Overexpression of an FTLD Mutant of Tau Disrupts the Tau Interactome and Enhances Network Excitability in Primary Cortical Neurons

Katriona Hole[1], Jonathan Brown[2], Jody Mason[1] & Robert Williams[1]

1. Department of Biology and Biochemistry, University of Bath, United Kingdom;
2. College of Medicine and Health, University of Exeter, United Kingdom

Twitter: @KatyHole

Email: klh64@bath.ac.uk

INTRODUCTION

- Frontotemporal lobar degeneration (FTLD) can be caused by autosomal dominant mutations in the *MAPT* gene (FTLD-Tau), such as P301L.
- Pathological tau is hyperphosphorylated and mislocalizes to the post-synaptic compartment where it can cause synaptic disruption.

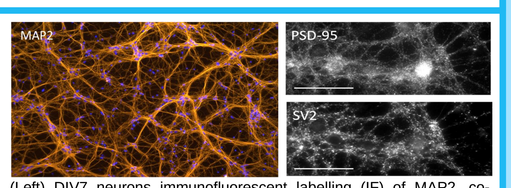
AIMS

1. Develop and characterise a neuronal model of tauopathy
2. Determine if there are any changes to network activity
3. Identify tau binding partners that might alter synaptic function

PRIMARY MOUSE CORTICAL NEURONS TRANSDUCED WITH AAVS CONTAINING eGFP-TAU CONSTRUCTS

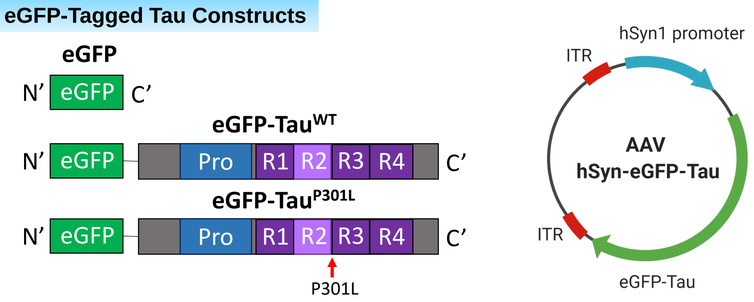
Primary Cortical Neurons

Primary cortical neurons from embryonic CD1 mice form extensive networks and active synapses in culture.

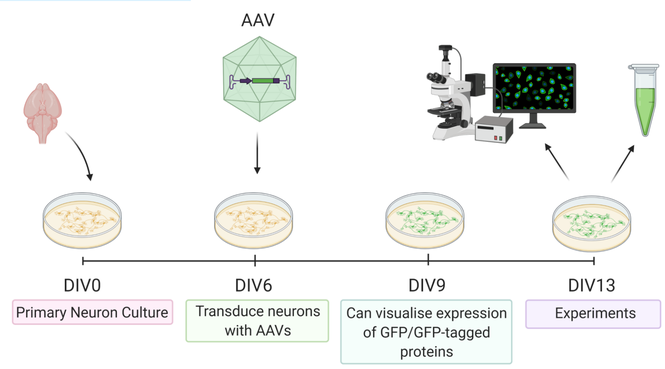


(Left) DIV7 neurons immunofluorescent labelling (IF) of MAP2, co-stained for DAPI (20X). (Right) DIV21 neurons following IF of the pre- and post-synaptic markers SV2 and PSD-95 respectively. Scale bar = 50µm

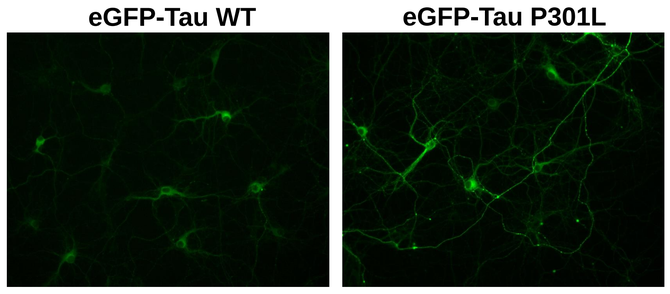
eGFP-Tagged Tau Constructs



Experimental Timeline

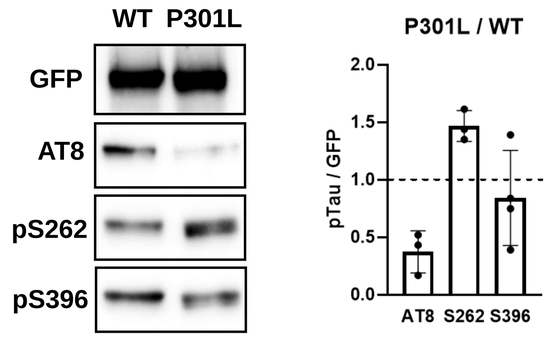


Transduced Neurons

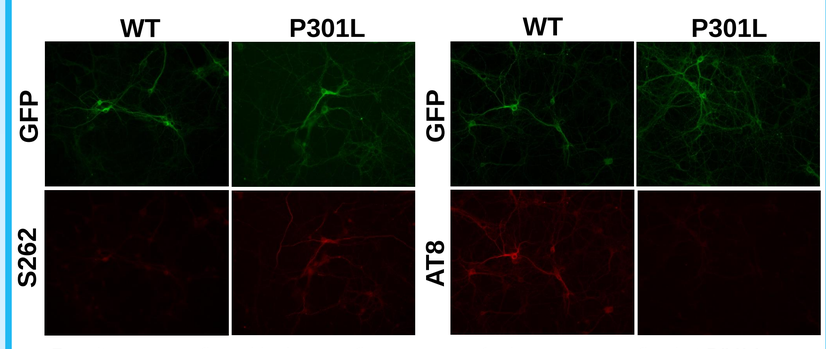


DIV14 primary cortical neurons overexpressing eGFP-tagged tau.
Note the increased levels of tau in neurons expressing eGFP-Tau P301L

P301L TAU IS DIFFERENTIALLY PHOSPHORYLATED TO WT TAU



P301L tau is more phosphorylated at Ser262 but less phosphorylated at AT8. Immunoblotting for different phospho-epitopes of tau, AT8 represents phosphorylation at Ser202/Thr205. Results were normalised to GFP (total tau) and then compared against WT tau. Error bars are +/- standard deviation, n = 3.



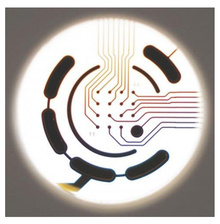
Results were confirmed by immunofluorescence of primary neurons fixed at DIV14.

IS NETWORK FUNCTION ALTERED IN PRIMARY CORTICAL NEURONS OVEREXPESSING eGFP-TAU P301L?

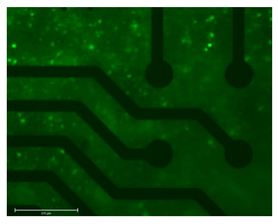
- Pathological tau is known to mislocalize to dendritic spines and disrupt synaptic function.
- Therefore we wanted to investigate whether network excitability was altered in this model.
- To do this, we used a multi-electrode array.

METHOD

- Primary neurons were plated onto a CytoView MEA plate at a density of 120,000 cells/well.
- Recordings were undertaken at DIV12.
- Baseline recordings were undertaken for 10 minutes.

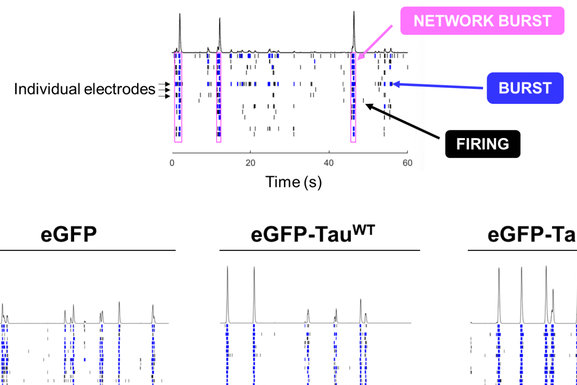


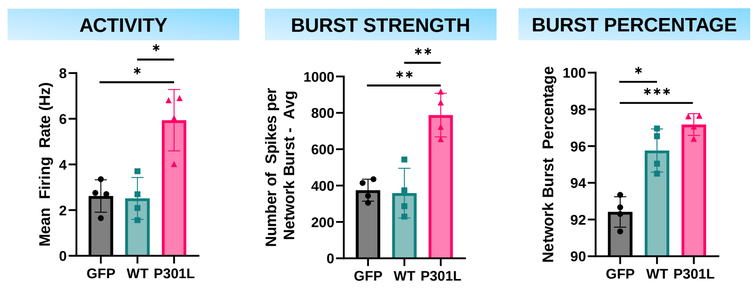
Each well contains 16 recording electrodes



Primary neurons overexpressing eGFP growing on the electrodes (scale bar = 275µm)

Maestro Pro MEA system (Axion Biosystems) was used to record electrical activity





Higher values suggest more frequent action potentials

Higher values suggest greater burst strength

What proportion of spikes contribute to network bursts?

KEY FINDINGS

- Overexpression of P301L tau **increases baseline excitability** in primary cortical neurons
- This could result in plasticity changes which make synapses vulnerable.

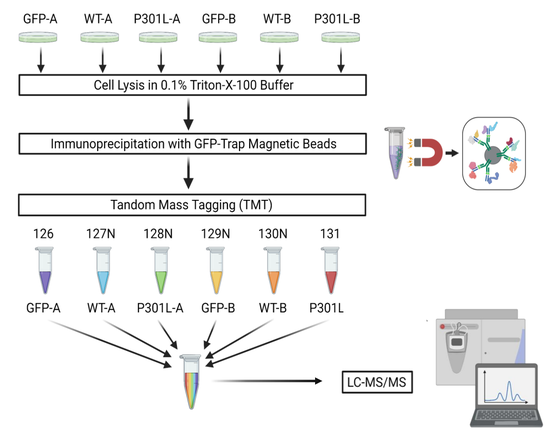
Overexpression of P301L tau increases network activity. Brown-Forsythe and Welch ANOVA tests with multiple comparisons. p* < 0.05, p** < 0.01, p*** < 0.001, p**** < 0.0001. Error bars are +/- standard deviation, n = 4.

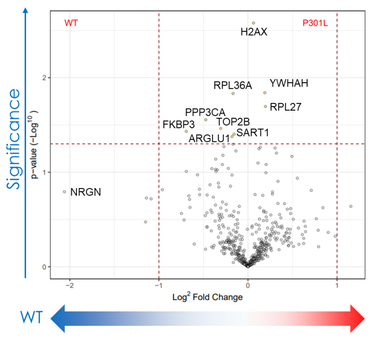
CAN THE TAU INTERACTOME EXPLAIN THE INCREASE IN BASAL EXCITABILITY?

In order to investigate how tau interactions might be affecting neuronal activity, a proteomics study was undertaken to identify the tau interactome.

METHOD

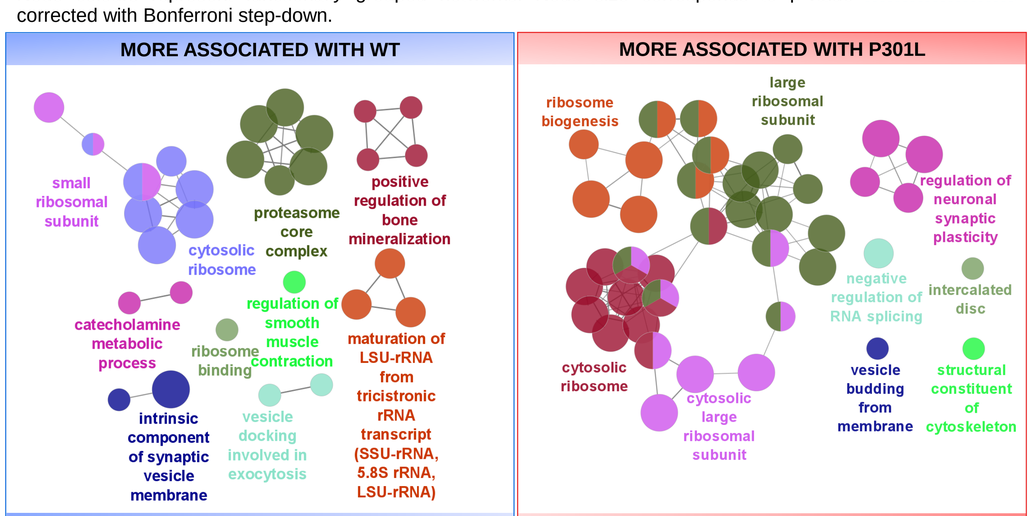
- A GFP-Trap was used to immunoprecipitate eGFP/eGFP-tagged tau and any proteins associated with it
- At the Bristol Proteomics Facility, samples were labelled by TMT to enable quantitative analysis and analysed by LC-MS/MS



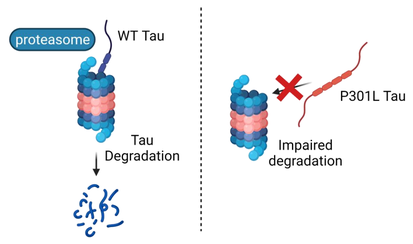


Differential binding of proteins to P301L tau. Volcano plot of proteins differentially bound to P301L tau over WT tau. Statistical significance was set at p<0.05, which is marked by the horizontal dashed line.

Differential association of proteins with WT and P301L tau. ClueGO Biological Process, Cellular Compartment and Molecular function analysis of proteins that were more associated with (left) WT tau over P301L tau or (right) P301L tau over WT tau in both repeats. Node colours represent functionally grouped networks. Node size corresponds to p-value corrected with Bonferroni step-down.



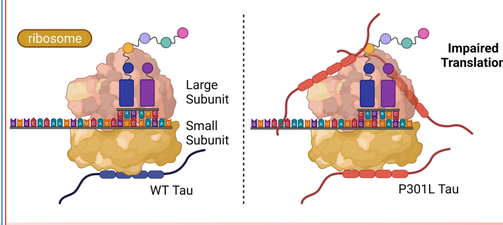
WT Tau Is More Associated With The Proteome



P301L tau has decreased association with the proteasome core complex compared to WT tau.

Impaired degradation of tau by the proteasome could be why we observe increased levels of P301L tau in our model.

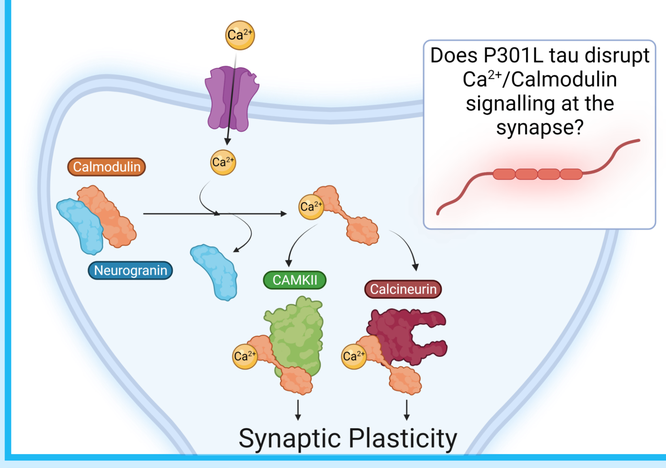
P301L Tau Is More Associated With The Ribosome

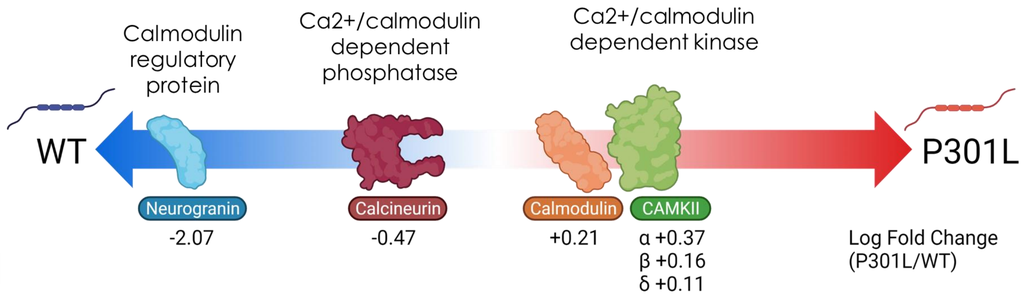


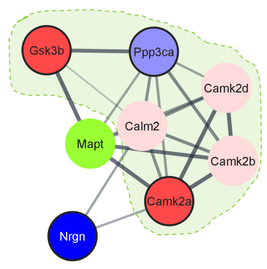
P301L tau has increased association with ribosomal subunits and proteins involved in ribosome biogenesis.

Pathological tau has been shown previously to impair translation. (Koren et al. 2020)

Tau Associates with Proteins Involved in Ca^{2+}/Calmodulin Signalling at the Synapse







STRING analysis of tau binding proteins associated with Ca^{2+}/Calmodulin signalling at the synapse. Node colours represent greater association with WT tau (blue) or P301L tau (red). Nodes with a black outline represent proteins that showed the same binding preference in both replicates. Direct binding partners of tau are highlighted with the green overlay.

Ppp3ca = Calcineurin, Calm2 = Calmodulin, Mapt = Tau, Nrgn = Neurogranin

CONCLUSION

In this model of tauopathy:

- P301L tau alters association with key Ca^{2+} dependent synaptic binding partners and enhances network excitability which could result in synaptic vulnerability
- Increased synaptic activity correlates with changes in tau phosphorylation, most notably an increase at pSer262

FUTURE WORK

- Link changes in synaptic proteome to enhanced excitability
- Investigate what effect pathological tau has on the Ca^{2+}/Calmodulin signalling pathway

ACKNOWLEDGEMENTS

This work was supported in part by grant MR/N0137941/1 for the GW4 BIOMED MRC DTP, awarded to the Universities of Bath, Bristol, Cardiff and Exeter from the Medical Research Council (MRC)/UKRI.

Figures were created with BioRender.com.

REFERENCES

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks.Bioinformatics. 2009, 25(8):1091-3.

Bindea G, Galon J, Mlecnik B, CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. Bioinformatics. 2013, 29(5):661-3.

Hole K, Staniaszek L, Balan Gayathri M, Mason J, Brown J & Williams R. (-)-Epicatechin inhibits progressive tau pathology in rTg4510 mice independent of direct actions at GSK3β. Frontiers in Neuroscience, 2021

Koren S, Galvis-Escobar S & Abisambra J. Tau-mediated dysregulation of RNA: Evidence for a common molecular mechanism of toxicity in frontotemporal dementia and other tauopathies. Neurobiology of Disease, 2020