergistic effect in cAMP–responsive element binding protein (CREB) and STAT3 activation that enforces neurite outgrowth [77]. In the retina, the CB_1 receptor induces growth cone collapse in a mechanism involving the intracellular trafficking of the deleted in colorectal cancer receptor [38]. Nerve growth factor-induced neurite outgrowth of PC12 cells is inhibited by CB_1 receptor modulation of Trk A/Rap1/B-Raf-mediated sustained ERK activation [72]. The recent demonstration that recruitment of the Gi-interacting protein GRIN (G-protein-regulated inducer of neurite outgrowth) upon CB_1 receptor activation can determine the signalling output of FGF stimulation, by allowing Sprouty-mediated inhibition of ERK signalling [46], may reconcile the apparent conflicting results of CB_1 receptors mediating a positive or inhibitory action in neurite outgrowth and ERK activation. In summary, further investigation on the role of recently described CB_1 receptor interacting proteins (i.e. CRIP1, AP3 and others) will shed light on cannabinoid signalling mechanisms [78] and may clarify the different neurodevelopmental actions of CB_1 receptor activity. Importantly, the different kinetics and intensity of signal transduction pathways engaged by the CB_1 receptor in a particular cellular context can induce different NP cell fate decisions, for example from proliferation and self-renewal (acute ERK activation) to neural differentiation (sustained ERK activation).

5. THE CB_1 CANNABINOID RECEPTOR AND NEURAL DIFFERENTIATION

The diversity of neurodevelopmental actions of the CB_1 receptor is suggestive of a regulatory role of the ECB system in neural cell differentiation and morphogenesis. CB_1 receptor activity has been associated to the regulation of different neural cell types' development, including neurons and glial cells. Genetic elimination of the CB_1 receptor at embryonic stages induces alterations of long-range subcortical axonal projections, but the particular mechanisms responsible for this deficit in CB_1 knockout cells are as yet unknown and may include: (i) defective VZ/SVZ pyramidal progenitor cell proliferation; (ii) impairment of radial migration; (iii) neuronal differentiation alterations; and (iv) axonal pathfinding disturbance. Inhibition of 2AG synthesis reduced vGlut1 expression and altered the expression of the glutamatergic synapse markers SNAP25 and synaptophysin [13]. However, this finding alone does not prove a regulatory role of CB_1 receptors in neuronal differentiation. CB_1 receptor expression increases with neuronal cell differentiation and thus increased or reduced CB_1 expression are likely to occur in parallel with changes in the expression of other neuronal markers. Although at embryonic stages CB_1 receptor ablation results in reduced neurogenesis [29,30], at postnatal stages manipulation of the ECB system interferes with astrocyte and oligodendrocyte development [27,79,80]. In these studies, altered neural cell populations upon CB_1 signalling manipulation are observed concomitantly with reduced progenitor cell proliferation. These observations raise the question of whether the CB_1 receptor tunes lineage selection of undifferentiated cells or acts by merely expanding specific NP populations.

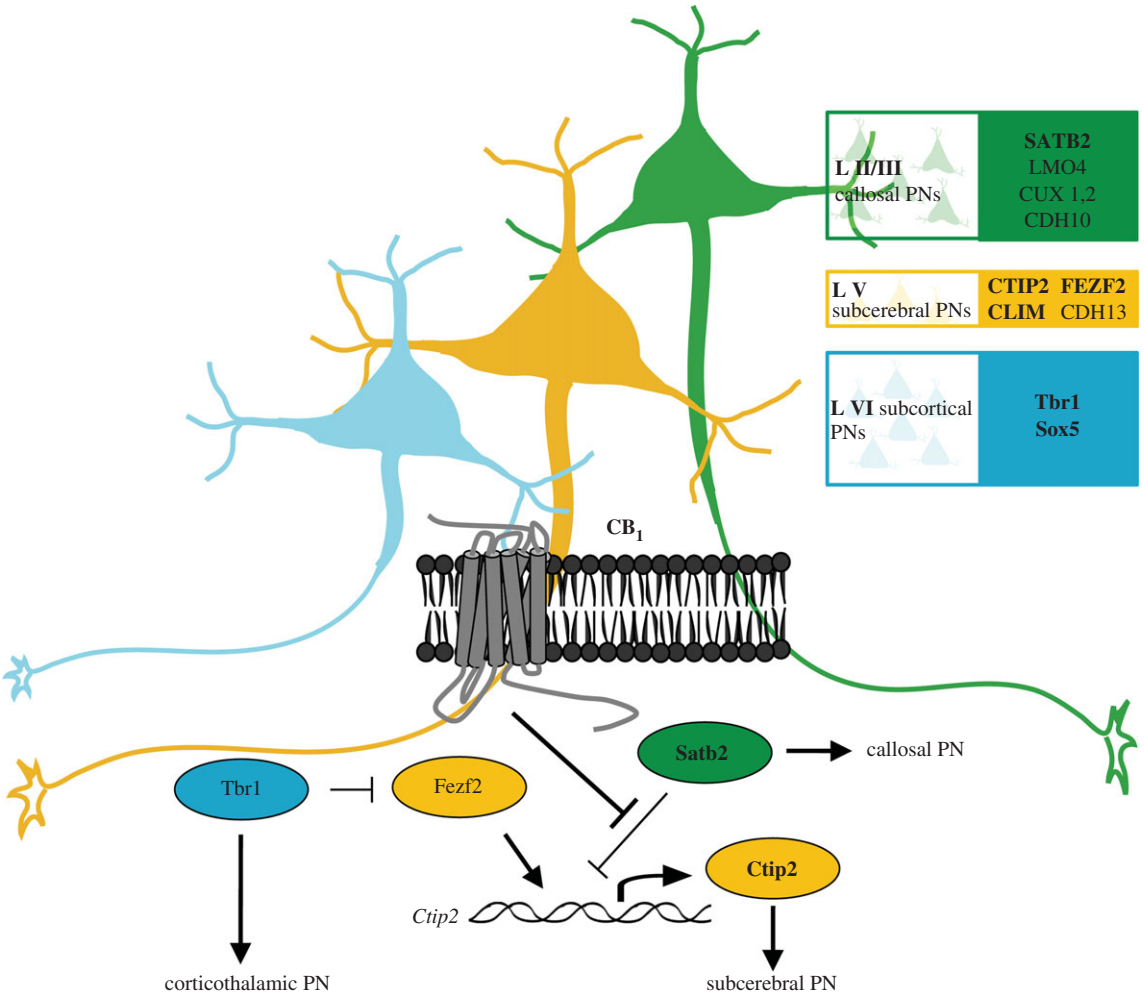

Figure 4. CB_1 cannabinoid receptor signalling and neuronal differentiation. CB_1 receptor activity in differentiating cortical neurons is coupled by as yet unknown mechanisms to the modulation of the neurogenic transcription factor code Ctip2-Satb2. CB_1 receptors are positively coupled to COUP-TF II interacting protein 2 (Ctip2) and negatively to Satb2-mediated repression of Ctip2. Thus, CB_1 receptor activity tunes the transcriptional neurogenic programme responsible for upper and lower cortical neuron differentiation. Transcription factors involved in cortical laminar specification regulated by CB_1 receptor are indicated in bold letters.

(a) *CB_1 cannabinoid receptor-mediated regulation of gene expression*

CB_1 receptor activation can regulate more than 20 transcription factors that are part of the gene expression signatures involved in NP maintenance, neuronal commitment and maturation [81]. CB_1 receptor signalling converges onto the activation of STAT3, a transcription factor responsible for gene expression regulation that is involved in cannabinoid-induced neurite outgrowth and ERK activation [76]. In neuroblastoma cells, CB_1 receptor-induced STAT3 activation relies on PI3K-dependent activation of the transcription factor Pax6 [81], a paired box family member essential for the generation of glutamatergic neurons and cortical neurogenesis [82]. In addition, CB_1 receptor prevents the inhibitory effect of breast cancer resistance associated on neuritogenesis [81]. During cortical development and pyramidal neurogenesis, CB_1 receptors are also able to modulate Pax6 and Tbr2 transcriptional activity in VZ/SVZ progenitors (Díaz-Alonso *et al.* 2012, unpublished results). Noteworthy, chronic administration of a Δ^9-tetrahydrocannabinol analogue severely disrupted chick neural development, and this was associated to gene expression changes of critical neurogenic transcription factors, including Krox20, Otx2, Pax6 and Sox2 [11]. Unfortunately, the involvement of the CB_1 receptor in these actions was not investigated. CB_1 receptor activity in differentiating cortical neurons is coupled by as yet unknown mechanisms [83] to the modulation of the neurogenic transcription factor code Ctip2-Satb2 (figure 4) [84]. CB_1 receptors are positively coupled to COUP-TF II interacting protein 2 (Ctip2) and negatively to Satb2-mediated repression of Ctip2. Thus, CB_1 receptor activity tunes the transcriptional neurogenic programme responsible for upper and lower cortical neuron differentiation, and CB_1 receptor inactivation results in reduced $Ctip2^+$ corticospinal projection neuron development that affects in turn motor function in adulthood [83].

The involvement of the CB_1 receptor in embryonic neuronal development [85], but also in postnatal astrogliogenesis [27] and oligodendrocyte survival and myelination [80,86], suggests that CB_1 receptor signalling could also target still unknown pro-gliogenic transcription factors [3]. ECB signalling may be involved in tumour-initiating stem cell decisions of

proliferation versus cell cycle exit and differentiation [87], and CB_1 receptor regulation of STAT3 is a likely candidate to mediate CBI regulation of astrogliogenesis [88]. In summary, the CB_1 receptor exerts a dual role, pro-neurogenic in some cases and progliogenic in others, thus indicating that differences in the intrinsic progenitor features and/or in the surrounding niche may be responsible for alternative CB_1 receptor-driven neurogenic outcomes.

6. PATHOPHYSIOLOGICAL IMPLICATIONS OF THE NEURODEVELOPMENTAL ROLE OF CB_1 CANNABINOID RECEPTORS

The neurodevelopmental role of the ECB system reveals that altered cannabinoid signalling, due to either hyper- or hypo-function of the CB_1 receptor, can exert long-lasting consequences in adult brain neuronal function by modifying the actively developing brain. Neurodevelopmental disorders can originate by subtle or severe alterations of various neurogenic processes, including neuronal generation, migration, maturation and connectivity that are responsible for adult brain dysfunction [89]. Among developmental disorders, cortical alterations constitute an important example of how embryonic deficits affect adult neurological function. As previously discussed, CB_1 receptor signalling plays a regulatory role in different neural cell fate processes involved in these pathologies. Genetic polymorphisms of cannabinoid receptors can induce subtle changes during development by influencing signalling strength or duration and later, when synaptic transmission ensues, by influencing the appropriate balance of neuronal activity. Likewise, mutations of ECB-metabolizing enzymes, including degrading (FAAH, ABHD6/12, MGL) or synthesizing enzymes (NAPE-PLD, DAGL), may result in less active enzymes that would increase or reduce ECB tone and signalling. In this regard, FAAH polymorphisms have been associated with drug abuse behaviours [90,91]. A recent proof of concept of this notion is the involvement of ABDH12 mutations that associate with the neurodegenerative disease polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract (PHARC) that occurs with concomitant demyelination and cerebellar ataxia [92].

CB_1 receptor signalling can be influenced as well by prenatal exposure to marijuana-derived cannabinoids or by contact with drugs targeting either directly or indirectly the ECB system. The neurobiological consequences of plant-derived cannabinoid intake on pre- and postnatal stages have been recently reviewed from the perspective of animal models and humans [6,93], and indicate that the brain burst period is of especial susceptibility. According to the developmental stage in which CB_1 receptor signalling is functional, its interference may affect different neural cell populations, including neuronal generation and specification (embryonic stages) [13,37], glial development (postnatal stages) [27,80] and neuronal maturation and connectivity [13,32,94]. Blockade of the CB_1 receptor when the neurogenic wave responsible for deep cortical neuronal generation is active affects corticospinal neuronal specification, thereby tuning subcerebral-versus callosal neuron-projections and thus skilled motor function in adulthood [83]. In addition, CB_1 receptor expression, first in white matter and later in postnatal grey matter, participates in whisker barrel map development of the somatosensory cortex, supporting the contribution of the CB_1 receptor for the appropriate integration of sensory information input [95]. In summary, the regulatory role of the CB_1 receptor in cortical development processes has the potential to exert significant impact on adult brain function [96,97].

Developmental interference of cannabinoid signalling can influence human emotion-, threat- and reward-related brain function at different levels [6,26]. Polymorphisms of the *CNR1* gene, which encodes the CB_1 receptor, may reduce or enhance G-protein-mediated signalling and have been associated to major depression, psychoses and schizophrenia [98,99]. Unexpectedly, polymorphisms of the CB_2 receptor-encoding gene, *CNR2*, may associate with depressive syndromes and schizophrenia [100]. Changes in the appropriate number, specification or migration of projection neurons and interneurons will result in modifications of neuronal activity that in turn will be followed by a more generalized neurochemical unbalance. The glutamatergic neuronal dysfunction hypothesis of schizophrenia [101] suggests that malfunction of the CB_1 receptors in pyramidal neurogenesis may contribute to the pathogenesis of psychoses or schizophrenia symptoms. Malfunction of the ECB system may be one of the causes underlying neuronal dysfunction, but alternatively the CB_1 receptor and ECB-metabolizing enzymes are also likely to adapt to aberrant neuronal homeostasis as an attempt to counteract the changes of neuronal transmission [102]. Thus, cortical glutamic acid decarboxylase 67 deficiency, a typical neurochemical marker of schizophrenia, results in lower CB_1 receptor expression. It remains unknown whether these kind of ECB system adaptations exert positive effects to cope with those alterations, or worsen the pathological processes.

(a) *Neurodevelopmental disorders: epileptogenesis*

One of the most common consequences of cortical development alterations is the appearance of epileptic foci due to alterations in neuronal excitability [89,103]. Considering the dual role of the CB_1 receptor in the generation and maturation of excitatory and inhibitory neurons it can be predicted that CB_1 receptor-dependent signalling alterations during development would impact the appropriate excitation/inhibition balance of the mature brain. Ablation of the CB_1 receptor interferes with cortical progenitor proliferation [29], the correct specification of upper/lower cortical neurons [83] and axonal growth and fasciculation [13,32]. Thus, unbalanced CB_1 receptor activity and its consequences in cortical pyramidal neurogenesis may elicit epileptic syndromes similar to those associated with cortical dysplasia, tuberous sclerosis or heterotopias [89]. Deletion of doublecortin, a microtubule-associated protein characteristic of migrating neuroblasts that is responsible for lissencephaly,

interferes with excitatory neuron radial migration [104], induces lamination alterations and has a profound impact on neuronal excitability [105]. These findings suggest that exacerbated excitotoxicity in CB_1-deficient mice [68] and the involvement of the ECB system in seizure threshold and epilepsy [106,107] may, at least in part, be due to developmental cortical alterations that result in unbalanced excitation/inhibition activity.

In addition to excitatory neuronal alterations, unbalanced generation of interneuron populations contribute to developmental epilepsies [103]. As the ECB system is involved in the development and morphogenesis of inhibitory neurons [15,20], it is likely that these developmental alterations may be responsible for changes in the susceptibility to epileptogenesis. Disruption of cortical interneuron development is known to exert GABAergic cell type-specific deficits, epilepsy and behavioural dysfunction [108,109]. Thus, the decrease in the number of interneurons and disruption of appropriate inhibitory synapse development observed in Dlx1-deficient mice, a homeodomain transcription factor essential during embryonic development for the production of forebrain GABAergic interneurons, is associated with a reduction of GABA-mediated inhibitory postsynaptic currents, electrographic seizures and cortical dysrhythmia *in vivo* [109]. Ablation of neurogenic transcription factors during development interferes with cortical excitation/inhibition balance and, for example, COUP-TFI knockout mice display altered balance of the development of medial versus caudal ganglionic eminence interneurons [110]. Whether the CB_1 receptor plays a role in the differentiation and development of the different interneuron populations is still unknown. However, defective CB_1 receptor function in $CCK^+vGlut3^+$ basket neuron development would conceivably affect the excitation/inhibition balance by interfering with interneuron-mediated inhibition. In agreement with this notion, experimental models of epilepsy result in predominant loss of $CCK^+CB_1^+$ basket interneurons [111], and indiscriminate loss of local-circuit hippocampal interneurons triggers network hyperexcitability, loss of CA1 pyramidal cells and hippocampal epileptiform seizures [112]. Chronic cannabinoid administration induces alterations of CCK^+ interneuron density in the hippocampus and cortex [15,20] that are likely to interfere with the balance of inhibition/excitation and thus may result in the development of epileptogenic foci.

Once neuronal activity is established, the absence or interference with CB_1-mediated neuromodulation would constitute a major mechanism for unbalanced neuronal activity through the disruption of excitatory and inhibitory activity [8,22]. CB_1 receptor engagement by retrograde ECB messengers is a key regulator of synaptic plasticity, both of inhibitory synapses (depolarization-induced suppression of inhibition and long-term depression of inhibitory transmission) and excitatory synapses (depolarization-induced suppression of excitation and long-term depression of excitatory transmission) [8,22,113]. Thus, CB_1 receptor blockade induces epileptic discharges that have been attributed to the absence of depolarization-induced suppression of GABA postsynaptic currents [114]. CB_1 receptors are involved in limbic hyperexcitability and fever-induced seizures through the potentiation of depolarization-induced suppression of inhibition in CCK^+ interneurons [105,115]. In addition, CB_1 receptors expressed solely in excitatory hippocampal vGlut1 neurons can allow protection from kainic acid-induced seizures [18,68]. It is important to note that, as within the early stages of brain development GABA is excitatory instead of inhibitory, CB_1 receptor activation and subsequent inhibition of GABA release would result in different outcomes depending on the developmental stage in which the ECB system function is altered.

7. CONCLUSIONS

Developmental neurobiology studies have started to elucidate the contribution of CB_1 receptor signalling in appropriate nervous system formation. These studies have underscored the active role of ECBs as local cues of neurogenic niches that, via the CB_1 receptor, drive progenitor cell proliferation/cell cycle progression, control neuronal migration and tune neuronal differentiation/specification. At early developmental stages, the CB_1 receptor and a precisely regulated ECB tone act as signalling cues in neurogenic niches [44,84]. CB_1 receptor activity exerts a critical regulatory role in different neural cell fate decisions, i.e. (i) cell cycle progression and proliferation; (ii) neural cell specification; and (iii) migration and morphogenesis. Dysfunction of the ECB system may be a determinant of seizure onset and epileptogenesis as a consequence of unbalanced excitatory and inhibitory neurotransmission [116,117]. At postnatal stages, acute or long-lasting CB_1 receptor-mediated neuromodulation upon cannabinoid exposure or altered ECB signalling interferes with neuronal maturation and tunes neuronal connectivity and developing circuits, which may in turn exert relevant consequences on adult neuronal function [6]. In summary, the CB_1 receptor exerts a key regulatory role in cortical developmental and this may have significant consequences in adult brain function, including the tuning of an appropriate balance of neuronal excitation/inhibition activity and the susceptibility to suffer neuropsychiatric disorders.

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