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The concept of alpha-synuclein as a prion-like protein: ten years after

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Abstract

Parkinson's disease is characterized by the loss of nigrostriatal dopaminergic signaling and the presence of alpha-synuclein aggregates (also called Lewy bodies and neurites) throughout the brain. In 2003, Braak and colleagues created a staging system for Parkinson's disease describing the connection between the alpha-synuclein pathology and disease severity. Later, they suggested that the pathology might initially be triggered by exogenous insults targeting the gut and olfactory system. In 2008, we and other groups documented Lewy pathology in grafted neurons in people with Parkinson's disease who had been transplanted over a decade prior to autopsy. We proposed that the Lewy pathology in the grafted neurons was the result of permissive templating or prion-like spread of alpha-synuclein pathology from neurons in the host to those in the grafts. During the following ten years, several studies described the transmission of alpha-synuclein pathology between neurons, both in cell culture and in experimental animals. Recent research has also begun to identify underlying molecular mechanisms. Collectively, these experimental studies tentatively support the idea that the progression from one Braak stage to the next is the consequence of prion-like propagation of Lewy pathology. However, definitive proof that intercellular propagation of alpha-synuclein pathology occurs in Parkinson's disease cases has proven difficult to secure. In this review, we highlight several open questions that currently prevent us from concluding with certainty that prion-like transfer of alpha-synuclein contributes to the progression of Parkinson's disease.

Keywords

α-synuclein; aggregation; transmission; Parkinson's disease

Introduction

People with Parkinson's disease (PD) progress over time with increasing motor and non-motor signs and symptoms due to the loss of striatal dopaminergic signaling and to the presence of alpha-synuclein-containing Lewy bodies and neurites. The importance of alpha-

Conflicts of Interest

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synuclein (α-syn) to PD pathophysiology is evident in both genetic and pathological realms. After initial discoveries of a mutation in the gene encoding α-syn, *SNCA*, that confers increased risk of developing PD (Polymeropoulos et al. 1997) and of the presence of α-syn-immunoreactivity in Lewy pathology (Spillantini et al. 1997), Braak and colleagues described staging of pathology in people with PD at autopsy (Braak et al. 2003a). In what was suggested to be the earliest phases of the disease (Braak stage 1 and 2, which actually precede the onset of the classical motor symptoms), α-syn-immunopositive pathology was found in two distinct brain areas, the dorsal motor nucleus of the vagal nerve and the anterior olfactory nucleus. Therefore, the authors proposed that these locations might be initiation sites that direct the spread of the pathology stereotypically throughout the brain (reviewed in (Rey et al. 2016c)). In the same timeframe, Hardy published a permissive templating theory regarding neurodegenerative diseases including Parkinson's (Hardy 2005).

Braak and colleagues developed a subsequent theory, in which nasal and gastric routes of entry might be used by a pathogen to gain access to neuronal populations. In this hypothesis, a neurotropic pathogen accesses olfactory and gastric environments, and enters the olfactory and gut epithelia. The authors first emphasized a potential neurotropic virus but also suggested briefly, in a passage of their paper that did not get as much attention, that the pathogen might be composed of fragments of misfolded α-syn (Braak et al. 2003b). From that point, infiltration in the gut to submucous plexus could lead to trans-synaptic travel of the pathogen along preganglionic parasympathetic fibers to the dorsal motor nucleus of the vagus nerve. Entry at the olfactory epithelium could induce transit of the pathogen to the olfactory bulb. Thus, in this hypothesis, an external pathogen might induce pathology in the proposed initiation sites identified by Braak and colleagues (reviewed in (Hawkes et al. 2007, 2009)).

These results and theory, in which prion-like mechanisms are proposed to underlie neurodegenerative disorders, fueled speculation about the interpretation of two long-term therapeutic transplantation studies published in 2008 (Kordower et al. 2008; Li et al. 2008). These authors discovered Lewy pathology at autopsy not only in the host neurons of the people with PD but also in young grafted neurons less than two decades of age. At the time, we hypothesized that prion-like transfer of α-syn might underlie the unexpected pathology (Li et al. 2008; Brundin et al. 2008). In fact, earlier work by El Agnaf and colleagues had demonstrated the presence of extracellular α-syn in human plasma and cerebrospinal fluid, thus α-syn could conceivably enter cells from extracellular space (El-Agnaf et al. 2003). These results and many subsequent *in vivo* studies gave support to the prion-like hypothesis of α-syn transfer from cell to cell (Table 1) (Desplats et al. 2009; Kordower et al. 2011; Luk et al. 2012b; Mougenot et al. 2012; Luk et al. 2012a; Ulusoy et al. 2013; Recasens et al. 2014; Holmqvist et al. 2014; Peelaerts et al. 2015; Paumier et al. 2015; Helwig et al. 2016; Koller et al. 2017; Ulusoy et al. 2017; Abdelmotilib et al. 2017).

In this review, we recognize that the spread of α-syn pathology from one cell to another and even one nervous system structure to another *in vivo* have already been extensively and convincingly summarized in prior articles and therefore will not be discussed in detail here again (Walker 2016; Dehay et al. 2016; Peelaerts and Baekelandt 2016; Goedert et al. 2017; Valdinocci et al. 2017; George and Brundin 2017; Hasegawa et al. 2017; Stopschinski and

Diamond 2017). However, whether this transmission of pathology is the explanation for Braak's pathological observations is yet unclear. Therefore, the aim of our article is to highlight and discuss some of the outstanding questions that can help define in the future whether prion-like transfer plays an important role in the progression of PD. Specifically we focus on three issues: do non-neuronal (i.e. glial) cells contribute to propagation of pathology; are certain brain areas selectively vulnerable to the prion-like propagation of α-syn aggregates and does the presence of α-syn aggregates explain the worsening and broadening of symptoms as PD progresses.

Do non-neuronal cells play a role in neuron to neuron transmission?

The major risk factor for PD is aging, but genetic predisposition and environmental insults also play key roles (Figure 1) (reviewed in (Hernandez et al. 2016; George and Brundin 2017; Stopschinski and Diamond 2017; Collier et al. 2017)). The brain environment during aging is characterized by inflammation which involves non-neuronal, proliferating, and circulating cells such as microglia, astrocytes, and oligodendrocytes (reviewed in (Chinta et al. 2013)). During aging, these non-neuronal cells release pro-inflammatory agents, which potentially compromises both the function and survival of the neurons that they support (Franceschi et al. 2007; Chung et al. 2009). An age-related elevated inflammatory status might be very important in the context of intercellular protein transfer, since microglia, oligodendrocytes and astroglia (which are all impacted by inflammatory stimuli) are suggested to modulate cell-to-cell transfer of α-syn (reviewed in (Lee et al. 2014)). In this regard, oligodendrocytes, astrocytes, and microglia can take up α-syn from the extracellular space in rodent and organotypic slice models (Reyes et al. 2014; Thakur et al. 2017; Loria et al. 2017). In addition, astrocytes appear to sequester and degrade α-syn assemblies (Loria et al. 2017). It is conceivable that inflammation-induced changes in glia impair both their efficacy to take up extracellular α-syn and their ability to degrade it. Indeed, a recent study has demonstrated that although astrocytes take up a significant amount of aggregated α-syn (i.e. α-syn oligomers) for subsequent degradation, their degradative capacity can become overwhelmed, resulting in limited clearance of α-syn and its associated toxic cellular effects (Lindström et al. 2017). Mechanistically, another study has suggested that overburdened astrocytes can transfer excess aggregated α-syn to other nearby astrocytes either through direct contact or through tunneling nanotubes (Rostami et al. 2017). Moreover, similar to neurons, astrocytes and oligodendrocytes utilize micro-vesicles or exosomes as another secretory mechanism for the bidirectional transfer of cellular organelles such as mitochondria, as well as toxic materials (Frühbeis et al. 2013; Hayakawa et al. 2016). It is possible that exosomes may have a role in the transfer of pathogenic α-syn between neuronal and non-neuronal cells. Indeed, Danzer and colleagues demonstrated that α-syn oligomers can be secreted to the extracellular space in exosomes of neurons and that extracellular exosome-containing α-syn oligomers can be taken up rapidly and induce significant toxicity (Danzer et al. 2012). Both neuron-derived and glia-derived exosomes containing α-syn that are secreted into conditioned medium can be taken up by glial cells and may seed the aggregation of intracellular proteins (Surgucheva et al. 2012; Chang et al. 2013; Chistiakov and Chistiakov 2017). Overall, the spread of excess aggregated α-syn

between neuronal and glial cells can contribute to increased propagation and cellular effects of the aggregated protein.

In addition, both astrocytes and microglia are also clearly capable of migrating over long distances in the adult brain and in the periphery. Animal experiments have suggested that microglia which carry engulfed aggregated tau (another dysfunctional protein similar to α-syn) can contribute to spread of tauopathy by migrating to other brain regions and releasing the aggregates there (Asai et al. 2015). Conceivably, both microglia and astrocytes could play similar roles in the long-distance spread of α-syn pathology. Long-distance transit is controversial, given emerging evidence that the spread of α-syn aggregates follows anatomical pathways in PD (see next section). However, as we elaborate upon in the next section, the notion that the spread of protein aggregates strictly follows neural tracts has also been recently questioned. Indeed, preformed aggregates of both α-syn and tau can spread beyond synaptic connections or anatomical pathways (de Calignon et al. 2012; Sacino et al. 2014a; Peeraer et al. 2015; Asai et al. 2015), suggesting that non-synaptic propagation mechanisms may also exist. Circulating glial cells, particularly microglia, may be key players in such long-distance spread of pathology. For instance, aggregated tau, like α-syn, can be carried by microglia and transduced into cells in other locations *in vivo*, promoting aggregated protein propagation (Asai et al. 2015). This form of aggregated protein propagation may be inhibited following the depletion of microglia (Asai et al. 2015). In addition, in some models of synucleinopathies, aggregated α-syn injected into the intestinal walls and peritoneal cavity propagates and seeds further aggregation in several parts of the brain (see Table 1). While the mechanism by which this aggregated α-syn is transmitted from the gut into the brain remains to be established, the vagal nerve has long been suggested as an entry site and a possible route by which α-syn may gain access into the brain from the periphery (Braak et al. 2003b). Although it remains to be supported, a role for microglia and other immune cells in such periphery-to-CNS propagation of aggregated α-syn cannot be discounted. Indeed, the transmission of α-syn from the periphery into the brain is often accompanied by increased microglial activity and neuroinflammation (Breid et al. 2016). To sum up, circulating glial cells may play various roles in the spread of excess aggregated α-syn, either over short or long distances.

With respect to the connectome, is there a difference in propagation from different brain areas and cell populations?

The connectome is a comprehensive brain map of the intricate synaptic connections formed between neurons. Functional connectivity changes (i.e. fMRI or specific tracer signal alterations) might track the overall and specific pathological changes in PD, including the spread of α-syn and Lewy pathology or cell death in the substantia nigra pars compacta and other relevant brain areas (Watabe-Uchida et al. 2012; Ogawa et al. 2014; Bellucci et al. 2016).

However, several gaps currently exist in the rationale that functional connectivity changes track specifically with most pathological changes that occur in PD (reviewed in (Surmeier et al. 2017)). Surmeier and coauthors posited that neurons in the substantia nigra pars

compacta and other vulnerable neurons possess common anatomical and physiological properties, which might explain not only the pattern of cell death in PD but also the arrangement of Lewy bodies and neurites (Surmeier et al. 2017). Indeed, an elevated vulnerability of substantia nigra pars compacta neurons in PD has long been considered, but other factors that might help connect pathology with functionality are still not verified (Surmeier et al. 2017). While work to fill in the gaps of knowledge continues in animal models of PD and in people with PD, we eagerly await a valid biomarker to track progression of pathology in human PD patients. Essentially, in the absence of a high-resolution *in vivo* imaging marker for aggregated α-syn (Eberling et al. 2013), it is not possible to define precisely how the Lewy pathology progresses in one individual, and instead currently we have to rely on cross sectional studies describing different patients of varying clinical stages and disease durations. Animal models of PD, with all their possible shortcomings, allow us to examine identically treated and genetically identical animals at different time points after triggering an experimental synucleinopathy, and therefore are the best option for increasing our understanding of how Lewy pathology spreads. Below, we review the animal model-based evidence of propagation of fibrillar α-syn from one brain area to another, and attempt to relate it to connectivity.

We begin with the most caudal site interrogated at this time – the intraperitoneal route of administration. Breid and colleagues injected fibrillar α-syn assemblies (PFFs) via intraperitoneal and intraglossal routes into A53T α-syn-expressing bigenic transgenic (M83$^{+/-}$:*Gfap*-luc$^{+/-}$) mice, and found that intraperitoneal injection of PFFs led to paralysis and the appearance of phosphorylated α-syn pathology in the central nervous system (Breid et al. 2016). Sargent and colleagues also used M83 transgenic mice in combination with intracerebral or systemic (intraperitoneal) injection of brain homogenates from sick mice to demonstrate that the type of inoculum and the genotype (hemizygous vs. homozygous) of the mouse determine the pathology load (phosphorylated α-syn) in the brain (Sargent et al. 2017). Finally, Ulusoy and colleagues performed injections of adeno-associated virus (AAV) 2/6 expressing human α-syn into rat midbrain, which led to the presence of human α-syn in vagal motor neurons and in gastric nerve endings of visceromotor vagal projections (Ulusoy et al. 2017). Taken together, all these results suggest that the dorsal motor nucleus of the vagal nerve could be critical in inducing development of pathology in rostral locations of the brain.

Additionally, the Di Monte group also performed more rostral injections of AAV2/6-human α-syn in the upper vagal nerve and the brain stem of rodents (Ulusoy et al. 2013, 2015; Helwig et al. 2016), and demonstrated that, in each model, phosphorylated- and thioflavin S-labeled α-syn lesions were present throughout the brainstem and forebrain of recipient mice, respectively. In animals where there was inadvertent toxicity due to the virus, which killed brainstem neurons, there was no progression of pathology, indicating that the presence of intact neural connections is a prerequisite for propagation of pathology (Ulusoy et al. 2015). Holmqvist and colleagues similarly demonstrated rostral presence of aggregated α-syn pathology in the dorsal motor nucleus of the vagal nerve after injection of a PD brain lysate into the intestinal wall (Holmqvist et al. 2014). In general, these results lend support to the idea that the dorsal motor nucleus of the vagal nerve is central to α-syn pathology transfer to and from the CNS. The evidence for involvement of dorsal motor nucleus of the vagal nerve

in the pathological “network” in PD is worth reviewing. It appears to be both a site of α-syn pathology in PD (Braak et al. 2003a) and a nucleus that contains possible vulnerable neurons with long and branched axons (Surmeier et al. 2017). More research is needed to verify that neurons within dorsal motor nucleus of the vagal nerve are indeed vulnerable to either cell death, or development of α-syn pathology, or both.

Next, we will examine the evidence that α-syn pathology transfers from or appears in the mid- and forebrain after introduction from exogenous sources. One of the first examples of host-to-graft transfer of α-syn in an animal model occurred after transplantation of neural stem cells into the hippocampus of a mouse expressing α-syn (Desplats et al. 2009). Soon after, two groups set up rodent models of protein uptake and host-to-graft transfer, and found that naïve transplanted rodent neurons import human α-syn in a variety of models (Hansen et al. 2011; Kordower et al. 2011; Angot et al. 2012). Other groups have injected patient material or oligomeric/fibrillar α-syn into hippocampus and substantia nigra and found aggregated α-syn lesions (Recasens et al. 2014; Peelaerts et al. 2015; Koller et al. 2017; Abdelmotilib et al. 2017). Even more severe α-syn pathology was found when α-syn was overexpressed and PFFs were injected into the nigra (Thakur et al. 2017). Similar aggregated α-syn results have been observed after injection of PFFs, patient material, and α-syn assemblies into the striatum (Luk et al. 2012a; Recasens et al. 2014; Peelaerts et al. 2015) and cortex (Osterberg et al. 2015). Finally, our group has also shown that PFFs injected into the olfactory bulb of mice led to olfactory deficits and widespread α-syn inclusions throughout the forebrain and midbrain (Rey et al. 2016b).

Regarding the “connectomic” spread of α-syn pathology to distinct brain regions from the injection site, we shall focus our discussion on findings emerging from animal models that received α-syn injections at four commonly investigated sites including: a) vagus nerve, b) substantia nigra, c) striatum, and d) cortex/olfactory bulb (see Table 1). In general, aggregated α-syn has been reported to spread from neurons in the vagus nerve (or medulla oblongata) along stereotypical neural tracts to more rostral brain areas such as pons, locus coeruleus, dorsal raphe, hypothalamus and amygdala twelve months after α-syn injection (Ulusoy et al. 2013, 2015; Helwig et al. 2016). From the substantia nigra, the aggregated protein similarly spreads along neural tracts to multiple brain areas such as amygdala, striatum, hippocampus, dentate gyrus, hypothalamus and the visual, motor, entorhinal, and cingulate cortex (Masuda-Suzukake et al. 2013, 2014; Peelaerts et al. 2015). Similar to those injections in the nigra, injections of aggregated α-syn into the striatum resulted in inclusion formation that spread to several brain areas that project afferent innervations to the striatum, such as the prefrontal, insular, cingulate and motor cortical areas, as well as the substantia nigra, amygdala and olfactory bulb (Luk et al. 2012a; Paumier et al. 2015; Bernis et al. 2015; Abdelmotilib et al. 2017). Moreover, intracerebral injections of α-syn into the cortical areas results in pathological spreading from the cortex to the striatum, thalamus, hypothalamus, locus coeruleus, raphe nucleus, reticular formation, cerebellum, and the spinal cord (Luk et al. 2012b; Mougenot et al. 2012; Watts et al. 2013). Being one of the main predicted entry sites for aggregated α-syn, the olfactory bulb may be quite critical for α-syn propagation (Rey et al. 2016c). Indeed, olfactory bulb injections trigger the spread of aggregated α-syn in an anatomical pattern across several brain areas including the frontal, entorhinal, perirhinal, and parietal cortex, as well as the striatum, amygdala, substantia

nigra, and hippocampus (Rey et al. 2016b). Overall, the findings from these studies clearly support the notion that pathological α-syn can spread from neuron to neuron and region to region. It is however, less clear from these *in vivo* studies, whether there is selective vulnerability and spread of α-syn pathology in some neuronal cell groups over others. What these *in vivo* studies appear to suggest is that aggregated α-syn, and for that matter Lewy pathology, might spread along defined neural tracts or interconnected brain networks. However, this has so far not been corroborated by data from clinical PD cases, and as outlined above, this will be difficult to do unless a sensitive and specific clinical imaging ligand that identifies α-syn aggregates with very high resolution is developed (Surmeier et al. 2017).

Arguments can be advanced that if indeed Lewy pathology spreads from neuron to neuron along defined interconnected tracts in clinical PD (as is the case in animal models), then from dopaminergic neurons in the substantia nigra pars compacta the pathology must spread to regions robustly innervating the nigral neurons (such as the substantia nigra pars reticulata, subthalamic nuclei and globus pallidus). Yet these regions often have limited pathology, if any (Surmeier et al. 2017). It would appear that Lewy pathology in clinical PD cases may exist in selective cell populations/brain areas, rather than having a widespread presence in the entire brain. Until clinical confirmation, therefore, we cannot say with certainty that the pathological spread of aggregated α-syn between interconnected brain regions from injection/seeding sites observable in the outlined animal models can adequately explain the selective propagation observed in clinical PD cases. Further studies addressing the question of selective propagation of pathological α-syn in both animal models and clinical PD cases may be required, together with more precise animal models and validated methods (Rey et al. 2016a). Assuming that aggregated α-syn could spread from one neuron to another and one brain area to another in both animal models and clinical PD, are there functional consequences or adequate explanations of PD progression? These questions are discussed in the next section.

Does the aggregation and propagation of α-syn correlate with increased PD pathology and symptomatology?

Neuronal loss is a major feature of clinical PD. Nigrostriatal dopaminergic neuronal cell loss, in particular, results in the motor features of PD (Cheng et al. 2010; Spillantini and Goedert 2017). In human PD patients, approximately 30% of nigral dopaminergic neurons, along with 50-60% of their synaptic terminals are lost at the time of onset of motor symptoms (Cheng et al. 2010; Spillantini and Goedert 2017). Major unresolved questions arise as to whether the progressive accumulation of α-syn aggregates contributes to neuronal cell loss in PD and what cellular mechanisms would underlie such a phenomenon (Lashuel et al. 2013; Wong and Krainc 2017). Although Lewy pathology is found in most cases of clinical PD, it is currently quite difficult to tease apart the contribution of only α-syn aggregates towards disease progression in PD patients. Thus, animal models are quite useful in this context for investigating the effect of α-syn aggregates independent of other factors that are also known to influence PD progression.

Several studies conducted on animal models of PD have linked aggregated recombinant α-syn with cytotoxicity. Such PD models often carry mutations in the α-syn gene that favor its aggregation such as the A53T, E46K and A30P point mutations detected in familial PD cases (Bisaglia et al. 2009). While some studies have examined the effect of α-syn aggregation in these point mutation models, others have further injected some of these models with α-syn oligomers and PFFs (Table 1). In particular, studies on animal models of PD and other synucleinopathies have primarily focused on the propagation of α-syn aggregates and therapeutic targeting of this propagation. Thus, much less attention has been given to the *in vivo* toxicity of α-syn aggregates. Regardless of this, there appears to be enough studies to provide a good picture of the effect of pathogenic α-syn aggregation *in vivo.* Importantly, in such *in vivo* studies, α-syn is often administered at higher levels than it is likely present even in a brain affected by a synucleinopathy and the toxic effects are assessed within that context. *Drosophila* models represent one of the simplest *in vivo* models used to show that the expression of human α-syn can indeed induce neuronal toxicity. Using such models for investigation, dopaminergic neuron cell loss has frequently been reported (Chen and Feany 2005; Haywood and Staveley 2006; Park and Lee 2006; Kontopoulos et al. 2006; Periquet et al. 2007), although the effects appeared to be quite modest. The nematode *C. elegans* has also been used to demonstrate that α-syn aggregates do not only spread from neuron to neuron but are also very toxic to dopaminergic neuronal cells and can cause motor deficits in these models (Petrucelli et al. 2002; Lakso et al. 2003; Kuwahara et al. 2006; Tyson et al. 2017). Using transgenic technology and viral vector delivery approaches, rodent and non-human primate mutant α-syn-induced aggregation models have also been generated. Studies introducing α-syn into these animal models using the traditional transgenics approach has produced conflicting results with some evidence for high α-syn toxicity in various parts of brain and the spinal cord, whereas other studies demonstrated moderate to no cell loss in the substantia nigra (Masliah et al. 2000; van der Putten et al. 2000; Kahle et al. 2000; Matsuoka et al. 2001; Richfield et al. 2002; Giasson et al. 2002; Lee et al. 2002). To provide more clarity, the viral vector delivery and stereotaxic PFF injection approaches were adapted to directly deliver α-syn to specific brain areas that are relevant to clinical PD such as the dorsal motor nucleus of the vagus nerve, substantia nigra, striatum and olfactory bulb (as discussed above). Data from this approach have been rather promising with most studies showing the deposition and spread of α-syn, and others demonstrating significant neuronal cell loss in relevant brain areas in mice and rats (Table 1) (Kirik et al. 2002; Giasson et al. 2002; Lo Bianco et al. 2002; Lauwers et al. 2003; Yamada et al. 2004; St Martin et al. 2007) and primates (Kirik et al. 2003; Eslamboli et al. 2007). For example, one study on adult marmosets and monkeys reported α-syn inclusion formation, 30-60% nigral and striatal dopaminergic cell loss, and severe motor impairments 16 weeks after viral vector mediated delivery of human α-syn into the substantia nigra. In these models, the nigrostriatal synucleinopathy developed slowly over time, reminiscent of clinical PD (Kirik et al. 2003). Compared to human PD however, the cell loss and behavioral deficits observed in most of these animal models may be less pronounced. Moreover, the relative toxicity of α-syn in these models is suggested to be detected much more rapidly than is the case in clinical PD (Surmeier et al. 2017). Despite the apparent limitations in studies on animal models, the important observation is that α-syn does not only propagate from neuron to neuron in these models but that it can also evoke toxicity in neuronal cells in the *in vivo*

context when present at non-physiological levels. It is however less clear if the toxicity of human α-syn in cellular and animal models are directly relevant in clinical PD.

To verify α-syn toxicity in clinical PD and to determine whether Lewy pathology precedes cellular dysfunction and neurodegeneration, a few studies have been conducted on PD patients and on postmortem tissues (these studies are rather challenging to perform in clinical cases due to current technological limitations). The findings from these studies have been inconclusive. In this regard, some of these studies have examined toxicity in brain areas having significant Lewy pathology and reported nigral dopamine cell loss (Halliday et al. 1996; Damier et al. 1999; Milber et al. 2012; Dijkstra et al. 2014), as well as a modest loss of glutamatergic neurons in the basolateral amygdala and thalamus (Henderson et al. 2000; Harding et al. 2002) and cholinergic neurons in the peduncolopontine nucleus (Halliday et al. 1990) in early clinical PD cases. In late clinical PD cases, there appears to be extensive neuronal cell loss in α-syn inclusion-rich areas including a significant loss of neurons in parts of the hypothalamus (Halliday et al. 1990; Kremer and Bots 1993; Thannickal et al. 2007; Fronczek et al. 2008). In contrast, some studies have failed to observe significant neuronal loss in brain areas that had α-syn inclusions and Lewy pathology including the motor cortex and neocortical areas (Halliday et al. 1990; Ansorge et al. 1997; MacDonald and Halliday 2002; Pedersen et al. 2005). In addition, one study reported no significant glutamatergic or GABAergic cell loss in the pedunculopontine nucleus (Halliday et al. 1990). There are also indications that some PD patients with widespread α-syn inclusions and Lewy pathology display no observable neuropsychiatric and motor symptoms (Jellinger 2009). In addition, there has been a report that significant neuronal cell loss and cellular dysfunction precede α-syn and Lewy pathology in some PD cases who are in Braak PD stages 1 and 2 (Milber et al. 2012). Taken together, the correlation between Lewy pathology and neuronal cell loss in clinical PD is not yet clear. Development of robust technology and biochemical approaches (e.g. biomarkers that can reliably track pathology development and disease progression in humans) may be useful in clarifying this. However, it must be mentioned that recent studies have identified polymorphs (i.e. different strains) of aggregated α-syn with the oligomeric and fibrillar strains being particularly toxic (Stöckl et al. 2013; Peelaerts et al. 2015; Walker 2016). Thus, not all α-syn inclusions may lead to pathology. It is currently uncertain if these polymorphs do exist in clinical PD, if it does it could at least explain in part the possible disconnect between Lewy pathology and neuronal cell loss reported in some clinical PD cases. In addition, α-syn levels that are too low to cause disease may also fail to result in observable pathological changes. So, while there could be detectable misfolded α-syn to seed aggregation in patients, there may not necessarily be enough to cause severe detectable neuronal cell loss and associated neurological dysfunction. Moreover, in humans, unlike in animal models, it is possible that it takes much longer for enough α-syn to aggregate to significant levels that could cause appreciable pathology (Tamgüney and Korczyn 2017).

Given that various lines of evidence from rodent models to human PD cases position aggregated α-syn as a potentially toxic protein in synucleinopathies, the logical question that emerges is how, and in which context would the aggregated protein be toxic to neuronal cells (for review, see (Wong and Krainc 2017)). The toxicity of α-syn may be induced through several mechanisms: a) binding and inhibition of lysosomal function (Cuervo et al. 2004;

Flavin et al. 2017), b) inhibition of proteasome activity (Tanaka et al. 2001; Stefanis et al. 2001; Petrucelli et al. 2002; Lindersson et al. 2004; Smith et al. 2005), c) induction of oxidative stress, Ca^{2+} dyshomeostasis and mitochondrial dysfunction (Cappai et al. 2005; Smith et al. 2005; Surmeier et al. 2017), and d) pathogenic redistribution of membrane proteins (Shrivastava et al. 2015; Mao et al. 2016; Shrivastava et al. 2017). In particular, α-syn oligomer and A53T overexpression in cultured cells significantly increase oxidative stress by enhancing the intracellular levels of reactive oxygen species (ROS) (Cappai et al. 2005; Smith et al. 2005; Reeve et al. 2015). The generation of ROS promotes mitochondrial and neuronal damage. In nigral neurons, a major source of ROS is the metabolism of dopamine. Interestingly, exposure of α-syn mutant cells to dopamine results in increased cell loss (Cappai et al. 2005). Perhaps, this could be related to the increased vulnerability of dopaminergic cells to α-syn induced toxicity. In addition, α-syn in A53T mutant cells may, in some conditions localize to the mitochondria, where it causes the release of cytochrome c from the mitochondria (Smith et al. 2005; Parihar et al. 2008). The accumulation of cytochrome c in the cytosol of cultured cells has been associated with increased activities of caspase 3 and 9 (Smith et al. 2005). Moreover, in line with its effect on mitochondrial homeostasis, the expression of α-syn in A53T mutants have been strongly associated with higher concentrations of mitochondrial Ca^{2+} levels and increased DNA damage (Martin et al. 2006; Chen et al. 2015). Abnormally high levels of mitochondrial Ca^{2+} triggers apoptosis. Therefore, α-syn may induce apoptotic cell degradation in a mitochondrial-linked pathway and caspase activation (Smith et al. 2005; Parihar et al. 2008), making mitochondria cellular targets of α-syn accumulation and neurotoxicity (Di Maio et al. 2016; Wong and Krainc 2017; Tapias et al. 2017). A mitochondrial-independent pathway has also been suggested, whereby α-syn oligomers are thought to interfere with the normal functions of cell membranes and form pore-like structures in lipid bilayers that leads to abnormal Ca^{2+} influx and subsequent neuronal cell damage (Tsigelny et al. 2012). In addition, strong interactions between fibrillar α-syn assemblies and the plasma membrane might disrupt the Na^{+} gradient (Shrivastava et al. 2015). Overall, it appears that multiple systems can be affected by α-syn aggregation and accumulation, which can trigger cellular pathways that results in neuronal cell loss. This view links primary protein aggregation to cellular targets of the protein. Therefore, determining which events are actually primary (i.e. α-syn aggregation *vs.* mitochondrial, proteasomal and lysosomal dysfunction) and which ones are secondary would be crucial for deciding the most important aspects of the cell death process that should be targeted to hinder disease progression (Wong and Krainc 2017; Brundin and Melki 2017; Surmeier et al. 2017).

Conclusions

Given that this field offers many reviews on the prion-like spread of α-syn pathology, we focused on unaddressed and under published questions. Our goal was to direct prion-like attention to the role of non-neuronal cells, the “connectomic” propagation, and whether α-syn pathology always begets dysfunction. Although we find that there is still a lack of tools to help connect pathology to dysfunction and disease state in clinical PD cases, we express optimism for the discovery of biomarkers that can reliably track pathology development and disease progression in humans.

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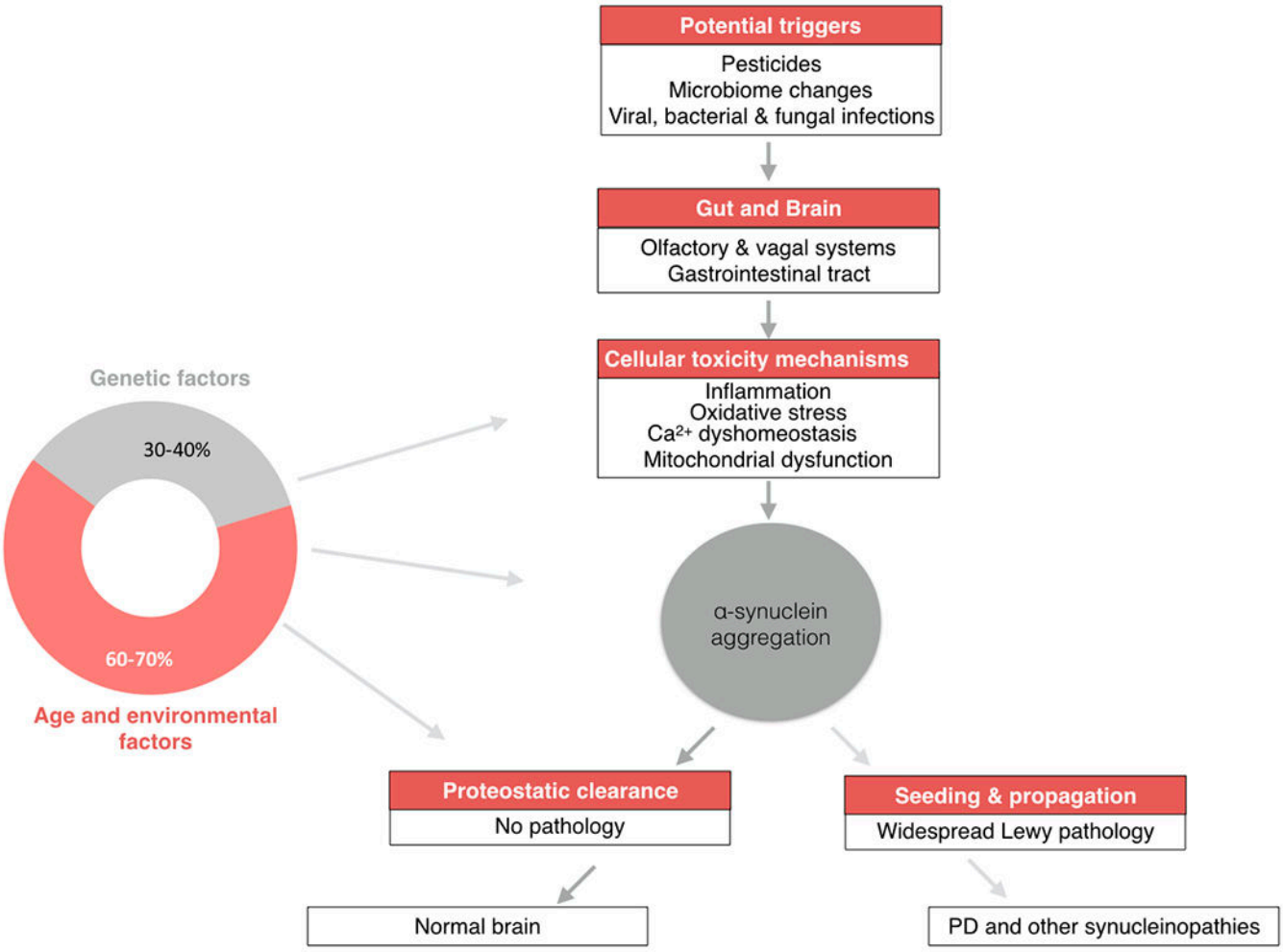

Figure 1: Mechanisms of α-syn aggregation and propagation.

(Left) Schematic showing presumed contributions of different factors to the development of Parkinson's disease and demonstrating how they influence various pieces of the pathway to Lewy pathology (Keller et al. 2012). (Right) Schematic of development of Lewy pathology by step-wise progression from potential triggers, proposed entry sites and mechanisms that may promote α-syn aggregation to the resultant effects of such aggregation. In a more likely scenario, the system can use proteostatic clearance successfully to remove aggregates. However, when proteostatic clearance is impaired and α-syn aggregation and accumulation proceeds unchecked, the initial aggregates may be transferred in a 'prion-like' manner and may seed further aggregation, resulting in widespread Lewy pathology that contributes to the development of PD and other synucleinopathies.

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Table 1:

Rodent and non-human primate models demonstrating α-syn transmission and neurologic dysfunction

Animal Model	Pathological α-syn* inoculation site	Timeline post-inoculation	α-syn pathology, neurodegeneration or neurologic dysfunction	Reference(s)
WT mice	Intranigral (rAAV of hA53T)	1, 3, 6 months	Yes	(St Martin et al. 2007)
WT rats	Intranigral (rAAV of hA53T)	Varying times up to ~6.75 months	Yes	(Kirik et al. 2002; Yamada et al. 2004)
WT mice	Intranigral, intraamygdala and intrastriatal (rAAV of A30P)	Varying times up to 12 months	Yes	(Lauwers et al. 2003)
WT rats	Intranigral (rAAV of hA53T, A30P)	0.75, 1.5, 5 months	Yes	(Lo Bianco et al. 2002)
WT rats	Intranigral and intrastriatal (rAAV of α-syn)	Varying times up to 18 months	Yes	(Peelaerts et al. 2015)
WT rats	Intranigral and intracerebral (rAAV of α-syn)	~1.25 months	Yes	(Kordower et al. 2011)
WT rats	Intranigral (rAAV of α-syn)	Varying times up to ~1 months	Yes	(Angot et al. 2012)
WT Marmosets	Intranigral (rAAV of hA53T)	~ 4 months	Yes	(Kirik et al. 2003)
Mice overexpressing hα-syn	Intrahippocampal (rAAV of α-syn)	Varying times up to 4 months	Yes	(Spencer et al. 2017)
WT rats	Vagus nerve (rAAV of α-syn)	Varying times up to ~4.5 months	Yes	(Ulusoy et al. 2013, 2015, 2017)
SNCA$^{-/-}$ mice	Vagus nerve (rAAV of α-syn)	Varying times up to 3 months	Yes	(Helwig et al. 2016)
WT Marmosets	Intracerebral (rAAV of hA53T)	Varying times up to 12 months	Yes	(Eslamboli et al. 2007)
WT mice and macaque monkeys	Intranigral and intrastriatal	Varying times up to ~17 months	Yes	(Recasens et al. 2014)
WT mice	Intranigral	15 months	Yes	(Masuda-Suzukake et al. 2013, 2014)
WT mice	Intrastriatal	1, 6 months	Yes	(Luk et al. 2012a)
WT rats	Intrastriatal	1, 2, 6 months	Yes	(Paumier et al. 2015)
WT rats and mice	Intrastriatal	~ 6 months	Yes	(Abdelmotilib et al. 2017)
#Tg(SNCA) Snca$^{0/0}$ mice	Intrastriatal	3, 6, 9 months	Yes	(Bernis et al. 2015)
WT mice	Intra-OB	Varying times up to 12 months	Yes (olfactory deficits)	(Rey et al. 2013, 2016b)
WT rats and mice	Intracerebral	Varying times up to ~1 months	Yes	(Reyes et al. 2014)
TgM83 (overexpressing A53T) mice	Intracerebral	1, 3 months	Yes	(Luk et al. 2012b)
TgM83$^{+/+}$ mice	Intracerebral	Varying times up to ~11 months	Yes	(Mougenot et al. 2012)
TgM83$^{+/+}$; TgM83$^{+/-}$ mice	Intracerebral	~4 months	Yes	(Prusiner et al. 2015)
TgM20 mice overexpressing hα-syn	Intracerebral	1, 2, 4, 8 months	Yes	(Sacino et al. 2013)

Animal Model	Pathological α-syn* inoculation site	Timeline post-inoculation	α-syn pathology, neurodegeneration or neurologic dysfunction	Reference(s)
hα-syn overexpressing mice	Intracerebral	Varying times up to ~13 months	Yes	(Hansen et al. 2011; Osterberg et al. 2015)
TgM83$^{+/+}$; TgM83$^{+/-}$ mice	Intracerebral	Varying times up to ~12 months	Yes	(Watts et al. 2013)
TgM83 (A53T mutant); TgM47 (E46K mutant) mice	Intracerebral and intrahippocampal	2, 4 months	Yes	(Sacino et al. 2014a)
hα-syn-overexpressing mice	Intrahippocampal	Varying times up to ~1 months	Yes	(Desplats et al. 2009)
WT mice	Intrahippocampal	2, 4 months	Yes	(Koller et al. 2017)
TgM83$^{+/+}$; TgM83$^{+/-}$; TgM20$^{+/-}$ mice	Intramuscular	Varying times up to 12 months	Yes	(Sacino et al. 2014b)
TgM83$^{+/+}$; TgM83$^{+/-}$ mice	Intraperitoneal and intracerebral	Varying times up to 14 months	Yes	(Sargent et al. 2017)
Tg(M83$^{+/-}$; *Gfap*-luc$^{+/-}$) mice	Intraperitoneal and intraglossal	Varying times up to 14 months	Yes	(Breid et al. 2016)
WT rats	Intestinal wall	12, 17, 48 hours	Yes	(Holmqvist et al. 2014)

WT, Wild type; hα-syn, human α-syn, rAAV, recombinant adeno-associated virus; hA53T, Human A53T; intra-OB, Intra-olfactory bulb.

*Pathological α-syn includes recombinant oligomers and PFFs, as well as brain extracts from sick animals or human patients.

#These mice express human wild-type α-syn on a mouse α-syn null background.