DMDA or manoalide, no such decreases occur (T. Kashiwagi, V.B. Meyer-Rochow, K. Nishimura and E. Eguchi, unpublished observations). These observations provide evidence that both phospholipase C, which is known to be abundant in the crustacean eye and eye-stalk, and is part of the transduction cascade[3], and phospholipase A_2 are activated in the photoreceptor cell by exposure to light (similar to the situation in the bovine retina[4]). An increase of free intracellular Ca^{2+} levels in the photoreceptors accompanies light adaptation[5], which can result in membrane damage, especially when the exposure to light is coupled with an increase in ambient temperature[2]. The decrease in PUFA levels, which is perhaps caused by its decomposition into chemically highly reactive fatty-acid species by activated lipoxygenases[6], could lead to the membrane damage observed. This effect is produced not only through PUFA-mediated effects on membrane fluidity and cytoskeletal protein interactions[7], but also through its effect on Ca^{2+} concentrations. As lipids react sensitively to an elevation in temperature, one might expect PUFA-mediated light-induced effects to be particularly pronounced in the vicinity of dark pigment granules, which act as Ca^{2+} stores and have been termed 'miniature fireballs', owing to their ability to dissipate absorbed energy as heat[8]. If this concept is correct, it offers one explanation of why photic damage does not occur in the pigmentless, small eighth rhabdom of the crayfish, despite affecting the remainder of the rhabdom[9].

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REVIEW

The endogenous cannabinoid system and brain development

Javier Fernández-Ruiz, Fernando Berrendero, Mari Luz Hernández and José A. Ramos

Cannabinoid receptors and their endogenous ligands constitute a novel modulatory system that is involved in specific brain functions, such as nociception, control of movement, memory and neuroendocrine regulation. Recently, it has also been suggested that this system is involved in brain development. Studies have used a variety of techniques to elucidate the effects of cannabinoids during development, as well as to characterize the presence of elements of the endogenous cannabinoid system (receptors and ligands) in the developing brain. Collectively, they suggest that endocannabinoids participate in brain development through the activation of second-messenger-coupled cannabinoid receptors.

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EXHAUSTIVE RESEARCH carried out during the past two decades has demonstrated the existence of an endogenous cannabinoid system in the CNS and also in the periphery, which consists of G-protein-coupled receptors and endogenous ligands (see Refs 1–5 for reviews). This novel system is thought to have modulatory actions in several neurobiological processes, as has been proposed from the anatomical distribution of binding and mRNA levels for the cannabinoid receptor in the brain[6,7] and from the well-known pharmacological effects of cannabinoid-related compounds[3–5]. Thus, the endogenous cannabinoid system could be involved in the control of movement, cognition, learning and memory, and pain relief (for reviews, see Refs 1–5).

On the basis of the pioneering studies of Walters and Carr[8,9], the neurotoxicological effects of plant-derived cannabinoids on rat brain development were largely examined in the past decade when these substances were fed to mothers during pregnancy or lactation, or both (for a review, see Ref 10). However, when these studies were initiated, it was not known that some years later, they might serve to provide the first indication that the endogenous cannabinoid system might have a role in brain development. In these studies, the perinatal exposure to synthetic or plant-derived cannabinoids was shown to modify the maturation of neurotransmitter systems and their related behaviors[10–13]. On completion of these first studies, secondary studies showed that these

effects were caused by the activation of cannabinoid receptors, which, as with endogenous cannabinoid ligands, are synthesized early in the developing brain[14–23]. As will be described, the atypical location of these elements in the developing brain is compatible with a specific role for the endogenous cannabinoid system in brain development. Finally, the use of cultures of fetal neuronal or glial cells has confirmed *in vitro* that some developing nerve cells contain cannabinoid receptors and that their activation produces several cellular effects[14,24–27].

Effects of cannabinoids on neurotransmitter maturation

Studies of the effects of cannabinoids in humans, although less exhaustive than studies of other drugs of abuse, have demonstrated that the consumption of marihuana by women during pregnancy or lactation, or both, affects the neurobehavioral development of their children (for a review, see Refs 28–30). In an attempt to explore these teratological effects observed in humans in more detail, the perinatal effects of cannabinoids were examined using rodents[8–13]. These studies were conducted by administering plant-derived cannabinoids during pregnancy (cannabinoids can be transferred from the mother to the offspring through the placental blood[13,31]) or lactation (cannabinoids are also present in the maternal milk[13,31]), or both. In all cases, cannabinoids reached the brain of fetuses and newborns in substantial amounts, presumably because of the immaturity of the blood–brain barrier at these early stages of development. The analysis of these pups provided valuable data on the effects of cannabinoids in brain development at different stages: fetal, early postnatal, peripubertal and adult. There were significant differences between results achieved in the different studies, according to experimental variables such as the onset of drug treatment and the dose of drug used[11–13]. For example, early administration, before or during organogenesis, as well as the use of very high cannabinoid doses, produced structural abnormalities that were even comparable to fetal alcohol syndrome (for a review, see Refs 11–13). However, this is a neurotoxic effect rather than one produced by the activation of the endogenous cannabinoid system and will not be addressed in this article. Other studies began drug treatment after organogenesis and used doses that were similar to the cannabinoid concentrations found in the blood of marihuana users. These studies demonstrated that plant-derived cannabinoids could mimic the effects of endogenous cannabinoids by altering the temporally ordered sequence of events that occurs during neurotransmitter development. A recent review gives a detailed description of how the administration of plant-derived cannabinoids during crucial prenatal and early postnatal periods of brain development can affect the maturation of specific neurotransmitters[10], and how these changes can result in long-term alterations in behavioral processes[10] (see Table 1 for a summary of effects and Ref. 10 for a more-detailed description).

This article will address only the studies carried out on one of the key proteins involved in the development of catecholaminergic neurons: tyrosine hydroxylase (TH). TH-containing neurons appear around gestational day (GD) 14 in rats[46] and seem to have as important a neurotrophic role[47] as other monoaminergic neurons (for a review, see Ref. 48). Endocannabinoids might have a role in the appearance or function, or both, of groups of TH-containing neurons, which exist only during development[49,50]. This would explain why pharmacological manipulations of TH, which are produced by plant-derived cannabinoids during perinatal life, might predictably have different consequences in not only the development of TH-containing neurons but also that of different neurons onto which these neurons make contact. Figure 1 shows how Δ^9-tetrahydrocannabinol (Δ^9-THC) is able to affect TH at different levels, gene expression, protein levels or activity, in the fetal brain in *in vivo* studies[33], and also *in vitro*, using cultured mesencephalic neurons obtained from GD14 fetuses[27]. These early effects of Δ^9-THC are likely to be mediated through the activation of cannabinoid receptors, as it has been found recently that they emerge and are operative early in brain development[14–23], and, in particular, that they are present in the cultured fetal mesencephalic neurons and are co-localized with TH (M.L. Hernández, F. Berrendero, I. Suárez, K. Mackie, J.A. Ramos and J.J. Fernández-Ruiz, unpublished observations). This observation is interesting because TH-containing neurons in the adult mesencephalon, which are located in the substantia nigra and ventral tegmental area, do not contain cannabinoid receptors[7], indicating the transient nature of this event (discussed below in more detail).

TABLE 1. Neurotransmitters and behavioral processes affected by perinatal exposure to cannabinoids in rodents[a]

Cannabinoid targets	Refs
Neurotransmitters	
Dopamine	8,9,32,33
5-HT	34,35
GABA	36
Opioid peptides	37–39
Behavioral processes	
Motor activity	13,40
Pain sensitivity	41
Drug-seeking behavior	38
Sexual behavior	42,43
Social interaction	43
Learning ability	13
Stress response	44
Neuroendocrine regulation	13,45

[a]See Ref. 10 for more details

Ontogenic development of elements of the endogenous cannabinoid system

In the absence of information about the existence of an endogenous cannabinoid system, the neuropharmacological effects of plant-derived cannabinoids, described in the preceding section, were interpreted as being related to the action of these compounds on a variety of molecular targets that are specific for a particular neurotransmitter. However, recent observations suggest that the effects of plant-derived cannabinoids in the developing brain are presumably produced by activating cannabinoid receptors and mimicking the natural functions of endocannabinoids during brain development. This assumption is supported by two facts. First, experiments performed by Fride and Mechoulam[51,52] showed that adult mice exposed perinatally to anandamide, the first endocannabinoid discovered, exhibit behavioral

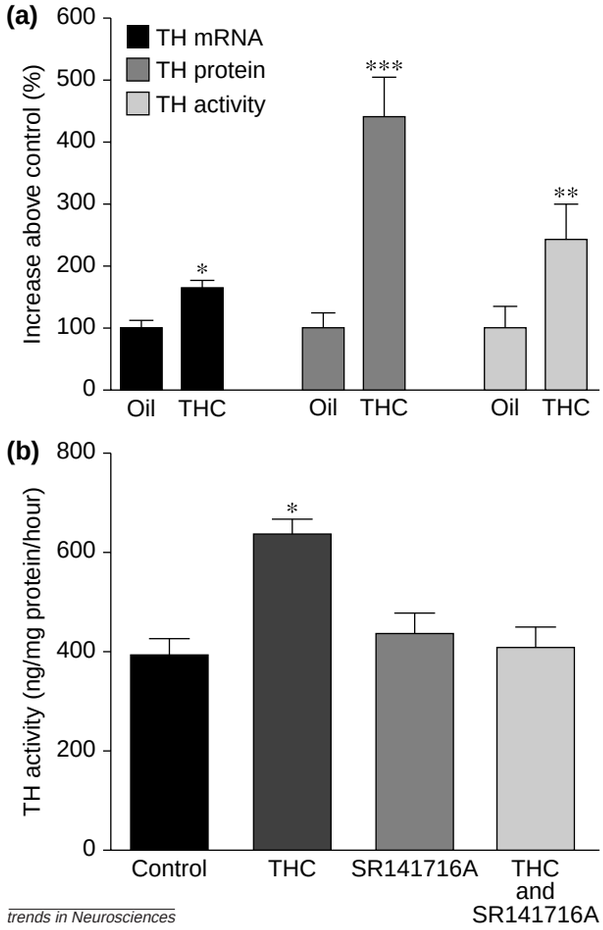

Fig. 1. ***Effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on tyrosine hydroxylase (TH) in the rat brain during the fetal period.*** **(a)** *shows the effects of prenatal Δ^9-THC exposure on TH mRNA levels, protein levels and TH activity in the brain of gestational-day 14 fetuses. All three are significantly increased compared with controls after Δ^9-THC treatment.* **(b)** *shows the effects caused by 24 h exposure to Δ^9-THC, or SR141716A (a specific CB_1-receptor antagonist), or both, on TH activity in cultured fetal mesencephalic neurons obtained from rat GD14 fetuses. TH activity was increased significantly only after treatment with Δ^9-THC alone. Values are means ± SEM and data were assessed by the appropriate statistical analysis (*P < 0.05, **P < 0.005, ***P < 0.0005).* Data in (a) are taken, with permission, from Ref. 33 and data in (b) are taken, with permission, from Ref. 27.

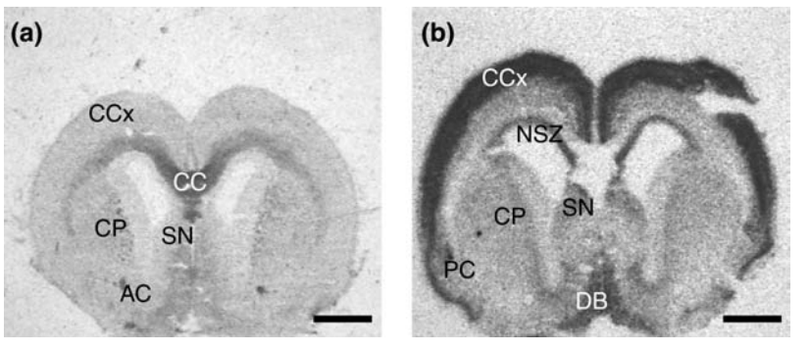

Fig. 2. ***CB_1-receptor binding.*** **(a)** *shows data obtained with [^{3}H]CP55940, a cannabinoid-receptor agonist that is currently used as a radioligand, and* **(b)** *shows CB_1-receptor mRNA levels in a representative slide-mounted brain section of gestational-day 21 fetal rat brain. In* (a) *there is high CB_1-receptor binding in the CC and AC, whereas in* (b) *high CB_1-receptor mRNA transcripts are seen in the CCx and NSZ. Abbreviations: AC, anterior commissure; CC, corpus callosum; CCx, cerebral cortex; CP, caudate putamen; DB, diagonal band: NSZ, neocortical subventricular zone; PC, piriform cortex; SN, septum nuclei. Scale bar, 1 mm.* Reproduced, with permission, from Ref. 14.

alterations similar to those caused by the administration of plant-derived cannabinoids. Second, the endogenous cannabinoid system (receptors and ligands) emerges at an early stage in brain development, during the fetal and early postnatal periods[12,14–23], and, therefore, the neuropharmacological effects produced by plant-derived cannabinoids administered during the perinatal period can be mediated by this canabinoid system. Several studies[14–23] addressed the ontogeny of the elements of the endogenous cannabinoid system in the human and rat brain, and found that they did not follow the classical pattern of a neuromodulatory system reaching its adult functionality. As will be detailed below, the ontogeny of the elements of the endogenous cannabinoid system, in particular the cannabinoid receptors, provides some characteristics that suggest a specific role for this system in the brain development, as also reported for some neurotransmitters or neuromodulators with recognized neurotrophic actions[46–50].

Ontogeny of cannabinoid receptors

The presence of cannabinoid-receptor binding was described for the first time in the developing rat brain in 1993 (Ref. 19). One year prior to this, it had already been demonstrated in the human brain by Mailleux and Vanderhaeghen[18]. Various studies extended this first demonstration[15–17], some of which analyzed the mRNA levels for the receptor[16,17], but they did not provide a detailed analysis of the distribution of cannabinoid receptors in the developing brain, and they only proved the existence of these receptors at postnatal ages. The first reports containing a detailed description, using autoradiographic techniques, of the distribution of cannabinoid-receptor binding[20], mRNA levels[14,21,22] and activation of signal-transduction mechanisms[14] in several brain structures in fetal rats appeared in 1997. Glass and co-workers[23] completed these studies by analyzing fetal and neonatal brains in humans.

With the use of autoradiographic techniques, cannabinoid-receptor binding and mRNA levels could be detected around GD11–GD14 (Refs 14,22). This interval is important because it corresponds with the fetal period during which most of the neurotransmitters appear (for a review, see Ref. 47). Cannabinoid receptors have been detected not only in studies of mRNA levels and receptor binding, but also by stimulation of GTP-binding proteins with a specific cannabinoid-receptor agonist[14,20], thus indicating that cannabinoid receptors appear to be functional at this stage and are already coupled to signal-transduction mechanisms. As mentioned above, the simple description of cannabinoid receptors in the developing brain does not imply that these receptors are involved in events related to brain development. The surprising transient distribution of these receptors in atypical and key regions of the developing brain, as compared with the adult brain[14,20,21], has led to this suggestion.

White-matter areas

CB_1-receptor binding and stimulation of GTP-binding proteins by cannabinoid-receptor agonists are prominent in white-matter areas, in particular in transverse or commissural fiber tracts (Figs 2 and 3) and in the white-matter areas of the midbrain and brainstem (Table 2)[14,20,21]. This phenomenon is apparent from GD16 to the first few days after birth (it is maximal at GD21), but practically disappears in the adult brain[14,20,21]. Figure 2 includes a representative autoradiogram for

CB_1-receptor binding in a brain section of GD21 fetuses; it shows a marked signal in the corpus callosum and also in the anterior commissure. Both structures are also markedly labelled in binding assays after stimulation of [^{35}S]GTPγS with WIN55212-2, a cannabinoid-receptor agonist (see Fig. 3). This contrasts with the low signal found in areas such as the caudate-putamen and the cerebral cortex (see Figs 2 and 3, and Table 2), two regions that contain higher densities of this receptor in the adult brain. Other fiber tracts, such as the stria terminalis, the stria medullaris, the fasciculum retroflexus, the fornix and others, also exhibit a high CB_1-receptor-binding levels[14,20,21]. With only the binding data available, it has been suggested that the appearance of CB_1-receptor binding in white-matter areas might indicate that CB_1 receptors are located either in axons that are migrating to reach their targets in the process of synapse formation, or in non-neuronal cells (astrocytes or oligodendrocytes) that accompany neuronal migration or axonal elongation[20]. However, it has been further shown, using *in situ* hybridization, that transverse or commissural fiber tracts, such as the corpus callosum and the anterior commissure, do not contain CB_1-receptor mRNA (Ref. 14; see Fig. 2), which indicates that CB_1 receptors are not located in the glial cells of these tracts, but in the elongating neuronal axons whose cell bodies are located at sites distant from the location of functioning receptors. The only exceptions are the midbrain and the brainstem, which are also rich in white matter and exhibit moderate levels of mRNA transcripts and CB_1-receptor binding[14]. However, RT–PCR has failed to show the presence of CB_1-receptor mRNA in cultures of fetal glial cells obtained from these regions (F. Berrendero, M.L. Hernández, J.A. Ramos and J.J. Fernández-Ruiz, unpublished observations).

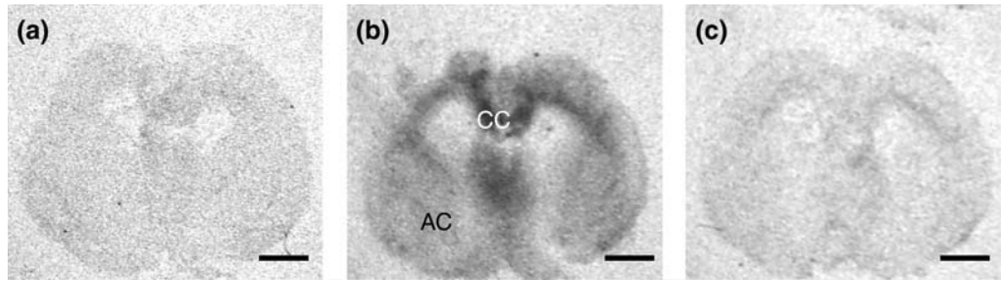

Fig. 3. ***Autoradiograms for WIN55212-2-stimulated [^{35}S]GTPγS binding in a representative slide-mounted brain section of gestational-day 21 fetal rat brain.*** **(a)** *shows [^{35}S]GTPγS binding in the absence of WIN55212-2, a specific-cannabinoid-receptor agonist, whereas* **(b)** *and* **(c)** *show WIN55212-2-stimulated [^{35}S]GTPγS binding in the absence and the presence of SR141716A, respectively, an antagonist of CB_1 receptors. Abbreviations: AC, anterior commissure; CC, corpus callosum. Scale bar, 1 mm.*

Cell-proliferative regions

The presence of mRNA transcripts for the CB_1 receptor in some forebrain areas, such as the subventricular zones of the striatum, nucleus accumbens and neocortex, which participate in the processes of neuronal and glial-cell proliferation (for a review, see Ref. 53), is the second relevant example of the atypical distribution of CB_1 receptors in the developing rat brain. As for binding in fiber tracts, the importance of this observation is its transient nature, as the presence of CB_1-receptor mRNA in these proliferative areas occurs mainly at GD21, but not before[14] or later[21]. Figure 2 includes a representative autoradiogram for CB_1-receptor mRNA levels in a brain section of GD21 fetuses, showing a marked signal in the subventricular zone of the neocortex, which contrasts with the low signal found in an area such as the caudate putamen. There is also a higher signal in the cerebral cortex at GD21 and early postnatal ages (see Table 2 and Fig. 2), which has been explained as being indicative of the presence of CB_1-receptor mRNA transcripts in neuronal cells migrating through the cortex to their final target sites[14]. These cells can also migrate through fiber tracts[54], which could explain why cannabinoid-receptor binding is found in fibers[14,20]. Moderate levels of CB_1-receptor-mRNA transcripts are also found in the midbrain and brainstem (see Ref 14 and Table 2), as mentioned above. In these cases, the levels of transcripts are significantly higher than those in the adult brain[14,21], which contrast with results obtained in the striatum and the cerebellum.

It can therefore be concluded from these studies that the transient nature of the occurrence of CB_1-receptor binding and mRNA synthesis in atypical areas might indicate the possible involvement of these receptors in developmental events, such as the process of proliferation and migration of neuronal or glial cells, the process of axonal elongation and synaptogenesis, and the formation of myelin. Alternatively, although cannabinoid receptors are located mainly in neuronal elements in the adult brain[55], during specific periods of development they can be also be found in some subpopulations of glial cells that have important roles in neuronal development (metabolic and trophic support, secretion of neurotrophic factors, guidance of neuronal migration and axonal elongation, and the formation of myelin).

TABLE 2. Ontogeny of CB_1-receptor binding and mRNA levels in the rat brain[a]

Brain regions	mRNA levels for the CB_1 receptor[b]/CB_1-receptor binding[c]					
	GD16	GD18	GD21	PND1	PND5	Adult
Cerebral cortex	+++/+	++++/+	+++++/+	+++++/+	+++++/+	++/++++
Caudate putamen	+/+	++/+	++/+	+++/+	+++/+	++++/+++++
Hippocampus	+++/+	++++/++	++++/++	+++++/++	++++/++	+++/+++++
Cerebellum	+++/+	++/+	+++/++	+++/+	+++/++	++++/+++++
Brainstem	+++/+	+++/+	+++/++	+++/+	++/+	+/+

[a]Constructed with data taken from Refs 14,20 and 21

[b]Arbitrary units of optical density: +, 0–25; ++, 25–50; +++, 50–75; ++++, 75–100; +++++, >100

[c]fmol/mg tissue: +, 0–30; ++, 30–60; +++, 60–90; ++++, 90–120; +++++, >120

Abbreviations: GD, gestational day; PND, postnatal day.

TABLE 3. Ontogeny of endocannabinoid content in the rat brain[a]

Endocannabinoid	Ages analyzed			
	GD21	PND1	PND5	Adult
Anandamide[b]	+	++	++++	+++++
N-arachidonoyl-phosphatidylethanolamine[c]	++	++	+++	+++++
2-arachidonoyl-glycerol[d]	++	+++++	++	++

[a]Constructed with data taken from Ref. 21.
[b]pmol/g wet weight: +, 0–3; ++, 3–6; +++, 6–9; ++++, 9–12; +++++, >12
[c]pmol/g wet weight: +, 0–15; ++, 15–30; +++, 30–45; ++++, 45–60; +++++, >60
[d]nmol/g wet weight: +, 0–2; ++, 2–4; +++, 4–6; ++++, 6–8; +++++, >8
Abbreviations: GD, gestational day; PND, postnatal day.

This hypothesis is concordant with data that have been obtained using neuronal or glial-cell cultures[14,24–26], which will be discussed below. Future studies will be required to support the potential presence of cannabinoid receptors in glial cells during development. The recent preparation of cannabinoid-receptor antibodies will allow double-labeling experiments with specific glial markers to be carried out.

The transient synthesis of cannabinoid receptors in atypical areas of the developing brain and their involvement in developmental events are also concordant with the fact that the maturation of two important pharmacological effects of endocannabinoids in adult individuals, motor depression and analgesia, are not fully developed until adulthood[52]. Thus, immature mice treated with anandamide exhibit only trends in these two pharmacological effects, as compared with the effects observed in adult mice. This indicates that although CB_1 receptors are present in the fetal and early postnatal brain[14,20,21], they reach their adult distribution and density in the areas that are responsible for motor and antinociceptive functions only at various stages after birth. The atypical location of binding sites and mRNA for these receptors during fetal and neonatal development therefore seems to be more related to a specific involvement in the molecular events that underlie brain development, whereas their localization in brain regions where the control of certain behaviors affected by endocannabinoids resides, seems to be an event that occurs later on, as suggested by Fride and Mechoulam[52].

Ontogeny of endocannabinoids

While the presence of CB_1 receptors in the developing brain has been known since 1992 (Ref. 18), the description of endocannabinoids was reported only very recently[21]. The absence of sensitive and rapid methods for the analysis of these compounds has probably been the reason for this delayed characterization. It has been demonstrated that both anandamide and 2-arachidonoyl-glycerol (2AG), as well as the anandamide precursor, *N*-arachidonoyl-phosphatidylethanolamine (NArPE), are present in the fetal rat brain at GD21 (Ref. 21). It is likely that endocannabinoids are present in the brain at earlier fetal ages, but it is difficult to demonstrate this with the present methods. The ontogenic pattern followed by these three compounds has been included in Table 3. It should be noted that data presented in Table 3 correspond to analysis of the whole brain, not of dissected regions, because of the aforementioned lack of sensitivity of present methods. The development of more-sensitive methods to measure endocannabinoids, as well as other presynaptic markers of endocannabinoid transmission, is therefore necessary and will hopefully be addressed over the next few years.

Effects of cannabinoids in cultured fetal nerve cells

The most-recent strategies used to demonstrate the involvement of the endogenous cannabinoid system in events related to neural development were based on the use of cultured fetal nerve cells. These studies have provided a conclusive demonstration that some subpopulations of neuronal or glial cells contain cannabinoid receptors[14,24–27] and that their activation has a role in the expression of key genes[24,27], and the regulation of energetic metabolism[26] and signaling mechanisms[25]. Figure 1 shows an example of the effects caused by activation of CB_1 receptors in cultured fetal mesencephalic neurons (where these receptors co-localize with TH; see above) in relation to the regulation of expression of the gene for TH (Ref. 27), an effect that has been also observed *in vivo*[33]. Cultured cortical and hippocampal neuronal cells also contain CB_1 receptors, as has been demonstrated conclusively using strategies such as PCR-mediated amplification of mRNA transcripts, analysis of agonist-stimulated [^{35}S]GTPγ*S* binding or agonist inhibition of forskolin-stimulated cAMP production[14].

Cannabinoid receptors could also be present in cultured glial cells[14,24–26], where their activation has been reported to produce arachidonic-acid mobilization[25], to increase basal and forskolin-inhibited glucose oxidation and phospholipid synthesis[26], and to induce *Egr1* (formerly *Krox24*) expression[24]. These effects are reversed by pertussis toxin or SR141716A, a CB_1-receptor antagonist, thus indicating involvement of a G_i/G_o-protein-coupled CB_1 receptor, although some evidence has recently been provided to show that glial cells contain a cannabinoid-receptor subtype that is distinct from the CB_1 receptor[14,56,57]. Involvement of sphingomyelin hydrolysis and mitogen-activated protein kinase stimulation has also been suggested[26]. On the basis of these results, Shivachar and co-workers proposed that the endogenous cannabinoid system might have a role in neuronal–glial signaling in the brain[25], so that anandamide released from neuronal cells might affect astrocyte function via the activation of cannabinoid receptors located in these cells. This is concordant with the recent observation that astrocytes can bind and take up [^{3}H]anandamide[58]. This endocannabinoid might participate in the control of gap-junctional communication in astrocytes[56], or, alternatively, it might have a role that is related to neuron–glial-cell adhesion molecules (NgCAMs). Both these processes are important for neural development (for reviews, see Refs 53 and 59).

Concluding remarks

Although further studies are necessary, the evidence presented in this article supports the view that, from an early age, the endogenous cannabinoid system has all the necessary elements to have a functional role in specific events related to brain development. The exact processes in which endocannabinoids are involved are not fully known and discovering these processes remains the major challenge for the future. However, evidence suggests that this role is exerted through mechanisms that are specific to the period of development and distinct from those that are functional in the adult brain. Thus, the perinatal administration of plant-derived cannabinoids, at doses that are physiologically relevant, affects, among others, the maturation of TH-containing

neurons, which suggests a possible role for endocannabinoids in the neurotrophic actions of these neurons. These effects are presumably mediated by the activation of CB_1 receptors, as binding, mRNA synthesis and activation of signal-transduction mechanisms for this receptor subtype, as well as significant amounts of endocannabinoids, have been detected in the fetal and early postnatal brain. It is of relevance that CB_1 receptors appear to be atypically distributed in the developing brain compared with the adult brain. The transient nature of this presence, together with the observation that the adult functions in which these receptors are involved, namely motor control and antinociception, mature later on, presumably suggests that the role of these atypically located CB_1 receptors is exclusively related to brain development. Studies using fetal-cell cultures suggest that CB_1-receptors are located in neuronal cells during development, although preliminary evidence for the existence of another receptor subtype that is operative in glial cells has been provided. As these cells have important roles in neuronal development (oligodendrocytes form myelin, and astrocytes not only provide metabolic and trophic support of neurons but are also involved in direct signaling to neurons), it can be concluded that endocannabinoids could be also involved in the functions of these cells in brain development.

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Calpain and caspase: can you tell the difference?

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Both necrotic and apoptotic neuronal death are observed in various neurological and neurodegenerative disorders. Calpain is activated in various necrotic and apoptotic conditions, while caspase 3 is only activated in neuronal apoptosis. Despite the difference in cleavage-site specificity, an increasing number of cellular proteins are found to be dually susceptible to these cysteine proteases. These include α- and β-fodrin, calmodulin-dependent protein kinases, ADP-ribosyltransferase (ADPRT/PARP) and tau. Intriguingly, calpastatin is susceptible to caspase-mediated fragmentation. Neurotoxic challenges such as hypoxia–hypoglycemia, excitotoxin treatment or metabolic inhibition of cultured neurons result in activation of both proteases. Calpain inhibitors can protect against necrotic neuronal death and, to a lesser extent, apoptotic death. Caspase inhibitors strongly suppress apoptotic neuronal death. Thus, both protease families might contribute to structural derangement and functional loss in neurons under degenerative conditions.

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APOPTOSIS, or programmed cell death, is a form of physiological cell death characterized usually, but not always, by the presence of DNA condensation in the nuclei, DNA fragmentation at the nucleosome linkage regions, cell shrinkage and the ultimate formation of apoptotic bodies[1]. It is used by the organism during development and at other stages to eliminate unwanted cells. Necrosis usually occurs when cells are injured by extreme physical stress or chemical challenges to the point where they are beyond repair, and is characterized by the presence of massive ion influx, mitochrondrial swelling, cell swelling, nonspecific DNA breakage in the nuclei and, ultimately, plasma-membrane rupture[1]. Unscheduled apoptosis might also be triggered when cells face some certain physical (for example, hypoxia) or chemical challenges (for example, toxins). It is also conceivable that other forms of cell death exist but have not yet been identified.

Apoptosis and necrosis in neurological and neurodegenerative disorders

About five years ago, it was generally accepted that acute degenerative neuronal death, such as that seen in cerebral ischemia, traumatic brain injury (TBI) and spinal-cord injury (SCI) were necrotic in nature. Linnik and colleagues were the first to challenge this idea by showing evidence for apoptosis in a rat focal-ischemia model (defined by the presence of DNA laddering)[2]. Since then, numerous reports have documented similar findings in either global ischemia or excitotoxicity models[3,4]. With respect to excitotoxicity, Bonfoco *et al.* reported that high and low levels of excitotoxin challenge to neuronal cultures resulted in necrosis and apoptosis, respectively (on the basis of nuclear morphology)[5]. Subsequently, evidence for apoptotic neuronal death was confirmed in experimental TBI (Ref. 6) as well as in SCI (Ref. 7). In parallel with this, apoptotic