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Review

LRRK2 G2019S Parkinson's Disease Mutation and Astrocyte–Neuron Crosstalk: How Diseased Astrocytes Program the Susceptibility to Inflammation and Oxidative Stress Predisposing to Nigrostriatal Degeneration

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Abstract

A complex interplay between genes and environmental factors is recognized to contribute to dopaminergic neuron death in Parkinson's disease (PD). Within the known genes associated with PD, there is Leucine-Rich Repeat Kinase (*LRRK2*), known as one of the most frequent causes of PD, of either familial or sporadic nature. On the other hand, the mechanisms involved in LRRK2 dysfunction that contribute to PD pathogenesis are not defined. Remarkably, earlier and more recent evidence corroborates *"a developmental hypothesis"* for PD pathogenesis. Here, genes, crucial transcription factors and developmental pathways linked to midbrain dopaminergic neuron (mDAn) vulnerability, interacting with early life events and key hormonal and inflammatory/anti-inflammatory signaling systems**,** are suspected to play important roles via their expression and/or intimate dialogue with astrocytes. In this work we put forward the hypothesis that early developmental astrocyte–neuron crosstalk dysregulation may predispose individuals to increased neuronal vulnerability later in life. Hence, given the vital role of astrocyte–neuron interactions during development and in the adult brain, we herein discuss a potential decisive role of LRRK2-diseased astrocytes as predisposing elements for PD susceptibility, asking the following: whether and how a malfunction of astrocyte–neuron crosstalk during development might in turn affect dopaminergic neuron vulnerability in adult life; what underlying mechanisms of LRRK2-astrocytes are involved; the extent to which astrocyte–microglia maladaptive interactions form part of such dysfunctional interplay; what the consequences are for programming the susceptibility of mDAns in adult life and ageing, with their implications for the ongoing understanding of PD pathology and therapeutic developments targeting the dynamics of astrocyte–neuron dialogue.

Keywords: astrocyte–neuron dialogue; Parkinson's disease; LRRK2; Nrf2/WNT anti-oxidant axis; development; neurodegeneration; neuroinflammation; neurorepair

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1. Introduction

There is significant evidence that Parkinson's disease (PD) may result from a dysfunctional inter-relation between a certain number of genes and factors from the

environment, that together synergize and contribute to the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc). However, PD's precise pathogenesis still remains unclear. Among others, there is a defective mitochondrial function, together with enhanced oxidative reactions associated with the presence of α-synuclein (α-syn) aggregates, identified both in neurons and in astrocytes [1–4]. The loss of midbrain dopaminergic neurons (mDAns) is selective and progressive, with a glial reaction connected to the degeneration of dopaminergic terminals in the corpus striatum (Cpu), finally resulting in the loss of dopamine (DA) [1,2]. The gradual DA depletion is responsible, in turn, for the progressive deterioration of motor function culminating in PD diagnosed symptoms, i.e., bradykinesia, rest tremor, rigidity and postural instability [1–6] (Figure 1).

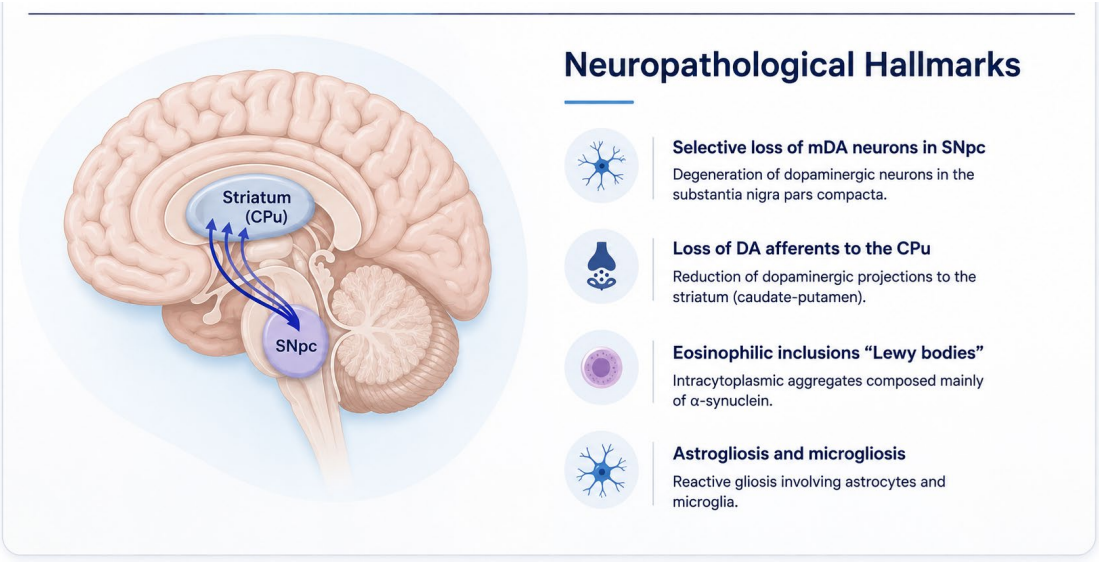


Figure 1. Nigrostriatal dopaminergic pathway and neuropathological hallmarks of Parkinson's disease (PD). At the top, a sagittal schematic cartoon of nigrostriatal dopaminergic (DAergic) neurons whose cell bodies originate from substantia nigra pars compacta (SNpc) and project to the corpus striatum (CPu), which includes the caudate and putamen nuclei. The major neuropathological hallmarks of PD are boxed on the right-hand side (for details see the text).

Within the genes associated with PD, there is Leucine-Rich Repeat Kinase (*LRRK2*, *PARK8*) whose mutations can contribute to both familial and sporadic PD [7–9]. LRRK2 mutations are recognized to "cause late-onset, autosomal dominant PD", which is comparable to idiopathic PD (iPD) [10–12]. Since its discovery, more than two decades ago, numerous *LRRK2* mutations were thought to modulate the risk for PD, with the "p.Gly2019Ser (G2019S) variant" recognized to be predominant [10–13]. In recent years, important developments in the physio/pathological function of LRRK2 have been examined, albeit the detailed function of LRRK2, and especially how it can be activated, still remain unaddressed [14].

Importantly, the weak penetrance of LRRK2 mutations, the reduced familial concordance, associating with the failure to document significant mDAn degeneration in LRRK2 mouse models of PD, have led to the suggestion that other events and/or factors of either genetic and/or environmental nature, are needed to cause mDAn death [15]. In fact, there are at least two recognized crucial conditions, such as brain ageing and inflammatory reactions, that are well known to play a vital role in mDAn degeneration [16,17], as recognized by a high number of both early and more recent publications in the field of PD and LRRK2-PD [13,15–17]. Hence, it is thought that the activation of the glial

cell compartment including both astrocytes and microglia may provide the cause of the onset and/or the progression of PD [16–28].

Of specific interest, in face of the well-recognized classical concept of "PD as an age-associated disorder", earlier and more recent evidence does corroborate "a developmental hypothesis" for PD pathogenesis [29–33]. Here, genes, crucial transcription factors and developmental pathways linked to mDAn vulnerability [33], interacting with early life events and key hormonal and inflammatory/anti-inflammatory signaling systems [20,34,35], are suspected to play important roles via their expression of and/or intimate dialogue with astrocytes, as developed in the next sections of this work. Overall, we put forward the hypothesis that early developmental astrocyte–neuron crosstalk dysregulation may predispose individuals to increased neuronal vulnerability later in life [36]. Two decades ago, work from our laboratory suggested that a malfunction in the crosstalk between the neuroendocrine and the immune systems through the hypothalamus–hypohyseal–adrenocortical and hypothalamus–hypophyseal–reproductive axes [20,34,35] during ontogeny may irreparably rebuild the function of astrocytes and «program» long-term consequences for the regulation of the immune response to oxidative and inflammatory stressors. These conditions may then increase susceptibility to the development of a number of pathologies of the endocrine, immune and central nervous systems, especially PD [20]. Multiple lines of more recent evidence further expand this conceptual model, underscoring astrocytes as critical elements of neuron–glia dialogue in both healthy and disease states [36–39].

Hence, as recently reviewed by Dr. Booth H.D.E. and coworkers [40], numerous genes recognized to play a role in PD development are significantly enriched in astrocytes, including *LRRK2*, among others [40], and they regulate various functions that include the regulation of the immune and oxidative stress response, the uptake of glutamate, mitochondrial and lysosomal functions, i.e., very similar pathways to those affected in PD [40–44]. Therefore, a dysfunction of astrocyte's biology has been linked to various NDs, including PD. Specifically regarding LRRK2, astrocytes have been deeply studied in order to document their potential role in *LRRK2*-mediated PD [45–55]. Generally, various studies have been carried out in "human induced pluripotent stem cell (hiPSC)-derived astrocytes and neurons from familial mutant LRRK2 G2019S PD patients and healthy individuals, as firstly discovered in the publication of Di Domenico and co [45]. Here, the authors unveiled a crucial and specific contribution of G2019S astrocytes to mDAn neurodegeneration through "a non-cell autonomous" dysregulation of bidirectional astrocyte–neuron crosstalk [45]. As reviewed in the present work, a number of impairments/alterations/deficits have been documented specifically in human iPSC- and mouse-derived G2019S mutant astrocytes. In particular, α-syn increased accumulation/reduced clearance [45,47], decreased the expression of the astrocytic neuroprotective factors transforming growth factor beta 1 (TGFβ1) and the matrix metallopeptidase 2 (MMP2) [46], and caused astrocyte atrophy [48], the inactivation of Sarco-Endoplasmic Reticulum Calcium ATPase (SERCA) [49], Ca^{2+} overload, unbalanced metabolism and mitochondrial dysfunction [46–50]. Additionally, an impairment of astrocyte-to-neuron crosstalk via extracellular vesicles [53] and a defective communication via mitochondrial transfer [55] were documented as potential dysfunctional mechanisms associated with *LRRK2* G2019S mutation [53,55]. An important aspect of astrocyte's function is the one linked to the formation and maintenance of the blood–brain barrier (BBB). Hence, de Rus Jacquet and coworkers [51] developed a system to study the interplay between astrocytes, inflammation and the integrity of the BBB [51]. Here, looking at patient-derived iPSC in association with the latest technologies, the authors generated a 3D human BBB chip [51], which showed a defect of LRRK2 G2019S astrocytes to support the formation of a functional capillary in vitro [51]. These studies

underscore the need to further our understanding of the mechanisms and significance of astrocyte LRRK2–G2019S mutation and its crosstalk with mDAns for PD development and/or progression.

In the CNS, astrocytes represent the greatest population of glial cells [56–58]. During development, the functions of astrocytes are well recognized, particularly their ability to control synaptogenesis, and their supportive and neuroprotective effects, according to region-specificity [37–39,59,60]. In particular, and as introduced by the pioneering studies of Dr P. Magistretti and co [61–64], astrocytes have a specific ability to regulate brain homeostasis thanks to their capacity "to regulate energy storage and defence against oxidative stress" [61–64], this being at the center of the neuroimmune signaling dialogue [61–64].

Especially, in the VM, astrocytes express specific growth and neurotrophic factors (GFs) as well as dopaminotrophic genes, including elements of the "wingless-related MMTV integration site (WNT)/β-catenin (WβC)" [26,65] pathway and its transcriptional regulatory networks, essential for the control of tissue morphogenesis and regeneration, and specifically involved in mDAn neurogenesis, mDAn survival, neuroprotection and repair [65–68]. Hence, several lines of evidence prompted us to present a novel "intrinsic WNT brain repair hypothesis" [25] motivated by data from the literature as well as by some redundancy of WβC activation after CNS injury and during neuroimmune interactions [25,26]. Here, we proposed the reactivation of silenced pathways under pathological conditions stimulated by WβC signaling activation to support neuronal survival and/or the regeneration in the lesioned or ageing brain [25,26,67–73]. Of specific interest, and as reviewed in Section 2, a reciprocal *LRRK2-WNT* signaling dialogue does occur, implicating disrupted WNT signaling in pathogenic LRRK2 mutations as a critical effector responsible for neurodegeneration observed in PD [Section 2].

Notably, astrocytes convey both beneficial and harmful effects according to a mutual crosstalk with microglial cells, which highlight the "*Dr Jekyll*/*Mr Hyde*" sides of glia, as recalled twenty years ago [20]. Here, astrocytes and microglia can promote neuroprotective or noxious effects in different physio/pathological states, which may include development, ageing and PD injury [20]. Therefore, astrocyte–microglia bidirectional reciprocal modulation is central in programming neurodegenerative disease onset and progression, with the "*To be or not to be inflamed*" dilemma still remaining a critical question of the inflammatory PD puzzle [20].

In particular, a highly important endogenous mechanism against oxidative stress and inflammation is carried out by a nuclear factor, i.e., Nrf2 (nuclear erythroid 2-related factor 2) and the interconnected anti-oxidant responsive element (ARE) axis [74–79]. By promoting the neutralization of ROS (reactive oxygen species), and by downregulating inflammation, Nrf2 supports the protection against a variety of stress-related disorders that have deleterious effects in various body tissues, specifically interlinked with antioxidant self-defenses and mitochondrial health [76–79]. Not surprisingly, in astrocytes, this system can govern the expression of numerous defense pathways, particularly neuroprotection [70–72,80–82] and the detoxification of ROS, RNS and xenobiotics; they can also provide for glutathione (GSH) synthesis and counteract inflammation, then having an impact on the selective region-specific neuronal susceptibility to a variety of insults [70–72,74–88]. Especially, in astrocytes, heme oxygenase1 (HO-1) expression is a prime mediator of both antioxidant and anti-inflammatory responses [70–72].

During the last three decades, our laboratory has focused on glial–neuron interactions during development, in the adult and ageing brain, using various rodent model systems [89,90], largely anticipating astrocytes' central position as convergent points of the "Neuroimmune Crossroad", and as key mediators of neural plasticity. In this scenario, and especially in light of this specific role of a physiological crosstalk between

mDAns and their partner glial cells, we aimed at reviewing the evidence in support of a decisive role of LRRK2-diseased astrocytes as predisposing elements for PD susceptibility, asking the following: whether, when and how a malfunction of this crosstalk might affect dopaminergic neuron vulnerability; what underlying mechanisms are involved; the extent to which astrocyte–microglia maladaptive interactions form part of such LRRK2-dysfunctional interplay; what the consequences are for programming the susceptibility of mDAns in the adult and/or ageing brain; whether LRRK2 is a novel actor within the Nrf2/HO1/WNT neuroprotective, survival and antioxidant axis. Addressing these and other related questions appears crucial to deciphering the involvement of astrocytes in LRRK2-diseased mechanisms.

2. LRRK2: A Multifunctional Protein Linking Neurodevelopment to Neurodegeneration

LRRK2 is recognized to play a number of important roles in various vital functions, both inside and outside the brain (Figure 2). As recently reviewed [91], the LRRK2 gene specifies a protein of 2527 amino acids with a molecular weight of 286 KDa [91]. Studies have indicated that the protein is part of the family of "ROCO" proteins, as documented in full detail elsewhere [91]. Of note, there is a "catalytic core" composed by the GTPase domain, which is accompanied by a serine/threonine kinase domain [91,92]. It has been documented that GTPase activity is required for the appropriate transfer of LRRK2 to its targets, where the kinase can phosphorylate its substrate [91]. Moreover, it has been shown that the catalytic core is bordered by "four protein motifs" that have been called armadillo (ARM), ankyrin repeat (ANK), leucine-rich repeat (LRR), and WD40 [91] (Figure 2). Notably, the functions of the major domains of LRRK2 have not been fully defined, but it is generally believed that the WD40 domain, LRR sequence and ANK sequence are involved in protein–protein interactions, as reviewed elsewhere [91,92].

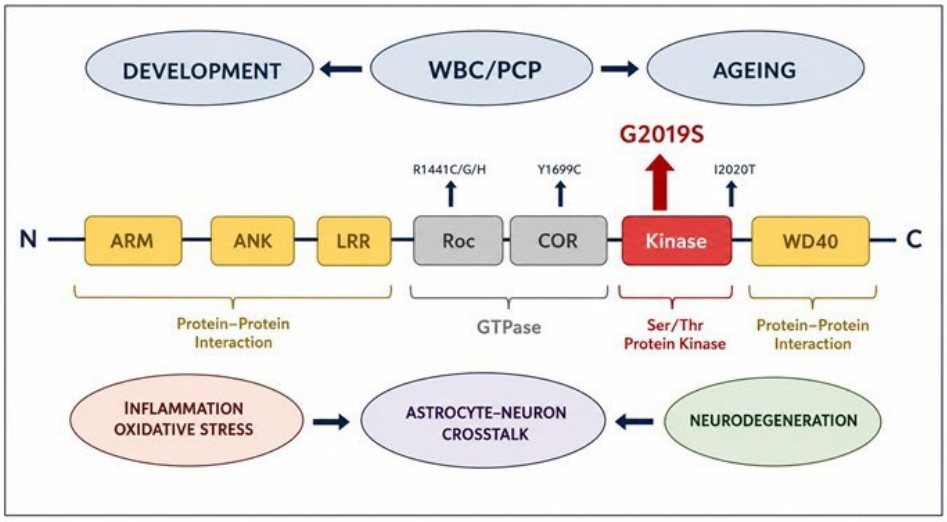


Figure 2. LRRK2 protein links neurodevelopment to neurodegeneration. A number of important systems interconnected with LRRK2 signaling and function are summarized. These include the LRRK2 link with development, the WNT/β–catenin (WβC) signaling system, the ageing process, the inflammatory and oxidative stress response, and neurodegenerative processes [70–73], all converging on astrocyte–neuron crosstalk modulation (detailed in the text). The different domains encoded by the LRRK2 protein and the recognized pathogenic mutations [91] are shown above. The WD40 domain, LRR sequence and ANK sequence are involved in protein–protein interactions (in yellow). The GTPase (Roc-COR) domain is shown in grey, with the black arrows indicating the R1441C/G/H and Y1699C described mutations [91]. The kinase domain is shown in red, and the red

arrow shows the LRRK2 G2019S mutation. ARM = armadillo repeats, ANK = ankyrin repeats, LRR = leucine-rich repeats, ROC = Ras of complex proteins, COR = C-terminal of ROC (for details see the text).

2.1. LRRK2/WNT Signalosome, Astrocytes and PD

The seminal studies of Drs. Sancho RM, Law BM and Harvey K [92], first reported the interaction of LRRK2 with the "Dishevelled family" of phosphoproteins (DVL1-3), which are key regulators of WNT signaling pathways [92]. WNT signaling is recognized to be important for axon guidance, synapse formation and neuronal maintenance [92].

After this first report, the LRRK2/WNT modulation in PD in the studies of K. Harvey's group underpinned the complex and critical LRRK2–WNT crosstalk in PD physiopathology [92–99], thereby stimulating research in the field in an increasing number of laboratories, including our own. Hence, the WβC (WNT/β-catenin) pathway has taken a prominent role in the context of mDA neurodegeneration and PD, as well as in post-lesion neurorepair processes and neuroimmune interactions [25,66–73,95–101]. Reportedly, following WNT stimulation, LRRK2 is recruited to membranes, where it binds to the WNT co-receptor LRP6, i.e., the low-density lipoprotein receptor-related protein 6 [93]. LRRK2 can then connect membrane and cytosolic components of WNT signaling [93]. Further studies of Dr Harvey's group have shown that changes in LRRK2 expression may influence pathway activity, while pathogenic LRRK2 mutants can reduce both signal strength and the LRRK2–LRP6 interaction [93,94]. Thus, decreased LRRK2-mediated WNT signaling resulting from decreased binding to LRP6 may cause the neurodegeneration observed in PD [94,97]. The authors hypothesized an additional role for LRRK2 in basal WNT signaling, as a component of the β-catenin destruction complex, and presented compelling evidence that the primary role of LRRK2 in the "canonical" WNT pathway is to repress β-catenin [94]. On the other hand, there is also a variant of *LRRK2* (i.e., R1398H) that is protective, and which increases GTPase and WNT signaling activity [96]. Together, these studies corroborate the intricate *LRRK2/WNT* relationship in PD [97]. Another prominent example of LRRK2/WNT-modulated pathways has been disclosed by Harvey's group [95], whereby the nuclear factor of activated T-cells (NFAT) signaling and WNT signaling may act as regulators of immune and nervous system development/maturation [95]. Intuitively, as the WNT signaling controls various cell functions and systems linked to both the development and the physiology of adult of mDAns [101–110], an early dysfunction of the intimate LRRK2–WNT relationship is likely to contribute to PD vulnerability. Notably, the midbrain dopaminergic neuronal system, which includes the DA cell bodies that degenerate in PD, has been shown to depend on WβC, specifically on the WNT1 member [33,102–104], whereby in the developing VM, both WβC and WNT/PCP (planar cell polarity) pathways function together to control several critical developmental processes, from establishing the floor plate domain to the maintenance of mature mDAns [33,102–112]. Of specific mention, the regional identity of astrocytes characterizes both their developmental and their neuroprotective dopaminergic WNT-linked functions [99–111].

Within this frame, it seems important to recall that in the developing VM, radial glial (Rgl) cells, i.e., the progenitors of mDAns, harbor the crucial signals required by neural precursors to acquire a DA phenotype [33,107]. In fact, brain region-specific identities of VM astrocytes rely on the expression of several developmental genes and transcription factors promoting the differentiation of NPCs into mDAns, among which WβC signaling displays a key role in the orchestration of mDAn development [33,65,102–111]. Hence, astrocytes cultured from the VM neurogenic niche (but not from the cerebral cortex, CX) facilitate mDAn differentiation and survival [65–73,111]. Especially, developmental pathways linked to the vulnerability of adult mDAns, as recently reviewed by Dr. Prakash,

N. [33], and contributing to mDAn critical functions, are expressed at least in part by VM astrocytes. The crucial roles for WβC signaling, extending from mDA neurogenesis to neuron maintenance, repair and regeneration, have been documented (summarized in Section 3), together with the ability of cultured VM-astrocytes to abundantly express not only WNT cytokines (WNT1, WNT4, WNT5a), but also spondin-2 (SPO-2), a secreted protein of the R-spondin family, which activates WβC signaling by preventing clearance of the Frizzled–LRP WNT receptor complex [111].

Remarkably, recapitulating the signals necessary for astrocyte differentiation in the human embryonic VM represents a significant technological advance and a platform for the study of region-specific properties of VM astrocytes under physiological, inflammatory and neurodegenerative conditions [112], including LRRK2 G2019S mutation (as summarized in Section 4).

Notably, pivotal PD mutations were documented to affect in a negative fashion WβC signaling, as well as iPSCs' capability to differentiate into mDAns [113], underscoring the link between PD mutations and downregulated WβC signaling.

Altogether, LRRK2 mutations/altered WNT signaling can affect the establishment and maturation of neural circuits [114,115], as well as the maturation of VM astrocyte–neuron interactions, thereby impacting on mDAn function during neurodevelopment and throughout life, and/or mDAn's predisposition to disease and/or death.

2.2. LRRK2 as a Multipurpose Homeostatic Regulator

Importantly, the expression of LRRK2 is species- and cell-type specific [52]. A number of studies have documented that LRRK2 expression is variable within neurons [52]. In particular, the higher expression was documented in striatal projection neurons, whereas a lower expression was present in striatal cholinergic and GABAergic interneurons, as well as ventral tegmental area (VTA) and SNpc DA neurons [31,52,116,117]. Interestingly, there is very low LRRK2 mRNA and protein in DA neurons, especially when compared to other PD-associated genes [52]. On the other hand, in human tissues, LRRK2 expression is greatest in peripheral tissues such as the lungs, spleens and kidneys [52]. Also, it has been shown that LRRK2 expression is high in peripheral blood mononuclear cells (PBMCs) and neutrophils [52,116,117]. Of specific note, LRRK2 is greatly expressed in non-neuronal brain cells, which include both astrocytes and microglia [52]. When considering the sub-cellular distribution, LRRK2 is most concentrated at membranous and vesicular structures, including synaptic vesicle and synaptosome cytosolic fractions, as well as lysosomes, endosomes, and importantly, the mitochondrial outer membrane [52,91,117–120].

Given its cell-specific localization, LRRK2 is involved in numerous signaling pathways. Principal cell functions include vesicular transport, autophagy, microtubule dynamics, mitochondrial function, and immune response [52,91,117–120]. Hence, the dysregulation of each of these pathways is well known to participate either directly and/or indirectly in mDAn degeneration [52,91,117–120]. An interesting LRRK2 feature is the novel LRRK2 link to the modulation of ciliogenesis [91,121] and centrosome cohesion [118]. In fact, both functions that are impaired in PD and LRRK2-PD are linked to the WNT pathway [118,121]. Another well-studied process affected in PD-linked LRRK2 mutations is the disruption of autophagic processes, which require lysosomal activity [52,91]. This condition may further cause mitochondrial dysfunction in multiple model systems, including patient-derived cell lines, interfering with mtDNA maintenance, mitochondrial dynamics, trafficking, and mitophagy by disrupting mechanisms controlling mitochondrial fusion and fission, as recently summarized [52,117–120].

Given that pathogenic *LRRK2* variants can upregulate LRRK2 kinase activity, there is ongoing research and clinical trials investigating the therapeutic potential of LRRK2

kinase inhibitors for the treatment of either LRRK2 PD and iPD, as reviewed elsewhere. Among the excellent reviews in the field, Kluss, J. H. et al. [122] provide the current status of LRRK2 kinase antagonists, along with their efficacy and overall safety, including the recommendations and critical issues extensively discussed together with recent technological advancements in the field [122].

2.3. The Vicious Cycle of LRRK2 Mutation, Oxidative Stress, Mitochondrial Dysfunction and PD

Studies have demonstrated that LRRK2's enhanced kinase activity is a critical characteristic of both familial and sporadic PD cases, which, in turn, may be involved in the regulation of crucial cellular pathways—in particular, mitochondrial processes [52,117–120]. Then, a dysfunction of these processes as a result of upregulated LRRK2 expression may affect mitochondrial dynamics, and can contribute to neuronal degeneration in PD [117–121]. Possibly, LRRK2 mutations may activate a vicious cycle of increased LRRK2 kinase activity and upregulating oxidative stress, in turn hyper-activating glial responses and mitochondrial dysfunction, according to age and dysfunctional astrocyte–microglia crosstalk, as observed in PD.

Notably, there is an ever-growing list of LRRK2-mediated cellular functions involved in a multitude of vital pathways. The dysfunctional LRRK2 protein can have important impacts in a cell- and tissue-specific way, leading to a significant dysregulation of tissue homeostasis, with severity and outcome that may vary according to the individual's genetics, sex, age and inflammatory status. Several recent reviews of the field have pinpointed different aspects of LRRK2 action, underscoring its multifaceted role in contributing to promoting mDAn degeneration [52,91,117–122]. Here we focus on astrocyte–neuron crosstalk, as one privileged target of LRRK2 mutation causing PD pathology, first asking how glial cells interact with mDAn's physio/pathology.

3. Astrocyte–Microglia–Neuron Tripartite Crosstalk: "Mr Hyde" Facet of Cell-to-Cell Dialogue

The principal glial actors, astrocytes and microglia, are recognized to preserve correct brain homeostasis, with both cell types contributing to PD onset and/or progression [18–24,123]. It is well known that upon brain injury, under some specific brain disease or during the ageing process, both astrocytes and microglia respond with an activation, defined as glial reactivity [56–58].

The field has been variously reviewed by both earlier (see the Introduction) and more recent publications and thesis dissertations [16,17,28,56,57,70,72,73,80,81,123–133]. Generally, in earlier times, glial activation was viewed as either a protective or a damaging condition. Its classification included the neurotoxic inflammatory astrocyte's (termed A1) and microglial (termed M1) states, and on the other hand, the "A2 (for astrocytes)/M2 (for microglia)" states, recognized to exert protective, supportive and/or anti-inflammatory functions. However, further molecular studies associated with the recognized functional effects of glial cells now indicate that this "dichotomous model is overly simplistic" [132–137], because it is now well accepted that both astrocytic and microglial cells can assume various reactive states that depend on a number of factors, including the specific brain region, the type of insult (acute or chronic), as well as various genetic and environmental interacting factors [101,112,123,130,132–137]. When activated, astrocytes are well known to show an increased hypertrophy, with a characteristic feature being represented by a significant upregulation of the typical astrocytic proteins, glial fibrillary acidic protein (GFAP) and S100 calcium-binding protein beta (S100β) [56].

Reactive astrocytes also express receptors participating in innate immunity (such as Toll-like receptors, TLR, besides others) and contribute to the control of astrocytic reaction under various stimuli, which include brain lesions, as well as the ageing process or toxic exposures [130–133]. Notably, when activated, astroglial cells can express receptors for both growth and survival factors, as well as for immune molecules, including cytokines and chemokines, and hormones, acting as crucial immune mediators in the neuron–glia dialogue [25,26,70–73,101,123,130,132].

It has been documented that under various conditions, such as the ageing process (both physiological and pathological), as well as under the condition of exposure of toxic agents, the tripartite astrocyte–microglia–neuron crosstalk in the VM can shift into having a detrimental influence, leading to neurotoxic effects onto the fragile mDAns [70–72], especially in the presence of the mitochondrial toxin MPTP (i.e., 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine). Here, after its conversion in VM astrocytes to its active metabolite MPP^+, and its delivery to mDAns via the DA transporter (DAT), MPP^+ inhibits complex I of the mitochondrial electron transport chain, resulting in the decrease in ATP in turn engendering mDAn death [2,72]. Associated with these processes, the VM microglial cell compartment also responds with significant microgliosis, leading to the generation of a multitude of inflammatory factors that include the cytokines, TNF-α and IL-1β, and various chemokines [21–27,130].

Especially, a key aspect is represented by age, given that astrocytes lose their functionality and turn into highly activated cells that trigger microglia, with a consequent and significant aggravation of astrocyte–microglia crosstalk [69,70]. Remarkably, as studied by a number of authors in the field [21–24,81], synergy between age and genetic and environmental factors such as toxic exposure can determine a greater microglial activation, resulting in the inhibition of the astrocytes' neuroprotection [69,70]. There are also other conditions able to shift astrocytes' protective potential, such as α-syn-positive inclusions present in reactive astrocytes, which may greatly contribute to mDAn deterioration [128,138].

Notably, increasing evidence documents a peripheral-to-central neuroimmune bidirectional crosstalk, linking both peripheral innate and adaptive immune responses in the pathophysiology of PD [27,28]. Particularly, besides CNS glial cells, other cells, from the periphery, can contribute to the neuroinflammatory processes in PD, as discussed elsewhere [27,28]. It has been documented that a malfunction of the brain–peripheral immune system may result in the increased infiltration of peripheral cells, thereby resulting in a greater glial activation, in turn contributing to aggravating the susceptibility and possibly the death of mDAns [27,28]. Of specific mention, the greatest expression of LRRK2 has been found in both innate and adaptive immune cells [27,28,52]. Moreover, a specific control of peripheral LRRK2 by endogenous and exogenous inflammatory factors has been documented, leading to the hypothesis that peripheral inflammation can contribute early in PD, as discussed in detail elsewhere [27,28].

Together, a dysfunctional bidirectional astrocyte–microglia crosstalk in the presence of and/or preceded by peripheral immune activation can perpetrate inflammation and oxidative stress in the PD brain, with consequences for surrounding neurons. Of particular note, however, astrocytes may play critical roles in directing glia–neuron crosstalk towards a protective anti-inflammatory dialogue, as documented in the next sections.

3.1. "Dr Jekyll" Facet of Astrocyte–Neuron Crosstalk: α-Syn, Tunneling Nanotubes (TNTs), Exosomes and Mitochondria Trafficking

One important feature of astrocyte's neuroprotection is their pivotal capacity to regulate α-syn toxicity. It has been shown that the cell-to-cell transfer of α-syn can take

place with various modalities—for example, through TNTs or exosomes or via mitochondrial transfer, also with a potential propagation of PD [128,137]. In fact, the role of astrocytes in α-syn capture is well recognized, whereby an efficient bidirectional transfer of α-syn between astrocytes and neurons can support neuroprotection [137]. On the other hand, in astrocytes, α-syn aggregates sequestered from the extracellular space can have deleterious consequences, as aberrant α-syn accumulation can induce astrocyte activation and may impair lysosomal function [128,137]. As reviewed by Ozoran and co [137], endogenous astrocytic α-syn levels are very dynamic, and they can vary upon exposure to different stimuli, with inflammatory factors mediating the upregulation of α-syn in astrocytes [137].

Among the various ways to communicate with each other, astrocytes-derived EVs represent an efficient method to transfer different cargoes including immune regulators, enzymes, mRNAs, or non-coding RNAs (including microRNAs). Given that EVs can be released by every type of cells, both at the periphery and in the brain, this mode of cell–cell interactions plays critical roles in a number of both physiological and pathological conditions, such as waste disposal, as well as the transfer of supportive or detrimental substances [53,73,138]. Hence, the secretion of astrocyte-derived EVs represents a likely path of astrocyte–neuron communication, thus providing a potential means to exert powerful neuroprotective functions [138,139]. Fascinatingly, such intercellular communication by astrocyte-to-neuron signaling appears region-specific and context-dependent [139]. For example, EVs derived from astrocytes of the PD-affected CNS regions, such as the VM and Str, have the capacity to contrast the death of neurons under the effect of MPP^+, through the protection of neuronal mitochondria [139]. In stark contrast, and as discussed in Section 4.6, in the work of de Rus Jacquet, A. and co., such a communication modality via astrocyte-derived EVs failed in the G2019S-affected PD model, resulting in neuron atrophy [53].

Importantly, the transfer of mitochondria from astrocytes to neurons has been recently documented to provide endogenous means of neuroprotection [140,141]. On the other hand, damaged mitochondria can cause a detrimental astrocytic response and activate inflammatory-dependent neuronal damage [128,140]. As recently reviewed by Jiao et al. (2026) [141], not only astrocytes but also microglia may donate mitochondria, thereby helping the fragile neurons to regain their bioenergetic capacity, resulting in a positive neuronal influence [141]. Notably, as discussed for astrocyte diversity and dual properties, mitochondria transfer is context- and genotype-dependent, thereby exerting opposing effects, propagating detrimental signals, and exacerbating tissue injury [55,128,141,142]. Remarkably, as recently documented by Amorin, P. and co. [142], the ageing process importantly disrupts mitochondrial function in human astrocytes [142]. Hence, using a doxorubicin-induced senescence model in primary human astrocytes, the authors show that astrocyte senescence transmits mitochondrial damage, thereby losing their capacity to sustain neuronal mitochondrial homeostasis [142].

Together, a number astrocytes' specialized defense functions are able to switch glial neurotoxic effects into protective and neuroreparative ones, again underscoring the beneficial astrocyte–neuron crosstalk, characterizing astrocytes' good side (Figure 3).

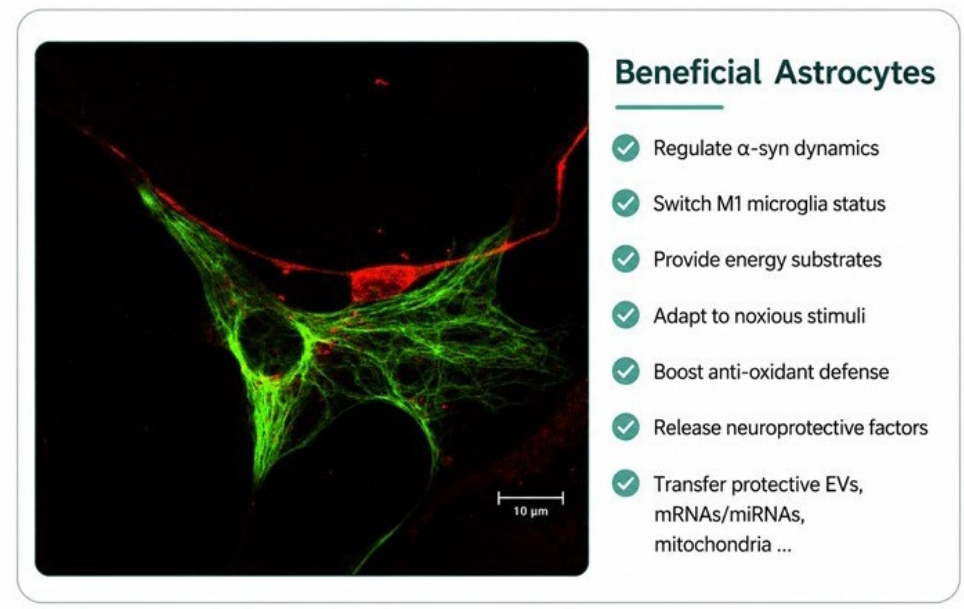


Figure 3. Beneficial astrocytes play crucial roles in promoting the neuroprotection of mDAns. A confocal image of a dual stained ventral midbrain astrocyte–neuron co-culture at 12 days in vitro showing a glial fibrillary acid protein (GFAP, in green) positive astrocyte "embracing" a tyrosine-hydroxylase (TH, in red)-stained dopaminergic neuron (image from astrocyte–neuron protocols of Marchetti B. et al. [34,35]). Some beneficial properties of midbrain astrocytes are summarized. For details see the text.

3.2. Astrocyte–Neuron Coupling, Brain Metabolic Homeostasis and Oxidative Stress

As anticipated in the Introduction, astrocytes provide energy substrates to neurons and support antioxidant defense mechanisms [61–64,88]. Hence, astrocytes are the main sites of brain glycogen storage, and produce lactate to meet the demands of neurons [61–64]. Here, lactate is critical for the control of neuronal plasticity [61–64,143,144]. Glycolysis represents another critical astrocyte actor supporting neuroprotection, given its vital role in consolidating a number of interactive metabolic signals, like glucose, lactate, and hormones [144]. Especially, pro-inflammatory cytokines can increase glycogenolysis in astrocytes as well as the oxidation of glucose [144]. Also, microglial-derived nitric oxide (NO) can induce, in turn, glycolytic genes through hypoxia-inducible factor 1 alpha (HIF1α) [145]. Then, in response to inflammatory stressors, there is an increase in astrocytic glycolysis resulting in the production of ATP and L-lactate, thereby contrasting the astrocyte's oxidative metabolism impaired by NO, also providing an energy substrate to neurons [145]. This glycolysis is considered as a two-edged sword: on the one hand it is able to support energy metabolism for neurons, but on the other it speeds up inflammatory reactions by stimulating A1-reactive astrocytes [142–145]. Importantly, with age, senescent astrocytes may lose their adaptive capacity, as documented in the recent work of Amorim et al. [142], showing abnormal and bioenergetically inefficient mitochondria, translating into reduced cellular metabolic activity, ATP depletion and impaired lactate production or release [142].

3.3. Astrocyte's Plasticity and Adaptive Functions

Notably, physio/pathological factors, such as hormones, immunoregulators including ROS, cytokines, and neurotransmitters, have been documented to control astrocytic metabolism [146]. Hence, there is a dynamic exchange of metabolites between astrocytes and neurons, which results in the modulation of neuronal activities [145]. Metabolic plasticity is also associated with the (re)activation of astrocytes [146]. It has been

suggested that "neurodegenerative" and "neuroprotective" subpopulations of reactive astrocytes metabolize distinct energy substrates, possibly causing different impacts on pathologic processes [146]. The pioneering studies of P. Magistretti and colleagues [61–64,143–145] documented that in basal conditions, lactate may regulate energy distribution via the astrocyte–neuron lactate shuttle. However, in the presence of a brain lesion or in neurodegenerative conditions, a dysfunctional "lactate reprogramming" occurs that may cause an impairment in mitochondrial energy coupling [146]. These conditions have been shown to increase further neuronal-detrimental effects via the induction of neuroinflammatory reactions, thereby modifying homeostasis [147–149]. Yet, the precise signaling and timing of the different cascades through which lactate modulates neurodegeneration are not clarified, but research on CNS lactate reprogramming may provide new standpoints on the mechanisms underlying NDs, for the development of specific therapies involving the modulation of lactate metabolism [148–151]. Within this frame, it has been clearly documented that in astrocytes, mitochondrial function is linked to redox regulation, in turn modulating astrocyte–neuron crosstalk, with consequences for neuroinflammatory reactions and for healthy responses in the long term [146–150].

Together, upon injury, an adaptation of astrocyte's metabolic function called "metabolic reprogramming" occurs, with lactate playing a principal role. The consequent dynamics are aimed at increasing the energy demands, connected to a switch to a novel status, with consequences for the progression of pathology [147–151].

3.4. Astrocyte Diversity Contribute to the Selective and Context-Dependent Neuronal Vulnerability

Growing evidence highlights a regional heterogeneity of astrocyte function, at both molecular and functional levels. Studies on the functional diversity of astrocytes suggest that such diversity may be derived from specialized populations [60,83,152,153]. Recent overviews on astrocyte heterogeneity [59,60] have documented both the origin of astrocyte's diverse populations as well as the mechanisms underlying the role of neurons contributing to their diversity [59,60,152]. Especially, recent studies show different astrocyte gene expressions as well as their morphological appearances within the different brain areas [152,153]. Interestingly, there are also comparable region-specific genes and functions connected with their regional heterogeneity [153]. Of note, astrocyte gene networks were identified and correlated with astrocyte morphology, together with "risk genes" correlating with pathological CNS disease [152,153].

Of particular relevance, as documented in the excellent recent review article of Zimmer and co. [83], within the individual astrocytes, there are different gene expressions, corresponding to different protein signatures, intracellular calcium and mitochondrial dynamics, resulting in a high level of organization and sophistication at a subcellular level [83].

Hence, in disease as well as under inflammatory conditions, astrocytes show regional and time-dependent responses, as observed by Hasel P and co. [153], documenting specific subpopulations of astrocytes with different transcriptional states under LPS challenge [154].

3.5. Astrocytes of the VM Display Unique Vulnerability and Neuroregulatory Properties in Response to Inflammation and Oxidative Stress

Particularly, in the VM, astrocytes represent a key source of neurotrophic, survival and neuroprotective factors, likely relating to their derivation from regionally patterned Rgl cells during the early development of mDAns [102–112], as discussed in Section 2. Then, some critical features of VM astrocytes retained in the adult and ageing VM are potentially implicated in mDAn plasticity/resilience [25,26,66,68,70,101]. Using TaqMan®

Low-Density-based Array (TLDA) for gene expression profiling in the acutely dissociated VM and Str of MPTP-treated mice, in vivo, we previously documented differential time-dependent and region-specific mRNAs' expressions [26], unveiling a major and long-lasting upregulation of the midbrain-patterning factor *WNT1,* and certain pro-inflammatory chemokines [26]. Next, a number of in vivo, ex vivo and in vitro experimental paradigms [25,26,66–73,100,101] documented the activation of WβC signaling in VM astrocytes, very likely contributing to limiting the degenerative process and to activating the spontaneous SNpc self-repair program.

Corroborating the VM astrocyte-specific inflammatory response, recent data demonstrate that hiPSC-derived VM astrocytes display unique changes in their secretome in response to inflammation [112]. Here, using the methods of Clarke et al. [59] for the differentiation of hiPSCs-derived VM astrocytes, Crompton, L.A. and co. [112] investigated how VM astrocyte's regional identity might influence their reactive phenotype. After a full characterization of their midbrain identity, the authors exposed VM astrocytes to a combination of inflammatory stimuli [59] and cytokines elevated in PD [112]. The secretome analysis of hiPSC-derived VM astrocytes using tandem mass tag (TMT) spectrometry demonstrated unique neuroinflammatory characteristics of the VM class of astrocytes in this experimental setting [112]. These, and other extensive results of this study, clearly indicate that the response of VM astrocytes to inflammatory stimuli is characterized "by regionally encoded changes in protein secretion profiles" [112], with implications for the development of immunomodulatory therapies for NDs, and in particular PD [112].

That VM astrocytes have differential characteristics and functions, as compared to either cortical or hippocampal astrocytes, was clearly documented by Xin and co. [155]. Here, VM astrocytes were shown to display unique histochemical, biochemical and molecular properties [155], thereby responding to various challenges in a region-specific fashion with potential consequences for their partner neuronal cells [70,84,155]. Notably, such VM astrocyte diversity crucially influences the microglial cellular compartment, with consequences for microglial–neuron and microglial–astrocyte interactions [130], as documented in the VM of aged and MPTP-induced PD mouse models [66–73], as well as in rotenone-induced brain region-specific glial dysfunction, recently underscored by Miyazaki, I. and co. [84].

Further evidence for a specific regional diversity in the nigrostriatal system stems from recent results documenting the neuroprotective activities of nigrostriatal astrocyte-derived EVs [139]. Here, EVs from both VM and Str astrocytes counteracted H_2O_2-induced caspase-3 activation, specifically in differentiated SH-SY5Y neurons, with EVs from chemokine-exposed astrocytes showing a higher protective effect [139]. As documented by the authors [139], high-resolution respirometry further revealed that nigrostriatal astrocyte EVs rescue the neuronal mitochondrial complex I function impaired by the neurotoxin MPP^+ [139]. Especially, only EVs from VM astrocytes fully restored ATP production, in differentiated SH-SY5Y neurons, further highlighting a regional and context-dependent diversity in the nigrostriatal system for the secretion and activities of astrocyte EVs [139].

It seems, then, plausible that differences in VM astrocytes at morphological, molecular, and functional levels, as observed with age, inflammation and PD, may significantly participate in shaping the different neuronal susceptibilities of mDAns [83,84,87,112,138,139,142,153–155].

In fact, within the nigrostriatal system, astrocytes display unique neuroprotective features given the recognized vulnerability of SNpc neurons. Here, cell-to-cell neuron-astrocyte crosstalk plays critical neuroprotective functions targeting oxidative stress [130].

In fact, astrocytes are principal elements promoting redox homeostasis in the brain [87] and especially in nigrostriatal neurons [72].

3.6. Redox Chemistry of Astrocyte–Neuron Crosstalk: Metabolic Interactions Between Neurons and Astrocytes Dictate Neuronal Vulnerability

Notably, oxidative and nitrosative stress are well recognized as major contributors to PD pathobiology [85–87], with significant evidence showing the upregulation of oxidative stress markers in PD and neurotoxicant-induced PD [70,72]. Earlier studies carried out in PD patients documented the greatly decreased levels of glutathione in the SN, associating with an upregulation of plasma lipid peroxidation and reduced antioxidant enzymes [85–87,156,157]. Here, the astrocyte's function appears critical under basal and acute stressful conditions, whereby VM astrocytes are recognized to promote neuroprotection thanks to their ability to clear the SN environment of ROS and RNS, and supply neurons with the necessary precursors for glutathione synthesis [156]. Importantly, upregulated levels of ROS can contribute to the formation of toxic dopamine metabolites/adducts that may trigger α-syn aggregation [72,86,130,157,158]. It has been suggested that the DA metabolic steps are themselves the cause of increased ROS and RNS, with a consequent neurotoxic cascade resulting in mDAn death [72]. It seems important to recall against this background that DA itself, via D1/D2-like receptors, has the capacity to counteract glial reactivity, thereby engendering an astrocyte-to-neuron self-protective loop [72].

An interlinked critical actor in DA metabolism contributing to mDAn degeneration is iron, via "ferroptosis", a mechanism connecting an excess of iron to neuronal loss. In fact, its pathologic accumulation may lead to increased oxidative stress through the Fenton reaction and the generation of ROS, causing an increased mDAn susceptibility to death [157,158]. Interestingly, recent evidence documents how genetic (i.e., *LRRK2* G2019S mutation) risk factors for PD may interact with ferroptosis pathways to cause mDAn death [157].

The critical role of VM astrocytes in shaping the toxic SNpc environment was documented in the mouse MPTP model of PD, upon the transplantation of adult subventricular zone (SVZ) stem neuroprogenitors as the mice age [69]. It seems important to underline that in this model, a great part of the transplanted neural stem cells differentiated into astrocytes within the lesioned SNpc [69]. Moreover, both the endogenous as well as the NSC-derived astrocytes activated nigrostriatal repair with the participation of WβC signaling [69]. Other recent results corroborating such a vital function of VM astrocytes arise from studies documenting that transplanting neonatal VM astrocytes within the injured SN of aged mice is able to counteract motor impairment and to promote antioxidant and anti-inflammatory effects via the WβC/Nrf2 axis, resulting in a nigrostriatal restoration [71]. These studies then corroborate that a "beneficial" glial–neuron crosstalk is a necessary condition for neuroprotection in the PD brain [25,26,66–73].

Overall, the SNpc microenvironment, DA receptor signaling and oxidative stress in the nigrostriatal system can determine a unique link for mDAn vulnerability in PD [70–73]. Within this frame, astrocytes' "ace in the hole" is represented by their Nrf2-ARE key machinery.

3.7. Astrocytes' Nrf2-Are Axis: A Crucial System for Neuron Homeostasis

In the brain, Nrf2 controls the activities of the majority of antioxidant systems used for the neutralization of ROS [77,87]. Interestingly, the antioxidant response can be modulated by genetic mutations, developmental transcription factors and signaling pathways, together with sexual and glucocorticoid hormones, then leading to differences in response depending on the brain region, genotype, cell state, age and sex [44,70–73].

Particularly, Nrf2 activation includes key components of the GSH antioxidant system. Hence, astrocytes are well recognized to provide a critical antioxidant support to their partner neuronal cells, thanks to Nrf2 after its binding to the antioxidant response element (ARE). On the other hand, the Nrf2 system is weak in neurons, but synaptic activity can control other neuronal antioxidant genes [87].

Notably, in cortical neurons, the levels of Nrf2 are reduced when compared to the level detected in astrocytes [87]. Also, neurons have been documented to express a higher concentration of "Cullin 3", involved in the degradation of Nrf2 [75–79,87]. On the contrary, Nrf2 activation is well recognized to promote protection against oxidative stressors thanks to the supply of antioxidants such as glutathione and HO-1 [74–79,130]. In physiological states, Nrf2 is degraded by its endogenous inhibitor Keap1. However, when excessive ROS are generated, Keap1 is oxidized, and the Nrf2 degradation process is inhibited [87]. In this condition, Nrf2 accumulates in the nucleus and triggers ARE genes to reduce ROS and xenobiotic accumulation, also abating inflammation. Of specific interest, Nrf2 is recognized to regulate mitochondrial function directly, thanks to its action on the oxidation of fatty acids, respiratory substrate availability and ATP synthesis, as well as acting upstream to regulate mitochondrial ROS production [74,77–79,87].

3.8. In Astrocytes Nrf2/WBC Signaling Interlinks Inflammation- and Metabolism-Related Pathways

As anticipated in previous studies [87], the Nrf2 system accomplishes different tasks in neurons and astrocytes, with specific transcription programs to induce redox homeostasis in the brain [87]. In fact, many studies have underlined the key role of the astrocytic Nrf2 system in the regulation of cellular antioxidant defense and mitochondrial health [77–79]. By contrast, with age or upon neurotoxic exposures, such as MPTP, there is a decrease in the Nrf2–ARE system [70,77,78,80–82]. Also, a synergy between α-syn expression associated with Nrf2 impairment has been documented to exacerbate the death of mDAns and to activate inflammatory reactions, early in PD [81]. The mechanisms underlying mDAn death include astrocytes' failure to trigger the Nrf2–ARE system, which can culminate in exacerbating ROS/RNS accumulation, and a number of studies have explored the therapeutic value of Nrf2 activation [74–81].

Remarkably, an interactive connection between the *Nrf2–ARE* system, glial inflammatory pathways and WβC signaling was recently unveiled [25,66,70–72]. As documented in Section 2, the WβC system is necessary not only to promoting mDAn neurogenesis [25,68], but also for the maintenance of physiological adult mDAns, and it contributes to regulating inflammation, thanks to the dynamic glia–neuron dialogue [25,66–72]. Of particular interest is the control exerted by *Nrf2* in the regulation of NSCs, especially under inflammation, MPTP exposure, as well as the ageing process, as recently reviewed [68,70–72]. There is also an important role for DA [72] in the control of VM glial activation, via an interaction of DA receptor signaling cascades with the *Nrf2*/*WNT* signaling axis [72].

Moreover, as documented by Gan and co. [159], the specific upregulation of Nrf2 in astrocytes (*GFAP-Nrf2*) of hSYN(A53T) mice has been documented to postpone the beginning of the disease to increase the lifetime of diseased mice [159]. This condition also associates with an extended survival of motor neurons, together with decreased oxidative reactions as well as glial activation [159]. Furthermore, *Nrf2* in astrocytes delayed autophagic and macroautophagic dysregulation, thereby indicating that *Nrf2* in astrocytes provides neuroprotection against hSYN(A53T)-mediated toxicity with the promotion of hSYN(A53T) degradation through the autophagy–lysosome pathway in vivo [159].

Interestingly, with age, there is a downmodulation of the *Nrf2-ARE/WβC* axis in VM astrocytes that results in the increased expression of various microglial inflammatory

cytokines at both the Str and SNpc levels [69,71], whereas the protective anti-inflammatory cytokines, IL-4 and IL-10, are decreased, resulting in increased oxidative stress and inflammation that may have detrimental effects on mDAns [69,71]. Within the important elements of the Nrf2 pathway, there is glycogen synthase kinase-3 (GSK3-3β), a serine/threonine kinase that interacts with both Nrf2 and WβC pathways [70–72]. For example, active GSK3-3β is recognized to phosphorylate a group of Ser residues in the Neh6 domain of mouse Nrf2 that results in Nrf2 degradation [160,161]. Of specific mention, GSK3β communicates with both the ERK/MAPK and the phosphatidylinositol 3-kinase/Akt (PI3K/AKT), also known as protein kinase B pathways, which have fundamental roles in mDAn death/survival [72].

Also, with age, in the VM, it has been documented that astrocyte-derived WNTs decrease, and contrastingly, certain endogenous antagonists of the WβC pathway, for example, Dickkopf1 (Dkk1), increase, thereby contributing to the reduced ability of neurons to survive and recover after an insult, also accompanied by a significant reduction in neurogenesis [67–72]. The crosstalk of Nrf2 and WβC/GSK-3β has been observed in experiments carried out both in vitro and in vivo, with ageing and MPTP mouse model systems documenting their interaction in the regulation not only of mDAn homeostasis, but also in immune regulation, as well as neurogenesis, as recently summarized [70–72].

In fact, and of specific interest, the resilience of anti-oxidant/anti-inflammatory Nrf2/ARE and WβC signaling pathways can be achieved by grafting young (2–10 post-natal days) VM astrocytes within the MPTP-lesioned SN of aged mice, corroborating the unique repair capacity of VM astrocytes [71]. Hence, as shown by Serapide and co. [71], the transplanted astrocytes lead to a significant recovery of mDAns associated with a functional recovery of DA neurons, as documented by the normalization of striatal DA concentration and the improvement of motor function in the transplanted mice, when compared to control (i.e., mock-grafted) mice [71]. These effects were accompanied by VM astrocytes' increased antioxidant capacity, via the activation of the Nrf2/HO1/WNT/β-catenin pathway [71], thus leading the authors to suggest that grafting VM astrocytes has the potential to "switch the SN unfriendly environment to a beneficial antioxidant/anti-inflammatory pro-survival milieu" [71]. Importantly, studies have shown that inflammatory microglia-derived factors can activate the Keap1/Nrf2/HO-1 system in astrocytes, as reported by Takahashi, S et al. [130 and references therein]. VM astrocytes may then play key roles in mDAn protection under neurotoxic/inflammatory stimuli, by triggering a protective glia–neuron crosstalk via HO-1, as exemplified in the triple astrocyte–microglia–neuron culture paradigm (Figure 4). Especially, microglia can communicate directly with astrocytes through WNT release to activate WβC signaling [25,26,70–72,129], thus engendering a complete Nrf2/HO-1/WβC neuroprotective feedback loop. Then, VM glia–neuron interactions are the crucial actors for mDAn protection and repair [69–73,80,130,159].

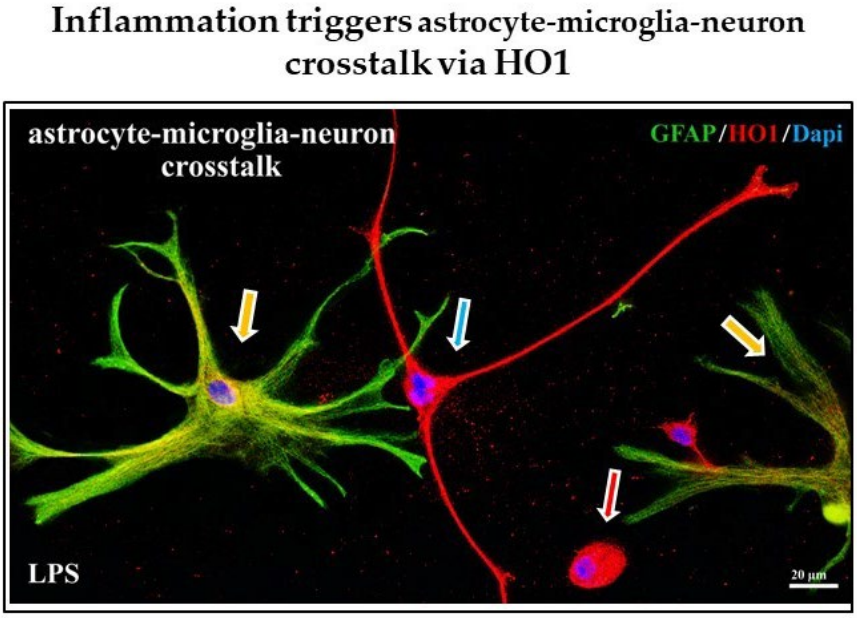


Figure 4. Inflammatory challenge upregulates neuroprotective HO-1 expression in astrocyte–microglia–neuronal cultures**.** Immunofluorescent (IF) image of a dual staining with glial fibrillary acid protein (GFAP, revealed in green) and Heme oxygenase 1 (HO-1, revealed in red) counterstained with the nuclear marker Dapi (in blue) in the in vitro setting of a triple astrocyte–microglia–neuron culture 24 h after a low (100 ng/ml) lipopolysaccharide (LPS) challenge, with HO-IF staining in GFAP-positive astrocytes showing a merged color (orange to yellow, arrows) and GFAP-negative cells expressing HO1-IF (in red) resembling activated microglia (red arrows) and neurons (blue arrow), highlighting astrocyte–microglia–neuron crosstalk [71].

Altogether, the growing body of research herein summarized provides compelling evidence of morphological, molecular and functional differences between VM astrocytes harboring essential properties for their partner mDAns during development, in the adult, ageing and lesioned brain. Therefore, VM astrocytes represent ideal targets for both PD- and LRRK2-diseased astrocytes, as summarized below. Given the vast array of essential pathways used, the following sections will discuss the different investigated impairments involved in LRRK2-affected astrocyte–neuron crosstalk, together with the outcomes for the vulnerable mDAns.

4. LRRK2 G2019s Impairs Astrocyte–Neuron Crosstalk

The involvement of LRRK2 kinase in various cellular pathways well anticipates that a disruption of LRRK2 in astrocytes may have harmful effects on neighboring neurons and microglial cells. Here, focusing on LRRK2 G2019S diseased astrocyte–mDAn crosstalk, we summarize the findings of research conducted during the last seven years, documenting the species-specific experimental systems and the *LRRK2* mutation studied (i.e., human iPSC-derived astrocytes and neurons from familial mutant *LRRK2* G2019S PD; mouse transgenic (TG) *LRRK2* G2019S and WT LRRK2). The different settings (monotypic astrocyte culture vs. dual or triple glia–neuron co-culture/mixed culture systems; or in vivo experimental conditions), brain regions (VM, Str, cerebral cortex, CX) and paradigms (toxic exposures, inflammatory challenges, LRRK2 antagonism) are also briefly summarized.

4.1. When and How the LRRK2 G2019S Mutation Affects Astrocyte–Neuron Crosstalk: Evidence for α-Syn Accumulation-Induced mDAn Degeneration

Compelling evidence of a key harmful *LRRK2* G2019S astrocyte–neuron interplay comes from the elegant studies of Di Domenico and co. (2019) [45]. In this study, to investigate whether the mutant *LRRK2* astrocytes might contribute to PD pathogenesis, the authors performed experiments on iPSC-derived astrocytes and neurons from the VM of *LRRK2* G2019S PD patients and healthy individuals (controls, Ctrls) [45]. After the characterization of VM astrocytes and the establishment of VM astrocyte–neuron co-culture models, the authors first documented the effects of *LRRK2* G2019S astrocytes expressing mutated *LRRK2* on the survival of Ctrl mDAns [45]. Hence, they showed alterations in the morphology of neurons, which included shorter neurites, associated to reduced neuron survival as compared to the co-cultures established with Ctrl astrocytes, which raised the question of a potentially detrimental molecule released by the LRRK2-affected astrocytes [45]. The possibility that α-syn might contribute to affecting mDAns in the cell culture experiments was next investigated by the authors, documenting that the G2019S mutation might impair the uptake of α-syn, but not its degradation [45]. Hence, Ctrl mDAns were shown to accumulate α-syn in the presence of PD astrocytes, whereas α-syn was almost undetectable in the cytoplasm of Ctrl mDAns, suggesting that α-syn from PD astrocytes might be transferred to Ctrl mDAns [45].

The ability of healthy astrocytes to rescue PD-mDAns from neurodegeneration was next studied, showing a partial recovery of neurite impairments when G2019S mDAns were cultured on the top of healthy astrocytes [45]. Specifically, the co-culture with Ctrl astrocytes prevented the accumulation of α-syn in G2019S mDAns [45]. Further evidence for a toxic role of α-syn was documented in experimental settings showing the direct uptake by mDAns of α-syn from conditioned medium [45]. Here, after the collection of the conditioned medium from PD α-syn-FLAG-tagged astrocytes and exposure of Ctrl mDAns, the authors further documented the neurotoxicity of PD astrocytes [45].

Given the key role of both proteasome and autophagic pathways for α-syn degradation, Di Domenico and co. [45] next documented that the lysosomal degradation of α-syn was significantly inhibited in G2019S-affected astrocytes, showing the disruption of chaperone-mediated autophagy (CMA) [45]. This effect suggests an almost complete inhibition of α-syn degradation by CMA in the mutated astrocytes [45]. Contrarily, and most importantly, treatment with a CMA activator was sufficient to restore α-syn to levels comparable to those in Ctrl cells [45]. However, in these conditions, there was only partial neuronal protection [45], thereby suggesting that, possibly, other non-α-syn-related factors secreted by PD astrocytes might contribute to mDAn degeneration [45].

Together, these and other results of this work suggest that *LRRK2*-mutated astrocytes can mediate toxicity in the course of PD via the transfer of toxic α-syn aggregates, resulting in mDAn degeneration [45].

4.2. LRRK2 G2019S Astrocytes Play a Decisive Role in Inhibiting mDAn Growth, Survival and Differentiation During Mouse Post-Natal Development [54,125]

The crucial role of *LRRK2* G2019S astrocytes for mDAn neuroprotection was investigated in our laboratory to deepen our understanding of astrocyte–neuron interactions during early post-natal development (Figure 5) [54,125]. To this end, we explored whether *LRRK2* G2019S mutation might impair VM astrocyte–neuron interaction, using WT-*LRRK2* and G2019S-*LRRK2* heterozygous [(C57BL/6J-TG [(LRRK2*G2019S)2AMjff/J)] mice prepared by crossing G2019S-*LRRK2* hemizygous females with WT C57BL/6J males [54,125]. Studying a number of co-culture settings and paradigms between VM astrocytes and mDAns, we documented that TH^+ axonal growth, processes number, and processes length were all sharply reduced in TG-*LRRK2* G2019S

astrocyte–neuron complexes vs. WT astrocyte–neuron counterparts during postnatal TH^+ neuron development [54,125]. By contrast, TG VM cultures depleted of TG astrocytes and exposed to WT VM astrocyte inserts displayed long, ramified processes [125]. On the other hand, G2019S astrocyte inserts powerfully inhibited WT neuron survival and growth, thus supporting the specificity of LRRK2 G2019S's astrocytic-harmful effect and corroborating the pro-survival effects of astrocytes on mDAns [45–55,125], as observed in our previous studies [26,34,35,66–73].

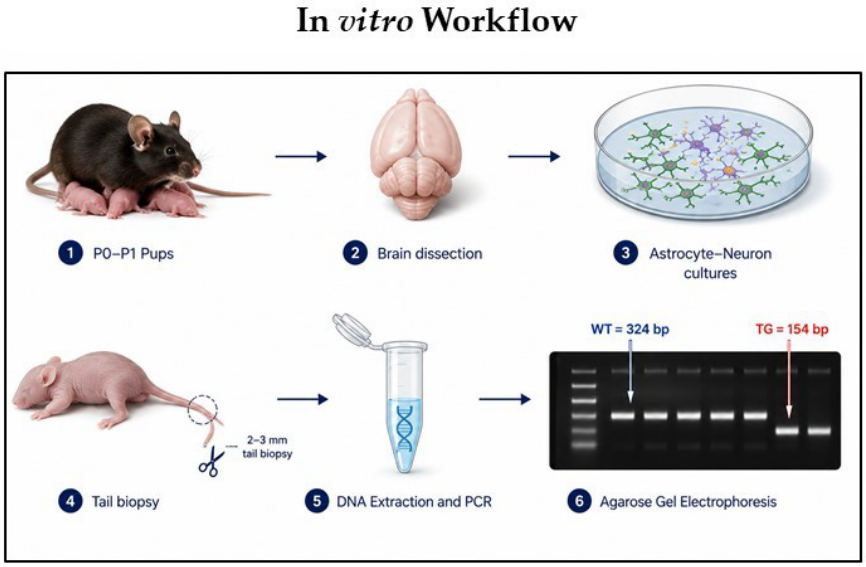


Figure 5. Schematic representation of the experimental design. 1. Newborn pups were sacrificed and 2. the ventral midbrain (VM) dissected; 3. primary astrocyte–neuron cultures were established from each isolated VM; 4. tails were collected from each pup for 5. DNA extraction and PCR; this was followed by 6. electrophoretic analysis. The day of birth was defined as postnatal day 0 (P0) and VM cultures were allowed to grow for different time intervals (7–18 days in vitro, DIV) [125].

Notably, as summarized in previous sections and Figure 4, there are important cell–cell interactions between astrocytes, microglial cells and neurons, but few studies have examined these conditions in the *LRRK2* G2019S mouse models. Whether and how a dysfunctional astrocyte–microglia–neuron dialogue underlying the impairment of astrocyte neuroprotection is observed in TG *LRRK2* G2019S glia–neuron cultures, when compared to WT counterparts, clearly needs to be further assessed.

4.3. RNA Sequencing Reveals Mmp2 and Tgfb1 Downregulation in LRRK2 G2019S Parkinson's IPSC-Derived Astrocytes [46]

In the studies of Booth and co. (2019) [46], iPSC-derived midbrain astrocytes were established from LRRK2 G2019S patients [46]. First, the authors showed LRRK2 expression in human midbrain astrocytes with comparable levels in cortical astrocytes and iPSC-derived mDAns, but with reduced levels measured in purified human microglia and oligodendrocytes [46]. In order to understand the effect of this mutation on astrocyte function, the authors compared the gene expressions of the mutated astrocytes as compared to those from healthy controls [46]. Here, the analyses documented a downregulation of important growth factors involved in the regulation of the ECM, also contributing to neuroprotection and immune reactivity [46]. Besides the ECM genes, TGFB1, which is known to inhibit glial inflammatory reactions [46], was also decreased [46]. Moreover, MMP2, which is recognized to degrade α-syn aggregates [46], was similarly downregulated [46]. These effects might explain the decreased neuroprotective

capacity of G2019S-affected astrocytes, possibly resulting from a more active inflammatory response, associated with their inability to degrade extracellular α-synuclein aggregates [46].

Overall, in this study, the authors introduce a novel deficit and propose a potential mechanism influencing the pathogenesis of LRRK2-PD via a transcriptomic dysregulation in midbrain astrocytes, with implications for neuroprotection in PD [46].

4.4. LRRK2 G2019S Mutant Astrocytes Affect ER and SERCA Activity [49]

In this study, Lee and co. (2019) [49] focused their attention on the role of a dysfunctional ER Ca^{2+} homeostasis and a potential SERCA malfunction leading to ER stress–mediated apoptosis [49]. Here, the authors studied G2019S-*LRRK2*-TG FVB and non-TG mice [49]. To this end, the G2019S-LRRK2-TG heterozygous mice were prepared by crossing G2019S-*LRRK2* heterozygous mice with WT mice [49]. Primary astrocytes were cultured from the cerebral cortices of 1-day-old non-TG and G2019S-*LRRK2*-TG heterozygous mice. The primary neurons were cultured from embryonic mouse cortices (E17). Additionally, organotypic brain slice cultures were prepared from postnatal day 7–8 non-TG and *LRRK2 G2019S* mice [49]. Notably, the 14–3-3 proteins (i.e., a family of seven conserved adapter proteins) have a recognized role in the localization of LRRK2 [49]. Given that the binding to LRRK2 is disrupted in some PD-related LRRK2 mutations, the authors investigated a potential alteration in the interaction of 14–3-3 proteins with LRRK2 in LRRK2 G2019S astrocytes [49]. This set of experiments documented that 14–3-3 proteins are involved in the localization of LRRK2 to ER [49].

An extensive series of additional studies was next carried out to determine the ER proteins that might interact with *LRRK2 G2019S*. Here, an increased LRRK2–SERCA2 interaction was documented in LRRK2 G2019S astrocytes [49]. A further set of analyses next documented that leucine-rich repeat (LRR) and carboxy-terminal of Roc (COR) domains of LRRK2 interacted with SERCA [49], in analogy with recognized studies showing that LRR and COR domains participate in protein–protein and intramolecular interactions [see 49,91]. Also, other studies showed that *LRRK2* and SERCA interactions in *LRRK2*–GS astrocytes can cause the dysregulation of SERCA, further leading to ER Ca^{2+} depletion, and possibly increased ER stress-induced cell death [49].

Collectively, these extensive series of important data suggest that in *cortical* astrocytes, *LRRK2 G2019S* may impair Ca^{2+} homeostasis, which determines cell survival, thereby contributing to the development of PD [49].

4.5. How Mutant LRRK2 Interferes with Astrocyte-Mediated α-Syn Clearance in Mouse Cortical and Striatal Astrocytes [47]

Alterations/impairments in astrocytic α-syn dynamics of LRRK2-diseased astrocytes have further attracted the attention of other laboratories and researchers in the field. For example, to explore α-syn dysfunctional dynamics in LRRK2-affected astrocytes, Streubel-Gallasch and co. (2021) used C57Bl/6J LRRK2 WT (*LRRK2*+/+) and knock-out (*LRRK2*−/−) mice, as well as LRRK2 G2019S knock-in mice, backcrossed on a C57Bl/6J background [47]. In this study, astrocytes were derived from mouse embryonic cortices (cortical stem cell-derived astrocytes), or from the striatum of postnatal mice between days 1 and 3 (primary striatal astrocytes) [47]. In some studies, Human H4 neuroglioma cells (immortalized H4 cells) were used [47]. The experimental protocol involved exposure to α-syn pre-formed fibrils (PFFs)/α-syn monomers and cell transfection, besides others [47]. In a first set of experiments, the authors used monotypic astrocyte cultures, depleted of microglia [47]. The α-syn uptake and degradation capacities were studied in cortical stem cell-derived astrocytes isolated from *LRRK2*−/− and *LRRK2* GS/GS mice [47].

The results of these experiments then suggested that the pathogenic mutation influences the uptake rather than the degradation of α-syn [47].

The high expression of *LRRK2* in the striatum also prompted the authors to carry out the study in isolated striatal astrocytes from the three genotypes, which further supported that the *LRRK2* G2019S pathological mutation modified the ability of striatal astrocytes to intracellularly accumulate exogenous α-syn [47]. Next, the functional aspects of the endo-lysosomal pathways (ELP) were investigated in mouse striatal astrocytes in the three genotypes using various methods [47]. In this detailed part of the study, the authors documented that both LRRK2 ablation and the G2019S pathological mutation caused a significant decrease in the number of lysosomal-like structures in astrocytic cells [47]. Using further techniques, the authors next corroborated that the G2019S mutation influences the morphology and number of the endo-lysosomal vesicles, but not the pH [47].

To identify LRRK2-specific effectors of astrocyte-mediated α-syn clearance using an unbiased approach, the authors then focused on a possible role of ANXA2 in regulating exogenous fibrillar α-syn internalization [47]. This thorough series of studies suggested that ANXA2 deficits might influence the astrocytic ability to store α-syn aggregates [47].

Together, these data and the other results of these interesting studies offer a more comprehensive understanding of the molecular mechanism underlying the impaired α-syn clearance in G2019S astrocytes, and prove the LRRK2 kinase's specific involvement. Such deficits, documented in mouse cortical stem cell-derived PD astrocytes, and post-natal PD striatal astrocytes, coupled to the described impairments summarized above in the VM astrocytes, then corroborated a common genotype-specific α-syn-detrimental trait of *LRRK2* G2019S astrocytes.

4.6. Is the Intercellular Communication via Exosome Altered in G2019s Astrocyte-mDAn Cultures? [53]

As summarized in the study of Rus Jacquet et al. [53], there is a difference in the composition of EVs isolated from the biofluids of PD patients as compared to those of healthy controls, which also contain proteins associated with PD, such as LRRK2 and αSyn [53]. Hence, in their work, the authors used LRRK2 G2019S astrocytes generated from patient-derived iPSCs, from PD patients with the LRRK2 G2019S mutation and its gene-corrected isogenic control [53]. A second independent pair of iPSCs prepared from a LRRK2 G2019S PD patient and a sex- and age-matched control was also studied, as reported [53]. Astrocytes were derived from NPCs patterned toward a midbrain fate, to generate a population of renewable midbrain NPCs that can differentiate into astrocytes or dopaminergic neurons, depending on exogenous cues [53]. The midbrain and floor plate identity of the NPCs was systematically validated for each iPSC-to-NPC differentiation by RT-qPCR [53].

To understand whether astrocyte-derived EV uptake by dopaminergic neurons is affected, the authors investigated the internalization of WT and LRRK2 G2019S astrocyte-derived exosomes by mDAn [53]. To this end, mDAns were co-cultured with either WT or G2019S astrocytes, although the authors did not observe a difference in the proportion of exosome-containing neurons. The results of this experimental series show that astrocyte-secreted exosomes are internalized by neurons, but in the presence of PD astrocytes, the pattern of uptake of exosomes is changed, suggesting, as reported in their publication, that the astrocyte-to-neuron shuttling of EV-enclosed proteins may be affected [53].

The functional consequences of the LRRK2 G2019S mutation in astrocyte–neuron interactions were next studied in 14-day-old co-cultures [53]. The results show an impairment of mDAn survival and neurite deficits [53]. Further studies suggest that the

morphological atrophy in dopaminergic neurons may result from a lack of neurotrophic support by the LRRK2 G2019S astrocyte secretome [53].

To study astrocytes' secretory pathways, dopaminergic neurons were cultured for 14 days in basal medium, or EV-free medium prepared from LRRK2 G2019S or isogenic control astrocytes [53]. The overall experimental series documented that WT astrocytes may release neurotrophic factors not only via direct release into the extracellular compartment, but also via the production and release of EVs, whereas these mechanisms are dysfunctional in the LRRK2 G2019S model of PD [53].

Performing a Gene Ontology (GO) analysis of downregulated genes in LRRK2 G2019S astrocytes next revealed certain impairments in some genes associated with cell growth and survival in mutant astrocytes [53]. Interestingly, among downregulated genes, *BIRC5*/survivin was downregulated [53]. Notably, survivin, via the WβC pathway, is a key mediator in NPCs' proliferation, whereas with age, NPC proliferation is decreased via a downregulation of WNTs genes in both the striatal subventricular zone and hippocampus [61,162]. These and other findings suggest a deficit of astrocytic prosurvival/neuroprotective pathways in *LRRK2*-mutant astrocytes, as observed in astrocytes of ageing mouse models [68–73].

Overall, these studies highlight that astrocyte-to-neuron signaling and trophic support mediated by EVs are dysregulated and contribute to *LRRK2* G2019S astrocyte-mediated dopaminergic neuron death [53].

4.7. Astrocytic Atrophy and Dysfunctional Mitochondrial Metabolism Are Pathological Features of LRRK2 Mutation [48]

In the study of Ramon-Gonzalez (2021) [48], astrocytes were derived from hiPSC of both PD patients carrying the LRRK2 G2019S mutation and healthy donors of a similar age [48]. Here, the authors documented a significant morphological difference between healthy astrocytes as opposed to PD astrocytes, whereby G2019S astrocytes showed a smaller cell body area and a lower process complexity, among other things, suggesting a reduced neuron supportive ability [48]. This effect appeared specific for astrocytes, as such morphological changes were not detected in *LRRK2* G2019 fibroblasts [48].

Studying the functional properties of PD and control astrocytes, the authors used the Seahorse technology to investigate mitochondrial metabolism in healthy and PD-affected astrocytes [48]. A simplified representation of the astrocytes' bioenergetic profile and the compounds used in the Seahorse live cell assay is summarized in Figure 6. After the determination of the mitochondrial oxygen consumption rate (OCRs), Ramon-Gonzalez and coworkers [48] documented, among other results, that PD astrocytes had a reduced OCR, as compared to healthy cells [48]. PD astrocytes also produced less ATP, associated with an increased glycolytic capacity [48]. Together, the data presented in the article Ramon-Gonzalez et al. [48] indicate that "PD astrocytes shift from oxidative phosphorylation to the aerobic glycolytic respiration" [48].

As a next step of their study, Ramos-Gonzalez and co. [48] asked whether mitochondrial malfunction was then associated with possible abnormal mitochondrial morphology by comparing intracellular distribution and the ultrastructure of the mitochondria in healthy and PD astrocytes [48]. As documented in their study [48], in healthy astrocytes, mitochondria were elongated and interconnected, whereas PD astrocytes had, aside from other results, fewer mitochondria, these being more fragmented and more rounded than in the control cells [48]. As to the astrocytic metabolic profile, the authors documented higher amounts of oxidized proteins in PD astrocytes when compared to the controls, suggesting a higher basal reactivity of PD astrocytes compared to healthy controls [48].

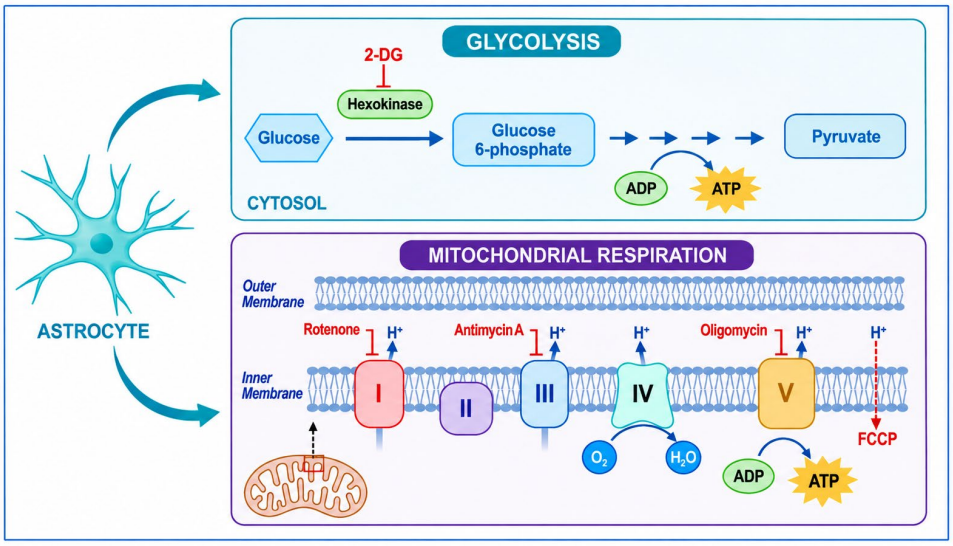


Figure 6. Simplified representation of bioenergetics in astrocytes using the Seahorse live cell assay. Cells generate energy in the form of ATP by a combination of two main metabolic pathways: glycolysis (above) and oxidative phosphorylation (below). The various compounds used to examine the bioenergetic profiles of cells are shown at their respective sites of action (in red). The glucose analog 2-DG cannot undergo conversion to glucose-6-phosphate, thereby competitively inhibiting glycolytic ATP production. The mitochondrial electron transport chain (ETC) consists of five enzyme complexes (I, II, III, IV, and V). Rotenone and antimycin A block complex I and complex III of the mitochondrial ETC, respectively, while oligomycin inhibits complex V by blocking its proton channel. FCCP is an uncoupling agent that permeabilizes the inner mitochondrial membrane to protons, forcing the mitochondria to increase the flow of electrons (and thus oxygen consumption) to maintain the membrane potential [125].

Collectively, these results thus indicate that LRRK2 G2019S mutation promotes a general mitochondrial dysfunction in astrocytes, which may contribute to neuronal demise via a reduced metabolic support and increased reactive status, leading to the failure of astrocyte neuroprotection [48] (Figure 7).

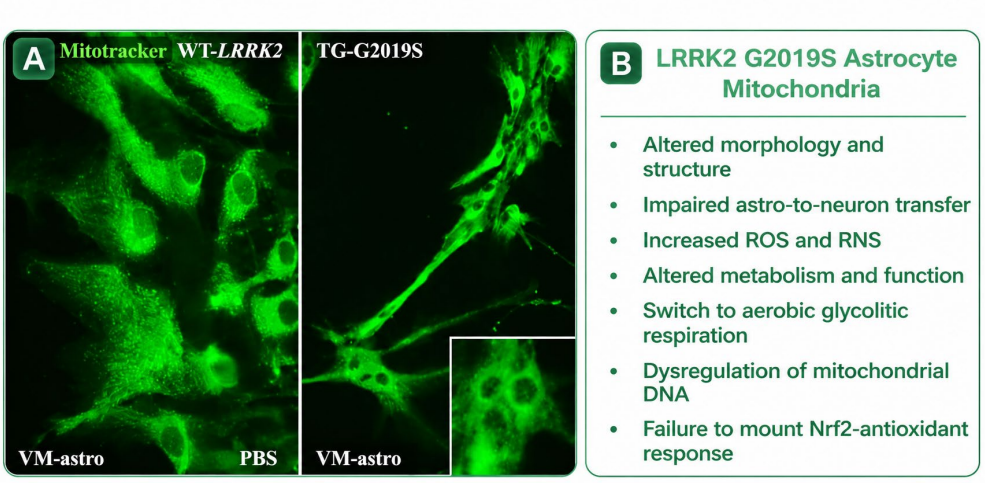


Figure 7. Mitochondrial properties of TG *LRRK2* G2019S astrocytes. (**A**) Representative images of mitochondrial distribution as visualized by staining with Mitotraker green in control (WT) and TG G2019S ventral midbrain (VM) astrocytes. Primary VM astrocyte cultures were treated with 100 nM of Mitotracker green (Molecular Probes/Invitrogen) for 30 min at 37 °C; they were washed and the mitochondria were visualized using confocal imaging [125]. In WT astrocytes, mitochondria appear

more homogenously distributed throughout the entire cytoplasm as compared to TG astrocytes. The inset represents a higher magnification of the field. Some impairments of mitochondrial function of *LRRK2* G2029S astrocytes summarized in the text are boxed in (**B**).

Studies carried out in our laboratory tested mouse TG LRRK2 G2019S and WT LRRK2 VM astrocytes both in basal and stimulated conditions with a low dose of LPS (100 ng/mL), and looked at mitochondrial bioenergetics using the Seahorse technology to analyze astrocyte mitochondrial OCR under basal and LPS-activated conditions [54,125]. Here, a decreased OCR was observed, especially under LPS [125], denoting the impairment of mitochondrial homeostasis in TG *LRRK2* G29019S astrocytes leading to a reduced adaptation capacity [125]. Such mitochondrial malfunction often associates with abnormal morphology [48], as herein observed when comparing WT and TG G2019S VM astrocyte cultures stained with Mitotracker green (Figure 7A), showing an impaired intracellular distribution of mitochondria in TG G2019S-dysfunctional astrocytes. Also, when exposed to LPS, TG *LRRK2* G2019S astrocytes showed an increased glycolytic activity with respect to the WT LRRK2 LPS-treated astrocyte cultures [125]. When the capacity of astrocytes to counterbalance mitochondrial impairment (i.e., "compensatory glycolysis") was studied [125], we documented a significant increase in glycolytic activity under the inflammatory challenge, possibly reflecting the need to equilibrate the metabolic impairment of the diseased TG G2019S astrocytes [143–145]. However, and as discussed in previous sections, such increased glycolysis may further activate inflammatory reactions by stimulating the already activated astrocytes [143–145], with potential harmful effects for mDAns. Notably, we found the exacerbated generation of ROS and RNS by TG G2019S astrocytes exposed to LPS, as above [54,125]. Also, we documented an upregulation of proinflammatory cytokines in the conditioned media (CM) of TG G2019S astrocytes when compared to the WT LRRK2 astrocyte counterparts [125], under a low inflammatory (LPS) and/or neurotoxic (MPP^+) challenge, at least in part counteracted by LRRK2 antagonism [125], supporting the excessive reactivity of G2019S-affected astrocytes [125]. Altogether, such a dysfunctional astrocyte response to inflammation and oxidative stress appears to be implicated in G2019S astrocytes' failure to exert neuroprotection.

4.8. LRRK2 G2019S Astrocytes Display Metabolic Alterations, Increased Reactivity and Disturbed Calcium Signaling [50]

In the same years, metabolic alterations were also assessed in the study of Sonninen and co. (2020) [51]. In this study, astrocytes were derived from the hiPSC of PD patients carrying the LRRK2 G2019S mutation, with one patient also carrying a GBA N370S mutation, as well as healthy subjects [50]. The authors documented, besides other things, an increased expression of α-syn detected with ICC, qRT-PCR for mRNA transcript and ELISA for protein levels [50]. The inflammatory response was studied by the authors after exposure to TNFα, IL-1β and IFNγ [50]. They found a greater secretion of both IL-6 and RANTES from PD astrocytes under TNFα and IL-1β when compared to control cells and isogenic controls, whereas in IFNγ-exposed cells, neither IL-6 nor RANTES were secreted [50]. Looking at gene expression under the same inflammatory challenges, both GFAP and LCN2 (lipocalin-2) expressions were significantly increased in PD astrocytes [50], supporting the enhanced reactive status of LRRK2 G2019S astrocytes [50]. Regarding the Ca^{2+} homeostasis, in LRRK2-diseased astrocytes under stimulation with Cresol, the amplitude of $[Ca^{2+}]$ responses to Cresol was greater in PD astrocytes as compared to controls [50]. When the metabolic activity of the cells was analyzed by Seahorse XF Technology, the authors found, besides other things, no difference in basal OCR levels, but both maximal and spare respiration were significantly decreased in the PD astrocytes,

indicating altered mitochondrial function [50]. Additionally, at the basal level, the healthy astrocytes were more glycolytic when compared to the PD cells [50]. Also, defects in mitochondrial DNA (mtDNA) copy number were observed, associated with alterations, among other things, of the astrocytes' polyamines putrescine and spermidine in PD1 astrocytes and their precursors in PD astrocytes [50].

Together, in this study, PD patient astrocytes displayed, among other things, an increased production of α-syn, greater astrocytic Ca2+ responses to activation, increased reactive inflammatory status upon cytokine challenge, mitochondrial alterations/defects, metabolic changes, and altered polyamine metabolism, all of which may contribute to PD advancement [50].

4.9. LRRK2 G2019S Mutation Contributes to a Dysfunctional Astrocyte-to-Neuron Mitochondrial Transfer [55]

As reviewed in previous sections, another critical aspect of astrocytes' beneficial function is their ability to transfer healthy mitochondria to surrounding cells, including neurons (Section 3.2) [140,141]. The potential dysfunction in astrocytes' delivery of healthy mitochondria to neurons was studied by Ding and co. [55]. For the generation of human-IPSC, human blood samples were collected from a PD patient with *LRRK2* G2019S mutation and a healthy subject [55]. Here, mDAns and astrocytes were differentiated and characterized, and their identity confirmed as described [55]. The established co-culture system between hiPSC-derived DA neurons and astrocytes documented that neurons with the *LRRK2* G2019S mutation displayed an increased sensitivity to environmental toxins [55], such as the PD neurotoxin rotenone [55].

Previous early and more recent studies, including our own [26,54,66–73], have demonstrated the protective role of VM astrocytes in co-culture with mDAns, under various harmful oxidative/inflammatory challenges, including rotenone exposure [55]. Therefore, the authors investigated the protective capacity of *LRRK2* G2019S astrocytes as compared to their non-affected astrocyte's counterparts in co-culture with mDAns [55]. When a co-culture of WT astrocytes with *LRRK2* G2019S-mutant DA neurons (Astrocyte healthy + Neuron *LRRK2* G2019S) was used, the authors found that the rotenone-induced damage to DA neurons was less severe than it was in the mutant co-culture system (Astrocyte *LRRK2* G2019S + Neuron *LRRK2* G2019S) [55]. Also, upon exposure to rotenone, the number of labeled astrocytic mitochondria in DA neurons decreased in a dose-dependent manner, indicating that *LRRK2* G2019S may reduce the mitochondrial transfer between astrocytes and DA neurons [55], thereby supporting a defect in the communication capacity of mutated astrocytes in their dialogue with mDAns [55].

The authors next investigated the potential roles of major SNARE proteins (key mediators of membrane fusion) in the regulation of mitochondrial exocytosis [55]. Particularly, the authors looked at Syntaxin 17 (STX17), a crucial mitochondrial protein located on the mitochondrial outer membrane, which is involved in the regulation of mitophagy, mitochondria–endoplasmic reticulum crosstalk, and other important functions, thanks to its role in mediating membrane fusion [55]. To investigate this issue, the authors used RNA interference technology to verify the effect of the knock down of STX17, VAMP3, and SNAP23 in astrocytes, then the quantification of astrocyte-derived mitochondria in ACM was assessed via flow cytometry [55]. The overall results suggest that STX17 is closely associated with the mitochondrial outer membrane and may be related to mitochondrial transfer [55]. Given the involvement of Drp1 (recognized to regulate mitochondrial fission), several studies were undertaken to examine whether, in mutant astrocytes, LRRK2 phosphorylation at Ser1292 (which is increased in mutant astrocytes) might play a role in Drp1 phosphorylation [55]. The authors used LRRK2 kinase inhibitor PF-06447475 and the Drp1 phosphorylation inhibitor DUSP6 in the

presence of rotenone, showing that the impairment on mitochondrial transfer can be reversed by LRRK2 antagonism and the Drp1 phosphorylation inhibitor *DUSP6*.

5. Astrocytes Carrying LRRK2 G2019S Mutation Display Increased Levels of Clusterin (Clu) via miR-22-5p, Morphological Impairments and Synapse Defects [163,164]

In a recent work, Filippini and co. (2025) [163] investigated the involvement of Clu in LRRK2 G2019S-mediated aggregated α-syn ingestion, together with the underlying molecular mechanisms [163]. Using the LRRK2 G2019S knock-in (KI) mouse strain, they first validated the inability of primary astrocytes (postnatal days 1–4, P1-P4) from cerebral cortices to exhibit a reduced capacity to ingest fibrillary α-syn. Then, they investigated whether LRRK2 G2019S might affect this pathway through the modulation of Clu [163]. A series of experiments carried out in brain slices and primary astrocytes from KI mice with the LRRK2 G2019S pathological mutation next documented increased levels of Clu protein compared to the respective WT counterparts [163]. Accordingly, they observed an opposite effect in brain slices and primary astrocytes from LRRK2 knock-out (KO) mice in comparison to their respective WT cultures. Additionally, they documented that LRRK2 controls Clu expression at the translation level through the action of miR-22-5p [163]. Moreover, they demonstrated that treatment with miR-22-5p mimic can improve the ability of LRRK2 G2019S-KI astrocytes to take up α-syn. Taken together, the authors indicate that the LRRK2-Clu pathway is involved in the ingestion of a-syn fibrils, and that the impairment of α-syn uptake in LRRK2 G2019S-KI astrocytes is associated with Clu levels [163], adding an additional feature of the commonly described α-syn pathology of G2019S astrocytes.

Wang, S. and co. recently documented in a pre-print article [164] whether and how LRRK2 G2019S might modulate the phosphorylation of the ERM proteins (Ezrin, Radixin, and Moesin), components of the perisynaptic astrocyte processes in a subset of mouse cortical astrocytes [164]. They found that ERM increased phosphorylation associated with the decreased morphological complexity of astrocytes, together with a decreased density and function of excitatory synapses [164]. As a proof of concept, reducing ERM phosphorylation levels in LRRK2 G2019S astrocytes restored both their morphology and the excitatory synapse density in the anterior cingulate cortex [164]. To determine how LRRK2 mutation impacts Ezrin interactome, the authors used an in vivo BioID proteomic approach, and found that astrocytic Ezrin interacts with Atg7, a master regulator of autophagy [164]. The Ezrin/Atg7 interaction is inhibited by Ezrin phosphorylation, thus diminished in LRRK2 G2019S astrocytes. Importantly, the authors indicated Atg7 function as a critical requirement to maintain proper astrocyte morphology [164].

6. Is the Nrf2/ARE System Involved in LRRK2-Affected Astrocytes?

As discussed in previous sections, a chief astrocytic beneficial actor for mDAn neuroprotection is Nrf2, with this aspect being touched on by different laboratories. Earlier studies of Skibinski and co-workers [165] expanded the biological role of Nrf2 by showing its ability to alleviate LRRK2- and α-Syn-induced neurodegeneration [165]. Here, specific *Nrf2* mechanisms are at play in improving neuronal survival against PD toxicity caused by both mutant LRRK2 and α-synuclein [165], including the Nrf2 reduction of the steady-state levels of α-Syn in part by increasing α-Syn degradation [165]. The studies of Lin and co (2016) [166] investigated the involvement of Nrf2 in the pharmacological reduction of G2019S PD, documenting the ability of lovastatin treatment to provide neuroprotection in the G2019S-induced PD model through the Akt/Nrf2 pathway [166]. In flies, Lin et al. [82], looking at Nrf2 phosphorylation and HO-1 expression levels,

showed that glial Nrf2 is inactivated in LRRK2 G2019S-affected flies, but it was activated by the herbal medicine *Gastrodia elata* Blume (GE), this being reported to have neuroprotective effects in toxin-induced PD models [82]. Hence, the G2019S flies treated with water extracts of GE (WGE) and its bioactive compounds, gastrodin and 4-HBA, displayed locomotion improvements and dopaminergic neuroprotection [82]. The genetic data indicate the impairment of the glia Nrf2 function of G2019S-affected flies. Also, Nrf2 depletion from glia eliminated the beneficial effects of WGE, whereas glial Nrf2 activation partially replaced the effect of WGE [82]. Collectively, the authors indicated that WGE-induced Nrf2 activation in glia, but not in neurons, protected dopaminergic neurons from degeneration and ameliorated impairments in locomotion [82].

To address the coordinated response of a panel of Nrf2 interactors and oxidative stress markers in *LRRK2* G2019S and WT *LRRK2* VM mouse astrocytes, in our studies, we concomitantly associated the response of a crucial NADPH oxidative stress transcript member, the subunit *gp91Phox* (phagocyte oxidase, also known as *Nox2*). Notably, *Nox2* chiefly contributes to oxidative stress and inflammation-dependent mDAn degeneration [21–24,71]. Interestingly, we found that *LRRK2* G2019S astrocytes of the VM fail to mount an *Nrf2*/*HO1*-driven anti-oxidant response, as observed in aged mice treated with MPTP. This effect associated with an exacerbated astro- and microgliosis with impaired mitochondrial function [71], suggesting that astrocytes' dysfunctional Nrf2–HO1 axis leads to reduced neuroprotective capacities, and this may participate in mDAn deterioration in PD and LRRK2-PD [70–72].

In the study of Kawakami and co. [167], the authors observed a reduced expression of *Nrf2* and of its target genes both in the LRRK2-TG mouse brain and in LRRK2-overexpressing SH-SY5Y cells [167]. Given the important role of active GSK-3β in LRRK2 phosphorylation and inactivation, it was further shown that the knockdown of GSK-3β recuperated Nrf2 expression in LRRK2-overexpressing SH-SY5Y cells, suggesting that a reduction in Nrf2 expression by LRRK2 may be part of the mechanism(s) in LRRK2-overexpression-induced vulnerability to oxidative stress in neuronal cells [167].

The study of Banerjee and co. (2023) [168] involved Nrf2-mediated glutathione machinery, glutamate uptake and metabolism, and ATP generation in astrocytes derived from LRRK2-I1371V PD patient iPSCs, a mutant variant, which is prevalent in East Asian populations, and healthy controls (HC) [168]. They found a significant decrease in the astrocytic ability to mitigate oxidative stress in terms of glutathione content in the mutant astrocytes, along with a reduction in the gene expression of the enzymes involved in glutathione machinery and Nrf2 expression [168]. Simultaneously, a significant decrease in glutamate uptake was observed in LRRK2-I1371V astrocytes, with the lower gene expression of glutamate transporters (SLC1A2 and SLC1A3) [168]. A reduction in the protein expression of SLC1A2 was also directly confirmed [168]. Enzymes catalyzing the generation of γ glutamyl cysteine (the precursor of glutathione from glutamate) and the metabolism of glutamate to enter the Krebs cycle (α-ketoglutaric acid) were impaired, with significantly lower ATP generation in LRRK2-I1371V astrocytes [168]. De novo glutamine synthesis via the conversion of glutamate to glutamine was also affected, indicating glutamate metabolism dysfunction in affected astrocytes [168]. The overall data demonstrate for the first time that the mutation in the LRRK2-I1371V allele causes significant astrocytic dysfunction with respect to Nrf2-mediated antioxidant machinery, ATP generation, and glutamate metabolisms [168].

Not only in astrocytes, but also in microglial cells, Nrf2 can play a counter-regulatory role in downregulating the exaggerated proinflammatory glia phenotype [169]. Within this frame, Liu and co. (2025) recently [170] presented novel findings demonstrating that LRRK2 regulates microglial neuroinflammation and ferroptosis through the p62–Keap1–Nrf2 signaling axis in the context of PD [170]. Using α-syn-stimulated BV2 microglial cells,

the authors found that LRRK2 inhibition significantly reduced the production of pro-inflammatory cytokines and enhanced the activation of the *p62–Keap1–Nrf2* pathway, thereby mitigating ferroptosis and oxidative stress [170]. Furthermore, it also conditioned the medium from LRRK2-inhibited microglia and promoted neuroprotective effects on cultured neurons, highlighting the therapeutic potential of targeting *LRRK2* in microglia [170]. Importantly, these in vitro findings were corroborated in the MPTP-induced PD mouse model, where LRRK2 inhibition led to diminished microglial activation, the decreased apoptosis of midbrain dopaminergic neurons, and the upregulation of the *p62–Keap1–Nrf2* pathway [170], suggesting that LRRK2 antagonism in microglia may represent a promising therapeutic strategy for PD [170].

In the studies of Weindel and co. (2026) [171], the authors used a differential gene expression analysis and found that the *Nrf2* pathway was increased in microglia of *LRRK2 KO* mice, associating with healthier mitochondria. On the other hand, the inhibition of LRRK2 kinase in peripheral macrophages had opposite effects, with reduced Nrf2 protein expression [171]. These and other data show that LRRK2 kinase function displays different roles in brain microglial cells vs. peripheral macrophages [171], underlying a different role for LRRK2 within central and peripheral immune cell populations [171].

Together these studies underscore the dysfunctional glial Nrf2–antioxidant axis in LRRK2 G2019S mutation as likely contributing to the inhibition of astrocytes' neuroprotective capacities, and participating in mDAn degeneration in PD and LRRK2-PD.

7. What Are the Consequences of Dysfunctional G2019S Astrocyte–Neuron Crosstalk in Programming the Vulnerability of mDAns in Adult Life?

From the summarized findings, it seems feasible that a disturbed astrocyte–neuron communication intersecting LRRK2 mutations during early life can have lifelong effects, as recalled in the Introduction [29–36], possibly increasing PD risk and/or modulating mDAn vulnerability to inflammatory/neurotoxic challenges in adult life and ageing.

For example, the early postnatal development corresponds to a developmental "critical period" (i.e., around 16–20 days post-natal life), a specific phase of active astrocyte maturation, whereby nigrostriatal neurons appear particularly vulnerable to mutant LRRK2 [31,114]. Hence, the timing of LRRK2 developmental expression in Str indicated that LRRK2 regulates the development, maturation and function of synapses, particularly during the post-natal developmental window [31]. By contrast, LRRK2 G2019S mutation impairs "vesicle recycling, trafficking and possibly protein synthesis during development of striatal circuits" [31,114], possibly also involving dysfunctional striatal astrocyte dynamics, as previously summarized [47]. In our in vivo studies, investigating the interaction between G2019S mutation and inflammation during the critical developmental window (post-natal days P14-16) or as the mice age (6 to 16 months), we found increased susceptibility to a subthreshold inflammatory challenge in TG -G2019S as compared to WT-LRRK2 mice, resulting in exacerbated gliosis associated with mDAn loss [125].

Together, the presented studies underscore that dysfunctional astrocyte–neuron crosstalk as a key event programming the vulnerability/predisposition to mDAn degeneration in later life, and future studies are needed to identify/establish the crucial mechanisms involved and their therapeutical potential.

8. Conclusions

Within the complex interplay between genetic and environmental factors participating in mDAn loss in PD, we herein discuss *LRRK2* mutations, particularly *LRRK2* G2019S mutation, recognized to cause "late-onset, autosomal dominant PD" [7], which is clinically and pathologically similar to idiopathic PD [7–17], with a main focus on astrocyte–neuron bidirectional crosstalk malfunction as a specific driver of mDAn vulnerability/death, thereby defining astrocytes as likely targets in PD and LRRK2-mediated PD [40–55].

We herein review the evidence corroborating "a developmental hypothesis" for PD pathogenesis, whereby genes, crucial transcription factors and developmental pathways linked to mDAn vulnerability [29–36] robustly interact with early life events and key hormonal and inflammatory/anti-inflammatory signaling systems [20,34,35], putting forward the hypothesis that dysfunctional astrocyte–neuron bidirectional crosstalk during neurodevelopment might mediate mDAn vulnerability later in life.

Hence, the multifunctional roles of LRRK2, from neurodevelopment to neurodegeneration, were briefly introduced, with a specific focus on the discovered interaction with key "WNT signaling proteins of the β-catenin destruction complex and DVL proteins" [92], essential actors for mDAn development [92–99]. In fact, critical PD mutations were demonstrated to negatively affect WβC signaling. Remarkably, LRRK2 mutations/altered WNT signaling present during brain development and beyond can exert significant effects on the establishment and maturation of neural circuits [28–32,100–115], as well as in the maturation of astrocyte–neuron interactions [36–39], thereby impacting mDAn function from neurodevelopment and throughout life, and/or on mDAn predisposition to disease and/or death.

Given that LRRK2's increased kinase activity is a crucial feature observed in both familial and sporadic PD cases, it is in turn implicated in modulating important cellular pathways, especially mitochondrial processes. Other processes include oxidative phosphorylation and ATP production, whereby a dysregulation of these processes likely results in compromised mitochondrial function in neurons and glial cells, then contributing to neuronal degeneration in PD [117–122]. Hence, LRRK2 mutation may engender a vicious cycle of increased LRRK2 kinase activity and exacerbated oxidative stress, in turn hyper-activating glial responses causing mitochondrial dysfunction, then resulting in neurodegeneration. These cascading interactions may be further perpetrated according to age and dysfunctional astrocyte–microglia crosstalk, as observed in PD.

Within this frame, we herein summarized some major aspects of astrocyte–microglial malfunction in PD, with a major focus on the dual Jekyll/Hyde facets of this dialogue [123–139], and attempted to overview the myriad of astrocytes' cell functions. The ample evidence indicating that astrocytes have extensive functional heterogeneity and respond to stimuli in a context-dependent manner was recapitulated [37–39,59,60], together with their crucial role in dictating neuronal vulnerability and resilience, whereas it was shown that diseased astrocytes undergo important functional changes that ultimately affect the viability of neurons [40–44,70–72,83,84].

Remarkably, astrocyte cell populations display a significant diversity within the different CNS regions and within subcellular compartments, especially within the VM [83,87,112,155]. Also, there is a broad cross-talk between energy and redox pathways in astrocyte–neuron dialogue [60–64], although with the ageing process such metabolic plasticity is lost, with detrimental consequences for VM astrocyte–neuron crosstalk [142]. Region-specific astrocyte gene networks then correlate with astrocyte morphology, together with "risk genes" associating with pathological CNS disease [152,153], altogether revealing a high degree of astrocyte organization and complexity.

Amazingly, astrocytes' plasticity and astrocyte–neuron reprogramming, with "beneficial" mitochondrial donation/transfer [140,141], clearly represent crucial aspects of the astrocytes' multifaceted functional properties [146–152,155] linked to their resilience upon injury. However, in diseased conditions such as NDs, astrocytes undergo transcriptional, functional and morphological changes, which can cause a severe inhibition of their global homeostatic functions. Especially, astrocytes' neuroprotective capacities are impaired, while they may gain destructive properties, with a negative impact on disease progression [123–125]. Particularly, under inflammatory conditions, astrocytes undergo specific changes as a function of a specific brain region, leading to differential neuronal outcomes [130–137,151–155].

Of primary interest, an important aspect is represented by astrocytes' ability to sequester α-syn aggregates from the extracellular space; a disruption of this mechanism leading to pathological accumulation can disrupt astrocytes' lysosomal machinery, inducing dysfunctional cell-to-cell transfer between astrocytes and neurons [128,137].

The critical role of astrocytes in modulating the exacerbated VM microenvironment in PD is supported after the transplantation of adult SVZ-NSCs within the SN of aged MPTP-treated mice [69]. Fascinatingly, a large part of the transplanted neural stem cells differentiated into astrocytes within the lesioned SNpc [69]. Here, both the NSC-derived astrocytes and the endogenous astrocytes activated intrinsic pathways directing and stimulating nigrostriatal repair [69]. That VM astrocytes, by themselves, are the key elements for mDAn survival, repair and regeneration was further underscored, documenting that by grafting VM astrocytes within the MPTP-lesioned SN of ageing mice exhibiting motor impairment induced beneficial antioxidant and anti-inflammatory effects leading to nigrostriatal restoration [70,71]. Together, these data highlight that a "beneficial" glial–neuron dialogue is a prerequisite to promote neuroprotection and activate neurogenesis in the Parkinsonian brain [25,67–73]. Particularly, the SNpc microenvironment, DA receptor signaling and oxidative stress in the nigrostriatal system can determine a unique link for mDAn vulnerability in PD [71]. Within this frame, astrocytes' Nrf2-ARE machinery and its crosstalk with the WβC signaling system represent key actors of neuron–astrocyte dialogue [69–72].

Given the multifunctional role of LRRK2 in critical cell functions, especially cell–cell communications, intuitively, pathological LRRK2 deeply influences astrocyte–neuron interactions and function, as herein reviewed. As studied, hiPSC- and mouse-derived *LRRK2* G2019S mutant VM and cortical astrocytes manifest several hallmarks of the disease. Hence, all major astrocyte functions herein documented are impaired/altered, with one of the impairments, i.e., α-syn increased accumulation/reduced clearance, described for the first time [45]. Then, LRRK2-affected astrocytes display the downregulation of a number of astrocytic neuroprotective factors, such as Ca^{2+} overload, unbalanced metabolism and mitochondrial dysfunction and astrocytes' hyper-reactivity, with enhanced transcriptomic and proteomic responses to inflammatory or mitochondrial toxic challenges, and aberrant ROS and RNS generation, in part antagonized by LRRK2-antagonists [45–55,125]. Especially, the dysfunctional astrocyte-to-neuron crosstalk was documented by the failure of G2019S astrocyte-derived EVs to transfer pro-survival, anti-apoptotic and neuroprotective factors, including *BIRC5* "survivin" [53], which is strictly linked to the WNT signaling machinery, altogether resulting in a severe impairment of astrocyte–neuron crosstalk and deficient mDAn survival and differentiation. Of special interest, the impaired mitochondrial donation of LRRK2 G2019S astrocytes resulted in impaired mDAn neuroprotection against rotenone-mediated neurotoxicity [55]. Importantly, the effects on mitochondrial transfer can be reversed by LRRK2 antagonism and the Drp1 phosphorylation inhibitor DUSP6 [55].

Additionally, an impairment of the BBB was documented by de Rus Jacquet [51], as LRRK2 G2019S astrocytes failed to support the formation of a functional capillary in vitro [51]. Recent studies show the ability of *LRRK2* mutations to disrupt iron homeostasis and to stimulate oxidative reactions and ferroptosis in human neurons and astrocytes [156], thereby exacerbating the redox chemistry of astrocyte–neuron interactions. Also, genetic data indicate the impairment of the glia Nrf2 function of G2019S flies [82], whereas by genetically manipulating the G2019S fly model, the authors indicated dopaminergic neuroprotection and ameliorated the impaired locomotion [82]. Additionally, a decreased expression of *Nrf2* and of its target genes in the *LRRK2*-TG mouse brain, and LRRK2 overexpressing SH-SY5Y cells, was seen [167]. Other recent studies [168] involved the Nrf2-mediated glutathione machinery, glutamate uptake/metabolism, and ATP generation in astrocytes derived from LRRK2-I1371V PD patient iPSCs, a mutant variant that is prevalent in East Asian populations, resulting in a significant decrease in the astrocytic ability to mitigate oxidative stress in terms of glutathione content in the mutant astrocytes. Along these lines, a reduction in the gene expression of enzymes involved in glutathione machinery and Nrf2 expression was documented [168]. These conditions together and combined with oxidative stress and the inflammatory exacerbation of *LRRK2* G2019S astrocytes severely impair astrocyte–neuron interactions.

Altogether, astrocytes of the midbrain dopaminergic niche, via a bidirectional crosstalk with mDAns from development to maturity, crucially contribute to neuronal health, survival and protection against inflammatory/oxidative stress insults. Then, a dysregulated neuron–astrocyte crosstalk during early development, as documented in G2019S astrocytes, may compromise neuron–glial neuroprotective dialogue, thereby predisposing the fragile mDAns to an increased susceptibility to inflammation and oxidative stress in adult life and ageing.

Can human brain organoids provide a proof of concept for testing astrocyte–neuron dysfunctional crosstalk during the development of PD?

In recent years, three-dimensional (3D) human brain organoids have demonstrated a great potential for in vitro disease modeling [29,172–177]. Studies have reported that midbrain organoids (MO) can recapitulate the physiology and complexity of the human brain, as they share comparable transcriptomic profile and cell-type diversity with the developing human midbrain. Ongoing studies in the field document gene expression profiles and protein changes that might underlie LRRK2-mutation-related phenotypes [172–174]. Specifically, in the excellent review article of L.M. Smits and J.C. Schwamborn [173], the development of human MO generation and its characterization were exhaustively documented, together with its potential to unravel the early phase of ND disease's development, especially PD and LRRK2-PD [173]. Also, the importance of a detailed characterization of astroglia, besides the neuronal population, and the integration of microglial cells appear crucial for modeling human midbrain development [112,173–176]. Of special interest, using autoptic brain samples and PD patient-specific 3D midbrain organoids and 2D astrocytes derived from induced pluripotent stem cell, the very recent (in press) work of Smits L and co. [177] shows that the LRRK2 G2019S mutation alters the transcriptomic profile of astrocyte subpopulations in MO, and impairs astrocytic specification, with the occurrence of a senescent phenotype [177]. These and other results of this work led the authors to propose a novel role of the LRRK2 G2019S mutation in impairing astrocytic differentiation and predisposing to senescence [177]

Together, these and other emerging studies on MO [175–178], along with human brain studies on midbrain patterned astrocytes complemented with both in vivo and in vitro mouse model systems, may advance our knowledge on astrocyte–neuron interactions in PD and LRRK2-PD, and corroborate early astrocyte–neuron crosstalk failure in G2019S PD.

Considering the overall studies herein summarized, along with an apology for not having cited other important publications due to space constraints, coupled with the emerging picture of astrocytes, some future directions are summarized below.

9. Future Directions

In recent years, a number of new techniques have opened up novel options for the study of astrocyte–neuron interactions to enhance our knowledge on astrocyte–neuron crosstalk, and in particular *LRRK2* G2019S mutant astrocytes. For example, a powerful means to prove the role of astrocytes in the developmental PD hypothesis is provided by transgenic animals with the conditional expression of astrocyte-specific LRRK2 G2019S; this model could help to answer the question about the role of mutated LRRK2 during various stages of mouse development. Hence, different *in vivo, ex vivo* and *in vitro* experimental settings and co-culture paradigms might be developed to test the role of the astrocyte-specific G2019S mutation in the development of astrocyte–neuron interactions during both early and adult life, as well as during ageing. Altogether, these suggestions further emphasize the need for advanced molecular, genetic, and physiological tools, as exemplified below,

Single-nucleus RNA-sequencing (snRNA-seq) can allow a high-resolution profiling of gene expression changes for neurons, astrocytes and microglial cells in various neurodegenerative disorders including PD, whereby it is possible to compare the astrocyte transcriptional changes with single-cell transcriptomic datasets [179]. Such techniques allow the identification of multiple transcriptionally distinct clusters of astrocytes, enabling the identification of cluster-specific gene expression changes in each disease context [179]. Hence differential changes in astrocyte subpopulations are predicted to result in both beneficial and detrimental outcomes, and these distinctions need to be considered for future therapeutic targeting design.

Maintaining mitochondrial health to preserve brain energetics. The research of Damodaran and co. (2026) [180] investigating the expression patterns of genes associated with mitochondrial function in the SN and DA neurons of individuals with PD is aimed at unveiling new potential therapeutic targets [180]. Using two independent expression array datasets, GO and pathway enrichment analyses allowed them to gain insights into the molecular mechanisms involved [180]. With this integrative analysis, novel mitochondrial gene candidates implicated in PD pathogenesis are uncovered, underscoring their potential as targets for therapeutic intervention.

Artificial intelligence (AI) platform studies aimed at identifying protective molecules. For example, some studies looked at candidate molecules to enhance mitophagy (i.e., a process that removes damaged mitochondria). Failure in mitophagy [181], as observed in *LRRK2* G2019S astrocytes, results in neurodegeneration, while enhancing mitophagy promotes the survival of mDAns. The study found that probucol, a lipid-lowering drug, effectively enhanced mitophagy. In vivo experiments with zebrafish and fly models showed that probucol improved survival, locomotor function, and dopaminergic neuron loss following mitochondrial damage [181]. Using an AI platform, the authors evaluated candidate molecules against a set of well-established mitophagy enhancers, showing that Probucol-mediated changes may prime the cell for a more efficient mitophagic response to mitochondrial damage [181].

Harnessing WNT signaling for astrocyte–neuron rescue. The work of Yang et al. (2025) [182], reporting that WNT and R-spondin (RSPO) proteins can be transported on engineered exosomes, is aimed at developing a panel of engineered exosomes with the active loading of individual 19 WNT and 4 RSPO ligands [182]. The authors used human liver progenitor cell cultures and identified WNT3–RSPO1 or WNT3A–RSPO1 combinations that could not only regulate liver progenitor cell fate, as described, but may

also be studied for developing neuron–astrocyte platforms for rescue/repair/regeneration in injured, diseased, and aged tissues.

Employing lentiviral luciferase biosensors in association with luciferase signaling activity, immunohistochemistry, qPCR and Western blot assays to study in vivo WNT signaling activity in LRRK2 KO and LRRK2 G2019S KI mice, as recently documented by Wetzel and co [95]. Here, region-specific expression changes in WNT and NFAT signaling components, with dysregulated WNT and NFAT signaling, were documented in a *LRRK2* G2019S knock-in PD model [95], with effects being predominantly observed at the protein level in the striatum and cerebral cortex of *LRRK2* KI mice. This study further built on knowledge of LRRK2 as a WNT and NFAT signaling protein, suggesting a role for mutant *LRRK2* in the dysregulation of NFAT, and canonical and non-canonical WNT signaling [95]. These critical advancements in the field may be used for expanding our knowledge in different physiopathological states, including development, ageing, inflammation and neurodegeneration.

Developing novel neuroprotective agents based on the activation of Nrf2 recently defined as a "guardian of health" [74] and a critical therapeutic target [74–82]. Dietary natural products may hold significant interest for the re-establishment of protective glia–neuron interactions to prevent and/or treat PD, as illustrated by Zhang and co. (2026) [183], showing the feasibility of an electrophilicity-based strategy in developing Nrf2-dependent neuroprotective agents "inspired" by dietary curcumin.

Computational glioscience to integrate mechanisms of neuron–astrocyte interactions into the standard neural network, for modeling and exploring global dynamics and emergent properties in brain systems [184]. For example, models were developed to study synchronization, information transfer, synaptic plasticity, and hyperexcitability, as well as classification tasks and hardware implementations [184]. Manninen and co. [180] focused on network models of at least two neurons interacting bidirectionally with at least two astrocytes that include astrocytic calcium dynamics [184]. In this study, the authors analyzed the evolution of these models and the biophysical, biochemical, cellular, and network mechanisms used to construct them [184]. Against this background, in this analysis, the authors propose how to systematically describe and categorize interaction schemes between cells in neuron–astrocyte networks, and present future perspectives [184].

Developing transformer-based deep learning models, as documented in the studies of Berson and co [185], such as "Cellformer", which resolves brain transcriptional data (RNA-seq) into cell type-specific RNA profiles that allow investigations of complex and heterogeneous phenotypes, such as cognitive resilience or brain resistance to NDs [185]. In this work, Berson, E. and co. identified astrocytes as the major cell mediator of Alzheimer's disease resilience across cerebral cortex regions, while excitatory neurons and oligodendrocyte progenitor cells were uncovered as the principal cell mediators of resistance, preserving neuron health [185].

Developing mitochondrial transfer/stimulation/transplantation therapies that are emerging as a crucial field for reprogramming neuron–astrocyte crosstalk in regeneration/repair [63,64,141,146–148,150,186,187]. Given the critical role of mitochondria in driving the shift of astrocytes from a quiescent to a reactive state, coupled with their dysfunctional regulation leading to metabolic failure, enhanced α-syn aggregation, amplifying neuroinflammation, ferroptotic vulnerability and oxidative stress, potential mitochondria-based therapies are currently being investigated [186,187].

Developing protocols for the generation of human MO for transplantation into the SN of PD mice, as reported by Chen, L.B. and co. [178]; developing human midbrain astrocyte platforms to potentiate the survival/neurotrophic/anti-inflammatory properties of VM astrocytes [188].

Developing bioengineered exosomes to enhance drug-loading efficacy and targeting specificity, reducing oxidative stress and ROS production, thus improving dopaminergic neuronal damage in PD, to be associated with biomarkers that monitor CNS redox status in vivo [189].

Finally, it seems important to underline some critical aspects and translational limitations of the herein-discussed models, especially the clinical translation of astrocyte-targeted therapies, which faces multiple challenges. For example, the identification of different astrocyte subpopulations for astrocyte region- and pathology-specific drug targeting is required, for which a continuous and more deep understanding of VM astrocyte biology appears necessary, in order to elucidate the complete degree and magnitude of astrocyte diversity and, most importantly, understand how it may influence neurodevelopment, as well as its translational relevance [83,112,182–184].The BBB may represent another critical obstacle that severely limits therapeutic options and clinical translation. Some novel technologies as well as innovative drug-delivery vehicles have been proposed to enhance drug-loading efficiency, targeting specificity and safety concerns [189]. Regarding LRRK2 antagonists, the efficacy and safety of chronically targeting LRRK2 kinase have recently been discussed, together with an update on additional approaches and current developments in the field [122].

Altogether, ongoing research on astrocyte biology and LRRK2 intersystem crosstalk with major astrocytic systems/signaling pathways and functions in PD, identifying the processes/mechanisms in VM astrocytes underlying selective vulnerability, coupled with technological advancements in the ND field, appears crucial for identifying early pathogenic mechanisms to improve PD diagnosis and treatment.

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Abbreviations

α-syn	Alpha synuclein
ANK	Ankyrin
ARM	Armadillo
BBB	Blood–brain barrier
CX	Cerebral cortex
CMA	Chaperone-mediated autophagy
Clu	Clusterin
COR	C-terminal of ROC
DA	Dopamine
DAT	Dopamine transporter

Dkk1	Dickkopf1
ER	Endoplasmic reticulum
FGF2	Fibroblast growth factor 2
FGF8	Fibroblast growth factor 8
FOXA2 or HNF3B	Forkhead box A2
GFAP	Glial fibrillary acidic protein
GDNF	Glial-derived neurotrophic factors
GFs	Growth factors
HO-1	Heme oxigenase 1
hiPSC	Human induced pluripotent stem cell
GTPase	Hydrolase enzyme of guanosine triphosphate
IF	Immunofluorescence
IL-1β	Interleukin 1 beta
IL4	Interleukin 4
IL10	Interleukin 10
IBA-1	Ionized calcium-binding adapter molecule 1
KO	Knock-out
LRR	Leucine-Rich Repeat
LPS	Lipopolysaccharide
(MT)-1	Metallothionein
MPTP	1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine
mDAns	Midbrain dopaminergic neurons
MO	Midbrain organoids
ND	Neurodegenerative disease
NeuN	Neuron-specific marker
NPCs	Neuroprogenitor cells
NO	Nitric-oxide
Nrf2	Nuclear factor erythroid 2-related factor 2
NR4A2 or NURR1	Orphan nuclear receptor
OCRs	Oxygen consumption rate
PD	Parkinson disease
PBMCs	Peripheral blood mononuclear cell
PCP	Planar cell polarity
ROC	Ras of complex protein
RNA-seq	RNA sequencing
RSPO	R-spondin
SERCA	Sarco-Endoplasmic Reticulum Calcium ATPase
S100β	S100 calcium-binding protein b
SPO-2	Spondin-2
StrO	Striatal organoids
TMT	Tandem mass tag
TLDA	TaqMan® Low-Density-based Array
TLR	Toll-like receptor
TGFβ1	Transforming growth factor beta1
TNF-α	Tumor necrosis factor alpha
TH	Tyrosine hydroxylase
VM	Ventral midbrain
VTA	Ventral tegmental area
WNT	Wingless-related MMTV integration site
WβC	β-catenin pathway

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