Focally Induced Tau pathology in APP_{SL} Mice Closer Mimics Alzheimer's Disease Spatio-Temporal Pathology

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BACKGROUND

AD brains are diagnosed under the co-existence of two main pathological hallmarks: (1) Neurofibrillary tangles (NFT) composed of hyperphosphorylated Tau aggregates, and (2) senile plaques comprising of insoluble β-amyloid (Aβ). Mentioned pathological events do not develop in the same brain region; while NFT production starts in the entorhinal (ERC) and transentorhinal cortex, Aβ plaque loading begins in the neocortex. Moreover, the onset of both pathologies is not triggered at the same time. Hence, we developed an inducible mouse model reproducing both spatio-temporal conditions aiming to obtain trustworthy results in AD preclinical studies.

MATERIALS AND METHODS

Recombinant Adeno-associated virus serotype 9/2 (AAV9/2) expressing human P301L Tau or empty vector was intracerebrally inoculated into the ERC of 3 months old male APP_{SL} mice and non-transgenic (Ntg) littermates.

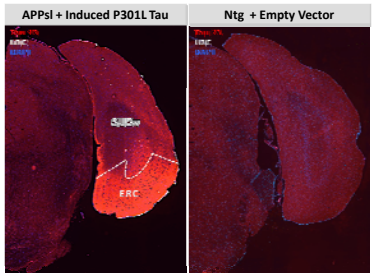


Figure 1. AAV-directed human Tau expression in ERC.
Surgery coordinates: AP:-4.2; ML:+/-3.4; DV:-4.5). ERC: Entorhinal cortex; SUBv: Subiculum.

RESULTS

Spread of Human P301L Tau to Connected Tissues

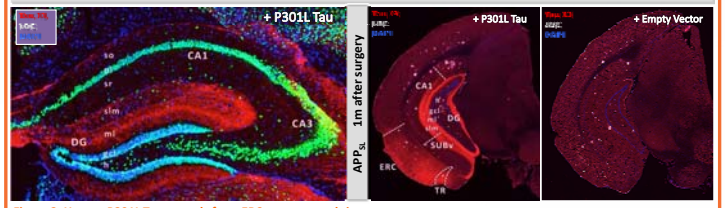


Figure 2. Human P301L Tau spreads from ERC to connected tissues.
Tau P301L focally injected in the ERC was found to spread to connected tissues already 1 month after injection. Immunofluorescence of human Tau in stratum lacunosum moleculare (slm) of CA1 and in the middle and outer molecular layer (ml) of the dentate gyrus (DG) suggest that Tau is transported via the lateral and medial perforant path, respectively. Time points analyzed: 1 and 6 months after injection. SUBv: subiculum; h: hilus; gcl: granule cell layer; so: stratum oriens; sr: stratum radiatum.

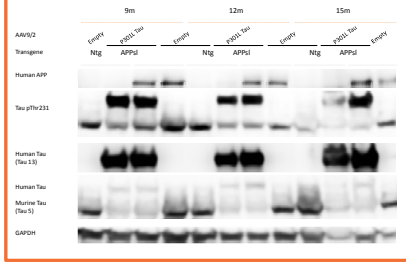


Figure 3. Concomitant expression of human P301L Tau and APP proteins.
Tau expression remained stable at least 12 months after surgery. Besides, it is increased at least 4-fold when compared to transgenic human APP protein.
On the other hand, Tau-5 antibody revealed possible down regulation of endogenous Tau when human Tau is present.
Higher amounts of Tau pThr231 are also present in Tau P301L injected animals in comparison to empty vector injected mice.

Sarkosyl soluble fraction from left hippocampus, pool of samples.

Tau Pathology: Pre-NFTs

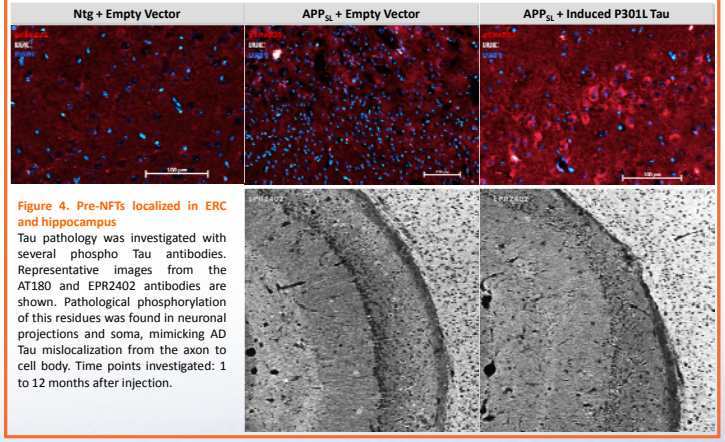


Figure 4. Pre-NFTs localized in ERC and hippocampus
Tau pathology was investigated with several phospho Tau antibodies. Representative images from the AT180 and EPR2402 antibodies are shown. Pathological phosphorylation of this residues was found in neuronal projections and soma, mimicking AD Tau mislocalization from the axon to cell body. Time points investigated: 1 to 12 months after injection.

Cognitive Impairment Worsens in APP_{SL} mice Inoculated with Human P301L Tau

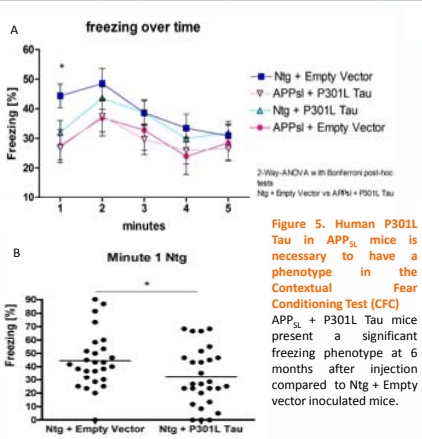


Figure 5. Human P301L Tau in APP_{SL} mice is necessary to have a phenotype in the Contextual Fear Conditioning Test (CFC)
APP_{SL} + P301L Tau mice present a significant freezing phenotype at 6 months after injection compared to Ntg + Empty vector inoculated mice.

However, a closer look exposes divergences during the first minute of test between Ntg groups. A) % of freezing over time. B) % of freezing during first minute of test. Mean + SEM (n=25+/-5). Statistical significance is indicated by *<0.05 as determined by Two-Way ANOVA (A) followed by Bonferroni post-hoc test or unpaired t-test (B).

Hints of increased cytotoxic Aβ in plasma

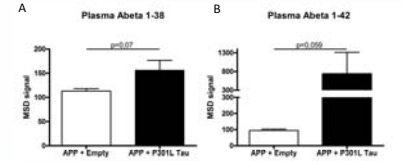
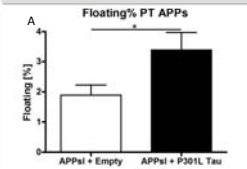
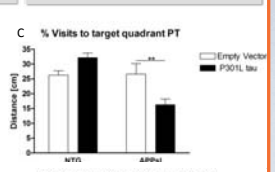


Figure 6. APP_{SL} mice inoculated with Tau P301L seem to present increased cytotoxic Aβ forms in the plasma.
MSD results from formic acid-processed plasma samples suggest that P301L Tau inoculated APP_{SL} animals display higher amounts of cytotoxic Aβ compared to empty vector injected APP_{SL} mice. A) MSD signal for Aβ 1-38. B) MSD signal for Aβ 1-42. Mean + SEM (n= 7+/-2). Unpaired t-tests.

6 months



9 months



12 months

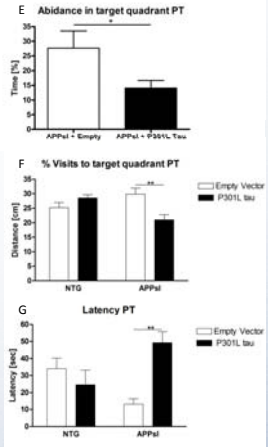


Figure 6. Cognitive impairment worsens over age in Morris Water Maze task (MWM) of longitudinally tested animals.
Thigmotaxic and floating behavior manifest during probe trial (PT) in APP_{SL} mice 6 months after injection. Statistically significant results are also found when analyzing memory loss 9 and 12 months after injection. Parameters such as time spent in or the number of visits to the target quadrant are altered. Moreover, latency to first visit area of absent platform is drastically increased in 15 months old P301L Tau injected mice. 6 months PT: (A) % floating. (B) % thigmotaxis. 9 months PT: (C) % of visits to target quadrant. (D) % time spent in target quadrant. 12 months PT: (E) % time spent in target quadrant. (F) % of visits to target quadrant. (G) Seconds needed to first cross the target zone. Mean + SEM (n=25+/-5). *p<0.05, **p<0.01, Two-Way ANOVA followed by Bonferroni post-hoc tests (C,F,G) and unpaired t-tests (A,B,D,E).

CONCLUSION

Histological and biochemical analysis revealed stable expression of human P301L Tau in **entorhinal cortex** and connected brain areas starting at 1 month after injection. Mice show a combination of the two main features of AD, a high density of senile plaques and pre-NFTs Tau. This new inducible mouse model is a promising tool which mimics spatio-temporal patterns of AD-related pathology.

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