THE PHYSIOLOGICAL FUNCTION OF TROPOMYOSIN 4.2 IN NEURONAL SIGNALING AND PATHOLOGICAL TAU UPTAKE

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ABSTRACT

Tropomyosins (Tpm) are master regulators of actin dynamics – a process crucial for synaptic plasticity and subsequently, learning and memory. Different Tpm isoforms have differential effects on filamentous actin dynamics in dendritic spines. As the Tpm4.2 isoform is highly expressed in the post-synapse[1], we sought to determine the physiological function of Tpm4.2 in neuronal signalling and synaptic plasticity using primary hippocampal neurons extracted from WT and Tpm4.2 KO mice (TP16). Using live calcium imaging and electrophysiology we report significant alterations in single cell kinetics of spontaneous spiking activity and significant impairments in neuronal connectivity in TP16 neurons compared with WT neurons - indicating an essential role for Tpm4.2 in neuronal signalling. Further, we identify significant alterations in TP16 receptor recycling at the post-synapse, suggesting that Tpm4.2 is essential for mechanisms underlying synaptic plasticity.

As Tpm4.2 is reported to play a role in bulk endocytosis, we seek to investigate its role in the trans-synaptic uptake of pathological tau protein. Using microfluidics and a tau AAV spreading construct, we identified alterations in trans-synaptic tau propagation in Tpm4.2 KO neurons compared with WT. In summary, our data suggest that Tpm4.2 plays a role in regulating actin dynamics at the synaptic compartment in neurons. To further elucidate mechanisms of pathological tau propagation, using the same microfluidic assay and tau AAV spreading construct, we report preliminary data that Amyloid precursor protein plays a role in pathological tau secretion from neurons.

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BACKGROUND

- Tpms are actin-associated proteins that regulate actin dynamics – a process crucial for neuronal development and function
- The Tpm4.2 isoform is present in growth cones and growing neurites during development however the physiological role of Tpm4.2 in neuronal growth, development and signaling has not yet been elucidated
- Tpm4.2 localizes at the post-synapse and plays a role in bulk endocytosis – suggesting potential involvement in pathological protein uptake
- Pathological tau protein propagates through the brain of patients with Alzheimer's disease (AD) and other tauopathies
- APP is involved in the regulation of neurite outgrowth and synaptic function and plasticity
- Amyloid Beta is a proteolytic product of APP and implicated in AD pathology

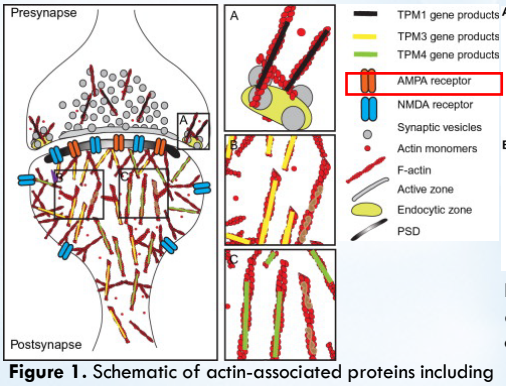


Figure 1. Schematic of actin-associated proteins including Tpm4 at a synapse. Adapted from Schevzov *et al.*, 2012

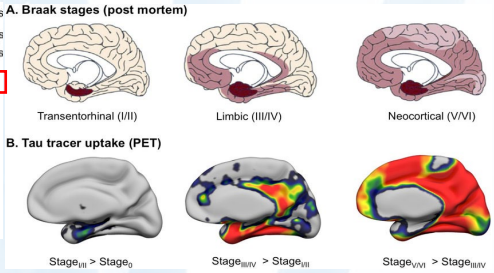


Figure 2. Schematic of Tau propagation in Alzheimer's disease. Adapted from Braak *et al.*, 2011 and Maass *et al.*, 2017

AIMS

1. Determine the physiological function of Tpm4.2 in neuronal signaling and synaptic plasticity
2. To investigate the role of Tpm4.2 in actin dyanamics
3. To investigate the role of APP in tau propagation

METHODS

Receptor internalisation assay

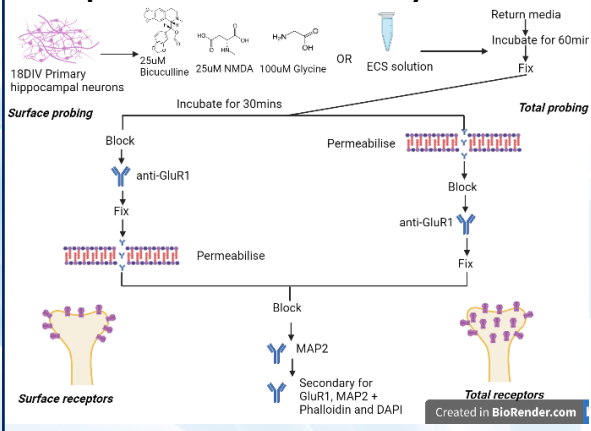


***Figure 3**. Receptor internalisation assay schematic*
Primary hippocampal neurons extracted from WT and TP16 transgenic mice were incubated for 30mins with Bicuculline, NMDA, Glycine or extracellular solution (ECS) or left untreated. Neurons were then fixed and immunolabelled to probe for surface or total GluR1 receptors. Neurons were imaged at 63x on a Zeiss AxioImager upright fluorescence microscope. GluR1 intensity in dendrites was then measured using ImageJ to obtain a ratio of surface to total receptors.

Calcium imaging

Primary hippocampal neurons extracted from WT or TP16 transgenic mice were transduced with Gcamp-eGFP-AAV and live imaging was performed at 12, 14, 16, 18 and 20DIV with a 5min time series with 500ms intervals.
Spontaneous spiking activity for single cells and network analyses were then conducted using MATLAB and Fluorosnnap software.

FRAP

Primary hippocampal neurons were transduced at 3DIV with AAV-hTpm4.2-mRuby2 and AAV-F-tractin-eGFP and imaged across two days (17-18DIV) using a Zeiss LSM880 inverted confocal microscope at 63x magnification. Intensity measurements in dendritic spines were taken prebleach, 3, 30, 60, 90 and 120secs after photobleaching. Rate of Fluorescence recovery was then fitted to one or two component exponential equations using a programming code written in MATLAB software.

Electrophysiology

Whole cell patch clamping was performed on primary neurons at 17 and 18DIV . Cells were perfused with extracellular solution which was pasued during recording on a Leica DM IL inverted microscope. After patching, 0.5uM TTX and 100uM picrotoxin was added. mEPSC recording were made at a holding potential of -70mV and measured using Axograph

Microfluidic assay

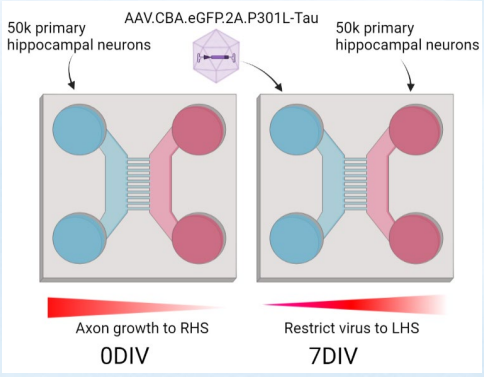
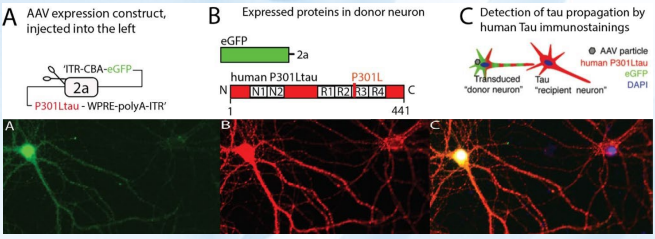


***Figure 4.** Schematic of microfluidic assay*
50,000 WT or APP KO primary hippocampal neurons were seeded into the left-hand side (LHS) of microfluidic devices. An osmotic gradient allowed axonal growth towards the right-hand side. At 7DIV 50,000 WT, TP16 or APP KO neurons were seeded into the RHS of the microfluidic and the AAV-P301L-2a-eGFP added into the LHS. The gradient was flipped to contain the AAV in the LHS. Neurons were fixed at 25DIV and immunolabelled for tau, eGFP, β3tubulin and DAPI. Positive neurons were counted using a Javascript code on ImageJ.

***Figure 5.** Schematic of tau spreading AAV construct adapted from Wegmann et al., 2015. 'Donor' neurons expressing eGFP (green) and tau (red) and 'recipient' neurons only expressing tau (red).*

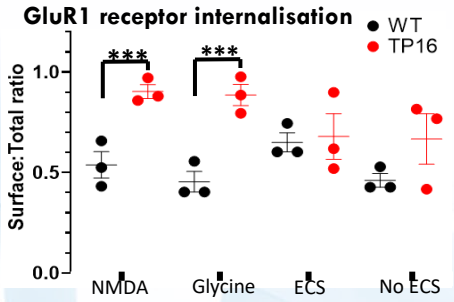


RESULTS

Effect of Tpm4.2 KO in neurons

Receptor recycling assay

Figure 6. *Effect of Tpm4.2 KO on GluR1 receptor recycling.* Stimulated TP16 neurons (red) demonstrate reduced receptor internalization compared with WT (black). These data indicate that Tpm4.2 is essential for GluR1 receptor recycling and plays a role in synaptic plasticity in mature neurons. ***p<0.001



Single cell calcium kinetics

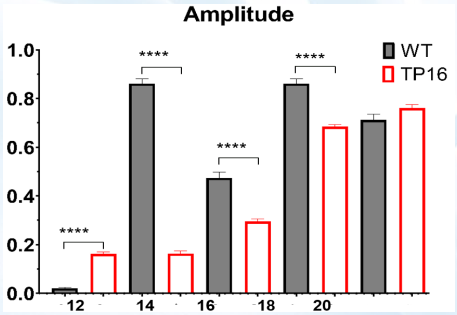
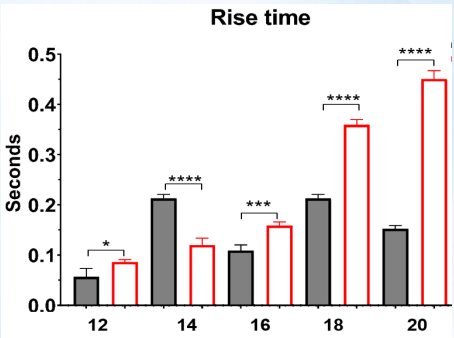
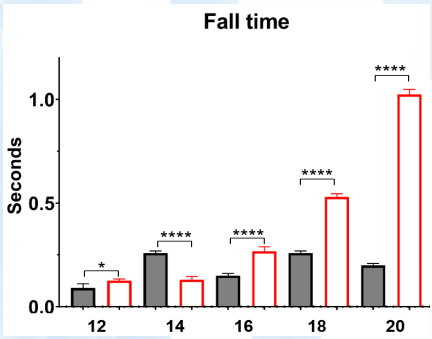


Figure 7. *Single cell kinetics from live calcium imaging of TP16 and WT neurons.* A) the amplitude of spontaneous spiking in single cells changes during development in TP16 neurons and is reduced in mature TP16 neurons compared with WT. Spike rise time (b) and fall time c) is increased in later stages of TP16 neuron development. .*p<0.05, **p<0.01, ***p<0.001

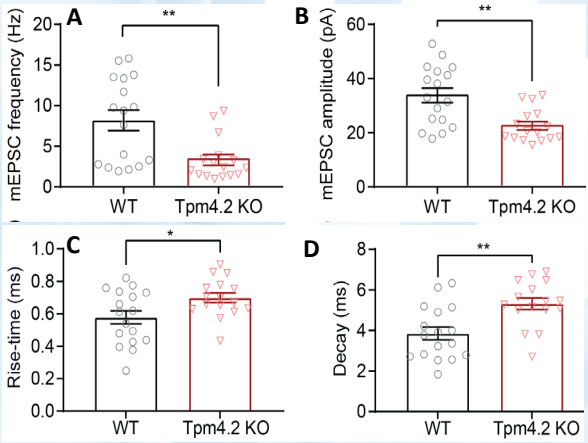




Whole cell patch clamping

Figure 8. *Tpm4.2 KO reduces miniature excitatory post-synaptic current (mEPSC) frequency and amplitude in primary neurons.* A) Mean mEPSC frequency and amplitude (b) were significantly decreased in Tpm4.2 KO neurons. C) Mean rise-time and d) decay time was increased in Tpm4.2 KO neurons compared to WT neurons.*p<0.05, **p<0.01

These data are consistent with live calcium imaging data, indicating Tpm4.2 plays a role in both stimulated and spontaneous firing activity.



RESULTS

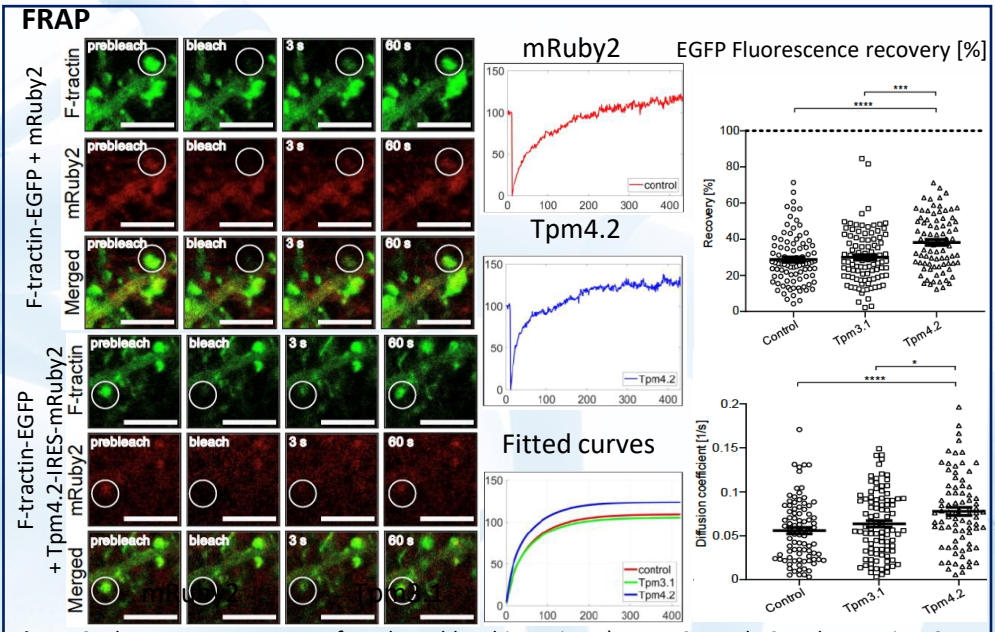


Figure 9. *Fluorescence recovery after photo-bleaching* using a) Tpm4.2-mRuby2 and F-tractin-eGFP. Tpm4.2 significantly increases recovery of F-actin pools to the dendritic spine after photobleaching indicating a role of Tpm4.2 in actin dynamics at the post-synapse. *p<0.05, ***p<0.001

Effect of Tpm4.2 and APP KO on Tau propagation

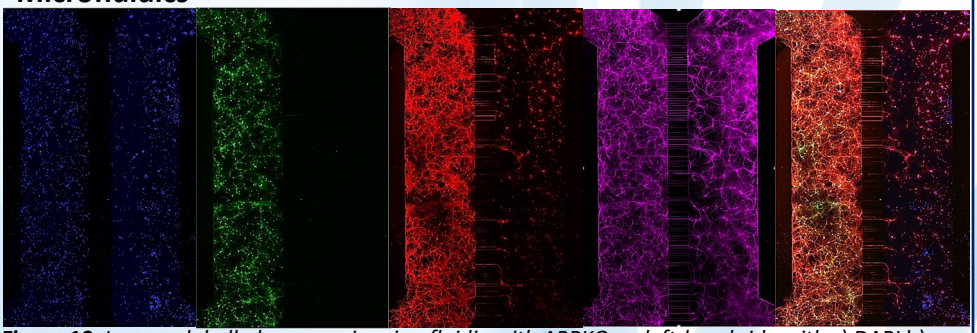


Figure 10. *Immunolabelled neurons in microfluidic with APPKO on left-hand side with* a) DAPI b) eGFP labelled AAV c) htau 555 d) B3 tubulin 647 e) merged image.

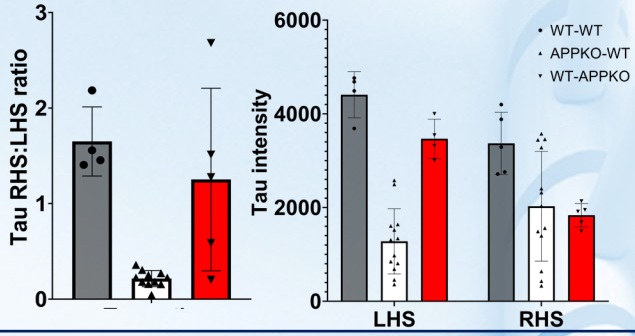


Figure 11. Tau secretion is affected by APP. APP KO neurons significantly alter human pathological tau secretion, suggesting a role of APP in increasing pathological trans-synaptic tau propagation in disease.

CONCLUSION

- Tpm4.2 KO reduced receptor internalization in primary hippocampal neurons signifying a role for Tpm4.2 in synaptic activity
- Tpm4.2 KO neurons had a lower spontaneous spike amplitude during development and longer rise and fall times
- Tpm4.2 is increased at the post-synapse and plays a role in facilitating F-actin pooling at the post-synapse
- Tpm4.2 reduces amplitude and increases both rise and fall time of neuronal firing
- APP increases pathological tau secretion and is implicated in facilitating pathological tau propagation in disease

REFERENCES

1. References: 1World Health Organisation (2020) Coronavirus disease 2019 (COVID-19) Situation Report (51).
2. Suzuki. M., Hotta, M., Nagase, A., Yamamoto, Y., Hirakawa, N., Satake, Y.,…Ikeda, M. (2020) The behavioral pattern of patients with frontotemporal dementia during the COVID-19 pandemic, Int Psychogeriatr, [1-4].