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Wnt/β-Catenin signaling pathway governs a full program for dopaminergic neuron survival, neurorescue and regeneration in the MPTP mouse model of Parkinson's disease

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Abstract

Wingless-type MMTV integration site (*Wnt*) signaling is one of the most critical pathways in developing and adult tissues. In the brain, *Wnt* signaling contributes to different neurodevelopmental aspects ranging from differentiation, axonal extension, synapse formation, neurogenesis and neuroprotection. *Canonical Wnt* signaling is mediated mainly by the multifunctional *β-catenin* protein which is a potent co-activator of transcription factors such as Lymphoid Enhancer Factor (LEF) and T Cell Factor (TCF). Accumulating evidence points to dysregulation of *Wnt/β-catenin* signaling in major neurodegenerative disorders. Here I focus on a "*Wnt/β-catenin-glial connection*" in Parkinson's disease (PD), the most common movement disorder characterized by the selective death of midbrain dopaminergic (mDAergic) neuronal cell bodies in the subtantia nigra pars compacta (SNpc) and gliosis. I will summarize the work of the last decade documenting that *Wnt/β-catenin* signaling in partnership with glial cells is critically involved in each step and at every levels in the regulation of nigrostriatal DAergic neuronal health, protection and regeneration in the MPTP mouse model of PD, focusing on *Wnt/β-catenin* signaling to boost a full neurorestorative program in PD.

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1. Introduction

Parkinson's disease (PD) is the most common movement disorder, and the second common aging-related neurodegenerative disease after Alzheimer's disease. It is characterized by the selective and progressive degeneration of midbrain dopaminergic (mDAergic) neurons of the 'substantia nigra pars compacta' (SNpc) and their projections into the caudate nucleus leading to substantial decreases in dopamine levels, the accumulation of pathological α-synuclein, which is a major component of Lewy bodies (LBs), and gliosis [1,2]. As the disease advances, the progressive loss of dopamine storage in striatum results in decreased motor function with symptoms that include resting tremors, rigidity, bradykinesia and postural instability, accompanied by progressive impairment of autonomic, cognitive and mood functions [1,2].

Several genes have been identified in the rare familial (about 5%) forms of the disease, but the majority of cases (almost 95%) are sporadic and likely represent a complex interplay between both genetic and environmental influences [3-6]. In particular, aging, inflammation and the hormonal background represent key factors programming the vulnerability to PD [7-21]. The mechanisms leading to the selective and progressive mDAergic neuron death in PD and experimentally-induced PD are not clarified, but oxidative stress and inflammation associated to molecular changes indicative of mitochondrial dysfunction and apoptosis have been defined in the parkinsonian brain [11-14,18-20,22-25].

So far, there is no cure for PD, and current treatments are centered on dopamine replacement therapy, albeit they only temporally alleviate the motor symptoms without stopping the ongoing neurodegeneration [1,2]. Therefore, an in-depth understanding of the molecular pathways chiefly involved in mDAergic neuron physiopathology is crucial for the development of neuroprotective and cell replacement therapies for PD.

Notably, the molecular mechanisms that govern neurogenesis and differentiation of mDA neurons have attracted intense investigations, with *Wingless-type MMTV integration site*

(*Wnt*)*/β-catenin* pathway being recognized as the central player in mDA neurogenesis [26-30]. The evolutionarily conserved *Wnt-β-catenin* pathway initiates a signaling cascade that is crucial during both normal embryonic development and throughout the life of the organism in almost every tissue and organ system. Within the central nervous system (CNS), *Wnt* signaling cascades orchestrate all facets of neuronal functions, including differentiation, neuron death/ survival, axonal extension, synapse formation and plasticity, neurotrophin transcription, neurogenesis and regeneration [31-35].

Wnts (Wnt1-Wnt19) interaction with their seven-pass transmembrane receptors of the Frizzled (Fzd) family (Fzd1-Fzd-10), trigger several signaling pathways, such as the so-called ‘‘*canonical*’’ *Wnt/β-catenin*, and the ‘‘*non-canonical*’’ Wnt/planar cell polarity (PCP) and Wnt/Ca^{2+} pathways [36,37, **Figure 1**].

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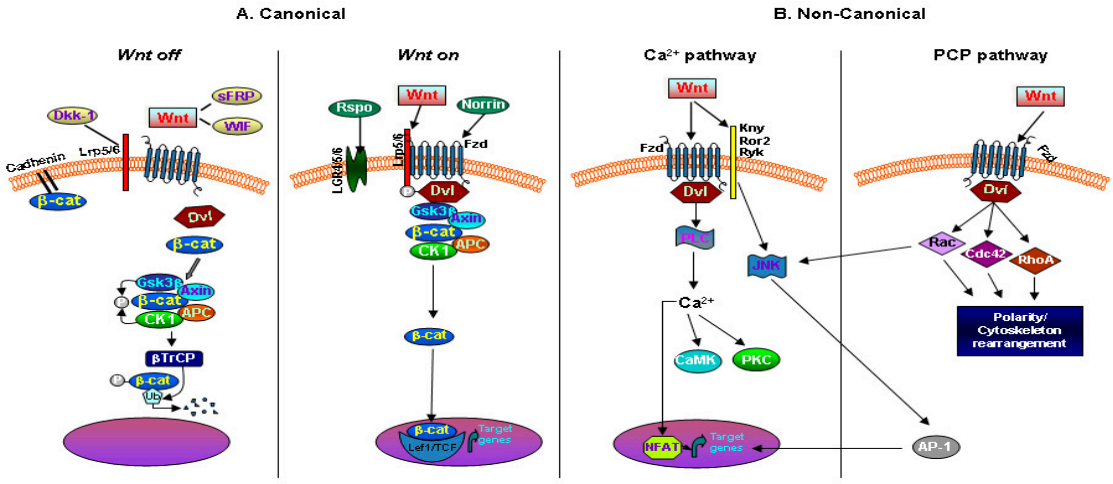

Figure 1. Canonical and non-canonical Wnt signalling cascades. Three Wnt-dependent pathways have been proposed: canonical Wnt/β-catenin pathway and noncanonical Wnt/PCP and Wnt/Ca^{++} pathways. **A:** In the Wnt/β-catenin pathway, the binding of Wnts to a receptor complex composed of members of the Fzd family and LRP promotes the inhibition of the APC/GSK-3β complex, and blockade of β-catenin phosphorylation by GSK-3β. Hypophosphorylated β-catenin then accumulates in the cytoplasm and is translocated to the nucleus, where it regulates target gene expression through partnerships with TCR/LEF1 family of transcription factors, resulting in changes in gene transcription. **B:** In noncanonical Wnt-Ca^{++} signalling pathway the binding of Wnts promotes Fzd-mediated activation of pertussis Toxin-sensitive heterotrimeric guanine nucleotide–binding proteins (G proteins). This, in turn, stimulates the release of Ca^{++} from intracellular stores, which leads to the activation of Ca^{++} dependent effector molecules. Several Ca^{++}-sensitive targets-protein kinase C (PKC), Ca^{++}-calmodulin-dependent protein kinase II (CamKII), and the Ca^{++}-calmodulin-sensitive protein phosphatase calcineurin have been identified downstream of the Wnt-Ca^{++} pathway. Targets of the Wnt-Ca^{++} pathway appear to interact with the Wnt-β-catenin pathway at multiple points. Additionally, Fzd receptors in association with Kny, Ror2 or Ryk receptors can activate JNK, promoting target gene expression through AP-1. In noncanonical Wnt/PCP pathway, the binding of Wnts activates RhoA/B, Cdc42 or Rac1. Dsh activates Rac1 and Rac1 can also activate JNK, resulting in the NFAT pathway. Modified from L'Episcopo et al. [50], with permission.

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A large variety of ligands and receptors are involved in Wnt signal transduction. Additionally, the cell tissue specificity of *Wnt/Fzd* interactions and the target genes implicated in a particular cellular context, coupled to the inherent neuronal properties and a specific physiopathological condition, may allow for a wide panel of possible outcomes [38-41]. Such a complex role of Wnt signaling anticipates that its dysfunction may lead to different diseases [40,41]. Earlier and more recent findings have provided compelling evidence that *Wnt1/β-catenin* signaling cascades play a central role in mDAergic development [26-30]. Studies focusing on genetic networks recapitulating the early signals for the development of mDA neurons identified *Wnt1* as a critical morphogen for mDA neurons, where activation of *Wnt1/β-catenin* signaling is required for mDA neuron specification [recently reviewed in 30]. In the adult brain, growing evidence accumulating from earlier and more recent investigations highlights that a *Wnt/Fzd/β-catenin* tone is involved in the maintenance of neuronal health, while its dysregulation associates with neuronal dysfunction and death [42-46].

Herein, I will trace the role of a dysfunctional *Wnt/β-catenin* signaling in PD. Hence, the demonstration of the specific involvement of *Wnt* signaling components in the adult PD-injured midbrain associating with mDAergic neuronal cell death and self-repair in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of basal ganglia injury, uncovered for the first time a novel role for *Wnt/β-catenin's* main actors in the maintenance and protection of mDA neurons in the adult and aged PD mesencephalon [47,48,]. Interestingly, neuroinflammation appeared to play unsuspected roles, since *Wnt1/β-catenin* signaling and MPTP-reactive astrocytes crosstalking with microglial cells, were unveiled as candidate components of the neurorescue pathways involved in nigrostriatal DAergic plasticity and in the regulation of neurogenesis [47-55]. Importantly, environmental PD neurotoxins and pesticides were shown to downregulate *Wnt/β-catenin* signaling in rodent, non human primate and human PD [56,57]. Accordingly, in genetic screens and in human

post-mortem studies Wnt's key components were shown to be altered [58,59]. Other studies, looking at methylation sequencing, revealed dysregulated Wnt signaling in PD [60].

As the importance of Wnt signaling in midbrain DAergic neurogenesis was underlined by the use of chemical Wnt activators for efficient generation of mDA neurons from cultured pluripotent stem cells (PSCs) [61,62], an increasing number of studies focusing on Wnt/β-catenin signaling genes has been reported. Notably, genome-wide analysis of gene expression identified Wnt signaling as an over-represented pathway in human induced pluripotent stem cell (hiPSC)-derived DA population [63], and activation of Wnt signaling was recognized to play an important role in deriving regionally homogeneous populations of NSCs and neurons [64], thereby greatly improving their scientific and therapeutic utility [64]. By contrast, pivotal PD proteins encoded by genes whose mutations have been linked to PD, were demonstrated to impact on *canonical Wnt/β-catenin* signaling [65-67] and to inhibit human induced pluripotent stem cells (iPSCs)' ability to differentiate into DAergic neurons [68], whereas pharmacological Wnt activation restored their developmental potential [68]. With the emerging role of micro-RNA (miRNA) regulatoy functions on major pathways involved in mDAergic neurodegeneration in PD [69-71], patient-specific dysregulation of mRNA/miRNAs expression is being revealed in PD [72], with an expected impact on Wnt signaling [41,72-74].

Besides genetic mutations, enviromnental risk factors for developing PD, namely aging, environmental neurotoxin exposure and inflammation, were shown to impair and/or dysregulate *Wnt/β-catenin* signaling in mDAergic neurons and neural/stem progenitor cells (NSCs), thereby predisposing to mDAergic neurons to apoptotic cell death and inhibiting the "*intrinsic"* mDA neurorepair potential with a severe impact on neurogenesis upon injury, while activation of Wnt/β-catenin signaling efficiently counteracted mDAergic toxity favoring neuroprotection and neurorestoration [47-55].

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Especially, the sustained and ectopic expression of *Wnt1* in genetically affected engrailed (*En1*) heterozygote (*En1*+/−) mice can induce a neuroprotective *Wnt1*-dependent gene cascade promoting the survival of *En1* mutant ($En1^{+/-}$ and $En1^{-/-}$ mice) mDA neurons, rescuing them from premature cell death [75]. Remarkably, within the ventral midbrain (VM), astrocyte-derived Wnts decline with age, whereas the expression of endogenous antagonists of *Wnt/β-catenin* signaling, including *Dickkopf1* (*Dkk1*), is up-regulated thereby contributing to the reduced neuronal survival and neurorepair capacity, and to the marked impairment of neurogenesis [47-55].

Of special importance, the inducible expression of *Dkk1*, resulting in deficient *Wnt* signaling, elicits synaptic degeneration in the adult striatum, associated to impaired motor coordination, which suggested that a dysfunction in *Wnt* signaling contributes to synaptic degeneration at early stages in neurodegenerative diseases [76]. Additionally, targeted deletion of *β-catenin* in DA neurons (DA-βcat KO mice), leads to alterations of motor and reward-associated memories and to adaptations of the DA neurotransmitter system [77]. Opposedly, investigations in canonical *Wnt* reporter mice, *BATGAL* and $Axin2^{LacZ}$, prooved the essential roles of *Wnt/β-catenin* signaling in mDAergic neurons and midbrain DAergic neuroprogenitors during degeneration, repair and regeneration of nigrostriatal neurons in the aged PD brain [55,78]. The growing field of Wnt signaling in neurodegeneration and regeneration was highlighted in a Special Issue "Wnt signaling cascades in neurodevelopment, neurodegeneration and regeneration", featuring the progresses achieved and the future challenges in the field [79]. So far, a wide panel of genetic, physiopathological, or neurotoxic conditions affecting mDA neurons in PD have shown to strongly impair canonical *Wnt/β-catenin* signaling, while an increasing number of pharmacological and/or immunomodulatory agents affording neuroprotection have been recognized to activate the canonical Wnt/β-catenin signaling pathway, promoting

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neuroprotection and immunomodulation, and counteracting the impairment of neurogenesis in PD injured brain [47-55, 80-89 **Figure 2**].

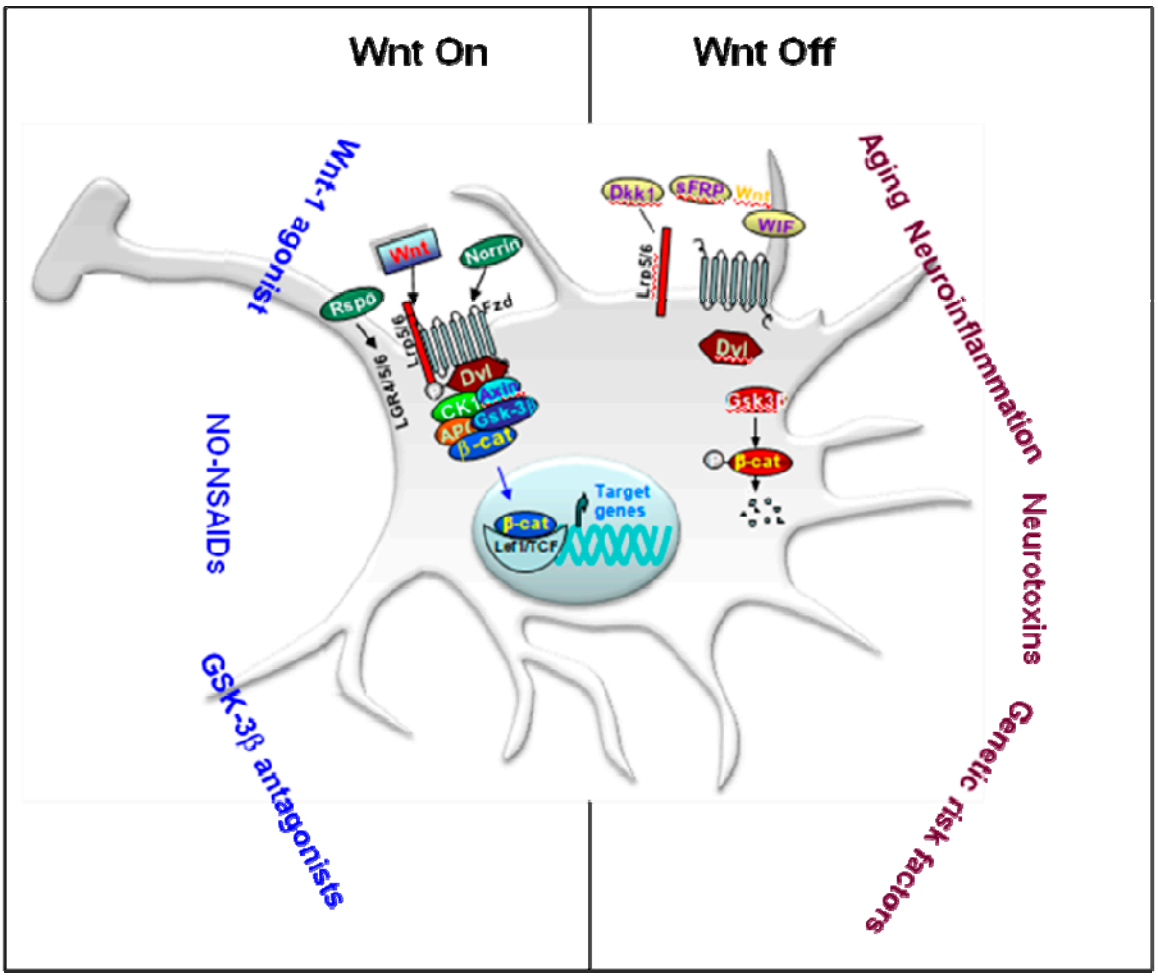

Figure 2. Schematic illustration of Wnt1/β-catenin signaling regulation of mDAergic neuron survival/death. Major environmental factors including aging, inflammation, neurotoxin exposure including PD neurotoxins (MPTP/MPP$^+$, 6-OHDA), pesticides (rotenone), increased oxidative load as a result of gowth factors (GFs) deprivation in synergy with genetic mutations, may antagonize Wnt/β-catenin signalling ("*Wnt off*") in mDA neurons. Up-regulation of active GSK-3β, then lead to β-catenin degradation and increased DA neuron vulnerability/degeneration/apoptosis. By contrast, in the intact midbrain canonical Wnt agonists, such as *Wnt1, Rspo* or *Norrin,* and activation of *Fzd-1* receptors also via exogenous Wnt/β-catenin activation such as GSK-3β antagonist, NO-NSAIDs treatments tors (*"Wnt on"*), contribute to maintain the integrity of mDA neurons via blockade of GSK-3β-induced phosphorylation (P) and proteosomal degradation of the neuronal pool of β-catenin. Stabilized β-catenin can translocate into the nucleus and associate with a family of transcription factors and regulate the expression of Wnt target genes involved in DA neuron survival/plasticity, neuroprotection and repair. β-catenin may also function as a pivotal defense molecule against oxidative stress,and can act as a coactivator for several nuclear receptors involved in the maintenance/protection of DA neurons. The hypothetical contribution of various endogenous Wnt agonists (Respondin, Rspo, Norrin) or antagonists (*Dkkopf, Dkk1, Wif*, frizzled-related proteins, SFRp) are also indicated. Modified from L'Episcopo et al. [50], with permission.

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Based on this background, I will highlight *Wnt/β-catenin* signaling and its crosstalk with survival and neuroinflammatory pathways as a pivotal actor for mDA neuronal maintenance, protection, repair and regeneration in mice affected by MPTP-induced nigrostriatal degeneration.

After a brief introduction of *Wnt/β-catenin* signaling, I will discuss its potential role in dictating mDAergic neuron vulnerability to major key risk factors such as aging, gene mutations, neurotoxin exposure and neuroinflammation, highlighting the potential therapeutical implications for PD in the light of recent published findings in the field.

2. The interactors of the *Wnt/β-catenin* signalling cascade: ligands, receptors, co-receptors and endogenous regulators

In the central nervous system (CNS), the glycoprotein ligand *Wnts*, control cell fate during embryonic development, self-renewal in adult neurogenic niches and neuronal integrity and homeostasis in adult brain [31-35 and references therein]. *Wnt* encode secretary glycoproteins to activate the *Wnt* signaling pathway [36]. *Wnt* signals are context-dependently transduced to the canonical and noncanonical pathways based on the expression profile of Wnt ligands, Wnt antagonists, the Frizzled (Fzd) family receptors, coreceptors, and the activity of cytoplasmic Wnt signaling regulators (for a detailed overview of Wnt proteins, receptor/co-receptors and signaling mechanisms [see 31-41, and references therein]. Wnt proteins share molecular and structural characteristics involving sequence identity of 39–46 kDa lipid-modified secreted glycoproteins containing 350–400 amino acids with a highly conserved pattern of 23–24 cysteine residues and several asparagines-(Wnt homepage:http://www.stanford.edu/~rnusse/wnt_window.html) linked glycosylation sites.

Generally classified into functional groups according to their ability to induce a secondary body axis in *Xenopus* embryos and to activate specific signaling cascades, Wnt proteins are

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essentially described as the *Wnt1* (including Wnt2, Wnt3, Wnt3a, Wnt8 and Wnt8a) and the Wnt5a (including Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, and Wnt11) classes, involving intracellular signaling pathways specifying *Wnt* signal transduction. The Wnt1 class has been generally assumed to signal into the cell via the ''*canonical*'' *Wnt/β-catenin*, whereas the Wnt5a class via the ''*non-canonical*'' Wnt/PCP and Wnt/Ca^{2+} pathways, albeit functional Wnt classification is a oversimplification, as there are cases/contexts where the same Wnt protein will activate different pathways depending on the presence of receptors **[Figure 1]**.

Common to all three pathways is binding of the Wnt ligand to the seven-pass transmembrane receptors of *Fzd* family, recently reviewed by Janda et al. [90]. The subsequent activation of one of these three alternative signaling pathways appears to depend on the specific complement of Fzd receptors and co-receptors of the Low-density lipoprotein receptor-related protein (Lrp) family encountered on the cell surface and the Wnt ligand activating these receptors [**Figure 1**].

As a key component of Wnt signaling, Dishevelled (Dvl/Dsh), a cytoplasmic multifunctional phosphoprotein, acts at the plasma membrane or in the cytoplasm in all three Wnt-Fzd signaling cascades. Depending on Wnt stimulation and receptor context, Dvl can inhibit the β-catenin destruction complex, thereby stabilizing β-catenin and activating canonical Wnt signaling, or Dvl can regulate some of the noncanonical branches of Wnt signaling [90,91]. Notably, the leucine-rich repeat kinase 2, *LRRK2*, a member of the leucine-rich repeat kinase family (also known as dardain), codified by PARK8 gene, interacts with Dvl proteins. *LRRK2* is recruited to membranes following Wnt stimulation, where it binds to the Wnt co-receptor Lrp6 in cellular models [see 65]. Pathogenic *LRRK2* mutations disrupted *Wnt* signaling, implicating binding to Lrp6-mediated *Wnt* signaling caused by reduced binding to Lrp6, as potential factor underlying neurodegeneration observed in PD (66). On the other hand, protective LRRK2 R1398H variant enhanced

GTPase and Wnt signaling activity, underlying the complexity of LRRK2-Wnt signaling crosstalk in PD [67]. Along this line, proteomic analysis of LRRK2 binding partners revealed interactions with multiple signaling components of the WNT/PCP pathway [94].

Other members, the transmembrane receptor Tyr kinases Ror2 and Ryk, as well as Fzd receptors can act independently of Lrp5 or Lrp6, function as receptors for Wnt and activate β-catenin-independent pathways **[Figure 1].**

Canonical Wnt pathway functions by regulating the amount of the transcriptional co-activator β-catenin that controls key developmental gene expression programs. β-catenin is a protein with dual functions in the cell, playing a role in both adhesion between cells as well as gene transcription via the *canonical Wnt* signaling pathway. In the absence of a Wnt ligand, i.e. in the *Wnt off* state, cytoplasmic β-catenin protein is constantly degraded by the action of the Axin complex, which is composed of the scaffolding protein Axin, the tumor suppressor *adenomatous polyposis coli* gene product (APC), *casein kinase 1* (CK1), and *glycogen synthase kinase 3β* (GSK-3β) **[Figure 1A]**. CK1 and GSK-β sequentially phosphorylate the amino terminal region of β-catenin, resulting in β-catenin recognition by β-Trcp, an E3 ubiquitin ligase subunit, and subsequent β-catenin ubiquitination and proteasomal degradation [91]. The continual elimination of β-catenin prevents β-catenin from reaching the nucleus, and Wnt target genes are thereby repressed by the DNA-bound T cell factor/lymphoid enhancer factor (TCF/LEF) family of proteins **[Figure 1A]**.

GSK-3β is a pleiotropic serine/threonine protein kinase that is active in a number of central intracellular signaling pathways, including Wnt/β-catenin pathway, thus impacting on a multitude of cellular functions including cellular proliferation, migration, neurogenesis, inflammation and apoptosis [95-98]. Of special mention for this subject, numerous apoptotic conditions antagonizing Wnt/β-catenin signaling can be facilitated by GSK-3β activation [97-98].

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The *Wnt/β-catenin* pathway is activated when a Wnt ligand binds to a seven-pass transmembrane Fzd receptor (i.e in *Wnt on* state) and its co-receptor, low-density lipoprotein receptor related protein 6 (Lrp6) or its close relative Lrp5 **[Figure 1A]**. The formation of a likely Wnt-Fz-Lrp6 complex together with the recruitment of the scaffolding protein Dvl results in Lrp6 phosphorylation and activation and the recruitment of the Axin complex to the receptors. Wnt signaling inhibits GSK-3β activity, thus increasing the amount of *β-catenin*, which enters the nucleus, and associates with T-cell factor/lymphoid enhancer binding factor (TCF/LEF) transcription factors, leading to the transcription of Wnt target genes involved in cell survival, proliferation and differentiation. Notably, the endolysosomal compartment can positively regulate Wnt signaling by sequestering GSK-3β inside microvesicular bodies (MVBs), thus diminishing the cytosolic availability of the β-catenin destruction complex [99]. By contrast, defective endolysosomal compartment, leading to reduced sequestration of GSK-3β may result in increased β-catenin degradation and downregulation of the canonical Wnt/β-catenin signaling, as observed in Gaucher's disease (GD) iPSC neuronal progenitors, leading to marked alteration of NSC-DAergic differentiation potential [68, see next sections].

The physiological role of canonical *Wnt/β-catenin* signaling is best exemplified during early brain development, when midbrain DAergic neurons originating from the ventral midline of the mesencephalon, require Wnt1 has the prime initial regulator of DAergic neurodevelopment, as it drives the initial origin for the midbrain DAergic progenitors, and consequently activates *Wnt/β-catenin* signaling, which is necessary to promote DAergic neurogenesis, whereas removal of β-catenin in tyrosine hydroxylase (TH)-positive neural progenitor cells in the ventral midbrain (VM) region negatively regulates mDAergic neurogenesis [see 26-29,100]. Here, β-catenin depletion interferes with the ability of committed progenitors to become DA neurons, resulting in adult animals with a significant decreased number of TH positive neurons in the adult ventral midbrain [100]. Notably,

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excessive Wnt signaling is also detrimental for mDA neuron production, adding to the general notion that morphogen dosage must be tightly regulated [see 26-30, 101].

In noncanonical Wnt-Ca^{++} signaling pathway, the binding of Wnts promotes Fzd-mediated activation of pertussis Toxin-sensitive heterotrimeric guanine nucleotide–binding proteins (G proteins) **[Figure 1B]**. This, in turn, stimulates the release of Ca^{++} from intracellular stores, which leads to the activation of Ca^{++} dependent effector molecules. Several Ca^{++}-sensitive targets-protein kinase C (PKC), Ca^{++}-calmodulin-dependent protein kinase II (CamKII), and the Ca^{++}-calmodulin-sensitive protein phosphatase calcineurin have been identified downstream of the Wnt-Ca^{++} pathway [38-419]. This leads to changes in cell movement and polarity and to the antagonism of the β-catenin pathway, at multiple points **[Fig. 1B]**.

Because Wnt/β-catenin is a powerful pathway, too much or too little might be detrimental, and thus, it must be tightly regulated. So far, various natural inhibitors/modulators of Wnt signaling pathway have been identified which can antagonize or regulate Wnt signaling pathway [Figure 1]. Generally, depending on their functional mechanism, Wnt signaling inhibitors are divided into two classes: secreted frizzled-related proteins (*sFRPs*) and Dickkopfs (*Dkks*) [see 102,103]. The members of sFrp class can bind to Wnts and thus regulate the association of Wnt ligands to their transmembrane receptors, inhibiting both canonical and noncanonical signaling pathways. The sFrp class constitutes sFRP family proteins: WIF-1 and Cerberus [Figure 1]. Members of the *Dkk* class, binds to LRP5/LRP6 component of the Wnt receptor complex to inhibit (*Dkk-1*, -2, -4) or activate (*Dkk-3*) canonical Wnt signaling [see 30], with one of the best characterized members being *Dkk1*. Besides antagonists, Wnt signaling pathway is also activated and regulated by some secreted proteins acting as agonists, for example, R-spondin (Rspo) [38-41].

Importantly, while *Sfrps* are considered Wnt signaling antagonists, recent studies have shown that specific family members can positively modulate Wnt signaling [102,103].

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Additionally, Wnt-Fzd binding and cooperation with particular co-receptors, such as LDL receptor-related protein 5/6 (Lrp5/6), receptor tyrosine kinase-like orphan receptor 1/2 or receptor-like tyrosine kinase, can define downstream signal specificity **[Figure 1B]**. For more comprehensive and historic perspective we refer readers to earlier and more recent reviews [32,41,104] .

Overall, the multiplicity of potential interactions between Wnts, their receptors and downstream effectors, together with their complements of endogenous positive and negative regulators, has exponentially increased the complexity of the signal transduction network. Signaling through each of the Wnt pathways, as well as crosstalk between them, all together play critical roles in all facets of nervous system development, and likely also contribute to adult CNS homeostasis. Not surprisingly, interruption of Wnt signaling leading to either hypo- or aberrant functioning may promote diverse pathogenic outcomes. Hence, dysregulation of the Wnt/Fzd system is associated with a variety of human hereditary diseases, and modulation of Wnt signaling is actively targeted for cancer, regenerative medicine, stem cell therapy,bone growth, and wound healing [40,41,104,105].

3. The MPTP mouse model of PD: inflammation mediates the effects of genes, age, gender and environmental exposures

One of the most highly compelling evidence for the potential contribution of environmental neurotoxicants and neuroinflammation in PD was revealed in humans who developed a parkinsonian syndrome after accidentally injecting themselves with the neurotoxicant MPTP [6]. Post-mortem analysis revealed clusters of reactive microglia around nerve cells. This finding was suggested to reflect an ongoing neurodegenerative process that persisted years after the initial toxic injury and that could have been perpetuated, at least in part, by chronic neuroinflammation [6]. Following this seminal discovery, MPTP was tested in various animal species, and showed its ability to recapitulate most, albeit not all, PD-like symptoms

[4,7,105]. So far, the MPTP mouse model is recognized as a valuable tool, and is widely used in rodent and cell models to understand the molecular mechanisms underlying dopaminergic neurodegeneration in sporadic PD [105].

The neurotoxin MPTP is converted into its active metabolite, 1-methyl-4-phenylpyridinium ion (MPP^+) in astrocytes, and selectively transported into striatal DAergic terminals via the DA transporter, DAT, where it induces oxidative stress, the opening of mitochondrial permeability transition pore (mPTP), the release of cytochrome C and the activation of caspases [98,105]. Of specific mention, mitochondrial damage due to Ca(2+) overload-induced opening of mPTP is believed to play a key role in selective degeneration of nigrostriatal dopaminergic neurons in PD [see 24,25 98]. In synergy with these early events accounting for approximately 10% of DAergic neuronal death, glial inflammatory mechanisms are believed to contribute to nigrostriatal DAergic degeneration [11-21,24,25,105].

Besides MPTP, several environmental toxicants such as herbicides and pesticides, related to rural living/occupation in agriculture, have been implicated as risk factors in PD [3,5,7]. For all of these toxins, it is important to underline the interaction between the period of exposure and age at time of exposure. For example, the effect of systemic exposure to MPTP will vary as a function of its schedule of administration (i.e. acute vs chronic) [105], and the age of the organism, with nigrostrial DAergic neurons of older individuals being more susceptible than DAergic neurons of young adult organisms [7,10]. On the other hand, toxic exposures that occur early in development (both single or combined exposures) could determine long-term pathology [see 7]. Importantly, there are also hormonal influences such as gender and the estrogen background, as well the stress hormones that contribute to the programming of mDAergic neuron vulnerability as part of gene-environment interactions vial glia-neuron crosstalk [7,12,15-17].

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Indeed, an increasing number of evidences from epidemiological, post-mortem, and animal studies suggest that innate inflammatory processes associated with glial cell activation coupled to an array of pro- and anti-flammatory mediators contribute to PD physiopathology [11-21]. As anticipated, aging represents the leading risk factor for the development of PD [8-10]. Hence, aging exacerbates inflammation and oxidative stress, which are the crucial hallmarks of MPTP, or 6-hydroxidopamine (6-OHDA)-induced PD, affecting plastic and regenerative responses (see 54 and references herein]. As a consequence, young adult rodents experience a time-dependent recovery/repair from MPTP insults, whereas aging mice fail to recover for their entire lifespan (see 54).

In fact, with advancing age, the compensatory potential or "adaptive capacity" of nigrostriatal DAergic neurons, believed to mask the ongoing mDA neurodegeneration in presyntomatic individuals, slowly diminishes, and the function of the nigrostriatal DAergic neurons progressively declines, leading to neurochemical, morphological and behavioural changes [8-10,107-110]. With aging, gene-environment interactions further reduce the brain's self-adaptive potential, including DAergic compensatory mechanisms, with harmful consequences for neuron-glia crosstalk, mDA neuron plasticity and repair [54].

4. Wnt/β-catenin signaling and gene-environment interactions in PD

Recent data from the study of genes linked to PD suggest a central importance of Wnt signaling pathways for the normal development and function of mDAergic neurons. The identification of at least 12 genes involved in familial PD, including α-synuclein, *SNCA*, *Parkin, Ubiquitin hydrolase*, PTEN-induced putative kinase, *DJ-1*, leucine-rich-repeat kinase, *LRRK2*, the vacuolar protein sorting 35 homolog gene, *VPS35*, and Glucocerebrosidase, *GBA*, linked to autosomal dominant late-onset PD, has provided new clues to the pathogenesis of PD. Hence, dysfunction of various Wnt-related genes have been found in patients and animal models of PD [47,48,56-60,65-68]. *PARK8, PARK2 and PARK17*, are directly related with the activity of Wnt signaling. *LRRK2* interacts with Dvl,

β-catenin, GSK-3β and Axin1 with a significant impact on Wnt activity, as recalled in section 1 [65-67]. On the other hand, *VPS35*, codified by *PARK17* gene [111], is an essential subunit of the retromer complex involved in recycling proteins from endosomes to the trans-Golgi system and plasma membrane [112]. Importantly enough, the loss of *VPS35* function alters the sorting and retrograde transport of the protein Wntless, an evolutionarily conserved transmembrane protein, essential for the secretion of multiple Wnt proteins [113]. Thus, mutation in *PARK17* can lead to a reduced secretion of Wnt agonists. Notably, Parkin protein, codified by *PARK2* gene, is involved in β-catenin ubiquitination and degradation, and its dysfunction results in β-catenin accumulation and up-regulation of the canonical pathway associated with dopaminergic neuronal death [101]. Additionally, hypermethylation resulting in downregulation of four Wnt and neurogenesis related genes, *FOXC1, NEURG2, SPRY1, and CTNNB1* was also found in cerebral cortex from PD patients [60].

GBA1, a most frequent genetic risk factor for PD [see 68, 114], encodes the lysosomal enzyme b-glucocerebrosidase (GCase) [68,115], with a reduced GCase activity resulting in accumulation of glucosylceramide and glucosylsphingosine in different tissues including the nervous system [see 68, 114-117 and references therein]. Recent reports showed that the endolysosomal compartment modulates canonical Wnt/β-catenin signaling [118 and references herein]. Of specific interest, using iPSCs-derived from patients with GBA1 mutation, Awad and co [68] showed a dramatic decrease in the survival of DAergic progenitors due to the interference with Wnt/β-catenin signaling. Consistent with mutant GBA1-dysfunctional Wnt signaling, NSCs also exhibited reduced expression of hindbrain progenitor markers and an increased expression of forebrain progenitor markers, which highlights the requirement for normal GCase activity during early stages of neuronal development. In this work, the authors therefore suggestd the Wnt/β-catenin pathway as a potential therapeutic target for neuronopathic GD [68].

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As a further level of Wnt impact on gene-environment interactions, most of the genes mutated in PD are expressed in immune cells [see 54,119], supporting a Wnt/neuroinflammatory connection in PD [47-55]. Reportedly, Wnt/β-catenin signaling has both positive and negative effects on local inflammatory pathways in the brain after neuronal damage induced by stroke, trauma and PD [se 49,54 and references herein]. Within this context, the genetic background and exposure to various neurotoxic or inflammatory challenges can promote a self-perpetuating cycle of microglial-mediated mDA neurotoxicity whereby dysfunctional astrocyte-microglia crosstalk contributes to increase mDAergic vulnerability [54,120-123]. Notably, such feedforward cycle of chronic activation of microglia and chronic damage of mDA neurons are likely to play a decisive role for the severity of nigrostriatal DA lesion and the overall detrimental effects of SNpc neurons and consequently, their capacity for neurorepair [see 54]. Therefore, different scenarios are suspected to contribute DAergic neuron degeneration in PD, and rely in the interaction between the specific genetic background and a combination of environmental risk factors engendering a cascade of neurotoxic effects, finally directing towards neuronal cell dysfunction and finally death. In this context, astrocytes (AS) and microglia (M) are the key players, mediating the effects of both genetic and environmental influences, including PD mutations, aging, hormones, endotoxins and neurotoxins, as a function of the host's-specific repertoire of genetic and environmental risk factors [see 12-21, and 54]. Notably, their uncontrolled activation (i.e. under inflammatory/neurotoxic exposure and upon brain injury) favor a switch towards the so-called AS-1 and M-1 harmful glial phenotype, that may directly affect neurons by releasing various molecular mediators, such as pro-inflammatory cytokines, reactive oxygen (ROS), and nitrogen species (RNS), which in turn perpetuate/exacerbate glial activation, resulting in increased mDA neuron vulnerability and cell death [54] [**Figure 3**]. Within this context, the pivotal role of Wnt signaling in directing to either neuroprotection/degeneration wil be discussed in the next section.

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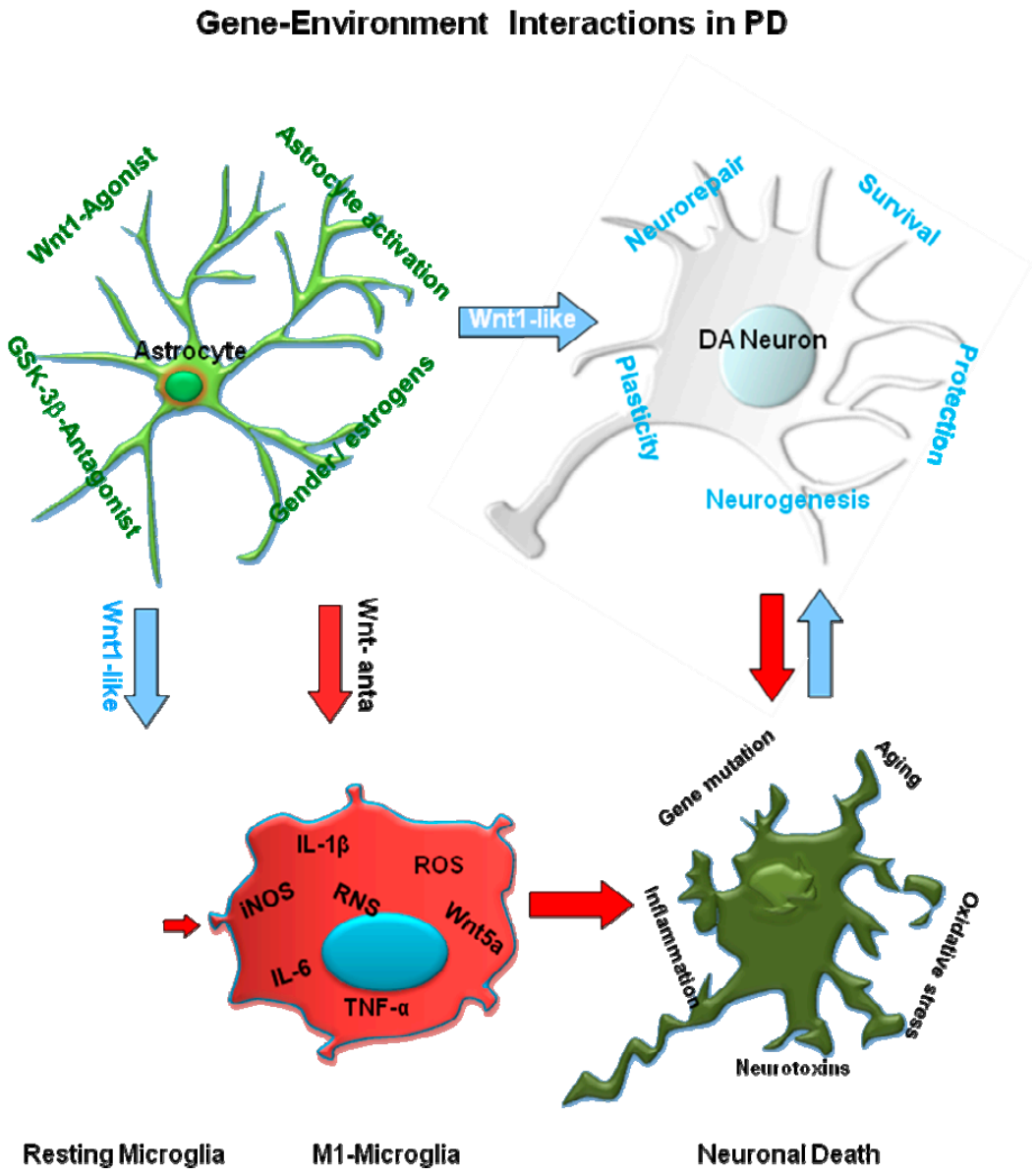

Figure 3. Schematic illustration of Wnt1/β-catenin signaling as a key player in glia-neuron crosstalk. A simplified scheme linking reactive astrocytes, mcroglia and Wnt/β-catenin signaling to mDAergic neuron survival/death is summarized. Upon injury, a number of conditons, including astrocyte activation, the genetic and hormonal background (i.e. gender and estrogens), endogenous and exogenous activators of Wnt/β-catenin signaling components (i.e., GSK-3β-antagonists), can promote astrocyte beneficial effects via the expression of a panel of growth/neurotrophic factors, particularly *Wnt1,* contributing to the survival, repair and neurorescue of DA neurons, via direct neuronal effects (see Legend to Figure. 2) and through the inhibition of the microglia-M1 activated phenotype. Activation of Wnt/β-catenin signaling can also promote neurogenesis from adult stem/neuroprogenitors. By contrast, aging, neurotoxin exposure (MPTP) and genetic mutations exacerbate microglia activation, with up-regulation of a wide variety of pro-inflammatory mediators including TNF-α, IL-1β, Wnt5a, iNOS-derived NO and reactive oxygen (ROS) and nitrogen (RNS) species. Neurotoxic injury or increased oxidative load as a result of gene-environment intercations may antagonize Wnt/β-catenin signaling in DA neurons by up-regulating active GSK-3β, leading to β-catenin degradation and increased DA neuron vulnerability. Modified from L'Episcopo et al. [50], with permission.

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5. A Wnt1 self-protective regulatory loop engaged by astrocyte-neuron crosstalk safeguards mDA neuron physiology in health and disease

Because astrocytes play key roles in the maintenance of brain homoestasis, energy metabolism, and in particular the defense against oxidative stress, an impairment of astrocyte-neuron crosstalk as a function of aging and neurodegenerative conditions may contribute to disease progression and impair the recovery process after mDA neuron injury. A body of evidences suggests that astrocytes play a vital role in the response of SNpc DA neurons to injury or inflammation, by scavenging excess of neurotoxic factors, removing dying cells and cellular debris, and stimulating repair processes, while impairment of astrocyte function as a result of ageing or exacerbated inflammation, may critically influence neurodegeneration and neurorepair [reviewed in 54]. Indeed, after injury, many adaptive changes occurring within the astroglial cell compartment may serve to increase the defense against oxidative stress, to reduce inflammation, to improve mitochondrial performance, to increase neurotrophic support, and/or to activate adult neurogenesis [54].

Hence, astrocytes are known to secrete both inflammatory and anti-inflammatory, as well as neurotrophic and survival factors, and play a critical role in modulating microglial activity. Importantly, glial fibrillary acidic protein (GFAP)-expressing astrocytes can contribute to cell genesis both as stem cells and as important cellular elements of the neurogenic microenvironment, with implications for self-recovery/neurorepair [see 54].

In 2011, a first series of *in vivo* and *in vitro* evidences implicated *Wnt/β-catenin* signaling and MPTP-reactive astrocytes “in situ” as a novel candidate component of neurorescue/repair pathways in MPTP-induced nigrostriatal dopaminergic plasticity [47,48]. First, wide gene expression analysis of 92 mRNA identified a major up-regulation of pro-inflammatory chemokines and Wnt1 during MPTP-induced DAergic degeneration and self-recovery [47]. Next, spatio-temporal analyses within the injured VM showed a dynamic interplay between prototypical proteins of canonical Wnt signaling pathway (e.g., Fzd-1

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receptor, β-catenin and GSK-3β), associated to DAergic degeneration/repair and glia activation [47]. Additionally, *in situ* hybridization histochemistry next revealed MPTP-reactive astrocytes as candidate source of Wnt1 [47]. *In vitro* experiments further suggested activated VM astrocytes as likely components of Wnt signaling pathway critically contributing to mDAergic neuroprotection against MPTP toxicity [47,48]. Moreover, factors derived from VM activated astrocytes, especially Wnt1, promoted neurogenesis and DAergic neurogenesis from neural stem/progenitor cells (NSCs) derived from the adult midbrain [36].

The presence of an intrinsic *Fzd-1/β-catenin* tone was highlighted using *in vitro* PD model systems, and various cytotoxic conditions, such as serum deprivation (SD), and 6-OHDA and MPP^+, the recognized neurotoxic compounds that mimick, both *in vivo* and *in vitro*, the biochemical characteristics of PD, namely oxidative stress and mitochondrial dysfunction [48]. Here, the three PD models confirmed the dramatic caspase3-dependent DAergic neuronal death, and identified an early and marked down-regulation of *Fzd-1* and *β-catenin* transcript and protein levels, as a result of the neurotoxic injury [47,48]. Importantly enough, the preventive application of *Wnt1* fully prevented neurotoxin-induced DAergic neuron death and efficiently reversed β-catenin downregulation, both at a mRNA and protein levels [48]. Further studies in both purified neurons and astrocyte-neuron co-culture paradigms, using pharmacological antagonism or RNA silencing along with functional studies in both intact and SN lesioned mice, clearly established an intrinsic *Wnt1/Fzd-1/β-catenin* tone critically contributing to the survival and protection of adult midbrain DA neurons [see 47,48] (Figure 2).

At the neuron-glial interface, astrocyte derived Wnt1 via Fzd-1 receptor likely transmits pro-survival signals in mDA neurons, possibly via blocking GSK-3β-induced degradation of β-catenin, which in turn incite cytoprotection/neurorepair [47-50]. Notably, exaggerated microglial pro-inflammatory status can still impair astrocyte anti-inflammatory functions

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and mDA neurorescue via inhibition of Wnt1 expression and downregulation of anti-oxidant/antiinflammatory cytoprotective proteins in astrocytes [47-55].

Together, these studies first introduced *Wnt/β-catenin* and its crosstalk with glial inflammatory pathways as a prominent actor in the neuron-glial scenario for the lifelong protection of the vulnerable mDAergic neuronal population [**Figure 3**].

6. Deficient Wnt signaling with age as a risk factor for nigrostriatal DAergic neuron integrity

The presence of a *Wnt/β-catenin* tone supervising the integrity of mDA neurons in the intact and PD injured brain of young adult mice was dramatically impaired with the aging process. Hence, with age, lack of Wnt1 expression in midbrain astrocytes in older as opposed to younger mice, and loss of β-catenin in mDA neurons correlated with failure to recover in response to acute MPTP injury, was first identified by 2011 [47,48]. Indeed, not only the astrocyte-dependent neuroprotective response was absent in aging mice midbrain, but major negative Wnt's inhibitors, including Dkk1 and GSK-3β showed a significant up-regulation, accompanied by the failure of mDAergic neurons to repair upon MPTP injury [47,48]. Notably, the pharmacological activation of *Wnt1/β-catenin* signaling via downregulation of GSK-3β, mimicked nigrostriatal recovery, thus establishing a functional link between Wnt signaling and DAergic plasticity [47,48]. Hence, these findings raised the possibility that astrocyte-derived Wnt signals might directly and/or indirectly participate to DAergic neuroplasticity observed upon MPTP exposure of young adult mice. Accordingly, blocking Wnt/Fzd signaling by intranigral infusion of the Wnt's antagonist, Dkk1, counteracted DAergic neuroprotection in young MPTP injured mice, thus mimicking aging-induced counteraction of mDAergic neurorepair [48]. Likewise, increased Dkk1 expression with age [48,55] and 6-OHDA-induced mDAergic neuron death [80]. In addition, a synergy between

aging, inflammation and neurotoxin (MPTP) treatment in down-regulating major components of canonical Wnt pathway was observed within the subventricular zone (SVZ) niche, resulting in altered Wnt/inflammatory crosstalk and dramatic impairment of neurogenesis [52,53].

Together, these results suggested that disruption of a key neurodevelopmental signaling pathway with age may predispose to loss of mDA plasticity via inhibition of Wnt/β-catenin signaling as a prelude for PD development and vulnerability [47,48].

Of specific interest, in the same years Okamoto and co [124] reported in 2011 a decreased Wnt3 release from aged hippocampal astrocytes regulating the age-associated decline of adult neurogenesis [124]; and in 2013, Seib et al [125] showed that the endogenous Wnt antagonist Dkk1 increased with age, resulting in the suppression of adult neurogenesis and proliferation, whereas Dkk1 knockout mice showed increased Wnt signaling, leading to enhanced neurogenesis and improved spatial memory [125].

The decline of Wnt signaling in the aging brain has been further corroborated by different lines of evidences [126-128]. In 2012, Miranda and co [126] observed a downregulation of Wnt agonists, which was most prominent for Wnt3 in astrocytes from13-month-old mice when compared with those from 3-month-oldmice [126]. A downregulation of several Wnt signaling-related molecules with age in mouse and rat brains were also reported by Hofmann et al. in 2014 [127], and Orellana et al., in 2015 [128]. In fact, the expression analysis of 84 Wnt-related genes showed a reduction of the Wnt dependent transcription factors Lef1 andTcf3 and a reduction in three Wnt ligand genes, Wnt2, Wnt4 andWnt9a, in old (36 months old) compared to young (5 months old) mice [127]. Accordingly, reduced activation of the canonical pathway was also observed in the hippocampus of old (24 monthsold) compared to young (4 months old) rats, where β-catenin is decreased in the nucleus, accompanied by an increase of active GSK3-β [128]. There is also a decrease in the activity of the canonical pathway in the hippocampus of the senescence accelerated SAMP8 mice,

since there is a decrease in the active β-catenin levels [129]. Remarkably, aging and neurotoxin exposure further amplify Wnt/β-catenin down modulation in SN tissues of 16-20 monh old mice, since the endogenous Wnt-antagonists, *Dkk1, sFrp2* and *Gsk3b* showed a striking up-regulation (of 6- to 14-fold) , in face of exacerbated levels of inflammatory and oxidative stress genes, including *IL-1β, TNF-α, IL-6, Nos2, NFkb* and the phagocyte NAPDH oxidase, *Phox,* that accompanied the failure of nigrostriatal DAergic repair [55].

By contrast, the key neuroprotective role of Wnt1 in the aged midbrain was highlighted in the recent study of Zhang and co in 2015 [75], in mice heterozygous for the homeodomain (HD) transcription factor (TF) Engrailed 1(En1+/− mice), and characterized by the age-dependent and slowly progressing degeneration of the mDAergic neurons [see 75 and Refs therein]. Here, the ectopic expression of Wnt1 in the adult En1+/Wnt1 VM activated a gene cascade that protected these genetically affected En1 heterozygote (En1+/−) neurons from their premature degeneration in the adult mouse VM. Hence, the direct Wnt1/β-catenin signaling targets Lef1, Lmx1a, Fgf20 and Dkk3, as well as the indirect targets Pitx3 (activated by LMX1A) and Bdnf (activated by PITX3), playing decisive roles in mDA neurodevelopment, were significantly up-regulated [75]. The authors also showed that the secreted neurotrophin BDNF and the secreted Wnt modulator Dkk3, but not the secreted growth factor FGF20, increased the survival of En1 mutant dopaminergic neurons *in vitro*, suggesting that the Wnt1-mediated signaling pathway and its downstream targets BDNF and Dkk3 might thus provide a useful means to treat certain genetic and environmental (neurotoxic) forms of human PD [75].

Furthermore, a major role of a physiological Wnt tone for synaptic maintenance and function in the adult striatum was recently uncovered [76]. The studies of Galli and co first demonstrated the expression of several Wnts, their receptors and modulators during synapse development and in the adult striatum [76]. The authors underscored a novel role for Wnt signaling in the maintenance/stability of excitatory and DAergic synapses in the adult

striatum [76]. Then, *in vivo* blockade of Wnt signaling by inducibly expressing the secreted Wnt antagonist Dkk1, resulted in a significant degeneration of DAergic cortico-striatal excitatory synapses in striatum and a decrease in glutamate release from cortico-striatal afferents [76]. Moreover, transgenic mice that overexpress Dkk1 in the hippocampus exhibit synapse loss, impaired long-term potentiation, enhanced long-term depression, and learning and memory alterations [130]. Notably, the effect of Dkk1can be reverted by Wnt agonists or by the inhibition of GSK-3β, thereby indicating that the endogenous Wnt proteins are critical to maintain synaptic connections in the adult brain [131].

Taken together, these results suggest that, in the aged brain, a general downregulation of Wnt signaling occurs with multiple consequences for the vulnerable nigrostriatal DAergic system; in addition synergy between various risk factors, including inflammation and neurotoxin exposure may further impair Wnt signaling with harmful effects for nigrostriatal functionality. Importantly, the ptential exists to revert some of these age-dependent changes, and will be discussed in the next section.

7.Wnt/β-catenin signaling is required to promote neurorepair and DAergic regeneration in ageing PD mouse model

In the adult brain, neural progenitors in neurogenic areas such as the subventricular zone of the lateral walls of the lateral ventricles (SVZ) and the subgranular zone (SGZ) of the hippocampus are in intimate contact with astrocytes which helps to generate an instructive "niche" that promotes neurogenesis [132-134). Notably, astrocyte-derived Wnts and *Wnt/β-catenin* signaling activation contribute to the regulation of adult neurogenesis [135]. During development, Wnt1/β-catenin activation control DAergic neurogenesis by maintaining the integrity of the neurogenic niche and promoting the progression from $Nurr1^{+}/TH^{-}$ post-mitotic DAergic neuroprogenitors to $Nurr1^{+}/TH^{+}$ neurons [136,137]. Recently, the adult

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midbrain aqueduct periventricular regions (Aq-PVRs) were shown to harbor neural stem/progenitor cells (mNSCs) with DAergic potential *in vitro* [138]. However, restrictive mechanisms *in vivo* are believed to limit their DAergic regenerative capacity [138,139].

Interestingly, *in vivo* studies in young adult mice revealed that MPTP-induced SNpc neuron death promoted a remarkable astrocyte-dependent remodeling within the Aq-PVR niche [78]. Fascinatingly, these cells had the morphology of radial glia, the stem cell population in the CNS that persist in the adult brain [see 140]. Hence, high levels of expression of Wnt/β-catenin genes, together with their Wnt-dependent transcription factor, Nurr1, associated with a remarkable time-dependent nigrostriatal DAergic histopathological and functional neurorestoration upon MPTP injury [78]. These results are in line with earlier and more recent evidence indicating that Wnt/ß-catenin signaling is required for radial glial neurogenesis and neuron regeneration following injury [see 140-142 and references herein]. Hence, using Cre-mediated lineage tracing to label the progeny of radial glia, Wnt/ß-catenin activation was necessary for progenitors to differentiate into neurons. Further, axonal regrowth after injury also required *Wnt/ß-catenin* signaling, suggesting coordinated roles for the pathway in functional recovery, and establishing Wnt/ß-catenin pathway activation as a necessary step in spinal cord regeneration [see 141,142] .

Canonical *Wnt/ß-catenin* signaling reporter mice are strains of transgenic mice with a *LacZ* transgene controlled by TCF/LEF consensus DNA binding elements and a minimal promoter [143]. The establishment of transgenic Wnt reporter mice and reliable antibodies allows the identification of cell types that contain functional Wnt signaling, express Fzd receptors and secrete Wnt ligands. Using transgenic (*BATGAL and Axin*) β-catenin reporter mice, *Wnt/ß-catenin* signaling activation was next demonstrated both on Aq-PVR-DA niche and mDAergic neurons in response to MPTP [78]. Hence, in young adult mice, spatio-temporal analyses further unveiled β-catenin signaling in predopaminergic ($Nurr1^{+}/TH^{-}$) and imperiled or rescuing DAT^{+} neurons during MPTP-induced DA neuron injury and self-

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repair [78]. Currently, the neurogenic potential of DA neurons in the adult midbrain is a highly-debated issue [78,144-146]. Recent findings showed newborn DAergic neurons mainly derived from the migration and differentiation of the NSCs in the Aq-PVRs and their adjacent regions upon 6-OHDA lesion [147], thus supporting the possibility of new DA neuron formation in response to SNpc DAergic neuron death [55,78].

As with the aging process Wnt signaling in the brain declines, this results in the impairment of Wnt-mediated self-protective, neuroreparative and neurorestorative DAergic mechanisms. Of special interest, in the aged PD mouse model, the changing properties of midbrain-Aq microenvironment, resulted in reduced DAergic neurogenic potential of Aq-NSCs via a loss of astrocytic *Wnt1* and a failure of Wnt/β-catenin signaling activation [78]. This effect, in turn, associated to the impairment of nigrostrial DAergic recovery from MPTP insult of aged mice for their entire lifespan [55,78].

Ex vivo and *in vitro* studies coupled to different co-coculture paradigms and a panel of experimental conditions, next indicated that both glial age and a decline of glial-derived factors, including Wnt1, were responsible for impaired NSC neurogenic potential within the SVZ and Aq-PVR niches [52,53,78]. Notably, aged NSCs still retained their neurogenic and DA differentiation potential when *Wnt/β-catenin* signaling was restored via “astrocyte rejuvenation”-induced Wnt1 expression or under *Wnt/β-catenin* activation regimens, such as GSK-3β antagonism, leading to DA neuron formation, *in vitro* [78]. *In vivo* studies further confirmed that the pharmacological activation β-catenin, *in situ,* with a specific GSK-3β antagonist promoted a significant degree of DAergic neurorestoration associated with reversal of motor deficit, with implications for neurorestorative approaches in PD [78].

The unique role of astroglial-derived Wnt1 in aged MPTP-injured mice was further supported ,very recently, following NSC transplantation in the injured SN, which promoted a remarkable time-dependent nigrostriatal DA neurorescue/repair [55]. Here, SVZ-derived adult NSCs transplanted in the aged MPTP-injured SN mainly differentiated into astrocytes

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re-expressing Wnt1. Especially, these NSC-derived astrocytes promoted a remarkable time-dependent endogenous nigrostriatal DAergic neurorepair [55]. Astrocyte-derived factors, especially Wnt1, were shown to act at different levels to rejuvenate the host microenvironment and to promote DAergic neurorestoration in the aged MPTP-injured brain [55].

Taken together, these results suggest the potential to restore mDA neuron functionality by activating *Wnt/β-catenin* signaling in endogenous Wnt-responsive sources, through either pharmacological/cellular approaches aimed at activating/recruiting endogenous progenitors and rescuing the imperiled/diseased DA neurons [55,78].

8. Targeting Wnt signaling as a "*Wn(t)dow*" of opportunity for mDAergic neurorescue

In last few years, an increasing number of pharmacological and immunomodulatory agents affording neuroprotection are being recognized as activators of the canonical Wnt/β-catenin signaling pathway. Different studies focused on the neuroprotective capacity of Wnt1-agonists and pharmacological inhibitors of GSK-3β. Hence, in 2013, Wei et al [81] supported the ability of exogenous Wnt1-induced activation of Wnt/β-catenin pathway, to protect SH-SY5Y cells against 6-OHDA-induced DA toxicity, and in 2015, Zhang and co [83] corroborated the protective role of enhancing β-catenin activity via GSK-3β inhibition to afford neuroprotection of PC12 cells against rotenone toxicity. Here, GSK-3β inhibitors LiCl and SB216763 leading to β-catenin stabilization afforded neuroprotection via the induction of the mDAergic transcription factor, orphan nuclear receptor, Nurr1 [83], crucially involved in the survival and maintenance of mDAergic neurons. Amongst others GSK-3β inhibitors, bromoinduru-30-oxime-(6-BIO) was shown to protect hippocampal neurons from the apoptotic effects of amyloid-β (Aβ) oligomers via a direct activation of Wnt/β-catenin pathway [148]. Interestingly enough, different classes of pharmacological agents including statins (simvastin) [85], opioids (pentazocine) [86], nicotinic receptor

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modulators [87], or derivative of natural products [88,89,149,150] amongst others, were reported to protect mDAergic neurons against apoptosis, in either *in vivo* or *in vitro* models of PD, via the activation of *Wnt/β-catenin* signaling pathway, thus supporting the critical role of this signaling system for the protection of mDAergic neurons against cytotoxicity .

Other studies indicated the potential of Wnt1-like agonist, such as Wnt1 inducible signaling pathway protein 1 (WISP1), a downstream target in the Wnt1 pathway, to block neurodegeneration [151-153]. WISP1, also known as CCN4, is a member of the six secreted extracellular matrix associated CCN family of proteins that mediate awide panel of critical functions including the ability to prevent apoptosis, control caspase activation, and oversee autophagy [see 151-153]. The neuroprotective mechanism of WISP1 was shown to involve pivotal pathways controlling neuronal death/survival, such as phosphoinositide 3 kinase/Akt1, apoptotic mitochondrial signaling and included Bad, Bax, Bim, and Bcl-xL [see 151-153]. Thus, targeting downstream pathways of Wnt1, such as WISP1, may represent ptential avenues for neurorepair upon CNS injury.

The interrelationships among inflammatory, survival, and Wnt/β-catenin signaling cascades also uncovered a complete regulatory loop impacting in both neurogenesis and neuronal outcome upon injury, protecting mDAergic neurons from loss in the MPTP mice model of PD through inflammatory inhibition, via activation of PI3K/p-Akt and Wnt1/Fzd1/β-catenin cell signaling pathways [47-55,88,89,154,155], thus prompting further investigations along these lines.

Together, these and other findings of the last few years support the indication of Wnt/β-catenin signaling as a critical final common pathway for mDAergic neurorescue, with an increasing interest in therapeutic targeting of this pathway [32,40,41,104]. Given the multitude of roles of Wnt/β-catenin signaling in the control of tissue homeostasis during development and disease, unraveling its complex biological roles through different tissues,

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at different ages and under different gene-environmental conditions will further increase our knowledge on PD physiopathology and identify novel therapeutic avenues.

Concluding remarks and future directions

A central challenge in the field of neurodegenerative disorders, is developing therapeutic strategies that boost neuroprotection, neurorepair and help endogenous regenerative programs in the injured brain. In this work, I summarized several lines of evidences supporting a key role for *canonical Wnt/Fzd-1/β-catenin* pathway contributing to the maintainance mDAergic neuron survival and functionality within the adult midbrain. Accumulating data from the studies of genes mutated in PD underscore the pivotal role of Wnt signaling pathways for the function of mDAergic neurons in health and disease states. Hence, new potential associations between the Wnt pathway and mitochondrial dynamics, lysosomal biogenesis, apoptosis, immunoregulation and cell cycle, affecting key functions in neuroprogenitors, post-mitic neurons and glial cells are starting to be unveiled.

Notably, *Wnt1*-induced neuroprotection is closely integrated with the astroglial response to oxidative stress and inflammation upon injury, and requires *Fzd-1* receptor and *β-catenin* stabilization to convey pro-survival signals to the nucleus, whose expression likely underlie the observed neuroprotection. Reactive astrocytes up-regulate Wnt/β-catenin signaling, and sustained Wnt/β-catenin activation might help in restraining excessive inflammation, thus further promoting neuroprotection. Additionally, VM astrocytes and *Wnt/β-catenin* signaling activation promote neurogenesis from adult NSCs. Hence, Wnt signaling at the neuroimmune interface plays a pivotal role in the regulation of neuroprogenitors, post-mitotic neurons, and central immune cell functions in PD. Pharmacological manipulation of microglial oxidative and nitrosative status, either *in vitro* or *in vivo*, can also activate abeneficial Wnt/β-catenin signaling, thus affording neuroprotection and induces a successful

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neurogenesis rescue. Therefore, modulating these astroglia changes may represent a powerful ground for novel therapeutic intervention strategies to prevent, delay progression, and/or ameliorate pathology.

Together, these studies suggest that Wnt-induced pathways are required for the regulation of a panel of processes linked to mDAergic neuroprotection, neurorepair and regeneration after injury, and include the re-expression of a genetic neurodevelopmental programs for DA neuroprogenitor acquisition of the mature mDAaergic phenotype, DA neuron formation and migration, as well as their survival and maintenance within an hostile microenvironment.

Given that the activity of Wnt signaling in the brain declines with the aging process, this results in the inhibition of Wnt-mediated self-protective, neuroreparative and neurorestorative mDAergic programs. Because *Wnt/β-catenin* signaling controls the expression of a wide panel of direct and indirect target genes, mis-regulation of this signaling cascade may be involved in various age-dependent diseases associated with impaired neurogenesis, including PD. Notably, the signaling mechanisms contributing to neuronal death and NSC impairment in the aged PD brain target the *Wnt/β-catenin* signaling pathway. Coupled to the increasing evidences on the key role of Wnt/β-catenin signaling cascades in neurodevelopment, neurodegeneration and regeneration, developing targeted in situ pharmacological interventions/cell manipulations that boost the inherent Wnt-dependent regenerative potential have implications for both restorative and regenerative approaches in PD.

Notably, given the multitude of roles of Wnt/β-catenin signaling in the control of tissue homeostasis and disease, there is an increasing interest in targeting of this pathway. From a therapeutic viewpoint, the Wnt signaling pathways presents several challenges to the development of a targeted neuroprotective drug, and further studies are still needed to elucidate the complex Wnt signaling circuitry. For example, the Wnt pathway itself is protective in different tissues, but the receptor context may influence the regulation of

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canonical Wnt signaling by agonist or antagonists, suggesting that a specific receptor component coupled to a specific ligand in the absence or the presence of endogenous inhibitors/activators, within a particular inflammatory context, finally mediate the tissue-/cell-specific response.

As a whole, the understanding of the intricate signaling networks of *Wnt/β-catenin* signaling is critical for the identification of new potential therapeutic targets and develop pharmacologic and cellular approaches for neurodegenerative diseases including PD.

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Conflict of interest

No conflict of interest to declare.

Abbreviations

Dkkopf (*Dkk*); dopamine, DA; dopaminergic (DAergic); DA transporter, DAT; Frizzled (Fzd); glial fibrillary acid protein (GFAP); glycogen synthase kinase-3β (GSK-3β); inducible nitric oxide (iNOS); interferon-γ (IFN-γ); interleukin-1α (IL-1α); interleukin-1β (IL-1β); LDL receptor-related protein 5/6 (Lrp5/6); leucine-rich repeat kinase 2, *LRRK2;* midbrain/hindbrain (MB); 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP); 1-methyl-

4-phenyl-1,2,3,6-tetrahydropyridine (MPP^+); nitric oxide (NO); NADPH oxidase (PHOX); peroxynitrite (ONOO-); Parkinson's disease (PD), Reactive oxygen species (ROS); reactive nitrogen species (RNS); 6-hydroxydopamine, 6-OHDA; stem/neuroprogenitor cell (NSC); Subtantia nigra pars compacta (SNpc); Subventricular zone, SVZ; Tyrosine hdroxylase (TH); Tumor necrosis factor α (TNF-α); Ventral midbrain (VM); *Wingless-type MMTV integration site 1(Wnt1)*.

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Figure Legends

Figure 1. Canonical and non-canonical Wnt signalling cascades. Three Wnt-dependent pathways have been proposed: canonical Wnt/β-catenin pathway and noncanonical Wnt/PCP and Wnt/Ca^{++} pathways. **A:** In the Wnt/β-catenin pathway, the binding of Wnts to a receptor complex composed of members of the Fzd family and LRP promotes the inhibition of the APC/GSK-3β complex, and blockade of β-catenin phosphorylation by GSK-3β. Hypophosphorylated β-catenin then accumulates in the cytoplasm and is translocated to the nucleus, where it regulates target gene expression through partnerships with TCR/LEF1 family of transcription factors, resulting in changes in gene transcription. **B:** In noncanonical Wnt-Ca^{++} signalling pathway the binding of Wnts promotes Fzd-mediated activation of pertussis Toxin-sensitive heterotrimeric guanine nucleotide–binding proteins (G proteins). This, in turn, stimulates the release of Ca^{++} from intracellular stores, which leads to the activation of Ca^{++} dependent effector molecules. Several Ca^{++}-sensitive targets-protein kinase C (PKC), Ca^{++}-calmodulin-dependent protein kinase II (CamKII), and the Ca^{++}-calmodulin-sensitive protein phosphatase calcineurin have been identified downstream of the Wnt-Ca^{++} pathway. Targets of the Wnt-Ca^{++} pathway appear to interact with the Wnt-β-catenin pathway at multiple points. Additionally, Fzd receptors in association with Kny, Ror2 or Ryk receptors can activate JNK, promoting target gene expression through AP-1. In noncanonical Wnt/PCP pathway, the binding of Wnts activates RhoA/B, Cdc42 or Rac1. Dsh activates Rac1 and Rac1 can also activate JNK, resulting in the NFAT pathway. Modified from L'Episcopo et al. [50], with permission.

Figure 2. Schematic illustration of Wnt1/β-catenin signaling regulation of mDAergic neuron survival/death. Major environmental factors including aging, inflammation,

neurotoxin exposure including PD neurotoxins (MPTP/MPP^+, 6-OHDA), pesticides (rotenone), increased oxidative load as a result of gowth factors (GFs) deprivation in synergy with genetic mutations, may antagonize Wnt/β-catenin signalling ("*Wnt off*") in mDA neurons. Up-regulation of active GSK-3β, then lead to β-catenin degradation and increased DA neuron vulnerability/degeneration/apoptosis. By contrast, in the intact midbrain canonical Wnt agonists, such as *Wnt1, Rspo* or *Norrin,* and activation of *Fzd-1* receptors also via exogenous Wnt/β-catenin activation such as GSK-3β antagonist, NO-NSAIDs treatments tors (*"Wnt on"*), contribute to maintain the integrity of mDA neurons via blockade of GSK-3β-induced phosphorylation (P) and proteosomal degradation of the neuronal pool of β-catenin. Stabilized β-catenin can translocate into the nucleus and associate with a family of transcription factors and regulate the expression of Wnt target genes involved in DA neuron survival/plasticity, neuroprotection and repair. β-catenin may also function as a pivotal defense molecule against oxidative stress,and can act as a coactivator for several nuclear receptors involved in the maintenance/protection of DA neurons. The hypothetical contribution of various endogenous Wnt agonists (Respondin, Rspo, Norrin) or antagonists (*Dkkopf, Dkk1, Wif*, frizzled-related proteins, SFRp) are also indicated. Modified from L'Episcopo et al. [50], with permission.

Figure 3. Schematic illustration of Wnt1/β-catenin signaling as a key player in glia-neuron crosstalk. A simplified scheme linking reactive astrocytes, mcroglia and Wnt/β-catenin signaling to mDAergic neuron survival/death is summarized. Upon injury, a number of conditons, including astrocyte activation, the genetic and hormonal background (i.e. gender and estrogens), endogenous and exogenous activators of Wnt/β-catenin signaling components (i.e., GSK-3β-antagonists), can promote astrocyte beneficial effects via the expression of a panel of growth/neurotrophic factors, particularly *Wnt1,* contributing to the survival, repair and neurorescue of DA neurons, via direct neuronal effects (see Legend to

Figure. 2) and through the inhibition of the microglia-M1 activated phenotype. Activation of Wnt/β-catenin signaling can also promote neurogenesis from adult stem/neuroprogenitors. By contrast, aging, neurotoxin exposure (MPTP) and genetic mutations exacerbate microglia activation, with up-regulation of a wide variety of pro-inflammatory mediators including TNF-α, IL-1β, Wnt5a, iNOS-derived NO and reactive oxygen (ROS) and nitrogen (RNS) species. Neurotoxic injury or increased oxidative load as a result of gene-environment intercations may antagonize Wnt/β-catenin signaling in DA neurons by up-regulating active GSK-3β, leading to β-catenin degradation and increased DA neuron vulnerability. Modified from L'Episcopo et al. [50], with permission.