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SPECIAL ISSUE

The endocannabinoid system and the regulation of neural development: potential implications in psychiatric disorders

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Abstract During brain development, functional neurogenesis is achieved by the concerted action of various steps that include the expansion of progenitor cells, neuronal specification, and establishment of appropriate synapses. Brain patterning and regionalization is regulated by a variety of extracellular signals and morphogens that, together with neuronal activity, orchestrate and regulate progenitor proliferation, differentiation, and neuronal maturation. In the adult brain, CB_1 cannabinoid receptors are expressed at very high levels in selective areas and are engaged by endocannabinoids, which act as retrograde messengers controlling neuronal function and preventing excessive synaptic activity. In addition, the endocannabinoid system is present at early developmental stages of nervous system formation. Recent studies have provided novel information on the role of this endogenous neuromodulatory system in the control of neuronal specification and maturation. Thus, cannabinoid receptors and locally produced endocannabinoids regulate neural progenitor proliferation and pyramidal specification of projecting neurons. CB_1 receptors also control axonal navigation, migration, and positioning of interneurons and excitatory neurons. Loss of function studies by genetic ablation or pharmacological blockade of CB_1 receptors interferes with long-range subcortical projections and, likewise, prenatal cannabinoid exposure induces different functional alterations in the adult brain. Potential implications of these new findings, such as the participation of the endocannabinoid system in the pathogenesis of neurodevelopmental disorders (e.g., schizophrenia) and the regulation of neurogenesis in brain depression, are discussed herein.

Keywords Cortical development · Endocannabinoid system · Neural progenitor · Neurogenesis

Abbreviations

AEA	*N*-arachidonoylethanolamine (anandamide)
2-AG	2-Arachidonoylglycerol
BDNF	Brain-derived neurotrophic factor
eCB	Endocannabinoid
DAGL	Diacylglycerol lipase
FAAH	Fatty acid amide hydrolase
GABA	Gamma-aminobutyric acid
Glu	Glutamic acid
MAGL	Monoacylglycerol lipase
NAPE-PLD	*N*-acyl-phosphatidylethanolamine phospholipase D
NP	Neural progenitor
PSA-NCAM	Polysialic acid neural cell adhesion molecule
THC	Δ^9-Tetrahydrocannabinol
SVZ/VZ	Subventricular/ventricular zone

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Introduction

The coordination and regulation of processes such as neural specification, neurogenesis, axonal growth, and patterning is required for effective brain development. The intrinsic properties of neural cells (e.g., different degree of potentiality in temporal and spatial brain niches) are crucial for their final destination into different neuronal phenotypes, which is modulated by extracellular signaling systems and gene expression signature programs that control neural tissue formation [85, 114]. Among the variety of mechanisms involved in the regulation of neural development, neuronal activity allows a fine-tuned crosstalk between developing circuits and neural cell generation. G-protein-coupled receptors, the most numerous protein family involved in signal transduction [30], mediate many effects of different neurotransmitters and neuromodulators [44]. Synaptic activity and the release of neurotransmitters like gamma-aminobutyric acid (GABA) and glutamic acid (Glu) are actively involved in instructing neural progenitor (NP) cell proliferation [48, 71] and influence neuronal development [66, 105]. The endocannabinoid (eCB) system is expressed since early stages of neural tissue formation [46], when active transcriptional regulation of proneurogenic and gliogenic factors orchestrate neural cell lineage commitment [14]. In the adult brain, the relevance of the eCB system becomes evident by the high expression levels of G-protein-coupled CB_1 receptors, which are comparable to those of classical neurotransmitter receptors [35]. The involvement of the eCB system in the regulation of neural plasticity largely resides on its neuromodulatory function, as CB_1 receptors exert a wide regulatory role in most types of synapses [25, 35, 47, 61]. In addition, cannabinoid signaling is involved in neurogenic processes (neuronal proliferation, specification, and maturation) and in the maintenance and survival of differentiated neural cells [37, 46, 61]. Here, we review the mechanisms involved in cannabinoid actions during cortical brain development, with particular emphasis on their regulatory role in NP cell proliferation, migration, and specification, and discuss the potential pathophysiological relevance in brain dysfunction of these processes.

Expression of the endocannabinoid system during brain development

Early studies on the expression of the eCB system during brain development, by using binding assays and mRNA in situ detection, allowed the identification of changes in CB_1 cannabinoid receptors in mice and rats [13, 104]. CB_1 receptors show a characteristic expression profile during embryonic development that is regulated along time in different areas [46] until the definitive pattern of the adult brain is acquired [35]. CB_1 receptors are particularly abundant in white matter areas of the embryonic brain and their levels progressively increase from early prenatal stages to adulthood in gray matter areas. The expression of CB_1 receptors in the developing nervous system correlates with neural differentiation [46], and thus differentiation is associated with increased CB_1 receptor levels. During neocortical development, high CB_1 receptor levels are distributed along the cortical plate starting in the intermediate zone with the appearance of the early neuronal marker Tuj1 [90]. Later, CB_1 receptors are distributed in cortical layers I–VI and their expression is ascribed to principal excitatory vGlut-1-positive neurons [90]. Cholecystokinin-expressing GABAergic interneurons, which migrate from the ventral telencephalon, also express CB_1 receptors [11, 89]. The functionality of the eCB system in both excitatory and inhibitory neurons during development is reproduced in the adult brain, in which CB_1 receptors are functional in cortical excitatory projecting neurons [62, 67, 77] and GABAergic interneurons [16, 116].

In addition to cells already committed to the neuronal lineage, NP and immature neural cells also express functional CB_1 receptors. CB_1 receptors are expressed in subventricular and ventricular zone (SVZ/VZ) progenitors, identified by the expression of the neuroepithelial marker nestin and the transcription factor Sox2 [1, 90]. In addition, intermediate progenitor cells, characterized by the expression of the transcription factor Tbr2 [52], are also targeted by eCBs [90]. CB_1 receptors are present in actively dividing cells, identified by BrdU labeling and the expression of endogenous cell cycle markers. In particular, expression of CB_1 receptors by postnatal radial glia-like cells and B-like type cells [2, 6], which are considered to constitute the neural stem cell population [29, 92, 115], could ensure the connection of a functional eCB system from embryonic to adult neurogenic areas [36]. It is important to note, however, that in the developing chick embryo CB_1 receptor expression follows neuronal differentiation and, at least in the spinal cord, is restricted to postmitotic neurons [9, 124]. The expression of different elements of the eCB signaling system in undifferentiated cells in vivo has been expanded to ex vivo and in vitro studies with neural progenitors and embryonic neural stem cells. Thus, CB_1 receptors are expressed in progenitor cells grown in neurospheres from different stages of brain development, starting at embryonic day 14.5 (E14.5), to early postnatal radial progenitors (postnatal day 2.5; P2.5) and adult neural progenitors from neurogenic zones [1, 3, 6, 57]. In addition, functional elements of the eCB system have also been described in immortalized human stem cells [107] and in NP cells of mesencephalic origin [24].

Since the earliest stages of development, eCB signaling acts as a crucial regulatory cue that controls embryonic preimplantation [122]. CB_1 receptors are expressed in the embryo

from the two-cell stage to the blastocyst, whereas the other cannabinoid receptor type, namely the CB_2 receptor, is present from the one-cell stage. CB_2 receptor expression in neuronal cells is highly restricted [7, 34], and only recently CB_2 receptors have been proposed to be functional in certain neuronal populations [95, 118] and NP/stem cells [6, 42, 86, 96]. The levels of the eCBs anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) are tightly regulated during embryo development and low AEA levels are required for proper implantation [113]. Thus, fatty acid amide hydrolase (FAAH) inactivation, which yields high AEA levels, and Δ^9-tetrahydrocannabinol (THC) administration, constrains embryo preimplantation and results in aberrant expression of the lineage-specification genes Cdx2, Nanog, and Oct3/4 [123]. In vitro studies with mouse embryonic stem cells revealed that embryoid body formation occurs in parallel with the induction of CB_1 and CB_2 receptors [56]. Whereas AEA is considered the key eCB ligand involved in the regulation of blastocyst implantation; 2-AG has been proposed to play a prominent role in embryoid body cell survival, chemotaxis, and hematopoietic differentiation [56].

The expression and functionality of the eCB system in developing human brain has also been investigated [82, 90, 121]. In the fetal brain, in situ hybridization and binding assays with radioactive ligands support a heterogeneous pattern of CB_1 receptors with different degrees of expression. There is a preferential limbic expression of CB_1 receptors with higher levels throughout the cerebral cortex, hippocampus, caudate nucleus, putamen, and cerebellar cortex. During the second trimester of development, intense signal for CB_1 receptors is evident in the hippocampal CA region and in the basal nuclear group of the amygdaloid complex. Interestingly, high densities of CB_1 receptors have also been detected during prenatal development in fiber-enriched areas that are practically devoid of these receptors in the adult brain [82]. Agonist-stimulated radioactive GTP binding studies evidence that CB_1 receptors are functionally coupled to heterotrimeric G protein signaling during brain development [82]. This early pattern of expression and functionality of CB_1 receptors, together with their transient and atypical localization in white matter areas during prenatal stages, suggest a specific role of the eCB system in human nervous system development. In agreement with the presence of CB_1 receptors in VZ and neighboring areas, subependymal layer progenitor cells in the adult brain also express CB_1 receptors [27]. The expression and functionality of the eCB system in the developing human brain will be discussed extensively in the contribution to this Special Issue by Justras-Aswad et al.

The origin of endocannabinoids in the neurogenic niche

Control of eCB levels is achieved by the balance between the extent of stimulation of their synthesis by neuronal activity (the major driving force for eCB production) and their rate of clearance via uptake and degradation [4, 47]. eCB generation from membrane lipid precursors can occur via the activity of different enzymes: *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD), α/β-hydrolase 4 and other enzymes for AEA [68, 94, 111], and *sn*-1-diacylglycerol lipase (DAGL) for 2-AG [15]. Cloning of DAGL revealed the existence of two isoforms (α and β) [15], whose specific functions remain to be elucidated. During embryonic development, DAGLβ appears earlier than DAGLα, being expressed by pyramidal neurons and inhibitory synapses [11, 90, 124]. The transition of DAGL microlocalization from axonal tracts (in the embryo) to dendritic fields (in the postnatal and adult brain) may be due to developmental changes in the requirement for 2-AG synthesis from the presynaptic to the postsynaptic compartment [15, 46]. Activation of metabotropic or ionotropic neurotransmitter receptors can increase intracellular calcium concentration and activate or recruit eCB-synthesizing enzymes [35, 47]. DAGLα in postsynaptic spines is functionally coupled to type 1 metabotropic glutamate receptors by Homer scaffold protein [59] and allows the production of 2-AG as a retrograde modulator targeting closely located presynaptic CB_1 receptors [117, 126]. On the other hand, expression of NAPE-PLD is apparent in dendritic spines of pyramidal cells by E18.5 but not at earlier times [11] and, similarly, is not detected during chick nervous system development [124]. NAPE-PLD in the adult brain seems to be predominantly located in intracellular organelles and calcium stores of presynaptic terminals [93]. Fine regulation of eCB availability in temporal and local axes is therefore achieved by the selective sub- and intercellular distribution of synthesizing/degrading enzymes and receptors [59, 93, 117], concerted or divergent regulatory mechanisms [101], and eCB interactions [4, 73]. Whereas 2-AG is the most abundant eCB in embryonic stages and AEA levels peak in the perinatal period, the described complexity of eCB metabolism makes necessary further studies to elucidate the relative importance of AEA, 2-AG, and other potential eCBs in the different events regulated by CB_1 receptors during neural development.

Regulation of neural progenitor proliferation by the endocannabinoid system

The dynamic expression of a functional eCB system during neural development, when neurogenic processes are most active, suggests that eCBs are involved in the regulation of important cell fate decisions. Studies of cannabinoid regulation of neurogenesis in knockout mice revealed that CB_1 receptors have reduced NP proliferation in the

hippocampus and SVZ [1, 55, 57]. Likewise, increased levels of eCBs in FAAH-deficient mice [26] were associated with enhanced hippocampal progenitor proliferation [2]. In vitro studies have shown that inhibition of FAAH and DAGL activities (with URB597 and RHC80267, respectively) increases NP/stem cell proliferation [1, 42, 106], suggesting a biological role for locally produced AEA and 2-AG (Fig. 1). During neocortical development, CB_1 receptors present in the SVZ/VZ participate in the regulation of pyramidal progenitor proliferation and pool size. Thus, BrdU pulse studies showed that in embryos of CB_1 receptor-deficient mice [78] neocortical NP proliferation is inhibited, whereas FAAH-deficient embryos display increased progenitor proliferation [2, 90]. These genetic evidences for the involvement of CB_1 receptors in NP proliferation are further supported by pharmacological manipulation in utero as well as in organotypical cultures. Administration of the CB_1-selective antagonist rimonabant (SR141716) to pregnant mice inhibited SVZ/VZ progenitor proliferation, whereas forebrain slices (E14.5) exposed to a synthetic cannabinoid agonist (HU-210) or FAAH inhibitor

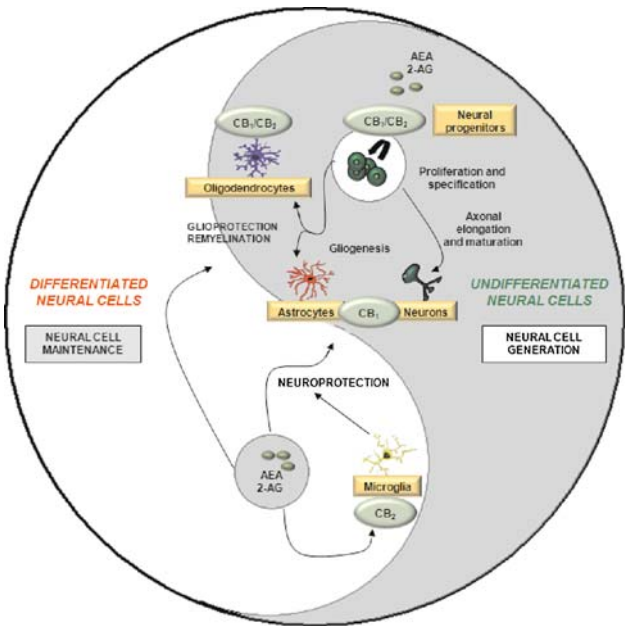

Fig. 1 The eCB system regulates neural cell generation and maintenance during embryonic development and in the adult brain. Cannabinoid receptors present in proliferative neural progenitors (*gray yang side*) are involved in self-renewal, cell proliferation, and pyramidal neuron specification. CB_1 receptors regulate radial migration of maturing neurons, axonal elongation, and pathfinding, which will ultimately control long-distance subcortical projections. In postnatal stages, CB_1 receptors are involved in the generation of the glial lineage including astrocytes and oligodendrocytes. In the adult brain (*white ying side*) CB_1 receptors exert a prosurvival effect that contributes to the protection of neuronal and glial cells. In addition, CB_2 receptors present in microglial cells exert a noncell autonomous regulatory role of neuronal function by controlling neuroimmune interactions

(URB597) had increased BrdU incorporation in the VZ/SVZ [39]. In postnatal stages, inhibited progenitor proliferation is still evident in CB_1-deficient mice [2, 90] and selective CB_1 receptor activation increased glial lineage cell proliferation of 3CB2-positive radial glia and Olig-2-positive oligodendroglial cells [6].

Role of CB_2 cannabinoid receptors in neural cell generation

In addition to CB_1 receptors, CB_2 receptors have also been implicated in progenitor cell fate decisions and neural survival [7, 34]. CB_2 receptors are present in embryonic undifferentiated NP and stem cells (based on RT-PCR, immunofluorescence, and pharmacological analyses [86, 96]) and their levels are strongly reduced in differentiated neuronal cells [34]. In postnatal SVZ, electron microscopy studies indicate that CB_2 receptors are present in polysialic acid neural cell adhesion molecule (PSA-NCAM)-positive cells [6]. Hippocampal progenitors from CB_2 receptor-deficient mice [21] have defective proliferation, whereas challenge with CB_2-selective agonists (HU-308 and JWH-133) increases NP proliferation and neurosphere generation [96]. CB_2-selective agonist administration increases hippocampal NP proliferation in vivo, an action that is not observed in the presence of a CB_2-selective antagonist (SR144528) or in CB_2 receptor knockout mice [42, 96].

The expression of CB_1 and CB_2 receptors in NP cells leaves open the question of whether they constitute equivalent or distinctive instructive mediators toward different neural lineages, e.g., neuronal or glial. Some evidences suggest that CB_2 receptors might be pro-neurogenic. Thus, hippocampal NP proliferation induced by kainic acid administration, which may ensue as an attempt to restore neuronal cell loss [70], is impaired in CB_2 receptor knockout mice [96]. In the postnatal brain, chronic CB_2 receptor activation with JWH-056 increased PSA-NCAM immunoreactivity, pointing to an increase in migrating neuroblasts [6], whereas enhanced MBP immunoreactivity and remyelinization in the external capsule of subcortical white matter occurred upon the administration of the CB_1/CB_2-mixed agonist WIN-55,212,2 [6]. The mechanism of CB_2 receptor-mediated regulation of NP proliferation is far from being fully understood, but some insights have already been provided. In vitro studies showed that CB_2 receptor-mediated NP cell proliferation and neurosphere generation relies on the activation of the extracellular signal-regulated kinase and the phosphatidylinositol 3-kinase/Akt pathways [96]. Cannabinoid regulation of NP cells and neurogenesis could be more relevant in situations of specific requirements such as the active stages of brain development, injury-induced neuronal loss, and neurodegenerative disorders. In line with this notion, pharmacological manipulation with WIN-55,212,2

or JWH-133 induced a robust NP proliferation response in the aged SVZ and an increased generation of newly born olfactory bulb neurons [42]. The impact of CB_1 receptors in those processes will be discussed in more detail below.

Besides the direct effect of CB_2 receptor activation in neural progenitors, it is likely that an important contribution to CB_2 receptor-mediated actions is made by noncell autonomous effects such as the regulation of neuroimmune interactions [7, 8]. Brain inflammation is associated with the inhibition of neurogenesis as a result of immune cell infiltration and excess of proinflammatory mediators [87]. Due to the prominent role of CB_2 receptors in attenuating the activation and recruitment of microglial and peripheral immune cells, which may exert a deleterious effect on neurons [32, 76, 97], CB_2 receptor engagement would be expected to contribute to the stimulation of neurogenesis (Fig. 1) [97, 112].

The endocannabinoid system in neural cell specification and brain patterning

The eCB system via CB_1 receptors is functional during cortical development from early embryonic stages, later during maturation of the postnatal brain and, finally, in the fine tuning of neural plasticity provided by adult neurogenesis. In addition to the expression of distinct elements of the eCB system (receptors, eCB-metabolizing enzymes, and eCBs ligands) by NPs and developing neuroblasts, mature postmitotic neurons posses high levels of CB_1 cannabinoid receptors, suggesting the requirement for CB_1 signaling in neuronal maturation and specification [46]. Recent evidences have indeed proven that the eCB system is an active player regulating cell specification in the developing neocortex for the two major neuronal populations: pyramidal projecting neurons and GABAergic interneurons [36, 46]. Radial migration and pyramidal neuronal layering show aberrant cortical distribution in CB_1 receptor- and FAAH-deficient mice at early postnatal stages [90]. FAAH inhibition or CB_1 receptor activation in organotypic cultures promoted radial migration from the SVZ/VZ to the upper cortical plate. Likewise, FAAH overexpression by embryo electroporation, and thus depletion of AEA and most likely other eCB-related species, resulted in impaired radial migration. Because the eCB system, in addition to regulate pyramidal cell development, is also involved in interneuron migration [10, 11, 89] (see below), the selectivity of the impairment of pyramidal cell morphogenesis by CB_1 receptors was tested by their conditional deletion in pyramidal progenitors [88]. CB_1 receptor deletion from glutamatergic cells was induced by Cre recombinase expression under the control of the Nex promoter, which drives the expression of a basic helix-loop-helix protein essential for pyramidal cell development [40]. Similar alterations in cortical formation were observed in complete and glutamatergic-conditional CB_1 knockout mice, corroborating the importance of CB_1 receptor signaling in pyramidal development [90]. At later stages of brain maturation, CB_1 deletion or pharmacological blockade by antagonist administration in utero resulted in aberrant corticofugal projections. Inhibition or deletion of CB_1 receptors induced corticothalamic misguidance of L1-NCAM pyramidal axons that failed to invade the dorsal striatum [90]. Likewise, exposure of chick embryo explants to the CB_1 receptor antagonist AM251 alters axonal growth of spinal cord trigeminal mesencephalic neurons, and similar findings were corroborated in zebrafish embryos by using CB_1 antisense morpholinos [124]. These observations are in agreement with the expression of CB_1 receptors in white matter areas during development and their colocalization with elongating axons identified with the GAP-43 marker [41]. Further support for an axonal growth-promoting role of eCBs has been obtained in vitro: AEA stimulates elongation of the leading axon while inhibiting nerve growth factor-induced neurite branching [90]. Moreover, DAGL-mediated 2-AG generation was proposed to mediate fibroblast growth factor-induced axonal growth of primary cerebellar neurons [125].

CB_1 receptors are also involved in the regulation of neuritogenesis and synaptogenesis. Studies with cell lines indicate that CB_1 receptor activation can induce either neurite outgrowth or retraction [49, 53, 58, 107, 128]. In neuroblastoma cell lines (e.g., Neuro2A), CB_1 receptor activation induces neurite outgrowth via Rap1, cytosolic tyrosine kinase src, Stat-3 [49, 58], and the regulation of a transcriptional signaling network that includes the transcription factor Pax-6 [20]. On the contrary, neurite retraction depends on the inhibition of Rap1/B-Raf-mediated sustained ERK activation [107] and the regulation of cytoskeletal dynamics via the monomeric G protein Rho [53]. Glutamatergic synapse establishment is also regulated by CB_1 receptors. In particular, inhibition of 2-AG synthesis in pyramidal cells reduced vGlut1 expression and altered the expression of the glutamatergic synapse markers SNAP25 and synaptophysin [90]. Other studies had previously shown that THC and WIN-55,212-2 inhibits cAMP-induced formation of new hippocampal synapses [64] but, in contrast, cannabinoids prevented synapse loss induced by neuronal activity [65].

The eCB system via CB_1 receptors regulates dentrite arborization and interneuron migration [10, 11]. In vivo, chronic THC administration during prenatal development altered cholecystokinin-positive cell density in the hippocampus [10]. In vitro, AEA stimulated cholecystokinin-positive interneuron migration in a CB_1 receptor-dependent manner. CB_1 receptors are enriched in filopodial tips and

axonal growth cones, and AEA inhibited both basal and brain-derived neurotrophic factor (BDNF)-induced neurite branching and elongation [11]. CB_1 receptors can transactivate trkB receptors in a src-dependent manner and this would favor interneuron migration [10], whereas neurite repulsion toward chemoattractive (e.g., BDNF) gradients relies on RhoA activation [11].

Overall, different studies support the notion that the eCB system plays a crucial role via CB_1 receptors in the control of neuronal migration, axonal elongation, and synaptogenesis. The precise impact of CB_1 receptor signaling depends on the neuronal maturation stage and may rely on differences in CB_1-signaling coupling, CB_1 receptor subcellular localization, and the activity of eCB-synthesizing enzymes.

Potential implications of cannabinoid regulation of neural development in psychiatric disorders

As discussed above, genetic and pharmacological studies have evidenced that eCBs and drugs targeting the eCB system can affect neuronal development and specification [37, 46]. These findings add to the reported impact of prenatal exposure to cannabinoids during gestation of animal models (Fig. 2) [5, 84, 102]. Although long-term administration of THC at high doses does not seem to induce neurotoxicity in rodents [110], cannabinoids can influence cognitive processes and emotional behavior by interfering with the fine-tune regulation of neuronal activity [12]. In fact, cannabis abuse has been related to several psychiatric disorders including anxiety, depression, cognitive impairment, psychosis, and schizophrenia [119]. It is important, however, to note that data derived from animal models based on the chronic administration of different cannabinergic drugs should be considered with caution when referring to the biological relevance of the signal transduction and cellular events regulated by the eCB system. In any case, it is conceivable that interfering with correct eCB signaling in the rapidly changing embryonic brain may contribute to some disorders of the adult nervous system, especially those considered to have a significant neural development component. Additionally, the potential use of cannabinergic drugs to palliate some of the symptoms of neuronal disorders may be supported by the role of the eCB system in the regulation of neuronal cell survival and plasticity.

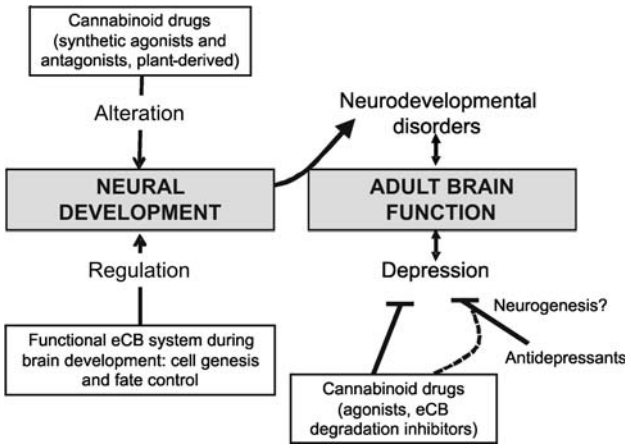

Fig. 2 Cannabinoid regulatory actions of neural development and brain function. During brain development the endocannabinoid (*eCB*) system exerts an endogenous role regulating neural cell fate and generation that may explain the alterations in the adult brain induced by the administration of exogenous cannabinoid drugs. The influence on adult neurogenesis of antidepressant drugs suggests the hypothesis that antidepressant cannabinoid action may, in part, be attributed to the regulation of neural cell generation. Likewise, defective eCB signaling and neurogenesis could be associated with depression

Cannabinoids, depression, and cognition

The neuromodulatory role of the eCB system and the high expression levels of CB_1 receptors in brain areas involved in the regulation of cognition and mood functions (e.g., amygdala, cortex, and hippocampus) point to the possible involvement of the eCB system in depressive syndrome and learning [54, 119]. The potential role of eCBs in cognition and the control of emotions [72, 119] is supported by the finding that AEA and 2-AG, as well as CB_1 receptor expression, are altered in animal models of depression [51] and postmortem brains of depressed humans [119]. Cannabinoid agonists induce antidepressive and anxiolytic-like effects in laboratory animals and humans (Fig. 2), but their potential therapeutic use is hampered by nondesired psychoactive effects induced upon CB_1 receptor activation. In this respect, a successful strategy may be the use of inhibitors of eCB uptake and degradation, which results in locally and temporally restricted increases of eCB levels [75]. Administration of the eCB reuptake inhibitor AM404 to rodents induces anxiolytic-like effects and, likewise, the inhibition of FAAH by URB597 enhances stress-coping and mood-related behavior [17, 60]. Moreover, the increased AEA levels as the result of FAAH inhibition could compensate the decrease in AEA in chronic unpredictable stress [51].

The identification and characterization of the molecular substrates of cognitive disorders and depression remains one of the major goals of modern neuroscience. The study of the mechanism of action of antidepressant drugs and stimuli has recently suggested the involvement of newly generated neurons in their effects [108]. Importantly, antidepressants are effective only after several days or even weeks of treatment, and thus their therapeutic potential cannot be fully explained by acute modulation of

neurotransmitter levels. Chronic fluoxetine treatment induces adult hippocampal neurogenesis, and ablation of NP proliferation by radiation or genetic strategies prevents antidepressant drug behavioral actions [33, 109]. Although controversy still exists about the requirement of neurogenesis in the effect of antidepressant drugs and stimuli [103], like in rodent models, antidepressants induce hippocampal neurogenesis in nonhuman primates [100]. The ability of cannabinoids to regulate adult neurogenesis suggests that the eCB system may account for some of their antidepressant actions through altered incorporation of newly born cells. CB_1 receptor activation induces NP proliferation in the hippocampus and SVZ [1, 57], which, in normal adult brain, correlates with increased astrogliogenesis rather than neurogenesis [2]. This outcome appears to be different after brain injury. Upon brain excitotoxicity, eCBs are produced on demand and exert a neuroprotective action, mediated at least in part by CB_1 receptor activation [43, 79]. Mice deficient in cannabinoid receptors had impaired NP proliferation after excitotoxicity, and neurogenesis was severely impaired in CB_1 knockout mice [3, 96]. In addition, chronic HU-210 administration induced hippocampal neurogenesis, and this seems to be involved in cannabinoid anxiolytic- and antidepressant-like actions [55]. Importantly, increasing the eCB tone by inhibiting eCB reuptake prevented stress-induced depression of neurogenesis [50]. The importance of BDNF in reducing anxiety and depression may explain, at least in part, the interaction of CB_1 receptor signaling with depression. BDNF is a crucial regulator of synaptic plasticity and its levels are elevated by antidepressants [81]. CB_1 receptor-deficient mice have lower BDNF levels, which makes them more susceptible to brain excitotoxicity [3, 63, 79, 99]. Likewise, THC administration increases BDNF levels in serum of humans [28] and brains of animal models [22]. Other growth factors which are important in NP proliferation, neurogenesis, and neuronal survival [98, 127], such as basic fibroblast growth factor, are also reduced in CB_1 receptor knockout mice [3].

The impact and consequences of adult neurogenesis may be different depending on the neurogenic area involved. In particular, dorsal hippocampal neurogenesis has been related to improved cognitive function by antidepressants, whereas ventral hippocampal neurogenesis may be more related to altered mood and emotion regulation [108]. In this context, it is still unknown whether cannabinoids regulate ventral hippocampal neurogenesis, which could contribute to understand some of the anxiolytic action of cannabinoids.

Cannabinoids and schizophrenia

Some epidemiological studies have associated increased psychotic episodes and a higher probability to develop schizophrenia with cannabis abuse [91]. In addition, alterations in the eCB system have been suggested to correlate with the severity and symptoms of this disorder. Increased levels of AEA were detected in cerebrospinal fluid from schizophrenic patients compared to healthy volunteers [38, 69]. AEA levels inversely correlated with psychotic symptoms, which may indicate that AEA release constitutes an adaptive mechanism to counteract the neurotransmitter deficits involved in psychoses. The existence of polymorphisms in the CB_1 receptor gene CNR1 that correlate with an increased probability to develop psychosis or some types of schizophrenia was also described [23, 80], although their relevance is under discussion [45]. In addition, alterations of CB_1 receptor density have been described in postmortem samples from schizophrenic patients [31]. Therefore, changes in eCB signaling and the ensuing consequences in neuronal activity and brain development might be important factors involved in the etiology of schizophrenia that may contribute to explain the impact of cannabis abuse in psychotic disorders. Importantly, defective adult hippocampal neurogenesis may participate in the pathogenesis of schizophrenia in humans [103].

The influence of the eCB system on crucial processes of neurodevelopment (Fig. 2), including neuronal specification, migration, and maturation, could also be involved to some of the alterations associated with schizophrenia. Gene polymorphisms for neuregulin 1 and its receptor Erb4 was associated to increased susceptibility to develop schizophrenia [83], and hypomorphic neuregulin-1 mice were more sensitive to the behavioral effects and increased c-fos expression evoked by THC administration [18, 19], suggesting that these or other related genetic factors may explain the potential link between cannabis consumption and psychosis or schizophrenia development. Likewise, in different models employed for the study of the etiology of schizophrenia or psychosis, such as maternal deprivation, social isolation, and others, changes in eCB signaling have been reported [74, 120]. Further studies based on detailed morphometric analyses and functional neural imaging could help clarify the involvement of the eCB system in neurodevelopment-mediated alterations of adult brain function.

Conclusions

New advances and improved procedures have allowed a better understanding of the molecular processes involved in correct nervous system formation. Thus, the cellular basis for developmental and adult brain disorders such as schizophrenia and depression is starting to be elucidated. The role of the eCB system in the regulation of neural cell survival and neuroprotection has been the subject of

intense research during the last years. In addition, recent findings on the role of the eCB system in neural development add to numerous evidences of the effect of prenatal cannabinoid exposure on adult brain function. The diversity of eCB actions in neural cells depends on a complex scenario in which different subcellular compartments (e.g., neural soma, axonal growth cones, and neurites) are involved. In addition, the existence of selective regulatory mechanisms of eCB synthesis and degradation is still under investigation. Therefore, further studies are necessary to elucidate the relative importance of the different events regulated by CB_1 receptors during neural development. Hopefully, in the near future, new findings of how cannabinoid signaling impacts neuronal differentiation (e.g., transcriptional and gene expression control mechanisms responsible for pyramidal and inhibitory neuronal specification), neuronal cell migration, and axonal guidance will contribute to understand the role of the eCB system in the development of brain disorders.

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