Specific vulnerability of neuronal populations & Transfer of alpha-synuclein in reconstructed neural networks

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Résumé

Alpha-synuclein (aSyn) aggregation spread in neural networks is thought to underlie the evolution of clinical symptoms in Parkinson's disease, as well as in other synucleinopathies. In a significant subset of Parkinson's disease patients, the spread of aSyn aggregation follows a conserved pattern known as "Braak staging". While it is not yet known how this spatial pattern emerges over time, anatomical observations support that sequentially touched regions are connected by axonal tracts, suggesting that aggregation propagates along neuroanatomical connectivity.
However, synaptic connectivity alone does not predict the spread of aggregation from regions affected early in the disease. Thus, intrinsic vulnerability of neuronal populations might modify the path of propagation, and neuron to neuron spreading might not be trans-synaptic, as is often assumed in the literature.

We first studied the determinants of intrinsic neuronal vulnerability to aggregation. For that, we exposed murine primary neuronal cultures prepared from different brain regions and from mice expressing various levels of aSyn to exogenously prepared fluorescent aSyn aggregates of the "Fibrils" type, previously characterized by Ronald Melki's team. We found out that while uptake of exogenous aggregates was similar in all the cultures, seeding of endogenous aSyn greatly differed. We then demonstrated that this difference in seeding could be explained mostly through differences in endogenous aSyn expression level. This study is the first to clearly demonstrate *in cellulo* that endogenous aSyn expression level is one of the main determinants of vulnerability to aSyn seeding.
We then sought to study the mechanisms of interneuronal propagation of aSyn aggregation *in vitro*. We chose to use microfluidic systems for the reconstruction of neural networks, which permit the selective exposure of a subpart of the network to exogenous aggregates.

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Most systems used in the literature contain 1) straight axonal microchannels which only allow the reconstruction of bidirectional neural networks, or 2) asymmetric microchannels which only partially orient axonal growth. These systems are not the most appropriate for the study of aSyn propagation in binary networks, as retrogradely growing axons from the second chamber might directly capture the aggregates, thus perturbating the study of interneuronal propagation mechanisms. We thus developed the first system allowing the reconstruction of fully oriented neural networks with functional synaptic connecitvity *in vitro*. We then demonstrated anterograde interneuronal propagation of exogenous aSyn aggregates in reconstructed neural networks. This system can be used to ask new questions about the fundamental mechanisms of aSyn aggregation spread in neural networks, as well as other neural pathogens.

Mots-Clés: alpha, synuclein, selective vulnerability, prion, like, microfluidics, neural networks, brain, on, a, chip, murine primary neuronal cultures