THE FREQUENCY OF TAU PROPAGATION FROM NEURON TO NEURON IS INCREASED IN MICE CARRYING THE AMYLOID PATHOLOGY

Sameer Nazeeruddin,[a] Pamela Valdés,[a] Francesca Capotosti,[b] Tanja Jürgens,[b] David T. Hickman,[b] Andrea Pfeifer,[b] Andreas Muhs,[b] Patrick Aebischer[a] and Bernard L. Schneider[a]
a, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland.
b, AC Immune SA, EPFL Innovation Park, Building B, 1015 Lausanne, Switzerland.
This work was supported by grants of the Swiss National Science Foundation (Sinergia 147660), and by the Swiss Commission for Technology and Innovation (CTI no. 17616.1 PFLS-LS).

Introduction:

Intraneuronal inclusions composed of misfolded and hyperphosphorylated Tau protein are a common pathological feature of Alzheimer's Disease (AD), Fronto-temporal Dementia (FTD) and other tauopathies. The distribution of the pathology correlates with cognitive decline and suggests that abnormal forms of the protein may propagate across neuronal networks. We used the interhemispheric connectivity between both hippocampi to determine if critical factors such as isoforms of Tau and Amyloid beta pathology found in AD have an impact on the propensity of the Tau protein to propagate. Understanding under which conditions the Tau protein can propagate could be key to finding prospective therapeutic strategies, which may limit the spreading of the Tau pathology in neurodegenerative diseases.

Methods:

Overexpression of Tau protein is obtained by stereotactic injection of adeno-associated viral (AAV) vectors in the right hippocampus. We injected either the AAV8-PGK-4R0N Tau WT vector or the AAV8-PGK-4R2N Tau WT vector in the CA3 area of the right hippocampus in 12 weeks old mice, with two time points, 1.5 and 3 months post vector injection.
To determine the effect of the Aβ pathology on the Tau propagation and Tau pathology, we injected the AAV8-PGK-4R0N Tau WT vector in the CA3 area of the right hippocampus of 12 weeks old 5xFAD and control mice. We compared at 1.5 and 6 months post vector injection, 17 5xFAD mice and 17 WT littermate mice.

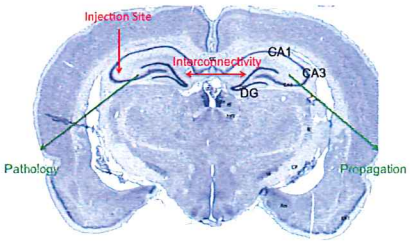


Fig. 1: Human Tau is overexpressed following unilateral injection in the CA3 region of the right hippocampus. We studied the development of the pathology in the ipsilateral injected side, assessed by AT8 and PHF-1 staining for Tau hyperphosphorylation and MC1 for Tau misfolding.
Hippocampal interconnectivity is used to assess Tau propagation to the contralateral non-injected hippocampus, and correlate the two observations. Propagation is assessed by HT7 staining for total human Tau.

Results:

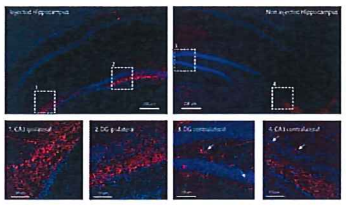


Fig. 2 : Coronal section of the hippocampus showing the expression of human WT Tau at 1.5 month after unilateral injection of AAV8-PGK-4R0NTauWT in the right CA3 area of a WT mouse. Infected neurons on the ipsilateral side show a high expression of human Tau stained with HT7. Note in the hilar region of the dentate gyrus and in the CA3, both in the contralateral non-injected hippocampus, the presence of several neurons that are positive for human Tau (arrows).

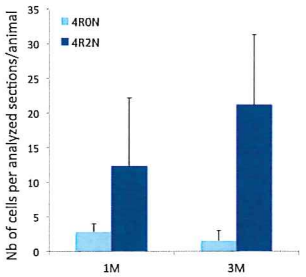
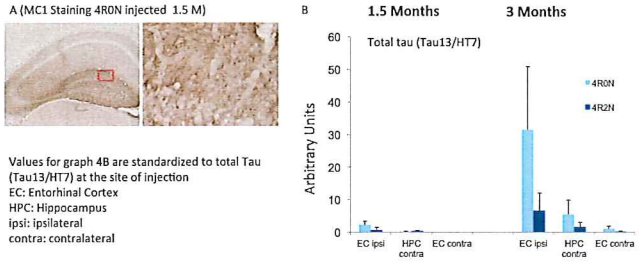


Fig. 3 : Total number of HT7 human Tau positive cells counted in the contralateral side on analyzed brain sections per animal.
Note that 4R2N Tau WT injected in WT mice tends to propagate more to distal neurons than the 4R0N Tau WT.
p=0.06 2-way ANOVA (N=5 per condition)



Values for graph 4B are standardized to total Tau (Tau13/HT7) at the site of injection
EC: Entorhinal Cortex
HPC: Hippocampus
ipsi: ipsilateral
contra: contralateral

Fig. 4: (A) Immunohistochemistry shows that pathological misfolding of tau (MC1) starts to appear at the injection side on the ipsilateral hippocampus at the level of axons and dendrites at 1.5 months.
(B) Total human tau is detected with AlphaLISA biochemical analysis (Tau13/HT7) and is found to accumulate in projection areas including the entorhinal cortex and the contralateral hippocampus in injected WT mice at three months post-vector injection.

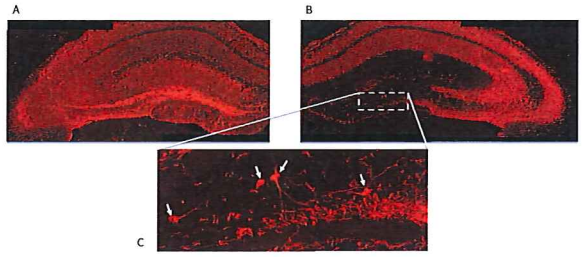


Fig. 5 : (A) Coronal section of the hippocampus showing the expression of human wild type Tau at 6 months after unilateral injection of AAV8-PGK-4R0N Tau WT in the right CA3 area of a 5xFAD transgenic mouse. Infected neurons on the ipsilateral side show a high expression of human Tau stained with HT7 in red. (B,C) Several neurons are positive for human Tau (arrows) in the contralateral non-injected hippocampus.

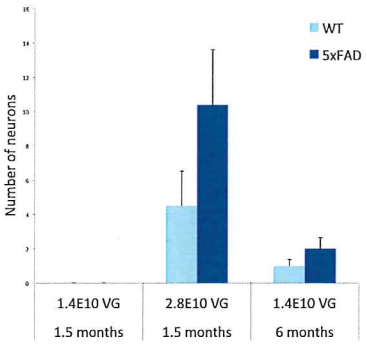


Fig. 7 : Number of human Tau positive neurons in the contralateral hippocampus. Note that the number of Tau positive neurons is higher in 5xFAD mice than in WT mice, as observed at 6 months in animals injected with 1.4E10 VG of AAV8-PGK-4R0N TauWT and already at 1.5 months with animals injected with the double dose of vector (2.8E10 VG).
The effect of 5xFAD is significant after a 3-way ANOVA and Tukey's post hoc analysis.
P=0.037

Conclusion:

- Isoform 4R2N tends to propagate more than isoform 4R0N towards distal neurons in the contralateral hippocampus.
- Misfolded forms of tau are mainly detected with MC1 by IHC near the site of vector injection, starting at 1.5 months post-injection. Hyperphosphorylation (PHF-1 and AT8) is not yet detectable at 1.5 months.
- The isoforms of tau have different propensity for propagation and development of pathological features such as misfolding, phosphorylation and aggregation.
- The rate of propagation of the Tau protein is higher in mice carrying the Aβ pathology, which indicates a possible cross-interaction between both pathologies. In Alzheimer's disease, this effect may contribute to the spread of the Tau pathology, which correlates closely to cognitive decline.