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Effects of α-synuclein on axonal transport

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ABSTRACT

Lewy bodies and Lewy neurites composed primarily of α-synuclein characterize synucleinopathies including Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). Despite decades of research on the impact of α-synuclein, little is known how abnormal inclusion made of this protein compromise neuronal function. Emerging evidence suggests that defects in axonal transport caused by aggregated α-synuclein contribute to neuronal dysfunction. These defects appear to occur well before the onset of neuronal death. Susceptible neurons in PD such as dopamine neurons with long elaborate axons may be particularly sensitive to abnormal axonal transport. Axonal transport is critical for delivery of signaling molecules to the soma responsible for neuronal differentiation and survival. In addition, axonal transport delivers degradative organelles such as endosomes and autophagosomes to lysosomes located in the soma to degrade damaged proteins and organelles. Identifying the molecular mechanisms by which axonal transport is impaired in PD and DLB may help identify novel therapeutic targets to enhance neuron survival and even possibly prevent disease progression. Here, we review the evidence that axonal transport is impaired in synucleinopathies, and describe potential mechanisms by which contribute to these defects.

1. Lewy neurites predominate in synucleinopathies

The proteinaceous inclusions known as Lewy Bodies (LBs), which characterize Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB), were first described by Fritz Heinrich Lewy in 1912 (Lewy, 1912). LBs are eosinophilic, spherical, or reniform dense inclusions in the neuronal soma. Lewy neurites (LNs) found in brains of PD and LBD patients were also described by Lewy. These inclusions have received less attention than LBs because they are not easily stained with acidophilic dyes. The first study to appreciate the density of LNs utilized immunohistochemistry for ubiquitin, a protein modification found in LBs and LNs, and revealed that LNs appear as a dense network in the hippocampus of DLB brains (Dickson et al., 1991). The discovery that α-synuclein is the main component of LBs and LNs (Spillantini et al., 1997) led to the development of sensitive α-synuclein antibodies, which revealed that Lewy neurites are a prominent feature of PD and DLB. These inclusions, which are far more abundant than LBs (Braak et al., 1999; Duda et al., 2002a; Braak et al., 2003), can appear as thick and club-shaped, short and stubby, or longer and thread-like. They appear throughout the nervous system including, but not limited to, cardiac sympathetic neurons, dorsal nucleus of the vagus nerve, substantia nigra pars compacta (SNpc), nucleus basalis, amygdala, hippocampus, and cortex.

Not only are LNs more abundant than LBs, their appearance precedes that of LBs (Braak et al., 1999; Braak et al., 2003). This is not surprising given that α-synuclein primarily resides at the presynaptic terminal (Maroteaux et al., 1988). Pathologic analyses of PD brains ranging from cases with mild pathology to severe pathology show, for example, that a plexus of LNs appears in the hippocampus at PD stage 3 of the evolution of PD pathology, whereas dense LBs only appear in the hippocampus at the latest stage 6 (Braak et al., 2003). The abundance of LNs correlates with the development of symptoms. In addition, Lewy neurites are the best correlate of cognitive symptoms in DLB (Irwin et al., 2012). More sensitive immunohistochemical techniques to differentiate aggregated α-synuclein from monomeric alpha-synuclein (Kramer and Schulz-Schaeffer, 2007; Spinelli et al., 2014) show that the majority of α-synuclein aggregates localize to the presynaptic terminal and axons and correspond with a reduction in dendritic spines in the postsynapse (Kramer and Schulz-Schaeffer, 2007). Thus,

Abbreviations: DLB, Dementia with Lewy Bodies; LB, Lewy Bodies; LN, Lewy neurites; LC, locus coeruleus; PD, Parkinson's disease; SNpc, substantia nigra pars compacta.

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identifying these early aggregates in presynaptic terminals and axons may provide an even better correlate of PD and DLB symptoms, and help reveal the mechanisms by which α-synuclein aggregate formation causes neuronal dysfunction.

2. Defects in axonal transport caused by α-synuclein aggregates may precede neurodegeneration

Pathological analyses of PD brains from over 50 years ago showed accumulations of vesicles along axons and near α-synuclein inclusion (Duffy and Tennyson, 1965; Forno and Norville, 1976; Watanabe et al., 1977; Hayashida et al., 1993). These early studies suggest that α-synuclein aggregates impair membrane traffic. Studies in multiple neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease implicate impaired axonal transport of vesicles as a major contributor to neurodegeneration (Chevalier-Larsen and Holzbaur, 2006; Hinckelmann et al., 2013; Millecamps and Julien, 2013). There are fewer studies implicating axonal transport defects as contributing to neurodegeneration in PD, but accumulating data suggest axonal transport defects precede degeneration (Chung et al., 2009; Chu et al., 2012; Lamberts et al., 2015). Understanding defects in neuronal function before neurodegeneration occurs is critical because therapeutic targets can be identified that can intervene before intractable cell death. One of the first studies to reveal pathologic α-synuclein aggregates in axon terminals in the hippocampus of PD and DLB showed they are associated with accumulations of synaptic vesicle proteins (Galvin et al., 1999). This study suggested that blocked axonal transport in neurons containing α-synuclein aggregates could cause a "dying back" of the axonal projections. Subsequent to this study, levels of the molecular motors associated with axonal transport were shown to be reduced in PD brains (Chu et al., 2012). Levels of kinesin, which facilitates anterograde axonal transport of cargo from the soma to presynaptic terminal, were dramatically reduced in the SNpc of PD brains well before loss of dopaminergic neurons. Levels of components of the dynein complex, which facilitates retrograde transport from the terminal back to the soma, were also reduced but only in late stage PD brains. The abundance of motor proteins was particularly low in neurons containing α-synuclein aggregates. These findings support that α-synuclein aggregate induced alteration in motor proteins can precede neurodegeneration in PD.

Animal models of abnormal α-synuclein help further dissect the contribution of axonal transport defects to neurodegeneration because they allow careful analyses of the time course in which defects emerge. For example, independent studies utilizing adeno associated viruses (AAV) induced overexpression of the A53T- or A30P-disease associated mutations in α-synuclein show reduced levels of kinesin and dynein in neurons with aggregated α-synuclein (Chung et al., 2009; Chu et al., 2012). Eight weeks after induction of A53T-α-synuclein, there is a substantial reduction in levels of kinesin isoforms without changes in other synaptic proteins or dopamine neuron loss. Kinesin levels are reduced in the striatum, but are increased in the SNpc, suggesting the motors are trapped in the soma and cannot bind to microtubule tracks to facilitate anterograde transport along the axon. Loss of nigral neurons was not apparent until 17 weeks after induction of A53T-α-synuclein, again supporting that defects in axonal transport emerge well before the onset of neuron death (Chung et al., 2009).

Some drawbacks of using in vivo animal models is that they do not provide the resolution to visualize axonal transport in live neurons, measure the kinetics of axonal transport, or easily dissect the molecular mechanisms that may contribute to potential defects. This is critical because axonal transport is a dynamic process. Distinct cargo show very different patterns of transport along the axon (Millecamps and Julien, 2013; Maday et al., 2014). For example, synaptic vesicle precursors travel predominantly in an anterograde direction from the cell body out to the presynaptic terminal. Late endosomes, autophagosomes, and signaling endosomes carrying neurotrophin receptors show predominantly retrograde transport from the presynaptic terminal back to the soma. Furthermore, transport of different cargos is mediated by distinct adaptor proteins. Kinesin motors facilitate anterograde transport; 38 kinesin superfamily members are expressed in the brain. Dynein, which facilitates retrograde transport, is a large motor complex composed of multiple subunits with multiple isoforms and requires activation by a large complex, dynactin. There are many distinct adaptors that interact with these molecular motors that regulate the specificity of cargo. For example, transport of mitochondria is regulated by the Mitochondrial Rho GTPase (Miro), and kinesin-1 (Macaskill et al., 2009; Wang and Schwarz, 2009). Axonal transport of signaling endosomes and late endosomes depends on dynein complexes specifically containing the intermediate chain IC-1B (Pfister, 2015). Axonal transport of these organelles is also controlled by Rab7 and Rab7 interactors such as and Snapin (Johansson et al., 2007; Cai et al., 2010). Dynein also forms a complex with adaptors Lis and *Nde*l, which play a role in late endosome axonal transport (Pandey and Smith, 2011). Overall, there is an increasing list of scaffolding proteins and adaptors that regulate transport of specific cargo along axons.

Recently, it was shown that exposure of neurons to fibrils made from recombinant α-synuclein can robustly induce endogenous α-synuclein to form inclusions that resemble Lewy bodies and Lewy neurites found in diseased brains (Fig. 1) (Volpicelli-Daley et al., 2011; Volpicelli-Daley et al., 2016). This is the first model that produces inclusions resembling LBs and LNs in primary neurons. This model provides the spatial resolution to determine subcellular neuronal compartments to which the inclusions localize, and provides the temporal resolution to examine inclusions from their initial formation to their spread throughout the neuron and consequent cell death. For example, after addition of fibrils to neurons, inclusions form first in axons (4–7 days after adding fibrils) and then spread to the soma and dendrites (10–14 days post-fibril addition), similar to findings from pathologic studies of PD brains (Volpicelli-Daley et al., 2011). Formation of inclusions in primary neurons also allows live cell imaging and quantitation of the kinetics of axonal transport of distinct organelles that move along the axon within milliseconds (Volpicelli-Daley et al., 2014). It was found that the presence of α-synuclein inclusions in axons at early time points, before neuron death, reduces velocities and mobilities of Rab7-positive late endosomes, TrkB neurotrophin receptors, and LC3-positive autophagosomes (Fig. 2). However, axonal transport of mitochondria and synaptic vesicle precursors remain normal. In addition, electron microscopy analyses showed that α-synuclein inclusions do not fill the axonal cytoplasm or grossly disrupt the microtubule tracts. This suggests that the α-synuclein inclusions do not impair axonal transport by occluding the axon, nor do the inclusions cause a generalized disruption of transport proteins such as kinesin or dynein. Axonal transport of endosomal organelles appears to be particularly affected by the presence of axonal Lewy neurite-like inclusions. However, it is possible that at longer time points after fibril addition, when the inclusions become more mature, transport of other organelles such as mitochondria may be impaired. Also, α-synuclein has been shown to act as a microtubule dynamase (Cartelli et al., 2016) and more sensitive biochemical techniques may reveal if the inclusions impact microtubule dynamics, causing disrupted axonal transport. Furthermore, it will be of great interest to determine if neurons particularly susceptible in PD, such as dopamine neurons, are more sensitive to defects in axonal transport.

3. Potential mechanisms by which α-synuclein aggregates impair axonal transport

The mechanisms by which α-synuclein aggregates impair axonal transport are unknown. Some rare neurodegenerative diseases are caused by mutations in motor proteins themselves such as (Zhao et al., 2001) in Charcot Marie Tooth disease, or KIF5A in spastic paraplegia (Reid et al., 2002). Mutations in motor proteins have not been identified in PD. However, it is possible that the α-synuclein aggregates may