Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: http://ees.elsevier.com

Advances in modelling alpha-synuclein-induced Parkinson's diseases in rodents: Virus-based models versus inoculation of exogenous preformed toxic species

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ARTICLE INFO

Keywords
Parkinson model
Rodent
Alpha-synuclein
Oligomer
Fibrils
PFF
Virus vector
AAV

ABSTRACT

Aggregates of alpha-synuclein (αSyn) have been described in Parkinson's disease (PD) patients, and recent evidence has suggested that the most toxic αSyn species in PD are small soluble aggregates including oligomers, prefibrils, protofibrils. The physiological function of αSyn is still highly debated, with a possible role in synaptic vesicle trafficking and release at the presynaptic compartment, and in the regulation of gene expression in the nucleus. Emerging evidence indicate that most of αSyn functions are related with the crucial ability to bind biological membranes, which is associated with structural conversion from a disordered monomer to an α-helical enriched structure. Conformational properties of αSyn can be modulated by a number of factors including post-translational modifications, gene duplication and triplication-driven overexpression, single point mutations, environmental changes, which affect membrane binding and the protein propensity to aggregate in toxic species. The recognized toxic role of αSyn in PD has laid the rational for purposing of αSyn-based, neuropathologically relevant preclinical models of PD. Different approaches have led to the establishment of transgenic models, viral vector-based models, and more recently models based on the intracerebral inoculation of exogenous αSyn preformed fibrils/oligomers. Here, we overview and compare viral vector-based models of αSyn overexpression and models obtained by direct intracerebral infusion of *in vitro* preformed αSyn species. The advantages and pitfalls associated with these different approaches are discussed.

1. Background

Parkinson's Disease (PD) is the second most common neurodegenerative disease after Alzheimer's Disease. Nowadays, PD is considered a synucleinopathy, a family of neurodegenerative diseases shearing the abnormal accumulation of misfolded, often largely phosphorylated, α-synuclein (αSyn) in neuronal and non-neuronal cells in the brain. Synucleinopathies including clinically different diseases such as PD, dementia with Lewy bodies, multiple system atrophy and pure autonomic failure, differ for the sites where the main protein deposition occur, however the factors leading to different clinical phenotypes remain elusive. The discovery that αSyn may form different aggregated structures *in vitro*, has suggested that differences in strains, but also different post-translational modifications, may account for a variety of clinical traits of synucleinopathies. In PD, neuropathological hallmarks are the progressive loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) (Lees et al., 2009) and the presence of deposits mainly containing fibrillar αSyn, namely Lewy Bodies (LB) (Spillantini et al., 1997; Tofaris et al., 2003). Cardinal symptoms of the disease are motor impairment including bradykinesia, resting tremors, rigidity and postural instability, and a plethora of non-motor symptoms such as anxiety, depression, sleep disorders and cognitive impairments related to damage in non- dopaminergic areas (Braak et al., 2003; Chaudhuri and Odin, 2010).

Aggregates of αSyn have been described in sporadic PD patients and have been genetically associated with inherited forms of PD, including point mutations in the SNCA gene, namely A53 T, A30 P, E46 K, H50Q, G51D and A53E (Appel-Cresswell et al., 2013; Kruger et al., 1998; Lesage et al., 2013; Pasanen et al., 2014; Petrucci et al., 2016; Proukakis et al., 2013; Singleton et al., 2003). Although the underlying mechanisms of αSyn neurotoxicity associated with PD are still largely unknown, its central neuropathological role is widely acknowledged, and has laid the rational for purposing of αSyn-based preclinical models of PD. These models have been developed through different approaches, leading to the establishment of transgenic, viral vector-based, and exogenous αSyn injection models (Koprich et al., 2017; Lo

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https://doi.org/10.1016/j.jneumeth.2020.108685
Received 27 December 2019; Received in revised form 5 March 2020; Accepted 10 March 2020
Available online xxx
0165-0270/

Bianco et al., 2002). Modelling the αSyn-related neuropathology of the disease clearly offers a superior tool to investigate new therapeutic targets and novel neuroprotective therapies if compared with classical toxin-based models, namely the 6-OHDA rat model, and the MPTP mouse model. Ideally, experimental models of PD should display: (i) face validity, reproducing most features of the disease; (ii) predictive validity, with progressive development of the neuropathology as in PD, and therapeutic responses comparable to the human disease; (iii) construct validity, reproducing the molecular aspects of the disease. Toxin-based models only partly meet these requirements at least in rodents, where the main caveat relates to the limited predictive and construct validity, while owning face validity. Toxin-based models are based on the ability of toxins to kill dopaminergic neurons with molecular mechanisms not necessarily relevant for PD (construct validity). The neurotoxins 6-OHDA acts acutely to kill neurons, failing to model the progressive pathology of PD and αSyn-related neuropathology. For these aspects, 6-OHDA models have been extremely successful in recognizing symptomatic targets and to develop treatments of PD symptoms and drug-induced motor complications (Cenci et al., 2011), but are not recommended to investigate neurodegenerative targets and to develop disease-modifying therapies. The other widely used neurotoxin MPTP offers consistent advantages over 6-OHDA in terms of construct validity and is highly used to test neuroprotective treatments, but fails to reproduce αSyn pathology. Moreover in mice, repeated MPTP administrations are required to guarantee the chronic presence of the neurotoxin in the brain, an essential condition to increment the neurodegeneration (Schintu et al., 2009). Of note, none of the treatments showing neuroprotection in toxin models has been translated to positive results in PD patients (Athauda and Foltynie, 2015). αSyn-based models, by recapitulating relevant pathological mechanisms of the human disease, may hold a higher construct and predictive validity, and may offer a more reliable tool to investigate the neuropathology and to develop novel neuroprotective therapies.

The first αSyn-based models have been obtained by genetic manipulation, and either overexpressed human WT αSyn, modelling SNCA multiplications, or human A53 T, A30 P mutant αSyn, modelling SNCA missense mutations (Hansen et al., 2013; Kurz et al., 2010; Richfield et al., 2002; Rieker et al., 2011; van der Putten et al., 2000). Few studies generated transgenic mice overexpressing mutant αSyn or its truncated form limited to dopaminergic neurons, under the control of the thyrosine hydroxylase (TH) promoter (Koprich et al., 2017; Richfield et al., 2002; Tofaris et al., 2006; Wegrzynowicz et al., 2019). Besides rodents, invertebrates such as the drosophila and the *C.elegans* have been used to develop αSyn-based models of PD. Although the drosophila does not have an αSyn gene ortholog, several models have been developed by overexpressing human WT or mutant αSyn in the fly (Feany and Bender, 2000; Ordonez et al., 2018). Likewise, a number of *C.elegans* models of PD that express αSyn have been generated (Cooper and Van Raamsdonk, 2018; Kuwahara et al., 2006; Lakso et al., 2003). In general, αSyn-based transgenic models in rodents have shown abnormalities at the striatal level, such as reduced TH-immunoreactivity and dopamine levels, without evident degeneration of dopaminergic cell bodies in the SNpc. Notably, in a recent study the transgenic overexpression of truncated αSyn in dopaminergic neurons resulted in several pathological features of PD, such as reduced dopamine levels and death of dopaminergic neurons, motor impairment, αSyn aggregation in the SNpc (Wegrzynowicz et al., 2019). Transgenic models have largely contributed to current knowledge on PD neuropathology, and have been successfully used to investigate the early stages of the disease and cognitive deficits associated with it, as extensively discussed elsewhere (Hansen et al., 2013; Hatami and Chesselet, 2015; Kurz et al., 2010; Richfield et al., 2002; Tofaris et al., 2006).

Thereafter, new models based on the overexpression of αSyn have been settled by using viral vectors as an alternative approach to reproduce PD-like pathology in rats and mice (Decressac et al., 2012). Based on the recent findings pointing to oligomers, protofibrils and fibrils as the most toxic forms of αSyn, PD models based on the exogenous inoculation of toxic αSyn species are currently under investigation (Barrett and Timothy Greenamyre, 2015).

Here, we will discuss viral vector-based models of αSyn overexpression and models obtained by direct intracerebral infusion of synthetic preformed oligomers and fibrils of αSyn. We will highlight the advantages and pitfalls that in our opinion are associated with the different approaches pursued to date.

2. Physiological role and conformational properties of αSyn

αSyn is a 14 kDa protein encoded by the gene SNCA, widely distributed throughout the body but highly expressed in neurons (Jakes et al., 1994; Maroteaux et al., 1988; Shibasaki et al., 1995). The physiological functions of αSyn are still highly debated. The localization in the presynaptic compartment and the association with synaptic vesicles has suggested a role in synaptic vesicle trafficking and release (Diao et al., 2013; Larsen et al., 2006; Maroteaux et al., 1988; Nemani et al., 2010), *via* a control of vesicle pool size, mobilization and endocytosis (Bendor et al., 2013; Faustini et al., 2018; Fusco et al., 2016; Lautenschlager et al., 2018; Vargas et al., 2014). Accordingly, αSyn interacts with presynaptic proteins such as VAMP-2 from the (SNARE)-complex (soluble N-ethylmaleimide- sensitive factor attachment protein receptor) promoting its assembly and synaptic release of neurotransmitters (Burre et al., 2010). In addition, the interaction of αSyn with the vesicular transporter of monoamines (VMAT2) has been described at the presynaptic compartment (Butler et al., 2015; Guo et al., 2008; Swant et al., 2011; Wersinger et al., 2006). In line with its presynaptic role, αSyn depletion *in vivo* decreased the reuptake of striatal dopamine (Chadchankar et al., 2011) and caused functional deficits of the nigrostriatal dopaminergic system (Abeliovich et al., 2000). Finally, a negative role of αSyn in the dopamine synthesis has been suggested (Baptista et al., 2003; Perez et al., 2002; Yu et al., 2004). All these evidences support the hypothesis that αSyn plays a key role in the regulation of dopaminergic transmission at presynaptic level.

Besides the presynaptic terminal, αSyn has been identified in other cellular compartments, such as endoplasmic reticulum and Golgi apparatus (Cooper et al., 2006; Thayanidhi et al., 2010), mitochondria (Devi et al., 2008; Li et al., 2007; Nakamura, 2013) and nucleus (Goncalves and Outeiro, 2013; Kontopoulos et al., 2006; Maroteaux et al., 1988; Mori et al., 2002). In mitochondria, αSyn has been proposed to improve ATP synthase efficiency and mitochondrial function (Ludtmann et al., 2016). Within the nucleus, αSyn expression levels were shown to increase under oxidative stress conditions and appear to regulate the expression of genes related to DNA repair and mitochondrial biogenesis (Paiva et al., 2017; Siddiqui et al., 2012). Other studies have suggested that αSyn may have chaperone-like activity, sharing some homology and binding a family of cytoplasmic chaperones to induce oxidative stress and neuronal death (Chandra et al., 2005; da Costa et al., 2000; Kanda et al., 2000; Ostrerova et al., 1999). αSyn has also been associated with apoptosis, by affecting the MAPK pathway (Iwata et al., 2001; Menges et al., 2017).

Most of these functions are associated with the ability of αSyn to bind biological membranes, which involves a dramatic structural conversion from an intrinsically disordered monomer in solution to a membrane-bound state that is enriched in α-helical structures (Bodner et al., 2009; Chandra et al., 2003; Davidson et al., 1998; Fauvet et al., 2012; Fusco et al., 2014, 2016; Fusco et al., 2018; Maltsev et al., 2013; McLean et al., 2000; Nuber et al., 2018; Snead and

Eliezer, 2014; Weinreb et al., 1996). This structural conversion is driven by 7 imperfect repeats of 11 residues located in the region 1–90 of the αSyn sequence and encoding for amphipathic class A2 lipid-binding α-helical segments. Other characteristic sequence properties of αSyn include the N-terminal 25 residues, having the role of membrane anchor (Bodner et al., 2009; Fusco et al., 2014), the hydrophobic non-amyloid beta component (NAC) segment (residues 61–95), which is believed to play a role in αSyn aggregation (Breydo et al., 2012), and the C-terminal acidic domain (96–140), containing several phosphorylation sites on Tyr-125, 133, 136 and Ser-129, crucial for protein-protein interactions (Burre et al., 2012, 2010; Woods et al., 2007), as well as for switching the conformational properties of the protein upon Ca^{2+} binding (Lautenschlager et al., 2018).

Conformational properties of αSyn and membrane binding can be modulated by post-translational modifications (PTM), including serine/tyrosine phosphorylation and tyrosine nitration. These PTMs have been observed in both the physiological and pathological contexts involving αSyn (Barrett and Timothy Greenamyre, 2015; Cariulo et al., 2019; Tenreiro et al., 2014). While phosphorylation may occur physiologically, only 4% of monomeric αSyn is phosphorylated under physiological condition, while extensively phosphorylated αSyn was found in synucleinopathy lesions, suggesting a pivotal role of this process in disease neuropathology (Anderson et al., 2006; Fujiwara et al., 2002).

3. Aggregation of αSyn

αSyn exhibits highly complex aggregation, with primary and secondary nucleation mechanisms observed depending on the experimental conditions (Buell et al., 2014). Membrane binding has also key relevance in the aggregation process of αSyn. It has indeed been shown that the interaction with lipid bilayers can modulate the kinetics and the mechanisms of αSyn aggregation, with effects ranging from inhibition to enhancement (Necula et al., 2003; Perrin et al., 2001; Zhu and Fink, 2003). The structural details of amyloid aggregates of αSyn are now known, including the characteristic cross-β spine motif and a unique orthogonal Greek key topology (Tuttle et al., 2016). Cryo-EM structures of αSyn fibrils have also revealed a crucial interface between protofilaments spanning the region 50–57 of the αSyn sequence (Guerrero-Ferreira et al., 2018). This region, which appears to stabilize the fibrillar structure of the protein, is highly relevant for hosting three pathological mutations of αSyn that are associated with early forms of PD (H50Q, G51D, A53 T), suggesting possible alterations of the properties of the fibrillar form in these αSyn variants.

Several factors may trigger the aggregation of αSyn *in vivo*, including overexpression due to gene duplication and triplication, single point mutations, post-translational modifications and environmental changes (Barrett and Timothy Greenamyre, 2015; Fujiwara et al., 2002; Kruger et al., 1998; Lesage et al., 2013; Polymeropoulos et al., 1997; Proukakis et al., 2013; Singleton et al., 2003; Uversky et al., 2001; Zarranz et al., 2004). Intense phosphorylation was found in transgenic mice over-expressing human mutant αSyn (Freichel et al., 2007; Kahle et al., 2002; Wakamatsu et al., 2007). Phosphorylation of Ser-129 has been associated with the accumulation of αSyn oligomers and exacerbated the deposition of inclusions (Anderson et al., 2006; Sugeno et al., 2008), with variable lifespan depending on intramolecular interactions of these species (Alam et al., 2019).

Although LBs contain αSyn in its fibrillar aggregates, several evidences suggested that the most toxic species are small aggregates that maintain a degree of solubility, including oligomers, prefibrils, protofibrils. These diffusible aggregates have been observed in degenerating areas in the PD brain (Karpinar et al., 2009; Sharon et al., 2003; Winner et al., 2011) and in biological fluids of PD patients (Majbour et al., 2016; Tokuda et al., 2010), suggesting that LBs may represent a protective mechanism that sequesters the toxic species from the cytoplasm (Bengoa-Vergniory et al., 2017; Bucciantini et al., 2002; Espa et al., 2019; Muchowski, 2002; Soto and Estrada, 2008). αSyn mutations that increase the propensity to form oligomeric species over fibrils displayed the most toxic effect in cell lines, primary neurons and in dopaminergic neurons of *C. -elegans* and Drosophila (Karpinar et al., 2009). This concept was further demonstrated *in vivo* by Winner and colleagues, showing that inoculation of αSyn variants that form oligomers caused the most severe dopaminergic loss in the rat SN, whereas the αSyn variants that rapidly formed fibrils were less toxic (Winner et al., 2011).

4. Toxic mechanisms of αSyn

4.1. αSyn oligomers/prefibrils interaction with neurons

Several mechanisms have been described to mediate the toxicity of αSyn oligomers against neurons (Villar-Pique et al., 2016). Toxic mechanisms may involve a direct interaction with neuronal membranes or subcellular organelles, as well as the interaction with glial cells, such as microglia and astroglia.

A widely accepted primary mechanism for the neurotoxicity of αSyn oligomers is the disruption of the membrane integrity (Stockl et al., 2012; van Rooijen et al., 2010), causing calcium influx into the cytoplasm (Angelova et al., 2016). Recent studies have identified the key elements for this mechanism (Fusco et al., 2017). In particular, it was shown that the disruption of biological membranes by these αSyn oligomers requires a highly lipophilic element, the N-terminal region that effectively anchors the oligomers onto the membrane surface, and a fibrillar core spanning segments of the NAC, which effectively inserts into the membrane interior and disrupts its integrity. The permeabilization of the cellular membrane has also been proposed to occur *via* annular pore-like oligomers in studies of mutational variants of αSyn (Danzer et al., 2007; Lashuel et al., 2002; Tosatto et al., 2012; van Rooijen et al., 2010; Volles et al., 2001). Indeed, the significant heterogeneity of αSyn oligomers is likely to be associated with a variety of multiple pathways of membrane binding and disruption, including the stabilization of pre-existing membrane defects, the alteration of membrane electrical properties, and the reduction of neuronal excitability (Chaudhary et al., 2016; Kaufmann et al., 2016). Notably, targeting the interaction of αSyn oligomers with neuronal membrane protected from αSyn neurotoxicity (Cascella et al., 2019; Ysselstein et al., 2017).

Intracellularly, αSyn oligomers may induce toxicity *via* the inhibition of tubulin polymerization and cytoskeletal damage (Chen et al., 2007), oxidative stress into the endoplasmic reticulum (Colla et al., 2012), mitochondrial dysfunction (Di Maio et al., 2016; Lindstrom et al., 2017; Nakamura, 2013; Plotegher et al., 2014; Prots et al., 2018) and impairment of the protein degradation systems (Emmanouilidou et al., 2010b; Tanik et al., 2013; Xilouri et al., 2009). Mitochondria are a main target of αSyn toxicity, as suggested by the presence of αSyn aggregates within mitochondria in dopaminergic neurons of PD brains and their correlation with complex I dysfunction (Devi et al., 2008). Transgenic mice overexpressing human αSyn A53 T display damaged mitochondria preceding neurodegeneration, that are defectively removed by the autophagic system (Chen et al., 2015). αSyn interact with mitochondria with the N-terminal region, resulting in the impairment of mitochondrial membrane potential and permeability, and increase of ROS levels (Sarafian et al., 2013; Shen et al., 2014). When the damage was investigated in relation with different αSyn species, oligomers specifically inhibited the mitochondrial protein influx (Di Maio et al., 2016) and disrupted the mitochondrial axonal transport (Prots et al., 2018), consistent with the observation of a structure-related toxicity against multiple cellular compartments.

The neuronal clearance mechanisms, including the autophagy-lysosomal pathway (ALP) and the ubiquitin-proteasome system, are targeted by αSyn (Stefanis et al., 2019; Xilouri et al., 2013). The PD brain displays a depletion of lysosomes and lysosomal markers in nigral neurons positive for αSyn inclusions (Chu et al., 2009; Dehay et al., 2010). Several studies have shown that ALP dysfunction increases the accumulation of aggregated αSyn, which in turn impairs the clearance machinery, suggesting a reciprocal deleterious relationship (Cuervo et al., 2004; Tanik et al., 2013). Moreover, mutant αSyn may inhibit the proteasome activity and induce the accumulation of ubiquitin-positive deposits (Stefanis et al., 2019; Tanaka et al., 2001). Notably, soluble αSyn oligomers specifically bind to proteasome particles and inhibit their function (Emmanouilidou et al., 2010b; Lindersson et al., 2004; Snyder et al., 2003).

Several studies have demonstrated that oligomers are the species mostly responsible for αSyn synaptic toxicity. In dopaminergic terminals, αSyn plays a key functional role in the presynaptic SNARE complex, cooperating with the protein synapsin III to regulate dopamine release (Longhena et al., 2018). In contrast, αSyn oligomers are synaptotoxic and negatively impact the neuronal signaling and excitability by targeting specific synaptic components (Choi et al., 2013; Diogenes et al., 2012; Kaufmann et al., 2016). *In vitro* studies demonstrated that αSyn oligomers displayed a negative impact on the development of long-term potentiation in hippocampal slices (Diogenes et al., 2012) and decreased the excitability of pyramidal neurons (Kaufmann et al., 2016). Moreover, the expression *in vivo* of the oligomer-prone variant E57 K induced synaptic dysfunction *via* the disruption of presynaptic vesicles (Rockenstein et al., 2014). Interestingly, synapsin III seems to be directly involved in the αSyn aggregation process, since αSyn overexpression in synapsin III KO mice neither led to deposition of aggregated or phosphorylated αSyn, nor to synaptic damage and degeneration (Faustini et al., 2018). Accordingly, synapsin III was found to colocalize with αSyn aggregates within LB of PD patients (Longhena et al., 2018). Recently, Spillantini and coworkers showed that, in transgenic mice expressing aggregation-prone truncated α-Syn, the progressive αSyn aggregation in striatal terminals was associated with the reduction of dopamine release, that was restored by aggregation inhibitors (Wegrzynowicz et al., 2019).

4.2. αSyn oligomers interaction with the glia

Importantly, αSyn oligomers released from neurons may spread in the brain and establish contacts with microglia and astroglia, which are the cells in charge of clearing extracellular protein aggregates (Emmanouilidou and Vekrellis, 2016; Stefanis et al., 2019; Yamada and Iwatsubo, 2018). Deficits in these clearing mechanisms may result in an increased concentration of the extracellular protein and consequent neurotoxicity. Moreover, the interaction with microglia induces an unbalanced activation of these cells ultimately leading to microglia-mediated neuronal toxicity. In addition, uptake of αSyn by astrocytes induces the production of pro-inflammatory mediators by these cells, likely contributing to the inflammatory milieu (Lee et al., 2010).

It has been proposed that αSyn, both as WT or mutational variants, behaves as a chemoattractant for microglia (Kim et al., 2009) and post-mortem studies found activated microglia in close contact with neurons showing αSyn deposits (Croisier et al., 2005). A number of *in vitro* studies have demonstrated that aggregated and mutated αSyn stimulate pro-inflammatory responses in microglia, and the release of pro-inflammatory mediators (Klegeris et al., 2008; Lee et al., 2010). Nevertheless, physiological levels of αSyn are essential to modulate microglial function and promote phagocytosis, since microglia from mice lacking αSyn show an exaggerated response to LPS, displaying a pro-inflammatory profile and impaired phagocytic function (Austin et al., 2006). Moreover, oligomer/protofibril forms of the protein hold a greater inflammatory potential when interacting with microglia than the native monomeric protein (Wilms et al., 2009; Zhang et al., 2005). Specific interaction of αSyn with microglia occurs mainly *via* the Toll-like receptors (TLRs), involving both the TLR2 and TLR4 subtypes, leading to NF-kB nuclear translocation and affecting the phagocytic function of these cells (Fellner et al., 2013; Stefanova et al., 2011). Accordingly, high levels of both the TLR2 and TLR4 were found in proximity of αSyn deposits in peripheral immune cells and in microglia (Doorn et al., 2014; Drouin-Ouellet et al., 2014). Although data are not univocal, several studies have demonstrated that the interaction with TLRs is also conformation-specific (Fellner et al., 2013; Kim et al., 2013). Oligomeric αSyn but not the monomer binds to microglia *via* TLR2, triggering the inflammatory cascade *via* NF-kB activation (Daniele et al., 2015; Kim et al., 2013). TLR4 are involved in microglial phagocytosis of extracellular αSyn (Stefanova et al., 2011). The phagocytic process seems to depend upon levels and type of αSyn although it has been proposed that αSyn, both as WT or mutational variants, behaves as a chemoattractant for microglia (Lee et al., 2008; Park et al., 2008). Park et al. (2008) showed that αSyn monomers stimulated while αSyn oligomers inhibited both basal and LPS-induced phagocytosis (Park et al., 2008). Microglia incubated with A53 T mutant αSyn displayed a pro-inflammatory profile which was associated with impaired phagocytic function (Rojanathammanee et al., 2011). In contrast, Roodveldt et al. (2010) showed that both WT and A53 T αSyn similarly promoted the phagocytosis in microglia, while the A30 P and E46 K αSyn induced the opposite effect (Roodveldt et al., 2010). Finally, the exposure to either soluble monomers or fibrillar or truncated αSyn increased the phagocytic activity of microglia, but fibrils induced a milder inflammatory response than the other forms (Fellner et al., 2013). Despite the discrepancies, these studies clearly highlight that the microglia phenotype and phagocytic function are differently affected by αSyn structural variants.

4.3. Seeding activity of αSyn oligomers

Increasing evidence suggests that oligomers of αSyn can induce misfolding and aggregation of protein monomers with a seeding effect (Kordower et al., 2008; Li et al., 2008, Olanow and Prusiner 2009). Several studies *in vitro* and *in vivo* suggested that the oligomeric forms of αSyn may induce the intracellular αSyn aggregation and in turn may promote the spreading of protein aggregates (Danzer et al., 2009; Hansen et al., 2011). Extending this concept, aberrant αSyn was able to induce misfolding in several endogenous proteins (Breydo et al., 2012; Guo et al., 2013; Peelaerts et al., 2015). Besides intracellular spreading, a further concern is the capability of a damaged cell to entangle nearby cells. Several mechanisms have been proposed to explain the cell-to-cell propagation, including passive diffusion (Ahn et al., 2006; Chandra et al., 2003; Grozdanov and Danzer, 2018), membrane pores (Stockl et al., 2013), exosomal transport (Emmanouilidou et al., 2010a), tunneling nanotubes (Abounit et al., 2016; Dieriks et al., 2017), and the transport through carrier proteins (Sung et al., 2001; Yang et al., 2017).

Interestingly the seeding activity of oligomers seems to be structure-specific. Long and stable αSyn oligomers promoted the aggregation of soluble αSyn more powerfully than low-molecular weight αSyn oligomers (Pieri et al., 2016). Accordingly, small annular oligomers displayed low seeding activity, despite the toxic activity *in vitro*, while larger oligomers induced protein seeding despite a low *in vitro* toxicity (Danzer et al., 2007). Of note, following phagocytosis of αSyn oligomers, failure in the degradation process within the phagolysosome may lead to their release into the cytoplasm, where oligomers may seed

the aggregation of αSyn from the physiological pool (Flavin et al., 2017; Freeman et al., 2013; Grozdanov and Danzer, 2018).

5. αSyn-based rodent models of Parkinson's disease

The transgenic models developed to overexpress either WT or pathological αSyn still represent an excellent tool to investigate pre-motor and cognitive symptoms associated with idiopathic or genetic PD (Hatami and Chesselet, 2015). In the last decade, the need of specifically investigate the seeding mechanism triggered by pathological αSyn and the consequent spreading pathology, and the need to investigate the role of αSyn in the later and more severe phases of PD, has motivated the researchers to develop alternative αSyn-based models of PD. Currently two approaches are being pursued, one of which involves the local overexpression of WT or mutant αSyn in specific brain regions *via* the inoculation of viral vectors to drive the protein expression, the other one involving the direct inoculation of exogenous aggregated αSyn, structured in different species such as fibrils, protofibrils and oligomers. Critical parameters when comparing the validity of the models are the extent and progression of the cell damage/loss at the inoculation site or distant sites and the occurrence of non-specific damage, together with the presence of the associated motor impairment and neuroinflammation, and their reproducibility by independent studies.

5.1. Virus-based models

Viral vector-based models of PD have rapidly become a widely used model to mimic PD neuropathology and represent a useful tool to investigate neuroprotective strategies. The choice of virus, animal and site of inoculation can differ from model to model. Both lentivirus and adenovirus have been used to overexpress αSyn into dopaminergic areas (Chung et al., 2009; Kirik et al., 2002; Lo Bianco et al., 2002; Winner et al., 2011), however the adeno-associated virus (AAV) is most commonly used to overexpress either WT or A53 T αSyn most likely because it allows to target neurons more specifically (Decressac et al., 2012; Kirik et al., 2002; Low and Aebischer, 2012; St Martin et al., 2007; Ulusoy et al., 2010). Among rodents, rats are mostly used for virus-based models, however an AAV-αSyn mouse model has been proposed as well (St Martin et al., 2007). As far as the site of virus inoculation the neuropathological effects of both intranigral and intrastriatal αSyn overexpression have been investigated. In particular the first pioneering viral vector-based studies date back to 2002, when at least three independent articles appeared, followed by similar short after, that used either lentiviral or first generation adenoviral vectors (LV and rAAV2/2, see also Table 1) to overexpress WT, A53 T or A30 P human αSyn within the SNpc (Chung et al., 2009; Kirik et al., 2002; Klein et al., 2002; Lo Bianco et al., 2002; Yamada et al., 2004) and the striatum (Kirik et al., 2002). By using a specific antibody against human αSyn, these studies have observed an extensive immunoreactivity (IR) in the injection area, either the SNpc (Chung et al., 2009; Kirik et al. 2002, Lo Bianco et al., 2002) or the striatum (Kirik et al., 2002). Moreover, αSyn IR extended from the injected SNpc to the SN reticulata, the ventral tegmental area and some adjacent nuclei as early as 3 weeks after the virus infusion, and to terminal fields including the striatum, accumbens and cingulate cortex, suggesting that αSyn was intra-axonally transported from the infusion site to terminals (Kirik et al., 2002). This was associated with neuropathological signs such as granular cytoplasmic inclusions positive for human αSyn that firstly appeared within SNpc neurons followed by striatal terminals. Importantly, these studies reproduced a progressive and specific loss of dopamine neurons in the SNpc and of striatal fibers, suggesting that the virus-based approach may mimic advanced stages of PD (Kirik et al., 2002; Lo Bianco et al., 2002).

However, these studies also highlighted how the nigral cell loss was extremely variable, ranging from 30 % to 80 %, and did not differ between WT and mutant αSyn-infused animals (Kirik et al., 2002). Moreover, despite the sometime great loss of DA neurons, rats did not

Table 1
Key Features of Virus-based models.

Model	cell loss in SNpc (%)*	loss of striatal DA and/or fibers	P-αSyn pathology	neuroinflammation	motor impairment
Virus-based					
1 st Generation	30–80 (3–27 weeks) (Kirik et al, 2002; Chung et al, 2009)	Yes (Kirik et al, 2002; Chung et al, 2009)	NR	Inflammatory citokines and activated microglia (Chung et al, 2009)	NS (Kirik et al, 2002)
rAAV2/2 LV	15–35 (3–20 weeks) (Lo Bianco et al, 2002)	Yes (Lo Bianco et al, 2002)	NR	NR	NR
2 nd Generation (rAAV1/2, rAAV2/5 and others)	28–80 (4–26 weeks) (Gorbatyuk et al, 2008; Koprich et al, 2011)	Yes (Gorbatyuk et al, 2008; Koprich et al, 2011)	NR	NR	Yes (Koprich et al, 2011)
3 rd Generation (rAAV2/6 or rAAV2/7)	20–80 (3–44 weeks) (Decressac et al, 2012; Van der Perren et al, 2015*)*	Yes (Decressac et al, 2012; Van der Perren et al, 2015*)*	Yes (Van der Perren et al, 2015)	NR	Yes (Decressac et al, 2012; Van der Perren et al, 2015)

*Progression of cell loss in SNpc (percentage of contralateral side). The time lapse (in weeks) for the neurodegeneration to occur is specified.
AAV = Adeno-associated virus; DA = Dopamine; LV = Lentivirus; NR = not reported; NS = not significant; P-αSyn = Phospho α-Synuclein; SNpc = Substantia nigra pars compacta.

show a significant motor impairment, which may reflect the high variability reported in neurodegeneration among animals (Kirik et al., 2002).

These studies have clearly demonstrated that the extent of αSyn-induced neuropathology depends on the level of protein expression, and most importantly that high levels of expression are necessary to induce a significant pathology with motor impairment (Koprich et al., 2011). Thereafter, a second generation of AAV vectors carrying a higher expression potential such as rAAV1/2, rAAV2/5, have been developed and tested by both intranigral and intrastriatal infusions (Table 1) (Gorbatyuk et al., 2008; Koprich et al., 2011). However, high titers of GFP-carrying vectors used as a control were toxic themselves, introducing a factor of non-specific toxicity (Koprich et al., 2017).

Nevertheless, this aspect offers the appealing opportunity to modulate the vector titer in order to reach a cell loss of various extent and to investigate different stages and aspects of the disease. To overcome this pitfall, subsequent studies have infused into the rat SNpc third generation AAV serotypes with higher transduction efficiency and transgene expression (rAAV2/6 and rAAV2/7, Table 1) (Van der Perren et al., 2011), with a modified vector construct, which included an enhancer element in order to potentiate αSyn expression (Decressac et al., 2012). These studies reported a significant loss of dopamine neurons in the SNpc, drop of striatal dopamine, and a frank motor impairment, within 4 months from infusion. Moreover, histopathological features, such as phosphorylated αSyn, were observed both in the SN and striatum of AAV-αSyn infused rats (Decressac et al., 2012; Van der Perren et al., 2015).

An intense and persistent neuroinflammatory milieu in mesencephalic areas is an important recognized hallmark in PD neuropathology. However, reports of the inflammatory response in the striatum and SN following AAV-αSyn infusion are still sporadic. One study showed that intranigral AAV-A53 T αSyn infusion induced an increase of markers of microglia reactivity and of inflammatory cytokines in the Str but not in the SN, that preceded the neurodegenerative event (Chung et al., 2009). Other studies reported that the intranigral infusion of AAV-WT αSyn induced a transient microglia activation in the SNpc in the absence of cell loss, that was slightly more persistent when dopamine neurons degenerated (Sanchez-Guajardo et al., 2010; Theodore et al., 2008). Interestingly, the injection of AAV-GFP as a control vector also induced some degree of microglia activation (Sanchez-Guajardo et al., 2010) suggesting that the high sensibility of microglia may strengthen the toxic effect of non-selective or weaker toxic tools. Altogether these studies concordantly suggest that microglial pathology precedes neuronal degeneration in these models. Intriguingly, the neuroinflammatory response may start in the projection area with respect to the AAV-αSyn infusion site, *i.e.* in the striatum when infusion is made within the SNpc (Chung et al., 2009). Several *in vitro* studies have shown that the inflammatory response to αSyn is structure-related as aggregated αSyn produced a stronger response than monomeric αSyn (Klegeris et al., 2008; Lee et al., 2010). Although seeding and spreading of toxic αSyn species from the infusion site have been poorly characterized in the AAV-αSyn models, it is conceivable that toxic aggregates of human αSyn are formed in the infusion site and are axonally transported to striatal terminals, were they are released extracellularly and interact with local microglia to trigger the inflammatory phenotype. These findings pose a question on the validity of AAV-αSyn models to reproduce the chronic inflammatory milieu typical of PD, and to investigate inflammatory-related neuropathological processes.

In synopsis, most recent AAV-αSyn models recapitulate several hallmarks of PD neuropathology such as the progressive loss of dopaminergic cells, the increasing motor impairment and the deposits of pathological αSyn within neurons, suggesting that they are reliable models in relation to face and construct validity, however a high variability of results has been considered. Notably, great advances have been made in recent years in modelling AAV-αSyn-induced neuropathology in monkeys (Eslamboli et al., 2007; Kirik et al., 2003; Koprich et al., 2016). These studies demonstrated the neurotoxic effect of AAV-WT αSyn and to a greater extent of AAV-A53 T αSyn inoculated into the monkey SN, showing a slow loss of dopaminergic neurons associated with the development of motor impairment, suggesting that the AAV-αSyn models may hold a pivotal translatability value and predictive validity.

As compared to transgenic models, viral-based αSyn overexpression offers some concrete advantages. The use of high titer viral vectors or highly efficient vectors yields higher gene expression and a more intense neurodegeneration, that enables to model the advanced disease stages better than transgenesis techniques; viral-based models can be established in rats, which allow a more accurate behavioral and histological evaluation than mice; local injection of viral vectors allows to target specific brain regions sparing non-dopaminergic areas; finally, infusion can be performed unilaterally in order to use the contralateral side as a control. As mentioned above, limitations of some AAV–αSyn models are represented by the inadequacy in reproducing some aspects of PD such as the chronic intense neuroinflammatory response, particularly in mesencephalic areas. The other methodological limitation is the need to express levels of the protein much higher than in human disease or physiological condition in order to induce a dopaminergic cell loss, and consequently a high variability in the degree of neurodegeneration can be observed. Moreover, this model requires to titer the virus for each new batch, that is to measure the number of viral particles per ml. In addition, level of expression does not seem to depend solely upon the number of particles injected, but also on the viral infectivity potential, suggesting that vector batches holding similar titer may result in different infectivity and potentially different expression levels (Sanchez-Guajardo et al., 2010).

An interesting finding that came out from studies in the virus-based model is that, differently from the human form, the overexpression of rat αSyn did not induce any cell damage despite the presence of αSyn deposits within neurons (Lo Bianco et al., 2002). *In vitro* studies have demonstrated that the rodent αSyn aggregates in amyloid fibrils more rapidly than the human WT or mutated A53 T/A30 P protein, which first aggregates in nonfibrillar soluble oligomers instead (Rochet et al., 2000). These findings are an evidence that soluble protein oligomers are the most toxic form of αSyn. Moreover, they suggest that the expression of a large amount of αSyn is needed in virus-based models in order to produce toxic oligomers responsible for the dopaminergic neurodegeneration.

5.2. Exogenous αSyn injection

These models are based on the unilateral or bilateral infusion of soluble oligomers of αSyn, or preformed fibrils (PFFs) of various length, or human brain tissue homogenate containing LB, into the SNpc (Abdelmotilib et al., 2017; Masuda-Suzukake et al., 2013; Peelaerts et al., 2015; Recasens et al., 2014; Thakur et al., 2017) striatum (Abdelmotilib et al., 2017; Blumenstock et al., 2017; Espa et al., 2019; Luk et al., 2012a; Paumier et al., 2015; Recasens et al., 2014), olfactory bulb (Rey et al., 2016) or more diffusely into the brain *via* intracerebroventricular (ICV) injection (Fortuna et al., 2017). Most of the studies addressing the properties of αSyn oligomers have been based on the generation of toxic oligomers *in vitro*, as the isolation of pure samples of oligomers *ex vivo* has revealed highly challenging. Some of the methods include the formation of highly pure oligomers using lyophilization steps in the incubation protocol. The resulting oligomers provide the advantage to be relatively homogeneous and induce neurotoxic effects to neuronal cells that are similar to those observed in cells carrying a triplication in the SNCA gene (Angelova et

al., 2016; Deas et al., 2016; Devine et al., 2011). Injections of oligomers/PFFs are typically made by stereotaxic surgery using a glass capillary or a steel needle connected to a Hamilton syringe. The main aim of these models has been to investigate the toxicity of αSyn strains in terms of seeding and spreading properties, and the results obtained have largely increased our knowledge of the mechanisms involved in these processes. Moreover, the oligomers or PFFs inoculation into dopaminergic areas has been performed with the specific aim of pursuing a valuable PD model that might reproduce the cardinal features of the disease, including cell loss, motor impairment, pathological deposits and neuroinflammatory response, or prodromal/non motor symptoms (Abdelmotilib et al., 2017; Espa et al., 2019; Rey et al., 2016; Thakur et al., 2017) (Table 2).

Upon neuron internalization, αSyn oligomers or fibrils may interact with endogenously expressed αSyn to seed new inclusions, and propagate within the cellular compartments in a retrograde or anterograde direction (Volpicelli-Daley et al., 2016). Fibrils may be transmitted to neighboring neurons or released from degenerating neurons triggering the spreading process. Results from these studies strongly suggest that structural and length differences in αSyn species are critical for determining the seeding potential and the spreading of pathology within the brain. Most soluble species, *i.e.* oligomers and short fibrils, hold the highest spreading property, however fibrils seem mostly responsible for the formation of intraneuronal p-αSyn deposits, which is the seeding hallmark (Abdelmotilib et al., 2017; Luk et al., 2012b; Masuda-Suzukake et al., 2013; Peelaerts et al., 2015). On the other hand, studies using preformed toxic species to model PD neuropathology have consistently shown that monomeric αSyn did not induce any αSyn pathology in any area and at any time (Luk et al., 2012b; Masuda-Suzukake et al., 2013). These studies have clearly demonstrated that the propagation of the αSyn pathology occurs in functionally interconnected areas and is time-dependent. Moreover, the spreading pattern was observed after the inoculation of αSyn PFFs in different brain areas, including the SNpc and striatum, confirming *in vivo* the concept that the propagation of toxic species may occur with a retrograde or anterograde direction, as previously demonstrated by *in vitro* studies (Luk et al., 2012b; Masuda-Suzukake et al., 2013; Volpicelli-Daley et al., 2016).

In SN-injected mice, the p-αSyn pathology was observed in the striatum and amygdala, both projection areas of the SN, and in the stria terminalis, which receives amygdala projections, suggesting that the pathology spreads unidirectionally through the neural circuit (Masuda-Suzukake et al., 2013). Of note, when the toxic species used for these models were of human origin, a mouse Ab was used to detect p-αSyn deposits (Masuda-Suzukake et al., 2013). In addition, while the human protein disappeared briefly after inoculation, the mouse pathological protein was detected up to 15 months after inoculation (Masuda-Suzukake et al., 2013). This suggests that the inoculated αSyn species converted endogenous αSyn into toxic forms, and supports the concept that the transmission of pathological αSyn, recruitment and conversion of the endogenous protein is critical for the progression of PD neuropathology in these models. The inoculation of LB-enriched fractions obtained from the SN of PD brains into the mice SN induced a slow spreading of p-αSyn in anatomically interconnected brain regions such as the striatum and cingulate, motor, somatosensory cortices (Recasens et al., 2014).

In striatum-injected mice or rats, PFFs were unilaterally inoculated by a single injection within the dorsal striatum at variable coordinates in mice (AP + 0.2–1.0 mm; ML + 1.85–2.0 mm; DV -2.6-3.0 mm from skull) (Abdelmotilib et al., 2017; Luk et al., 2012b),or rats (AP + 0.7–1.6 mm; ML + 2.4–3.0 mm; DV -4.2–5.5 mm from skull or beneath the dura) (Abdelmotilib et al., 2017; Paumier et al., 2015) or by two injections within the dorsomedial and dorsolateral striatum (Paumier et al., 2015). In this model, the spreading of p-αSyn was observed with sequential involvement of interconnected areas, including pre-frontal, insular, cingulate and motor cortical regions (layers IV and V), olfactory bulb and amygdala, followed by ventral striatum, thalamus, occipital cortex and SNpc.

WT mouse α-syn PFFs or WT human α-syn PFFs have been infused directly into the mouse olfactory bulb in order to investigate the relationship between αSyn deposits and prodromal symptoms of PD, namely olfactory dysfunction, and to characterize the spreading of p-αSyn pathology in the early disease stages, based on Braak hypothesis

Table 2
Key Features of Exogenous αSyn inoculation-based models.

Model	cell loss in SNpc (%)*	loss of striatal DA and/or fibers	P-αSyn pathology	neuroinflammation	motor impairment
Exogenous α-synuclein - based					
SNpc infusion	25–50 (3–68 weeks) (Abdelmotilib et al, 2017; Recasens et al, 2014; Thackur et al., 2017)	Yes (Abdelmotilib et al, 2017; Recasens et al, 2014; Thackur et al., 2017)	Yes (Abdelmotilib et al, 2017; Masuda-Suzukake et al, 2013; Peelaerts et al, 2015; Recasens et al, 2014; Thackur et al., 2017)	Transient microglial activation (Thakur et al, 2017) astrogliosis (Recasens et al, 2014)	Yes (Recasens et al, 2014)
Str infusion	15–35 (8–24 weeks) (Luk et al, 2012a; Paumier et al, 2015)	Yes (Luk et al, 2012a; Paumier et al, 2015)	Yes (Abdelmotilib et al, 2017; Blumenstock et al, 2017; Luk et al, 2012a, 2012b; Paumier et al, 2015)	NS (Blumenstock et al, 2017)	Yes (Luk et al, 2012a)
OB infusion (Rey et al, 2016)	NO	NO	Yes	NS	NS
ICV infusion (*Fortuna et al, 2017*)	45 (6 weeks)	Yes	NR	NR	Yes
Combined Models	41-70 (3–24 weeks) (Espa et al, 2019; Peelaert et al, 2015; Thakur et al, 2017)	Yes (Peelaert et al, 2015; Thakur et al, 2017)	Yes (Espa et al, 2019; Peelaert et al, 2015; Thakur et al, 2017)	Transient microglial activation. p-αSyn in Iba $^{+}$ cells (Thakur et al., 2017)	Yes (Peelaert et al, 2015; Thakur et al, 2017)

*Progression of cell loss in SNpc (percentage of contralateral side). The time lapse (in weeks) for the neurodegeneration to occur is specified.
AAV = Adeno-associated virus; DA = Dopamine; ICV; Intracerebroventricular; LV = Lentivirus; NR = not reported; NS = not significant; OB = Olfactory Bulb; P-αSyn = Phospho α-Synuclein; SNpc = Substantia nigra pars compacta; Str = Striatum.

(Braak et al., 2003; Rey et al., 2019, 2016). In this model p-αSyn pathology appeared first in olfactory regions directly connected with the olfactory bulb (1 month after infusion). At later time points pathology spread to non-olfactory regions located 1 synapse (3 months after infusion) and 2 synapses (6 months after infusion) away from the infusion nucleus, such as the central amygdala and the dentate gyrus, and to more distant widespread regions such as the SN and locus coeruleus at later time points (12 months after infusion) (Rey et al., 2016). While mice PFFs-injected into the olfactory bulb did not display any motor abnormality, they exhibited a progressive alteration of odor detection, detectable as early as 1-month post injection.

Few studies have started to use the PFFs model to address cognitive symptoms of PD, by investigating the correlation between the accumulation of p-αSyn aggregates in cortical areas and dendritic spines abnormalities in the same area or cognitive impairment. One study addressed this issue by infusing PFFs into the dorsal striatum (coordinates relative to the bregma: +0.2 mm anterior, +2.0 mm from midline, +2.6 mm beneath the dura), reporting that spine density in cortical layers I, IV, V was lowered in presence of αSyn aggregates (Blumenstock et al., 2017). Another study infused both the viral vector coding for human wild-type αSyn bilaterally *via* a double injection into the prelimbic and infralimbic cortex, and PFFs bilaterally into the rostromedial striatum (Espa et al., 2019). This study reported a significant deficit in working memory, attention, and inhibitory control, demonstrating a role of pathological αSyn in non-motor symptoms (Espa et al., 2019).

Although the seeding and spreading properties of oligomers and PFFs were consistent across different works, a recent study reported the different seeding and spreading propensity of 5 αSyn strains when injected *in vivo* in the olfactory tubercle, confirming the structure-dependent toxicity (Rey et al., 2019). Moreover, contrasting results have been observed in relation to the neurodegenerative and proinflammatory properties of αSyn species (Table 2). The intracerebroventricular infusion of αSyn oligomers induced non-motor symptoms such as olfactory deficits and motor symptoms accompanied by dopaminergic loss in mice (Fortuna et al., 2017). In some instances, intrastriatal infusion of toxic αSyn, either oligomers or PFFs, did not induce the degeneration of nigrostriatal neurons, despite the presence of PD pathology epitomized by p-αSyn deposits (Masuda-Suzukake et al., 2013, Peelaerts et al., 2015). In other studies, the infusion of PFFs into the dorsal striatum lead to a gradual and partial degeneration of nigral dopamine neurons projecting to the dorsal striatum, detectable 6 months post-infusion and associated with a deterioration of motor performance in the rotarod test (Luk et al., 2012b).The intranigral infusion of PFFs or oligomers was sufficient itself to induce a significant loss of dopamine neurons of the SNpc 3–6 months later in some studies (Abdelmotilib et al., 2017, Thakur et al., 2017) but not in others (Peelaerts et al., 2015). Both oligomeric and PFFs species increased or accelerated the cell loss when infused into AAV-αSyn treated rats (Peelaerts et al., 2015, Thakur et al., 2017). Interestingly, similarly to deposits formation the extent of cell loss was strictly dependent upon the length of PFFs infused into the SN, with shorter 49−29 nm PFFs displaying the most toxic outcome (Abdelmotilib et al., 2017).

On the last years, the AAV-induced human αSyn expression was combined with the injection of PFFs in the SNpc (Thakur et al., 2017). PFFs were inoculated into the SNpc 4 weeks after the infusion of low dose AAV within the same area but in a different site, leading to the formation of αSyn aggregates and to dopaminergic degeneration associated with motor deficits (Thakur et al., 2017). Despite the long and complex methodological procedure that involves a double stereotaxic surgery, such a combined AAV/PFF-based approach has been successful in reproducing both neuropathological and symptomatic features of PD (Table 2). It is worth to note that in this case the nigral cell loss appeared as early as 3 weeks after PFFs inoculation, which may represent an advantage to faster results but a limit when reproducing the slow disease progression (Thakur et al., 2017).

The neuroinflammatory response to oligomers or PFFs inoculation has been poorly investigated and various results have been reported (Table 1). In general, PFFs-induced PD pathology without neurodegeneration failed to induce any inflammatory response in the infused area, although microglia and astroglia proliferation was only investigated at 15 months post-infusion (Masuda-Suzukake et al., 2013). Moreover, αSyn aggregates were not found within microglia cells after PFFs inoculation in this study (Masuda-Suzukake et al., 2013). In contrast, when the inoculation of PFFs into the SNpc induced PD pathology and neurodegeneration, it was associated with a transient microglial activation, which was exacerbated by previous inoculation of AAV- αSyn, and which tended to remit 3 months post-infusion (Thakur et al., 2017, Wilms et al., 2009). Microglia positive for p-αSyn were detected in the SN of AAV + PFFs inoculated rats for a few weeks after infusion (Thakur et al., 2017). Interestingly, the inflammatory response induced by the inoculation of short oligomers into the SNpc was intense, unbalanced and lasted for several months (Boi et al., in preparation). Although more studies are necessary to clarify the interrelationship between αSyn species and microglia *in vivo*, it is plausible that short oligomers are the most proinflammatory species, in accord with *in vitro* observations (Park et al., 2008). Moreover, the poor pathological microgliosis observed after PFFs inoculation may account for the absence (Masuda-Suzukake et al., 2013) or scarce (Thakur et al., 2017) nigral cell loss observed after PFFs inoculation.

All together these studies strongly suggest that the toxicity of αSyn is structure-dependent both in terms of seeding/spreading properties and neurogenerative properties. Exogenous αSyn inoculation models are a great tool to investigate αSyn seeding and spreading pathology and are promising models to evaluate approaches interfering with these processes. Evidence accumulated so far indicates that these models display face validity, recapitulating motor and non-motor symptoms of PD although with some variability, and construct validity by modeling cellular mechanisms of PD. Importantly, the intranigral or intrastriatal inoculation of PD-derived LB extracts, and the intrastriatal injection of αSyn PFFs in monkeys, induced a progressive nigrostriatal neurodegeneration, providing evidence for translatability and potential predictive validity of αSyn PFFs-based models (Chu et al., 2019; Recasens et al., 2014). A practical advantage of this model is the easiness of manipulation of oligomers/PFFs as compared to the virus-based technique, permitting an approach to the model by laboratories worldwide. Moreover, the exogenous preparations of the toxic species, *i.e.* oligomers and PFFs, offers the advantage of a more rigorous control of the amount injected and intracerebral concentration of these species, offering a better starting point for the development of a highly standardized PD model. Alike virus-based models, exogenous αSyn inoculation can be made unilaterally, although unilateral injection of aSyn oligomers/PFFs may produce bilateral pathology, and therefore caution should be made in using the contralateral side as a control. The inoculation of oligomers/PFFs can be made in specific areas and thus it is useful to investigate pathological properties and effects of toxic αSyn species, in relation to both cardinal symptoms and non-motor symptoms of PD. The long time required to develop the neuropathology and the motor symptomatology after oligomers/PFFs infusion reproduces the slow progression of human neuropathology, useful when testing disease-modifying agents, although this may be considered a disadvantage in the practical use of this model. A limitation of oligomers and PFFs is their poor stability and the tendency to dissociate, therefore requiring particular care in the storage and handling, and the need to program the intracerebral infusion within 10 days from preparation (Polinski et al., 2018). Finally, as discussed above, not all oligomeric/PFFs species hold the same toxicity, and the mechanism of structure-related toxicity is still largely unclear. In synthesis, further investigations are required to definitely

establish the exogenous αSyn inoculation as a valid and standardized preclinical model of PD; however, it is predictable that it will become a widely used approach because the many advantages over the other available models.

Declaration of Competing Interest

None.

References

Abdelmotilib, H, Maltbie, T, Delic, V, Liu, Z, Hu, X, et al., 2017. Alpha-Synuclein fibril-induced inclusion spread in rats and mice correlates with dopaminergic Neurodegeneration. Neurobiol. Dis. 105, 84–98.

Abeliovich, A, Schmitz, Y, Farinas, I, Choi-Lundberg, D, Ho, W H, et al., 2000. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25, 239–252.

Abounit, S, Bousset, L, Loria, F, Zhu, S, de Chaumont, F, et al., 2016. Tunneling nanotubes spread fibrillar alpha-synuclein by intercellular trafficking of lysosomes. EMBO J. 35, 2120–2138.

Ahn, K J, Paik, S R, Chung, K C, Kim, J, 2006. Amino acid sequence motifs and mechanistic features of the membrane translocation of alpha-synuclein. J. Neurochem. 97, 265–279.

Alam, P, Bousset, L, Melki, R, Otzen, D E, 2019. Alpha-synuclein oligomers and fibrils: a spectrum of species, a spectrum of toxicities. J. Neurochem. 150, 522–534.

Anderson, J P, Walker, D E, Goldstein, J M, de Laat, R, Banducci, K, et al., 2006. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J. Biol. Chem. 281, 29739–29752.

Angelova, P R, Ludtmann, M H, Horrocks, M H, Negoda, A, Cremades, N, et al., 2016. Ca2+ is a key factor in alpha-synuclein-induced neurotoxicity. J. Cell. Sci. 129, 1792–1801.

Appel-Cresswell, S, Vilarino-Guell, C, Encarnacion, M, Sherman, H, Yu, I, et al., 2013. Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. Mov. Disord. 28, 811–813.

Athauda, D, Foltynie, T, 2015. The ongoing pursuit of neuroprotective therapies in Parkinson disease. Nat. Rev. Neurol. 11, 25–40.

Austin, S A, Floden, A M, Murphy, E J, Combs, C K, 2006. Alpha-synuclein expression modulates microglial activation phenotype. J. Neurosci. 26, 10558–10563.

Baptista, M J, O'Farrell, C, Daya, S, Ahmad, R, Miller, D W, et al., 2003. Co-ordinate transcriptional regulation of dopamine synthesis genes by alpha-synuclein in human neuroblastoma cell lines. J. Neurochem. 85, 957–968.

Barrett, P J, Timothy Greenamyre, J, 2015. Post-translational modification of alpha-synuclein in Parkinson's disease. Brain Res. 1628, 247–253.

Bendor, J T, Logan, T P, Edwards, R H, 2013. The function of alpha-synuclein. Neuron 79, 1044–1066.

Bengoa-Vergniory, N, Roberts, R F, Wade-Martins, R, Alegre-Abarrategui, J, 2017. Alpha-synuclein oligomers: a new hope. Acta Neuropathol. 134, 819–838.

Blumenstock, S, Rodrigues, E F, Peters, F, Blazquez-Llorca, L, Schmidt, F, et al., 2017. Seeding and transgenic overexpression of alpha-synuclein triggers dendritic spine pathology in the neocortex. EMBO Mol. Med. 9, 716–731.

Bodner, C R, Dobson, C M, Bax, A, 2009. Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy. J. Mol. Biol. 390, 775–790.

Braak, H, Del Tredici, K, Rub, U, de Vos, R A, Jansen Steur, E N, Braak, E, 2003. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 24, 197–211.

Breydo, L, Wu, J W, Uversky, V N, 2012. Alpha-synuclein misfolding and Parkinson's disease. Biochim. Biophys. Acta 1822, 261–285.

Bucciantini, M, Giannoni, E, Chiti, F, Baroni, F, Formigli, L, et al., 2002. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416, 507–511.

Buell, A K, Galvagnion, C, Gaspar, R, Sparr, E, Vendruscolo, M, et al., 2014. Solution conditions determine the relative importance of nucleation and growth processes in alpha-synuclein aggregation. Proc. Natl. Acad. Sci. U. S. A. 111, 7671–7676.

Burre, J, Sharma, M, Tsetsenis, T, Buchman, V, Etherton, M R, Sudhof, T C, 2010. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329, 1663–1667.

Burre, J, Sharma, M, Sudhof, T C, 2012. Systematic mutagenesis of alpha-synuclein reveals distinct sequence requirements for physiological and pathological activities. J. Neurosci. 32, 15227–15242.

Butler, B, Saha, K, Rana, T, Becker, J P, Sambo, D, et al., 2015. Dopamine transporter activity is modulated by alpha-synuclein. J. Biol. Chem. 290, 29542–29554.

Cariulo, C, Martufi, P, Verani, M, Azzollini, L, Bruni, G, et al., 2019. Phospho-S129 alpha-synuclein is present in human plasma but not in cerebrospinal fluid as determined by an ultrasensitive immunoassay. Front. Neurosci. 13 (889).

Cascella, R, Perni, M, Chen, S W, Fusco, G, Cecchi, C, et al., 2019. Probing the origin of the toxicity of oligomeric aggregates of alpha-synuclein with antibodies. ACS Chem. Biol. 14, 1352–1362.

Cenci, M A, Ohlin, K E, Odin, P, 2011. Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease. CNS Neurol. Disord. Drug Targets 10, 670–684.

Chadchankar, H, Ihalainen, J, Tanila, H, Yavich, L, 2011. Decreased reuptake of dopamine in the dorsal striatum in the absence of alpha-synuclein. Brain Res. 1382, 37–44.

Chandra, S, Chen, X, Rizo, J, Jahn, R, Sudhof, T C, 2003. A broken alpha -helix in folded alpha -Synuclein. J. Biol. Chem. 278, 15313–15318.

Chandra, S, Gallardo, G, Fernandez-Chacon, R, Schluter, O M, Sudhof, T C, 2005. Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 123, 383–396.

Chaudhary, H, Iyer, A, Subramaniam, V, Claessens, M M, 2016. Alpha-synuclein oligomers stabilize pre-existing defects in supported bilayers and propagate membrane damage in a fractal-like pattern. Langmuir 32, 11827–11836.

Chaudhuri, K R, Odin, P, 2010. The challenge of non-motor symptoms in Parkinson's disease. Prog. Brain Res. 184, 325–341.

Chen, L, Jin, J, Davis, J, Zhou, Y, Wang, Y, et al., 2007. Oligomeric alpha-synuclein inhibits tubulin polymerization. Biochem. Biophys. Res. Commun. 356, 548–553.

Chen, L, Xie, Z, Turkson, S, Zhuang, X, 2015. A53T human alpha-synuclein overexpression in transgenic mice induces pervasive mitochondria macroautophagy defects preceding dopamine neuron degeneration. J. Neurosci. 35, 890–905.

Choi, B K, Choi, M G, Kim, J Y, Yang, Y, Lai, Y, et al., 2013. Large alpha-synuclein oligomers inhibit neuronal SNARE-mediated vesicle docking. Proc. Natl. Acad. Sci. U. S. A. 110, 4087–4092.

Chu, Y, Dodiya, H, Aebischer, P, Olanow, C W, Kordower, J H, 2009. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions. Neurobiol. Dis. 35, 385–398.

Chu, Y, Muller, S, Tavares, A, Barret, O, Alagille, D, et al., 2019. Intrastriatal alpha-synuclein fibrils in monkeys: spreading, imaging and neuropathological changes. Brain 142, 3565–3579.

Chung, C Y, Koprich, J B, Siddiqi, H, Isacson, O, 2009. Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy. J. Neurosci. 29, 3365–3373.

Colla, E, Jensen, P H, Pletnikova, O, Troncoso, J C, Glabe, C, Lee, M K, 2012. Accumulation of toxic alpha-synuclein oligomer within endoplasmic reticulum occurs in alpha-synucleinopathy in vivo. J. Neurosci. 32, 3301–3305.

Cooper, J F, Van Raamsdonk, J M, 2018. Modeling parkinson's disease in C. Elegans. J. Parkinsons Dis. 8, 17–32.

Cooper, A A, Gitler, A D, Cashikar, A, Haynes, C M, Hill, K J, et al., 2006. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313, 324–328.

Croisier, E, Moran, L B, Dexter, D T, Pearce, R K, Graeber, M B, 2005. Microglial inflammation in the parkinsonian substantia nigra: relationship to alpha-synuclein deposition. J. Neuroinflammation 2 (14).

Cuervo, A M, Stefanis, L, Fredenburg, R, Lansbury, P T, Sulzer, D, 2004. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science 305, 1292–1295.

da Costa, C A, Ancolio, K, Checler, F, 2000. Wild-type but not Parkinson's disease-related ala-53 --& Thr mutant alpha -synuclein protects neuronal cells from apoptotic stimuli. J. Biol. Chem. 275, 24065–24069.

Daniele, S G, Beraud, D, Davenport, C, Cheng, K, Yin, H, Maguire-Zeiss, K A, 2015. Activation of MyD88-dependent TLR1/2 signaling by misfolded alpha-synuclein, a protein linked to neurodegenerative disorders. Sci. Signal. 8, ra45.

Danzer, K M, Haasen, D, Karow, A R, Moussaud, S, Habeck, M, et al., 2007. Different species of alpha-synuclein oligomers induce calcium influx and seeding. J. Neurosci. 27, 9220–9232.

Danzer, K M, Krebs, S K, Wolff, M, Birk, G, Hengerer, B, 2009. Seeding induced by alpha-synuclein oligomers provides evidence for spreading of alpha-synuclein pathology. J. Neurochem. 111, 192–203.

Davidson, W S, Jonas, A, Clayton, D F, George, J M, 1998. Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. J. Biol. Chem. 273, 9443–9449.

Deas, E, Cremades, N, Angelova, P R, Ludtmann, M H, Yao, Z, et al., 2016. Alpha-synuclein oligomers interact with metal ions to induce oxidative stress and neuronal death in parkinson's disease. Antioxid. Redox Signal. 24, 376–391.

Decressac, M, Mattsson, B, Lundblad, M, Weikop, P, Bjorklund, A, 2012. Progressive neurodegenerative and behavioural changes induced by AAV-mediated overexpression of alpha-synuclein in midbrain dopamine neurons. Neurobiol. Dis. 45, 939–953.

Dehay, B, Bove, J, Rodriguez-Muela, N, Perier, C, Recasens, A, et al., 2010. Pathogenic lysosomal depletion in Parkinson's disease. J. Neurosci. 30, 12535–12544.

Devi, L, Raghavendran, V, Prabhu, B M, Avadhani, N G, Anandatheerthavarada, H K, 2008. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. J. Biol. Chem. 283, 9089–9100.

Devine, M J, Ryten, M, Vodicka, P, Thomson, A J, Burdon, T, et al., 2011. Parkinson's disease induced pluripotent stem cells with triplication of the alpha-synuclein locus. Nat. Commun. 2 (440).

Di Maio, R, Barrett, P J, Hoffman, E K, Barrett, C W, Zharikov, A, et al., 2016. Alpha-Synuclein binds to TOM20 and inhibits mitochondrial protein import in Parkinson's disease. Sci. Transl. Med. 8, 342ra78.

Diao, J, Burre, J, Vivona, S, Cipriano, D J, Sharma, M, et al., 2013. Native alpha-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. Elife 2, e00592.

Dieriks, B V, Park, T I, Fourie, C, Faull, R L, Dragunow, M, Curtis, M A, 2017. Alpha-synuclein transfer through tunneling nanotubes occurs in SH-SY5Y cells and primary brain pericytes from Parkinson's disease patients. Sci. Rep. 7, 42984.

Diogenes, M J, Dias, R B, Rombo, D M, Vicente Miranda, H, Maiolino, F, et al., 2012. Extracellular alpha-synuclein oligomers modulate synaptic transmission and impair LTP via NMDA-receptor activation. J. Neurosci. 32, 11750–11762.
Doorn, K J, Moors, T, Drukarch, B, van de Berg, W, Lucassen, P J, van Dam, A M, 2014. Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. Acta Neuropathol. Commun. 2, 90.
Drouin-Ouellet, J, St-Amour, I, Saint-Pierre, M, Lamontagne-Proulx, J, Kriz, J, et al., 2014. Toll-like receptor expression in the blood and brain of patients and a mouse model of Parkinson's disease. Int. J. Neuropsychopharmacol. 18.
Emmanouilidou, E, Vekrellis, K, 2016. Exocytosis and spreading of normal and aberrant alpha-synuclein. Brain Pathol. 26, 398–403.
Emmanouilidou, E, Melachroinou, K, Roumeliotis, T, Garbis, S D, Ntzouni, M, et al., 2010. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. J. Neurosci. 30, 6838–6851.
Emmanouilidou, E, Stefanis, L, Vekrellis, K, 2010. Cell-produced alpha-synuclein oligomers are targeted to, and impair, the 26S proteasome. Neurobiol. Aging 31, 953–968.
Eslamboli, A, Romero-Ramos, M, Burger, C, Bjorklund, T, Muzyczka, N, et al., 2007. Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. Brain 130, 799–815.
Espa, E, Clemensson EKH, Luk K C, Heuer, A, Bjorklund, T, Cenci, M A, 2019. Seeding of protein aggregation causes cognitive impairment in rat model of cortical synucleinopathy. Mov. Disord..
Faustini, G, Longhena, F, Varanita, T, Bubacco, L, Pizzi, M, et al., 2018. Synapsin III deficiency hampers alpha-synuclein aggregation, striatal synaptic damage and nigral cell loss in an AAV-based mouse model of Parkinson's disease. Acta Neuropathol. 136, 621–639.
Fauvet, B, Mbefo, M K, Fares, M B, Desobry, C, Michael, S, et al., 2012. Alpha-Synuclein in central nervous system and from erythrocytes, mammalian cells, and Escherichia coli exists predominantly as disordered monomer. J. Biol. Chem. 287, 15345–15364.
Feany, M B, Bender, W W, 2000. A Drosophila model of parkinson's disease. Nature 404, 394–398.
Fellner, L, Irschick, R, Schanda, K, Reindl, M, Klimaschewski, L, et al., 2013. Toll-like receptor 4 is required for alpha-synuclein dependent activation of microglia and astroglia. Glia 61, 349–360.
Flavin, W P, Bousset, L, Green, Z C, Chu, Y, Skarpathiotis, S, et al., 2017. Endocytic vesicle rupture is a conserved mechanism of cellular invasion by amyloid proteins. Acta Neuropathol. 134, 629–653.
Fortuna, J T S, Gralle, M, Beckman, D, Neves, F S, Diniz, L P, et al., 2017. Brain infusion of alpha-synuclein oligomers induces motor and non-motor Parkinson's disease-like symptoms in mice. Behav. Brain Res. 333, 150–160.
Freeman, D, Cedillos, R, Choyke, S, Lukic, Z, McGuire, K, et al., 2013. Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. PLoS One 8, e62143.
Freichel, C, Neumann, M, Ballard, T, Muller, V, Woolley, M, et al., 2007. Age-dependent cognitive decline and amygdala pathology in alpha-synuclein transgenic mice. Neurobiol. Aging 28, 1421–1435.
Fujiwara, H, Hasegawa, M, Dohmae, N, Kawashima, A, Masliah, E, et al., 2002. Alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat. Cell Biol. 4, 160–164.
Fusco, G, De Simone, A, Gopinath, T, Vostrikov, V, Vendruscolo, M, et al., 2014. Direct observation of the three regions in alpha-synuclein that determine its membrane-bound behaviour. Nat. Commun. 5 (3827).
Fusco, G, Pape, T, Stephens, A D, Mahou, P, Costa, A R, et al., 2016. Structural basis of synaptic vesicle assembly promoted by alpha-synuclein. Nat. Commun. 7 (12563).
Fusco, G, Chen, S W, Williamson, P T F, Cascella, R, Perni, M, et al., 2017. Structural basis of membrane disruption and cellular toxicity by alpha-synuclein oligomers. Science 358, 1440–1443.
Fusco, G, Sanz-Hernandez, M, De Simone, A, 2018. Order and disorder in the physiological membrane binding of alpha-synuclein. Curr. Opin. Struct. Biol. 48, 49–57.
Goncalves, S, Outeiro, T F, 2013. Assessing the subcellular dynamics of alpha-synuclein using photoactivation microscopy. Mol. Neurobiol. 47, 1081–1092.
Gorbatyuk, O S, Li, S, Sullivan, L F, Chen, W, Kondrikova, G, et al., 2008. The phosphorylation state of Ser-129 in human alpha-synuclein determines neurodegeneration in a rat model of Parkinson disease. Proc. Natl. Acad. Sci. U. S. A. 105, 763–768.
Grozdanov, V, Danzer, K M, 2018. Release and uptake of pathologic alpha-synuclein. Cell Tissue Res. 373, 175–182.
Guerrero-Ferreira, R, Taylor, N M, Mona, D, Ringler, P, Lauer, M E, et al., 2018. Cryo-EM structure of alpha-synuclein fibrils. Elife 7.
Guo, J T, Chen, A Q, Kong, Q, Zhu, H, Ma, C M, Qin, C, 2008. Inhibition of vesicular monoamine transporter-2 activity in alpha-synuclein stably transfected SH-SY5Y cells. Cell. Mol. Neurobiol. 28, 35–47.
Guo, J L, Covell, D J, Daniels, J P, Iba, M, Stieber, A, et al., 2013. Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. Cell 154, 103–117.
Hansen, C, Angot, E, Bergstrom, A L, Steiner, J A, Pieri, L, et al., 2011. Alpha-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. J. Clin. Invest. 121, 715–725.
Hansen, C, Bjorklund, T, Petit, G H, Lundblad, M, Murmu, R P, et al., 2013. A novel alpha-synuclein-GFP mouse model displays progressive motor impairment, olfactory dysfunction and accumulation of alpha-synuclein-GFP. Neurobiol. Dis. 56, 145–155.
Hatami, A, Chesselet, M F, 2015. Transgenic rodent models to study alpha-synuclein pathogenesis, with a focus on cognitive deficits. Curr. Top. Behav. Neurosci. 22, 303–330.
Iwata, A, Maruyama, M, Kanazawa, I, Nukina, N, 2001. Alpha-Synuclein affects the MAPK pathway and accelerates cell death. J. Biol. Chem. 276, 45320–45329.
Jakes, R, Spillantini, M G, Goedert, M, 1994. Identification of two distinct synucleins from human brain. FEBS Lett. 345, 27–32.
Kahle, P J, Neumann, M, Ozmen, L, Muller, V, Jacobsen, H, et al., 2002. Hyperphosphorylation and insolubility of alpha-synuclein in transgenic mouse oligodendrocytes. EMBO Rep. 3, 583–588.
Kanda, S, Bishop, J F, Eglitis, M A, Yang, Y, Mouradian, M M, 2000. Enhanced vulnerability to oxidative stress by alpha-synuclein mutations and C-terminal truncation. Neuroscience 97, 279–284.
Karpinar, D P, Balija, M B, Kugler, S, Opazo, F, Rezaei-Ghaleh, N, et al., 2009. Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. EMBO J. 28, 3256–3268.
Kaufmann, T J, Harrison, P M, Richardson, M J, Pinheiro, T J, Wall, M J, 2016. Intracellular soluble alpha-synuclein oligomers reduce pyramidal cell excitability. J. Physiol. (Paris) 594, 2751–2772.
Kim, S, Cho, S H, Kim, K Y, Shin, K Y, Kim, H S, et al., 2009. Alpha-synuclein induces migration of BV-2 microglial cells by up-regulation of CD44 and MT1-MMP. J. Neurochem. 109, 1483–1496.
Kim, C, Ho, D H, Suk, J E, You, S, Michael, S, et al., 2013. Neuron-released oligomeric alpha-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat. Commun. 4 (1562).
Kirik, D, Rosenblad, C, Burger, C, Lundberg, C, Johansen, T E, et al., 2002. Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. J. Neurosci. 22, 2780–2791.
Kirik, D, Annett, L E, Burger, C, Muzyczka, N, Mandel, R J, Bjorklund, A, 2003. Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new primate model of Parkinson's disease. Proc. Natl. Acad. Sci. U. S. A. 100, 2884–2889.
Klegeris, A, Pelech, S, Giasson, B I, Maguire, J, Zhang, H, et al., 2008. Alpha-synuclein activates stress signaling protein kinases in THP-1 cells and microglia. Neurobiol. Aging 29, 739–752.
Klein, R L, King, M A, Hamby, M E, Meyer, E M, 2002. Dopaminergic cell loss induced by human A30P alpha-synuclein gene transfer to the rat substantia nigra. Hum. Gene Ther. 13, 605–612.
Kontopoulos, E, Parvin, J D, Feany, M B, 2006. Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. Hum. Mol. Genet. 15, 3012–3023.
Koprich, J B, Johnston, T H, Huot, P, Reyes, M G, Espinosa, M, Brotchie, J M, 2011. Progressive neurodegeneration or endogenous compensation in an animal model of Parkinson's disease produced by decreasing doses of alpha-synuclein. PLoS One 6, e17698.
Koprich, J B, Johnston, T H, Reyes, G, Omana, V, Brotchie, J M, 2016. Towards a non-human primate model of alpha-synucleinopathy for development of therapeutics for parkinson's disease: optimization of AAV1/2 delivery parameters to drive sustained expression of alpha synuclein and dopaminergic degeneration in Macaque. PLoS One 11, e0167235.
Koprich, J B, Kalia, L V, Brotchie, J M, 2017. Animal models of alpha-synucleinopathy for Parkinson disease drug development. Nat. Rev. Neurosci. 18, 515–529.
Kordower, J H, Chu, Y, Hauser, R A, Freeman, T B, Olanow, C W, 2008. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat. Med. 14, 504–506.
Kruger, R, Kuhn, W, Muller, T, Woitalla, D, Graeber, M, et al., 1998. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat. Genet. 18, 106–108.
Kurz, A, Double, K L, Lastres-Becker, I, Tozzi, A, Tantucci, M, et al., 2010. A53T-alpha-synuclein overexpression impairs dopamine signaling and striatal synaptic plasticity in old mice. PLoS One 5, e11464.
Kuwahara, T, Koyama, A, Gengyo-Ando, K, Masuda, M, Kowa, H, et al., 2006. Familial Parkinson mutant alpha-synuclein causes dopamine neuron dysfunction in transgenic Caenorhabditis elegans. J. Biol. Chem. 281, 334–340.
Lakso, M, Vartiainen, S, Moilanen, A M, Sirvio, J, Thomas, J H, et al., 2003. Dopaminergic neuronal loss and motor deficits in Caenorhabditis elegans overexpressing human alpha-synuclein. J. Neurochem. 86, 165–172.
Larsen, K E, Schmitz, Y, Troyer, M D, Mosharov, E, Dietrich, P, et al., 2006. Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. J. Neurosci. 26, 11915–11922.
Lashuel, H A, Petre, B M, Wall, J, Simon, M, Nowak, R J, et al., 2002. Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J. Mol. Biol. 322, 1089–1102.
Lautenschlager, J, Stephens, A D, Fusco, G, Strohl, F, Curry, N, et al., 2018. C-terminal calcium binding of alpha-synuclein modulates synaptic vesicle interaction. Nat. Commun. 9 (712).
Lee, H J, Suk, J E, Bae, E J, Lee, S J, 2008. Clearance and deposition of extracellular alpha-synuclein aggregates in microglia. Biochem. Biophys. Res. Commun. 372, 423–428.
Lee, E J, Woo, M S, Moon, P G, Baek, M C, Choi, I Y, et al., 2010. Alpha-synuclein activates microglia by inducing the expressions of matrix metalloproteinases and the subsequent activation of protease-activated receptor-1. J. Immunol. 185, 615–623.
Lees, A J, Hardy, J, Revesz, T, 2009. Parkinson's disease. Lancet (London, England) 373, 2055–2066.
Lesage, S, Anheim, M, Letournel, F, Bousset, L, Honore, A, et al., 2013. G51D alpha-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. Ann. Neurol. 73, 459–471.

Li, W W, Yang, R, Guo, J C, Ren, H M, Zha, X L, et al., 2007. Localization of alpha-synuclein to mitochondria within midbrain of mice. Neuroreport 18, 1543–1546.

Li, J Y, Englund, E, Holton, J L, Soulet, D, Hagell, P, et al., 2008. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat. Med. 14, 501–503.

Lindersson, E, Beedholm, R, Hojrup, P, Moos, T, Gai, W, et al., 2004. Proteasomal inhibition by alpha-synuclein filaments and oligomers. J. Biol. Chem. 279, 12924–12934.

Lindstrom, V, Gustafsson, G, Sanders, L H, Howlett, E H, Sigvardson, J, et al., 2017. Extensive uptake of alpha-synuclein oligomers in astrocytes results in sustained intracellular deposits and mitochondrial damage. Mol. Cell. Neurosci. 82, 143–156.

Lo Bianco, C, Ridet, J L, Schneider, B L, Deglon, N, Aebischer, P, 2002. Alpha -Synucleinopathy and selective dopaminergic neuron loss in a rat lentiviral-based model of Parkinson's disease. Proc. Natl. Acad. Sci. U. S. A. 99, 10813–10818.

Longhena, F, Faustini, G, Varanita, T, Zaltieri, M, Porrini, V, et al., 2018. Synapsin III is a key component of alpha-synuclein fibrils in Lewy bodies of PD brains. Brain Pathol. 28, 875–888.

Low, K, Aebischer, P, 2012. Use of viral vectors to create animal models for Parkinson's disease. Neurobiol. Dis. 48, 189–201.

Ludtmann, M H, Angelova, P R, Ninkina, N N, Gandhi, S, Buchman, V L, Abramov, A Y, 2016. Monomeric alpha-synuclein exerts a physiological role on brain ATP synthase. J. Neurosci. 36, 10510–10521.

Luk, K C, Kehm, V, Carroll, J, Zhang, B, O'Brien, P, et al., 2012. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338, 949–953.

Luk, K C, Kehm, V M, Zhang, B, O'Brien, P, Trojanowski, J Q, Lee, V M, 2012. Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. J. Exp. Med. 209, 975–986.

Majbour, N K, Vaikath, N N, Eusebi, P, Chiasserini, D, Ardah, M, et al., 2016. Longitudinal changes in CSF alpha-synuclein species reflect Parkinson's disease progression. Mov. Disord. 31, 1535–1542.

Maltsev, A S, Chen, J, Levine, R L, Bax, A, 2013. Site-specific interaction between alpha-synuclein and membranes probed by NMR-observed methionine oxidation rates. J. Am. Chem. Soc. 135, 2943–2946.

Maroteaux, L, Campanelli, J T, Scheller, R H, 1988. Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J. Neurosci. 8, 2804–2815.

Masuda-Suzukake, M, Nonaka, T, Hosokawa, M, Oikawa, T, Arai, T, et al., 2013. Prion-like spreading of pathological alpha-synuclein in brain. Brain 136, 1128–1138.

McLean, P J, Kawamata, H, Ribich, S, Hyman, B T, 2000. Membrane association and protein conformation of alpha-synuclein in intact neurons. Effect of Parkinson's disease-linked mutations. J. Biol. Chem. 275, 8812–8816.

Menges, S, Minakaki, G, Schaefer, P M, Meixner, H, Prots, I, et al., 2017. Alpha-synuclein prevents the formation of spherical mitochondria and apoptosis under oxidative stress. Sci. Rep. 7 (42942).

Mori, F, Tanji, K, Yoshimoto, M, Takahashi, H, Wakabayashi, K, 2002. Immunohistochemical comparison of alpha- and beta-synuclein in adult rat central nervous system. Brain Res. 941, 118–126.

Muchowski, P J, 2002. Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones? Neuron 35, 9–12.

Nakamura, K, 2013. alpha-Synuclein and mitochondria: partners in crime? Neurotherapeutics 10, 391–399.

Necula, M, Chirita, C N, Kuret, J, 2003. Rapid anionic micelle-mediated alpha-synuclein fibrillization in vitro. J. Biol. Chem. 278, 46674–46680.

Nemani, V M, Lu, W, Berge, V, Nakamura, K, Onoa, B, et al., 2010. Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. Neuron 65, 66–79.

Nuber, S, Rajsombath, M, Minakaki, G, Winkler, J, Muller, C P, et al., 2018. Abrogating native alpha-synuclein tetramers in mice causes a L-DOPA-Responsive motor syndrome closely resembling parkinson's disease. Neuron 100, 75–90 e5.

Olanow, C W, Prusiner, S B, 2009. Is Parkinson's disease a prion disorder? Proc. Natl. Acad. Sci. U. S. A. 106, 12571–12572.

Ordonez, D G, Lee, M K, Feany, M B, 2018. Alpha-synuclein induces mitochondrial dysfunction through Spectrin and the actin cytoskeleton. Neuron 97, 108–124 e6.

Ostrerova, N, Petrucelli, L, Farrer, M, Mehta, N, Choi, P, et al., 1999. Alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. J. Neurosci. 19, 5782–5791.

Paiva, I, Pinho, R, Pavlou, M A, Hennion, M, Wales, P, et al., 2017. Sodium butyrate rescues dopaminergic cells from alpha-synuclein-induced transcriptional deregulation and DNA damage. Hum. Mol. Genet. 26, 2231–2246.

Park, J Y, Paik, S R, Jou, I, Park, S M, 2008. Microglial phagocytosis is enhanced by monomeric alpha-synuclein, not aggregated alpha-synuclein: implications for Parkinson's disease. Glia 56, 1215–1223.

Pasanen, P, Myllykangas, L, Siitonen, M, Raunio, A, Kaakkola, S, et al., 2014. Novel alpha-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. Neurobiol. Aging 35 (2180), e1–e5.

Paumier, K L, Luk, K C, Manfredsson, F P, Kanaan, N M, Lipton, J W, et al., 2015. Intrastriatal injection of pre-formed mouse alpha-synuclein fibrils into rats triggers alpha-synuclein pathology and bilateral nigrostriatal degeneration. Neurobiol. Dis. 82, 185–199.

Peelaerts, W, Bousset, L, Van der Perren, A, Moskalyuk, A, Pulizzi, R, et al., 2015. Alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature 522, 340–344.

Perez, R G, Waymire, J C, Lin, E, Liu, J J, Guo, F, Zigmond, M J, 2002. A role for alpha-synuclein in the regulation of dopamine biosynthesis. J. Neurosci. 22, 3090–3099.

Perrin, R J, Woods, W S, Clayton, D F, George, J M, 2001. Exposure to long chain polyunsaturated fatty acids triggers rapid multimerization of synucleins. J. Biol. Chem. 276, 41958–41962.

Petrucci, S, Ginevrino, M, Valente, E M, 2016. Phenotypic spectrum of alpha-synuclein mutations: new insights from patients and cellular models. Parkinsonism Relat. Disord. 22 (Suppl 1), S16-20.

Pieri, L, Madiona, K, Melki, R, 2016. Structural and functional properties of prefibrillar alpha-synuclein oligomers. Sci. Rep. 6 (24526).

Plotegher, N, Gratton, E, Bubacco, L, 2014. Number and Brightness analysis of alpha-synuclein oligomerization and the associated mitochondrial morphology alterations in live cells. Biochim. Biophys. Acta 1840, 2014–2024.

Polinski, N K, Volpicelli-Daley, L A, Sortwell, C E, Luk, K C, Cremades, N, et al., 2018. Best practices for generating and using alpha-synuclein pre-formed fibrils to model parkinson's disease in rodents. J. Parkinsons Dis. 8, 303–322.

Polymeropoulos, M H, Lavedan, C, Leroy, E, Ide, S E, Dehejia, A, et al., 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276, 2045–2047.

Prots, I, Grosch, J, Brazdis, R M, Simmnacher, K, Veber, V, et al., 2018. Alpha-Synuclein oligomers induce early axonal dysfunction in human iPSC-based models of synucleinopathies. Proc. Natl. Acad. Sci. U. S. A. 115, 7813–7818.

Proukakis, C, Dudzik, C G, Brier, T, MacKay, D S, Cooper, J M, et al., 2013. A novel alpha-synuclein missense mutation in Parkinson disease. Neurology 80, 1062–1064.

Recasens, A, Dehay, B, Bove, J, Carballo-Carbajal, I, Dovero, S, et al., 2014. Lewy body extracts from Parkinson disease brains trigger alpha-synuclein pathology and neurodegeneration in mice and monkeys. Ann. Neurol. 75, 351–362.

Rey, N L, Steiner, J A, Maroof, N, Luk, K C, Madaj, Z, et al., 2016. Widespread transneuronal propagation of alpha-synucleinopathy triggered in olfactory bulb mimics prodromal Parkinson's disease. J. Exp. Med. 213, 1759–1778.

Rey, N L, Bousset, L, George, S, Madaj, Z, Meyerdirk, L, et al., 2019. Alpha-Synuclein conformational strains spread, seed and target neuronal cells differentially after injection into the olfactory bulb. Acta Neuropathol. Commun. 7 (221).

Richfield, E K, Thiruchelvam, M J, Cory-Slechta, D A, Wuertzer, C, Gainetdinov, R R, et al., 2002. Behavioral and neurochemical effects of wild-type and mutated human alpha-synuclein in transgenic mice. Exp. Neurol. 175, 35–48.

Rieker, C, Dev, K K, Lehnhoff, K, Barbieri, S, Ksiazek, I, et al., 2011. Neuropathology in mice expressing mouse alpha-synuclein. PLoS One 6, e24834.

Rochet, J C, Conway, K A, Lansbury, P T, Jr., 2000. Inhibition of fibrillization and accumulation of prefibrillar oligomers in mixtures of human and mouse alpha-synuclein. Biochemistry 39, 10619–10626.

Rockenstein, E, Nuber, S, Overk, C R, Ubhi, K, Mante, M, et al., 2014. Accumulation of oligomer-prone alpha-synuclein exacerbates synaptic and neuronal degeneration in vivo. Brain 137, 1496–1513.

Rojanathammanee, L, Murphy, E J, Combs, C K, 2011. Expression of mutant alpha-synuclein modulates microglial phenotype in vitro. J. Neuroinflammation 8 (44).

Roodveldt, C, Labrador-Garrido, A, Gonzalez-Rey, E, Fernandez-Montesinos, R, Caro, M, et al., 2010. Glial innate immunity generated by non-aggregated alpha-synuclein in mouse: differences between wild-type and Parkinson's disease-linked mutants. PLoS One 5, e13481.

Sanchez-Guajardo, V, Febbraro, F, Kirik, D, Romero-Ramos, M, 2010. Microglia acquire distinct activation profiles depending on the degree of alpha-synuclein neuropathology in a rAAV based model of Parkinson's disease. PLoS One 5, e8784.

Sarafian, T A, Ryan, C M, Souda, P, Masliah, E, Kar, U K, et al., 2013. Impairment of mitochondria in adult mouse brain overexpressing predominantly full-length, N-terminally acetylated human alpha-synuclein. PLoS One 8, e63557.

Schintu, N, Frau, L, Ibba, M, Garau, A, Carboni, E, Carta, A R, 2009. Progressive dopaminergic degeneration in the chronic MPTPp mouse model of Parkinson's disease. Neurotox. Res. 16, 127–139.

Sharon, R, Bar-Joseph, I, Frosch, M P, Walsh, D M, Hamilton, J A, Selkoe, D J, 2003. The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron 37, 583–595.

Shen, J, Du, T, Wang, X, Duan, C, Gao, G, et al., 2014. alpha-Synuclein amino terminus regulates mitochondrial membrane permeability. Brain Res. 1591, 14–26.

Shibasaki, Y, Baillie, D A, St Clair, D, Brookes, A J, 1995. High-resolution mapping of SNCA encoding alpha-synuclein, the non-A beta component of Alzheimer's disease amyloid precursor, to human chromosome 4q21.3--&q22 by fluorescence in situ hybridization.. Cytogenet. Cell Genet. 71, 54–55.

Siddiqui, A, Chinta, S J, Mallajosyula, J K, Rajagopolan, S, Hanson, I, et al., 2012. Selective binding of nuclear alpha-synuclein to the PGC1alpha promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: implications for Parkinson's disease. Free Radic. Biol. Med. 53, 993–1003.

Singleton, A B, Farrer, M, Johnson, J, Singleton, A, Hague, S, et al., 2003. Alpha-Synuclein locus triplication causes Parkinson's disease. Science 302 (841).

Snead, D, Eliezer, D, 2014. Alpha-synuclein function and dysfunction on cellular membranes. Exp. Neurobiol. 23, 292–313.

Snyder, H, Mensah, K, Theisler, C, Lee, J, Matouschek, A, Wolozin, B, 2003. Aggregated and monomeric alpha-synuclein bind to the S6' proteasomal protein and inhibit proteasomal function. J. Biol. Chem. 278, 11753–11759.

Soto, C, Estrada, L D, 2008. Protein misfolding and neurodegeneration. Arch. Neurol. 65, 184–189.

Spillantini, M G, Schmidt, M L, Lee, V M, Trojanowski, J Q, Jakes, R, Goedert, M, 1997. Alpha-synuclein in lewy bodies. Nature 388, 839–840.

St Martin, J L, Klucken, J, Outeiro, T F, Nguyen, P, Keller-McGandy, C, et al., 2007. Dopaminergic neuron loss and up-regulation of chaperone protein mRNA induced by targeted over-expression of alpha-synuclein in mouse substantia nigra. J. Neurochem. 100, 1449–1457.

Stefanis, L, Emmanouilidou, E, Pantazopoulou, M, Kirik, D, Vekrellis, K, Tofaris, G K, 2019. How is alpha-synuclein cleared from the cell? J. Neurochem. 150, 577–590.

Stefanova, N, Fellner, L, Reindl, M, Masliah, E, Poewe, W, Wenning, G K, 2011. Toll-like receptor 4 promotes alpha-synuclein clearance and survival of nigral dopaminergic neurons. Am. J. Pathol. 179, 954–963.

Stockl, M, Claessens, M M, Subramaniam, V, 2012. Kinetic measurements give new insights into lipid membrane permeabilization by alpha-synuclein oligomers. Mol. Biosyst. 8, 338–345.

Stockl, M T, Zijlstra, N, Subramaniam, V, 2013. Alpha-Synuclein oligomers: an amyloid pore? Insights into mechanisms of alpha-synuclein oligomer-lipid interactions. Mol. Neurobiol. 47, 613–621.

Sugeno, N, Takeda, A, Hasegawa, T, Kobayashi, M, Kikuchi, A, et al., 2008. Serine 129 phosphorylation of alpha-synuclein induces unfolded protein response-mediated cell death. J. Biol. Chem. 283, 23179–23188.

Sung, J Y, Kim, J, Paik, S R, Park, J H, Ahn, Y S, Chung, K C, 2001. Induction of neuronal cell death by Rab5A-dependent endocytosis of alpha-synuclein. J. Biol. Chem. 276, 27441–27448.

Swant, J, Goodwin, J S, North, A, Ali, A A, Gamble-George, J, et al., 2011. Alpha-Synuclein stimulates a dopamine transporter-dependent chloride current and modulates the activity of the transporter. J. Biol. Chem. 286, 43933–43943.

Tanaka, Y, Engelender, S, Igarashi, S, Rao, R K, Wanner, T, et al., 2001. Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. Hum. Mol. Genet. 10, 919–926.

Tanik, S A, Schultheiss, C E, Volpicelli-Daley, L A, Brunden, K R, Lee, V M, 2013. Lewy body-like alpha-synuclein aggregates resist degradation and impair macroautophagy. J. Biol. Chem. 288, 15194–15210.

Tenreiro, S, Reimao-Pinto, M M, Antas, P, Rino, J, Wawrzycka, D, et al., 2014. Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease. PLoS Genet. 10, e1004302.

Thakur, P, Breger, L S, Lundblad, M, Wan, O W, Mattsson, B, et al., 2017. Modeling Parkinson's disease pathology by combination of fibril seeds and alpha-synuclein overexpression in the rat brain. Proc. Natl. Acad. Sci. U. S. A. 114 E8284-E93.

Thayanidhi, N, Helm, J R, Nycz, D C, Bentley, M, Liang, Y, Hay, J C, 2010. Alpha-synuclein delays endoplasmic reticulum (ER)-to-Golgi transport in mammalian cells by antagonizing ER/Golgi SNAREs. Mol. Biol. Cell 21, 1850–1863.

Theodore, S, Cao, S, McLean, P J, Standaert, D G, 2008. Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. J. Neuropathol. Exp. Neurol. 67, 1149–1158.

Tofaris, G K, Razzaq, A, Ghetti, B, Lilley, K S, Spillantini, M G, 2003. Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. J. Biol. Chem. 278, 44405–44411.

Tofaris, G K, Garcia Reitbock, P, Humby, T, Lambourne, S L, O'Connell, M, et al., 2006. Pathological changes in dopaminergic nerve cells of the substantia nigra and olfactory bulb in mice transgenic for truncated human alpha-synuclein(1-120): implications for Lewy body disorders. J. Neurosci. 26, 3942–3950.

Tokuda, T, Qureshi, M M, Ardah, M T, Varghese, S, Shehab, S A, et al., 2010. Detection of elevated levels of alpha-synuclein oligomers in CSF from patients with Parkinson disease. Neurology 75, 1766–1772.

Tosatto, L, Andrighetti, A O, Plotegher, N, Antonini, V, Tessari, I, et al., 2012. Alpha-synuclein pore forming activity upon membrane association. Biochim. Biophys. Acta 1818, 2876–2883.

Tuttle, M D, Comellas, G, Nieuwkoop, A J, Covell, D J, Berthold, D A, et al., 2016. Solid-state NMR structure of a pathogenic fibril of full-length human alpha-synuclein. Nat. Struct. Mol. Biol. 23, 409–415.

Ulusoy, A, Decressac, M, Kirik, D, Bjorklund, A, 2010. Viral vector-mediated overexpression of alpha-synuclein as a progressive model of Parkinson's disease. Prog. Brain Res. 184, 89–111.

Uversky, V N, Li, J, Fink, A L, 2001. Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular NK between Parkinson's disease and heavy metal exposure. J. Biol. Chem. 276, 44284–44296.

Van der Perren, A, Toelen, J, Carlon, M, Van den Haute, C, Coun, F, et al., 2011. Efficient and stable transduction of dopaminergic neurons in rat substantia nigra by rAAV 2/1, 2/2, 2/5, 2/6.2, 2/7, 2/8 and 2/9.. Gene Ther. 18, 517–527.

Van der Perren, A, Toelen, J, Casteels, C, Macchi, F, Van Rompuy, A S, et al., 2015. Longitudinal follow-up and characterization of a robust rat model for Parkinson's disease based on overexpression of alpha-synuclein with adeno-associated viral vectors. Neurobiol. Aging 36, 1543–1558.

van der Putten, H, Wiederhold, K H, Probst, A, Barbieri, S, Mistl, C, et al., 2000. Neuropathology in mice expressing human alpha-synuclein. J. Neurosci. 20, 6021–6029.

van Rooijen, B D, Claessens, M M, Subramaniam, V, 2010. Membrane interactions of oligomeric alpha-synuclein: potential role in Parkinson's disease. Curr. Protein Pept. Sci. 11, 334–342.

Vargas, K J, Makani, S, Davis, T, Westphal, C H, Castillo, P E, Chandra, S S, 2014. Synucleins regulate the kinetics of synaptic vesicle endocytosis. J. Neurosci. 34, 9364–9376.

Villar-Pique, A, Lopes da Fonseca, T, Outeiro, T F, 2016. Structure, function and toxicity of alpha-synuclein: the Bermuda triangle in synucleinopathies. J. Neurochem. 139 (Suppl 1), 240–255.

Volles, M J, Lee, S J, Rochet, J C, Shtilerman, M D, Ding, T T, et al., 2001. Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. Biochemistry 40, 7812–7819.

Volpicelli-Daley, L A, Kirik, D, Stoyka, L E, Standaert, D G, Harms, A S, 2016. How can rAAV-alpha-synuclein and the fibril alpha-synuclein models advance our understanding of Parkinson's disease? J. Neurochem. 139 (Suppl. 1), 131–155.

Wakamatsu, M, Ishii, A, Ukai, Y, Sakagami, J, Iwata, S, et al., 2007. Accumulation of phosphorylated alpha-synuclein in dopaminergic neurons of transgenic mice that express human alpha-synuclein. J. Neurosci. Res. 85, 1819–1825.

Wegrzynowicz, M, Bar-On, D, Calo, L, Anichtchik, O, Iovino, M, et al., 2019. Depopulation of dense alpha-synuclein aggregates is associated with rescue of dopamine neuron dysfunction and death in a new Parkinson's disease model. Acta Neuropathol..

Weinreb, P H, Zhen, W, Poon, A W, Conway, K A, Lansbury, P T, Jr., 1996. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. Biochemistry 35, 13709–13715.

Wersinger, C, Rusnak, M, Sidhu, A, 2006. Modulation of the trafficking of the human serotonin transporter by human alpha-synuclein. Eur. J. Neurosci. 24, 55–64.

Wilms, H, Rosenstiel, P, Romero-Ramos, M, Arlt, A, Schafer, H, et al., 2009. Suppression of MAP kinases inhibits microglial activation and attenuates neuronal cell death induced by alpha-synuclein protofibrils. Int. J. Immunopathol. Pharmacol. 22, 897–909.

Winner, B, Jappelli, R, Maji, S K, Desplats, P A, Boyer, L, et al., 2011. In vivo demonstration that alpha-synuclein oligomers are toxic. Proc. Natl. Acad. Sci. U. S. A. 108, 4194–4199.

Woods, W S, Boettcher, J M, Zhou, D H, Kloepper, K D, Hartman, K L, et al., 2007. Conformation-specific binding of alpha-synuclein to novel protein partners detected by phage display and NMR spectroscopy. J. Biol. Chem. 282, 34555–34567.

Xilouri, M, Vogiatzi, T, Vekrellis, K, Park, D, Stefanis, L, 2009. Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One 4, e5515.

Xilouri, M, Brekk, O R, Stefanis, L, 2013. Alpha-Synuclein and protein degradation systems: a reciprocal relationship. Mol. Neurobiol. 47, 537–551.

Yamada, K, Iwatsubo, T, 2018. Extracellular alpha-synuclein levels are regulated by neuronal activity. Mol. Neurodegener. 13 (9).

Yamada, M, Iwatsubo, T, Mizuno, Y, Mochizuki, H, 2004. Overexpression of alpha-synuclein in rat substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-synuclein and activation of caspase-9: resemblance to pathogenetic changes in Parkinson's disease. J. Neurochem. 91, 451–461.

Yang, Y, Qin, M, Bao, P, Xu, W, Xu, J, 2017. Secretory carrier membrane protein 5 is an autophagy inhibitor that promotes the secretion of alpha-synuclein via exosome. PLoS One 12, e0180892.

Ysselstein, D, Dehay, B, Costantino, I M, McCabe, G P, Frosch, M P, et al., 2017. Endosulfine-alpha inhibits membrane-induced alpha-synuclein aggregation and protects against alpha-synuclein neurotoxicity. Acta Neuropathol. Commun. 5 (3).

Yu, S, Zuo, X, Li, Y, Zhang, C, Zhou, M, et al., 2004. Inhibition of tyrosine hydroxylase expression in alpha-synuclein-transfected dopaminergic neuronal cells. Neurosci. Lett. 367, 34–39.

Zarranz, J J, Alegre, J, Gomez-Esteban, J C, Lezcano, E, Ros, R, et al., 2004. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann. Neurol. 55, 164–173.

Zhang, W, Wang, T, Pei, Z, Miller, D S, Wu, X, et al., 2005. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB J. 19, 533–542.

Zhu, M, Fink, A L, 2003. Lipid binding inhibits alpha-synuclein fibril formation. J. Biol. Chem. 278, 16873–16877.