**Toxicity assessment of the hyperphosphorylated Tau protein on the neuronal network connectivity**

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Over the past years, small species of Tau protein have been increasingly investigated in the field of Alzheimer’s Disease (AD) considering their propensity to induce a synaptic toxicity in neurons before larger aggregates appear. Although scientists got more insight into their effects, their studies usually implied non-physiological conditions with recombinant proteins purified from bacteria ensuring a good concentration but missing post-translational modifications yet well-known to play a key role in AD.

It leads us to the optimisation of the purification in mammalian cells to retrieve normal (Tau) and hyperphosphorylated (P-Tau) proteins with a proper concentration that also required the adaptation of the Multiple Reaction Monitoring (MRM) technique. We are currently dealing to define the phosphorylation and O-glycosylation sites by mass spectrometry but in the meantime, we obtained preliminary information by using antibodies targeting unique phospho-epitopes in SDS-PAGE that showed a higher phosphorylation occupation at the Serine 396 for the P-Tau compared to Tau. Furthermore, a native gel electrophoresis was performed to confirm that the proteins remain in a soluble state.

Another major purpose of our project was to identify the mechanisms involved in the internalisation and the processing of proteins, once added in the extracellular space of cortical cultures. These ones have the particularity to possess a TEV site resulting from the purification allowing us to distinguish it from the endogenous Tau in immunocytochemistry. We succeeded to observe both proteins inside a small proportion of cells but more likely in their aggregated form; monomers being too small to be detected after background removal. Besides, a LAMP1 staining has been achieved to see if there is any increasing or decreasing in the signal that would suggest a degradation via the lysosomal pathway.

The third part of this study refers to the acute toxicity of the proteins on the neuronal network connectivity according to the application of a lower (12ng) or a higher (60ng) quantity. Firstly, we proceeded to an immunocytochemistry including pre- and post-synaptic antibodies to quantify synapses by colocalization. Secondly, we looked to the calcium signal dynamic in living cells either with Fluor-4 (not cell-type specific) or GCaMP (neuron-specific). Finally, we will quantify the dendritic spines and sort it by morphology by the addition of DiI.

All together, these results should allow us to say if, once released in the extracellular space, the hyperphosphorylated form of the protein induces toxic events in adjacent neurons.

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