Reduced gliotransmitter release from astrocytes mediates tau-induced synaptic dysfunction in cultured hippocampal neurons

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Tau is a microtubule-associated protein exerting several physiological functions in neurons, however increasing evidence demonstrate the pivotal role of tau in Alzheimer's disease (AD) pathogenesis [1]. Hyperphosphorylated and misfolded tau accumulates intraneuronally and acts destroying axons, thus contributing to neuronal loss in AD. Tau protein has been also found in extracellular medium. We and other groups demonstrated that extracellular oligomeric tau (ex-oTau) exerts a strong synaptotoxic action [1,2]. Indeed, it may cross neuronal membranes and impair synaptic plasticity at the CA3-CA1 synapse in mouse hippocampal brain slices, as well as hippocampal-dependent memory in mice [2], thus suggesting that ex-oTau synaptotoxicity may depend on internalization and intracellular accumulation in neurons. However, tau has also been found accumulated in cells others than neurons, such as astrocytes and microglia [3]. Astrocytes have the ability of orchestrate synaptic plasticity and synaptic transmission. As part of the "tripartite synapse" they respond to synaptic activity and regulate synaptic transmission by buffering excess of glutamate and releasing gliotransmitters such as glutamate, ATP and D-serine in a Ca^{2+}-dependent fashion.

Here we report novel evidence that ex-oTau is abundantly and rapidly accumulated in astrocytes where they disrupt intracellular Ca^{2+} signaling and Ca^{2+}-dependent release of gliotransmitters, especially ATP. Consequently, synaptic vesicle release, the expression of pre- and post-synaptic proteins, and miniature Excitatory Post Synaptic Current (mEPSC) frequency and amplitude were reduced in neighbouring neurons. Notably, tau uploading from astrocytes required the amyloid precursor protein, APP.

Indeed, we found that: **i)** oTau accumulates more rapidly and abundantly in astrocytes than in neurons while it exerts its toxicity at synaptic level (Fig. 1 and 3). In fact, after 1-h ex-oTau application synaptic vesicular release (studied by FM1-43 imaging) and basal synaptic transmission (studied by patch-clamp recordings) were significantly depressed, and the expression of the synaptic proteins synaptophysin, synapsin and GluR1(studied by Western Blot) was reduced; **ii)** 1-h ex-oTau application reduces ATP-induced intracellular Ca^{2+} waves (studied by Ca^{2+} imaging) and Ca^{2+}-dependent gliotransmitter release, with ATP being the most affected one (Fig. 2). In fact, ATP levels (quantified by HPLC) were reduced by 73±7% respect to vehicle-treated cultures (from 93±27 to 28±13 nM; $P<0.01$). Conversely, ADP levels increased significantly following tau treatment (+28±13%; $P<0.05$); **iii)** synaptotoxic effects induced by oTau are reverted by exogenous ATP (Fig. 3); **iv)** when oTau is applied to wild-type neurons grown on astrocytes unable to upload tau (APP-KO) it does not exert any synaptotoxic effects (Fig. 4).

Collectively, our findings suggests that astrocytes play a critical role in the synaptotoxic effects of tau via reduced gliotransmitter availability, and that astrocytes are major determinants of tau pathology in AD.

References: [1] Guerrero-Muñoz MJ et al. 2015. Front Cell Neurosci. 9:464; [2] Fá M et al. 2016. Sci Rep. 6:19393; [3] Kahlson&Colodner 2015. J Exp Neurosci. 9: 43–50;

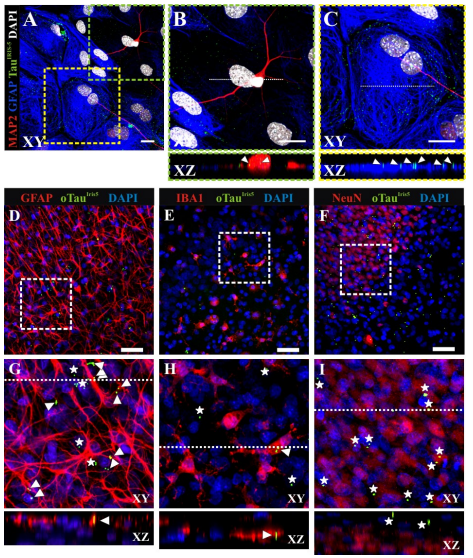

Figure 1. *Tau protein enters more efficiently astrocytes than neurons*

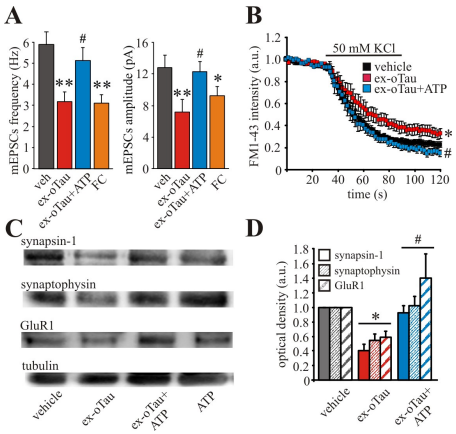

Figure 3. *Tau treatment affects basal synaptic transmission and its action is reverted by ATP*

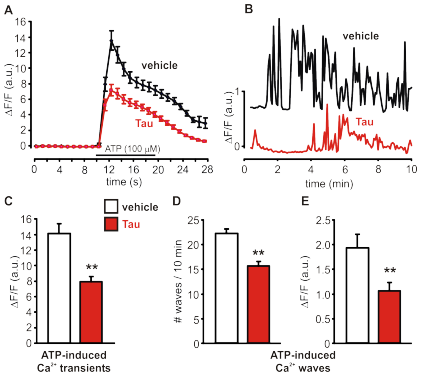

Figure 2. *Tau treatment affects ATP-induced intracellular Ca^{2+} transients and Ca^{2+} waves.*

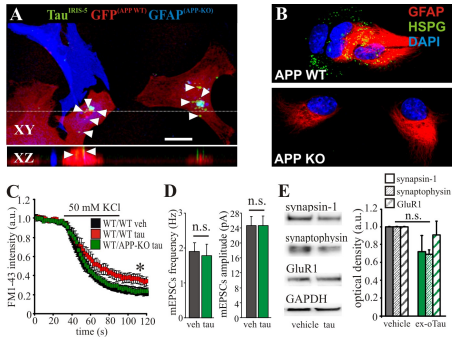

Figure 4. *Ex-oTau synaptotoxicity depends on its ability to be uploaded from astrocytes.*