Functional ultrasound imaging can detect vascular changes in Tau P301S mouse model of Alzheimer's disease

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1 INTRODUCTION

Tauopathies are neurodegenerative disorders characterized by the accumulation of abnormal tau protein leading to cognitive and/or motor dysfunction. Evidence suggests that tauopathy mouse models develop changes in blood vessels morphologies and density as well as altered cerebral blood flow that could be linked to cognitive deficits in Alzheimer´s disease. Here we assess changes in neurovascular coupling in Tau P301S mouse (PS19) using functional ultrasound (fUS) and its relationship to cognitive deficits and histological changes.

2 METHODS

At the age of 6 months, hemizygous (TauTg) P301S male mice and their WT littermates (nTg, n=15-18/group) were subjected to fUS imaging of the brain at D1 and D35, including resting state functional connectivity (RFC), somatosensory stimulation and acetazolamide challenge (ACZ, 30mg/kg, *i.v.*). At 7 months of age, radial arm water maze (RAWM, D28-30) and contextual fear conditioning (CFC, D31-D33) were used to assess behavioral impairments. At the end of the study (7 months of age, D35), brains were histologically assessed for microgliosis and synaptic density. All animal in the study were used in compliance with license for animal experiment approved by National Animal Experiment Board, following national and international regulations.

Experimental Groups:
1. nTg (n = 18)
2. TauTg (n = 15)

3 RESULTS

In the radial arm water maze, a significant genotype effect was observed between the nTg and TauTg mice in latency, with TauTg mice taking longer to reach the platform compared to nTg mice (Figure 1). nTg and TauTg animals displayed a similar pattern of CFC freezing, but a significant difference could be observed in the last 30 s interval of Day 2 (Figure 2).

Functional ultrasound imaging showed a genotype effect in all measurements, namely RFC, somatosensory stimulation and pharmacological challenge at D1 (Figure 3, 4). The somatosensory stimulation was in line with RFC data showing clear genotype effect on the somatosensory cortex, with the TauTg group having lower response in comparison to nTg group (Figure 3A). The vascular reactivity was assessed after ACZ challenge and showed a strong vascular reaction in nTg mice (~35% increase in power doppler signal, Figure 3B). Due to ACZ-related mortality in TauTg mice, the D42 timepoint ACZ challenge was not performed. The strongest phenotype difference in FC between nTg and TauTg was observed in decreased sensory-motor cortex as well as in cortico-hippocampal and to lesser extent in thalamic connectivity values (Figure 4).
The fUS data collected on D35 was characterized by lower signal-to-noise ratio compared to D1 data. Similar trends were observed in RFC but did not reach statistically significance between the groups. Due to lower signal-to-noise ratio at D35 somatosensory fUS, the stimulation failed to produce detectable responses in all groups, and thus no genotype effect was observed (data not shown).

IHC analysis at 7 mo of age showed increased CD68 % area values in the hippocampus, amygdala, piriform cortex, and fimbria in TauTg mice as compared to nTg group (Figure 5). TauTg had reduced levels of synaptic density compared to nTg group (Figure 6).

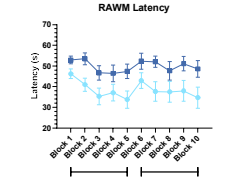

Figure 1. RAWM latency to platform. nTg (n = 18), non-carrier animals; TauTg (n = 15), hemizygous animals. Statistical analysis was performed using two-way ANOVA followed by Sidak's post hoc test. Significant main effect of Genotype ($F_{1, 390}$ = 41.27, p < .0001). Graph shows mean ± SEM.

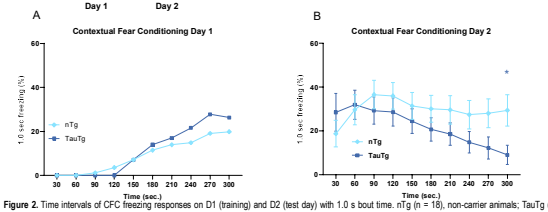

Figure 2. Time intervals of CFC freezing responses on D1 (training) and D2 (test day) with 1.0 s bout time. nTg (n = 18), non-carrier animals; TauTg (n = 15), hemizygous animals. Statistical analysis was performed using two-way RM ANOVA followed by Sidak's post hoc test. Comparison of group differences at the last time interval (Day 2, 300 s) was done using Mann Whitney test. *p < .05. Figure A shows mean and figure B shows mean ± SEM.

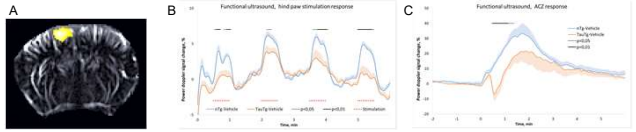

Figure 3. fUS of somato-sensory (hindpaw) stimulation (A – activation map and B – fUS signal time series) and vascular challenge with acetazolamide (C) at D1.
nTg (n = 13), non-carrier animals; TauTg (n = 13), hemizygous animals. Vascular challenge was performed in a subgroup of animals (n = 6 per group) due to poor tolerability. Statistical significances between timepoints: dashed black line p < 0.05, thick solid black line p < 0.01 (one-way ANOVA). Graph shows mean ± SEM.

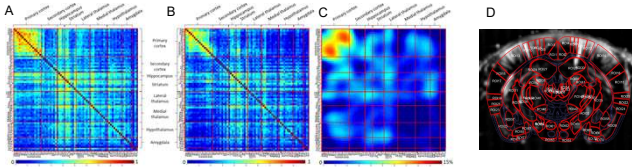

Figure 4. Early (D1) resting state fUS. Functional connectivity matrix of nTg (A) and TauTg (B) with group differences (C). Graph shows median positive correlation coefficient values between bilateral brain regions based on the stereotaxic coordinates and the Paxinos atlas of the mouse brain (D).

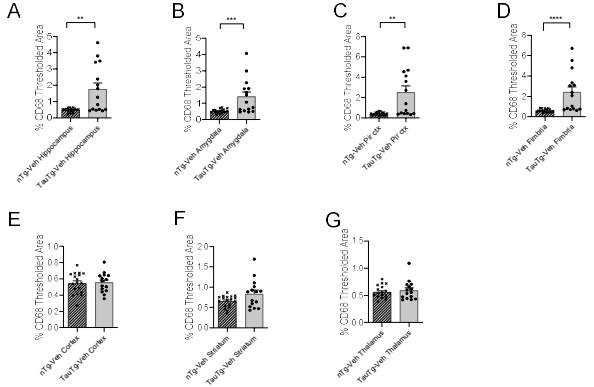

Figure 5. % CD68 of thresholded area, hippocampus (A), amygdala (B), piriform cortex (C), fimbria(D), cortex (E), striatum (F) and thalamus (G) at the age of 7 mo. nTg (n = 18), non-carrier animals; TauTg (n = 15), hemizygous animals. **p<0.01, ***p<0.001, ****p<0.0001, Mann-Whitney test (A-D); Student's *t*-test (E-G). Graph shows mean ± SEM

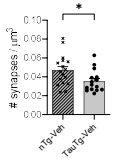

Figure 6. Synaptic density (A), presynaptic density (B) and post synaptic density (C) at the age of 7 mo. nTg (n = 18), non-carrier animals; TauTg (n = 15), hemizygous animals. *p<0.05, **p<0.01 Student's *t*-test. Graph shows mean ± SEM

4 SUMMARY

Functional Ultrasound (fUS) is a powerful tool for functional imaging of preclinical central nervous system disease models. It can provide functional information from the vasculature without invasive procedures.
Here we confirmed vascular changes in P301S mouse model accompanied by mild decline in cognition and effects on brain neuroinflammation and synaptic density. At the age of 6 months, resting state functional connectivity, particularly thalamo-cortical, intra-cortical and cortico-striatal connectivity, in the TauTg group was decreased as compared to nTg animals, accompanied by decreased responses to somatosensory stimulation and vasodilatation challenge, confirming the importance of vascular component of pathophysiology in P301S mouse model.
Immunohistochemical analysis revealed a genotype effect as seen in increased CD68 immunoreactivity and decreased synaptic density in TauTg mice compared to nTg counterparts.