UNTANGLING ALZHEIMER'S DISEASE HALLMARKS IN SENSORY SYSTEMS OF RODENT MODELS

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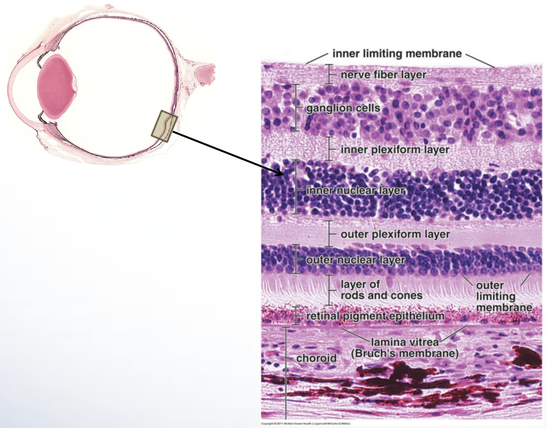
BACKGROUND

Alzheimer´s disease (AD) is the most common form of neurodegenerative dementia. Major hallmarks of the disease are: (1) extracellular plaque deposits of the β-amyloid peptide (Aβ) and (2) intracellular neurofibrillary tangles of hyperphosphorylated tau. Published research suggests an association between AD and functional impairments of sensory systems. In fact, the occurrence of Tau-mediated glaucoma has been recently reported, as well as AD protein-associated neuropathology in sensory systems (M. Chiasseu et al., 2016). The current study is designed to analyze features of AD-related pathological changes in mouse retina of an AD animal model and addresses suitable biomarkers for early screening tests of AD.

MATERIALS AND METHODS

Eyes from the rodent AD model TMHT and non-transgenic control mice, aged 6 and 12 months, were collected. TMHT mice express the longest human tau isoform Tau441 (2N4R) with two mutations, V337M and R406W, under regulatory control of the neuron specific murine Thy-1 promoter. Tissue was cryosectioned and histologically labelled by multichannel immunofluorescence (Zeiss AxioImager Z1 microscope) to analyze different neuronal and neuropathological markers using the following antibodies: ChAT (Millipore), GAD67 (1G102, Millipore), TH (Novus Biologicals), GFAP (Dako), IBA1 (Synaptic Systems), pThr231 Tau (PHF-6, Biolegend). All samples were digitized and immunofluorescent labelling was quantified by image analysis.



RESULTS

Analysis of different neuronal and neuropathological markers in the retina of TMHT and ntg mice showed mostly a signal increase in TMHT mice compared to ntg controls (Fig. 1A-F). Quantification of ChAT immunoreactive area (IR) revealed a significant increase of the IR area at the age of 6 and 12 months (Fig. 1A). TMHT mice showed no significant differences in GAD67 levels (Fig. 1B). The TH signal was significantly lower in 6 months old TMHT mice compared to ntg controls (Fig 1C). Representative images of ChAT, GAD67 and TH labeling are shown in Figure 2. Analysis of GFAP expression levels showed a highly significant increase in TMHT mice of both age groups compared to ntg controls. Furthermore, GFAP labeling revealed a significant decrease over age in TMHT mice (Fig 1D). IR area of Iba1 showed a significant increase in TMHT mice at the age of 6 and 12 months compared to ntg controls. In addition, there was a significant decrease over age in TMHT mice, similar to the observed decrease of GFAP labeling (Fig. 1E). 12 months old TMHT mice showed significantly increased pTau Thr231 levels compared to ntg mice. Additionally, TMHT mice showed a significant increase of pTau over age (Fig. 1F). Representative images of GFAP, Iba1 and pTau Thr231 are shown in Figure 3.

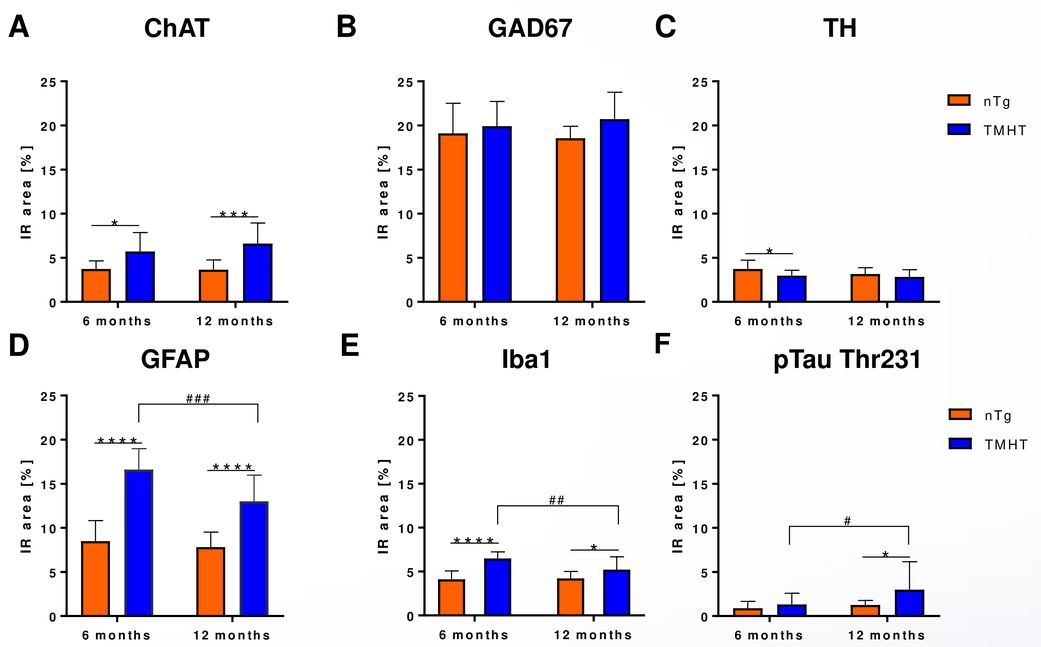


Figure 1. ChAT, GAD67, TH, GFAP, Iba1 and pTau Thr231 expression in mouse retina:
A: Choline acetyltransferase (ChAT) to label cholinergic cells; B: Glutamic acid decarboxylase 67 (GAD67) to label GABAergic cells; C: Tyrosine hydroxylase (TH) to label catecholaminergic cells; D: Glial fibrillary acidic protein (GFAP) to label astroglia; E: Ionized calcium-binding adapter molecule 1 (Iba1) to label microglia; F: Phosphorylated Tau at Thr 231 (pTau Thr231). n = 12-19 per group; mean + SEM; A-F: Two-way-ANOVA followed by Sidak's multiple comparisons *posthoc* test.

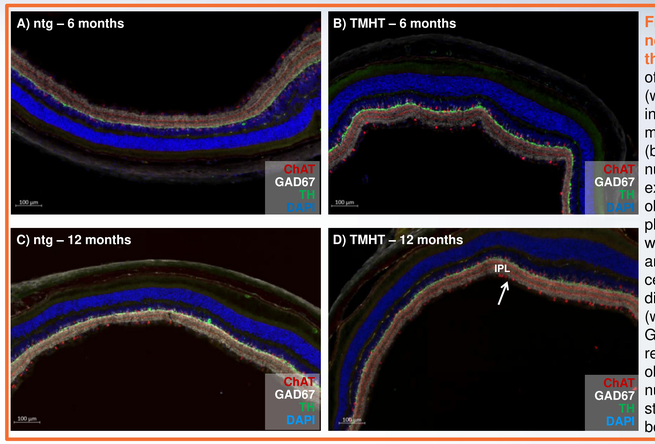


Figure 2: Detection of neurotransmitters in the retina. Co-labeling of ChAT (red), GAD67 (white) and TH (green) in ntg (**A**,**C**) and TMHT mice (**B**,**D**). DAPI (blue) to stain cell nuclei. Peak expression of ChAT is observed in the inner plexiform layer (IPL) where cholinergic amacrine and ganglion cell dendrites form two distinct synaptic strata (white arrow in **D**). GAD67 signal is restricted to IPL. TH is observed in the inner nuclear layer (INL), stratifying mainly at the border to the IPL.

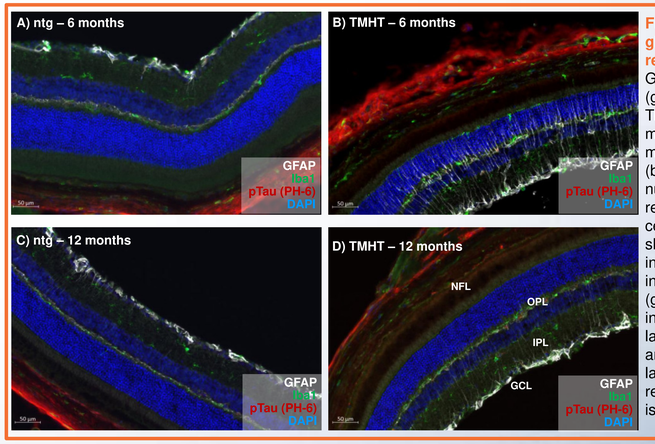


Figure 3: Detection of gliosis and tau in retina. Co-labeling of GFAP (white), Iba1 (green) and pTau Thr231 (red) in ntg mice (**A**,**C**) and TMHT mice (**B**,**D**). DAPI (blue) to stain cell nuclei. GFAP signal is restricted to ganglion cell layer (GCL) showing a significantly increased expression in TMHT mice. Iba1 (green) is expressed in the GCL, nerve fiber layer (NFL), and IPL and outer plexiform layer (OPL). The region labelled by pTau is the NFL.

CONCLUSION

The AD model employed in this study, together with the specific antibodies tested, provide us with a powerful tool to analyze neuropathology in the retina of TMHT mice. In fact, this study shows that there is a difference in the expression of the various neurotransmitters and neuropathological markers analyzed in TMHT compared to non-transgenic mice, not only at the age of 6 but also at 12 months. Further studies will now focus on the visual cortex of the animals analyzed in order to receive a detailed characterization of the visual system neuropathology.

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