Dual Perspectives

Dual Perspectives Companion Paper: Prying into the Prion Hypothesis for Parkinson's Disease, by Patrik Brundin and Ronald Melki

Parkinson's Disease Is Not Simply a Prion Disorder

D. James Surmeier,[1] José A. Obeso,[2,3] and Glenda M. Halliday[4,5]

[1]Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, [2]CINAC, HM Puerta del Sur, Hospitales de Madrid, Mostoles and CEU-San Pablo University, 28938 Madrid, Spain, [3]Network Center for Biomedical Research on Neurodegenerative Diseases, Instituto Carlos III, 28029 Madrid, Spain, [4]Brain and Mind Centre, Sydney Medical School, University of Sydney, Sydney, 2006 New South Wales, Australia, and [5]School of Medical Sciences, University of New South Wales and Neuroscience Research Australia, Sydney, 2052 New South Wales, Australia

The notion that prion-like spreading of misfolded α-synuclein (α-SYN) causes Parkinson's disease (PD) has received a great deal of attention. Although attractive in its simplicity, the hypothesis is difficult to reconcile with postmortem analysis of human brains and connectome-mapping studies. An alternative hypothesis is that PD pathology is governed by regional or cell-autonomous factors. Although these factors provide an explanation for the pattern of neuronal loss in PD, they do not readily explain the apparently staged distribution of Lewy pathology in many PD brains, the feature of the disease that initially motivated the spreading hypothesis by Braak and colleagues. While each hypothesis alone has its shortcomings, a synthesis of the two can explain much of what we know about the etiopathology of PD.

Key words: **aging; alpha-synuclein; calcium; mitochondria; neurodegeneration; neuron; selective vulnerability; synapse**

Introduction

Clinical Parkinson's disease (cPD) is the most common form of a broad class of movement disorders called parkinsonism (Postuma et al., 2015). The cardinal motor manifestations of cPD are attributable to the progressive loss of dopaminergic neurons in the SNc (Hornykiewicz, 2002). In addition to neuronal loss, a defining feature of cPD is the appearance of proteinaceous, α-synuclein (α-SYN) rich inclusions, called Lewy pathology (LP), exclusively in neurons.

While rigorous determination of the regional loss of neurons in postmortem tissue has been difficult, the advent of immunocytochemical techniques allowing the localization of aggregated forms of α-SYN has propelled the study of LP forward. It is always easier to see what is gained than what is lost. Nearly two decades ago, Braak and colleagues used these approaches to compare brains taken from asymptomatic individuals and cPD patients at various times after diagnosis. This exercise led them to hypothesize that LP spreads into the brain from either the olfactory bulb or the dorsal motor nucleus of vagus (DMV) in the caudal medulla, two brain regions with axons extending to body surface (Kosaka et al., 1984; Braak et al., 2003; Beach et al., 2009). It was conjectured that, at these interfaces, LP-inducing, environmental pathogens or infectious agents invaded axon terminals, were retrogradely transported, and then trans-synaptically spread to other neurons (Hawkes et al., 2007). With time, these LP-inducing agents were thought to slowly propagate through the brain connectome, leading to widespread neuronal dysfunction and death (Braak et al., 2004).

This sounds like a neuropathological tsunami, which is essentially the way it is depicted in many reviews (Braak et al., 2004). However, this is misleading. Even in late-stage cPD brains, LP has a discrete, patch-like distribution (Beach et al., 2009; Dijkstra et al., 2014; Surmeier et al., 2017b). Moreover, within each of the nuclei or regions manifesting LP, its distribution is sparse and confined to particular cell types (Braak and Del Tredici, 2009; Dugger and Dickson, 2010). For example, within the DMV, only cholinergic and catecholaminergic neurons ever exhibit LP, whereas GABAergic neurons never do (Kingsbury et al., 2010). A similar discrete distribution of LP is seen in other regions, such as the pedunculopontine nucleus, basal forebrain, and cerebral cortex (Wakabayashi et al., 1995; Hall et al., 2014). It is of some note that GABAergic neurons, regardless of where they are, appear to be resistant to LP. In all of these cases, the percentage of neurons exhibiting LP is small (<15%) and relatively constant over the disease course, even in the absence of neuronal loss (Greffard et al., 2010; Parkkinen et al., 2011; Milber et al., 2012; Dijkstra et al., 2014; Iacono et al., 2015).

The proposition that this distributed pathology evolves over time in a predictable way that is causally related to symptoms clearly is attractive. It was an extraordinary example of inductive reasoning because the human data upon which the hypothesis

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Correspondence should be addressed to Dr. D. James Surmeier, Department of Physiology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611. E-mail: j-surmeier@northwestern.edu.

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was built had significant limitations. The most important of these was that the postmortem data did not provide any "hard" longitudinal information; that is, it did not show how LP pathology within individual brains evolved as a function of time and disease state. This relationship had to be inferred from a reasonable, but untested, set of assumptions. As a consequence, it is not surprising that subsequent studies have found that only approximately half of cPD patients have brains with a pattern of LP that is consistent with the Braak staging model (Kalaitzakis et al., 2008; Jellinger, 2009b; Halliday et al., 2012). Some cPD patients have no discernible LP at all (Berg et al., 2014). Moreover, attempts to correlate Braak LP staging with clinical state have been unsuccessful (Jellinger, 2009b).

For the sake of argument, let us set aside those brains that do not conform to Braak staging (we can suppose they are some form of "atypical" cPD for the time being). Is it plausible that the globally and regionally heterogeneous pattern of LP seen in these brains is a consequence of retrograde, trans-synaptic spread of a pathogen from the DMV or olfactory bulb? In principle, what would be needed to evaluate this hypothesis is the retrograde synaptic connectome of neurons in the nuclei from which the pathology is thought to spread. Unfortunately, this type of information is only now being generated in transgenic mice (not humans) using techniques, such as monosynaptic rabies virus mapping (Wall et al., 2010). Nevertheless, there are some data that are relevant if it is assumed that mice and men are approximately similar in the wiring of their brains (an assumption that is generally supported by the experimental literature). Consider the SNc; this is clearly an important node in the network of LP in the cPD brain. If a pathogen is passed from SNc dopaminergic neurons to neurons synapsing upon them, then the probability that this happens should be directly related to the number of synapses formed by the innervating neuron (i.e., the probability of one getting the flu is proportional to the number of times you come into contact with someone who has it). If this is the case, then basal ganglia nuclei (substantia nigra pars reticulata, globus pallidus, subthalamic nucleus, and striatum) should be prominent sites of late stage LP. These nuclei robustly innervate dopaminergic neurons in the SNc (Watabe-Uchida et al., 2012; Ogawa et al., 2014). Yet, none of these regions has any discernible postsynaptic LP, ever. The striatal Lewy neurites seen relatively early in the disease are undoubtedly degenerating dopaminergic axons (Halliday et al., 2011).

Consider the locus ceruleus (LC), another prominent site of LP for which there are connectomic data (Schwarz et al., 2015). Again, there is no correlation between the strength of synaptic connectivity and the probability of manifesting LP at any stage of the disease. The most prominent synaptic inputs to the LC are from the cerebellum and the medial reticular formation. Neither have any significant LP in cPD patients. So, if a pathogen spreads as hypothesized by Braak and others, its spread (or its propensity to induce LP) must be governed by some other factor. It cannot be governed by synaptic connectivity alone.

The other major caveat of the Braak hypothesis is that the relationship between LP, neuronal dysfunction, and neuronal death was, and remains, uncertain. Braak's conjecture was that LP was a harbinger of death and dysfunction. In contrast to LP, there have been relatively few rigorous studies of neuronal death in PD. This is hard to do. In brains with LP confined to the caudal medulla, there is a significant loss of SNc DA neurons in the ventral tier of the SNc (Milber et al., 2012; Dijkstra et al., 2014). There is not any substantial neurodegeneration in the other regions, most notably those that had LP. In the brains of recently diagnosed cPD patients, DA neurons in the ventral tier of the SNc

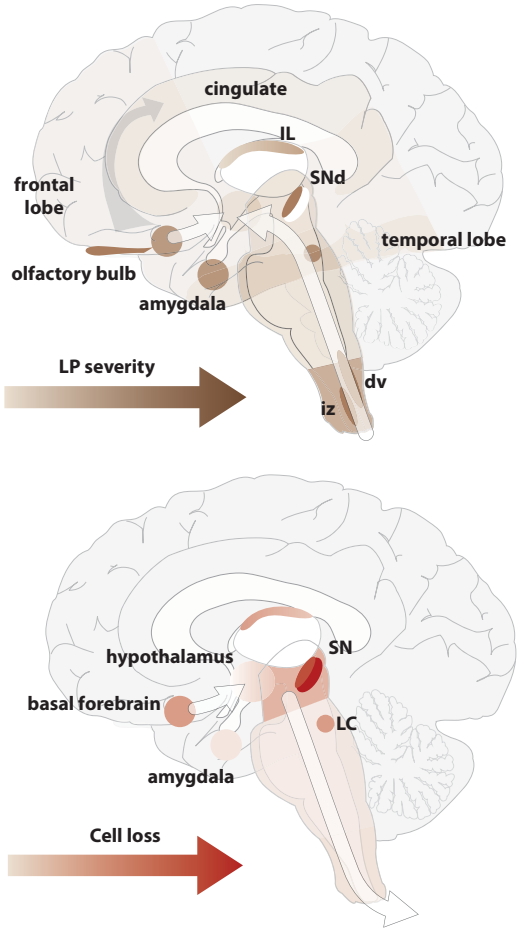

Figure 1. Top, The selective regions containing LP and the severity and conceptual progression based on cross-sectional postmortem data from patients at different stages of Parkinson's disease. Bottom, The selective regions with neuronal cell loss, and the severity and conceptual progression based on cross-sectional postmortem data from patients at different stages of PD. Although there is some overlap in the regions identified with LP and neuronal loss, the severity and regions affected over the disease course indicate different progression patterns, and these patterns are independent of the major projections of the regions affected, suggesting that prion propagation through neuronal connections is unlikely as a simplistic mechanism. IL, Intralaminar thalamus; SNd, dorsal tier of the substantia nigra pars compacta; dv, dorsal motor nucleus of the vagus nerve; iz, intermediate reticular zone.

are nearly gone (Halliday et al., 1996; Damier et al., 1999), and neuronal loss is apparent in a handful of other regions. For example, cholinergic neurons in the pedunculopontine nucleus are lost, but not glutamatergic or GABAergic pedunculopontine nucleus neurons (Halliday et al., 1990b). There also is modest loss of glutamatergic neurons in the intralaminar nuclei of the thalamus and the basolateral amygdala (Henderson et al., 2000; Harding et al., 2002). Thus, in the early stages of PD, there is not a compelling correlation between LP and neuronal loss.

With clinical progression, neuronal death is found in other regions, particularly those with LP (Halliday et al., 1990b; Kremer and Bots, 1993; Thannickal et al., 2007; Fronczek et al., 2008; Jellinger, 2009a). But there are plenty of exceptions (Halliday et al., 1990a; Ansorge et al., 1997; MacDonald and Halliday, 2002; Pedersen et al., 2005). Thus, both early and late in the disease, the correlation between LP and neuronal loss is poor (Fig. 1).

Braak redux

The recognition that the pathology in PD is distributed and not restricted to the SNc by Braak and others fundamentally changed

thinking about cPD pathogenesis. But the proposition that LP spreads through the brain connectome from well-defined nuclei and is solely responsible for the pathophysiology underlying cPD symptoms was inconsistent with much of the literature, so it languished, until recently.

Two set of observations have resurrected Braak's conjecture. One piece comes from histological analysis of fetal transplants into the striatum of PD patients. Most (but not all) of these studies revealed that, after only a decade or so, a few dopaminergic neurons exhibited proteinaceous inclusions that strongly resembled LP (Kordower et al., 2008; Li et al., 2008; compare Mendez et al., 2008). This was interpreted as spread of LP from the host to the graft. Although there is no doubt that α-SYN can be taken up by neurons from the extracellular space (Desplats et al., 2009; Hansen et al., 2011), the proposition that LP per se spread from the host to the graft was quite speculative and left several basic questions unanswered. For example, from where in the host did the seeding pathology spread? In late-stage PD patients, there is not any discernible LP in the host striatum, meaning it would have had to spread from some distant, nonsynaptically coupled site. Another question was why the Lewy-like pathology was restricted to dopaminergic neurons in the graft (compare Ahn et al., 2012), sparing neighboring GABAergc neurons? And why does the fraction of neurons displaying α-SYN pathology not increase with the duration the graft is in the host (Cooper et al., 2009; Kurowska et al., 2011; Li et al., 2016)? Could it not simply be the case that living in a graft is stressful and that the cumulative effect of this environment on already stressed (see below) dopaminergic neurons is proteostatic dysfunction and α-SYN accumulation?

A more compelling argument for spreading comes from experiments where α-SYN fibrils have been directly injected into the brain of mice and monkeys. In contrast to monomeric α-SYN (Kirik et al., 2003; Maingay et al., 2006; Ulusoy et al., 2013), synthetic, preformed α-SYN fibrils (PFFs) injected into the mouse striatum can propagate to synaptically connected, neighboring structures, creating Lewy-like pathology (Luk et al., 2012; Masuda-Suzukake et al., 2013; Peelaerts et al., 2015). In monkeys, proteins extracted from human brains with LP (that would contain α-SYN fibrils and other LP proteins) also can retrogradely propagate from the striatum after injection (Recasens et al., 2014). More recent work has shown spreading from the olfactory bulb (Rey et al., 2016). Although there are a lot of questions about these experiments and their interpretation (Sacino et al., 2016; Uchihara and Giasson, 2016; Walsh and Selkoe, 2016), they do demonstrate that extracellular α-SYN fibrils can be taken up, retrogradely (and possibly anterogradely) transported, and induce Lewy-like pathology (and cell death). Moreover, because endogenous α-SYN is recruited to the intracellular PFF aggregates and is necessary for the spreading of pathology (Volpicelli-Daley et al., 2011, 2014; Luk et al., 2012; Masuda-Suzukake et al., 2013; Peelaerts et al., 2015; compare Helwig et al., 2016), the PFFs have been likened to prions (Olanow and Brundin, 2013; Brettschneider et al., 2015). The hypothesis that prion-like fibrillary α-SYN drive pathogenesis in PD is attractive in many ways, as it posits a conceptually simple mechanism that, taken at face value, explains the evolution of LP in patients and captures the essence of the original Braak idea.

But is this what happens in PD? In principle, a prion-like process should follow one of two rules: a nearest neighbor rule or a synaptic connectivity rule. The nearest neighbor rule, whereby the probability of manifesting LP is directly related to the physical proximity to an initial seeding site, clearly is not consistent with the pattern of LP in PD patients. LP does not simply fill up the brain; it has a discrete distribution. Does the spread follow a simple synaptic connectivity rule? As described above, the available data argue that it does not. Hence, if LP spreads in PD, then there must be some other determinant of spreading in addition to just connectivity.

Neuronal phenotype and PD pathogenesis

An alternative to the Braak hypothesis is that neuronal death and LP are driven by cell-autonomous or regionally autonomous mechanisms, not a propagated pathogen. One of the features of the brain that distinguishes it from other organs is its incredible cellular diversity. Neurons, in particular, vary enormously in their size, shape, and function. The neurons at risk in PD may have a phenotype that renders them particularly vulnerable to factors known to cause the disease, age, genetic mutations, and environmental toxins.

What do we know about the phenotype of neurons that are at risk in PD? Almost all of the work on this topic has focused on SNc dopaminergic neurons whose loss is responsible for the core motor features of PD. While the SNc dopaminergic neuron may be the "poster child" for the disease, Braak and colleagues have shown that PD stretches well beyond them. Any general theory of PD pathogenesis must explain this feature.

Many (if not all) of the neurons that degenerate or manifest profound LP in PD seem to have a set of shared traits. The most notable and best characterized of these shared traits is a long and highly branched axon with an extraordinary number of transmitter release sites. This diffuse axonal arbor helps them coordinate the activity in large networks, such as the basal ganglia or the spinal cord. For example, SNc DA neurons in the rodent have axons that branch profusely in the striatum and possess as many as 200,000 vesicular release sites (Matsuda et al., 2009). This branching is similar in primates (Parent and Parent, 2006). Why might a long and highly branched axon be problematic? There are several hypotheses that have been proposed (Venda et al., 2010; Bolam and Pissadaki, 2012; Hunn et al., 2015), but only one has compelling experimental support at this point. This hypothesis posits that the bioenergetic demands of sustaining electrical excitability in a highly branched axon leads to mitochondrial oxidant stress. Indeed, it has been shown that *in vitro* mitochondrial oxidant stress is higher in SNc DA neuron axons than in the axons of less vulnerable VTA DA neurons and that reducing the size of the arbor (by manipulating axon guidance signals) decreases this stress (Pacelli et al., 2015). That said, it is puzzling that neurons, such as striatal cholinergic interneurons (Zhou et al., 2002), which have axons that are similar in complexity to those of an SNc dopaminergic neuron, seem to be resistant to whatever is going on in PD.

In addition to having a long axon, many vulnerable neurons share a set of physiological traits (Surmeier et al., 2017b). *In vivo*, at-risk neurons that have been studied are tonically active (Surmeier et al., 2012). Typically, the action potentials of these neurons are slow and broad, which maximizes Ca^{2+} entry and promotes slow rhythmic activity (Bean, 2007). In that subset of neurons studied in depth, the slow, rhythmic activity (2–10 Hz) is autonomously generated and accompanied by slow oscillations in intracellular Ca^{2+} concentration that are triggered by the opening of plasma membrane Cav1 and Cav3 Ca^{2+} channels and release of Ca^{2+} from intracellular, ER stores (Nedergaard et al., 1993; Wolfart and Roeper, 2002; Puopolo et al., 2007; Guzman et al., 2010; Morikawa and Paladini, 2011; Goldberg et al., 2012; Sanchez-Padilla et al., 2014; Matschke et al., 2015). In these cells, the diffusion of Ca^{2+} in the cytosol is unimpeded by the expres-

sion of Ca^{2+} buffering proteins, such as calbindin (Foehring et al., 2009; Goldberg et al., 2012; Sanchez-Padilla et al., 2014). This combination of features, broad spikes, pacemaking, low intrinsic Ca^{2+} buffering, and cytosolic Ca^{2+} oscillations (not any one) is what appears to distinguish vulnerable neurons.

The slow Ca^{2+} oscillations in at-risk neurons subserve two complementary functions. First, they help maintain the slow tonic spiking in these neurons (Nedergaard et al., 1993; Puopolo et al., 2007; Putzier et al., 2009). Second, they promote Ca^{2+} entry into mitochondria, oxidative phosphorylation (OXPHOS), and the production of ATP (Guzman et al., 2010; Sanchez-Padilla et al., 2014; Llorente-Folch et al., 2015). In principle, this feedforward control of OXPHOS helps to ensure that bioenergetic needs are met (Budd and Nicholls, 1998; Balaban, 2009) and that intracellular ATP levels do not fall into a range that would trigger protective activation of K-ATP channels and cessation of ongoing activity (Dragicevic et al., 2015). Even temporary cessation of activity in neuronal networks necessary to mobilize sensory and motor systems directing escape or attack behavior would lessen the chances of survival in an unpredictable environment. As a consequence, there should have been strong evolutionary pressure to design neurons in these "too important to fail" networks with this type of feedforward control mechanism.

There are two obvious downsides of this design. First, stimulating OXPHOS in the absence of strong ATP demand increases the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Votyakova and Reynolds, 2001; Guzman et al., 2010; Goldberg et al., 2012; Sanchez-Padilla et al., 2014). ROS and RNS damage proteins, lipids, and DNA, particularly in mitochondria. Sustained oxidant stress could be a major factor underlying declining mitochondrial function in at-risk neurons with age (Reeve et al., 2014). ROS and RNS also exacerbate the impact of genetic mutations and environmental toxins affecting mitochondria (Gegg and Schapira, 2016), as well as increase the propensity of α-SYN to aggregate (Gupta et al., 2008). The second downside is that it results in sustained elevations in cytosolic Ca^{2+} concentration. Ca^{2+} promotes α-SYN aggregation both directly (Rcom-H'cheo-Gauthier et al., 2014) and indirectly through activation of calpain and calcineurin (Dufty et al., 2007; Caraveo et al., 2014; Diepenbroek et al., 2014). Elevated cytosolic Ca^{2+} also impairs lysosomal motility and turnover of misfolded proteins (Gómez-Sintes et al., 2016), potentially synergizing with other defects in proteasomal/autophagic function to increase the likelihood of LP (Wong and Cuervo, 2010). Thus, by design, these vulnerable neurons appear to reside close to mitochondrial and degradative "tipping points."

But do all of the neurons at risk in PD conform to this model? It is unclear. In-depth analysis has only been performed in SNc, LC, and DMV neurons. While much of the brainstem data are consistent with a shared phenotype, more in-depth phenotyping needs to be done. However, healthy, young telencephalic neurons are not phenocopies of SNc dopaminergic neurons. That said, many of the telencephalic regions at-risk in PD (and AD) are part of a "default" network, which manifests high resting activity, albeit of synaptic origin (Andrews-Hanna et al., 2007). It is possible that, in aged, late-stage PD patients, network dysfunction (Hammond et al., 2007; Ko et al., 2013) triggers adaptations that bring these neurons and networks phenotypically closer to other at-risk neurons. Cav1 Ca^{2+} channels, which are key determinants of the SNc phenotype, could be a major factor in this process. Sustained Ca^{2+} entry through Cav1 channels in forebrain neurons has long been associated with aging-related cognitive decline and AD (Disterhoft et al., 1994; Thibault et al., 2007). Moreover, in PD patients, Cav1 Ca^{2+} channels are upregulated in limbic and motor cortices (Hurley et al., 2013, 2014).

Can the phenotype of at-risk neurons account for LP staging? The simple answer is no, at least at this point in time. From what we currently know about cell-autonomous risk factors, LP should appear in the SNc before it does in the DMV. Barring the emergence of some other cell-autonomous factor that drives LP, the most parsimonious explanation of the LP pattern in PD is that there is spreading of α-SYN pathology, as posited by Braak and colleagues and the proponents of the prion model, but that spreading is limited to a subset of neurons whose phenotype renders them susceptible to spreading, a proposition that is very consistent with the phenotype outlined above.

What is better explained by cell-autonomous factors is the sequence of cell death in PD (Fig. 2). The earliest known loss of neurons in PD is the SNc. These neurons are at one extreme of the anatomical, physiological, and molecular spectrum of vulnerable neurons as we currently understand it (Sulzer and Surmeier, 2013; Poulin et al., 2014; Anderegg et al., 2015; Brichta et al., 2015; Surmeier et al., 2017a), exhibiting the highest basal levels of mitochondrial oxidant stress and free cytosolic Ca^{2+} of any cell examined. Mitochondria and intracellular Ca^{2+} are linchpins of all three major death cascades (apoptotic, autophagic, and necrotic) (Nagley et al., 2010). In human SNc, there are telltale signs of sustained mitochondrial oxidant stress with aging and PD, such as mitochondrial DNA deletions (Bender et al., 2006, 2008). Against this backdrop, it makes sense that genetic mutations that compromise mitochondrial oxidant defenses, biogenesis, or quality control cause the preferential loss of SNc dopaminergic neurons and early onset forms of PD (Lin and Farrer, 2014; Kumaran and Cookson, 2015; Mullin and Schapira, 2015; Beilina and Cookson, 2016). The tipping point for these neurons also could be reached by other genetic mutations that indirectly compromise mitochondrial function (McCoy and Cookson, 2012; Mullin and Schapira, 2013; Brini et al., 2014; Guardia-Laguarta et al., 2015; Beilina and Cookson, 2016; Gegg and Schapira, 2016).

It also is important to acknowledge that other forms of α-SYN may be more toxic than LP and contribute to pathogenesis and cell death in PD (Ingelsson, 2016). Soluble, oligomeric forms of α-SYN clearly can induce cell death when present in sufficient quantities. Given that Ca^{2+} and ROS/RNS promote α-SYN aggregation (see above), toxic oligomers, and proto-fibrils could be more likely to form in nominally vulnerable neurons, effectively synergizing with mitochondrial and lysosomal dysfunction to trigger cell death (Fig. 2). New strategies for visualization of oligomeric forms of α-SYN (Roberts et al., 2015) should allow this possibility to be tested.

Another factor that has long been hypothesized to put SNc neurons specifically at risk is DA (Sulzer, 2007; Zucca et al., 2017). Elevated cytosolic Ca^{2+}, α-SYN, and DA in SNc DA neurons could be a particularly toxic combination, especially in axon terminals and dendrites (Mosharov et al., 2009; Dryanovski et al., 2013; Caraveo et al., 2014; Brimblecombe et al., 2015). Indeed, striatal DA axon terminals appear to be lost early in the development of PD, preceding the loss of DA cell bodies (Kordower et al., 2013). In this regard, the inference that levodopa therapy does not accelerate disease progression (Fahn, 2005) might be wrong if the primary site of DA toxicity is the axon terminal, terminals that are largely gone by the time levodopa therapy is usually started.

If cell-autonomous factors are critical to the evolution of PD, then "normalizing" one or more of these factors should slow disease progression. As outlined above, Ca^{2+} entry through Cav1 Ca^{2+} channels appears to be a major driver of mitochondrial

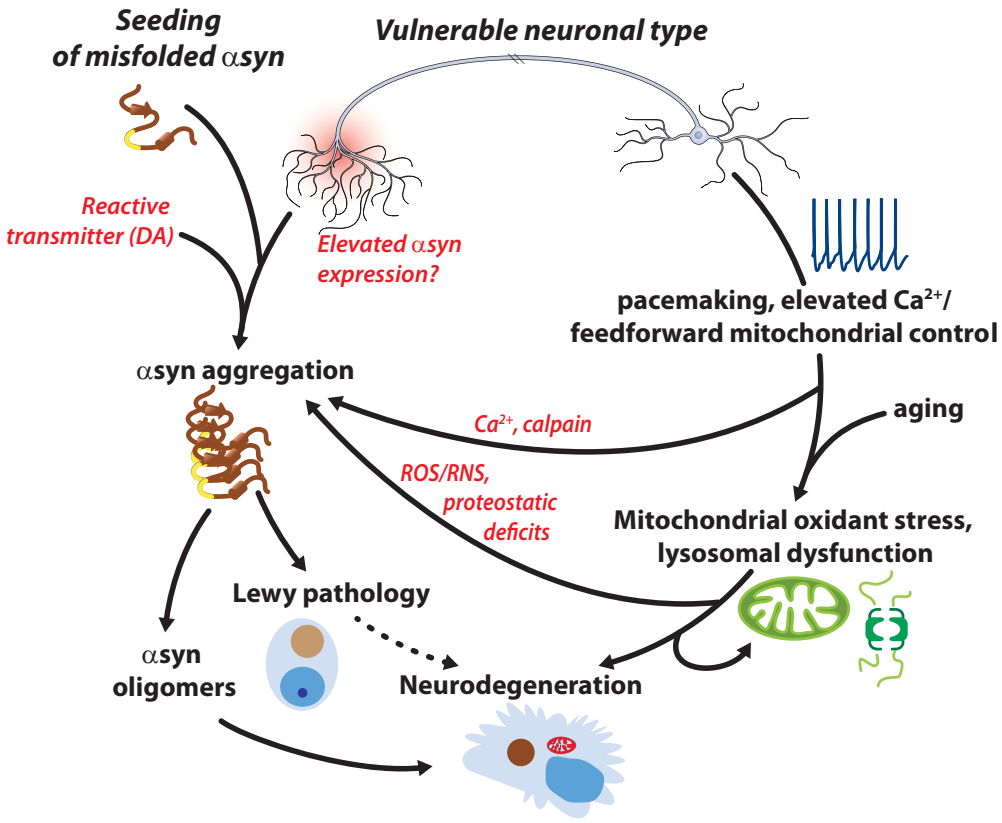

Figure 2. Schematic summary of the factors potentially driving LP and neurodegeneration in PD. The vulnerable neuronal phenotype has a long, highly branched axon, which could lead to elevated expression of α-SYN, as well as increase transmission sites for misfolded α-SYN. Both of these factors could promote α-SYN aggregation, oligomer formation, LP, and possibly neurodegeneration. In parallel, pacemaking, elevated cytosolic Ca^{2+}, and mitochondrial oxidant stress could put vulnerable neurons at risk, both by promoting mitochondrial and lysosomal dysfunction with aging as well as by promoting α-SYN aggregation (through elevated ROS/RNS, Ca^{2+}, and calpain activation, proteostatic deficits). Other potential factors, such as a reactive neurotransmitter (e.g., dopamine), also could contribute.

oxidant stress in all of the at-risk neurons examined to date. Moreover, these channels can be targeted. Dihydropyridines are FDA-approved, selective negative allosteric modulators of Cav1 channels that have good brain bioavailability (Striessnig et al., 1998; Anekonda et al., 2011; Surmeier et al., 2017a). Because dihydropyridines are voltage-dependent negative allosteric modulators that bind to and inhibit channels only when the plasma membrane is depolarized for sustained periods of time (as in pacemaking neurons), they should effectively blunt Ca^{2+} entry only in a small subset of healthy neurons, precisely the pacemaking neurons at risk in PD. Moreover, at FDA-approved doses, the inhibition of Cav1 channels is decidedly partial (Ilijic et al., 2011). Epidemiological studies have consistently found that the use of dihydropyridines is associated with a decreased risk of developing PD (Becker et al., 2008; Ritz et al., 2010; Pasternak et al., 2012; Lee et al., 2014; Gudala et al., 2015); their use even seems to slow progression after diagnosis (Marras et al., 2012). The combination of preclinical and clinical data implicating Cav1 channels in PD pathogenesis motivated the National Institutes of Health to mount a 5 year, Phase III, disease modification clinical trial in early stage PD patients with the dihydropyridine isradipine that will be completed in 2018.

In conclusion, the prevailing view of PD etiology is that LP spreads in the brain through synaptically coupled networks, driving cell death, and clinical manifestations. However, the distribution of pathology in PD brains and recent connectomics are not consistent with this simple model. Moreover, the relationship between LP, neuronal dysfunction, and death remains uncertain. If LP spreads trans-synaptically in PD, the processes must be gated by cell- or region-autonomous mechanisms. Indeed, at-risk neurons appear to share a set of traits that would not only make them more vulnerable to α-SYN pathology, but would make them more vulnerable to age, as well as toxins and genetic mutations associated with the disease. Although the relative roles of neuronal design and propagated pathology in the etiology of PD remain to be determined, it is clear that both factors need to be considered.

Response by Dual Perspectives Companion Authors–Patrik Brundin and Ronald Melki

Having read the article by Surmeier and colleagues, we conclude that there is more that unites than divides our views! Indeed, we agree with the fundamental conclusion, i.e., "... the most parsimonious explanation of the Lewy pathology pattern in Parkinson's disease is that there is spreading of α-SYN pathology ... but that spreading is limited to a subset of neurons whose phenotype renders them susceptible to spreading ..."

Surmeier and colleagues highlight multiple factors that might govern the selective susceptibility, but which is the primary reason that some cells develop Lewy pathology (LP) and others do not remains to be clarified. One overlooked factor is differences in the lysosomal autophagy system between different neuronal populations; this might

govern whether neurons effectively degrade α-synuclein (α-SYN) or allow amplification to take place (Lopes da Fonseca et al., 2015). Another option is that receptors that, at least in part, mediate uptake of aggregation-prone species of α-SYN are differentially expressed in different neuronal populations (Shrivastava et al., 2015; Mao et al., 2016).

We respectfully disagree with Surmeier and colleagues on a few points. For example, we do not agree that LP in grafts in patients with Parkinson's disease has only been seen in dopamine neurons. Indeed, studies of grafts have focused on pigmented (putative dopaminergic) neurons because the presence of melanin indicated they were definitely graft-derived. However, contrary to Surmeier and colleagues' claim, Ahn et al. (2012) reported that LP was also present in GABAergic neurons inside a patient graft. Furthermore, LP is present inside the striatum of patients with advanced Lewy body disease (Duda et al., 2002) (i.e., the same structure that is innervated by graft-derived axons in patients). This supports the idea that LP inside grafted neurons is the consequence of misfolded α-SYN being taken up by terminals in the host striatum and retrogradely transported to cell bodies inside the graft. Surmeier and colleagues also suggested that the fraction of grafted neurons displaying α-SYN did not increase with time after surgery. We think the contrary has been demonstrated. Chu and Kordower (2010) summarized that patients who died between 18 months and 4 years after surgery had no LP in their grafts, and 1 patient who died after 14 years exhibited LP in 5%–8% of grafted pigmented neurons. In the Swedish series of patients (operated with a different technique), the relationship between proportion of pigmented neurons showing LP and time since surgery was 2% at 12 years, 5% at 16 years, and 11%–12% at 24 years (Li et al., 2008, 2010, 2016). Finally, regarding the direction of axonal transport (the retrograde direction being emphasized by Surmeier and colleagues) and possible need for synaptic contacts discussed by Surmeier and colleagues, we think that experiments have clearly shown that α-SYN assemblies are transported anterogradely and retrogradely with similar efficiency, and that they can propagate after transport independently of both synapses and cell-to-cell contacts (Freundt et al., 2012; Brahic et al., 2016).

In conclusion, we think that the first decade of the "prion hypothesis" for Parkinson's disease has generated several exciting findings and that prion-like seeding definitely has a role in disease pathogenesis. We also believe there is much more to explore and understand. Hopefully, the coming decade will lead to a deeper understanding that can trigger the development of new therapies.

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