Evidence for dopaminergic axonal degeneration as an early pathological process in Parkinson's disease.

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Abstract

Parkinson's disease is a common neurodegenerative disorder presenting with a variety of motor and non-motor symptoms. The motor symptoms manifest as a result of the progressive degeneration of midbrain dopaminergic neurons. The axons of these neurons project to the striatum as the nigrostriatal pathway, which is a crucial part of the basal ganglia circuitry controlling movement. In addition to the neuronal degeneration, abnormal intraneuronal α-synuclein protein inclusions called Lewy bodies and Lewy neurites increase in number and spread throughout the nervous system as the disease progresses. While the loss of midbrain dopaminergic neurons is well-established as being central to motor symptoms, there is an increasing focus on the timing of nigrostriatal degeneration, with preclinical evidence suggesting that early axonal degeneration may play a key role in the early stages of Parkinson's disease. Here we review recent evidence for early midbrain dopaminergic axonal degeneration in patients with Parkinson's disease, and explore the potential role of α-synuclein accumulation in this process, with a focus on studies in human populations at the imaging, post-mortem, cellular and molecular levels. Finally, we discuss the implications of this for neurotrophic factors as therapies for Parkinson's disease.

Key words:

Parkinson's disease; Midbrain; Axon; Degeneration; Alpha-synuclein; Patients.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder that affects 1-2% of the population over the age of 60 [1-3]. PD is characterized by motor deficits including bradykinesia, akinesia and resting tremor, and a range of non-motor symptoms that worsen over time [4-6]. One of the key neuropathological hallmarks of the disease is the severe loss of midbrain dopaminergic (mDA) neurons in the substantia nigra (SN), whose axons project to the striatum as part of the nigrostriatal pathway [7, 8]. The degeneration of the nigrostriatal pathway leads to a reduction of striatal dopamine, resulting in the characteristic motor features that are the basis of clinical diagnosis [4]. PD is also characterized by the presence of abnormal intraneuronal protein inclusions composed of α-synuclein within cell soma and neurites, called Lewy bodies and Lewy neurites respectively. Lewy bodies and Lewy neurites increase in number and spread to multiple locations throughout the nervous system as the PD progresses [9-14]. While the loss of mDA neurons is central to the motor symptoms of PD, there is an increased focus on when and where this degeneration begins, with much preclinical evidence suggesting that early axonal degeneration may play a key role (for an excellent review see [15]). Here we review recent the evidence for early mDA axonal degeneration in PD patients, and with a focus on studies in human populations at the imaging, post-mortem, cellular and molecular levels. We also review the evidence for α-synuclein accumulation in this process and discuss the implications of this for neurotrophic factor trials in PD.

2. Evidence from imaging studies for early mDA axonal degeneration in PD.

In the human brain, the majority of dopaminergic neurons are found in the midbrain, with approximately 200,000 to 420,000 TH-positive neurons located bilaterally in the adult human SNpc [16-19]. Evidence for early axonal degeneration in PD patients comes from imaging studies that have used tracers to measure the activity of vesicular monoamine transporter type

2 (VMAT2), aromatic L-amino-acid decarboxylase (AADC) or the dopamine transporter (DAT), all of which are expressed in or on mDA axonal terminals (as well as in mDA neuronal soma), and are used as proxy measures of mDA axonal and somal integrity [20, 21]. For a comprehensive meta-analysis of imaging studies of striatal dopamine in PD see [22]. In this review, we focus predominantly on studies that have directly compared the striatum and the SN in the early stages of PD, in order to examine the evidence for nigrostriatal axonal pathology in PD patients.

In a study by Hsiao et al. [23], VMAT2 imaging was carried out by measuring uptake of 18F-DTBZ using PET in specific brain regions of patients with mild (n=22), moderate (n=20) and advanced PD (n=11), as defined by modified Hoehn-Yahr (mH-Y) staging; these were compared to healthy age-matched controls (n=17) [23]. For the purposes of this review on early axonal changes, we focus on the mild PD group, since they had a mean disease duration of 5 years, compared to durations of 14 and 15.5 years in the moderate and advanced PD groups, respectively [23]. In this mild PD group, VMAT2 activity in the posterior putamen was most affected, showing a 63-76% reduction compared to controls, whereas in the SN, there was a reduction of 13-29% compared to controls. This supports the idea that early loss of mDA axonal terminals in the striatum may be central to the initial progression of PD (Fig. 1A, B). This conclusion is supported by another PET study, which compared DAT signal loss in the putamen and the SN of PD patients. Fazio et al. used ^{8}F-FE-PE2I PET imaging to measure DAT activity in 10 early-stage PD patients, with a mean disease duration of three years, compared to 10 age-matched controls [24]. It is worth noting that of the 10 PD patients, one had a disease duration of 12 years, with the other nine having a mean disease duration of 2.1 years. The binding of ^{8}F-FE-PE2I was significantly lower in PD patients than in controls, and in agreement with the Hsiao et al. study [23], the putamen was most affected. Compared to controls, there was a three-fold reduction in ^{8}F-FE-PE2I signal in the putamen, and a 1.5-fold

reduction in the SN, of PD patients [24]. These two PET imaging studies support the hypothesis that loss of striatal axonal terminals of nigrostriatal dopamine neurons precedes the loss of their nigral cell bodies in PD. An alternative explanation for the greater loss of dopamine-related signals in the putamen than in the SN is that the loss of a low number of nigral dopamine neurons correlates with a larger loss of striatal terminals. This is supported by a study by Matsuda and colleagues, which showed that individual dopamine neurons of the rat SN innervate a large area of the striatum, with each neuron sending very dense and highly spread axonal arborsiations to synapse on a large number of striatal neurons [25]. If this is also the case in the human brain, then the more extensive loss of DAT-related signals measured by PET in the striatum compared to the SN could suggest that the earliest event in PD pathology is a loss of a small number of nigral dopamine cell bodies, with an corresponding loss of their extensive axonal arborisations in the striatum.

The two PET studies on PD patients described above support the proposal that nigrostriatal damage occurs early in PD pathology and may lead to alterations in nigrostriatal connections *in vivo*. Evidence for this nigrostriatal disconnection comes from a recent integrated *in vivo* imaging study by Caminiti and colleagues, on patients with early-stage idiopathic PD. Specifically they used [^{11}C]FeCIT PET imaging to measure presynaptic DAT activity in the nigrostriatal and mesolimbic systems of 36 patients (mean age 57.5 ± 12.6 years) with a mean disease duration of less than two years, as well as in 14 age-matched controls [26]. They reported a substantial reduction of presynaptic DAT availability in the dorsal putamen, less reduction in the dorsal caudate, and limited reduction in the SN [26]. The longitudinal nature of the progression of pre-synaptic dopaminergic deficiency in the early stages of PD is highlighted by recent DAT imaging data from the Parkinson's Progression Markers Initiative (PPMI) study [27]. This study recruited recently-diagnosed PD patients (n=423) and showed that the reduction in DAT binding (compared to baseline) progressed from 10 to 16 to 26

percent in the caudate, and from 14 to 19 to 31 percent in the putamen after 1, 2 and 4 years, respectively [27]. Moreover, the annualized changes in DAT binding were greatest in the first year than in subsequent years [27]. These data show that there is a rapid progression of a presynaptic dopaminergic deficiency in early-stage PD.

Intriguingly, Caminiti and colleagues also performed a dopamine network analysis, to examine resultant changes in patterns of mDA connectivity *in vivo* [26]. In agreement with the DAT measurements, this analysis showed a severe reduction in connectivity between the SN and the dorsal putamen. Such functional mDA disconnection in the nigrostriatal pathway is also supported by a study by Theisen et al., who used probabilistic tractography to examine structural connectivity between the putamen and SN in a cross sectional study of 40 PD patients (mean age 65.3 ± 9.6 years, mean disease duration 6 years), and 44 age-matched controls [28]. Based on diffusion-weighted dated, they estimated that anatomical striato-nigral connectivity was generally lower in PD patients than in controls [28]. Collectively, these data suggest that there is a more profound loss of mDA striatal axonal terminals at the early stages of PD.

3. Post-mortem evidence for early mDA axonal degeneration in PD.

The evidence from the imaging studies described above showing a loss of mDA axonal terminal early in PD would suggest that in post-mortem samples, there should be a greater reduction of mDA striatal innervation than of nigral mDA neurons. To address this, an important study by Kordower et al. analysed the extent of nigrostriatal degeneration in post-mortem samples from PD patients with various disease durations [29]. Specifically, they examined 28 PD brains with disease duration ranging from 1 to 27 years, as well as nine age-matched control brains. In one case at one-year post-diagnosis, there was a mild reduction in the putamen of immunoreactivity for tyrosine hydroxylase (TH) and DAT, two markers of mDA neurons. This was more pronounced in cases of three years disease duration. By four

years post-diagnosis, there was minimal DAT and TH-positive axons remaining in the striatum, and these exhibited morphological features consistent with ongoing degeneration. Quantitation of the loss of striatal innervation revealed a 35-75% loss of TH and DAT immunoreactivity at 1 to 3 years post diagnosis [29]. This is similar to the 63-76% reduction in VMAT2 activity in the posterior putamen in patients with a mean disease duration of five years reported in Hsiao et al. imaging study [23].

In addition to the axonal loss, Kordower et al. found variable but marked reduction (ranging from 50-90%) of the number of TH-stained mDA neurons in the SN, even at the early stages of the disease [29]. Other post mortem studies have found a graded loss of nigral DA neurons in early-stage PD, and in incidental Lewy body disease (ILBD), which is considered to be a presymptomatic stage of PD [30, 31]. For example, Dijkstra et al reported a 20% lower nigral neuronal density in patients with ILBD and a 56% lower density in PD patients, compared to controls [32]. Another post mortem study found a 40% loss of nigral DA neurons in ILBD patients, and a 82% loss in PD patients, compared to controls [33]. Similar results were reported by Milber et al (40% loss in ILBD patients and 67% loss in PD), who also found that mDA cell number did not decline over the Braak stages of ILBD [34]. Overall these findings suggest a premotor period with limited nigral neuronal loss in the SN, followed by a steep decline in nigral mDA neurons between Braak stages 3 and 4, coinciding with the onset of motor symptoms [35].

Kordower et al noted that a residual population of mDA neurons remained in the SN even decades after diagnosis, despite the almost complete absence of DAT and TH-positive fibers in the putamen that was detectable at four years after diagnosis [29]. This is an important finding, as it suggests that axonal regeneration may be possible even from a relatively small pool of residual neurons. The potential of this for striatal reinnervation is highlighted by an elegant study by Matsuda and colleagues, who labelled the axonal arbors of individual

nigrostriatal mDA neurons in Wistar rats and showed that a single nigrostriatal mDA axon covered on average 2.7% of the striatum volume [25]. These authors estimated that the entire striatum is influenced by a relatively small percentage of the total pool of mDA neurons in the SN [25]. Given that a residual population of mDA neurons remain in the SN for decades after PD diagnosis [29], a focus on identifying strategies for axonal regeneration may offer new opportunities for disease-modifying therapies.

4. Evidence from induced pluripotent stem cells for axonal degeneration in PD.

Since α-synuclein was first described by Spillantini and colleagues as the main component of Lewy bodies [36], it is well-established that this protein plays a central role in PD [37-40]. There is a large body of work on pre-clinical modelling of α-synucleinopathy which gives important insights into the mechanisms of α-synuclein-induced neurodegeneration, leading to potential platforms for therapeutic development [41-44]. The first evidence supporting a role for α-synuclein in PD came with the demonstration that a G209A mutation in the α-synuclein (SNCA) gene, leading to an A53T substitution in α-synuclein protein, led to autosomal-dominant, early-onset PD [45]. Subsequent studies identified triplications [46] and duplications [47] of the SNCA gene, which were also associated with autosomal-dominant PD. Subsequent reports established SNCA as a definitive susceptibility gene for sporadic PD [44, 48-50]. These studies collectively showed that α-synuclein is linked to both familial and sporadic PD, with preclinical work suggesting that it may play a central role in mDA axonal degeneration [51].

Studying the role of α-synuclein in axonal degeneration in humans is considerably more difficult, however work using induced pluripotent stem cells (iPSCs) is now shedding new light in this regard. iPSCs are pluripotent stem cells that can be generated and reprogrammed from adult somatic cells and differentiated into neurons [52-55]. Consequently iPSCs and neurons, can be generated from the somatic cells of patients with sporadic or familial PD [56]. This has

paved the way for studies on α-synuclein's role in mechanisms of disease pathology including axonal degeneration. iPSC-derived neurons generated from patients with SNCA triplication have been reported to show lower neuronal connectivity and spine formation than neurons derived from controls [57]. These features have been demonstrated in iPSC-derived mDA neurons from patients with SNCA triplication and from those carrying the LRRK2 (G2019S) mutation [58-60]. Another study showed that iPSC-derived mDA neurons carrying SNCA triplication or the LRRK2 (G2019S) mutation exhibited abnormalities in neurite length, along with evidence for axonal degeneration that included axonal blebbing and fragmentation [61].

An important recent study reported significantly higher α-synuclein load in iPSC-derived mDA neurons from two patients with early-onset PD with the p.A53T SNCA mutation, compared to controls [62]. Furthermore, the PD iPSC-derived neurons contained the pathological form of α-synuclein within Lewy-like neurites, exhibited α-synuclein aggregation, and developed α-synuclein-positive varicosities, neurite swelling and fragmentation [62]. These neuropathological changes in the PD iPSC-derived neurons could be prevented by treatment with small molecules that reduce α-synuclein toxicity by interfering with oligomer formation [62]. These findings are supported by the fact that knockdown of α-synuclein can prevent decreases in neurite number and in total neurite length in iPSC-derived mDA neurons from patients with SNCA triplication [57]. Moreover, the p.A53T SNCA PD iPSC-derived neurons had an impaired ability to form synapses [62], which is fully supported by work from the Spillantini group showing that transgenic mice expressing human α-synuclein develop synaptic dysfunction in striatal DA terminals and an age-dependent reduction in DA release [63, 64]. These studies are important as they provide a causal link between α-synuclein pathology and synaptic and axonal degeneration in human mDA neurons carrying SCNA genetic alterations which are strongly associated with PD (Fig. 1C).

5. Molecular changes in the PD brain linked to mechanisms of axonal growth.

The morphological evidence for axonal degeneration in early PD supports the hypothesis that changes in gene expression patterns in the human brain can be associated with molecular pathways known to be critical for axonal growth. To date, 18 studies have examined the SN transcriptome in PD patients (for comprehensive review see [65]). Evidence supporting the hypothesis that there are changes in molecular pathways linked to axonal growth in early PD, came from a study by Dijkstra et al. [66], who carried out a transcriptome analysis on post mortem SN tissue from patients including from those characterised as Braak stage 1 and stage 2 (n=5), which is early-stage PD [12], compared to age-matched non-demented controls (n=8). In patients at Braak stage 1-2, there was a significant down-regulation of molecular pathways linked to axonal guidance signalling, and of molecular pathways linked to maintenance of the cytoskeleton [66]. This supports the morphological evidence described above, by showing that there are dysfunctional molecular pathways linked to axonal maintenance in the early stages of PD. Further evidence came from a study carried out at the single-cell level, on patients with sporadic PD (n=10) and from controls matched for age and post-mortem interval (n=9) [66]. In this study, Simunovic and colleagues performed transcriptome level analysis of single human mDA neurons, using laser microdissection to analyse differential gene expression. They reported a strong down-regulation compared to controls, of genes known to play key roles in axonal growth and guidance, including *TUBA1A* (6-fold decrease) [67], *TUBB2A* [68] (12-fold decrease) and *TUBB2B* (4-fold decrease) [69]. At the network level, differential protein expression has also been reported in a study that used proteomic analysis on human SN tissue and on age-matched neurologically-intact controls [70]. The differentially-expressed proteins included those known to be involved in a range of pathogenic processes, including cytoskeletal dysfunction [70, 71]. In addition a proteome analysis study on SN from PD patients reported reduced expression of two proteins, neurofilament-medium and neurofilament-light [72],

which are critical for axonal growth and maintenance [71]. While changes in axonal proteins is to be expected as a result of ongoing neurodegeneration, the specificity of these changes for the early stages of PD is notable, and is highlighted by the study of Dijkstra et al. [66], in which transcriptome analysis of the SN from PD patients revealed that the strongest changes in genes linked to axonal guidance signalling were seen at Braak stages 1 and 2, compared to Braak stages 3, 4, 5 and 6 [66]. These data are consistent with the theory that in the early stages of PD there is an initial and progressive loss of mDA striatal axonal terminals.

The above-described studies on molecular changes in SN tissue from PD patients are supported by transcriptome studies on iPSCs-derived neurons (Fig. 1C). Specifically, Kouroupi et al. performed global gene-expression profiling of PD iPSC-derived neurons generated from patients carrying the A53T SNCA mutations, and compared them to controls [62]. A bioinformatics analysis of differentially-expressed genes revealed significant perturbations in gene networks linked to cytoskeletal organization, neuronal differentiation and maturation [62]. This is supported by a study examining global gene expression in mDA-derived neurons from three control and three cell lines with PD genetic mutations (*PARKIN*c.255delA, *LRRK2* G2019S, *SNCA* triplication), which found alterations in certain groups of genes, including those associated with synaptic transmission and nervous system development [61]. Similar changes in global gene expression have been reported in iPSC-derived mDA neurons generated from patients with genetic (*LRRK2* G2019S) or sporadic PD, in which differentially-expressed genes included those involved in axogenesis and axon guidance [73]. Collectively, these data suggest dysregulation of gene networks linked processes important for neuronal structure is a feature of early-stage PD.

6. Implications of axonal degeneration for neurotrophic factor therapy.

Disease-modifying therapies for PD are still a major unmet clinical need. Neurotrophic

factors are proteins that have potential to slow the progression of PD. Despite showing early promise in studies on animal models of PD, clinical trials using glial cell line-derived neurotrophic factor (GDNF) protein or adeno-associated viral vector (AAV)-neurturin (NRTN) have been unsuccessful (for reviews, see [74-76]). There has been much debate on possible reasons for the lack of translation of the robust neuroprotective properties of GDNF and NRTN from animal models to human PD. The main theories revolve around optimal patient selection, limited dosage and/or diffusion, and surgical issues. Even after the application of technical advances such as convection-enhanced delivery and gene therapy, the clinical response to GDNF and NRTN has not been as robust as was expected from pre-clinical experiments. This low response of the nigrostriatal system in patients could be due to the low number of mDA neurons or in the diseased brain, or to defects in the capacity of these neurons to respond to GDNF and NRTN.

Many investigators have hypothesised that the main reason for the failure of the clinical trials was that the patients enrolled were at advanced disease stages [75-79]. Support for this theory comes from scrutiny of the clinical data from individual participants in the recent AAV-NRTN clinical trials. Segregation of data from the Phase 2b trial showed that participants who received AAV-NRTN within five years of diagnosis benefitted most, while those diagnosed more than ten years before the trial experienced no clinical improvement [80, 81]. Further post hoc analysis of data from an earlier Phase 2a trial of AAV-NRTN [82] showed that the reported clinical benefit could be attributable solely to those patients who were within five years of diagnosis, whereas none of the treatment effect was contributed by those ten years or more post-diagnosis [81]. These data illustrate that the patients enrolled in the GDNF and NRTN trials to date have been too far advanced in terms of disease stage, to have been likely to benefit from these potential disease-modifying treatments.

The evidence for axonopathy occurring during early-stage PD may explain at least partly

explain the lack of efficacy. As well as removing the main source of endogenous trophic support and signalling for mDA neurons, early degeneration of nigrostriatal axons affects the ability of these cells to respond to exogenous neurotrophic factors. Neurotrophic factor signalling relies on communication between neuronal cell bodies and their axonal terminals in distal target structures and depends on efficient axonal transport (for review see [83]). In each of the clinical trials, apart from the most recent NRTN one, which administered to both putamen and SN [80], neurotrophic factors were delivered solely to the putamen. At advanced disease stages, there may be insufficient axons remaining to allow adequate levels of neurotrophic factor to be retrogradely transported to the nigral cell bodies. Although only conducted on a few subjects, post mortem studies have clearly demonstrated that participants in the GDNF [84] and AAV-NRTN [81] clinical trials had substantial loss of dopaminergic axonal terminals in the putamen.

In addition to the dying-back of a large proportion of the nigrostriatal pathway that occurs in PD, it is possible that the remaining axons, which are themselves undergoing the degenerative process, may not be fully functional in terms of their ability to transport proteins such as neurotrophic factors. Indeed, there is substantial evidence for defects in axonal transport occurring prior to physical degeneration of axons [85-87]. Any defect or inefficiency in axonal transport could impact on the efficacy of neurotrophic factors administered to the putamen, by severely reducing the amount that can reach the nigral cell bodies (Fig. 1D). This idea, which may account, at least partially, for the low therapeutic response seen in the clinical trials, is supported by data from post mortem studies on patients from the GDNF and AAV-NRTN trials. In contrast to animal studies, which reported robust expression of GDNF/NRTN in SN neurons following intrastriatal injection of recombinant GDNF/AAV-NRTN, very little GDNF/NRTN expression was detected in the SN of patients [77, 81]. This suggests that the remaining axons in the PD brain may be unable to effectively transport proteins such as GDNF

and NRTN to their cell bodies, where they can mediate their survival-promoting actions. The mechanisms involved in impaired axonal transport in the PD brain are not fully understood, but may involve intraneuronal α-synuclein accumulation. Studies on the α-synuclein rat model of PD, which recapitulates human disease neuropathology more closely than the neurotoxin-based preclinical models used in the early studies on GDNF and NRTN, suggest that α-synuclein accumulation can affect axonal transport, by disrupting the levels of proteins that are necessary for this process [87]. Specifically, α-synuclein overexpression in the adult rat SN resulted in accumulation of proteins associated with retrograde transport and concomitant decreases in proteins required for anterograde transport, reflecting a shift in the balance of axonal transport towards the retrograde direction [87]. Another study found downregulation of the Ret receptor (a critical component of the signalling pathways of both GDNF and NRTN) in AAV-α-synuclein-injected rats and hypothesised that reduced availability of Ret at axonal terminals accounted for the deficit in axonal transport and consequent failure of GDNF to induce neuroprotective effects [88]. It is important to note that, in addition to accumulation of α-synuclein, dramatic and persistent axonal pathology has been reported in the AAV-α-synuclein rat model [89], rendering this an appropriate model for future studies on disease-modifying therapies for PD, in particular those exploring the potential for axonal regeneration.

In addition to limiting the transport of neurotrophic factors from striatal terminals to cell bodies, axonal degeneration may lead to alterations in the responsiveness of the brain to these factors. For example, lack of endogenous target-derived trophic support may result in changes in the expression of neurotrophic factor receptors, which could limit the ability of these neurons to respond to exogenously-administered factors. There is evidence for downregulation of Ret in the human PD brain [88]. The combination of a lower number of nigrostriatal axons, defects in the ability of these remaining axons to transport trophic factors from putamen to SN, as well as limited expression of receptors, may explain the low efficacy of GDNF and NRTN after

administration to patients with extensive nigrostriatal axonopathy. There will be significant challenges in achieving success with neuroprotective therapy on patients with moderate to advanced PD. In future trials, the recruitment of patients at an earlier disease stage, when the integrity of the nigrostriatal system is not so severely compromised, will be important. However, while many ongoing trials continue to specifically exclude subjects diagnosed less than five years previously, while including those at longer and advanced disease stages, there is a critical need to consider adjunct therapies that can enhance the efficacy of neurotrophic factors. Strategies aimed at promoting axonal regeneration may provide an environment in the PD brain that is more responsive to exogenously-administered neurotrophic factors, allowing them to act with increased efficacy.

One strategy aimed directly at axonal regeneration has been described by Burke and colleagues, who reported restoration of striatal dopaminergic innervation in an adult rat model of PD following AAV-mediated upregulation of the activity of Akt kinase, a critical regulator of axonal growth during development [90]. In further elegant experiments using tract tracing, they showed that this striatal reinnervation was due to growth of new nigrostriatal axons within the medial forebrain bundle, rather than to local sprouting in the striatum, or to upregulation of TH expression [91]. Strategies such as this, aimed at stimulating the re-growth of damaged axons, which occurs early in the disease process, may have a large positive impact on the advancement of neurotrophic factor therapy for PD. Since the early loss of axons and their integrity is likely to be a limiting factor for neurotrophic factor therapy, combining axon growth stimulants with neurotrophic factors has potential to achieve a higher degree of efficacy than either approach alone.

7. Future Perspectives

Despite the limited benefits achieved to date in clinical trials, there remains reason for optimism

that the early promise of neurotrophic factors for PD therapy may yet result in clinical success. Many researchers believe that the primary end-points of these trials were set at too early a stage, and that the longer-term follow-up of the patients will provide significant clinical improvements. Signs of a delayed neurotrophic effect were already seen in the Phase 2a AAV-NRTN trial [82]. The eagerly-awaited data from the latest Bristol GDNF trial, expected to be published this year, may provide further enlightenment. Patient selection, in terms of both age and disease stage, will be critical. This highlights the importance of early diagnosis of PD, probably based on biomarkers which have not yet been identified or validated, to enable treatment while the nigrostriatal pathway remains relatively intact. Testing of Ret-independent neurotrophic factors such as GDF5 and CDNF will also be important [75]. Strategies aimed at inducing the growth of new axons from mDA neurons may be used in combination with neurotrophic factors [91] to optimise the efficacy of this approach. Based on the data presented here, also critical will be studies aimed at elucidating the mechanisms involved in axonal degeneration during early-stage PD, and the development of strategies to intervene in this process, to enable regeneration of mDA axons.

If such strategies are successful in achieving axonal regeneration, it will be necessary for these new axons to grow in an appropriate manner to their striatal targets, in order to achieve functional restoration of the circuitry. The growth of the nigrostriatal pathway is guided by multiple spatially and temporally distributed chemoattractive and chemorepulsive cues [92]. There is evidence from studies on animal models of PD that axons of mDA neurons transplanted into the adult rat SN are capable of growing to and integrating into the host striatum in an anatomically and functionally correct manner [93, 94]. This suggests that the appropriate guidance cues remain in the denervated rat brain. However, it is not known whether such guidance cues remain in the human PD brain. There is some evidence for polymorphisms in genes coding for guidance cues being linked to PD susceptibility [for review see ref [95]],

but there is no clear data regarding the levels of permissive or repulsive guidance cues for mDA neurons in the human parkinsonian brain. Strategies involving exogenous application of GDNF as a chemoattractant [94], or the degradation of endogenous inhibitory molecules such as chondroitin sulfate proteoglycan [96], have been used to enhance and direct axonal outgrowth to the striatum from fetal dopaminergic neurons transplanted into the adult rat SN. Similar approaches could be used to optimise the success of future attempts to achieve regrowth of degenerating mDA neurons.

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Figures and Figure Legend:

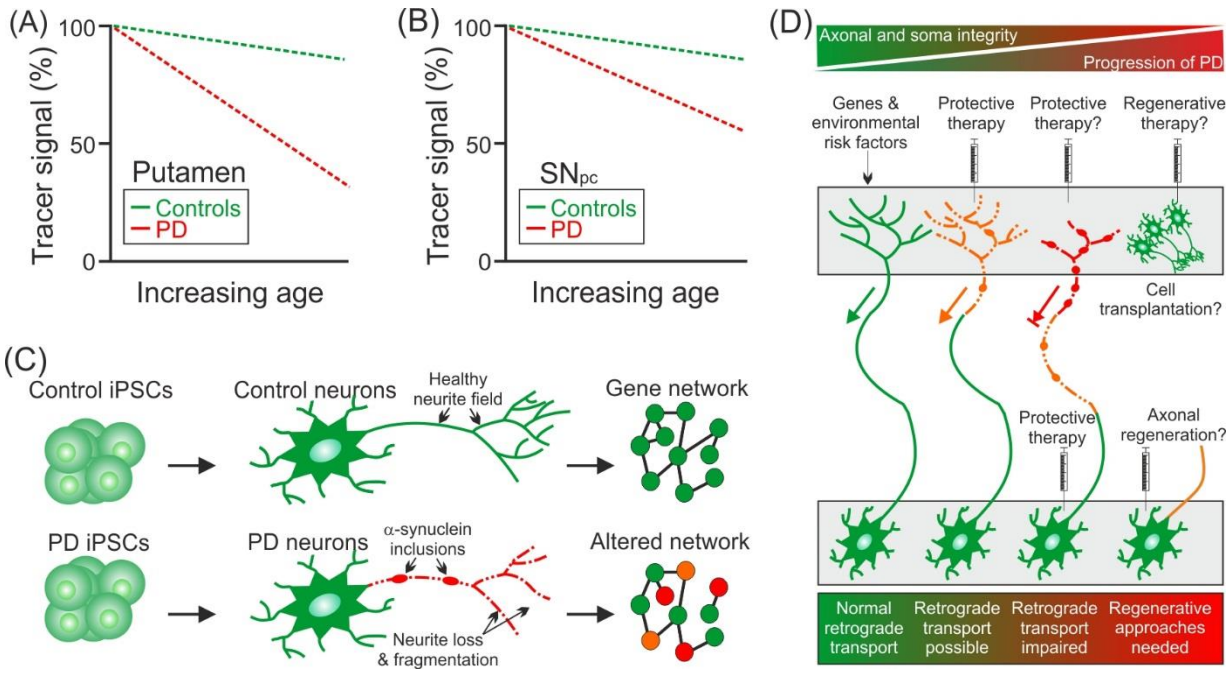

Figure 1: Schema depicting the evidence for axonal degeneration in PD.

Imaging studies that have used tracers to measure midbrain dopaminergic axonal and somal integrity have reported a more profound loss of **(A)** striatal axonal terminals than of **(B)** midbrain dopaminergic neuronal soma, at the early stages of PD. **(C)** Induced pluripotent stem cell-derived neurons from patients with sporadic or genetic PD display neurite pathology and degeneration including changes in associated gene networks, which have also been reported in post-mortem studies. **(D)** Schema depicting the progressive nature of axonal degeneration in PD, and the implications of this for therapeutic approaches.