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Marijuana, *Spice* ‘herbal high’, and early neural development: implications for rescheduling and legalization

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Abstract

Marijuana is the most widely used illicit drug by pregnant women in the world. *In utero* exposure to ⁹-tetrahydrocannabinol (⁹-THC), a major psychoactive component of marijuana, is associated with an increased risk for anencephaly and neurobehavioural deficiencies in the offspring, including attention deficit hyperactivity disorder (ADHD), learning disabilities, and memory impairment. Recent studies demonstrate that the developing central nervous system (CNS) is susceptible to the effects of ⁹-THC and other cannabimimetics, including the psychoactive ingredients of the branded product ‘*Spice*’ branded products. These exocannabinoids interfere with the function of an endocannabinoid (eCB) system, present in the developing CNS from E12.5 (week 5 of gestation in humans), and required for proliferation, migration, and differentiation of neurons. Until recently, it was not known whether the eCB system is also present in the developing CNS during the initial stages of its ontogeny, i.e. from E7.0 onwards (week 2 of gestation in humans), and if so, whether this system is also susceptible to the action of exocannabinoids. Here, we review current data, in which the presence of an eCB system during the initial stage of development of the CNS is demonstrated. Furthermore, we focus on recent advances on the effect of cannabimimetics on early gestation. The relevance of these findings and potential adverse developmental consequences of *in utero* exposure to ‘high potency’ marijuana, *Spice* branded products and/or cannabinoid research chemicals during this period is discussed. Finally, we address the implication of these findings in terms of the potential dangers of synthetic cannabinoid use during pregnancy, and the ongoing debate over legalization of marijuana.

Keywords

marijuana; synthetic cannabinoids; *Spice*; neural development; animal model; pregnancy; legalization; rescheduling

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Introduction

Marijuana, the crude drug derived from *Cannabis sativa* L. pistillate inflorescence, is the most prevalent illicit drug consumed by pregnant women, with a usage rate of 11%, as measured by serum metabolites.^[1] Classic animal studies have shown that during gestation, marijuana's predominant psychoactive constituent, Δ^9 -THC,^[2] crosses the fetoplacental barrier and accumulates in fetal tissues, particularly in the developing brain, with the potential to harm the development of the central nervous system (CNS).^[3–5] Human studies demonstrate that gestational exposure to Δ^9 -THC is associated with an increased risk for anencephaly (a non-sustaining life condition in which the brain fails to form; Figure 1A^[6]), and neurobehavioural deficiencies in the offspring.^[7,8] In addition to traditional marijuana, new markets of *synthetic marijuana* have recently emerged^[9–12] (Figure 1): these markets are distributed mainly under the branded name 'Spice', and include products such as Spice gold, Kronik Pineapple Express, Marley Extra Strength, etc.; in addition to the brand *Spice*, there are other herbal blends for which the claim is made that they have a composition similar to that of *Spice*: these include K2, Genie, Yucatan Fire, Skunk, Sence, Smoke, ChillX, Highdi's Almdröhner, Earth Impact, Gorillaz, Galaxy Gold, Space Truckin, Solar Flare, Moon Rocks, Blue Lotus, Aroma, Scope, Sky, OG Potpourri etc.^[9–12] Finally a market of "cannabinoid research chemicals", which fall under the category of "designer drugs", also termed 'legal high' and which claim to have marijuana-like effects, is now surfacing on various websites.^[9–12]

The risk of human gestational exposure to these novel drugs is so far completely unknown. In this review, we attempt to discuss the potentially adverse effects of exocannabinoids, including Δ^9 -THC, synthetic cannabinoids found in *Spice* branded products, and other cannabinoid research chemicals, on CNS development during pregnancy in both human and animal models. First, we review what is currently known in terms of the potency of marijuana; second, we review the mechanisms, by which Δ^9 -THC interacts with the eCB system in the adult CNS. We also describe the emergence of the *Spice* brand of *synthetic marijuana*, and discuss potential new markets for cannabinoid research chemicals. Third, we review the literature indicating the presence of eCB system in the embryo/fetus during CNS development and the potential effects of gestational exposure to Δ^9 -THC, to psychoactive components detected in *Spice* branded products, and to cannabinoid research chemicals on CNS development.

Prevalence of 'high potency' marijuana

The potentially adverse effects of marijuana abuse during pregnancy are aggravated by the fact that the mean potency of marijuana, in terms of its Δ^9 -THC content, has increased substantially in the past 30 years. Data collected from seized samples between 1985 and 2008 show a steadily increasing trend in the mean potency of marijuana, with a Δ^9 -THC content of 2.8% in 1985, 3.4% in 1993, and 5.8 to 9.3% in 2008 in marijuana, and a Δ^9 -THC content of 7.3% in 1985, 5.8% in 1993, and 11.7% in 2008 in *Sinsemilla* (i.e. the flowering tops of unfertilized female plants with no seeds),^[13,14] with individual 2008 *Sinsemilla* samples reaching up to 37.2% Δ^9 -THC content. Similarly, Δ^9 -THC content in hashish (dried cannabis resin and compressed flowering buds) averages 28.2% in 2008 samples, compared

to 2.3% in the 1970s, with some hashish samples composed of up to 66% Δ^9 -THC.^[13] In the last 25 years, there has been an alarmingly steady increase in the availability of “high potency” marijuana, i.e. marijuana consisting of high Δ^9 -THC content (i.e. 9% or higher) versus “low potency” marijuana, i.e. marijuana with low Δ^9 -THC content (less than 3%): In 1989, only 1.8% of seized marijuana samples were composed of high Δ^9 -THC content, compared to 52.6% of seized marijuana samples composed of low Δ^9 -THC content; by contrast, in 2007, 37% of seized marijuana samples were composed of high Δ^9 -THC content.^[13] From the studies above, we can infer that, since pregnant women are now exposed to the availability of high potency marijuana, the developing CNS of embryo/fetus is potentially exposed to high levels of Δ^9 -THC. Furthermore, marijuana is becoming the focus of intense biotechnological research.^[15] Initial steps in the production of Δ^9 -THC have been undertaken with the synthesis of Δ^11 -THC through the use of yeast-based expression systems^[16] and transgenic tobacco hairy roots^[17] (Figure 1C). Δ^11 -THC can then be readily transformed into psychoactive Δ^9 -THC through heat decarboxylation within a laboratory setting^[18] with the potential of opening a new era of extensive marijuana production.

Δ^9 -THC interferes with the eCB system in the adult CNS

Overview of the eCB system in adult CNS

In the adult CNS, Δ^9 -THC exerts its effects by interfering with the eCB signalling system. This system is responsible for modulating synaptic neurotransmitter release to regulate motor control, memory, and other brain functions, reviewed in Pertwee,^[19] Mackie,^[20] and Kano *et al.*,^[21] and consists primarily of endogenous ligands (eCBs), such 2-arachidonoyl glycerol (2-AG) and N-arachidonoyl ethanolamide (AEA; anandamide).^[22–24] These eCBs are synthesized and released upon demand from postsynaptic neuronal compartments, where they act as retrograde messengers at presynaptic terminals to stimulate cannabinoid (CB) receptor 1 and 2 (CB₁ and CB₂).^[23–25] Activation of CB₁ receptor elicits changes in transcription, translation, cell motility, shape, proliferation, cell fate, and differentiation of neurons, as well as modulation of neurotransmitter release in many excitatory and inhibitory synapses, within the basal ganglia, hippocampus, amygdala, cerebral cortex, tectum, substantia nigra, nucleus accumbens, ventral tegmentum area (VTA) and cerebellum.^[19–21,26,27] These changes are shown to be mainly mediated through CB₁ receptor-coupled G_{i/o} protein activation, which results in inhibition of adenylate cyclase activity, resulting in inhibition of cAMP and decreased activity of cAMP-dependent protein kinase (PKA), and activation of MAPK (ERK1/2, JNK and p38) and other signalling pathways.^[28–31] These events in turn result in phosphorylation of nuclear transcription factors and other cytosolic targets, as well as modulation of Ca²⁺ and K⁺ channels, events which together lead to the changes mentioned above including modulation of glutamatergic, GABAergic, opioidergic, serotonergic and dopaminergic neurotransmission, as well as regulation of cell fate.^[19–21,26–28,32–34]

The eCB system also comprises endocannabinoid ligands (eCBs) 2-AG and AEA, which act as full agonist and partial agonist at the CB₁ receptor, respectively.^[19,20] Several other eCBs have been identified lately and their physiological roles are yet to be delineated. The eCB system is also composed of enzymes, which are responsible for the metabolism of eCBs.

The synthesis of 2-AG mainly occurs via *sn*-1-selective diacylglycerol lipases α and β (DAGL α/β) and it is degraded by monoglyceride lipase (MAGL) and α/β -hydrolase domain-containing serine hydrolases (ABHD6/12).^[35–38] The synthesis of AEA occurs mainly by NAPE-specific phospholipase D (NAPE-PLD) and it is degraded via fatty acid amide hydrolase (FAAH).^[39,40]

Δ^9 -THC interferes with the eCB system in the CNS

Δ^9 -THC, a major psychoactive component of marijuana, exerts its action mainly through activation of CB₁ receptor in the CNS, and mimics many effects of 2-AG and AEA. It acts as a partial agonist or a full agonist depending on the area of the CNS under study.^[19,20] For instance, Δ^9 -THC acts as a full agonist at CB₁ receptors on GABAergic and glutamatergic axonal terminals in hippocampal neurons and inhibiting GABA and glutamate release.^[41–44] The distribution of CB₁ receptor in the adult CNS accounts for the psychoactive properties of Δ^9 -THC, since it encompasses regions implicated in the actions of Δ^9 -THC^[45] (Table 1). Due to its psychoactive effects on the CNS, Δ^9 -THC is currently classified as a Schedule I drug under the Controlled Substances Act.^[46]

Synthetic marijuana and Δ^9 -THC analogues found in the brand *Spice* branded products

Potency of cannabimimetics found in *Spice* branded products

Blends falling under the brand *Spice*, such as Spice Gold, 2Spice, Mojo, etc, are composed of herbal mixes sprayed with potent Δ^9 -THC analogues (or *cannabimimetics*). The blends are then inhaled via smoke, producing psychoactive effects similar to the ones observed with high-potency marijuana. Since these compounds are extremely potent Δ^9 -THC analogues, they have the potential to cause deleterious effects on the CNS.^[9–12,47–62] Cannabimimetics found in *Spice* branded products are CB₁ receptor agonists, originally manufactured for laboratory research use, with varying degrees of potency.

Some of these cannabimimetics have *extremely high potency*, with potency ranging from 40 to 660 fold higher than Δ^9 -THC, and include AM694 (500x more potent than Δ^9 -THC),^[51] AM-2201,^[51] (\pm)-CP-47,497-C8,^[52–55,57,58] (–)-CP-55,940,^[49,50,55,57] HU-210 (660x more potent than Δ^9 -THC),^{[24,54,56,59]–[61]} and others^[62] (Table 2). The potential cytotoxicity of these compounds was recently investigated in neuronal cell line NG 108–15. These potent cannabimimetics are found to induce apoptosis via CB₁ activation, through caspase-3 mediated mechanisms.^[57] Furthermore, (\pm)-CP-47,497-C8 (as well as JWH-018 and JWH-073) is able to inhibit neurotransmission in cultured hippocampal neurons.^[53,58] Some cannabimimetics in this group, such as HU-210, have neuroprotective effects in primary neurons of the adult cortex. These effects are mediated via PI3K/Akt signalling.^[59] In addition, HU-210 has neurogenic properties, i.e. proliferative effects, in neural stem/progenitor cells (NS/PCs) derived from E17 and adult hippocampus.^[60] These neuroproliferative effects are mediated via activation of PI3K/Akt and ERK1/2 pathways.^[60,61]

Other cannabimimetics found in *Spice* branded products have *relatively high potency*, with potency ranging from 4- to 20-fold higher than Δ^9 -THC, and include CP-47,497,^[52,54,55,57,63–65] JWH-018,^[53,58,62,66–69] and JWH-073,^[50,57,69,70] amongst others^[62] (Table 2). Notably, JWH-018 is the cannabimimetic most commonly found in current *Spice* blends^[47–50] JWH-018 is found in all blends analyzed in Table 2 (i.e. from blend 1, which corresponds to Tai High Afgan Kush, to blend 21, which corresponds to Magic Gold); By contrast, other extremely potent synthetic cannabinoids are only found in one of the *Spice* blends mentioned herein, as is the case for (\pm)-CP-47,497-C8 and HU-210, which are found only in blend 18 [Spice Gold] (Table 2). JWH-018 activates multiple CB₁ receptor signalling pathways, including ERK1/2 in CB₁ receptor expressing HEK and CHO cells.^[58,67] JWH-018 usage in humans is currently not detectable with the immunoassay screening methods employed for detecting marijuana use from urine specimens; determination of the parent drug in serum, or its metabolites in urine, is performed by gas chromatography–mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS).^[54,68,69,71–75] Peak concentrations of JWH-018 vary between 5 and 35 $\mu\text{g/L}$ depending on the *Spice* blend used.^[68] Some cannabimimetics found in *Spice* blends have potency similar to that of Δ^9 -THC, as is the case for WIN 55,212-2.^[76–78] A note to mention that for some cannabimimetics commonly found in *Spice* brands, there is no current available data in terms of their potency; such is the case for the RCS class of cannabimimetics, including RCS-04, RCS-4-C4 homolog and RCS-8.^[47,79]

Most of the synthetic cannabinoids are found in different concentrations within any given *Spice* branded product. Due to their considerable potency in comparison with Δ^9 -THC, typical doses of any synthetic cannabinoid may be less than a 1 mg/g of *Spice* blend: JWH-018 is found in all *Spice* branded products analyzed to date, with a concentration of 0.2% to 3% of the total content of *Spice* content analyzed.^[47–50] (\pm)-CP-47,497-C8 is reported to range between 1–17 mg/g, JWH-018 ranges between 2 and 36 mg/g and JWH-073 ranges between 6 and 23 mg/g following analysis of up to 46 products of different brands, including *Spice*.^[50,80] As far as the duration of effects of CP-47,497-C8 and JWH-018 following human consumption in comparison to Δ^9 -THC, the effects seems to be considerably longer for CP-47,497-C8 (5–6 h) and shorter for synthetic cannabinoid JWH-018 (1–2 h), as reported in a self-experiment;^[81] this corroborates the data in terms of potency of these synthetic cannabinoids compared to Δ^9 -THC, which are 5x and 48x at CB₁ receptor (Table 2).

Current status on the scheduling of cannabimimetics found in *Spice* branded products

As of 1 March 2011, and with the exception of HU-210, which already falls under Schedule I,^[82] most of the cannabimimetics found in current K2 and *Spice* branded products, were placed in Schedule I on a temporary basis (one-year ban), under the US House of Representatives (HR) Bill 1254.^[83] Exempt of this proposed Bill and for reasons unknown, were a CB₁ agonists (\pm)-CP-55,940 (about 70 times more potent than Δ^9 -THC;^[49]), and WIN 55,212-2, amongst others. There are ample debates as a result of passage of this Bill, including arguments that it would criminalize numerous substances without any scientific or medical evidence to support doing so, or without evidence that criminalization would actually reduce demand or prevent deaths.^[84] Others argue that, ‘the Federal Government

(then followed by the State Government) should ban this entire class of substances, including the derivatives, analogues and homologues. Otherwise we will continue to see years of this ‘dance’ where the Government bans a small subset of synthetic cannabinoids, followed by the makers of *Spice*/K2 coming out with a new version of their product containing cannabinoids not yet banned’.^[85] As of March 2012, the HR 1254 Bill had received a six-month extension period; it is currently up to individual states to provide Legislature as regards banning of cannabimimetics found in *Spice*.^[86] For instance, as of April 2012, in the JWH series of cannabimimetics, only JWH-018 and JWH-073 are under US Drug Enforcement Agency (DEA) ban (with DEA numbers 7118 and 7173, respectively) and the remaining JWH compounds found in *Spice* blends, remaining on temporary ban.^[87] It is important to mention that the composition of *Spice* blends constantly changes and these chemicals could be found in higher abundance and/or include novel, more potent cannabimimetics in future blends, without adequate DEA regulation under current DEA standards.^[87,88]

Potential novel markets with cannabinoid research chemicals

In addition to marijuana and *Spice* cannabimimetics, there is now potential for a novel market, of cannabinoid *research chemicals*. These include (1) full and potent CB₁ receptor agonists, such as O-2545-HCl (K_i=1.5 nM) and others, currently available from various websites,^[89–92] with potency 10- to 40-fold higher than ⁹-THC (Table 3); as well as (2) inhibitors of the enzymes responsible for 2-AG and AEA degradation, currently not marketed on non-research websites. By preventing the degradation of eCBs such as 2-AG and AEA, these inhibitors enhance the effects of these eCBs on CB₁ receptors in the CNS^[93–123] (Table 4). These cannabinoid research chemicals, currently mostly used in laboratory research, as is the case for FAAH inhibitor URB597, which has antidepressant and anxiolytic activity in animal models,^[121–123] have the potential to be exploited as street drugs in future *Spice* blends or otherwise. None of these research chemicals is currently under DEA Schedule I, and therefore these chemicals are readily available to the general population on a legal basis; most importantly, their potentially detrimental effects on the CNS are currently unknown.

⁹-THC, cannabimimetics and adult CNS: beneficial effects in neuropsychiatric diseases

Marijuana, marijuana-derived ⁹-THC, and some of the cannabimimetics listed above, have been studied in animal models and clinical trials, (and in some cases, approved for patient use in some countries), for their potential therapeutic, neuroprotective and neuroregenerative properties in psychotic, mood and neurodegenerative disorders.^[124] For instance, HU-210 treatment exerts anxiolytic- and antidepressant-like effects, possibly through its stimulating effect on hippocampal neurogenesis in the adult rat.^[61] Inhibitors of FAAH (URB597) (Table 4) and AEA transporter (AM-404) have shown to exert antidepressant and anxiolytic effects in rodent models.^[121,122,125] Importantly, only lower doses of CP-55,940 and WIN 55212–2 have been shown to exert anxiolytic effect while ⁹-THC (0.25–10 mg/kg) produced a dose-dependent anxiogenic effect.^[122] Sativex[®] (a preparation derived from cannabis leaf), is approved for the treatment of specific symptoms such as, spasticity and pain of multiple sclerosis patients, in various countries (excluding the USA).^[126] In pilot studies, Nabilone[®] (a Schedule II synthetic cannabinoid), is effective in alleviation of

Huntington's Disease (HD), presumably through its effects on GABAergic, dopaminergic and glutamatergic systems present in the brain areas known to degenerate in HD.^[127]

Ontogeny of the eCB system: pre-neuronal phase of CNS development

In this section, we review findings that components of the eCB system are present during early (i.e. pre-neuronal) CNS development of the developing embryo, a phase of CNS development which occurs at days 16–22 gestation in human, and during which the basic scaffold for the CNS is established with the formation of forebrain, midbrain and hindbrain (Table 5).

Overview of pre-neuronal phase of CNS development

It is during the pre-neuronal phase of CNS development that the basic scaffold for brain structures such as the cerebral cortex, amygdala, hippocampus, tectum, cerebellum and basal ganglia originate, from a simple neuroepithelium, the neural plate^[128,129] (Figure 2A). This is the earliest recognizable form of the CNS and appears at mouse E7 (chick HH4, day 16 gestation in human) (Table 5). The neural plate is subdivided into presumptive territories for the different precursors of the forming CNS, the forebrain, the midbrain and the hindbrain^[130] (Figure 2A). A crease progressively appears along the midline of the neural plate, and deepens until its sides arch over and fuse with each other to form the neural tube, the anterior segment of which will form the CNS. As the embryo develops, the anterior neural tube becomes divided into presumptive areas for the forebrain, midbrain and hindbrain (chick HH11, mouse E8.0, day 22 gestation in human, Figures 2B and 2C). These events together constitute the pre-neuronal phase of embryonic CNS development^[129–131] (in subsequent development, i.e. during the neuronal phase, these areas will further differentiate to become the domains of the brain mentioned in Table 1; thus, the forebrain will form the amygdala, mediodorsal thalamus etc, the midbrain will form the reticular formation, tectum etc, and the hindbrain will give rise to the cerebellum, pons etc) (Table 1)). Interestingly, chick (Figures 2A–2D), mouse and human (Figures 2E–2H) embryos share the same developmental cascades, as well as similar basic morphology during early stages of CNS development.^[132–136]

Ontogeny of the eCB system

Recent studies demonstrate that components of the eCB system are present in the developing CNS at its earliest stages of development, i.e. during the pre-neuronal phase. There are so far no available studies on the eCB system in human gestation weeks 2 to 4; Our current knowledge on this question comes from animal studies at equivalent developmental stages. Recently obtained data on eCB levels during the pre-neuronal phase of CNS development come from studies employing mass spectrometry and liquid chromatography: Both 2-AG and AEA are detectable in chick embryos at stages HH9–11, with levels of 2-AG of 1.1 nmol/g tissue and levels of AEA of 3.6 pmol/g tissue (earlier stages were not investigated in this study).^[137] These stages are equivalent to stage E7.75–8 in mouse and the third week of gestation in human (Table 5). The qPCR, RT-PCR and immunohistological studies also demonstrate that some of the enzymes required for 2-AG and AEA metabolism in adult CNS, such as DAGL α/β , NAPE-PLD, MAGL and FAAH, are also found in embryonic CNS

at pre-neuronal stages in chick HH3-11 and mouse E7.5-E9.5.^[137] Although the enzymatic activities of these enzymes have not yet been examined, these preliminary results corroborate the findings that 2-AG and AEA are also detectable at pre-neuronal stages.

CB₁ receptor mRNA is present in the chick embryo from the earliest stages of CNS development, i.e. from stage HH3⁻ onwards (equivalent to the second week of gestation in human).^[137] CB₁ receptor mRNA and protein are subsequently expressed in the neural plate from HH4-5; during neurulation (stages HH8-10 in chick) and formation of the brain primordia (HH11-13 in chick), CB₁ receptor is expressed in presumptive forebrain, midbrain and hindbrain (Figures 3A–3D) in addition to other tissues. A note to mention that these CB₁ expressing regions are the very same areas that will give rise to the adult brain structures mentioned earlier in Table 1. Similarly, CB₁ receptor is expressed in the neural plate and brain primordia precursors of mouse E7.5-9.5 embryos, in addition to other tissues.^[137] In zebrafish, CB₁ receptor mRNA is detected from the three-somite stage onwards (equivalent to mouse E7.5),^[138] corroborating earlier studies in rat^[139] and chick^[140,141] (reviewed in Psychoyos *et al.*^[133]). Pharmacological studies on the ability of the CB₁ receptor agonist (-)-CP-55,940 to stimulate [³⁵S]GTPγS binding in neural membranes derived from stage HH9-11 chick embryos, indicate that CB₁ receptors are functionally active from the earliest stages of CNS development onwards, i.e. during the pre-neuronal phase^[137] (Figure 3E). Most importantly, this last set of findings demonstrates that the developing brain of HH9-11 chick embryos (corresponding to the third week of gestation in human) is responsive to the effects of (-)-CP-55,940, a synthetic cannabinoid commonly found in Spice Gold.

Potential role for the eCB system during pre-neuronal development of the CNS

Together, these data suggest a possible function for the eCB system at this earliest phase of *in utero* CNS development. This role is so far unknown, although it is clearly discernible from the role of the eCB system during neuronal development (reviewed below); such function(s) for the eCB system in early, pre-neuronal development of the future CNS, could potentially include, modulation of cell fate and gene transcription, resulting in the establishment of a neuronal scaffold within the neural tube, anteroposterior (AP), dorsoventral (DV) and mediolateral (ML) patterning of the forebrain, midbrain and hindbrain, and differentiation and/or apoptosis within the neuroepithelium of these three brain subdivisions, to generate the secondary vesicles which will subsequently form the brain. Clearly, this is currently an open question, and studies in this field of eCB research are warranted. An important point here is that an eCB system is indeed present in early neural development, and that this system is amenable to potential interference by exocannabinoids such as ⁹-THC, cannabimimetics found in *Spice* branded products, and other cannabinoid research chemicals (see below).

The eCB system during neuronal development

We have reviewed recent findings, which suggest that the eCB system is present during the initial, pre-neuronal phase of embryonic CNS development. So far, the function of the eCB system during the pre-neuronal phase of CNS development is not well characterized. On the other hand, the eCB system is well understood and extensively characterized in the later

stages of CNS development, i.e. during the neuronal phase; we have therefore summarize its functions briefly herein, since most findings have been extensively reviewed elsewhere.^[142–148]

Function of the eCB system during neuronal development of the CNS

During the neuronal phase of CNS development (from mouse E9.5 and week 4.5 human gestation), the forebrain differentiates into telencephalon and diencephalon; the telencephalon will then develop into amygdala, globus pallidus (part of basal ganglia), cerebral cortex and hippocampus; the diencephalon will become epithalamus, thalamus, hypothalamus, and pineal gland amongst others; the midbrain will differentiate into striatum (caudate and putamen), substantia nigra, tectum and VTA, and the hindbrain will give rise to cerebellum, medulla oblongata and pons^[131] (Table 1). During neuronal development, the eCB system is required for the correct establishment of neuronal diversity and connectivity within the developing amygdala, cerebral cortex, caudate nucleus, hippocampus, nucleus accumbens, cerebellum and putamen; in these areas, the eCB system is implicated in neurogenesis, neuronal migration, dendritogenesis, axon guidance, synaptogenesis, lineage specification and gliogenesis^[145–148] (Table 6). As far as components of the eCB system. in mouse E10.5 and chick HH17, levels of 2-AG are 2.8 nmol/g and 2.1 nmol/g respectively; subsequently, levels of 2-AG reach 10 ng/g at mouse E14.5. At the end of gestation (mouse E18 and chick HH44), 2-AG levels are 17.6 nmol/g and 9.4 nmol/g respectively. Levels of AEA are also detectable in the embryonic CNS, albeit at much lower concentrations than 2-AG.^[137,149,150]

In humans, the earliest stage of investigation is 14 weeks gestation, where CB₁ receptor is expressed with preferential expression in the cerebral cortex, hippocampus, caudate nucleus, and putamen.^[151] By 19–20 weeks, intense expression is evident in CA2–CA3 of hippocampus and in the basal nuclear group of the amygdala.^[152,153] In the developing mouse brain, CB₁ receptor is expressed at E10.5.^[137] During subsequent neuronal development, CB₁ receptors are expressed in early neural progenitors in hippocampus and cortex,^[154,155] with receptor levels increasing throughout neuronal specification and synaptogenesis, and CB₁ being progressively localized to developing axonal projections.^[152,153,156,157] In cultured mesencephalic neurons of E12 mouse embryos, CB₁ receptors co-localize with tyrosine hydroxylase (TH) containing neurons.^[158] At E12.5, CB₁ receptor protein is localized in Cajal-Retzius cells and newly differentiated postmitotic glutamatergic neurons of the mouse telencephalon^[156] and to the subpial area of the ganglionic eminence and marginal zone of the neocortex.^[159] From E13.5–E21, abundant CB₁ receptor protein is detected in several long-range axonal tracts including corticofugal tracts such as corticothalamic and corticospinal tracts.^[156,157] From E16 onwards in rat, CB₁ receptors are also highly expressed in hippocampus during the initiation of gliogenesis.^[160] Using cultured chick cerebral cortex neurons derived from E3 embryos (HH18–20), Nilsson *et al.* demonstrated that CB₁ receptor agonist and *Spice* constituent (–)-CP-55,940, was able to reduce cAMP activity, and that this reduction is blocked by CB₁ receptor antagonist AM251, indicating the presence of functional CB₁ receptors in the cultures.^[161] Finally, pharmacological studies demonstrate the ability of CB₁ receptor agonist and *Spice* constituent WIN 55212–2, to stimulate [³⁵S]GTPγS binding in various areas of rat E16.5

(including cortex, midbrain and brainstem), rat E18 (same areas and also hippocampus) and in human 19 weeks gestation.^[152] Together, the above studies indicate that CB₁ receptors are functionally active during neuronal development^[160] similar to the situation at pre-neuronal stages.

Enzymes required for 2-AG and AEA metabolism, including DAGL α/β , NAPE-PLD, MAGL and FAAH, are present in the developing CNS at the earliest stages of neuronal development (as early as E10.5 and HH17),^[137] and in the presumptive cortical and hippocampal neurons of E14.5, E16.5 and E18.5 embryos,^[149,162] suggesting that these enzymes play an essential role in determining the availability of 2-AG and AEA in the developing brain. In particular, DAGL α/β protein levels peak at E14.5 and E16.5, and then dramatically decrease at P1. In contrast, MAGL levels transiently decrease at E18.5.^[149]

Functional studies: the eCB system and neuronal circuitry during cortex and hippocampus development

During corticogenesis (i.e. formation of the cortex), eCBs constitute a novel class of morphogens required for the genesis, proliferation, migration, and axonal guidance of neocortical pyramidal cells. In the telencephalon of mouse E12, the eCB system is functional in the ventricular zone (VZ) / subcortical proliferative ventricular zone (SVZ), to control the commitment and proliferation of pyramidal cell progenitors, and the radial migration of immature pyramidal cells^[157] (Table 6). Later during gestational development, the eCB system is responsible for the pyramidal cells of the newly formed cortical plate to initiate the elongation and fasciculation of their long-range glutamatergic axons.^[157] In the developing cortex of postnatal (P) 2 rat embryos, eCBs inhibit lineage commitment and differentiation program of neural progenitor cells into mature neurons, via attenuation of ERK pathway by CB₁ receptor, and promote astroglial differentiation.^[163] Furthermore, eCBs are responsible for controlling interneuron specification and migration in the developing cortex of rat E19 embryos, by regulating BDNF/TrkB and glutamatergic receptor signalling.^[164] In the developing hippocampus of E17 rat embryos, eCBs promote neurogenesis via ERK1/2 MAPK.^[61] The eCB system also inhibits network-driven synapse loss between hippocampal neurons in cultures of rat E18 hippocampal neurons.^[165-168] Finally, CB₁ signalling is required for FGF-dependent axonal growth and fasciculation of cerebellar in zebrafish^[169,170] (Table 6).

The eCB system and the ontogeny of neurotransmitter systems

In addition to its role in shaping neuronal circuitry in the developing embryo, the eCB system is also responsible for modulating the ontogeny of various neurotransmitter systems during development, mostly the catecholaminergic (i.e. dopaminergic) and opioidergic systems. The eCB system is responsible for regulating the expression of genes encoding for key components involved in the synthesis of these neurotransmitters, namely, tyrosine hydroxylase (responsible for dopamine synthesis) and pro-enkephalin (PENK) mRNA, a precursor of enkephalin A.^[145,158] Additional studies demonstrate that the eCB system is also involved in the maturation of serotonergic,^[171] GABAergic,^[164,165] and glutamatergic (NMDA receptor,^[172]) systems. These functions are summarized in Table 6.

Exposure TO Δ^9 -THC and other cannabimimetics during the pre-neuronal phase of CNS development

In this section we review current knowledge on the effects of Δ^9 -THC and other cannabimimetics, such as those found in *Spice* branded products and cannabinoid research chemicals, on gestational early CNS development.

Increased risk for anencephaly following peri-conceptual exposure to marijuana in humans

In utero exposure to marijuana during the peri-conceptual period (i.e. 1 to 4 weeks of gestation) is associated with an increased risk for anencephaly, a non life-sustaining condition in which the forebrain fails to form^[7] (Figure 1A): A study performed by the US National Birth Defects Prevention Center, which included 10241 infants with major congenital malformations and 4967 infants without major congenital malformations born between 1997 and 2003, found a clear correlation between gestational marijuana exposure and the risk of anencephaly: periconceptual marijuana use (first trimester) was found to be associated with an increased risk of anencephaly. Restricting the analysis to marijuana use in the first month after conception, during which the neural tube closes, confirmed this finding. Marijuana use in the other months of the peri-conceptual period was not associated with an increased risk of anencephaly.^[7] It is noteworthy that this study was carried out using data collected in the period 1997–2004. During this time, Δ^9 -THC content in marijuana averaged 5.2% (varying between 4.5% and 6.4%), compared to 3.1% in the period 1983–1994, and 5.8 to 9.3% in 2008.^[13] From these results, we can predict that the risk of infants born with anencephaly will increase in the coming years, considering that not only the number of childbearing women potentially exposed to marijuana has increased, but also has the Δ^9 -THC content found in marijuana preparations.

In utero exposure to the synthetic cannabinoids found in *Spice* branded products and to other cannabimimetics in human and animal models

So far, nothing is known as whether cannabimimetics found in *Spice* branded products, such as JWH-018 and others, have the potential to cross the placental barrier following human consumption in pregnant users, and if it does, what the implications on CNS development might be. Our only knowledge is inferred from animal studies, which will be discussed herein. Classical studies on marijuana embryotoxicity describe cases of anencephaly and exencephaly (an early stage of anencephaly) in rodent models.^[173–177] Furthermore, experiments with chick embryo report anencephaly and other CNS malformations following exposure to the potent cannabimimetic research chemical O-2545-HCl ($K_i = 1.5$ nM)^[178] (Figure 4A,B). In embryos treated with low dose of O-2545-HCl, the neural folds fail to elevate and to fuse, a phenotype comparable to exencephaly in rodent systems. In embryos treated with moderate dose of O-2545-HCl, the brain is poorly segmented into forebrain, midbrain and hindbrain primordia, in a phenotype comparable to anencephaly in rodents. These studies also reveal that O-2545-HCl also affects gene expression in the developing CNS. The spatial and temporal expression of genes required for correct establishment of brain primordia (*Otx2*, *Krox20* and *Pax6*) is altered in embryo treated with this

cannabimimetic Figure 4A,B). Finally, these studies also show that the time point most susceptible to the deleterious effects of O-2545-HCl coincides with the formation of the neural plate, equivalent to days 15–19 gestation in human.

Thus, it is possible that severe marijuana consumption in users who have conceived and are at their 15th–19th day post-conception, results in aberrant embryonic CNS development (i.e. an embryo in which the neural plate fails to develop, in a phenotype similar to that observed in chick following cannabimimetic exposure). This aberrant phenotype could easily be misinterpreted as lack of implantation in human with the conclusion that, Δ^9 -THC is deemed devoid of any detrimental effects, except ‘lack of implantation’, a phenotype which has been described in mice.^[179,180] It is also possible that less severe consumption of marijuana at 15–19 days after conception might result in implantation of embryos partly lacking anterior neural tissue and developing anencephalic phenotype; in principle, this hypothesis corroborates the findings concerning the association between an increased risk of anencephaly and marijuana consumption during the peri-conceptual period in human^[7] described earlier. Another variable is that with some human studies, subjects might be selected after pregnancy is confirmed, and therefore it is not possible to investigate the possibility that exposure to marijuana early in gestation, is associated with lethality for severely malformed fetuses. What is certain is that, at early stages of pregnancy (15–19 days after conception), most women are unaware of their pregnancy and of the potential risks of concomitant use of marijuana and/ or potential cannabimimetics such as those found in *Spice* brands.

There is some evidence that CB₁ and other components of the eCB system are present in chick embryos at pre-neuronal stages,^[137] i.e. at stages during which, cannabimimetic O-2545-HCl is shown to exert its detrimental effects. At these pre-neuronal stages, the chick embryo expresses CB₁ receptors which are responsive to the effects of CB₁ agonist and Spice Gold constituent (–)-CP-55,940 (Figure 3E). It is therefore possible that cannabimimetics, such as O-2545-HCl or others, exert their deleterious effects on pre-neuronal development by interfering with an eCB system of hitherto unknown function, present in the embryo at the earliest stages of CNS ontogeny. Because the basic molecular mechanisms that control early CNS development are evolutionary conserved amongst species^[133–136] the knowledge gained by analyzing the mechanism of cannabimimetic mediated embryotoxicity in animal models is applicable to our understanding of the effects of Δ^9 -THC, and potentially other cannabimimetics, in human.

Exposure to Δ^9 -THC and other cannabimimetics during the neuronal phase of CNS development

Neurobehavioural deficiencies following exposure to marijuana during human pregnancy

In humans, gestational marijuana exposure is associated with a plethora of neurobehavioural deficiencies including visual behavioural alterations in neonates;^[181] mental, motor and neurobehavioral deficiencies,^[182] as well as aggressive behavior and attention problems^[183] in 18-month olds; lower scores in verbal and memory domains^[184] in 3 year olds; lower performance at intelligence tests^[185] and social behavioral disturbances^[186] at age 6–7;

decrease in learning abilities and in academic achievements,^[187] neuropsychological problems,^[188] ADHD (inattention, impulsivity)^[189] and depressive symptoms^[190] at age 10; as well as long-term abnormal cognitive and behavioral function in young adults.^[191,192] It is not known whether the neurobehavioural deficiencies observed in the offspring following gestational Δ^9 -THC exposure, stem from developmental defects in the cognitive and emotional centres in which the eCB system is present during neuronal development, i.e. cortex, hippocampus, amygdala and nucleus accumbens. In addition, there is no data available at the moment on the possible outcome of pregnancy following exposure to cannabimimetics and other research chemicals during gestation, although we can predict symptoms similar to those observed following Δ^9 -THC exposure. Some human studies and several rodent studies have provided a partial answer to these questions and will be reviewed in the following section.

Human studies on the effect of Δ^9 -THC on neurotransmitter synthesis

Gestational exposure to Δ^9 -THC results in interference with the ontogeny of various neurotransmitter systems during development, mostly the catecholaminergic and opioidergic systems, leading to abnormal neuronal circuitry during development in the centres in which these neurotransmitters are required, and consequent neurobehavioural abnormalities in the offspring. In humans, there is evidence that *in utero* marijuana exposure impairs dopamine D2 mRNA expression in the amygdala and in the nucleus accumbens of 18–22 weeks gestation (Figure 4C,D).^[193] It is possible that defective dopamine D2 signalling in the amygdala and nucleus accumbens following Δ^9 -THC exposure, results in abnormal neuronal circuitry in those areas, which are cognitive and emotional centres. This in turn would lead to abnormal outcome in the offspring, such as the neurobehavioural deficiencies observed by Fried *et al.*,^[181] Richardson *et al.*,^[188] and Noland *et al.*,^[189] and the neuropsychiatric disorders observed by Stein *et al.*,^[188] and Gray *et al.*^[189] Similarly, gestational Δ^9 -THC exposure increases μ opioid receptor mRNA in the amygdala, decreases κ opioid receptor mRNA in the mediodorsal thalamus and decreases PENK mRNA levels in the striatum of 18–22 weeks gestation.^[194] As is the case for dopamine D2, it is possible that defective opioidergic signalling in centres required for the wiring of emotion, impulsivity and attention, result in defective neuronal circuitry in those centres, and subsequent neurobehavioural defects. Exposure to Δ^9 -THC could also affect other neurotransmitter systems during gestation, such as opioidergic, GABAergic, and glutamatergic (NMDA) systems as well as others, although this possibility has not been investigated so far. A note to mention that so far nothing is known on possible effects of gestational exposure to *Spice* branded products and to cannabinoid research chemicals in humans (all our current knowledge on this issue stems from animal studies, reviewed below).

Animal studies: Effect of Δ^9 -THC, *Spice* cannabimimetics and other research chemicals during neuronal development

Animal studies examining the effects of gestational exposure to Δ^9 -THC, cannabimimetics found in *Spice* branded products and to other research chemicals on gestational neurotransmitter signalling, gestational development of neurons, and subsequent neurobehavioural development in the offspring and/or adult animal will be summarized

herein. Δ^9 -THC and *Spice* constituent WIN 55,212-2 both induce deficits in memory and learning, as well as alterations in motor activity, exploratory behaviour, emotional hyperactivity, social interaction, neuroendocrine control, stress responses, nociception, anxiogenic-like profile, and heroin seeking profiles in rat offspring and/or adult exposed during gestational E12.5-E20.^[146,155,194–198]

1. Interference with neurotransmitter synthesis: At the molecular level, these neurobehavioural deficiencies are associated with impaired neurotransmitter synthesis and signalling in the areas of the developing CNS associated with cognitive circuitry, emotional behaviour, impulsivity, reward, addiction and movement and attention/multitasking (i.e. amygdala, cortex, dorsal striatum/caudate putamen, hippocampus, mediodorsal thalamus amongst others) (Table 7). Such examples include impaired dopamine function in the striatum, substantia nigra and VTA, via increase of tyrosine hydroxylase activity,^[155,158,199–201] impaired glutamatergic receptor function and signalling in the cerebellum,^[172] cortex,^[195,197] and hippocampus,^[196] and impaired noradrenergic signalling in the hippocampus^[196] and the cortex,^[197] all of which were accompanied by neurobehavioural deficiencies in the offspring. For instance, gestational exposure to WIN 55,212-2 in rat at E5-20 results in deficient glutamatergic neurotransmission in the offspring cortex and hippocampus, i.e. in the brain centres required for higher cognitive functions such as memory and learning; these biochemical deficiencies are associated with neurobehavioural impairment in the adult, including memory impairment, alterations in LTP, and emotional reactivity.^[195,196] Gestational exposure to Δ^9 -THC and other cannabimimetics also results in perturbations in the GABAergic, opioidergic and serotonergic systems during neuronal development associated with neurobehavioural deficiencies in the offspring.^[171,193,194,202–206] For instance, Δ^9 -THC inhibits PENK mRNA expression in the nucleus accumbens, caudal putamen and rostral dorsal striatum during early neurodevelopment in rodent. This is associated with long-lasting neurobiological impairments in neuronal systems linked with opioid/reward/ stress limbic function in the offspring,^[194,203,204] suggesting that impairment of PENK signalling during gestation via exposure to Δ^9 -THC. This might result in deficient circuitry in nucleus accumbens, and henceforth, aberrant limbic function in the offspring. Interestingly, PENK is highly expressed in proliferating neuronal and glial progenitors in rat at E14, its levels of expression later decrease sharply and are hardly detectable until E2. This suggests that this neurotransmitter might be responsible for proliferation and commitment of neuronal precursors within the developing cortex,^[206] a function, which could also be potentially, impeded following gestational Δ^9 -THC exposure. As far as interference with serotonergic system in rats exposed gestationally to Δ^9 -THC, lower serotonin levels were noted in the diencephalon, hippocampus, midbrain raphe nuclei and septum in these offspring, and neurobehavioural deficiencies were noted in adult animals^[171] (Table 7).
2. Interference with neuronal circuitry: In addition to their ability to interfere with neurotransmitter synthesis during neuronal development, Δ^9 -THC and

cannabimimetics also impede with the formation of neuronal circuitry in the developing embryo, perhaps by modulating the expression of genes encoding for neuron-glia cell adhesion molecules, molecules which are required for cell proliferation, neuronal migration or axonal elongation. In cultured hippocampal neurons derived from E17-E18 embryos, the cannabimimetic and *Spice* constituent WIN 55,212-2 inhibits new synapse formation^[168] and dendritogenesis,^[156] the latter via reduction of both length and number of primary dendrites, while CB₁ antagonist AM-281 exerted opposite effects (Figure 4E–G). CB₁ receptor was found to translocate from the axonal termini to the somatic compartment of hippocampal neurons in E16.5 embryos following exposure to cannabimimetic (–)-CP-55,940,^[156] which is also a compound commonly found in *Spice* blends.⁹ Similar results are observed in hippocampal interneurons derived from rat neonates exposed to ⁹-THC during gestation. ⁹-THC was found to interfere with the specification and migration of these interneurons, which failed to migrate within the hippocampus and remained within the strata radiatum, lacunosum-moleculare of the CA1-CA3 subfields.^[164,165] At the molecular level, HU-210, a synthetic cannabinoid commonly found in *Spice* blends, was found to have (1) neuroregenerative effects (i.e. proliferative) in cultures of E17 hippocampal neurons, via CB₁/ERK1/2 MAPK activation^[61] and proliferative effects in granule cell precursors (GCPs) derived from the cerebellum of P4 pups, via CB₁/AKT/glycogen synthase kinase-3β/β-catenin activation.^[205] Together, the above studies suggest that disturbance of neuronal development in cortex, hippocampus and possibly amygdala and nucleus accumbens, following gestational cannabimimetic exposure, might in part result in disruptions in neurotransmitter signalling, as well as interference with neuronal morphogenesis and proper circuitry. These aberrations would in turn lead to subtle defects in cognitive, neurobehavioural and emotional processing in the offspring, which is the phenotype we observe in the offspring born to marijuana users. Recent studies in zebrafish are of particular interest in illustrating this point whereas previous studies focused on behaviour of neurons/axons at the earliest E14 in rat brain slices following treatment with cannabimimetics, this study used 1 to 4 cell stage embryos, in other words a period corresponding to peri-implantation in human (week 1 of gestation): In CB₁-morpholino treated zebrafish embryos, the medial longitudinal fascicule, which corresponds to the anterior and posterior commissures of the forebrain in human, and which normally runs along the AP axis as segmented tight bundles of axons, appears clearly disorganized, spreading along the ML and DV axis of the CB₁-morpholino treated embryo, suggesting that these embryos are receiving the wrong cues/or fail to receive cues upon CB₁ receptor inactivation.^[170] The anterior and posterior commissures of the forebrain are responsible for transferring information between the two cerebral hemispheres to coordinate localized functions in the adult, such as memory establishment^[207] and visual discrimination,^[208] both functions which are impaired in offspring following gestational exposure to marijuana.^[7,156,182,184,209]

Future perspectives

To summarize, (1) the eCB system is present from conception onwards in the developing CNS; (2) Δ^9 -THC and other cannabimimetics interfere with the eCB system to cause anencephaly and neurobehavioural deficiencies in the offspring; and (3) pregnant users are now potentially exposed to high potency marijuana and to a new market for 'synthetic marijuana' i.e. *Spice*, a market to which drug enforcement regulations are struggling to adapt, and for which the risks on pregnancy and the developing fetus are not yet understood.

In view of these findings, awareness should be brought to the following: (1) the necessity to better inform potential pregnant users on the availability of high potency marijuana which significantly differs from the marijuana inhaled in the 70s' and 80s', and of synthetic marijuana such as that found in *Spice* branded products); (2) the necessity to inform users who are planning to conceive, since detrimental effects of marijuana (and potentially cannabimimetics found in *Spice* branded products), potentially occur already at periconceptional age (from conception to week 4 of pregnancy); and (3) the necessity to revise Bills and Legislations in terms of the specificity of banned research chemicals, and to broaden these legislations, as to include potential novel cannabimimetics and cannabinoid research chemicals.

Concluding remarks

The argument that marijuana is a "harmless" drug is no longer valid. Although some of the cannabimimetics and constituents of marijuana appear to be useful in some conditions, the recent advances in registry and statistical evaluation of effects, which now take into account confounding variables, has enabled us to clearly affirm that marijuana is detrimental to pregnancy. This is enhanced by the recent discovery of an eCB system in the developing embryo, a system of which the function is impeded following maternal exposure to marijuana. Most alarmingly, Δ^9 -THC content of marijuana has increased from 1.3% in the 1970s to an average content of 8.2% in modern preparations, with some preparations containing up to up to 37.2% Δ^9 -THC.^[13] Marijuana has regained its popularity from the 1970s, especially amongst teens/young adults, where it has regained its social and cultural status as the most popular drug of abuse; As a result, this poses not only a potential risk for the fetus of pregnant teen/young adults, but also for teens in general.^[210-212] Clearly, additional awareness should be provided to teens and young adults in particular, concerning the health deficits caused by marijuana, especially given the current debates on rescheduling, legalization and decriminalization of marijuana based on its medical applications.^[213,214] Finally, very little is known on the potentially detrimental effects of gestational exposure to the psychoactive constituents of *Spice* blends and to cannabinoid research chemicals and further studies are warranted.

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Appendix

ABHD6/12	α/β -hydrolase domain-containing serine hydrolases 6 and 12
AEA	<i>N</i> -arachidonylethanolamide
2-AG	2-arachidonoylglycerol
AP	anteroposterior
CNS	central nervous system
DAGLα	sn-1 specific diacylglycerol lipase, alpha
DV	dorsoventral
eCB	endocannabinoid
ERK1/2	extracellular-signal-regulated kinase 1/2
FAAH	fatty acid amide hydrolase
HD	Huntington's Disease
HH	Hamburger and Hamilton
K_i	dissociation constant
LC-MS	liquid chromatography-mass spectrometry
MAGL	monoacylglycerol lipase
ML	mediolateral
NAPE-PLD	<i>N</i> -acyl phosphatidyl ethanolamine phospholipase D
NAT	Ca ²⁺ -dependent <i>N</i> -acyltransferase
PKA	protein kinase A
NBDP	National Birth Defects Prevention
NS/PCs	neural stem/progenitor cells
PENK	pro-enkephalin
P	postnatal
qPCR	real-time quantitative RT-PCR
RT-PCR	reverse transcription polymerase chain reaction
SVZ	subcortical proliferative ventricular zone
⁹-THC	⁹ -tetrahydrocannabinol
¹-THC	¹ -tetrahydrocannabinol
THCAS	cDNA encoding tetrahydrocannabinol synthase
VZ	ventricular zone
VTA	ventral tegmentum area

AACOCF3	1,1,1-Trifluoro-6Z,9Z,12Z,15Z-heneicosatetraen-2-one
AA-5HT	N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-5,8,11, 14-eicosatetraenamide
ACEA	N-(2-chloroethyl)-5Z,8Z,11Z, 14Z-eicosatetraenamide
ACPA	N-(cyclopropyl)-5Z,8Z,11Z, 14Z-eicosatetraenamide
AM-251	N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AM-281	1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide
AM-404	4-hydroxyphenylarachidonamide
AM-694	1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole
AM-1235	[1-(5-fluoropentyl)-6-nitro-1H-indol-3-yl]-1-naphthalenyl-methanone
AM-2201	[1-(5-fluoropentyl)-1H-indol-3-yl]-1-naphthalenyl-methanone
AM-2232	3-(1-naphthalenylcarbonyl)-1H-indole-1-pentanenitrile
AM-2233	2-iodophenyl[1-[(1-methyl-2-piperidinyl)methyl]-1H-indol-3-yl]-methanone
(±)-CP-47497	rel-5-(1,1-dimethylheptyl)-2-[(1R,3S)-3-hydroxycyclohexyl]phenol
(-)-CP-55,940	2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl) phenol
HU-210	(6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol
JNJ 1661010	N-phenyl-4-(3-phenyl-1,2,4-thiadiazol-5-yl)-1-piperazinecarboxamide
JWH-018	1-pentyl-3-(1-naphthoyl)indole
JWH-073	naphthalen-1-yl-(1-butylindol-3-yl)methanone
JWH-250	2-(2-Methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone
JWH-398	(4-chloronaphthalen-1-yl)(1-pentylindolin-3-yl)-methanone
JZL 184	4-[bis(1,3-benzodioxol-5-yl)hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophenyl ester
LY 2183240	5-[(1,1'-biphenyl)-4-yl methyl]-N,N-dimethyl-1H-tetrazole-1-carboxamide
MAFP	(5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraenyl-methyl ester phosphonofluoridic acid
Nabilone	(6aR,10aR)-rel-1-hydroxy-6,6-dimethyl-3-(2-methyloctan-2-yl)-(dibenzo[b,d]pyran-1-ol)

NADA	N-[2-(3,4-dihydroxyphenyl)ethyl]-5Z,8Z,11Z,14Z-eicosatetraenamide
NAM	1-(5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraen-1-yl-1H-pyrrole-2,5-dione
O-2545-HCl	(6aR,10aR)-6a,7,10, 10a-tetrahydro-3-[5-(1H-imidazol-1-yl)-1,1-dimethylpentyl]-6, 6,9-trimethyl-6H-dibenzo[b,d]pyran-1-ol
PF 750	N-phenyl-4-(3-quinolinylmethyl)-1-piperidinecarboxamide
PF 3845	N-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidine-carboxamide
PIA	N-(1-methylethyl)-hexadecanamide
RCS-04	1-pentyl-3-[(4-methoxy)-benzoyl]indole
RCS-4-C4 homolog	(4-methoxyphenyl)(1-butyl-1H-indol-3-yl)-methanone
RCS-8	1-cyclohexylethyl-3-(2-methoxyphenylacetyl)indole
SR141716	N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride
ST4070	1-biphenyl-4-ylethenyl piperidine-1-carboxylate
9-THC	(-)-(6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol
URB597	[3-(3-carbamoylphenyl)-phenyl] N-cyclohexylcarbamate
URB602	biphenyl-3-ylcarbamic acid cyclohexyl ester

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Figure 1.

A, A typical case of anencephaly in human. Source: Wikimedia.org; **B**, Various forms of *synthetic marijuana* containing synthetic cannabinoids: Popular brands include *Spice* (of which *Spice Tropical Synergy* and *Spice Diamond* are shown here); other brand names include *Yucatan Fire*, *Smoke*, *Chill X* and *Space* (of which some blends are shown here) as well as others. Because there are no current FDA or DEA regulations, these products can be sold as “proprietary blend” and not list ingredients on the package. Source UNODC;^[9]**C**, Synthesis of Δ^9 -THC using transgenic tobacco hairy roots expressing *THCAS*.^[17] (reproduced with permission from Wikimedia.org (A),^[6] UNODC (B)^[9] and the American Society for Biochemistry and Molecular Biology (C).^[17]

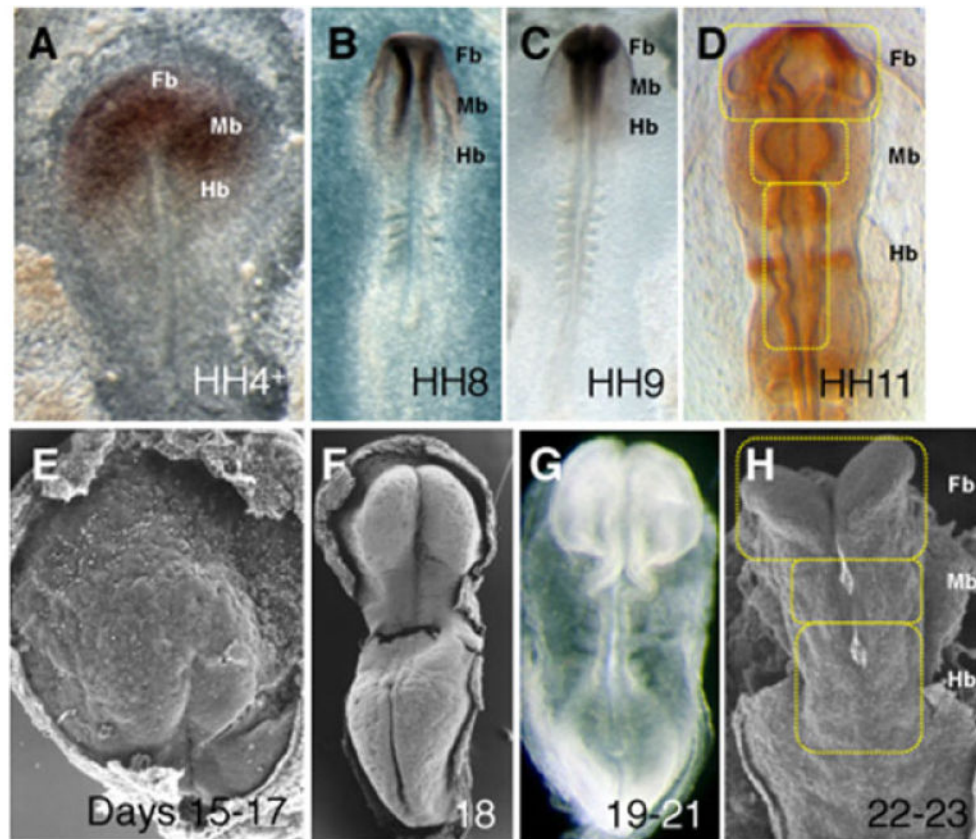


Figure 2.

Pre-neuronal development of the CNS in chick and human embryos: **A-D**, chick embryos at pre-neuronal stages: **A**, Neural plate in chick at stage HH4⁺: presumptive territories for the forebrain (Fb), midbrain (Mb) and hindbrain (Hb) of the CNS; The forebrain and anterior midbrain territories express the neural marker *Otx2*; **B**, Neurulation, stage HH8 (**B**) and HH9 (**C**); **D**, Subdivisions of the developing CNS brain primordia, stage HH11; these 3 primitive vesicles will subsequently develop into the domains of the brain described in Table 1, during neuronal stages; In **D**, the embryo expresses neural marker *En1*. **E-H**, human embryos at pre-neuronal stages: **E**, 15–17 days; **F**, 18 days, **G**, 19–21 days, and **H**, 22–23 days (Table 5 indicates stage comparison). At pre-neuronal stages, chick (**A-D**), mouse (not shown) and human (**E-H**) embryos share the same basic morphology. (**A-D**) adapted from Psychoyos *et al.*^[133] with permission from Bentham Open; (**E-H**) reproduced with permission from Prof. K. Sulik (University of Tennessee).

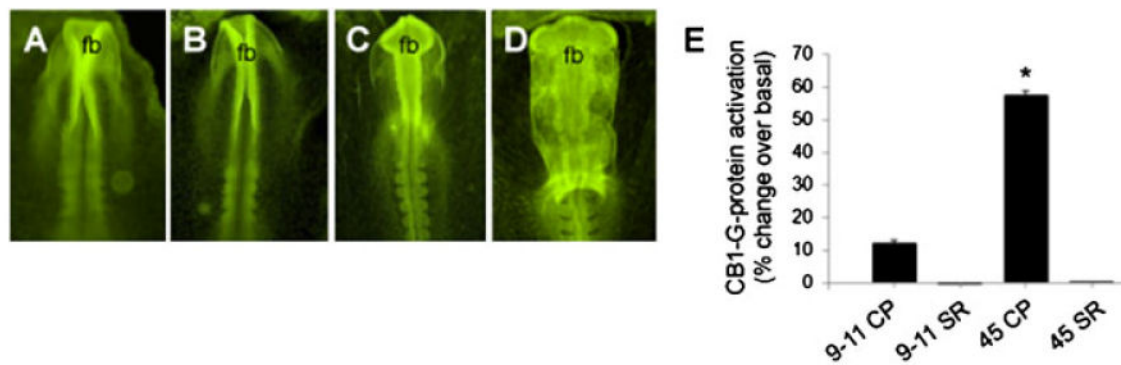


Figure 3. Functional studies on CB₁ receptor in chick at pre-neuronal stages of CNS development, using CB₁ agonist and potent Spice synthetic cannabinoid (-)-CP-55,940: **A-D**, whole mount immunohistochemistry with CB₁ receptor antibody in chick. The CB₁ receptor antigen is homogeneously distributed throughout the embryo, with intense localization in the anterior neural folds (A, B) and brain primordia, including the forebrain (fb) (C, D). **E**, Binding studies with CB₁ receptor in chick. CP-55,940-stimulated [³⁵S]GTPγS binding in membranes derived from stage HH9-11 and stage HH45 chick embryonic brain. A 12% increase in G-protein activation over basal levels is found in the brain primordia of stage HH9-HH11 embryos (9–11 CP). This effect is completely reversed following the addition of a CB₁ antagonist SR141716A (9–11 SR). Similarly, in stage HH45 embryos, a 57% increase in G-protein activation (45 CP) is reversed following the addition of SR141716A (45 SR). Data presented as mean ± SEM. *Indicates a statistical difference (p < .05) from all other groups. CP; CP-55,940, SR; SR141716A. Reproduced with permission from from Bentham Open^[133] and John Wiley & Sons, Inc.^[137]

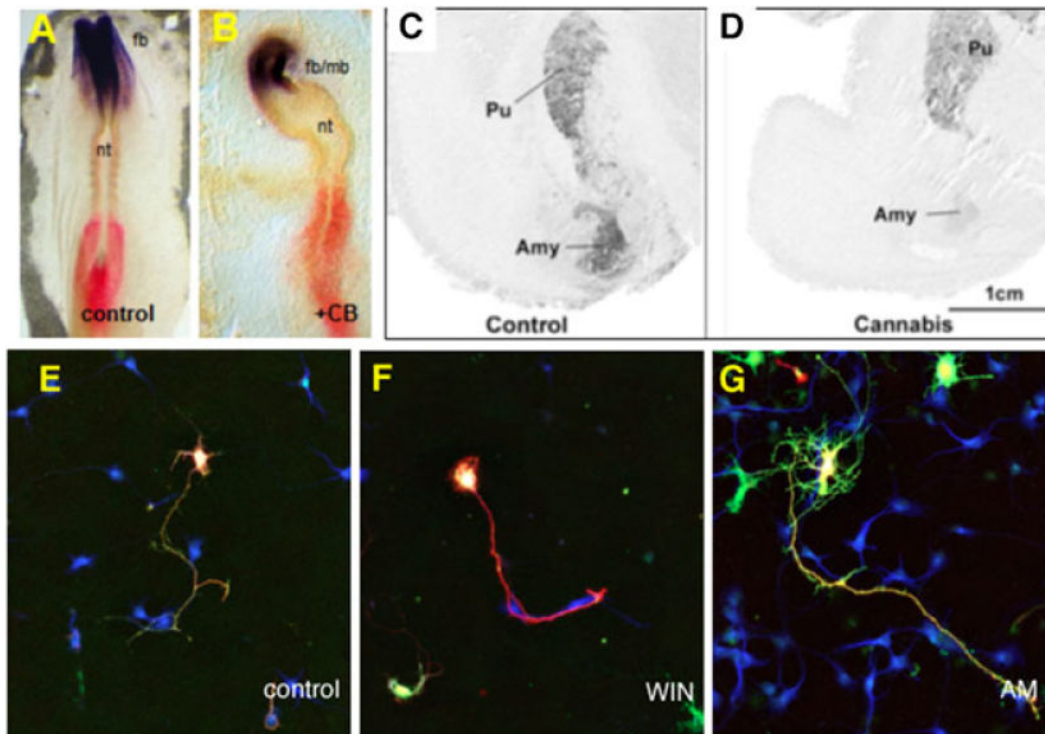


Figure 4. Effect of cannabimimetics at pre-neuronal and neuronal stages of CNS development: **A-B**, Effect of potent cannabimimetic O-2545-HCl (+CB; Table 4) on CNS development at pre-neuronal stages in chick, equivalent to days 17–19 in human. Exposure to O-26545-HCl at HH4 results in anencephaly observed at HH8, as evidenced by disruption in the expression pattern of *Otx2*, a marker for forebrain (fb) and midbrain (mb) at pre-neuronal stages;^[133,178] Note that the neural tube (nt), i.e. the future spinal cord, is also disrupted. **C-D**, Human studies on the effect of gestational exposure to ⁹-THC on the mRNA expression of dopamine D2 in the amygdala (Amy) and Putamen (Pu) of 18–22 weeks gestation embryos. ⁹-THC is shown to down-regulate the expression of D2 mRNA;^[193] **E-G**, Effect of WIN 55,212-2, a research chemical found in some *Spice* preparations, on cultured hippocampal neurons derived from E17 rat embryos: WIN 55,212-2 is shown to 2 inhibits dendritogenesis, via reduction of both length and number of primary dendrites (F), while CB₁ antagonist AM-281 exerts opposite effects (G).^[156] Reproduced with permission from John Wiley & Sons, Inc.,^[178] Elsevier,^[193] Wiley-Blackwell (E-G)^[156] and Bentham Open.^[135]

Table 1

CB₁ receptor containing regions in the adult CNS, associated functions and embryonic origin of these regions in the forebrain, midbrain and hindbrain primordia of the CNS at the pre-neuronal stages of embryonic CNS development

Brain region with CB ₁ receptors	Functions associated with this region	Pre-neuronal origin
Amygdala	Emotional behavior, fear, memory, aggression	Forebrain
Basal ganglia	Movement control, reward, addiction	
Cerebral cortex	Higher cognitive functions	
Hippocampus	Learning, memory, fear, anger, motivation	
Hypothalamus	Body housekeeping functions	
Dorsal striatum i.e. caudate and putamen (part of basal ganglia)	Impulsivity, decision-making, especially to action selection and initiation	
Ventral striatum i.e. nucleus accumbens	Reward, addiction, movement, pleasure, laughter, aggression, fear via modulation of opioid system	
Mediodorsal thalamus	Attention/multi-tasking	Midbrain
Reticular formation	Relays onto motor neurons	
Substantia nigra (part of basal ganglia)	Motor control, reward seeking, learning, addiction	
Tectum	Auditory and visual reflexes	
Ventral tegmental area	Reward, addiction, movement	Hindbrain
Cerebellum	Motor control	
Medulla oblongata	Breathing	
Pons	Sleep, arousal, sensory input from eyes, ears and touch	

Table 2

Potency of cannabimimetics found in major *Spice* branded products: Major ‘*Spice*’ (or ‘herbal high’) psychoactive constituents are listed, together with their alternative names: ^a Cannabicyclohexanol, CCH; ^b AM678; ^c RCS-04 butyl analogue, SR-19, BTM-4, E-4; ^d SR-18, BTM-8. The major *Spice* branded products mentioned herein are (1) Tai High Afghan Kush, (2) Lazy J, (3) Puff The Philosophers Stone, (4) Puff Super Strength, (5) Kronic Pineapple Express, (6) Marley Extra Strength 1.5, (7) Euphoric Blends White Rhino, (8) Euphoric Blends Big Bang, (9) Euphoric Blends Bubble Gum, (10) High Dro, (11) Kronic Tropical Explosion, (12) Kronic Purple Haze, (13) Electric Puha Ganja Guru Delta, (14) Kronic Skunk, (15) Space V2 Herbal Incense, (16) Spice Diamond, (18) Spice Gold, (19) Blueberry Posh, (20) Black Mamba, (21) Magic Gold; data compiled from.^{19,48,68,69]} Fold potency indicates potency relative to ⁹-THC in terms of affinity to CB₁ receptor, as measured by K_i activity (the lower the K_i value, the higher the affinity for CB₁ receptor)

Compound	Specificity	K _i , CB ₁ (nM)	Fold potency	<i>Spice</i> branded product	Cannabimimetic activity in adult CNS	Refs
<i>Extremely high potency compared to ⁹-THC: (40–660 fold):</i>						
AM-694	Potent selective CB ₁ agonist (also weaker CB ₂ agonist)	0.08	500x	7-9	?	51
AM-2201	Potent, non-selective CB ₁ /CB ₂ agonist	1.0	40x	1, 10	?	51
(±)-CP-47497-C8 ^a	Potent selective CB ₁ agonist	0.83	48x	18	Induces cell death in neuronal cell line NG108-15; inhibits neurotransmission in cultured hippocampal neurons	52-55,57,58
(-)-CP-55,940	Potent, non-selective CB ₁ /CB ₂ agonist	0.58	70x	18	Induces cell death in neuronal cell line NG108-15	49,50,55,57
HU-210	Full non-selective agonist at CB ₁ /CB ₂	0.06	660x	18	Promotes adult hippocampus neurogenesis (NS/PCs neurosphere proliferation via PI3K signaling); neuroprotective effect in primary CNS neurons via PI3K signaling	24,54,56,59-61
JWH 210	Potent, non-selective CB ₁ /CB ₂ agonist	0.46	87x		?	62
<i>High potency, compared to ⁹-THC: (4 to 20 fold):</i>						
(±)-CP-47,497	Potent selective CB ₁ agonist	2.2	18x		Induces cell death in neuronal cell line NG108-15	52,54,55,57,63-65
JWH-018 ^b	Very potent selective CB ₂ agonist (also potent CB ₁ agonist)	9.0	5x	1-21	Induces ERK1/2 MAPK phosphorylation in CB ₁ receptor expressing HEK and CHO cells; inhibits postsynaptic currents via CB ₁ receptor in cultured hippocampal neurons	53,58,62,66-69
JWH-073	Potent selective CB ₁ agonist (also weaker CB ₂ agonist)	8.9	5x	2-9, 14–16, 21	Induces cell death in neuronal cell line NG108-15; Inhibits neurotransmission in cultured hippocampal neurons	50,57,69,70
JWH-250	Potent selective CB ₁ agonist (also weaker CB ₂ agonist)	11	4x	7-9, 13-16	?	62

Compound	Specificity	K _i CB ₁ (nM)	Fold potency	Spice branded product	Cannabinimetic activity in adult CNS	Refs
JWH-398	Very potent non-selective CB ₁ /CB ₂ agonist	2.3	17x	?	?	62
<i>Potency similar to that of ⁹-THC:</i>						
WIN 55,212-2	Potent selective CB ₂ agonist (also potent CB ₁ agonist)	62.3	0.63x	?	Modulates NMDA receptor mediated release of Ca ²⁺ in hippocampal slices	76-78
<i>No data on potency:</i>						
RCS-4	?	?		3-6, 11, 12	?	47,49
RCS-4-C4 homolog ^c	?	?		3-6	?	47,49
RCS-08 ^d	?	?		?	?	47,49

Table 3

Potent cannabimimetic research chemicals: These are currently available to research laboratories, but also accessible to the general population through various websites, for which there are currently no regulations as far as legality, and no Schedule under DEA. The effects of these chemicals following potential human consumption are currently unknown

Compound	Potency and selectivity	K_i at CB_1 (nM)	Fold potency	Refs
O-2545-HCl	potent CB_1/CB_2 agonist	1.5	26x	90
ACEA	potent and highly selective CB_1 agonist	1.4	29x	91
ACPA	potent and selective CB_1 agonist	2.2	18x	91
AM-1235	potent and selective CB_1 agonist	1.5	26x	66
AM-2232	potent CB_1/CB_2 agonist	0.3	133x	66
AM-2233	full agonist at CB_1	2.8	14x	80

Table 4

Inhibitors of endogenous eCB degradation, including MAGL, FAAH, and ABHD6 inhibitors; these cannabinoid research chemicals could potentially be used as potent 'street drugs', since they are known to inhibit 2-AG and AEA degradation in animal models and pharmacological studies. As is the case for cannabimimetics listed in Table 3, there are no Schedule regulations on these compounds, or known effects in human

Compound	Specificity	IC ₅₀ (μM)	Observations	Refs
URB602	Weak reversible MAGL inhibitor	223	Reduces MAGL activity to 73% and elevates 2-AG levels in hippocampal slices at 100 μM	93,94
URB602 carbamate analogue 16	MAGL inhibitor	?	Reduces MAGL activity to 26% at 100 μM in hippocampal slices	95
NAM	Irreversible MAGL inhibitor	0.14	Electively prevents metabolic inactivation of 2-AG by ~85% in mouse cerebellum <i>in vitro</i>	96-98
JZL 184	Irreversible MAGL inhibitor	0.008	Blocks hydrolysis of 2-AG <i>in vivo</i> in the mouse brain	99,100
(±)-oxiran- hexanoate	Reversible dual MAGL/FAAH inhibitor	4.1 _{MAGL} 5.1 _{FAAH}		101
(2R)-(-)-oxiran acetate	Reversible dual MAGL/FAAH inhibitor	2.4 _{MAGL} 0.3 _{FAAH}		101
URB602 carbamate analogue 12	Reversible dual MAGL/FAAH inhibitor	0.006 _{MAGL} 0.012 _{FAAH}		102
URB602 carbamate analogue 26	Reversible dual MAGL/FAAH inhibitor	2.1 _{MAGL} 1.0 _{FAAH}	Also a highly potent blocker of AEA uptake (0.082 μM) by RBL2H3 cells; reduce 2-AG hydrolysis by these cells at [0.030 μM]	102
WWL 70	Potent ABHD6 inhibitor	0.07		103,104
UCM710	Potent dual ABHD6 and FAAH inhibitor	2.4 _{ABHD6} 4.0 _{FAAH}	Augments levels of 2-AG and AEA in neurons; no effect on MAGL	105
PMSF	Non-specific irreversible amidase (incl. FAAH) inhibitor	290		106
AACOCF3	cPLA2 and FAAH inhibitor	0.008		107
AA-5-HT	?	5.6		108
JNJ 1661010	Reversible FAAH inhibitor	0.012	Brain penetrant and active <i>in vivo</i>	109
LY 2183240	FAAH inhibitor	0.013	Also a highly potent blocker of AEA uptake (270 pM)	110
MAFP	Potent, irreversible FAAH inhibitor	0.003		111,112
NADA	FAAH inhibitor	0.4	Also a potent CB ₁ agonist (K _i = 0.25 μM)	113
PIA	FAAH inhibitor	4.89		114
PF 3845	FAAH inhibitor	0.23		115
PF 750	Irreversible FAAH inhibitor	0.016	Orally active, acts within the CNS	116,117
ST4070	Reversible FAAH inhibitor	0.033		118,119
VER-156084	FAAH inhibitor	0.1-10		120
URB597	FAAH inhibitor	0.0046		121-123

Table 5

Stage comparison between chick, mouse, and human at pre-neuronal stages of embryonic CNS development. Comparison between chick (HH), mouse, (E), and human (days, weeks) embryonic stages is shown for neural plate formation, neurulation (i.e. formation of the neural folds), and brain primordia formation (i.e. formation of the forebrain, midbrain and hindbrain vesicles). The data is compiled from Carnegie Stages Comparison^[132] and Psychoyos *et al.*^[133]

Species	Neural plate	Neurulation	Brain primordia
Chick (HH)	4	5 8	10 11 12 13
Mouse (E)	7	7.25 7.5	7.75 8 8.25 9
Human (days)	16	18 20	21 22 24 28
Human (wks)	2	2.5 2.5	3 3 3 3.5 4

Table 6
Summary of the functions of the eCB system is shown in animal models at various stages of neuronal development of the CNS

Species	Stage	Function of the eCB during neuronal development of the CNS	Refs
<i>Developing cortex (forebrain derivative):</i>			
Rat	E5-20	Maturation of glutamatergic system	172
Mouse	E12	Pyramidal cell specification; radial migration of immature pyramidal cells in VZ/SVZ	157
Mouse	E14.5	Establishment of long-range axonal connections from pyramidal cells in VZ/SVZ	157
Rat	E19	Interneuron specification and migration via regulation of BDNF/TrkB and GABA receptor	164,165
Rat	E21	Maturation of serotonergic system	171
Rat	P2	Inhibits neurogenesis via attenuation of ERK and promotes astroglial differentiation	163
<i>Developing hippocampus (forebrain derivative):</i>			
Rat	E17	Promotes neurogenesis	61
Rat	E17-E18	Inhibits synapse loss between hippocampal neurons in models for neurodegenerative diseases; protects hippocampal neurons from excitotoxicity; modulates synaptogenesis in intact E17 hippocampus	165–168
<i>Developing telencephalon (forebrain derivative):</i>			
Rat	E14	Modulation of TH activity (catecholaminergic system)	158
Rat	E14	Modulation of PENK mRNA (opioidergenic system)	145
<i>Developing cerebellum (hindbrain derivative):</i>			
Zebrafish	P1	Required for FGF-dependent axonal elongation and fasciculation of cerebellar neurons	169,170

Table 7

Summary of known effects of Δ^9 -THC and other cannabimimetics on neuronal development of the CNS, subdivided on effects in neuronal circuitry, and effects on neurotransmitter signalling. All experiments were performed in rat

Compound	Stage	Area	Effect on neuronal development of developing CNS in rat	Refs
Δ^9 -THC, WIN-55,212-2	E14	Mesencephalic neuron cultures	Modulates the activity of TH	155,158,199-201
Δ^9 -THC	E5-P20	Cerebellum	Modulates transcription of glutamate receptor subunits	172
Δ^9 -THC, WIN 55,212-2	E15-P9	Cortex	Reduces levels of glutamatergic neurotransmitters	195,197
WIN55,212-2	E5-20	Hippocampal neuron cultures	Reduction in glutamate outflow	196
Δ^9 -THC, WIN 55,212-2	E15-P9	Cortex, amygdala	Reduces levels of noradrenergic neurotransmitters	193,197
Δ^9 -THC	–	Nucleus accumbens, caudal putamen and rostral dorsal striatum	Inhibition of PENK mRNA expression	194,202-204
HU-210	P4	Cerebellar neurons	GABA mRNA expression	205
Δ^9 -THC	–	Diencephalon, hippocampus, midbrain raphe nuclei and septum	Reduces serotonin levels	171