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α-Synuclein Elevation in Human Neurodegenerative Diseases: Experimental, Pathogenetic, and Therapeutic Implications

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Abstract The discovery of α-synuclein has had profound implications concerning our understanding of Parkinson's disease (PD) and other neurodegenerative disorders characterized by α-synuclein accumulation. In fact, as compared with pre-α-synuclein times, a "new" PD can now be described as a whole-body disease in which a progressive spreading of α-synuclein pathology underlies a wide spectrum of motor as well as nonmotor clinical manifestations. Not only is α-synuclein accumulation a pathological hallmark of human α-synucleinopathies but increased protein levels are sufficient to trigger neurodegenerative processes. α-Synuclein elevations could also be a mechanism by which disease risk factors (e.g., aging) increase neuronal vulnerability to degeneration. An important corollary to the role of enhanced α-synuclein in PD pathogenesis is the possibility of developing α-synuclein-based biomarkers and new therapeutics aimed at suppressing α-synuclein expression. The use of in vitro and in vivo experimental models, including transgenic mice overexpressing α-synuclein and animals with viral vector-mediated α-synuclein transduction, has helped clarify pathogenetic mechanisms and therapeutic strategies involving α-synuclein. These models are not devoid of significant limitations, however. Therefore, further pursuit of new clues on the cause and treatment of PD in this post-α-synuclein era would benefit substantially from the development of improved research paradigms of α-synuclein elevation.

Keywords Parkinson's disease · Risk factor · Synuclein · Therapeutics · Transgenic · Viral vector

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Introduction

Since the first description of the disease in the early nineteenth century, several milestones have marked the history of Parkinson's disease (PD). They include (1) the characterization of Lewy bodies and Lewy neurites as pathognomonic inclusions in the brain of PD patients in the early twentieth century [1] and (2) the development of L-DOPA for the symptomatic treatment of motor dysfunction in the 1960s [2]. Another historical turning point was the report of the first familial form of autosomal dominant parkinsonism linked to a single-point mutation in the α-synuclein gene published in 1997 [3]. The implications of this discovery have been far more profound than originally thought. Research on α-synuclein has led to a completely new understanding of the disease process and opened new horizons of disease prevention and therapeutics. Thus, 1997 could be considered the year that separates the "old" from a "new" PD (Fig. 1).

The initial consequence of the α-synuclein discovery was a realization that genetic factors could themselves cause familial parkinsonian syndromes that, albeit rare, reproduce clinical and pathological features typical of idiopathic (and sporadic) PD. Shortly after 1997, however, the role of α-synuclein in pathogenetic processes became even more intriguing, extending well beyond the exceptional situation of patients carrying α-synuclein mutations. In all PD patients, antibodies targeting α-synuclein were found to immunoreact against Lewy bodies and Lewy neurites, ultimately indicating that α-synuclein is a primary component of these intraneuronal inclusions [4]. Spurred by the seminal work of Braak and colleagues over the past 10 years, neuropathologists have come to recognize that in most instances, the distribution and progression of α-synuclein-immunoreactive inclusions follow a predictable consistent pattern [5, 6]. Regions in the brainstem are affected first, preceding an upward spreading of the pathology that finally reaches cortical brain areas. These observations are at

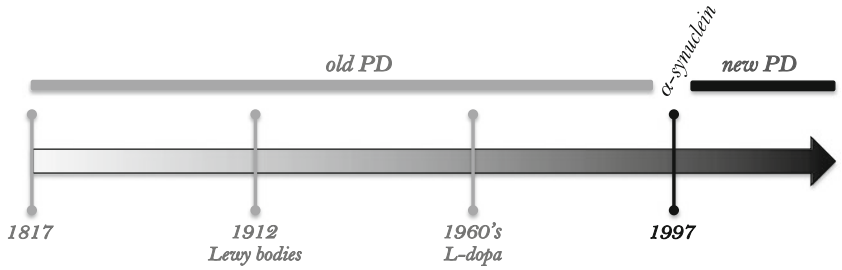

Fig. 1 Milestones of PD history. After the first description of PD in 1817, disease history has featured important milestones, such as the identification of Lewy bodies and Lewy neurites in 1912 and the introduction of L-DOPA treatment in the 1960s. With the discovery of α-synuclein in 1997, our view of the disease has significantly changed and, for this reason, 1997 could be considered the year of transition from the "old" to a "new" PD

the basis of a re-classification of PD in pathological stages, ranging from stages 1 and 2 (limited α-synuclein accumulation in asymptomatic individuals) to stages 3 and 4 (involvement of midbrain regions with symptomatic manifestations of motor abnormalities) and lastly stages 5 and 6 (widespread pathology and increasing nonmotor clinical signs) [5]. Most recently, research on α-synuclein has led to another surprising outcome: abnormally aggregated α-synuclein has been found within neurons of the peripheral nervous system, such as neurons of the submucosal Meissner plexus in the enteric nervous system [7, 8]. It is worth noting that this intraneuronal accumulation is likely to be an early pathological event since enteric α-synuclein-containing inclusions were present in symptomatic PD patients as well as nonsymptomatic individuals with CNS lesions limited to Braak stages 1 and 2 [7].

What then distinguishes the old vs. new, post-α-synuclein PD? A comparative list is shown in Table 1. Perhaps the most remarkable difference relates to the extent of disease process. In the pre-α-synuclein age, our view of the disease was very much "nigrostriatal centric". The degeneration of dopaminergic neurons in the substantia nigra was considered the primary pathological hallmark of the disease and was the main focus of research on pathogenetic mechanisms and new therapeutic approaches. It is now obvious that PD is a "whole-brain", "whole-body" disease that manifests itself with symptoms related to the loss of nigral dopaminergic neurons (motor impairment) as well as an array of other symptoms reflecting, for example, mood, cognitive, and autonomic dysfunction (e.g., depression, bradyphrenia, and constipation). It is not that the latter clinical manifestations were unknown or completely ignored in the past. Rather, the observation of widespread α-synuclein lesions provides a pathological basis for understanding the ample constellation of nonmotor PD symptoms, making them a more integral part of the clinical picture. A comparison of the old and new PD revels other significant advancements, such as the classification of "α-synucleinopathies" that not only includes PD but also other neurodegenerative disorders such as dementia with Lewy bodies and multiple system atrophy; interestingly, diffuse α-synuclein pathology is a common feature shared by all of these diseases [9]. Moreover, new research strategies for unraveling neurodegenerative mechanisms and new opportunities for developing preventive and therapeutic intervention have emerged in the post-α-synuclein age. These important consequences of the discovery of α-synuclein will be discussed next in greater detail.

Pathogenetic Mechanisms of PD: The Importance of Elevated α-Synuclein

If α-synuclein is directly involved in PD development, information concerning its biological and toxic properties

Table 1 Comparison of features of the (old) pre- and (new) post-α-synuclein PD

	Old PD	New PD
Pathology/pathogenesis	Nigrostriatal centric	Whole-brain, whole-body disease
Clinical features	Focus on motor impairment	Complex clinical syndrome with motor and nonmotor manifestations
Mechanisms	Dopamine toxicity and dopaminergic cell vulnerability (e.g., oxidative stress)	New interest in α-synuclein toxic potential and mechanisms such as neuronal protein mishandling
Therapeutic targets	Dopamine replacement (symptomatic) and nigrostriatal protection	Anti-α-synuclein disease-modifying intervention
Biomarkers	Indicators of loss of dopaminergic function/integrity	Assays of α-synuclein levels and evaluation of early progressive α-synuclein pathology

PD Parkinson's disease

would be of the utmost importance to elucidate neurodegenerative mechanisms. The normal function of α-synuclein is only in part understood. Its synaptic location together with electrophysiological findings supports an involvement in neurotransmission and plasticity [10, 11]. Furthermore, α-synuclein has recently been found to bind to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein synaptobrevin 2/VAMP2; by doing so, it would play a role in synaptic vesicle release regulated by SNARE-complex assembly [12].

From the standpoint of toxic properties, the autosomal dominant inheritance of α-synuclein mutations suggests that a gain rather than loss of toxic function underlies α-synuclein pathology. Particular attention has been given to conformational changes of α-synuclein that would promote its tendency to aggregate into oligomeric and fibrillar structures [13, 14]. Protein aggregation would be an obvious critical step toward the formation of Lewy bodies and Lewy neurites. It could also be a mechanism linked to neurodegenerative effects via the production of toxic α-synuclein species; the nature of these species is highly debated, however, with some evidence supporting fibrillar aggregates while other, perhaps stronger data suggesting toxic soluble oligomers [13, 15, 16]. Besides protein aggregation, a variety of other mechanisms have been implicated in α-synuclein-mediated neuronal death, including mitochondrial injury, perturbation of lysosomal function and an imbalance in calcium homeostasis [17–19]. It is conceivable that these neurodegenerative pathways may also be interrelated. For example, the generation of oligomeric α-synuclein species could result in the formation of ion-permeable membrane pores, which may in turn promote calcium perturbation [15]. Overall, experimental work over the past 10 years has identified toxic properties and potential mechanisms of α-synuclein toxicity; it is also fair to admit, however, that the precise sequence of events underlying α-synuclein-induced neurodegeneration remains unsolved.

Evidence from human genetic studies has provided perhaps one of the most relevant clues concerning (1) the pathogenetic role of α-synuclein in neurodegenerative diseases and (2) strategies that could be used to target α-synuclein for therapeutic purposes. As already noted, changes in its amino acid sequence trigger α-synuclein pathogenicity and, indeed, three distinct single-point mutations have been identified in familial cases of parkinsonism [3, 20, 21]. As importantly, human parkinsonism has been associated with multiplication mutations of the α-synuclein gene resulting in elevated protein expression [22, 23]. Thus, increased "normal" α-synuclein is itself a cause of pathologic/toxic events, a notion bearing widespread implications. For example, the relationship between enhanced α-synuclein concentration and increased tendency of the protein to aggregate, supported by both in vitro and in vivo data [13, 24], could well be a critical pathogenetic mechanism for inclusion formation and neuronal injury. Another important corollary to the notion that a lifelong α-synuclein elevation causes neurodegenerative processes is the likelihood that other conditions associated with less severe and/or more transient α-synuclein increases may also promote α-synuclein pathology and thus enhance disease risk. Finally, the toxic potential of elevated α-synuclein suggests that therapeutic strategies aimed at controlling intraneuronal protein levels could prevent disease or alleviate its progression in patients affected by PD and other α-synucleinopathies.

Risk Factors for α-Synucleinopathies: The Role of Elevated α-Synuclein

That α-synuclein expression is likely to affect PD onset, severity and risk is supported by a variety of genetic evidence. In families with parkinsonian patients harboring multiplication of the chromosomal locus (4q21) containing the α-synuclein gene (*SNCA*), severity of the clinical phenotype has been reported to be dependent upon gene dosage; indeed, *SNCA* triplication was associated with earlier-onset and graver disease than allele duplication [23, 25, 26]. Genome-wide association studies comparing PD patients and controls have revealed *SNCA* as a major disease risk locus [27, 28]. Furthermore, genetic variation in the *SNCA* promoter region, such as variability in the length of the dinucleotide repeat sequence REP1, appears to modulate PD risk [29]. Of note, *SNCA* REP1 changes affect α-synuclein levels in the blood and brain, further supporting the concept that PD risk is increased and decreased by elevated and lowered α-synuclein, respectively [30].

The pathogenesis of sporadic PD is most likely multifactorial, with genetic and environmental factors contributing to a varying extent in different individuals [31]. Also, aging represents an unequivocal disease risk factor since PD incidence is very rare in young and middle-aged people and increases progressively after the fifth decade of life [32]. As described in the previous paragraph, genetic modulation of α-synuclein expression occurs and is likely to affect PD risk. Is there any evidence that elevated α-synuclein may play a mechanistic role in promoting disease development after environmental insults and as a result of normal aging? The likely contribution of toxic challenges to PD pathogenesis is supported by clinical, epidemiological, and experimental findings. For example, specific environmental conditions, such as agricultural work and head trauma, and certain toxic agents, such as the herbicide paraquat, have been associated with enhanced disease risk in a significant number of investigations (though not all) [33, 34]. An intriguing possibility linking neuronal damage and α-synuclein is suggested by studies using animal models in

which neurodegenerative changes are caused by toxic exposures. Treatment of mice or nonhuman primates with the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been one of the most widely used PD models since the early 1980s; at that time, a parkinsonian syndrome was described in young drug addicts who accidentally injected themselves with illicit substances contaminated with MPTP [35]. Interestingly, nigrostriatal damage induced by MPTP in animal models (both mice and monkeys) is accompanied by a dramatic α-synuclein upregulation [36, 37]. In monkeys, this increased expression is longer lasting than in mice and results in α-synuclein modifications (i.e., nitration, phosphorylation, and aggregation) that resemble changes in PD brains [38].

MPTP is not the only toxic agent affecting α-synuclein expression. Similar effects, i.e., intraneuronal α-synuclein elevation and aggregation, have been reported in mice injected with paraquat [39, 40]. Paraquat-induced changes are of particular relevance, given its potential role and the putative involvement of pesticide exposure in PD pathogenesis. Indeed, a recent epidemiological investigation has reported interactions between *SNCA* REP1 genotypes and paraquat exposure [41]. In line with earlier work, the shorter repeat length REP1 259 genotype appeared to confer protection against PD, whereas the longer repeat length REP1 263 genotype was associated with a mild increase in PD risk. Data also suggested, however, that paraquat exposure counteracted the protective effect of the REP1 259 genotype while the REP1 263 genotype increased susceptibility to the herbicide and enhanced PD risk in younger onset patients [41].

Traumatic brain injury is another condition in which gene–environmental interactions may co-operate to modulate PD risk via changes in α-synuclein expression. Goldman and colleagues reported that, when participants in two independent case–control studies were genotyped for *SNCA* REP1 and asked about head injuries, head trauma was strongly associated with increased PD risk only in individuals with longer repeat length REP1 [42]. Laboratory work further confirms a relationship between brain damage and α-synuclein since levels of α-synuclein were transiently enhanced in aged but not young rats after experimental traumatic brain injury [43].

A general consideration arising from these findings is that elevated α-synuclein seems to be a consistent feature of neurons subjected to toxic or traumatic damage. The significance and consequences of this upregulation (levels of both α-synuclein mRNA and protein are increased after MPTP or paraquat administration) remain unclear. An interesting possibility, which is suggested by the normal role of α-synuclein in facilitating neurotransmission and synaptic plasticity, is that injury-induced α-synuclein elevation represents a response of neurons aimed at maintaining and restoring proper synaptic function. It is also possible, however, that an exaggerated α-synuclein response may be triggered, for example, by a particularly sustained toxic insult and/or in individuals with increased α-synuclein levels due to genetic variations in the *SNCA* promoter region. Under these circumstances, α-synuclein changes would not contribute to the recovery process but rather could amplify and perpetuate pathological damaging events.

The risk for human α-synucleinopathies is enhanced in the older population, justifying interest in the relationship between normal aging and α-synuclein pathophysiology. Advanced age is characterized by an impairment of neuronal activities, such as protein degradation, which could conceivably promote or exacerbate α-synuclein's toxic potential [44]. In addition, age-related changes in α-synuclein expression have been described in the primate brain and could explain, at least in part, the high vulnerability of specific neuronal populations to degenerate in PD. Dopaminergic cells in the substantia nigra pars compacta (SNpc) are distinctively targeted by neurodegenerative processes as indicated, for example, by their greater susceptibility to die in PD as compared with dopaminergic neurons in the nearby ventral tegmental area (VTA) [45, 46]. It is therefore quite remarkable that an age-dependent increase in α-synuclein protein characterizes dopaminergic neurons in the SNpc but not the VTA of humans and nonhuman primates [47, 48]. Furthermore, elevated α-synuclein within nigral neurons is associated with a decrease in tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis, suggesting a relationship between α-synuclein accumulation and loss of dopaminergic function/integrity [47, 48]. More recent findings also revealed the presence of nitrated and phosphorylated α-synuclein in the SNpc of old monkeys; since these posttranslational modifications of α-synuclein are typical of PD, their occurrence within older dopaminergic neurons further support the possibility that age-related α-synuclein changes contribute to enhanced disease vulnerability [49]. A final consideration relates to the discrete effect of aging in regard to different animal species. In contrast to observations in the primate brain, levels of α-synuclein significantly decline with age in the rodent CNS, as assessed in various brain regions including the substantia nigra [50]. Thus, an age-dependent α-synuclein elevation only occurs within dopaminergic neurons in humans and monkeys, underlying a unique feature of the primate substantia nigra that may predispose it to α-synuclein pathology and neurodegeneration.

Taken together, evidence from genetic studies (e.g., genetic variations in the *SNCA* promoter region), investigations into toxic insults (e.g., MPTP-induced α-synuclein upregulation), and observations in the aging brain (e.g., age-related elevation of nigral α-synuclein) are all consistent with the interpretation that increased α-synuclein levels could represent a common mechanism underlying the

effects of disease risk factors in PD and other human α-synucleinopathies.

Elevated α-Synuclein as a Therapeutic Target and a Disease Biomarker

New therapeutic strategies targeting α-synuclein could be designed to counteract putative toxic properties of the protein, such as its propensity to aggregate [51]. On the other hand, the gain of toxic function associated with increased α-synuclein suggests that another, perhaps more direct approach should be evaluated, i.e., controlling intraneuronal protein levels. Different strategies have been proposed and tested to modulate α-synuclein expression. Knowledge of the likely role of *SNCA* REP1 in *SNCA* transcriptional regulation prompted Chiba-Falek and colleagues to screen for proteins capable of binding to the *SNCA* REP1 DNA element [52]. One of the identified proteins was poly(ADP-ribose) transferase/polymerase-1 (PARP-1), which, by physically interacting with *SNCA* REP1, also reduced the transcriptional activity of the *SNCA* promoter. In a separate study, downregulation of α-synuclein expression was achieved in the rat substantia nigra by injection of adeno-associated viral (AAV) vectors carrying hammerhead ribozymes directed against α-synuclein [53]. This treatment was also shown to partially protect against the loss of nigral dopaminergic neurons caused by injection of 1-methyl-4-phenylpyridinium ion (the toxic metabolite of MPTP) into the medial forebrain bundle.

Another promising strategy to silence α-synuclein would be to take advantage of the RNA interference (RNAi) pathway. Several lines of evidence support the feasibility and effectiveness of this approach targeting α-synuclein expression in both rodents and nonhuman primates. Relatively less unequivocal, however, are data concerning the safety of RNAi-mediated α-synuclein silencing. Short hairpin (sh) RNA against α-synuclein delivered via a lentiviral vector was able to suppress ectopic expression of human α-synuclein in the rat striatum and, in mice, a 2-week infusion of α-synuclein small interfering RNA (siRNA) decreased levels of endogenous α-synuclein in the hippocampus [54, 55]. A naked siRNA molecule directed against monkey α-synuclein was also found to reduce intraneuronal levels of both α-synuclein mRNA and protein when directly infused into the substantia nigra of squirrel monkeys [56]. No overt toxicity was reported as a consequence of α-synuclein suppression in these three studies. For example, in the monkey model, lack of adverse effects was indicated by the number of nigral dopaminergic neurons and the concentration of striatal dopamine that remained unchanged between the siRNA-injected vs. untreated sides of the brain [56]. These findings are apparently at odds with data published by Gorbatyuk and colleagues who reported a significant loss of dopaminergic neurons when two α-synuclein siRNAs embedded as shRNAs in AAV vectors were unilaterally injected into the rat substantia nigra pars compacta [57]. The fact that toxic effects were observed in only one out of four studies suggests that suppression of α-synuclein is unlikely to cause tissue damage by itself. Rather, the mechanisms (e.g., viral vectors) and tools (e.g., specific RNAi molecules) by which this silencing is achieved may trigger adverse side effects that need to be evaluated by comprehensive preclinical investigations. These studies should also be performed in different animal species and different strains of animals since recent work indicates, for example, that the sensitivity of mice to specific shRNA compounds varies depending on their distinct genetic background [58].

Another important caveat concerning α-synuclein-silencing intervention is raised by two considerations. First, α-synuclein is an abundant CNS protein with important physiological properties that would be negatively affected by complete protein depletion (see above). Secondly, experimental evidence suggests that, under specific circumstances, α-synuclein expression may even be associated with neuroprotective effects [59, 60]. These observations should not, in our view, discourage the testing of new therapeutics but rather underscore the importance of a more nuanced approach. For example, the extent and duration of α-synuclein silencing are likely to be important factors, and it could be argued that partial α-synuclein suppression may represent a safer therapeutic target; less-than-complete depletion may still protect against the deleterious consequences of α-synuclein accumulation while being less likely to impair the normal function of the protein [56].

It is now clear that PD pathology in the form of α-synuclein inclusions begins well before the onset of the motor symptoms that bring patients to their physicians and allow physicians to make a clinical diagnosis. Thus, for PD therapeutics (including modulators of α-synuclein expression) to be as effective as possible, a more timely disease diagnosis is warranted. An elusive critical step to achieve early diagnosis has been the identification of PD biomarkers. In pre-α-synuclein times, research efforts focused on the challenging task to identify indicators of nigrostriatal degeneration measurable in peripheral tissues (e.g., blood or cerebrospinal fluid) or with sophisticated live brain imaging techniques (e.g., positron emission tomography). New opportunities and significant advancements in the search for PD biomarkers have been prompted by studies on α-synuclein (Table 1). First, these studies have revealed a whole-body involvement of PD pathology, making it a more realistic endeavor to identify systemic indicators of the disease. As importantly, assessment of α-synuclein levels and accumulation in peripheral tissues could itself provide a means to achieve early diagnosis and to monitor disease

progression. Interesting, though not always consistent, results have been obtained assaying α-synuclein in the cerebrospinal fluid (CSF). Data reported by Hong and colleagues revealed a reduction of α-synuclein levels in PD patients as compared with controls and patients with Alzheimer's disease; lower CSF α-synuclein concentration may reflect a decreased release of the protein due to its intraneuronal accumulation in PD [61]. Measurements of modified forms of α-synuclein, such as phosphorylated α-synuclein and α-synuclein oligomers, have also been proposed as valuable indicators of disease status [62, 63]; for example, Tokuda et al. reported a significant increase in α-synuclein oligomers and oligomers/total α-synuclein ratio in the CSF from PD cases [63]. Another very promising approach consists in evaluating α-synuclein pathology in specimens of the autonomic nervous system. In particular, PD-related α-synuclein accumulation has been described and seems to be a rather consistent and early feature in colonic biopsies. Robust staining for α-synuclein was present in nerve fibers of the colonic submucosa from untreated PD patients with only mild disability [64]. Furthermore, the degree of neuritic α-synuclein pathology in biopsies from PD patients positively correlated with L-DOPA-unresponsive symptoms, further suggesting a relationship between colonic α-synuclein accumulation and progression of clinical disease manifestations [65]. Taken together, these findings strongly support the potential significance of assaying peripheral α-synuclein levels/pathology to obtain early PD diagnosis and to monitor disease progression.

Experimental Models of Elevated α-Synuclein

The prominent role that elevated α-synuclein plays in PD pathogenesis and as a target for disease-modifying intervention underscores the importance of further investigations using suitable experimental tools. Increased levels of α-synuclein characterize a variety of model systems ranging from in vitro cell cultures, *Drosophila melanogaster*, *Caenorhabditis elegans*, mice, rats, and monkeys [40, 66–70]. Features of these models are quite variable depending, for example, on (1) the extent and degree of expression, (2) the cells/tissues targeted for α-synuclein elevation (e.g., dopaminergic neurons vs. other neuronal cell populations), and (3) whether the protein is overexpressed on the background of endogenous α-synuclein (e.g., transgenic mice) or is expressed in organisms that lack an α-synuclein homolog in their genome (e.g., *Drosophila*). Here, we will focus on mouse and rat models of α-synuclein overexpression and, in particular, discuss advantages and pitfalls of α-synuclein transgenics and rats with viral-mediated α-synuclein transduction.

Masliah and colleagues were the first to develop and characterize transgenic mouse lines in which wild-type human α-synuclein was expressed under the regulatory control of the platelet-derived growth factor-β (PDGF) promoter [24]. In 2000, they reported an intraneuronal accumulation of α-synuclein-immunoreactive inclusions in various brain regions (e.g., deeper layers of the neocortex and hippocampus) of these animals. Subsequent studies in PDGF-α-synuclein mice also revealed that transgene accumulation was not limited to neurons but also occurred within glial cells [71]. Since 2000, a number of different lines of transgenic mice overexpressing either wild-type or mutated human α-synuclein have been generated and evaluated experimentally; overexpression of A53T, A30P, or E46K α-synuclein is aimed at reproducing the effects of mutant forms of the protein that are associated with familial parkinsonism in humans. Transgenic animals have also been developed that overexpress mouse α-synuclein, modified forms (e.g., truncated) of the protein or double proteins (e.g., α-synuclein together with ß-synuclein) [60, 72–74]. A variety of factors influence the occurrence, nature, distribution, and severity of α-synuclein lesions, with important roles played by the transgene promoters and the degree of α-synuclein overexpression (2- to 30-folds higher than the endogenous levels) in different transgenic mouse lines.

Models driven by the TH promoter, in which α-synuclein occurs within catecholaminergic neurons, have been used to determine the toxic/pathological effects of α-synuclein elevation on nigral dopaminergic neurons. Somewhat unexpectedly, these mice display no dopaminergic deficits or a very subtle loss of dopaminergic function [75, 76]. The use of non-TH promoters has allowed investigations into the pathological consequences of α-synuclein elevation in neurons throughout the central and peripheral nervous system. This is of particular relevance and could be considered a potential advantage of these transgenic models given our current evidence of diffuse α-synuclein pathology in PD brains (see above). For instance, mice overexpressing human α-synuclein under the thymocyte differentiation antigen 1 (Thy1) promoter exhibit extensive α-synuclein accumulation in a variety of CNS regions. The pattern of expression/pathology seems to vary in different lines of Thy1-α-synuclein mice, involving areas such as the neocortex, hippocampus, olfactory system and, in some animals, the spinal cord [71, 77, 78]. Young adult Thy1-α-synuclein mice also develop sensorimotor and olfactory deficits that reproduce PD features (e.g., anosmia) and are not reversed by L-DOPA administration; the latter finding supports a nondopaminergic basis of α-synuclein-induced behavioral abnormalities in these animals [79, 80].

Both cognitive and motor deficits characterize a conditional mouse model driven by the calcium/calmodulin-dependent protein kinase IIα promoter, in which α-synuclein

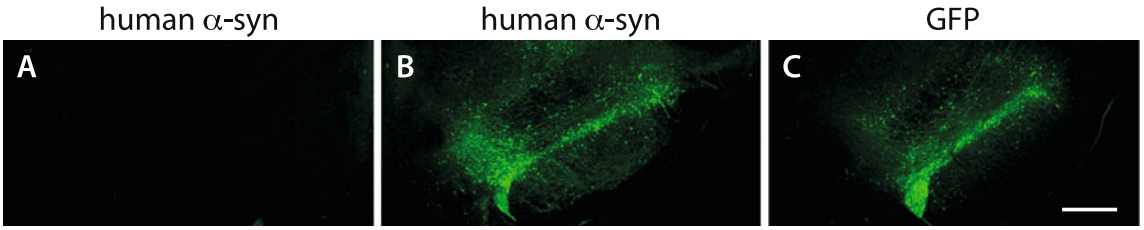

Fig. 2 AAV-mediated transgene expression in the rat midbrain. AAV6 vectors carrying wild-type human α-synuclein or green fluorescent protein (*GFP*) were unilaterally injected into the right rat midbrain. Representative midbrain sections were immunostained with (1) an antibody that specifically recognizes human α-synuclein (**a**, **b**) or (2) an antibody against GFP (**c**). Lack of α-synuclein immunoreactivity in the noninjected (intact) side of the brain (**a**) contrasts with a robust transgene expression (human α-synuclein or GFP) in the right (injected) substantia nigra (**b**, **c**). *Scale bar*= 500 μm

is overexpressed predominantly in the olfactory bulb, cortex, and basal ganglia [81]. This tet-off conditional model provided evidence that the progression of behavioral deficits could indeed be halted by turning off α-synuclein expression. Widespread and robust α-synuclein pathology (i.e., α-synuclein- and ubiquitin-positive inclusions) in brain regions such as the cerebellum, midbrain, and brainstem is observed in transgenic mice overexpressing mutated α-synuclein (A53T α-synuclein) generated using the murine prion protein promoter (PrP) [82]. Transgene expression, as assessed by in situ hybridization, was also observed in the substantia nigra of these mice in the absence, however, of overt pathological changes. Although severe motor impairment (bradykinesia, ataxia, dystonia, and paralysis) characterizes PrP-α-synuclein animals, it is noteworthy that no abnormalities in markers of dopaminergic function/integrity (e.g., striatal dopamine levels) could be detected. Thus, behavioral changes most likely result from α-synuclein expression and pathology within nondopaminergic neurons; particularly relevant in this respect is the observation of extensive lesions in the spinal cord [82]. In summary, α-synuclein transgenics represent valuable experimental tools to investigate mechanisms and consequences of α-synuclein accumulation within a variety of neuronal cell populations as well as within glial cells. The latter feature in PDGF-α-synuclein animals is reminiscent of the glial lesions observed in patients affected by certain α-synucleinopathies such as multiple system atrophy [83, 84].

While it is true that PD is no longer considered simply a disease of the nigrostriatal system, it also remains unquestionable that nigral dopaminergic neurons are preferential targets of neurodegenerative processes and their loss underlies the development of highly debilitating motor symptoms. Investigations into the relationship between α-synuclein accumulation, nigrostriatal pathology, and dopaminergic cell vulnerability to death will therefore continue to be an important component of future research endeavors. One of the most surprising findings of studies with α-synuclein transgenics is the relative sparing of nigral cells from the toxic consequences of protein overexpression. No clear explanation is presently available for this lack of damaging effects that somewhat restricts, however, the use of these models. In part to circumvent this limitation and to induce α-synuclein elevation in targeted brain regions (including the substantia nigra), alternative models have been developed by injecting α-synuclein-carrying viral vectors into the brain of rodents or monkeys. Viral-mediated α-synuclein expression is faster than the generation of transgenic mouse lines; it also allows evaluation of the effects of protein accumulation

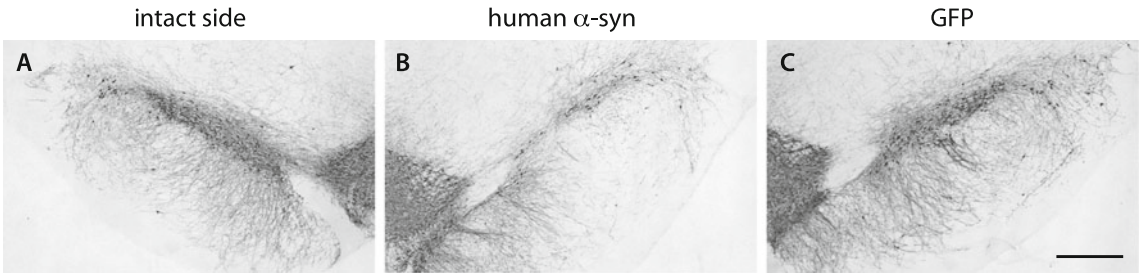

Fig. 3 Loss of tyrosine hydroxylase-immunoreactive neurons as a consequence of α-synuclein overexpression. AAV6 vectors carrying wild-type human α-synuclein or green fluorescent protein (*GFP*) were unilaterally injected into the right rat midbrain. Representative midbrain sections from α-synuclein (**a**, **b**) and GFP (**c**) overexpressing animals were immunostained with an antibody against tyrosine hydroxylase, a marker of dopaminergic neurons. A marked reduction of tyrosine hydroxylase-positive cells and fibers characterizes the substantia nigra injected with AAV/α-synuclein (**b**) as compared with the noninjected side of the brain (**a**) and the substantia nigra injected with AAV/GFP (**c**). *Scale bar*=500 μm

directly in the adult brain, thus reducing the possibility that adaptive changes due to developmental transgene expression may interfere with the model's phenotype.

Both lentiviral and AAV vectors have been successfully used to transduce α-synuclein within nigral neurons. One of the most noteworthy differences between the two strategies relates to the lower tropism of lentivirus toward dopaminergic cells. Indeed, maximal transduction efficiency has been reported to be approximately 50 % with lentivirus and 80–90 % with AAV [85]. As a consequence, neurodegeneration is also more pronounced in models using the latter (50–80 % cell loss) than the former (approximately 30 %) vectors. The most commonly used AAV capsid serotypes that, after midbrain injection, are capable of inducing a discrete transduction in the substantia nigra are AAV1, AAV2, AAV5, and AAV6 [70, 86–89]; in contrast, other vector serotypes such as the AAV7 lead to a more widespread protein expression that is relatively less specific to the substantia nigra [90]. The use of neuron-specific promoters such as synapsin 1 has been reported to enhance transduction efficacy in the substantia nigra compared with more ubiquitous promoters such as the cytomegalovirus one [91].

Figure 2 shows robust transgene expression at 5 weeks after a single injection of AAV6 vector encoding for (1) synapsin 1 (promoter), (2) wild-type α-synuclein, and (3) the enhancer element woodchuck hepatitis posttranscriptional regulatory element (WPRE) into the rat brain. Significant (approximately 50 %) neurodegeneration, as assessed by the loss of TH-immunoreactive cells (Fig. 3) and Nissl-stained neurons (data not shown), develops over a period of 4–8 weeks postinjection. As a control, administration of the same titer of viral particles carrying the gene for green fluorescent protein (GFP) with the same vector design (i.e., synapsin 1 promoter and WPRE enhancer element) induces strong transgene expression but only minimal neuronal cell loss (approximately 10 %) (Figs. 2 and 3). Beside neurodegeneration, other important features of these viral models include the formation of α-synuclein-positive cytoplasmic inclusions, accumulation of phosphorylated α-synuclein and axonal pathology [69, 92, 93]. The morphology of dystrophic axons observed in α-synuclein overexpressing rats and primates is similar to that described in brains from PD patients [4, 5, 94]. Furthermore, the appearance of dystrophic swollen striatal projections is accompanied by alterations in axonal transport, dopaminergic synthesis, vesicle exocytosis, and synaptic function [69, 95]. In most instances, models of virus-mediated α-synuclein expression do not display significant behavioral deficits. This is likely due to the fact that nigrostriatal damage and, in particular, striatal dopamine depletion do not usually reach the degree of severity necessary for behavioral manifestations. In line with this interpretation, a recent report presented a detailed characterization of a rat model of α-synuclein overexpression generated using a more efficient AAV vector construct characterized by a WPRE enhancer element [69]. When AAV6-α-synuclein rats with and without WPRE were compared, L-DOPA-sensitive motor behavioral deficits were only observed in the presence of the enhancer and were associated with increased transgene expression and a more significant reduction of markers of dopaminergic neurotransmission.

In summary, a variety of models of α-synuclein elevation have become available over the past several years. It is also important to recognize, however, that these models do not necessarily capture the complexity of features and pathogenetic processes underlying PD and other α-synucleinopathies. Hence, future work will need to focus on improving current experimental tools and developing more accurate model systems. One example of a novel approach is the use of induced pluripotent stem cells isolated from patients with sporadic or genetic parkinsonism [96]. In the meantime, while planning PD-related research, the choice of a specific paradigm should match as accurately as possible the experimental question that is being addressed. The selection may also be dictated by the need to reproduce discrete aspects of α-synuclein pathology/toxicity (e.g., protein aggregation) that may be featured in certain but not other models. Improved research tools and a sensible use of our current paradigms will play a critical role in pursuing important new clues on the cause and treatment of PD in this post-α-synuclein era.

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Conflict of interest The authors declare that they have no conflict of interest.

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