

BRAIN COMMUNICATIONS

SCIENTIFIC COMMENTARY

A toxic subset of soluble α -synuclein species in dementia with Lewy body

This scientific commentary refers to ‘Analysis of α -synuclein species enriched from cerebral cortex of humans with sporadic dementia with Lewy bodies’, by Sanderson *et al.* (<https://doi.org/10.1093/braincomms/fcaa010>).

For decades researchers have sought to find cures for health conditions that affect the brain (as imagined by the artist Peter Rogers in Fig. 1). Dementia with Lewy body (DLB) and Parkinson’s disease are two of the several brain diseases, collectively called synucleinopathies. They comprise a group of chronic neurological disorders characterized by intracellular inclusions of α -synuclein (α Syn) and the progressive and irreversible neurodegeneration. The physiological effects of these diseases do not manifest until mid-to-late adulthood. Similar to other dementias, there is no cure for DLB. The insidious symptoms of DLB are vague, and the initial changes that may drive α Syn dysfunction are completely unknown (McKeith *et al.*, 2017).

In this issue, Sanderson *et al.* (2020) took a step towards unveiling dysfunctional α Syn contributing to the sporadic DLB pathology, by providing compelling evidence of the existence of neurotoxic soluble α Syn species in the human brain. This small protein (140 amino acids), which is abundant in presynaptic terminals in a healthy brain, is the main component of Lewy body deposits and Lewy neurites in synucleinopathies. Like other amyloidogenic proteins, α Syn has structural characteristics that

make it prone to aggregate; the protein is arranged into three distinct domains that likely contribute to its aggregation and deposition. For instance, familial forms of Parkinson’s disease are associated with mutations in the N-terminal domain of α Syn. In addition, crystal structure analysis revealed that residues 68–78 and 47–56 from the central core are prone to form β -sheets, which are characteristics of amyloid assemblies. Furthermore, C-terminally truncated forms of α Syn appeared to aggregate faster than the full-length protein. These observations suggest that the different regions of the protein somehow aggregate compromising cell function and survival.

In the DLB brain, as well as Parkinson’s disease, the regional staging of α Syn deposits suggests a spatio-temporal progression of the pathology. The transfer of dysfunctional α Syn between interconnected neurons predicts the pattern of Lewy pathology and neurodegeneration in Parkinson’s disease (Del Tredici and Braak, 2020). The first direct evidence of α Syn spreading in the human brain has come from the development of Lewy pathology in grafts of patients with Parkinson’s disease receiving foetal human midbrain neurons (Hansen *et al.*, 2011). Nevertheless, these large aggregates do not appear to be the main cause of neuronal deterioration. For instance, amyloid plaques considered for long time as the main toxic structure in Alzheimer’s disease do not correlate with cognitive decline and neurodegeneration. These large extracellular deposits can be even found

in normally aging people, thus questioning the role of amyloid plaques in Alzheimer’s disease. Similarly, in Alzheimer’s disease and other neurodegenerative diseases, post-mortem analysis of older individuals revealed extensive Lewy pathology with no evident Parkinson-like symptoms. All of these findings point to a different conformation that might be responsible for neuronal impairment.

It is now well accepted based on a strong body of evidence that oligomers, rather than the large fibrils, are likely to be involved in neurodegeneration. The α Syn oligomers are small soluble toxic intermediates, which are en route to fibrils formation and recently shown to be the end product of the aggregation process *in vitro* (Breydo and Uversky, 2015). These unstable, recombinant and/or brain-derived oligomers have shown to be toxic when applied extracellularly to the cells in culture or injected into the mouse brain. The dynamic properties, structural diversity and limited suitable methods to analyse oligomers represent a challenge. Only a handful of studies have provided evidence of these soluble oligomers in the human brain. A key challenge in the field that we need to overcome on the road to develop effective therapeutics is to develop a more thorough picture of which oligomeric forms of α Syn are toxic and how they confer this toxicity. Sanderson *et al.* (2020) have helped address this challenge by examining α Syn oligomers isolated from human disease tissue and their toxic effects in human

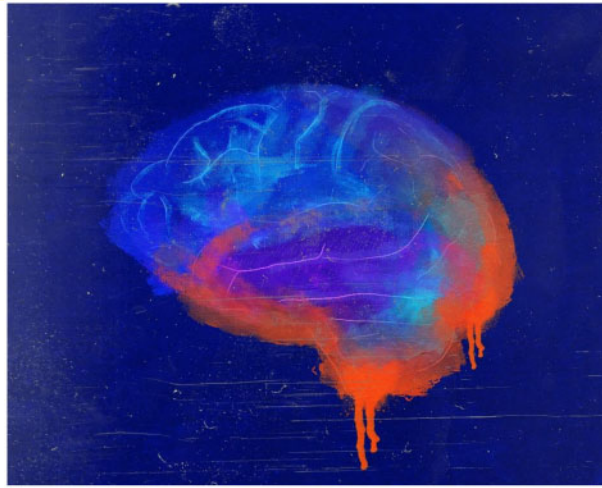


Figure 1 The brain under attack. Image reproduced by permission of the artist. Copyright Peter Rogers.

induced pluripotent stem cell-derived neurons.

The work from Sanderson *et al.* (2020) broadens previous work from others and provides evidence for the presence of soluble neurotoxic α Syn aggregates in DLB brain tissue. As previously shown, α Syn species can be detected using specific tools designed to recognize these unstable intermediates. As such, the use of conformational-dependent antibodies has proven to be valuable to identify oligomers in synucleinopathies (Sengupta *et al.*, 2015; Castillo-Carranza *et al.*, 2018).

Following a different approach, Sanderson *et al.* (2020) developed a method to investigate α Syn species in a cohort of patients with a neuropathological diagnosis of DLB and also control brains, by using a sequence of non-denaturing biochemical fractionation followed by enzyme-linked immunosorbent assay (ELISA) analysis. They measured absolute α Syn by ELISA and found enriched α Syn species in the cytosolic fraction of the DLB cortex, which was lower in the membrane-associated fraction. Apparently, the levels of α Syn in the cytosolic fraction corresponded with the degree of Lewy neuropathology. The subcellular redistribution of membrane-bound α Syn to the cytosolic compartment coincided with a recent report showing the abundance of vesicle-bound α Syn in Lewy bodies (Burre

et al., 2012.; Shahmoradian *et al.*, 2019). In other words, the shift of α Syn from the membrane to cytosol may suggest a loss of α Syn physiological function. This is a critical protein required for the survival of distinct neuronal populations, which is also involved in the compartmentalization, storage, and release of synaptic vesicles, required for neurotransmission. If accurate, these findings may support the hypothesis that α Syn loses its function in the DLB cortex.

Sanderson *et al.* (2020) then analysed the soluble fraction using non-denaturing size-exclusion chromatography fractionation followed by ELISA quantitation and found an increase in the soluble high molecular weight α Syn species (~2 MDa to 440 kDa). Although soluble high molecular weight α Syn aggregates were found in both control and DLB cortex, permeabilization assays showed that only α Syn species from DLB cases destabilized lipid membranes compared with the control brains. Their findings suggested that soluble α Syn aggregates obtained from DLB brains exert cytotoxic effects by permeabilizing membranes. These findings are consistent with the concept that soluble intermediates display toxic properties. Oligomers are known to vary in size (dimers, trimers, multimers), shape (granular, globulomers, annular) and β -sheet content (Breydo and Uversky,

2015); thus, it is possible that one or more distinct α Syn species are responsible for the effects observed here.

Going beyond the soluble α Syn species, the authors investigated the effects of sonicated detergent-insoluble α Syn on human induced pluripotent stem cell neurons. They determined the impact of insoluble α Syn by measuring morphological changes inflicted to the neurites. Although the effects of insoluble α Syn to induced pluripotent stem cells are clear, recent work has proven that sonication of recombinant fibrils from distinct amyloidogenic protein can break into short fibrils and oligomers among other conformations (Ghag *et al.*, 2018), thus representing a source of distinct species. Given that the protein aggregation is a dynamic process, this could suggest that the toxic effects observed here may be linked to the oligomeric foci.

The work from Sanderson *et al.* (2020) provides important information regarding α Syn species involved in DLB while introducing a new perspective in respect to the distribution of pathology in the brain tissues used in this work. The microheterogeneity within the same tissue represents a confounding factor that should be taken into consideration, thus highlighting the need for a better sampling in research.

The search for clues that will help to understand the pathology of the disease

and the earlier brain changes in DLB before the symptoms develop is still underway. Without a doubt, the techniques described by Sanderson *et al.*, in this issue of *Brain Communications*, provided valuable information to better understand the contribution of α Syn species to DLB pathology and related synucleinopathies.

Diana L. Castillo-Carranza
Health Science Department, Medical
School, Universidad de Monterrey,
Monterrey, Mexico

Correspondence to: Diana L. Castillo-Carranza, PhD, Health Science Department, Medical School, Universidad de Monterrey, Av. Ignacio Morones Prieto 4500 Pte., 66238 Monterrey, NL, Mexico.
E-mail:diana.castillo@udem.edu

doi:10.1093/braincomms/fcaa016

Funding

This work was supported by the Consejo Nacional de Ciencia y

Tecnología (CONACYT) (Fondo Sectorial 202164).

Competing interests

The author declares no competing interests.

References

- Breydo L, Uversky VN. Structural, morphological, and functional diversity of amyloid oligomers. *FEBS Lett* 2015; 589: 2640–8.
- Burre J, Sharma M, Sudhof TC. Systematic mutagenesis of α -synuclein reveals distinct sequence requirements for physiological and pathological activities. *J Neurosci* 2012; 32: 15227–42.
- Castillo-Carranza DL, Guerrero-Muñoz MJ, Sengupta U, Gerson JE, Kaye R. α -Synuclein oligomers induce a unique toxic tau strain. *Biol Psychiatry* 2018; 84: 499–508.
- Del Tredici K, Braak H. To stage, or not to stage. *Curr Opin Neurobiol* 2020; 61: 10–22.
- Ghag G, Bhatt N, Cantu DV, Guerrero-Munoz MJ, Ellsworth A, Sengupta U, et al. Soluble tau aggregates, not large fibrils, are the toxic species that display seeding and cross-seeding behavior. *Protein Sci* 2018; 27: 1901–1909.
- Hansen C, Angot E, Bergström A-L, Steiner JA, Pieri L, Paul G, et al. α -Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J Clin Invest* 2011; 121: 715–25.
- McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor J-P, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB consortium. *Neurology* 2017; 89: 88–100.
- Sanderson J, De S, Jiang H, Rovere M, Jin M, Zaccagnini L, et al. Analysis of α -synuclein species enriched from cerebral cortex of humans with sporadic dementia with Lewy bodies. *Brain Commun* 2020; doi: 10.1093/braincomms/fcaa010.
- Sengupta U, Guerrero-Muñoz MJ, Castillo-Carranza DL, Lasagna-Reeves CA, Gerson JE, Paulucci-Holthausen AA, et al. Pathological interface between oligomeric alpha-synuclein and tau in synucleinopathies. *Biol Psychiatry* 2015; 78: 672–83.
- Shahmoradian SH, Lewis AJ, Genoud C, Hench J, Moors TE, Navarro PP, et al. Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. *Nat Neurosci* 2019; 22: 1099–1109.