UNIVERSITÀ
DI PAVIA

PhD IN BIOMEDICAL SCIENCES

DEPARTMENT OF BRAIN AND BEHAVIORAL SCIENCES

UNIT OF NEUROPHYSIOLOGY

NEUROPROTECTIVE AND SYMPTOMATIC EFFECTS OF CANNABIDIOL IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

PhD Tutor: Prof. Cristina Tassorelli

PhD dissertation of
Claudio Giuliano

a.a. 2018/2021

Contents

INTRODUCTION

1. Parkinson’s disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the slow and progressive degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc). This pathology represents the second most common neurodegenerative disorder, after Alzheimer's disease. It occurs mostly in sporadic form (about 90% of cases), but there are also familial forms that correspond to 5-10% of cases (Xiromerisiou et al. 2010). Aging is the major risk factor in sporadic forms: PD affects about 1-3% of the population over the age of 65 and 4-5% of those over 80 years old (Dexter and Jenner 2013; Bellou et al. 2016). As worldwide life expectancy has increased, these percentage data are expected to increase steadily (World Population Prospects 2019: Highlights). Epidemiological studies show that exposure to environmental factors such as living in a rural area, well water use, exposure to pesticides, and heavy metals may serve as a risk factor for developing PD (Priyadarshi et al. 2001).

In addition to age, genetics, and environment, biological sex also appears to play a role in disease development (Cerri, Mus, and Blandini 2019). The incidence of the disease is higher in men than in women (Elbaz et al. 2002), although the latter ones show a higher mortality rate and a more rapid progression of the disease (Dahodwala et al. 2018). In addition, women exhibit different symptomatology and response to therapies, both pharmacological and surgical, than men (Georgiev et al. 2017). These differences are largely attributable to the neuroprotective role played by estrogen, as demonstrated by the similar incidence of disease between postmenopausal women and men (Cerri, Mus, and Blandini 2019).

1.1 Diagnosis

A PD diagnosis is primarily based on clinical history and physical examination. History can include prodromal features (eg, rapid eye movement sleep behavior disorder, hyposmia, constipation), characteristic movement difficulty (eg, tremor, stiffness, slowness), and psychological or cognitive problems (eg, cognitive decline, depression, anxiety). Examination typically demonstrates bradykinesia with tremor, rigidity, or both. Dopamine transporter single-photon emission computed tomography

can improve the accuracy of diagnosis when the presence of parkinsonism is uncertain (Berg et al. 2014).

For clinically established PD, individuals must also satisfy at least 2 of the 4 supporting criteria: (1) resting tremor, (2) a dramatic improvement with dopaminergic therapy (eg, carbidopa-levodopa), (3) the presence of levodopa-induced dyskinesias, or (4) the presence of either olfactory loss (Berg et al. 2014).

	PD[71]	DLB[72]	MSA[73]	PSP[74]	CBD[75]
Dementia		+			
Apraxia					+
Akinesia	+	+	+	+	+
Rigidity	+	+	+	+	+
Tremor	+	+			
Gait disorder		+	+	+	+
Falls		+		+	+
Dysarthria		+	+	+	+
Dysphagia		+		+	
Gaze palsy				+	
Autonomic failure		+			

PD=Parkinson's disease; DLB=dementia with Lewy bodies; MSA=multisystem atrophy; PSP=progressive supranuclear palsy; CBD=corticobasal degeneration. + denotes features present in more than 70% of patients in post-mortem series.

Table 1: **Frequency of clinical characteristics in parkinsonian disorders**

Figure 1: Frequency of clinical characteristics in parkinsoniam disorders (Tolosa, Wenning, and Poewe 2006).

The correct diagnosis is often complicated by the fact that the motor symptoms are in many ways similar to those found in other forms of degenerative parkinsonism (Figure 1) (Tolosa, Wenning, and Poewe 2006). Diagnostic confirmation of the disease can therefore only occur through postmortem analysis of autopsy brain samples from the patient. The characteristic pathognomic feature in PD is the presence of Lewy bodies, protein aggregates formed by alpha-synuclein, phosphorylated proteins, and ubiquitin (Figure 2) (Braak et al. 2003).

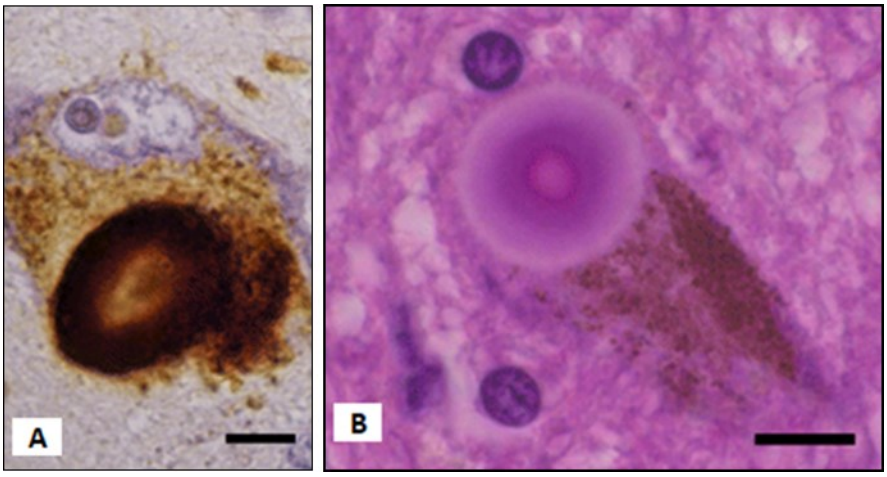

Figure 2: Lewy bodies in substantia nigra dopaminergic neurons labeled by anti-alpha-synuclein antibodies (A) and hematoxylin-eosin reaction (B) (Wakabayashi et al. 2013).

1.2 Clinical features

Parkinson disease causes motor and nonmotor symptoms. Motor symptoms include: tremor, bradykinesia, rigidity and postural instability. The tremor, which initially develops unilaterally, is typically observed at rest and is reduced during voluntary movements. Bradykinesia consists of slowness of movements both in their planning phase and in their complete execution. This symptom involves walking with small steps, micrographs, facial hypomimics and progressive hypophonia. Muscle stiffness affects all muscle districts and manifests as resistance to passive limb movements (Reich and Savitt 2019). Lastly, postural instability manifests as the patient's difficulty in maintaining balance, due to alterations in postural reflexes. The appearance of typical motor symptoms is preceded by a latent phase of the disease, which can last up to a few years, characterized by the presence of nonmotor disorders (Reichmann 2017) (Figure 3).

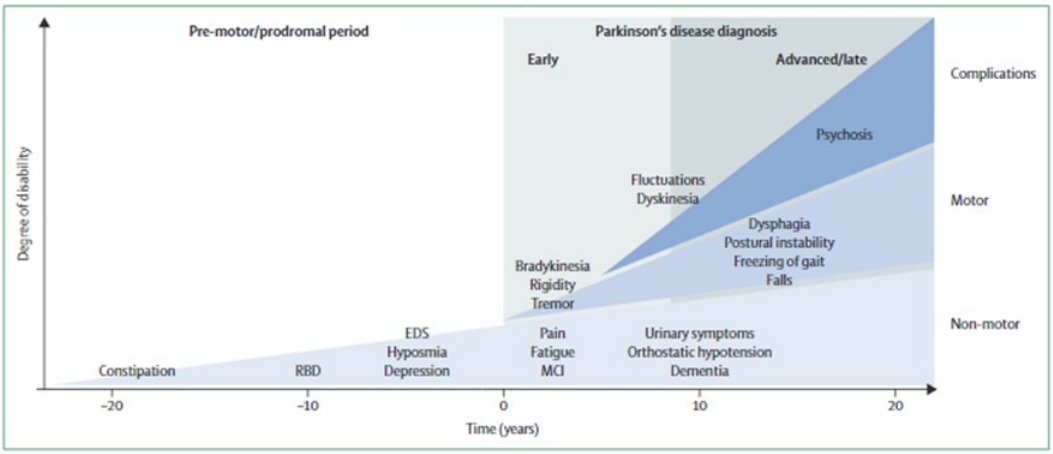

Figure 3: Temporal stages of Parkinson's disease symptomatology (Kalia and Lang 2015).

Nonmotor symptoms affect many organ systems, such as gastrointestinal and genitourinary systems, and are heterogeneous. Individuals diagnosed with PD typically have gradual development of nonmotor symptoms for years before movement symptoms begin. These prodromal nonmotor features include rapid eye movement sleep behavior disorder, loss of smell, constipation, urinary dysfunction, orthostatic hypotension, excessive daytime sleepiness, and depression (Berg et al. 2014). Moreover, several studies suggest that the progression of PD-associated symptomatology is linked to the progressive propagation of pathological forms of

alpha-synuclein through the central and peripheral nervous system, by a prion-like mechanism (Angot et al. 2010; Woerman et al. 2015). Specifically, the sporadic form of PD could originate from two sites - neurons in the nasal cavity and intestine - and from there spread to the central nervous system via the olfactory and vagus nerve pathways, respectively (Hawkes, Del Tredici, and Braak 2007, 2009). Non-motor and motor symptoms would then be the result of alpha-synuclein deposition in specific areas.

1.3 Therapy

Current therapeutic strategies for the treatment of PD are mainly focused on the control of motor symptoms. While improving the quality of life of patients, these approaches are not able to counteract the evolution of the degenerative process that characterizes the disease. The main pharmacological and surgical strategies adopted for the treatment of PD are described below.

1.3.1 Pharmacological therapy

The main target of PD pharmacological therapy is to maintain appropriate levels of dopamine in the brain in order to reduce motor symptoms (Youdim 2010). The major pharmacological compound used is 3,4-dihydroxyphenylalanine or L-DOPA. L-DOPA is a neutral amino acid produced from tyrosine by the enzyme tyrosine hydroxylase and is dopamine's precursor. This compound is administered in combination with peripheral DOPA-decarboxylase enzyme inhibitors, carbidopa and benserazide, in order to block its peripheral catabolism and maximize its distribution in the brain (Fahn 2008). Once in the brain, L-DOPA is picked up by surviving dopaminergic neurons and converted to dopamine. This may explain why the efficacy of L-DOPA has a tendency to decrease over the course of disease progression. Another strategy to maximize L-DOPA levels centrally involves the use of dopamine catabolic pathway inhibitors, such as monoamine oxidases B and catechol-O-methyltransferases (Figure 4).

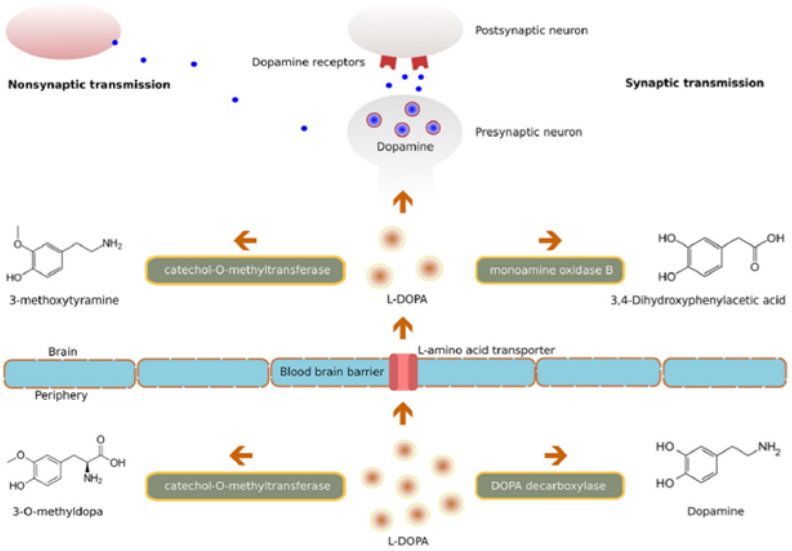

Figure 4: Molecular mechanism of L-DOPA. (Muthuraman et al. 2018).

Despite its therapeutic efficacy, prolonged treatment with L-DOPA may present some side effects including hypotension, arrhythmia, gastrointestinal disturbances, hair loss, confusion, and behavioral and emotional disturbances (Olanow, Lees, and Obeso 2008). In addition, more than 50% of patients treated with L-DOPA develop motor complications called dyskinesias. Dyskinesias are characterized by both dystonic (slow) and choreic (fast) involuntary movements that affect various muscles of the body including facial, neck, tongue, jaw, limbs, hands, chest, and abdomen. In addition to dyskinesias, treatment with L-DOPA can cause other complications such as the "wearing off" effect (reduction in the duration of the therapeutic effect of L-DOPA with the passage of time) or the appearance of "on/off" fluctuations in L-DOPA activity. The latter are characterized by the transition from "on" periods, in which there is normal motor activity in the patient, to "off" periods, in which motor activity is reduced (Schwartz and Sabetay 2012).

For this reason, clinicians often prefer to postpone L-DOPA therapy by favoring the use of dopamine agonists to treat PD in the early stages (Clarke and Guttman 2002). This class of drugs provides continuous stimulation of striatal dopaminergic receptors, resulting in over-regulation of postsynaptic activity (Blandini and Armentero 2014).

However, it has been reported that treatment with dopaminergic agonists can also cause significant side effects such as impulse control disorders, cardiovascular dysfunction, and sleep disorders.

1.3.2 Surgical treatment

Deep brain stimulation is the main surgical treatment used in the treatment of PD. It is based on the stereotactic implantation of an electrode in pre-defined areas of the brain to generate high and continuous frequency stimulation in order to regulate abnormal impulses. The electrode is connected to a pacemaker which is placed in the patient's subclavicular region. The most common stimulation target is the subthalamic nucleus, which plays a central role in the basal nucleus circuit and is known to be hyperactive in PD (Fasano, Daniele, and Albanese 2012). This technique improves motor symptoms (Limousin et al. 1995), allowing a reduction in the daily dosage of L-DOPA (Caudle and Zhang 2009). However, its application is limited to a restricted number of patients who possess specific requirements such as the absence of cognitive deficits and psychiatric disorders, an age of less than 75 years and an unsatisfactory response to pharmacological treatment (Bronstein et al. 2011). Deep brain stimulation limitations are the absence of real functional recovery and the re-appearance of symptoms following stimulator shutdown (Olanow, Lees, and Obeso 2008).

2. Etiopathogenesis of Parkinson's disease

PD is a multifactorial disorder in which environmental and genetic factors play a key role (Gan-Or et al. 2015). Although the etiology is still unclear, it is known that factors such as mitochondrial dysfunction, neuroinflammation, oxidative stress, and abnormal protein aggregation contribute to the neurodegenerative process in the nigrostriatal pathway (Blandini 2013) (Figure 5).

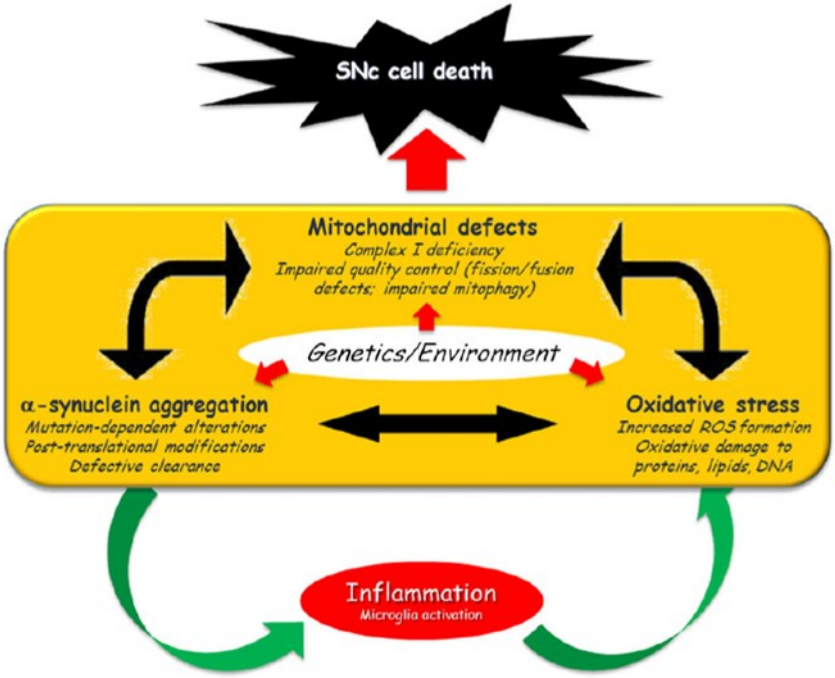

Figure 5: Schematic representation of the main pathogenic mechanisms of PD (Blandini 2013).

2.1 Genetic factors

Most cases are sporadic but about 10–15% of patients have a positive family history of PD (Blandini and Armentero 2012). The main autosomal dominant forms are linked to mutations in the following genes (Monin 2019) (Figure 6):

- SNCA, is the first gene identified in familial forms of PD, which encodes for the protein alpha-synuclein. Point mutations in this gene are rare and associated with a disease with late onset. On the contrary, duplications and triplications of the gene are more frequent and determine the appearance of a form of disease with early onset and rapid progression.
- LRRK2, is a 51 exons gene that encodes for the protein Leucine-rich repeat kinase 2 involved in several cellular signaling pathways and in particular in the

regulation of endosomal trafficking. More than forty missense variants of this gene have been identified, of which six are pathogenic and recurrent. It represents the most common form of PD with autosomal dominant transmission and the clinical phenotype associated with it is the most similar to sporadic PD.

Instead, autosomal recessive forms include mutations in the following genes:

- PARKIN, is the gene encoding for the protein parkin, a ubiquitin-ligase, which regulates protein degradation mediated by the ubiquitin-proteasome system. In case of mitochondrial damage this protein can translocate from cytosol to mitochondria, where it is phosphorylated by PINK1, and promotes autophagic degradation of damaged mitochondria, a process defined as mitophagy. In patients with this mutation, the disease shows an early onset, a slow progression, and an increased susceptibility to the development of L-DOPA-induced dyskinesias.
- PINK1, is the gene encoding for the mitochondrial PTEN-induced putative kinase1. As for mutations in the PARKIN gene, the disease has an early onset, slow evolution and good response to treatment with L-DOPA.
- DJ1 is the gene that encodes for the protein, which plays a protective role against oxidative stress (Mukherjee et al. 2015). In patients carrying this mutation, the disease shows an early onset, slow progression, and a good response to L-DOPA (Ibanez et al. 2003).

Symbol	Gene locus	Name of gene	Role in PD
Park 1	4q21–22	SNCA	Confirmed
Park 2	6q25.2–q27	Parkin	Confirmed
Park 3	2p13	Unknown	Unconfirmed
Park 4	4q21–q23	SNCA triplication	----
Park 5	4p13	UCLH1	Unconfirmed but possible
Park 6	1p35–p36	Pink1	Confirmed
Park 7	1p36	DJ1	Confirmed
Park 8	12q12	LRRK2/dardarin	Confirmed
Park 9	1p36	ATP13A2	Confirmed
Park 10	1p32	Unknown	Confirmed
Park 11	2q36–27	Unknown	Unconfirmed
Park 12	Xq21–q25	Unknown	Confirmed
Park 13	2p12	HtrA2	Confirmed
Park 14	22q14.1	PLA2G6	Confirmed
Park 15	22q12–q13	FBX07	Confirmed
Park 16	1q32	Unknown	Confirmed
Park 17	16q11.2	VPS35	Confirmed
Park 18	3q27.1	EIF4G1	Unconfirmed

Figure 6: Gene loci associated with Parkinson's disease (Wagh et al. 2017).

The identification of genes involved in familial forms of PD has highlighted a number of mechanisms involved in the pathogenesis of the disease (Figure 7), which will be better described in the following paragraphs.

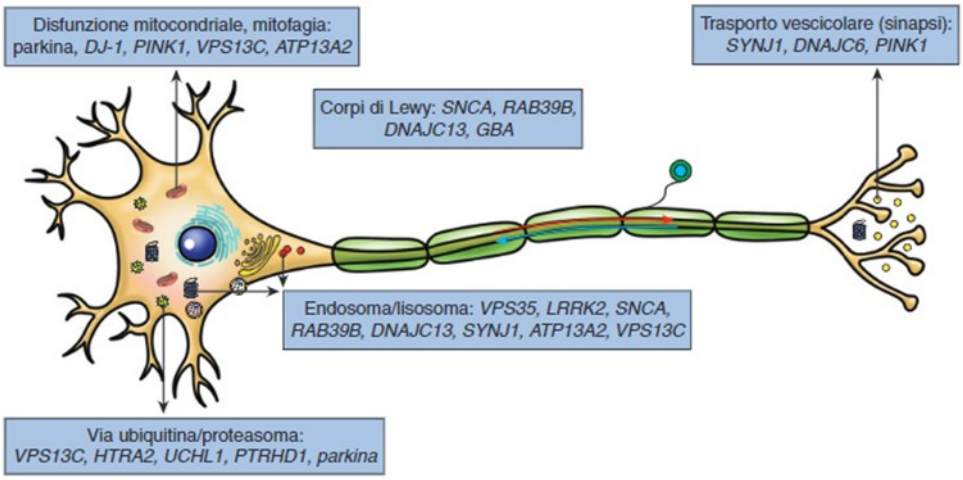

Figura 7: Role of proteins associated with the major familial forms of PD (Monin 2019).

There are also a number of atypical forms of familial PD, in which the disease may present with a variable pathophysiology, which are summarized in the figure 8.

Symbol	Gene locus	Disorder	Inheritance	Gene	Status and remarks	Mode of identification
PARK9	1p36	Kufor-Rakeb syndrome; atypical PD with dementia, spasticity, and supranuclear gaze palsy	AR	*ATP13A2*	Confirmed; but complex phenotype that would not be mistaken for early-onset or classical parkinsonism	Linkage analysis
PARK10	1p32	Classical PD	Risk factor	Unknown	Confirmed susceptibility locus; gene unknown since first described in 2002	Linkage analysis
PARK11	2q36-27	Late-onset PD	AD	Unknown; not *GIGYF2*	Not independently confirmed; possibly represents a risk factor; gene not found since first described in 2002	Linkage analysis
PARK12	Xq21–q25	Classical PD	Risk factor	Unknown	Confirmed susceptibility locus; possibly represents a risk factor; gene not found since first described in 2003	Linkage analysis
PARK13	2p12	Classical PD	AD or risk factor	*HTRA2*	Unconfirmed	Candidate gene approach
PARK14	22q13.1	Early-onset dystonia-parkinsonism	AR	*PLA2G6*	Confirmed	Linkage analysis (homozygosity mapping)
PARK15	22q12–q13	Early-onset parkinsonian-pyramidal syndrome	AR	*FBX07*	Confirmed	Linkage analysis
PARK16	1q32	Classical PD	Risk factor	Unknown	Confirmed susceptibility locus	Genome-wide association studies
PARK17	16q11.2	Classical PD	AD	*VPS35*	Confirmed	Exome sequencing
PARK18	3q27.1	Classical PD	AD	*EIF4G1*	Unconfirmed; recently published (Chartier-Harlin et al. 2011)	Linkage analysis

AD, autosomal dominant; AR, autosomal recessive.

Figure 8: PARK-designated atypical PD-related loci (Monin 2019).

2.2 Protein aggregation and deficits in proteolytic systems

Alpha-synuclein (αSyn) is a small protein comprising 140 amino acids with three domains: an N-terminal domain (aa 1–65), a nonamyloid-β component of plaques

(NAC) domain (aa 66–95), and a C-terminal domain (aa 96–140) (Jakes, Spillantini, and Goedert 1994). Although the function of αSyn is still unclear, recent studies showed that the normal physiological function of αSyn involves roles in compartmentalization, storage, and recycling of neurotransmitters (Reich and Savitt 2019). In addition, αSyn is associated with the physiological regulation of certain enzymes and is thought to increase the number of dopamine transporter molecules. Neurotransmitter release and interaction with the synaptic SNARE- (soluble N-ethylmaleimide-ensitive factor attachment protein receptors) complex are partly mediated by its role as molecular chaperone (Burre et al. 2010). In pathological states, αSyn becomes misfolded, aggregates and accumulates in neuronal inclusion bodies, Lewy bodies, seen in PD and other synucleinopathies (Spillantini et al. 1998). The abnormal accumulation of this protein within Lewy bodies suggested the existence of alterations in cellular degradation pathways, such as the ubiquitin-proteasome system and the autophagy system, in PD (Figure 9).

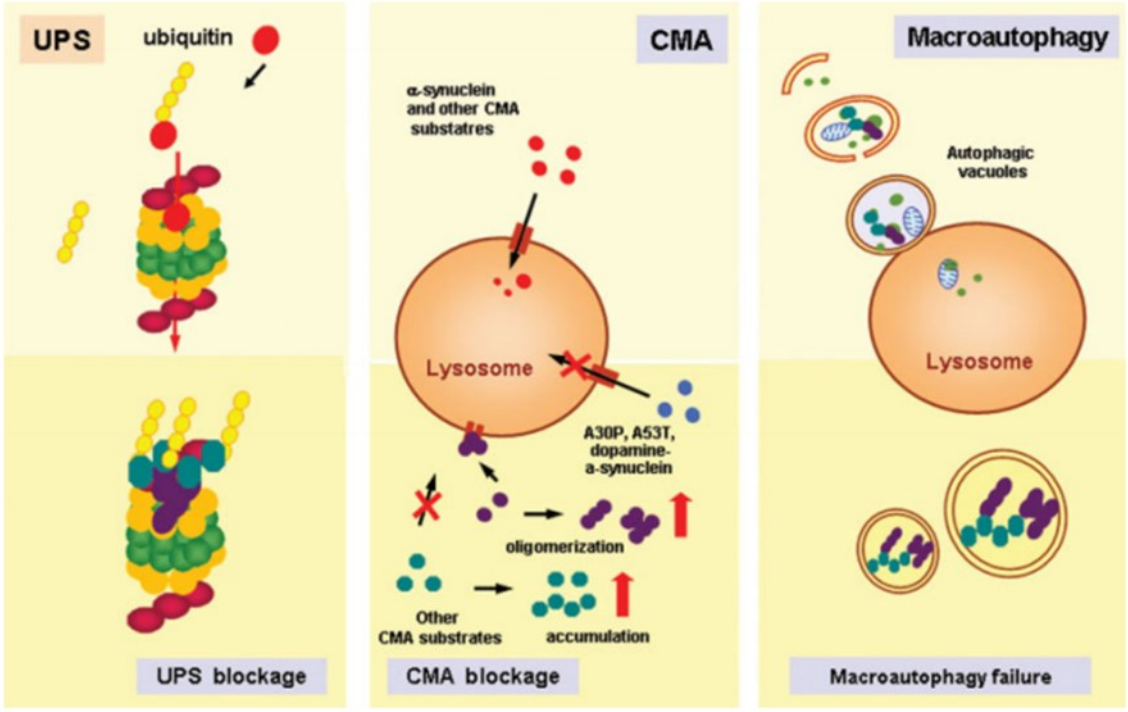

Figure 9: Cellular degradation systems of alpha-synuclein (Cuervo, Wong, and Martinez-Vicente 2010).

The ubiquitin-proteasome system is a non-lysosomal abnormal protein degradation pathway in which the protein to be degraded is polyubiquitinated to be recognized by the proteolytic complex. It consists of a 20S subunit with proteolytic action and a 19S subunit with regulatory action (Lilienbaum 2013) (Figure 10).

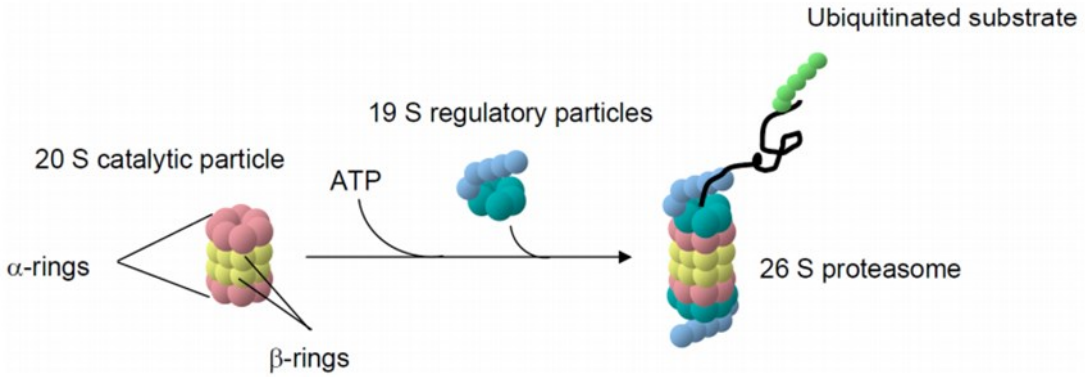

Figure 10: Structure and function of the ubiquitin-proteosome system (Lilienbaum 2013).

The involvement of the ubiquitin-proteasome system in the pathogenesis of PD is supported primarily by the discovery of mutations in the genes encoding for the proteins Parkin and UCH-L1, which are implicated in the proper functioning of this proteolytic system and associated with familiar PD. In addition, in vivo studies have shown that the administration of proteasome inhibitors in adult rats, led to the appearance of motor deficits associated with the disease (McNaught et al. 2004). Similarly, alterations in the autophagy system have been found in PD, particularly in chaperone-mediated autophagy (CMA) and macroautophagy, both of which are involved in αSyn degradation. Many of the canonical mutations associated with PD are in fact linked to autophagy systems. One of the most common LRRK2 mutations, G2019S, has a negative impact on CMA (Orenstein et al. 2013). In addition, accumulation of pathological alpha-synuclein variants can in turn interfere with the normal functions of the CMA translocation complex resulting in accumulation of substrates in the cytosol and impaired neuronal function (Cuervo, Wong, and Martinez-Vicente 2010).

2.3 Mitochondrial dysfunction

Mitochondria are cellular organelles, mainly deputed to produce energy in the form of ATP through the process of oxidative phosphorylation (Mattson, Gleichmann, and Cheng 2008). One of the first observations regarding the existence of a relationship between PD and mitochondrial deficits dates back to the discovery of cases of PD in subjects using a synthetic drug, contaminated with 1-methyl-4-phenyl1,2,3,6tetrahydropyridine (MPTP) (Langston and Ballard 1983). MPTP is a toxin capable of crossing the blood-brain barrier and reaching brain tissue where it is rapidly converted to its toxic form, 1-methyl4phenylpyridinium (MPP+), by the enzyme monoamine oxidase B within glial cells. The MPP+ metabolite, due to its high affinity for the dopamine transporter, is able to go into the dopaminergic neuron causing inhibition of complex I of the mitochondrial respiratory chain and, consequently, cell death (Heikkila et al. 1984; Javitch et al. 1985). Further evidence supporting the role of dysfunction of mitochondrial activity in PD comes from the observation that some genes implicated in familial forms of PD encode for proteins, such as Parkin and PINK1, implicated in the control of mitochondrial integrity and function (Henchcliffe and Beal 2008; Van Laar and Berman 2009). Finally, a relationship between mitochondria and αSyn has also been observed. Indeed, this protein has been identified at the level of the mitochondrial membrane (Li et al. 2007) and has been shown to alter the fusion and fission processes of mitochondria (Gui et al. 2012; Xie and Chung 2012), resulting in altered mitochondrial mobility, energy availability, and increased oxidative stress (Van Laar and Berman 2009).

VDAC1, a channel located on the outer mitochondrial membrane, levels were found to be reduced in sporadic PD patient nigral neurons in association with αSyn aggregations and is therefore implicated as a component of overall mitochondrial dysfunction in sporadic PD (Chu et al. 2014). Aggregated αSyn affects proteostasis by impairing the function and trafficking between ER, Golgi and the autophagy-lysosomal system, as well as impacting on mitochondrial functions including energy production, calcium and iron buffering and ROS production (Yasuda, Nakata, and Mochizuki 2013).

2.4 Oxidative stress

Oxidative stress is the result of an imbalance between the production and detoxification, by endogenous antioxidant systems, of reactive oxygen species (Jiang, Sun, and Chen 2016). Reactive oxygen species, including superoxide anion ($O2^{-}$), hydroxyl radical (OH-), and hydrogen peroxide (H2O2), are molecules possessing a mismatched electron in the last orbital, which gives them a marked instability (Holmstrom et al. 2013; Ludtmann et al. 2014; Wu, Cui, and Klaassen 2011; Subramaniam and Chesselet 2013). Their formation occurs under physiological conditions during electron transfer along the mitochondrial transport chain. However, excessive levels of these molecules can cause alteration of mitochondria and induce oxidative damage to lipids, proteins, and DNA, compromising their integrity and function (Zhang et al. 2014). An early evidence supporting the role of oxidative stress in the pathogenesis of PD is the PARK7 gene, implicated in a familial form of the disease, encodes for the DJ-1 protein, which has antioxidant potential and contributes to the activation of endogenous antioxidant defenses (Levy, Malagelada, and Greene 2009) (Figure 11). Mutations in the PARK7 gene lead to the loss of function of DJ-1 which, therefore, would contribute to increase oxidative stress with critical consequences for the survival of dopaminergic neurons. The existence of a relationship between oxidative stress and PD also derives from the post-mortem analysis of brains of patients affected by this disease in which it was observed a reduced activity of antioxidant systems such as glutathione peroxidase, the presence of high levels of iron and intracellular calcium, increased activity of the enzyme monoamine oxidase B and increased autoxidation of dopamine itself (Obeso et al. 2000; Foley and Riederer 2000; Lim et al. 2012; Hermida-Ameijeiras et al. 2004; Youdim and Bakhle 2006). Ultimately, it has been shown that alpha-synuclein may also contribute to the increase in reactive oxygen species since its overexpression has been observed to induce increased oxidative stress in the perinuclear and dendritic compartments (Brennan et al. 2009; Dryanovski et al. 2013).

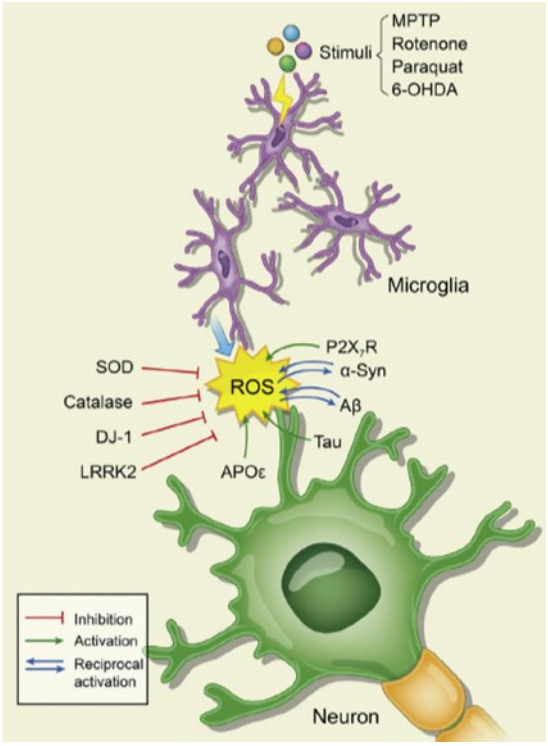

Figure 11: Role of major PD-associated proteins in the production of reactive oxygen species (Jiang, Sun, and Chen 2016).

2.5 Neuroinflammation

Neuroinflammation is an inflammatory process affecting the nervous system. It is characterized by activation of glial cells, lymphocytic infiltration and release of inflammatory factors into the extracellular space (Fuzzati-Armentero, Cerri, and Blandini 2019).

One of the first proofs of the relationship between neuroinflammation and PD came from the observation of relevant microglial activation, accompanied by increased levels of pro-inflammatory cytokines, in autopsy specimens of PD patients (McGeer et al. 1988). Studies in *in vivo* models of PD have subsequently demonstrated the involvement of the inflammatory process in the degeneration of SNc dopaminergic neurons (He, Appel, and Le 2001; Depino et al. 2003; Gao and Hong 2008).

The two glial populations involved in the neuroinflammatory response are microglia and astrocytes. Microglial cells are resident immune cells in the central nervous system. They are generated during embryogenesis and are similar to peripheral macrophages in both their function and monocytic origin. Under physiological conditions they exhibit long, branching processes and small cell bodies and perform

innumerable functions (Figure 12), such as maintaining homeostasis, clearing and remodeling synapses, and repairing tissue damage, thereby promoting neuronal survival (Sominsky, De Luca, and Spencer 2018).

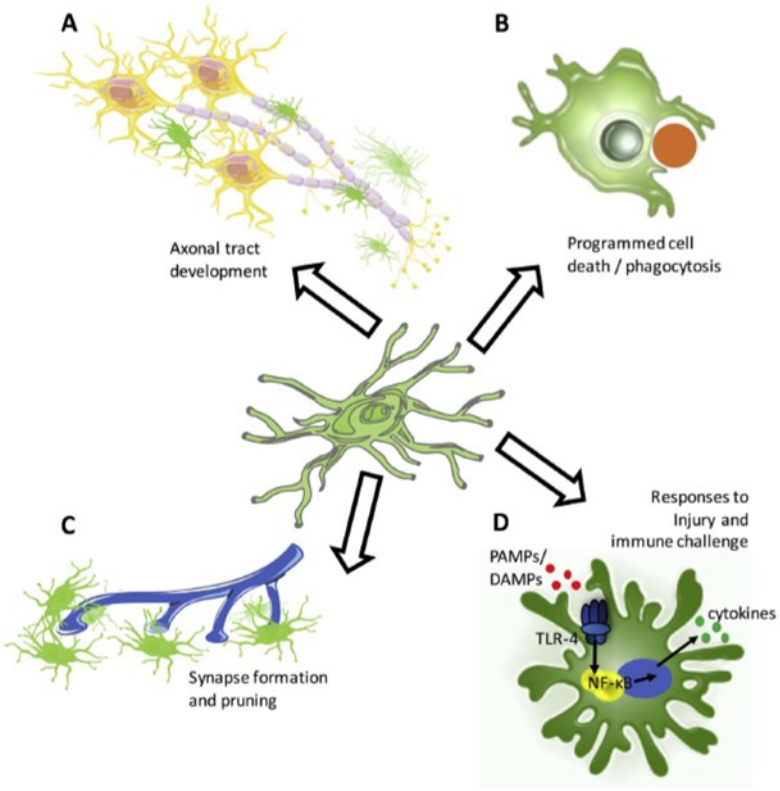

Figure 12: The different functions of microglia cells in the central nervous system (Sominsky, De Luca, and Spencer 2018).

Astrocytes are a class of neural cells of ectodermal, neuroepithelial origin that sustain homeostasis and provide for defense of the central nervous system. Astrocytes maintain molecular homeostasis of the CNS by transporting major ions and protons, by removing and catabolizing neurotransmitters, and by releasing neurotransmitter precursors and scavengers of reactive oxygen species. Astrocytes sustain neurotransmission by supplying neurons with neurotransmitter precursors and control cellular homeostasis through embryonic and adult neurogenesis. Astrocytes regulate metabolic homeostasis through synthesizing glycogen and supplying neurons with energy substrates. Astrocytes in the guise of glia limitans form the pial cover of the CNS, control blood-brain barrier and act as chemosensors, thus contributing to systemic homeostasis (regulation of energy balance, blood pH and Na concentration).

Under pathological conditions, glial cell activation is manifested not only as an increase in the number of activated cells, but the cells undergo morphological and functional changes. This phenomenon, termed polarization, leads to the development of two distinct cellular phenotypes with opposing characteristics (Joers et al. 2017; Liddelow and Barres 2017):

- The cytotoxic phenotype, identified with M1 or A1 (microglia and astrocytes, respectively), characterized by the release of proinflammatory cytokines such as IL-1beta, IL-2, IL-6, TNF-alpha, IFN-gamma, nitric oxide, reactive oxygen species, and chemokines such as CCL2 and CXCL10 (Chhor et al. 2013; Franco and Fernandez-Suarez 2015; Koyama 2014);
- The cytoprotective phenotype (M2 or A2) associated with the release of anti-inflammatory cytokines such as IL-4 and IL-10, TGF-beta (Koyama 2014; Benarroch 2013; Franco and Fernandez-Suarez 2015);

Glial cells can influence each other, promoting polarization toward one phenotype or the other. In particular, it has been observed that activated microglia induce the polarization of astroglia toward the cytotoxic phenotype through the release of the cytokines IL-1alpha, TNF-alpha, and C1q (Liddelow et al. 2017) (Figure 13). Other factors involved in the pathogenesis of PD, such as αSyn accumulation, may also act on the polarization process. It has been shown that in the early stages of PD, the accumulation of αSyn, induces the polarization of microglia toward the anti-inflammatory M2 phenotype. However, the persistence of this condition and the progression of the degenerative process, unbalances the polarization towards the pro-inflammatory M1 phenotype, contributing to the evolution of the pathology (Blandini 2013; Tang and Le 2016).

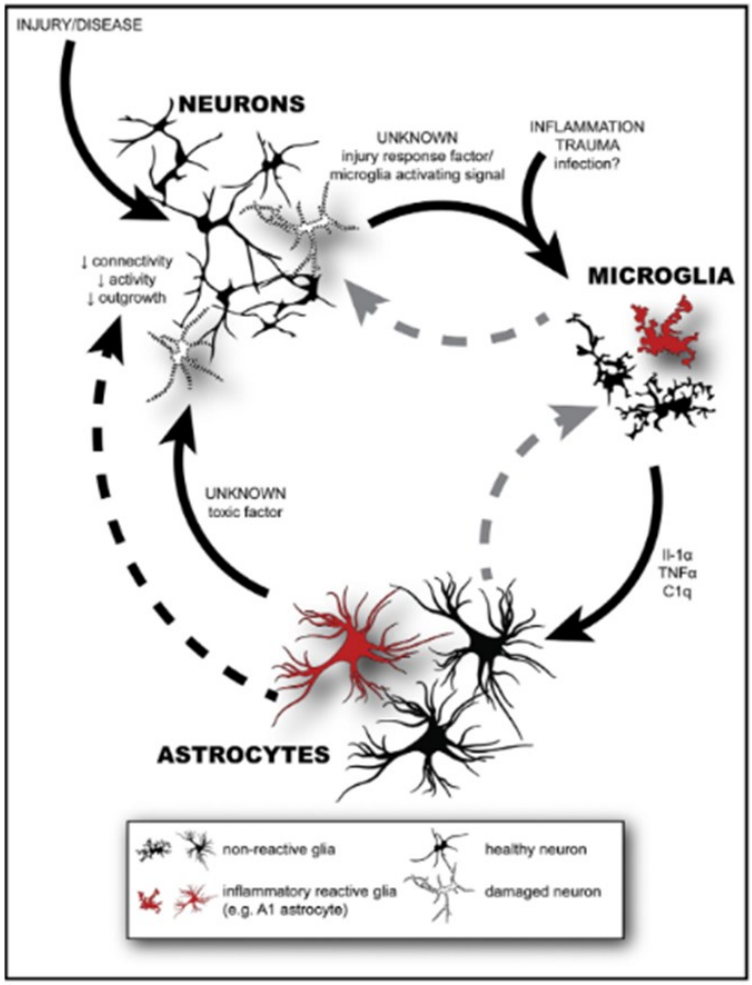

Figure 13: Relationship and contribution of microglia and astrocytes to the neuroinflammatory process (Liddelow and Barres 2017).

3. Rodent models of Parkinson's Disease

Although it is accepted that PD does not result exclusively from dopaminergic degeneration of the nigrostriatal pathway, the latter is a pre-requisite for animals to exhibit behavioral deficits reminiscent of the motor dysfunction of PD. In preclinical PD research, rodent species offer cost-effective models of both motor and cognitive-affective dysfunction. There are two categories of models: toxic models, which are based on the administration of specific neurotoxins, and transgenic models, which are mainly based on the induction of expression of genes associated with familial forms of PD. Both classes of animal models have specific characteristics and limitations, so the choice of which model to adopt depends on the purpose of the project. In this chapter we examine parkinsonian rodent models obtained through systemic or intracerebral administration of toxins. All of these toxins cause the death of dopaminergic neurons primarily through oxidative stress and mitochondrial dysfunction, however, with a significant contribution from the neuroinflammatory process (Blandini and Armentero 2012).

The model based on the administration of 6-hydroxydopamine (6-OHDA) is one of the most widely used models in PD research. 6-OHDA does not cross the blood-brain barrier, thus it is necessary stereotactic administration into the brain parenchyma. This toxin, being a hydroxylated analogue of dopamine, is taken up within dopaminergic neurons via the dopamine transporter where, inducing cell death induced by oxidative stress (Blandini and Armentero 2012). Depending on the point of infusion of the toxin along the nigrostriatal pathway, two distinct levels of injury are obtained. If infusion of 6-OHDA occurs at the level of the SNc or medial forebrain fasciculus (cell bodies and/or axons of dopaminergic neurons), rapid and nearly complete injury (90-100%) is achieved. Alternatively, the toxin can be injected in the striatum (terminals of dopaminergic neurons), causing a retrograde progressive (4 weeks) and incomplete (50-70%) lesion of the same neurons. This gradual progression of the lesion creates a "4-wk concept," defining a therapeutic time window that can be exploited to evaluate the effectiveness of treatments aimed to counteract or modify the progression of nigrostriatal damage and related motor deficits (Deumens, Blokland, and Prickaerts 2002). The lesion induced by 6-OHDA is highly reproducible. Moreover, it is associated with the appearance of a neuroinflammatory response that develops in parallel with the neurodegenerative process (Steyers and Miller 2014). The 6-OHDA

lesion model has been shown to be useful in assessing and studying motor and cognitive symptoms (Owen 1995) . In fact, it was developerd a wide battery of behavioral tests such as: cylinder test, rotarod, and apomorphine tests for the assessment of motor performance (Truong et al. 2006; Landers, Kinney, and van Breukelen 2014); and the forced-swimming test, morris water maze test, and social recognition test for the assessment of cognitive impairments (Porsolt et al. 1978; Miyoshi et al. 2002; Prediger et al. 2006; Tadaiesky et al. 2008).

The second most used model of PD is based on the administration of MPTP, a neurotoxin identified in the early 80s in the United States and whose mechanism of action has been described in previous chapters (see section 2.3). The lesion, in primates, is produced by intra-carotid injection of the neurotoxin causing the onset of parkinsonism which represents, from a symptomatic point of view, the form of experimental PD closest to the idiopathic disease of man. In mice, the administration of MPTP occurs through multiple intraperitoneal injections, following treatment protocols that allow to obtain lesions of different size and evolution. Similar to 6-OHDA administration, MPTP-induced injury is also accompanied by the onset of a relevant inflammatory process and results in both motor and cognitive impairment (Perry, Nicoll, and Holmes 2010; Perry and Holmes 2014).

There are also models based on the administration of pesticides, such as rotenone and paraquat, both of which are systemically administered. Rotenone is an organic insecticide used in agriculture that is known to be one of the most potent inhibitors of mitochondrial complex I (Betarbet et al. 2000). Systemic administration of rotenone causes selective degeneration of nigrostriatal neurons and subsequent dopaminergic denervation of the corpus striatum. Unlike the other models, this is the only one in which it is possible to observe the presence of intracytoplasmic inclusions in dopaminergic neurons similar to Lewy bodies. However, this model is not widely used because of its poor reproducibility and the variability of the response to the toxin.

Paraquat is a herbicide, which accesses the cytoplasm of dopaminergic neurons through the use of the neutral amino acid transporter. Once in the neuron, it induces the formation of reactive oxygen species and reduces the efficiency of antioxidant systems (Grant, Lantos, and Parkinson 1980; Barbeau et al. 1985). Chronic treatment with paraquat induces a neuronal loss of approximately 20-30%. This animal model

shows reduced motor activity and increased aggregation and deposition of alpha-synuclein (Manning-Bog et al. 2002; Cicchetti, Drouin-Ouellet, and Gross 2009).

4. New therapeutic strategies: the importance of natural-derived compounds

The simplest definition for a "natural compound" is a small molecule that is produced by a biological source, including plant extracts and products of microbial or animal origin (Mignani et al. 2018). Different studies have demonstrated the therapeutic efficacy of naturally derived products in a wide range of disease conditions, including neurodegenerative diseases (Guo et al. 2017; Solayman et al. 2017; Mirza-Aghazadeh-Attari et al. 2020). Some natural molecules have been shown to be potentially applicable in PD, thanks to their anti-apoptotic, anti-inflammatory, antioxidant and protein folding activity properties (Li et al. 2019). For instance, polyphenols, natural molecules present in different plant-derived foods including fruits, vegetables, cereals, tea, and wine. In particular, it has been demonstrated at the brain level the ability of these compounds to counteract the neurodegenerative process by enhancing endogenous antioxidant and anti-inflammatory responses and promoting cell survival and differentiation (Chen et al. 2000; Molina et al. 2003; Shen et al. 2007; Spencer 2007; Mythri, Harish, and Bharath 2012). Furthermore, polyphenol treatment has been observed to result in increased dopaminergic tone at the striatal level, which is associated with improved motor performance in animal models of PD (Patil et al. 2014; Lv et al. 2012; Khan et al. 2012; Giuliano et al. 2020).

Palazzi and collaborators (2018) demonstrated the anti-amyloidogenic power in vitro of oleuropein aglycone, the main olive oil polyphenol. The authors reported that oleuropein aglycone is able to interact and stabilize αSyn monomers, hampering the growth of oligomers (with cytotoxic properties) and favouring the growth of stable and harmless aggregates (Palazzi et al. 2018). In another mouse model of PD based on MPTP administration, Prema and coworkers (2015) demonstrated the neuroprotective and symptomatic potential of lycopene, an aliphatic hydrocarbon carotenoid. Specifically, the authors observed an increase in brain dopamine levels, a reduction in the extent of apoptotic process and oxidative stress induced by neurotoxin following treatment with this compound (Prema et al. 2015). Anti-apoptotic properties have also been demonstrated for ginsenoside Re, a natural molecule belonging to the steroidal saponins, which has been shown to exert a neuroprotective action on dopaminergic neurons by modulating the expression levels of Bcl-2 and Bax proteins and reducing caspase-3 activation (Xu et al. 2005).

4.1 Cannabinoids

Cannabinoids are compounds, both natural and synthetic, capable of interacting with cannabinoid receptors and exerting effects similar to those produced by *Cannabis sativa*. To date, more than 100 naturally occurring cannabinoids or phytocannabinoids have been identified (Figure 14). The most well-known among these compounds is delta-9-tetrahydrocannabinol (THC), famous for its therapeutic and psychoactive properties, and cannabidiol (CBD), a non-psychoactive cannabinoid able to modulate the action of THC in the brain, limiting its psychoactive and sedative effects and simultaneously prolonging the duration of its therapeutic effects (Zuardi 2008).

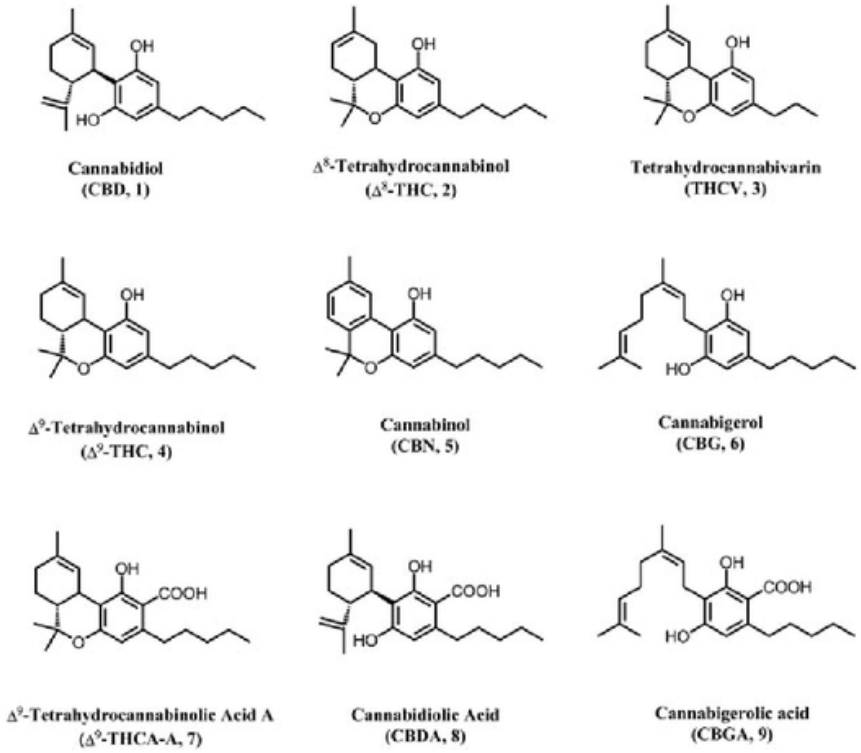

Figure 14: Structures of major cannabinoids in *Cannabis sativa*.

Insights into the mechanism of action of THC led to identification of the cannabinoid receptors in the 1990s and, consequently, of endogenous ligands of these receptors, which became known as endocannabinoids (Devane et al. 1992; Mechoulam et al. 1970; Sugiura et al. 1995; Di Marzo and Fontana 1995). There are at least two types of cannabinoid receptors, CB1 and CB2, both coupled to G-proteins. CB1 receptors are mainly present in the central nervous system and CB2 receptors are highly expressed in the immune system and in certain peripheral tissues (Matsuda et al. 1990; Munro,

Thomas, and Abu-Shaar 1993). Subsequently, other receptor classes that were able to interact with cannabinoid compounds were identified, including receptor-channels that result in transient changes in potential, "orphan" receptors (GPR55, GPR18, GPR3, GPR6), and nuclear receptors activated by peroxisomal proliferators (PPARγ) (Kendall 2017). Receptors and their endogenous ligands - anandamide and 2-arachidonoylglycerol - and the enzymes involved in their metabolism constitute the endocannabinoid system (Raitio et al. 2005). The endocannabinoid system is involved in several processes such as pain control, food intake, neuronal development, reproduction, and gut motility, suggesting its possible use as a therapeutic target in a wide range of diseases (Fowler 2012). In particular, the involvement of this system in the modulation of neuronal activity and neuroinflammatory response has been demonstrated (Galiegue et al. 1995; Klein, Friedman, and Specter 1998; Klein et al. 2003). The potential involvement of the endocannabinoid system in neurodegenerative diseases dates back to the early 2000s, when increased expression of the CB2 receptor was identified in microglial cells in the hippocampus and entorhinal cortex of patients with Alzheimer's disease, and subsequently also in subjects with multiple sclerosis, amyotrophic lateral sclerosis and PD (Benito et al. 2007; Garcia et al. 2015). The phenomenon observed in these patients could be the result of a compensatory mechanism aimed at attenuating the development of the neuroinflammatory process (Bie et al. 2018). This effect was associated with a reduction in neurodegenerative processes. In particular, an attenuation of the neuroinflammatory process and a reduction in the loss of dopaminergic neurons was observed following chronic treatment with delta-9-tetrahydrocannabivarin, a potent CB2 receptor partial agonist, in animal models of PD (Garcia et al. 2011). Similarly, treatment with the CB1 agonist HU-210 in an in vitro model of PD promoted cell survival. Moreover, this neuroprotective effect was enhanced when neuronal cultures were exposed to conditioned media from mixed glial cultures (70% astrocytes/30% microglia) treated with the same agonist, suggesting that this effect is largely due to the ability of this compound to regulate glial influence on neurons (Lastres-Becker et al. 2005).

4.1.1 Cannabidiol

Cannabidiol (CBD) is a natural cannabinoid, isolated in 1940 from cannabis plants (Mechoulam et al. 1970). It is the major non-psychoactive cannabinoid and occurs naturally in appreciable amounts in the plant leaves and flowers, accounting for up to 40% of the plant's extracts obtained from newly developed varieties poor in Δ9 -THC (Andre, Hausman, and Guerriero 2016). CBD is a weak activator of cannabinoid receptors type 1 (CB1) and type 2 (CB2) due to its structural conformation (Reggio, Panu, and Miles 1993) (Figure 15).

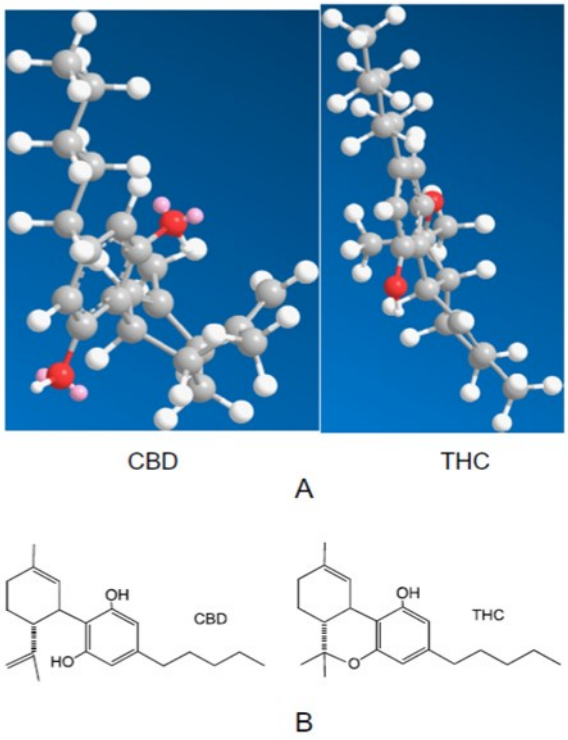

Figure 15: The minimal energy conformations of CBD and Δ9-THC (Burstein 2015)

Actually, CBD may also act as a negative allosteric modulator of the CB1 receptor, and as an inverse agonist of the CB2 receptor (Pertwee 2008).

Furthermore, this compound is able to interact on other receptors, including transient receptor potential (TRP) channels, vanilloid, ankyrin and melastatin receptors (Muller, Morales, and Reggio 2018; Kossakowski et al. 2019; Campos and Guimaraes 2009; Galaj et al. 2020). Notably, several studies over the years have demonstrated the involvement of TRPV1 receptors in the anxiolytic, anticonvulsant and anticancer effects of CBD (Fonseca, Correia-da-Silva, and Teixeira 2018; Gray et al. 2020;

Campos and Guimaraes 2009). Moreover, another study supports the benefits of CBD treatment, which was able to induce apoptosis in breast cancer cells through direct or indirect activation of TRPV1 (Ligresti et al. 2006). CBD can also bind the peroxisome proliferator-activated receptor (PPARγ) (Figure 16) and is able to increase the physiological levels of the endocannabinoid anandamide by inhibiting the enzymes responsible for its hydrolysis (Bisogno et al. 2001; Navarrete et al. 2009; Nomura et al. 2011; Peres et al. 2018). CBD also binds some G protein-coupled orphan receptors (GPR). In particular, it has been reported to act as an antagonist at GPR55, and as an inverse agonist at GPR3, GPR6 and GPR12 (Atalay, Jarocka-Karpowicz, and Skrzydlewska 2019). Finally, CBD may be an agonist at serotonin (5-hydroxytryptamine, 5-HT) receptors 1a (Russo et al. 2005), and at the adenosine A2A receptors (Ribeiro et al. 2012). CBD induces anti-inflammatory and antioxidant responses through modulation of these receptors (O'Sullivan et al. 2009; Rajan et al. 2016; Giacoppo et al. 2017). Indeed, CBD has been shown to reduce oxidative metabolism in polymorphonuclear leukocytes (Mabou Tagne et al. 2019) and H2O2-treated nucleus pulposus cells (Chen et al. 2016), and furthermore reduces oxidative stress parameters in aged pancreatic cells. In addition, CBD has the proclivity to improve cell viability following H2O2 treatment (Chen et al. 2016). Valvassori and collaborators (2013) observed an increased activity of the respiratory chain and creatine kinase in the brain in CBD-treated rats. It has also been demonstrated to be effective in reducing levels of pro-inflammatory cytokines, such as IL-1β, TNF-α, IFN-β, IFN-γ, IL-17, and IL-6, and increasing levels of the anti-inflammatory cytokines IL-4 and IL-10 (Watzl, Scuderi, and Watson 1991; Weiss et al. 2006; Rajan et al. 2016). The efficacy of CBD has also been demonstrated in models of neurodegenerative diseases. Due to its action on PPARγ receptors, CBD treatment promoted hippocampal neurogenesis by reducing reactive gliosis and levels of pro-inflammatory molecules, in a mouse model of Alzheimer's disease (Esposito et al. 2011). Clinical studies have also shown the efficacy of this molecule in movement disorders, such as PD (Farooqui and Farooqui 2011; Sanchez-Lopez et al. 2012; Niranjan 2014). CBD reduced psychotic symptoms without worsening motor function or inducing adverse effects (Zuardi et al. 2009). Chagas and collaborators demonstrated that CBD was able to reduce the frequency of REM sleep behaviour

disorder events, without improving PD patients' motor function or their general symptom score (Chagas, Eckeli, et al. 2014). The authors suggest that this effect could be related to the anxiolytic, antidepressant and antipsychotic properties of CBD (Chagas, Zuardi, et al. 2014).

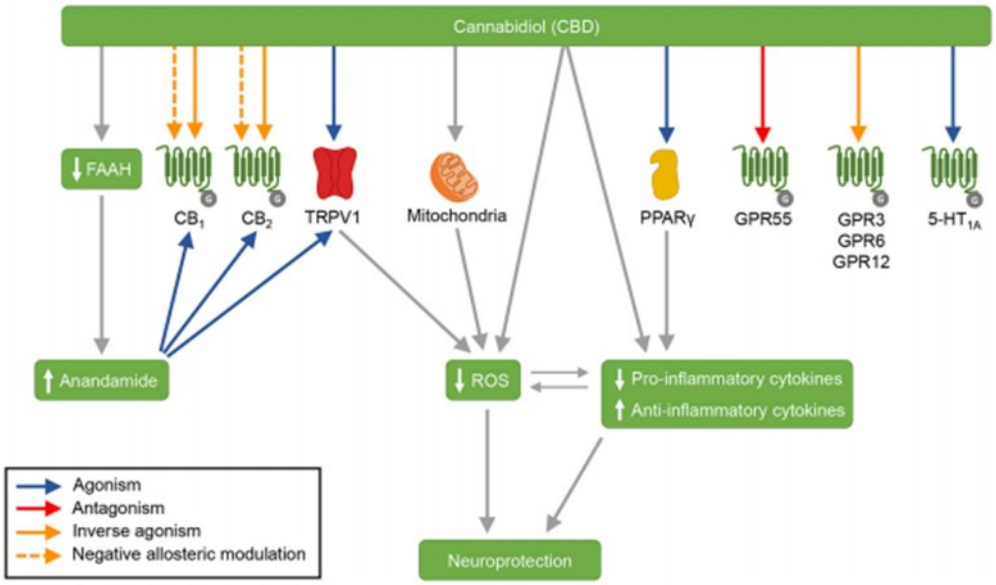

Figure 16: Cannabidiol's mechanisms of action. (Peres et al. 2018).

AIMS

Identifying new therapeutic strategies able to perform a neuroprotective effect is a major research challenge in the PD field. Several studies show that neuroinflammation is one of the main factors contributing to the neurodegenerative process that characterises the disease. Consequently, strategies capable of shutting down or modulating the neuroinflammatory response in a cytoprotective way may be able to promote neuronal survival motor functions recovery.

The aim of present study was to evaluate the effects of chronic treatment with CBD on:

- **PD-associated neurodegeneration** by assessing the prercentage of degeneration of dopaminergic neurons tyrosine hydroxylase-positive in the nigrostriatal pathway;
- **Neuroinflammatory processes** associated with the 6-OHDA-induced lesion in the substantia nigra *pars compacta*. In particular, it was evalutated the glial cells activation and polarization (biochemical and morphologica phenotype) levels;
- **Peculiar motor deficits** of the 6-OHDA rat model, performing a standard behaviural tests battery (Cylinder, Apomorphine-induced rotational and rotarod tests);

Moreover, a potential mechanism of CBD effect was investigated in this model. In particular, the attention was focused on the TRPV1-ciliary neurotrophic factor pathway. According to the literature, this pathway is one of the main involved in the neuroprotection-TRPV1 agonists induced on dopaminergic neurons in analogous animal models of PD.

MATERIALS AND METHODS

1. Experimental design

CBD (Purity: 99.5% (HPLC)—Linnea SA, Riazzino, TI, Switzerland) was prepared by isolation and purification from *Cannabis sativa* L. aerial parts. Prolonged decarboxylation allowed the conversion of the natural acidic form cannabidiolic acid to CBD. The procedure required refining steps with final crystallization.

We used a 6-OHDA rat model of PD to investigate the potential neuroprotective effects of CBD on the neurodegenerative process, neuroinflammation and motor deficits associated with the disease. For this reason, we establish a chronic treatment with CBD (10 mg/kg daily, i.p.; the choice of dosage and route of administration will be discussed in the discussion chapter) for 28-d (4 wk) starting on the first day after 6-OHDA infusion. The sample sizewas calculated to provide a statistical power of 80% and a confidence range of 95%. Basal motor activity was assessed by behavioural tests on the day before infusion of the neurotoxin, while the efficacy of CBD treatment on motor symptoms associated with nigrostriatal degeneration was evaluated four weeks after 6-OHDA infusion. Male Sprague-Dawley rats (176g-200g on arrival), were divided into the following experimental groups after microinfusion of 6-OHDA:

- CBD group: animals treated with CBD 10 mg/kg/day, in Tween 80-saline 1:16, i.p.;
- Control group: animals treated with vehicle, Tween 80-saline 1:16, i.p.

Each rat received 1ul of solution (vehicle+CBD or vehicle alone) per gram of body weight.

1.1 Surgery procedure

Rats (Charles River, Calco, LC, Italy) were anaesthetized with Sodium thiopental (50 mg/kg, i.p.) and placed in a stereotaxic frame (Stoelting, Chicago, IL, USA). They received a unilateral injection of 6-OHDA (20 µg/3 µL in saline/0.02% ascorbic acid; Sigma, St. Louis, MO, USA) into the right striatum (1.0 mm anterior, 3.0 mm lateral and 5.0 mm ventral, with respect to bregma and dura). All animal experiments were carried out with strict observance of protocols and guidelines approved by Local Committee, Italian Minister of Health and European Union legislation (Permit Number: 7/2019-PR of 8 January 2019 in compliance with article 31, D.Lgs.n. 26/2014).

1.2 Behavioral Evaluation

Three behavioural tests were performed to evaluate the effects of CBD chronic treatment on motor deficits induced by 6-OHDA infusion: cylinder test, apomorphine-induced rotational test and rotarod test. For this analysis, 9 to 20 animals for each group were used. All tests were performed during the light phase (10:00–16:00), in full compliance with the directive of the European community.

1.2.1 Cylinder test

The test evaluates the asymmetry in the use of the forepaws to support the weight of the body against the walls of a cylindrical container, during the exploratory behavior. Under normal conditions the animal does not express any preference in the use of the right or left front paw, whereas following unilateral injury (6-OHDA-induced) the animal shows a preference in the use of the paw ipsilateral to the lesion(Brooks and Dunnett 2009).

Rats were placed individually in a glass cylinder (21 cm diameter, 34 cm height) for 5 min, and their behavior was recorded by video camera. The number of wall contacts made by the rat with the left or right forepaw was counted and preference of paw use was calculated using the following formula:

$$P = \frac{\text{Ipsilateral}}{(\text{Ipsilateral} + \text{Controlateral})} - \frac{\text{Controlateral}}{(\text{Ipsilateral} + \text{Controlateral})}$$

1.2.2 Apomorphine-induced rotational test

This test is widely used as a functional index of nigrostriatal lesion. In particular, the gradual denervation of the nigrostriatal pathway induced by the neurotoxin results in receptor hypersensitivity when exposed to a dopaminergic agonist (apomorphine)(Ambrosi et al. 2010). The animals were injected with apomorphine (0.5 mg/kg dissolved in saline with 0.2% ascorbic acid, i.p.). The procedure induces a rotational motor response toward the opposite side to that of the 6-OHDA induced lesion. This response was evaluated using an automatic rotameter connected to the rat by means of an plastic belt for 30 min (Bioseb, Largo, FL, USA) and calculated by subtracting the total number of ipsilateral rotations from the total number of contralateral rotations.

1.2.3 Rotarod test

This test is widely used to evaluate the motor coordination of rodents(Brooks and Dunnett 2009). Following training to familiarize the animal with the task and the instrument, two speed protocols were performed:

A) Constant motion: 3 trials of 120 seconds for each animal at 12 RPM.
B) Accelerated motion: 3 trials of 180 seconds for each animal at a rate that gradually increases from 4 to 20 RPM.

Before the infusion of 6-OHDA the animal tends to perform the 3 trials of each protocol for the whole time period. While following injury the animal develops motor deficits particularly related to the contralateral limb resulting in a reduction in total time.

1.3 Animal sacrifice, brain cutting and preservation

Animals were deeply anesthetized with Sodium thiopental (150 mg/kg, i.p.) and transcardially perfused with saline and ice-cold 4% paraformaldehyde (Merck, Darmstadt, Germany). Brains were rapidly removed, postfixed for 24 h in the same fixative and subsequently transferred in solutions of sucrose at increasing concentrations (up to 30%). Brains were then cut in serial coronal sections (40 μm) containing both the striatum and the substantia nigra pars compacta (SNc) using a microtome (Histo-line Laboratories) and underwent immunohistochemical staining.

1.4 Neurodegeneration process assessment

The nigrostriatal lesion was assessed by immunohistochemistry directed against the dopaminergic marker tyrosine hydroxylase (TH) on coronal sections of both SNc and striatum (6 to 8 animals for each group). Briefly, sections were processed with a rabbit anti-TH primary antibody (1:2000, Chemicon AB152) and a biotinylated anti-rabbit immunoglobulin G secondary antibody (1:500, Vector Laboratories, San Francisco Bay Area, CA, USA) and revealed using a commercial kit based on the avidin-biotin technique (Vectastain ABC Elite kit, Vector Laboratories). Reaction products were developed using nickel-intensified 30-30- diaminobenzedine tetrahydrochloride for 1 min (DAB Substrate Kit for Peroxidase, Vector Laboratories).

1.5 Neuroinflammatory process assessment

Microglia and astrocyte activation and polarization in the SNc was assessed by triple immunofluorescent staining directed against (a) the Cluster of Differentiation molecule 11 b (CD11b) (1:300, Serotec MCA275 R) for microglia or the Glial Fibrillary Acidic Protein (GFAP; 1:1000, Sigma, USA, G3893) for astrocytes, (b) the Cluster of Differentiation molecule 32 (CD32, 1:300, Santa Cruz, Dallas, TX, USA, sc-28842) or CD206 (1:300, Santa Cruz, USA, sc-48758) for assessing cytotoxic (M1-A1) or neuroprotective (M2-A2) phenotypes, respectively) TH for SNc localization (1:200, Novusbio NB300-110). Alexa Fluor 350 (1:150, Thermo Fisher, Dallas, TX, USA), 488 and 594 (1:300, Thermo Fisher, Waltham, MA, USA) were used as secondary antibodies. For this analysis, 5 to 8 animals for each group were used.

1.6 TRPV1-CNTF pathway assessment

Transient Receptor Potential Vanilloid 1 (TRPV1) (1:1000, Alomone Labs, Israel, ACC-030) and ciliary neurotrophic factor (CNTF) (1:500, Millipore, Burlington, MA, USA, MAB338) levels in glial cells were assessed by double immunofluorescent staining. Alexa Fluor 350 (1:150, Thermo Fisher, Dallas, TX, USA), 488 and 594 (1:300, Thermo Fisher, Waltham, MA, USA) were used as secondary antibodies. For this analysis, 4 to 5 animals for each group were used.

1.7 Image Analysis

Image analysis was performed using an AxioSkop2 microscope, equipped with Apotome 2, connected to a computerized image analysis system (AxioCam MR5, Zeiss, Gina, Germany) with dedicated software (AxioVision Rel 4.2, Zeiss, Germany). The striatal degeneration was expressed as the percentage of striatal volume deprived of TH immunoreactivity, with respect to the entire striatal volume. The number of TH-positive neurons in the SNc was counted bilaterally on every four sections throughout the entire nucleus by unbiased stereology using the optical fractionator method (Stereo Investigator System 9.03.2, Microbrightfield Inc., Williston, VT, USA). The results were expressed as the percentage of TH-positive neurons in the lesioned SNc compared with the intact hemisphere. Cell count of microglia and astrocytes was performed by analyzing three different SNc sections, chosen according to rostrocaudal coordinates. Cell density was assessed by counting CD11b- or GFAP-positive cells

from a stack of 16 pictures (in a 0.04 mm frame, 1 mm-thick, 40× magnification) taken from three discrete areas of the same SNc section. The analysis of microglia or astrocytes polarization was performed by evaluating the percentage of CD32-positive (M1-A1) and CD206-positive cells (M2-A2) of the total microglia cells or astrocytes. The microglia process length and number of endpoints were quantified using skeleton plugin in FIJI (NIH, Bethesda, MD, USA) (Young and Morrison 2018). The results obtained were expressed as a percentage of branch length (μm)/cell and number of endpoints/cell in the lesioned SNc compared with the intact hemisphere. Colocalization analyses for TRPV1 and CNTF in glial cells were accomplished by using the EzColocalization plugin in FIJI (Stauffer, Sheng, and Lim 2018). Metric matrix used in this analysis was the threshold overlap score (TOS).

1.8 Western Blot

Animals were sacrificed by decapitation and SNc area was rapidly removed and frozen on dry ice, and stored at −80 ◦C. Protein lysate was obtained by re-suspending SNc in ice-cold lysis buffer (CelLytic, Sigma, USA) containing diluted phosphatase (1:10, Roche, Monza, Italy) and protease inhibitors (1:25, Roche, Italy). After centrifugation, the supernatant was collected and protein concentration was measured using a Bicinchoninic Acid Protein Assay (Sigma, USA). Protein lysates were run on 10% gels, transferred onto nitrocellulose membranes (Biorad, USA) and western blot was performed. Membranes were blocked (Odyssey blocking buffer, LiCor, USA) and incubated overnight with the following primary antibodies: anti-GFAP (1:2000, Sigma, USA, G3893), anti-TRPV1 (1:500, Santa Cruz, USA); anti-CNTF (1:500, Millipore, Germany, MAB338). As secondary antibodies, anti-mouse 1:10000 secondary IgG HRP-Conjugated were used. Image analysis of western blots was performed using Azure 600 azure biosystems and software (LiCor, Biosciences, Rockville, MD, USA) and signal was normalized with the corresponding GFAP signal. For this analysis, 2 to 4 animals for each group were used.

1.9 Statistical Analysis

The results are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism 8 (GraphPad software, San Diego, CA, USA). Comparisons between groups were made using Student's t-test unpaired two-tailed. Statistical significance was set at $p < 0.05$.

RESULTS

1. Cannabidiol Treatment Attenuates Nigrostriatal Degeneration and Improves Motor Performance

The potential effects of CBD treatment on PD progression were investigated by using the unilateral intrastriatal 6-OHDA-lesion model. The 6-OHDA injection in the striatum causes a partial and gradual degeneration of the nigrostriatal pathway, providing a therapeutic window (28 days) useful to evaluate the neuroprotective efficacy of new therapeutic strategies(Blandini and Armentero 2012). In this study, the 6-OHDA injection induced a 75% loss of dopaminergic striatal terminal and 70% loss of dopaminergic neurons in the SNc after 28 days (Figure 17). Animals treated with CBD showed a 21% significant reduction of the striatal terminal degeneration (Figure 17A; $p = 0.012$) and cell body loss in the SNc (Figure 17B; $p = 0.008$) in comparison with control group. CBD also ameliorated 6-OHDA-induced motor deficits as assessed by behavioral tests. Lesioned animals showed a prevalent use of the forepaw ipsilateral to the lesioned side over the contralateral, "parkinsonized" forepaw at cylinder test. Interestingly, animals treated with CBD displayed a significantly lower preference (34%; $p = 0.032$) in the use of the ipsilateral limb than control rats, indicative of a behavioral rescue (Figure 18A). Analogously the apomorphine-induced rotational behavior, a functional index of nigrostriatal lesion in 6-OHDA animal models, was significantly reduced (54%) ($p = 0.044$) in the animals treated with CBD (Figure 18B). Last, the rotarod test was performed to evaluate the motor coordination of rodents. While the analysis of baseline (pre-lesion) motor activity did not reveal any difficulty to stay in balance on the rotating bar in both experimental groups, after nigrostriatal lesion, animals exhibit a reduction (about 50%) in the motor performance as shown in the control group. CBD treatment did not ameliorate motor performance at this test (Figure 18C).

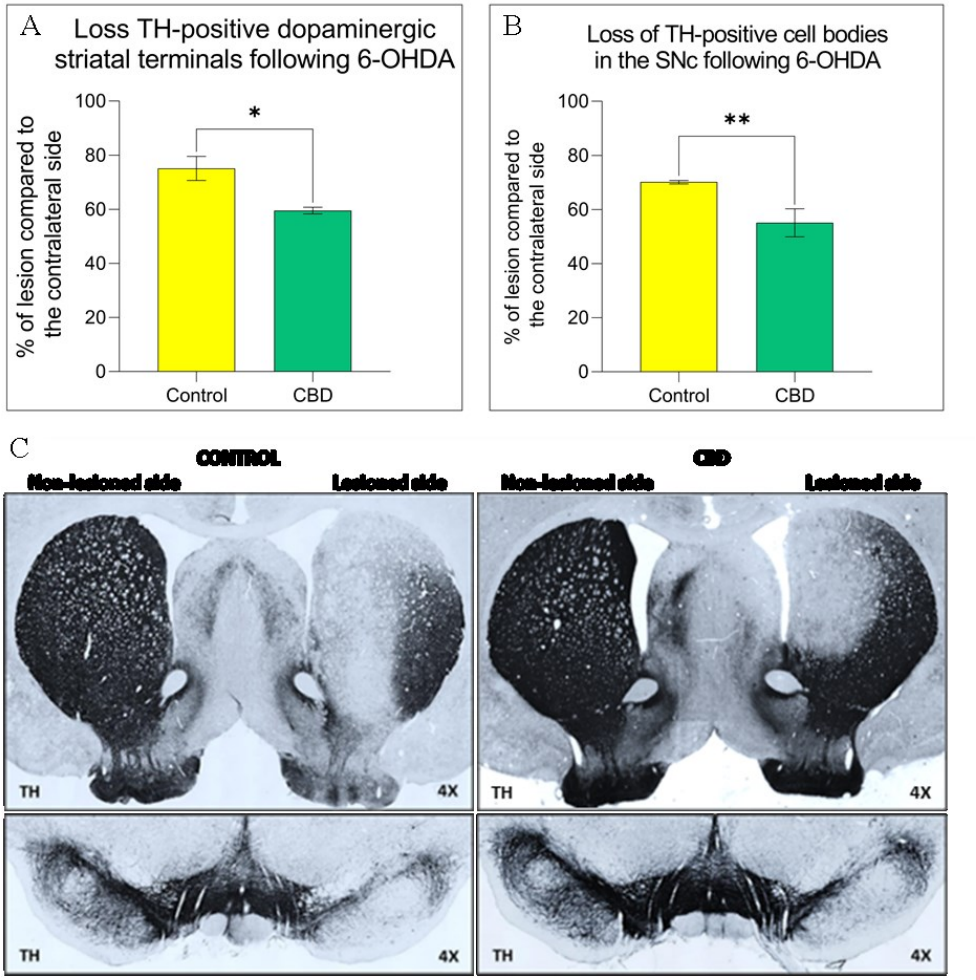

Figure 17: Effect of CBD treatment on the neurodegenerative process in the nigrostriatal pathway. The graphs show the percentage of lesion of (A) in the striatum. (B) in the SNc. (C) Representative images of the nigrostriatal damage in both experimental groups. Results are expressed as mean ± SEM. * $p < 0.05$ vs. control $t = 2947$, $df = 12$. ** $p < 0.01$ vs. control $t = 3102$, $df = 13$, Student's t-test unpaired two-tailed. N = 6 to 8 in each group.

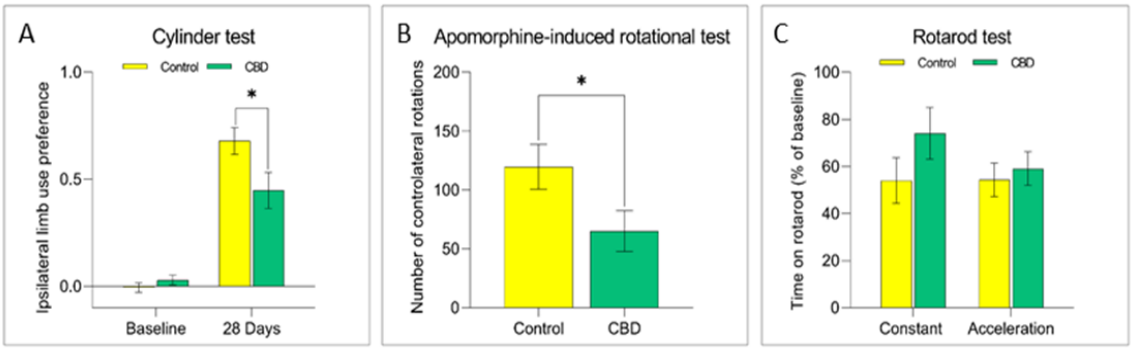

Figure 18: Effects of chronic treatment with CBD on motor behavior of 6-OHDA lesioned rats. The graphs show the motor performance evaluated using the (A) Cylinder, (B) Apomorphine-induced rotational and (C) Rotarod test in both experimental groups. Results are expressed as mean ± SEM. * $p < 0.05$ vs. control $t = 2218$, $df = 39$ (Cylinder), $t = 2109$, $df = 28$ (Apomorphine), Student's t-test unpaired two-tailed. N = 9 to 20 in each group.

2. Cannabidiol Modulates the Neuroinflammatory Process in the SNc through a Preferential Action on Astrocytes

The 6-OHDA-induced neurodegenerative process is accompanied by a neuroinflammatory response in the SNc characterized by the increase in glial cells density (microglia and astrocytes) and a change towards a pro-inflammatory phenotype (Giuliano et al. 2020).

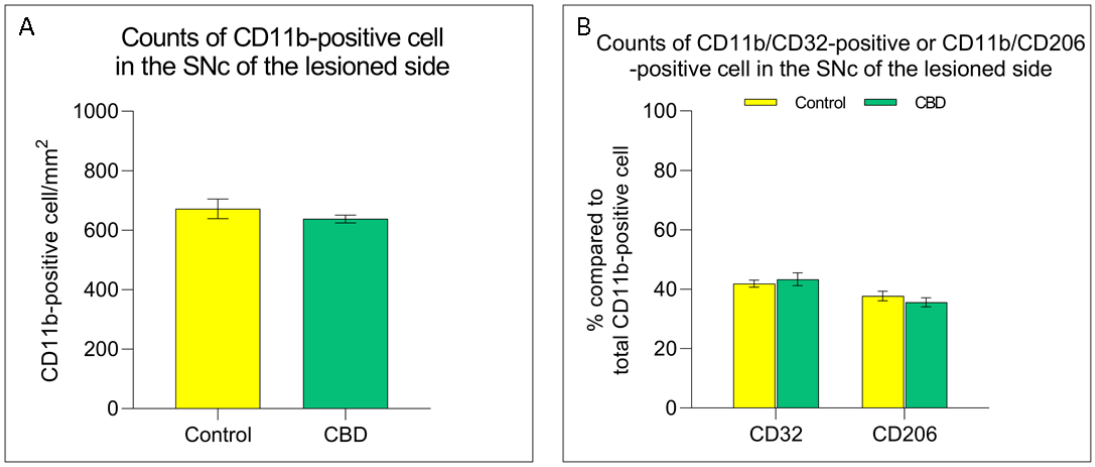

Figure 19: Immunomodulatory effect of chronic treatment with CBD on microglia activation and polarization in the SNc. Com-parison of (A) CD11b+ cell density and (B) microglia polarization state in the SNc in both experimental groups. The results are expressed as mean ± SEM. N = 6 to 8 in each group.

No differences in the number of CD11b+ cells/mm and in microglia cell polarization towards the cytotoxic M1 (CD11b+/CD32+) or cytoprotective M2 phenotype (CD11b+/CD206+) were observed in animals treated with CBD compared to controls, as shown in Figure 19 and 20. As we did not observe any effects on microglia density and phenotype, potential changes in microglia morphology after CBD treatment have been investigated. CBD-treated animals showed an 8% increase in the number of microglia endpoints ($p = 0.010$) (Figure 21A, C), accompanied by an increment in the length of microglia branches (Figure 21B, C), but the effect was not statistically significant ($p = 0.082$).

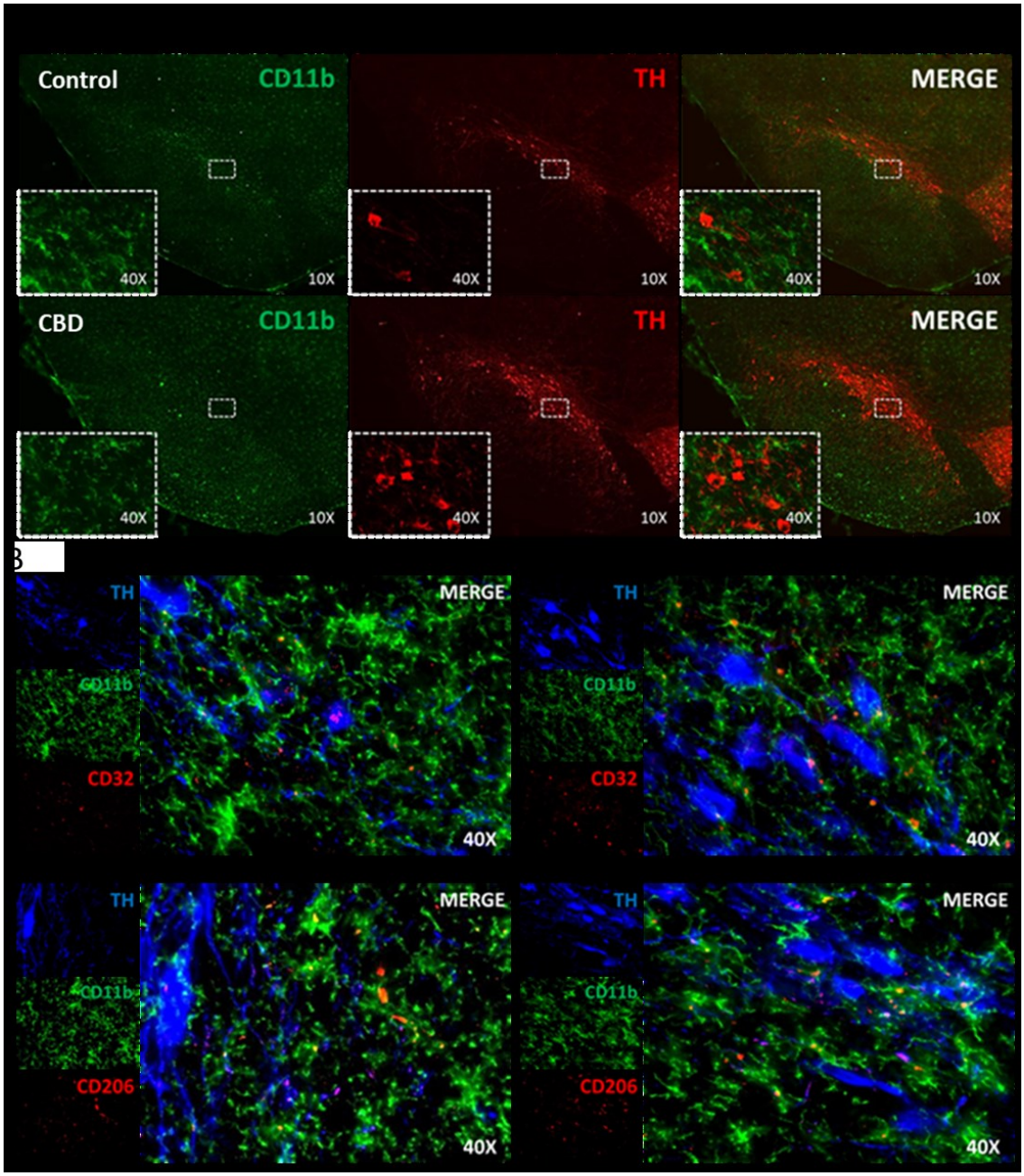

Figure 20: Representative images of the microglia activation (A) and polarization (B) in the SNc in both experimental groups.

Unlike microglial cells, the density of GFAP+ cells was significantly reduced (14%; p = 0.011) in the SNc of animals treated with CBD, as shown in Figure 22A and 23A. Nevertheless, the treatment with CBD did not affect the phenotype of these cells. Indeed, no differences in the number of GFAP+ cell polarized towards the cytotoxic A1 (GFAP+/CD32+) or cytoprotective A2 (GFAP+/CD206+) phenotype were observed in animals treated with CBD compared to controls (Figure 22B and 23B).

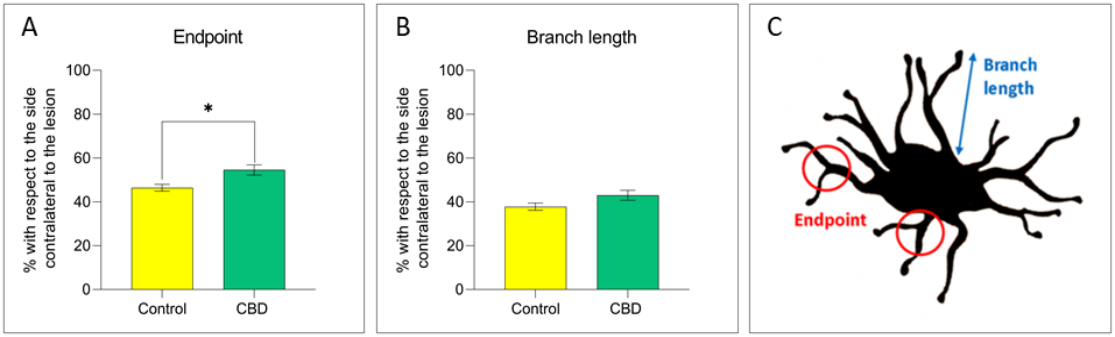

Figure 21: The graphs show the percentage of the (A) number of endpoints/cell and (B) branch length/cell (µm) in the SNc lesioned side with respect to the unlesioned side in both experimental groups. (C) Parameters analyzed to study microglial morphology. Results are expressed as mean ± SEM. * $p < 0.05$ vs. control $t = 2992$, $df = 13$, Student's t-test unpaired two-tailed. N = 5 to 8 in each group.

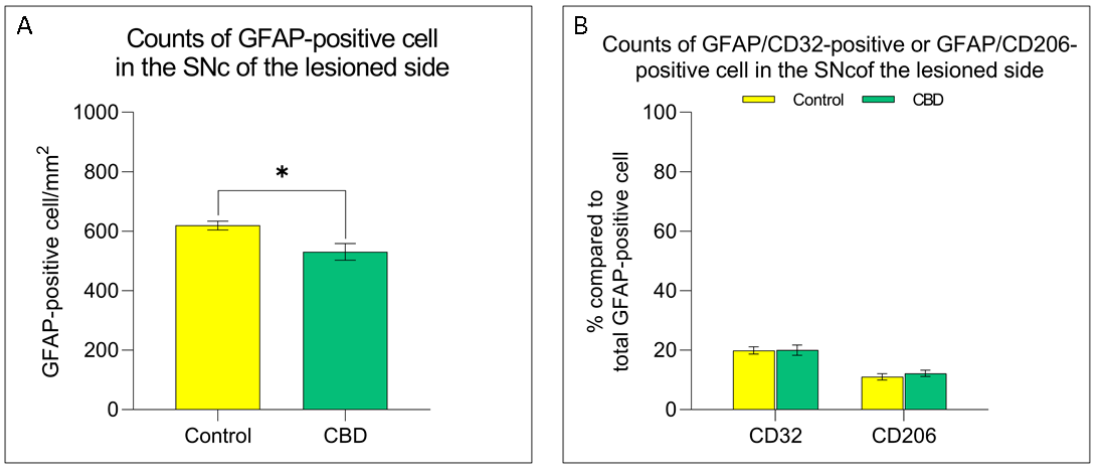

Figure 22: Immunomodulatory effect of chronic treatment with CBD on astrocytes activation and polarization in the SNc. (A) Comparison of GFAP+ cell density in the SNc in both experimental groups. (B) Comparison of astrocyte polarization in SNc in both experimental groups. Results are expressed as mean ± SEM. * $p < 0.05$ vs. control $t = 2937$, $df = 13$, Student's t-test unpaired two-tailed. N = 7 to 8 in each group.

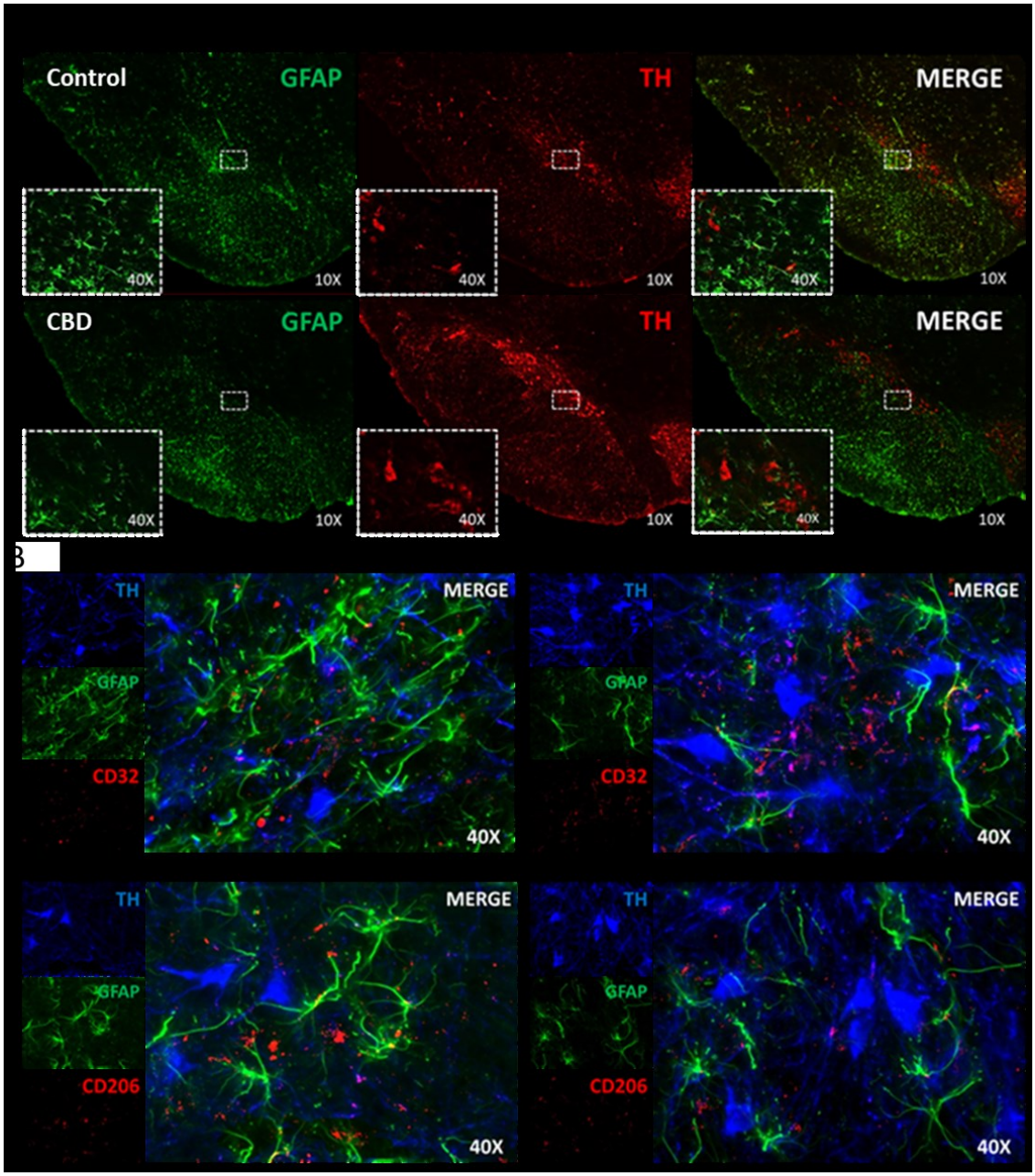

Figure 23: Representative images of the astrocyte activation (A) and polarization (B) in the SNc in both experimental groups.

3. Cannabidiol Induces the Activation of TRPV1-CNTF Cascade in the Astrocytes

In order to scrutinize the potential molecular mechanisms underlying CBD modulation of astrocytes, we focused our attention on TRPV1 activation, being CBD a TRPV1 agonist (Bisogno et al. 2001). In CBD-treated animals, increased expression levels of TRPV1 receptor were observed by both Western Blot and immunohistochemical analysis in astrocytic cells compared with the control group (WB: 81%, p = 0.059; IHF: 61%, p = 0.063) (Figure 24A and 25A-C). In contrast, no differences in TRPV1 receptor expression levels on microglial cells between two experimental groups were found (Figure 25A). Western Blot and colocalization analysis showed that the increased astrocytic TRPV1 expression in CBD-treated animals is accompanied by a rise in the levels of the ciliary neurotrophic factor (CNTF) compared to control animals, with a difference bordering on statistical significance (WB: 122%, p = 0.080; IHF: 67%, p = 0.052) (Figure 24B and Figure 25B-D).

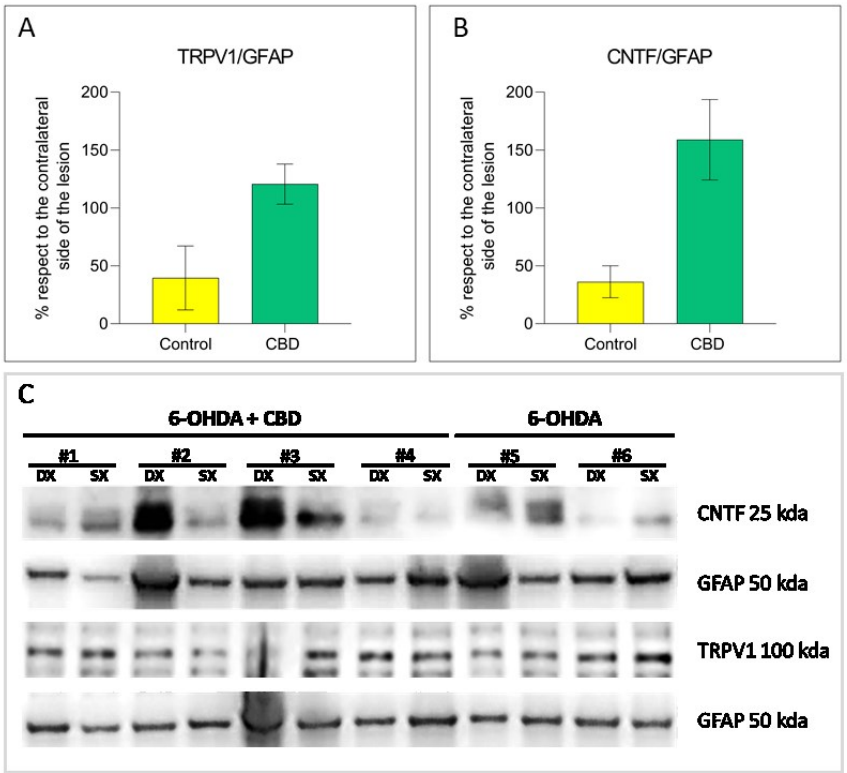

Figure 24: TRPV1 and CNTF astrocytic expression in the substantia nigra. (A) Quantification of TRPV1 expression in astrocytes. (B) Quantification of CNTF expression in astrocytes. (C,D) Representative images of Western Blot for TRPV1, CNTF and GFAP expression in the lesioned (Dx) and unlesioned (Sx) SNc, in both experimental groups. Results are expressed as mean ± SEM. N = 2 to 4 in each group.

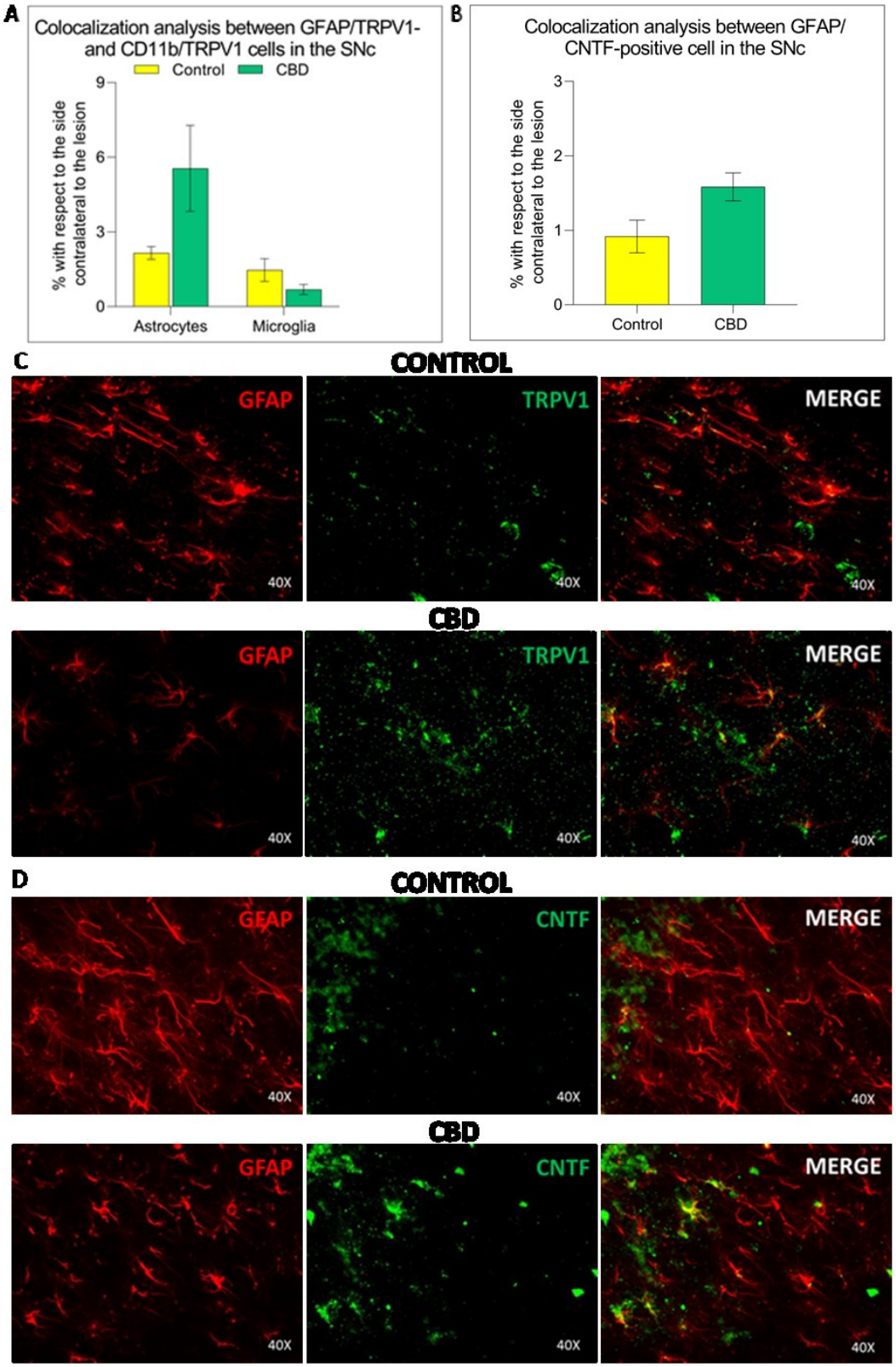

Figure 25: TRPV1 and CNTF glial expression in the substantia nigra. (A) Quantification of TRPV1 expression in astrocytes and mi-croglia. (B) Quantification of CNTF expression in astrocytes. (C,D) Representative images of the TRPV1 and CNTF expression in both glial subpopulations in the SNc, in both experimental groups. The results are expressed as mean ± SEM. N = 4 to 5 in each group.

DISCUSSION AND CONCLUSIONS

Current pharmacological treatments of PD are essentially focused on alleviating the characteristic motor symptoms by compensating the loss of dopamine in the nigrostriatal pathway, without affecting the progression of the disease. Therefore, the identification of new therapeutic strategies capable of slowing down or counteracting the neurodegenerative process that characterizes PD is one of the major challenges in this field. Natural compounds, especially phytocannabinoids, represent potential candidates in the treatment of neurodegenerative diseases. In particular, CBD, one of over 100 phytocannabinoids identified in *Cannabis sativa*, shows anti-inflammatory and antioxidant actions, which make it a potential and promising candidate in the PD context (Bisogno et al. 2001; Farooqui and Farooqui 2011; Niranjan 2014; Mecha et al. 2013; Gomes et al. 2015; Watt and Karl 2017). In this study, we evaluated the neuroprotective and symptomatic efficacy of chronic treatment with CBD in a rat model of PD, based on the unilateral intrastriatal infusion of 6-OHDA. Animals treated with CBD showed a significant reduction of the striatal terminal degeneration and cell body loss in the SNc in comparison with the control group. Interestingly, the increased survival of dopaminergic neurons was accompanied by a recovery of motor performance. The results of this study agree with previous findings, further supporting the neuroprotective and symptomatic action of CBD treatment in different in vivo models of neurodegeneration (Lastres-Becker et al. 2005; Garcia-Arencibia et al. 2007; Silveira et al. 2014; Mori et al. 2017). In contrast to pre-clinical studies, the results on motor symptoms obtained in clinical trials are not as straightforward. CBD treatment exhibited beneficial effect on most non-motor symptoms of PD patients, such as psychosis and sleep disorders, without any notable efficacy on motor symptoms (Zuardi 2008; Zuardi et al. 2009; Chagas, Zuardi, et al. 2014). This discrepancy could be due to the dosages of CBD, which are lower than the one chosen in the present study. This hypothesis is supported by the results of a recent clinical study, in which PD patients were treated with escalating doses of CBD until the target dose of 20 mg/kg/day (per os), which is much higher than doses used in previous studies (Leehey et al. 2020). In this study a beneficial effect was observed on tremor, nocturnal sleep, and emotional and behavioral dyscontrol (Leehey et al. 2020). Another factor to be considered is the administration route, which also differs between pre-clinical and clinical studies. In the exploratory, as well as in preclinical studies,

administration routes that provide higher bioavailability of the drug—such as intraperitoneal and intravenous route- are commonly used to prove the efficacy of a drug (Al Shoyaib, Archie, and Karamyan 2019). Therefore, the oral administration at relatively low doses used in the abovementioned clinical trials may have reduced the bioavailability of CBD and possibly some of its effects. It is noteworthy that the choice of pharmacological administration by intraperitoneal injection adopted in the present study was based on the properties of purified CBD (crystallised form) since this highly lipophilic form made the oral administration impossible. Moreover, based on previous pre-clinical studies (Lastres-Becker et al. 2005; Garcia-Arencibia et al. 2007; Garcia et al. 2010) we employed a dosage which demonstrated beneficial effects in combination with a good tolerability profile. Last, we cannot exclude that the symptomatic improvement, observed in our study, may be attributed to starting the treatment shortly after the lesion induction, while the current clinical studies administered CBD after a diagnosis is made (i.e., about 50% of dopaminergic neurons in the brain are already destroyed (Jaber et al. 1996). Although this could represent a limitation of our study, this treatment paradigm was essential to explore the neuroprotective effects of this drug. In addition, chronic CBD treatment led to a decrease of the number of reactive astrocytes in the SNc without affecting microglial activation, as demonstrated by the comparable number of CD11b positive microglial cells in animals treated with CBD and controls. However, it should be noted that CBD-treated animals show an increase in the number of microglia endpoints and branch length, an index of the resting state of these cells. Indeed, under physiological conditions, microglia cells are characterized by extensive and branched processes whereas, under pathological conditions, these cells undergo morpho logical changes characterized by a reduction in both the number and the length of its ramifications (Baek et al. 2018; Kim et al. 2019). Therefore, CBD did not reduce the extent of microglial response in the SNc, but it induced a moderate restoration of the resting condition in these cells. No straightforward phenotypic variations towards the cytotoxic or cytoprotective phenotype were instead observed after CBD treatment in both glia cell populations. These results confirm previous studies showing an immunomodulatory effect of CBD on glial cells (Iuvone et al. 2004; Esposito et al. 2006; Hayakawa et al. 2008; Schiavon et al. 2014; DiSabato, Quan, and Godbout

2016), characterized by the reduction in the number of active cells and in the consequent release of pro-inflammatory factors, such as TNFα, COX-2 and iNOS (Mori et al. 2017; Castillo et al. 2010). These effects could account for the therapeutic action exerted by CBD in this study. In order to further investigate the potential molecular mechanisms underlying the action of CBD, the expression levels of TRPV1 vanilloid receptor in the SNc were evaluated. Indeed, together with its antagonistic activity on cannabinoid CB1 and CB2 receptors (Pertwee 2008), CBD can act as an agonist of the TRPV1 vanilloid receptor, playing a key role in the activation of anti-inflammatory response (Bisogno et al. 2001; O'Sullivan et al. 2009; Giacoppo et al. 2017; Rajan et al. 2016). The assessment of the expression levels of TRPV1 in glial cells in the SNc highlighted a notably increased expression of the TRPV1 receptor in astrocytes of CBD-treated animals, but not in microglial cells. CBD-mediated activation of TRPV1 in the astroglial cells was accompanied by a marked increase in CNTF levels compared with the control group. Nam and collaborators (2015) showed that the activation of the TRPV1 astrocyte receptor is responsible for the neuroprotective and symptomatic effects observed in an animal model of PD based on MPP+ administration. In particular, capsaicin-induced activation of the TRPV1 receptor on the astrocytes enhances the endogenous neuroprotective response promoted by these glial cells, through the production and release of the ciliary neurotrophic factor (CNTF) (Nam et al. 2015). Ciliary neurotrophic factor is a member of the interleukin-6 (IL-6) cytokine family that is almost exclusively expressed in the nervous system (Stockli et al. 1989) where it is released by astrocytes, which increase its production following brain injury (Nam et al. 2015; Baek et al. 2018; Kang et al. 2012; Zhao et al. 2017). In addition to promoting adult neurogenesis (Kang et al. 2012), CNTF showed to increase survival of neurons after injury (Kang et al. 2012; Hagg, Varon, and Louis 1993) and improve cognitive and memory function in rodent models (Garcia et al. 2010). As CNTF receptor expression in the SNc dopaminergic neurons is known (Nam et al. 2015; Lee, Hofmann, and Kirsch 1997), and according our preliminary results, we hypothesize that CNTF released by the astrocytes after CBD-induced TRPV1 activation might promote the survival of dopaminergic neurons, resulting in a recovery of motor performance as shown by recent studies (Nam et al. 2015; Baek et al. 2018; Staff 2015). The presented results concerning the TRPV1-

CNTF mechanism are considered preliminary data. Indeed, future studies are necessary to validate the involvement of this pathway as mechanism of action of CBD in this animal model.

In conclusion, the present study demonstrated the neuroprotective, anti-inflammatory and symptomatic effects of CBD treatment in an animal model of PD, potentially via the activation of astrocytic TRPV1-CNTF pathway (Figure 26). Although it cannot be excluded that other signaling pathways can contribute to the abovementioned effects —according to the pleiotropic action of CBD—the results of this study overall support the therapeutic potential of this phytocannabinoid as disease-modifying and symptomatic treatment for PD.

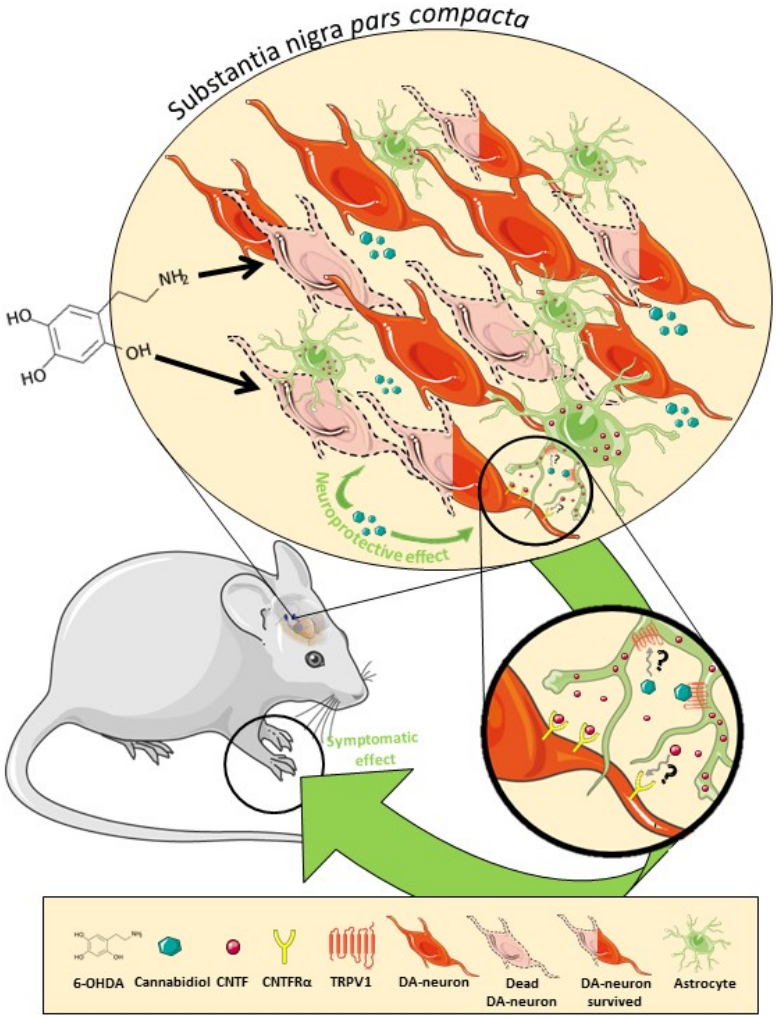

Figure 26: The present study demonstrated the neuroprotective and symptomatic effects of CBD treatment in an animal model of PD, potentially via the activation of astrocytic TRPV1-CNTF pathway.

REFERENCES

Al Shoyaib, A., S. R. Archie, and V. T. Karamyan. 2019. 'Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies?', *Pharm Res*, 37: 12.

Ambrosi, G., M. T. Armentero, G. Levandis, P. Bramanti, G. Nappi, and F. Blandini. 2010. 'Effects of early and delayed treatment with an mGluR5 antagonist on motor impairment, nigrostriatal damage and neuroinflammation in a rodent model of Parkinson's disease', *Brain Res Bull*, 82: 29-38.

Andre, C. M., J. F. Hausman, and G. Guerriero. 2016. '*Cannabis sativa*: The Plant of the Thousand and One Molecules', *Front Plant Sci*, 7: 19.

Angot, E., J. A. Steiner, C. Hansen, J. Y. Li, and P. Brundin. 2010. 'Are synucleinopathies prion-like disorders?', *Lancet Neurol*, 9: 1128-38.

Atalay, S., I. Jarocka-Karpowicz, and E. Skrzydlewska. 2019. 'Antioxidative and Anti-Inflammatory Properties of Cannabidiol', *Antioxidants (Basel)*, 9.

Baek, J. Y., J. Y. Jeong, K. I. Kim, S. Y. Won, Y. C. Chung, J. H. Nam, E. J. Cho, T. B. Ahn, E. Bok, W. H. Shin, and B. K. Jin. 2018. 'Inhibition of Microglia-Derived Oxidative Stress by Ciliary Neurotrophic Factor Protects Dopamine Neurons In Vivo from MPP(+) Neurotoxicity', *Int J Mol Sci*, 19.

Barbeau, A., L. Dallaire, N. T. Buu, J. Poirier, and E. Rucinska. 1985. 'Comparative behavioral, biochemical and pigmentary effects of MPTP, MPP+ and paraquat in Rana pipiens', *Life Sci*, 37: 1529-38.

Bellou, V., L. Belbasis, I. Tzoulaki, E. Evangelou, and J. P. Ioannidis. 2016. 'Environmental risk factors and Parkinson's disease: An umbrella review of meta-analyses', *Parkinsonism Relat Disord*, 23: 1-9.

Benarroch, E. E. 2013. 'Microglia: Multiple roles in surveillance, circuit shaping, and response to injury', *Neurology*, 81: 1079-88.

Benito, C., J. P. Romero, R. M. Tolon, D. Clemente, F. Docagne, C. J. Hillard, C. Guaza, and J. Romero. 2007. 'Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis', *J Neurosci*, 27: 2396-402.

Berg, D., R. B. Postuma, B. Bloem, P. Chan, B. Dubois, T. Gasser, C. G. Goetz, G. M. Halliday, J. Hardy, A. E. Lang, I. Litvan, K. Marek, J. Obeso, W. Oertel, C. W. Olanow, W. Poewe, M. Stern, and G. Deuschl. 2014. 'Time to redefine PD? Introductory statement of the MDS Task Force on the definition of Parkinson's disease', *Mov Disord*, 29: 454-62.

Betarbet, R., T. B. Sherer, G. MacKenzie, M. Garcia-Osuna, A. V. Panov, and J. T. Greenamyre. 2000. 'Chronic systemic pesticide exposure reproduces features of Parkinson's disease', *Nat Neurosci*, 3: 1301-6.

Bie, B., J. Wu, J. F. Foss, and M. Naguib. 2018. 'An overview of the cannabinoid type 2 receptor system and its therapeutic potential', *Curr Opin Anaesthesiol*, 31: 407-14.

Bisogno, T., L. Hanus, L. De Petrocellis, S. Tchilibon, D. E. Ponde, I. Brandi, A. S. Moriello, J. B. Davis, R. Mechoulam, and V. Di Marzo. 2001. 'Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide', *Br J Pharmacol*, 134: 845-52.

Blandini, F. 2013. 'Neural and immune mechanisms in the pathogenesis of Parkinson's disease', *J Neuroimmune Pharmacol*, 8: 189-201.

Blandini, F., and M. T. Armentero. 2012. 'Animal models of Parkinson's disease', *FEBS J*, 279: 1156-66.

———. 2014. 'Dopamine receptor agonists for Parkinson's disease', *Expert Opin Investig Drugs*, 23: 387-410.

Braak, H., K. Del Tredici, U. Rub, R. A. de Vos, E. N. Jansen Steur, and E. Braak. 2003. 'Staging of brain pathology related to sporadic Parkinson's disease', *Neurobiol Aging*, 24: 197-211.

Brennan, A. M., S. W. Suh, S. J. Won, P. Narasimhan, T. M. Kauppinen, H. Lee, Y. Edling, P. H. Chan, and R. A. Swanson. 2009. 'NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation', *Nat Neurosci*, 12: 857-63.

Bronstein, J. M., M. Tagliati, R. L. Alterman, A. M. Lozano, J. Volkmann, A. Stefani, F. B. Horak, M. S. Okun, K. D. Foote, P. Krack, R. Pahwa, J. M. Henderson, M. I. Hariz, R. A. Bakay, A. Rezai, W. J. Marks, Jr., E. Moro, J. L. Vitek, F. M. Weaver, R. E. Gross, and M. R. DeLong. 2011. 'Deep brain stimulation for Parkinson disease: an expert consensus and review of key issues', *Arch Neurol*, 68: 165.

Brooks, S. P., and S. B. Dunnett. 2009. 'Tests to assess motor phenotype in mice: a user's guide', *Nat Rev Neurosci*, 10: 519-29.

Burre, J., M. Sharma, T. Tsetsenis, V. Buchman, M. R. Etherton, and T. C. Sudhof. 2010. 'Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro', *Science*, 329: 1663-7.

Burstein, S. 2015. 'Cannabidiol (CBD) and its analogs: a review of their effects on inflammation', *Bioorg Med Chem*, 23: 1377-85.

Campos, A. C., and F. S. Guimaraes. 2009. 'Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids', *Prog Neuropsychopharmacol Biol Psychiatry*, 33: 1517-21.

Castillo, A., M. R. Tolon, J. Fernandez-Ruiz, J. Romero, and J. Martinez-Orgado. 2010. 'The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosine receptors', *Neurobiol Dis*, 37: 434-40.

Caudle, W. M., and J. Zhang. 2009. 'Glutamate, excitotoxicity, and programmed cell death in Parkinson disease', *Exp Neurol*, 220: 230-3.

Cerri, S., L. Mus, and F. Blandini. 2019. 'Parkinson's Disease in Women and Men: What's the Difference?', *J Parkinsons Dis*, 9: 501-15.

Chagas, M. H., A. L. Eckeli, A. W. Zuardi, M. A. Pena-Pereira, M. A. Sobreira-Neto, E. T. Sobreira, M. R. Camilo, M. M. Bergamaschi, C. H. Schenck, J. E. Hallak, V. Tumas, and J. A. Crippa. 2014. 'Cannabidiol can improve complex sleep-related behaviours associated with rapid eye movement sleep behaviour disorder in Parkinson's disease patients: a case series', *J Clin Pharm Ther*, 39: 564-6.

Chagas, M. H., A. W. Zuardi, V. Tumas, M. A. Pena-Pereira, E. T. Sobreira, M. M. Bergamaschi, A. C. dos Santos, A. L. Teixeira, J. E. Hallak, and J. A. Crippa. 2014. 'Effects of cannabidiol in the treatment of patients with Parkinson's disease: an exploratory double-blind trial', *J Psychopharmacol*, 28: 1088-98.

Chen, C., R. Yu, E. D. Owuor, and A. N. Kong. 2000. 'Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and

caspases by major green tea polyphenol components during cell survival and death', *Arch Pharm Res*, 23: 605-12.

Chen, J., C. Hou, X. Chen, D. Wang, P. Yang, X. He, J. Zhou, and H. Li. 2016. 'Protective effect of cannabidiol on hydrogen peroxideinduced apoptosis, inflammation and oxidative stress in nucleus pulposus cells', *Mol Med Rep*, 14: 2321-7.

Chhor, V., T. Le Charpentier, S. Lebon, M. V. Ore, I. L. Celador, J. Josserand, V. Degos, E. Jacotot, H. Hagberg, K. Savman, C. Mallard, P. Gressens, and B. Fleiss. 2013. 'Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia in vitro', *Brain Behav Immun*, 32: 70-85.

Chu, Y., J. G. Goldman, L. Kelly, Y. He, T. Waliczek, and J. H. Kordower. 2014. 'Abnormal alpha-synuclein reduces nigral voltage-dependent anion channel 1 in sporadic and experimental Parkinson's disease', *Neurobiol Dis*, 69: 1-14.

Cicchetti, F., J. Drouin-Ouellet, and R. E. Gross. 2009. 'Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models?', *Trends Pharmacol Sci*, 30: 475-83.

Clarke, C. E., and M. Guttman. 2002. 'Dopamine agonist monotherapy in Parkinson's disease', *Lancet*, 360: 1767-9.

Cuervo, A. M., E. S. Wong, and M. Martinez-Vicente. 2010. 'Protein degradation, aggregation, and misfolding', *Mov Disord*, 25 Suppl 1: S49-54.

Dahodwala, N., K. Shah, Y. He, S. S. Wu, P. Schmidt, F. Cubillos, and A. W. Willis. 2018. 'Sex disparities in access to caregiving in Parkinson disease', *Neurology*, 90: e48-e54.

Depino, A. M., C. Earl, E. Kaczmarczyk, C. Ferrari, H. Besedovsky, A. del Rey, F. J. Pitossi, and W. H. Oertel. 2003. 'Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease', *Eur J Neurosci*, 18: 2731-42.

Deumens, R., A. Blokland, and J. Prickaerts. 2002. 'Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway', *Exp Neurol*, 175: 303-17.

Devane, W. A., L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, and R. Mechoulam. 1992. 'Isolation and structure of a brain constituent that binds to the cannabinoid receptor', *Science*, 258: 1946-9.

Dexter, D. T., and P. Jenner. 2013. 'Parkinson disease: from pathology to molecular disease mechanisms', *Free Radic Biol Med*, 62: 132-44.

Di Marzo, V., and A. Fontana. 1995. 'Anandamide, an endogenous cannabinomimetic eicosanoid: 'killing two birds with one stone'', *Prostaglandins Leukot Essent Fatty Acids*, 53: 1-11.

DiSabato, D. J., N. Quan, and J. P. Godbout. 2016. 'Neuroinflammation: the devil is in the details', *J Neurochem*, 139 Suppl 2: 136-53.

Dryanovski, D. I., J. N. Guzman, Z. Xie, D. J. Galteri, L. A. Volpicelli-Daley, V. M. Lee, R. J. Miller, P. T. Schumacker, and D. J. Surmeier. 2013. 'Calcium entry and alpha-synuclein inclusions elevate dendritic mitochondrial oxidant stress in dopaminergic neurons', *J Neurosci*, 33: 10154-64.

Elbaz, A., J. H. Bower, D. M. Maraganore, S. K. McDonnell, B. J. Peterson, J. E. Ahlskog, D. J. Schaid, and W. A. Rocca. 2002. 'Risk tables for parkinsonism and Parkinson's disease', *J Clin Epidemiol*, 55: 25-31.

Esposito, G., D. De Filippis, R. Carnuccio, A. A. Izzo, and T. Iuvone. 2006. 'The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells', *J Mol Med (Berl)*, 84: 253-8.

Esposito, G., C. Scuderi, M. Valenza, G. I. Togna, V. Latina, D. De Filippis, M. Cipriano, M. R. Carratu, T. Iuvone, and L. Steardo. 2011. 'Cannabidiol reduces Abeta-induced neuroinflammation and promotes hippocampal neurogenesis through PPARgamma involvement', *PLoS One*, 6: e28668.

Fahn, S. 2008. 'The history of dopamine and levodopa in the treatment of Parkinson's disease', *Mov Disord*, 23 Suppl 3: S497-508.

Farooqui, T., and A. A. Farooqui. 2011. 'Lipid-mediated oxidative stress and inflammation in the pathogenesis of Parkinson's disease', *Parkinsons Dis*, 2011: 247467.

Fasano, A., A. Daniele, and A. Albanese. 2012. 'Treatment of motor and non-motor features of Parkinson's disease with deep brain stimulation', *Lancet Neurol*, 11: 429-42.

Foley, P., and P. Riederer. 2000. 'Influence of neurotoxins and oxidative stress on the onset and progression of Parkinson's disease', *J Neurol*, 247 Suppl 2: II82-94.

Fonseca, B. M., G. Correia-da-Silva, and N. A. Teixeira. 2018. 'Cannabinoid-induced cell death in endometrial cancer cells: involvement of TRPV1 receptors in apoptosis', *J Physiol Biochem*, 74: 261-72.

Fowler, C. J. 2012. 'Anandamide uptake explained?', *Trends Pharmacol Sci*, 33: 181-5.

Franco, R., and D. Fernandez-Suarez. 2015. 'Alternatively activated microglia and macrophages in the central nervous system', *Prog Neurobiol*, 131: 65-86.

Fuzzati-Armentero, M. T., S. Cerri, and F. Blandini. 2019. 'Peripheral-Central Neuroimmune Crosstalk in Parkinson's Disease: What Do Patients and Animal Models Tell Us?', *Front Neurol*, 10: 232.

Galaj, E., G. H. Bi, H. J. Yang, and Z. X. Xi. 2020. 'Cannabidiol attenuates the rewarding effects of cocaine in rats by CB2, 5-HT1A and TRPV1 receptor mechanisms', *Neuropharmacology*, 167: 107740.

Galiegue, S., S. Mary, J. Marchand, D. Dussossoy, D. Carriere, P. Carayon, M. Bouaboula, D. Shire, G. Le Fur, and P. Casellas. 1995. 'Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations', *Eur J Biochem*, 232: 54-61.

Gan-Or, Z., A. Mirelman, R. B. Postuma, I. Arnulf, A. Bar-Shira, Y. Dauvilliers, A. Desautels, J. F. Gagnon, C. S. Leblond, B. Frauscher, R. N. Alcalay, R. Saunders-Pullman, S. B. Bressman, K. Marder, C. Monaca, B. Hogl, A. Orr-Urtreger, P. A. Dion, J. Y. Montplaisir, N. Giladi, and G. A. Rouleau. 2015. 'GBA mutations are associated with Rapid Eye Movement Sleep Behavior Disorder', *Ann Clin Transl Neurol*, 2: 941-5.

Gao, H. M., and J. S. Hong. 2008. 'Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression', *Trends Immunol*, 29: 357-65.

Garcia-Arencibia, M., S. Gonzalez, E. de Lago, J. A. Ramos, R. Mechoulam, and J. Fernandez-Ruiz. 2007. 'Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties', *Brain Res*, 1134: 162-70.

Garcia, C., C. Palomo-Garo, M. Garcia-Arencibia, J. Ramos, R. Pertwee, and J. Fernandez-Ruiz. 2011. 'Symptom-relieving and neuroprotective effects of the phytocannabinoid Delta(9)-THCV in animal models of Parkinson's disease', *Br J Pharmacol*, 163: 1495-506.

Garcia, M. C., V. Cinquina, C. Palomo-Garo, A. Rabano, and J. Fernandez-Ruiz. 2015. 'Identification of CB(2) receptors in human nigral neurons that degenerate in Parkinson's disease', *Neurosci Lett*, 587: 1-4.

Garcia, P., I. Youssef, J. K. Utvik, S. Florent-Bechard, V. Barthelemy, C. Malaplate-Armand, B. Kriem, C. Stenger, V. Koziel, J. L. Olivier, M. C. Escanye, M. Hanse, A. Allouche, C. Desbene, F. T. Yen, R. Bjerkvig, T. Oster, S. P. Niclou, and T. Pillot. 2010. 'Ciliary neurotrophic factor cell-based delivery prevents synaptic impairment and improves memory in mouse models of Alzheimer's disease', *J Neurosci*, 30: 7516-27.

Georgiev, D., K. Hamberg, M. Hariz, L. Forsgren, and G. M. Hariz. 2017. 'Gender differences in Parkinson's disease: A clinical perspective', *Acta Neurol Scand*, 136: 570-84.

Giacoppo, S., F. Pollastro, G. Grassi, P. Bramanti, and E. Mazzon. 2017. 'Target regulation of PI3K/Akt/mTOR pathway by cannabidiol in treatment of experimental multiple sclerosis', *Fitoterapia*, 116: 77-84.

Giuliano, C., F. Siani, L. Mus, C. Ghezzi, S. Cerri, B. Pacchetti, C. Bigogno, and F. Blandini. 2020. 'Neuroprotective effects of lignan 7-hydroxymatairesinol (HMR/lignan) in a rodent model of Parkinson's disease', *Nutrition*, 69: 110494.

Gomes, F. V., R. Llorente, E. A. Del Bel, M. P. Viveros, M. Lopez-Gallardo, and F. S. Guimaraes. 2015. 'Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol', *Schizophr Res*, 164: 155-63.

Grant, H., P. L. Lantos, and C. Parkinson. 1980. 'Cerebral damage in paraquat poisoning', *Histopathology*, 4: 185-95.

Gray, R. A., C. G. Stott, N. A. Jones, V. Di Marzo, and B. J. Whalley. 2020. 'Anticonvulsive Properties of Cannabidiol in a Model of Generalized Seizure Are Transient Receptor Potential Vanilloid 1 Dependent', *Cannabis Cannabinoid Res*, 5: 145-49.

Gui, Y. X., X. Y. Wang, W. Y. Kang, Y. J. Zhang, Y. Zhang, Y. Zhou, T. J. Quinn, J. Liu, and S. D. Chen. 2012. 'Extracellular signal-regulated kinase is involved in alpha-synuclein-induced mitochondrial dynamic disorders by regulating dynamin-like protein 1', *Neurobiol Aging*, 33: 2841-54.

Guo, B. J., Z. X. Bian, H. C. Qiu, Y. T. Wang, and Y. Wang. 2017. 'Biological and clinical implications of herbal medicine and natural products for the treatment of inflammatory bowel disease', *Ann N Y Acad Sci*, 1401: 37-48.

Hagg, T., S. Varon, and J. C. Louis. 1993. 'Ciliary neurotrophic factor (CNTF) promotes low-affinity nerve growth factor receptor and CD4 expression by rat CNS microglia', *J Neuroimmunol*, 48: 177-87.

Hawkes, C. H., K. Del Tredici, and H. Braak. 2007. 'Parkinson's disease: a dual-hit hypothesis', *Neuropathol Appl Neurobiol*, 33: 599-614.

———. 2009. 'Parkinson's disease: the dual hit theory revisited', *Ann N Y Acad Sci*, 1170: 615-22.

Hayakawa, K., K. Mishima, K. Irie, M. Hazekawa, S. Mishima, M. Fujioka, K. Orito, N. Egashira, S. Katsurabayashi, K. Takasaki, K. Iwasaki, and M. Fujiwara. 2008. 'Cannabidiol prevents a post-ischemic injury progressively induced by cerebral ischemia via a high-mobility group box1-inhibiting mechanism', *Neuropharmacology*, 55: 1280-6.

He, Y., S. Appel, and W. Le. 2001. 'Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum', *Brain Res*, 909: 187-93.

Heikkila, R. E., F. S. Cabbat, L. Manzino, and R. C. Duvoisin. 1984. 'Effects of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine on neostriatal dopamine in mice', *Neuropharmacology*, 23: 711-3.

Henchcliffe, C., and M. F. Beal. 2008. 'Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis', *Nat Clin Pract Neurol*, 4: 600-9.

Hermida-Ameijeiras, A., E. Mendez-Alvarez, S. Sanchez-Iglesias, C. Sanmartin-Suarez, and R. Soto-Otero. 2004. 'Autoxidation and MAO-mediated metabolism of dopamine as a potential cause of oxidative stress: role of ferrous and ferric ions', *Neurochem Int*, 45: 103-16.

Holmstrom, K. M., L. Baird, Y. Zhang, I. Hargreaves, A. Chalasani, J. M. Land, L. Stanyer, M. Yamamoto, A. T. Dinkova-Kostova, and A. Y. Abramov. 2013. 'Nrf2 impacts cellular bioenergetics by controlling substrate availability for mitochondrial respiration', *Biol Open*, 2: 761-70.

Ibanez, P., G. De Michele, V. Bonifati, E. Lohmann, S. Thobois, P. Pollak, Y. Agid, P. Heutink, A. Durr, A. Brice, and Group French Parkinson's Disease Genetics Study. 2003. 'Screening for DJ-1 mutations in early onset autosomal recessive parkinsonism', *Neurology*, 61: 1429-31.

Iuvone, T., G. Esposito, R. Esposito, R. Santamaria, M. Di Rosa, and A. A. Izzo. 2004. 'Neuroprotective effect of cannabidiol, a non-psychoactive component from *Cannabis sativa*, on beta-amyloid-induced toxicity in PC12 cells', *J Neurochem*, 89: 134-41.

Jaber, M., S. W. Robinson, C. Missale, and M. G. Caron. 1996. 'Dopamine receptors and brain function', *Neuropharmacology*, 35: 1503-19.

Jakes, R., M. G. Spillantini, and M. Goedert. 1994. 'Identification of two distinct synucleins from human brain', *FEBS Lett*, 345: 27-32.

Javitch, J. A., R. J. D'Amato, S. M. Strittmatter, and S. H. Snyder. 1985. 'Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6 - tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity', *Proc Natl Acad Sci U S A*, 82: 2173-7.

Jiang, T., Q. Sun, and S. Chen. 2016. 'Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson's disease and Alzheimer's disease', *Prog Neurobiol*, 147: 1-19.

Joers, V., M. G. Tansey, G. Mulas, and A. R. Carta. 2017. 'Microglial phenotypes in Parkinson's disease and animal models of the disease', *Prog Neurobiol*, 155: 57-75.

Kalia, L. V., and A. E. Lang. 2015. 'Parkinson's disease', *Lancet*, 386: 896-912.

Kang, S. S., M. P. Keasey, J. Cai, and T. Hagg. 2012. 'Loss of neuron-astroglial interaction rapidly induces protective CNTF expression after stroke in mice', *J Neurosci*, 32: 9277-87.

Kendall. 2017. 'Cannabinoid Pharmacology', *Advances in Pharmacology*, 80.

Khan, M. M., S. S. Raza, H. Javed, A. Ahmad, A. Khan, F. Islam, M. M. Safhi, and F. Islam. 2012. 'Rutin protects dopaminergic neurons from oxidative stress in an animal model of Parkinson's disease', *Neurotox Res*, 22: 1-15.

Kim, K. I., J. Y. Baek, J. Y. Jeong, J. H. Nam, E. S. Park, E. Bok, W. H. Shin, Y. C. Chung, and B. K. Jin. 2019. 'Delayed Treatment of Capsaicin Produces Partial Motor Recovery by Enhancing Dopamine Function in MPP(+)-lesioned Rats via Ciliary Neurotrophic Factor', *Exp Neurobiol*, 28: 289-99.

Klein, T. W., H. Friedman, and S. Specter. 1998. 'Marijuana, immunity and infection', *J Neuroimmunol*, 83: 102-15.

Klein, T. W., C. Newton, K. Larsen, L. Lu, I. Perkins, L. Nong, and H. Friedman. 2003. 'The cannabinoid system and immune modulation', *J Leukoc Biol*, 74: 486-96.

Kossakowski, R., E. Schlicker, M. Toczek, J. Weresa, and B. Malinowska. 2019. 'Cannabidiol Affects the Bezold-Jarisch Reflex via TRPV1 and 5-HT3 Receptors and Has Peripheral Sympathomimetic Effects in Spontaneously Hypertensive and Normotensive Rats', *Front Pharmacol*, 10: 500.

Koyama, Y. 2014. 'Signaling molecules regulating phenotypic conversions of astrocytes and glial scar formation in damaged nerve tissues', *Neurochem Int*, 78: 35-42.

Landers, M. R., J. W. Kinney, and F. van Breukelen. 2014. 'Forced exercise before or after induction of 6-OHDA-mediated nigrostriatal insult does not mitigate behavioral asymmetry in a hemiparkinsonian rat model', *Brain Res*, 1543: 263-70.

Langston, J. W., and P. A. Ballard, Jr. 1983. 'Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine', *N Engl J Med*, 309: 310.

Lastres-Becker, I., F. Molina-Holgado, J. A. Ramos, R. Mechoulam, and J. Fernandez-Ruiz. 2005. 'Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease', *Neurobiol Dis*, 19: 96-107.

Lee, M. Y., H. D. Hofmann, and M. Kirsch. 1997. 'Expression of ciliary neurotrophic factor receptor-alpha messenger RNA in neonatal and adult rat brain: an in situ hybridization study', *Neuroscience*, 77: 233-46.

Leehey, M. A., Y. Liu, F. Hart, C. Epstein, M. Cook, S. Sillau, J. Klawitter, H. Newman, C. Sempio, L. Forman, L. Seeberger, O. Klepitskaya, Z. Baud, and J. Bainbridge. 2020. 'Safety and Tolerability of Cannabidiol in Parkinson Disease: An Open Label, Dose-Escalation Study', *Cannabis Cannabinoid Res*, 5: 326-36.

Levy, O. A., C. Malagelada, and L. A. Greene. 2009. 'Cell death pathways in Parkinson's disease: proximal triggers, distal effectors, and final steps', *Apoptosis*, 14: 478-500.

Li, J., X. Long, J. Hu, J. Bi, T. Zhou, X. Guo, C. Han, J. Huang, T. Wang, N. Xiong, and Z. Lin. 2019. 'Multiple pathways for natural product treatment of Parkinson's disease: A mini review', *Phytomedicine*, 60: 152954.

Li, W. W., R. Yang, J. C. Guo, H. M. Ren, X. L. Zha, J. S. Cheng, and D. F. Cai. 2007. 'Localization of alpha-synuclein to mitochondria within midbrain of mice', *Neuroreport*, 18: 1543-6.

Liddelow, S. A., and B. A. Barres. 2017. 'Reactive Astrocytes: Production, Function, and Therapeutic Potential', *Immunity*, 46: 957-67.

Liddelow, S. A., K. A. Guttenplan, L. E. Clarke, F. C. Bennett, C. J. Bohlen, L. Schirmer, M. L. Bennett, A. E. Munch, W. S. Chung, T. C. Peterson, D. K. Wilton, A. Frouin, B. A. Napier, N. Panicker, M. Kumar, M. S. Buckwalter, D. H. Rowitch, V. L. Dawson, T. M. Dawson, B. Stevens, and B. A. Barres. 2017. 'Neurotoxic reactive astrocytes are induced by activated microglia', *Nature*, 541: 481-87.

Ligresti, A., A. S. Moriello, K. Starowicz, I. Matias, S. Pisanti, L. De Petrocellis, C. Laezza, G. Portella, M. Bifulco, and V. Di Marzo. 2006. 'Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma', *J Pharmacol Exp Ther*, 318: 1375-87.

Lilienbaum, A. 2013. 'Relationship between the proteasomal system and autophagy', *Int J Biochem Mol Biol*, 4: 1-26.

Lim, K. L., X. H. Ng, L. G. Grace, and T. P. Yao. 2012. 'Mitochondrial dynamics and Parkinson's disease: focus on parkin', *Antioxid Redox Signal*, 16: 935-49.

Limousin, P., P. Pollak, A. Benazzouz, D. Hoffmann, J. F. Le Bas, E. Broussolle, J. E. Perret, and A. L. Benabid. 1995. 'Effect of parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation', *Lancet*, 345: 91-5.

Ludtmann, M. H., P. R. Angelova, Y. Zhang, A. Y. Abramov, and A. T. Dinkova-Kostova. 2014. 'Nrf2 affects the efficiency of mitochondrial fatty acid oxidation', *Biochem J*, 457: 415-24.

Lv, C., T. Hong, Z. Yang, Y. Zhang, L. Wang, M. Dong, J. Zhao, J. Mu, and Y. Meng. 2012. 'Effect of Quercetin in the 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-Induced Mouse Model of Parkinson's Disease', *Evid Based Complement Alternat Med*, 2012: 928643.

Mabou Tagne, A., F. Marino, M. Legnaro, A. Luini, B. Pacchetti, and M. Cosentino. 2019. 'A Novel Standardized *Cannabis sativa* L. Extract and Its Constituent Cannabidiol Inhibit Human Polymorphonuclear Leukocyte Functions', *Int J Mol Sci*, 20.

Manning-Bog, A. B., A. L. McCormack, J. Li, V. N. Uversky, A. L. Fink, and D. A. Di Monte. 2002. 'The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein', *J Biol Chem*, 277: 1641-4.

Matsuda, L. A., S. J. Lolait, M. J. Brownstein, A. C. Young, and T. I. Bonner. 1990. 'Structure of a cannabinoid receptor and functional expression of the cloned cDNA', *Nature*, 346: 561-4.

Mattson, M. P., M. Gleichmann, and A. Cheng. 2008. 'Mitochondria in neuroplasticity and neurological disorders', *Neuron*, 60: 748-66.

McGeer, P. L., S. Itagaki, B. E. Boyes, and E. G. McGeer. 1988. 'Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains', *Neurology*, 38: 1285-91.

McNaught, K. S., D. P. Perl, A. L. Brownell, and C. W. Olanow. 2004. 'Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease', *Ann Neurol*, 56: 149-62.

Mecha, M., A. Feliu, P. M. Inigo, L. Mestre, F. J. Carrillo-Salinas, and C. Guaza. 2013. 'Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors', *Neurobiol Dis*, 59: 141-50.

Mechoulam, R., A. Shani, H. Edery, and Y. Grunfeld. 1970. 'Chemical basis of hashish activity', *Science*, 169: 611-2.

Mignani, S., J. Rodrigues, H. Tomas, M. Zablocka, X. Shi, A. M. Caminade, and J. P. Majoral. 2018. 'Dendrimers in combination with natural products and analogues as anti-cancer agents', *Chem Soc Rev*, 47: 514-32.

Mirza-Aghazadeh-Attari, M., E. M. Ekrami, S. A. M. Aghdas, A. Mihanfar, S. Hallaj, B. Yousefi, A. Safa, and M. Majidinia. 2020. 'Targeting PI3K/Akt/mTOR signaling pathway by polyphenols: Implication for cancer therapy', *Life Sci*, 255: 117481.

Miyoshi, E., S. Wietzikoski, M. Camplessei, R. Silveira, R. N. Takahashi, and C. Da Cunha. 2002. 'Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP-induced mesencephalic dopaminergic lesions', *Brain Res Bull*, 58: 41-7.

Molina, M. F., I. Sanchez-Reus, I. Iglesias, and J. Benedi. 2003. 'Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver', *Biol Pharm Bull*, 26: 1398-402.

Monin, ML. 2019. 'Basi molecolari della malattia di Parkinson', *EMC - Neurologia*, 19: 1-10.

Mori, M. A., E. Meyer, L. M. Soares, H. Milani, F. S. Guimaraes, and R. M. W. de Oliveira. 2017. 'Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia', *Prog Neuropsychopharmacol Biol Psychiatry*, 75: 94-105.

Mukherjee, U. A., S. B. Ong, S. G. Ong, and D. J. Hausenloy. 2015. 'Parkinson's disease proteins: Novel mitochondrial targets for cardioprotection', *Pharmacol Ther*, 156: 34-43.

Muller, C., P. Morales, and P. H. Reggio. 2018. 'Cannabinoid Ligands Targeting TRP Channels', *Front Mol Neurosci*, 11: 487.

Munro, S., K. L. Thomas, and M. Abu-Shaar. 1993. 'Molecular characterization of a peripheral receptor for cannabinoids', *Nature*, 365: 61-5.

Muthuraman, M., N. Koirala, D. Ciolac, B. Pintea, M. Glaser, S. Groppa, G. Tamas, and S. Groppa. 2018. 'Deep Brain Stimulation and L-DOPA Therapy: Concepts of Action and Clinical Applications in Parkinson's Disease', *Front Neurol*, 9: 711.

Mythri, R. B., G. Harish, and M. M. Bharath. 2012. 'Therapeutic potential of natural products in Parkinson's disease', *Recent Pat Endocr Metab Immune Drug Discov*, 6: 181-200.

Nam, J. H., E. S. Park, S. Y. Won, Y. A. Lee, K. I. Kim, J. Y. Jeong, J. Y. Baek, E. J. Cho, M. Jin, Y. C. Chung, B. D. Lee, S. H. Kim, E. G. Kim, K. Byun, B. Lee, D. H. Woo, C. J. Lee, S. R. Kim, E. Bok, Y. S. Kim, T. B. Ahn, H. W. Ko, S. Brahmachari, O. Pletinkova, J. C. Troconso, V. L. Dawson, T. M. Dawson, and B. K. Jin. 2015. 'TRPV1 on astrocytes rescues nigral dopamine neurons in Parkinson's disease via CNTF', *Brain*, 138: 3610-22.

Navarrete, C. M., B. L. Fiebich, A. G. de Vinuesa, S. Hess, A. C. de Oliveira, E. Candelario-Jalil, F. J. Caballero, M. A. Calzado, and E. Munoz. 2009.

'Opposite effects of anandamide and N-arachidonoyl dopamine in the regulation of prostaglandin E and 8-iso-PGF formation in primary glial cells', *J Neurochem*, 109: 452-64.

Niranjan, R. 2014. 'The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes', *Mol Neurobiol*, 49: 28-38.

Nomura, D. K., B. E. Morrison, J. L. Blankman, J. Z. Long, S. G. Kinsey, M. C. Marcondes, A. M. Ward, Y. K. Hahn, A. H. Lichtman, B. Conti, and B. F. Cravatt. 2011. 'Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation', *Science*, 334: 809-13.

O'Sullivan, S. E., Y. Sun, A. J. Bennett, M. D. Randall, and D. A. Kendall. 2009. 'Time-dependent vascular actions of cannabidiol in the rat aorta', *Eur J Pharmacol*, 612: 61-8.

Obeso, J. A., M. C. Rodriguez-Oroz, M. Rodriguez, J. L. Lanciego, J. Artieda, N. Gonzalo, and C. W. Olanow. 2000. 'Pathophysiology of the basal ganglia in Parkinson's disease', *Trends Neurosci*, 23: S8-19.

Olanow, C. W., A. Lees, and J. Obeso. 2008. 'Levodopa therapy for Parkinson's disease: challenges and future prospects', *Mov Disord*, 23 Suppl 3: S495-6.

Orenstein, S. J., S. H. Kuo, I. Tasset, E. Arias, H. Koga, I. Fernandez-Carasa, E. Cortes, L. S. Honig, W. Dauer, A. Consiglio, A. Raya, D. Sulzer, and A. M. Cuervo. 2013. 'Interplay of LRRK2 with chaperone-mediated autophagy', *Nat Neurosci*, 16: 394-406.

Owen. 1995. 'Dopamine-dependent frontostriatal planning deficits in early Parkinson's disease.', *Neuropsychology,*, 9(1), 126–140.

Palazzi, L., E. Bruzzone, G. Bisello, M. Leri, M. Stefani, M. Bucciantini, and P. Polverino de Laureto. 2018. 'Oleuropein aglycone stabilizes the monomeric alpha-synuclein and favours the growth of non-toxic aggregates', *Sci Rep*, 8: 8337.

Patil, S. P., P. D. Jain, J. S. Sancheti, P. J. Ghumatkar, R. Tambe, and S. Sathaye. 2014. 'Neuroprotective and neurotrophic effects of Apigenin and Luteolin in MPTP induced parkinsonism in mice', *Neuropharmacology*, 86: 192-202.

Peres, F. F., A. C. Lima, J. E. C. Hallak, J. A. Crippa, R. H. Silva, and V. C. Abilio. 2018. 'Cannabidiol as a Promising Strategy to Treat and Prevent Movement Disorders?', *Front Pharmacol*, 9: 482.

Perry, V. H., and C. Holmes. 2014. 'Microglial priming in neurodegenerative disease', *Nat Rev Neurol*, 10: 217-24.

Perry, V. H., J. A. Nicoll, and C. Holmes. 2010. 'Microglia in neurodegenerative disease', *Nat Rev Neurol*, 6: 193-201.

Pertwee, R. G. 2008. 'The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin', *Br J Pharmacol*, 153: 199-215.

Porsolt, R. D., G. Anton, N. Blavet, and M. Jalfre. 1978. 'Behavioural despair in rats: a new model sensitive to antidepressant treatments', *Eur J Pharmacol*, 47: 379-91.

Prediger, R. D., L. C. Batista, R. Medeiros, P. Pandolfo, J. C. Florio, and R. N. Takahashi. 2006. 'The risk is in the air: Intranasal administration of MPTP to rats reproducing clinical features of Parkinson's disease', *Exp Neurol*, 202: 391-403.

Prema, A., U. Janakiraman, T. Manivasagam, and A. J. Thenmozhi. 2015. 'Neuroprotective effect of lycopene against MPTP induced experimental Parkinson's disease in mice', *Neurosci Lett*, 599: 12-9.

Priyadarshi, A., S. A. Khuder, E. A. Schaub, and S. S. Priyadarshi. 2001. 'Environmental risk factors and Parkinson's disease: a metaanalysis', *Environ Res*, 86: 122-7.

Raitio, K. H., O. M. Salo, T. Nevalainen, A. Poso, and T. Jarvinen. 2005. 'Targeting the cannabinoid CB2 receptor: mutations, modeling and development of CB2 selective ligands', *Curr Med Chem*, 12: 1217-37.

Rajan, T. S., S. Giacoppo, R. Iori, G. R. De Nicola, G. Grassi, F. Pollastro, P. Bramanti, and E. Mazzon. 2016. 'Anti-inflammatory and antioxidant effects of a combination of cannabidiol and moringin in LPS-stimulated macrophages', *Fitoterapia*, 112: 104-15.

Reggio, P. H., A. M. Panu, and S. Miles. 1993. 'Characterization of a region of steric interference at the cannabinoid receptor using the active analog approach', *J Med Chem*, 36: 1761-71.

Reich, S. G., and J. M. Savitt. 2019. 'Parkinson's Disease', *Med Clin North Am*, 103: 337-50.

Reichmann, H. 2017. 'Premotor Diagnosis of Parkinson's Disease', *Neurosci Bull*, 33: 526-34.

Ribeiro, A., V. Ferraz-de-Paula, M. L. Pinheiro, L. B. Vitoretti, D. P. Mariano-Souza, W. M. Quinteiro-Filho, A. T. Akamine, V. I. Almeida, J. Quevedo, F. Dal-Pizzol, J. E. Hallak, A. W. Zuardi, J. A. Crippa, and J. Palermo-Neto. 2012. 'Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: role for the adenosine A(2A) receptor', *Eur J Pharmacol*, 678: 78-85.

Russo, E. B., A. Burnett, B. Hall, and K. K. Parker. 2005. 'Agonistic properties of cannabidiol at 5-HT1a receptors', *Neurochem Res*, 30: 1037-43.

Sanchez-Lopez, F., I. Tasset, E. Aguera, M. Feijoo, R. Fernandez-Bolanos, F. M. Sanchez, M. C. Ruiz, A. H. Cruz, F. Gascon, and I. Tunez. 2012. 'Oxidative stress and inflammation biomarkers in the blood of patients with Huntington's disease', *Neurol Res*, 34: 721-4.

Schiavon, A. P., L. M. Soares, J. M. Bonato, H. Milani, F. S. Guimaraes, and R. M. Weffort de Oliveira. 2014. 'Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice', *Neurotox Res*, 26: 307-16.

Schwartz, M., and S. Sabetay. 2012. 'An approach to the continuous dopaminergic stimulation in Parkinson's disease', *Isr Med Assoc J*, 14: 175-9.

Shen, S. Q., Y. Zhang, J. J. Xiang, and C. L. Xiong. 2007. 'Protective effect of curcumin against liver warm ischemia/reperfusion injury in rat model is associated with regulation of heat shock protein and antioxidant enzymes', *World J Gastroenterol*, 13: 1953-61.

Silveira, J. W., A. C. Issy, V. A. Castania, C. E. Salmon, M. H. Nogueira-Barbosa, F. S. Guimaraes, H. L. Defino, and E. Del Bel. 2014. 'Protective effects of cannabidiol on lesion-induced intervertebral disc degeneration', *PLoS One*, 9: e113161.

Solayman, M., M. A. Islam, F. Alam, M. I. Khalil, M. A. Kamal, and S. H. Gan. 2017. 'Natural Products Combating Neurodegeneration: Parkinson's Disease', *Curr Drug Metab*, 18: 50-61.

Sominsky, L., S. De Luca, and S. J. Spencer. 2018. 'Microglia: Key players in neurodevelopment and neuronal plasticity', *Int J Biochem Cell Biol*, 94: 56-60.

Spencer, J. P. 2007. 'The interactions of flavonoids within neuronal signalling pathways', *Genes Nutr*, 2: 257-73.

Spillantini, M. G., R. A. Crowther, R. Jakes, M. Hasegawa, and M. Goedert. 1998. 'alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies', *Proc Natl Acad Sci U S A*, 95: 6469-73.

Staff, Plos One. 2015. 'Correction: Activation of CNTF/CNTFRalpha signaling pathway by hRheb(S16H) transduction of dopaminergic neurons in vivo', *PLoS One*, 10: e0126450.

Stauffer, W., H. Sheng, and H. N. Lim. 2018. 'EzColocalization: An ImageJ plugin for visualizing and measuring colocalization in cells and organisms', *Sci Rep*, 8: 15764.

Steyers, C. M., 3rd, and F. J. Miller, Jr. 2014. 'Endothelial dysfunction in chronic inflammatory diseases', *Int J Mol Sci*, 15: 11324-49.

Stockli, K. A., F. Lottspeich, M. Sendtner, P. Masiakowski, P. Carroll, R. Gotz, D. Lindholm, and H. Thoenen. 1989. 'Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor', *Nature*, 342: 920-3.

Subramaniam, S. R., and M. F. Chesselet. 2013. 'Mitochondrial dysfunction and oxidative stress in Parkinson's disease', *Prog Neurobiol*, 106-107: 17-32.

Sugiura, T., S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, and K. Waku. 1995. '2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain', *Biochem Biophys Res Commun*, 215: 89-97.

Tadaiesky, M. T., P. A. Dombrowski, C. P. Figueiredo, E. Cargnin-Ferreira, C. Da Cunha, and R. N. Takahashi. 2008. 'Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease', *Neuroscience*, 156: 830-40.

Tang, Y., and W. Le. 2016. 'Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases', *Mol Neurobiol*, 53: 1181-94.

Tolosa, E., G. Wenning, and W. Poewe. 2006. 'The diagnosis of Parkinson's disease', *Lancet Neurol*, 5: 75-86.

Truong, L., H. Allbutt, M. Kassiou, and J. M. Henderson. 2006. 'Developing a preclinical model of Parkinson's disease: a study of behaviour in rats with graded 6-OHDA lesions', *Behav Brain Res*, 169: 1-9.

Van Laar, V. S., and S. B. Berman. 2009. 'Mitochondrial dynamics in Parkinson's disease', *Exp Neurol*, 218: 247-56.

Wakabayashi, K., K. Tanji, S. Odagiri, Y. Miki, F. Mori, and H. Takahashi. 2013. 'The Lewy body in Parkinson's disease and related neurodegenerative disorders', *Mol Neurobiol*, 47: 495-508.

Watt, G., and T. Karl. 2017. 'In vivo Evidence for Therapeutic Properties of Cannabidiol (CBD) for Alzheimer's Disease', *Front Pharmacol*, 8: 20.

Watzl, B., P. Scuderi, and R. R. Watson. 1991. 'Influence of marijuana components (THC and CBD) on human mononuclear cell cytokine secretion in vitro', *Adv Exp Med Biol*, 288: 63-70.

Weiss, L., M. Zeira, S. Reich, M. Har-Noy, R. Mechoulam, S. Slavin, and R. Gallily. 2006. 'Cannabidiol lowers incidence of diabetes in non-obese diabetic mice', *Autoimmunity*, 39: 143-51.

Woerman, A. L., J. Stohr, A. Aoyagi, R. Rampersaud, Z. Krejciova, J. C. Watts, T. Ohyama, S. Patel, K. Widjaja, A. Oehler, D. W. Sanders, M. I. Diamond, W. W. Seeley, L. T. Middleton, S. M. Gentleman, D. A. Mordes, T. C. Sudhof, K. Giles, and S. B. Prusiner. 2015. 'Propagation of prions causing synucleinopathies in cultured cells', *Proc Natl Acad Sci U S A*, 112: E4949-58.

Wu, K. C., J. Y. Cui, and C. D. Klaassen. 2011. 'Beneficial role of Nrf2 in regulating NADPH generation and consumption', *Toxicol Sci*, 123: 590-600.

Xie, W., and K. K. Chung. 2012. 'Alpha-synuclein impairs normal dynamics of mitochondria in cell and animal models of Parkinson's disease', *J Neurochem*, 122: 404-14.

Xiromerisiou, G., E. Dardiotis, V. Tsimourtou, P. M. Kountra, K. N. Paterakis, E. Z. Kapsalaki, K. N. Fountas, and G. M. Hadjigeorgiou. 2010. 'Genetic basis of Parkinson disease', *Neurosurg Focus*, 28: E7.

Xu, B. B., C. Q. Liu, X. Gao, W. Q. Zhang, S. W. Wang, and Y. L. Cao. 2005. 'Possible mechanisms of the protection of ginsenoside Re against MPTP-induced apoptosis in substantia nigra neurons of Parkinson's disease mouse model', *J Asian Nat Prod Res*, 7: 215-24.

Yasuda, T., Y. Nakata, and H. Mochizuki. 2013. 'alpha-Synuclein and neuronal cell death', *Mol Neurobiol*, 47: 466-83.

Youdim, M. B. 2010. 'Why do we need multifunctional neuroprotective and neurorestorative drugs for Parkinson's and Alzheimer's diseases as disease modifying agents', *Exp Neurobiol*, 19: 1-14.

Youdim, M. B., and Y. S. Bakhle. 2006. 'Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness', *Br J Pharmacol*, 147 Suppl 1: S287-96.

Young, K., and H. Morrison. 2018. 'Quantifying Microglia Morphology from Photomicrographs of Immunohistochemistry Prepared Tissue Using ImageJ', *J Vis Exp*.

Zhang, J., A. Malik, H. B. Choi, R. W. Ko, L. Dissing-Olesen, and B. A. MacVicar. 2014. 'Microglial CR3 activation triggers long-term synaptic depression in the hippocampus via NADPH oxidase', *Neuron*, 82: 195-207.

Zhao, Z., J. Wang, L. Wang, X. Yao, Y. Liu, Y. Li, S. Chen, T. Yue, X. Wang, W. Yu, and Y. Liu. 2017. 'Capsaicin Protects Against Oxidative Insults and Alleviates Behavioral Deficits in Rats with 6-OHDA-Induced Parkinson's Disease via Activation of TRPV1', *Neurochem Res*, 42: 3431-38.

Zuardi, A. W. 2008. 'Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action', *Braz J Psychiatry*, 30: 271-80.

Zuardi, A. W., J. A. Crippa, J. E. Hallak, J. P. Pinto, M. H. Chagas, G. G. Rodrigues, S. M. Dursun, and V. Tumas. 2009. 'Cannabidiol for the treatment of psychosis in Parkinson's disease', *J Psychopharmacol*, 23: 979-83.

ABROAD EXPERIENCE

During the last year of my PhD I did a period of research abroad in the laboratory of Jun.-Prof. Dr. Michela Deleidi "Mitochondria & Inflammation in Neurodegenerative Diseases" is a research lab at the German Center for Neurodegenerative Disease (Tübingen).

Research in this laboratory concentrates on the molecular mechanisms of neurodegeneration, with a particular focus on Parkinson's disease (PD). To this end, they developed models with human pluripotent stem cells and long-term cultures of organoids combined with single cell-omics to identify the role of disease related genetic variants and validate targets with diagnostic and therapeutic potential. Another line of research concentrates on the role of GBA1 mutations in Parkinson's disease. GBA1 mutations represent the most common risk factor for Parkinson's disease identified to date. Ongoing research in our laboratory focuses on the mechanistic pathways involved in GBA1-linked neurodegeneration, with a particular interest in mitochondrial function and autophagy.

Therefore, I chose this laboratory in order to expand my academic knowledge in the world of in vitro research, which is a fundamental part of preclinical studies. Here I was able to learn about the following cell models and methods:

Cell models in S1-2 condition:

- Human embryonic kidney 293 cells;
- Human induced pluripotent stem cells (iPSCs) and Human iPSC-derived cells
    - Neural progenitor cells;
    - Astrocytes;
    - Dopaminergic neurons;
    - Cortical neurons;

Methods:

- Proximity ligation assay;
- Reverse transcriptase-polymerase chain reaction;
- Western Blot;
- Immunocytochemistry and immunofluorescence;
- Confocal microscopy;
- Seahorse XF Cell Mito Stress Test;
- Luminex® Multiplex Assays;

Here is a brief description of the main project I followed during my experience abroad:

STUDY OF THE MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE (UPRMT) IN ALPHA-SYNUCLEIN-INDUCED CELLULAR STRESS IN INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED MICROGLIA, ASTROCYTES AND NEURONS

Protein aggregation and mitochondrial dysfunction are hallmarks of aging and neurodegenerative diseases, and strategies targeting such pathological pathways may help halt neuronal loss. Unlike other cell organelles, the mitochondria has its own DNA. Mitochondrial DNA comprises only 37 genes, the most important of which encode the enzyme complexes of the respiratory chain. The rest of the proteins essential for mitochondrial function are transcribed from the nuclear genome, synthesized in the cytoplasm and then imported into the mitochondria. Here, mitochondria provide the correct folding of proteins encoded by both the mitochondrial and nuclear genome. When homeostasis is compromised by misfolded proteins and consequently their accumulation, leads to mitochondrial stress, which could endanger the survival of the whole cell. Indeed, to restore homeostasis and to promote recovery from the stress condition, mitochondria have developed a mechanism called mitochondrial unfolded protein response (UPR^{mt}). Activation of this pathway promotes increased levels of transcription factors, mitochondrial chaperones, and enzymes in order to recover mitochondrial functions. Studies in C.elegans indicate that UPR^{mt} activation is linked to enhanced protection of dopamine neurons. However, the actual role of UPR^{mt} in Parkinson's disease (PD) is still unclear. The investigation of human UPR^{mt} has been hampered by the lack of relevant in vitro models.

To overcome this limitation and investigate the cell-type specific role of mitochondrial stress responses, we developed human induced pluripotent stem cell (iPSC)-derived models of UPR^{mt}. Using iPSC-derived neurons, astrocytes, and microglia, we found that the pharmacological inhibition of the mitochondrial protease LONP1 induces UPR^{mt} activation and leads to the upregulation of genes linked to cytosolic protein quality control, mitochondrial metabolism, and neurodegenerative disease pathways. Additionally, our results show an early and prominent activation of UPR^{mt} pathways in iPSC-derived astrocytes and microglia compared to iPSC-derived neurons. Interestingly, the inhibition of the mitochondrial protease LONP1 promotes the

intracellular accumulation of α-synuclein and cellular damage in α-synuclein preformed fibril-treated iPSC-derived astrocytes and neurons, suggesting an essential role of mitochondrial proteostasis in toxic protein clearence by neurons and glial cells, with relevance for PD. We are currently investigating the molecular mechanisms underlying such cell-type specific mitochondrial stress responses and their role in α-synuclein pathology.