Detection of Vascular Tau Pathology in Atherosclerotic Mouse Model

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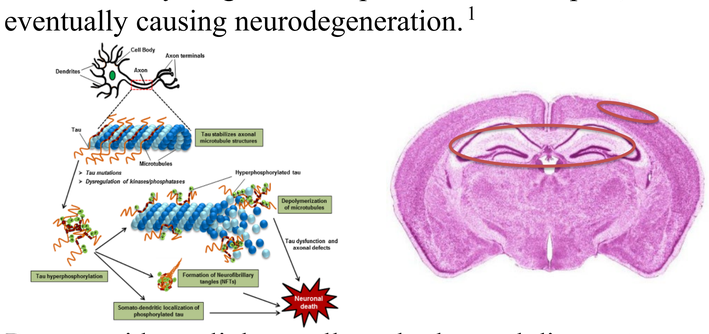
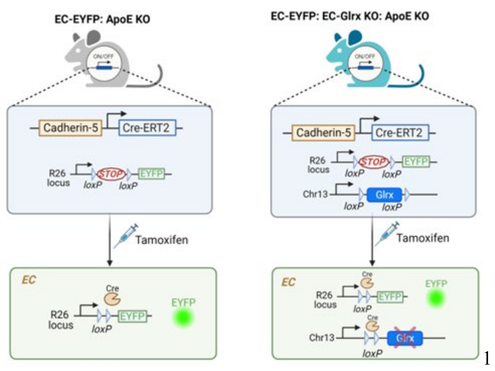
Background

- Vascular dementia describes problems associated with memory loss, cognitive impairment.
- The tau hypothesis: abnormal phosphorylation of tau forms neurofibrillary tangles and impairs axonal transport, eventually causing neurodegeneration.[1]



- Recent evidence links small cerebral vessel disease to vascular dementia.[2]
- Abnormal tau accumulates in specific brain regions involved in memory (hippocampus & entorhinal cortex).[2]
- ROS drives tau phosphorylation, which interacts with fibrinogen, promotes vascular damage.



- **Fig. 1 ApoE$^{-/-}$ background**: Cdh5(PAC)-CreERT2 mice (Taconic #13073) with R26-stop-EYFP reporter mice (JAX #006148), enabling tamoxifen-inducible EYFP labeling

Methods

Sample Preparation:

- Floating brain sections from the region of Bregma -1.0 to -2.8mm: wild-type (WT), Glutaredoxin-1 (Glrx1) knockout, WT with treatment, and Glrx1 knockout with treatment.
- Perfusion with 4% paraformaldehyde (PFA) in PBS

Treatment protocol:

- Angiotensin II infusion, high lipid diets; Glrx1 knockout

Immunofluorescent Staining:

- Washing solution: TBS+0.3% Triton-X
- Phosphorylated tau at Serine 202/Threonine 205 immunostained with AT8 antibody.
- Imaging through Keyence (BZ-X800 Model) Analyzer: DAPI, EYFP (GFP), NeuN (594 nm), AT8 (647 nm) channels; constant exposure time for AT8
- 20X magnification; image stitching, and Cell Profiler analysis used to fraction of cells with NeuN & AT8 signal

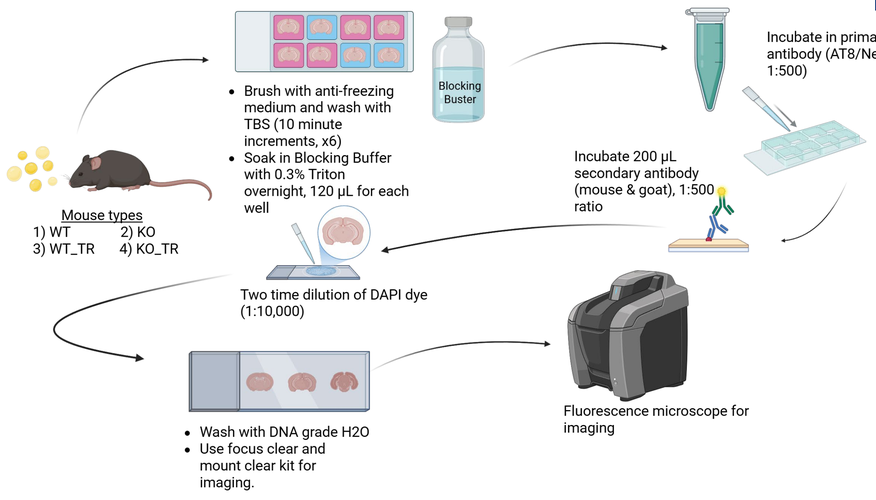
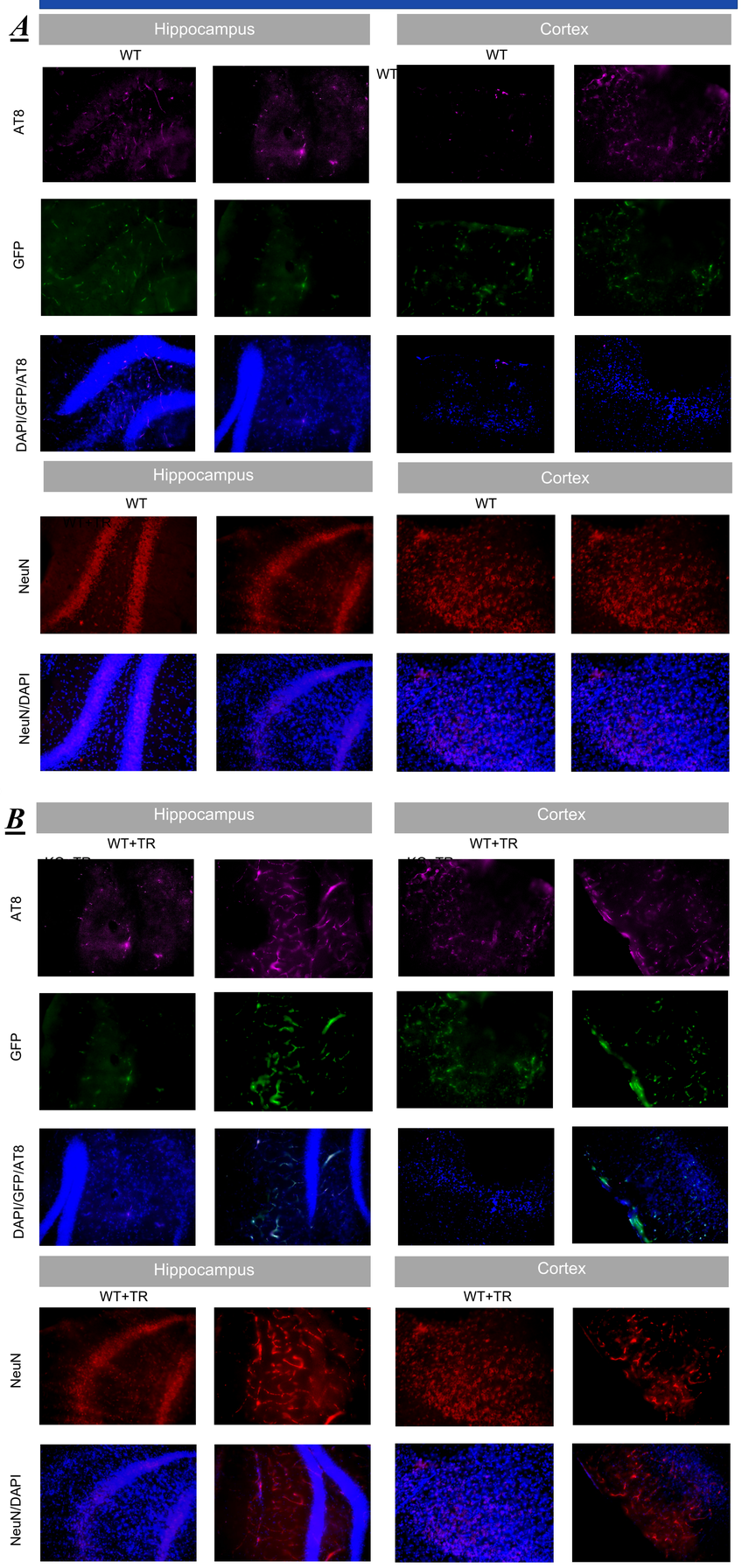


Fig. 2 Purpose of staining: EYFP and NeuN positive cells indicate endothelial and neuronal cells, allowing visualization of spatial distribution and vascular deposition.

Results



Analysis of Results

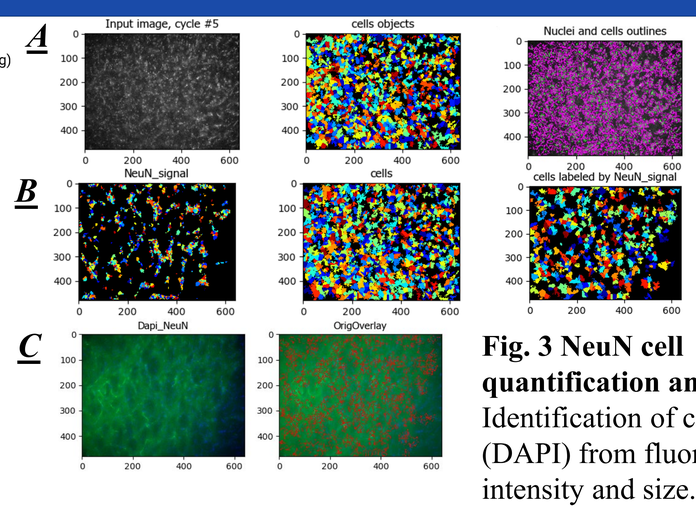


Fig. 3 NeuN cell quantification analysis ***A*** Identification of cells (DAPI) from fluorescence intensity and size.

B Identification of NeuN signal (not shown) mapped to cells; overlay of cells expressing NeuN signal. ***C*** Overlay shown of DAPI & NeuN with color/red represents NeuN positive cells. Same methods used for AT8

Results

Fig. 4 Treatment induced phosphorylated tau buildup: Tau and GFP in ApoE$^{-/-}$ Mice ***A*** Immunostaining of treatment mice AT8 (Cy5, magenta), GFP (Green), and DAPI (Blue) in CTX and HP regions. Comparison of ApoE-/- mice with or without AngII infusion and high lipid diet. ***B*** Comparison of ApoE-/- mice with or without Glrx1 knockout. In both images, NeuN (TxRed) staining shows concentration and distribution of nucleus.

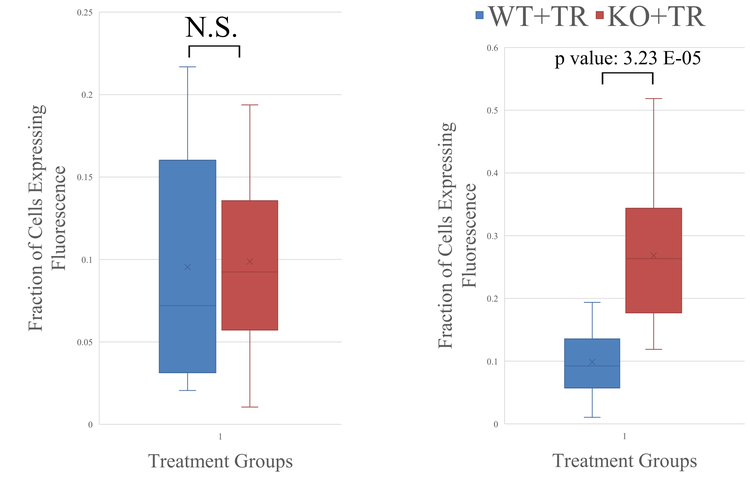


Fig. 5 Graphical Representation AT8 After Treatment & Knockout. ***A*** p value= 0.909656301 (>0.05, not statistically significant). ***B*** p value= 3.23034E-05 (<0.05).

WT	KO	WT+TR	KO+TR
0.55027	0.52747	0.46963	0.50481

Fig. 6 Mean decimal of NeuN Positive Cells

p value between WT and WT+TR = 0.0441 (<0.05). p value between WT + TR and KO+TR = 0.2159 (>0.05).

Conclusion/Discussion

Two statically significant conclusions through T-testing: AT8 after Knockout & NeuN value after treatment.

Averages align with expected results, but other comparisons did not yield statistically significant differences ($p > 0.05$), suggesting either minimal effect size, or insufficient sample size.

Further research into colocalization of GFP & AT8 provides insight into spatial distribution of tau protein.

References

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Figures created by BioRender

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