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Review

Cannabinoids and endocannabinoids as therapeutics for nervous system disorders: preclinical models and clinical studies

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https://doi.org/10.4103/1673-5374.382220

Date of submission: March 10, 2023

Date of decision: June 8, 2023

Date of acceptance: July 1, 2023

Date of web publication: August 14, 2023

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Abstract

Cannabinoids are lipophilic substances derived from Cannabis sativa that can exert a variety of effects in the human body. They have been studied in cellular and animal models as well as in human clinical trials for their therapeutic benefits in several human diseases. Some of these include central nervous system (CNS) diseases and dysfunctions such as forms of epilepsy, multiple sclerosis, Parkinson's disease, pain and neuropsychiatric disorders. In addition, the endogenously produced cannabinoid lipids, endocannabinoids, are critical for normal CNS function, and if controlled or modified, may represent an additional therapeutic avenue for CNS diseases. This review discusses *in vitro* cellular, *ex vivo* tissue and *in vivo* animal model studies on cannabinoids and their utility as therapeutics in multiple CNS pathologies. In addition, the review provides an overview on the use of cannabinoids in human clinical trials for a variety of CNS diseases. Cannabinoids and endocannabinoids hold promise for use as disease modifiers and therapeutic agents for the prevention or treatment of neurodegenerative diseases and neurological disorders.

Key Words: anandamide; cannabidiol; cannabinoid; endocannabinoid; epilepsy; multiple sclerosis; neurodegeneration; neuroprotection; tetrahydrocannabinol

Introduction

The psychotropic and medicinal properties of the plant, Cannabis sativa, have been known to humans for thousands of years (Ibeas Bih et al., 2015). In the last 20 years, however, the medicinal properties of cannabinoids have been the focus of much research and important discoveries have been made. Cannabinoids are compounds that directly or indirectly influence the activity of cannabinoid receptors and include phytocannabinoids (plants) and synthetic cannabinoids. Phytocannabinoids such as Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are present in Cannabis species (reviewed in Sarne and Mechoulam, 2005). There is not currently a strong consensus on what function phytocannabinoids like THC and CBD have in plant biology, but it is widely accepted that their presence may serve as a protective strategy to ward off herbivores (Chapman, 2004).

Another type of cannabinoids, endogenous cannabinoids or endocannabinoids (eCBs), are lipids produced de novo in a variety of plant species and in animal species including humans. Mammals produce eCBs that are essential for normal physiological function. The best characterized endocannabinoids include 2-arachidonoylglycerol (2-AG) and *N*-acylethanolamine (NAE), *N*-arachidonoylethanolamine or anandamide (AEA). NAEs are synthesized in organisms ranging from bacteria to plants to vertebrates. Several NAEs play an important role in seedling development in plants and in the protective response against environmental stress (Chapman, 2004). Because plants do not have cannabinoid receptors, they are not referred to as eCBs in the plant literature.

In vertebrates, they are generated in numerous cell types, including neurons (Sarne and Mechoulam, 2005). NAE and eCB levels are increased in response to cerebral ischemia and trauma and can trigger neuroprotection. The neuroprotective activities of eCBs and cannabinoids make them suitable candidates as potential therapeutics for a variety of conditions (Sarne and Mechoulam, 2005).

Early studies with THC and synthetic cannabinoids, including levonantradol (CP 50,556-1) and desacetyl levonantradol (CP 54939), were used to help identify the presence of a cannabinoid receptor in cell-free *in vitro* radioligand binding assays (Howlett et al., 1984). Several phytocannabinoids and synthetic CB1 receptor agonists were found to bind a G protein-coupled receptor that inhibited adenylate cyclase activity (Howlett et al., 1984; Devane et al., 1988; Matsuda et al., 1990). The inhibition of adenylyl cyclase was specific for psychoactive cannabinoids. The cloning and expression of a gene encoding a G protein-coupled receptor have been identified in cell lines, tissues and brain regions that possess binding sites (Matsuda et al., 1990).

Endocannabinoids are produced by mammalian cells in response to a variety of stimuli including excitotoxicity, ischemia, inflammation, and trauma and target cannabinoid receptor 1 (CB1), cannabinoid receptor 2 (CB2), transient receptor potential V1 (TRPV1), G protein-coupled receptor 55 (GPR55) and peroxisome proliferator antigen receptors (PPARs) (Hohmann et al., 2019; Bouskila et al., 2021; Lana et al., 2022; Landucci et al., 2022; **Table 1**). The prototypical eCBs are 2-AG and AEA. 2-AG is a full CB1 agonist, whereas AEA is a partial agonist at CB1 and full agonist at TRPV1 and GPR55. The evolutionary evidence available suggests that eCBs have existed long before the emergence of CB1/2 receptors and TRPV1 channels that they activate. Similarly, the phytocannabinoid, THC, is a non-selective full agonist of CB1 and CB2 and it does activate TRPV1 and GPR55. CBD, on the other hand, is not believed to activate CB1 or CB2 at normal physiological concentrations, but it still activates TRPV1 and GPR55 (Hohmann et al., 2019; Bouskila et al., 2021). In addition, phytocannabinoids have a phenolic ring structure not found in eCBs that exhibit antioxidant activity.

Synthetic cannabinoids have been synthesized with selectivity for CB1 or CB2 or other targets such as TRPV1. In addition, inhibitors of the enzymes that degrade endocannabinoids, such as fatty acid amide hydrolase (FAAH), *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) and monoacylglycerol lipase (MAGL), may be potential therapeutics by regulating eCB levels. Dual selectivity has also been achieved with synthetics (Nagayama et al., 1999; Solbrig et al., 2010). Synthetics may also exhibit different pharmacokinetics than phytocannabinoids and may be more resistant to enzymatic degradation.

Recently, phytocannabinoids have been prescribed with limited medical use for a variety of health issues, including epilepsy, pain and cancer-related anorexia (**Table 2**). This new therapeutic approach may improve some

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Funding: *This work was supported in part by grants from the National Institute on Aging and National Eye Institute [EY030747 (3R01EY030747-02S2) and EY031248 (3R01EY031248-02S1)] of the National Institutes of Health (to PK). Additional support by the Felix and Carmen Sabates Missouri Endowed Chair in Vision Research and the Vision Research Foundation of Kansas City is gratefully acknowledged.*

How to cite this article: *Duncan RS, Riordan SM, Gernon MC, Koulen P (2024) Cannabinoids and endocannabinoids as therapeutics for nervous system disorders: preclinical models and clinical studies. Neural Regen Res 19(4):788-799.*

Table 1 | **Endocannabinoids covered in this review**

Endocannabinoid	Abbreviation	Target	Reference
N-arachidonoyl-ethanolamine (anandamide)	AEA	TRPV1, GPR55, CB1, CB2	Leweke et al., 2007; Webb et al., 2008; Montgomery et al., 2016; Cui et al., 2017; Campbell et al., 2021
2-Arachidonoylglycerol	2-AG	CB1, CB2	Cabral et al., 2008; Hohmann et al., 2019; Campbell et al., 2021
N-palmitoylethanolamine	PEA	GPR55, GPR119, PPARα, TRPV1, FAAH	Webb et al., 2008; Duncan et al., 2009; Garg et al., 2010; Hohmann et al., 2019; Im, 2021
N-linoleylethanolamine	LEA	GPR119, TRPV1, PPARs	Syed et al., 2012
N-linoleoylethanolamine	LLEA	GPR119, PPARs	Duncan et al., 2010; Garg et al., 2011

2-AG: 2-Arachidonoylglycerol; AEA: arachidonoylethanolamide; CB1/CB2: cannabinoid receptor 1/cannabinoid receptor 2; FAAH: fatty acid amide hydrolase; GPR119: G-protein-coupled receptor 119; GPR55: G-protein-coupled receptor 55; LEA: linoleoylethanolamine; LLEA: linoleoylethanolamine; PEA: palmitoylethanolamine; PPARγ: peroxisome proliferator antigen receptor γ; TRPV2: transient receptor potential V1 or vanilloid receptor.

Table 2 | **Phytocannabinoids and synthetic cannabinoids/cannabinoid signaling compounds covered in this review**

Cannabinoid	Abbreviation	Type	Target	Reference
Δ9-Tetrahydro-cannabinol	THC	phyto	CB1/CB2, direct antioxidant	Howlett et al., 1984; Hampson et al., 1998; Baker et al., 2000; van der Stelt et al., 2001; Cabral et al., 2008; Kozela et al., 2013; Aguado et al., 2021; Landucci et al., 2021; Lana et al., 2022
Cannabidiol	CBD		CB2, GPR55, PPARγ, TRPV2, 5HTA1, direct antioxidant	Hampson et al., 1998; Jones et al., 2010; Ruiz-Valdepeñas et al., 2011; Kozela et al., 2013; Sun et al., 2017; da Silva et al., 2018; Hohmann et al., 2019; Bouskila et al., 2021; Landucci et al., 2021, 2022; Lana et al., 2022; Patel et al., 2022
Levonantradol (CP 50,556-1)	CP50,556-1	synth	CB1/CB2 agonist	Howlett et al., 1984
Decacetyllevonantradol (CP 54939)	CP54939		CB1/CB2 agonist	Howlett et al., 1984
R(+) WIN 55212-2	WIN 55212-2		CB1/CB2 agonist	Nagayama et al., 1999; Baker, et al., 2000; Bilsland et al., 2006; Solbrig et al., 2010;
CP55940	CP55940		CB1/CB2 agonist	Cabral et al., 2008
BAY 38-7271	BAY 38-7271		CB1/CB2 agonist	Mauler et al., 2003
SR141716	SR141716		CB1 antagonist	Nagayama et al., 1999; van der Stelt et al., 2001; Spyridakos et al., 2022
JWH-133	JWH-133		CB2 agonist	Baker, et al., 2000; Wang et al., 2018; Çakır et al., 2019
VCE-004.8	VCE-004.8		CB2/PPARγ agonist	Navarrete et al., 2022
AM1710	AM1710		CB2 agonist	Spyridakos et al., 2022
Dexabinol (HU-211)	HU-211		NMDA receptor antagonist	Shohami et al., 1993
URB597	URB597		FAAH inhibitor	Celorrio et al., 2016; Wang et al., 2021
N-arachidonoyl-phenolamine (AM404)	AM404		eCB transport inhibitor	Saliba et al., 2019

5-HT1A: 5-Hydroxytryptamine 1A; CB1/CB2: cannabinoid receptor 1/cannabinoid receptor 2; CBD: cannabigerol; eCB: endocannabinoid; FAAH: fatty acid amide hydrolase; GPR55: G-protein-coupled receptor 55; NMDA: *N*-methyl-D-aspartate; PPARγ: peroxisome proliferator antigen receptor γ; THC: Δ9-Tetrahydro-cannabinol; TRPV2: transient receptor potential V1 or vanilloid receptor.

symptoms of certain diseases, but the psychoactive properties can make the drug unattractive for many people. There is a significant focus on CBD in neurological diseases, as CBD does not activate cannabinoid receptors except at very high concentrations *in vitro*, and therefore does not have the psychotropic effects of THC (Ibeas Bih et al., 2015). This has prompted researchers to find cannabinoids, or cannabinoid mimetics that can alleviate symptoms of disease but not negatively affect the cognitive function of the patient. Cannabinoids can be chemically modified in various ways, resulting in drugs that may alter their selectivity, stability, and efficacy.

Many types of cell and animal models have been utilized to determine the molecular, cellular, and physiological function of cannabinoids and eCBs. Early studies on cannabinoids utilized simple cellular model systems, such as cell lines and primary cells and rodent models, for studying the pharmacokinetic, pharmacodynamic and behavioral properties of cannabinoids. For example, neural progenitor cell-derived neurons, such as ReN cells, are becoming more common for studying human neuronal development and neurodegeneration. ReN cells are easily differentiated, eliminating the need to use transformed cell lines and primary neuronal models (Duncan et al., 2022). In recent years, new experimental models are being implemented that circumvent the limitations of single cell and whole animal models. Some of these models include 3-dimensional cell cultures, *ex vivo* cultures and organotypic explant cultures. Organotypic cultures have been widely used due to the preservation of tissue architecture and the maintenance of native cellular function (Jones et al., 2010; Landucci et al., 2022).

These experimental models have allowed scientists to determine the receptors, enzymes, and signaling pathways that cannabinoids affect, and have supported the idea that cannabinoids are generally safe, have physiological effects on multiple organ systems and are readily metabolized and removed from tissues (**Table 3**). This review focuses on the preclinical experimental models that have been utilized to understand the mechanism of how cannabinoids function in the central nervous system, how new models are becoming available to improve and speed up research on cannabinoids, and the current state of clinical trials on cannabinoids for various human diseases.

Search Strategy

Results from the search strategies using the titles of each sub-heading as search terms and selection criteria in PubMed (accessed May 6, 2023) for all available years are being presented subsequently.

Cannabinoids in Neuroprotection – Cell and Tissue Models

Cannabinoids encompass a diverse range of molecules and elicit diverse functions in the CNS such as neuronal development, function, and viability (Sarne and Mechoulam, 2005). *In vitro* studies have uncovered a role of endocannabinoids in tissue development in the CNS. Better understanding the role they play on development is important as the recreational use of cannabis has a possible effect on neuronal development (Galve-Roperh et al., 2013).

A balance between enzymes involved in eCB biosynthesis and degradation regulating the optimal eCB concentration elicit neuroprotection in ischemic, inflammatory, and traumatic injuries (Sarne and Mechoulam, 2005). Several *in vitro* studies have determined that cannabinoids and eCBs exhibit neuroprotective properties either via a CB1/CB2 receptor-dependent or CB1/CB2-independent mechanism (Nagayama et al., 1999; van der Stelt et al., 2001; Marsicano et al., 2002; Mauler et al., 2003; Duncan et al., 2009). A few ubiquitous pathophysiological processes occur in the CNS, including excitotoxicity, oxidative stress, and inflammation. The combination of these pathophysiological processes can contribute to the development and/or progression of several neurodegenerative diseases. As such, studies have been conducted to determine whether cannabinoids and/or eCBs are neuroprotective against these pathophysiological processes.

Excitotoxicity

Excitotoxicity is a neuronal injury that occurs from over activation of ionotropic glutamate receptors in neurons leading to excessive intracellular calcium concentrations that trigger cell death. Diseases that exhibit excitotoxicity as a pathophysiological event include seizures, ischemic stroke, and trauma, but excitotoxicity can play a role in many other neurological disorders and diseases.

Phytocannabinoids can elicit neuroprotection in models of excitotoxicity. CBD and THC protect rat cortical neurons against glutamate toxicity produced by ionotropic glutamate receptor over-activation independent of cannabinoid receptor function (Hampson et al., 1998). Cannabinoids also inhibit peroxide-induced oxidative damage like other antioxidant compounds and have been shown to be more effective than ascorbate or α-tocopherol against glutamate toxicity (Hampson et al., 1998).

Organotypic cultures are *ex vivo* model systems consisting of a piece of tissue, particularly from an organ, that is maintained in culture long enough for experimental treatments or interventions to be performed. Studies utilizing explant or organotypic culture systems have been proved useful in determining whether cannabinoids/endocannabinoids can reduce excitotoxicity in *ex vivo* models of epileptic activity. CBD attenuated kainite mediated CA3 injury in rat organotypic hippocampal slice cultures (OHSCs)

Table 3 | **Neuropathology/neuroprotection studies using *in vitro*/*ex vivo* models**

Pathology	Model	Pathology	eCB/CB	Target	Reference
Excito-toxicity	Rat cortical neurons	Glutamate toxicity	CBD, THC	AO function	Hampson et al., 1998
	OHSCs	4-Aminopyridine-induce seizures	CBD	Not determined	Jones et al., 2010
		NMDA-induced excitotoxicity	2-AG, PEA	GPR55, GPR18 and GPR119 (2-AG); PPARα (PEA)	Hohmann et al., 2019
		NMDA-induced excitotoxicity	AM-404	Reduced glutamate release increase in 2-AG, AEA (via presynaptic CB1R?)	Saliba et al., 2019
		Kainate excitotoxicity	CBD	5HT1A, TRPV1/2, PPARγ	Landucci et al., 2022
	Retina explants	Glutamte excitotoxicity	LLEA	Not determined	Duncan et al., 2010
Oxidative Stress	HT-22, cerebellar granule cells	Glutamate-, peroxide-induced oxidative stress	CBD, THC, WIN 55212, CP55940	Direct AO effect	Marsicano et al., 2002
	HT-22 cells	Peroxidative stress	PEA	Unknown- possibly Akt activity	Duncan et al., 2009
Ischemia	HT-22 cells	OGD	CBD	Not determined	Sun et al., 2017
	Primary cortical neurons	OGD	*N*-stearoyl-tyrosine	Reduced FAAH activity, inhibition of anandamide transporter	Cui et al., 2017
	Primary cortical neurons	OGD	WIN 55212-2	CB1/CB2-independent	Nagayama et al., 1999
	OHSCs	OGD	THC, CBD	PPARγ, 5-HT1A and TRPV2	Lana et al., 2022
		OGD	Bedrocan®, THC, CBD	CBD neuroprotection involved PPARγ, 5-HT1A and TRPV2 THC toxicity involved CB1	Landucci et al., 2021
Inflammation	OHSCs	Secondary to kainate excitotoxicity	CBD	5-HT1A, TRPV1/2, and PPARγ	Landucci et al., 2022
		Secondary to NMDA excitotoxicity	2-AG, AEA, THC and AM630	Inhibited microglial chemotaxis (2-AG) via CB2	Cabral et al., 2008
		LPS	CBD	Inhibited LPS-mediated vasodilation, reduced leukocyte margination	Ruiz-Valdepeñas et al., 2011
Infection	Amoeba	Infection	eCBs	Inhibited amoeba chemotaxis & growth via CB2	Dey et al., 2010
AD/PD	HEK293	Hyperphosphorylated tau	JWH133	Decreased tau phosphorylation	Wang et al., 2018
	STHdhQ7/Q7 cells	thapsigargin	CBD	Increased cell viability	Patel et al., 2022

2-AG: 2-Arachidonoylglycerol; 5-HT1A: 5-hydroxytryptamine 1A; AD: Alzheimer's disease; AEA: arachidonoylethanolamide; AO: Antioxidant; CB: cannabinoid; CB1/CB2: cannabinoid receptor 1/cannabinoid receptor 2; CBD: cannabigerol; eCB: endocannabinoid; FAAH: fatty acid amide hydrolase; LLEA: linoleoylethanolamine; LPS: lipopolysaccharide; OGD: oxygen-glucose deprivation; OHSCs: organotypic hippocampal slice cultures; PD: Parkinson's disease; PEA: palmitoylethanolamine; PPARγ: peroxisome proliferator antigen receptor γ; THC: Δ9-Tetrahydro-cannabinol; TRPV2: transient receptor potential V1 or vanilloid receptor.

while THC worsened hippocampal damage. CBD neuroprotection was inhibited by antagonists of receptors, 5-hydroxytryptamine 1A (5-HT1A), TRPV1/2, and PPARγ, suggesting that these are targets for CBD (Landucci et al., 2022).

The impact of CBD treatment was determined *in vitro* with multielectrode array recordings utilizing an Mg^{2+}-free and 4-aminopyridine epilepsy model in OHSCs (Jones et al., 2010). Using the Mg^{2+}-free model, CBD was shown to reduce amplitude of local field potential (LFP) burst (in dentate gyrus (DG) and CA1 hippocampal regions) and decrease duration of LFP bursts and increase frequency of LFP burst (in all regions). In a 4-aminopyridine model of epilepsy, CBD treatment reduced amplitude (in CA1), duration of burst (in DG and CA3) and frequency of bursts (all regions) (Jones et al., 2010).

Like phytocannabinoids, eCBs can elicit neuroprotection against *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity. 2-AG or palmitoylethanolamine (PEA) reduced microglial cell activation and neuronal damage after excitotoxicity in OHSCs (Hohmann et al., 2019). 2-AG activation of abnormal cannabidiol (abn-CBD) receptors, which include GPR55, GPR18 and GPR119, led to neuroprotection while PEA neuroprotection was mediated by PPARα. While 2-AG and PEA themselves were neuroprotective, co-application was not (Hohmann et al., 2019). PEA and 2-AG had opposing activities on nitric oxide generation. No changes in microglial proliferation occurred after treatment (Hohmann et al., 2019). PPARα distribution was affected by the addition of PEA or 2-AG, but the expression of PPARα was unaltered.

Like phytocannabinoids and endocannabinoids, synthetic cannabinoids can elicit neuroprotection against excitotoxicity. The activity of *N*-arachidonoylphenolamine (AM404), an AEA/NAE transporter, on NMDA-mediated Ca^{2+} release was measured in hippocampal slices (Saliba et al., 2019). AM404 reduced NMDA-mediated cell death by reducing glutamate release, Ca^{2+} signaling, and interleukin-1β expression (Saliba et al., 2019).

In glaucoma, the neuroprotection of retinal ganglion cells (RGCs) has been an approach to decrease the severity of the disease. Excitotoxicity is one of the contributors to RGC death and treatment of retinal explants with *N*-linoleoylethanolamine (LLEA) reduced RGC apoptosis due to glutamate excitotoxicity (Duncan et al., 2010). Taken together, the studies discussed above and others provide strong evidence that CBD and eCBs, and possibly THC, in some cases, provide neuroprotection against excitotoxicity.

Oxidative stress

Oxidative stress, caused by excessive reactive oxygen species, is implicated in many diseases. While this section focuses on the effects of cannabinoids and eCBs in models of oxidative stress, the results of the studies reviewed may be relevant to several of the neurodegenerative diseases discussed below. Endocannabinoids cannot act as direct antioxidants due to their molecular structure, but phytocannabinoids have a multi-ring structure containing a phenolic group that can act as a direct antioxidant.

The mouse hippocampal cell line HT22 has been used for several years to study glutamate- and peroxide-induced oxidative stress. The phenolic structure appears to be required for the antioxidant activity of cannabinoids (Marsicano et al., 2002). Results from CB1-overexpressing HT22 cells and cultured cerebellar granule cells indicate that the antioxidant neuroprotective effects of cannabinoids are independent of CB1 (Marsicano et al., 2002). Endocannabinoid NAEs protect neuronal cells from oxidative stress by activating pro-survival signaling pathways (Duncan et al., 2009). Palmitoylethanolamine (PEA) protected HT22 cells from oxidative stress via phosphorylation and nuclear translocation of Akt and upregulation of ERK1/2 expression (Duncan et al., 2009). The protective effects described in this study were CB2 receptor independent.

Many neuronal cell lines have limitations including a lack of a phenotype similar to primary neurons. The aforementioned human neural stem cell derived line, ReN, can grow in the presence of growth factors and then be differentiated with growth factor withdrawal. ReN cells have been used to measure how oxidative stress mediates the expression of multiple proteins involved in cannabinoid signaling (Duncan et al., 2022). Mild, non-lethal, oxidative stress upregulates the expression of CB1, CB2, FAAH, NAAA and NAPE-PLD, supporting the idea that they can defend against oxidative stress in neurons derived from progenitor cells (ReN cells). Oxidative stress in ReN cells results in the nuclear localization of FAAH, an enzyme that degrades eCBs (Duncan et al., 2022).

Hypoxia/oxygen-glucose deprivation

CBD neuroprotective potential was determined using an oxygen-glucose deprivation and reperfusion (OGD/R) paradigm in a mouse hippocampal neuronal cell line (Sun et al., 2017). During reperfusion, CBD treatment prevented OGD/R-mediated cell death, reduced ROS production and lipid peroxidation. CBD improves basal respiration, oxygen consumption rate, glucose utilization, and spare respiratory capacity (Sun et al., 2017). *N*-stearoyltyrosine, an AEA analogue, exhibited neuroprotection against OGD-mediated injury accompanied by reduced FAAH activity and inhibition of the anandamide transporter (Cui et al., 2017). In addition, the synthetic CB1/2 agonist, R(+)-WIN 55212-2, protected cultured cortical neurons from hypoxia and glucose deprivation *in vitro* and this effect was non-stereoselective and was not affected by antagonists of CB1 and CB2 receptors (Nagayama et al., 1999).

Organotypic hippocampal slice cultures from rat brains have been widely used as an explant model to study ischemic injury. CBD or THC activity in rat OHSCs subjected to OGD was determined (Lana et al., 2022). Expression of neuronal TRPV2 was diminished after OGD, but was elevated in activated microglia (Lana et al., 2022). CBD augmented the expression of TRPV2, reduced microglia phagocytosis, and amplified microglia following OGD (Lana et al., 2022). THC had opposing effects to those of CBD as it exacerbated the ischemic effects. CBD elicited neuroprotection generated in part by TRPV2 channels. Incubation

with the Bedrocan® cultivar extract, which has a low concentration of CBD or incubation with THC, increased CA1 injury elicited by OGD (Landucci et al., 2021). Δ9-THC toxicity was blocked by antagonists of CB1. CBD administration, on the other hand, mediated neuroprotection that was inhibited by PPARγ, 5-HT1A and TRPV2 antagonists (Landucci et al., 2021). Altogether, these studies suggest that CBD and eCBs provide neuroprotection against OGD.

Inflammation/infection

CB1 is expressed at basal levels in the CNS, while CB2 is upregulated during inflammation, particularly in microglia, which exerts an important function during inflammation (Cabral and Griffin-Thomas 2008). Cannabinoid receptors may play a role in the prevention of neuropathological diseases, such as multiple sclerosis, Alzheimer's disease, traumatic head injury, amyotrophic lateral sclerosis (ALS) and HIV encephalitis (reviewed in Cabral and Griffin-Thomas 2008). CB2R is upregulated during inflammation in brain tissue, predominantly in microglia, and primary brain microvascular endothelial cells (BMVECs) (Cabral et al., 2008; Ramirez et al., 2012). The addition of CB2 agonist increased transendothelial electrical resistance and upregulated tight junction proteins. CB2 agonists reduced vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 surface expression in brain microvascular endothelial cells when exposed to proinflammatory conditions (Ramirez et al., 2012).

In rat OHSCs, CBD inhibited microglial activation from an M0 transition toward an M2 phenotype (Landucci et al., 2022). Constitutive expression of CB1 is low while CB2 expression is elevated. Elevated expression of CB2 is associated with primed microglia. 2-AG can initiate microglial chemotaxis via CB2 (Cabral et al., 2008). These data suggest that CB2 may be therapeutic target to prevent inflammation in the CNS.

In vitro, treatment with a dual CB2/PPARγ agonist, VCE-004.8, inhibited microvascular endothelial cell barrier dysfunction under proinflammatory conditions (Navarrete et al., 2022).

Several eCBs reduce the growth of amoebae at low doses (Dey et al., 2010). THC and CP55940 reduce the chemotactic activity of microglia to *Acanthamoeba culbertsoni*, the cause of granulomatous amoebic encephalitis, via CB2R activation (Cabral et al., 2008). THC can also reduce the *in vitro* growth of some pathogenic amoebae and worsens amoebic encephalitis in animal models (Dey et al., 2010). Overall, in addition to reducing inflammation, CB2 activation may reduce the severity of amoebic encephalitis.

Neurodegenerative diseases

In HEK293 tau cells, the CB2 agonist, JWH133, decreases tau phosphorylation, but the effect is blocked by AMPK inhibition (Wang et al., 2018). In transfected HEK-293T cells, CB2-orexin receptor complexes have been identified (Raïch et al., 2022). The OX1R antagonist, SB334867, augments activation of CB2 by JWH133, which is also observed in transfected HEK-293T cells and microglia, and even more prominent in the APPSw/Ind animal model of Alzheimer's disease (Raïch et al., 2022). This study suggests that CB2 may be a potential therapeutic target for treating some pathophysiological aspects found Alzheimer's disease.

The aggregation of misfolded endoplasmic reticulum (ER) proteins is a pathophysiological feature common to many neurodegenerative diseases. The unfolded protein response and ER stress lead to apoptosis. In a study by Patel et al. (2022), mouse STHdhQ7/Q7 striatal cells were exposed to thapsigargin, an ER stress inducer, and/or CBD. And the cells pretreated with CBD prior to exposure to thapsigargin showed increased cell viability (Patel et al., 2022).

R6/2 mice express fragment of the human huntingtin (HTT) protein with multiple CAG repeats which makes them a useful mouse model to study Huntington's disease (HD). PET resonance spectroscopy analysis of *ex vivo* striatal cultures from R6/2 mice exhibited changes in predictive indicators typical in HD patients, some of which were prevented by administration of the nabiximols oromucosal spray (THC and CBD in a 1:1 ratio) (Valdeolivas et al., 2017).

Cannabinoids in Neuroprotection – Animal Models

Excitotoxicity and seizures

Animal studies are useful to determine whether cannabinoids can elicit neuroprotection against excitotoxicity (**Table 4**). In a longitudinal study with magnetic resonance imaging, THC decreased excitotoxic nerve cell damage in neonatal rats intracerebrally injected with ouabain, a Na^+/K^+-ATPase inhibitor and inducer of seizure activity (van der Stelt et al., 2001). THC diminished the volume of edema and reduced neuronal damage (van der Stelt et al., 2001). Co-administration of the CB1 antagonist SR141716 with THC blocked the THC-mediated neuroprotection. In addition, THC-treated rats showed a reduction in the volume of astrogliotic tissue (van der Stelt et al., 2001).

Animal studies are also critical to determine whether eCBs can reduce epileptic activity in models of epilepsy. Temporal lobe epilepsy is often characterized by epileptic foci in the limbic system, a precipitating injury, a period of latency, and sclerotic lesions in the hippocampus causing neuronal network remodeling (Curia et al., 2008).

CBD effects were determined *in vivo* using the pentylenetetrazole model of generalized seizures. CBD produced anticonvulsant effects with decreased incidence of severe seizures and mortality (Jones et al., 2010). In the pilocarpine model, status epilepticus is followed by a latent period followed by the development of spontaneous recurrent seizures (Curia et al., 2008). The use of these models could prove useful in studying the efficacy of cannabinoids and eCBs in epilepsy.

Synthetic cannabinoids are being tested for their ability to treat multiple neurodegenerative diseases. The benefits of developing and using synthetic cannabinoids against disease include their potential specificity (selectivity) for a particular target protein and their potential superior distribution and resistance to degradation compared to phytocannabinoids. HU-211, a synthetic non-psychotropic cannabinoid, exhibits NMDA receptor antagonism which can reduce seizure activity (Shohami et al., 1993).

Oxidative stress

Oxidative stress is a ubiquitous pathophysiological condition that occurs when oxidative damage reaches a point where the cell's antioxidant system cannot keep up. Oxidative stress occurs in most diseases and is co-pathology of excitotoxicity, inflammation and trauma. Iron can catalyze the generation of highly reactive hydroxyl radicals from reactive oxygen species. Iron overload in the brain induces oxidative stress and memory deficiency in adult rats. da Silva et al. (2018) investigated whether iron overload during the neonatal period resulted in long-term consequences in memory function in adult rats. Postnatal male rats received iron carbonyl which induced the expression of several pro-apoptotic proteins and CBD administration prevented the iron-mediated effects (da Silva et al., 2018).

Hyperglycemia can induce endocannabinoid system (ECS) perturbations and preclinical studies suggest that the ECS can affect oxidative stress, inflammation and tissue injury mediated by diabetes (Gruden et al., 2016). The neuroprotective properties of CB1 inhibition and CB2 activation were determined in diabetes-mediated (using a streptozotocin (STZ)-induced diabetes model) retinal toxicity (Spyridakos et al., 2022). CB2R activation (with AM1710), CB1 inhibition (with SR141716), or dual treatment (SR141716/AM1710) reduced diabetes-induced nitrative stress, a form of free radical stress. Each type of single exposure protected RGC axons and decreased vascular permeability, while CB2 activation (AM1710) alone prevented all toxic effects (Spyridakos et al., 2022).

Ischemia and stroke

Animal studies have proved useful for determining whether eCBs are neuroprotective in models of ischemic stroke (**Table 4**). The middle cerebral artery occlusion (MCAO) model in rats has been utilized extensively to induce ischemia-reperfusion injury *in vivo*. Use of this model led to the discovery of many potential neuroprotectants including cannabinoids and eCBs. Several studies with NAE eCBs have revealed a neuroprotective effect in stroke models. For example, PEA administration prior to or following stroke diminished cortical and subcortical infarct volume and was associated with improved neurological function (Garg et al., 2010). The neuroprotection was CB1 and VR1 independent. AM404 administration to prevent NAE uptake prevented PEA-elicited neuroprotection, indicating that its effects are mediated through an intracellular mechanism or via TRV1 activation or COX1/2 inhibition (Garg et al., 2010). PEA decreased the number of apoptotic cells and decreased expression of NFκB and inducible nitric oxide synthase.

The non-cannabinoid NAE, LLEA, was tested for its ability to protect against ischemia-reperfusion injury in a rat MCAO model. LLEA administration prior to ischemia/reperfusion (I/R) injury led to decreased cortical infarct volume and improved neurological deficit score (Garg et al., 2011). The plant derived NAE, lauroylethanolamine, improved neurological deficits without reducing lesion size (Garg et al., 2011). Using a lipidomics approach, alterations in the composition and concentration of NAE intermediates were measured in a rat model of cerebral ischemia (Kilaru et al., 2011). Ischemia resulted in elevated levels of PEA and stearoylethanolamine, NAPE, and free fatty acid species. PEA pretreatment decreased infarct volume, neurological deficiencies, and the composition of free fatty acids in ischemic tissues of rats (Kilaru et al., 2011).

Synthetic cannabinoids have been tested in animal models of stroke to determine if they exhibit neuroprotective properties. CB1 agonist (WIN 55212-2) administration reduced the loss of hippocampal neurons following transient global cerebral ischemia and it decreased infarct volume following focal cerebral ischemia elicited by MCAO in rats (Nagayama et al., 1999). The WIN 55212-2-mediated neuroprotection was inhibited by the CB1 receptor antagonist, SR141716A (Nagayama et al., 1999). The CB1 receptor agonist, BAY 38-7271, elicited significant neuroprotection when administered after subdural hematoma (Mauler et al., 2003). BAY 38-7271 generated cortical and striatal neuroprotection in the rat MCAO model decreasing intracranial pressure (Mauler et al., 2003).

FAAH inhibitors prevent the degradation of eCBs leading to an increase in their concentrations. A vascular dementia animal model was created by utilizing bilateral common carotid artery occlusion. Treatment of animals with the FAAH inhibitor, URB597, prevented the reduction in growth factor expression and spatial memory and learning deficiencies induced by bilateral common carotid artery occlusion (Wang et al., 2021).

Table 4 | **Cannabinoid studies in animal models**

Disease model	Animal	Disease model	eCB/CB	Target/mechanism	Reference
Seizures	Rat	Pentylenetetrazole	CBD	Not determined	Jones et al., 2010
	Rat (neonatal)	Ouabain	THC	CB1-mediated neuroprotective effect	van der Stelt et al., 2001
Oxidative stress	Rat (postnatal to adult)	Iron overload (iron carbonyl)	CBD	Prevented loss of memory function	da Silva et al., 2018
	Rat	STZ-induced oxidative stress	SR141716, AM1710	CB1 inhibition (SR141716) and/or CB2 activation (AM1710) reduced nitrative stress	Spyridakos et al., 2022
Stroke	Rat	MCAO	PEA	Neuroprotection was CB1-/VR1-independent but AM404 prevented protection	Garg et al., 2010
	Rat	MCAO	LLEA	Decreased cortical infarct volume and improved neurological deficit score	Garg et al., 2011
	Rat	MCAO	WIN 55512-2, SR141716A	Neuroprotection elicited by CB1 agonist (WIN 55512-2), was inhibited by CB1 antagonist (SR141716A)	Nagayama et al., 1999
	Rat	MCAO	BAY 38-7271	Generated neuroprotection; decreased intracranial pressure	Mauler et al., 2003
	Rat	BCCAO	URB597	FAAH inhibition improved spatial memory and learning; prevented neuronal death	Wang et al., 2021
Inflammation	Mouse	LPS injection	CBD	Inhibited vasodilation, reduced leukocyte margination, blocked TNFα, COX-2 and iNOS upregulation, and maintained integrity of the blood-brain barrier	Ruiz-Valdepeñas et al., 2011
	Mouse	Human PBL/HIVE immunodeficient mice	Gp1a	Gp1a is a CB1 agonist; decreased human cell penetration into the mouse brain	Gorantla et al., 2010
	Rat (neonatal)	Ouabain	THC	THC reduced astogliosis	van der Stelt et al., 2001
	Rat	Borna virus infection	WIN 55,212	Protected proliferating striatal progenitor cells, inhibition of microglia activation; augmented striatal progenitor cell proliferation/survival; increased number of proliferating oligodendrocyte progenitor cells and reduced phagocytic cells	Solbrig et al., 2010
Traumatic brain injury	Mouse	Controlled cortical impact	VCE-004.8	Promoted neovascularization, blocked infiltrations of immune cells, and reduced neuroinflammation and inhibited neuronal death	Navarrete et al., 2022
	Rat	Closed concussive injury	BAY 38-7271	Neuroprotection	Mauler et al., 2003
	Rat	Closed concussive injury	HU-211	Improved motor function in a head trauma model	Shohami et al., 1993
Multiple sclerosis	Mouse	Experimental autoimmune encephalitis (EAE)	THC & CBD	THC and CBD reduced IL-6 and IL-17, elevated immunosuppressive cytokine, IL-10; THC and CBD decrease the Th17 phenotype	Kozela et al., 2013
	FAAH KO mouse	Chronic mouse EAE model	Elevated eCBs	Displayed greater clinical remission compared to wild type mice	Webb et al., 2008
	Mouse	Experimental allergic uveitis model	WIN 55,212, CP55,940, SR141716	CB1 agonists elicited neuroprotection	Pryce et al., 2003
	C57Bl/6 mouse	Cuprizone-induced demyelination	THC	Regeneration of oligodendrocytes, remyelination of white matter, and recovery of motor function	Aguado et al., 2021
	Mouse	Chronic relapsing experimental allergic encephalomyelitis	WIN 55,212, THC, methanandamide and JWH-133	Ameliorated both tremor and spasticity	Baker et al., 2000
Alzheimer's disease	Mouse	$CB2^{-/-}$ mice	n.a.	Tau hyperphosphorylation, mitochondria dysfunction, and memory dysfunction	Wang et al., 2018
	Rat	Okadaic acid (OKA) induced spatial memory impairment	JWH-133	Prevented the increase in caspase-3, Aβ, phosphorylated tau, and IL-1β in cortical and hippocampal regions	Çakır et al., 2019
Parkinson's disease	Mouse	MPTPp	URB597	Inhibited motor impairment via CB1/CB2	Celorrio et al., 2016
	Monkey	MPTP, L-DOPA	n.a.	MPTP & L-DOPA treatments altered the expression of CB1 & 2-AG metaboloc enzymes	Rojo-Bustamante et al., 2018
AMD	Mouse	SOD1G93A mutant mice	WIN55,212-2	WIN55,212-2 (CB1/CB2 agonist) slows disease progression	Bilsland et al., 2006
Huntington's disease	Mouse	R6/2 mouse	Nabiximols	Reduced clasping behavior	Valdeolivas et al., 2017
Pain	Rat	Sciatic nerve constriction; inflammatory pain	CBD	Reduced hyperalgesic effect via TRPV1	Costa et al., 2007
	Rat	Arthritis pain	CBD	Transdermal delivery of CBD reduced inflammation and pain	Hammell et al., 2016
	Mouse	Neuropathic pain	CBD-NLC-gel	Nasal delivery of CBD-nano-structured lipid carrier complex	Matarazzo et al., 2020
Neuropsychiatric disorders	Mouse	Chronic stress model	CBD	Anxiolytic effects, increase in hippocampal neurogenesis	Schiavon et al., 2016
	Mouse	Olfactory bulbectomy model of depression	CBD	Increase in glutamate and serotonin in the ventromedial prefrontal cortex via 5HT1A inhibition	Linge et al., 2015

Aβ: Amyloid β; AMD: age-related macular degeneration; BCCAO: bilateral common carotid artery occlusion; CB: cannabinoid; CBD: cannabidiol; EAE: experimental autoimmune encephalitis; IL-1β: interleukin-1β; IL-10: interleukin-10; IL-17: interleukin-17; IL-6: interleukin-6; KO: knockout; L-DOPA: levodopa; LLEA: *N*-linoleoylethanolamine; LPS: lipopolysaccharide; MCAO: middle cerebral artery occlusion; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPTPp: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid; PEA: *N*-palmitoylethanolamine; STZ: streptozotocin; Th17: T helper cell 17; THC: Δ9-Tetrahydro-cannabinol.

Infection, inflammation and autoimmunity

The ECS plays a role in neurotropic infections and neuroinflammation. CB1 was upregulated in brains with HIV encephalitis and CB2 was upregulated in the preferentially in the white matter (Cosenza-Nashat et al., 2011). CB1 was expressed in neurons, and both CB1 and CB2 were expressed in meningeal macrophages and subpial glia. In brains with HIV encephalitis, CB1 was expressed in microglia and perivascular cells, while CB2 was upregulated in astrocytes, microglia, and perivascular macrophages (Cosenza-Nashat et al., 2011). Chronic viral CNS infections can induce a reduction in prefrontal cortex (PFC) and striatal activity (Solbrig et al., 2010). Chronic HIV-1 infection damages cognitive and motor functions because of leukocyte entry into cerebral perivascular regions, causing blood-brain barrier and neuronal injury (Gorantla et al., 2010). The ECS regulates immune functions as well as neuronal and glial activity. Human PBL/HIVE immunodeficient mice exhibited microglial activation (positive for Iba-1 staining) and elevated CB2 expression in the brain. The CB2 agonist, Gp1a, decreased the penetration of human cells into the mouse brain (Gorantla et al., 2010). Cannabinoids exhibit beneficial effect on vascular endothelial cell function, which is important for blood-brain barrier function and neuronal viability. The use of bacterial lipopolysaccharide (LPS) has been used to elicit some of the effects of bacterial blood infections and septic shock.

For example, CBD inhibited LPS-mediated vasodilation in pia vessels and reduced leukocyte margination (Ruiz-Valdepeñas et al., 2011). CBD blocked LPS-mediated upregulation of TNFα, COX-2 and iNOS and it maintained the integrity of the blood-brain barrier (Ruiz-Valdepeñas et al., 2011).

A common model of CNS-immune system interactions is rats infected with Borna disease virus. Borna disease virus in rats leads to cortical and subcortical infection with motor dysfunction. Borna disease virus infection of the hippocampus inhibits neurogenesis (Solbrig et al., 2008). CB1/2 agonist, WIN 55,212, treatment protected proliferating striatal progenitor cells via inhibition of microglia activation. Proliferating progenitor cell populations were reduced in rats with Borna disease virus. WIN 55,212-2 augmented striatal progenitor cell proliferation and survival. WIN 55,212-2 increased the number of proliferating oligodendrocyte progenitor cells and reduced phagocytic cells (Solbrig et al., 2010).

The myelin oligodendrocyte glycoprotein model of experimental autoimmune encephalomyelitis (EAE) in rats has been useful in identifying potential neuroprotective strategies in demyelinating diseases such as multiple sclerosis. The release of the proinflammatory cytokine, interleukin (IL)-17, from T-helper 17 cells leads to chemokine release and subsequent neutrophil infiltration which can cause local tissue damage. THC and CBD decrease the Th17 phenotype which is increased in Multiple Sclerosis (Kozela et al., 2013). Reactivation of myelin oligodendrocyte glycoprotein 35-55-specific T cells generating EAE resulted in increases in IL-6 and IL-17. These increases in IL-6 and IL-17 could be attenuated by CBD and THC. CBD pretreatment also elevated the concentration of the immunosuppressive cytokine, IL-10 (Kozela et al., 2013). CB1 deficient mice are more susceptible to inflammation and exhibit neurodegeneration in EAE and CB1 agonists elicit neuroprotection against inflammation in an experimental allergic uveitis model (Pryce et al., 2003). Since FAAH regulates the concentration of some endogenous eCBs, FAAH inhibition or knockout may elicit anti-inflammatory and neuroprotective effects (Webb et al., 2008). AEA, PEA and oleoylethanolamine concentrations were increased in mice lacking FAAH displayed greater clinical remission in a chronic EAE model compared to wild type mice (Webb et al., 2008).

THC administration can increase the regeneration of oligodendrocytes, remyelination of white matter, and recovery of motor function (Aguado et al., 2021). THC also initiates axonal remyelination in organotypic cerebellar cultures. THC remyelinating activity was dependent on the differentiation of oligodendrocyte precursor via CB1 (Aguado et al., 2021). In mouse models, cannabinoid receptor agonists reduce spasticity associated with multiple sclerosis (MS) (Baker, et al., 2000). THC administration can prevent the onset of a chronic relapsing experimental allergic encephalomyelitis model of MS in rodents. Overall, these animal studies provide ample evidence that cannabinoids may be potential therapies in the treatment of MS.

Age-related neurodegenerative diseases

The eCB system may play a role in the development or progression of some neurodegenerative diseases. For example, CB2 may play a role in Alzheimer's disease (AD) pathology (Wang et al., 2018). $CB2^{-/-}$ mice exhibit tau hyperphosphorylation, mitochondria dysfunction, and memory dysfunction similar to what is observed in AD. Agonists of AMP-activated protein kinase reversed most changes caused by CB2 deficiency in mice (Wang et al., 2018). In AD animal models, the CB2-orexin receptor (CB2-OX1) complex is elevated in microglia where OX1R inhibition increases the neuroprotective activity of CB2 (Raïch et al., 2022).

In the Okadaic acid (OKA) induced spatial memory impairment rat model, caspase-3, amyloid β, phosphorylated tau, and IL-1β levels were elevated in cortical and hippocampal regions (Çakır et al., 2019). Treatment with the CB2 agonist, JWH-133, prevented the increase in these proteins and improved spatial memory (Çakır et al., 2019).

ECS components are found in the basal ganglia and dopamine depletion alters their function. Inhibition of monoacylglycerol lipase and subsequent elevation of 2-AG reduces glial activation and elicits neuroprotection in a chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration mouse model of Parkinson's disease (Celorrio et al., 2016). The FAAH inhibitor, URB597, was administered to mice treated with the dopaminergic neuron toxin, MPTP and probenecid. URB597 inhibited motor impairment by MPTP and probenecid and were prevented by CB1 and CB2 inhibitors and were absent in CB1/2 deficient animals. Other FAAH antagonists exhibited similar effects (Celorrio et al., 2016).

Levodopa-induced dyskinesia (LID) is a major side effect during the treatment of Parkinson's disease. The basal ganglia nuclei and striatum were dissected from two groups of levodopa treated parkinsonian monkeys which exhibited dyskinesia (Rojo-Bustamante et al., 2018). There were changes in CB1 and 2-AG metabolic enzyme expression which indicates that they may be involved in and may be a therapeutic target for levodopa-induced dyskinesia (Rojo-Bustamante et al., 2018).

Genetic diseases of the CNS

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with selective loss of motor neurons in the spinal cord, brainstem, and motor cortex. Post-symptomatic treatment with WIN55,212-2 slows disease progression in SOD1 mutant mice (SOD1G93A) (Bilsland et al., 2006). In addition, genetic knockout of FAAH blocked the onset of disease in post-symptomatic SOD1G93A mice, but it did not increase lifespan. Knockout of CB1, however, had no impact on disease onset but increased lifespan, suggesting that the neuroprotective effects may be through a CB1-independent mechanism (Bilsland et al., 2006).

Cannabinoids are also neuroprotective in models of HD (Valdeolivas et al., 2017). The R6/2 mouse strain is a model for HD as they exhibit poor performance in rotarod tasks and increased clasping behavior suggestive of dystonia. Nabiximols given to R6/2 mice during the initial motor symptom onset provided some improvements. Treatment with nabiximols reduced clasping behavior but had no effect on rotarod performance (Valdeolivas et al., 2017). Positron emission tomography carried out on R6/2 mice show decreased basal ganglia metabolism which was reversed by treatment with nabiximols.

Traumatic brain injury

Systemically administered PEA stabilized in rats the amplitude of cortical spreading depression, which can be caused by traumatic brain injury, indicating that mechanism other than direct modulation of neuronal excitability, such as control of inflammation, could be relevant to eCB activity in the CNS (Richter et al., 2016). In a rat traumatic brain injury model, administration of the CB1/CB2 agonist, BAY 38-7271, was neuroprotective (Mauler et al., 2003). The synthetic cannabinoid, HU-211 improved motor function in a head trauma model by increasing beam walking ability and reducing blood-brain barrier breakdown and cerebral edema (Shohami et al., 1993).

Similarly, in a model of closed concussive injury, the CB2/PPARα dual agonist, VCE-004.8, reduced cerebral edema by maintaining blood-brain barrier integrity and prevented initial motor deficiencies following injury (Navarrete et al., 2022). VCE-004.8 promotes neovascularization, blocked infiltrations of immune cells, and reduced neuroinflammation and inhibited neuronal death in the affected area (Navarrete et al., 2022).

Vision

The ECS is found in most tissues of the eye and is important for the visual information processing in numerous species, including humans. Cannabinoid receptors are expressed in both the glutamatergic and GABAergic pathways in the monkey retina (Bouskila et al., 2021). The eCB system mediates normal retinal function. Typical (canonical) cannabinoid receptors are comprised of CB1 and CB2 while atypical (non-canonical) cannabinoid receptors include GPR55 and TRPV1 (Bouskila et al., 2021).

Intravitreal administration of the CB1 inverse agonist, AM251, or the CB2 inverse agonist, AM630, increased ERG photopic (light) and scotopic (dark) responses while the GPR55 agonist, lysophosphatidylglucoside, elevated the scotopic response while the GPR55 antagonist, CID16020046, reduced the scotopic response (Bouskila et al., 2021). Agonists of GPR55 can enhance night vision in monkey rod photoreceptors and augment TRPV1 to maintain contrast perception by enhancing horizontal cell lateral inhibition (Bouskila et al., 2021).

Numerous studies have determined the neuroprotective properties of phytocannabinoids, and eCBs, in retinal diseases (Rapino et al., 2018). The presence of eCBs and respective receptors was identified in the retina of many species and their role in visual processing has been determined, with an emphasis on retinal neurodegeneration and neuroprotection (Rapino et al., 2018).

Endogenous administration of eCBs has been used to determine neuroprotective effects in a variety of models. A PEA emulsion in sterile corn oil was administered via depot injection into young DBA/2 mice, a commonly utilized mouse model of glaucoma, to increase tissue levels of PEA. PEA concentrations were elevated in blood serum, heart, brain, and retina. Elevated concentrations of other NAEs were also detected likely due to the entourage effect of PEA, as NAE levels in the retina can be controlled therapeutically (Grillo et al., 2013; Montgomery et al., 2016). These data suggest that systemic administration of PEA via depot injection may present a potential therapeutic strategy for increasing NAE-mediated neuroprotection.

Endocannabinoid receptors, CB1 and CB2 receptors, GPR55, and TRPV1, are differentially distributed in the retina of the vervet monkey (Bouskila et al., 2021). By activating the CB1 receptor in the monkey retina, cells were protected from AMPA excitotoxicity. By activating the CB2 receptor in Müller cells of the monkey retina, cytokines, nitric oxide, and reactive oxygen species production can be regulated (Bouskila et al., 2021).

A study quantified NAEs and three oxylipin derivatives in retinas from young and aged DBA/2Crl mice (Montgomery et al., 2016). AEA concentration was reduced and the AEA oxylipin derivative, 15(S)-HETE ethanolamide, was elevated in aged mice. Depletion of AEA by 15-lipoxygenase and subsequent elevation of 15(S)-HETE ethanolamine suggests a possible role in the reduced visual function in glaucomatous mice (Montgomery et al., 2016). Overall, these studies suggest that the ECS and cannabinoids may be potential therapeutic targets in retinal diseases.

Pain

In animal models of arthritis, cannabinoids reduce joint injury (Mbvundula et al., 2006). CBD exhibits anti-inflammatory and immunomodulatory activity and may elicit therapeutic benefits on chronic sciatic nerve constriction (neuropathic pain) and inflammatory pain (Costa et al., 2007). The anti-hyperalgesic effects of CBD in animals with neuropathic pain were blocked by capsazepine, a TRPV1/vanilloid receptor antagonist. CBD reduced prostaglandin E(2) (PGE(2)) and lipid peroxides, but not the activation of NFκB

and tumor necrosis factor alpha (TNFalpha) expression (Costa et al., 2007). CBD reduces inflammation and pain, but because of its low oral bioavailability, topical CBD application has been used to improve plasma levels by averting first pass metabolism. Transdermal CBD gel decreased joint swelling, pain, and thickening of the synovial membrane (Hammell et al., 2016). Paw withdrawal latency improved to near normal levels and there were decreases in inflammatory proteins in the spinal cord (Hammell et al., 2016).

The delivery strategy for CBD has been studied in animal models of pain. For example, a positively charged nanostructured lipid carrier (NLCs) for the nasal delivery of CBD was developed (Matarazzo et al., 2021). Two formulation, CBD-NLC (a solution) and CBD-NLC-gel (a gel), were tested and both exhibited high mucoadhesion and similar *in vitro* drug release profiles. Intranasal administration of the CBD-NLC formulation generated a greater and longer lasting antinociception in animals with neuropathic pain compared to treatment with the CBD solution. These results suggest that gelling hydrogels are poor vehicles for lipophilic drugs including CBD, while cationic CBD-NLC shows potential for nasal delivery of CBD (Matarazzo et al., 2021). Altogether, in animal models of pain, CBD showed promise in reducing neuropathic and inflammatory pain and new CBD delivery strategies may make CBD treatments in humans more useful.

Neurogenesis in depression

CBD plays a role in synaptic plasticity, promotes neurogenesis and has positive effects on reducing some psychotic-, anxiety- and depressive-like behaviors (Campos et al., 2016). Antidepressant effects arise in part from the promotion of neurogenesis (Schiavon et al., 2016). CBD treatment exhibits anxiolytic-like effects in chronically stressed animals and is associated with an increase in hippocampal neurogenesis. In non-stressed Swiss mice, repeated low dose CBD administration increased neurogenesis in the DG and sub ventricular zone (SVZ), but high dose CBD administration reduced neurogenesis (Schiavon et al., 2016). CBD administration to olfactory bulbectomy model of depression (OBX) in mice reduced hyperactivity and anhedonia suggestive of antidepressant activity (Linge et al., 2015). CBD treatment elevated glutamate and serotonin levels in the ventromedial prefrontal cortex and this was blocked by 5-HT1A receptor inhibition (Linge et al., 2015).

Neural regeneration and regenerative medicine approaches

Stem cell-based therapies have become a promising strategy for replenishing damaged cells in tissues and are especially attractive for the use in replacing non-regenerative cells such as neurons (Zimmermann et al., 2018). As a result, it is important to determine the roles that cannabinoids and eCBs play in cellular growth, differentiation and migration.

CB1 and CB2 are expressed in stem cells and neural progenitor cells (NPCs) early in embryonic development and are critical for NPC proliferation and neuronal development (Galve-Roperh et al., 2013). The expression of CB1 increases and CB2 decreases during neuronal differentiation. CBRs are also expressed in mesodermal and mesenchymal stem cells, indicating that the eCB system could be an important therapeutic target in human diseases (Galve-Roperh et al., 2013). eCB signaling is involved in neural progenitor cell proliferation and subsequent neurogenesis. In HiB5 hippocampal NPCs, activation of CB2 with HU-308 led to AKT-mTOR1 activation and NPC proliferation (Palazuelos et al., 2012).

The role that eCBs, CB1, and monoacylglycerol play in Müller glia transition to progenitor-like cells was investigated in mouse and chick models (Campbell et al., 2021). The endogenous CB1 agonists, 2-AG and AEA, induced the formation of Müller glia progenitor cells. NFκB expression in damaged mouse retinas was decreased by CB1 activation but not in retinal microglia (Campbell et al., 2021).

Increased endogenous 2-AG generation in neurosphere-derived neuroblasts initiates surges of neuroblast motility that move further distances and exhibit less frequent turning as well as a decrease in neuron-to-neuron contacts (Turunen et al., 2018). Differentiation of SVZ and DG neurons was promoted by activation of CB1 and/or CB2 via endogenous BDNF (Ferreira et al., 2018). CB2 activation with HU-308 led to NPC proliferation in embryonic cortical slices (Palazuelos et al., 2012). In SVZ cells, BDNF effects were blocked via CB1 or CB2, while in DG cells it was via CB2 (Ferreira et al., 2018). In mouse hippocampal organotypic slices, activation of CB1 promotes the formation and stabilization of inhibitory boutons (Liang et al., 2021). Inhibitory bouton development was mediated by elevated cAMP concentrations and activated stimulatory G-proteins. PKA inhibition blocked CB1 receptor-mediated bouton development indicating that axonal CB1 receptors may be coupled to different second messengers (Liang et al., 2021).

Neural stem cells (NSCs) contain a functional ECS, including CB1. Using triple-transgenic mice with conditional inactivation of CB1 in NCSs, it was determined that CB1 deficiency in NSCs leads to reduced stem cell proliferation and fewer neurons in newborns (Zimmermann et al., 2018). Neuronal differentiation reduced the degree of dendritic maturation suggesting a postsynaptic function of CB1. Impaired neurogenesis in NSC-specific CB1 knockouts led to decreased hippocampal long-term potentiation (Zimmermann et al., 2018). Human astrocyte spheroids, derived from the D384 astrocyte cell line, were treated with the synthetic cannabinoid, MAM-2201 which altered the cell growth, morphology, and viability (De Simone et al., 2023). Since stem cell therapy holds promise as a new therapeutic paradigm for the treatment of human diseases, determining the role of cannabinoids and their receptors in stem cells has provided opportunities to potentially improve stem cell therapy.

Cannabinoids in Human Disease

A systematic review of preclinical studies with CBD and neurological disorders was carried out where CBD molecular targets were explored for their possible use in neurological therapeutics with variable results (Ibeas Bih et al., 2015). While over 65 molecular targets for CBD were reported, few were likely targets for CBD activity in neurological disorders. It was concluded that CBD was unlikely to elicit effects in neurological disorders via alteration of the ECS. Furthermore, other reported molecular targets of CBD are likely not relevant due to its use at high concentrations.

The therapeutic potential of cannabinoids exhibit variability in the quality of evidence depending upon on the type of cannabinoid studied (**Table 5**). CBD exhibits a therapeutic effect for epilepsy, MS and Parkinsonism while there is a moderate degree evidence for dronabinol (synthetic THC) use in chronic pain, appetite and Tourette. Likewise, there is a moderate degree of quality evidence for the use of nabiximols for chronic pain, spasticity and sleep (Bilbao and Spanagel, 2022). eCBs have been studied for their potential neuroprotective and anti-inflammatory effects in Parkinson's disease and Alzheimer's disease, respectively (Cooray et al., 2020).

Although cannabinoids and NAEs exhibit neuroprotective properties in rodent models of ischemic stroke, human clinical studies and clinical trials have not fully supported their use in patients. One clinical study found that intracerebral hemorrhage patients with cannabinoids present in their urine toxicology screen exhibit better outcomes such as milder IHC and less disability at discharge (Di Napoli et al., 2016).

In a major double-blind, placebo-controlled clinical trial, Comprehensive Rimonabant Evaluation Study of Cardiovascular ENDpoints Outcomes (CRESCENDO), the CB1 inhibitor, Rimonabant, was tested to determine if it exhibits therapeutic properties in cardiovascular disease and stroke patients (Topol et al., 2010). There were 18,695 participants from 974 hospitals across 42 countries that were randomly assigned to the Rimonabant versus placebo control group. The trial was prematurely discontinued, however, due to concerning reports of an increase in neuropsychiatric side effects and suicides (Topol et al., 2010). While this study was designed to measure an effect of blocking cannabinoid receptors, it did not address whether cannabinoids, or activation of CB1, would have a positive effect on stroke similar to that observed in animal models.

Epilepsy and other seizure syndrome diseases

CBD elicits anticonvulsant effects in animal models of epilepsy and decreased the frequency of seizure in limited human trials (Jones et al., 2010). To date, there have been numerous studies of CBD as an add-on treatment with traditional anti-seizure medications for drug-resistant forms of epilepsy. Studies were initially performed on children and young adults due to the prevalence of drug resistant epilepsy diseases in pediatric populations for diseases like Dravet syndrome before progressing to adult populations (Devinsky et al., 2016, 2017, 2019; Szaflarski et al., 2018).

A prospective study was carried out to assess the effect of CBD treatment on interictal epileptiform discharge (IED) frequency in children with drug-resistant epilepsy (Klotz et al., 2021). The rate of IEDs at three months was reduced compared to baseline. In addition, sleep microstructure was irregular in 56.5% of recordings, but CBD enhanced sleep in 84.6% of those cases (Klotz et al., 2021). The safety of a CBD transdermal gel was determined in children with encephalopathies and evaluated to measure frequency of seizures, sleep, and quality of life. CBD treatment twice daily was associated with a decrease in the frequency in tonic-clonic seizures (TCS) and focal impaired awareness seizures (FIAS) (Scheffer et al., 2021). Patients exhibited a monthly seizure reduction of 58% at 5 months and 43.5% over the study duration. There were noted improvements in social engagement, sleep, and cognitive function (Scheffer et al., 2021). Another trial studied the effectiveness of two concentrations of CBD delivered via a transdermal patch placed on the skin to adults with drug-resistant focal epilepsy. At 12 weeks, there was no decrease in the number of seizures per month. However, when the study was extended into an open-label period 60.8% of the participants exhibited at least a 50% decrease in seizures (O'Brien et al., 2022). Pharmacogenetic variation is related to CBD efficacy and may affect the expression of CBD target proteins in treatment-resistant epilepsy. In one study, enrolled patients were genotyped and associations between gene variants and CBD tolerability and seizure reduction were determined (Davis et al., 2021). Patients with the aldehyde oxidase (AOX1) rs6729738 CC gene variant (OR 6.69) or the diamine oxidase (ABP1) rs12539 variant (OR 3.96) were more responsive to CBD, while carriers of the solute carrier 15A1 (SLC15A1) rs1339067 TT variant had reduced likelihood of CBD response (OR 0.06) (Davis et al., 2021). The ABCC5 rs3749442 variant was associated with a reduced CBD effectiveness and greater sedation. The study also uncovered that SLC15A1 rs1339067 reduced the expression of GPR18 (an abnormal CB receptor) in white matter, and ABCC5 rs3749442 reduced hippocampal 5-hydroxytryptamine receptor 3E expression (Davis et al., 2021).

CBD is effective in reducing seizures in tuberous sclerosis complex (TSC) patients and other neurological syndromes involving seizures, but little is currently understood regarding its ability to decrease interictal epileptiform activity (Klotz et al., 2021). CBD treatment in human patients TSC experiencing seizures showed positive, significant effects. The largest double-blind, placebo-controlled trial to date showed that for two different high-dose regimens there was a 48.6% reduction in primarily focal seizures per month as opposed to a 26.5% reduction per month in the placebo group (Thiele et al., 2021).

Table 5 | **Human clinical studies and trials on cannabinoids**

Disease	eCB/CB	Trial	Outcome	Reference
Stroke	Rimonabant (SR141716)	Comprehensive Rimonabant Evaluation Study of Cardiovascular ENDpoints Outcomes (CRESCENDO) trial	Trial discontinued due to side effects	Topol et al., 2010
Epilepsy	CBD	Prospective study	Reduced interictal epileptiform discharge frequency; improved sleep	Klotz et al., 2021
		Nonrandomized controlled trial in children aged 3 to 18 years	CBD transdermal gel reduced frequency of tonic-clonic seizures; improved social engagement, sleep, and cognitive function	Scheffer et al., 2021
		Open-label extension trial	CBD transdermal patch led to 60.8% of the participants exhibiting ≥ 50% decrease in seizures	O'Brien et al., 2022
		Genetic study- gene variants	Genetic associations between gene variants, CBD tolerability, seizure reduction were determined	Davis et al., 2021
Tuberous sclerosis complex seizures	CBD	Double-blind, placebo-controlled trial	Reduction in primarily focal seizures/month; 50% of patients had 50% reduction in seizures	Thiele et al., 2021, 2022
	CBD	Randomized, placebo-controlled, phase 3 trial	CBD-mediated decline in seizures emerged on day 6 and by day 10	Wu et al., 2022
Lennox-Gastaut syndrome seizures	CBD	GWPCARE5 open label extension trial	CBD led to 48–71% median decrease in atonic seizures and 48–68% decrease in all seizures	Patel et al., 2021
Multiple sclerosis	THC/CBD oro-mucosal spray	20 MS patients (10 with and 10 without neuropathic pain) with clinical and neurophysiological assessment	Nabiximols reduced pain rating and improved quality of life	Russo et al., 2016
		Pilot, single center, open, and prospective study	Positive subjective effect on MS-related spasticity	Contin et al., 2018
		SAVANT RCT study (*post hoc* analysis)	Reduced the average spasticity and pain severity scores in all subgroups	Meuth et al., 2020
Alzheimer's disease	THC, synthetic cannabinoids	Meta-analysis of clinical studies	Trend toward improvement in agitation with synthetic cannabinoids	Ruthirakuhan et al., 2019
Parkinson's disease	THC, CBD	119 patients evaluated in outpatient clinic	THC had no effect; possible CBD effect in improving quality of life in PD patients	Chagas et al., 2014
Huntington's disease	Cannabinoids	7 early-onset HD patients	Reduction in dystonia and motor symptoms; led to improvement in fine motor skills and gait	Saft et al., 2018
	CBD	Double-blind, randomized cross-over design; 15 neuroleptic-free patients with HD	No effect on chorea severity	Consroe et al., 1991
Pain	Inhaled cannabis vapor (Bedrocan, Bediol, Bedrolite & placebo)	Randomized, placebo-controlled, crossover trial	Bediol reduced spontaneous pain scores	van de Donk et al., 2019
	Inhaled cannabis vapor (THC/CBD)	Randomized crossover trial with individuals with sickle cell disease	No reduction in pain or in pain interference ratings, but improvement in mood	Abrams et al., 2020
	THC (oral)	Randomized placebo-controlled crossover study with evoked pain tasks	THC reduced pain from electrical and pressure stimuli and altered calmness and alertness	van Amerongen et al., 2018
	Oral THC-rich cannabis oil	Double-blind, randomized, placebo-controlled trial with women with fibromyalgia pain	Decrease in the Fibromyalgia Impact Score (reduced pain)	Chaves et al., 2020
	Inhaled THC (metered dose)	Randomized, double-blinded, placebo-controlled, cross-over trial with 27 patients with chronic pain	Decrease in pain intensity	Almog et al., 2020
	Aerosolized THC	Pain from diabetic peripheral neuropathy	THC reduced pain ratings at moderate levels	Wallace et al., 2020
	Sublingual THC administration	Measurement of functional brain changes and THC improvement of chronic neuropathic pain	Decreased in pain associated with anterior cingulate cortex-sensorimotor cortex and dorsolateral prefrontal cortex function	Weizman et al., 2018
	Intravenous THC	Exploratory randomized, double-blind, placebo-controlled, cross-over study using evoked pain in healthy participants	No analgesic effects in hyperalgesia or induced thermal, electrical, chemical, or mechanical pain	Schindler et al., 2020
	Oral CBD	Double-blind, placebo-controlled, clinical study in healthy subjects	No effect on pain threshold/ tolerance in cold pressor test; increased pain ratings	Arout et al., 2022
	oral CBD	Random, double-blinded, placebo-controlled trial on pain associated with surgical procedures	Pain score reduced on first day after procedure; improved patient satisfaction with pain control	Alaia et al., 2022
	Topical CBD (with shea butter)	Therapeutic activity of CBD for thumb basal joint arthritis pain	Improvement in patient-reported visual analogue scale pain rating	Heineman et al., 2022
	Synthetic CBD (add-on therapy)	Randomized, double-blind, placebo-controlled trial for for osteoarthritis or psoriatic arthritis pain	Exhibited no therapeutic effects; did not improve depression, anxiety, or sleep quality	Vela et al., 2022
	Topically CBD oil	Randomized, placebo-controlled, crossover trial for neuropathic pain	Decreased intense and sharp pain, cold and itch	Xu et al., 2020
Neuropsychiatric disorders	CBD	Double-blind, placebo-controlled Pavlovian fear-conditioning study	Improved consolidation of extinction learning	Das et al., 2013
	THC and CBD	Regional brain activation, electrodermal activity, and anxiety ratings to determine effects of THC & CBD on anxiety	THC exacerbated anxiety; CBD decreased anxiety	Fusar-Poli et al., 2009
	Transdermal CBD gel (ZYN002)	Measure effectiveness of transdermal CBD gel, ZYN002, in children with FXS	Decreased the anxiety, depression, and mood scores	Heussler et al., 2019
	CBD	Add-on CBD treatment administered in participants on antidepressant medication	Reduced in anxiety and depression scores after several weeks	Berger et al., 2022
	CBD	Administered to patients with psychosis followed by functional magnetic resonance imaging during a verbal learning exercise	Led to a trend toward a decrease in psychotic symptoms	O'Neill et al., 2021
	AEA	Cerebrospinal fluid (CSF) measured from schizophrenic patients	AEA concentrations in CSF negatively correlated with psychotic symptoms	Leweke et al., 2007

AEA: *N*-arachidonoylethanolamine; CB: cannabinoid; CBD: cannabidiol; eCB: endocannabinoid; FXS: Fragile X syndrome; HD: Huntington's diseases; MS: multiple sclerosis; THC: Δ9-Tetrahydro-cannabinol.

More than 80% of patients, caregivers and physicians indicated an improvement in TSC patient's conditions after 26 weeks of CBD treatment in the form of 25 mg/kg/d CBD treatment (Thiele et al., 2022). Roughly 85% of patients with TSC experience epileptic seizures with initial onset commonly occurring in the first two years of life. Greater than 60% of TSC patients exhibit treatment-resistant epilepsy, which is linked to neurodevelopmental disorders (Thiele et al., 2022). CBD treatment of patients in the study produces a 54% reduction compared to the baseline. Epileptic seizure reduction increased to 61-68% for patients who completed 37–48 weeks of CBD treatment. 50% of patients had a 50% reduction in TSC-associated seizures and 25% of patients had a 75% reduction across a 12-week period. Greater than 5% of patients were free of TSC-associated seizures during this period (Thiele et al., 2022).

The timing of CBD treatment onset was determined in patients with drug-resistant epilepsy related to TSC. Data analyzed from a randomized, placebo-controlled, phase 3 trial revealed that seizure decline between CBD and placebo emerged on day 6 and by day 10, it exhibited a ≥ 50% responder rate (Wu et al., 2022).

As mentioned earlier, CBD is efficacious in reducing seizures in patients with Dravet syndrome and TSC (Klotz et al., 2021; Patel et al., 2021). Lennox-Gastaut syndrome is another epilepsy-associated encephalopathy that is also frequently treatment resistant. CBD showed therapeutic potential for the treatment of seizures associated with Lennox-Gastaut syndrome. Patients who completed the RCT enrolled in an open-label extension trial (GWPCARE5) where they received CBD (Epidiolex) oral solution daily over 2 weeks (Patel et al., 2021). CBD administration led to a 48–71% median reduction in atonic seizures and a 48–68% reduction in all seizures for up to a 156-week period. Eighty-seven percent or more of caregivers or patients described improvements in the patient's condition (Patel et al., 2021).

Multiple sclerosis

In clinical studies, oral THC administration improved muscle spasticity in MS patients (Killestein et al., 2002). The role of nabiximols oromucosal spray (THC and CBD in a 1:1 ratio) in alleviating neuropathic pain in MS patients was determined utilizing clinical or neurophysiological assessment (Russo et al., 2016). Using cannabinoids to treat pain in MS is promising as the side-effects associated with other therapies make them unsuitable for chronic use. Nabiximols are likely to act on the α3 subunit of the glycine receptor, which also play an important role in antinociception (Russo et al., 2016). Post-mortem analysis of the brains of MS patients exhibited reduced expression of CB1 and CB2, suggesting that THC or CBD may be neuroprotective in MS. Nabiximols were also assessed in clinical trials as a therapeutic for spasticity in MS (Pagano et al., 2022).

A prospective study was administered in MS patients chronically treated with THC/CBD to determine the association between plasma concentrations of THC/CBD and clinical effects of THC/CBD administration (Contin et al., 2018). Patients self-rated their spasticity using the Numerical Rating Scale (NRS) during blood draws. Patients' NRS scores decreased following drug administration and an inverse correlation was measured between NRS scores and plasma levels of THC and CBD. THC/CBD treatment had no effect on postural measurements and motor tests (Contin et al., 2018).

A post-hoc analysis on SAVANT RCT study data was administered to determine the effect of add-on THC:CBD oromucosal spray on spasticity severity and duration, status of disability, and the evolution of pain severity over a 12-week period in treatment-resistant MS patients (Meuth et al., 2020). Nabiximols treatment reduced the average spasticity and pain severity scores in all subgroups (Meuth et al., 2020).

Alzheimer's, Parkinson's and Huntington's diseases

Cannabinoid effects on agitation in patients with AD were determined (Ruthirakuhan et al., 2019). A meta-analysis of clinical studies revealed that cannabinoids, as a combined group, had no effect on agitation, but there was a trend toward an improvement in agitation with synthetic cannabinoids over THC (Ruthirakuhan et al., 2019). Cannabinoids exhibited a greater effect on agitation with more pronounced cognitive dysfunction. Cannabinoids had no neuropsychiatric effect, but there was an improvement in patients with a lower BMI. There may be a benefit of synthetic cannabinoids on agitation associated with AD (Ruthirakuhan et al., 2019).

The ECS is expressed at high levels in the basal ganglia making cannabinoid receptors of interest when considering movement control and motor inhibition, especially in PD. In brain samples from post-mortem patients with PD, there was lowered expression of CB1 in the basal ganglia (Sanchez et al., 2018). In clinical studies, THC was not found to have any beneficial effect on patients with PD, but daily administration of CBD was found to alleviate some motor symptoms of PD with an improved quality of life for PD patients (Sanchez et al., 2018).

Cannabinoids may also be a possible therapy for patients with dystonia of a different origin (Saft et al., 2018). Early onset HD patients treated with cannabinoids exhibited a reduction in dystonia and motor symptoms and led to an improvement in fine motor skills and gait. Patients treated with cannabinoids also exhibited reduced apathy and irritability (Saft et al., 2018). The effects of CBD on chorea severity, however, suggest that it has no clinically significant effect (Consroe et al., 1991).

Traumatic brain injury

The efficacy of oromucosal nabiximols as an adjunct therapy was assessed over a 12-week period in children with spasticity resulting from cerebral palsy/traumatic CNS injury (Fairhurst et al., 2020). Nabiximols had no effect on the severity of spasticity or secondary endpoints including sleep quality, pain, comfort, and depression compared to the placebo group (Fairhurst et al., 2020).

Currently, no drugs have been identified that improve severe head injury outcomes. The dual CB1/CB2 agonist, KN38-7271, is neuroprotective in animal models of head trauma (Firsching et al., 2012). A randomized, double-blind, placebo-controlled clinical trial was carried out to measure the potential efficacy of KN38-7271 in patients with severe head trauma (Firsching et al., 2012). KN38-7271 was provided within 4.5 hours of the traumatic injury. Survival rates within a month of the injury increased in the KN38-7271 group compared to the placebo group, but its effect on survival was absent after 6 months. In addition, intracranial and cerebral perfusion pressures were reduced by KN38-7271 (Firsching et al., 2012).

Traumatic spinal cord injury has been difficult to treat as neuronal regeneration is reduced by apoptosis. An injectable hydrogel was established for the local administration of CBD using a method derived from a carboxymethylcellulose- and chitosan-based system (Zhang et al., 2022). This hydrogel can be injected into the spinal cavity as a liquid and gelled within minutes. Its mechanical properties are like that of the spinal cord. CBD-loaded hydrogels sustain CBD delivery for a few days to reduce apoptosis in spinal cord injury by improving mitochondrial biogenesis and increasing neurogenesis, both *in vivo* and *in vitro*, compared to other hydrogels, leading to motor function recovery (Zhang et al., 2022).

Pain

Human studies with cannabinoids and their effects on pain have been carried out for decades. Both THC and CBD appear to affect pain associated with specific conditions including arthritis and chronic neuropathies. Inhaled vaporous cannabis has been tested in clinical trials for its potential pain modifying effects. The effectiveness of THC on fibromyalgia pain has been tested in clinical trials with promising results. In a randomized, placebo-controlled, crossover trial, the analgesic activity of inhaled vaporized varieties of cannabis was measured in fibromyalgia patients. Four varieties of cannabis were examined: Bedrocan (with 22.4-mg THC/< 1-mg CBD), Bediol (with 13.4-mg THC/17.8-mg CBD), Bedrolite (with 18.4-mg CBD/< 1-mg THC) and a placebo version lacking THC or CBD (van de Donk et al., 2019). None of the cannabis varieties affected pain responses, except for Bediol which reduced spontaneous pain scores. Varieties containing high THC elicited an elevation in pressure pain threshold compared to placebo (van de Donk et al., 2019). Another randomized crossover clinical trial determined the efficacy of inhaled cannabis vapor (4.4% Δ-9-THC/4.9% CBD) on chronic pain relief in individuals with sickle cell disease (Abrams et al., 2020). Participants inhaled cannabis (or vaporized placebo cannabis) three times daily. There was no reduction in pain or in pain interference ratings (Brief Pain Inventory and visual analog scale (VAS) score) between cannabis and placebo groups, except for a reduction in interference with mood (Abrams et al., 2020).

Orally delivered THC has been utilized in human clinical studies for its therapeutic potential in reducing pain, although THC delivered this way is subjected to first-pass metabolism. There are a couple of studies carried out that determined the effect of THC on evoked (experimenter-administered) pain. In a randomized placebo-controlled crossover study, a sequence of evoked pain tasks was utilized to determine potential analgesic effects (van Amerongen et al., 2018). The pain tasks included heat, cold pressure, electrical, and inflammatory pain, where participants were orally administered THC or placebo. THC administration led to a reduction in pain from electrical and pressure stimuli and it altered calmness and alertness (van Amerongen et al., 2018). A double-blind, randomized, placebo-controlled clinical trial was carried out for an eight-week period to measure the potential efficacy of THC-rich cannabis oil on symptoms in women with fibromyalgia (Chaves et al., 2020). After an 8-week period, the group receiving daily oral cannabis drops exhibited a decrease in the Fibromyalgia Impact Score compared to placebo and day one baseline score. This result suggests that THC-rich oil can improve pain symptoms associated with fibromyalgia in women (Chaves et al., 2020).

THC inhalation has been studied in human trials as an optimal way of delivering THC in patients experiencing chronic pain. A clinical trial was conducted to determine the analgesic and cognitive effects of precise inhaled THC doses in participants with chronic pain (Almog et al., 2020). THC doses elicited a decrease in pain intensity (determined by VAS score) compared to baseline and placebo. There were also no measured decrements in cognitive function (determined by Cambridge Neuropsychological Test Automated Battery) (Almog et al., 2020). Another study was carried out in subjects with diabetic peripheral neuropathy receiving 1%, 4%, or 7% aerosolized THC dose (or placebo) and exposed to pain tests (Wallace et al., 2020). Participants were provided aerosolized cannabis (or placebo) and pain intensity and cognitive performance was measured. THC administration resulting in reduced pain ratings at moderate, but not low or high, levels of THC. THC dose-dependently affected one out of three cognitive tests (Wallace et al., 2020). Another trial was carried out to characterize the functional brain changes involved in Δ-9-THC improvement of chronic neuropathic pain (Weizman et al., 2018). Functional interaction between the anterior cingulate cortex (ACC) and pain-related network activity was measured. Sublingual THC administration decreased pain which was also associated with diminished anterior cingulate cortex-sensorimotor cortex functional connectivity and in an area correlated with pain reduction, the dorsolateral prefrontal cortex (Weizman et al., 2018).

In another exploratory randomized, double-blind, placebo-controlled, cross-over study, the efficacy of intravenous THC on several painful stimuli was measured in healthy human participants (Schindler et al., 2020). THC exhibited no analgesic effects in capsaicin-elicited hyperalgesia or experimentally induced thermal, electrical chemical or mechanical pain (Schindler et al., 2020).

Unlike THC, CBD is not believed to be a cannabinoid receptor agonist at physiological concentrations. Therefore, reports of the efficacy of CBD in pain relief suggest that its effects may be through a different mechanism of action than THC. Oral CBD has been tested in clinical studies for its potential effects in pain reduction. A double-blind, placebo-controlled, clinical study determined the analgesic effects of acute oral CBD in healthy subjects (Arout et al., 2022). CBD exhibited no effect on pain threshold and tolerance in the cold pressor test. In fact, CBD increased pain ratings and exhibited only a modest improvement in mood (Arout et al., 2022). The efficacy of CBD was also determined in pain associated with surgical procedures. A random, double-blinded, placebo-controlled trial was conducted to measure the analgesic activity of orally absorbed CBD in patients who experienced arthroscopic surgery (Alaia et al., 2022). On the first day, the pain score was reduced in patients receiving CBD but was absent on the following day. Patient satisfaction with pain control was greater in the CBD group, but after one week of CBD administration, the improvements in VAS scores, or satisfaction with pain control the CBD group disappeared (Alaia et al., 2022).

Topical CBD delivery was studied in trials to determine if it elicits local (non-systemic) effects. A human clinical trial was conducted to measure the therapeutic activity of CBD for thumb basal joint arthritis pain (Heineman et al., 2022). Patients were treated twice daily for 2 weeks with topical CBD with shea butter or shea butter alone. CBD treatment led to an improvement in patient-reported VAS pain. There were similar reported improvements in grip and pinch strength and range of motion (Heineman et al., 2022). In another randomized, double-blind, placebo-controlled trial, the effectiveness of a synthetic CBD as add-on analgesic therapy for osteoarthritis or psoriatic arthritis was conducted over a 12-week period (Vela et al., 2022). The synthetic CBD compound exhibited no therapeutic effects, nor did it improve depression, anxiety, or sleep quality scores (Vela et al., 2022). In a randomized, placebo-controlled, crossover trial, the effectiveness of topically delivered CBD oil for improving neuropathic pain was examined (Xu et al., 2020). The Neuropathic Pain Scale was utilized to evaluate changes in pain throughout the treatment interval. CBD administration led to a decrease in intense and sharp pain, cold and itch (Xu et al., 2020).

Neuropsychiatric disorders

CBD exhibits efficacy in neuropsychiatric disorders. CBD effects on extinction learning and consolidation were determined in a double-blind, placebo-controlled Pavlovian fear-conditioning study (Das et al., 2013). Subjects were conditioned to a colored box by using electric shocks in one scenario and were extinguished in a second scenario. CBD was given following before or after extinction (Das et al., 2013). At recall, subjects were exposed to colored boxes and conditioning contexts before and after electric shock exposure. CBD given after extinction improved consolidation of extinction learning. CBD given at either time caused a trend toward a reduction in reinstatement, suggesting that CBD can improve consolidation of extinction learning and that it may be useful as an adjunct treatment for anxiety disorders (Das et al., 2013).

Regional brain activation, electrodermal activity, and anxiety ratings were used to determine the effects of THC and CBD on anxiety (Fusar-Poli et al., 2009). THC exacerbated anxiety, intoxication, and psychotic symptoms, while CBD led to a trend toward a decrease in anxiety. THC administration increased fluctuations in electrodermal activity during the processing of fearful faces, but this was reduced after CBD administration (Fusar-Poli et al., 2009). CBD reduced the regional brain activation in the amygdala and cingulate cortex during the processing of fearful face stimuli, while THC mainly altered activation in frontal and parietal areas. The effect of CBD was associated with the concurrent decrease in fluctuations of electrodermal activity (Fusar-Poli et al., 2009).

Fragile X syndrome (FXS) causes multiple developmental and neuropsychiatric impairments (Heussler et al., 2019). A study was carried out to measure the effectiveness of transdermal CBD gel, ZYN002, administration in children with FXS. ZYN002 administration decreased the anxiety, depression, and mood scores by 12 weeks and reduced the Aberrant Behavior Checklist-Community for FXS and Pediatric Anxiety Rating Scale scores as well as scores for quality of life (Heussler et al., 2019).

Add-on CBD treatment was administered in participants on antidepressant medication (Berger et al., 2022). Subjects receiving add-on CBD exhibited reductions in anxiety and depression scores after several weeks (Berger et al., 2022).

The efficacy of a single oral dose of CBD was determined in patients with psychosis (O'Neill et al., 2021). Shortly after receiving CBD, patients were subjected to functional magnetic resonance imaging during a verbal learning exercise. CBD effects on brain region activation were measured in the prefrontal, mediotemporal, and striatal regions. Subjects with psychosis exhibited specific changes in mediotemporal and prefrontal region activity during verbal encoding. Functional connectivity between mediotemporal and striatal regions was also higher during verbal recall (O'Neill et al., 2021). CBD reduced the psychosis-associated activities in these regions and led to a trend toward a decrease in psychotic symptoms, suggesting that CBD may be effective in reducing symptoms of psychosis.

Cerebrospinal fluid (CSF) from schizophrenic patients contains higher AEA concentrations than from healthy individuals (Leweke et al., 2007). AEA concentrations in CSF are negatively correlated with psychotic symptoms. Schizophrenic low-frequency cannabis consumers have elevated CSF AEA concentrations compared to high-frequency users (Leweke et al., 2007). Although CSF AEA concentrations and symptoms of schizophrenia were inversely correlated, schizophrenic individuals with frequent cannabis exposure exhibit reduced CNS AEA signaling (Leweke et al., 2007). This study suggests that eCBs may play a role in schizophrenia and that eCBs may represent a potential therapeutic option for neuropsychiatric disorders.

Conclusions and Future Directions

Cannabinoid administration elicits powerful effects on many organ systems including the CNS and some of these effects are therapeutic. The major drawback of some cannabinoids, particularly THC and other CB1 agonists, is the complex side effect profile. On the one hand, THC and THC-like synthetics hold some promise in pain and anxiety, but they induce significant cognitive dysfunction. On the other hand, the psychotropic effects of THC and similar synthetics may be partly responsible for their therapeutic effects by altering the perception of pain and anxiety. One of the major challenges for future research is designing synthetic cannabinoids that elicit positive effects of CB1 activation in peripheral neurons and in specific brain regions, but without significant cognitive effects.

The non-psychotropic cannabinoid, CBD, holds perhaps more promise as a therapeutic for some neurodegenerative diseases. The challenge for CBD therapeutics may lie in the controlled delivery to the proper site of action. Studies have clearly demonstrated the safety of CBD, so the design of CBD analogs that are more stable and more potent is a likely future development. Finally, eCBs hold promise for positively affecting CNS dysfunction and degeneration. One approach already studied is the inhibition of enzymes that metabolize eCBs, resulting in higher concentrations at the site where they are induced. Another step forward may also be the development of eCB analogs that exhibit higher potency and that are more resistant to degradation for the treatment of neurodegenerative diseases. Cannabinoid and endocannabinoid research will continue for decades to come, with certain breakthroughs very likely to occur.

Acknowledgments: *We gratefully acknowledged the support of Megan Florance. We also thank Margaret, Richard, and Sara Koulen for generous support and encouragement.*

Author contributions: *PK and RSD conceived and designed the manuscript. All authors contributed to the writing, editing, review of the manuscript, and approved the submitted version.*

Conflicts of interest: *The authors declare no conflicts of interest.*

Data availability statement: *Not applicable.*

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C-Editors: Zhao M, Wang L; T-Editor: Jia Y