UNIVERSIDADE FEDERAL DE SANTA CATARINA – CAMPUS ARARANGUÁ
DEPARTAMENTO DE CIÊNCIAS DA SAÚDE
CURSO DE FISIOTERAPIA

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RODRIGO SEBBEN PAES

Endocannabinoid system as potential targets for neurodegenerative diseases

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Trabalho de conclusão do Curso II de Graduação em Fisioterapia do Centro de Ciências, Tecnologias e Saúde, Campus Araranguá, da Universidade Federal de Santa Catarina para a obtenção do título de bacharel em Fisioterapia.

Orientadores: Profa. Dra. Melissa Negro-Dellacqua e Prof. Dr. Rafael Cypriano Dutra

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Endocannabinoid system as a potential target for neurodegenerative diseases

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Abstract

Neurodegenerative diseases (ND) represent a critical social and economic problem worldwide and are becoming highly prevalent because of the increasing average age of the population. ND is characterized by exacerbated neural death which leads to motor, cognitive or behavioral diss representing an important cause of disability and mortality. Both its development and maintenance have been investigated over the last decades. In most of them, there is a chronic neuroinflammatory state establishment commonly associated with pathological protein aggregation, as evidenced in AD, PD and HD. This neuroinflammatory state is recognized by microglial hyperactivation, inflammatory cytokines storm and neural death, compromising the neurological environment. Accordingly, in the last decades, intensive efforts have been put into investigating the main pathways involved in its development and new pharmacological approaches to management it. Since the ’80s, when cannabinoid receptors (CB1R and CB2R) were discovered, the endocannabinoid system modulation has been investigated in many diseases context in order to the millenary medicinal use of cannabis. Nowadays, have already been described the different roles played by components that make up it, such as antitumoral, regulation of neurotransmission in different brain regions, inflammatory modulation, and regulation of glial cells. In this context, this work aimed to provide a summarized translational review of the most recent studies about the involvement of the ECS in NDs development and maintenance, as well as a therapeutic target.

Keywords: Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, neuroinflammation, immunoinflammatory response.

Introduction

Neurodegenerative diseases (ND) are characterized by progressive loss of selective populations of neurons, which main pathological hallmark is protein aggregation which often leads to the increase in reactive oxygen species (ROS), synaptic dysfunction, and mitochondrial dysfunction, endoplasmic reticulum stress, and neuroinflammation (1–3). These features can be verified in the *postmortem* brain of patients and are also useful to classify the ND according to anatomic distribution (3–5). In general, despite each particularity within pathology, ND shares fundamental clinical signs and symptoms such as some motor, cognitive or behavioral disorders derivate from the exacerbated neural death in specific regions of the brain.

In pathological conditions, there is overproduction and aggregation of some proteins throughout the brain, such as Tau tangles and Amyloid-beta (Aβ) plaques in Alzheimer's disease (AD) progress, and alpha-synuclein (α-Syn) aggregate, known as Lewy bodies, in Parkinson's disease (PD) and Lewy body dementia (LBD) (1). This abnormal protein aggregation leads to neuron and glial dysfunction followed by neuron death and a chronic neuroinflammation state. This abnormal protein build-up and neurotoxic environment may affect different regions throughout according to the specific protein accumulation (1). In the central nervous system (CNS) context, the first line of defense is microglial cells who are in charge to maintain a safe environment for neurons (Figure 1). Under physiological conditions, it occurs properly, but in pathological situations, this regulation is compromised, affording a hyperactivation of microglia leading to a dysfunctional neural environment (6).

Currently, NDs are a social and economic burden due to their several implications on independence and life quality for those affected by them. Nowadays, the approaches available to NDs treatment promote symptomatic relief but do not able to prevent neurodegeneration or revert it. Therefore, the knowledge of the pathophysiological pathways of these disorders and new therapies is imperative. Since the '80s, when cannabinoid receptors type 1 and type 2 (CB1R and CB2R, respectively) were discovered, the endocannabinoid system modulation has been investigated in many disorders context in order to the millenary medicinal use of cannabis.

Nowadays, the different roles played by components that make up it have already been described, such as antitumoral, regulation of neurotransmission in different brain regions, inflammatory modulation, and regulation of glial cells. In this context, this work aimed to provide a summarized translational review of the most recent studies about the involvement of the endocannabinoid system (ECS) in NDs development and maintenance, as well as a therapeutic target.

Endocannabinoid system and cannabinoids

The *Cannabis sativa* era has a long and outstanding history dating about 4000 years ago in Asia, in which many folks consumed *Cannabis sativa* for religious, hedonic, and medicinal purposes (7). Meanwhile, only in 1964 Mechoulam and Gaoni got an important advance in knowledge about the main component of *Cannabis*, succeeding in isolating and characterizing the Δ9-tetrahydrocannabinol (THC), the main psychoactive compound of *Cannabis*, and later cannabidiol (CBD), the main non-psychoactive compound of the plant (8). This event marked the beginning of the knowledge about the effects of cannabinoids on human physiology, and it allowed Howlett's group, in 1988, breakthrough the knowledge about ECS, through the discovery of cannabinoid receptor type-1 (CB1R) (9). Subsequently, in 1990, Matsudo and collaborates discovered and cloned the cannabinoid receptor type-2 (CB2R). Finally, in 1992, Devane and colleagues discovered that mammalian tissues could synthesize and release cannabinoid receptor ligands, and described the first endocannabinoid (EC), the anandamide (AEA), which can interact with both CBRs (7). Altogether, these finds aid the knowledge involving the cannabinoid's effects on ECS, and consequently on human physiology.

The activities currently tied to ECS range from behavioral effects to immunomodulatory activity and it basically consists of i) endogenous cannabinoids, which have as main molecules AEA and 2-arachidonoyl glycerol (2-AG), ii) two G protein-coupled receptors (GPCR) CB1R and CB2R, and iii) enzymes involved in syntheses and degradations of EC (7,10). Both AEA and 2-AG are arachidonic acid derivates, and their biosynthesis is catalyzed by *N*-acylphosphatidylethanolamine (NAPE)-specific phospholipase D-like hydrolase (NAPE-PLD)

and α/β-Diacylglycerol lipase, respectively (10). These molecules are considered retrograde messengers, because are synthesized by a respective enzyme, on-demand, in the postsynaptic neuron, being later released at the synaptic cleft where bind in both CBRs (7,9). More recently, has been defined multiple target receptors to EC beyond CB1R and CB2R, such as transient receptor potential cation channel subfamily V members 1 and 4 (TRPV1 and TRPV4, respectively) Peroxisome proliferator-activated receptor gamma (PPAR-□), N-Arachidonyl glycine receptors 18 and 55 (GPR18 and GPR55, respectively), among others (9–11). Once binding in their respective receptor, EC modulates neurotransmitter release by several pathways, the main is by voltage-gated Ca^{2+} shutdown and adenylyl cyclase inhibition, leading to gamma-aminobutyric acid (GABA) and glutamate release inhibition (9,10) (Figure 2). Lastly, the EC is degraded at postsynaptic neurons by respective enzymes (9). The most knowledgeable enzymes accountable for the degradation of EC are fatty acid amide hydrolase (FAAH) in AEA and monoacylglycerol lipase (MAGL) for 2-AG (9). It is important to mention that some other molecules, called cannabimimetic ligands, can interact with ECS receptors, such as D-limonene, β-caryophyllene, α-caryophyllene, and linalool, among others (7).

The distribution of ECS in the human body has been proved through the understanding of the several effects of cannabinoids on human physiology, mainly through the contribution to several systems and tissues, such as the immunologic and peripheral nervous system, and gut and cardiovascular tissues (10–12). Initially, the CBRs were divided into central receptors (CB1R) and peripheral receptors (CB2R), but in the last decades, it has been proved the presence of both receptors within CNS, where the CB2R has an important role in microglia and astrocyte regulation (13). Furthermore, presence both CBR in organelles such as mitochondria, lysosomes and endoplasmic reticulum has been demonstrated (9,14). The presence of CBR in intracellular compartments may be closely related to cellular physiology mostly regarding the bioenergetic process of the cell as well as ROS levels, while these are expressed in the mitochondrial membrane (1,14,15). Complementary was demonstrated that CBRs expressed on mitochondria play important role in memory formation through modulation of intra-mitochondrial G-protein signaling (14).

Additionally, neurotransmitter modulation provided by CBR, mostly CB1R, activation may represent an important piece in mood, cognition, locomotor activity, and microglia activation. Therefore, any disturbance in this complex system may contribute to alterations in neurophysiology. Some studies have demonstrated that ablation of MAGL, leads to an increase in 2-AG levels, suppresses neuroinflammation, and consequently reduced the amyloid plaque formation within the brain of transgenic mice model of AD (16). Additionally, several clinical and pre-clinical studies showed upregulation of CB2R in brain regions richly in Aβ plaques fortifying the relationship between ECS and NDs development, such as AD (13,17). As commented above, CBRs, mainly CB2R are quite distributed in microglia and are fundamental to defining their profile of it in M1 or M2 profile. In most NDs, the persistent low-grade inflammatory state is a common find, and it is closely related whit greater expression of M1 microglia associated with lower expression of M2 profile (17). Moreover, the interrelation between brain-gut is growing, and several pieces of evidence have shown that disbalances in any of these may contribute to the development of many neurologic and psychiatric disorders such as PD and depression. This may be confirmed through recent work that suggests the relationship between lower content of 2-AG in the hippocampus and gut microbiota dysbiosis in depressive mice model (18). Altogether, these data show the substantial role played by ECS in neurophysiology and its relationship with ND development. The next sections will describe the pathophysiology of AD, PD and Huntington's disease HD, and the possible involvement of ECS in its development.

Alzheimer's diseases (AD)

Dementia is a cognitive impairment able to impact meaningly the people life quality. It is the most common cause of disability and mortality worldwide affecting more than 40 million people and promoting an important social and economic burden (72). Into the dementia, context is AD, the most cause of dementia worldwide, accounting for more than 55% of all dementia cases (72). The disease was reported for the first time in 1907 by Alois Alzheimer, which described the case of Auguste Deter, a 51-year-old woman with cognitive disturbance, disorientation, delusions, and

other behavioral changes. Since then, several advances in knowledge about physiopathology, diagnosis, and treatment have been made. Nowadays, AD is characterized by gradual loss of episodic memory with accompanying functional impact and complex behavioral changes (73). Some of the earliest symptoms manifest years before receiving a clinical diagnosis of dementia, including changes in mood, anxiety, and sleep (74,75). After, the progression to later-stage symptoms, such as impaired judgment, disorientation, and confusion; aggression and agitation; and neuropsychiatric symptoms, such as delusions and hallucinations, are significant warning signs for the clinical diagnosis (76). Over the last decades, has been established that genetic, as well as environmental factors, may aid in its onset (72,77). The pathological hallmarks of AD are deposition of Aβ protein in the brain, chronic neuroinflammation, and neuro atrophy, leading to an important cognitive impairment, even as behavioral changes (77,78). Currently, is known that pathological changes in neural tissue begin decades before the symptomatic stage of the disease. The aberrant Aβ aggregation, furthering senile plaque buildup is the most reinforced pathologic feature of AD's brain. It happens basically owing to an imbalance in Aβ production by amyloid precursor protein (APP) cleavage, and Aβ clearance leading to increase in Aβ amount throughout the brain. These senile plaques promote an excessive activation of microglia promoting the increase in inflammatory mediators and free radicals, leading to the onset of a chronic neuroinflammatory state (77,79). In addition to that, over two last decades, the amyloid ß oligomers (AβOs) have been recognized as a fundamental piece in AD pathogenesis, it is known as the amyloid ß oligomers hypothesis. Firstly, the AβOs were regarded only as a precursor to Aβ plaques generation, it did not regard as root agents in AD's pathophysiology (79). Meantime, today, the AβOs are considered the most toxic form of Aβ, and its presence in neural tissue is regarded as crucial to AD onset (79). A classical illustration of the role played by AβOs on AD pathogenicity is the Osaka familial AD mutation of Aβ, known for several cognitive impairments, is a familial AD form, which shows low levels of senile plaques, while showing high levels of AβOs, agreeing with AβO hypothesis (80). Complementary, the AβOs presence is recognized as

an important agent in aberrant tau phosphorylation, higher oxidative stress, neuron death and plasticity dysfunction (79). Nowadays, the AβOs are regarded as a potential therapeutic and early diagnostical target whereas its shaping is verified in early stages of AD, including before Aβ plaques buildup. Another crucial feature in AD is dysfunctions in Tau protein, which play an important role in microtubules stabilization within neurons, but in pathologic conditions, its functionality may be disrupted leading to synaptic and neural structural commitment (2). In physiological conditions, tau protein undergoes several post-translational modifications such as phosphorylation, glycosylation, nitration, and ubiquitination (81). However, in pathological conditions, these processes are changed leading to conformational and functional disbalance. In the AD context, hyperphosphorylation has already been established as the main alteration related to tau protein. The AβOs accumulation, Aβ plaques buildup, and tau hyperphosphorylation are the pathologic hallmark of AD and play a root role in inflammation and neuron death that happens during AD progress. Furthermore, the cholinergic signaling, as well as the cholinergic neurons population, is importantly reduced in several brain regions, such as the cortex, basal forebrain (highlighting basalis of Meyenert), and hippocampus (82–84). This reduction is due to intense Aß deposition in these regions (85) and is thought as the main mechanism involved in the cognitive decline of AD. Nowadays, acetylcholine (ChS) management, mainly through acetylcholinesterase inhibition, is the most usual therapeutic strategy in AD treatment. Acetylcholinesterase inhibition can ameliorate cognitive symptoms and enhance the quality of life in patients with mild to several AD (86). However, only 40% of patients are responsive to these pharmacological approaches (85). Lately, the efforts are being directed to developing technics able to promote early diagnosis, making it possible to avoid the distinct neuron death that occurs in AD and consequently avoid cognitive impairment. In this context, hereafter will be discussing the recent findings on the crosstalk between ECS and AD development.

ECS in AD

In CNS, the CB1R is the most abundant G protein-coupled receptor, playing an important role in brain functionality, mostly in memory, mood, motor control, and pain perception (86). It is expressed majority in the cerebellum, cortex, hippocampus, and basal ganglia (86,87). While CB2R in the CNS is expressed, mainly, in microglia and astrocytes, playing important roles in neuron proliferation, differentiation, and survival, as well as in M1/M2 microglia's phenotypes dynamic changes, shaping the immune phenotype in the brain (88,89). Generally, in healthy conditions, CB2R is lowly expressed in CNS (88). Nonetheless, there is an upregulation of CB2R in the amyloid-associated neuroinflammation state, mostly in regions with high amyloid deposition such as the cortex, hippocampus, brain stem, and thalamus in different AD-like animal models (90–92). The microglia-Aβ protein interaction is very important to maintain the equilibrium between Aβ production and clearance (6,93). Nonetheless, an increase in Aβ density in the brain further a pathological activation of the innate immune system leading to overactivation of microglia and overproduction of inflammatory cytokines (6,93,94). Indeed, both animal models and those affected by AD have shown an intensified shift from M2 to M1 microglia in the brain, leading to an inflammatory phenotype (89). This supports the idea of the therapeutic potential of both endocannabinoids and phytocannabinoids targeting microglia for neuroprotection and possible prevention of neurodegeneration strategies. In agreement, Esposito and colleagues reported that CBD treatment in rats exerted a marked anti-inflammatory effect through selective PPARγ activation that occurs upstream of CBD-mediated NFκB inhibition (95). In addition, the authors demonstrated that CBD markedly downregulates reactive gliosis by reducing pro-inflammatory molecules and cytokine release that strongly occurs in Aβ neurotoxicity. Indeed, the interaction of CBD at the PPARγ site results in a profound inhibition of reactive gliosis by the reduction of both GFAP and S100B protein expression together with a marked decline of pro-inflammatory molecules and cytokine release (95). S100B is an astroglial-derived neurotrophin that plays a crucial role in the pro-inflammatory cytokine cycle and the promotion of APP to cleave Aβ42, and therefore their reduction demonstrates CBD's neuroprotective properties. On the other hand, learning and memory impairment can be considered the most relevant side effects of

cannabinoids, effects mainly mediated by CB1R through their activation (49). Nonetheless, recently has been compared the transgenic load with the severity in the 5xFAD AD-like animal model and was showed imbalances in CB1R, CB2R, and GPR55 in the hippocampus as well as impairment in memory and an increase in neuroinflammatory response, both in heterozygote and homozygote animals, demonstrating that the transgenic load clearly influences the severity of these alterations (96). Furthermore, the CB2R expression is directly related to neuroinflammation intensity, indicating an important role performed by CBR in the mitigation of the chronic inflammatory state of NDs (97). Accordingly, recently was investigated the effects of URB597, a specific FAAH inhibitor, in BV-2 cells incubated with $A\beta_{25-35}$. The $A\beta_{25-35}$ incubation increased the microglial activation shifting it from resting to activated phenotype leading to an increase in IL-1β, TNF, and iNOS levels. On other hand, the URB597 treatment was capable to prevent it. Moreover, the URB597 treatment increased TGF-β, IL-10 and Arg-1 expression, as well as prevented the cell death induced by $A\beta_{25-35}$ inoculation (98). Likewise, Tanaka and colleagues have shown that both genetic and pharmacological inhibition (with PF3845 and URB597) of FAAH were able to decrease the expression of prostaglandin G2 and pro-inflammatory cytokines (99). These effects were not affected by CBR antagonism thus, the eCBs may use other pathways to promote its anti-inflammatory effects (99). Furthermore, have shown a decrease both in APP and neuritic plaques amount in 5xFAD mice from genetic inactivation of FAAH (100). However, was not verified a decrease in proinflammatory cytokines amount in the hippocampus (100). The dual inhibition MAGL/FAAH has been discussed as a pathway to promote more similar effects to CB1R agonists in neuroinflammation (86,101). Newly, Bajaj and colleagues investigated the effects of JZL-195, a dual FAAH/MAGL inhibitor, in a sporadic AD model induced by streptozotocin (STZ) (86). The JZL-195 treatment reversed the increase of Aβ amount in the hippocampus as well the IL-6 and TNF induced by STZ. Even though, the treatment increased HSP-70 levels and CAT/SOD activity in the animal's brain (86). These immune alterations are observed beyond the CNS in AD patients. Recently, Chiurchiù and colleagues have shown an imbalance in CBRs expression in peripheral immune cells of AD patients, in which both receptors'

expressions were significantly lower in B-lymphocytes of AD patients' blood, as well the FAAH expression was increased in monocytes (102). Furthermore, was verified a relationship between mini-mental state examination (MMSE) score and peripherical CB2R expression, in which higher levels of CB2R were observed in the patients showing progressively higher scores and those whose FAAH was higher a negative correlation was observed, presenting the worst scores (102). Findings that justify the correlation of FAAH with AD, inasmuch as its levels progressively increased along with disease severity. These finds suggest a link between peripherical immune alterations and the progression of clinical features of AD.

Beyond the solid knowledge about the anti-inflammatory activity of ECS, it has also been shown to play an important role in learning and memory, thus its dysregulation might be related to the cognitive impairment in AD. The 5xFAD animal model shows a decrease in long-term potentiation (LTP) in CA3-to-CA1 hippocampal, a crucial region to learning and memory, than wild-type (WT) animals (103). In this context, has been shown that the inactivation of FAAH increases the LTP in CA3-to-CA1 hippocampal synapses (103). However, the effect was sustained after CB1R inhibition indicating an alternative pathway beyond CB1R by which the eCBs modulate the hippocampal synapses (103). Another central pathological feature both in AD patients and animal models are dendritic density reduction mostly in the hippocampus (103). In the 5xFAD mouse model, it has been reported to play an important role in animals' cognition. In this way, was investigated the effect of FAAH inhibition on dendritic density in this model was verified that the genetical ablation of FAAH restored the dendritic density in the hippocampus. Furthermore, in the same study, the genetic inhibition of FAAH performed a higher microglial activation as well as upregulation of mRNA TREM2 expression in 5xFAD FAAH-null mice than 5xFAD mice (103). In addition, CBD might also promote neurite outgrowth by indirectly stimulating CB1 and CB2 receptors to trigger different signal transduction pathways. The activation of CB1 receptors expressed on neuronal somata increases extracellular signal-regulated kinase (ERK) activity and induces brain-derived neurotrophic factor (BDNF) expression (104). The CB2 receptor is coupled to Gi/o protein in the CNS, and through the activation of adenylate

cyclase reduces the cAMP concentration and ignites the PI3K/Akt and ERK pathways to promote the growth of neurites (105). According to the important roles played by ECS in neural homeostasis its imbalance has been shown as an important piece in ND development, as well as a highly differentiated complex system.

Moreover, thinking of a possible gateway to the development of treatments through some cannabimimetic ligands, beyond the Cannabis plant, could show promising therapeutic effects and still possibly rule out many of the important adverse effects found in current treatments. In this way, a discussion about some studies turned to confirm the effect of terpenes and terpenoids will be shown as an innovative alternative to AD, for treatment or prevention. Thus, Lee and colleagues, demonstrated in the Drosophila AD model, that α-pinene may increase cortical acetylcholine production through choline acetyltransferase (ChAT) activity and exert antioxidant effects in the hippocampus as a mechanism that contributes to its pro-cognitive effects (106). This response could be important when considering the cholinergic hypothesis of Alzheimer's disease, where Aβ plaques cause a significant loss of cholinergic neurons (postsynaptic muscarinic M1 receptors and presynaptic muscarinic M2 receptors) in the nucleus basalis of Meynert, diminished ChAT transcription and activity, and loss of cholinergic synapses (including in the hippocampus), often related to cognitive dysfunctions similar to those observed in dementia (106,107). In another model of Drosophila AD (elav-GAL4>UAS-Aβ422X), Linalool (LI) also showed protective effects against Aβ42 toxicity in a dose-dependent manner and did not affect the survival of wild-type flies at the concentrations used in AD model flies (108). Yuan and colleagues also demonstrated that LI significantly reduced Aβ42-induced cell death in the developing eye discs and suppressed Aβ42-induced cell death in the larval brain. However, the neuroprotective effect was not achieved by inhibiting Aβ42 accumulation or aggregation, suggesting that the protective effects of LI against Aβ42 toxicity are related to its antioxidant effect, at least in part through the decreased ROS levels (108). Corroborating with these findings, Xu and colleagues demonstrated LI was able to ameliorate the cognitive deficits of mice induced by Aβ1-40, and its neuroprotective effect might be related to the role against Aβ induced oxidative stress and apoptosis depending on

Nrf2/HO-1 signalling, involving mitochondrial dysfunction, caspase activation, and fragmentation. Moreover, results demonstrated that the hippocampal injury and cell apoptosis rate were reversed by LI, as well the Aβ1-40 activated cleaved caspase-3 and cleaved caspase-9 expression were also decreased (109). Also, Cheng and colleagues showed that the higher doses of β-caryophyllene (BCP), another terpenoid, prevented cognitive impairment in APP/PS1 mice, and this positive cognitive effect was associated with reduced Aβ burden and neuroinflammation manifested as decreased gliosis (110). In this sense, Askari and Shafiee-Nick report on the immunomodulatory properties of BCP on LPS-induced the inflammatory state of primary mice microglia and M1/M2 imbalance (111). As result, low doses of BCP provide anti-inflammatory activities through the CB2 receptor and in addition, PPAR-γ receptor-mediated. Furthermore, it has been shown that BCP protects glial cells against glutamate excitotoxicity and abrogates hypoxia-induced activation of BV-2 microglial cells through activation of CB2 receptor and reduction in the levels of inflammatory cytokines TNF-α, IL-1β and IL-6 as well as inhibition of NF-κB (111). Notably, cannabinoid-mediated anti-inflammatory actions involve suppression of inflammatory cytokines, and modulation of TNF and NF-κB, all pathways in which terpenoids have been demonstrated to be effective. Cannabimimetic has the potential to be a very attractive anti-inflammatory molecule that mainly works through the CBR2, but evidence shows that it definitely can go beyond that, and may as an alternative to AD control. Despite these findings, more studies are expected, as animal models as clinical trials, to confirm the effects of terpenes and terpenoids in AD progression.

In the AD context, as mentioned above, several studies have shown the relevance of ECS in microglial activity, phagocytoses regulation, Aβ deposition, and neuroinflammation. Therefore, altogether, these data present the ECS as a promising research object regarding AD, both for the development of new treatment approaches and early diagnosis.

Parkinson's disease (PD)

Parkinson's disease is the most common movement disorder in the world, first described in 1817 by James Parkinson, it was characterized as a neurological illness consisting of resting tremors and a peculiar form of progressive motor disability. With an annual incidence of 1% of adults older than 60 years, PD is the second most common neurodegenerative disease (after Alzheimer's disease), affecting about 4 million people worldwide (19,20). It is known that the development of symptomatic PD is slightly delayed in women, about 2 years, compared with men, and that twice as many men as women will develop the disease throughout life (21). Moreover, the prevalence of disease in industrialized countries is estimated at 0.3%, which could be increased in rural areas with exposure to pesticides (22). Young-onset PD affects 5–10% of patients, in which the first symptom arrives between 21 and 40 years old (19).

The classic findings of PD are motor symptoms, basically composed of three principal features, rest tremor, rigidity, and bradykinesia (19). Normally, the first symptom observed in 70% of PD patients is the resting tremor in one extremity, usually unilateral, with a frequency of 3–5 Hz which worsens with anxiety, contralateral motor activity, and during ambulation (19,22). Bradykinesia is the most disabling symptom of early PD and refers to a slowing of movement and the simplification of complex motor tasks (23). It initially manifests as difficulties with fine motor tasks such as buttoning, using utensils, and handwriting. Other manifestations of bradykinesia include loss of spontaneous movements and gesturing, manifested as hypomimia of PD (22). Also, spontaneous swallowing is reduced, and the mechanics of swallowing are affected, resulting in sialorrhea. Gait becomes slower, with shuffling, and turning is en bloc, alternating movement becomes difficult and there is frequent "freezing." The patient can no longer turn on a pivot and shows the difficulty in initiating gait, hesitation in turning or arriving at an obstacle (19,22,23). The non-motor symptoms are as challenging as motor symptoms, and normally include autonomic dysfunctions, cognitive abnormalities, dysautonomia, and mood disorders such as anxiety, depression, apathy, and sleep syndromes (24,25). Autonomic dysregulation is the most common symptom in parkinsonian syndrome and generally is manifest by constipation, urinary urgency and frequency, and orthostatic hypotension (26). Another common symptom is dysautonomia, present

in 90% of all PD patients, which is a very early symptom that maintains for many years (22). Dementia develops in about 40% of individuals with PD and depression affects nearly half of patients, in which women feel more melancholy, and men have more apathy and decreased libido (19,22). Also, disturbed sleep is common and has several different causes, including nocturnal stiffness, nocturia, depression, restless legs syndrome, and rapid eye movement (REM) sleep behaviour disorder (19).

The essential pathological feature of PD is the loss of dopaminergic neurons through apoptosis and autophagy process within the CNS, with the most affected area containing neurons that project from the *substantia nigra pars compacta* (SNpc) to the caudate putamen (Figure 3). This loss of dopamine neurotransmission may precede by two decades or more of the primary motor symptoms (22,27,28). In this sense, clinical signs of PD are evident when about 80% of striatal dopamine and 50% of nigral neurons are lost (19). Other brain regions are also affected by these neuronal losses, including the *locus ceruleus*, *nucleus basalis* of Meynert, pedunculopontine nucleus, *raphe nucleus*, and the dorsal motor nucleus of the vagus, amygdala, and hypothalamus (27). In these affected areas are observed Lewy bodies, abnormal intracellular aggregates which contain different proteins including α-Syn and ubiquitin, that impair optimal neuron functioning (22,27). Moreover, it is accepted that PD progression could affect other extra-nigral dopaminergic, cholinergic and serotoninergic tracts, leading to non-motor symptoms (28).

The acquired knowledge about PD until now remains unclear and at times controversial. While 5–10% of PD cases are of genetic origin, most cases remain idiopathic and are probably a result of multiple factors acting together (19). Current theories around PD include mitochondrial dysfunction, inflammation, abnormalities in protein handling, and oxidative stress associated with the aging process (22). Furthermore, some risk factors are associated with environmental toxins, pesticides, heavy metals, traumatic lesions, and bacterial or viral infections (29,30), all of which are intimately associated with neuroinflammation.

Neuroinflammation has been frequently linked to neurodegeneration, but despite the association between both being sustained, the predecessor process remains unanswered. In fact,

neuroinflammatory mechanisms contribute to both neuroprotective and neurotoxic functions. Under physiological conditions, microglia, the principal innate immune cells in the brain, represent the first line of defence and constantly surveil the brain parenchyma to maintain CNS homeostasis through a cascade of inflammatory processes (31). However, these glial cells can be activated by pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), such as secreted factors from damaged neurons or protein aggregates, increasing the expression of toll-like receptors (TLRs) and several pro-inflammatory mediators, which consequently activate peripheral immune cells, leading to persistent neuroinflammation (28,31,32). Also, microglia can act on the endothelial cells of the blood-brain barrier (BBB) triggering an increase in vascular permeability and inducing brain infiltration by circulating leukocytes (28,33). The increased levels of pro-inflammatory cytokines as interleukin 1 beta (IL-1β), interleukin 2 (IL-2), tumour necrosis factor (TNF), interleukin 6 (IL-6), interferon-gamma (IFN-γ), transforming growth factor-beta (TGF-β), and macrophage inflammatory protein 1 beta (MIP-1β) highlights the existence of a specific inflammatory signature of PD, principally when associated with lower levels of interleukin 9 (IL-9), a pleiotropic cytokine with pro-inflammatory and regulatory functions (34).

Besides microglia, astrocytes have also an important role in the neuropathology of PD (34,35). It metabolically supports neurons through cytoplasmic extensions which directly connect it's with blood vessels (28). In this way, astrocytes contribute to the development and plasticity of the CNS, participate in tissue repair provide energy to neurons, and maintain brain homeostasis with maintenance and permeability of the BBB (28,31,34). In healthy individuals, astrocytes are heterogeneously distributed within the mesencephalon. However, in PD, reactive astrocytes experience gene expression changes as well as morphological rearrangement following a specific distribution pattern and an elevation in its number (28,31,34,35). In this sense, Chinta and colleagues showed that during normal aging occurs a natural increase in astrocytic senescence, but Parkinsonian SNpc tissues displayed elevated expression of the senescence marker p16INK4a, and several SASP factors included the protease matrix metalloproteinase-3 (MMP-3) and the pro-

inflammatory cytokines, which could contribute to the development of sporadic PD (36). Normally, astrocytes may undergo senescence in diseased brain tissues and this process probably occurs through the TNF released which bind to specific receptors expressed by dopaminergic neurons, such as tumour necrosis receptors 1 and 2 (TNFR1 and TNFR2, respectively), and activate proapoptotic programs (28). Furthermore, upregulation of calcium-binding protein S100b, which is primarily expressed by astrocytes and acts as a cytokine may increase the expression of inducible nitric oxide synthase (iNOS) which, in turn, may result in the activation of the pro-inflammatory enzyme cyclo-oxygenase-2 (COX-2) in microglia as well as increased production of nitric oxide (NO) and superoxide radicals, directly or indirectly causing neuronal cell death (31,37).

This feature strengthens the relationship between oxidative stress, neuroinflammation and PD. In fact, elevated levels of several inflammatory mediators and oxidized biomolecules (4-hydroxynonenal, oxidative cholesterol metabolites, 8-oxoG), expose dopaminergic neurons to high levels of oxygen and nitrogen reactive species (ROS and RNS, respectively) favouring the aggregation and oxidative modification of several proteins, including α-Syn (28,38). TGF-β is the key regulator of neuro-inflammation as it controls the switch of microglia from protective to deleterious (39). In microglia, ROS are mainly produced by multi-subunit enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) acts as microbicides as well as signalling molecules (28,39). There are seven isoforms of NOX, NOX1 and NOX2 especially relevant in PD. It has been shown that in patients with PD, NOX1 and NOX2 expression can be increased in substantia nigra, stimulating and eventually contributing to dopaminergic damage (28,40). Moreover, increased ROS activates the redox-sensitive nuclear factor kappa beta (NF-kβ), which promotes more neuroinflammation which results in a vicious circle of neuronal damage, and can be translated into functional deficits, such as cognitive impairment (41). It has become clear that the disease progresses following a characteristic pattern of pathological changes throughout the brain and despite remaining unanswered regarding who came first, inflammation is known to deteriorate and accelerate the progression of the disease's pathogenicity.

ECS and PD

Dopaminergic responsiveness has been proposed as a key perspective in PD treatment and for this reason, drugs that enhance intracerebral dopamine concentrations or stimulate dopamine receptors remain the mainstay of symptomatic therapy. These drugs include levodopa, dopamine agonists, monoamine oxidase type B inhibitors, and amantadine (27,42). Normally, most patients started on dopamine agonist therapy will need the addition of levodopa combined with carbidopa or benserazide within 5 years, aiming to prevent peripheral conversion to dopamine by dopa-decarboxylase (19). In contrast, this late indication of levodopa is due to drug-induced adverse reactions should be always regarded, principally when considering that about a quarter to half of the patients taking even low-dose levodopa develop motor complications, such as dyskinesia and wearing-off or end-of-dose fluctuations, in long-term use of this drug. Moreover, other side effects are frequently observed, like nausea, daytime somnolence, hallucinations, and edema (19,27). For this reason, the major goal of the current PD research is the development of disease-modifying drugs, considering that the available therapies only treat symptoms, and do not that slow or stop the underlying neurodegenerative process.

In this sense, PD progression has been shown to be associated with multiple molecular changes in the brain and the alleged involvement of the ECs in dopaminergic neuron degeneration is evident in different reports. CBRs are highly expressed in the basal ganglia (BG) (*caudate nucleus*, anterior dorsal putamen, and external segment of the *globus pallidus*) circuit of both animals and humans (43). The BG are subcortical structures that regulate the initiation, execution, and orientation of the motor activity. Interestingly, CBRs have been shown to inhibit neurotransmitter release, while endocannabinoids (eCBs) regulate dopaminergic transmission in the nigrostriatal pathway (43,44). Also, analysis performed in *post-mortem* brain samples from patients with PD described a lowered expression of CB1R in different areas of the BG. These observations suggest that EC-mediated depression of synapses in the indirect pathway plays a significant role in the control of movement (43–45).

There is also evidence that CB1R expression is increased in patients with PD (10,46,47) and imaging in rats and patients has revealed CB2R upregulation (10,48). This increase in CB1R density can be considered as the compensatory response that occurs due to dopamine depletion in PD (49). Moreover, animal models have shown an exacerbation of the PD pathology in CB2R knockout mice arising the enhanced microglial activation (44,50), and that a biphasic dysregulation of CB1R results in hypoactivity in pre-symptomatic and early PD patients besides hyperactivity at disorder later stages (10,51). Of note, aberrant expression or dysregulation of these receptors are linked with PD development and progression, either as a compensatory response to dopamine depletion or an exacerbation of microglial activation, promoting such as movement disorders or hypoactivity in pre-symptomatic and early PD patients. Another preclinical trial of 6-hydroxydopamine (6-OHDA)-lesioned rats demonstrated that chronic levodopa treatment significantly increased CB1R mRNA expression in the denervated striatum (44,52). Also, enhanced levels of AEA in the striatum and decreased activity of FAAH were reported in the same induce model (53). Cerebrospinal fluid samples from patients at different stages of PD were also tested and indicated more than a two-fold rise in the levels of AEA (53,54). However, it is known that an elevation in the levels of the endogenous cannabinoid via inhibition of its main degradative enzyme could promote consolidation of fear extinction memories and protects against anxiogenic effects of stress (55). In this way, an MPTP-lesioned mice model, exhibited protective effects of URB597, an inhibitor of FAAH, resulting in inhibited dopaminergic neuronal death, decreased microglial immunoreactivity, and improved motor alterations (56). Also, Lastres-Becker and colleagues also demonstrated a decrease in dopamine depletion and tyrosine hydroxylase expression with a daily administration of CBD (3 mg/kg) for 14 days, within the striatum of rats that received 6-OHDA (57). These neuroprotective effects were associated with an upregulation of mRNA levels of Cu2+/Zn superoxide dismutase, a key enzyme necessary for the endogenous control of oxidative stress. In this way, Sánchez e Suárez demonstrated that the CB2R agonist β-caryophyllene was able to induce neuroprotection by virtue of its anti-inflammatory and antioxidant properties (53,58). Also, activation of CB2 reduced dopamine depletion in 6-OHDA-

treated rats (10,59). In MPTP treatment in marmosets and 6-OHDA treatment in rats, CB1 agonists ameliorated levodopa-induced dyskinesia (10,60,61). Moreover, Gutiérrez-Valdez and colleagues demonstrated that co-administration of levodopa and CB1R antagonist rimonabant in MPTP-lesioned rats, decreased LID severity and partially preserved the dopaminergic cells without affecting the anti-parkinsonian (62). This anti-dyskinetic effect probably occurred by a GABA-induced signal transmission, enhanced by cannabinoid agonists through inhibition of its uptake at glutamatergic synapses and suppression of excitation produced by N-methyl D-aspartate (NMDA) receptors and alfa-amino3-hydroxy-5-methyl-4-isoxazole propionic receptor (AMPA) on dopaminergic neurons (49,63). The database involving ECS and PD is compound of robust and diversified pre-clinical studies that show the relevance to invest in this new treatment strategy for PD patients. These studies embrace the regulation of neuroinflammation to the regulation of dopaminergic synapses, as well as the presence of CBR in crucial areas in the control of movement.

Discussing the literature on clinical research, an observational study showed that smoked Cannabis was well tolerated and improved motor (tremor, rigidity, and bradykinesia) and non–motor symptoms (pain relief and improved quality of sleep) in parkinsonian patients (64). In a subsequent clinical trial, PD patients have been treated for at least 6 weeks with increasing doses of CBD. As a result, CBD improved mobility, emotional well-being, cognition, communication, and provided pain relief, but failed to produce any difference in the total motor score compared to placebo-treated patients (65). This effect might be related to the anxiolytic, antidepressant, and antipsychotic properties of CBD (65). In another pilot study, both THC and nabilone, a synthetic analog of THC but with more predictable side effects and less euphoria, reduced levodopa-induced dyskinesia (LID) in PD (66). In line with this, beneficial effects of nabilone on anxious mood and night-time sleep problems were observed in a double-blind trial (67). To a better understanding, nabilone may improve sleep, and alleviate pain and mood disorders via modulation of monoaminergic, GABA-ergic, glutamatergic neurons and opioid signalling in nociception and mood processing (68). On the other hand, patients treated with nabilone commonly reported worsening symptoms of postural dizziness as an adverse event of the treatment (67). However,

Chagas and colleagues described the beneficial effects of treatment with CBD in the reduction of symptoms of REM sleep behavior disorder (RBD) in PD. In this case, all patients treated with CBD had a prompt, substantial and persistent reduction in the frequency of RBD-related events. Interestingly demonstrating, regarding symptoms after drug discontinuation, a return with the same frequency and intensity of baseline after the treatment was interrupted (69). Moreover, several studies have investigated whether the modulation of the ECS could represent a potential tool to alleviate levodopa-induced abnormal involuntary movements (AIMs). In this sense, in another clinical trial conducted by Zuardi and colleagues, PD patients have been treated for at least 4 weeks with CBD in addition to their usual therapy. Authors observed a rapid onset of antipsychotic effect in the PD patients, probably attributed to changes in dopaminergic neurotransmission in areas related to the production of psychotic symptoms (70).

Based on these approaches, nowadays, most drugs used in the treatment of PD act in the dopaminergic system, and little is known about the role of other neurotransmitter systems in the disease. The ECS seems to be an important target of investigation with relevant gaps to be filled. Moreover, the overactivity of the ECS in PD patients and shared pathways of the cannabinoid and dopaminergic systems in the basal ganglia as presented in these studies justify its use in PD patients. Despite the Movement Disorder Society Evidence-Based Medicine Committee recommendations for treatments of PD published in 2018 concluded that there was insufficient evidence to support the use of CBD for the treatment of PD (71), cannabinoids are considered to be safe and seem to be well tolerated in clinical trials and routine use in other indications. For this reason, more clinical trials are needed to elucidate the possible effectiveness, possible side effects, as well as the therapeutic window for motor and non-motor PD symptoms, and the mechanisms involved in the therapeutic potential of ECS in PD, given that most studies of EC targeting have been preclinical. Moreover, is necessary to clarify whether the developing new neuro therapies from cannabinoids can be fully realized and what is the beneficial potential of the use of cannabinoids as an additional treatment option for symptoms not concerning motor control of PD, even though as a combined treatment with levodopa.

Huntington's disease

Huntington's disease (HD) is an inherited autosomal dominant disease characterized by a triplet repeat expansion in the Huntingtin protein gene (HTT) (112). Worldwide, 4-10 per 100,000 people are affected by HD, prominently in east Europe and America (113). The mean age of diagnosis is 40 years when normally the motor symptoms are onset, before then, they are healthy and have no detectable clinical abnormalities, but patients could become symptomatic at any time between the ages of 1 and 80 years (113). This healthy period merges imperceptibly with a pre-diagnostic phase when patients show subtle changes in personality, cognition, and motor control, called may have a "prodromal" phase. (114,115). Herein, individuals might become irritable or disinhibited; multitasking activities become difficult and forgetfulness and anxiety mount (115). Also in this phase, chorea is one of the most striking features of HD. Defined as short-lived, involuntary, excessive movements, which are semi-purposeful, chorea initially causes an occasional small-amplitude movement of the distal extremities and face are seen, then spread and involve more proximal regions, increasing in amount and amplitude, leading to writing difficulties, eating, and maintaining balance (114,116). As the disease progresses, bradykinesia, akinesia, rigidity and impaired postural reflexes dominate and can be caused by sustained muscle contractions and increased muscle tone (114). Impaired postural reflexes result in difficulties with gait and fall, especially on uneven terrain. Other motor features that may arise include tics such as blinks, sniffs, head jerks, grunts and snorts. (116). Cognitive dysfunction in HD often spares long-term memory but impairs executive functions, such as organizing, planning, checking, or adapting alternatives (114,115). Mood disorders affect directly the patients; depression has a prevalence of 40% leading the suicide being the second most common cause of death in HD patients (117). Other neuropsychiatric symptoms include irritability and aggression, both related to frontal-lobe dysfunction (116). However, the neural and metabolic alterations have been known to start a few years before the onset of motor symptoms, but so far, the exact timeline of its onset is unknown

(118). The HTT integrity is important to embryonic neural development, transcriptional regulation, and synapse regulation, among other roles (112,113). Therefore, alterations in its activity and morphology compromise neuronal homeostasis leading to a high shrinkage of the brain, highlighting a loss of cortical volume and degradations of the *striatum*, in which about 95% of GABAergic medium spiny projection neurons are lost (113,119). This prominent neuronal loss leads to motor, cognitive, and behavioral impairment patients are affected by mutations in the HTT gene (120). The knowledge about HD physiopathology has improved in the last few decades, but at this time the available treatments are not able to promote recovery of neurodegeneration induced by misfolded proteins, while some pharmacological approaches might attenuate the severity of symptoms in HD, such as the ameliorate of chorea trough Deutetrabenazine and Tetrabenazine treatment.

The mutant huntingtin aggregates buildup mostly in the neural nucleus and cytoplasm is thought as the centrepiece of HD pathogeneses. So far, the exact origin of neurotoxicity caused by mutant HTT (mHTT) is not completely understood but has been shown these aggregates, and fragments derived from them, promote a wide array of dysfunctional alterations in neuronal homeostasis such as reduction in BDNF expression, decrease in ATP production, increase in ROS production, impairment of ubiquitin-proteosome system, and impairment in axonal transport (113,119). In addition to neuronal alterations, has been shown an overactivation both of microglia and astrocyte, followed by an increase in pro-inflammatory cytokines expression in the brain promotes the onset of a neurotoxicity environment throughout the brain (113,119,121). Indeed, has been delineated an increased activation of glial cells years before the onset of the clinical symptoms (122–124). It is thought that the microglia are a double-edged sword in HD's physiopathology because it is important both to its role in the clearance of mHTT aggregates and trophic activity in the brain. In contrast, its overactivation also can provide exacerbated levels of TNF, IL-1β, and TGF-β1 in the brain (124–127) setting an inflammatory and neurotoxicity state. Thereby, the modulation of immune cells within the CNS might be an approach to be investigated more deeply in HD physiopathology.

ECS and HD

The improvement of our knowledge about the alterations that start before the onset of the symptoms in HD is extremely important to enable the earlier diagnosis and improve the treatment of the disease. Like many other NDs, HD has an important immunologic component in its physiopathology and alterations in ECS activity throughout the brain, such as overexpression of inflammatory cytokines and exacerbated activation both of microglia and astrocytes (113,121,122), combined with an increase of CB2R expression throughout the brain (124). In 3NP mice, is observed a neural death mainly in Nissl-stained cells, locomotor impairments, and an increase in inflammatory markers, reproducing some of the features of human HD. In this context, was investigated the effects of cannabigerol (CBG), a phytocannabinoid, in 3NP mice. The treatment with CBG was able to protect the neuron loss and the locomotor impairment induced by intoxication (128). Moreover, the CBG significantly decreased the upregulation of proinflammatory markers induced by intoxication (128). Also, Granja and colleagues demonstrated that CBG and VCE-003.2, a novel CBG derivative, have been shown to reduce the inflammatory molecules TNF-α, IL-1β, IL-6, Macrophage Inflammatory Protein (MIP-1α), and Prostaglandin E2 (PGE2) in rat microglial cells treated with LPS, and both compounds reduce glutamate-induced oxidative cell death in mouse hippocampal cells (129). In agreement, Valdeolivas and colleagues showed, in 3-nitropropionate–treated mice, that CBG prevented striatal neuron death, reduced markers of inflammation, and improved motor deficits (128). More recently, in 2021, Echeverry and colleagues investigated the effects of CBG and CBD on neurotoxicity in neural cell cultures in the H2O2 model and the rotenone model of oxidative damage (130). In both models, authors found that the protective effects were lost when CBG and CBD were administered with a 5-HT1A antagonist, WAY-100635, but were unchanged with CB1 and CB2 receptor antagonists (130), suggesting that the protective effects of CBG and CBD against oxidative neurotoxicity are derived from a 5-HT1A receptor-mediated process. Also, Laprairie and colleagues demonstrated in a cell culture involving HD, that mutant STHdQ111/Q111 striatal-

derived cell lines treated with CBD, demonstrated an increase in cell survival rates by 40%, while reducing the membrane permeability and the rates of cell death (131). Moreover, in another HD in vitro model, CBD promoted upregulating the gene expression of the proteins implicated in cell survival (131). Altogether, these data demonstrate a protective effect of CBG, VCE-003.2 and CBD against pathological features in different *in vivo* models of HD disease, through ECS modulation. Previous studies showed that CB1R is highly expressed in presynaptic boutons in the medium spiny projection of the striatum. Even more, have been shown alterations in the expression and activity of CBR in CNS, mostly in the striatum, both in patients and animal models of HD, even before the onset of motor symptoms (118,131–136). Mievis and colleagues showed that genetic deletion of CB1R in the HD mouse model worsens the phenotype of the disease (133). Additionally, the genetic rescue of CB1R in R6/2 HD mice was sufficient to prevent excitatory synaptic density loss in the striatum, setting a positive correlation between loss of CB1R and reduction of glutamatergic synapses in the striatum. However, the genetic CB1R rescue did not change the HD's motor impairment (134). Likewise, the positive allosteric modulators (PAM) of CB1R promote a change in receptor configuration, enhancing the receptor affinity for its orthostatic ligands. In this way, these results support the conception that loss of CB1R is a major pathogenic event in HD and that pharmacological strategies aimed at promoting CB1R signalling may result in therapeutic benefits. Moreover, in another study, in the transgenic R6/2, CAG repeat length Huntington chorea mouse model, CB2R expression was shown to be elevated in the hippocampus, brain, striatum, and cerebellum (137). The authors also demonstrated that CB2R-deficient mice exhibited a faster onset of motor deficits and increased severity of the symptoms (137–140). Taken together, compounds that selectively activate CB2R might be utilized as a potential therapeutic agent in the treatment of HD, the extent of which will most likely depend on the degree of disease severity at the time of drug administration. The major challenge regarding CB2R is related to the selective targeting of the brain CB2Rs without influencing peripheral CB2R. Recently, the effect of GAT211, GAT228, and GAT229, three CB1R PAMs, was evaluated in vivo and vitro models of HD. As the main finds the study showed the positive activity of GAT229

both in cell viability (*in vitro*) and enhancement in locomotor impairment of HD (*in vivo*) (132). A recent clinical trial conducted by Saft and colleagues demonstrated that in seven patients suffering from early-onset HD with dystonia as the leading syndrome and five of them in advanced disease stages, the treatment with cannabinoids in all cases lead to an improvement of motor symptoms, mainly driven by the improvement of dystonia (141). In addition, changes in behavior with less irritability, less apathy and hypersalivation were reported. As a result, as patients as patient relatives, reported functional improvements and thus improvement in quality of life (141). In this sense, by analyzing the post-mortem brains of patients suffering from HD, a decrease in CB1R expression was observed. This reduction was also seen in genetic and phenotypic models of the disease. Moreover, an up-regulation of CB1R may lead to an improvement in motor dysfunction, highlighting the idea that an early reduction of these receptors is of high importance in HD development (142). For this reason, is proposed that a protective effect could be obtained by activating CB1R, while a reduction of these receptors can negatively influence the disease. Altogether these data support an important role of ECS disbalance in HD pathophysiology even before symptoms onset and put it in the spotlight of future investigations as a new therapeutic approach.

Conclusions

Throughout this review, we were able to cover and demonstrate the real importance of ECS in many impairments observed in the ND presented. The disbalance of immune response and the pathological hallmarks modulated by up or down-regulation of CBRs in the CNS makes clear the actuation of ECS as a treatment target. Future investigations are needed to show the deep connection between neural death, locomotor impairments, and an increase in inflammatory markers that derivates regulation of CBRs in the CNS, reproducing some of the features of ND. Moreover, clinical trials are requested to elucidate the possible effectiveness and mechanisms involved in ECS's therapeutic potential, given that most studies have been preclinical. Remains necessary to clarify whether the developing new neuro therapies from cannabinoids can be fully

realized alone or the beneficial potential of combined treatment with drugs that already exist is the best way to prograde.

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Conflicts of Interest

The authors declare no conflict of interest.

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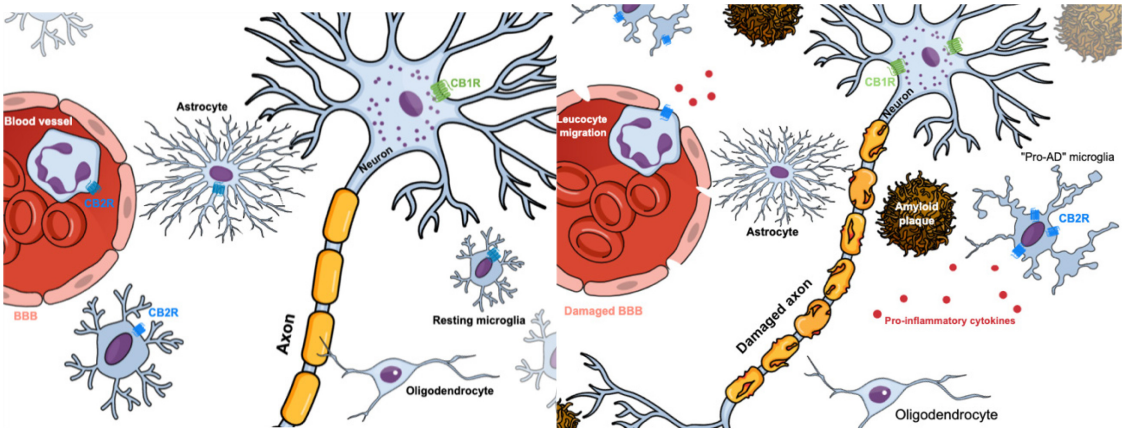
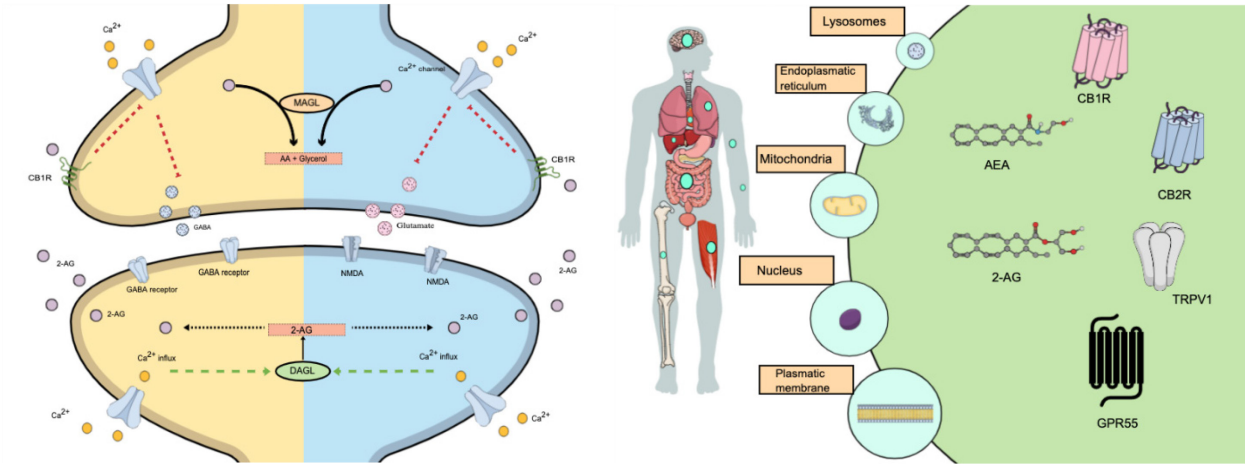
Figure captions



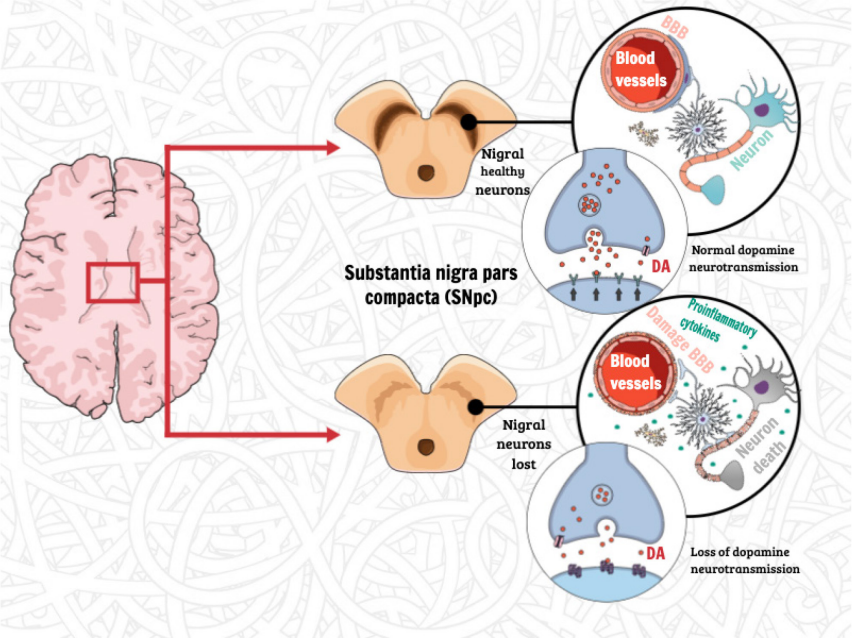
1. **Figure 1.** Neuroinflammation process during the development of the neurodegenerative disease - Under physiological conditions, microglia and astrocytes ensure the stability of the brain microenvironment, which is crucial to promoting both immunity and nutrition balance to neuron activity, such as BBB maintenance and synaptic support. In this context, both the CBRs play a role in immunity balance being a root piece in microglia shift (M1 <-> M2), which is very important to set up a homeostatic brain environment. However, under

pathological challenges, such as over NDs conditions, several alterations are noted in its functionality, such as the increase of pro-inflammatory cytokines levels (such as IL-1 beta, IL-6, INF-gamma, and TNF-alfa) which build up a chronic neuroinflammatory and neurotoxic state. Moreover, the excessive glutamatergic release also promotes several damages throughout the BBB and peripherical leucocytes migrating through the damaged BBB intensifying the neurotoxic environment. Lastly, the expression of CBRs in neuropathological conditions is much larger than in healthy conditions, instancing the importance of this system in the physiopathology of neurodegenerative diseases.



2. **Figure 2.** Endocannabinoid system distribution and function in the human body - (**A**) Since the CB1R was discovered in the 1980s the ECS distribution and roles have been intensely investigated. Nowadays, it is known that the ECS is distributed throughout many tissues besides the brain, giving it many physiological functions. Among the tissues where ECS compounds can be found are the gut, cardiovascular system, lungs, bones, and muscles. This vast distribution in the body gives this system several roles from immunity control to synaptic control. Have recently been demonstrated the presence of CBRs besides the plasmatic membrane, such as in the mitochondria external membrane, lysosomal membrane, and the endoplasmatic reticulum. Thus, the ECS also regulates the electro

transporter chain, lysosomal activity, nuclear factors expression, and calcium release from the endoplasmatic reticulum. Among the main compounds of ECS that can be cited the CB1R and CB2R, TRPV1, GPR55, and the most investigated eCBs, AEA and 2-AG. (**B**) The endocannabinoid system has been defined as a retrograde signaling system that consists of the endocannabinoids, mainly anandamide (N-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG), the corresponding biosynthesis enzymes and transporter molecules, and receptors highlighting both cannabinoid receptors (CB1R and CB2R), TRPV1, and GPR55. The eCB's release is induced by calcium influx in the postsynaptic neuron terminal, increasing the neuron activity and activating the eCBs biosynthesis enzymes. In the image is illustrated a classical didactic representation of retrograde signaling performed by eCBs, which are demonstrating the release of 2-AG in synaptic cleft followed by its interaction whit CB1R and subsequent inhibition of both glutamatergic and gabaergic neurotransmission, by inhibition of calcium influx in pre-synaptic neuron terminal. The presence of MAGL, the main enzyme involved in 2-AG degradation, in presynaptic neurons and the DAGL, involved in the 2-AG synthesis, presence in the postsynaptic terminal corroborates with retrograde signaling theory.



3. **Figure 3.** The pathological feature of PD - The critical loss of dopaminergic neurons through apoptosis and autophagy process within the CNS, mostly reaches the areas containing neurons that project from the substantia nigra pars compacta (SNpc) to the caudate-putamen. In this sense, only when more than half of these dopamine neurons are lost will clinical signs of the disease be evident. Contributing to this neuron loss, microglia, which under physiological conditions represent the first line of defence and constantly maintain CNS homeostasis, are activated and increase the expression of toll-like receptors (TLRs) and several pro-inflammatory mediators, which activate peripheral immune cells, leading to persistent neuroinflammation. Further, microglia can act on the endothelial cells of the blood-brain barrier (BBB) triggering an increase in vascular permeability and inducing brain infiltration by circulating leukocytes. Besides microglia, astrocytes also have an important role in brain infiltration, directly connecting neurons to blood vessels, increasing the expression of inducible nitric oxide synthase (iNOS) which, in turn, may result in the activation of the pro-inflammatory enzyme cyclo-oxygenase-2 (COX-2) in

microglia as well as increased production of nitric oxide (NO) and superoxide radicals, directly or indirectly causing neuronal cell death.