FACULTAD DE
MEDICINA UNIVERSIDAD
DE CANTABRIA

# GRADO EN MEDICINA

## TRABAJO FIN DE GRADO

**Cannabinoides en la enfermedad de Parkinson**

Cannabinoids in Parkinson's disease

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TFG-Anexo III

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**ABSTRACT**

Parkinson's disease is a chronic neurodegenerative disease that affects the world's population and consists in the progressive loss and degeneration of dopaminergic neurons in the brain. This causes motor symptoms such as bradykinesia, rest tremor, postural disturbances, and rigidity. This disease is also characterized by non-motor symptoms such as sleep disturbances, cognitive deficits, and psychiatric disorders such as psychosis, depression, and anxiety. The efficacy of its pharmacological treatment is limited and comes with significant side effects. Moreover, there is little clinical evidence proving efficient therapeutic strategies to improve the symptomatology. This is why there is an important need to find better alternatives and therapeutic approaches. Recent studies have proven that the endocannabinoid system has a significant regulatory function in the basal ganglia within the brain, and it is involved in the pathogenesis of Parkinson's disease. Therefore, investigating these biochemical interactions and other involved pathways may render a possible and effective new pharmacological strategy. Cannabidiol is a phytocannabinoid obtained from the plant *Cannabis sativa*, but lacking the euphoriant and psychotic effect of $\Delta^9$-tetrahydrocannabidiol. Recent studies have shown that this compound acts through the endocannabinoid pathways and thus, could have therapeutic effects in treating Parkinson's disease–symptoms. The fact that the endocannabinoid system plays a central role in regulating basal ganglia activity, as well as the modifications taking place in its signalling pathways, provide support for the development of pharmacological compounds targetting the endocannabinoid system, and highlights the importance of adding new insights into these interactions.

*Keywords: Parkinson's disease, non-motor symptoms, endocannabinoid system, cannabidiol, $\Delta^9$-tetrahydrocannabidiol.*

**RESUMEN**

La enfermedad de Parkinson es una enfermedad neurodegenerativa crónica que afecta a la población mundial y consiste en una pérdida progresiva y degeneración de las neuronas dopaminergicas en el cerebro. Esto causa síntomas motores como bradiquinesia, temblor de reposo, alteraciones posturales y rigidez. Esta enfermedad está además caracterizada por síntomas no motores como alteraciones del sueño, déficits cognitivos, y trastornos psiquiátricos como psicosis, depresión y ansiedad. La eficacia de su tratamiento farmacológico es limitada y produce efectos secundarios significativos. Además hay poca evidencia clínica que demuestre estrategias terapéuticas eficientes para mejorar la sintomatología. Por esta razón hay una necesidad importante de buscar mejores alternativas y enfoques terapéuticos. Estudios recientes han demostrado que el sistema endocannabinoide tiene una importante función reguladora en los ganglios basales y está involucrado en la patógenesis de la enfermedad de Parkinson. Por tanto, investigar estas interacciones bioquímicas y otras rutas involucradas puede suponer una nueva posible y efectiva

estrategia farmacológica. Cannabidiol es un fitocannabinoide obtenido de la planta *Cannabis sativa*, pero no posee el efecto eufórico y antipsicótico del $\Delta^9$-tetrahydrocannabidiol. Estudios recientes han demostrado que este compuesto actúa a través de las rutas endocannabinoides y por tanto, podría tener un efecto terapéutico para tratar los síntomas de la enfermedad de Parkinson. El hecho de que el sistema endocannabinoide juegue un rol central regulando la actividad de los ganglios basales, así como las modificaciones que tienen lugar en sus rutas reñalizadoras, apoya el desarrollo de compuestos que tengan como diana el sistema endocannabinoide, y subraya la importancia de añadir nuevas investigaciones sobre estas interacciones.

*Palabras clave: enfermedad de Parkinson, síntomas no motores, sistema endocannabinoide, cannabidiol, $\Delta^9$-tetrahydrocannabidiol.*

# INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disease of the central nervous system (CNS) that mainly affects the motor system. PD is a common disease amongst our world population, being the second most usual neurodegenerative disorder, just after Alzheimer's disease. It typically occurs in people aged over 65, presenting an incidence of 1-2% among people within this age group *(Connolly and Lang, 2014).* It is more prevalent in men than it is in women, at a ratio of about 3:2 *(Kalia et al., 2015).* It is characterised by motor disorders, such as rest tremor, bradykinesia, stiffness, slowness of movement and postural disturbances, which also produces walking difficulty. PD also causes non-motor symptoms such as sleep disturbances, constipation, urinary incontinence, sexual dysfunction, and orthostatic hypotension. In the advanced stages of the disease, more critical symptoms also appear, that is, cognitive deficits, dementia, and psychiatric disorders such as psychosis, depression, and anxiety *(Crippa et al., 2019).*

The etiology of this disease remains unknown, but it is thought to result from a complicated combination of both genetic and environmental factors. It has been proved that people with a family member affected with PD are more likely to suffer the disease themselves. Moreover, there seems to be also an increased risk in people who are exposed to certain pesticides, as well as those who have had previous head injuries *(Kalia et al., 2015).* However, there is a lower risk of presenting this disorder among tobacco smokers, and studies have shown the significant protective effect of coffee and tea against PD *(Barranco Quintana et al., 2009).*

The pathophysiology of the disease results primarily from abnormalities in the brain basal ganglia function. The basal ganglia include the striatum (caudate nucleus and putamen), the external and internal pallidal segments (GPe, GPi), the subthalamic nucleus (STN), and the substantia nigra with its pars reticulata (SNpr) and pars compacta (SNpc) *(Galvan et al., 2008).* The substantia nigra, specifically its pars compacta, is connected to the striatum projecting dopaminergic neurons, sending waves of dopamine, which are responsible for the production of movement.

The abnormalities in PD consist of progressive and selective degeneration and death of these dopaminergic neurons projected from the SNpc to the striatum *(Han et al., 2019).* This neuronal degeneration appears to be related to mitochondrial dysfunction, neuroinflammation and oxidative stress, which results in the destruction of the nigrostriatal tract, and the deficit of dopamine arriving at the striatum, thus, creating PD's characteristic motor symptoms, such as bradykinesia, muscle rigidity, and tremors *(Crippa et al., 2019)*. Furthermore, the dopaminergic neurons that do survive are left with the accumulation of α-synuclein protein, forming the Lewy bodies, which is known to be the hallmark of this disease (*see figure 1*).

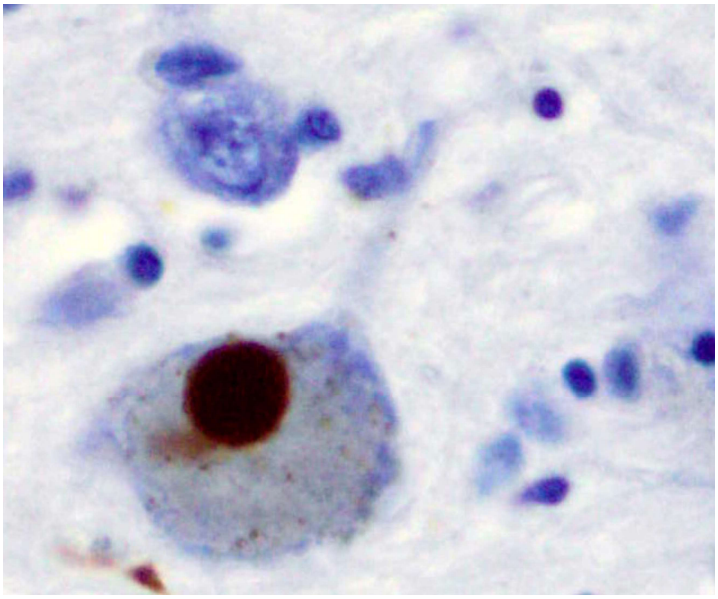

**Figure 1.** A Lewy body (stained brown) in a brain cell of the substantia nigra in Parkinson's disease. The brown color is the positive immunohistochemical staining for α-synuclein. From *Han et al., 2019*.

Given that the motor symptoms of Parkinson's disease are due to the deficit of dopamine in the basal brain, the main treatment is to administer the precursor levodopa (L-dopa) which can pass through the blood-brain barrier. Once in the brain, it is readily converted to dopamine and it temporarily diminishes the motor symptoms of PD. Other pharmacological treatments are also dopamine degradation inhibitors [dopa-decarboxylase inhibitors, monoamine oxidase (MAO) inhibitors, and catechol-O-methyl transferase (COMT) inhibitors] *(Crippa et al., 2019).* Though levodopa is considered the most effective therapy for motor symptoms of PD, it also comes with drawbacks. After chronic treatment with this drug, the patient might develop involuntary movements called dyskinesias, thus decreasing its therapeutic use, as well as presenting fluctuations on its effectiveness. Moreover, patients are also treated with other medications to palliate their non-motor symptoms which are not alleviated with dopaminergic drugs, such as antidepressants, anxiolytics, sedatives, and antipsychotics, which also come with other adverse reactions *(Crippa et al., 2019)*. The handling of these non-motor symptoms is one of the most difficult challenges in the treatment of PD. Therefore, current researches focus on the development of new drugs that can treat PD effectively, that is, restoring dopaminergic transmission, producing as few non-motor symptoms as possible, plus avoiding the development of dyskinesias *(Han et al., 2019).*

The discovery of the endocannabinoid system (ECS) in the central nervous system has triggered an avalanche of experimental studies, involving physiological and pathological functions *(Pacher and Kunos, 2013)*. These studies have suggested that the ECS regulates a huge range of physiological functions, including mood, cognition, motor control, and pain, and that modulating the activity of this system presents

therapeutic promises for a wide range of diseases, including neurodegenerative disorders, such as Parkinson's disease *(Han et al., 2019).*

The ECS is modulated by endogenous and exogenous cannabinoids extracted from *Cannabis sativa* (the marijuana plant). Cannabidiol (CBD) is one of the 100 cannabinoids obtained from *Cannabis sativa*, that lack of psychotomimetic properties. The different cannabinoids obtained from this plant have been widely used throughout the history of medicine, for specific and limited indications related to pain, wasting disorders, and chemotherapy-induced nausea and vomiting, but not any further due to their socially undesirable psychoactive properties *(Pacher and Kunos, 2013).*

Cannabinoids are found not only to produce neuroprotection but also to present positive effects in relieving motor symptoms of PD. Furthermore, CBD reveals diverse actions in the central nervous system that may have a role in easing non-motor effects of PD, including anxiolytic, antipsychotic, antidepressant, and sleep effects *(Crippa et al., 2019).* In this review, I provide a comprehensive overview of the ECS, the cannabinoid receptors and their targeted pathways, and present clinical evidence implicating the ECS in human disease, as well as the treatment strategies that target the ECS in PD.

**PARKINSON'S DISEASE**

The causes of PD are yet unknown, though it has been proposed that there might be some risk factors included in the genetic and toxic domain *(Bartels et al., 2009)*, involving a complex puzzle of genes, environment, and aging-related processes. In fact, only a minority of cases seem to be related to well-defined genetic or environmental causes, whereas a combination of mostly unknown genetic and environmental factors is considered to account for the vast majority of cases *(Klein et al., 2007).*

The PD affects several regions of the brain, including the pigmented nuclei in the midbrain and brainstem, the olfactory tubercle, the cerebral cortex, and elements of the peripheral nervous system *(Braak et al., 2006)*. The earliest and most striking disabilities resulting from these changes are motor impairments that, together, are called 'parkinsonism'. These include poverty of movements (akinesia), slowness of movement (bradykinesia), muscle stiffness (rigidity), and tremor at rest *(Lang et al., 1998)*.

The main characteristic of this disorder is the progressive loss and degeneration of dopamine-containing neurons in the midbrain, specifically in the SNpc, projecting to the striatum, which is the main input station of the basal ganglia neural circuit *(Lang et al., 1998)*. All this consequently results in the deficiency of dopamine in brain areas that receive dopaminergic inputs from those neurons, specifically the post-commissural putamen and other basal ganglia regions *(Galvan et al., 2008)*. This loss is irreversible, and leads to basal ganglia circuit deficits and its disorganisation, which is

normally responsible for motor functions, hence the result of the movement dysfunctions that characterize this syndrome *(Balapal et al., 2017)*.

**BASAL GANGLIA CIRCUIT**

Parkinsonism is considered to result primarily from abnormalities of basal ganglia function. The basal ganglia consist of a group of five principal subcortical nuclei. These include the neostriatum (caudate nucleus and putamen), the external and internal pallidal segments (GPe, GPi), the subthalamic nucleus (STN), and the substantia nigra with its pars reticulata (SNr) and pars compacta (SNc). They participate in anatomically and functionally segregated loops that involve specific thalamic and cortical areas (*see figure 2A*).

The neostriatum is the main input nucleus of the basal ganglia, as it receives excitatory glutamatergic cortical inputs from all functional subdivisions of the neocortex and a prominent input directly from the thalamic nuclei.

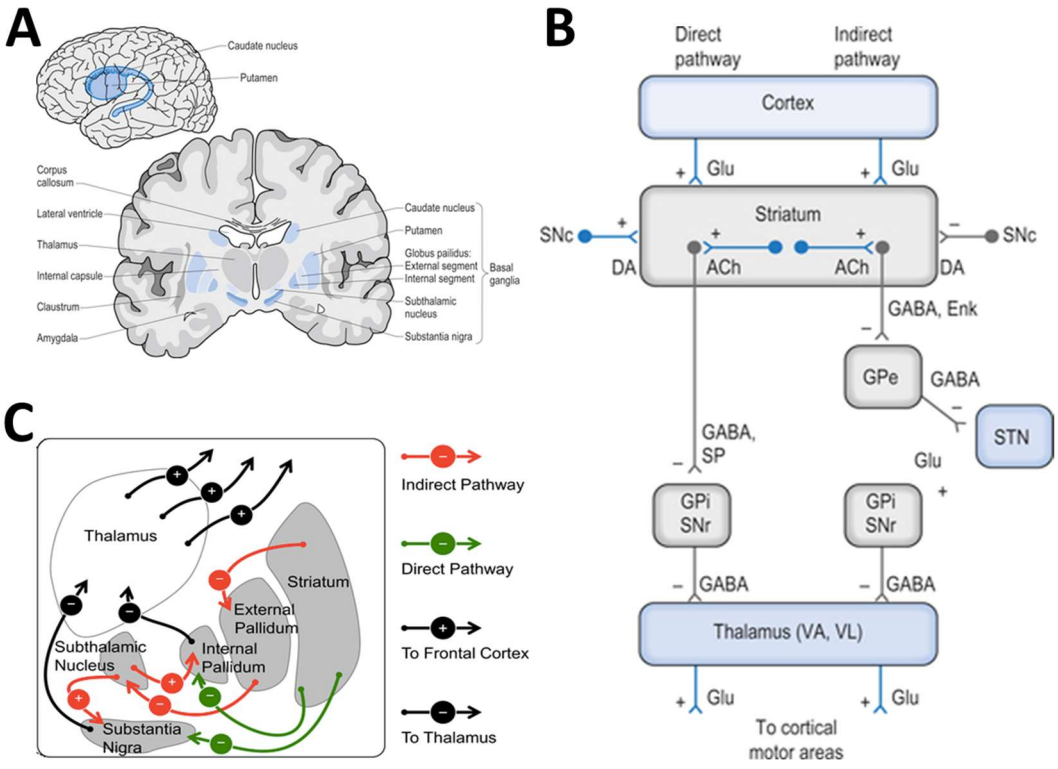


**Figure 2.** Overview of basal ganglia circuit. (A) Human coronal brain section showing the basal ganglia nuclei *(Clinical gate, 2015)*. (B and C) Basal ganglia circuitry showing the direct and indirect pathways. From *Rice, 2017.*

It contains a variety of different neurons including medium-spiny projection neurons, large cholinergic neurons, and small interneurons. Medium-spiny projection neurons (MSNs), which comprise about 90-95% of the neurons in the neostriatum, and release gamma-aminobutyric acid (GABA). Cortical neural signals are processed by a striatal network comprising GABAergic and cholinergic interneurons. These MSNs, receive the majority of the cortical input to the neostriatum and provide the only striatal output of the neostriatum *(Kawaguchi et al., 1993)*. Thus all output from the neostriatum to the globus pallidus and substantia nigra is inhibitory.

There are two predominant pathways from the neostriatum to the output nuclei of the basal ganglia: the globus pallidus internus and the substantia nigra pars reticulata:

a) The *direct pathway*, in which the striatal MSNs project output neurons that synapse on the neurons of the GPi and/or on the neurons in the SNpr. These projections release GABA, which acts as inhibitors on the target neurons in the GPi and the SNpr (*see figures 2B*).
b) The *indirect pathway*, in which the axons from the neurons of the neostriatum project to neurons in the GPe, where they release GABA, which inhibit the neurons of the GPe. The neurons of the GPe in turn, project to the neurons located in the subthalamic nucleus (STN), where they release GABA and act to inhibit the output neurons of the subthalamic nuclei. These STN neurons, on their hand, project to the neurons of the GPi via the subthalamic fasciculus where they release glutamate. The subthalamic output neurons are the only excitatory neurons in the basal ganglia circuits *(Alexander et al., 1986)* (*see figure 2B*).

The neurons in the GPi project axons via the anterior thalamic fasciculus to the ventral lateral and ventral anterior nuclei of the thalamus. These projections are mainly associated with motor control functions of the body below the head and neck. They also project to the intralaminar nuclei and the mediodorsal nuclei of the thalamus. These projections are largely associated with limbic activities *(Chusid, 1982)*. The neurons in the SNpr also project to the ventral anterior and ventrolateral nuclei of the thalamus and are associated with motor control of the head and neck.

The neurons in the substantia nigra pars compacta release dopamine. They project to the neostriatum where they have complex modulatory effects on the output neurons of the neostriatum. The net effect of the SNpc release of dopamine in the neostriatum is an excitation of the output neurons of the direct pathway and an inhibition of the output neurons of the indirect pathway *(Parent et al., 1998).*

The thalamus receives all this information and, at the same time, projects back to the frontal cortex, in an excitatory neuron synapse, thus completing the cortico-basal ganglia-thalamo-cortical loop (*see figure 2C*).

The physiological effect on MSNs of dopamine coming from the SNpc is complex and is still far from being completely understood. It is known though, that the stimulation of dopamine receptors results in different effects depending on the degree of membrane depolarization, and which receptor is activated *(Di Filippo et al., 2008)*.

D1 receptors are coupled to Gs proteins and stimulate several downstream effectors such as the enhancement of the N-methyl-D-aspartate receptor (NMDA) receptor-mediated currents. On the other hand, D2 receptors are coupled to Gi/o proteins and act by reducing neuronal excitability and neuronal response by glutamatergic inputs *(Surmeier et al., 2007)*. D1 receptors are located predominantly in the MSNs of the direct pathway, whereas D2 receptors are mainly expressed by the MSNs of the indirect pathway (*see figure 3*). Thus, dopamine acts finely regulating the balance between both pathways *(Di Filippo et al., 2008)*. Indeed, when a subpopulation of striatal neurons is activated, it inhibits a subpopulation of pallidal neurons and thus indirectly removes the tonic inhibition from a particular target motor center, thereby activating its motor program.

The progressive loss of midbrain dopaminergic neurons occurring in PD leads to lower striatal levels of dopamine and thus to the alteration of the equilibrium between the direct and the indirect basal ganglia pathways, leading to GPi overactivity and thus to an over-inhibition of the motor thalamus *(Bezard et al., 2001)*. The inhibition of the motor thalamus, in turn, acts as a "brake" on the activity of the supplementary motor cortex resulting in the onset of the parkinsonian syndrome.

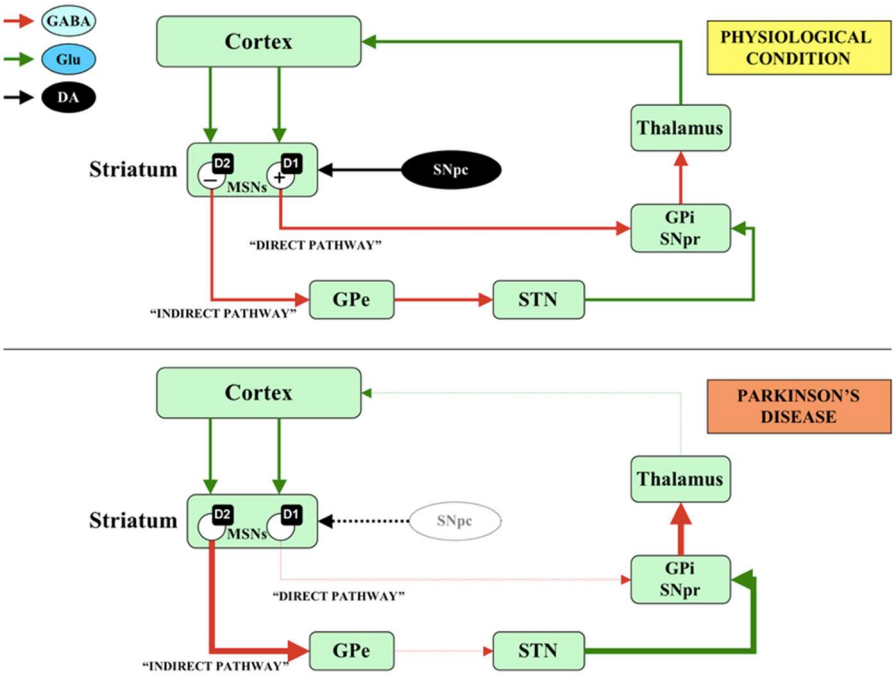


**Figure 3**. The Basal Ganglia circuit under physiological conditions (up) and Parkinson's disease (down). GABAergic connections are represented in red, and glutamatergic connections are in green. DA, dopamine; GABA, gamma-aminobutyric acid; Glu, glutamate; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; SNpc, substantia nigra pars compacta; SNpr, substantia nigra pars reticulata; STN, subthalamic nucleus. From *Di Filippo et al., 2010.*

## THE ENDOCANNABINOID SYSTEM

The ECS is a neuromodulatory system that plays important role in the CNS, development, and synaptic plasticity. This system is thought to control the regulation of physiological functions such as movement, memory and learning, cognition, neuroendocrine secretion, appetite, emesis, regulation of body temperature, pain, and immune system modulation *(Croxford et al., 2005)*. The discovery of their ability, to modulate synaptic neurotransmission, has been key to target these molecules as therapeutic agents in diseases where inappropriate neurotransmission induces disease pathology, including neurodegenerative disorders.

The ECS is formed by various components, including: cannabinoid (CB) receptors, their endogenous ligands (endocannabinoids) and the enzymes that regulate the synthesis and degradation of endocannabinoids *(Lu and Mackie, 2016).* The receptors are CB1 and CB2. They belong to the large family of G protein-coupled receptors (GPCRs) *(Castillo et al., 2012).* They are present in both the CNS and the periphery. Signaling downstream of these CB receptors is significantly involved in a wide variety of standard functions, as well as several pathological functions of the CNS *(Balapal et al., 2017).*

Endocannabinoids are key modulators of the synaptic function. Both endogenous and exogenous cannabinoids perform their effect through their interactions with cannabinoid receptors. The most studied endogenous cannabinoids are N-arachidonoyl-ethanolamine (AEA) and 2-arachidonoyl-glycerol (2-AG). They are mainly located in the brain and periphery. By activating these receptors, they regulate and perform different actions *(Castillo et al., 2012).* Though they present similarities in their chemical structure, these endocannabinoids are synthesized and degraded by different enzymatic pathways, which develop different physiological roles *(Lu and Mackie, 2016)*. Their mechanism of action consists of a retrograde mode of action, where they are liberated postsynaptically, after neuronal depolarization, suppressing the neurotransmitter release in both excitatory and inhibitory synapses. This performance is regulated by calcium influx *(Saito et al., 2013).* The enzymes that take part in these processes, synthesizing and metabolizing the endocannabinoids are the fatty acid amide hydrolase (FAAH) and the monoacylglycerol lipase (MAGL).

### The endocannabinoid ligands

Endocannabinoids are long-chain polyunsaturated fatty acids derivated from membrane phospholipids, especially arachidonic acid. They can activate different receptors through their biosynthetic and catabolic pathways. The best studied endogenous cannabinoids are N-arachidonoyl-ethanolamine (AEA) and 2-arachidonoyl-glycerol (2-AG) (*see figure 4*).

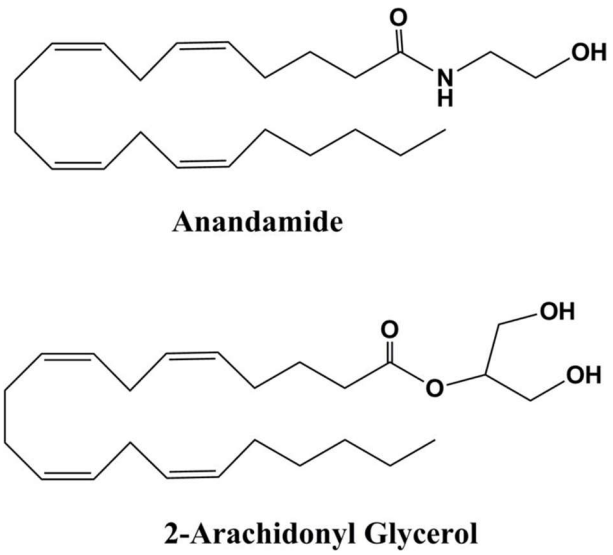


**Figure 4**. Chemical structure of the first endocannabinoids identified. From *Balapal et al., 2017*.

The first endogenous cannabinoid receptor agonist identified was N-arachidonoyl-ethanolamine, also known as anandamide (AEA). It was first isolated from porcine brain *(Devane et al., 1992),* and exhibits a higher affinity to CB1 than to CB2 receptor. It is found in high concentrations in brain areas involved in the processing and execution of body movements, such as the basal ganglia. The higher levels of AEA are found in the hippocampus, thalamus, striatum, and brain stem, where it exists a high density of CB1 receptors. Peripherally, AEA is expressed in structures such as the spleen, kidney, skin, and uterus *(Felder et al., 1996).* Its physiological effects respond to the activation of different receptors, including CB1, CB2, and TPRV1 *(Devane et al., 1992)*.

The second identified endocannabinoid ligand, 2-arachidonoyl-glycerol (2-AG), was originally isolated from the canine intestinal tissue, and later in spleen and pancreas, which brought up the suggestion of it being a peripheral ligand *(Mechoulam et al., 1995)*. Later, it was found also in the brain, in quantities even higher than AEA *(Sugiura et al., 1995).* In the brain, the higher levels are found in the hippocampus, striatum, and brain stem, as well as in the spinal cord. 2-AG is also present in high density in the CNS although it has a lower affinity for CB1 receptors than AEA. 2-AG acts as an agonist in both CB1 and CB2 receptors, remarking its higher affinity for the latter *(Bisogno et al., 1999),* and also presents a higher half life than AEA.

**Synthesis and degradation of endocannabinoids**

The endocannabinoids are lipophilic and are synthesized on demand from membrane phospholipids, without storage in vesicles *(Barapal et al., 2017)*. They are synthesized

and released from the postsynaptic neuron, and bind to its correspondent receptor located in the presynaptic membrane, activating it to inhibit the neurotransmitter release *(Basavarajappa et al., 2018)*. Then, they are removed from the synaptic junction by process of cellular transport followed by hydrolysis. Finally, they are inactivated and degraded in postsynaptic neurons by different enzymes: anandamide is hydrolyzed by the fatty acid amide hydrolase (FAAH) and 2-AG is hydrolyzed by the monoacyclygerol lipase (MAGL) *(Balapal et al., 2017)*. This retrograde signaling provides a mechanism for inhibitory feedback to regulate the neurotransmitter release in the brain.

Although both ligands have similarities in structure and ways of action, their synthetic pathways are completely different and mediated by different enzymes *(Saito et al., 2013)*.

**AEA:** AEA is synthesized and released by a calcium-dependent mechanism. Its synthesis is produced from a phospholipidic precursor which is present in the cell membrane, the phosphatidyl-ethanolamine (PEA), which by the action of N-acetyltransferase (NAT) enzyme, gives place to N-acylphosphatidylethanolamine (NAPE) (*see figure 5*). This molecule then is hydrolyzed, in a process mediated by the phospholipase D, to yield AEA *(Di Marzo et al., 2008).* AEA is then released into the synaptic cleft to be rapidly inactivated by reuptake and subsequently undergoes intracellular enzymatic degradation. FAAH metabolizes AEA to arachidonic acid, and ethanolamine leading to rapid clearance of AEA from extracellular compartments *(Di Marzo et al., 2008).*

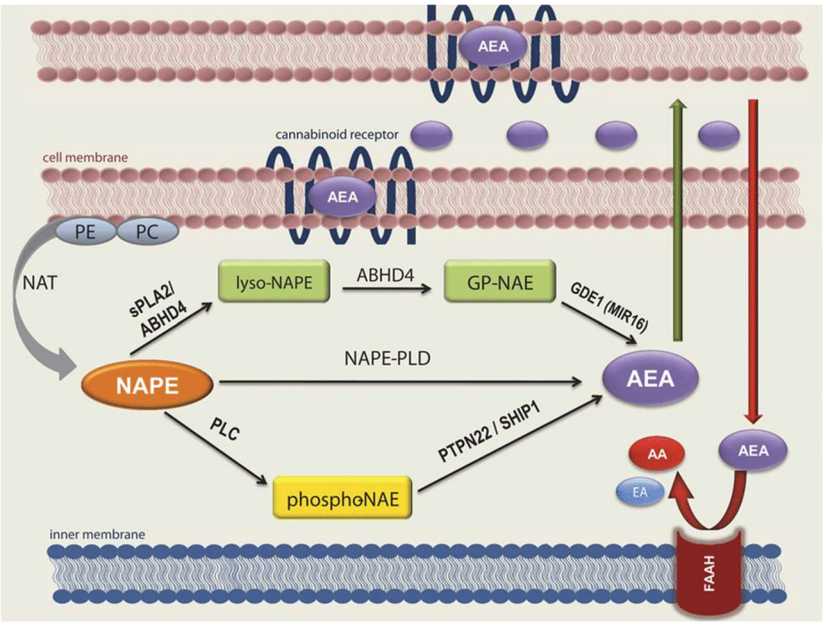

**Figure 5.** A schematic enzymatic pathway of the oxidation of the endogenous cannabinoid Anandamide. From *Hollenberg, 2010*.

**2-AG**: its biosynthesis is initiated in the membrane by a calcium-dependent mechanism that activates the phospholipase C (PLC), whose action is to transform phosphatidyl-inositol into diacylglycerol (DAG) *(Di marzo et al., 2008).* This compound, through the hydrolysis catalyzed by diacylglycerol lipase (DAGL), produces 2-AG *(Pacher et al., 2006)*. 2-AG is also synthesized through the conversion of 2-arachidonoyl lysophosphatidic acid (LPA) by a phosphatase. 2-AG activates the CB1 receptors with greater efficacy than does AEA. Like AEA, 2-AG is inactivated by reuptake and subsequently undergoes intracellular enzymatic degradation by the MAGL *(Di Marzo et al., 2008; Beltramo et al., 2000).*

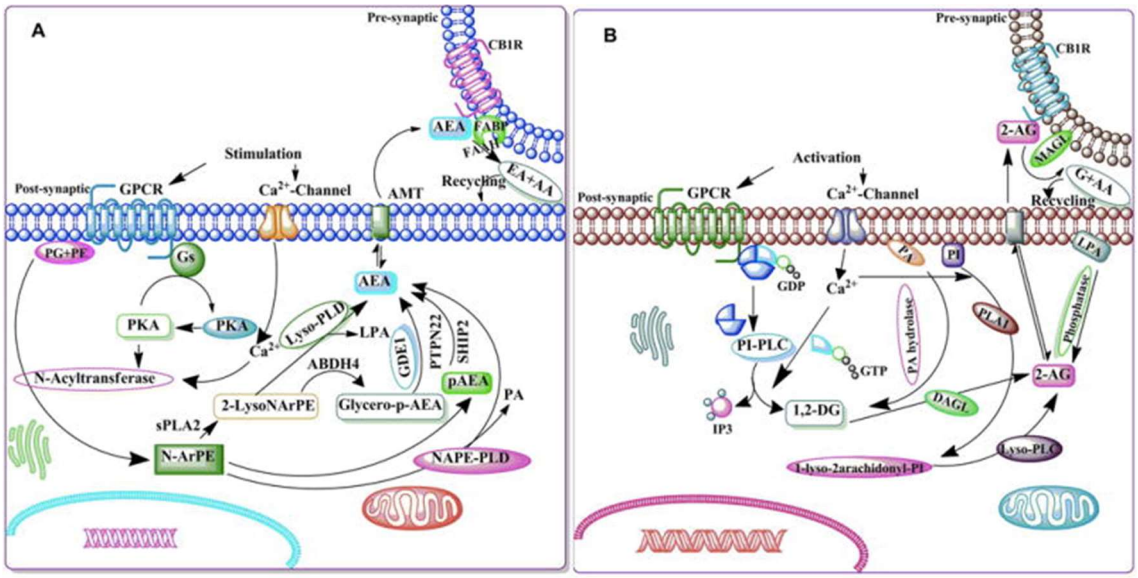


**Figure 6**. A schematic enzymatic pathway that regulates catabolism of AEA (A) and 2-AG (B). From *Barapa. et al., 2017*.

In addition, there are other alternative synthesis pathways. From arachidonic acid and ethanolamine, AEA can be formed, by the action of the enzyme FAAH, as long as both molecules are found in high concentrations. 2-AG can also be synthesized from PI by the action of different enzymes as the phospholipase A (PLA) and the liso-phospholipase C (liso-PLC) (*see figure 6*).

In terms of its degradation, this can take part through different processes. AEA has a very short life span and its metabolization can be principally produced by three different mechanisms *(Danert et al., 2004)*:

1. Hydrolysis produced by FAAH, which separates AEA into arachidonic acid and ethanolamide *(Cravatt et al., 1996).*
2. Oxidation performed by the hepatic enzyme cytochrome P450 (CYP450), producing twenty active metabolites *(Snider et al., 2007)*.
3. Oxydation mediated by cyclooxygenase 2 (COX-2), in which AEA is transformed into the prostaglandins E2-ethanolamide and D2-ethanolamide *(Kozak et al., 2002)*.

Furthermore, it has been described the effect of the lipooxygenase (LOX) on AEA to produce 5/11/12/15-H(p)-AEA *(Danert et al., 2004).*

Regarding 2-AG's degradation, although it has been less studied, it is known that once its function is completed, it is metabolized by the enzyme MAGL, transforming 2-AG into arachidonic acid and a glycerol molecule *(Pacher et al., 2006)*. Besides, *in vivo* studies have demonstrated the capacity of FAAH degrading this compound, though with less affinity than for AEA *(Lambert et al., 2005).* Furthermore, it has been shown the paths by which COX-2 can metabolize 2-AG to produce different prostaglandins (E2, D2, F2alpha) and tromboxanes *(Kozak et al., 2002).*

Overall, though it was thought that these two endocannabinoid ligands acted through different synthetic and metabolic pathways, it has been studied and proven that the degradation pathways they both follow are not that further apart from one another, but instead, they result to be relatively similar.

**Endocannabinoid receptors and signaling**

The physiological effect of the endocannabinoid ligands is mediated mainly by two receptors: CB1 and CB2 *(Howlett et al., 2009).* They both belong to the family of G-protein coupled receptors (GPCRs) that mediate almost all the actions of exogenous and endogenous cannabinoids *(Castillo et al., 2012)*.

CB1 receptors contribute to control the activity of a wide variety of cell functions such as cell differentiation, survival, and propagation, and the AC/cAMP cascade plays an important role in intracellular mechanisms *(Han et al., 2019).*

On the other hand, the CB2 receptors are poorly expressed in the brain compared to the CB1 and are typically found in the immune system. CB2 receptors are limited to specific neuronal cells and are abundant in activated astrocytes and microglia *(Stella et al., 2010)*. CB2 receptors are also highly expressed in various cells of the immune system, such as monocytes, NK cells, B-lymphocytes and macrophages, while the expression in T-lymphocytes is lower *(Graham et al., 2010).* CB2 receptors are found in other peripheral immune organs including the spleen, thymus and human skin. Consequently, they are believed to play an important role in immune responses. In

addition, some studies revealed that these receptors might be activated in microglial cells of pathologic human brains, and genetic studies demonstrated that they do appear in mice, being activated in neural pathology and inflammation *(Javed et al., 2016)*. Conclusively, it is suggested that CB2 receptors might participate in neuroinflammation which gradually develops into the induction of neurodegeneration. This suggests that cannabinoid related compounds can be a potential therapeutic target for the treatment of the neurodegenerative inflammatory processes *(Benito et al., 2008)*. Also, some selective CB2 receptors agonists have been proposed as therapeutics to the central nervous and immune system *(Galiegue et al., 1995)*.

***Retrograde Endocannabinoid Signaling.*** The principal mechanism by which endocannabinoids regulate synaptic function, involving both short and long term forms of plasticity at both excitatory and inhibitory synapses, is through retrograde signaling. However, there is evidence this is not the only mechanism of action by which they act, but also a non-retrograde manner *(Castillo et al., 2012).* Furthermore, apart from mediating synaptic plasticity, it has been proven the ECS is itself subject to plastic changes. It presents multiple points of interaction with other neuromodulatory and signaling systems, that have just been identified.

In the retrograde signalling, the postsynaptic activity leads to the production of an endocannabinoid (ECB) that moves backward across the synapse, binds to presynaptic CB1 receptors and suppresses neurotransmitter release (*see figure 7A*). In addition, it has been described a new procedure that involves a non-retrograde or autocrine manner. Through this mechanism, they can modulate the neural function and synaptic transmission by engaging the transient receptor potential vanilloid type 1 (TRPV1) and also binding to receptors CB1 located on or within the postsynaptic cell (*see figure 7B*). Lastly, other studies indicate that ECB can also signal via astrocytes to indirectly modulate presynaptic or postsynaptic function (*see figure 7C*).

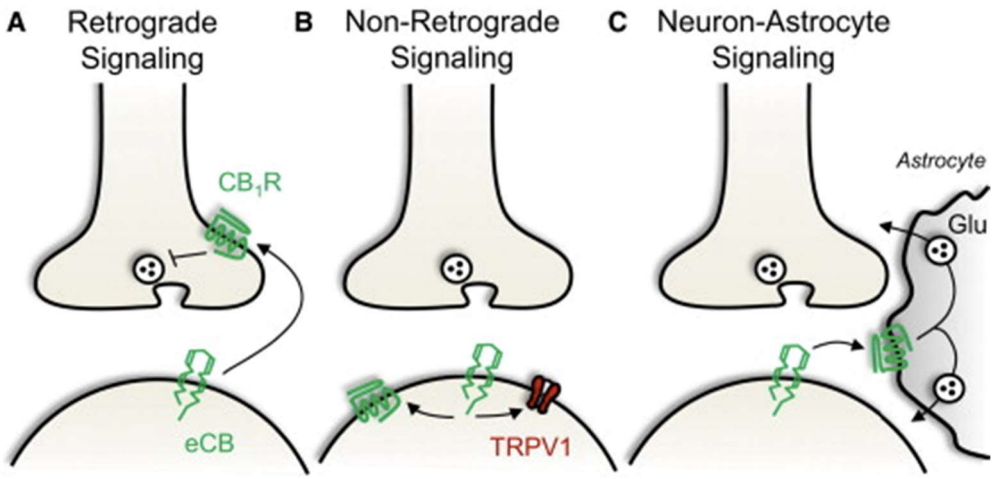


**Figure 7.** Three different ECS signaling in neurons synapse. From *Castillo et al., 2012.*

It was discovered that ECBs mediate short-term synaptic plasticity known as depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE) *(Ohno-Shosaku et al., 2001).* Shortly after, it was shown that ECBs also mediate presynaptic forms of long-term depression at both excitatory and inhibitory synapses *(Gerdeman et al., 2002; Chevaleyre et al., 2003)*. Since this discovery, ECBs have been characterized as retrograde messengers, reporting multiple examples of short and long term forms of synaptic plasticity throughout the brain *(Kano et al., 2009)*.

***Nonretrograde Endocannabinoid Signaling.*** Apart from the CB1 and CB2 receptors, there has been evidence found that other receptors also perform similar physiological roles, participating as well in ECB signaling: the endovanilloid system with its TRPV1 channels. They too may be a potential therapeutic target due to their diverse pharmacological characteristics *(Pertwee et al., 2010)*.

TRPV1 is a polimodal transient receptor potential ion channel largely expressed in afferent peripheral sensory neurons. Its activation regulates synaptic transmission associated with pain sensation *(Caterina et al., 2001).* In addition to their expression in the periphery, TRPV1 channels have been found in the CNS, where they appear to regulate synaptic function.

The endocannabinoid and the endovanilloid system have a close relationship. It is remarkable how TRPV1 can also bind lipophilic substances, therefore being able to bind some endogenous cannabinoids such as AEA. AEA does not only act on CB1 receptors as a partial agonist, but also acts as a full agonist on the TRPV1 channels *(Smart et al., 2000)*.

Recent studies reveal that AEA acting on TRPV1 mediates a postsynaptic form of LTD (Figure 10A). This TRPV1-mediated postsynaptic LTD has been observed in the D2 receptor located in spiny neurons of the nucleus accumbens, the dentate granule cells, and the bed nucleus of the stria terminalis *(Puente et al., 2011).* The physiology underlying this mechanism is the activation of receptor mGluR5 in the postsynaptic neuron, which presumably via PLC and $Ca^{2+}$ release from intracellular stores, promotes the synthesis of AEA, which will then activate TRPV1 channels. In addition, this signaling relies on AMPA receptor endocytosis. These findings add up to the notion that AEA can act as an intracellular messenger *(Di Marzo et al., 2005)*, but its way of action differs depending on which receptor binds to. While CB1 receptor activation brings about the inhibition of $Ca^{2+}$ influx and the suppression of the neurotransmitter discharge, the activation of TRPV1 receptor channels favours $Ca^{2+}$ influx and neurotransmitter release *(Vaughan et al., 2000).*

The nonretrograde ECB signaling has been observed in other contexts. The activation of a subtype of cortical GABAergic interneuron triggers a CB1 receptor-dependent postsynaptic hyperpolarization, which reduces its excitability, therefore inhibiting neuronal signaling (Figure 10B) *(Bacci et al., 2004).* This process consists of a slow action, involving rising of intracellular $Ca^{2+}$, mobilization of 2-AG, and activation of the

CB1 receptor, which then couples to a G-protein that activates a $K^+$ channel. This form of autocrine signaling was also observed in a fraction of the medium layer of neocortical pyramidal neurons *(Marinelli et al., 2009).*

Another endocannabinoid receptor that has been found is the G-protein coupled receptor 55 (GPR55) *(Sharir et al., 2012)*. Though the interaction of the endocannabinoid system with GPR55 remains poorly known, it has been shown that a number of cannabinoids target at GPR55 *(Han et al., 2019)*. This receptor acts through β-arrestin2 (βarr2), and its internalization, following activation of lysophosphatidylinositol (LPI). AEA acts as an agonist, enhancing the agonist effect at low concentrations and inhibiting it at high concentrations *(Sharir et al., 2012)*. GPR55 has a significant expression in the striatum, thus probably participating in motor behaviours. It is important to outstand that studies in mice lacking GPR55 showed that their motor behaviours were impaired, suggesting that these receptors have a modulatory role in motor activity.

**PARKINSON'S DISEASE AND ENDOCANNABINOID SYSTEM**

The long-term treatment with L-dopa produces motor fluctuations and dyskinesias in most patients (>90%) within 5-10 years of treatment initiation. For this reason, other non-dopaminergic targets are being studiesd for the treatment of the disease *(Lang et al., 2004)*.

The ECS represents an interesting potential drug target in PD treatment. In the striatum, the endocannabinoid, glutamatergic, GABAergic and dopaminergic signaling systems profoundly interact in order to modulate basal ganglia neural network dynamics and long-term forms of synaptic plasticity. This interaction between ECBs, dopaminergic, and glutamatergic signals converging onto striatal projecting neurons represent the basis for the potential use of drugs targetting the ECS as a therapeutic strategy in PD. Moreover, since immune mechanisms and neuroinflammation are among the factors that have been implicated in PD pathogenesis, the CB2 receptor-mediated immunomodulatory effects of the ECBs might represent another useful target for drug development *(Di Filippo et al., 2008)*.

Several anatomical, biochemical, and electrophysiological studies have demonstrated that the components of the ECS are highly expressed at different levels in the basal ganglia neural circuit and thus critically modulate motor functions *(Brotchie et al., 2003)*.

CB1 receptors are expressed at MSNs, both in their dendrites and in their presynaptic axon terminals innervating the external and internal segments of the globus pallidus and the SNpr (*see figure 8*). They are also present at the corticostriatal excitatory glutamatergic terminals and in the excitatory projections from the STN to the GPi/SNpr and SNpc. Indeed, the activation of presynaptic CB1 receptors at corticostriatal terminals reduces glutamate release, both through the direct and indirect pathway,

and therefore, inhibits the excitatory response of glutamate. At the same time, in the output basal ganglia nuclei (GPi and SNpr), CB1 receptors activation inhibits both glutamate release from STN afferents and GABA release from striatal afferents, creating a balance between excitatory and inhibitory responses. In the direct pathway, CB1 receptors activation seems to decrease GABA release from striatal afferents that innervates dopaminergic neurons of the SNpc. This reduction results in an increased firing of these cells projecting to the thalamus *(Brotchie et al., 2003)*.

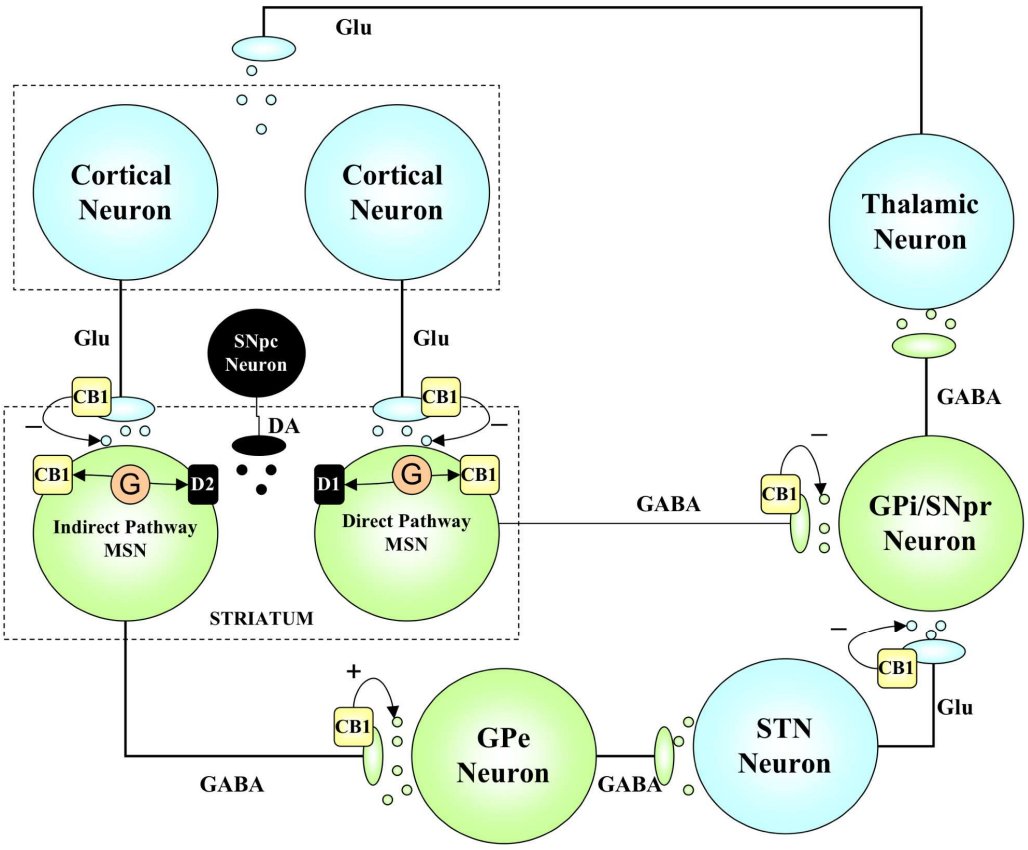


**Figure 8.** ECB signalling is also bi-directionally linked to dopaminergic signalling within the basal ganglia, specifically within the cortico-striatum synapses. From *Brotchie et al., 2003*.

Additionally, it has also been demonstrated the presence of transient receptor potential vanilloid type 1 (TRPV1) in dopaminergic nigral neurons and a functional role of these receptors in the modulation of synaptic transmission with the SNpc *(Marinelli et al., 2007)*.

According to this evidence, it is thought that ECBs may significantly modulate the physiological function of the basal ganglia network. The presence of components of the ECS in different neural structures, as well as their interactions with dopaminergic, glutamatergic, and GABAergic neurotransmitter signaling systems, entails these

elements as ideal targets for the search of non-dopaminergic pharmacological therapies for Parkinson's disease *(Di Filippo et al., 2008)*.

## THE THERAPEUTIC ROLE OF CANNABINOID DRUGS IN PARKINSON'S DISEASE

It has been proved that within the basal ganglia neural circuit and in particular in the striatum, synapses are able to undergo long-lasting functional and morphological modifications, as a result of the repeated activation of neuronal pathways. This capacity is known as synaptic plasticity.

The long-term depression (LTD) and long-term potentiation (LTP) of the efficacy of synaptic transmission have also been demonstrated to occur at striatal synapses of MSNs, following the repeated stimulation of the corticostriatal pathway. In order to process these corticostriatal synapse mechanisms, aside from the endocannabinoid receptors, also the stimulation of dopamine receptors is required *(Calabresi et al., 2007).*

It is remarkable to point out that in PD patients, these synapses are impaired, both in the striatum and in the motor cortex. According to this evidence, the impairment of synaptic plasticity in the basal ganglia neuronal circuit has been suggested as the hallmark of the basal ganglia network abnormalities that occur during this neurodegenerative disease *(Morgante et al., 2006).*

Endocannabinoid ligands, such as AEA have been proved to participate in the regulation of striatal neural circuits at the corticostriatal synapses, thus inducing the LTD. They seem to be released by striatal MSNs following membrane depolarization after the stimulation of the cortical neurons by the intracellular calcium elevation and D2 receptor stimulation. This provokes the release of AEA to the synaptic cleft, where acts as a retrograde messenger activating presynaptic CB1 receptors and thus inducing the long-lasting depression of excitatory glutamatergic transmission *(Di Marzo et al., 1994).*

Studies using experimental models of PD showed that ECB-dependent LTD was lost at indirect pathway MSNs synapses. This loss of ECBs-dependent striatal LTD at corticostriatal synapses might be then considered as the main event leading to the alteration of the balance between the direct and the indirect basal ganglia pathways *(Di Filippo et al., 2008)*. However, the administracion of quinpirole, a D2 receptor agonist, or URB597, an inhibitor of FAAH, the degradative enzyme of the endocannabinoid ligand AEA decreases catalepsy and increases locomotor activity in the same experimental PD models *(Kreitzer et al., 2007)*. This suggests the correlation of inducing an ECB-mediated form of synaptic plasticity at corticostriatal synapses with the improvement of PD motor symptoms.

Furthermore, both animal and human studies demonstrated that the ECB system undergoes neurochemical changes during PD, including downregulation of CB1 receptors in the early stages of the disease and the upregulation of these receptors, as

well as CB2 receptors, in the intermediate and more advanced phases of the disease *(Di Marzo et al., 2000; Giacoppo et al., 2014; Stampanoni Bassi et al., 2017)*.

Pre-clinical studies performed in the last 20 years show that, depending on the PD stage and the different sub-areas of the basal ganglia involved, cannabinoids (natural and synthetic) can regulate the neurochemical changes in the glutamate and GABA neural system through activation or inhibition of CB1 and CB2 receptors *(Gómez-Gálvez et al., 2016)*.

Given the knowledge of the degeneration of dopaminergic neurons of the SNpc and the subsequent striatal dopamine deficiency being the key event in the disorder of PD and the cause of most of the motor features of the disease, therapeutic strategies aim to prevent further dopaminergic cell death, thus slowing the disease progression *(Clarke, 2004)*. Several factors seem to be implicated in the pathogenesis of cell death in PD, including oxidative stress, mitochondrial dysfunction, ubiquitin-proteasome dysfunction, inflammation, excitotoxicity, and apoptosis *(Jiang et al., 2018)*.

Several mechanisms have been proposed as responsible for the possible therapeutic effects of endocannabinoids in PD, including antioxidant, anti-excitotoxic, and anti-inflammatory effects, inhibition of anandamide hydrolysis, and other mechanisms of action that are independent of cannabinoid receptors, such as the modulation of the receptor channel TRPV1 and the G protein-coupled receptor 55 (GPR55).

Recent studies have shown that compounds with a specific activity in CB1 and CB2 receptors render significant promises to treat PD. The next sections review the preclinical and clinical studies of these therapies targetting this neurodegenerative progression.

**Neuroinflammation**

Neuroinflammation is the basic hallmark that leads to degeneration and loss of the dopaminergic neurons in PD. This degeneration is associated with substantial activation of microglia *(Orr et al., 2002)*. Using stimuli to mimic Parkinson's disease in animal models helped to discover the changes in cellular tissue among them, and revealed a consistent pattern implicating microglia as the effector for the selective degeneration of dopaminergic neurons. When microglia are activated, a series of physiological and biochemical reactions may occur, such as the production of pro-inflammatory cytokines, the recruitment of immune system cells in injured brain tissue, and the functional changes of brain vascular endothelium.

Studies in rats, causing lesions of nigrostriatal dopaminergic neurons by local application of 6-hydroxydopamine *(García-Arencibia et al., 2007)* demonstrated that the THC and CBD were neuroprotective in PD, presumably because of their antioxidant properties. They found that CBD was able to recover dopamine depletion when it was administered immediately after the lesion, but failed to do it when the treatment started 1 week later. They also examined the neuroprotective effects of some cannabinoid-based compounds, with selectivity for elements of the cannabinoid

signaling system. They used the CB1 receptor agonist arachidonyl-2-chloroethylamide (ACEA), the CB2 receptor agonist HU-308, the non-selective CB1/CB2 agonist WIN55,212-2, and the inhibitors of the endocannabinoid inactivation AM404 and UCM707. Results showed that HU-308 produced a small recovery in dopamine depletion, which suggests the possible involvement of CB2 but not CB1 receptors. This study indicates that the cannabinoids provide neuroprotection against the progressive degeneration of nigrostriatal dopaminergic neurons in PD and that the activation of CB2, but not CB1 receptors, might contribute to some extent *(García-Arencibia et al., 2007)*.

Another study has proved that CBD and THC possess anti-oxidant and anti-apoptotic effects, as well as anti-inflammatory properties. The mechanism underlying this effect starts with the inhibition of p38MAP kinase phosphorylation, which can induce the nuclear factor-kappa B (NF-κB) inhibitory signaling, thus avoiding the nuclear translocation of NF-κB, and inhibit the expression of the proinflammatory genes. These contribute to the reduction of the expression of the inflammatory enzymes and cytokines, such as the inducible nitric oxide synthase (NOS) or the cyclooxygenase (COX-2) *(Esposito et al., 2006)*.

Although the anti-inflammatory effects of cannabinoids are more commonly associated with CB2 receptors in brain immune cells, some studies revealed that CB1 receptors also play a significant role in animal models *(Zoppi et al., 2010)*, using wild-type and CB1 knockout mice exposed to immobilization or acoustic stress. The selective CB1 agonist ACEA was able to demonstrate a reduction in pro-inflammatory molecules such as tumor necrosis factor-α (TNF-α). Furthermore, targetting the primary brain glutamate transporter in astrocytes, it was found that activated CB1 receptors enhanced the glutamate glial uptake and the glutamate astroglial transporter, the excitatory amino acid expression, as well as a downregulation of anti-inflammatory pathways L-PGDS/15d-PGJ2/peroxisome proliferator-activated receptor γ (PPARγ), which also leads to the inhibition of the activity of TNF-α. As a result, there is a reduction of the expression of pro-inflammatory enzymes such as COX-2 and NOS *(Zoppi et al., 2010)* *(see figure 9)*.

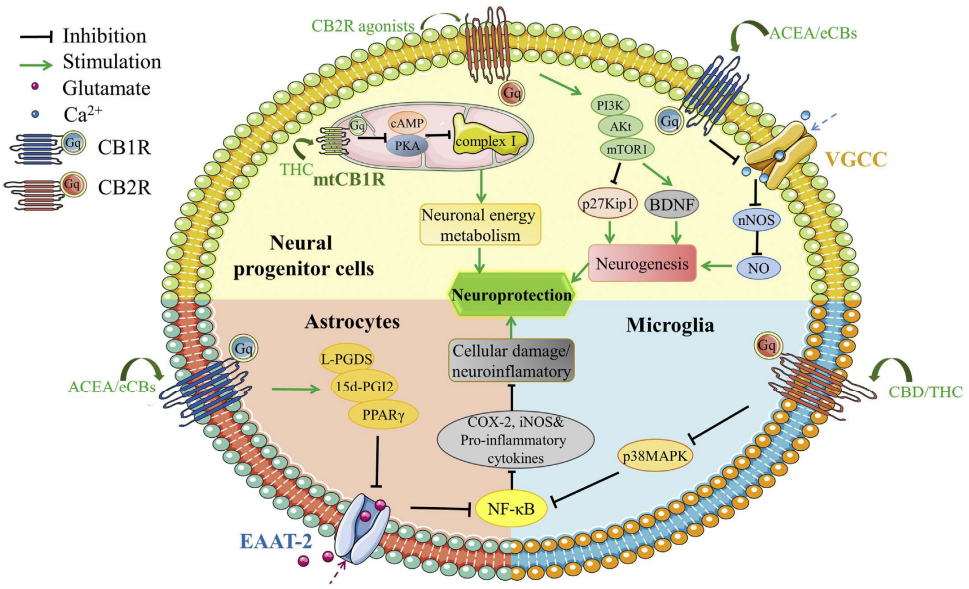


**Figure 9**. The intracellular mechanism of LTD pathway mediated by ECS. From *Han et al., 2019.*

## Oxidative stress

It is generally known that PD patient's brain has a high level of oxidative stress in their SNpc, compared to that of normal brains with no disease. This oxidative stress contributes to the cascade that leads to dopamine cell degeneration *(Jenner, 2003)*. Moreover, this stress is intimately linked to other components of the degenerative process, such as mitochondrial dysfunction, excitotoxicity, nitric oxide (NO) toxicity, and inflammation. Oxidative damage to lipids, proteins, and DNA occurs in PD, and the production of toxic products from this damage, such as 4-hydroxynonenal (HNE), can react with proteins to impair cell viability. Also, altered ubiquitination and degradation of proteins are implicated in dopaminergic cell death in PD. Oxidative stress can impair these processes directly, and products of oxidative damage such as HNE can damage the 26S proteasome. This impairment of the proteasomal function leads to free radical generation and oxidative stress *(Jenner, 2003)*.

THC shows a high affinity for CB1 and CB2 receptors, whereas CBD presents little affinity. This helps to increase some beneficial effects of THC as it reduces the psychoactivity of THC, enhancing its tolerability and widening THC's therapeutic window. Some reports show that CBD and THC are potent antioxidants, which display antioxidant activity and decrease neurotoxic levels of glutamate in rat cortical neuron cultures, without activating cannabinoid receptors *(Hampson et al., 1998)*. In addition, CBD has a positive effect on upregulating mRNA levels for Cu,Zn-superoxide dismutase (SOD1), which renders a key enzyme in endogenous defenses against oxidative stress *(García-Arencibia et al., 2007).*

A study was performed to prove the neuroprotective effect of THC in models of PD. Cannabinoids and modulatory compounds were co-administered with toxins for 48 h

and the effects on cell death, viability, apoptosis, and oxidative stress were assessed *(Carrol et al., 2012).* The results found were a CB1 receptor up-regulation in response to the PD-relevant toxins: $MPP^+$, lactacystin and paraquat, and a protective effect of THC against all three toxins. Moreover, the antioxidant cannabinoids, nabilone, and CBD, as well as the natural antioxidants α-tocopherol and butylhydroxytoluene, were not able to display the same neuroprotection as THC. This direct neuronal protective effect of THC may be mediated through PPARγ activation *(Carrol et al., 2012)*.

All the other studies performed strongly contributed to the fact that cannabinoids may display antioxidant effects on neurodegenerative pathologies, independent of cannabinoid signaling *(Fernández-Ruiz et al., 2012)*.

In terms of organelles, mitochondria seem to play a significant role, and mutations in mitochondrial genes have been found to produce neurological diseases. Mitochondria-mediated oxidative stress, perturbed calcium homeostasis, and apoptosis may contribute to the pathogenesis of neurological diseases, including PD *(Mattson et al., 2008)*. Recent studies reported the appearance of low levels of CB2 receptors on brain mitochondrial membranes, which suggests a direct correlation between them and the mitochondrial functions in the brain. Results showed that if mitochondrial fractions are enriched and purified, CB1 receptor agonists reliably decrease respiration in brain mitochondria *(Hebert-Chatelain et al., 2014)*. Exogenous cannabinoids such as THC can activate these receptors on the mitochondrial membranes of mice and then decrease cAMP concentrations and PKA activity. This process seems to participate in repairing neuronal mitochondria and modulating neuronal energy metabolism.

**Excitotoxicity**

Excitotoxicity refers to a process where excessive or long-term activation of the NMDA receptor by the excitatory amino acid neurotransmitter glutamate, leading to neuronal necrosis, apoptosis and cell death. This can happen in chronic neurodegenerative disorders such as PD *(Doble, 1999)*.

Anandamide (AEA), a partial agonist of CB1 receptor, can act directly at these NMDA receptors and decrease the NMDA-induced calcium influx. Studies performed in mice, using CB1/CB2 agonist WIN-55,212-2 and AEA, showed that they also reduce the NMDA-mediated release of $Ca^{2+}$ from intracellular stores *(Fagan et al., 2014)*. Apart from this, WIN-55,212-2 was also found to decrease the intracellular accumulation of α-synuclein. This regulation requires the CB1 receptor, and the NR1 subunit of the NMDA receptor to be coupled to the histidine triad nucleotide-binding protein 1 (HINT1) *(Sánchez-Blázquez et al., 2013)*.

Regarding the CB2 receptor, reports have shown that mice lacking CB2 receptors showed a higher occurrence of clonic-tonic seizures after NMDA receptor agonist quinolinic acid was administered *(Palazuelos et al., 2009)*. This higher sensitivity to excitotoxicity was reflected in an increased brain oedema volume. Analyzing the damaged striatum confirmed the enhanced activation of glial cells in CB2 receptor-deficient mice, and inflammatory markers such as NOS, interleukin 6 (IL-6) and TNF-α.

Furthermore, stimulation of the CB2 receptor by the selective CB2 agonist HU-308 showed that these oedema was significantly reduced after excitotoxicity. Lastly, cannabinoid administration attenuated the loss of striatal GABA levels and improved motor performance after excitotoxicity *(Palazuelos et al., 2009).*

Lastly, mutations in the PARK2 gene (also named parkin) cause hereditary PD. This gene regulates the function and stability of excitatory glutamatergic synapses *(Helton et al., 2008)*. Under normal conditions, the postsynaptic expression of parkin diminishes excitatory synaptic transmission and causes a marked loss of these excitatory synapses onto hippocampal neurons. Conversely, in PD, the expression of parkin mutants or a deficiency of endogenous parkin enhances synaptic efficacy and triggers a proliferation of glutamatergic synapses, which leads to increasing susceptibility to synaptic excitotoxicity *(Helton et al., 2008)*. These findings support the theory that cannabinoid receptors displaying excitotoxicity play a crucial role in the pathophysiology of PD.

**Neurogenesis**

This term refers to the generation of new neurons that takes place throughout adulthood within the brain. The subventricular zone (SVZ) provides new neurons for the olfactory bulb (OB), and the subgranular zone (SGZ) of the hippocampus produces new granule neurons for the dentate gyrus *(Horgusluoglu et al., 2016)*. In animal models of PD, it was discovered that this neurogenesis was severely affected. It has been reported that this disruption in olfactory neurons processing in PD patients may contribute to some of the non-motor symptoms of PD, such as depression, anxiety, or hyposmia *(Sullivan et al., 2013)*.

Also, studies performing the genetic deletion or the pharmacological blockade of the CB1 and CB2 receptors, confirmed their role in the proliferation of neural stem cells in PD patients (Jiang et al., 2005). The selective CB1 agonist ACEA, the non-selective agonist WIN-55,212-2, or the inhibition of FAAH, stimulate progenitor cell proliferation in vivo in the SVZ. In contrast, CB1 and CB2 antagonists reduce the proliferation of these new neurons *(Marxreiter et al., 2013)*.

These findings suggest the fact that the CB1 and CB2 agonists might help to counteract the observed disruption in neurogenesis that is associated with PD, and could provide a useful therapeutic target tackling this domain.

**Levodopa-Induced dyskinesias**

It is well known that the most effective treatment for Parkinson's disease patients is replacing the dopamine that is lost by this neuronal degradation, with the dopamine precursor L-3,4-dihydroxyphenylalanine (L-dopa or levodopa). However, long-term administration of this treatment bears some important adverse effects, which are known as levodopa-induced-dyskinesias, and consist of motor complications, such as

myoclonus jerks and abnormal involuntary movements *(Bastide et al., 2015)*.

The mechanisms involved in the appearance of these motor complications are not well known yet, but modifications in the activity of different neurotransmitter pathways seem to play an important role. The management of levodopa-induced dyskinesias (LID) is one of the main challenges in the treatment of Parkinson's disease patients, due to the lack of effective pharmacological treatments *(Rojo-Bustamante et al., 2018)*.

LID are associated with the activation of D1 and D2 receptors, which are expressed in striatal projection neurons, downstream changes in proteins and abnormalities within the system, all of which produce alterations in the neuronal firing pattern between the basal ganglia and the cortex *(Bastide et al., 2015)*.

It has been proposed and confirmed by studies the alteration of ECS in basal ganglia during the active phase of LID. These studies have been focused mainly on the CB1 receptor. Cannabinoid receptor agonists, such as WIN55,212–2, can attenuate LID by reducing the striatal glutamate release, inhibiting D1 receptor-mediated effects and enhancing GABA levels in the lateral or external segment of the globus pallidus *(Brotchie, 2003)*. A study used the combined administration of AEA (CB1 receptor agonist), with the FAAH inhibitor URB597 and the TRPV-1 antagonist capsazepine *(Morgese et al., 2007)*. The result showed a significant reduction of the abnormal involuntary movements induced by L-dopa. This neuroprotective effect was mediated by the activation of PPARγ receptors. Furthermore, another study used CBD in combination with a TRPV1 antagonist, and also showed a reduction in dyskinesias, by acting on CB1 and PPARγ receptors *(Dos-Santos-Pereira et al., 2016)*.

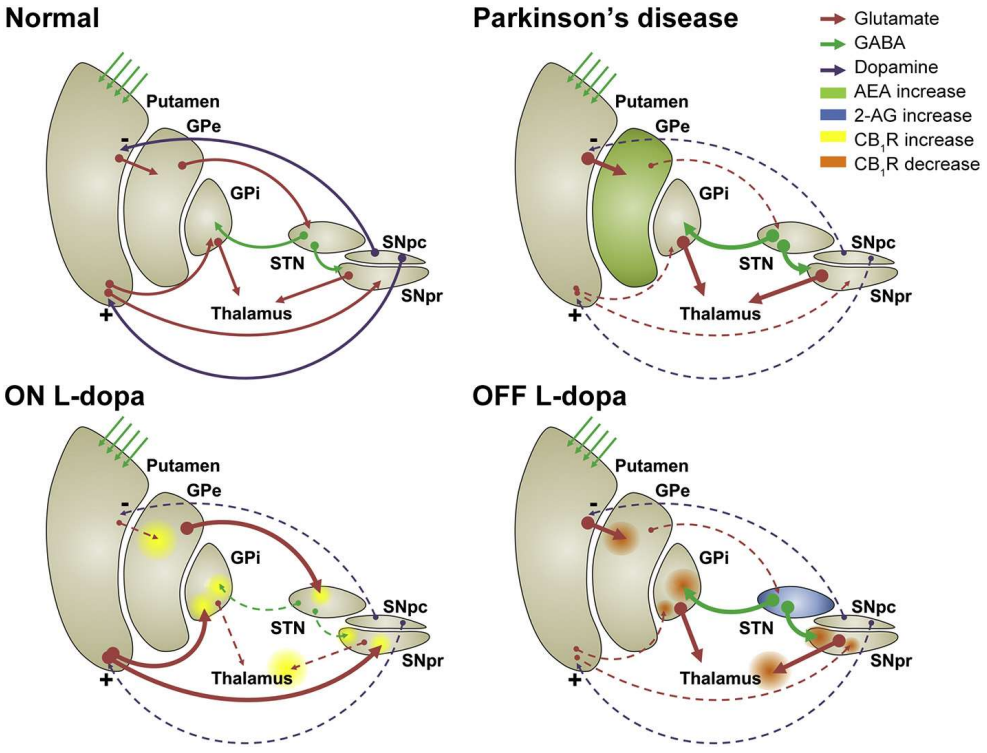

**Figure 10**. Modulation in the expression of the ECS elements in the basal ganglia of non-human primates under parkinsonian conditions *(Rojo-Bustamante et al., 2018)*. *AEA: anandamide; 2-AG: 2-arachidonoyl glycerol; $CB_1R$: cannabinoid receptor type 1; GPi: internal segment of the globus pallidus; GPe: external segment of the globus pallidus; SNpc: Substantia nigra pars compacta; SNpr: Substantia nigra pars reticulata; STN: subthalamic nucleus.*

Pharmacological studies performed in mice support the statement that confirmed the participation of the ECS in LID, and proved both CB1 receptor agonists and antagonists show anti-dyskinetic properties *(Sieradzan et al., 2001; Fox et al., 2002; Ferrer et al., 2003)*. There have been hypotheses proposing that the elements of the ECS would be altered in the basal ganglia nuclei during the active phase of dyskinesia, such as the CB1 receptor and the 2-AG synthesizing and degrading enzymes *(Rojo-Bustamante et al., 2018)*. To prove this, a study was performed in dyskinetic monkeys, to which L-dopa was administered, and results were registered in two groups, differentiating those OFF L-dopa (in the absence of LID), and those ON L-dopa (during the active phase of LID). The most consistent alteration in basal ganglia nuclei that was discovered was the increased mRNA and protein expression of CB1 receptor during the active phase of LID (ON L-dopa), and the decrease of mRNA and protein CB1 receptor's expression when dyskinetic animals were OFF L-dopa. The most significant alteration detected was in the AEA synthesizing and degrading enzymes, which suggested that AEA levels might be increased in the GPe *(Rojo-Bustamante et al., 2018).* This would be the result of a compensatory mechanism to counteract the increased GABAergic input from the putamen and the glutamatergic input from the STN. Nevertheless, regarding the CB2 receptor, changes in its expression were not detected (*see figure 10*).

These specific changes in CB1 receptor expression render a possible therapeutic target to treat the active phase of dyskinesias. However, further investigation is needed to demonstrate this potential non-dopaminergic strategy for the treatment of dyskinesias.

Targeted effects on the ECS may provide the desired benefit to PD. This is why, modulating this system, through selectively targetting cannabinoid signaling pathways with specific drugs, can reduce abnormalities in PD patients and provide neuroprotective effects. Several neurotransmitter systems have been proposed as potential targets for drug development, such as glutamate or dopamine signaling. The modulation of the ECS by pharmacological compounds which selectively influence either CB1 or CB2 receptors is potentially able to produce beneficial effects on PD patients, by reducing immunomodulation, neuroinflammation, excitotoxicity, and synaptic alterations. All this taken together could result in both symptomatic and neuroprotective beneficial effects in PD patients *(Di Filippo et al., 2008; Han et al., 2019)* (*see figure 11*).

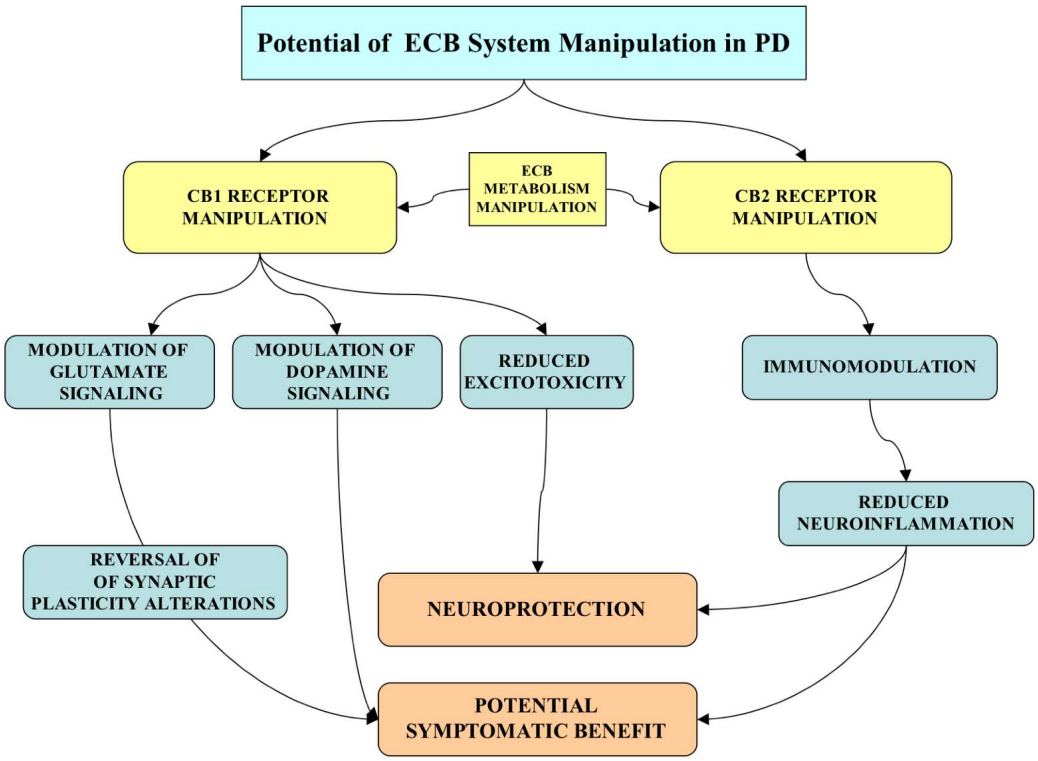


**Figure 11.** The potential therapeutic potential of endocannabinoid system manipulation in PD *(Di Filippo et al., 2008)*.

Conclusively, the whole endocannabinoid system can be therapeutically targeted to treat neurodegenerative diseases, including Parkinson's disease. Not only this but also the alterations occurring in the brain of patients with neurodegerative disorders have a significant relation with the cannabinoid system (its ligands, receptors, synapse transmission), displaying a remarkable intervention for Parkinson's disease pathophysiology. As a result, many researchers have promptly devoted their investigations to dig into this subject, and published numerous studies on the matter. The next sections review the preclinical and clinical studies of therapies in PD.

## CLINICAL STUDIES ON THE EFFECTS OF CANNABINOIDS IN PARKINSON'S DISEASE

Since 2014, several reviews have been published about the possible therapeutic effects of cannabinoids in neurological diseases in general, as well as in motor disorders in particular *(Martinez et al., 2012; Gutierrez-Valdez et al., 2013; Giacoppo et al., 2014)*. However, most of these reviews only focus on behavioural and neurochemical effects in preclinical models. To start with, there was a systematic review based on two randomized controlled trials *(Catlow et al., 2015; Fernández-Ruiz*

*et al., 2015)* that stated that oral cannabis extract was probably ineffective for treating levodopa-induced dyskinesias in patients affected from Parkinson's disease.

After that publication, several studies were performed, using each two randomized controlled trials, evaluating the effects of cannabinoids in PD patients. They assessed the effects on CB1 receptors using various drugs: the synthetic THC analogue, and CB1 agonist nabilone *(Sieradzan et al., 2001; Fox et al., 2002)*; cannabis standardized extract *(Carroll et al., 2004),* and synthetic CB1 receptor antagonist rimonabant *(Mesnage et al., 2004)*. The information for each study is presented in *Table 1*.

The first study is a randomized, double-blind, placebo-controlled, cross-over trial, with a sample of 7 patients. Both nabilone and placebo induced a postural fall in systolic blood with significant reductions in levodopa-induced dyskinesia, but two patients were withdrawn after nabilone treatment due to vertigo and symptomatic postural hypotension. Nabilone also induced other side effects (n=5) such as sedation, "floating sensation", dizziness, hyperacusia, partial disorientation, and visual hallucinations *(Sieradzan et al., 2001)*.

The second study is also a double-blind, randomized, placebo, controlled, crossover study. Here they treated 15 patients, with 0.03 mg/kg nabilone, all with generalized and segmental primary dystonia. They did not find any significant differences in the reduction of dystonia between nabilone and placebo groups. Nevertheless, four patients stated they found improvements in dystonia severity 2-3 days after nabilone treatment. Two patients were withdrawn from the study after presenting postural hypotension and marked sedation. Nabilone was well tolerated, and no other side effects were observed *(Fox et al., 2002)*.

**Table 1**. Randomized controlled trials with CB1 receptor agonists/antagonists in Parkinson's disease and dystonia: *RCT: randomized controlled trials; PD: Parkinson's disease (Crippa et al., 2019)*.

| Study design /drug | Patients | Main results | References |
|---|---|---|---|
| RCT, nabilone (single oral dose: 0.03 mg/kg) | PD (n=7) | Significant reductions in levodopa-induced dyskinesia. Well tolerated. | *Sieradzan et al., 2001* |
| RCT, nabilone (single oral dose: 0.03 mg/kg) | Primary dystonia (n=15) | No significant reduction in dystonia. Well tolerated. | *Fox et al., 2002* |
| RCT, cannabis extract2.5 mg THC/1.25 mg CBD per capsule) | PD (n=19) | No significant reduction in dystonia. Well tolerated. | *Carroll et al., 2004* |

| | | | |
|---|---|---|---|
| RCT, rimonabant (single oral dose, 20 mg) | PD (n=8) | No significant reduction in dystonia. Well tolerated. | *Mesnage et al., 2004* |

In the third study, also double-blind, randomized, placebo-controlled, crossover study, in PD patients, placebo capsules were compared to capsules of a standardized ethanolic cannabis extract (2.5 mg THC and 1.25 mg CBD per capsule). However, no significant reduction in dystonia was observed between the drug and the placebo. The cannabis extract was well tolerated, but some adverse effects were also found (dry mouth, drowsiness, dizziness, constipation, paranoia, vivid dreams and nightmares, confusion, panic attacks, and poor concentration). The incidence of these side effects was higher as the doses of the cannabis extract increased *(Carroll et al., 2004)*.

In the last study (a double-blind, randomized, placebo-controlled, and parallel-arm study), eight PD patients were included, all presenting motor fluctuations and levodopa-induced dyskinesias. They received either the CB1 antagonist rimonabant (20 mg, single dose) or placebo for at least 6 months. There were no significant differences between treatments *(Mesnage et al., 2004)*.

These clinical studies indicate that the direct modulation of the CB1 receptors, either with agonists or antagonists, does not provide clear evidence in efficacy. The reason might be the few studies that have been performed, the small sample size, and the dose range used.

## CANNABIDIOL AND PARKINSON'S DISEASE

### Preclinial studies

Many preclinical studies were performed to investigate the antiparkinsonian properties of CBD *(see review Crippa et al., 2019)*. The first preclinical study looking into this field was published in 2005 *(Lastres-Becker et al., 2005)*, and since then many more were performed *(Lastres-Becker et al., 2005; García-Arencibia et al., 2007; García et al., 2011; Carroll et al., 2012; Casarejos et al., 2013; Gomes et al., 2013; Peres et al., 2016; Dos Santos-Pereira et al., 2016)*. Four of these studies used a classic rat animal model, administrating the neurotoxin 6-hydroxydopamine (6-OHDA), a toxin that causes dopamine depletion and a reduction of tyrosine hydroxylase (TH) activity in the striatum and of TH-mRNA levels in the substantia nigra, among other biochemical changes. The information and characteristics of these studies are shown in *Table 2*.

Some studies using CBD (3 mg/kg) reported significant reductions in neurotoxic effects, probably mediated by cannabinoid receptor-independent antioxidant and anti-inflammatory properties *(Lastres-Becker et al., 2005; García-Arencibia et al., 2007)*. CBD's neuroprotective effects were related to an upregulation of mRNA levels of Cu and Zn-superoxide dismutase *(García-Arencibia et al., 2007)*. This enzyme regulates oxidative stress, therefore the antioxidant effects of CBD might be through a

cannabinoid receptor-independent pathway. Other authors replicated these same results with chronic CBD administration (during 14 days), though with was a slight difference in this one, consisting on the involvement of the CBD-enriched cannabis extract administration (4.63 mg/kg). This extract is made up of 64.8% CBD, 2.3% THC, 1.1% cannabigerol, 3.0% cannabichromene, and 1.5% other phytocannabinoids. The chronic treatment resulted in a significant reduction in the neurotoxic effects *(Garcia et al., 2011)*.

The administration of this CBD-extract for 30 days to mice *(Casajeros et al., 2013)*, induced remarkable reductions in stress-related behaviours, auto- and hetero-aggression, stereotypy, levels of free radicals in the limbic system, gliosis in cortex and hippocampus, and the inducible nitric oxide synthase (iNOS) levels in the cortex. They also showed significant increases in the ratio of reduced/oxidized glutathione in the limbic system and in the cortical levels of complex IV cytochrome c oxidase enzyme, as well as in autophagy. Finally, it is important to point out, that these mice presented a reduction of Tau and amyloid protein deposition in the cortex and hippocampus. These improvements in oxidative stress display big importance for the pathophysiology of PD.

Other studies used human neuroblastoma cells exposed to three toxins that replicate some biochemical alterations related to PD: 1-methyl-4-phenylpyridinium (MPP+), that reduced mitochondrial activity; paraquat, that produced free radicals; and lactacystin, that inhibited ubiquitin-proteasome system (UPS). Also, the administration of MPP+ and lactacystin produced a significant up-regulation of CB1 receptors. The dose of CBD administered was 0.01-1.0 µM, and no significant protective effects were found *(Carroll et al., 2012)*.

**Table 2**. Preclinical studies of CBD effect in Parkinson's disease *(Crippa et al., 2019)*.

| Model/species | CBD dose | Main results | References |
|---|---|---|---|
| 6-OHDA/rats | 3 mg/kg | Significant reductions in neurotoxic effects | *Lastres-Becker et al., 2005* |
| 6-OHDA/rats | 3 mg/kg | Significant reductions in neurotoxic effects | *García-Arencibia et al., 2007* |
| 6-OHDA/rats | 4.63 mg/kg (CBD extract) for 14 days | Significant reductions in neurotoxic effects | *García C. et al., 2011* |
| Multisystemic neurological disease/mice | 4.63 mg/kg (CBD extract) for 30 days | Significant reductions in stress, aggression, stereotypy, free radicals, gliosis, iNOS, tau, and amyloid deposition<br><br>Significant increases in the ratio of reduced/oxidized glutathione, | *Casajeros et al., 2013* |

| | | | |
|---|---|---|---|
| | | complex IV, autophagy | |
| MPP+, paraquat, lactacystin/human neuroblastoma cells | 0.01-1.0 µM | Non-significant effect | *Carroll et al., 2012* |
| 6-OHDA/L-dopa/mice | 15-60 mg/kg for 3 days | CBD alone: nonsignificant effect<br>CBD+capsazepine: significant reduction in involuntary movements and COX-2 and NF-κB expression | *Dos Santos-Pereira et al., 2016* |
| Haloperidol, NOS inhibitor, CB1 receptor agonist/mice | 5-60 mg/kg | Significant attenuation of catalepsy | *Gomes et al., 2013* |
| Reserpine/rats | 0.5-5 mg/kg | Significant reductions in catalepsy, chewing movements, and memory/learning deficits | *Peres et al., 2016* |

It is important to note that these studies only assessed the neuroprotector effect of CBD, not its symptomatic or antiparkinsonian effects. In this regard, other studies analysed the effect on symptomatic motor complications.

In this sense, Dos Santos-Pereira and colleagues tested the effect of CBD alone, and in combination with capsazepine (antagonist of the TRPV-1 receptor); administering a dose range of CBD of 15-60 mg/kg for 3 days. These findings were assessed in the 6-OHDA mouse model which present L-dopa-induced dyskinesias after 21 days of treatment. No significant effects were observed with the administration of CBD alone, while when combined with capsazepine, it induced a significant reduction in L-dopa-induced involuntary movements. Moreover, this combination also showed a relevant reduction in the expression of pro-inflammatory markers, such as COX-2 and NF-κB. Furthermore, it was found that all these effects were blocked by antagonists of CB1 and PPARγ receptors, which suggests that the neuroprotective effects of CBD in association with a TRPV-1 antagonist are produced by the anti-inflammatory properties mediated by CB1 and PPARγ receptors *(Dos Santos-Pereira et al., 2016)*.

Another study used the drug-induced catalepsy test in mice, which has been commonly used to study motor impairments related to changes in the striatal function. The catalepsy was induced by haloperidol (a D2 receptor antagonist), L-nitro-N-arginine (a selecive nitric oxide synthase inhibitor), and WIN55,212 (a CB1/CB2 receptor agonist), and they were treated with CBD (dose of 5, 15, 30 or 60 mg/kg). All three drugs induced catalepsy, that was reverted dose-dependently by CBD *(Gomes et al., 2013)*.

Finally, in a study using the repeated administration of reserpine to rats to induce motor impairments and cognitive disorders. The authors used different CBD doses of 0.5 or 5 mg/kg, and found significant reductions in reserpine-induced-catalepsy and chewing movements, but not in locomotor activity. Additionally, the lowest dose (0.5

mg/kg) also showed a remarkable improvement in memory and learning déficits *(Peres et al., 2016)*.

Taking into account all these preclinical data, CBD shows improvement in motor, biochemical alteration, and cognitive symptomatology related to PD in animal models. However, there are still some drawbacks, such as the limited number of studies performed in this subject and the heterogeneous methodology that has been used (the different CBD doses and the presence of other phytocannabinoids, such as the CBD-enriched cannabis extract). Also, most of the preclinical studies mentioned used a single model of motor symptoms using 6-OHDA, with few studies assessing other aspects of PD.

**Clinical studies**

After preclinical studies, more research has been made on PD patients, effectively showing results on the status of the ECB system that seems to follow the same general pattern as the one observed in rats. Indeed, studies in animals and in humans indicate that the nigro-striatal lesion is associated with an increase in CB1 receptors in the basal ganglia, as well as an increased efficacy of the activation on the CB1 receptor *(Lastres-Becker I., et al., 2001)*.

However, there is little investigation directly involving PD patients. Three clinical trials have evaluated the efficacy, tolerability, and safety of CBD on PD patients with symptoms *(Zuardi et al., 2009; Chagas et al., 2014a; Chagas et al., 2014b*). The main characteristics of these studies are presented in Table 3.

The first study consists of an open-label pilot study performed on six patients with the diagnosis of PD and psychosis *(Zuardi et al., 2009)*. Due to the antipsychotic and neuroprotective effects of CBD, the researchers evaluated CBD on PD patients with psychosis. In order to achieve this, they established inclusion criteria that included PD patients who have had psychotic symptoms for at least 3 months before entering the study, and that could not be controlled with antiparkinsonian drugs. All patients received CBD in flexible doses, starting with an oral dose of 150 mg/day and increasing up to 400 mg/day for 4 weeks. The results showed significant reductions in psychotic symptoms in the CBD treated group. Also, CBD did not worsen the motor function and had no side effects; therefore suggesting that CBD may be effective, safe, and well-tolerated for the treatment of psychosis in PD.

**Table 3.** Clinical trials on CBD in Parkinson's disease *(Crippa et al., 2019)*. *PD: Parkinson's disease, ADL: activities of daily living, RCT: randomized controlled trial, PDQ-39: Parkinson's Disease Questionnaire; RBD: sleep behaviour disorder*

| Study design / dose | Patients | Main results | References |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Open-label<br><br>150-400 mg/day for 4 weeks | PD patients with psychotic symptoms (n=6) | Significant improvements in psychotic and global symptoms<br><br>Well tolerated | *Zuardi et al., 2009* |
| RCT<br><br>75 or 300 mg/day for 6 weeks | PD patients without dementia or comorbid psychiatric conditions (n=21) | Significant improvements on well-being and quality of life, ADL (PDQ-39)<br><br>Well tolerated | *Chagas et al., 2014a* |
| Case series<br><br>75 or 300 mg/day for 6 weeks | PD patients with RBD (n=4) | Significant improvements in sleep symptoms<br><br>Well tolerated | *Chagas et al., 2014b* |

The second study was a randomized, double-blind, placebo-controlled, parallel-group trial *(Chagas et al., 2014a)*. Here they selected 21 patients without dementia or comorbid psychiatric conditions, who were assigned to three groups each who were treated with placebo, CBD 75 mg/day, or CBD 300 mg/day, for 6 weeks. Participants were assessed regarding motor and general PD symptoms, well-being and quality of life, and possible neuroprotective effects. Regarding the latter, no significant differences were found. However, the groups treated with placebo and CBD 300 mg/day presented significant improvements in the total score of the Parkinson's Disease Questionnaire (PDQ-39), and also on activities of daily living (ADL). No adverse reactions were observed. These results suggest a significant improvement in their well-being and quality of life.

The third and last study was developed on four PD patients that had a diagnosis of rapid eye movement disorder while sleeping *(Chagas et al., 2014b)*. REM sleep behaviour disorder (RBD) is a parasomnia characterized by the loss of muscle atonia during REM sleep associated with nightmares and active behaviour during dreaming. To enter the study, patients needed to have been assessed by a neurologist specialized in sleep disorders and have presented at least two episodes of complex sleep-related behaviours per week. Three of the patients received the low CBD dose of 75 mg/day and the other received 300 mg/day, for 6 weeks. CBD was well tolerated and no side effects were found. Results showed a significant reduction in the frequency of RBD symptoms, which included swearing, talking, yelling, pushing, kicking, punching, etc. In addition, three patients had no symptoms at all after the treatment, and the other patient had only one symptom per week. Not only this disappearance of symptoms was observed, but also it is important to remark that after the CBD treatment was interrupted, sleep disturbances symptoms returned to the same frequency and intensity as they had before the CBD treatment.

**Efficacy in non-motor symptoms**

It is well know that PD also causes non-motor symptoms that include psychosis, anxiety and depression, sleep disorders, cognitive decline, and quality of life (see figure 12). In this section, we are going to develop these possible therapeutic uses.

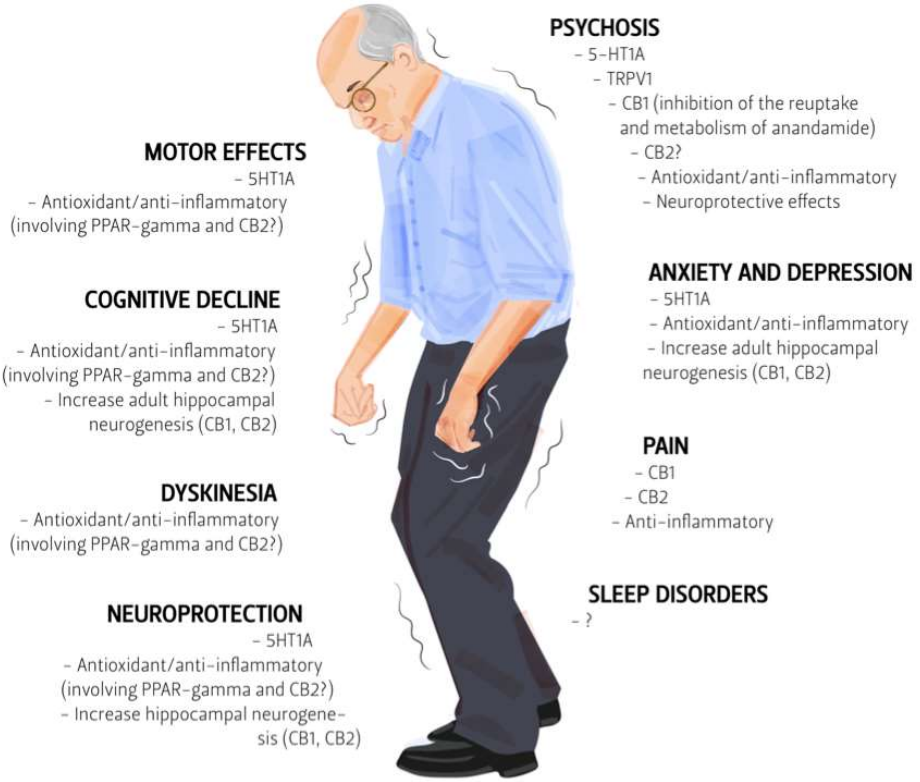


**Figure 12**. Non-motor Parkinson's disease conditions and its possible related mechanisms *(Crippa et al., 2019)*.

✓ **Psychosis**

Psychosis is a usual non-motor symptom occurring in PD patients, affecting nearly one-third of the patients, especially during the late stages of the disease *(Zuardi et al., 2009)*. The antipsychotics currently used in clinic have as their main mechanism of action the blockade of the dopamine receptors, which interferrs with the classical antiparkinsonian treatment and can even potentiate the parkinsonian symptoms (i.e. the extrapyramidal symptoms).

Studies have demonstrated that CBD is able to improve psychotic symptoms. In addition, a study reproduced psychotic-like symptoms using high doses of THC and effectively prevented them when administering CBD *(Zuardi et al., 2012)*. CBD seems to have a pharmacological profile similar to that of atypical antipsychotic drugs. CBD's antipsychotic effect in the ECS seems to be mediated through the inhibition of the FAAH, which would increase the levels of AEA, and the effective activation of the

vanilloid TRPV1 and 5-$HT_{1A}$ receptors. Moreover, CBD prevented human experimental psychosis and was effective in case reports and clinical trials among patients with schizophrenia, and also showed antipsychotic effects in healthy volunteers *(Zuardi et al., 2012)*.

However, there are still few clinical trials with CBD administration to psychotic patients, and the ones that were published had small cohort and/or were of short duration *(Crippa et al., 2019)*. Therefore, taking this into account, and given that the mechanism of action of the antipsychotic drugs is still not fully understood, there is still considerable uncertainty on CBD's efficacy and safety on psychosis.

- ✓ **Anxiety and depression**

These, together with apathy, are the most prevalent psychiatric symptoms in PD, appearing in more than 30% of patients *(Blessing et al., 2015)*. CBD can modulate anxiety and depressive symptoms, due to its anxiolytic, panicolytic, anticompulsive, and antidepressive effects *(Blessing et al., 2015)*. It presents a broad pharmacological profile, including interactions with several receptors known to regulate fear and anxiety-related behaviours. Its mechanism of action involving the anxiolytic and antidepressant effects appear to act specifically through CB1, and the 5-$HT_{1A}$ receptors *(Schier et al., 2012)*. Regarding human studies, the anxiolytic effects of CBD administration (300-600 mg) were demonstrated in both healthy volunteers and in patients diagnosed with social anxiety *(Blessing et al., 2015)*. CBD reduced the THC-induced anxiety when administered simultaneously with this compound, but had no effect when administered alone. Also, CBD helped to extinguish fear memories in healthy volunteers. In addition, other mechanisms involved in the anxiolytic effects of CBD, may also regulate the PPARγ receptor and the G protein-coupled receptor 55 (GPR55), as well as the TRPV1 receptor. All this has been shown to mediate the anxiolytic effects, reduce stress, and enhance fear extinction *(Schier et al., 2012)*.

However, like psychosis, the pathophysiology underlying these symptoms is not fully understood, and the number of clinical trials and evidence is still scarce, therefore it is important to emphasize the need for further study in the putative use of CBD treatment of anxiety disorders. Besides, current preclinical and human findings mostly involve acute doses of CBD, so further studies are required to assess chronic dosing of CBD.

- ✓ **Sleep disturbances**

Insomnia, restless leg syndrome, reduced quality of sleep, and rapid eye movement sleep behaviour disorder or REM behaviour disorder (RBP) is also one of the non-motor symptoms occurring in PD patients *(Chagas et al., 2014a)*. The pathophysiology is not well known, though it is thought to be produced by neurochemical alterations in cholinergic, GABAergic, and serotonergic neurons in brain areas, which control the

sleep-wake cycle. These, together with dopaminergic neurotoxicity would be the reason for these sleep disorders.

Preclinical studies on rodents show contradictory results on the CBD effect on sleep *(Murillo-Rodríguez et al., 2014)*. For instance, some found sedative-hypnotic effects and improvement of sleep in insomniacs, whereas others found a reduction of sleep and an increase in wakefulness. The reason for these contradictory results is thought to be due to methodological differences: dose discrepancies, route of administration, the vehicle used, duration of the treatment, etc.

Clinical studies on humans investigating CBD effects on sleep are few. In healthy volunteers with a regular sleep cycle, 600 mg of CBD induced sedative effects *(Linares et al., 2018)*, and in subjects with insomnia, acute use of CBD (160 mg/day) produced an increase in total sleep time and less frequent awakenings. However, at lower doses (15 mg/day) co-administered with THC (15 mg/day) increased wakefulness *(Nicholson et al., 2004)*. All these studies involved an acute CBD administration, whereas the study previously mentioned *(Chagas et al., 2014a)* investigated the effects of chronically administered CBD (75-300 mg per day for 6 weeks), and showed significant reductions in PD's sleep disturbances when administering CBD for several weeks.

- ✓ **Cognitive decline**

Cognitive dysfunction includes deficits in attention, information processing, verbal fluency, and episodic memory. This, together with dementia, renders the most significant and important cognitive non-motor symptoms of PD, occurring in up to 40% of patients *(Crippa et al., 2019)*.

We have already discussed the neuroprotective effects of CBD in several animal models, specifically on nigrostriatal dopaminergic neurons, and involving anti-inflammatory and antioxidant actions, through $5HT_{1A}$ receptors, PPARγ receptor, and CB1 and CB2 receptors. Also, CBD has the potential to improve cognitive functions such as learning and memory. To date, the studies investigating cognitive dysfunction in PD only showed beneficial results in animals *(Peres et al., 2016).*

However, in humans, CBD does not seem to affect psychomotor functions or cognition *(Crippa et al., 2019)*. The neuroprotective effects of CBD in humans have not been clearly demonstrated in cognition or in biomarkers of neuroprotection in PD patients specifically. As a matter of fact, this could suppose a real non-relation between CBD administration and the improvement of cognitive dysfunction in PD's patients or it could be explained by the large limitation that the small number of volunteers entails, as well as the short duration of the trials. One way or another, it is clear that large-scale trials are needed and it is important to emphasize the potential beneficial effects that CBD could produce on cognition and neuroprotection, thus remarking the urge for further research.

## CONCLUSION

The only effective treatment used for PD, L-DOPA, produces significant side effects and its efficiency decreases over time. For this reason, the research of pharmacological drugs able to improve PD symptoms is urgent and clear. The ECB is involved in the pathophysiology of PD, especially through its CB1 and CB2 receptors, and could represent an ideal therapeutic target in order to treat this neurodegenerative disorder. Modulating this system with selectively targetting compounds and pathways could reduce alterations in patients with PD. However, this knowledge is still far from being totally proven and thus, unable to be thoroughly used for therapeutic strategies. The reason for this is that the preclinical and clinical studies that have been so far performed present still a number of drawbacks, such as the few samples included, the little evidence there has been shown, the short duration of the treatment, and the few studies that have been made. Taking all this into account, it is a huge step to have discovered these mechanisms and how they work, but there is still a need to remark the importance of further research.

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