


Review

# Molecular Targets of Cannabidiol in Experimental Models of Neurological Disease

Serena Silvestro <sup>†</sup> , Giovanni Schepici <sup>†</sup>, Placido Bramanti and Emanuela Mazzon <sup>\*</sup>

Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Centro Neurolesi “Bonino-Pulejo”, Via Provinciale Palermo, Contrada Casazza, 98124 Messina, Italy; serena.silvestro@irccsme.it (S.S.); giovanni.schepici@irccsme.it (G.S.); placido.bramanti@irccsme.it (P.B.)

<sup>\*</sup> Correspondence: emanuela.mazzon@irccsme.it; Tel.: +39-090-6012-8172

<sup>†</sup> These authors contributed equally to this work.

Academic Editor: Francesca Cardona

Received: 16 October 2020; Accepted: 5 November 2020; Published: 7 November 2020



**Abstract:** Cannabidiol (CBD) is a non-psychoactive phytocannabinoid known for its beneficial effects including antioxidant and anti-inflammatory properties. Moreover, CBD is a compound with antidepressant, anxiolytic, anticonvulsant and antipsychotic effects. Thanks to all these properties, the interest of the scientific community for it has grown. Indeed, CBD is a great candidate for the management of neurological diseases. The purpose of our review is to summarize the *in vitro* and *in vivo* studies published in the last 15 years that describe the biochemical and molecular mechanisms underlying the effects of CBD and its therapeutic application in neurological diseases. CBD exerts its neuroprotective effects through three G protein coupled-receptors (adenosine receptor subtype 2A, serotonin receptor subtype 1A and G protein-coupled receptor 55), one ligand-gated ion channel (transient receptor potential vanilloid channel-1) and one nuclear factor (peroxisome proliferator-activated receptor  $\gamma$ ). Moreover, the therapeutic properties of CBD are also due to GABAergic modulation. In conclusion, CBD, through multi-target mechanisms, represents a valid therapeutic tool for the management of epilepsy, Alzheimer’s disease, multiple sclerosis and Parkinson’s disease.

**Keywords:** cannabidiol; molecular mechanisms; neurological diseases; neuroprotective effects

## 1. Introduction

Neurological diseases are complex conditions affecting millions of people around the world [1]. These disorders can have different etiologies; indeed, they can be caused by genetic or environmental factors [2–5]. Although for some of these pathologies treatment that can delay or control the clinical symptoms is available, they remain incurable diseases.

Phytocannabinoids, such as CBD, represent a new class of compounds characterized by beneficial effects in various neurodegenerative and psychiatric diseases [6,7].

CBD is extracted from *Cannabis sativa*, and, together with the psychoactive  $\Delta^9$ -tetrahydro-cannabinol ( $\Delta^9$ -THC), they represent the main neuroactive components of the plant. Unlike  $\Delta^9$ -THC that induces psychotropic effects, CBD is the main non-psychotropic compound present in the plant [8,9].

CBD shows a relatively low toxicity and dependence profile [10]. For these reasons, the role of CBD as adjuvant therapy is being evaluated in those conditions in which the available treatments are not satisfying. Furthermore, CBD has a broad spectrum of therapeutic properties, such as anxiolytic [7,11], neuroprotective [7,12–15], antidepressant [16], anti-inflammatory [17–19] and immunomodulating activities [20,21]. The neuroprotective effects of CBD are due to its antioxidant and anti-inflammatory activities and the modulation of a large number of brain biological targets, such as receptors and channels, involved in the development and maintenance of neurodegenerative diseases [22]. CBD can

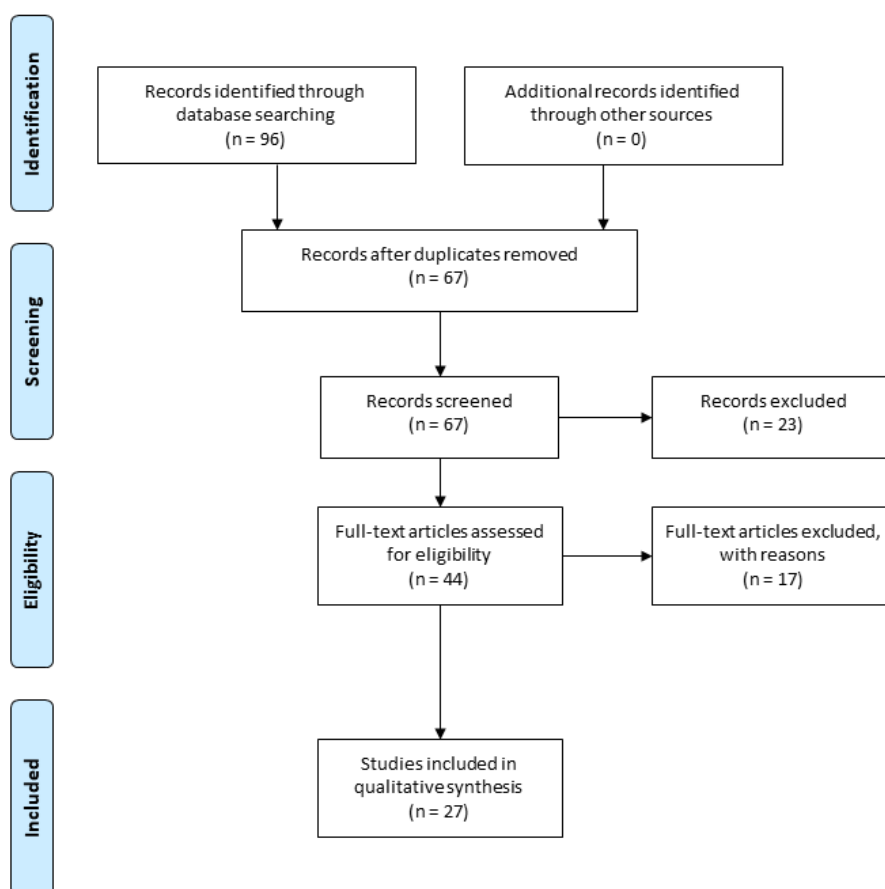
exert its antioxidant action directly by the modulation of oxidative stress or indirectly through molecules targets associated with the redox system such as nuclear factor erythroid 2-related factor 2 (Nrf2), implicated in the transcription of genes that encodes for antioxidant proteins, such as superoxide dismutase (SOD) and glutathione (GSH) peroxidase [23,24]. CBD can increase the activity of GSH peroxidase and reductase, favoring the reduction of malondialdehyde and thus preventing oxidative stress [25]. Moreover, thanks to its ability to reduce reactive oxygen species (ROS), CBD maintains the correct GSH levels, necessary for the antioxidant activity of vitamins A, C and E [26]. CBD is a regulator of the expression of nitrotyrosine and inducible nitric oxide synthase (iNOS), thus promoting the reduction of the production of ROS [27]. Moreover, CBD exerts its anti-inflammatory action, modulating the release of proinflammatory cytokines such as interleukin-6 (IL-6) and interleukin 1- $\beta$  (IL-1 $\beta$ ) and interacting with transcription factors such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), nuclear factor  $\kappa$ B (NF- $\kappa$ B) or peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) [28–30]. CBD also performs its anti-inflammatory action by regulating the transient receptor potential (TRP) channels such as transient receptor potential vanilloid (TRPV) Type 1 and 2, a non-selective cationic channel whose activation allows the entry of Ca<sup>2+</sup> [31]. Indeed, in the neuroinflammatory conditions, an increase of the density and sensitivity of TRPV1 was demonstrated. Conversely, the binding of the CBD with TRPV1 leads to the desensitization of these channel receptors and a consequent reduction of neuroinflammation, thus explaining the neuroprotective properties of CBD [32].

Therefore, in recent years, the scientific community has shown interest in this compound due to its neuroprotective effects in several neurological disorders, including Parkinson's disease [33,34], Alzheimer's diseases [35–37] and epilepsy [38]. Additionally, CBD shows other actions such as antidepressant, antipsychotic, antiepileptic and analgesic effects, as highlighted in preclinical studies and human clinical trials [7,39–41].

The purpose of this review is to describe the molecular mechanisms associated with the efficacy of CBD in neurological diseases. In the present review, the experimental studies highlighting the CBD's mechanisms of action in neurological disorders are summarized.

## 2. Methodology

In this review, the articles published from 2004 to 2020 are considered. Specifically, the bibliography research in PubMed was performed using the following keywords: "cannabidiol", "neurological disease", "adenosine receptors", "serotonin receptors", "transient receptor potential", "TRPV receptors", "GPR55 receptors", "peroxisome proliferator-activated receptor- $\gamma$ ", "PPAR $\gamma$  receptors" and "GABA receptors". In this way, 67 articles were found, as shown in the Prisma flow diagram (Figure 1). Articles that evaluate the biochemical and molecular mechanisms underlying the effects of CBD and its therapeutic application in neurological diseases are considered.

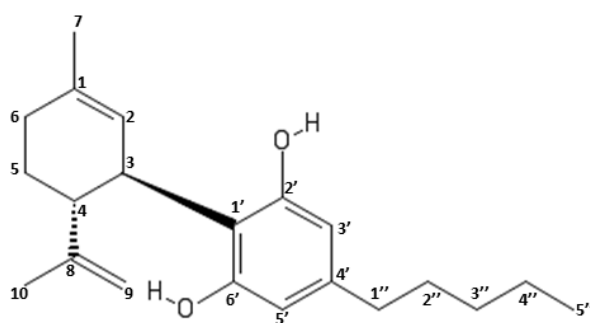


**Figure 1.** Prisma flow diagram illustrating the selection methodology of the preclinical studies used for the writing of the review. Duplicate articles were excluded from the total of the studies recorded. Instead, articles that evaluate the biochemical and molecular mechanisms underlying the effects of CBD and its therapeutic application in neurological diseases are considered (The PRISMA Statement is published in [42]).

### 3. Chemical Properties of Cannabidiol

CBD was first isolated in 1940 from Mexican marijuana by Roger Adams and from Indian charas by Alexander Todd [43,44]. However, its crystalline structure was determined in 1977 (Jones et al. 1977) [45]. CBD 2-[(1*R*,6*R*)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol is a terpenophenol containing 21 carbon atoms, with the formula  $C_{21}H_{30}O_2$ . CBD is a cyclohexene which is substituted in position 1 by a methyl group, by a 2,6-dihydroxy-4-pentylphenyl group at position 3 and with a prop-1-en-2-yl group at position 4 [46]. The aromatic ring and the terpene ring are almost perpendicular to each other. In the chemical nomenclature, its chemical numbering is determined by the terpene ring (Figure 2).

The reactivity of CBD is mainly due to the methyl group in position C-1 of the cyclohexane ring, to the hydroxyl groups present in the aromatic ring in position C-2' and C-6' and to the pentyl chain present in position C-4'. The hydroxyl groups are also capable of binding threonine, tyrosine and glutamic acid [47]. Moreover, CBD, thanks to its hydroxyl groups in the aromatic ring, can exert an antioxidant action by inactivating the free radicals [48].



**Figure 2.** Chemical Structure and numbering system of Cannabidiol (CBD).

#### 4. Cannabidiol Mechanism of Action

Unlike other cannabinoids, CBD has a poor affinity for cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2); however, it acts as a non-competitive negative allosteric modulator of CB1 [49]. It has recently been shown that CBD is a CB1R-negative allosteric modulator of  $\Delta^9$ -THC and the 2-arachidonoyl-glycerol (2-AG), this evidence may explain some of the *in vivo* effects of this nonpsychoactive phycompond [50]. Furthermore, CBD modulates the tone of endocannabinoids by inhibiting cellular uptake of the endocannabinoids arachidonylethanolamide (AEA) [51]. AEA, CB1, CB2 and 2-AG constitute the endocannabinoid system (ECS) responsible for the synthesis and degradation of endocannabinoids [52]. Therefore, CBD can lead to increased levels of AEA, thus interacting with CB receptors [53]. AEA is involved in different biological processes such as mood regulation, pain sensation and appetite. The synthesis of AEA and 2-AG is regulated by increased of intracellular calcium ( $\text{Ca}^{2+}$ ). Moreover, AEA and 2-AG are, respectively, metabolized by fatty acid amide hydrolase (FAAH) and monoglyceride lipase [54]. The increase of intracellular  $\text{Ca}^{2+}$  induces the production and release into the synaptic space of AEA and 2-AG that acts as retrograde synaptic messengers [55,56]. Indeed, these endocannabinoids acting on CB1 at a presynaptic level block the release by neuronal terminals of neurotransmitters such as  $\gamma$ -aminobutyric acid (GABA), dopamine, glutamate, serotonin (5-HT), norepinephrine and acetylcholine [57,58].

However, it was shown that CBD has more affinity for 5-HT receptors, non-endocannabinoid G protein-coupled receptors (GPCRs) and other targets such as enzymes and ion channels [53]. Indeed, some anti-inflammatory and immunosuppressive effects of CBD may be partly mediated by 5-HT and adenosine receptors (ARs) which are not considered part of the ECS. CBD acts as a 5-HT<sub>1A</sub> agonist, as a partial agonist of 5-HT<sub>2A</sub> and as a non-competitive antagonist of 5-HT<sub>3A</sub> [59–61]. Furthermore, CBD is capable of activating the ARs [62]. Moreover, CBD acts as a GPR55 antagonist and as an agonist of TRPV1 and TRPV2 [63]. It has already been reported that CBD exerts its anti-inflammatory and neuroprotective effects also due to its ability to activate PPAR $\gamma$  [17,64]. Furthermore, it is a positive allosteric modulator of GABA<sub>A</sub> receptors, thereby exerting its anticonvulsant, analgesic and anxiolytic properties [65]. However, despite all this evidence, the molecular mechanisms underlying the effects of CBD remain complex.

#### 5. Pharmacokinetic Properties of Cannabidiol

The pharmacokinetics and observed effects of CBD are related to the formulation and route of administration [66].

CBD can be administered orally, inhaled and vaporized [67]. CBD administered by inhalation is effectively absorbed into the lungs from the circulating blood, showing similar pharmacokinetics to the intravenous route [68]. Administered by inhalation, CBD reaches peak plasma concentrations in 5–10 min and shows bioavailability of 31%. However, the need for specialized equipment for these routes of administration limits the development of this mode of delivery [69]. On the contrary, oral administration shows a variable pharmacokinetic profile, probably due to the poor solubility of

CBD in water [70]. Furthermore, the maximum plasma concentrations are lower than those reached when the drug is administered by inhalation. Indeed, CBD in human showed an oral bioavailability of 6% [71]. The administration of CBD by the oro-mucosal and sublingual route shows a less variable pharmacokinetic profile than the oral administration. Instead, when CBD is administered intravenously, it quickly passes the blood–brain barrier (BBB) to distribute in the brain, adipose tissue and other organs [68]. Moreover, thanks to its liposolubility, CBD forms aggregates which can be released slowly into adipose tissue [72].

CBD is metabolized in the liver by cytochrome P450 enzymes (CYPs) such as CYP2C19 and CYP3A4, CYP1A1, CYP1A2, CYP2C9 and CYP2D6 and is converted to 7-hydroxycannabidiol (7-OH-CBD) [73]. After hydroxylation, CBD is further metabolized in the liver and subsequently excreted, mainly in feces and urine. CBD exhibits a half-life of 18–32 h and a clearance of 57.6–93.6 L/h [69].

CBD is well tolerated and shows a good safety profile at therapeutic dosages. However, some studies have shown that CBD possesses strong inhibitory activity against CYP2C, CYP2D6 and CYP3A isoforms [74].

## 6. Molecular Targets of CBD for Application in Neurodegenerative Diseases

The neuroprotective effects of CBD based on its anti-inflammatory and antioxidant action are directed to the modulation of receptors and channels involved in neurodegenerative diseases [22]. It is known that CBD interacts with many non-endocannabinoid signaling systems such as G protein coupled-receptors, TRPV1 and PPAR $\gamma$  [75]. Additionally, CBD therapeutic potential possibly derives from its GABAergic modulation [65].

### 6.1. GPCRs

#### 6.1.1. Adenosine Receptors

ARs ( $A_1R$ ,  $A_{2A}R$ ,  $A_{2B}R$  and  $A_3R$ ) are GPCRs stimulated by endogenous adenosine, involved in several physiological and pathological processes [76]. The stimulation of  $A_1R$  and  $A_3R$  by adenosine leads to the activation inhibiting G ( $G_{i/o}$ ) proteins with the consequent inhibition of adenylate cyclase and intracellular reduction of cyclic adenosine monophosphate (cAMP). Instead,  $A_{2A}R$  and  $A_{2B}R$  promote the G protein activation and the subsequent increase of cAMP [77]. ARs activation involves the modulation of second messengers and additional signaling mechanisms such as phospholipase C; the protein kinase C dependent on  $Ca^{2+}$  involved in cell communication; phosphoinositide 3-kinases/protein kinase B (PI3K/Akt) signaling involved in cell proliferation, growth and differentiation; and the activation of ion channels and regulation of  $Ca^{2+}$  [76,78]. ARs are located in immune cells, blood vessels, astrocytes, microglia, corpus striatum and spinal cord [79]. ARs can affect both in the central nervous system (CNS) and peripheral tissues, thus could represent a useful tool for the development of new neuroprotective strategies [79,80].

Although CBD exerts its beneficial action through several signaling pathways, its anti-inflammatory effects seem to involve  $A_{2A}R$  [81]. The anti-inflammatory effect of CBD can be directly mediated by  $A_{2A}R$  whose activation induces the regulation of the immune response, as well as a reduction of proinflammatory cytokines [82,83]. CBD can improve the adenosine signaling, leading to an increase of extracellular adenosine and a consequent reduction of the neuroinflammation [81]. Mecha et al. demonstrated the anti-inflammatory effects of CBD, through  $A_{2A}R$  activation, in a viral model of multiple sclerosis. They showed in mice infected with Theiler's murine encephalomyelitis virus (TMEV) that CBD (5 mg/kg), administered via intraperitoneal (i.p.) daily, for seven days, led to a reduction of leukocyte migration in the blood and of the inflammatory response. Moreover, CBD treatment induces the downregulation of the levels of adhesion expression of vascular cells molecule-1 (VCAM-1), chemokine ligand 2 (CCL2) and chemokine ligand 5 (CCL5). Likewise, CBD reduced IL-1 $\beta$  and microglia activation, thus demonstrating its immunosuppressive and neuroprotective action. In addition, CBD has also improved motor deficits, especially in the chronic phase of the disease. To confirm the action of

CBD on A<sub>2A</sub>R the animals were treated with ZM241385 (5 mg/kg), a selective A<sub>2A</sub>R antagonist, at the time of TMEV infection and 30 min before CBD treatment. ZM241385, especially in the early stages, attenuated some of the anti-inflammatory effects of CBD, such as the inhibition of the expression of VCAM-1, the infiltration of immune cells and the reduction of immunoreactivity. Moreover, it was also demonstrated that ZM241385 (5 μM) with a dose-dependent mechanism, antagonizes A<sub>2A</sub>R blocking completely the inhibitory action of CBD on the release of VCAM-1. Conversely, the single administration of ZM241385 did not affect TMEV mice. Therefore, the results suggest that the A<sub>2A</sub>R mediated anti-inflammatory effects of CBD could be useful for the management of inflammatory diseases such as multiple sclerosis [18].

The involvement of A<sub>2A</sub>R in neuroprotective effects of CBD was also demonstrated *in vitro* on hypoxic-ischemic immature brain of mice. In this study, Castillo et al. showed the effects of CBD in forebrain sections of C57BL6 mice incubated in absence of oxygen and glucose and treated for 15 min with CBD or vehicle. CBD treatment (100 μM) has significantly reduced acute brain damage and apoptosis, evaluated through the decrease in the efflux of lactate dehydrogenase. In the same way, CBD led to a reduction of glutamate and increase of caspase-9. CBD has also reduced the neuroinflammation leading to the reduction of IL-6, TNF-α, cyclooxygenase-2 (COX-2) and iNOS. Moreover, it was shown that the administration of SCH58261, an A<sub>2A</sub>R antagonist, or AM630, a CB2 antagonist, abolished the neuroprotective effects of CBD. The possible affinity of CBD to CB2 receptors is prompted by the effect of AM630 on CBD neuroprotection. Therefore, the study demonstrated how the neuroprotective effects of CBD can be mediated by A<sub>2A</sub>R and CB2 receptors [28]. The neuroprotective effects of CBD were also demonstrated by Martin-Moreno et al. *in vitro* and *in vivo* model of Alzheimer's disease. CBD inhibited ATP-induced intracellular Ca<sup>2+</sup> increase in cultured N13 cells and primary microglial cells. The use of ZM241385, an A<sub>2A</sub> receptor antagonist, reversed the effects of CBD on intracellular Ca<sup>2+</sup> in N13 microglial cells and primary rat microglial. This result confirms the implication of A<sub>2A</sub>R in the action of CBD. *In vivo*, CBD at a dose of 20 mg/kg was able to prevent cognitive impairment induced by amyloid-β (Aβ). Indeed, after *i.p.* administration for three weeks, CBD reduced the expression of the gene encoding for IL-6, a proinflammatory cytokine. Therefore, in light of these results, CBD, interacting with A<sub>2A</sub>R, could be a useful approach for Alzheimer's disease [84].

Instead, Magen et al. evaluated the A<sub>2A</sub>R-mediated therapeutic effects of CBD in the experimental model of hepatic encephalopathy induced by bile duct ligation. After four weeks of treatment, CBD (5 mg/kg), administrated *i.p.* daily, induced a reduction of expression of the TNF-α-receptor 1 gene in the hippocampus. Conversely, increased the expression of the brain-derived neurotrophic factor (BDNF) gene. CBD exerts these effects through an indirect modulation of the A<sub>2A</sub>Rs. Indeed, the use of its antagonist ZM241385 (1 mg/kg) reversed the effects of CBD, confirming the involvement of the A<sub>2A</sub>Rs. In this way, the chronic treatment with CBD, through the indirect activation of the A<sub>2A</sub>Rs, improved the cognitive and motor function of the rats with hepatic encephalopathy. As demonstrated by these studies, CBD, probably inhibiting the reuptake of adenosine, exerts an indirect modulation of ARs [82].

In conclusion, the results of these studies show that CBD, through A<sub>2A</sub>R activation, exerts anti-inflammatory effects in models of multiple sclerosis, hypoxic-ischemic damage, Alzheimer's disease and hepatic encephalopathy (Table 1).



**Table 1.** Neuroprotective effects of CBD in different neurological diseases through the activation of the A<sub>2A</sub>Rs.

In Vitro and in Vivo Models	CBD Dose	Treatments	Biological/Pharmacological Effect	Neurological Diseases	Ref.
Female SJL/J mice	5 mg/kg	Once-daily during Days 1–7 post-infection	CBD attenuated the activation of microglia downregulating the expression of VCAM-1, CCL2 and CCL5 and the proinflammatory cytokine IL-1 $\beta$ . Moreover, CBD improved motor deficits in the chronic phase of the disease	multiple sclerosis	[18]
Newborn C57BL6 mice	0.1–1000 $\mu$ M	15 min. pre-incubation	CBD reduced acute brain damage and apoptosis. Moreover, it induced a reduction concentration of glutamate and IL-6 and decreased the expression of TNF- $\alpha$ , COX-2 and iNOS.	hypoxic-ischemic brain damage	[28]
Primary Rat Microglial and N13 Microglial Cells and C57Bl/6 mice	20 mg/kg	Once-daily during the first week, then 3 days/week for 2 weeks	CBD inhibited ATP-induced intracellular Ca <sup>2+</sup> increase in cultured N13 and primary microglial cells and A <sub>2A</sub> receptors may be involved in this mechanism. In vivo, CBD reduced the gene expression of proinflammatory cytokine IL-6 and prevented cognitive impairment induced by A $\beta$ .	Alzheimer's disease	[84]
Female Sabra mice	5 mg/kg	Every day for 4 weeks	CBD reduced the expression of the TNF- $\alpha$ -receptor 1 gene in the hippocampus. Conversely, enhanced the expression of the BDNF gene. Moreover, CBD, through the indirect activation of the A <sub>2A</sub> R, improved the cognitive and motor function of the rats with Hepatic Encephalopathy.	hepatic encephalopathy	[82]

CBD, cannabidiol; VCAM-1, vascular cell adhesion molecule-1; CCL-2, chemokine ligand 2; CCL-5, chemokine ligand 5; IL-6, interleukin-6; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; A<sub>2A</sub>R, adenosine 2A receptors; CB2, cannabinoid receptors type 2; A $\beta$ ,  $\beta$ -amyloid; BDNF, brain-derived neurotrophic factor.

### 6.1.2. 5-HT Receptors

5-HT receptors are activated by serotonin and involved in the release of neurotransmitters and hormones, thus regulating many of the processes that occur in the nervous system. The family of receptors 5-HT<sub>1</sub> is coupled to G<sub>i/o</sub> proteins and to adenylate cyclase which leads to the production of cAMP. In particular, the 5-HT<sub>1A</sub> receptor inhibits the Ca<sup>2+</sup> channel and activates a ligand-dependent potassium (K<sup>+</sup>) channel [85]. 5-HT<sub>1A</sub> receptors are associated to GPCR and they modulate neurotransmission through K<sup>+</sup> and Ca<sup>2+</sup> channels. Moreover, the 5-HT<sub>1</sub> receptors were classified into five subtypes (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub> and 5-HT<sub>1F</sub>), which are located in different areas of the brain at pre- and post-synaptic level [86]. It was demonstrated that the main neuroprotective effects of CBD are related to the 5-HT<sub>1A</sub> receptor. Russo et al. demonstrated that CBD while showing a low-affinity agonism towards the 5-HT<sub>1A</sub> receptor could enhance 5-HT<sub>1A</sub>-mediated neurotransmission [59].

Mishima et al. explored the 5-HT<sub>1A</sub> receptors-mediate neuroprotective effects of CBD, in mice with middle cerebral artery (MCA) occlusion. Mice received 3 or 10 mg/kg of CBD immediately before and 3 h after occlusion. CBD, at a dose of 3 mg/kg, significantly decreased the infarct volume induced by MCA occlusion. However, treatment with CBD (3 mg/kg) plus WAY100135 (10 mg/kg; a 5-HT<sub>1A</sub> antagonist) inhibited the effects of CBD. This data suggested the involvement, at least in part, of 5-HT<sub>1A</sub> receptors in the neuroprotective effects of CBD against cerebral ischemia [87].

Gomes et al. evaluated the beneficial effects of CBD, through the facilitation of the 5-HT<sub>1A</sub> receptors, in motor-related striatal disorders, such as Parkinson's disease. For the study, catalepsy was induced in mice, using pharmacological mechanisms, in order to test the motor function disorders. Thirty minutes before receiving the drugs that induce catalepsy, mice were treated with CBD 5, 15, 30 or 60 mg/kg (i.p.). The pretreatment with CBD attenuated the cataleptic effects, in a dose-dependent manner. To explain the mechanism of action by which this phytocannabinoid exerts its anticataleptic action, mice were treated intraperitoneally with WAY100635 (0.1 mg/kg), a 5-HT<sub>1A</sub> receptor antagonist, 30 min before of the treatment with CBD (30 mg/kg). The administration of WAY100635 prevented the anticataleptic effect of CBD. Therefore, CBD exerting its anticataleptic action, through a mechanism that involves 5-HT<sub>1A</sub> receptors, could be a possible therapeutic tool in Parkinson's disease [88]. Moreover, Sonogo et al. evaluated the 5-HT<sub>1A</sub> receptors-mediated anticataleptic effect of CBD. In this study, the researchers induced catalepsy, in male Swiss mice, with haloperidol (0.6 mg/kg). The pre-treatment with CBD (15–60 mg/kg) i.p., prevented the catalepsy. To understand the mechanism of action, it was demonstrated that administration i.p. of WAY100635 (0.1 mg/kg) reduced the anticataleptic effect of CBD and its action on the expression of c-FOS. Moreover, it was shown that the administration of bilateral injections of CBD (60 nmol) into the dorsal striatum, followed by treatment with haloperidol (0.6 mg/kg), reduced the catalepsy, in a similar way to systemic administration. These data suggest that this compound, via activation of 5-HT<sub>1A</sub> receptor, could represent a therapeutic opportunity for the treatment of striatal disorders such as Parkinson's disease [89].

Instead, Pelz et al. evaluated the role of the 5-HT<sub>1A</sub> receptors in the anticonvulsant effect of CBD. In this study, to induce the experimental model of generalized seizure, male Wistar Kyoto rats were given a single i.p. injection of 85 mg/kg pentylenetetrazole (PTZ), to induce seizures. Mice received CBD at a dose of 100 mg/kg 60 min before induction of seizures. The results show that CBD significantly reduced the proconvulsant activity induced by PTZ. In particular, CBD significantly reduced seizure severity and the number of animals exhibiting seizure activity and prevented the severe consequences of seizures. Serotonergic signaling is known to be involved in seizure susceptibility and CBD shows a binding affinity for both 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>. To test the mechanism of action used by CBD to exert these anticonvulsant effects, mice were pretreated with WAY 100635 (1 mg/kg), a 5-HT<sub>1A</sub> antagonist, or MDL-100907 (0.3 mg/kg), a 5-HT<sub>2A</sub> antagonist. Contrary to what might be expected, pretreatment with WAY 100635 and MDL-100907 did not reduce the anticonvulsant effect of CBD. However, this study does not prove that CBD exerted its effects through the 5-HT<sub>1A</sub> or 5-HT<sub>2A</sub> receptors [90].



CBD has anxiolytic and analgesic effects and it is known that, at least in part, the anxiolytic effects of CBD depend on the activation of 5-HT<sub>1A</sub> mediated neurotransmission. De Gregorio et al. evaluated these effects using rats subjected to the spared nerve injury for 24 days to induce a neuropathic pain. The animals were treated acutely with increasing intravenous (i.v.) CBD doses (0.1–1.0 mg/kg). As CBD is poorly soluble in water, it was prepared in a vehicle of ethanol/Tween 80/0.9% saline (3:1:16). Acute CBD treatment reduced the activating activity of 5-HT neurons in the dorsal raphe nucleus. Additionally, to simulate the drug regimen used by patients using CBD to treat chronic neuropathic pain and anxiety, the animals were treated with subcutaneous injections of CBD (5 mg/kg) for seven days. Treatment with CBD for one week decreased mechanical allodynia and anxiety-like behavior, which increased following the spared nerve injury. Furthermore, CBD normalized the activity of 5-HT neurons in the dorsal raphe nucleus. To investigate the mechanism used by CBD, the animals were subjected to single injections of WAY 100635 (0.3 mg/kg; i.v.), 5-HT<sub>1A</sub> antagonist, capsazepine (1 mg/kg; i.v.), a TRPV1 antagonist and AM 251 (1 mg/kg; i.v.), to CB1 receptor antagonist. Capsazepine treatment completely reduced the antiallodynic effect of CBD, while WAY100635 reduced this effect partially. Instead, the anxiolytic effect of CBD was blocked following treatment with WAY100635. Therefore, it is possible to conclude that treatment with CBD in low doses protects 5-HT neurotransmission, exerts antiallodynic effects through the activation of TRPV1 and anxiolytic properties through the activation of 5-HT<sub>1A</sub> receptors. In this way, CBD results as a possible candidate for treating neuropathic pain and behavior disorders [91].

Instead, Magen et al. evaluated the 5-HT<sub>1A</sub> receptors-mediated therapeutic effects of CBD on hepatic encephalopathy induced by bile duct ligation in a model of chronic liver disease. Mice subjected to bile-duct ligation were treated with CBD (5 mg/kg) administrated i.p. every day for 28 days. CBD treatment improved cognitive impairments and motor function. After four weeks of treatment, CBD induced a reduction of expression of the TNF- $\alpha$ -receptor 1 gene in the hippocampus. Conversely, increased the expression of the BDNF gene. To verify other mechanisms of action used by CBD to exert these beneficial effects, the animals were co-treated with WAY 100635. Co-administration of CBD and WAY 100635 reversed the effects of CBD, confirming the involvement of the 5-HT<sub>1A</sub> receptors [83]. The same positive effects of CBD in the cognitive and locomotor deficits were observed in another model of hepatic encephalopathy induced by injection of thioacetamide. One day after thioacetamide-administration, the animals were given a single dose of 5 mg/kg i.p. Treatment with CBD improved neurological and motor function, assessed, respectively, two and three days after the induction of liver damage. While eight days after the induction of hepatic insufficiency, CBD significantly ameliorated cognitive deficits, impaired following thioacetamide. However, 12 days after treatment with thioacetamide, CBD normalized the 5-HT levels in the brain and induced an improvement in liver function. In this way, the chronic treatment with CBD, through the indirect activation of the 5-HT<sub>1A</sub> receptors, improved the cognitive and motor function of the rats with hepatic encephalopathy [92].

In conclusion, the findings of these studies suggest that the neuroprotective effects of CBD are mediated, at least in part, by 5-HT<sub>1A</sub> receptors. In this way, CBD protects against cerebral ischemia and ameliorates the motor-related striatal damage in experimental models. In the same way, it improved the cognitive and motor function in an in vivo model of hepatic encephalopathy. Moreover, CBD exhibited anxiolytic properties through the activation of 5-HT<sub>1A</sub> receptors, in experimental models of anxiety-like behavior disorders (Table 2).

**Table 2.** Neuroprotective effects of CBD in different neurological diseases through the activation of the 5-HT<sub>1A</sub>.

In Vivo Models	CBD Dose	Treatments	Biological/Pharmacological Effect	Neurological Diseases	Ref.
MCA occlusion male mice	3 or 10 mg/kg	Before and 3 h after damage	CBD, at dose of 3 mg/kg, significantly reduced the infarct volume induced by MCA occlusion, at least in part, through the 5-HT <sub>1A</sub> receptor.	cerebral ischemia	[87]
Male Swiss mice	5, 15, 30 or 60 mg/kg	30 min before receiving the drugs that induce catalepsy	Pretreatment with CBD reduced the cataleptic effects, in a dose-dependent manner, through the 5-HT <sub>1A</sub> receptor.	striatal disorders	[88]
Male Swiss mice	15–60 mg/kg or 60 nmol	30 min before or 2.5 h after receiving the drugs that induce catalepsy	Pretreatment with CBD reduced the cataleptic effects, in a dose-dependent manner, through the 5-HT <sub>1A</sub> receptor.	striatal disorders	[89]
Male Wistar Kyoto rats	100 mg/kg	60 min before induction of seizures	CBD significantly mitigated PTZ-induced seizure.	seizure disorders	[90]
Adult male Wistar rats	0.1–1.0 mg/kg and 5 mg/kg	Acute treatment with cumulative injections of CBD every 5 min and repeated treatment with 5 mg/kg/day for 7 days	CBD (5 mg/kg) protects nerve injury-induced deficits in dorsal raphe nucleus 5-HT neuronal activity. Moreover, CBD exerts antiallodynic effects through the activation of TRPV1 and anxiolytic properties through the activation of 5-HT <sub>1A</sub> receptors.	allodynia and anxiety-like behavior	[91]
Female Sabra mice	5 mg/kg	28 days	CBD, through the 5-HT <sub>1A</sub> receptor activation, improved cognition and motor function, which were impaired by bile-duct ligation. Moreover, in the animal model of hepatic encephalopathy, CBD also reduced neuroinflammation, increasing expression of the BDNF genes and reducing TNF- $\alpha$ receptor 1 gene expression.	hepatic encephalopathy	[83]
Female Sabra mice	5 mg/kg	Single dose	CBD ameliorated cognitive impairments and locomotor activity. Moreover, CBD restored the 5-HT levels in the brain and improved the liver function.	hepatic encephalopathy	[92]

CBD, cannabidiol; MCA, middle cerebral artery; 5-HT<sub>1A</sub>, serotonin 5-hydroxytryptamine<sub>1A</sub>; PTZ, pentylenetetrazole; BDNF, brain-derived neurotrophic factor; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

### 6.1.3. GPR55

The CBD also exhibits a high affinity towards the G-protein-coupled receptor 55 (GPR55), a class of receptors implicated in the synaptic transmission. GPR55 is a G protein-coupled receptor widely expressed in the immune and nervous systems. GPR55 is involved in the modulation of parameters and cell processes such as blood pressure and bone density modulation, cell migration and proliferation, inflammation, neuropathic pain, energy balance and antiepileptic action [93]. GPR55 is a seven-transmembrane receptor that acts as a G protein inducing the intracellular increase of  $Ca^{2+}$  and the phosphorylation of the extracellular receptor-activated kinases (ERK) protein, which in turn is involved in proliferation, differentiation and cytoskeletal modulation [93]. GPR55 was found at the post-synaptic level in endothelial cells and at the pre-synaptic level in the hippocampus where it appears to increase the release of vesicular glutamate [94,95]. The facilitating effect of GPR55 contrasts the action of CB1 receptors, inhibiting neurotransmitter release. On the contrary, CBD can suppress GPR55 activation, thus increasing the release of neurotransmitters [96]. This mechanism, at least in part, elucidates the anti-convulsive effect of cannabinoids towards epileptic pharmaco-resistant patients [97]. In this regard, Kaplan et al. evaluated the GPR55-mediated antiepileptic properties of CBD in a mouse model of genetically-induced Dravet syndrome (DS). CBD (100 mg/kg or 200 mg/kg) was administered intraperitoneally, twice daily for one week. The acute treatment of CBD, in a manner dependent on its concentration, reduced the thermally-induced seizures and significantly decreased the rate of spontaneous seizures. Additionally, CBD treatment ameliorated hyperactivity due to disease. Moreover, the mouse model of genetically-induced DS showed a decrease in the GABA inhibitory transmission. The CBD treatment restored the excitability of neurons inhibitors in the dentate gyrus of the hippocampus, which represents an important zone for convulsions propagation. To confirm the involvement of GPR55 in anticonvulsant effects of CBD, mice were treated with a CID16020046 (10  $\mu$ M), a GPR55 antagonist. The treatment with CID16020046 abolished the beneficial effects of CBD in inhibitory. Thus, this result suggests that the therapeutic effects of CBD are mediated through GPR55. Therefore, GPR55 could be an important therapeutic target for the treatment of epilepsy [98].

GPR55 can be involved in motor function. Celorrio et al. studied the effects of CBD and abnormal-CBD on the modulation of GPR55, in an experimental model of Parkinson's disease. To induce Parkinson's model, adult male C57BL/6 mice were treated, for five weeks, with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg) and probenecid (250 mg/kg), a drug capable of reducing the renal disposal capacity of MPTP and its metabolites. MPTP mice were treated with CBD (5 mg/kg) and abnormal-CBD (5 mg/kg), administered chronically for five weeks. Abnormal-CBD is a synthetic CBD isomer that owns a high affinity towards GPR55. The study demonstrated that abnormal-CBD prevented the motor deficits MPTP-induced, while CBD did not produce a significant effect in motor behavior tests. However, both CBD and abnormal-CBD induced morphological changes in the microglia, probably due to an anti-inflammatory response. The anti-parkinsonian effect of abnormal-CBD was also confirmed in cataleptic mice induced by i.p. injection of haloperidol (1 mg/kg). Instead, the administration of CBD (5 mg/kg) did not show anti-cataleptic effects but rather abolished the action of abnormal-CBD. To confirm the involvement of GPR55 in the results obtained, it was also shown that the treatment with PSB1216 (10 mg/kg), a GPR55 antagonist, abolished the effect of abnormal-CBD. Conversely, the treatment with GPR55 agonists such as CID1792197 and CID2440433, similarly to abnormal-CBD, showed anti-cataleptic effects. Therefore, the study demonstrated that compounds able to activate GPR55 could be beneficial in combating PD [99].

The anti-inflammatory effects of CBD mediated by GPR55 were also tested by González-García et al. in experimental autoimmune encephalomyelitis (EAE) mice, a model of multiple sclerosis. The experimental model was induced in female C57BL/6J mice through i.p. injection of encephalitogenic cells cultured with Myelin Oligodendrocyte Glycoprotein peptide 35–55 (25  $\mu$ g/mL) and interleukin-12 (25 ng). After the induction of the model, animals were treated with CBD (5–10 mg/kg) or CBD (50 mg/kg) i.p. The study showed an improvement in the disease already at low doses of CBD (5 mg/kg).

In addition, it was shown an improvement of the disease with no signs of toxicity also at the high doses of CBD (50 mg/kg). EAE induction caused a significant reduction in the levels of CB1 and the levels were not restored by CBD treatment. On the contrary, CB2 was found in scarce levels in healthy control mice, whereas its expression was significantly increased in the EAE mice. CBD induced a significant reduction of CB2 expression in the treated animals. In the same way, the high level of EAE-induced GPR55 was reduced after CBD treatment. Although the role of GPR55 in EAE is not fully understood, its function for the disease can be very important. Indeed, mice with genetic deletion of GPR55 showed a less severe form of EAE. Therefore, the action of CBD as an antagonist of the GPR55 receptor could be useful to counteract the disease [100].

In conclusion, via the antagonist action of the GPR55 receptor, CBD carries out its anti-inflammatory effects in experimental models of DS, Parkinson's disease and EAE disease (Table 3).

## 6.2. TRP

TRP is a family of ion channels mainly located on the plasma membrane of many animal cells. CBD could interact with TRP, thus modulating the inflammation [101]. Interestingly, CBD is a potent and selective agonist of TRPV1, a TRP channel from the vanilloid subfamily. TRPV1 is a non-selective cationic channel present on sensory tissues such as skin, lungs, heart and blood vessels. TRPV1 activation induces the release of neuropeptides involved in pain perception, neuroinflammation, and regulation of body temperature [102]. Indeed, this receptor coupled to G proteins is characterized by six transmembrane domains which can be activated by capsaicin and stimuli such as a low pH, heat (>43 °C) and phytocannabinoids [101]. TRPV1 receptors are mainly expressed in the ganglia of dorsal roots in the spinal cord, while in the CNS they located in the hypothalamus and hippocampus [103]. It is known that TRPV1 antagonists possess analgesic properties. However, the activation of TRPV1 receptors by some agonists, such as capsaicin, leads to the entry of  $\text{Ca}^{2+}$  and  $\text{Na}^{+}$  with consequent desensitization of the channel [104]. Consequently, the increase of  $\text{Ca}^{2+}$  induced by capsaicin activates the calcineurin protein which dephosphorylates TRPV1 and other proteins in the voltage-gated  $\text{Ca}^{2+}$  channels also involved in the nociceptive transmission. Thus, the receptor desensitization in response to capsaicin can render the TRPV1 channel insensitive to further painful stimuli [105]. In the same way, CBD agonist action towards TRPV1 induces the desensitization of these channels [106,107]. Therefore, at least in part, the anti-nociceptive and antihyperalgesic actions of CBD appear to be mediated by activation, dephosphorization and strong desensitization of TRPV1 channels [108].

Costa et al. showed the effects of CBD and the mechanisms associated with antihyperalgesic action in a mouse model of acute inflammation. CBD (10 mg/kg) administered orally 2 h after the induction of the model abolished the thermal hyperalgesia induced by carrageenan (0.1 mL). The CBD co-administration with capsazepine (2 mg/kg), a synthetic capsaicin antagonist, suppressed the carrageenan-induced hyperalgesia. Capsazepine at the higher dose (10 mg/kg) inhibited the CBD-induced antihyperalgesic action. These data suggest how CBD can exert its antihyperalgesic effect by directly involving TRPV1. Therefore, CBD could represent a valid therapeutical tool in the treatment of pathological conditions such as neuropathy [31].

Moreover, CBD is also able to activate TRPV2, transient receptor potential ankyrin 1 (TRPA1) and antagonize also the transient receptor potential cation channel subfamily M member 8 (TRPM8) [101]. CBD through TRP channels, involved in the proliferation and release of proinflammatory cytokines, can regulate  $\text{Ca}^{2+}$  in immune and inflammatory cells [24].

**Table 3.** Neuroprotective effects of CBD in different neurological diseases through the antagonize activation of the GPR55.

In Vitro and in Vivo Models	CBD Dose	Treatments	Biological/Pharmacological Effect	Neurological Diseases	Ref.
<i>Scn1a</i> mutant mice	10, 20, 100 or 200 mg/kg	Twice daily for 1 week	Acute treatment of CBD decreased thermally-induced seizures and reduced the rate of spontaneous seizures. Moreover, the low doses of CBD ameliorated the autism-type social interaction deficits in the mouse model of genetically-induced DS. CBD also increased the GABA inhibitory transmission which was impaired in DS. These therapeutic effects of CBD are mediated through GPR55.	DS	[98]
Adult male C57BL/6 mice	5 mg/kg	5 days a week for 5 weeks	Abnormal-CBD, but not CBD, ameliorated MPTP-induced motor damage. Instead, both compounds significantly reduced the density of microglial cells in the cell body. In the haloperidol-induced catalepsy mouse model, abnormal-CBD also showed anti-cataleptic effects, through the GPR55-activation.	Parkinson's disease	[99]
Male and female C57BL/6 mice	5–10 and 50 mg/kg	Increasing doses from 5 to 10 mg/kg three times per week, or daily, at a dose of 50 mg/kg, for 23 days	CBD, both at low and high doses, ameliorated the EAE disease. Moreover, CBD treatment reduced the vitality of encephalitogenic cells, levels of IL-6, production of ROS with consequent decrease of the apoptosis process. Additionally, it decreased the levels of GPR55 receptors in the CNS.	EAE disease	[100]

CBD, cannabidiol; DS, Dravet syndrome; GABA,  $\gamma$ -aminobutyric acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; EAE, experimental autoimmune encephalomyelitis; ROS, reactive oxygen species; CNS, central nervous system.

The effects of CBD and its affinity for the TRPV2 receptors were also demonstrated by Luo et al. in human brain endothelial cells forming the BBB. CBD promoted a long-lasting increase of intracellular  $\text{Ca}^{2+}$  level, especially at 15  $\mu\text{M}$  dose. After 24 h of incubation, CBD treatment (0.1, 0.3, 1, 3 and 10  $\mu\text{M}$ ), in a dose-dependent manner, induced the cell growth of hCMEC/D3 cells, and, after 4 h, it significantly enhanced cell migration. Instead, after 7 or 24 h, CBD significantly increased the tubulogenesis of hCMEC/D3 cells. Additionally, after 72 h to seeding, treatment with 1  $\mu\text{M}$  CBD increased trans-endothelial resistance in human Primary Brain Microvascular Endothelial Cells (hPBMECs) Monolayers. To demonstrate the possible involvement of TRPV2 in CBD-induced effect, cells were pretreated, 5 min before added CBD, with ruthenium red, a nonspecific TRP antagonist, or with tranilast, a selective TRPV2 inhibitor. Ruthenium red and tranilast suppressed the CBD-induced long-lasting increase of intracellular  $\text{Ca}^{2+}$  levels. The same results were also obtained in silencing cells with TRPV2 siRNA. These data highlight the role of TRPV2 in the CBD mechanism of action. Therefore, CBD, due to the high affinity of TRPV2, could be a potential pharmacological tool to regulate the BBB characteristic [109].

Nabissi et al. evaluated the role of CBD to contrast the proliferation of glioblastoma, through activation of TRPV2. In this study, the authors showed that CBD (10  $\mu\text{M}$ ) improved the action of cytotoxic agents able to contrast the proliferation of glioblastoma. CBD, through activation of TRPV2 and the consequent entry of  $\text{Ca}^{2+}$ , improved the action of chemotherapy drugs. To confirm the agonize effect of CBD on TRPV2, it was performed deletion of the TRPV2 poredomain. Since the poredomain of the TRP channels is important for  $\text{Ca}^{2+}$  entry, the authors demonstrated that the deletion of this region prevents CBD-induced influx of  $\text{Ca}^{2+}$  by reducing drug absorption and cytotoxic effects. Therefore, with this result, it has been shown that CBD co-administered together with chemotherapeutic agents, activating TRPV2, enhances drug absorption and cytotoxic activity in human glioma cells. Consequently, the administration of chemotherapy drugs together with CBD could improve the efficacy of therapy useful to counteracting the glioma cells [110].

Moreover, CBD, through the activation of the TRPV, is able to promote  $\text{PI}_3\text{K}/\text{Akt}$  signaling, which in turn inhibits the glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ). The GSK-3 $\beta$  inhibition induces an increase of the Wnt/ $\beta$ -catenin pathway, thus exerting a neuroprotective action against the oxidative stress and neurotoxicity induces of  $\text{A}\beta$  in the Alzheimer's disease [54]. In line with this evidence in an in vitro model of Alzheimer's disease, CBD treatment suppressed the hyperphosphorylation of tau protein-mediated to  $\beta$ -catenin and GSK-3 $\beta$ , in  $\text{A}\beta$ -stimulated PC12 neuronal cells [111]. Moreover, CBD decreased  $\text{A}\beta$  levels in SH-SY5Y cells transfected with the amyloid precursor protein (SH-SY5Y<sup>APP+</sup>) [64], and, in an Alzheimer's disease mouse model, CBD administration ameliorated cognitive impairment [112]. In this context, our research group, in a previous study, investigated the involvement of TRPV2 in the molecular CBD's mechanism of action, comparing the expression profiles of human gingival mesenchymal stem cells treated with CBD (5  $\mu\text{M}$ ) to those without treatment. The results of the transcriptomic analysis show that CBD decreased the expression of genes related to Alzheimer's disease. Conversely, CBD upregulated genes coding for the  $\text{PI}_3\text{K}$  subunits and for AKT1. Thus, CBD, through modulation of  $\text{PI}_3\text{K}/\text{Akt}$  signaling, is capable of regulating GSK3 $\beta$  activity and consequently improve hallmarks of Alzheimer's disease. To study how CBD modulated the  $\text{PI}_3\text{K}/\text{Akt}$  signaling, human gingival mesenchymal stem cells were treated with antagonists for CB1R (SR141716A), CB2R (AM630), or TRPV1 (capsazepine) receptors. Noteworthy, only the pretreatment with capsazepine reversed the CBD-mediated effects. Therefore, CBD, by TRPV1 activation, promoted the  $\text{PI}_3\text{K}/\text{Akt}$  pathway that inactivates GSK3 $\beta$ . Thus, CBD could reduce Alzheimer's hallmarks [37].

The results of the studies show that CBD activates and rapidly desensitize TRPV1, inducing antihyperalgesic effects. Moreover, TRPV1 activation induced the  $\text{PI}_3\text{K}/\text{Akt}$  pathway signaling, which can reduce Alzheimer's hallmarks. Instead, CBD, through activation of TRPV2, enhanced cell proliferation and improved the action of chemotherapy drugs (Table 4).



**Table 4.** Neuroprotective effects of CBD in different neurological diseases through the activation of the TRPV receptors.

In Vitro and in Vivo Models	CBD Dose	Treatments	Biological/Pharmacological Effect	Neurological Diseases	Ref.
Male Wistar rats	10 mg/kg	2 h after the induction of model	CBD inhibited the carrageenan-induced hyperalgesia through the desensitization of the TRPV1 receptor	Hyperalgesia	[31]
hPBMECs and hCMEC/D3 Cells	0.1, 0.3, 1, 3, 10 and 15 $\mu$ M	7 or 24 h of incubation	CBD, in a dose-dependent manner, led a last-lasting increase in intracellular $Ca^{2+}$ level, through activation of TRPV2. In this way, CBD, enhanced cell proliferation, cell migration and tubulogenesis in human brain endothelial cells.	-	[109]
U87MG glioma cell line	10 $\mu$ M	Cells were treated with different doses of CBD for 1 day or co-treated with CBD 10 $\mu$ M and chemotherapeutic drugs for 6 h.	CBD, through activation of TRPV2 and the consequent entry of $Ca^{2+}$ , improved the action of chemotherapy drugs enhancing drug absorption and ameliorated cytotoxic activity in human glioma cells.	-	[110]
human Gingival Mesenchymal Stem Cells	5 $\mu$ M	24 h of incubation	CBD, through TRPV1 desensitization, promoted the $PI_3K/Akt$ pathway signaling, which can reduce Alzheimer's hallmarks.	Alzheimer's disease	[37]

CBD, cannabidiol; hPBMECs, human primary brain microvascular endothelial cell.

### 6.3. PPAR $\gamma$ Receptors

PPAR $\gamma$  represents a member of the nuclear receptor family and is also a transcription factor modulated by a ligand that regulates the expression levels of genes involved in inflammation, trophic factors production, redox equilibrium, metabolism of glucose and lipids [24,113,114]. PPAR $\gamma$  is also a ubiquitin E3 ligase consisting of several residues of lysine, including Lysine48 capable of generating polyubiquitin chains [115]. The polyubiquitin linked to Lysine48 of PPAR $\gamma$  is responsible for proteasomal degradation of p65, which in turn leads to the inhibition of the inflammatory pathway mediated by NF- $\kappa$ B. Indeed, the activation of PPAR $\gamma$  inhibits the transcription of proinflammatory genes, cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6, thus preventing the NF- $\kappa$ B signaling pathway [35]. Therefore, PPAR $\gamma$  agonists such as CBD, inhibiting the transcription of downstream genes mediated by NF- $\kappa$ B can perform an anti-inflammatory action through a molecular mechanism regulated by GSK3 $\beta$  [116].

This mechanism of action was evaluated by Scuderi et al. in SH-SY5Y<sup>APP+</sup> cells, an in vitro model of Alzheimer's disease. SH-SY5Y<sup>APP+</sup> cells were treated with CBD ( $10^{-9}$ – $10^{-6}$  M) for 24 h. Treatment with CBD reduced the expression of the APP protein, as well as its ubiquitination, thus leading to the reduction of A $\beta$  and neuronal apoptosis. To demonstrate the selective involvement of PPAR $\gamma$  in mediating CBD activity in SH-SY5Y<sup>APP+</sup> cells, CBD was co-administered with MK886 (3  $\mu$ M) or GW9662 (9 nM), selective antagonists of PPAR $\alpha$  and PPAR $\gamma$ , respectively. The results show that treatment with GW9662 (9 nM) led to the almost complete lack of efficacy of CBD, thus confirming that, the neuroprotective role of this phytocannabinoid, involved the PPAR $\gamma$  receptors [64]. The neuroprotective effect of CBD through the activation of PPAR $\gamma$  was observed in an experimental study of Alzheimer's disease. Esposito et al. showed both in vitro and in vivo the properties of PPAR $\gamma$  agonists and non-agonists on neurotoxicity induced by A $\beta$ . Cultures primary of rat astrocytes were induced with A $\beta$  (1  $\mu$ g/mL). The treatment with CBD ( $10^{-9}$ – $10^{-7}$  M), in a concentration-dependent manner, reduced the effect of A $\beta$  mediated through the inhibition of NF- $\kappa$ B. On the contrary, the treatment with GW9662 (9 nM), a PPAR $\gamma$  antagonist, was shown to reverse the anti-inflammatory effect of CBD. To confirm the results obtained with CBD in reactive gliosis, Esposito et al. performed the study in vivo. Male Sprague-Dawley rats were induced with A $\beta$  (10  $\mu$ g/mL). After the induction of the Alzheimer's disease model, the animals were treated for 15 days with CBD (10 mg/kg; i.p.). The data obtained show that CBD preserved from the neuronal damage induced by A $\beta$  and also led to a reduction of gliosis and glial fibrillary acidic protein. Conversely, the administration of GW9662 (10 mg/kg), the antagonist PPAR $\gamma$ , has completely reversed the neuroprotective effects of CBD. Therefore, the results obtained both in vitro and in vivo confirm the important role of PPAR $\gamma$  in mediating the neuroprotective actions of CBD in experimental models of Alzheimer's disease [17]. The role of PPAR $\gamma$  in neuroprotective effects of CBD was also demonstrated by Hughes et al. The hippocampal slices of C57/black 6 mice induced with soluble oligomeric A $\beta_{1-42}$  were treated with CBD 30 min before to the addition of A $\beta_{1-42}$ . The treatment with CBD improved the synaptic transmission and the potentiation long-term in the hippocampus, thereby preserving it from cognitive deficits induced by A $\beta_{1-42}$ . To understand the mechanism of action of CBD, WAY 100635, (300 nM; 5-HT<sub>1A</sub> antagonists), ZM241385 (100 nM; A<sub>2A</sub>R antagonist), AM 251 (2  $\mu$ M; CB1 inverse agonist) or GW9662 (2  $\mu$ M; PPAR $\gamma$  antagonist) was added to the perfusate 30 min before of CBD. It was shown that only the treatment with GW9662 attenuated the neuroprotective effects of CBD. In addition, this study suggested that CBD, at least in part, through interaction with PPAR $\gamma$ , could be a therapeutic potential for the treatment of Alzheimer's disease [117].

The anti-inflammatory effect of CBD was also shown in tardive dyskinesia, a disease characterized by chronic use of drugs capable of reducing or blocking the dopaminergic neurotransmission, which in turn induces the abnormal and repetitive involuntary movements that mainly involve the orofacial region [118]. In this context, Sonogo et al. investigated the PPAR $\gamma$ -mediated protective effect of CBD in vivo and in vitro models of dyskinesia haloperidol-induced. Swiss mice were received two daily i.p. injections of CBD (60 mg/kg) for 21 days and 30 min later were treated with haloperidol (2 and 3 mg/kg). The behavioral analysis showed that CBD treatment prevented dyskinesia induced by haloperidol and reduced the oxidative stress and activation of microglial and inflammatory cytokine (IL-1 $\beta$  and

TNF- $\alpha$ ) in the corpus striatum. Moreover, it was shown to increase the expression of peroxisome proliferator-activated receptor- $\gamma$  coactivator 1- $\alpha$ , a co-activator of PPAR $\gamma$ , thereby confirming PPAR $\gamma$  as a molecular target of CBD. To confirm the involvement of PPAR $\gamma$  in the effect of CBD, animals received the injection of GW9662 (2 mg/kg), 30 min after CBD. The administration of GW9662, inhibited the positive effect of CBD on dyskinesia. Moreover, to confirm the involvement of PPAR $\gamma$ , the authors also performed an in vitro study. Primary microglial cells were pretreated with GW9662 (0.1, 1 and 10  $\mu$ M), 30 min after treatment with CBD (10  $\mu$ M) and 4 h after the cells were stimulated with lipopolysaccharide (10 ng/mL). The in vitro study confirmed the results obtained in vivo, showing the involvement of PPAR $\gamma$  in the neuroprotective effects of CBD [119].

Instead, Dos-Santos-Pereira et. evaluated the PPAR $\gamma$ -mediated effects of CBD on L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia, a mouse model of Parkinson's disease. Male C57/BL6 mice were treated with 6-hydroxydopamine (6-OHDA) neurotoxin, which induces hallmarks of Parkinson's disease. To induce dyskinesia, 6-OHDA-lesioned animals were treated with L-DOPA for 21 days. Subsequently, 15 min before L-DOPA administration, mice received CBD (15, 30 and 60 mg/kg) i.p. for three days. CBD alone was not able to prevent the L-DOPA-induced dyskinesia. To understand the CBD's mechanism of action, it was co-administrated with capsazepine (1 or 5 mg/kg), a TRPV-1 antagonist, or with arachidonoyl-serotonin (AA-5-HT; 5 mg/kg), an enzyme responsible for inhibiting anandamide metabolism FAAH and TRPV-1. Co-administration with CBD and capsazepine, through an increase in AEA and an antagonism of TRPV1 receptors, leads to the reduction of dyskinesia. Additionally, co-treatment of CBD with AM 251 (1 mg/kg), a CB1 antagonist, or with GW9662 (4 mg/kg), an antagonist of PPAR $\gamma$  receptors, reversed the anti-dyskinetic effect of CBD and capsazepine. In conclusion, CBD together with capsazepine, through the interaction with CB1 and PPAR $\gamma$  receptors, could be a valid therapeutic strategy to prevent the L-DOPA-induced dyskinesia in patients with Parkinson's disease [120]. Hind et al. also demonstrated the role of PPAR $\gamma$  in mediating the neuroprotective effects of CBD in experimental models of ischemic damage. To induce ischemic damage, the cells were simulated using oxygen-glucose deprivation. CBD treatment (100 nM, 1  $\mu$ M and 10  $\mu$ M) was administered either before or immediately after the induction of ischemic damage. CBD (10  $\mu$ M) reduced the increase of BBB permeability following the ischemic damage. To assess the CBD's mechanism of action, cells were treated with AM 251 (100 nM; CB1 receptors antagonists), AM630 (100 nM; CB2 receptors antagonist), capsazepine (1  $\mu$ M; TRPV1 channels antagonists), GW9662 (100 nM; PPAR $\gamma$  antagonist), SCH58261 (100 nM; A<sub>2A</sub>Rs antagonist) and WAY100135 (300 nM; 5-HT<sub>1A</sub> receptors antagonist). The neuroprotective effect of CBD was abolished by the administration of GW9662 (100 nM) and partially reduced by WAY100135 (300 nM). To confirm the involvement of PPAR $\gamma$  and 5-HT<sub>1A</sub> receptors underlying the neuroprotective effect of CBD, cells were also treated with pioglitazone (a PPAR $\gamma$  agonist) and 8-OH-DPAT (an agonist of 5-HT<sub>1A</sub> receptors). The treatment with these receptors showed similar effects of CBD on the permeability induced by ischemic damage. Therefore, the study reported useful results on the neuroprotective effect of CBD in the permeability of the BBB, through the activation of PPAR $\gamma$  and 5-HT<sub>1A</sub> [121].

In addition, in an experimental model of multiple sclerosis, PPAR $\gamma$  exerted an important role in mediating the effects of CBD. Giacoppo et al. showed the effects of CBD in C57BL/6 mice with EAE immunized with Myelin Oligodendrocyte Glycoprotein peptide 35–55 (300  $\mu$ g). Fourteen days after the induction of the EAE model, the mice were treated daily with CBD (10 mg/kg) i.p. The results of the treatment demonstrate that CBD restored the PI<sub>3</sub>K/Akt/mTOR pathway, which was downregulated after EAE induction. CBD treatment has also led to the reduction of inflammatory cytokines interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin 17 (IL-17) and significantly increased the levels of PPAR $\gamma$ . These results suggest that, at least in part, the effects of CBD could be related to the increased level of PPAR $\gamma$ . In conclusion, CBD, through both enhanced PPAR $\gamma$  and modulation of the PI<sub>3</sub>K/Akt/mTOR pathway, could be an interesting therapeutic target for multiple sclerosis [122].

In conclusion, the data obtained from these studies underline the implication of PPAR $\gamma$  in the anti-inflammatory effects of CBD, in experimental models of Alzheimer's disease, Ischemic stroke, Parkinson's disease and EAE disease (Table 5).

**Table 5.** Neuroprotective effects of CBD in different neurological diseases through the activation of the PPAR $\gamma$ .

In Vitro and in Vivo Models	CBD Dose	Treatments	Biological/Pharmacological Effect	Neurological Diseases	Ref.
SH-SY5Y <sup>APP+</sup>	10 <sup>-9</sup> –10 <sup>-6</sup> M	24 h	CBD reduced the expression of the APP protein, as well as its ubiquitination, thus leading to the reduction of A $\beta$ and neuronal apoptosis. These CBD's effects were mediated by PPAR $\gamma$ activation.	Alzheimer's disease	[64]
Cultures primary of astrocytes rat and male Sprague-Dawley rats	10 <sup>-9</sup> –10 <sup>-7</sup> M for in vitro study; 10 mg/kg, for in vivo study.	Daily for 15 days	In the in vitro study, CBD in a concentration-dependent manner reduced the effect of A $\beta$ mediated through the inhibition of NF- $\kappa$ B. In addition, in vivo, CBD ameliorated neuronal damage induced by A $\beta$ and led to a reduction of gliosis and glial fibrillary acidic protein. CBD exerts these effects through PPAR $\gamma$ activation.	Alzheimer's disease	[17]
Hippocampal slices from C57Bl/6 mice	10 $\mu$ M	30 min before to the addition of A $\beta$	The treatment with CBD improved the synaptic transmission and the potentiation long-term in the hippocampus slice of C57/black 6 mice, thereby preserving it from cognitive deficits induced by A $\beta$ <sub>1-42</sub> . CBD exerts these effects, at least in part, through interaction with PPAR $\gamma$ .	Alzheimer's disease	[117]
Primary microglial cultures from brain of male and female newborn C57/BL6 mice and Swiss mice	60 mg/kg; for in vivo study; 10 $\mu$ M for in vitro study	Two daily injections 30 min before received haloperidol for 21 days	In mice, CBD treatment prevented dyskinesia induced by haloperidol. Moreover, in the corpus striatum, CBD reduced oxidative stress, activation of microglial, inflammatory cytokine (such as IL-1 $\beta$ and TNF- $\alpha$ ) and increased anti-inflammatory cytokine IL-10. It was demonstrated that PPAR $\gamma$ is a molecular target of CBD. In the same way, it was also confirmed the effect of CBD through PPAR $\gamma$ on lipopolysaccharide-stimulated microglial cells.	Tardive dyskinesia	[119]
Male adult C57/BL6 mice	15, 30 and 60 mg/kg	15 min before the L-DOPA administration for three days	CBD alone was not able to prevent the L-DOPA-induced dyskinesia. The co-treatment with CBD and capsazepine, through the interaction with CB1 and PPAR $\gamma$ receptors, ameliorate dyskinesia.	Parkinson's disease	[120]
Human brain microvascular endothelial cell and human astrocyte co-cultures modeled	100 nM, 1 and 10 $\mu$ M	Either before or immediately after the induction of ischemic damage	CBD (10 $\mu$ M) prevented the enhance of BBB permeability following the ischemic damage induced by oxygen-glucose deprivation, through the activation of PPAR $\gamma$ and 5-HT <sub>1A</sub> receptors.	Ischemic stroke	[121]
Male C57BL/6 mice	10 mg/kg	Daily treated, approximately 14 days after disease induction, for 14 days	CBD treatment ameliorated the clinical evidence of disease in EAE mice. CBD restored the PI <sub>3</sub> K/Akt/mTOR pathway that was downregulated after EAE induction. Moreover, CBD reduced inflammatory cytokines IFN- $\gamma$ and IL-17 significantly and increased the levels of PPAR $\gamma$ . Probably, the anti-inflammatory effects of CBD are linked to the increased of PPAR $\gamma$ .	EAE disease	[122]

CBD, cannabidiol; SH-SY5Y<sup>APP+</sup>, SH-SY5Y cells transfected with the amyloid precursor protein; A $\beta$ , amyloid- $\beta$ ; IL-1 $\beta$ , interleukin 1- $\beta$ ; IL-6, interleukin 6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; L-DOPA, L-3,4-dihydroxyphenylalanine; BBB, blood–brain barrier; IFN- $\gamma$ , interferon- $\gamma$ ; IL-17, interleukin-17; EAE, experimental autoimmune encephalomyelitis.

#### 6.4. GABA Receptors

GABA is the principal inhibitory neurotransmitter of the CNS. This receptor binds three classes of type A receptor: GABA<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub>. Among these, the GABA<sub>A</sub> receptor subfamily appears to be involved in many neurological diseases [123–125]. Deficits of this receptor are responsible for several neurological disorders such as Huntington's disease [126], cognitive alterations [3], epileptic disorders [127], drug addiction [128], chronic stress and anxiety [129]. For these reasons, GABA<sub>A</sub> receptor represents an interesting target for new compounds. CBD is known to potentiate GABA<sub>A</sub>-mediated inhibitory currents by acting on the GABA<sub>A</sub> receptor [65]. In accordance with this evidence, our research team in a previous work suggested that CBD might be able to decrease neuronal excitability in NSC-34 motor neuron-like cells through enhancing the expression of genes linked in GABA release and increasing the GABA<sub>A</sub> receptor gene expression [130].

Recently, CBD has been shown to be a promising compound in the treatment of patients with drug-resistant DS [131–133]. In this context, Ruffolo et al. studied the effects of CBD on GABA<sub>A</sub> receptor-mediated neuronal transmission using human cortical tissue obtained from patients with DS and tuberous sclerosis complex (TSC). Cell membranes obtained from cortical tissue of patients were transplanted in *Xenopus oocytes* to perform experiments. To evaluate the effect of CBD on GABA currents, the cells were preincubated for 10 s with CBD (5  $\mu$ M) before the co-application of GABA and CBD. CBD enhanced the amplitude of the GABA-evoked current, in cortical tissue of patients with DS. A similar effect was also obtained using cortical tissues of patients with TSC. These results highlight that CBD, increasing the average amplitude of the currents evoked from GABA, could be a new therapeutic discovery in drug-resistant DS [134]. In light of this evidence, in 2018, the US Food and Drug Administration, approved CBD to treat DS and Lennox–Gastaut syndrome, two drug-resistant epileptic syndromes [135].

In the treatment of these drug-resistant epileptic syndromes, CBD is often co-administered with common antiepileptic drugs, such as clobazam (CLB). However, several clinical studies have highlighted the existence of drug–drug interaction between CBD and CLB. In these studies, it was observed that CBD causes an increase in plasma concentrations of both CLB and its active metabolite, *N*-desmethyloclobazam (N-CLB) [136–138]. Similar to CBD, CLB and N-CLB are positive allosteric modulators of GABA<sub>A</sub> receptors [139–141]. In view of these this data, Anderson et al., in vivo and in vitro, explored the pharmacodynamic and pharmacokinetic interactions of these compounds highlighted the involvement of GABA<sub>A</sub> receptors. For the study, the researchers used mice with heterozygous loss of function *SCN1A* (*Scn1a*<sup>+/-</sup>), a genetic model of DS. CBD was administrated i.p. 45 min before to receive CLB (0.1–10 mg/kg). The combined treatment of CBD and CLB resulted in an increase of anticonvulsant effect against seizures compared to the administration of the single compound. In this regard, the researchers investigated the novel pharmacodynamic mechanism where CBD and CLB together enhanced inhibitory GABA<sub>A</sub> receptor activation using *Xenopus oocytes* expressing GABA<sub>A</sub> receptors. For the in vitro study, GABA treatment (15  $\mu$ M) was performed three times with a washout period of 7–12 min between GABA applications. Subsequently, CBD (10  $\mu$ M) was co-applied with GABA, for 60 s. The results show that CBD and CLB exert their anticonvulsant action by enhancing the activity of the GABA<sub>A</sub> receptor. This pharmacological interaction between CBD and GABAergic drugs, like CLB, could explain the anti-seizures effect of CBD [142].

In another study, Aso et al. showed that chronic co-administration of  $\Delta^9$ -THC and CBD improves cognitive deficits in transgenic *A $\beta$ PP/PS1* mice, a model of Alzheimer's disease. Interestingly, the positive effects of co-administration of these two compounds may be related to a reduction in glutamate ionotropic receptors AMPA Type Subunits 2/3 and an increase in GABA<sub>A</sub> receptors. Therefore, this study further confirms the involvement of cannabinoids in excitatory and inhibitory neural activity [143]. These studies demonstrated that CBD, enhancing inhibitory GABA<sub>A</sub> receptor activation, could be an interesting approach for conditions such as epilepsy (Table 6).

**Table 6.** Neuroprotective effects of CBD in different neurological diseases through positive allosteric modulation of GABA<sub>A</sub> receptors.

In Vitro and in Vivo Models	CBD Dose	Treatments	Biological/Pharmacological Effect	Neurological Diseases	Ref.
Surgical human DS and TSC cortical tissue in <i>Xenopus oocytes</i>	5 $\mu$ M	Pre-incubation of cells of 10 s before the co-application of GABA and CBD	CBD, through positive modulation of GABA <sub>A</sub> receptors, enhanced the amplitude of the GABA-evoked current, in brain tissues of patients with DS and TSC.	DS and TSC	[134]
Male and female <i>Scn1a</i> <sup>+/-</sup> mice and <i>Xenopus oocytes</i> expressing GABA <sub>A</sub> receptors	12 mg/kg or 100 mg/kg for in vivo study; 10 $\mu$ M for in vitro study	In in vivo study, CBD was administrated i.p. 45 min before CLB; in in vitro study CBD (10 $\mu$ M) was co-applied with GABA, for 60 s	CBD significantly increased the concentrations of CLB and its active metabolite N-CLB, both in the plasma and in the brain. Co-administration of both compounds significantly increased the anticonvulsant effect. CBD and CLB exert their anticonvulsant action by enhancing the activity of the GABA <sub>A</sub> receptor.	DS	[142]

CBD, cannabidiol; GABA,  $\gamma$ -aminobutyric acid; DS, Dravet syndrome; TSC, tuberous sclerosis complex; *Scn1a*<sup>+/-</sup>, heterozygous loss of function *SCN1A*; CLB, clobazam; N-CLB, N-desmethyloclobazam.



## 7. Conclusions

The neuroprotective properties of CBD are performed via several mechanisms of action. CBD mainly exerts these effects through multiple biological targets. Specifically, CBD, through A<sub>2A</sub>R activation, exerts anti-inflammatory effects in animal models of Alzheimer's disease and multiple sclerosis. Moreover, at least in part, it exerts neuroprotective effects by the activation of 5-HT<sub>1A</sub> receptors. In experimental models of Parkinson's disease and DS, CBD performs its beneficial effects antagonizing the GPR55 receptor. Noteworthy, CBD through the TRPV1 activation could modulate PI<sub>3</sub>K/Akt signaling, thus ameliorating the Alzheimer's hallmarks. Additionally, the neuroprotective properties of CBD in Alzheimer's and Parkinson's diseases are also mediated by the interaction with PPAR $\gamma$ . Instead, CBD, enhancing the inhibitor GABA transmission, could be an interesting tool to treat epilepsy condition. The preclinical evidence reviewed here, linked with the already reported safety profile of CBD in humans, highlights that CBD represents a new opportunity for the treatment of several neurological diseases. However, further studies are needed to elucidate the molecular mechanisms underlying the properties of CBD and identify new molecular targets.

**Author Contributions:** Conceptualization, E.M. and P.B.; writing—original draft preparation, S.S. and G.S.; and writing—review and editing, E.M. and P.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by a Current Research Funds 2020, Ministry of Health, Italy.

**Acknowledgments:** The authors would like to thank the Ministry of Health, Italy.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

CBD	cannabidiol
$\Delta^9$ -THC	$\Delta^9$ -tetrahydro-cannabinol
Nrf2	nuclear factor erythroid 2 – related factor 2
SOD	superoxide dismutase
GSH	glutathione
ROS	reactive oxygen species
iNOS	inducible nitric oxide synthase
IL-6	interleukin-6
IL-1 $\beta$	interleukin-1 $\beta$
TNF- $\alpha$	tumor necrosis factor $\alpha$
NF- $\kappa$ B	nuclear factor $\kappa$ B
PPAR $\gamma$	peroxisome proliferator-activated receptor $\gamma$
TRP	transient receptor potential
TRPV	transient receptor potential vanilloid
CB1	cannabinoid receptor type 1
CB2	cannabinoid receptor type 2
2-AG	2-arachidonoyl glycerol
AEA	arachidonylethanolamide
ECS	endocannabinoid system
Ca <sup>2+</sup>	calcium
FAAH	fatty acid amide hydrolase
GABA	$\gamma$ -aminobutyric acid
5-HT	Serotonin
GPCRs	G protein-coupled receptors
ARs	adenosine receptors
BBB	blood–brain barrier
CYPs	cytochrome P450 enzymes
7-OH-CBD	7-hydroxycannabidiol
G <sub>i/o</sub>	inhibiting G

cAMP	cyclic adenosine monophosphate
PI <sub>3</sub> K/Akt	phosphoinositide 3-kinases/protein kinase B
CNS	central nervous system
TMEV	Theiler's murine encephalomyelitis virus
i.p.	intraperitoneal
VCAM-1	vascular cell adhesion molecule-1
CCL2	chemokine 2
CCL5	chemokine 5
COX-2	cyclooxygenase-2
A $\beta$	amyloid- $\beta$
BDNF	brain-derived neurotrophic factor
K <sup>+</sup>	potassium
MCA	middle cerebral artery occlusion
PTZ	pentylentetrazole
i.v.	intravenous
GPR55	G protein-coupled receptors 55
ERK	extracellular receptor-activated kinases
DS	Dravet syndrome
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
EAE	experimental autoimmune encephalomyelitis
TRPA1	transient receptor potential ankyrin 1
TRPM8	transient receptor potential cation channel subfamily M member 8
hPBMECs	human Primary Brain Microvascular Endothelial Cells
GSK-3 $\beta$	glycogen synthase kinase 3 $\beta$
SH-SY5Y <sup>APP+</sup>	SH-SY5Y cells transfected with the amyloid precursor protein
L-DOPA	L-3,4-Dihydroxyphenylalanine
6-OHDA	6-hydroxydopamine
AA-5-HT	arachidonoyl-serotonin
IFN- $\gamma$	interferon- $\gamma$
IL-17	interleukin-17
TSC	tuberous sclerosis complex
CLB	clobazam
N-CLB	N-desmethyloclobazam
<i>Scn1a</i> <sup>+/-</sup>	heterozygous loss of function <i>SCN1A</i>

## References

1. WHO. *WHO Methods and Data Sources for Global Burden of Disease Estimates 2000–2011*; Department of Health Statistics and Information Systems: Geneva, Switzerland, 2013; Available online: [http://www.who.int/healthinfo/statistics/GlobalDALYmethods\\_2000\\_2011.pdf?ua=1](http://www.who.int/healthinfo/statistics/GlobalDALYmethods_2000_2011.pdf?ua=1) (accessed on 6 November 2020).
2. Roy, M.; Tapadia, M.G.; Joshi, S.; Koch, B. Molecular and genetic basis of depression. *J. Genet.* **2014**, *93*, 879–892. [[CrossRef](#)] [[PubMed](#)]
3. Lopizzo, N.; Bocchio Chiavetto, L.; Cattane, N.; Plazzotta, G.; Tarazi, F.I.; Pariante, C.M.; Riva, M.A.; Cattaneo, A. Gene–environment interaction in major depression: Focus on experience-dependent biological systems. *Front. Psychiatry* **2015**, *6*, 68. [[CrossRef](#)] [[PubMed](#)]
4. Nowicka, N.; Juranek, J.; Juranek, J.K.; Wojtkiewicz, J. Risk factors and emerging therapies in amyotrophic lateral sclerosis. *Int. J. Mol. Sci.* **2019**, *20*, 2616. [[CrossRef](#)] [[PubMed](#)]
5. Ascherio, A.; Munger, K. Epidemiology of multiple sclerosis: From risk factors to prevention, *Semin. Neurol.* **2016**, *36*, 103–114. [[CrossRef](#)] [[PubMed](#)]
6. Fernández-Ruiz, J.; Sagredo, O.; Pazos, M.R.; García, C.; Pertwee, R.; Mechoulam, R.; Martínez-Orgado, J. Cannabidiol for neurodegenerative disorders: Important new clinical applications for this phytocannabinoid? *Br. J. Clin. Pharm.* **2013**, *75*, 323–333. [[CrossRef](#)]

7. Campos, A.C.; Moreira, F.A.; Gomes, F.V.; Del Bel, E.A.; Guimaraes, F.S. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos. Trans. R. Soc. B Biol. Sci.* **2012**, *367*, 3364–3378. [[CrossRef](#)]
8. Mechoulam, R.; Gaoni, Y. Hashish—IV: The isolation and structure of cannabinolic cannabidiolic and cannabigerolic acids. *Tetrahedron* **1965**, *21*, 1223–1229. [[CrossRef](#)]
9. Pertwee, R.G. Pharmacological actions of cannabinoids. In *Cannabinoids*; Springer: Heidelberg, Germany, 2005; pp. 1–51.
10. Fiani, B.; Sarhadi, K.J.; Soula, M.; Zafar, A.; Quadri, S.A. Current application of cannabidiol (CBD) in the management and treatment of neurological disorders. *Neurol. Sci. Off. J. Ital. Neurol. Soc. Ital. Soc. Clin. Neurophysiol.* **2020**, *41*, 3085–3098. [[CrossRef](#)] [[PubMed](#)]
11. Zuardi, A.W.; Cosme, R.; Graeff, F.; Guimarães, F. Effects of ipsapirone and cannabidiol on human experimental anxiety. *J. Psychopharmacol.* **1993**, *7*, 82–88. [[CrossRef](#)] [[PubMed](#)]
12. Campos, A.; Brant, F.; Miranda, A.; Machado, F.; Teixeira, A. Cannabidiol increases survival and promotes rescue of cognitive function in a murine model of cerebral malaria. *Neuroscience* **2015**, *289*, 166–180. [[CrossRef](#)]
13. Silveira, J.W.; Issy, A.C.; Castania, V.A.; Salmon, C.E.; Nogueira-Barbosa, M.H.; Guimarães, F.S.; Defino, H.L.; Del Bel, E. Protective effects of cannabidiol on lesion-induced intervertebral disc degeneration. *PLoS ONE* **2014**, *9*, e113161. [[CrossRef](#)] [[PubMed](#)]
14. Schiavon, A.P.; Soares, L.M.; Bonato, J.M.; Milani, H.; Guimaraes, F.S.; de Oliveira, R.M.W. Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. *Neurotox. Res.* **2014**, *26*, 307–316. [[CrossRef](#)] [[PubMed](#)]
15. Kwiatkoski, M.; Guimaraes, F.S.; Del-Bel, E. Cannabidiol-treated rats exhibited higher motor score after cryogenic spinal cord injury. *Neurotox. Res.* **2012**, *21*, 271–280. [[CrossRef](#)] [[PubMed](#)]
16. Linge, R.; Jiménez-Sánchez, L.; Campa, L.; Pilar-Cuéllar, F.; Vidal, R.; Pazos, A.; Adell, A.; Díaz, Á. Cannabidiol induces rapid-acting antidepressant-like effects and enhances cortical 5-HT/glutamate neurotransmission: Role of 5-HT<sub>1A</sub> receptors. *Neuropharmacology* **2016**, *103*, 16–26. [[CrossRef](#)]
17. Esposito, G.; Scuderi, C.; Valenza, M.; Togna, G.I.; Latina, V.; De Filippis, D.; Cipriano, M.; Carratù, M.R.; Iuvone, T.; Steardo, L. Cannabidiol reduces A $\beta$ -induced neuroinflammation and promotes hippocampal neurogenesis through PPAR $\gamma$  involvement. *PLoS ONE* **2011**, *6*, e28668. [[CrossRef](#)] [[PubMed](#)]
18. Mecha, M.; Feliú, A.; Iñigo, P.; Mestre, L.; Carrillo-Salinas, F.; Guaza, C. Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: A role for A<sub>2A</sub> receptors. *Neurobiol. Dis.* **2013**, *59*, 141–150. [[CrossRef](#)] [[PubMed](#)]
19. Napimoga, M.H.; Benatti, B.B.; Lima, F.O.; Alves, P.M.; Campos, A.C.; Pena-dos-Santos, D.R.; Severino, F.P.; Cunha, F.Q.; Guimarães, F.S. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. *Int. Immunopharmacol.* **2009**, *9*, 216–222. [[CrossRef](#)]
20. Malfait, A.; Gallily, R.; Sumariwalla, P.; Malik, A.; Andreakos, E.; Mechoulam, R.; Feldmann, M. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9561–9566. [[CrossRef](#)]
21. Kozela, E.; Pietr, M.; Juknat, A.; Rimmerman, N.; Levy, R.; Vogel, Z. Cannabinoids  $\Delta^9$ -tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF- $\kappa$ B and interferon- $\beta$ /STAT proinflammatory pathways in BV-2 microglial cells. *J. Biol. Chem.* **2010**, *285*, 1616–1626. [[CrossRef](#)]
22. Mannucci, C.; Navarra, M.; Calapai, F.; Spagnolo, E.V.; Busardo, F.P.; Cas, R.D.; Ippolito, F.M.; Calapai, G. Neurological Aspects of Medical Use of Cannabidiol. *Cns. Neurol. Disord. Drug Targets* **2017**, *16*, 541–553. [[CrossRef](#)]
23. Peres, F.F.; Lima, A.C.; Hallak, J.E.C.; Crippa, J.A.; Silva, R.H.; Abilio, V.C. Cannabidiol as a Promising Strategy to Treat and Prevent Movement Disorders? *Front. Pharm.* **2018**, *9*, 482. [[CrossRef](#)]
24. Atalay, S.; Jarocka-Karpowicz, I.; Skrzydlewska, E. Antioxidative and Anti-Inflammatory Properties of Cannabidiol. *Antioxidants* **2019**, *9*, 21. [[CrossRef](#)]
25. Costa, B.; Trovato, A.E.; Comelli, F.; Giagnoni, G.; Colleoni, M. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *Eur. J. Pharm.* **2007**, *556*, 75–83. [[CrossRef](#)] [[PubMed](#)]

26. Pan, H.; Mukhopadhyay, P.; Rajesh, M.; Patel, V.; Mukhopadhyay, B.; Gao, B.; Hasko, G.; Pacher, P. Cannabidiol Attenuates Cisplatin-Induced Nephrotoxicity by Decreasing Oxidative/Nitrosative Stress, Inflammation, and Cell Death. *J. Pharm. Exp.* **2009**, *328*, 708–714. [[CrossRef](#)] [[PubMed](#)]
27. Esposito, G.; De Filippis, D.; Maiuri, M.C.; De Stefano, D.; Carnuccio, R.; Iuvone, T. Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in  $\beta$ -amyloid stimulated PC12 neurons through p38 MAP kinase and NF- $\kappa$ B involvement. *Neurosci. Lett.* **2006**, *399*, 91–95. [[CrossRef](#)] [[PubMed](#)]
28. Castillo, A.; Tolon, M.R.; Fernandez-Ruiz, J.; Romero, J.; Martinez-Orgado, J. The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB2 and adenosine receptors. *Neurobiol. Dis.* **2010**, *37*, 434–440. [[CrossRef](#)] [[PubMed](#)]
29. Jean-Gilles, L.; Gran, B.; Constantinescu, C.S. Interaction between cytokines, cannabinoids and the nervous system. *Immunobiology* **2010**, *215*, 606–610. [[CrossRef](#)] [[PubMed](#)]
30. Jastrzab, A.; Gegotek, A.; Skrzydlewska, E. Cannabidiol Regulates the Expression of Keratinocyte Proteins Involved in the Inflammation Process through Transcriptional Regulation. *Cells-Basel* **2019**, *8*, 827. [[CrossRef](#)]
31. Costa, B.; Giagnoni, G.; Franke, C.; Trovato, A.E.; Colleoni, M. Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br. J. Pharm.* **2004**, *143*, 247–250. [[CrossRef](#)] [[PubMed](#)]
32. Rajan, T.S.; Giacoppo, S.; Iori, R.; De Nicola, G.R.; Grassi, G.; Pollastro, F.; Bramanti, P.; Mazzon, E. Anti-inflammatory and antioxidant effects of a combination of cannabidiol and moringin in LPS-stimulated macrophages. *Fitoterapia* **2016**, *112*, 104–115. [[CrossRef](#)] [[PubMed](#)]
33. Santos, N.A.G.; Martins, N.M.; Sisti, F.M.; Fernandes, L.S.; Ferreira, R.S.; Queiroz, R.H.C.; Santos, A.C. The neuroprotection of cannabidiol against MPP<sup>+</sup>-induced toxicity in PC12 cells involves trkA receptors, upregulation of axonal and synaptic proteins, neuritogenesis, and might be relevant to Parkinson's disease. *Toxicol. In Vitro* **2015**, *30*, 231–240. [[CrossRef](#)] [[PubMed](#)]
34. Chagas, M.H.N.; Zuardi, A.W.; Tumas, V.; Pena-Pereira, M.A.; Sobreira, E.T.; Bergamaschi, M.M.; dos Santos, A.C.; Teixeira, A.L.; Hallak, J.E.; Crippa, J.A.S. Effects of cannabidiol in the treatment of patients with Parkinson's disease: An exploratory double-blind trial. *J. Psychopharmacol.* **2014**, *28*, 1088–1098. [[CrossRef](#)] [[PubMed](#)]
35. Vallée, A.; Lecarpentier, Y.; Guillevin, R.; Vallée, J.-N. Effects of cannabidiol interactions with Wnt/ $\beta$ -catenin pathway and PPAR $\gamma$  on oxidative stress and neuroinflammation in Alzheimer's disease. *Acta Biochim. Et Biophys. Sin.* **2017**, *49*, 853–866. [[CrossRef](#)]
36. Watt, G.; Karl, T. In vivo evidence for therapeutic properties of cannabidiol (CBD) for Alzheimer's disease. *Front Pharm.* **2017**, *8*, 20. [[CrossRef](#)] [[PubMed](#)]
37. Libro, R.; Diomedea, F.; Scionti, D.; Piattelli, A.; Grassi, G.; Pollastro, F.; Bramanti, P.; Mazzon, E.; Trubiani, O. Cannabidiol Modulates the Expression of Alzheimer's Disease-Related Genes in Mesenchymal Stem Cells. *Int. J. Mol. Sci.* **2017**, *18*, 26. [[CrossRef](#)]
38. Hussain, S.A.; Zhou, R.; Jacobson, C.; Weng, J.; Cheng, E.; Lay, J.; Hung, P.; Lerner, J.T.; Sankar, R. Perceived efficacy of cannabidiol-enriched cannabis extracts for treatment of pediatric epilepsy: A potential role for infantile spasms and Lennox–Gastaut syndrome. *Epilepsy Behav.* **2015**, *47*, 138–141. [[CrossRef](#)] [[PubMed](#)]
39. Devinsky, O.; Cilio, M.R.; Cross, H.; Fernandez-Ruiz, J.; French, J.; Hill, C.; Katz, R.; Di Marzo, V.; Jutras-Aswad, D.; Notcutt, W.G. Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia* **2014**, *55*, 791–802. [[CrossRef](#)]
40. Bergamaschi, M.M.; Queiroz, R.H.C.; Chagas, M.H.N.; De Oliveira, D.C.G.; De Martinis, B.S.; Kapczinski, F.; Quevedo, J.; Roesler, R.; Schröder, N.; Nardi, A.E. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* **2011**, *36*, 1219–1226. [[CrossRef](#)]
41. Zhornitsky, S.; Potvin, S. Cannabidiol in humans—the quest for therapeutic targets. *Pharmaceuticals* **2012**, *5*, 529–552. [[CrossRef](#)]
42. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G.; Group, P. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* **2009**, *6*, e1000097. [[CrossRef](#)]
43. Adams, R. Marijuana: Harvey lecture, February 19, 1942. *Bull. New York Acad. Med.* **1942**, *18*, 705.
44. Todd, A. The chemistry of hashish. *Sci. J. R. Coll. Sci.* **1942**, *12*, 37.

45. Jones, P.G.; Falvello, L.; Kennard, O.; Sheldrick, G.; Mechoulam, R. Cannabidiol. *Acta Cryst. Sect. B Struct. Cryst. Cryst. Chem.* **1977**, *33*, 3211–3214. [[CrossRef](#)]
46. Morales, P.; Reggio, P.H.; Jagerovic, N. An Overview on Medicinal Chemistry of Synthetic and Natural Derivatives of Cannabidiol. *Front Pharm.* **2017**, *8*, 422. [[CrossRef](#)] [[PubMed](#)]
47. Elmes, M.W.; Kaczocha, M.; Berger, W.T.; Leung, K.; Ralph, B.P.; Wang, L.Q.; Sweeney, J.M.; Miyauchi, J.T.; Tsirka, S.E.; Ojima, I.; et al. Fatty Acid-binding Proteins (FABPs) Are Intracellular Carriers for Delta (9)-Tetrahydrocannabinol (THC) and Cannabidiol (CBD). *J. Biol. Chem.* **2015**, *290*, 8711–8721. [[CrossRef](#)]
48. Borges, R.S.; Batista, J.; Viana, R.B.; Baetas, A.C.; Orestes, E.; Andrade, M.A.; Honorio, K.M.; da Silva, A.B.F. Understanding the Molecular Aspects of Tetrahydrocannabinol and Cannabidiol as Antioxidants. *Molecules* **2013**, *18*, 12663–12674. [[CrossRef](#)] [[PubMed](#)]
49. Laprairie, R.B.; Bagher, A.M.; Kelly, M.E.M.; Denovan-Wright, E.M. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br. J. Pharm.* **2015**, *172*, 4790–4805. [[CrossRef](#)]
50. Morales, P.; Goya, P.; Jagerovic, N.; Hernandez-Folgado, L. Allosteric modulators of the CB1 cannabinoid receptor: A structural update review. *Cannabis Cannabinoid Res.* **2016**, *1*, 22–30. [[CrossRef](#)]
51. Leweke, F.; Piomelli, D.; Pahlisch, F.; Muhl, D.; Gerth, C.; Hoyer, C.; Klosterkötter, J.; Hellmich, M.; Koethe, D. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl. Psychiatry* **2012**, *2*, e94. [[CrossRef](#)]
52. Campos, A.C.; Fogaca, M.V.; Sonogo, A.B.; Guimaraes, F.S. Cannabidiol, neuroprotection and neuropsychiatric disorders. *Pharm. Res.* **2016**, *112*, 119–127. [[CrossRef](#)]
53. Ibeas Bih, C.; Chen, T.; Nunn, A.V.; Bazelat, M.; Dallas, M.; Whalley, B.J. Molecular Targets of Cannabidiol in Neurological Disorders. *Neurotherapeutics* **2015**, *12*, 699–730. [[CrossRef](#)] [[PubMed](#)]
54. Cassano, T.; Villani, R.; Pace, L.; Carbone, A.; Bukke, V.N.; Orkisz, S.; Avolio, C.; Serviddio, G. From Cannabis sativa to Cannabidiol: Promising Therapeutic Candidate for the Treatment of Neurodegenerative Diseases. *Front Pharm.* **2020**, *11*, 124. [[CrossRef](#)] [[PubMed](#)]
55. Kano, M.; Ohno-Shosaku, T.; Maejima, T. Retrograde signaling at central synapses via endogenous cannabinoids. *Mol. Psychiatr.* **2002**, *7*, 234–235. [[CrossRef](#)] [[PubMed](#)]
56. Freund, T.F.; Katona, I.; Piomelli, D. Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* **2003**, *83*, 1017–1066. [[CrossRef](#)]
57. Pertwee, R.G.; Ross, R.A. Cannabinoid receptors and their ligands. *Prostaglandins Leukot. Essent. Fat. Acids* **2002**, *66*, 101–121. [[CrossRef](#)] [[PubMed](#)]
58. Szabo, B.; Schlicker, E. Effects of cannabinoids on neurotransmission. *Handb. Exp. Pharm.* **2005**, 327–365.
59. Russo, E.B.; Burnett, A.; Hall, B.; Parker, K.K. Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem. Res.* **2005**, *30*, 1037–1043. [[CrossRef](#)]
60. Yang, K.-H.; Galadari, S.; Isaev, D.; Petroianu, G.; Shippenberg, T.S.; Oz, M. The nonpsychoactive cannabinoid cannabidiol inhibits 5-hydroxytryptamine3A receptor-mediated currents in *Xenopus laevis* oocytes. *J. Pharm. Exp.* **2010**, *333*, 547–554. [[CrossRef](#)]
61. Rock, E.; Bolognini, D.; Limebeer, C.; Cascio, M.; Anavi-Goffer, S.; Fletcher, P.; Mechoulam, R.; Pertwee, R.G.; Parker, L.A. Cannabidiol, a non-psychoactive component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT1A somatodendritic autoreceptors in the dorsal raphe nucleus. *Br. J. Pharm.* **2012**, *165*, 2620–2634. [[CrossRef](#)]
62. Gonca, E.; Darici, F. The effect of cannabidiol on ischemia/reperfusion-induced ventricular arrhythmias: The role of adenosine A1 receptors. *J. Cardiovasc. Pharm.* **2015**, *20*, 76–83. [[CrossRef](#)]
63. Izzo, A.A.; Borrelli, F.; Capasso, R.; Di Marzo, V.; Mechoulam, R. Non-psychoactive plant cannabinoids: New therapeutic opportunities from an ancient herb. *Trends Pharm. Sci.* **2009**, *30*, 515–527. [[CrossRef](#)] [[PubMed](#)]
64. Scuderi, C.; Steardo, L.; Esposito, G. Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of  $\beta$  amyloid expression in SHSY5YAPP+ cells through PPAR $\gamma$  involvement. *Phytother. Res.* **2014**, *28*, 1007–1013. [[CrossRef](#)] [[PubMed](#)]
65. Bakas, T.; van Nieuwenhuijzen, P.S.; Devenish, S.O.; McGregor, I.S.; Arnold, J.C.; Chebib, M. The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABAA receptors. *Pharm. Res.* **2017**, *119*, 358–370. [[CrossRef](#)] [[PubMed](#)]



66. Newmeyer, M.N.; Swortwood, M.J.; Barnes, A.J.; Abulseoud, O.A.; Scheidweiler, K.B.; Huestis, M.A. Free and Glucuronide Whole Blood Cannabinoids' Pharmacokinetics after Controlled Smoked, Vaporized, and Oral Cannabis Administration in Frequent and Occasional Cannabis Users: Identification of Recent Cannabis Intake. *Clin. Chem.* **2016**, *62*, 1579–1592. [[CrossRef](#)]
67. Mechoulam, R.; Parker, L.A.; Gallily, R. Cannabidiol: An overview of some pharmacological aspects. *J. Clin. Pharm.* **2002**, *42*, 11s–19s. [[CrossRef](#)]
68. Grotenhermen, F. Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin. Pharm.* **2003**, *42*, 327–360. [[CrossRef](#)] [[PubMed](#)]
69. Fasinu, P.S.; Phillips, S.; ElSohly, M.A.; Walker, L.A. Current Status and Prospects for Cannabidiol Preparations as New Therapeutic Agents. *Pharmacotherapy* **2016**, *36*, 781–796. [[CrossRef](#)] [[PubMed](#)]
70. Huestis, M.A. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. *Handb. Exp. Pharm.* **2005**, 657–690. [[CrossRef](#)]
71. Gaston, T.E.; Friedman, D. Pharmacology of cannabinoids in the treatment of epilepsy. *Epilepsy Behav.* **2017**, *70*, 313–318. [[CrossRef](#)]
72. Bergamaschi, M.M.; Queiroz, R.H.; Zuardi, A.W.; Crippa, J.A. Safety and side effects of cannabidiol, a Cannabis sativa constituent. *Curr. Drug Saf.* **2011**, *6*, 237–249. [[CrossRef](#)]
73. Zundulka, O.; Dovrtelova, G.; Noskova, K.; Turjap, M.; Sulcova, A.; Hanus, L.; Jurica, J. Cannabinoids and Cytochrome P450 Interactions. *Curr. Drug Metab.* **2016**, *17*, 206–226. [[CrossRef](#)]
74. Jiang, R.R.; Yamaori, S.; Okamoto, Y.; Yamamoto, I.; Watanabe, K. Cannabidiol Is a Potent Inhibitor of the Catalytic Activity of Cytochrome P450 2C19. *Drug Metab. Pharm.* **2013**, *28*, 332–338. [[CrossRef](#)] [[PubMed](#)]
75. Premoli, M.; Aria, F.; Bonini, S.A.; Maccarinelli, G.; Gianoncelli, A.; Pina, S.D.; Tambaro, S.; Memo, M.; Mastinu, A. Cannabidiol: Recent advances and new insights for neuropsychiatric disorders treatment. *Life Sci.* **2019**, *224*, 120–127. [[CrossRef](#)] [[PubMed](#)]
76. Dal Ben, D.; Lambertucci, C.; Buccioni, M.; Marti Navia, A.; Marucci, G.; Spinaci, A.; Volpini, R. Non-Nucleoside Agonists of the Adenosine Receptors: An Overview. *Pharmaceuticals* **2019**, *12*, 150. [[CrossRef](#)]
77. Kamp, T.J.; Hell, J.W. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. *Circ. Res.* **2000**, *87*, 1095–1102. [[CrossRef](#)] [[PubMed](#)]
78. Borea, P.A.; Gessi, S.; Merighi, S.; Vincenzi, F.; Varani, K. Pharmacology of Adenosine Receptors: The State of the Art. *Physiol. Rev.* **2018**, *98*, 1591–1625. [[CrossRef](#)]
79. Jacobson, K.A.; Gao, Z.G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discov.* **2006**, *5*, 247–264. [[CrossRef](#)] [[PubMed](#)]
80. Chen, J.F.; Eltzhig, H.K.; Fredholm, B.B. Adenosine receptors as drug targets—what are the challenges? *Nat. Rev. Drug Discov.* **2013**, *12*, 265–286. [[CrossRef](#)]
81. Carrier, E.J.; Auchampach, J.A.; Hillard, C.J. Inhibition of an equilibrative nucleoside transporter by cannabidiol: A mechanism of cannabinoid immunosuppression. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7895–7900. [[CrossRef](#)]
82. Magen, I.; Avraham, Y.; Ackerman, Z.; Vorobiev, L.; Mechoulam, R.; Berry, E.M. Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. *J. Hepatol.* **2009**, *51*, 528–534. [[CrossRef](#)] [[PubMed](#)]
83. Magen, I.; Avraham, Y.; Ackerman, Z.; Vorobiev, L.; Mechoulam, R.; Berry, E.M. Cannabidiol ameliorates cognitive and motor impairments in bile-duct ligated mice via 5-HT1A receptor activation. *Br. J. Pharm.* **2010**, *159*, 950–957. [[CrossRef](#)] [[PubMed](#)]
84. Martin-Moreno, A.M.; Reigada, D.; Ramirez, B.G.; Mechoulam, R.; Innamorato, N.; Cuadrado, A.; de Ceballos, M.L. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: Relevance to Alzheimer's disease. *Mol. Pharm.* **2011**, *79*, 964–973. [[CrossRef](#)]
85. Raymond, J.R.; Mukhin, Y.V.; Gettys, T.W.; Garnovskaya, M.N. The recombinant 5-HT1A receptor: G protein coupling and signalling pathways. *Br. J. Pharm.* **1999**, *127*, 1751–1764. [[CrossRef](#)]
86. Bevilacqua, L.; Ardenghi, P.; Schroder, N.; Bromberg, E.; Schmitz, P.K.; Schaeffer, E.; Quevedo, J.; Bianchin, M.; Walz, R.; Medina, J.H.; et al. Drugs acting upon the cyclic adenosine monophosphate protein kinase A signalling pathway modulate memory consolidation when given late after training into rat hippocampus but not amygdala. *Behav. Pharm.* **1997**, *8*, 331–338. [[CrossRef](#)] [[PubMed](#)]



87. Mishima, K.; Hayakawa, K.; Abe, K.; Ikeda, T.; Egashira, N.; Iwasaki, K.; Fujiwara, M. Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine<sub>1A</sub> receptor-dependent mechanism. *Stroke* **2005**, *36*, 1077–1082. [[CrossRef](#)]
88. Gomes, F.V.; Del Bel, E.A.; Guimaraes, F.S. Cannabidiol attenuates catalepsy induced by distinct pharmacological mechanisms via 5-HT<sub>1A</sub> receptor activation in mice. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2013**, *46*, 43–47. [[CrossRef](#)]
89. Sonogo, A.B.; Gomes, F.V.; Del Bel, E.A.; Guimaraes, F.S. Cannabidiol attenuates haloperidol-induced catalepsy and c-Fos protein expression in the dorsolateral striatum via 5-HT<sub>1A</sub> receptors in mice. *Behav. Brain Res.* **2016**, *309*, 22–28. [[CrossRef](#)]
90. Pelz, M.C.; Schoolcraft, K.D.; Larson, C.; Spring, M.G.; Lopez, H.H. Assessing the role of serotonergic receptors in cannabidiol's anticonvulsant efficacy. *Epilepsy Behav.* **2017**, *73*, 111–118. [[CrossRef](#)] [[PubMed](#)]
91. De Gregorio, D.; McLaughlin, R.J.; Posa, L.; Ochoa-Sanchez, R.; Enns, J.; Lopez-Canul, M.; Aboud, M.; Maione, S.; Comai, S.; Gobbi, G. Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain. *Pain* **2019**, *160*, 136–150. [[CrossRef](#)] [[PubMed](#)]
92. Avraham, Y.; Grigoriadis, N.; Poutahidis, T.; Vorobiev, L.; Magen, I.; Ilan, Y.; Mechoulam, R.; Berry, E. Cannabidiol improves brain and liver function in a fulminant hepatic failure-induced model of hepatic encephalopathy in mice. *Br. J. Pharm.* **2011**, *162*, 1650–1658. [[CrossRef](#)]
93. Marichal-Cancino, B.A.; Fajardo-Valdez, A.; Ruiz-Contreras, A.E.; Mendez-Diaz, M.; Prospero-Garcia, O. Advances in the Physiology of GPR55 in the Central Nervous System. *Curr. Neuropharmacol.* **2017**, *15*, 771–778. [[CrossRef](#)]
94. Kremshofer, J.; Siwetz, M.; Berghold, V.M.; Lang, I.; Huppertz, B.; Gauster, M. A role for GPR55 in human placental venous endothelial cells. *Histochem. Cell Biol.* **2015**, *144*, 49–58. [[CrossRef](#)]
95. Sylantsev, S.; Jensen, T.P.; Ross, R.A.; Rusakov, D.A. Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5193–5198. [[CrossRef](#)] [[PubMed](#)]
96. Ryberg, E.; Larsson, N.; Sjögren, S.; Hjorth, S.; Hermansson, N.O.; Leonova, J.; Elebring, T.; Nilsson, K.; Drmota, T.; Greasley, P. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharm.* **2007**, *152*, 1092–1101. [[CrossRef](#)] [[PubMed](#)]
97. Jones, N.A.; Hill, A.J.; Smith, I.; Bevan, S.A.; Williams, C.M.; Whalley, B.J.; Stephens, G.J. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *J. Pharm. Exp.* **2010**, *332*, 569–577. [[CrossRef](#)]
98. Kaplan, J.S.; Stella, N.; Catterall, W.A.; Westenbroek, R.E. Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 11229–11234. [[CrossRef](#)]
99. Celorrio, M.; Rojo-Bustamante, E.; Fernandez-Suarez, D.; Saez, E.; Estella-Hermoso de Mendoza, A.; Muller, C.E.; Ramirez, M.J.; Oyarzabal, J.; Franco, R.; Aymerich, M.S. GPR55: A therapeutic target for Parkinson's disease? *Neuropharmacology* **2017**, *125*, 319–332. [[CrossRef](#)]
100. Gonzalez-Garcia, C.; Torres, I.M.; Garcia-Hernandez, R.; Campos-Ruiz, L.; Esparragoza, L.R.; Coronado, M.J.; Grande, A.G.; Garcia-Merino, A.; Sanchez Lopez, A.J. Mechanisms of action of cannabidiol in adoptively transferred experimental autoimmune encephalomyelitis. *Exp. Neurol.* **2017**, *298*, 57–67. [[CrossRef](#)]
101. De Petrocellis, L.; Ligresti, A.; Moriello, A.S.; Allara, M.; Bisogno, T.; Petrosino, S.; Stott, C.G.; Di Marzo, V. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br. J. Pharm.* **2011**, *163*, 1479–1494. [[CrossRef](#)]
102. Ross, R.A. Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharm.* **2003**, *140*, 790–801. [[CrossRef](#)] [[PubMed](#)]
103. Vyklicky, L.; Novakova-Tousova, K.; Benedikt, J.; Samad, A.; Touska, F.; Vlachova, V. Calcium-dependent desensitization of vanilloid receptor TRPV1: A mechanism possibly involved in analgesia induced by topical application of capsaicin. *Physiol. Res.* **2008**, *57*, 59–68.
104. Jara-Oseguera, A.; Simon, S.A.; Rosenbaum, T. TRPV1: On the road to pain relief. *Curr. Mol. Pharm.* **2008**, *1*, 255–269. [[CrossRef](#)]
105. Novakova-Tousova, K.; Vyklicky, L.; Susankova, K.; Benedikt, J.; Samad, A.; Teisinger, J.; Vlachova, V. Functional changes in the vanilloid receptor subtype 1 channel during and after acute desensitization. *Neuroscience* **2007**, *149*, 144–154. [[CrossRef](#)]

106. Planells-Cases, R.; Garcia-Sanz, N.; Morenilla-Palao, C.; Ferrer-Montiel, A. Functional aspects and mechanisms of TRPV1 involvement in neurogenic inflammation that leads to thermal hyperalgesia. *Pflug. Arch. Eur. J. Phys.* **2005**, *451*, 151–159. [[CrossRef](#)]
107. Srivastava, M.D.; Srivastava, B.I.; Brouhard, B. Delta9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. *Immunopharmacology* **1998**, *40*, 179–185. [[CrossRef](#)]
108. Bisogno, T.; Hanus, L.; De Petrocellis, L.; Tchilibon, S.; Ponde, D.E.; Brandi, I.; Moriello, A.S.; Davis, J.B.; Mechoulam, R.; Di Marzo, V. 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. Pharm.* **2001**, *134*, 845–852. [[CrossRef](#)]
109. Luo, H.L.; Rossi, E.; Saubamea, B.; Chasseigneaux, S.; Cochois, V.; Choublier, N.; Smirnova, M.; Glacial, F.; Perriere, N.; Bourdoulous, S.; et al. Cannabidiol Increases Proliferation, Migration, Tubulogenesis, and Integrity of Human Brain Endothelial Cells through TRPV2 Activation. *Mol. Pharm.* **2019**, *16*, 1312–1326. [[CrossRef](#)] [[PubMed](#)]
110. Nabissi, M.; Morelli, M.B.; Santoni, M.; Santoni, G. Triggering of the TRPV2 channel by cannabidiol sensitizes glioblastoma cells to cytotoxic chemotherapeutic agents. *Carcinogenesis* **2013**, *34*, 48–57. [[CrossRef](#)] [[PubMed](#)]
111. Esposito, G.; De Filippis, D.; Carnuccio, R.; Izzo, A.A.; Iuvone, T. The marijuana component cannabidiol inhibits  $\beta$ -amyloid-induced tau protein hyperphosphorylation through Wnt/ $\beta$ -catenin pathway rescue in PC12 cells. *J. Mol. Med.* **2006**, *84*, 253–258. [[CrossRef](#)]
112. Cheng, D.; Low, J.K.; Logge, W.; Garner, B.; Karl, T. Chronic cannabidiol treatment improves social and object recognition in double transgenic APP. (swe)/PS1a Delta E9 mice. *Psychopharmacology* **2014**, *231*, 3009–3017. [[CrossRef](#)] [[PubMed](#)]
113. Cai, W.; Yang, T.; Liu, H.; Han, L.J.; Zhang, K.; Hu, X.M.; Zhang, X.J.; Yin, K.J.; Gao, Y.Q.; Bennett, M.V.L.; et al. Peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ): A master gatekeeper in CNS injury and repair. *Prog. Neurobiol.* **2018**, *163*, 27–58. [[CrossRef](#)]
114. Surgucheva, I.; Surguchov, A.  $\Gamma$ -synuclein: Cell-type-specific promoter activity and binding to transcription factors. *J. Mol. Neurosci. Mn* **2008**, *35*, 267–271. [[CrossRef](#)] [[PubMed](#)]
115. Hou, Y.Z.; Moreau, F.; Chadee, K. PPAR  $\gamma$  is an E3 ligase that induces the degradation of NF- $\kappa$ B/p65. *Nat. Commun.* **2012**, *3*, 1–11. [[CrossRef](#)]
116. O'Sullivan, S.E. An update on PPAR activation by cannabinoids. *Br J. Pharm.* **2016**, *173*, 1899–1910. [[CrossRef](#)]
117. Hughes, B.; Herron, C.E. Cannabidiol Reverses Deficits in Hippocampal LTP in a Model of Alzheimer's Disease. *Neurochem. Res.* **2019**, *44*, 703–713. [[CrossRef](#)]
118. Aia, P.G.; Revuelta, G.J.; Cloud, L.J.; Factor, S.A. Tardive dyskinesia. *Curr. Treat. Options Neurol.* **2011**, *13*, 231–241. [[CrossRef](#)] [[PubMed](#)]
119. Sonogo, A.B.; Prado, D.S.; Vale, G.T.; Sepulveda-Diaz, J.E.; Cunha, T.M.; Tirapelli, C.R.; Del Bel, E.A.; Raisman-Vozari, R.; Guimaraes, F.S. Cannabidiol prevents haloperidol-induced vacuos chewing movements and inflammatory changes in mice via PPAR $\gamma$  receptors. *Brain Behav. Immun.* **2018**, *74*, 241–251. [[CrossRef](#)]
120. Dos-Santos-Pereira, M.; da-Silva, C.A.; Guimaraes, F.S.; Del-Bel, E. Co-administration of cannabidiol and capsazepine reduces L-DOPA-induced dyskinesia in mice: Possible mechanism of action. *Neurobiol. Dis.* **2016**, *94*, 179–195. [[CrossRef](#)]
121. Hind, W.H.; England, T.J.; O'Sullivan, S.E. Cannabidiol protects an in vitro model of the blood-brain barrier from oxygen-glucose deprivation via PPAR $\gamma$  and 5-HT1A receptors. *Br J. Pharm.* **2016**, *173*, 815–825. [[CrossRef](#)]
122. Giacompo, S.; Pollastro, F.; Grassi, G.; Bramanti, P.; Mazzon, E. Target regulation of PI3K/Akt/mTOR pathway by cannabidiol in treatment of experimental multiple sclerosis. *Fitoterapia* **2017**, *116*, 77–84. [[CrossRef](#)]
123. Sigel, E.; Steinmann, M.E. Structure, function, and modulation of GABA(A) receptors. *J. Biol. Chem.* **2012**, *287*, 40224–40231. [[CrossRef](#)] [[PubMed](#)]
124. Rabow, L.E.; Russek, S.J.; Farb, D.H. From ion currents to genomic analysis: Recent advances in GABAA receptor research. *Synapse* **1995**, *21*, 189–274. [[CrossRef](#)]
125. Cifelli, P.; Ruffolo, G.; De Felice, E.; Alfano, V.; van Vliet, E.A.; Aronica, E.; Palma, E. Phytocannabinoids in Neurological Diseases: Could They Restore a Physiological GABAergic Transmission? *Int. J. Mol. Sci.* **2020**, *21*, 723. [[CrossRef](#)] [[PubMed](#)]

126. Holley, S.M.; Galvan, L.; Kamdjou, T.; Dong, A.; Levine, M.S.; Cepeda, C. Major Contribution of Somatostatin-Expressing Interneurons and Cannabinoid Receptors to Increased GABA Synaptic Activity in the Striatum of Huntington's Disease Mice. *Front. Synaptic Neurosci.* **2019**, *11*, 14. [CrossRef]
127. Palma, E.; Ruffolo, G.; Cifelli, P.; Roseti, C.; Vliet, E.A.V.; Aronica, E. Modulation of GABAA Receptors in the Treatment of Epilepsy. *Curr. Pharm. Des.* **2017**, *23*, 5563–5568. [CrossRef]
128. Olsen, R.W.; Liang, J. Role of GABAA receptors in alcohol use disorders suggested by chronic intermittent ethanol (CIE) rodent model. *Mol. Brain* **2017**, *10*, 45. [CrossRef] [PubMed]
129. Bambico, F.R.; Li, Z.; Oliveira, C.; McNeill, S.; Diwan, M.; Raymond, R.; Nobrega, J.N. Rostrocaudal subregions of the ventral tegmental area are differentially impacted by chronic stress. *Psychopharmacol. (Berl.)* **2019**, *236*, 1917–1929. [CrossRef]
130. Gugliandolo, A.; Silvestro, S.; Chiricosta, L.; Pollastro, F.; Bramanti, P.; Mazzon, E. The Transcriptomic Analysis of NSC-34 Motor Neuron-Like Cells Reveals That Cannabigerol Influences Synaptic Pathways: A Comparative Study with Cannabidiol. *Life* **2020**, *10*, 227. [CrossRef]
131. Perucca, E. Cannabinoids in the Treatment of Epilepsy: Hard Evidence at Last? *J. Epilepsy Res.* **2017**, *7*, 61–76. [CrossRef]
132. Tang, R.R.; Fang, F. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N. Engl. J. Med.* **2017**, *377*, 699.
133. Devinsky, O.; Cross, J.H.; Laux, L.; Marsh, E.; Miller, I.; Nabhout, R.; Scheffer, I.E.; Thiele, E.A.; Wright, S.; Cannabidiol in Dravet Syndrome Study, G. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N. Engl. J. Med.* **2017**, *376*, 2011–2020. [CrossRef] [PubMed]
134. Ruffolo, G.; Cifelli, P.; Roseti, C.; Thom, M.; van Vliet, E.A.; Limatola, C.; Aronica, E.; Palma, E. A novel GABAergic dysfunction in human Dravet syndrome. *Epilepsia* **2018**, *59*, 2106–2117. [CrossRef] [PubMed]
135. FDA, U.S. FDA approves first drug comprised of an active ingredient derived from marijuana to treat rare, severe forms of epilepsy. *Silver Spring: Us Food Drug Adm.* **2018**. Available online: <https://www.fda.gov/news-events/press-announcements/fda-approves-first-drug-comprised-active-ingredient-derived-marijuana-treat-rare-severe-forms> (accessed on 16 December 2019).
136. Geoffrey, A.L.; Pollack, S.F.; Bruno, P.L.; Thiele, E.A. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia* **2015**, *56*, 1246–1251. [CrossRef]
137. Hess, E.J.; Moody, K.A.; Geoffrey, A.L.; Pollack, S.F.; Skirvin, L.A.; Bruno, P.L.; Paolini, J.L.; Thiele, E.A. Cannabidiol as a new treatment for drug-resistant epilepsy in tuberous sclerosis complex. *Epilepsia* **2016**, *57*, 1617–1624. [CrossRef]
138. Gaston, T.E.; Bebin, E.M.; Cutter, G.R.; Liu, Y.; Szaflarski, J.P.; Program, U.C. Interactions between cannabidiol and commonly used antiepileptic drugs. *Epilepsia* **2017**, *58*, 1586–1592. [CrossRef]
139. Nakajima, H. A pharmacological profile of clobazam (Mystan), a new antiepileptic drug. *Nihon Yakurigaku Zasshi. Folia Pharm. Jpn.* **2001**, *118*, 117–122. [CrossRef] [PubMed]
140. Sankar, R. GABA A receptor physiology and its relationship to the mechanism of action of the 1,5-benzodiazepine clobazam. *Cns. Drugs* **2012**, *26*, 229–244. [CrossRef] [PubMed]
141. Jensen, H.S.; Nichol, K.; Lee, D.; Ebert, B. Clobazam and its active metabolite N-desmethyloclobazam display significantly greater affinities for  $\alpha$  2-versus  $\alpha$  1-GABA A-receptor complexes. *PLoS ONE* **2014**, *9*, e88456. [CrossRef]
142. Anderson, L.L.; Absalom, N.L.; Abelev, S.V.; Low, I.K.; Doohan, P.T.; Martin, L.J.; Chebib, M.; McGregor, I.S.; Arnold, J.C. Coadministered cannabidiol and clobazam: Preclinical evidence for both pharmacodynamic and pharmacokinetic interactions. *Epilepsia* **2019**, *60*, 2224–2234. [CrossRef]
143. Aso, E.; Andres-Benito, P.; Ferrer, I. Delineating the Efficacy of a Cannabis-Based Medicine at Advanced Stages of Dementia in a Murine Model. *J. Alzheimers Dis.* **2016**, *54*, 903–912. [CrossRef] [PubMed]

**Sample Availability:** Not available.

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).