Chapter 6
Cannabinoids as Regulators of Neural Development and Adult Neurogenesis

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Abbreviations

2-AG	2-arachidonoylglycerol
AEA	Anandamide
Ca^{2+}	Calcium
CB_1	Cannabinoid receptor type 1
CB_2	Cannabinoid receptor type 2
CBD	Cannabidiol
DAGL	Diacylglycerol lipase
DG	Dentate gyrus
E/I	Excitation/inhibition
ECB	Endocannabinoids
MAGL	Monoacylglycerol lipase

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NP Neural progenitor
SGZ Subgranular zone
SVZ Subventricular zone
THC Δ^9-tetrahydrocannabinol

6.1 Cannabinoids

The term cannabinoid was first used to describe a class of substances with similar chemical structures extracted from the plant *Cannabis sativa*. More than 100 cannabinoids have been identified in this plant, including Δ^9-tetrahydrocannabinol (THC), the one responsible for its main psychological effects, and cannabidiol (CBD), the major non-psychotomimetic compound [1]. The observation that the activity of psychoactive cannabinoids was intrinsically related to its chemical structure [2] raised the hypothesis that cannabinoid receptors would be present in the organism. In the late 1980s, the endocannabinoid (ECB) system started to be described with the identification of a specific receptor for THC in the central nervous system (CNS, [3]) that was subsequently cloned and named cannabinoid CB_1 receptor [4].

CB_1 receptors are now considered the most abundant metabotropic receptor in the mammals' CNS and are also present in peripheral tissues. The CB_1 receptors are widely expressed in presynaptic terminals, where they regulate the release of several neurotransmitters (e.g., GABAe, glutamate, serotonin, acetylcholine, dopamine) [5, 6]. A second cannabinoid receptor, named CB_2, was described in 1993 by Munro and colleagues [7]. Although initially thought to be expressed mainly in cells of the hematopoietic and immune systems, more recent studies have challenged this notion demonstrating that CB_2 receptors may be expressed in neurons and is present in microglia and neural stem cells [8–10]. Of note, despite their different localization and, apparently, functions, both CB_1 and CB_2 receptors are coupled to a $G_{i/o}$ protein [11].

In addition to CB_1 and CB_2 receptors, their endogenous ligands (termed endocannabinoids) were also isolated in mammals. The most extensively investigated are those derived from arachidonic acid, arachidonoyl ethanolamide (anandamide-AEA), and 2-arachidonoyl glycerol (2-AG), which are degraded by specific enzymes (Fig. 6.1, [12, 13]). AEA and 2-AG can also interact with other receptors such as proliferator-activated receptors (PPAR- α and γ). Moreover, AEA interacts with GPR55 and the Transient Receptor Potential Vanilloid Type 1 (TRPV1) [14].

Cannabinoids decrease neurotransmitter release by inhibiting calcium (Ca^{2+}) and activating potassium channels [15]. They also affect short-term neuronal activity by reducing the depolarization-induced suppression of inhibition (DSI), mainly in GABAergic synapses, and the depolarization-induced suppression of excitation (DSE), in synapses that release glutamate and the neuropeptide cholecystokinin [16–18]. Moreover, cannabinoids display neuroprotective actions, being involved in the control of glutamate-induced excitotoxicity [19], and are critical regulators of neurodevelopment and adult neurogenesis [20].

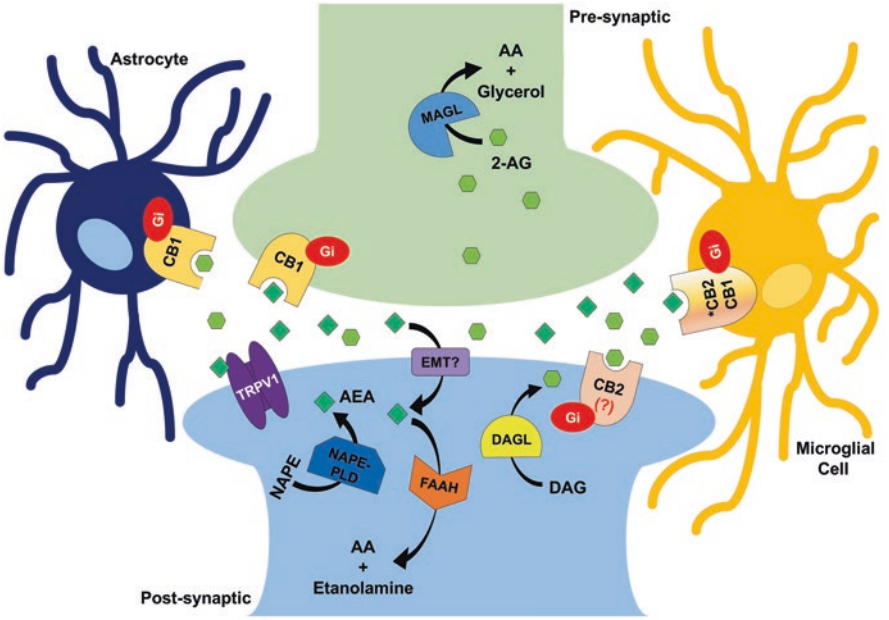

Fig. 6.1. Schematic representation of the endocannabinoid system in the brain. (*?*) Putative expression of CB2 receptor in neurons. *Microglial cells express CB1(constitutive) and CB2 (activated state) receptors. Endocannabinoids are produced in astrocytes, microglia, and neurons

In this chapter, we summarize the main pieces of evidence indicating that cannabinoid signaling on neural stem/progenitor cells affects their proliferation, maturation, and survival. These effects can modify CNS functions, being a potential new avenue for the development of novel therapeutic strategies for neurodegenerative and psychiatric disorders.

6.2 The Neurodevelopmental Role of the Endocannabinoid System

An extensive literature has addressed the consequences of developmental exposure to phytocannabinoids, mostly THC, and also to potent synthetic cannabinoid agonists. These studies have demonstrated that exposure of the immature nervous system to THC, in perinatal stages and/or the adolescence, is associated to numerous behavioral alterations [21]. Experimental evidence indicates that the developing brain is more sensitive to exogenous cannabinoid-induced plastic adaptations. These findings prompted the search of the neurobiological substrate of phytocannabinoid actions.

6.2.1 Expression of the Endocannabinoid System

The ECB system is present and functional since early stages of development, including the primordium of the nervous system, as well as in the restricted neurogenic areas of the adult brain (the hippocampal subgranular zone-SGZ and subventricular zone-SVZ). Along neuronal differentiation, CB_1 and CB_2 receptors show opposite patterns of expression, being increased and decreased, respectively [10, 22]. CB_1 receptors are expressed, although at low levels, in neuroepithelial progenitor cells from early embryonic stages, and their levels increase along neural differentiation [20]. In addition, CB_1 is enriched in white matter areas in embryonic stages, until the acquisition of its final expression pattern in the adult nervous system [23]. In vivo, CB_1 receptor levels are associated with higher expression of differentiation markers of various neuronal lineages. CB_1 receptor activity is more prominent in differentiated pyramidal projection neurons, interneurons, or cholinergic neurons than in their respective undifferentiated progenitor cells [20]. Little is known about the mechanisms controlling CB_1 receptor expression during neurodevelopment. CB_1 is induced during neuronal differentiation by neurotrophins such as brain-derived neurotrophic factor (BDNF) [24]. In mature GABAergic interneurons, CB_1 expression is controlled by the 67-kDa isoform GABA-synthesizing enzyme glutamate decarboxylase [25] and in striatal neurons is regulated by the transcription factor REST via RE1 sites [26].

The CB_1 receptor regulation by ECBS during development is poorly understood. 2-AG and AEA can be synthesized on-demand by surrounding differentiated neurons in response to neuronal activity. In addition, ECB can be produced in a paracrine/autocrine manner by neural progenitors (NPs) [27, 28]. The extracellular or intrinsic mechanisms responsible for ECB production in active neurogenic niches are not entirely understood. NPs produce and release the two major ECB compounds, namely, AEA and 2-AG, in response to increased intracellular Ca^{2+} concentration, and the ECB tone contributes to basal and stimulus-induced NP proliferation via CB_1 receptors [27, 29, 30]. 2-AG levels in neurogenic niches are precisely regulated by diacylglycerol lipase (DAGL) and monoacylglycerol lipase (MAGL) activity. Ablation of DAGLα, but not of the β isoform, interferes with hippocampal and SVZ-derived neurogenesis [31] and pharmacological inhibition of DAGL activity in NP cultures reduces cell proliferation [32]. NPs express FAAH, the primary enzyme involved in AEA degradation, and its genetic ablation or pharmacological inhibition promote NP proliferation [27, 33].

The role of extracellular signaling cues promoting ECB production is solely known for 2-AG generation, whereas signals driving AEA levels remain elusive, as the expression pattern of NAPE-PLD (N-acyl phosphatidylethanolamine phospholipase D) and FAAH (fatty acid amide hydrolase) enzymes responsible for AEA synthesis and degradation, respectively, during brain development remains unknown. Fibroblast growth factor (FGF) in coordination with neural cell adhesion molecule increases 2-AG levels via DAGL coupled with PLCγ activation. Alternatively, NGF via TrkA enhances 2-AG production during neurite outgrowth of cholinergic neurons by controlling the levels of MAGL [14]. In NPs, the high expression levels of DAGLα have been shown to rapidly decrease along their differentiation into GABAergic neuronal

cells [34], through a mechanism that relies on the regulation of the transcriptional regulator specificity protein 1. On the contrary, retinoic acid-induced neuronal-like differentiation of neuroblastoma cells increases first DAGLα expression and later DAGLβ [35].

A variety of neuroactive molecules acting via ionotropic and metabotropic receptors have the potential to engage ECB generation via increased Ca^{2+} levels or G_q-PLC activation. These responses may occur after neurotransmitter-mediated neuronal activity and are also associated with spontaneous neuronal activity during cortical development. However, the contribution of spontaneous neuronal activity (during brain development) or neuronal synaptic activity (in adult neurogenic niches) in NP cell fate regulation, via ECB production, remains unknown. In addition to CB_1 receptors, CB_2 receptor activity regulates NP cell proliferation, cell cycle maintenance, and neural differentiation [10, 32, 36]. Whereas CB_2 receptor regulation clearly regulates stem/progenitor cell responses, its expression levels and the identity of neural cells expressing it remain obscure.

6.2.2 Cannabinoid Signaling Consequences in the Developing Brain

6.2.2.1 Proliferation

The first pieces of evidence for an active role of cannabinoid signaling in NP cells came from studies on the regulation of adult neurogenesis by pharmacological cannabinoid manipulation or genetic ablation of the CB_1 receptor [20, 37]. These studies evidenced that ablation of CB_1 receptor expression reduced hippocampal and SVZ NP cell proliferation in vivo. Likewise, CB_1 receptor absence in vitro inhibits self-renewal and NP proliferation [27]. Recent findings suggest that the positive role of CB_1 receptor signaling in adult neurogenesis is reminiscent of its role in NP proliferation and identity during cortical development Fig. 6.2a [38].

CB_1 receptor signaling controls neural cell fate decisions during CNS development by regulating the expression of genes responsible for neural identity [39]. In differentiating neuroblasts, CB_1 activation regulates the homeodomain containing transcription factor Pax6 post-translationally via PI3K/Akt-dependent phosphorylation, and this is in turn responsible for its positive actions in neurite outgrowth [40]. In addition, CB_1 activation increases Pax6 expression in cortical progenitors, driving the expansion towards basal intermediate progenitors by inducing the expression of the transcription factor Tbr2/eomes [38].

6.2.2.2 Neuronal Differentiation and Morphogenesis

CB_1 receptor signaling also affects neuronal differentiation acting in post-mitotic cells and, in an independent manner of its regulatory role, in undifferentiated NPs. CB_1 signaling activates NP cell proliferation and pro-survival signaling pathways

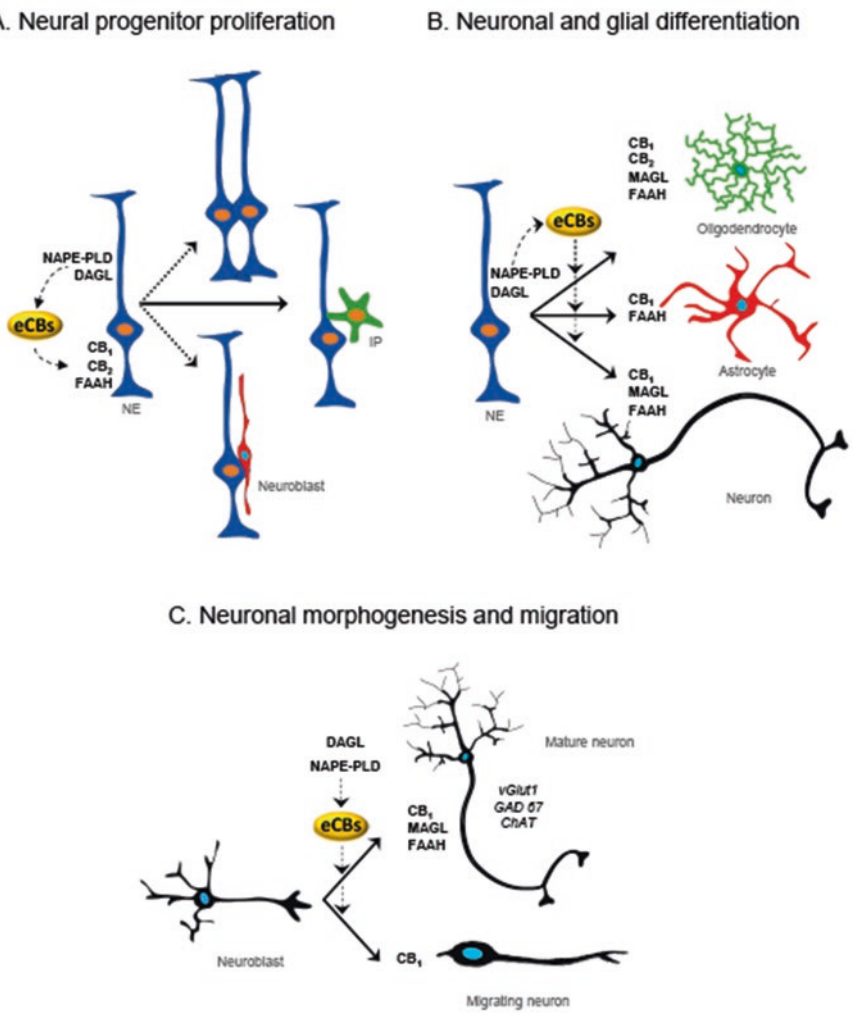

Fig. 6.2. The endocannabinoid system exerts a regulatory role on neural cell fate at different levels. Cannabinoid signaling regulates (**a**) NP proliferation and identity of progenitor cells, (**b**) neuronal and glial differentiation, and (**c**) neuronal morphogenesis and migration

that contribute to the regulation of cell cycle maintenance and the switch between cell proliferation and differentiation/migration. On the other hand, post-mitotic conditional CB_1 receptor ablation does not affect cortical progenitor expansion but only neuronal differentiation (Fig. 6.2b) [41]. CB_1 regulates the balance between the expression of Ctip2 and Satb2, two transcriptional regulators that are involved in the decision switch of deep- versus upper-layer cortical neurons. Ctip2 drives deep-layer cortical neuronal identity and corticospinal connectivity, whereas Satb2 is involved in intracortical projection neurons selectively arising from upper cortical

layers [42]. Deletion of CB_1 during mouse cortical development lowered Ctip2 expression and generation of deep-layer V neurons, and this is reflected in the reduced ability for skilled motor activity of CB_1-deficient mice [39].

Cannabinoid signaling also exerts a crucial regulatory role in axon guidance and morphogenesis (Fig. 6.2c) [14]. CB_1 receptor located in axon growth cones of differentiating neurons induces its collapse in response to DAGL-derived 2-AG, [43]. A tight spatiotemporal regulation of 2-AG availability has been suggested accordingly to the differential subcellular localization of 2-AG metabolizing enzymes [44]. MAGL is enriched in tubulin-consolidating axon shafts while DAGL accumulates in actin-rich motile axon tips, thus generating a 2-AG gradient that triggers axonal growth cone collapse. In cortical and retinal neurons, CB_1 regulates axonal growth cone by controlling the plasma membrane localization of the Dcc (deleted in colorectal cancer) receptor [45], whereas in GABAergic interneurons the monomeric G protein RhoA is involved [43]. CB_1 receptor regulation of growth cone collapse and neurite retraction relies on its ability to regulate actomyosin cytoskeleton via RhoA/ROCK signaling and Rac1/WAVE complex [46, 47].

CB_1 receptor regulation of growth cone dynamics is responsible for its role in the establishment of long-range subcortical projections. Ablation or pharmacological blockade of CB_1 receptors in utero alters corticothalamic projections and induces axon fasciculation deficits [48]. The complementary expression pattern of DAGL in thalamocortical axons and of MAGL in corticothalamic and thalamocortical developing axons contribute to the generation of spatially restricted 2-AG pools. It has therefore been suggested a potential role for 2-AG as one of the molecules responsible for the timely developmental coordination between corticothalamic and thalamocortical projection "hand-shaking" [49]. The CB_1 receptor thus exerts an acute/short-term regulation of growth cone signaling in neurite tips, as well as long-lasting changes in neurogenic gene expression that affect neuronal wiring and connectivity.

In postnatal stages, cannabinoid receptor activity regulates astroglial and oligodendroglial differentiation (Fig. 6.2b). CB_1 receptor activity increases astroglial differentiation and GFAP expression in the developing cortex [50]. In oligodendrocyte progenitor cells CB_1 and CB_2 activation promotes the expression of Olig-2 in a PI3K/Akt/mTORC1-dependent manner [51], and their activation by 2AG or WIN55,212-2 administration favors white matter recovery and oligodendrocyte differentiation [52, 53].

Noteworthy, ECB signaling in oligodendrocytes via CB_2 receptors can contribute to neuron axon pathfinding by modulating Slit/Robo signaling in corticothalamic neurons expressing CB_1 receptor [54].

6.3 Pathological Implications of Cannabinoid Signaling in the Developing Brain

The neurodevelopmental role of the ECB system and its ability to regulate neural cell fate has important implications in regard to its potential contribution to neurodevelopmental disorders. Likewise, exposure to plant-derived cannabinoids,

cannabinergic drugs interacting with the ECB system (i.e., modulators of ECB synthesis and degradation), or pollutants interfering with the ECB system can induce functional alterations in the adult progeny. Extensive literature exists regarding the consequences of cannabinoid-exposure during adolescence indicating that this is a critical period of susceptibility to deleterious actions produced by these compounds [21]. Less is known about the consequences of prenatal cannabinoid administration or embryonic manipulation of cannabinoid signaling [54, 55]. Cannabinoid-induced alterations of the nervous system development have been demonstrated in different experimental models. In early embryonic chick development, administration of a THC analogue disrupts neurogenesis and affects brain, somite and spinal cord primordium development, indicating that the ECB system is active in early cell fate decisions of neural tube progenitor cells [56]. In pregnant rats, administration of WIN-55,212-2 during the gestational period induces changes in dorsal pallial migrating neuroblasts and marginal zone interneurons [57]. Unfortunately, the impact of WIN-55,212-2 treatment in the progeny's brain was not investigated.

6.3.1 Neuronal Hyperexcitability and Epileptogenesis

Constitutive absence of CB_1 receptors in null mice results in increased seizure susceptibility that is mostly attributed to the lack of the neuromodulatory role of presynaptic CB_1 receptors [58]. In addition, the neurodevelopmental alterations associated with the loss of CB_1 receptors in early stages, i.e., during embryonic development when synaptic activity is still absent or emerging, can shed new light on the cellular mechanisms responsible for epileptogenesis and the appropriate balance of excitation/inhibition (E/I). Alterations of neurogenesis and changes of excitatory and inhibitory neuronal cell populations are, therefore, essential for coordinated activity. Considering the evidence that the ECB via CB_1 receptors regulates both excitatory projection neuron specification and GABAergic interneuron morphogenesis and local microcircuits, these alterations can contribute to the higher susceptibility and severity to seizures as a consequence of CB_1 signaling manipulation. In agreement, embryonic THC administration exerts a deleterious impact in deep-cortical layer projection neurons and increases seizure susceptibility via CB_1 receptors [59]. In this study, the impact of THC in interneurons and particularly in CCK basket cells was not investigated, but selective neuronal lineage rescue of CB_1 receptor expression [60] revealed that CB_1 receptors expressed in projection neurons and the GABAergic lineage contribute to seizure susceptibility. Likewise, prenatal THC administration, by interfering with cytoskeleton stability via c-Jun N-terminal kinase and Superior Cervical Ganglion 10/stathmin-2 protein levels, decreases Schaffer collateral-induced long-term depression and perisomatic basket cell surrounding pyramidal cell somata [61]. Interference with the correct generation of different neuronal subpopulations can be responsible for embryonic THC-induced E/I unbalance. In addition to CB_1

receptor regulation of neuronal differentiation, cannabinoid signaling actions in neuronal migration can contribute to developmental epileptogenesis. Genetic ablation of CB_1 receptors during cortical development exerts a radial migration blockade that results in ectopic projection neurons resembling subcortical band heterotopias (Díaz-Alonso, de Salas-Quiroga, Galve-Roperh, personal communication). Noteworthy, transient CB_1 receptor knockdown restricted to embryonic stages exerts long-lasting migration blockade that persists in the adulthood and induces increased seizure susceptibility. The promigratory role of CB1 receptors during brain development (Fig. 6.2c) is in agreement with the described role of the ECB system regulating neuroblasts migration in the adult rostral migratory stream [62]. These findings support the notion that cannabinoid signaling controls the appropriate E/I balance by additional mechanisms to the canonical CB_1 receptor neuromodulation.

6.3.2 Neuropsychiatric Disorders

Experimental evidence described herein reveals that defective ECB signaling or developmental exposure to phytocannabinoids can induce alterations in neuronal number, specification and functional properties, or morphological changes that may be responsible not only for seizure susceptibility but also for neuropsychiatric actions of cannabinoid signaling. The neurobiological substrate responsible for the emotional, social interaction, and cognitive changes induced by phytocannabinoid consumption or by an unbalanced ECB signaling during brain development remains largely unknown [54, 55]. In agreement with previous evidence of CB_1 regulation of CCK development, a recent study showed that embryonic THC administration correlated with selective changes in the development of CCK basket cells, but not other interneuron populations. Embryonic THC administration compromised feedforward and feedback inhibition in the progeny [63]. The persistent inhibitory deficits in the adult progeny was associated with deficient social interaction, but not increased anxiety, as reported in many studies where THC was administered in the adolescent period [21]. The impact of THC in CCK development raises the hypothesis of a potential interaction between cannabinoid signaling and autism. Noteworthy, autism-related mutations of neuroligin 3 are associated with changes in CB_1 constitutive activity [64]. THC administration during adolescence, but not later, interferes with GABA maturation and functionality in the prefrontal cortex, highlighting the importance of developmental actions in cannabinoid effects [65]. On the other hand, CB_1 receptor blockade in the adult can counteract several phenotypic markers of the Fragile X model (based on the loss of fragile X mental retardation protein FRMP) [66]. The consequences of manipulating CB_1 receptor signaling during brain development in autism models remain to be investigated. Furthermore, the role of CB_1 in interneuron developmental changes underlying the pathogenesis of schizophrenia constitutes an expanding field of research [67].

6.4 Adult Neurogenesis

At the beginning of the twentieth century, independent researchers reported what they believed to be the first description of mitotic figures in the adult nervous system of mammals [68]. However, this finding was not recognized because of the accepted dogma based on Santiago Ramon y Cajal's view that, reflecting the limitations of the techniques available at that time, it was impossible to identify dividing neurons in the adult brain [69].

For more than 100 years, evidence of adult neurogenesis was denied, as the accepted view was that this process could only happen during embryonic periods, stopping just after birth. In the early 1960s, Joseph Altman, a scientist of the Massachusetts Institute of Technology, using tritiated thymidine administered intraperitoneally in adult rats, reported that "a proliferative region of granule cells was identified in the dentate gyrus of the hippocampus" [70, 71]. Almost 15 years later, Dr. Michael Kaplan presented additional evidence that new neurons are added in specific regions of the young and adult rat brain, including the neocortex, hippocampal formation, and olfactory bulb [72–74]. However, it was the work of [75], which reported that new neurons are indeed generated in the hippocampus of adult humans that established one of the most exciting recent fields in neuroscience: adult neurogenesis.

Adult neurogenesis is a complex process that evolves from the initial division of precursor cells until the effective differentiation and generation of a new functional and integrated neuron. In the words of Dr. G. Kempermann: "Neurogenesis is a process, not an event.". It can be more precisely defined as an in vivo process that involves cell division, survival (not all cells that divide will survive), migration, differentiation, and maturation [76–78]. Neural proliferative capacity has been reported in different brain regions, such as the hypothalamus and the cell layers surrounding the third ventricle [79]. However, the best characterized neurogenic areas in the adult brain are the SVZ of the lateral walls of the lateral ventricle and SGZ of the dentate gyrus (DG) of the hippocampal formation [80]. Both regions have a resident population of neural stem/progenitor cells that can originate neurons, astrocytes, and oligodendrocytes [81].

Despite the half-century of research separating the initial findings of Altman from our current knowledge, the particular function/physiological role of adult neurogenesis, as well as the key regulators of this process, remain under debate. So far, it seems to be a consensus that experience modulates neurogenesis in the adult brain either positively or negatively. Voluntary exercise or enrichment environment enhances proliferation in neurogenic niches [82]. Conversely, chronic stress exposure decreases neurogenesis. However, due the different neurobiological nature of the two main neurogenic niches, it is reasonable to infer that neurogenesis in SVZ and SGZ might be recruited differently and consequently exerts distinct or complementary roles on brain functions [77].

In the SVZ, neurogenesis is regulated by the olfactory experience of the animals [83, 84]. Odor exposure can increase the survival of newborn neurons and improve

memory in a learned odor discrimination task, suggesting that neurogenesis in the olfactory bulb is recruited during learning and memory processes related to olfactory stimulation [85]. However, due to the relevance of the hippocampus for several brain functions and its implication on the genesis of neuropsychiatric disorders, much closer attention has been paid to SGZ than SVZ neurogenesis [86, 87].

Hippocampal neurogenesis is proposed to be important for at least some forms of learning and memory. Positive associations between them have been replicated by independent groups in rodents and humans [88–90]. For example, voluntary running and exposure to enriched environments improve learning and memory process with a concomitant increase in cell proliferation and survival of new DG generated neurons [82, 91, 92].

In addition, decreased adult hippocampal neurogenesis has been associated with psychiatric disorders such as anxiety, schizophrenia, and mood disorders. Stressful experiences that can precipitate symptoms of anxiety and mood disorders downregulate hippocampal neurogenesis [33, 93, 94]. Snyder et al. [95] showed that impaired SGZ, but not SVZ, neurogenic capacity facilitates stress-induced depressive-like symptoms and disrupt the essential negative feedback of hippocampus in hypothalamic-pituitary-adrenal (HPA) axis [95]. Adult hippocampal neurogenesis has also been implicated in the mechanism of pattern separation [96, 97]). Pattern separation is a complex concept that involves CA3 region as an associative network between a spatial location and a situation or an object that allows completion of memory during recall [98]. It has been hypothesized that this event is highly regulated by new neurons formed in the DG. In addition, several authors have demonstrated that neurogenesis is relevant for the perception of an event as stressful or not [99]. In the light of psychiatric conditions that involve an initial exposure to a traumatic event, such as posttraumatic stress disorder, the intact capacity of DG to produce new neurons has been associated with a poor ability of fear discrimination and overgeneralization (Besnard and Sahay 2015).

Of note, drugs used in the clinical practice for the treatment of psychiatric disorders, such as antidepressants or lithium, normalize or even facilitate hippocampal neurogenesis [94, 100]. Moreover, compounds with therapeutic potential for psychiatric conditions, such as cannabinoids, also impacts positively in adult hippocampal neurogenesis [33, 101].

6.4.1 Cannabinoids and Adult Neurogenesis

Several independent groups around the world have demonstrated the importance of the ECB system in the modulation of different steps required for neurogenesis: cell proliferation, differentiation, maturation, and survival (Fig. 6.3, [37, 86]). Indeed, activation of CB receptors regulates intracellular pathways involved in cell proliferation, differentiation, survival, and the integration of new cells in already established circuitries, such as the MEK/ERK/CREB and PI3K/Akt/mTOR and BDNF production [14, 37]. Also, voluntary exercise seems to increase adult hippocampal

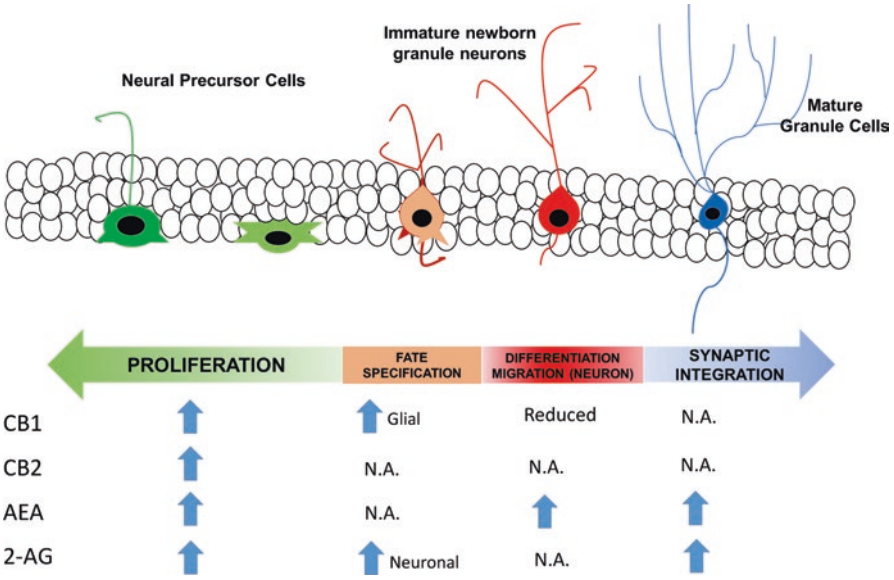

Fig. 6.3. Complex modulation of the endocannabinoid system during the process of adult hippocampal neurogenesis. *Blue arrows* facilitation of the formation of new cells/new neurons, *N.A* data not available or inconclusive. Based on in vivo studies

neurogenesis through a facilitation of CB_1-mediated neurotransmission. Finally, a positive association between cannabinoid-induced neurogenesis and behavioral improvement has been observed in animal models of anxiety, psychosis, depression, and memory impairment (as further discussed in item 1.5 of this chapter). Chronic (10 days), but not the acute administration of HU-210, a synthetic cannabinoid, induces neurogenesis in mice. A very similar picture is found after repeated administration of WIN55,212-2, a CB_1/CB_2 agonist [27, 32, 101, 102].

The two main compounds of the plant *Cannabis sativa*, THC and CBD, also affect adult hippocampal neurogenesis. Repeated treatment with CBD for 15-days prevented β-amyloid-induced neurotoxicity via activation of the proliferator-activated receptor-γ (PAAR-γ), suggesting a mechanism for CBD neuroprotective effects [103]. Wolf et al. [30] suggested that chronic treatment with CBD (42 days) decreases cell proliferation but stimulates cell survival. These responses were mediated by CB_1 receptors, as CBD effects were absent in CB_1 receptor knockout mice. Also, repeated CBD (30 mg/kg) treatment for 14 days prevented a stress-induced decrease in cell survival and differentiation in mice. In non-stressed mice, CBD increased the number of double-labeled BrdU/NeuN cells in the dentate gyrus [33]. These results were associated with increased levels of AEA, but not 2-AG, in the hippocampus of mice treated with CBD [33]. On the other hand, THC, a partial CB_1 receptor agonist, decreased proliferation and, at the same time, spatial memory [30].

The participation of ECB in the modulation of neurogenesis has also been investigated. For example, hippocampal cell proliferation is increased in FAAH deficient

mice and in animals treated with URB597, an FAAH inhibitor [27]. On the other hand, the ECB uptake inhibitor, AM404, reversed the trimethylthiazoline (TMT)-induced decrease of neurogenesis [104]. Finally, the genetic ablation of the enzyme responsible for 2-AG synthesis reduced cell proliferation, the number of doublecortin (a neuroblast marker) positive cells, and decreased the survival of newborn cells in the DG [31, 105].

The facilitation of CB_2 signaling also influences adult neurogenesis. Repeated administration of HU-308, a CB_2 receptor agonist, during 5 days, induces neural precursor cells proliferation in the DG. This effect seems to recruit Akt/mTORC1 pathway [36]. In the opposite way, the administration of CB_2 inverse agonist (JTE907) or antagonists (SR144528 or AM630) reduces cell proliferation and the number of BrdU labeled cells in the SVZ and SGZ [10, 32, 36]. The involvement of CB_2 receptors in these results was confirmed by the failure of a CB_2 agonist to induce any change in neurogenesis in animals deficient for this receptor [10, 36].

In the case of studies using pharmacological and genetic regulation of CB_1 receptors, the results are controversial. CB_1-deficient mice exhibit low rates of proliferation, astrogliogenesis, and neurogenesis in the DG and SVZ [27]. Also, repeated administration of the CB_1 antagonists/inverse agonists, SR141716A, and AM251, decreased neurogenesis in some studies [106]. Other groups, nevertheless, suggested that these drugs facilitate neurogenesis [30, 104, 107]. Interestingly, the effects of some of these cannabinergic drugs were preserved in CB_1 but not in $TRPV_1$-deficient mice [107]. These discrepancies may be related to the use of different animal species, strain or gender, cannabinergic drugs, and doses employed. In addition, contradictory results may be the consequence of different BrdU-administration schedule, and time-point of analysis, which may induce alternative interpretations. For example, Wolf et al. [30] found increased cell proliferation 1 and 24 h after treatment with AM251, but a decrease in cell maturation 48 h and 7 days later.

6.4.2 Neurogenesis, Cannabinoids, and Neuropsychiatric/ Neurodegenerative Disorders: What's the Correlation?

Considering that the ECB system modulates adult neurogenesis and that this process is impaired in neuropsychiatric and neurodegenerative disorders, it is plausible that cannabinoids may induce beneficial or detrimental effects in the brain and influence behavior by controlling newly generated neuron-induced plasticity. Cannabinoids are effective in modulating neurogenesis in various animal models of depression, anxiety disorders, Alzheimer's disease, and cerebral ischemia. Some of these studies are not only based on associative results, but suggest causality, once the direct ablation of hippocampal neurogenesis by different methods prevented the therapeutic effects induced by distinct cannabinoids tested.

Acute treatment with AM404, an ECB uptake inhibitor, reversed the trimethylthiazoline-induced decrease of hippocampal cell proliferation and pro-

moted anxiolytic-like effect [104]. In the same sense, sub-chronic treatment with the CB_1/CB_2 agonist HU210 induced anxiolytic- and antidepressant-like effects accompanied by an increase in neurogenesis [101]. Although a controversial finding, authors suggested that neurogenesis ablation through hippocampal X-ray irradiation prevented HU210-induced behavioral responses [101]. In agreement, repeated injections of CBD reversed the anxiogenic-like responses and the neurogenesis impairment produced by chronic stress in a CB1-dependent manner [33]. These effects were completely lost after ganciclovir administration to transgenic mice that express thymidine kinase under the control of the GFAP promoter, a method used to ablate only adult dividing precursor cells. In accordance, a recent study showed that the enhancement of 2-AG-induced neurotransmission by the MAGL inhibitor, JZL184, also prevented the anxiogenic- and pro-depressive-like effects, as well as the decrease in neurogenesis, induced by chronic stress [108]. Strengthening this hypothesis, the antidepressant-like effect produced by a single injection of the CB_1 antagonist SR141716A was lost after sub-chronic administration of the drug, probably due to the reduction in neurogenesis observed in these animals [106].

Several studies in the literature show that (1) neurogenesis is altered in some neurodegenerative diseases, and (2) cannabinoids can improve behavioral responses, as memory impairment, and brain damage, in animal models of these disorders. For example, Esposito et al. [103] showed that chronic administration of CBD in rats that previously received β-amyloid injection in the hippocampus, an animal model of Alzheimer's disease, decreases reactive gliosis, neuronal damage and facilitates adult hippocampal neurogenesis through PPAR-γ receptors. Also, cannabinoids can ameliorate age-related reduction in neurogenesis, suggesting that these compounds could replenish damaged/death neurons during neurodegeneration [32, 102]. In the middle cerebral artery occlusion rat model, widely used to evaluate cerebral ischemic injury, daily injections of oleoylethanolamide, a monounsaturated analog of anandamide, improved the spatial cognitive impairment concomitant to an increase in BDNF and hippocampal neurogenesis [109]. Also, CB_2 receptor regulation counteracts alcohol-induced decline in neurogenesis [110].

Taken together, these pieces of evidence suggest that cannabinoids could exert anxiolytic- and antidepressant-like effects as well as neuroprotection through an enhancement of adult neurogenesis. New studies using cannabinergic drugs that modulate the ECB tone in long-term studies of animal models of mood, cognitive, or neurodegenerative disorders are urgently needed to clarify these important aspects.

6.5 Conclusions and Perspectives

In this chapter, we have presented evidence indicating that cannabinoids exert an important neurodevelopmental regulatory role on and mediate plastic events in the adult brain (Figs. 6.2 and 6.3). Important unanswered questions, however, remain.

For example, is the modulation of neurogenesis by endocannabinoid signaling always positive, or can it be deleterious in some pathological conditions? What are the precise mechanisms by which cannabinoid regulate neurogenesis, neurodevelopment, and cell fate? What is the role of non-cannabinoid mediated mechanisms (e.g., TRPV1, GPR55, PPAR-γ receptors) in cannabinoid modulation of neurogenesis? What intracellular pathways are involved? These open questions indicate that we are only at the beginning of our journey. However, the results so far clearly support the perspective that new knowledge in this area could bring important contributions to the therapy of neuropsychiatric and neurodevelopmental disorders.

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