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CURRENT OPINION

Cannabinoid-Based Therapies and Brain Development: Potential Harmful Effect of Early Modulation of the Endocannabinoid System

Patrícia Schonhofen[1,2,3] · Ivi Juliana Bristot[1,2,3] · José Alexandre Crippa[3,4] · Jaime Eduardo Cecílio Hallak[3,4] · Antônio Waldo Zuardi[3,4] · Richard B. Parsons[5] · Fábio Klamt[1,2,3]

Abstract

The endocannabinoid retrograde signaling pathway is widely expressed in the central nervous system, where it plays major roles in regulating synaptic plasticity (excitatory and inhibitory) through long-term potentiation and long-term depression. The endocannabinoid system (ECS) components—cannabinoid receptors, endocannabinoids and synthesis/degradation enzymes—are expressed and are functional from early developmental stages and throughout adolescent cortical development, regulating progenitor cell fate, neural differentiation, migration and survival. This may potentially confer increased vulnerability to adverse outcomes from early cannabinoid exposure. Cannabidiol (CBD) is one of the most studied exogenous cannabinoids, and CBD-enriched *Cannabis* extracts have been widely (and successfully) used as adjuvants to treat children with refractory epilepsy, and there is even a Food and Drug Administration (FDA)-approved drug with purified CBD derived from *Cannabis*. However, there is insufficient information on possible long-term changes in the central nervous system caused by cannabinoid treatments during early childhood. Like the majority of cannabinoids, CBD is able to exert its effects directly and indirectly through the ECS, which can perturb the regulatory processes mediated by this system. In addition, CBD has a large number of non-endocannabinoid targets, which can explain CBD's effects. Here, we review the current knowledge about CBD-based therapies—pure and CBD-enriched *Cannabis* extracts—in studies with pediatric patients, their side effects, and their mechanisms of action regarding the central nervous system and neurodevelopment aspects. Since *Cannabis* extracts contain Δ^9-tetrahydrocannabinol (Δ^9-THC), we consider that pure CBD is possibly safer for young patients. Nevertheless, CBD, as well as other natural and/or synthetic cannabinoids, should be studied in more detail as a therapeutic alternative to CBD-enriched *Cannabis* extracts for young patients.

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✉ Fábio Klamt
fabio.klamt@ufrgs.br

[1] Laboratory of Cellular Biochemistry, Department of Biochemistry, ICBS/UFRGS, 2600 Ramiro Barcelos St, Porto Alegre, RS 90035-003, Brazil

[2] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, ICBS/UFRGS, Porto Alegre, RS 90035-003, Brazil

[3] National Institutes of Science and Technology-Translational Medicine (INCT-TM), Porto Alegre, Brazil

[4] Neuroscience and Behavior Department, Faculty of Medicine of Ribeirão Preto, Ribeirão Preto, SP, Brazil

[5] Institute of Pharmaceutical Science, King's College London (KCL), London SE1 9NH, UK

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Key Points

Cannabidiol (CBD) targets the endocannabinoid system directly via cannabinoid receptor type 1 (CB_1) receptors or indirectly by regulating endocannabinoid levels, in both developing and mature brains.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is believed to be responsible for the majority of the potential harmful effects of CBD-enriched *Cannabis* extracts, although further direct evaluation of the effects of CBD upon brain development is necessary.

For young patients, pure CBD, both synthetic or plant derived, produced in accordance with good manufacturing practices (GMP-grade), is recommended as a therapeutic option instead of CBD-enriched *Cannabis* extracts, and a recently CBD-based product (Epidiolex®) was approved by the Food and Drug Administration (FDA) for the treatment of Dravet and Lennox-Gastaut syndromes.

There is a lack of trials of chronic administration of CBD-based therapies with long-term follow-up periods; conducting such trials would allow a more realistic comparison of the effects of these therapies with those of current treatment options.

1 Introduction

The plant *Cannabis sativa* has been used for medicinal purposes for thousands of years by different cultures [1]. *Cannabis* extract contains more than 80 components, of which Δ^9-tetrahydrocannabinol (Δ^9-THC) (the main psychoactive ingredient) and cannabidiol (CBD) are the most abundant [2, 3]. These compounds were first identified several decades ago [4], but it is only more recently that the discovery of cannabinoid receptors and their endogenous homologues, the endocannabinoids [5] such as *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) [6], has occurred. Together with their related enzymes, endocannabinoids and their receptors form the endocannabinoid system (ECS) (Fig. 1) [7]. Cannabinoids—both endogenous and plant derived—target the G protein-coupled cannabinoid receptor type 1 (CB_1), which is widely expressed in the nervous system, and cannabinoid receptor type 2 (CB_2), which is mainly expressed in immune cells [8, 9]. Presently, it is proposed that the ECS has roles in the pathological mechanisms of several psychiatric disorders, including schizophrenia [10]. Besides, cannabinoids such as CBD also interact with a variety of non-endocannabinoid mechanisms, including numerous classical ion channels, receptors, transporters, and enzymes, as reviewed recently [11].

The effects of isolated cannabinoids and *Cannabis* extracts in different diseases have been studied for many years [12]. In the United States, recent medical and recreational marijuana legalization increased *Cannabis* accessibility and use [13]. Additionally, despite widely known deleterious effects during central nervous system development, medical marijuana usage by minors, with the consent from a legal guardian and certification from a physician, is approved [14]. Marijuana-derived products have their main effects against childhood severe epilepsies, including Dravet and Lennox-Gastaut syndromes. These early onset disorders are characterized by frequent, refractory seizures and neurodevelopmental delays, which lead to impaired quality of life in these individuals. This scenario compels families to seek alternative treatment methods, such as CBD-based therapies, which include pure synthetic or plant-derived CBD and CBD-enriched *Cannabis* extracts. In children, plant-derived, pharmaceutical-grade isolated CBD has been tested in clinical trials in patients with such syndromes [15–17], and this drug (Epidiolex®) has recently been approved in the USA as an orphan drug for those syndromes. Clinical trials with synthetic isolated CBD are ongoing (clinicaltrials.gov website). In addition, reports on the use of different forms of *Cannabis* extracts in children with epilepsy have also been published [18–20]. However, only few adequately powered, placebo-controlled, randomized studies have evaluated the safety and efficacy of CBD-based therapies in children [21]. Nevertheless, most of these therapies have been reported to have a greater reduction in convulsive seizure frequency than placebo, being associated, however, with higher rates of adverse events [22].

The constituents of the ECS, receptors and endocannabinoids, are expressed and are functional from very early developmental stages, whereby they regulate inhibitory and excitatory synapses. Even during adolescence, the brain and the ECS undergo active development, which may confer increased vulnerability to adverse long-term outcomes from early cannabinoid exposure [23]. Endocannabinoids have been shown to regulate cortical development throughout life in humans, and exogenous cannabinoids can alter cortical development of both the somatosensory and the prefrontal cortex [24].

Nevertheless, the current widespread use of CBD-based therapies in children and young adults, without sufficient studies on the potential consequences regarding neuronal and other systems' development, is of concern to the scientific and medical communities. One area of particular concern is the uncontrolled amount of Δ^9-THC present in such extracts. Moreover, in 2017, an ad hoc committee of the National Academies of Sciences, Engineering, and Medicine presented a report regarding the health effects of *Cannabis*

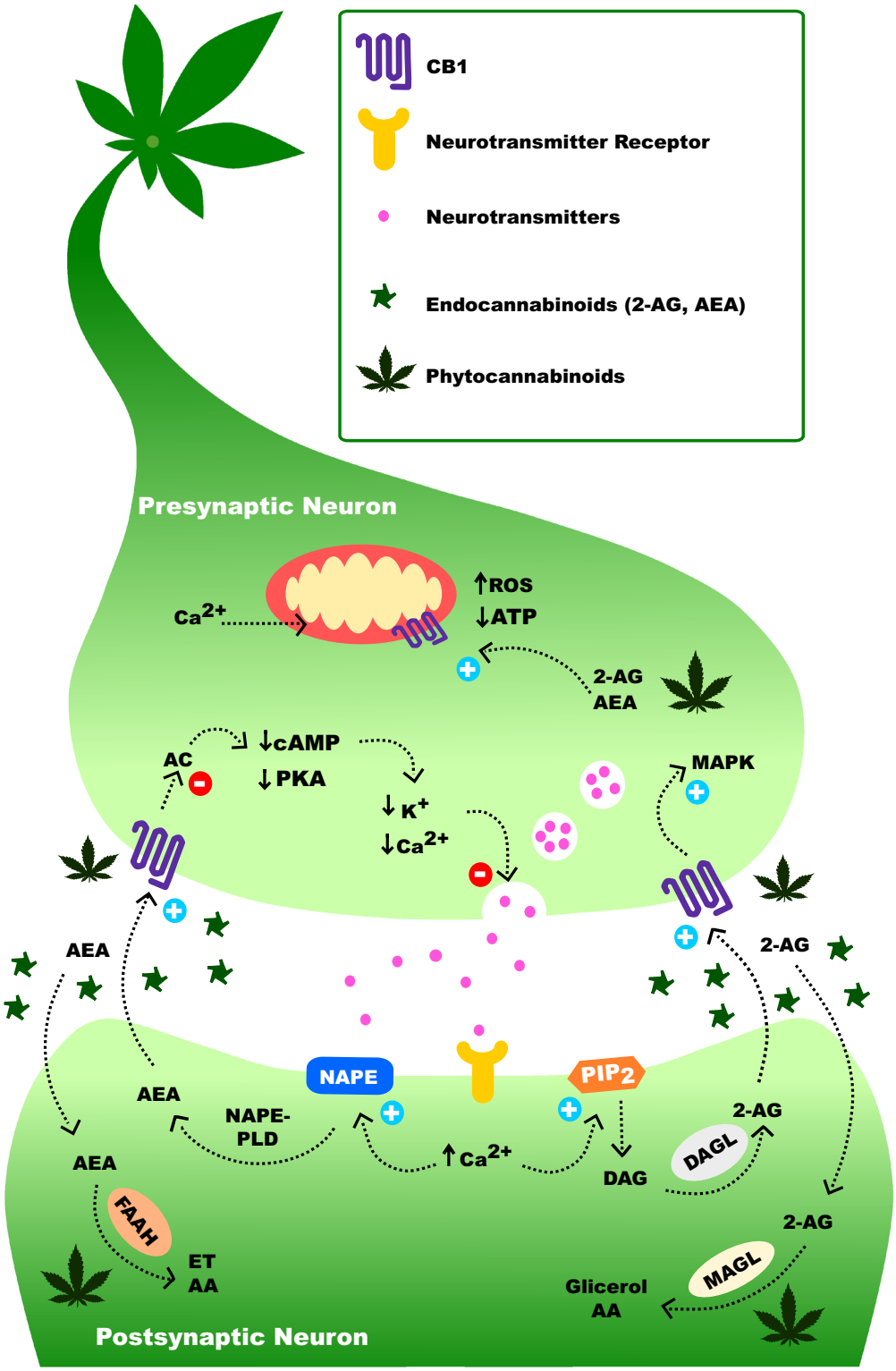

Fig. 1 Retrograde endocannabinoid signaling. Endocannabinoids are produced on demand in the post-synaptic neuron, released in the synaptic cleft and activate CB_1 receptors in the pre-synaptic neuron. *2-AG* 2-arachidonoylglycerol, *AA* arachidonic acid, *AC* adenylyl cyclase, *AEA* anandamide, *ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate, *CB_1* cannabinoid receptor type 1, *DAG* diacylglycerol, *DAGL* diacylglycerol lipase, *ET* ethanolamine, *FAAH* fatty acid amide hydrolase, *MAGL* monoacylglycerol lipase, *MAPK* mitogen-activated protein kinase, *NAPE* *N*-arachidonoylphosphatidylethanolamine, *NAPE-PLD* *N*-arachidonoylphosphatidylethanolamine phospholipase-D, *PIP_2* phosphatidylinositol biphosphate, *PKA* protein kinase A, *ROS* reactive oxygen species

and CBD use, which revealed no or insufficient evidence to either support or refute the use of such compounds as an effective treatment for epilepsy [25]. Hence, this article reviews the current knowledge about the use of CBD-based therapies in pediatric patients, the alleged side effects, and the mechanisms of action regarding the central nervous system and neurodevelopmental aspects. We highlight that CBD administration before adulthood must be carefully evaluated, and the use of pure CBD and/or synthetic cannabinoids as a preferential alternative to *Cannabis* extracts for children and young adults needs to be studied further.

2 The Endocannabinoid System

Most cannabinoids exert their therapeutic properties upon the central nervous system primarily via the ECS, although there are other known targets [26]. Here, we discuss their effects upon the ECS. Endocannabinoid signaling plays crucial roles in various aspects of both the underdeveloped and the mature brain [27]. Therefore, disturbances in this system may disrupt neural development.

The classical ECS signaling pathway is shown in Fig. 1 (for review see [10]). In the mature brain, the ECS modulates synapses (excitatory and inhibitory) through the release of endocannabinoids AEA and 2-AG. These act as retrograde messengers, their release by the postsynaptic neuron activating CB_1 receptors in the pre-synaptic neuron, leading to decreased release of neurotransmitters into the synaptic cleft [10, 28, 29]. This process is initiated by increased Ca^{2+} influx caused by neurotransmission in the postsynaptic neuron, which activates endocannabinoid synthesis from its precursors in the plasma membrane. AEA is generated from phospholipase D-mediated hydrolysis of the membrane lipid *N*-arachidonoylphosphatidylethanolamine (NAPE), while 2-AG originates from the diacylglycerol lipase-mediated hydrolysis of diacylglycerol (DAG), derived mainly from membrane-localized phosphatidylinositol biphosphate (PIP_2). AEA and 2-AG diffuse towards the pre-synaptic terminals and, like exogenous cannabinoids such as Δ^9-THC, bind to and activate the pre-synaptic, G protein-coupled CB_1 receptors. This binding triggers the activation and release of Gi/Go proteins from the CB_1, inhibiting adenylyl cyclase (AC) and thus decreasing cyclic adenosine monophosphate (cAMP) formation and subsequent protein kinase A (PKA) activity. These events lead to opening of inwardly rectifying K^+ channels, causing a hyperpolarization of the pre-synaptic terminal, and closing of Ca^{2+} channels, arresting the release of stored neurotransmitters. Finally, AEA and 2-AG re-enter the post- or pre-synaptic terminals, where they are catabolized respectively by fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL), to yield either arachidonic acid (AA) and ethanolamine (ET) in the case of AEA, or AA and glycerol for 2-AG. The transport of endocannabinoids through the plasma membrane is still not completely understood. Although some studies have proposed the existence of an endocannabinoid transporter, the trafficking of AEA, which has been most extensively studied, is proposed to occur through facilitated membrane transport followed by intracellular shuttling and sequestration [30].

Additionally, CB_1 receptor activation leads to stimulation of mitogen-activated protein kinase (MAPK) activity, a mechanism by which cannabinoids affect synaptic plasticity, cell migration, and possibly neuronal growth [23]. In mature neurons, the MAPK cascade, which leads to the activation of extracellular signal-regulated kinases (ERK), is stimulated by excitatory glutamatergic signaling. Subsequently, ERK activity regulates two processes that underlie changes in synaptic transmission—the activity of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and structural plasticity [31]. ECS retrograde signaling mediates synaptic plasticity through three classical mechanisms: depolarization-induced suppression of inhibition or excitation, metabotropic-induced suppression of inhibition or excitation, and endocannabinoid-mediated, short-term depression or long-term depression (STD/LTD) [10]. Also, CB_1 agonists can prevent long-term potentiation (LTP) of synaptic transmission, but the influence of endogenously formed cannabinoids on hippocampal LTP remains ambiguous [32]. Both LTP and LTD have roles in learning and neural development [24].

Thus, the central component of the ECS in neurons is the CB_1 receptor (Fig. 1). In the central nervous system, CB_1 is particularly enriched in the cortex, hippocampus, amygdala, basal ganglia outflow tracts, and cerebellum. This distribution corresponds to the most prominent behavioral effects of *Cannabis* and helps to predict neurological and psychological effects of ECS manipulation [33]. CB_1 receptors are also observed in intracellular compartments such as the mitochondrial surface, where they are able to activate G protein-dependent signaling and modify intracellular levels of adenosine triphosphate (ATP), Ca^{2+}, and reactive oxygen species, all of which impact upon synaptic transmission [34].

In the developing nervous system and the remaining neurogenic areas in the adult brain (the hippocampal subgranular zone and subventricular zone), the ECS exerts a regulatory role on neural progenitor cell survival, proliferation, differentiation and migration via CB_1 [35, 36], thus possibly affecting the formation of adult specialized tissues [37]. Recently, the ECS has also been shown to regulate proliferation and differentiation of mesoderm-derived hematopoietic and mesenchymal stem cells, with a key role in determining the formation of several cell types in peripheral tissues [38].

The importance of the ECS during embryonic development has been investigated through many experimental

models and approaches, mainly focusing upon the deleterious effect of early Δ^9-THC administration. For example, Δ^9-THC administration to pregnant mice interfered with sub-cerebral projection neuron generation, thereby altering corticospinal connectivity, and produced long-lasting alterations in the fine motor performance and seizure susceptibility of the adult offspring. These deleterious consequences were solely attributed to Δ^9-THC's ability to disrupt the neurodevelopmental role of CB_1 signaling [39].

During adolescence, the ECS has a role in the development of the cortex, amygdala, hippocampus and hypothalamus, and exogenous cannabinoids have long-term effects on cognition, anxiety and stress-related behaviors, leading to mood disorders and substance abuse [24]. At this age, cannabinoids may produce abnormal LTD in prefrontal cortex by disrupting LTD mediated by metabotropic glutamate receptors and CB_1 [40]. The ECS maintain the homeostasis of prefrontal cortex interactions with the amygdala and hippocampus, which are responsible for behaviors such as emotional memory and anxiety-related behaviors. Endocannabinoids are required for the normal stress response, a process which matures during adolescence [24]. Besides, as the prefrontal cortex is the last brain region to finish development after adolescence, the abundance of CB_1 receptors may explain the negative effects of *Cannabis* use in this age range [27]. Finally, endocannabinoids are necessary for the normal regulation of neuronal excitation and inhibition; hence, disturbances in this delicate equilibrium likely result in changes in the balance of excitation/inhibition in individual neurons and networks, processes which are necessary for normal cortical development [24].

For therapeutic purposes, regarding the mature central nervous system, the ECS has been shown to modulate anxiety, depression, neurogenesis, reward, cognition, learning, and memory [23]. Moreover, its retrograde signaling acts to regulate seizure activity and neuronal hyper-excitability—cannabinoids have shown CB_1 activity in experimental models of seizure and epilepsy [41, 42]. However, the use of CB_1 agonists such as Δ^9-THC, or even *Cannabis* extract, as a therapeutic strategy is unfeasible because of their psychoactive effects, abuse potential and development of tolerance [42]. On the other hand, antagonism of CB_1 can also exacerbate seizure activity in the epileptic phenotype [43].

Thus, the modulation of the ECS as a therapeutic approach is challenging because its blockage or its exacerbation could lead to undesired outcomes, especially during neuronal development. More studies are required to clarify its physiological functions and to predict the effect of CB_1 agonists and antagonists, both in adult and pediatric patients, to support its targeting for therapeutic purposes.

3 Therapeutic Uses and Mechanisms of Action of Cannabidiol (CBD)

Cannabis causes many psychotropic effects, mainly mediated by Δ^9-THC agonism of CB_1 [44], which makes it unlikely to be used *in natura*. On the other hand, experimental studies have demonstrated several therapeutic properties of isolated cannabinoids in a number of in vitro and in vivo models [45]. Here, we discuss the therapeutic uses of the most prominent of these cannabinoids, CBD, and its mechanisms of action, highlighting its activity towards the CB_1 receptor.

Although only a limited number of studies have focused upon CBD, recently, it has been shown to be a potent anti-inflammatory and antioxidant agent and to attenuate the memory-impairing effects produced by Δ^9-THC, amongst other effects [23]. This opens a wide range of possible therapeutic uses in neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, and cerebral ischemia [46]. Moreover, CBD is anti-emetic [47], has antitumoral properties against many types of cancer [48], and is also suggested to have antipsychotic, anxiolytic and antidepressant effects [49]. Finally, as already mentioned above, numerous studies have shown CBD to have anticonvulsive properties [50].

CBD has been reported to have a large number of possible molecular targets other than the ECS in a wide range of medical conditions, raising the possibility of significant off-target effects [26]. For instance, CBD is described as a full serotonin 1A ($5\text{-}HT_{1A}$) receptor agonist, a weak partial $5\text{-}HT_{2A}$ agonist and a non-competitive $5\text{-}HT_{3A}$ antagonist [51]. The ability of CBD to activate the A_{1A} adenosine receptor has also been reported [52]. CBD may play a role in the regulation of T-type calcium channels and the activity of nuclear peroxisome proliferator-activated receptor-γ (PPARγ), both of which have been implicated in seizure activity [53]. Other molecular targets have also been studied, among them the PPARγ nuclear receptors [54], glycine receptors [55], $GABA_A$ receptors [56], and transient receptor potential (TRP) channels [57]. Studies focused on the possible epigenetic regulation of skin differentiation genes by CBD revealed that it can act as a transcriptional repressor, controlling cell proliferation and differentiation through DNA methylation [58]. Hence, the molecular mechanistic basis for the effects of CBD appears to be complex and thus remains to be fully elucidated.

Although current evidence suggests that CBD does not directly interact with the ECS except in vitro at supraphysiological concentrations [11], it can also indirectly act as agonist or antagonist of the CB_1 receptor. In the nanomolar range [below the reported affinity (K_i) for CBD to these receptors], CBD can antagonize the pharmacological effects

of CB_1 agonists such as Δ^9-THC and AEA, despite having low direct affinity in the micromolar range for CB_1 in vitro [59, 60]. McPartland et al. reviewed in vitro and ex vivo mechanistic studies of CBD and found one study that reported slight agonism and one study that reported slightly inverse agonism comprising binding to the inactive form of the receptor, blocking agonist effects, both of which occurred at high concentrations of CBD (≥ 10 µM) [59]. Surprisingly, in some mechanistic studies, the effects of CBD could be reversed by CB_1 receptor inverse agonists, or were absent in CB_1 receptor knockout mice [59]. This suggests that CBD may exert indirect agonism, comprising enhancement of the effect of a receptor's agonist without having any direct agonist effect itself, at CB_1 receptors—either augmenting CB_1 constitutional activity [61] or augmenting endocannabinoid tone through inhibition of AEA hydrolysis, inhibition of the putative AEA transporter and increase of 2-AG levels [59].

Recent evidence supports the hypothesis that CBD also binds to an allosteric site on CB_1 receptors that is functionally distinct from the orthosteric site for its agonists. CBD reduced the potency and efficacy of CB_1 agonists at concentrations lower than the predicted affinity of CBD for the orthosteric site of CB_1 receptors [62]. The presence of this allosteric site is still to be directly demonstrated due to difficulties in the resolution of the crystallographic structure of this receptor [63]. Despite such methodological issues, in vitro pharmacological experiments have demonstrated that, at very low concentrations, CBD is a negative allosteric modulator of CB_1 [62].

Therefore, depending on the conditions, CBD seems to be able to interact both directly and indirectly with the CB_1 receptor via the regulation of endocannabinoid levels. Thus, since the ECS has a broad spectrum of physiological functions during neural development, it is reasonable to assume that CBD is potentially able to interfere with processes regulated by CB_1 when administered in infants. In fact, depending on the dosage and the clinical condition, potential CBD activity over CB_1 (agonism or antagonism) results in different outcomes—either therapeutic or harmful [27]; therefore its use must be very carefully considered in such ages. Besides, as CBD has effects on other targets at lower concentrations, the mechanisms underlying its therapeutic properties are not yet clearly understood [42].

4 Studies with CBD-Enriched *Cannabis* Extracts and Pure CDB in Pediatric Patients

Currently, CBD is clinically used in association with Δ^9-THC in a *Cannabis*-based preparation (Sativex®) that contains equimolar content of both, for the management of neuropathic symptoms associated with multiple sclerosis [64]. Relieve of spasticity and pain have been reported for multiple sclerosis patients that smoke *Cannabis*, but for these patients, structural magnetic resonance imaging (MRI) scans have suggested reduced brain volume is associated with cognitive impairment [65]. Likewise, in recreational users, *Cannabis* has been shown to result in volumetric gray matter and white matter structural changes in the brain, in particular, in the hippocampus and the amygdala [66], further evidence that *Cannabis* (smoked and possibly in extracts) can be harmful in adult brain.

In 2016, GW Pharmaceuticals reported the first results of pure CBD (Epidiolex®) in phase III clinical trials for use in treatment-resistant seizure disorders, including Lennox-Gastaut and Dravet syndromes [17, 22]. More recently, the same authors have released further results from a randomized, double-blind, placebo-controlled trial using pure CBD [67, 68]. Moreover, CBD-enriched *Cannabis* extract is still widely used as a therapeutic option. In this section, we review the available data on clinical trials, case reports and parental surveys available from January 2000 to May 2018. We focused on literature containing data about isolated CDB administration and relevant oral *Cannabis* extracts with high CBD content in pediatric and young patients, as well as relevant studies in adult volunteers.

The use of common *Cannabis* extracts is not recommended in children and adolescent patients because of the potential for deleterious effects. Fetal development is affected by prenatal maternal *Cannabis* use, while during infancy there is a negative impact upon cognitive and behavioral outcomes [69]. Early exposure to cannabinoids, mainly Δ^9-THC, can impair all stages of memory, from encoding to consolidation and retrieval [70]. Additionally, *Cannabis* usage during adolescence increases the risk of developing psychotic disorders such as schizophrenia later in life [71, 72]. Nevertheless, these effects are mainly associated with Δ^9-THC, and CBD is able to counteract such effects [73]. This indicates that pure CBD would be a better therapeutic option instead of CBD-enriched or common *Cannabis* extracts. Careful consideration and attention should be taken when using CBD-enriched *Cannabis* extracts, in particular, within pediatric contexts. In a recent case report, for example, two children presented typical symptoms of Δ^9-THC intoxication (inappropriate laughter, ataxia, reduced attention, and eye redness) after using a CBD-enriched *Cannabis* extract. The extract was replaced by the same dose of purified CBD, resulting in decreased intoxication symptoms and seizure remission [74].

Table 1 summarizes the main findings in children and young adult patients treated with pure CBD and CBD-enriched *Cannabis* extract. As most studies that established safety and dose tolerance were performed in adults, they were also reviewed (see the electronic supplementary

Table 1 Clinical trials using pure CBD published until May 2018 with children and young patients

Author/year	Design	Target/aim	Age (years)	Number of subjects included	Dose and administration route	Duration of treatment	Main results	Adverse/undesired effects
Thiele et al., 2018 [67]	Randomized, double-blind, placebo-controlled trial	Lennox-Gastaut syndrome	2–55	171	20 mg/kg/day orally	14 weeks	Reduction in seizure frequency of 43.9% in the CBD group and 21.8% in the placebo group	86% of CBD group: diarrhea, somnolence, pyrexia, decreased appetite, and vomiting; 14% withdrew from the study; 1 patient died in the CBD group, but unrelated to treatment
Devinsky et al., 2018 [68]	Double-blind, placebo-controlled trial	Lennox-Gastaut syndrome	2–55	225	10 or 20 mg/kg/day orally, in 2 equally divided doses daily	14 weeks	Reduction in seizure frequency was 41.9% in the 20-mg group, 37.2% in the 10-mg group, and 17.2% in the placebo group	Somnolence, decreased appetite, and diarrhea; 6 patients in the 20-mg CBD group and 1 patient in the 10-mg CBD group withdrew from the study; 14 patients had elevated liver aminotransferase concentrations
Devinsky et al., 2018 [16]	Randomized, dose-ranging safety trial	Safety and pharmacokinetics of CBD in Dravet syndrome	4–10	34	5, 10, or 20 mg/kg/day orally in 2 equally divided doses daily	3-week treatment, 10-day taper, and 4-week follow-up periods	Content of CBD and its metabolites increased proportionally with dose; CBD did not affect concomitant antiepileptic drugs	Pyrexia, somnolence, decreased appetite, sedation, vomiting, ataxia, and abnormal behavior; 6 patients taking CBD and valproate developed elevated transaminase levels
Devinsky et al., 2017 [22]	Randomized controlled trial	Dravet syndrome	2–18	120	Titrated up to 20 mg/kg/day twice a day orally	14 weeks	Reduction in convulsive seizure frequency; patients' overall condition improved 62%	Diarrhea, vomiting, fatigue, pyrexia, somnolence, and abnormal results on liver-function tests; 15% discontinued treatment; no significant reduction in non-convulsive seizures

Table 1 (continued)

Author/year	Design	Target/aim	Age (years)	Number of subjects included	Dose and administration route	Duration of treatment	Main results	Adverse/undesired effects
Gofshteyn et al., 2017 [101]	Open-label trial	Febrile infection-related epilepsy syndrome	3–8	7	Titrated up to 25 mg/kg/day orally	Acute and chronic treatment after status epilepticus for 4 weeks and 48 months	Cessation of the status epilepticus, with 100% reduction in all seizures in an acute patient; 90.9% decrease in frequency at 4 weeks and a 65.3% decrease at 48 weeks in chronic patients; reduction of other antiepileptic drugs	Dizziness, decreased appetite and weight loss, and nausea/vomiting; 4 of 7 developed a persistent tremor, but this was believed to be secondary to underlying central nervous system pathology; all living subjects continued to have cognitive impairment
Kaplan et al., 2017 [102]	Open-label trial	Sturge-Weber syndrome	2–19	5	Titrated up to intolerance or to 25 mg/kg/day orally	48 weeks	Seizure frequency decreased in 4 of 5 subjects; motor and cognitive improvements	Temporary increased seizures in 3 subjects, and behavioral issues in 2 subjects
Devinsky et al., 2016 [17]	Open-label trial	Treatment-resistant epilepsy	1–30	214	Titrated until intolerance or to 25 or 50 mg/kg/day orally	12 weeks	Motor seizures reduced in 36.5%; 4% were free of all motor seizures at the end of the treatment; 4 weeks after the end of treatment, 11% of patients were free of all motor seizures and 7% were free of all seizures; 37% had a reduction of 50% or more; 22% had a response of 70% or more; 8% had a response of 90% or more	79% of the safety group: somnolence, decreased appetite, diarrhea, fatigue, convulsion, increased appetite, status epilepticus, lethargy, weight increased, weight decreased, drug concentration increased; 11 patients withdrew the study; 1 death not related to CBD occurred

Table 1 (continued)

Author/year	Design	Target/aim	Age (years)	Number of subjects included	Dose and administration route	Duration of treatment	Main results	Adverse/undesired effects
Geffrey et al., 2015 [78]	Clinical trial	Drug interactions in pediatric epilepsy	4–19	13	5–25 mg/kg/day orally with clobazam	36 weeks	70% had a 50% or more decrease in seizures; enhanced blood levels of clobazam; reduction of clobazam doses for 77% of subjects	Side effects were reported in 77% of subjects: drowsiness, ataxia, irritability, restless sleep, urinary retention, tremor, and loss of appetite

Clinical trials performed until May 2018 with pure, synthetic or plant-derived CBD, presenting dosages and duration of treatments, main results and adverse effects. References and their corresponding results are presented according to date of publication in descending order

CBD cannabidiol

material, Supplementary Table 1). The majority of published articles focused on neurological and neuropsychiatric conditions. In adult volunteers, CBD presented few adverse events and appeared to be safe, although its effectiveness was not always confirmed. In most of these studies, CBD was administered in a single dose. A recent article on the safety and tolerability of pure CBD in 34 children between 4 and 10 years old with Dravet syndrome showed that CBD did not alter plasma antileptic drug levels, when randomized into different dosages or placebo for 3 weeks of treatment followed by a 4-week follow-up period [16]. The main adverse effects were pyrexia, somnolence, decreased appetite, sedation, vomiting, ataxia, and abnormal behavior. As observed in the abovementioned studies and reviewed by Wong and Wilens (2017) [13], the methodological quality of those clinical studies varied significantly (e.g., studies lacking control groups; limited by small sample size). Studies are also heterogeneous in the dosage and duration of treatment, and many lacked any long-term follow-up reviews to identify potential adverse effects [13]. This variability in employed protocols makes it difficult to evaluate the real benefits and risks of CBD-based therapies.

Until a few years ago, the suggested beneficial outcomes of CBD-based therapies for pediatric patients were based mainly on case reports and surveys of parents with epileptic children (see the electronic supplementary material, Supplementary Table 2). Such anecdotal studies were the first to report improvement in the general condition of children with refractory epilepsies with *Cannabis* extracts, and so they attracted the interest of the scientific community for cannabinoid-based treatments. Many surveys of parents of children with refractory seizures who self-administered CBD-enriched *Cannabis* extracts have been published in the last few decades. One such survey, involving a small cohort of patients, showed that 42% of children had a greater than 80% reduction in seizure frequency [75]. Another survey, using a larger cohort of 75 pediatric patients, reported that 38% of children achieved a greater than 50% reduction in seizures [20]. An online survey of 117 parents of children with epilepsy reported that 85% of children had a reduction in seizure frequency, whilst 14% reported complete freedom from seizures after CBD-enriched *Cannabis* treatment [19]. These surveys, even though not controlled, reported general improvements in cognitive and motor function in patients undergoing CBD-based therapies, along with some mild side effects.

On the other hand, not all studies have reported favorable results (e.g., CBD-enriched *Cannabis* extract resulted in no improvement in the general condition or seizure relief of an 18-year-old male with severe refractory epilepsy) [76]. Moreover, case reports and parent surveys rarely describe side effects or even drug administration issues. For this reason, clinical trials are indispensable for investigating both

the therapeutic and toxicological aspects of CBD-based therapies, as well as standardizing drug administration protocols to allow direct study comparisons.

However, as anecdotal studies have stimulated a growing interest in the anticonvulsive properties of CBD, pure CBD or CBD-enriched *Cannabis* extracts are now being tested in controlled clinical trials, with relevant positive outcomes thus far reported (Table 1). Such studies are still somewhat limited in number; however, a brief survey on clinicaltrials.gov website reported at least 20 clinical trials that are currently recruiting young patients or already in progress [77]. An open-label clinical trial of 214 patients (aged 1–30 years) with severe, intractable, childhood-onset, treatment-resistant epilepsy investigated the efficacy and safety of pure CBD. Patients in the efficacy analysis group reported a median reduction in monthly motor seizures of 36.5% compared to the placebo group. Adverse events were reported in 79% of the safety analysis group, and serious adverse events were reported in 30% of patients, including one death—a sudden, unexpected death due to the patient's epilepsy which was determined as unrelated to CBD. Twelve percent of patients had severe adverse events possibly related to CBD use, the most common of which was status epilepticus (6%). Three percent of patients discontinued treatment because of an adverse event [17].

A randomized, placebo-controlled, clinical trial of pure CBD reported a significant reduction in total seizures of all types. Although there was no significant reduction in non-convulsive seizures, the trial did demonstrate a greater reduction in convulsive seizure frequency, with 62% of patients reporting an improvement in overall condition, with 5% of patients becoming seizure-free. Adverse events included diarrhea, vomiting, fatigue, pyrexia, somnolence, and abnormal liver function tests [22]. This report, however, did not evaluate possible drug–drug interactions between CBD and clobazam, of which 65% of patients enrolled on the study were prescribed. CBD can increase plasma clobazam concentrations [78]; hence, the beneficial effects of CBD may have arisen indirectly due to the increased pharmacological effects of clobazam and not as a direct pharmacological effect of CBD itself.

In 2018, a randomized, double-blind, placebo-controlled trial encompassing 24 clinical sites in the USA, the Netherlands, and Poland was published. In this study, pure CBD (20 mg/kg/day) or placebo was administered to patients with treatment-resistant Lennox-Gastaut syndrome (aged 2–55 years) for 14 weeks. Of the 171 randomly assigned patients who received CBD ($n=86$) or placebo ($n=85$), 14 patients in the CBD group and one in the placebo group discontinued study treatment. The monthly drop in seizure frequency was reduced by 43.9% in the CBD group and 21.8% in the placebo group. Adverse events, which were mostly mild or moderate, occurred in 86% of patients in the CBD group and in 69% of patients in the placebo group [67].

Another recent double-blind, placebo-controlled trial, in which 225 patients with the Lennox-Gastaut syndrome (age range of 2–55 years) were randomly assigned to receive CBD at 10, 20 mg/kg/day, or placebo administered in two equally divided doses daily for 14 weeks, showed significant decreases in seizure frequency [68]. Seizure frequency decreased by 41.9% in the 20-mg CBD group, 37.2% in the 10-mg CBD group, and 17.2% in the placebo group. Six patients in the 20-mg CBD group and one patient in the 10-mg CBD group were withdrawn from the trial because of adverse events. Fourteen patients who received CBD (9%) had elevated plasma liver aminotransferase levels. The most common adverse events among the patients in the CBD groups were somnolence, decreased appetite, and diarrhea; these events occurred more frequently in the higher-dose group. Yet, even in these two recent clinical trials, although they are scientifically relevant and reliable, a longer treatment and follow-up period was missing.

In general, in pediatric patient clinical trials, the most common side effects reported were either mild (somnolence, fatigue, altered appetite, weight gain/loss, diarrhea and other gastrointestinal disturbances, irritability) or serious (drowsiness/dizziness, ataxia, tremor, mental sedation), with severe adverse effects such as increased seizure frequency and worsening seizure phenotype also being observed. Alimentary effects can be explained by the presence of the ECS in the gastrointestinal tract, where it has effects on motility, inflammation and immunity, intestinal and gastric acid secretion, nociception and emesis pathways, and appetite control [79]. In the brain, ECS modulates several brain functions, such as memory, mood, food intake, pain perception and the sleep–wake cycle [80], which may explain, at least partially, the central nervous system-mediated adverse effects observed in clinical trials. Besides, as discussed above, other cannabinoids present in *Cannabis* extracts as well as CBD are able to interact and possibly disturb the important roles played by the ECS during neurodevelopmental stages.

It is likely that non-endocannabinoid targets of CBD may explain some of the positive and adverse effects observed [11]. For example, in a mouse model of Dravet syndrome, the beneficial effects of CBD on inhibitory neurotransmission were mimicked and blocked by an antagonist of the orphan G protein-coupled receptor 55 (GPR55), suggesting that the therapeutic effects of CBD are mediated through this lipid-activated, G protein-coupled receptor and thus identify it as a third cannabinoid receptor [81].

A careful case-to-case evaluation on the risk/benefit balance of CBD usage must be taken, as in the most serious cases, repetitive infantile seizures can cause severe developmental, cognitive and motor impairment. These are obviously more detrimental than the adverse effects and possible

neurodevelopmental implications of CBD; hence, CBD may be an attractive therapeutic option in these cases.

Finally, CBD therapy does not always work for all patients. Also, some of the studies used CBD-enriched *Cannabis* extracts, which contain Δ^9-THC. Even controlled clinical trials investigating pure CBD used mostly short treatment periods and short follow-up periods, which will not reveal the possible long-term effects of CBD and possible developmental adverse effects. Hence, more clinical trials, with larger population sizes and longer chronic pure CBD administration, are warranted in order to clarify under which conditions it is worthwhile and safe to use. In addition, it is still unknown how CBD acts on hormones, hepatic enzymes, and drug transporters, along with its interactions with other drugs [12].

5 CBD During Development: Effects in Cell Culture and Animal Models of the Developing Brain

Despite the increasing use of CBD-based therapies in children and adolescents whose brains are still developing, most in vitro and in vivo studies use mature cells or adult animal models and are thus not faithful mimics of the juvenile central nervous system. Experiments with immature animals or cells have greater potential for identifying CBD's effects and the molecular mechanisms by which such effects are mediated with greater relevance to juveniles. However, few studies have evaluated the developmental phases which are equivalent to human central nervous system development. Here, we present some of the recent studies using pure CBD in relevant cellular and animal models of the developing brain.

In a genetic mouse model of Dravet syndrome, caused by loss-of-function mutations in the voltage-gated sodium channel NaV1.1, CBD treatment from postnatal day 21 to 27 decreased the duration and severity of thermally induced seizures and the frequency of spontaneous seizures. Lower doses of CBD also improved autistic-like social interaction deficits [81]. This mouse model represents a very specific cause of children refractory epilepsy, a single mutation in a sodium channel subunit, and its positive outcomes must be considered carefully when extrapolated to other pathologies.

Single-dose administration of CBD to newborn piglets shortly after hypoxia ischemia had a protective effect upon neurons and astrocytes, preserved brain activity, prevented seizures and improved neurobehavioral performance [82, 83]. In newborn rat brains, CBD administration also prevented necrotic and apoptotic cell death in an in vivo model of hypoxia ischemia damage [84], and rescued neuron function after sciatic nerve transection [85]. However, both studies used a single dose of CBD at a very specific moment, namely immediately after an intensive brain injury, to evaluate its acute effects. Thus, these results may not be representative of long-term treatments with CBD.

Although recent literature has primarily searched for potential protective and therapeutic effects of CBD, a recent research paper has reported negative effects. Zebrafish, exposed from blastula through to larval stage to micromolar concentrations of Δ^9-THC (1–16 µM) or CBD (0.25–4 µM), presented similarity in dysmorphologies to both compounds (i.e., edemas, curved axis, eye/snout/jaw/trunk/fin deformities, swim bladder distention, and behavioral abnormalities), whilst the LC_{50} (lethal concentration 50—concentration to kill 50% of the population) for CBD was nearly seven times lower than that for Δ^9-THC. The authors also reported teratogenic effects of low concentrations of CBD [86]. In contrast, other research found no malformation in development of zebrafish embryos exposed to CBD 20–300 µg/L, although the maximal dosage caused delay in embryo hatching. Besides, they were temporarily more active than control. The authors discussed that the effects observed are intimately related to the CB_1 receptor [87]. Again, the chosen doses may be responsible for the difference in results observed in these two studies. Additionally, 10 µM of Δ^9-THC, but not 10 µM of CBD, arrested the development of pre-implantation mouse embryos [88].

Notwithstanding that very few studies offer insight into CBD toxicity, some deleterious effects have been reported for CBD in vitro and in vivo. These include alterations in cell viability, reduced fertilization capacity, and inhibition of hepatic drug metabolism and drug transporters [89]. Our research group showed in a study using an in vitro model of human neurons (human neuroblastoma SH-SY5Y cells differentiated with retinoid acid) that a sublethal dose of CBD with antioxidant activity did not exhibit neuroprotection against the neurotoxic effect of glycolaldehyde, methylglyoxal, 6-hydroxydopamine, and hydrogen peroxide in terminally differentiated neurons. When SH-SY5Y cells undergoing neuronal differentiation were exposed to the same dose of CBD, besides the lack of neuroprotection and antioxidant activity, CBD potentiated the neurotoxicity induced by all redox-active drugs tested [90]. These results suggest a possible hidden negative effect of CBD during neuronal development, reinforcing the observation that effective dosages for CBD and the resulting pathologies observed can vary widely according to the experimental model used.

Thus, pure CBD presents both positive and deleterious effects in animal and cellular models of early stages of development. We recommend that the therapeutic use of CBD and other cannabinoids during brain developmental stages must be always supported by experimental studies in appropriate cellular and animal models, with special attention to the therapeutic window of CBD. It is particularly important

to consider that the effect of CBD in humans follows an inverted U-shaped dose–effect curve pattern of effectiveness as observed in many animal studies [91, 92].

6 Therapeutic Perspectives

Although a number of physiological effects of CBD in the brain have been identified, the mechanism(s) underlying its therapeutic properties in neurological diseases and during neurodevelopment are not yet clearly understood. Depending on the experimental model, the dosage used and the protocol, CBD can act upon CB_1 as an agonist, as an antagonist of endogenous ligands, or as an allosteric modulator, as well as acting upon non-endocannabinoid targets. Nevertheless, Δ^9-THC, which is able to interact with the ECS, is present in CBD-enriched *Cannabis* extracts used in some studies. Since the ECS performs primordial functions during embryonic development and neurodevelopment, in addition to neurogenesis in adults, it makes sense to hypothesize that any molecule that disturbs ECS activity, such as Δ^9-THC (and potentially CBD), might disrupt the processes regulated by this cellular signaling system.

Regarding CBD therapeutic use for the treatment of children, there are several positive results in clinical trials and case reports in children with refractory epilepsy. However, for CBD-enriched *Cannabis* extracts the controversial effects of Δ^9-THC points to a possible risk of adverse effects for its use in young patients. *Cannabis* has been associated with development of psychotic symptoms later in life, and a recent publication was able to establish a causal role of *Cannabis* use during adolescence and the emergence of such symptoms in the subsequent year [72]. Such effects are attributed to Δ^9-THC activity on CB_1. As CBD has low affinity for CB_1, although it interferes in other steps of ECS signaling, this cannabinoid may be preferable and safer. Thus, formulations containing Δ^9-THC should be avoided. Moreover, adverse effects of CBD and its extracts—even though they are mainly not severe—as well as absence of therapeutic effects were also reported. Seizure reduction has a significant effect on the patient's quality of life, but the need to take into account other changes that CBD could cause in social behavior, cognitive function, or motor skills is also important. Another concern is that the use of CBD-based therapies for pediatric epilepsy and anxiety (see Table 1 and Supplementary Table 2), together with the common belief that natural products are always harmless, could represent a precedent for its use to treat other neurological diseases. It is not completely clear how CBD affects children's brain development and how it could represent a risk of developing diseases later in adulthood. Thus, despite evidence for potential benefits in pediatric patients, pediatricians and families must balance the decision to use CBD with the associated risks [13]. An evaluation must occur on a case-to-case basis, with, at each instance, consideration of the damage to the patient that may arise from uncontrolled epileptic seizures, the adverse effects of the established antiepileptic drugs and the uncertainties in the effects of CBD during brain development.

Recently, natural and synthetic derivatives of CBD have attracted the attention of both industry and academia. Indeed, some of these molecules are being studied for a variety of purposes, most of them aiming to improve the potency, efficacy, or pharmacokinetic properties of CBD [93]. For instance, a natural CBD derivative, cannabidiolic acid (CBDA), does not have an effect on inhibition of AEA uptake, while keeping the low CB_1 affinity [93]. Thus, CBDA probably does not interfere in ECS signaling, which lowers the risk for adverse effects during brain development. The conversion of oral CBD into Δ^9-THC in an acidic environment (e.g., the stomach) is another concern, although it has not been observed in vivo thus far [94]. A novel CBD derivative, HU-444, is a potential novel drug which cannot be converted by acid cyclization into a Δ^9-THC–like compound. In vitro, HU-444 has an anti-inflammatory activity, leading to the suppression of tumor necrosis factor-α production and amelioration of liver damage, whilst not causing Δ^9-THC–like effects in mice [95]. Another synthetic cannabinoid, HU-320, produced strong anti-inflammatory and immunosuppressive effects in an in vivo model of collagen-induced arthritis [95].

For the generation of another class of CBD derivatives, the introduction of the dimethylheptyl (DMH) alkyl chain in the (−)-DMH-CBD series did not alter the lack of CB_1 and CB_2 receptor affinity [96]. (−)-DMH-CBD analogs have displayed anxiolytic, analgesic, anti-inflammatory, and antiproliferative effects in diverse assays [93]. (−)-DMH-CBD has been shown to have anti-inflammatory and antiproliferative properties in human acute myeloid leukemia [97]. Interestingly, (−)-7-OH-DMH-CBD exhibited potent inhibition of electrically evoked contractions of the mouse vas deferens that was not mediated through CB_1, CB_2, TRPV1, opioid, or α2-adrenergic receptors [98, 99].

Measurements of the binding affinities for the CB_1 and CB_2 cannabinoid receptors yielded unexpected outcomes of some CBD enantiomers. Contrary to naturally occurring (−)-CBD analogs, some synthetic derivatives, such as (+)-CBD, H2-CBD, H4-CBD, and HU-465, bind to CB_1, and several of them have shown interesting pharmacological properties for various pathologies [93]. However, as CB_1 activity is not desirable for an antiepileptic drug, because of all the ECS roles at developmental stages, such derivatives might not be an alternative in these cases. Thus, different CBD derivatives vary in their pharmacological and therapeutic properties, as well as naturally occurring

cannabinoids, evidencing the need for a better understanding of their mechanism of action.

7 Conclusion

As *Cannabis* extracts contain Δ^9-THC, which has psychoactive effects and is a CB_1 agonist and may potentially disturb the ECS processes during brain development, pure GMP-grade CBD, synthetic or plant derived, is probably a safer option for use in pediatric and juvenile patients. Recently, a CBD oral solution purified from a *Cannabis* extract and developed and tested by GW Research has been approved by the Food and Drug Administration (FDA) of the United States as an adjuvant in the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in patients 2 years of age and older. According to the released document, the approval was based on CBD's effectiveness in preclinical and clinical trials and due to its mechanisms of action (low CB_1 affinity, reduction of neuronal hyperexcitability and inflammation) [100].

However, since CBD can potentially affect the ECS also, further studies are recommended in order to clarify its mechanisms of action and developmental implications. Besides, longer chronic treatment and follow-up periods are recommended in clinical trials and animal studies in order to evaluate CBD's long-term effects, as well as the most effective dosage and the age which the therapeutic use of pure CBD is not only effective but safe.

At the moment, we consider that CBD is recommended as the last option for the treatment of non-responsive epileptic children. For other neurological or psychiatric diseases, such as childhood anxiety, there is insufficient evidence to support the effectiveness of CBD. Besides, we suggest that more studies should use adequate experimental models to focus on pure CBD, in order to establish its safe and effective dosage and therapeutic targets, as well as synthetic CBD derivatives, aiming to identify a CBD analog with therapeutic properties, but with fewer risks to the developing brain.

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Compliance with Ethical Standards

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Conflict of interest AWZ, JECH and JAC are co-inventors of the patent "Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023" Def. US no. Reg. 62193296; 29/07/2015; INPI on 19/08/2015 (BR1120150164927). The University of São Paulo has licensed the patent to *Phytecs Pharm* (USP Resolution No. 15.1.130002.1.1). The University of São Paulo has an agreement with *Prati-Donaduzzi* (Toledo, Brazil) to "develop a pharmaceutical product containing synthetic cannabidiol and prove its safety and therapeutic efficacy in the treatment of epilepsy, schizophrenia, Parkinson's disease, and anxiety disorders." JECH and JAC have received travel support from and are medical advisors of BSPG-Pharm. AWZ is medical advisor of BSPG-Pharm. PS, FK, IJB, RBP declare no conflics of interest.

References

1. Cassol-jr OJ, Comim CM, Silva BR, Hermani F V, Constantino LS, Felisberto F, et al. Treatment with cannabidiol reverses oxidative stress parameters, cognitive impairment and mortality in rats submitted to sepsis by cecal ligation and puncture. Brain Res [Internet]. 2010;1348:128–38. 10.1016/j.brainres.2010.06.023.
2. Karniol IG, Shirakawa I, Kasinski N, Pfeferman A, Carlini EA. Cannabidiol interferes with the effects of delta 9—tetrahydrocannabinol in man. Eur J Pharmacol [Internet]. 1974;28:172–7.
3. Grlic L. A comparative study on some chemical and biological characteristics of various samples of cannabis resin. Bull Narcot. 1976;14:37–46.
4. Gaoni Y, Mechoulam R. Isolation and structure of Δ^+-tetrahydrocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc. 1971;93:217–24.
5. Howlett AC, Blume LC, Dalton GD. CB(1) cannabinoid receptors and their associated proteins. Curr Med Chem. 2010;17:1382–93.
6. Luchicchi A, Pistis M. Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids. Mol Neurobiol. 2012;46:374–92.
7. Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, International Union of Basic and Clinical Pharmacology, et al. LXXIX. Cannabinoid receptors and their ligands: beyond CB 1 and CB 2. Pharmacol Rev. 2010;62:588–631.
8. Pertwee RG. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. Philos Trans R Soc B Biol Sci. 2012;367:3353–63.
9. Di Marzo V, De Petrocellis L. Why do cannabinoid receptors have more than one endogenous ligand? Philos Trans R Soc B Biol Sci. 2012;367:3216–28.
10. Lu HC, MacKie K. An introduction to the endogenous cannabinoid system. Biol Psychiatry. 2016;79:516–25.
11. Ibeas Bih C, Chen T, Nunn AVW, Bazelot M, Dallas M, Whalley BJ. Molecular targets of cannabidiol in neurological disorders [Internet]. Neurotherapeutics. 2015. https://doi.org/10.1007/s13311-015-0377-3.

12. Iffland K, Grotenhermen F. An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. Cannabis Cannabinoid Res. 2017;2:139–54.
13. Wong SS, Wilens TE. Medical cannabinoids in children and adolescents: a systematic review. Pediatr Am Acad Pediatr. 2017;140:e20171818.
14. Health Reform and State Health Legislative Initiatives. State medical marijuana laws. 2018. http://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx#. Accessed 18 Jan 2018.
15. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. Lancet Neurol [Internet]. 2015;15:270–8. https://doi.org/10.1016/S1474-4422(15)00379-8.
16. Devinsky O, Patel AD, Thiele EA, Wong MH, Appleton R, Harden CL, et al. Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. Neurology [Internet]. 2018;90:e1204–11.
17. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. Lancet Neurol [Internet]. 2016;15:270–8.
18. Tzadok M, Uliel-Siboni S, Linder I, Kramer U, Epstein O, Menascu S, et al. CBD-enriched medical cannabis for intractable pediatric epilepsy. Seizure [Internet]. 2016;35:41–4.
19. Hussain SA, Zhou R, Jacobson C, Weng J, Cheng E, Lay J, et al. Perceived efficacy of cannabidiol-enriched cannabis extracts for treatment of pediatric epilepsy: a potential role for infantile spasms and Lennox-Gastaut syndrome. Epilepsy Behav [Internet]. 2015;47:138–41. https://doi.org/10.1016/j.yebeh.2015.04.009.
20. Press CA, Knupp KG, Chapman KE. Parental reporting of response to oral cannabis extracts for treatment of refractory epilepsy. Epilepsy Behav [Internet]. 2015;45:49–52.
21. O'Connell BK, Gloss D, Devinsky O. Cannabinoids in treatment-resistant epilepsy: a review. Epilepsy Behav. 2017;70:341–8.
22. Devinsky O, Cross JH, Laux L, Marsh E, Miller I, Nabbout R, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med [Internet]. 2017;376:2011–20.
23. Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annu Rev Psychol. 2013;64:21–47.
24. Dow-Edwards D, Silva L. Endocannabinoids in brain plasticity: cortical maturation, HPA axis function and behavior. Brain Res [Internet]. 2017;1654:157–64.
25. Definitions of weights of evidence. The health effects of cannabis and cannabinoids: the current state of evidence and recommendations for research. 2017. http://nationalacademies.org/hmd/~/media/Files/Report%20Files/2017/Cannabis-Health-Effects/Cannabis-conclusions.pdf. Accessed 12 June 2018.
26. Morales P, Hurst DP, Reggio PH. Molecular targets of the phytocannabinoids: a complex picture. Prog Chem Org Nat Prod. 2017;103:103–31.
27. Meyer HC, Lee FS, Gee DG. The role of the endocannabinoid system and genetic variation in adolescent brain development. Neuropsychopharmacology. 2017;43:21–33.
28. Alger BE. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol [Internet]. 2002;68:247–86.
29. Velasco G, Sánchez C, Guzmán M. Towards the use of cannabinoids as antitumour agents. Nat Rev Cancer [Internet]. 2012;12:436–44.
30. Fowler CJ. Transport of endocannabinoids across the plasma membrane and within the cell. FEBS J. 2013;280:1895–904.
31. Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci [Internet]. 2004;5:173–83. https://doi.org/10.1038/nrn1346.
32. Silva-Cruz A, Carlström M, Ribeiro JA, Sebastião AM. Dual influence of endocannabinoids on long-term potentiation of synaptic transmission. Front Pharmacol [Internet]. 2017;8:921.
33. Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol. 2005;168:299–325.
34. Djeungoue-Petga M-A, Hebert-Chatelain E. Linking mitochondria and synaptic transmission: the CB1 receptor. BioEssays [Internet]. 2017;39:1700126.
35. Harkany T, Guzmán M, Galve-Roperh I, Berghuis P, Devi LA, Mackie K. The emerging functions of endocannabinoid signaling during CNS development. Trends Pharmacol Sci [Internet]. 2007;28:83–92.
36. Díaz-Alonso J, Aguado T, Wu C-S, Palazuelos J, Hofmann C, Garcez P, et al. The CB(1) cannabinoid receptor drives corticospinal motor neuron differentiation through the Ctip2/Satb2 transcriptional regulation axis. J Neurosci [Internet]. 2012;32:16651–65.
37. Habayeb OMH, Taylor AH, Bell SC, Taylor DJ, Konje JC. Expression of the endocannabinoid system in human first trimester placenta and its role in trophoblast proliferation. Endocrinology. 2008;149:5052–60.
38. Galve-Roperh I, Chiurchiù V, Díaz-Alonso J, Bari M, Guzmán M, Maccarrone M. Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. Prog Lipid Res [Internet]. 2013;52:633–50. https://doi.org/10.1016/j.plipres.2013.05.004.
39. de Salas-Quiroga A, Díaz-Alonso J, García-Rincón D, Remmers F, Vega D, Gómez-Cañas M, et al. Prenatal exposure to cannabinoids evokes long-lasting functional alterations by targeting CB_1 receptors on developing cortical neurons. Proc Natl Acad Sci [Internet]. 2015;201514962. https://doi.org/10.1073/pnas.1514962112.
40. Lovelace JW, Corches A, Vieira PA, Hiroto AS, Mackie K, Korzus E. An animal model of female adolescent cannabinoid exposure elicits a long-lasting deficit in presynaptic long-term plasticity. Neuropharmacology [Internet]. 2015;99:242–55.
41. Gaston TE, Friedman D. Pharmacology of cannabinoids in the treatment of epilepsy. Epilepsy Behav. 2017;70:313–8.
42. Blair RE, Deshpande LS, DeLorenzo RJ. Cannabinoids: is there a potential treatment role in epilepsy? Expert Opin Pharmacother. 2015;16:1911–4.
43. Braakman HMH, van Oostenbrugge RJ, van Kranen-Mastenbroek VHJM, de Krom MCTFM. Rimonabant induces partial seizures in a patient with a history of generalized epilepsy. Epilepsia. 2009;50:2171–2.
44. Silveira MM, Adams WK, Morena M, Hill MN, Winstanley CA. Δ9-Tetrahydrocannabinol decreases willingness to exert cognitive effort in male rats. J Psychiatry Neurosci [Internet]. 2017;42:131–8.
45. Kaur R, Ambwani SR, Singh S. Endocannabinoid system: a multi-facet therapeutic target. Curr Clin Pharmacol. 2016;11:110–7.
46. Fernández-Ruiz J, Sagredo O, Pazos MR, García C, Pertwee R, Mechoulam R, et al. Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? Br J Clin Pharmacol. 2013;75:323–33.
47. Parker LA, Rock EM, Limebeer CL. Regulation of nausea and vomiting by cannabinoids. Br J Pharmacol. 2011;163:1411–22.
48. Massi P, Solinas M, Cinquina V, Parolaro D. Cannabidiol as potential anticancer drug. Br J Clin Pharmacol. 2013;75:303–12.

49. Crippa JAS, Zuardi AW, Hallak JEC. Therapeutical use of the cannabinoids in psychiatry. Rev Bras Psiquiatr. 2010;32(Suppl 1):S56–66.
50. Campbell CT, Phillips MS, Manasco K. Cannabinoids in pediatrics. J Pediatr Pharmacol Ther. 2017;22:176–85.
51. Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ, et al. Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT(1A) somatodendritic autoreceptors in the dorsal raphe nucleus. Br J Pharmacol [Internet]. 2012;165:2620–34.
52. Gonca E, Darıcı F. The effect of cannabidiol on ischemia/reperfusion-induced ventricular arrhythmias. J Cardiovasc Pharmacol Ther. 2015;20:76–83.
53. Cilio MR, Thiele EA, Devinsky O. The case for assessing cannabidiol in epilepsy. Epilepsia. 2014;55:787–90.
54. Hind WH, England TJ, O'Sullivan SE. Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPARγ and 5-HT1A receptors. Br J Pharmacol. 2016;173:815–25.
55. Xiong W, Cui T, Cheng K, Yang F, Chen S-R, Willenbring D, et al. Cannabinoids suppress inflammatory and neuropathic pain by targeting α3 glycine receptors. J Exp Med. 2012;209:1121–34.
56. Bakas T, van Nieuwenhuijzen PS, Devenish SO, McGregor IS, Arnold JC, Chebib M. The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABAA receptors. Pharmacol Res. 2017;119:358–70.
57. De Petrocellis L, Ligresti A, Moriello AS, Allarà M, Bisogno T, Petrosino S, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol [Internet]. 2011;163:1479–94.
58. Pucci M, Rapino C, Di Francesco A, Dainese E, D'Addario C, Maccarrone M. Epigenetic control of skin differentiation genes by phytocannabinoids. Br J Pharmacol. 2013;170:581–91.
59. McPartland JM, Duncan M, Di Marzo V, Pertwee RG. Are cannabidiol and Δ(9) -tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol. 2015;172:737–53.
60. Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. Br J Pharmacol [Internet]. 2007;150:613–23.
61. Sagredo O, Pazos MR, Satta V, Ramos JA, Pertwee RG, Fernández-Ruiz J. Neuroprotective effects of phytocannabinoid-based medicines in experimental models of Huntington's disease. J Neurosci Res. 2011;89:1509–18.
62. Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright EM. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. Br J Pharmacol. 2015;172:4790–805.
63. Hua T, Vemuri K, Nikas SP, Laprairie RB, Wu Y, Qu L, et al. Crystal structures of agonist-bound human cannabinoid receptor CB1. Nature [Internet]. 2017;547:468–71. https://doi.org/10.1038/nature23272.
64. Fernández Ó. THC:CBD in daily practice: available data from UK, Germany and Spain. Eur Neurol [Internet]. 2016;75:1–3.
65. Romero K, Pavisian B, Staines WR, Feinstein A. Multiple sclerosis, cannabis, and cognition: a structural MRI study. NeuroImage Clin [Internet]. 2015;8:140–7.
66. Weinstein A, Livny A, Weizman A. Brain imaging studies on the cognitive, pharmacological and neurobiological effects of Cannabis in humans: evidence from studies of adult users. Curr Pharm Des [Internet]. 2017;22:6366–79.
67. Thiele EA, Marsh ED, French JA, Mazurkiewicz-Beldzinska M, Benbadis SR, Joshi C, et al. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2018;391:1085–96.
68. Devinsky O, Patel AD, Cross JH, Villanueva V, Wirrell EC, Privitera M, et al. Effect of cannabidiol on drop seizures in the Lennox-Gastaut syndrome. N Engl J Med [Internet]. 2018;378:1888–97. https://doi.org/10.1056/NEJMoa1714631.
69. Huizink AC. Prenatal cannabis exposure and infant outcomes: overview of studies. Prog Neuropsychopharmacol Biol Psychiatry. 2014;52:45–52.
70. Ranganathan M, D'Souza DC. The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl) [Internet]. 2006;188:425–44.
71. Bossong MG, Niesink RJM. Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. Prog Neurobiol [Internet]. 2010;92:370–85.
72. Bourque J, Afzali MH, Conrod PJ. Association of Cannabis use with adolescent psychotic symptoms. JAMA Psychiatry [Internet]. 2018. https://doi.org/10.1001/jamapsychiatry.2018.1330.
73. Niesink RJM, van Laar MW. Does cannabidiol protect against adverse psychological effects of THC? Front Psychiatry [Internet]. 2013;4:130.
74. Crippa JAS, Crippa ACS, Hallak JEC, Martín-Santos R, Zuardi AW. Δ9-THC intoxication by cannabidiol-enriched Cannabis extract in two children with refractory epilepsy: full remission after switching to purified cannabidiol. Front Pharmacol [Internet]. 2016;7:359.
75. Porter BE, Jacobson C. Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. Epilepsy Behav [Internet]. 2013;29:574–7.
76. Rosemergy I, Adler J, Psirides A. Cannabidiol oil in the treatment of super refractory status epilepticus. A case report. Seizure [Internet]. 2016;35:56–8.
77. Seizures TCA, Results S, Available NR, States U, Specialists N, States U. ClinicalTrials.gov Search Results 06/15/2018; 2018. P. 7–8.
78. Geffrey AL, Pollack SF, Bruno PL, Thiele EA. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. Epilepsia [Internet]. 2015;56:1246–51. https://doi.org/10.1111/epi.13060.
79. Gotfried J, Kataria R, Schey R. Review: the role of cannabinoids on esophageal function-what we know thus far. Cannabis Cannabinoid Res [Internet]. 2017;2:252–8.
80. Prospéro-García O, Amancio-Belmont O, Becerril Meléndez AL, Ruiz-Contreras AE, Méndez-Díaz M. Endocannabinoids and sleep. Neurosci Biobehav Rev [Internet]. 2016;71:671–9.
81. Kaplan JS, Stella N, Catterall WA, Westenbroek RE. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. Proc Natl Acad Sci [Internet]. 2017;114:11229–34.
82. Alvarez FJ, Lafuente H, Rey-Santano MC, Mielgo VE, Gastiasoro E, Rueda M, et al. Neuroprotective effects of the nonpsychoactive cannabinoid cannabidiol in hypoxic-ischemic newborn piglets. Pediatr Res [Internet]. 2008;64:653–8.
83. Lafuente H, Alvarez FJ, Pazos MR, Alvarez A, Rey-Santano MC, Mielgo V, et al. Cannabidiol reduces brain damage and improves functional recovery after acute hypoxia-ischemia in newborn pigs. Pediatr Res [Internet]. 2011;70:272–7.
84. Pazos MR, Cinquina V, Gómez A, Layunta R, Santos M, Fernández-ruiz J, et al. Neuropharmacology cannabidiol administration after hypoxia e ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. 2012;63:776–83.
85. Perez M, Benitez SU, Cartarozzi LP, Del Bel E, Guimarães FS, Oliveira ALR. Neuroprotection and reduction of glial reaction by

cannabidiol treatment after sciatic nerve transection in neonatal rats. Eur J Neurosci [Internet]. 2013;38:3424–34.
86. Carty DR, Thornton C, Gledhill JH, Willett KL. Developmental effects of cannabidiol and Δ9-tetrahydrocannabinol in zebrafish. Toxicol Sci [Internet]. 2017;162:137–45.
87. Valim Brigante TA, Abe FR, Zuardi AW, Hallak JEC, Crippa JAS, de Oliveira DP. Cannabidiol did not induce teratogenicity or neurotoxicity in exposed zebrafish embryos. Chem Biol Interact [Internet]. 2018;291:81–6.
88. Paria BC, Das SK, Dey SK. The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling. Dev Biol [Internet]. 1995;92:9460–4.
89. Bergamaschi MM, Queiroz RHC, Zuardi AW, Crippa JAS. Safety and side effects of cannabidiol, a Cannabis sativa constituent. Curr Drug Saf [Internet]. 2011;6:237–49.
90. Schönhofen P, de Medeiros LM, Bristot IJ, Lopes FM, De Bastiani MA, Kapczinski F, et al. Cannabidiol exposure during neuronal differentiation sensitizes cells against redox-active neurotoxins. Mol Neurobiol. 2015;52:26–37.
91. Zuardi AW, Rodrigues NP, Silva AL, Bernardo SA, Hallak JEC, Guimarães FS, et al. Inverted U-shaped dose-response curve of the anxiolytic effect of cannabidiol during public speaking in real life. Front Pharmacol [Internet]. 2017;8:259.
92. Genaro K, Fabris D, Arantes ALF, Zuardi AW, Crippa JAS, Prado WA. Cannabidiol is a potential therapeutic for the affective-motivational dimension of incision pain in rats. Front Pharmacol [Internet]. 2017;8:391.
93. Morales P, Reggio PH, Jagerovic N. An overview on medicinal chemistry of synthetic and natural derivatives of cannabidiol. Front Pharmacol [Internet]. 2017;8:422. https://doi.org/10.3389/fphar.2017.00422/full.
94. Nahler G, Grotenhermen F, Zuardi AW, Crippa JAS. A conversion of oral cannabidiol to Delta9-tetrahydrocannabinol seems not to occur in humans. Cannabis Cannabinoid Res [Internet]. 2017;2:81–6. https://doi.org/10.1089/can.2017.0009.
95. Haj CG, Sumariwalla PF, Hanu L, Kogan NM, Yektin Z, Mechoulam R, et al. HU-444, a novel, potent anti-inflammatory, nonpsychotropic cannabinoid. J Pharmacol Exp Ther [Internet]. 2015;355:66–75.
96. Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol [Internet]. 2001;134:845–52.
97. Juknat A, Pietr M, Kozela E, Rimmerman N, Levy R, Coppola G, et al. Differential transcriptional profiles mediated by exposure to the cannabinoids cannabidiol and Δ9-tetrahydrocannabinol in BV-2 microglial cells. Br J Pharmacol. 2012;165:2512–28.
98. Fride E, Ponde D, Breuer A, Hanus L. Peripheral, but not central effects of cannabidiol derivatives: mediation by CB(1) and unidentified receptors. Neuropharmacology [Internet]. 2005;48:1117–29.
99. Pertwee RG, Thomas A, Stevenson LA, Maor Y, Mechoulam R. Evidence that (-)-7-hydroxy-4′-dimethylheptyl-cannabidiol activates a non-CB(1), non-CB(2), non-TRPV1 target in the mouse vas deferens. Neuropharmacology [Internet]. 2005;48:1139–46.
100. CBD-OS for the treatment of Lennox-Gastaut syndrome and Dravet syndrome FDA advisory committee meeting briefing document. https://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/peripheralandcentralnervoussystemdrugsadvisorycommittee/ucm604738.pdf. Accessed 11 July 2018.
101. Gofshteyn JS, Wilfong A, Devinsky O, Bluvstein J, Charuta J, Ciliberto MA, et al. Cannabidiol as a potential treatment for febrile infection-related epilepsy syndrome (FIRES) in the acute and chronic phases. J Child Neurol. 2017;32:35–40.
102. Kaplan EH, Offermann EA, Sievers JW, Comi AM. Cannabidiol treatment for refractory seizures in sturge-weber syndrome. Pediatr Neurol. 2017;71:18–23.e2.