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The Polarised Life of the Endocannabinoid System in CNS Development

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Endocannabinoids (eCBs) play an important role in fine-tuning neurotransmission and have recently been shown to play an important role in brain development. The spatiotemporal expression of cannabinoid receptors and endocannabinoid-metabolising enzymes during development guides major developmental processes including neurogenesis, cell differentiation, cell migration, neuronal specification and synaptogenesis. Furthermore, pharmacological experiments and transgenic animal models have shown the impact of disrupted eCB signalling on normal brain development and revealed the danger of both cannabis abuse and exposure to cannabinoid drugs during embryonic development, childhood and adolescence. In this review, we focus on the dynamic expression of eCB components and the physiological role eCBs play during brain development.

Introduction

Recent developments in the cannabinoid arena have rapidly increased our comprehension of the multifunctionality of the endocannabinoid (eCB) system in the periphery and the central nervous system. In particular, the identification of an active eCB system in the developing brain and temporal changes in this system during brain development has led to recent discoveries that show the importance of eCB signalling in most of the developmental processes that shape the brain. It is important to fully understand the role of the eCB system during brain development in order to assess the devastating consequences of prenatal cannabis exposure or the toxic effects of exogenously applied cannabinoid receptor ligands or enzyme inhibitors on the developing brain. In this review, we focus on the spatiotemporal distribution and dynamics of the eCB system throughout early life and emphasise the role of the eCB system in the major developmental processes that shape the central nervous system.

The Endocannabinoid System

CB_1R distribution in the developing CNS

The classified cannabinoid receptors, CB_1R and CB_2R, act primarily through activation of Gi/o proteins and are activated by Δ^9-tetrahydrocannabinol and the major endocannabinoids anandamide (arachidonoylethanolamide) and 2-arachidonoylglycerol.[1] The abundant expression of CB_1R in neurons[2] has led scientists to investigate many aspects of CB_1R pharmacology and receptor functions in the CNS. In the prenatal brain, CB_1R is the most studied component of the eCB system. CB1R mRNA can be detected as early as embryonic day 11 in a subset of cells in the neural tube.[3] Detailed analysis of CB_1R mRNA levels by using in-situ hybridisation revealed temporal variation in CB_1R expression in the forebrain, brainstem and cerebellum during brain development;[4] this points to a functional divergence and the importance of the eCB system in guiding distinct brain areas through attaining neuronal complexity and diversity.

In the developing rodent neocortex, CB_1R mRNA expression reaches its maximum during gestation day 16.5, and gradually declines towards birth.[5,6] Furthermore, the presence of CB_1R protein has been detected in early neural progenitors[7–9] and in the axons and growth cones of developing cortical projection neurons.[6,10–12] Moreover, [^{3}H]CP55940 autoradiography and [^{35}S]GTPγS binding studies with WIN55212-2, potent CB_1R agonists (Table 1), revealed high CB_1R binding in the corpus callosum and corticospinal tract during this embryonic stage.[13] The specific targeting of functional CB_1R to developing axons suggests a role of eCBs in the establishment and organisation of neuronal networks. During late gestation and early postnatal life, cortical CB_1R is predominantly expressed in interneurons,[10] thus resulting in increasing CB_1R binding and WIN55212-2-induced [^{35}S]GTPγS binding within the neocortex. In contrast, CB_1R binding in the pyramidal tract and internal capsula is markedly reduced during late gestation and early postnatal development.[13]

In the developing rat brainstem, the CB_1R mRNA level gradually decreases from gestation day 21 towards adulthood, while in the cerebellum, CB_1R expression continuously increases, reaching maximum expression during adulthood.[4] These spatiotemporal changes in expression are in line with changes in the relative intensity of [^{3}H]CP55940 binding and WIN55212-2-induced [^{35}S]GTPγS binding within these brain areas (Figure 1).

Shifts in temporal expression from mid-gestation (week 20) to adulthood are also evident in the developing human brain.[14] [^{3}H]CP55940 binding and agonist-stimulated [^{35}S]GTPγ autoradiography in the hippocampus, cerebellum white matter

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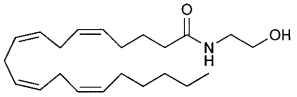
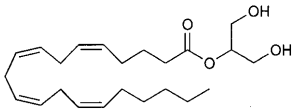
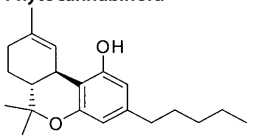
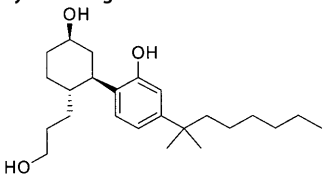
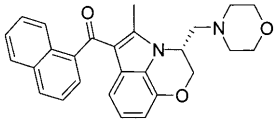
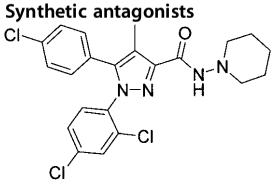
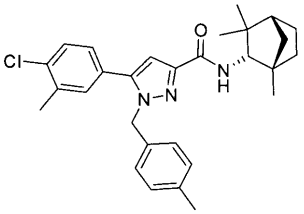
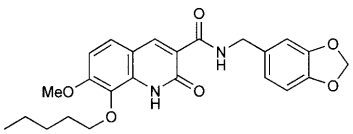
Table 1. Binding and activity profile of key cannabinoids at CB_1R, CB_2R and GPR55 receptors.

Cannabinoids	Ligand	Affinity (K_i [nM]) CB_1R	CB_2R	Selectivity	Efficacy (E_{max} [%]) CB_1R	CB_2R	GPR55	Potency (EC_{50} [nM]) CB_1R	CB_2R	GPR55
	Anandamide	90–543	370–2000	3	30–100/**66**	38–64/**58**	**73**	80–200/**30**	>1000/**27**	**18**
	2-AG	58–470	145–1400	3	80/**92**	60/**87**	**99**	430/**520**	780/**620**	**3**
Phytocannabinoid										
	Δ^9-THC	53	75	1.4	40–75/**61**	20–63/**67**	**92**	2–16/**6**	42–>1000/**0.4**	**8**
Synthetic agonists										
	CP55940	0.5–3	0.5–3	1	45–83/**100–140**	55–90/**100**	**100**	1–4/**0.2–2**	0.72–2.5/**0.3**	**5**
	WIN55212-2	2–123	0.3–4	6.7–30	40–75/**100–130**	45–80/**97**	–	7–44/**18–80**	0.6–12/**1**	**30000**
Synthetic antagonists										
	SR141716A	12	700–13200	60–1100	**−20 to −47**	**−65**	–	**0.8–8**	**1000**	–
	SR144528	50–10000	**0.6–6**	25–1780	0/**−30**	**−50 to −69**	–	**6**	**8–10**	–
	JTE-907	2370	40	66	4	300	–	5000	100	–

K_i values for human CB_1R and human CB_2R transfected cells,[1,25,67] activity values determined by cAMP production (normal font),[1,25] activity values determined by [^{35}S]GTPγS binding (in bold).[1,25,67,68]

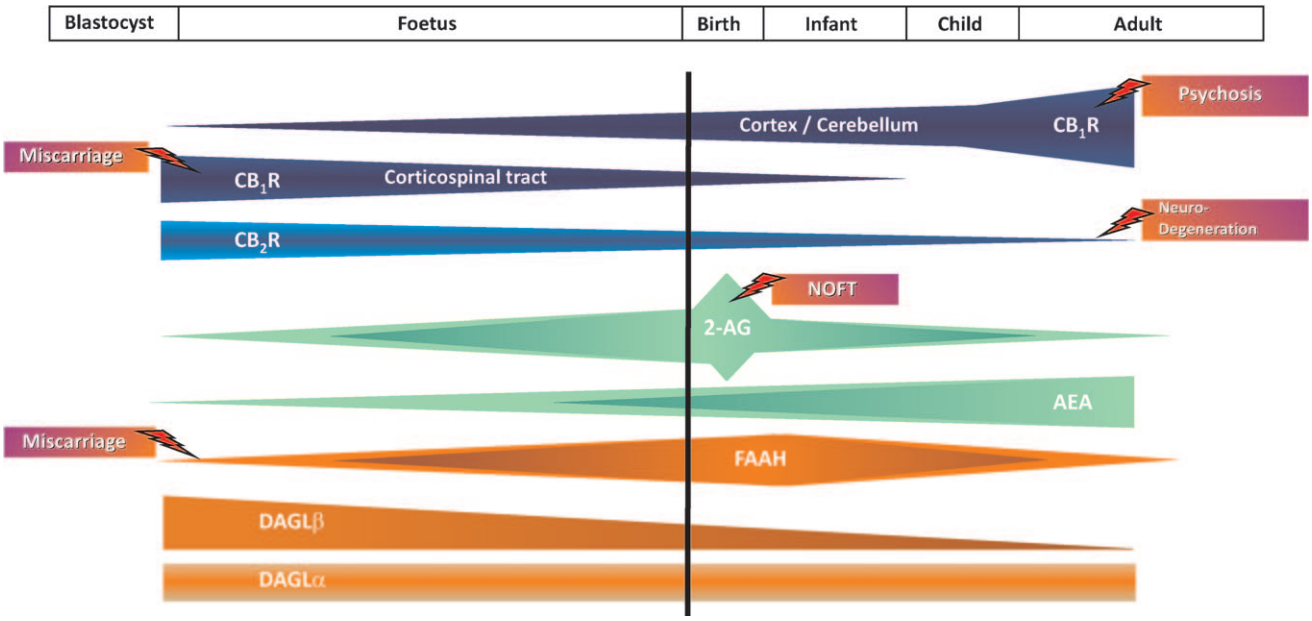


Figure 1. The polarised life of the endocannabinoid system. Temporal changes in the expression of endocannabinoid system constituents and changes in endocannabinoid levels are illustrated at different developmental stages. Note the differential expression of CB_1R in the corticospinal tract versus the cortex and cerebellum. At early developmental stages, cannabis consumption increases the risk for miscarriage, while at adolescent age it enhances the appearance of psychiatric disorders. CB_2R expression appears to decline with brain development. Up regulation of CB_2R expression at adulthood is associated with neurodegenerative diseases. The level of 2-AG (nmol g^{-1}) exceed that of AEA (pmol g^{-1}) in the developing brain. A peak in 2-AG level right after birth has been recorded and is thought to promote CB_1R activation that enables proper suckling. Inhibition of CB1R receptor signalling is suggested to promote nonorganic failure to thrive (NOFT). The expression level of anandamide-degrading enzyme FAAH peaks during early postnatal life and gradually decreases during childhood, coinciding with the increased synthesis of AEA. DAG lipases are expressed throughout development of the CNS and adulthood. DAGLα expression is constant through brain development, while the expression of DAGLβ is most abundant in embryonic brain tissue.

areas and, to a lesser extent, in the neocortex are elevated during major developmental events that shape these structures and their connections.[15]

Even though the spatial and temporal distribution of CB_1R expression and distribution supports receptor-binding results, it should be stated that CP55940 and WIN55212-2 are nonselective CB_1R/CB_2R agonists with an affinity for GPR55.[16–19] The nature of [^{3}H]CP55940 binding and WIN55212-2-induced [^{35}S]GTPγS binding should therefore be carefully interpreted as "solely" CB_1R indicator. Addition of SR141716A, a CB_1R antagonist and possible GPR55 antagonist,[18] reversed most WIN55212-2-induced [^{35}S]GTPγS binding in the human brain,[15] and almost completely abolished WIN55212-2-induced [^{35}S]GTPγS binding in the rat brain,[13] thus supporting the abundant expression of CB_1R in the developing brain. However, WIN55212-2-induced [^{35}S]GTPγS binding in the presence of a CB_1R antagonist[13] and in CB_1R knock-out mice[20] implies the existence of another WIN55212-2 binding receptor in the CNS.

CB_2R distribution in the developing CNS

During embryogenesis, CB_2R mRNA is mainly expressed in the liver and is undetectable in brain tissue by in situ hybridisation[3] or real-time PCR.[21] In the adult rodent brain, CB_2R expression is detected in a subset of brainstem neurons,[22] cerebellar neurons[23] and neural stem cells in the subventricular zone.[24] More data suggesting sparse CB_2R expression in the rodent brain come from displacement binding results from CB_2R antagonists (Table 1). Nanomolar concentrations of SR144528 displaced [^{3}H]CP55940 binding by up to 20% in the cerebellum of both adolescent mice[25] and rats,[26] and JTE-907 displaced 20% of [^{3}H]CP55940 binding in the adult mouse cerebellum at nanomolar concentrations.[25] These data support the expression of both CB_1R and CB_2R in the rodent cerebellum. However, it cannot be excluded that a receptor similar to CB_2R is responsible for [^{3}H]CP55940 binding and SR144528/JTE-907 displacement. In conclusion, there is a general consensus on the expression of CB_2R on macrophages and the role of the CB_2R in the central immune system.[27] However, the expression of CB_2R in neurons and neuronal progenitors and the role of this receptor in the development of the central nervous system remain unclear.

GPR55 distribution in the CNS

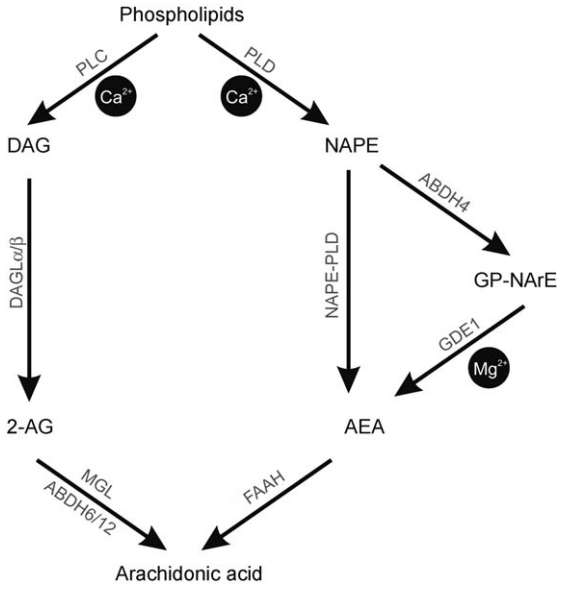
The current pharmacology of cannabinoid receptors is facing a challenging extension in which orphan GPCR receptors appear to be activated by a subset of cannabinoid ligands. This has prompted a revision of the current opinion on cannabinoid receptor pharmacology in the adult and developing CNS. More recently an orphan receptor (GPR55), coupled to $G_q/G_{12/13}$ proteins,[16–19] has been shown to have affinity for AEA (Table 1);[16,18,28] and might constitute a novel cannabinoid receptor.[29] High (5 μM) concentrations of Δ^9-THC have been shown to activate Ca^{2+} influx[18] through G_q protein while nanomolar concentrations could activate G_{13} proteins through

this receptor.[16] Cannabidiol, another cannabis constituent, has been shown to antagonise GPR55 signalling.[16] Quantitative PCR revealed that the GPR55 level in the brain is lower than that of CB_1R. In the cerebellum, the GPR55 mRNA level is 100 times lower, while in the hippocampus it is 10 times lower than that of CB_1R.[16] However, in the cortex, striatum, hypothalamus and brain stem, the GPR55 mRNA level is only half of CB_1R mRNA expression.[16] The coupling of GPR55 to a signalling pathway that differs from that of CB_1R and CB_2R suggests the existence of a more delicate regulation of endocannabinoid signalling in these brain regions.[28]

Endocannabinoid metabolism

Endocannabinoids are endogenously produced phospholipid derivatives (Scheme 1) that are produced on demand and exert biological activity through stimulation of cannabinoid receptors.[30] So far five eCBs have been identified *N*-arachidonoylethanolamine (AEA), 2-arachidonoyl-glycerol (2-AG), noladin, virhodamine and *N*-arachidonyl-dopamine, of which AEA and 2-AG are the most studied.[31]

2-AG is synthesised from diacylglycerol by *sn*-1-diacylglycerol lipases. The synthesis of DAG by phospholipase C is regulated by Ca^{2+} availability and is therefore the rate-limiting factor in the activity-dependent biosynthesis of 2-AG.[32] Two related



Scheme 1. Endocannabinoid synthesising and degrading pathways. Phospholipids are the lipid precursors of both 2-arachidonoylglycerol and anandamide. 2-AG production requires activity-dependent (Ca^{2+}) synthesis of diacylglycerol by phospholipase C, which is converted to 2-AG by DAG lipases α and β. 2-AG can by hydrolysed by monoglyceride lipase, or $\alpha\beta$-hydrolase 6 or 12 to form arachidonic acid. Synthesis of *N*-acylphosphatidyl ethanolamines by phospholipase D is believed to underpin activity-dependent (Ca^{2+}) AEA production. NAPE was believed to be directly converted to AEA by NAPE-hydrolysing phospholipase D, but recently an alternative pathway that includes the synthesis of glycerophospho-*N*-arachidonoylethanolamine from NAPEs by α/β-hydrolase 4 and the phosphodiesterase 1-catalysed Mg^{2+}-dependent conversion of GP-NArE to AEA has been suggested. Endocannabinoid levels are regulated by expression of synthesising and degrading enzymes and the availability of precursors and cofactors.

genes coding for DAGL(α and β) have been identified and shown to be highly conserved throughout evolution with their highest expression in the central and peripheral nervous systems.[33] Upon release, 2-AG is mainly hydrolysed by monoglyceride lipase,[34] and, to a lesser extent, by α/β-hydrolase 6 and 12.[35]

The catalytic pathway responsible for the generation of AEA from phospholipids was long believed to be mediated by Ca^{2+}-dependent phospholipase D activity and the conversion of *N*-acylated phosphatidylethanolamines by NAPE-hydrolysing phospholipase D.[36] Recently, an alternative pathway via the synthesis of the AEA precursor glycerophospho-*N*-arachidonoyl-ethanolamine (GP-NArE) from NAPEs by α/β-hydrolase 4 and the Mg^{2+}-dependent catalysed conversion of GP-NArE to AEA by phosphodiesterase 1 (GDE1) has been proposed.[37] Fatty acid amide hydrolases 1 and 2 have been identified as the enzymes responsible for degrading AEA into arachidonic acid in the brain;[38] this is supported by the increased levels of AEA in transgenic mice lacking this enzyme ($FAAH^{-/-}$).[39]

The synthesis and degradation of eCBs depends on the expression and distribution of the responsible enzymes. Both 2-AG and AEA levels show temporal variation during brain development. 2-AG synthesis gradually increases during embryonic development, peaks in the CNS immediately after birth and normalises during postnatal development.[40] Meanwhile, brain AEA levels gradually increase during postnatal development reaching a maximum in adulthood (Figure 1). The spatiotemporal dynamics of the key enzymes responsible for the synthesis and degradation of eCBs during brain ontogeny have only been partly unravelled. DAGLs are present in the brain during early development, and expression is maintained throughout life. While total DAGLα protein levels seem constant during brain development, the expression of DAGLβ appears to be most abundant in embryonic brain tissue.[10,33] DAGLs are distributed to the developing axons of pyramidal neurons,[6] while in the adult brain DAGLα is mainly localised in dendrites.[41] This indicates a different role of the eCB system in developing and mature neurons. Expression of the proposed AEA-synthesising enzyme NAPE-PLD is low during embryonic development, and can be detected in GABA-ergic and glutamatergic processes in the developing cortex.[10] The expression and distribution of the newly identified AEA-synthesising enzymes ABDH4 and GDE1 in the adult and embryonic brain have not been identified yet, but NAPE-PLD expression steadily increases after birth, coinciding with a marked increase in AEA levels.[42]

Not only the distribution and expression of enzymes regulates the production of eCBs. The availability of precursor molecules and cations (Ca^{2+} and Mg^{2+}) also varies during different stages of brain development. For example the level of phosphatidylcholine, a precursor of DAG, is high during mid gestation in the developing guinea pig brain at the stage dominated by axon targeting and myelination processes.[43] In rat brain tissue, the concentration of magnesium regulator of GDE1 activity, is highest at postnatal day 5 and gradually decreases during the first weeks of life.[44] However, to what extent fluctuations in these factors influence eCB biosynthesis remains un-

certain and largely depends on the local concentration in cellular compartments that give rise to the production of eCBs. Furthermore, extracellular eCB levels depend on the rate of degradation. No information on the distribution of 2-AG-degrading enzymes during embryonic and early postnatal life is available, but the expression of the AEA-hydrolysing enzyme FAAH gradually increases during postnatal life in the rodent hippocampus.[45] This coincides with increased levels of AEA during postnatal life and suggests a role of FAAH in controlling AEA signalling (Figure 2).

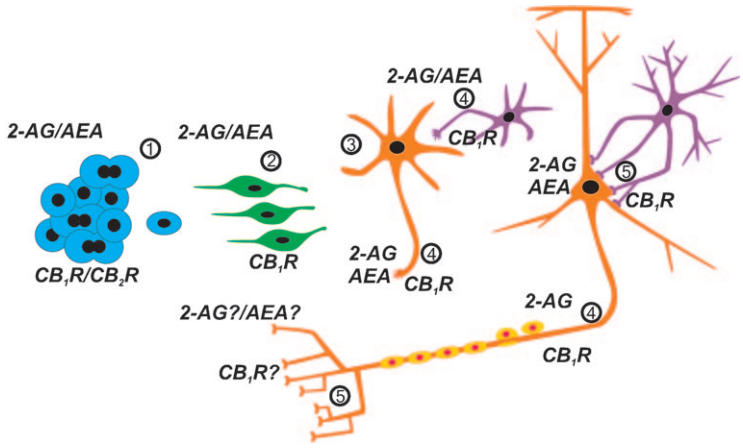


Figure 2. eCB signalling in corticogenesis. The main cell populations in the cerebral cortex are glutamatergic pyramidal neurons and GABA-ergic interneurons. Both types of neurons are born in different proliferative zones. GABA-containing interneurons are generated in the subcortical telencephalon and undergo long-range migration into the neocortex. The role of eCBs in (1) cortical interneuron progenitor proliferation and (2) the early phase of migration is unknown, and the existence of an eCB signalling system within these young interneurons has not been confirmed. However, from the moment these neurons turn radially, leaving the superficial and deep migratory streams, CB_1R expression becomes apparent. (4) eCBs guide the formation of synaptic connections between GABA-ergic interneurons and pyramidal neurons by influence growth cone turning. Pyramidal neurons are generated in the ventricular and subventricular zone, and proliferation (1) of these progenitors is regulated by eCB signalling. Both CB_1R and CB_2R are expressed in cortical progenitors (1), and both 2-AG and AEA are produced in their microenvironment. Newly formed neurons undergo radial migration to populate the neocortex (2). Both AEA- and 2-AG-synthesising enzymes are present in the developing neocortex, and eCBs stimulate radial migration. Once neurons reach their final destination, cells undergo further neuronal specification (3). The formation of distinct neuronal connections is essential for information processing and involves (4) axon targeting and (5) synaptogenesis. Endocannabinoids control the targeting and fasciculation of long-range projections of pyramidal neurons to subcortical areas. The distribution of CB_1R to both the growth cones and axon shafts suggest an action of eCBs on both axon path finding and axon collateral formation.

The Roles of the eCB System in Brain Development

The development of the brain involves the birth of cells that populate the brain (neurogenesis), migration of the newly formed cells to their destination, neuronal specification and the formation of adequate connections with neighbouring or distal neurons.

Neurogenesis

The activation of CB_1R and CB_2R expressed on neural stem cells has been shown to promote proliferation through activation of phosphoinositide-3 kinase/Akt and ERK1/2 signalling pathways in vitro.[7,46] Both CB_1R and CB_2R inverse-agonists/antagonists (SR141716A and SR144528) arrest basal proliferation rate, leading to apoptosis.[46] Furthermore, neural stem cells express DAGLs and FAAH[7] and have the capacity to synthesise AEA and 2-AG.[11,47] Deletion of FAAH ($FAAH^{-/-}$) increases levels of AEA, stimulating neurogenesis in proliferation zones in the developing and adult brain.[6,7] The roles of CB_1R and CB_2R, their complex interaction in the regulation of neural proliferation and how their relative expression determines cell fate remains poorly understood. Deletion of CB_1R or pharmacological inhibition of its function inhibits proliferation in neurospheres in vitro[7,24,46] and in proliferative zones in vivo in both the developing[6,7] and adult brain.[24] Treatment of neurospheres with both CB_1R and CB_2R agonists increases the proliferation rate in vitro.[7,46] In the adult brain, increased stimulation of the CB_1R did not result in increased neurogenesis, whereas stimulation of the CB_2R markedly increased the number of proliferating cells in the subventricular zone of 6-month-old mice.[24] The relative expression of CB_1R on neural stem cells is low, but undergoes robust up-regulation after initial differentiation into a neuron.[6] Simultaneously, CB_2R expression appears to be down-regulated upon commitment to a neuronal lineage.[46] This suggests that the low expression level of CB_1R is required to maintain a proliferative state, but that the rate of proliferation is regulated by alterations in eCB levels that signal through CB_2R.

Neuronal migration

Cannabinoids are known to modulate the motility of hematopoietic stem cells,[48] lymphocytes,[49] macrophages,[50] natural killer cells[51] and tumour cells.[49,52] In the developing central nervous system cells are born in proliferative zones and actively migrate to their final destination. During corticogenesis pyramidal progenitors, born in the ventricular/subventricular zone, radially migrate into the cortical plate and populate distinct cortical layers.[53] Deletion of the CB_1R arrests newly born neurons in the deep cortical layers, whereas elevated levels of AEA through deletion of FAAH markedly increase the penetration of newly formed neurons into the cortical plate ex vivo.[6] Interneurons populating the hippocampus and cortex are born in the ganglionic eminence and undergo long distance migration. A subset of newly formed interneurons in the ganglionic eminence express CB_1R, and give rise to the population of CB_1R-expressing cholecystokin (CCK) and reelin-/calretinin-positive interneurons in the cortex and hippocampus.[9] Stimulation of these newborn neurons with AEA and WIN552122-2 alters the motility in vitro, and prenatal exposure to Δ^9-THC increases the number of CCK-expressing interneurons in the rat hippocampal CA3 region in vivo.[5] This could indicate a role of CB_1R in the regulation of long-distance migration in the developing

brain. The onset of CB_1R expression in cortical interneurons is unknown, but CB_1R becomes apparent in a subset of cortical interneurons from the time they radially deviate from the superficial and deep migratory streams. Selective deletion of CB_1R in cortical GABA-ergic interneurons ($CB_1R^{f/f;Dlx5/6-Cre}$) did not affect the total number of different subsets of GABA-ergic interneurons in the cortex.[10] However, a more detailed analysis of the distribution of interneuron subsets in the entire hippocampus and in different cortical layers in CB_1R knock-out mice and mice prenatally exposed to Δ^9-THC is needed to fully understand the role of eCBs in the control of interneuron migration within these brain structures.

Neuronal specification

An important step in brain development is the incorporation of newborn neurons into neuronal networks. This requires neurochemical specification and the formation of adequate connections with neighbouring or distal cells. Cortical interneurons include multiple phenotypes that express different sets of proteins that are required for their specialised functions. Fate-mapping studies have identified several transcription factors (e.g., Dlx1/2/5/6, Nkx2.1, lhx6, ER81, Pax6, Arx) involved in the cortical interneuron development.[54] Furthermore, CB_1R activation has been shown to regulate the expression of a large number of transcription factors including CREB, Stat3 and Pax6.[55] In theory, this would suggest a possibility of eCBs' directly regulating cell fate by altering the expression of transcription factors. However, neuronal specification is mainly driven by epigenetic control of transcription, and any possible role of eCBs in regulating cell fate depends on the coincidence of a functioning eCB loop at critical decision points during neuronal development.

Axonal targeting and synaptogenesis

In the neocortex, the fine-tuned connections between pyramidal neurons and GABA-ergic interneurons underscore adequate information processing. This not only involves the number of synaptic connections between and within subsets of cortical neurons,[56] but also the distribution of synapses on the soma and dendrites. CB_1R is expressed in both GABA-ergic interneurons and glutamatergic projection neurons during axonal development and synaptogenesis.[5,6,10] Both CB_1R autoradiography[13] and immunohistochemistry[6,10,12] show distribution of CB_1R to elongating axons in the corpus callosum and corticospinal tract between GD14.5 and GD18.5 in the rodent brain. CB_1R expression is down-regulated when pyramidal axons reach their final destination and synapses are formed. Elevated CB_1R expression coincides with axonal targeting of DAG lipases; this suggests axonal synthesis of 2-AG.[6] CB_1R autoradiography and agonist-induced [^{35}S]GTPγS binding[13] suggest that functional CB_1Rs are present not only in the navigating growth cones, but also on the axonal shaft. This could indicate a role of eCBs both in axon guidance and in suppression of axon collateral formation. Pyramidal neuron-specific deletion of CB_1R ($CB_1R^{f/f,NEX-Cre}$) or in utero infusion of the CB_1R antagonist SR141716A resulted in mistargeting and abnormal fasciculation of corticothalamic axons.[6] These data are supported by in vitro experiments that show increased elongation of the primary axon and suppression of axonal branching in embryonic primary cortical neurons stimulated with AEA.[6] Interestingly, when CB_1R–GFP was over-expressed in primary hippocampal neurons in vitro, it seemed to negatively regulate neurite growth.[12] This could indicate that the total expression and subcellular distribution of CB_1R determines the cellular response to eCB, and could underpin the different physiological responses to eCBs by different populations of CB_1R-expressing neurons. However, it should be stated that the modified and EGFP-tagged constructs used in these experiments could influence CB_1R signalling and alter cellular responses to eCBs. Also in CB_1R-expressing GABA-ergic interneurons stimulation with AEA inhibits brain-derived neurotrophic factor (BDNF)-induced neurite growth.[5] Furthermore, CB_1R is present in the axonal growth cone of GABA-ergic interneurons. Stimulation of CB_1R induced RhoA-dependent growth cone repulsion in vitro. Conditional CB_1R knockout in GABA-ergic neurons ($CB_1R^{f/f;Dlx5/6-Cre}$) in mice led to impaired postsynaptic target selection that altered the distribution and density of vesicular glutamate transporter 3 and vesicular GABA transporter containing perisomatic terminals on L2/3 pyramidal cells.[10] Endocannabinoids are not only involved in corticogenesis, but a role of eCBs in the establishment of other intraregional neuronal connections is evident. Pharmacological blockade or deletion of the CB_1R also results in abnormal axon growth and fasciculation deficits in zebra fish spinal cord and chick mesencephalon.[11] The delayed increase in CB_1R expression in the rodent cerebellum compared to other brain areas[4,13] coincides with the major developmental processes of this brain structure.[57] This could indicate a role of CB_1R in the maturation of Purkinje cells or the innervation of climbing fibres from the inferior olive that continue postnatally.

These findings indicate the importance of the eCB system in the developing brain, and the role of endocannabinoids in almost all aspects of neuron development from neurogenesis to synaptogenesis. Disturbance of eCB signalling has a large impact on normal brain development, in particular inhibition of CB_1R signalling during critical developmental stages has irreversible consequences on the formation of functional neuronal networks.

Δ^9-THC-Induced Developmental Impairments

A functional eCB signalling loop already exists during early embryogenesis,[57] and Δ^9-THC-induced disturbance of the endocannabinoid tone can inhibit implantation of the blastocyst in the uterus leading to miscarriage.[58]

In cases of a full-term pregnancy, continuous maternal cannabis abuse leads to long-lasting alterations in several CNS functions in offspring,[59] which can even be initiated by a very low (0.001 mg kg^{-1}) single dose of Δ^9-THC.[60] Prenatal exposure to Δ^9-THC does not affect eCB concentrations and CB_1R affinity in the adult brain, but impairs CB_1R mediated down stream signalling in the hippocampus and slightly elevates CB_1R response

in the striatum, areas involved in learning and motor activity, respectively.[61] Moreover, a longitudinal study on the developmental effects of prenatal cannabis consumption in humans has shown that high-risk but not low-risk cannabis consumption during pregnancy affects children's mental score during the first year, and affects verbal communication for up to 2–3 years. However, effects on the visual system and higher cognitive abilities that require attention and working memory appear to last into adolescence.[62]

Human brain development continues postnatally, and during adolescence many brain regions still undergo remodelling. These brain areas include the prefrontal cortex and other limbic brain areas involved in higher cognitive functions and emotions. During puberty and adolescence, synaptic connections in the prefrontal cortex are refined through a loss of excitatory drive[63] and an increase in dopaminergic input.[64] The neuropsychiatric consequences of regular cannabis abuse during adolescence can be long-lasting, and can induce anxiety, depression and psychosis.[65] This increased vulnerability is partly due to the physiological effects of Δ^9-THC on neurotransmission, but also involves structural alterations that lead to abnormal brain plasticity and connectivity that are not yet fully understood.[66]

Conclusions and Future Directions

Recent developments in neuroscience have highlighted the important role of the eCB system in fine-tuning signal transduction and guiding brain development. Our understanding of the physiological roles of the endocannabinoid system during normal brain development has increased tremendously over the last years, and catastrophic consequences of pharmacological disturbance of eCB signalling during embryonic development are evident. New challenges are to further refine our knowledge of the expression of eCB molecules in the brain, their global distribution, and temporal expression and integration within basic physiological systems and developmental processes. Also the pharmaceutical industry faces the challenge of redirecting their strategies in order to produce new therapeutic approaches with the same potency as the recently developed receptor ligands, but with the ability to target specific systems, thereby restricting the drug action to isolated eCB systems and avoiding unwanted side effects.

Abbreviations

2-AG: 2-arachidonoylglycerol; ABDH4, -6 and -12: α/β-hydrolases 4, 6 and 12; AEA: arachidonoylethanolamide; CB_1R: CB_1 cannabinoid receptor; CB_2R: CB_2 cannabinoid receptor; Δ9-THC: Δ9-tetrahydrocannabinol; DAG: diacylglycerol; DAGL: *sn*-1-diacylglycerol lipases; eCB: endocannabinoid; FAAH: fatty acid amide hydrolase; GD: gestational day; GP-NArE: glycerophospho-*N*-arachidonoylethanolamine; MGL: monoglyceride lipase; NAPEs: *N*-acylated phosphatidylethanolamines; PLC: phospholipase C; PLD: phospholipase D; PND: postnatal day.

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