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The usual suspects, dopamine and alpha synuclein, conspire to cause neurodegeneration

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Abstract

Parkinson's disease (PD) is primarily a movement disorder driven by the loss of dopamine-producing neurons in the substantia nigra (SN). Early identification of the oxidative properties of dopamine implicated it as a potential source of oxidative stress in PD, yet few studies have investigated dopamine neurotoxicity *in vivo*. The discovery of PD-causing mutations in α -synuclein, and the presence of aggregated α -synuclein in the hallmark Lewy body pathology of PD, revealed another important player. Despite extensive efforts, the precise role of α -synuclein aggregation in neurodegeneration remains unclear. We recently manipulated both dopamine levels and α -synuclein expression in aged mice, and found that only the combination of these two factors caused progressive neurodegeneration of the SN and an associated motor deficit. Dopamine modified α -synuclein aggregation in the SN, resulting in greater abundance of α -synuclein oligomers and unique dopamine-induced oligomeric conformations. Furthermore, disruption of the dopamine- α -synuclein interaction rescued dopaminergic neurons from degeneration in transgenic *C. elegans* models. In this Perspective, we discuss these findings in the context of known α -synuclein and dopamine biology, review the evidence for α -synuclein oligomer toxicity and potential mechanisms, and discuss therapeutic implications.

Keywords

dopamine; alpha-synuclein; oligomers; Parkinson's disease

Introduction

As the global population ages, the number of people suffering from Parkinson's disease (PD) is predicted to rise significantly.^{1,2} PD is a progressive neurodegenerative disorder, primarily characterized by motor signs including resting tremor, muscle rigidity, and slow

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movement (bradykinesia). These motor features were first described by James Parkinson in 1817, and later discovered to result from the death of dopamine-producing neurons in the substantia nigra pars compacta (SN).^{3–5} The vulnerability of dopaminergic neurons, coupled with the known chemistry of dopamine oxidation and its potential for inducing oxidative stress, led to the early hypothesis that dopamine contributes to neurodegeneration in PD.^{6–9} Despite this longstanding notion, the neurotoxicity of dopamine is debated.

In the last twenty years, the protein α -synuclein has emerged as a central player in PD pathogenesis. In 1997, an alanine-to-threonine (A53T) missense mutation in the α -synuclein gene, *SNCA*, was identified in families with autosomal dominant, early-onset PD.¹⁰ Shortly thereafter, aggregated α -synuclein was found within Lewy bodies, the pathological lesions that characterize PD, even in sporadic (non-familial) forms of the disease.¹¹ Since these discoveries, additional *SNCA* mutations have been linked to PD,^{12–16} and meta-analysis of genome-wide association studies has identified 28 independent risk variants across 24 *SNCA* loci, including 6 new loci that confer substantial cumulative risk for PD.¹⁷ Increasing copy number of *SNCA* is correlated with both earlier age of disease onset and greater severity of clinical phenotype, indicating that elevated expression of wild-type α -synuclein is also detrimental.^{18–20}

While PD is rarely inherited (~10% of all cases),²¹ the identification of familial genetic mutations has allowed for the development of genetic animal and cellular models, greatly advancing our understanding of the molecular mechanisms underlying the sporadic disorder. Despite extensive research into the role of α -synuclein in disease, several key questions remain, including: Which species of aggregated α -synuclein are toxic? How do these toxic species form in dopaminergic neurons, and what events ultimately lead to neuronal demise?

In this Perspective, we discuss the evidence for dopamine and α -synuclein each playing a causative role in PD, and highlight our recent findings²² that suggest the convergence of these two factors may be a critical event in PD pathogenesis.

Potential pathways of dopamine neurotoxicity

Despite the clinical benefit of supplementing dopamine levels in PD patients, it has long been suspected that dopamine may contribute to the disease process.^{7–9} Dopamine has many fates within the synapse, including release into the synaptic cleft, reuptake into the presynaptic terminal for recycling, degradation by enzyme-catalyzed oxidation, non-enzymatic auto-oxidation, and deposition into neuromelanin, the pigmented structure for which the SN is named.²³

Dopamine synthesis begins with the rate-limiting step of converting L-tyrosine to L-DOPA, which is catalyzed by tyrosine hydroxylase (TH). L-DOPA is converted to dopamine by aromatic amino acid decarboxylase (AADC), and dopamine levels are ultimately self-regulating through feedback inhibition of TH.^{24–25} Upon synthesis, dopamine is transported into synaptic vesicles by vesicular monoamine transporter 2 (VMAT2). The acidic environment (pH ~5.6) within the vesicles prevents the oxidation of dopamine.^{26–27} Alternatively, cytosolic dopamine is degraded by enzyme-catalyzed oxidation, which may

produce potentially toxic aldehydes and peroxides. Both in the nerve terminal and extracellularly, monoamine oxidase (MAO) catalyzes the oxidative deamination of dopamine into 3,4-dihydroxyphenylacetaldehyde (DOPAL), generating hydrogen peroxide as a byproduct. DOPAL, a recognized neurotoxin,^{28–29} is further oxidized to the non-toxic 3,4-dihydroxyphenylacetate (DOPAC) by aldehyde dehydrogenase.²³

Dopamine that is not degraded or packaged into synaptic vesicles is able to either auto-oxidize or oxidize by transition metals, such as iron, in the cytosol. Oxidation of dopamine generates electron-deficient quinones and reactive, partially reduced oxygen species.^{6,30,31} Further oxidation and polymerization of dopamine leads to the formation of neuromelanin, an insoluble granular pigment thought to be protective in that it sequesters reactive dopamine derivatives.³¹ Formation of neuromelanin can also be facilitated by the reaction of the dopamine-*o*-quinone with cysteine, forming 5-*S*-cysteinyl-dopamine, which upon further oxidation to benzothiazine structures is deposited within the neuromelanin granules.³²

Oxidative stress occurs when the rate of formation and concentration of oxidants exceeds the antioxidant buffering capacity of the cell. Dopamine quinones, and oxidants generated by enzymatic or transition metal-catalyzed reactions of hydrogen peroxide, can damage all major classes of macromolecules, potentially leading to cellular dysfunction and death.^{7–9} Several studies have identified markers of oxidative stress in postmortem PD brain tissue. In the SN, polyunsaturated fatty acid content was reduced compared with other brain regions and tissue from controls, while products of lipid peroxidation including malondialdehyde and 4-hydroxynonan-3-ol protein adducts were increased.^{33,34} Lewy bodies exhibit robust immunoreactivity for protein nitration, including specifically nitrated α -synuclein,^{35,36} and oxidatively modified nucleic acids are also present in SN neurons.³⁷ Nucleophiles and antioxidant enzymes that remove the products of dopamine oxidation may therefore play an important role in protecting dopaminergic neurons from oxidative stress.

***In vivo* models of dopamine toxicity**

Early studies investigated whether dopamine is toxic *in vivo* by injecting relatively high concentrations of dopamine (up to 1 μ mol) into the striatum of rats. Within one week of dopamine injection, loss of dopaminergic nerve terminals and formation of protein cysteinyl-dopamine adducts were documented. The size of the lesion correlated with the concentration of dopamine that was injected, and co-administration of glutathione or ascorbate mitigated toxicity and decreased adduct formation.^{38–40} Similarly, rats injected into the SN with aminochrome (an oxidation product of dopamine) showed evidence of neurodegeneration and motor impairment.^{41,42} Based on structural similarity to dopamine, unilateral or bilateral injections of 6-hydroxydopamine in animals causes dopaminergic neuron loss, which leads to motor phenotypes. Although 6-hydroxydopamine injection does not induce protein aggregation and formation of protein inclusions, α -synuclein null mice showed less SN dopaminergic loss than wild type littermates after striatal 6-hydroxydopamine injection. The protection was limited to SN dopaminergic neurons and did not affect striatal dopamine levels.⁴³ These studies suggest that acute elevation of dopamine, its oxidized derivatives, or structurally related compounds can be neurotoxic.

Pharmacological and genetic approaches that dysregulate endogenous dopamine have in some cases shown detrimental effects. Whereas a complete knockout of VMAT2 is lethal,⁴⁴ transgenic mice expressing 5% of normal VMAT2 levels are viable and did not have SN cell loss.⁴⁵ In another report, these mice eventually developed a mild and progressive degenerative phenotype along with elevated levels of cysteinyl-catechols.⁴⁶ Amphetamines are highly toxic to dopaminergic nerve terminals, in part by the redistribution of dopamine from synaptic vesicles into the cytosol.^{47,48} Mice deficient in VMAT2 exhibit greater sensitivity to methamphetamine,^{49,50} and conversely, overexpression of VMAT2 is protective against methamphetamine-induced damage.⁵¹ In addition, elevation of cytosolic dopamine by overexpression of the dopamine transporter (DAT) in SN neurons⁵² or by ectopic DAT expression in striatal neurons⁵³ resulted in neurodegeneration and oxidative stress.

The effects of raising dopamine levels long-term has remained unknown. In order to investigate this, we injected the SN of non-transgenic mice with a lentivirus encoding the human TH gene.²² Two residues, Arg-37 and Arg-38, in the N-terminal regulatory domain of TH are required for feedback inhibition by dopamine.⁵⁴ We mutated these residues to Glu-37, Glu-38 (TH-RREE) in order to increase the catalytic activity of TH, and measured a 52% increase in striatal dopamine at 5 months post-injection. To our surprise, chronic enhancement of dopamine levels did not cause dopaminergic nerve terminal or cell body loss, and the animals maintained robust motor coordination and balance. TH-RREE injection did cause hyperactivity in non-transgenic mice,²² consistent with an increase in synaptic dopamine release.^{55,56} Oxidative stress may be prevented if dopamine storage, release, and re-uptake mechanisms remain tightly coupled with dopamine levels. It is possible that the excess dopamine was well-tolerated due to compensatory upregulation of metabolic enzymes and transporters. In line with this, we observed significantly increased DAT levels in these mice.²² Collectively, these studies indicate that high concentrations of dopamine can be acutely toxic, whereas chronic elevation or dysregulation of dopamine can in some cases be mitigated by protective mechanisms.

α -Synuclein, a key contributor to PD pathology

While dopamine toxicity has been implicated in PD for several decades, the protein α -synuclein emerged as a key player in the disease more recently. Synuclein proteins were first discovered in cholinergic vesicles and nuclei purified from the electric organ of *Torpedo*.⁵⁷ Syn1, later renamed α -synuclein, was first characterized in rat brains probed with sera against electric organ samples.⁵⁸ A component of the α -synuclein sequence was detected as the non-amyloid- β component of amyloid plaques (NAC) in Alzheimer's disease, but was not attributed to α -synuclein until the full-length protein was isolated from human brain.^{59,60} Interest in α -synuclein increased greatly following its identification in Lewy bodies and the discovery of an α -synuclein point mutation in kindreds with early-onset familial PD.^{10,11}

Sequence and structure

The primary sequence of the 140 amino acid α -synuclein (molecular mass of 14,460.16 kDa) contains three potentially functional domains. The N-terminal domain (amino acids

1-60), which is frequently acetylated⁶¹⁻⁶³ is characterized by lysine-rich imperfect repeats predicted to form amphipathic α -helices upon lipid interaction.^{57,64} All α -synuclein point mutations (A30P, E46K, H50Q, G51D, A53E, A53T) that cause autosomal dominant forms of PD occur in the N-terminal domain,^{10,12-16,65} suggesting that disruption of this region is pathogenic. Amino acids 61-95 compose the hydrophobic NAC domain, containing a sequence (amino acids 71-82) that is both necessary and sufficient for α -synuclein aggregation into β -sheet-rich fibrils similar to those observed in PD patient brain.^{66,67} The C-terminal domain is largely unstructured both when α -synuclein is bound to lipids and aggregated into fibrils,^{68,69} and C-terminal truncation increases the rate of α -synuclein aggregation.^{70,71} The C-terminal domain is the site of many posttranslational modifications of α -synuclein, including phosphorylation at serine 129, which was first detected in Lewy body extracts and is used as a marker of pathologically aggregated α -synuclein.⁷²

The native conformation of α -synuclein has been the subject of numerous studies and intense debate. Early results from biochemical analysis of monomeric α -synuclein indicated an apparent molecular weight of 57-58 kDA, greater than would be expected for a globular protein of 140 amino acids.⁷³ Subsequent analyses failed to detect significant secondary structure, suggesting that the monomer may have an extended conformation and unusual mobility in assays that approximate protein size.⁷⁴ In 2011, two groups published results indicating that α -synuclein exists natively as multimers with tetramers as the most abundant conformation.^{75,76} However, subsequent in-cell NMR analysis of α -synuclein in *E. coli* failed to detect tetramers and found limited secondary structure, prompting further study.⁷⁷ Evidence from human brain tissue suggests that α -synuclein forms multimeric conformers, which are heat-sensitive and therefore metastable.⁷⁸ These results have given rise to the hypothesis that a dynamic equilibrium exists between disordered monomers and metastable multimers. The A53T familial mutation has been shown to reduce the levels of physiological multimers, suggesting that disruption of this equilibrium may promote α -synuclein aggregation.⁷⁹ Furthermore, the dynamic interplay between α -synuclein monomers and multimers has been shown to regulate its physiological roles in the synapse.^{80,81}

Physiological function

When first identified, synucleins were localized in the nucleus and presynapse of neurons.⁵⁷ Subsequent studies have failed to detect nuclear synucleins, but have reinforced their presence in presynaptic terminals.^{58,64,82-84} Expression of synelfin, later renamed α -synuclein, was found to increase during zebra finch song learning, suggesting a role in neurotransmission or long-term potentiation.⁶⁴

The identification of α -synuclein in the presynapse, and its implication in neuronal plasticity, led to examination of neuronal function in α -synuclein knockout mice. These mice were found to lack a gross developmental or functional phenotype.⁸⁵ Striatal brain slices from α -synuclein knockout mice displayed higher levels of dopamine release in response to repeated stimuli than those from wild-type mice, suggesting α -synuclein may play a role in regulating neurotransmitter release. Subsequent studies on synuclein double (α and β) and triple (α , β , and γ) knockout mice revealed a high degree of redundancy within the family.^{86,87} Only the triple knockout showed a profound phenotype, which included age-

related neurological deficits, premature mortality, and motor deficits consistent with models of dopamine neuron loss.⁸⁶

Evidence suggests that α -synuclein modulates vesicular trafficking during neurotransmission. The presence of α -synuclein in the synapse accelerates the kinetics of individual exocytotic events, and this function is disrupted by PD-causing mutations.⁸⁸ While α -synuclein knockout leads to increased vesicular reserve pool in presynaptic terminals,⁸⁴ α -synuclein overexpression leads to accumulation of vesicles at the active zone.⁸⁹ α -Synuclein has been shown to regulate the function of SNARE proteins involved in vesicular fission and fusion,⁹⁰ which is further supported by direct physical interaction between α -synuclein and the v-SNARE synaptobrevin 2.⁸⁶ In mammalian cells and purified *in vitro* systems, α -synuclein dose-dependently facilitates SNARE complex assembly,⁸⁶ and can act as a molecular chaperone.^{90,91} α -Synuclein also facilitates vesicle endocytosis following neurotransmitter release into the synapse.⁹² Collectively, the data highlight the importance of α -synuclein in presynaptic function.

α -Synuclein aggregation in disease

In PD, α -synuclein is deposited into intracellular, proteinaceous inclusions known as Lewy bodies.^{4,93} Aggregated α -synuclein is also found in the analogous structures in neuronal projections, termed Lewy neurites.^{66,94} Lewy bodies classically appear as a dense core surrounded by a diffuse halo, and are comprised of typical amyloid-like fibrils, which are rich in β -pleated sheets and produce characteristic signals upon binding Thioflavin dyes.^{95,96} By electron microscopy, α -synuclein filaments from diseased brain appear unbranched, are typically 5-10 nm wide, and can reach hundreds of nm in length.⁶⁶ Extensive investigations into α -synuclein aggregation using recombinant protein *in vitro* have reproduced many disease-associated features.⁹⁷⁻¹⁰² Furthermore, PD-linked α -synuclein mutations alter its aggregation properties.^{97,99,103-106}

Efforts to characterize the distribution of Lewy pathology during the course of PD have produced somewhat conflicting results. Braak and colleagues defined six stages of PD based on postmortem analysis of inclusion pathology, and suggested that the disease may originate in the enteric nervous system.^{107,108} According to this scheme, Lewy pathology may spread from the gut up through the vagal nerve, to the dorsal motor nucleus of the vagus, one of the earliest brain regions found to bear inclusions. The anterior olfactory nucleus, raphe nuclei, and locus coeruleus also exhibit pathology in the early stages of PD. As PD advances, the midbrain develops inclusions particularly in the SN, and in late stages, cortical regions such as sensory association areas become affected.¹⁰⁷ However, the origin of pathology in the enteric nervous system has come under debate, given difficulties replicating these findings in large autopsy cohorts.¹⁰⁹ Beach and colleagues have outlined a new staging scheme, which begins with pathology in the olfactory bulb, and progresses to involve brainstem regions and ultimately the neocortex.¹¹⁰

The spread of α -synuclein aggregation during the course of the disease is suggestive of prion-like transmission of pathogenic α -synuclein species, for which there is now extensive evidence.¹¹² Several animal models based on the concept of injecting preformed α -synuclein fibril fragments (PFFs) into the brain or peripherally have reinforced the concept

of prion-like transmission,^{113–118} and revealed potential receptors such as LAG3 (lymphocyte-activation gene 3)¹¹⁹ and Fc γ RIIB.¹²⁰ Exogenous application of PFFs in mice caused aggregation of endogenous α -synuclein followed by dopamine neuron demise.¹¹³ Moreover, time-resolved imaging of α -synuclein inclusion-bearing neurons and neighboring neurons without inclusions indicated a selective degeneration of inclusion-bearing cells, providing evidence that α -synuclein aggregation causes neurodegeneration.¹¹⁵

Modeling α -synuclein toxicity in transgenic mice

Despite the clear association between neurodegeneration and α -synuclein aggregation in human disease and the success in replicating prion-like propagation of α -synuclein pathology in PFF models, it has been difficult to recapitulate these features in transgenic mouse models. In mice expressing human wild-type or familial mutants (A30P, E46K, or A53T) of α -synuclein, the SN was consistently spared of both neurodegeneration and inclusion pathology, despite the development of Lewy body-like inclusions in other brain regions.^{121–127} Similarly, targeting wild-type, A30P, or A53T human α -synuclein expression specifically to dopaminergic neurons using the TH promoter was insufficient to produce either nigrostriatal degeneration or Lewy body-like α -synuclein pathology.^{128–130} Greater success inducing dopaminergic cell death has been achieved using bacterial artificial chromosome transgenic mice expressing human wild-type α -synuclein¹³¹ and by conditional expression of human wild-type or A53T α -synuclein.^{132,133}

In order to test if elevating dopamine levels could promote α -synuclein toxicity, we used a well-characterized A53T transgenic mouse model, in which the SN does not develop α -synuclein inclusions and does not undergo neuronal cell loss.¹²¹ We injected TH-RREE lentivirus into the SN of A53T mice. In stark contrast to non-transgenic TH-RREE mice, elevation of dopamine levels in A53T mice resulted in previously undescribed, progressive nigrostriatal degeneration with locomotor impairment.²² Within 2.5 months after lentiviral injection, dopaminergic innervation to the striatum (assessed by VMAT2 staining) had decreased by 25%, while cell bodies in the SN remained intact. By 5 months post-injection, denervation had progressed to 62% loss of VMAT2-positive fibers, with a corresponding decrease in DAT levels. At this point there was also a significant 25% reduction in the number of neurons in the SN. These degenerative changes occurred along with an eventual decrease in striatal dopamine, and significantly reduced ambulation.²² Thus, elevating dopamine levels in A53T mice (but not non-transgenic mice) recapitulates key features of PD, suggesting that dopamine dysregulation may be an important factor in α -synuclein-mediated toxicity.

Our findings are consistent with previous studies showing a synergistic toxic effect of dopamine and α -synuclein. Mosharov et al. elegantly showed in rodent midbrain cultures that elevating cytosolic dopamine levels by L-DOPA treatment resulted in neurotoxicity. Pharmacological inhibition of L-DOPA conversion to dopamine, overexpression of VMAT2, or the absence of α -synuclein abrogated the exogenous L-DOPA-induced neurotoxicity. Moreover, L-DOPA treatment induced neuromelanin synthesis in SN-derived neurons but not in dopamine neurons derived from the ventral tegmental area, which were not sensitive to L-DOPA-induced neurotoxicity, suggesting the oxidation of cytosolic dopamine accounts

for the observed toxicity.¹³⁴ In another study, viral expression of α -synuclein in the SN of VMAT2-deficient mice (which have diminished handling of cytosolic dopamine) led to a significant increase in dopaminergic neurodegeneration compared with either α -synuclein expression or VMAT2 deficiency alone. This exacerbated toxicity was rescued by knockdown of TH, further supporting the notion of dopamine and α -synuclein acting together to induce neurotoxicity.¹³⁵ Our mouse model is also consistent with a recent study in which increasing either α -synuclein or dopamine levels in mouse midbrain neurons was required to recapitulate degenerative phenotypes observed in PD patient-derived neurons.¹³⁶

The A53T TH-RREE mouse model offers new opportunities to study aspects of PD, including the progressive nature of dopaminergic neuron death. In A53T TH-RREE mice, dopaminergic nerve terminals undergo degeneration prior to cell bodies, and terminal loss worsens by the time cell body loss is detected.²² This sequence of events is supported by existing evidence in humans that synapses are an early site of the disease process.^{137,138} Autopsy reports as well as PET imaging studies in living PD patients suggest that, at the time of disease onset, the loss of striatal nerve terminals exceeds that of dopaminergic cell bodies.¹³⁹ Using proteomic approaches in a model of endogenous α -synuclein aggregation,^{113,140} we documented selective changes in proteins and signaling pathways that participate in critical functions of neuronal synaptic activity prior to significant dopaminergic neuron death. Collectively, several lines of evidence indicate that α -synuclein toxicity in the synapse causes a ‘dying back’ of axons and a retrograde cascade of events that ultimately leads to neuronal demise.

α -Synuclein oligomers as a potential point of convergence

In 2001, Lansbury and colleagues conducted a screen for compounds that inhibit α -synuclein aggregation into amyloid-like fibrils *in vitro*. Of the 15 candidates identified as potent modifiers of aggregation, 14 were catecholamines. Dopamine was found to kinetically arrest the formation of fibrils at the level of oligomers.¹⁴¹ Many studies have subsequently confirmed that dopamine stabilizes α -synuclein oligomers *in vitro*.^{142–150} However, the effects of dopamine on α -synuclein aggregation *in vivo* were not investigated until recently.²² Being that α -synuclein oligomers are increasingly thought to be toxic species in disease,¹⁵¹ it is of critical importance to investigate whether dopaminergic neurons are specifically vulnerable due to the interaction of dopamine and α -synuclein.

Evidence for α -synuclein oligomer toxicity

α -Synuclein aggregation generates diverse intermediate species prior to the formation of insoluble fibrils and deposition into inclusions, and consequently it has been difficult to determine which aggregated species mediate disease. While it was originally thought that mature inclusion bodies cause neurodegeneration, this hypothesis has come under debate. Lewy body burden in the brain does not correlate well with either neuron death or the severity of clinical features,¹⁵² and Lewy bodies have been found in the brains of asymptomatic individuals.^{153,154} Moreover, some familial PD patients lack Lewy pathology, as in autosomal recessive forms of PD caused by mutations in *Parkin*, *PINK1*, and *DJ-1*,¹¹¹ and in some cases of dominantly inherited PD caused by *LRRK2* mutation.¹⁵⁵

Growing evidence suggests that oligomeric intermediates may be neurotoxic. Soluble, high molecular weight oligomers of α -synuclein have been extracted from human brain tissue and are increased in PD patients.¹⁵⁶ Higher levels of α -synuclein oligomers are also quantified in PD patient CSF and plasma compared with healthy controls.^{157,158} Animal and cellular model systems support a causative role for α -synuclein oligomers in disease. Viral expression of artificial α -synuclein variants with enhanced oligomerization in the rat SN resulted in the loss of dopaminergic neurons.¹⁵⁹ Similarly, artificial mutants with impaired β -sheet structure and increased propensity to form oligomers caused degeneration of dopaminergic nerve terminals when expressed in worms and overt dopaminergic cell loss in flies.¹⁶⁰ In another study, oligomer extracts from A53T α -synuclein transgenic mice were found to be toxic when applied to primary cortical cultures, while extracts immunodepleted of α -synuclein were not.¹⁶¹ Recombinant α -synuclein oligomers generated *in vitro* have also been shown to be neurotoxic in culture,¹⁶² and α -synuclein oligomer formation is accelerated by the A53T and A30P familial PD mutations.¹⁶³

The interaction of dopamine and α -synuclein *in vitro*

Extensive evidence from cultured cells and cell-free systems indicates that dopamine inhibits the aggregation of α -synuclein even at substoichiometric concentrations and oxidation of dopamine is required for the inhibition.^{141–150} Consistent with these findings, elevating dopamine levels in SH-SY5Y cells expressing A53T α -synuclein reduced the number of cells containing large intracellular aggregates and increased levels of soluble oligomers. The appearance of oligomers was prevented by antioxidant treatment.^{164,165} The interaction of dopamine and α -synuclein is non-covalent, reversible, and dependent on residue E83 and the 125-YEMPS-129 motif in the C-terminus of α -synuclein.^{141,144–147,164,165}

The toxicity of dopamine-induced α -synuclein oligomers is largely unexplored. Evidence from studies conducted *in vitro* suggests that these species are able to inhibit their own degradation and that of other substrates by chaperone-mediated autophagy,¹⁶⁶ and may also reduce neurotransmitter release by inhibiting SNARE complex formation.¹⁶⁷ While there is no evidence that increasing dopamine in SH-SY5Y cultures produces toxic α -synuclein oligomers,^{164,168} exogenous application of recombinant dopamine-stabilized oligomers has been reported to reduce viability and neurotransmitter release in PC12 cells.^{143,167} A30P α -synuclein oligomers generated in the presence of dopamine reduced neurite number and length in primary mesencephalic and cortical cultures.¹⁴⁶ In addition, the toxicity of endogenous cytosolic dopamine in cultured human fetal dopaminergic neurons was linked to the accumulation of soluble α -synuclein protein complexes.¹⁶⁹

***In vivo* evidence of toxic dopamine-induced α -synuclein oligomers**

In order to test the effects of dopamine on α -synuclein aggregation *in vivo*, we examined α -synuclein in the SN of A53T TH-RREE mice and age-matched A53T controls that had been injected with an empty lentiviral vector.²² Consistent with prior reports,¹²¹ the A53T control mice lacked inclusion pathology in the SN, and increasing dopamine levels did not induce pathology or alter levels of insoluble α -synuclein.²² Instead, elevating dopamine levels caused a reduction in α -synuclein monomer, and a corresponding increase in total steady-state levels of oligomers, which were detected by Western blot following enrichment by

native size exclusion chromatography. Interestingly, raising dopamine levels also resulted in different conformations of oligomers. While both A53T TH-RREE and A53T control mice harbored soluble oligomers with Stokes radii of up to 65 Å and apparent molecular weights ranging from 36 to 80 kDa (similar to previous observations¹⁶¹) A53T TH-RREE mice additionally possessed larger oligomer species, with Stokes radii of 72-122 Å. These high Å oligomers were detected exclusively in A53T TH-RREE mice, using multiple α -synuclein antibodies by both western blotting and immunoelectron microscopy.²²

In addition to having distinct conformations, dopamine-induced oligomers derived from A53T TH-RREE mice were also functionally modified. In contrast to oligomers extracted from control mice, oligomers from A53T TH-RREE mice were unable to seed the aggregation of recombinant α -synuclein *in vitro*.²² These findings suggest that the toxicity associated with dopamine-induced oligomers is not dependent on seeding. Indeed, the relationship of seeding competency and neurodegeneration is not clear. Studies have demonstrated neurotoxicity of both seeding-competent and seeding-incompetent α -synuclein oligomers, as well as lack of toxicity for both types of oligomers.^{161,162} In PFF models, while prion-like propagation of α -synuclein aggregates is clearly associated with neurodegeneration, the precise conformations of the toxic species are unknown and may be oligomeric. It is possible that seeding is a critical mechanism for the spread of pathology, while toxicity depends on specific conformations and interaction with cell type-specific factors. For example, as α -synuclein is aggregating, interaction with dopamine within neurons of the SN may induce toxic conformations that lead to cell death. At present, the exact α -synuclein species with which dopamine interacts is unknown, although the presence of oxidized dopamine only in high molecular weight protein complexes extracted from A53T TH-RREE mice suggests that oligomers may be the likely target.²²

To further test if disrupting the interaction of dopamine and α -synuclein rescues neurons from degeneration, we used the genetically tractable small model organism, *C. elegans*. Expression of wild-type or A53T mutant α -synuclein in dopaminergic neurons in *C. elegans* has been shown to cause neuronal dysfunction and death, as well as motor deficits.¹⁷⁰⁻¹⁷³ Increasing cytosolic dopamine in wild-type α -synuclein-expressing worms by mutating the VMAT2 worm homolog accelerate neurodegeneration. Conversely, worms expressing wild-type α -synuclein and a non-functional homolog of TH, preventing dopamine synthesis, were protected from neuronal loss.¹⁷³ These studies are entirely consistent with our findings in mice that indicate dopamine and α -synuclein act together to induce neuronal cell death. We first generated transgenic *C. elegans* expressing human A53T α -synuclein in dopaminergic neurons, and documented neurodegeneration, which was exacerbated when combined with overexpression of CAT-2, the worm homolog of TH.²² Remarkably, when we expressed A53T α -synuclein with mutation of the C-terminal 125-YEMPS-129 motif that is required for dopamine-mediated stabilization of oligomers,^{144,165} the worms showed complete resistance to the neurotoxicity of CAT-2 expression.²² These results provide strong evidence that inhibiting the interaction of dopamine and α -synuclein, and thus preventing the effects of dopamine on α -synuclein oligomers, is neuroprotective.

Therapeutic implications

Since the discovery that striatal dopamine is severely depleted in PD patients,¹⁷⁴ followed immediately thereafter by the first clinical use of L-DOPA to replenish dopamine levels,¹⁷⁵ L-DOPA has become a staple of PD treatment. Undeniably, the resulting symptomatic relief greatly improves patients' quality of life. However, due to the notion that dopamine may be neurotoxic, there has been a widespread practice of 'DOPA sparing' that delays administration of the drug until more advanced stages of disease.¹⁷⁶ At present, there is no conclusive evidence indicating that L-DOPA therapy accelerates PD progression.¹⁷⁷ A number of studies have been conducted in PD patients to test the question of L-DOPA toxicity, including trials based on clinical outcome,^{178–180} neuroimaging markers,^{179,181,182} and postmortem analyses.¹⁸³ With the exception of the neuroimaging studies, which found that L-DOPA was associated with an accelerated decline in dopaminergic markers,^{179,181,182} the consensus has been that L-DOPA either does not affect PD progression or in some cases may actually be neuroprotective. Unfortunately, the results of these studies are called into question by the significant limitations in their designs and methodologies, and the issue of L-DOPA toxicity remains unresolved.¹⁷⁷ By the time PD patients manifest motor signs and receive L-DOPA treatment, a significant number of dopaminergic neurons in the SN have already been lost.¹³⁹ While a role for L-DOPA in advancing neurodegeneration cannot be ruled out, our findings in mouse and worm models²² are instead more relevant to the etiology of PD, offering a potential mechanism for the initial demise of dopaminergic neurons.

Concluding remarks

PD may begin when disturbances in the native state of α -synuclein, i.e. through genetic mutations, increased expression levels, or age-related deterioration of protein quality control, allow α -synuclein to become misfolded and aggregation-competent. α -Synuclein may then aggregate into oligomers and fibrils, eventually forming Lewy body inclusions and Lewy neurites. Region-specific factors may determine whether toxic species are able to form and induce neurodegeneration. In the SN, oxidized dopamine in the cytosol may interact with α -synuclein oligomers resulting in particularly damaging conformations. Similar mechanisms may drive neurodegeneration in the locus coeruleus, which produces the catecholamine norepinephrine that was also shown to inhibit α -synuclein fibrillization *in vitro*.¹⁴¹ Non-catecholaminergic regions that degenerate in PD, including the dorsal motor nucleus of the vagus and the nucleus basalis of Meynert, may contain other region-specific factors that upon interaction with α -synuclein induce neurotoxic species. The ventral tegmental area, a dopaminergic region that is largely spared in PD,¹³⁴ may not succumb to the prion-like spread of α -synuclein aggregation and therefore may not degenerate despite producing dopamine.

The mechanisms by which dopamine-induced α -synuclein oligomers may induce cell death are not known, although evidence points to several possibilities (Figure 1). Recombinant α -synuclein oligomers can have ring-like morphologies and are able to permeabilize synthetic vesicles potentially through a pore-forming mechanism.^{163,184–186} Consistent with aberrant membrane interactions, α -synuclein oligomers (in one study, with annular morphology

confirmed) were shown to increase intracellular calcium levels and cause cell death when applied exogenously to primary neuronal cultures and SH-SY5Y cells.^{162,187} In another report, α -synuclein oligomers were able to disturb calcium homeostasis and induce mitochondrial dysfunction when incubated with isolated mitochondria *in vitro*.¹⁸⁸ α -Synuclein oligomers, including dopamine-modified species, were also demonstrated to bind to the mitochondrial receptor TOM20 preventing protein import,¹⁸⁹ and cause an increase in mitochondrial permeability transition pore opening.¹⁹⁰ Other potential mechanisms of oligomer toxicity include impairment of microtubule-kinesin transport,¹⁹¹ and inhibition of SNARE complex assembly, the latter of which was specifically demonstrated with dopamine-induced oligomer species.¹⁶⁷ Thus, α -synuclein oligomers arising from the pathological interaction with dopamine may cause synaptic dysfunction and cell death through a variety of mechanisms, and may represent an important new target in the design of disease-modifying therapies for PD.

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Figure 1: Proposed model for the formation of dopamine-induced α -synuclein oligomers and potential mechanisms of toxicity

In the dopaminergic nerve terminal, dopamine is synthesized from the amino acid tyrosine and packaged into synaptic vesicles by the vesicular monoamine transporter, VMAT2. The release of dopamine into the synaptic cleft is followed by re-uptake through the dopamine transporter, DAT. Oxidized cytosolic dopamine may interact with α -synuclein (α -Syn) during the aggregation of α -synuclein monomers into soluble oligomers and insoluble fibrils. Oxidized dopamine promotes the formation of 'off-pathway' α -synuclein oligomers, which may be neurotoxic by disrupting cytoskeletal organization and signaling, altering membrane permeability and ion homeostasis, inhibiting SNARE complex assembly, disrupting synaptic vesicle membranes, and causing mitochondrial dysfunction. AADC, aromatic amino acid decarboxylase; L-DOPA, L-3,4-dihydroxyphenylalanine; TH, tyrosine hydroxylase.