Investigation of tau pathology in a P301S tau transgenic mouse model of Frontotemporal dementia

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A thesis submitted to Macquarie University in fulfillment of the requirements for the degree of Doctor of Philosophy

2019

Macquarie University

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Acknowledgements

First and foremost, I would like to thank my primary supervisor Prof. Lars Ittner for his constant support, encouragement and mentorship throughout my PhD. I am really fortunate to work alongside such a great mind and researcher who always inspired me to go a step further and opened up my mind and heart to the fascinating world of research and allowed me to find my real passion.

I would also like to thank my associate supervisor Dr. Janet van Eersel for all the encouragement, patience and guidance throughout the years and beyond. I wouldn't be where I am today if it wasn't for you. You really have been the greatest teacher I could have ever wished for and someone I have always looked up to. Thank you for all you've done and for keeping up with me! I am really looking forward to working with you in the future.

Thank you also to my two besties in the lab, Julz and Annika for all the laughter, love, support and most importantly for your friendship. You really are one of the reasons why we feel home away from home and I am super grateful that we have found each other.
I would also like to thank the other members of the Dementia Research Centre, especially Yazi, Astrid, Josi and Arne for all your help, encouragement and support in so many different ways. You all are great and unbelievably talented, and it has always been a pleasure working and laughing with you.

Thank you as well to my beautiful family in Germany, especially to my sister Jagoda and my parents Brigitte and Gregor Przybyla for your unconditional love, support and encouragement way beyond my PhD. Thank you for always being there for me and for teaching me to pursue my dreams, even if that meant to move to the other side of the world. You've always believed and encouraged me, and I will always be grateful for all you've done for me!

Last, but not least I would like to thank my husband Boris for going with me on this great adventure- without you this thesis wouldn’t have been possible. Thank you for all the prepared lunches and dinners, late night pick-ups and for your unconditional love and support throughout. Thank you for always believing in me, for being there for me when I needed you the most, for laughing at my bad jokes and most importantly thank you for loving me for who I am. You are my greatest hero!
This thesis is dedicated to you!

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)_____ Date: _____30/06/2019_____

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Publications during Candidature

1. **Przybyla M**, van Eersel J, van Hummel A, van der Hoven J, Harasta A, Müller J, Gajwani M, Mueller T, Stevens C H, Power J, Housley G, Karl M, Kassiou M, Ke Y D, Ittner A, Ittner L M (2019). Onset of hippocampal network aberration and memory deficits in P301S tau transgenic mice is linked to an immediate early gene signature, *in prep.* for submission to *Neuron*

2. van der Hoven J, van Hummel A, **Przybyla M**, Asih Prita R, Ke Y D , Ittner A, van Eersel J, and Ittner L M (2019). Contribution of endogenous antibodies to learning deficits and astrocytosis in human P301S mutant tau transgenic mice. Science Reports, *in revision*.

3. Watt G, **Przybyla M**, Van Eersel J, Ittner A, Ittner LM and Karl T (2019). Novel behavioural characteristics of male human P301S mutant tau transgenic mice - a model for tauopathy. Journal of Neuroscience, *submitted*.

4. Brettle M, Stefen H, Djordjevic A, Fok S, Chan J, van Hummel A, Van Der Hoven J, **Przybyla M**, Volkerling A, Ke Y D, Delerue F, Ittner L M, Fath T (2019). Developmental expression of mutant PFN1 in motor neurons impacts neuronal growth and motor performance of young and adult mice. *Frontiers in Molecular Neuroscience*.

5. Lei, M, Teo J D, Couttas T A, Duncan T, Song H, McEwen H P, Chesworth R, Bertz J, **Przybyla M**, Ittner L M, Fath T, Garner B, Ittner A, Karl T, Don A S (2019). Loss of sphingosine kinase 2 in Alzheimer's disease impedes amyloid deposition but enhances hippocampal volume loss and demyelination. *Journal of Neuroscience*, *in revision*.

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10. Schledde B, Galashan F O, **Przybyla M**, Kreiter A, Wegener D (2017). Task-specific, dimension-based attentional shaping of motion processing in monkey area MT. *Journal of Neurophysiology.*

11. Ittner A, Chua S W, Bertz J, Volkerling A, van der Hoven J, Gladbach A, **Przybyla M**, Bi M, van Hummel A, Stevens C H, Ippati S, Suh L S, Macmillan A, Sutherland G, Krill J, Silva A P G, Mackay J, Poljak A, Delerue F, Ke Y D, Ittner L M (2016). Site-specific phosphorylation of tau inhibits amyloid-β toxicity in Alzheimer's mice. *Science*.

12. **Przybyla M**, Stevens C H, van der Hoven J, Harasta A, Bi M, Ittner A, van Hummel A, Hodges J R, Piquet O, Karl T, Kassiou M, Ke Y D, Ittner L M, van Eersel J (2016). Disinhibition-like behaviour in a P301S mutant tau transgenic mouse model of frontotemporal dementia. *Neuroscience Letters*.

Publications included in this Thesis

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I. **Przybyla M***, Stevens C H*, van der Hoven J, Harasta A, Bi M, Ittner A, van Hummel A, Hodges J R, Piquet O, Karl T, Kassiou M, Ke Y D, Ittner L M, van Eersel J (2016). Disinhibition-like behavior in a P301S mutant tau transgenic mouse model of frontotemporal dementia. *Neuroscience Letters*.

*contributed equally

II. **Przybyla M**, van Eersel J, van Hummel A, van der Hoven J, Harasta A, Müller J, Gajwani M, Mueller T, Stevens C H, Power J, Housley G, Karl M, Kassiou M, Ke Y D, Ittner A, Ittner L M (2019). Onset of hippocampal network aberration and memory deficits in P301S tau transgenic mice is linked to an immediate early gene signature, *in prep.* for submission to *Neuron*

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	I.	**II.**
STUDY DESIGN	**MP**, CS, LI	**MP**, LI
PLANNING OF EXPERIMENTS	**MP**, CS, LI	**MP**, LI
DATA COLLECTION	**MP**, CS, JvH, AH, AvH, JvH, MB	**MP**, JvE, JvH, AvH, CS, AH, AI
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MANUSCRIPT REVIEW AND EDITING	**MP**, CS, LI, JvE, OP, YK, MK, TK, JH	**MP**, JvE, LI, JP, GH, TK, AI, YK, MK
ADDITIONAL EXPERIMENTS DURING REVISION	**MP**	

List of Abbreviations

ΔK280	deletion mutation within exon 10 at amino acid position 280
ΔN296	Deletion mutation within exon 10 at amino acid position 296
0N, 1N or 2N	zero, one or two amino- terminus inserts
3R or 4R	three or four microtubule binding repeats
AD	Alzheimer´s disease
ALS	amyotrophic lateral sclerosis
ANOVA	analysis of variance
APOE	apolipoprotein E
APOE-ε2	one of the three major apolipoprotein E isoforms
APOE-ε4	one of the three major apolipoprotein E isoforms
APP	amyloid precursor protein
APP	gene encoding APP
APP23	transgenic mice expressing human APP bearing the swedish (KM670/671NL) mutation
Arc	activity-regulated cytoskeleton-associated
Aβ	abeta amyloid
$A\beta_{40}$	amyloid-β_{40}- peptide
$A\beta_{42}$	amyloid-β_{42}- peptide
BSA	bovine Serum Albumin
bvFTD	Behavioural varaint FTD
C57BL6	C57 black 6, a common inbred mouse strain
°C	celsius degree
C9ORF72	chromosome 9 open reading frame 72
C9ORF72	gene encoding chromosome 9 open reading frame 72. Mutations in this gene are associated with ALS and FTD/MND
CA1	cornu ammonius region 1 (hippocampus)
CaMKII	calcium/calmodulin-dependent protein kinase II
CBD	Corticobasal degeneration

cDNA	complementary deoxyribonucleic acid
Cdk2	cyclin-dependent kinases 2
Cdk5	cyclin-dependent kinases 5
CK1	casein kinase 1
CK2	Casein kinase 2
CNS	central nervous system
C-terminus	carboxy terminus
D280K	deletion mutation in exon 10 of the tau gene
D296N	deletion mutation in exon 10 of the tau gene
DAPI	4',6-diamidino-2-phenylindole, fluorescent dye that binds DNA
DNA	deoxyribonucleic acid
E0-E16	Exon 0- Exon 16
E342V	tau mutation within exon 12 at amino acid position 342, changing it from glutamate to valine
EEG	electroencephalography
i.e.	for example
EPM	Elevated plus maze
EtOH	ethanol
E-NFTs	extracellular neurofribrillary tangles
FAD	familial Alzheimer’s disease
FTD	frontotemporal dementia
FTDP-17	frontotemporal dementia with parkinsonism linked to chromosome 17
FTLD-FUS	frontotemporal lobar degeneration with FUS positive pathology
FTD/MND	frontotemporal dementia with motor neuron disease
FTLD-tau	frontotemporal lobar degeneration with tau positive pathology
FTLD-TDP	frontotemporal lobar degeneration with TDP-43 positive pathology
FTLD-UPS	frontotemporal lobar degeneration with ubiquitin positive pathology

FUS	fused in sarcoma protein
FUS	gene encoding the fused in sarcoma protein
Fyn	non-receptor tyrosine kinases Fyn
G272V	tau mutation within exon 9 at amino acid position 272, changing it from glycine to valine
G389R	tau mutation within exon 13 at amino acid position 389, changing it from glycine to arginine
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GSK-3-β	glycogen- synthase kinase-3-β
IF	immunofluorescence staining
IHC	immunohistochemistry
I-NFTs	Intracellular neurofibrillary tangles
JNK	c-Junk N.- terminal kinase
K3	K369I mutant tau transgenic mouse
K257T	tau mutation within exon 9 at amino acid position 257, changing it from lysine to threonine
K369I	tau mutation within exon 12 at amino acid position 369, changing it from lysine to isoleucine
KO	Knock out
KXGS	KXGS motifs targeted by non- proline directed kinases within the microtubule binding domain
L266V	tau mutation within exon 9 at amino acid position 266, changing it from leucin to valine
L284L	Silent tau mutation within exon 10 at amino acid position 284
MAP1	microtubule-associated protein 1
MAP2	microtubule-associated protein 2
MAP4	microtubule-associated protein 4
MAPK	Mitogen- activated protein kinase
MAPT	Microtubule-associated protein tau
MAPT	gene encoding tau
MBD	microtubule-binding-domain
MND	motor neuron disease

MTs	Microtubules
MWM	Morris Water Maze
N279K	missense tau mutation within exon 10, at amino acid position 279, changing it from asparagine to lysine
N296H	missense tau mutation within exon 10 at amino acid position 296, changing it from asparagine to histidine
N296N	silent tau mutation within exon 10 at amino acid position 296
NFTs	Neurofibrillary tangles
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
OF	Open Field
P301L	tau mutation at amino acid position 310, changing it from proline to leucin
P301S	tau mutation at amino acid position 310, changing it from proline to serine
p38	p38-kinase
PBS	phosphate buffered saline
PD	Parkinson's disease
PDPK	proline-directed kinases that phosphorylate serine and threonine motifs
PFA	paraformaldehyde
PGRN	progranulin
PGRN	gene encoding for the protein progranulin. Mutations in this gene are associated with amyotrophic lateral sclerosis
PHF	Paired helical filaments
PHF-1	antibody against tau phosphorylated at epitopes Ser396 and Ser404
PiBs	Pick bodies
PiD	Picks disease
PKA	cyclic- AMP- dependent kinase
PNFA	Progressive nonfluent aphasia
PPA	Collective term for PNFA and SD

pR5	P301L mutant tau transgenic mice
PS-1	gene encoding Presenilin-1
PS-2	Gene encoding Presenilin-2
PSP	progressive supranuclear palsy
PTMs	Post-translational modifications
qPCR	quantitative PCR
R5H	tau mutation within Exon1 at amino acid position 5, changing it from arginine to histidine
R5L	tau mutation within Exon1 at amino acid position 5, changing it from arginine to leucin
R406W	tau mutation within Exon 13 at amino acid position 405, changing it from arginine to tryptophan
Rev	reverse
RNA	ribonucleic acid
RNAseq	RNA sequencing
mRNA	messenger RNA
rpm	revolutions per minute
S320F	tau mutation within exon 11 at amino acid position 320, changing it from serine to phenylalanine
S305N	Missense tau mutation within exon 10 at amino acid position 305, changing it from serine to asparagine
S305S	Silent tau mutation within exon 10 at amino acid position 305
SD	standard deviation
SF	Straight filaments
Src kinases	non-receptor tyrosine kinases Src
Tauopathies	Umbrella term for neurodegenerative disorders that are characterized by tau pathology
Tau13	antibody against human tau
TAU58/2	P301S mutant tau transgenic mice
tau-KO mice	tau deficient mice

TARDPB	gene encoding the transactive response DNA binding protein **43** kDa. Mutations in this gene are associated with amyotrophic lateral sclerosis
TDP-43	transactive response DNA binding protein **43** kDa
Thy.1	Thymus cell antigen 1
TREM2	Triggering receptor expressed on myeloid cells 2
V337M	tau mutation at amino acid position 337, changing it from valine to methionine
VCP	valosin-containing protein/p97
VCP	gene encoding for the valosin-containing protein/p97. Mutations in this gene are associated with amyotrophic lateral sclerosis
WT	Wild type

Abstract

Alzheimer´s disease (AD) and frontotemporal dementia (FTD) are two of the most prevalent causes of dementia. Both neurodegenerative disorders are characterized by intracellular neurofibrillary tangles (NFT), composed of hyperphosphorylated protein tau (Gotz and Ittner, 2008, Arendt et al., 2016). Under physiological conditions, tau promotes and regulates microtubule dynamics, which emphasizes the important role of tau in the development, maintenance and function of neurons and its contribution to neuronal viability (Bunker et al., 2006). However, in the pathogenesis of neurodegenerative disorders tau is abnormally phosphorylated, dissociates from microtubules and may thereby contribute to microtubule breakdown and axonal transport dysfunction. Following this, tau undergoes secondary modifications and accumulates into insoluble NFTs.

With the identification of FTD mutations in the gene encoding tau, *MAPT*, a direct linkage between tau gene mutations and neurodegenerative disorders was established and further emphasized that tau dysfunction *per se* is sufficient to cause neurodegeneration and cognitive decline. Since then, transgenic mice carrying pathogenic *MAPT* mutations have been generated which have provided new insights into the pathomechanistic role of tau in disease and have significantly contributed to the understanding of mechanisms underlying the pathophysiology of AD and related tauopathies.

Recently, we characterized a novel tau transgenic mouse line, known as TAU58/2. These mice express the human tau P301S mutation under the control of the Thy1.2 promoter, which in mice restricts the transgene expression to neurons. The TAU58/2 transgenic mice recapitulate essential features of AD, FTD and related tauopathies, including the presence of hyperphosphorylated human tau, abundant NFT formation throughout the brain and spinal cord and neuronal spheroid formation, prior to tau deposition. In addition, histopathological markers were accompanied by an early onset of progressive motor deficits, including a decline in motor strength, balance and coordination. However, our original study primarily focused on the motor deficits of the TAU58/2 and did not determine whether expression of P301S mutant human tau also affected other functional outcomes. Therefore, this thesis aims to determine

the role of P301S mutant human tau in the development of behavioural, functional and cognitive deficits in the TAU58/2 transgenic mouse line.

The first part of this thesis focusses on the question of whether the expression of P301S mutant human tau impacts on other aspects of behaviour, aside from motor impairments. Here, I determined that TAU58/2 mice develop progressive and early onset disinhibition-like behaviour and increased motor activity when subjected to the elevated plus maze (EPM) and the Open field apparatus, respectively. Further, I determined that these behavioural changes are accompanied by early and progressive tau deposition in the amygdala. Interestingly, significant tau pathology and atrophy of the amygdala has been observed in early stages of FTD and related tauopathies. Considering the importance of the amygdala in controlling behaviour, early onset of pathology and behavioural changes in the EPM in TAU58/2 mice suggests a contributing role to the clinical presentation of FTD.

The second part of this thesis characterizes the effects of transgenic P301S mutant human tau expression on neuronal network function in the murine hippocampus. Here, I found that the onset of progressive spatial learning deficits in TAU58/2 transgenic mice were paralleled by deficits in long-term potentiation (LTP) and neuronal network aberrations using electrophysiological and electroencephalography (EEG) recordings, respectively. Further, gene-expression profiling at onset of deficits in TAU58/2 mice revealed a signature of immediate early genes (IEG) that is consistent with neuronal network hypersynchronicity. Finally, I determined that increased IEG activity was confined to neurons harboring tau pathology, providing a cellular link between aberrant tau and network dysfunction. Taken together, our data suggests that tau pathology drives neuronal network dysfunction through hyperexcitation of individual, pathology-harboring neurons as a major contributor to memory deficits. Both studies provide new insights into the pathomechanistic role of tau in disease and may thereby assist in the identification of new targets for future translation into therapy.

1. Introduction

1.1 Dementia and Neurodegeneration

Over the past century, life expectancy of the human population has steadily increased due to significant improvements in medical health care, sanitation, nutrition and housing (Jamison, 2006, Niccoli and Partridge, 2012). Presently, we are confronted with a demographic change culminating in a rapidly growing aged population globally. However, longevity raises considerable socioeconomic consequences, since aging is known as the most common risk factor for neurodegenerative diseases, with dementia being its greatest burden (Yankner et al., 2008, Winblad et al., 2016).

Today, approximately 50 million people are affected by dementia worldwide (Querfurth and LaFerla, 2010, Alzheimer's Disease International, 2018), with nearly 10 million people being diagnosed with dementia every year (World Health Organization, 2017, December), resulting in one new case every three seconds (Alzheimer's Disease International, 2018). In fact, considering the predicted increase in life expectancy, there will be at least a triplication of dementia patients by 2050 (Alzheimer's Association, 2019, Alzheimer's Disease International, 2018).

Consequently, understanding, deciphering and preventing neurodegenerative illness, in particular dementia, is of major importance (Gotz et al., 2012).

1.2 Alzheimer´s disease

Alzheimer´s disease (AD) is the most common form of dementia, accounting for 60-80% of all dementia cases (Alzheimer's Association, 2019). AD is mainly characterized by the impairment and degeneration of neurons, resulting in progressive memory loss and cognitive decline (Gotz et al., 2012).

Depending on disease etiology, two types of AD can be differentiated; The vast majority (>95%) of AD cases are considered to be sporadic and typically affect people after the age of 65. These cases are therefore referred to as sporadic or late-onset AD

forms. Although a purely genetic linkage has not been reported in these cases, genetic predispositions were highlighted as possible risk factors (Blennow et al., 1994, Blennow, 2004, Lambert et al., 2005). In this regard, apolipoprotein E (APOE), in particular the ε4 allele, is probably the most prevalent genetic risk factor for sporadic, late-onset Alzheimer's disease (Verghese et al., 2011, Liu et al., 2013). Further, several studies have shown, that APOE-ε4 increases the pathogenic effects of amyloid beta (Aβ) - one of the two hallmark lesions found in AD - by inhibiting its clearance and/or stimulating Aβ aggregation and its deposition (LaDu et al., 1994, Strittmatter et al., 1993, Sanan et al., 1994). Consequently, carriers of one or two APOE-ε4 allele(s) have a respective 3- or 12-fold higher risk of developing AD, whilst i.e. the APOE-ε2 variant of APOE was shown to negatively correlate with AD (Verghese et al., 2011).

The second type of AD, referred to as familial AD (FAD), accounts for 1- 5 % of all AD cases, and is typically associated with an earlier disease onset, usually affecting people in their 30s to 50s (Ulrich et al., 2017). FAD features autosomal dominant patterns of inheritance, represented by pathogenic mutations in genes encoding the amyloid precursor protein (APP), presenilin-1 (PSEN1), or presenilin-2 (PSEN2) (Lambert et al., 2005, Morrissette et al., 2009). In addition, more recently discovered mutations in the gene encoding for TREM2, are thought to be primarily associated with a higher risk of developing late-onset AD (Jonsson et al., 2013, Guerreiro et al., 2013) and FTD (Paloneva et al., 2002).

Both familial and sporadic types of AD display the typical neuropathological hallmarks of AD (**Figure 1**): extracellular amyloid-β-plaques, mainly composed of amyloid-beta- peptides ($Aß_{40}$, $Aß_{42}$); and intracellular neurofibrillary tangles (NFTs), consisting of tau protein inclusions (Gotz et al., 2011).

Aβ is a polypeptide ranging from 38-43 amino acids in length. It is generated by sequential proteolytic cleavage of APP (Glenner and Wong, 1984, Masters et al., 1985), which is thought to be involved in neuronal plasticity and synapse regulation (Turner et al., 2003, Priller et al., 2006). Proteolytic APP processing is characterized by two consecutive cleavage steps. First, extracellularly, by β-secretase, which generates the amino terminus of Aβ and second, by γ-secretase (a complex containing PSEN1 and PSEN2), which dictates its length, and also determines the hydrophobic properties of its C-terminus which has an impact on the self-aggregation propensities of each Aβ peptide (Gotz and Ittner, 2008, Gotz et al., 2008a). Of the resulting Aβ

peptides, $A\beta_{40}$ is the more prevalent one (90%) whereas $A\beta_{42,}$ is far more prone to self-aggregation, oligomerization and fibril formation and therefore considered to be crucial to the early stages of fibril deposition into plaques.

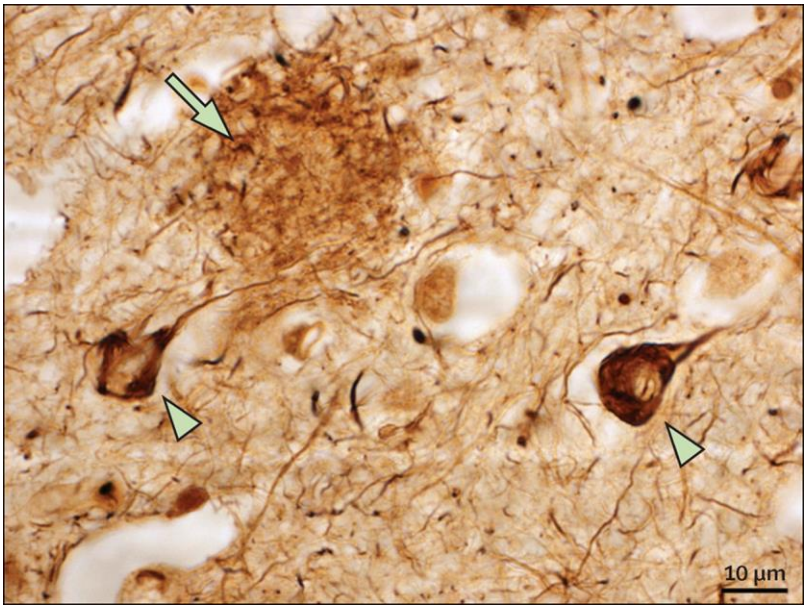

Figure 1: Neuropathological hallmarks of Alzheimer's disease

Cortical, post-mortem tissue from an AD patient, showing an extracellular amyloid-β plaque (arrow), composed of aggregated Aβ-fibrils, and neurofibrillary tangles (arrowheads), composed of hyperphosphorylated tau. Both neuropathological hallmarks of AD are labelled with Bielschowsky silver staining (image obtained with permission from (Winblad et al., 2016)).

However, unlike previous assumptions, APP processing by β-secretase and the generation of $A\beta_{42}$-fragments in particular, does not necessarily have to be pathogenic, since this part of APP processing takes place under normal physiological conditions i.e. during hippocampal long-term potentiation, (Garcia-Osta and Alberini, 2009, Li et al., 2009, Puzzo et al., 2011) although less frequent than in disease (Fukumoto et al., 2002). Moreover, several studies have shown, that short exposure to picomolar levels of $A\beta_{42}$ positively modulated synaptic plasticity and memory function in vivo (Puzzo et al., 2008, Koppensteiner et al., 2016) and in vitro (Koppensteiner et al., 2016), whilst prolonged exposure to $A\beta_{42}$ or increased levels of $A\beta_{42}$ towards nanomolar

concentrations were shown to facilitate deficits in synaptic transmission and neuronal loss (Puzzo et al., 2008, Koppensteiner et al., 2016).

In fact, the crucial role of $A\beta_{42}$ and its contribution to AD is rather thought to result from APP misprocessing and/or reduced Aβ clearance, leading to an elevated $A\beta_{42}$ over $A\beta_{40}$ production, which is thought to result in gradual accumulation of Aβ to oligomers, polymers and finally to amylogenic fibrils (toxic-gain-of-function).

Although, numerous *in vitro* and *in vivo* experiments point to Aβ-oligomers and insoluble forms of Aβ as being the toxic species in AD, none of the therapeutic interventions targeting those species have been successful or have improved clinical outcomes in AD patients (reviewed in (Brothers et al., 2018)). The lack of successful disease-modifying strategies over the past two decades is one reason why the focus of research has shifted towards the other potential treatment candidate: tau, which in the form of NFTs is the second hallmark lesion of AD. Another reason for the increasing interest in tau as a potential treatment target is that, even though Aβ deposition may be closely associated with the onset of AD, the temporal and spatial distribution and propagation of tau, but not Aβ pathology, strongly correlates with the extent of cognitive and clinical symptoms in AD (Serrano-Pozo et al., 2011, Ittner and Ittner, 2018). Additionally, Roberson and others showed that tau may play a crucial role in the development of Aβ-toxicity, since reduction of tau levels improved Aβ-induced deficits in a mouse model of AD. In fact, crossing Aβ-producing transgenic mice onto a tau-knockout background, rescued lethality and memory deficits (Roberson et al., 2007), suggesting that tau acts as a mediator of Aβ-toxicity (Ittner et al., 2010, Ke et al., 2012). However, tau pathology does not only play an important role in AD but is also central to a number of other neurodegenerative diseases- commonly referred to as tauopathies- one of which is FTD, the second most common form of dementia (Ratnavalli et al., 2002, Gotz et al., 2008a).

1.3 Frontotemporal dementia

Frontotemporal dementia is a clinically, genetically and pathologically diverse group of neurodegenerative disorders (Seltman and Matthews, 2012). Unlike AD, FTD is typically characterized by an early disease onset and is in this context the second most common form of dementia, accounting for up to 50% of all dementia cases presenting

before the age of 60 (Ratnavalli et al., 2002, Gotz et al., 2008a, Gotz et al., 2008b). Whilst the disease onset usually affects people between the age of 40-60 years, FTD has also been reported in patients as early as 20 or as late as 80 years of age (Loy et al., 2010, Sorbi et al., 2012). FTD patients present with progressive atrophy of the prefrontal and anterior temporal cortices, resulting in wide phenotypic variations of symptoms within the spectrum of FTD, which is unfortunately often the cause of misdiagnosis (Piguet et al., 2004, Cardarelli et al., 2010).

Whilst AD is mainly associated with memory impairments and cognitive decline, clinical symptoms of FTD can range from profound behavioural and/or language impairments, to apathy, disinhibition, hyperactivity and aphasia. Further, executive dysfunctions, stereotypical behaviour, such as a lack of appropriate and social emotions and insomnia with severely fragmented sleep (McCarter et al., 2016) are additional, common clinical features within the spectrum of FTD (Weder et al., 2007, Gotz and Ittner, 2008, McCarter et al., 2016).

However, depending on the most predominant clinical feature present, FTD can be further classified into three clinical syndromes: a behavioural variant (bvFTD) and two language variants, namely semantic dementia (SD) and progressive non-fluent aphasia (PNFA) (Rabinovici and Miller, 2010), both of which are often summarized under the term primary progressive aphasia (PPA) (Convery et al., 2019). Of these clinical syndromes, bvFTD is the most common FTD variant, accounting for approximately 50% of all cases (Johnson et al., 2005). Patients with bvFTD typically present with early and progressive changes in personality and behaviour, which often manifests in a mixture of disinhibition and apathy but may also include stereotypic behaviour and a loss of sympathy (Rabinovici and Miller, 2010, Convery et al., 2019). While the initial presentation of bvFTD is associated with personality and behavioural changes, cognitive decline and/or executive dysfunctions may appear at later stages of the disease (Rabinovici and Miller, 2010, Riedl et al., 2014). As opposed to bvFTD, the clinical picture of the two FTD language variants are mainly characterized by progressive decline in speech and language functions (Convery et al., 2019). Whilst SD mainly presents with an impairment in word comprehension and progressive decline in conceptual knowledge (Landin-Romero et al., 2016), PNFA rather manifests in non-fluent, “effortful” speech, agrammatism and/or deficits in speech production (Rabinovici and Miller, 2010, Convery et al., 2019). Furthermore, as the disease

progresses patients with PPA may present with behavioural changes similar to bvFTD (**Figure 2**) (Bozeat et al., 2000).

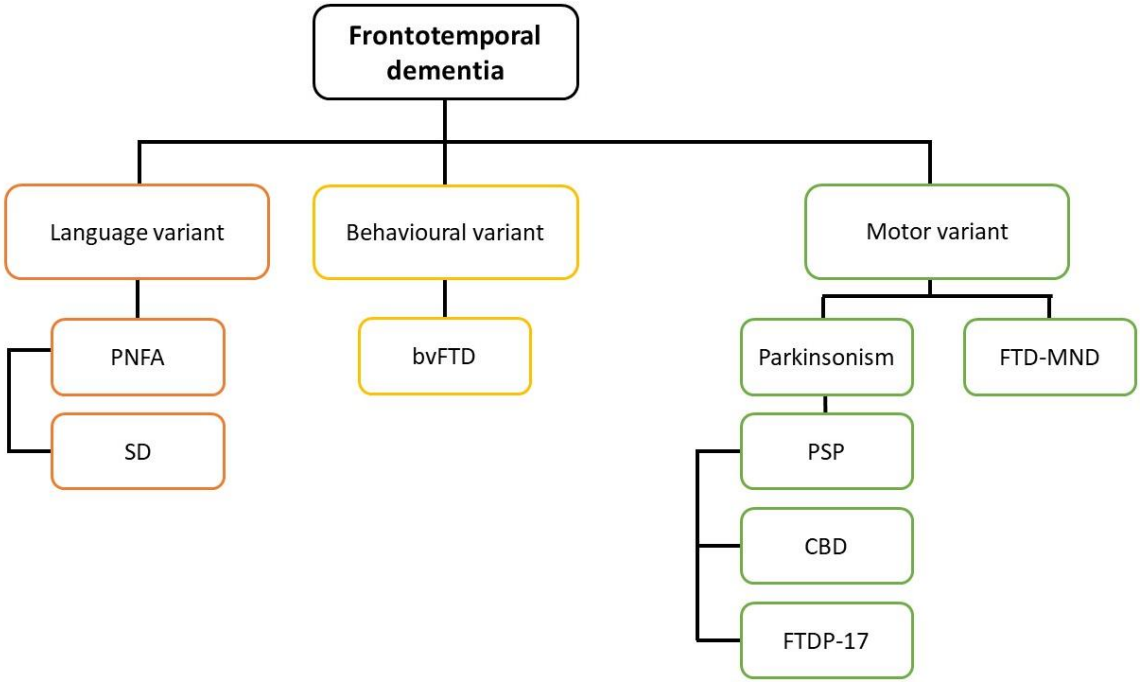

Figure 2: Clinical classification of frontotemporal dementia

Depending on the most prominent phenotype, FTD can be further classified into three syndromes: a behavioural variant (bvFTD), which is mainly associated with personality and behavioural changes, and two language variants, semantic dementia (SD) and progressive non-fluent aphasia (PNFA). Both language variants of FTD manifest in progressive decline of speech and language function. However, late disease stages may also show behavioural changes reminiscent of bvFTD. Besides behavioural and language dysfunctions, FTD may develop in tandem with movement disorders including motor neuron disease (FTD-MND), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). Notably, as the disease progresses, clinical phenotypes may converge or overlap. For example, patients may present features reminiscent of more than one disease, which reflects the complexity of the FTD-spectrum and may further explain the difficulties of differential diagnosis (image adapted with permission from (Park and Chung, 2013)).

Besides behavioural and/or language impairments, FTD may develop in tandem with motor neuron disease (FTD/MND, or FTD with ALS), resulting in progressive muscle

weakness, fasciculation and spasticity, since this spectrum affects the motor neurons in the spinal cord, brain stem and cerebral cortex (Warren et al., 2013, Ferrari et al., 2014). Additionally, FTD may also be associated with other movement abnormalities, including dystonia and spontaneous, asymmetric limb movements (alien limb), which are characteristic features of corticobasal degeneration (CBD), whilst axial rigidity and supranuclear palsy are commonly associated with progressive supranuclear palsy (PSP) (Sha et al., 2006) (**Figure 2**). Notably, patients may show symptoms reminiscent of more than one clinical phenotype. For example, a patient diagnosed with CBD may also show behavioural changes that are similar to those observed in bvFTD or may present language dysfunctions similar to PNFA. Furthermore, as the disease progresses clinical phenotypes may converge, so that patients initially diagnosed with bvFTD may develop movement abnormalities reminiscent of CBD at a later disease stage, reflecting the phenotypic complexity of the FTD-spectrum (Rabinovici and Miller, 2010, Fernandez-Matarrubia et al., 2015).

In addition to clinical symptoms, common neuropathological features of FTD are abnormal, disease specific protein inclusions in neurons and glial cells, which, as the disease progresses, affect additional brain regions in a stereotypic manner (Gotz et al., 2012). Depending on the type of proteinaceous inclusions in the brain, FTD can be further classified neuropathologically into four subgroups, referred to as frontotemporal lobar degeneration (FTLD) subtypes: i) FTLD with ubiquitin and TDP-43-positive accumulations, termed FTLD-TDP, ii) FTLD with tau-positive inclusions, termed FTLD-tau iii) FTLD with inclusions positive for fused in sarcoma, termed FTLD-FUS and iv) FTLD with ubiquitin-positive inclusions (FTLD-UPS). However, the most prevalent pathologies associated with FTLD are TDP-43 and tau inclusions (**Figure 3**), which account for approximately 90% of all underlying pathologies found within the clinical FTD spectrum (Mohandas and Rajmohan, 2009).

Whilst TDP-43 accumulations are the most prevalent feature of ALS and FTD/MND (Neumann et al., 2006), intracellular tau inclusions are histopathological hallmarks of several neurodegenerative diseases- collectively referred to as tauopathies (Lee and Leugers, 2012). Apart from AD, other common tauopathies are PSP, CBD and Pick's disease (PiD), with the latter usually being associated with bvFTD or PNFA, whilst movement disabilities are less common clinical features found in PiD patients (Piguet et al., 2011a). Although FTLD-tau subgroups share common pathological features, including cortical atrophy of the frontal and temporal lobes,

severe neuronal loss and intracellular tau accumulations in neurons and glia, each FTD-syndrome has distinct tau-associated lesions, varying in structure, appearance and distribution (Mackenzie and Neumann, 2016). For example, PiD is characterized by cytoplasmatic neuronal lesions known as Pick bodies (PiBs). These inclusions are

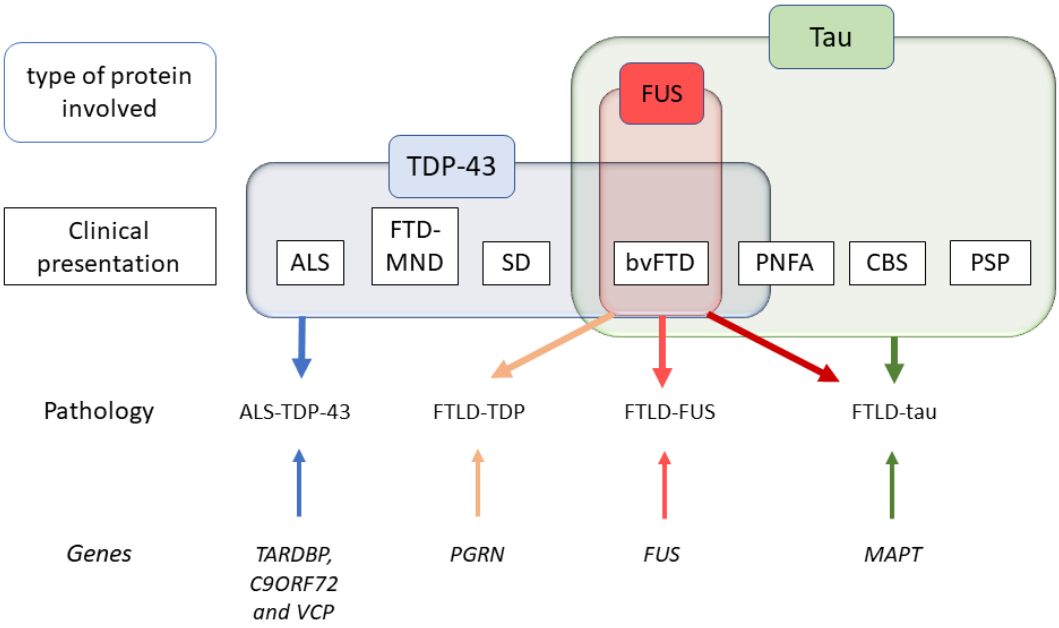

Figure 3: Clinical, neuropathological and genetic spectrum of FTD

FTD is a clinically, genetically and pathologically diverse group of neurodegenerative diseases characterized by distinct proteinaceous inclusions in the brain. Interestingly, the same misfolded protein may underline several clinical presentations and contrariwise the same clinical phenotype may result from different misfolded proteins and/ or genetic mutations. Besides TDP-43 (blue) which is a key pathology in ALS and FTD/MND, tau protein (green) inclusions are found in the majority of FTD-cases, including tauopathies. Notably, mutations in *FUS* may also be causative of ALS and FTD/MND, however this detail is not shown in this scheme.
ALS, amyotrophic lateral sclerosis; FTD/MND, frontotemporal dementia with motor neuron disease; SD, semantic dementia; bvFTD, behavioural variant FTD, PNFA, progressive-nonfluent aphasia; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; *TARDBP*, gene encoding the transactive response DNA binding protein 43 kDa; *C9ORF72*, gene encoding chromosome 9 open reading frame 72; *VCP*, gene encoding for the valosin-containing protein/p97, *PGRN*, gene encoding for progranulin; *FUS*, gene encoding for the fused in sarcoma protein; *MAPT*, tau encoding gene. (image adapted with permission from (Villemagne et al., 2015, Meeter et al., 2017).

composed of straight tau filaments and are predominately found in the frontal and temporal cortices and in the hippocampus of PiD patients (Probst et al., 1996, Gotz et al., 2019). On the other hand, PSP presents with spherical globose NFTs and glial tau inclusions in the form of tufted astrocytes (Mackenzie and Neumann, 2016), whilst the hallmark lesions of CBD are tau-immunoreactive astrocytic plaques and pre-tangles which are most prevalent in the cerebral cortex and in the white matter of CBD patients (Dickson et al., 2002). These broad phenotypic manifestations as well as clinical and histopathological similarities of FTD subtypes, reflect the complexity of the FTD spectrum and may further explain the difficulties to classify and diagnose each disease as a separate entity. Especially when keeping in mind, that a definite diagnosis can only be confirmed at autopsy, since there are presently no reliable tools for a definite diagnosis *antemortem* (Bigio, 2013, Ling and Macerollo, 2018) .

On average, patients with FTD are institutionalized approximately one year after diagnosis, which is likely due to the complexity of behavioural impairments and personality changes associated with FTD, that greatly affect daily living (Hodges et al., 2003).

The majority of FTD cases are sporadic. However, FTD is thought to have a strong genetic component as approximately 25-50% of patients present with a positive familial history of dementia, which in some cases result from an autosomal dominant pattern of inheritance (Van Deerlin et al., 2003, Weder et al., 2007, Mackenzie and Neumann, 2016).

In 1994, Wilhelmsen found the first evidence for a genetic cause in FTD cases (Wilhelmsen et al., 1994) when he discovered a linkage between familial FTD and the chromosome 17, a region containing tau. Following this discovery, additional mutations in the tau gene (MAPT) were identified, resulting in the umbrella term of “Frontotemporal dementia with parkinsonism linked to chromosome 17” (FTDP-17) to accommodate these genetic FTD cases (Foster et al., 1997). Moreover, those findings established that mutations in the gene encoding tau (*MAPT*) segregate with disease phenotypes and thereby provided clear evidence that tau dysfunction *per se* is sufficient to cause neurodegeneration and cognitive decline (Spillantini et al., 2000, Wszolek et al., 2005).

1.3.1 FTD with parkinsonism linked to Chromosome 17

To date more than 51 pathogenic mutations associated with FTDP-17 or related tauopathies, have been identified (Goedert et al., 2012), whereby mutations in *MAPT*, represent approximately 7-50% of familial FTD cases (Rosso and van Swieten, 2002, Goldman et al., 2004). Presently, approximately 100-200 families have been diagnosed with FTDP-17, although the exact prevalence remains unclear (Wszolek et al., 2005, Wszolek et al., 2006). Even though mutations in *MAPT* are a common underlying cause of FTDP-17, its clinical presentation may highly vary within and between the families, but usually manifests in behavioural changes, cognitive impairments, and loss of executive functions (Spillantini et al., 1998a). Whilst parkinsonism and muscle atrophy are less common features, each symptom reflects the progression of neuronal lesions and degeneration in certain brain regions (Kar et al., 2005, Wszolek et al., 2005).

Further, since FTDP-17 is a subtype of FTLD-tau, patients share common neuropathological manifestations including progressive neuronal loss in the frontal and/or temporal cortices, astrocytic gliosis in the white and grey matter (Gotz et al., 2001) and abundant neuronal lesions, composed of hyperphosphorylated tau. However, similar to FTLD-tau, intracellular tau lesions may present with a large variability within and between families. For example, even carries of the same *MAPT* mutation may present with lesions that vary in their physical structure, initial location, distribution throughout the brain and may even be composed of different tau isoforms (van Swieten et al., 2004).

However, even though the nomenclature of FTDP-17 is still widely used to describe genetic forms of FTLD independently of sporadic FTLD-tau cases, recent evidence suggests that *MAPT* mutation carriers should rather be classified as familial forms of sporadic FTLD-tau to reflect the shared characteristic pathologies with sporadic FTLD-tau forms and to further enable the discrimination between *MAPT* and *GRN* mutations in FTLD patients (Forrest et al., 2018). In this study Forrest and colleagues assessed human cases carrying various *MAPT* mutations (K257T, S305S, P301L, IVS10+16 and R406W) and compared their neuropathological features to four sporadic FTLD-tau subtypes, including CBD, PSP, PiD and globular glial tauopathies (GGT). Interestingly, hallmark molecular and morphological characteristics of cases with sporadic PiD resembled those found in K257T mutation carriers. Similar carries

findings were reported for the other sporadic cases, indicating that genetic forms of FTLD-tau are on a disease continuum with sporadic FTLD-tau cases and therefore FTDP-17 is not a separate clinical entity. The authors concluded that revising the traditional neuropathological classification of FTLD-tau could improve our understanding of the underlying pathomechanisms in sporadic cases (Forrest et al., 2018),

1.4 The structure and physiological function of tau

Tau was first identified in 1975 as a protein that modulates microtubule (MT) assembly (Cleveland et al., 1977, Weingarten et al., 1975). Since then, research focusing on tau quickly progressed, as mutations in the gene encoding tau were found to be causative of neurodegenerative diseases (Wilhelmsen et al., 1994).

Tau belongs to the family of microtubule-associated proteins (MAP1, MAP2, MAP4 and tau) and accounts for 80% of total MAPs in human neuronal tissue (Weingarten et al., 1975, Cleveland et al., 1977). As neurons develop, tau segregates primarily into axons (Ittner et al., 2011), whilst i.e. MAP4 is mainly localized to peripheral tissues (Kavallaris et al., 2008).

Tau is constitutively expressed throughout the central nervous system (CNS) but has also been described in non-neuronal tissue, including kidneys, testis, lungs (Gu et al., 1996) and fibroblasts (Ingelson et al., 1996). In the CNS, tau was identified in oligodendrocytes, perineuronal glial cells (LoPresti et al., 1995), in the somatodendritic compartment and dendritic spines (Ittner et al., 2010), but is significantly more enriched in axons, where it is traditionally thought to bind to microtubules and modulate their assembly (Lee et al., 2001). However, the interaction between tau and MTs is not static, but rather thought to be a highly dynamic equilibrium of attachment to and detachment from MTs (Igaev et al., 2014, Janning et al., 2014). Further, this interaction seems to be determined by the phosphorylation state of tau, which is modulated by the balanced action of kinases and phosphatases (Ittner and Ittner, 2018). So that i.e. phosphorylation of tau by specific kinases reduces its binding affinity to MTs, and hence negatively regulates the ability of tau to assemble MTs. In this regard, tau is thought to contribute either directly or indirectly, to structural and regulatory cellular functions, such as the maintenance of cell polarity and cell

morphology (Mandelkow et al., 2000). Further, tau has been implicated in the regulation of intracellular trafficking, cell signaling and axonal outgrowth during cell development and may thereby play a crucial role in the functioning and viability of neurons and their axonal extensions (Trinczek et al., 1999, Buee et al., 2000, Mandelkow et al., 2000).

In addition to its well characterized role as a microtubule-associated protein, tau was identified in the nucleus (Loomis et al., 1990), associated to the plasma membrane (Brandt et al., 1995), and the actin cytoskeleton (Yamauchi and Purich, 1993). However, more recently it was discovered that tau is also present at the post-synapse, both under physiological and pathological conditions (Ittner et al., 2010). At the post-synapse, tau interacts with Src kinases, like Fyn, which then localizes to the dendritic compartment. Here, Fyn was shown to phosphorylate subunit two of the NMDA receptor, resulting in an interaction with the postsynaptic density protein 95 (PSD-95). This cascade seems to strengthen excitotoxic glutamate signaling, which significantly increased Aβ-associated toxicity, as shown in APP transgenic mice (APP23) (Gotz et al., 2008b), where this interaction results in excitotoxicity and premature mortality (Gotz et al., 1995, Ittner et al., 2010, Ittner and Gotz, 2011). Accordingly, a tau-dependent increase in Fyn at the post-synapse was shown to enhance neuronal circuit aberrations and thereby significantly exacerbate Aβ-toxicity. In contrast, tau-deficiency, a downregulation of endogenous tau levels and/or decreased levels of Fyn in the dendritic spines, not only attenuated this process, but lead to an improvement in memory function and survival (Ittner et al., 2010, Haass and Mandelkow, 2010). Moreover, a study by Ittner and colleagues from 2016 showed that tau does not only act as a mediator of Aβ-toxicity, but may also have a protective, physiological function at the post-synapse (Ittner et al., 2016). In this study, specific phosphorylation of tau at T205, which is mediated by p38γ, was shown to prevent excitotoxic signaling, resulting in decreased Aβ-toxicity in early stages of AD. Consequently, depletion of p38γ, appeared to boost excitotoxicity and significantly exacerbated memory deficits and premature lethality in APP23 transgenic mice, whereas increasing p38γ activity was shown to mitigate these effects. Overall, this study showed for the first time, that site specific phosphorylation of tau may have beneficial effects in the context of Aβ-induced neuronal death and challenges the dogma that tau phosphorylation in AD is purely pathogenic (Ittner et al., 2016).

A variety of physiological functions of tau have been proposed over the last few years, some of which were revealed using tau-deficient mice (tau-KO mice) (Ke et al., 2012, Bakota et al., 2017). With regards to the interplay between tau, Fyn and Aβ, studies using tau-KO mice have been instrumental and have markedly contributed to a better understanding of tau's physiological functions and the role of tau in disease (Ke et al., 2012). Furthermore, these studies also questioned the long-lasting assumption that tau may stabilize MT-assembly, as tau-KO mice only show a very subtle increase in the MT-disassembly-rate (reviewed in (Bakota et al., 2017)). In fact, most studies using tau-deficient mice showed that tau depletion during neuronal development may be compensated by an increased expression of other microtubule-associated proteins, in particular MAP1A, which was found to be increased by up to 2-fold in developing and adult mice (Harada et al., 1994, Fujio et al., 2007).

To date, several tau-KO mice have been published (reviewed in (Ke et al., 2012, Bakota et al., 2017)). However, studies utilizing these mice have sometimes reported inconsistent, or even conflicting results, and most surprisingly did not reveal major indispensable functions of tau (Ittner and Ittner, 2018). For example, a study by Kimura and colleagues reported that tau-deficiency, both *in vivo* and in acute tissue slices *in vitro*, compromised NMDAR-dependent long-term depression (LTD), whilst long-term potentiation (LTP) seemed not to be affected (Kimura et al., 2014). In contrast, another study found severe impairments in LTP after tau depletion, indicating that tau is required for signal transmission and synaptic plasticity and further suggesting that tau-deficiency may result in a loss of physiological function (Ahmed et al., 2014). Another example are the behavioural phenotypes of tau-deficient mice, with some studies reporting motor and/or cognitive deficits (Ikegami et al., 2000, Lei et al., 2014), while results from other groups indicate otherwise (Harada et al., 1994, Dawson et al., 2001, van Hummel et al., 2016). These conflicting outcomes may be due to a number of confounding factors (reviewed in (Bakota et al., 2017)). Firstly, the deficits may be explained by different constructs and genetic backgrounds used to generate these tau-deficient strains. Secondly, some behavioural changes observed in these mice seem to be mostly age-related rather than a result of tau depletion, as they seem to occur at highly advanced ages. Reported differences in behavioural and/or cognitive outcomes between tau-KO strains may also result from methodical differences between studies, i.e. in the preparation of brain slices used for LTP/LTD recordings or differences in behavioural testing procedures. Finally, as mentioned above, other members of the

MAP family seem to be able to compensate for tau deficiency, which may explain why some research groups have reported no overt phenotype in tau-KO mice (Harada et al., 1994, van Hummel et al., 2016, Tan et al., 2018).
Nonetheless, studies using tau-deficient mice have markedly increased our understanding of tau's physiological and pathological functions and may thereby open up new avenues for tau-targeting treatments in AD and FTD (Ittner and Ittner, 2018).

In the human brain, tau is encoded by a single gene, namely *MAPT*, which is located on the long arm of chromosome 17q21 (**Figure 4A**). *MAPT* consists of 16 exons (E) (Gendron and Petrucelli, 2009, Kolarova et al., 2012), whereby E0 and E14 are transcribed but not translated (Wang and Mandelkow, 2016). Whilst E1, E4, E5, E7, E9, E11, E12 and E13 are constitutive domains of *MAPT*, E2, E3 and E10 are subject to alternative splicing, hence their inclusion or exclusion determines the distinct tau isoforms (Buee et al., 2000, Gendron and Petrucelli, 2009). Accordingly, alternative splicing of *MAPT* results in six major tau isoforms (**Figure 4B**), ranging from 352-441 amino acid residues in length (Ballatore et al., 2007, Gotz et al., 2012).
These tau isoforms vary with respect to the number of microtubule-binding repeats (either 3R or 4R), depending on the absence or presence of E10, and by the presence or absence of one or two N-terminal inserts (resulting in 0N, 1N or 2N), which are determined by E2 and E3 (Hong et al., 1998, Morris et al., 2011, Ittner et al., 2011). Isoforms including E10 are commonly referred to as 4R (4 repeat) tau isoforms while those that exclude E10 are known as 3R (3 repeat) tau isoforms (Lee and Leugers, 2012). In this regard, alternative splicing of E10 is of particular interest, as distinct splice variants of tau are associated with distinct FTLD-tau subgroups. For example, 4R-tau is the main constituent of tau inclusions found in CBD and PSP patients, whilst tau-immunoreactive neuronal ovoid lesions found in PiD predominantly, but not exclusively, contain 3R-tau variants (Lee et al., 2001, Dickson et al., 2011).

With respect to the primary structure, each tau isoform consists of four functional domains: (1) a negatively charged N-terminal projection domain; (2) a proline- rich domain; (3) the microtubule-binding domain (MDB), and (4) the carboxyl-terminal (C-terminal)-region. Each tau protein domain has been attributed distinct physiological functions. For example, whilst the N-terminal projection domain is thought to interact with cytoskeletal elements to ascertain the spacing between MTs

(Buee et al., 2000), the MBD regulates microtubule polymerization. The region between repeat 1 and 2 (R1 and R2, inter-region), which is unique to 4R-tau, seems to be the most potent in promoting microtubule assembly (Brandt and Lee, 1993).

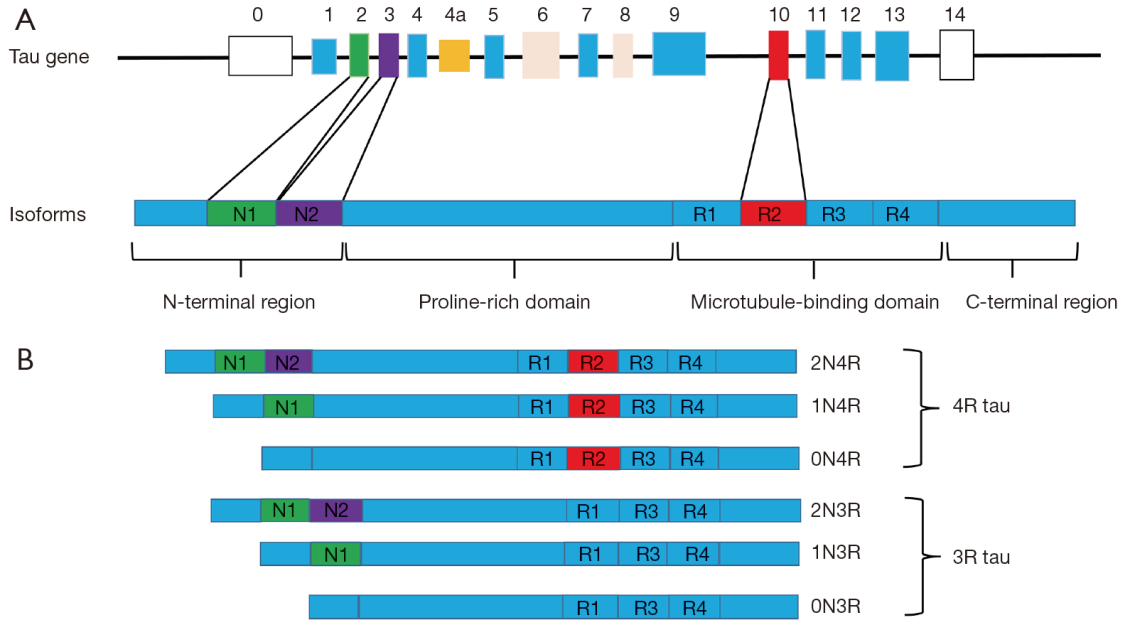

Figure 4: *MAPT* and the six tau isoforms found in the adult human brain

Human tau is encoded by *MAPT* (A), which is located on the long arm of chromosome 17q21 and comprises 16 Exons (E). E0 and E14 are non-coding regions (white), whilst E4a (displayed here in yellow), E6 and E8 (pink) are exclusively transcribed in peripheral tissue and are therefore not expressed in the human brain (Guo et al., 2017). Each tau isoform consists of four regions: The N-terminal region, a proline-rich domain, a microtubule binding domain (MBD) and the C-terminal region. The longest tau isoform (2N4R) contains two inserts, N1 and N2, which are both located in the N-terminal region and are encoded by E2 and E3 respectively, whilst E10 encodes for insert R2, which is located in the MBD. Alternative splicing of E2, E3 and E10 gives rise to six major tau isoforms, that are expressed under physiological conditions in the human brain. (B) Splice variants differ in their number of microtubule-binding repeats (either 3 or 4 repeats, labelled as R1-R4), and in the number of N-terminal inserts (either 0, 1, or 2 inserts, shown in green and purple) (Ballatore et al., 2007). Whilst the microtubule-binding domain comprises the carboxyl terminal half of the protein, the N-terminal half of tau includes the proline-rich domain and is termed the projection domain (image obtained with permission from (Gao et al., 2018)).

The structural variability amongst splice variants implies precise and distinctive physiological functions of each individual tau isoform. In support of this, *in vitro* studies indicate that 4R-tau variants have a greater MT-binding affinity than 3R-tau isoforms and are thus more efficient in promoting microtubules assembly. This effect might be due to the presence of an additional MT-binding repeat (Lee et al., 2001) but may also result from the above mentioned R1-R2 inter-region (Brandt and Lee, 1993). Moreover, some isoforms display distinct spatial and temporal expression patterns during development and maturation (Buee et al., 2000). For example, only the shortest tau isoform (0N3R) is expressed during fetal stages after which it is downregulated in the adult brain, whilst the other tau isoforms (1N3R, 2N3R, 0N4R, 1N4R and 2N4R) are present throughout adulthood (Goode et al., 2000). In fact, the expression of the fetal tau isoform seems to coincide with the formation of neuronal processes and synapses in the brain, suggesting that the change in isoform expression from the fetal to the adult pattern might be a crucial step in neuronal development and may thereby also play a role in synaptic plasticity (Arendt et al., 2016). On the other hand, expression levels of 4R-tau isoforms were found to be increased in the globus pallidus (Majounie et al., 2013), compared to other brain regions, whereas lowest expression levels of the fetal tau isoform were found in the cerebellum (McMillan et al., 2008).

Notably, in the human brain, expression levels of tau isoforms are tightly regulated, so that balanced splicing of E10 results in approximately equal amounts of 3R and 4R-tau isoforms. Deviations from this ratio (i.e. by increasing the amount of 4R-tau, which can result from intronic *MAPT* mutations associated with FTD) are implicated in the development of several tauopathies (Hutton et al., 1998, D'Souza and Schellenberg, 2005).

1.4.1 Post-translational phosphorylation of tau

Tau is subject to a large number of post-translational modifications (PTMs), which regulate its physical structure and determine physiological functions of tau. The most prevalent PTMs include phosphorylation, isomerization, glycosylation, ubiquitination, acetylation and oxidation (Steiner et al., 1990, Spillantini et al., 1998b, Poorkaj et al., 2001, Miyasaka et al., 2005). This wide range and diversity of tau modifications implies that tau function is highly regulated (Morris et al., 2011).

The most commonly described PTM of tau is phosphorylation. The phosphorylation status of tau is highly dependent on the balanced action of kinases and phosphatases (Mietelska-Porowska et al., 2014). Several studies indicate that abnormal phosphorylation of tau reduces its affinity to microtubules, and eventually initiates missorting and pathological accumulation of tau in disease. Hence, aberrant tau phosphorylation is commonly recognized as a crucial step in the pathogenesis of neurodegenerative diseases (Duka et al., 2013, Mietelska-Porowska et al., 2014).
On the other hand, increased tau phosphorylation has been described as an important regulatory mechanism, naturally occurring during early development (Yu et al., 2009) and hibernation (Arendt et al., 2003, Su et al., 2008). Another study found highly increased levels of abnormally phosphorylated tau during mitosis in tau transfected neuroblastomas cells, which were similar to those found in AD patients (Illenberger et al., 1998). Finally, site-specific phosphorylation of tau was shown to attenuate Aβ-toxicity in an APP-transgenic mouse model of AD, indicating that tau phosphorylation may also have a protective, physiological function at the post-synapse (Ittner et al., 2016). In conclusion, these studies suggest that increased phosphorylation of tau may not only determine its localization, solubility and biological function but may also affect the interaction of tau with other molecules and further indicate that tau phosphorylation *per se* may not be sufficient to trigger neurodegeneration and cognitive decline (Luna-Muñoz et al., 2013).

The longest tau isoform (2N4R) contains 85 potential phosphorylation sites, (45 serine, 35 threonine and 5 tyrosine), whereby most of them are located within the proline-rich domain and the C-terminal region harboring the MBD (Gendron and Petrucelli, 2009). Some of these phosphorylation sites are considered to be physiological, whilst others are ‘de-novo’ phosphorylated or phosphorylated to a higher degree during neurodegenerative illness (Ittner et al., 2011). The difference between these stages is determined by the amount of phosphates per mole of tau. For instance, on average 2-3 moles of phosphate are found per mole of tau under normal physiological conditions. In disease state however, this ratio is remarkably increased, resulting in 6-10 moles of phosphate per mole of tau, typically affecting multiple phosphorylation sites (Ksiezak-Reding et al., 1992, Kopke et al., 1993, Alonso Adel et al., 2004). This state is referred to as “hyperphosphorylation” (Goedert et al., 1992) and is considered to be critical for the interplay of tau and microtubules.

1.4.1.1 Tau kinases

Three classes of protein kinases contribute to tau phosphorylation. The first are proline-directed that phosphorylate serine and threonine motifs (PDPK) outside the MBD. The second are non-proline directed and target KXGS-motifs within or near the MBD (Avila et al., 2004b, Zempel et al., 2010). The third class contains protein kinases that specifically target tyrosine residues and includes kinases such as Fyn, Src, Syk and Abl (Martin et al., 2011).

PDPKs include the glycogen-synthase-kinase-3-β (GSK3-β), the cyclin-dependent kinases 2 and 5 (cdk2, cdk5), the mitogen-activated protein kinase (MAPK) and a number of stress-activated protein kinases, namely the c-Jun N-terminal kinase (SAPK/JNK) and p38 kinase (p38), (Goedert et al., 1997, Buee et al., 2000, Ferrer et al., 2005, Gendron and Petrucelli, 2009). However, despite the fact that increased activity of those kinases is thought to result in aberrant tau phosphorylation and decreased tau-MT interaction, there is particularly significant evidence that two PDPKs, namely GSK3-β and cdk5, play a key role in tau hyperphosphorylation in disease (Ishiguro et al., 1993, Wagner et al., 1996, Ishiguro et al., 1988, Iqbal and Grundke-Iqbal, 2008). For instance, both kinases were shown to co-purify with microtubules and have been found to phosphorylate tau at disease-specific sites (Gendron and Petrucelli, 2009). Moreover, the amount, activity and distribution of GSK3-β was found to correlate with disease progression and tangle burden in AD patients (Pei et al., 1997). Furthermore, inhibition of GSK3-β in a mouse model of AD was shown to decrease tau pathology and axonal degeneration (Noble et al., 2005). Additionally, tau phosphorylation by GSK3-β, particularly at the C-terminus, was shown to significantly promote self-aggregation and deposition of tau (Liu et al., 2007). Moreover, recent findings in young transgenic mice implicate a dynamic crosstalk between cdk5 and GSK3-β, in which upregulation of cdk5 activity was demonstrated to negatively regulate GSK3-β (Plattner et al., 2006). However, this inhibitory effect is lost in aged transgenic mice, where GSK3-β activity was shown to be highly upregulated, leading to aberrant tau phosphorylation at disease-associated sites (Plattner et al., 2006). Furthermore, several studies suggest, that pre-phosphorylation of tau, which is referred to as priming, may induce subsequent phosphorylation of tau by another kinase. This mechanism, has also been proposed for cdk5 and GSK3-β, in which priming of tau by cdk5 was found to result in subsequent phosphorylation of tau

by GSK3-β (Ishiguro et al., 1993). These findings imply that tau is an *in vivo* substrate of GSK3-β (Johnson and Stoothoff, 2004) and further suggest a central role of GSK3-β in mediating tau phosphorylation, whilst cdk5 rather acts as modulator of tau phosphorylation through the inhibition of GSK3-β (Plattner et al., 2006, Mietelska-Porowska et al., 2014).

Unlike PDPKs, non-proline-directed kinases, including the microtubule-affinity-regulating kinase (MARK), the cyclic-AMP-dependent kinase (PKA), the casein kinase 1 and 2 (CK1 and CK2), the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and the mammalian homologue of PAR1 tend to phosphorylate tau residues within the MBD, which is thought to result in decreased binding affinity of tau to MTs (Gendron and Petrucelli, 2009). For instance, phosphorylation of KXGS motifs (in particular S262 and S356) by MARK and PKA was shown to compromise tau-MT interaction, leading to conformational changes of tau which may strongly impact on its biological function (Gustke et al., 1992, Augustinack et al., 2002, Fischer et al., 2009). In addition, priming of tau by PKA (Liu et al., 2004, Mietelska-Porowska et al., 2014), CK1 (Hanger et al., 2009) and MAPK/PAR-1 (Nishimura et al., 2004) was found to result in subsequent phosphorylation of tau by GSK3-β at several AD-specific sites.

1.4.1.2 Tau phosphatases

Several serine/threonine phosphatases (PPA) were shown to contribute to tau dephosphorylation *in vitro* (Gong et al., 2005), including PP1, PP2A, PP2B and PP5. However, the most commonly described PPA is protein phosphatase 2A, which accounts for 70% of cellular phosphatase activity in the human brain (Liu et al., 2005). PP2A has been shown to dephosphorylate tau at multiple sites, suggesting that PP2A might be implicated in the regulation of tau phosphorylation (Sontag et al., 1996, Liu et al., 2005). Furthermore, both activity and expression levels of PP2A have been found to be significantly decreased in the brains of AD patients (Sontag et al., 2004, Liu et al., 2005), implying that deregulation of PP2A may underline, or at least contribute to aberrant tau phosphorylation in disease (Gong et al., 2005). Consistent with these findings, inhibition of PP2A was shown to induce hyperphosphorylation, tau accumulation (Gong et al., 2000) and cognitive decline *in vivo* (Sun et al., 2003). However, given that PP2A also regulates the activity of a number of kinases, the

proposed mechanism by which PP2A inhibition leads to tau hyperphosphorylation may also occur indirectly, namely through the activation of kinases like CAMKII and MAPK that are no longer negatively regulated by PP2A (Pei et al., 1997, Bennecib et al., 2001).

Presently it remains unclear, whether aberrant tau phosphorylation results from over-activation of kinases or might be due to the downregulation of phosphatases. Alternatively, priming of tau *in vivo* may render it a better substrate, increasing the possibility of subsequent tau phosphorylation at additional sites, a theory which possibly explains the mechanism by which pathological phosphorylation and deposition of tau occurs (Blum et al., 2015). In fact, it seems to be a combination of several pathogenic events that prime sequential downstream processes, eventually leading to hyperphosphorylation and deposition of tau (Trojanowski and Lee, 1995, Bertrand et al., 2010).

1.4.2 Pathological function of tau

Presently, there are still controversial hypotheses about how aberrant tau phosphorylation occurs and how it compromises neuronal functioning in disease. Nevertheless, increasing evidence suggests that a disruption to its physiological phosphorylation converts tau from a biologically functional and soluble molecule into a toxic entity, promoting its oligomerization and accumulation into intracellular, neurofibrillary inclusions, named NFTs (**Figure 5**) (Kopke et al., 1993, Schneider et al., 2004, Wang et al., 2013).

It is commonly believed, that pathological phosphorylation of tau negatively regulates the tau-MT interaction, resulting in an increased detachment of tau from MTs. This process may then compromise the ability of tau to regulate MT-assembly and contribute to the disruption of axonal transport (loss of physiological function) (Drechsel et al., 1992, Buee et al., 2000, Ballatore et al., 2007, Ke et al., 2012). On the other hand, subsequent dissociation of tau from MTs is thought to deregulate the dynamic equilibrium of tau (soluble, unbound tau vs. tau attached to MTs), resulting in critically high levels of unbound tau in the cytoplasm (Ballatore et al., 2007). This imbalance

may then favour pathogenic conformational changes, misfolding and missorting of tau from the axon to the somatodendritic compartment (Fischer et al., 2009), which further

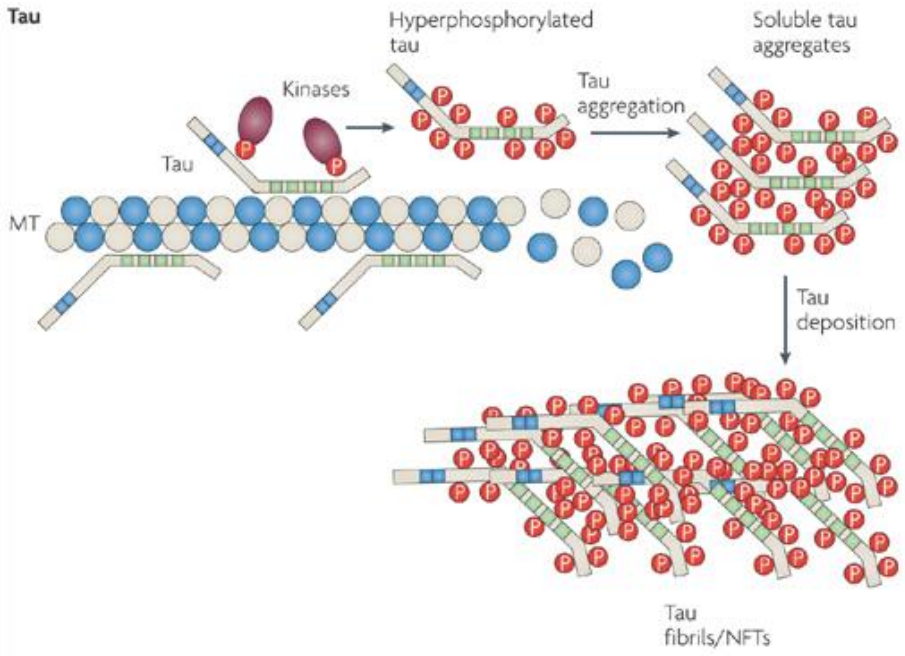

Figure 5: Pathological phosphorylation of tau

Under physiological conditions tau is primarily localized to the axon, where it regulates and promotes microtubule-assembly. In disease state, however, tau becomes aberrantly phosphorylated at multiple sites (referred to as hyperphosphorylation), dissociates from microtubules, causing them to depolymerize. Hyperphosphorylated tau then accumulates into soluble aggregates eventually leading to the formation of insoluble neurofibrillary tangles (NFTs), (image adapted with permission from (Gotz and Ittner, 2008)).

compromises microtubule integrity and may thereby induce synaptic dysfunction (Thies and Mandelkow, 2007, Ballatore et al., 2007, Hoover et al., 2010, Ittner et al., 2010). Additionally, pathogenic tau monomers within the cytosol are thought to be highly interactive and may sequester other molecules such as normal tau, MAP1 and MAP2 (Alonso et al., 1994, Iqbal and Grundke-Iqbal, 2008), making them more resistant to degradation by the ubiquitin-proteasome system (Lee et al., 2001, Wang et al., 2013). Finally, the likelihood that cytosolic tau might be targeted by additional PTMs at this stage is significantly increased and is thought to further compromise tau-

MT interaction and/or result in an increased propensity of tau to self-aggregate into oligomeric structures. Consequently, these pathogenic processes are thought to initiate and promote tau accumulation and oligomerization into NFTs and are therefore considered to be critical stages underlying tau pathogenesis in disease. (Kuret et al., 2005, Ballatore et al., 2007, Wang et al., 2013).

However, these findings would imply that abnormally increased levels of soluble tau *per se* are sufficient to induce conformational changes of tau, and contribute to impaired neuronal functioning and toxicity prior to the actual deposition of tau (Yoshiyama et al., 2007, van Eersel et al., 2009, Mietelska-Porowska et al., 2014). The hypotheses that soluble pathogenic tau species, rather than tau deposits are the toxic species underlying neuronal toxicity has been controversially discussed in seminal studies. For instance, it has been reported that tau, which deposits into NFTs, shows reduced propensity to sequester other molecules. This would suggest, that tau oligomerization into filaments might be induced as a self-defense response by affected neurons (Iqbal and Grundke-Iqbal, 2005, Alonso Adel et al., 2006). On the other hand, a recently published study utilizing inducible tau transgenic mice demonstrated, that the formation of NFTs still occurred, even after the transgene-expression was significantly lowered and improvements in memory function and neuronal loss were detected (Santacruz et al., 2005). This implies that NFTs may not be sufficient nor causative of neuronal degeneration and cognitive decline and further highlighted the detrimental and neurotoxic role of monomeric/oligomeric unbound tau in disease (Santacruz et al., 2005, Hanger et al., 2009).

The formation of NFTs is considered to be a multi-step phenomenon, initiated by the detachment of tau from microtubules, concomitant increase of cytosolic tau monomers, followed by conformational changes and misfolding of tau. Once tau is polymerized, small, non-fibrillary tau deposits, termed “pre-tangles” are formed (Ballatore et al., 2007). Pre-tangles are composed of diffuse, granular deposits, that are typically distributed perinuclear and in proximal process of affected neurons (**Figure 6A**) (Maccioni and Perry, 2009). Subsequent polymerization and aggregation results in the inclusion of tau deposits into insoluble, more organized bead-like structures (**Figure 6B**). These structures are referred to as paired-helical filaments and straight-filaments (PHFs and SFs respectively) (Grundke-Iqbal et al., 1986), which are thought to be the

main constituents of NFTs, neutrophil threads and neuritic plaques (gain of toxic function) (Tolnay and Probst, 2003, Finder, 2010).

While seminal disease-associated lesions are characterized by straight filament pathology (Perry et al., 1991), PHFs are predominantly present at later disease stages, (Gruden et al., 2008), where they make up approximately 95% of neuronal inclusions found in neurodegenerative diseases (Koechling et al., 2010). The most prevalent feature of PHF is their pleated β-sheet structure, which is determined by two identical strands, that are helically twisted around each other (von Bergen et al., 2000, Giannetti et al., 2000, von Bergen et al., 2001, Ballatore et al., 2007). Both SFs and PHFs are composed of hyperphosphorylated tau, whereby all splice variants of tau can be present in their derivative form (Goedert et al., 1989, Goedert et al., 1992). The remaining structure of PHFs, is determined by the amino- and carboxyl-terminal region of tau, which forms the so called fuzzy coat, whilst the core of PHFs is formed by the MBD of tau (**Figure 6C)** (Crowther et al., 1989, Crowther et al., 1992, Goedert et al., 1992, Fitzpatrick et al., 2017).

A final polymerization step may then bundle the elongated PHFs into intracellular NFTs (I-NFTs, **Figure 6D**), which are morphologically characterized by their flame-shape structure (Maccioni and Perry, 2009, Mena and Luna-Munoz, 2009, Mietelska-Porowska et al., 2014). However, as the inclusion progresses, filaments and NFT-structure start to be proteolytically degenerated and abolished, eventually resulting in neuronal cell death. The remaining physical structure is referred to as an extracellular neurofibrillary tangle (E-NFT) or ghost tangle (**Figure 6E**) which is characterized by a dominant core (arrow), whilst the filaments are exposed into the extracellular space (Maccioni and Perry, 2009, Mena and Luna-Munoz, 2009).

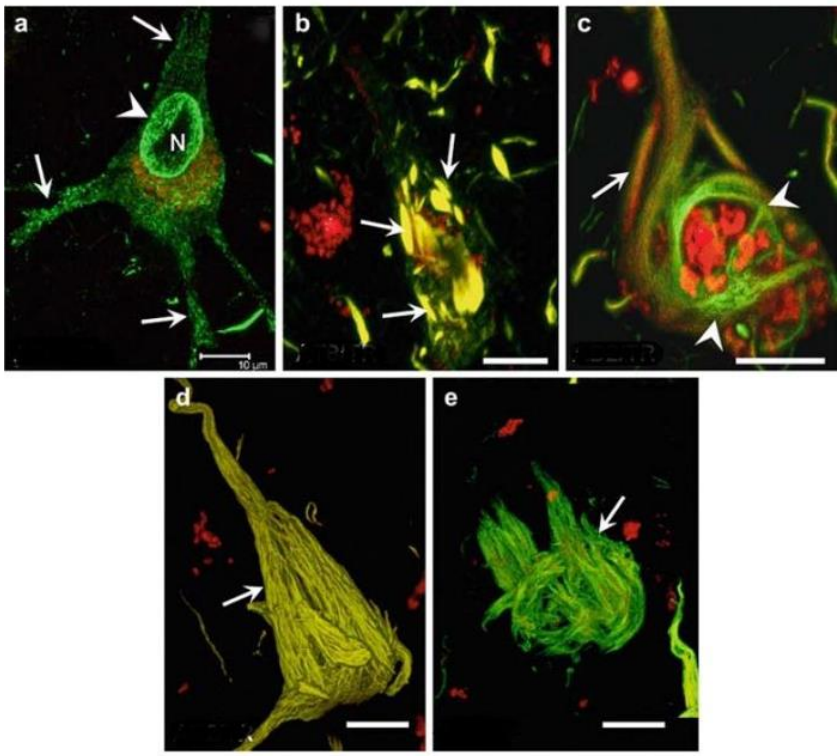

Figure 6: Fibrillization and deposition of tau into NFTs

The process of tau fibrilization into NFTs is characterized by distinct morphological changes of tau, starting with the pre-tangle stage (a). Pre-tangles are characterized by small, non-fibrillary tau deposits, which are found throughout the perinuclear area (arrowhead) and proximal processes of affected neurons (arrows). Subsequent polymerization then initiates the transition of non-fibrillar tau deposits into more organized, bead-like structures (arrows, b). Those structures are termed paired helical filaments (PHFs) and straight filaments (SF). As the polymerization progresses (arrows), filaments bundle around the nucleus (arrowheads) and are organized into intracellular neurofibrillary tangles (c) termed (I-NFT). I-NFTs (d, arrow) are thought to represent early stages in NFT formation and show the characteristic flame-like structure (arrows). Following this, filaments start to be exposed into the extracellular space, indicating neuronal cell death. This structure is a characteristic feature of extracellular neurofibrillary tangles (E-NFTs, arrow) or ghost tangles (e). Cell structures were visualized utilizing the following antibodies: AT8 (a, b), AD2 (c, d) and 423 (e) (Image modified with permission from (Mena and Luna-Munoz, 2009, Maccioni and Perry, 2009))**.**

In conjunction with the recent hypotheses, that aberrantly increased levels of soluble tau species may give rise to cognitive decline and neuronal loss, there is also evidence

that the formation of tangles may contribute to neurotoxicity in a loss of physiological function (decreased tau-MT interaction) and/or gain of toxic function (increased formation of fibrils) manner (Braak and Braak, 1991, Trojanowski and Lee, 2005, Lee and Trojanowski, 2006).

Firstly, the typical anatomical spreading of NFTs throughout the brain was shown to nicely correlate with disease severity, neuronal loss and cognitive dysfunctions present in neurodegenerative disorders (Arriagada et al., 1992, Bierer et al., 1995, Augustinack et al., 2002, Ballatore et al., 2007, Craddock et al., 2012, Gendreau and Hall, 2013). Secondly, similar to pathogenic tau species, NFTs have been proposed to interfere with MT-polarization, which is thought to compromise MT-assembly and potentially result in intraneuronal transport dysfunctions (loss of physiological function) in affected neurons (Lee and Trojanowski, 2006). Consequently, the antero- and retrograde transport of proteins and substances throughout the cell, would no longer be sufficient to maintain cell viability (Lee and Trojanowski, 2006, Reddy, 2011) eventually leading to synaptic deprivation and cell death (Trojanowski and Lee, 2005).

In addition, some studies showed an inverse correlation between the number of ghost tangles and surviving neurons in highly vulnerable brain regions of AD patients (Cras et al., 1995, Fukutani et al., 1995). These findings imply that NFTs may be released by degenerating neurons where they are thought to sequester other cellular structures or may initiate the formation of new inclusions in surrounding neurons (Cras et al., 1995, Gomez-Ramos et al., 2006). Moreover, ghost tangles appear to be very resistant to proteolytic removal by microglia, which may further negatively regulate and compromise neuronal functioning. Therefore, these studies suggest that NFTs may either directly or indirectly interfere with neuronal functioning and even contribute to tau-induced neuronal death (Cras et al., 1995, Avila et al., 2004a, Gomez-Ramos et al., 2006).

Although the above studies suggest NFTs as being the toxic species that underlines neuronal degeneration, more recent studies clearly challenge this viewpoint and instead suggest that NFT formation is neither necessary nor sufficient to cause neurodegeneration and cognitive decline (Wang and Mandelkow, 2016). One observation that supports this notion is provided by the incidence in which PHFs, the main components of NFTs, occur. Only 10% of PHFs were shown to actually deposit

in NFTs, whilst more than 90% of PHFs are found in other neuronal structures including dystrophic neurites (Mitchell et al., 2000). Further, neuronal loss in the superior temporal sulci of AD-patients was shown to exceed the number of fibrillar inclusions by more than seven-fold (Gomez-Isla et al., 1997), implying that the corpus of neurons may degenerate, without ever developing NFTs (Gomez-Isla et al., 1997, Spires-Jones et al., 2008). In addition, a mathematical prediction model by Morsh and colleagues estimated that neurons may survive for decades whilst carrying NFTs (Morsch et al., 1999), which clearly contradicts the previously assumed causality between neuronal loss and NFT-induced toxicity (Gendreau and Hall, 2013). In line with these findings are observations from a drosophila model of tauopathies, in which wild type and mutant tau expression was sufficient to induce AD-like symptoms, in the absence of NFTs (Wittmann et al., 2001). Likewise, findings were reported in several tau-inducible transgenic mouse lines, in which synapse degeneration, neuronal loss, and cognitive dysfunctions were detected prior or even in the absence of fibrillar inclusions (Santacruz et al., 2005, Sydow et al., 2011, Van der Jeugd et al., 2012). Furthermore, whilst the dynamic processes leading to tau-induced impairments could clearly be ameliorated in the mouse models, the formation of fibrillar inclusions remained. In conclusion, these studies suggest that soluble, rather than fibrillar tau inclusions are the species to blame and further suggest that NFTs *per se* are not as toxic, as the processes that lead to NFT-formation (Santacruz et al., 2005, Sydow et al., 2011).

1.4.3 Mutations in *MAPT*

Since the first report of a direct link between mutations in *MAPT* and FTDP-17 (Wilhelmsen et al., 1994, Hutton et al., 1998), more than 50 mutations in over 100 families have been described to date (Pittman et al., 2006). Moreover, these mutations were not only identified in FTDP-17 individuals, but could also be linked to other tauopathies, including CBD, PSP and PiD (Pickering-Brown et al., 2000, Poorkaj et al., 2002, Ros et al., 2005, Kouri et al., 2014).

The majority of *MAPT* mutations are either silent, missense or deletion mutations (Goedert and Spillantini, 2000, Pittman et al., 2006) clustered within (E9-12) or near the MBD (E13). Alternatively, they belong to the family of intronic mutations,

located downstream of E10, although two mutations in E1 of tau have recently been identified (Hayashi et al., 2002). Whilst some mutations have been described in all six tau isoforms (i.e. R5H, G272V, K257T, L266V, E342V, K369I, V337M, G389R and R406W), mutations in E10 (ΔK280, N279K L284L, N296H, N296N, ΔN296, P301L, P301S, P301T, S305N and S305S) are thought to only affect 4R-tau isoforms (**Figure 7**) (Hutton et al., 1998, Mirra et al., 1999, Bugiani et al., 1999, Goedert and Spillantini, 2000, Ingram and Spillantini, 2002).

On the other hand, *MAPT* mutations can also be classified according to their functional properties and the location of their effect, which can either be at protein level (modulating tau-MT interaction) and/or at RNA level (targeting the splicing of pre-

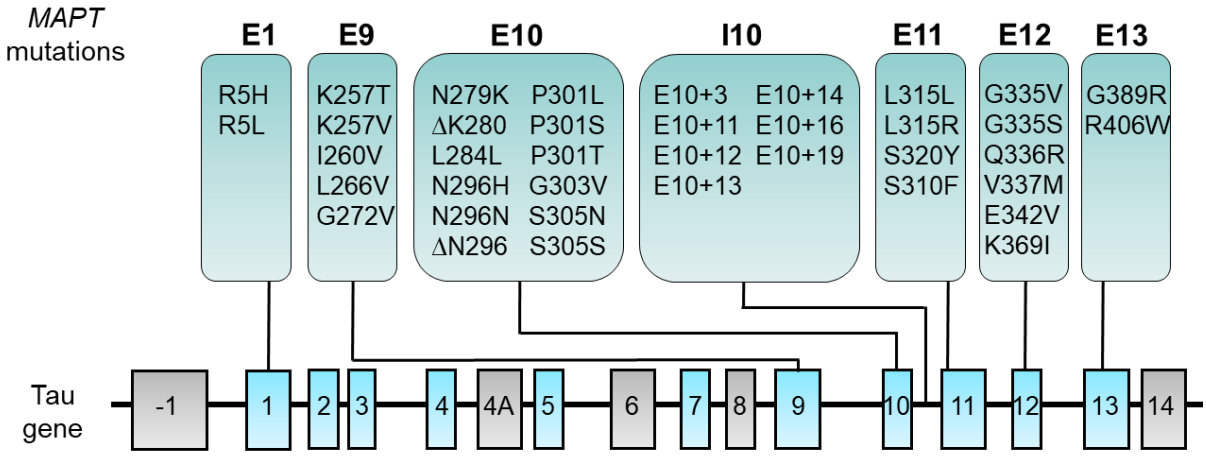

Figure 7: Mutations in *MAPT*

Since the first report of a *MAPT* mutation in FTDP-17 (Wilhelmsen et al., 1994, Hutton et al., 1998), more than 50 mutations in over 100 families have been described to date (Pittman et al., 2006). In addition to FTDP-17 cases, MAPT mutations have also been identified in other tauopathies, including CBD, PSP and PiD (Pickering-Brown et al., 2000, Poorkaj et al., 2002, Ros et al., 2005, Kouri et al., 2014) whilst presently no tau mutations have been found in patients with AD. Most *MAPT* mutations underlying FTDP-17 primarily cluster around E9 to E12 or have been linked to the intronic region between E10 and E11 of the tau gene (image modified with permission from (Brunden et al., 2009).

mRNA) (Buee et al., 2000, Ingram and Spillantini, 2002, D'Souza and Schellenberg, 2005). The first group of mutations, which affects tau protein function, include missense and deletion mutations within E9, E11, E12 and E13 of tau. These mutations were found to negatively regulate the affinity of tau to MTs, resulting in the disruption of tau-MT interaction (Ingram and Spillantini, 2002, D'Souza and Schellenberg, 2005, Gendron and Petrucelli, 2009). This particular feature has also been described for some mutations within E10, including the P301L and P301S *MAPT* mutations (Hasegawa et al., 1998). Furthermore, in vitro evidence indicates that this first class of mutations, in particular P301L and P301S, may have a direct effect on heparin-induced assembly of tau into filaments, meaning that these mutations positively correlate with tau accumulation and hence are thought to increase the deposition of tau into NFTs (Nacharaju et al., 1999, Goedert et al., 1999, Goedert and Spillantini, 2000). The reason for these functional outcomes may be due to the fact that first class mutations mainly occur in the MBD of tau, which plays an important role in tau-MT interaction (Butner and Kirschner, 1991, Lee and Rook, 1992, Trinczek et al., 1995), and was further found to be an essential constituent of PHFs (Wischik et al., 1988). Accordingly, this first class of mutations is thought to compromise tau function and increase its accumulation into NFTs and may thereby contribute to tau-mediated toxicity (Gendron and Petrucelli, 2009).

On the other hand, the second class of mutations is represented by deletion (Δ280K, Δ296N), intronic (E10+3, E10+11, E10+12, E10+13, E10+14, E10+16, E10+19), missense (L284L, N296N, S305S) and some silent mutations (N279K, N296H, S305N) (Poorkaj et al., 1998, Hutton et al., 1998, Buee et al., 2000, Yoshida et al., 2002, D'Souza and Schellenberg, 2005). These mutations are mainly located within or near E10 and are thought to target the *MAPT* transcript by primarily enhancing alternative splicing of E10 (Gendron and Petrucelli, 2009).

Keeping in mind that an inclusion of E10 primarily results in 4R-tau isoforms, increased splicing of E10, either by strengthening of splicing enhancers or weakening of E10 splicing silencers (Bugiani et al., 1999), would significantly increase 4R-tau expression levels (D'Souza and Schellenberg, 2005). In fact, increased amounts of 4R-tau splice variants have been described in several neurodegenerative disorders including AD and FTD (Hutton et al., 1998, Yasojima et al., 1999, Hyman et al., 2005), suggesting that a disruption to the physiological 4R:3R ratio could result in neurodegeneration (Spillantini et al., 1998b, Gendron and Petrucelli, 2009). On the

other hand, 4R-tau isoforms were shown to have distinct physiological functions in the developing hippocampus (Sennvik et al., 2007), such that alterations to tau isoform expression levels may result in detrimental consequences including abnormal modulations of the cytoskeleton and deregulation of neuronal differentiation (Deshpande et al., 2008). Finally, given that each tau isoform interacts with MTs at different binding-sites (Goode and Feinstein, 1994), an excessive increase of one specific tau isoform (in this case 4R isoforms) may result in a shortage of available MT-binding sites (Heutink, 2000). This process may then result in abnormally increased levels of 4R-tau isoforms in the cytosol, which have been associated with tau-MT deregulation and neurotoxicity (Gendron and Petrucelli, 2009). For these reasons, second-class mutations are thought to increase neuronal vulnerability and may thereby result in, or at least contribute to, neurodegeneration (Liu and Gong, 2008).

One particular *MAPT* mutation, namely the P301S mutation, will be discussed in more detail, as it is essential to the basis of the present studies. The P301S mutation, is a missense mutations, which results from a transition of nucleotide C to T in the codon encoding the amino acid 301, located within E10 (Spillantini et al., 2000, D'Souza and Schellenberg, 2005). This nucleotide transition results in a substitution of the amino acid proline (P) to serine (S) affecting all 4R-tau transcripts. The P301S mutation belongs to the first class mutations, which are, as previously described, thought to compromise tau-MT interaction by affecting and decreasing both the affinity and binding capacity of tau to MTs (Hasegawa et al., 1998, Hutton et al., 1998, Hong et al., 1998, Spillantini et al., 2000, Brandt et al., 2005). Accordingly, the P301S pathogenic mutation is thought to increase the amount of soluble, cytosolic tau, which facilitates the polymerization of soluble tau into filaments (Hutton et al., 1998) and may further render tau more prone to hyperphosphorylation and aggregation (Nacharaju et al., 1999, Delobel et al., 2002, Fischer et al., 2007). Additionally, it has been suggested that missense mutations may also negatively regulate tau degradation which may further fuel the formation of tau into filamentous structures (Hutton et al., 1998, Yen et al., 1999). Resulting tau inclusions mainly consists of narrow, twisted filaments, which are predominantly composed of 4R-tau isoforms (Hutton et al., 1998, Hong et al., 1998, Ingram and Spillantini, 2002, Brandt et al., 2005, Kar et al., 2005) and are present in both glial and neuronal cells (Ingram and Spillantini, 2002).

Individuals carrying the P301S mutation show highly diverse clinical phenotypes across patients, reflecting the complexity of neuronal modifications and resulting malfunctions in affected individuals (Foster et al., 1997, Yasuda et al., 2000, Lossos et al., 2003, Casseron et al., 2005). For instance, whilst an early-onset of rapidly progressing parkinsonism, symptoms of FTD and epileptic seizures were described in a German patient (Sperfeld et al., 1999), a three generation case study of a Jewish family revealed personality changes and progressive motor and cognitive deficits as the most predominate clinical symptoms (Lossos et al., 2003). Additionally, Bugiani and colleagues described distinct clinical pictures in two family members, both of which were carriers of the P301S mutation. Whilst the parent showed characteristic features of FTD, their child presented with symptoms reminiscent of CBD. Hence, these case reports indicate that the same gene mutation may result in a variety of distinct clinical presentations (Foster et al., 1997, Bugiani et al., 1999).

Despite the large phenotypic variabilities amongst patients, P301S mutation carriers do display, at least to some extent, clinical similarities. For instance, all human cases described so far, were characterized by an early disease onset, typically ranging between 25 and 40 years of age and rapidly progressing disease symptoms (Bugiani et al., 1999, Ingram and Spillantini, 2002, Kumar and Weatherall, 2008).

1.5 Animal models of tauopathies

Considering the last decades of dementia research, it becomes apparent how important animal models have been and still are for the understanding and deciphering of fundamental pathomechanisms underlying neurodegenerative diseases (Gotz and Gotz, 2009, Jucker, 2010, Gotz et al., 2012). In fact, questions regarding disease initiation, progression and severity cannot easily be addressed in humans, as a definite diagnosis can only be confirmed at autopsy (Love, 2004, Cayton, 2007). Furthermore, disease-associated changes in molecular and cellular structures have been shown to start decades prior to clinical presentation (Do Carmo and Cuello, 2013), which clearly illustrates the need for salient disease models that allow researchers to emulate the processes leading to tau-mediated neurotoxicity. Additionally, although cell lines, flies and worms might be able to recapitulate some disease features, they fail to reflect the complexity of the disease spectrum. This may include substantial components of

disease-specific pathological processes that may induce neuronal vulnerability, or characteristic behavioural features, presented in individuals (Forman, 2004, Tovar et al., 2009). Finally, it is important to note that utilized disease models should not only represent snap shots of current disease states, but rather reflect disease progression over time, similar to age-related disorders (Do Carmo and Cuello, 2013).

The majority of these aspects are fulfilled in rodents, in particular transgenic mouse models. To date, transgenic mice are, by far, the most widely used *in vivo* tool to study disease-associated mechanisms, allowing the identification of potential targets or new concepts for future translation into therapies (Hock and Lamb, 2001, Gotz and Ittner, 2008). In comparison to other disease organisms, transgenic mice offer several advantages. Firstly, they enable the introduction, overexpression and/or deletion of human genes, which can be utilized to assess the functional role of disease-specific genes in an *in vivo* setting or may even provide insights into the traits of susceptible genes (Libby et al., 2005, Fuster-Matanzo et al., 2011). Secondly, laboratory animals are usually inbred strains, meaning that they are characterized by a highly homogeneous genetic composition across animals. This feature allows increased reproducibility of research outcomes and further facilitates high throughput studies (Vandamme, 2014). Thirdly, recent studies indicate that transgenic animals have already successfully contributed to the identification and validation of disease-modifying factors (Do Carmo and Cuello, 2013, Yiannopoulou and Papageorgiou, 2013), which for instance may underline phenotypic variabilities amongst disease carriers (Kearney, 2011). Finally, the genetic and physiological properties of mice are comparable to those present in humans (approximately 97% genetic homology (Harper, 2010)), thus any insights gained could potentially be translated into human clinical trials (Nicoll et al., 2003, Jucker, 2010). For instance, active immunization against Aβ was demonstrated to reduce cerebral amyloidosis in APP-transgenic mice (Schenk et al., 1999, Bard et al., 2000, Brody and Holtzman, 2008). These positive outcomes were instantly translated into clinical trials where they were shown to be effective in immunized patients, present with moderate AD symptoms (Holmes et al., 2008, Boche et al., 2008). Moreover, post-mortem analysis of immunized patients suggested that active immunization not only resulted in reduced plaque load, but also negatively correlated with amyloid-associated neuropathology, which was consistent with previous findings in transgenic mice (Holmes et al., 2008, Serrano-Pozo et al., 2010). However, above mentioned clinical trials needed to be terminated after a subset

of patients unexpectedly developed encephalitis (Orgogozo et al., 2003). These side effects were not initially predicted during preclinical trials, only later was it reported that some mice developed similar symptoms under rare circumstances (Furlan et al., 2003, Lee et al., 2005).

Unfortunately, the long history of failed or halted clinical trials in the dementia field not only questions the translatability of preclinical trials but also challenges the validity of currently utilised animal models (Onos et al., 2016). However, although animal models may have their limitations, it needs to be pointed out that most translational failures in the clinic are rather attributed to inadequately conducted preclinical trials and their misinterpretation, rather than to the incomplete nature of the models themselves (Jucker, 2010). This implies that improvements in clinical translation can only be achieved by critical and careful evaluation, validation and appropriate interpretation of preclinical research outcomes (Dawson et al., 2018). Nevertheless, when used accordingly, animal models, in particular transgenic mice, are valuable and indispensable tools to dissect the pathophysiological features of tau-mediated diseases. Finally, as mentioned above, animal models have already successfully contributed to a better understanding of disease-associated mechanisms (Santacruz et al., 2005, Ittner et al., 2010, Ittner et al., 2016).

The first tau transgenic mouse model, expressing the longest human tau isoform (4R2N) was established over two decades ago (Gotz et al., 1995). Whilst these tau transgenic mice presented with pre-tangles, tau hyperphosphorylation and human tau expression in neurons, dendrites and axons, the disease characteristic NFT pathology could not be achieved (Gotz et al., 1995). Following this, several other groups utilized more efficient promoters to drive transgene expression (Spittaels et al., 1999, Ishihara et al., 1999), however, tangles were still absent in aged mice (Ishihara et al., 2001), highlighting the limitations of previously used expression approaches.

Following the discovery of pathogenic mutations in *MAPT*, NFT pathology was finally achieved, in both neurons and glia (Gotz and Gotz, 2009). Moreover, the identified pathogenic FTDP-17 mutations enabled the generation of more specific disease models, including the JNPL3 tau transgenic mice, which expressed the most common FTDP-17 mutation, P301L. These mice recapitulated several disease-associated features, including the formation of tangles in neurons and astrocytes and a significant reduction in the numbers motor neurons, which seemed to positively

correlate with neuronal loss in these mice (Lewis et al., 2000). Moreover, the phenotypic changes present in JNPL3 transgenic mice suggest that the human P301L mutation may decrease tau affinity to MTs and thereby negatively regulate tau-MT interaction in these tau transgenic mice (Lewis et al., 2000, Koechling et al., 2010).

Subsequently, another mutant human P301L mouse model was introduced, namely the pR5 tau transgenic mice. In these mice, transgene expression was driven by the neuron specific Thy1.2 promoter, resulting in pronounced mutant tau expression in the hippocampus and amygdala and abnormally phosphorylated tau at disease specific sites (Gotz et al., 2001). Additionally, pR5 mice presented with distinct memory impairments (Pennanen et al., 2006), mature tangles and neuronal loss, suggesting that distinct processes may contribute to neurodegenerative diseases (Gotz et al., 2001).

In addition to constitutive disease models (i.e. JNLP3 and pR5), in which the transgene expression is not controllable but rather continuously expressed, the generation of mice using inducible expression systems was initiated (Santacruz et al., 2005, Mocanu et al., 2008). The advantage of these expression systems lies in the ability to regulate the transgene expression by determining the exact region (tissue/cell type) and time point at which the transgene expression is initiated and/or terminated (Saunders, 2011). One sophisticated example of an inducible system is the rTg4510 mouse model, which was first introduced by Santacruz and colleagues (Santacruz et al., 2005). The rTg4510 tau transgenic mice express the P301L FTDP-17 mutation and were initially generated to determine the role of NFTs in tau-mediated neurotoxicity (Santacruz et al., 2005). During this study, transgene expression was turned on first, resulting in progressive age-related NFT pathology, neuronal loss, brain atrophy and cognitive decline. However, once the transgene expression was turned off, a significant decrease in mutant tau protein levels and memory improvements were detected, whilst tangles still continued to accumulate (Santacruz et al., 2005). This study provided the first *in vivo* evidence that NFTs *per se* may neither be necessary nor sufficient to cause neurodegeneration and cognitive decline and further suggested that soluble rather than fibrillar tau are the toxic species underlying neurodegeneration (Santacruz et al., 2005).

Another tau transgenic model, which nicely resembles features of FTLD-tau, is the K369I (K3) transgenic mouse model. These mice express the 1N4R-tau isoform

containing the *MAPT* mutation K369I, which was previously identified in a patient with PiD (Neumann et al., 2001). In these mice, mutant tau is expressed in the cortex, hippocampus and cerebellum where it was found to be hyperphosphorylated at multiple sites and accumulated in pathogenic inclusion resembling both NFTs and Pick bodies. Unlike previous tau disease models, transgene expression in K3 mice was also present in the substantia nigra, which may account for the early-onset of parkinson-like symptoms, including bradykinesia, abnormalities in gait, resting tremor and postural instability (Ittner et al., 2008). Interestingly, these motor dysfunctions could be improved with L-Dopa treatment in young, but not old transgenic K3 mice, resembling aspects of parkinsonism found in FTD. Finally, K3 mice developed selective dysfunctions in the anterograde axonal transport of certain cargos, prior to the degeneration of dopaminergic neurons (Ittner et al., 2008). These findings are in line with previous studies, indicating that tau may contribute to axonal transport dysfunctions, prior to its deposition (Spittaels et al., 1999, Stamer et al., 2002). Finally, this, and other studies, suggest that tau-mediated axonal transport dysfunctions may underline a central pathomechanism in AD and related tauopathies (Trojanowski et al., 2005, Stokin and Goldstein, 2006, Thies and Mandelkow, 2007).

Of particular relevance for this study are tau transgenic mouse models expressing the P301S *MAPT* mutation, as they provide the basis for the following studies. The most commonly utilised P301S tau transgenic mouse models will therefore be discussed in more detail.

Allen and colleagues introduced the first P301S tau transgenic mouse line, expressing the shortest human tau isoform under the control of the murine Thy1.2 promoter (Allen et al., 2002). These mice develop a striking neurological phenotype, resembling muscle weakness and tremor, but most dominantly severe paraparesis. These neurological features may be attributed to the substantial degeneration of motor neurons in the anterior horn of the spinal cord (Allen et al., 2002). Additionally, these mice present with abundant tau hyperphosphorylation at diseases-associated sites and fibrillar tau inclusions in several brain regions including the spinal cord (Allen et al., 2002). Finally, most fibrillar inclusions contained twisted ribbon filaments, reminiscent of the pathological features previously found in cases of FTDP-17. However, whether the abundance of tau pathology in the spinal cord contributed or even induced motor neuron atrophy in the spinal cord, remains to be established.

Notably, due to the striking neurological phenotype present in these mice, behavioural and/or functional assessments might not be feasible (Allen et al., 2002).

Another mutant P301S transgenic mouse model established by Yoshiyama and colleagues (commonly referred to as PS19) developed pronounced neuronal and synaptic loss, hippocampal atrophy and microglial activation as most prevalent features (Yoshiyama et al., 2007). However, whilst neuronal loss and atrophy presented by eight months of age, microglial activation and synaptic dysfunction were described as early manifestations of disease. Interestingly, the latter occurred even prior to tau aggregation, indicating that pathological processes, other than NFT formation, may drive neuronal dysfunction and degeneration in the PS19 tauopathy mouse model (Yoshiyama et al., 2007). Moreover, immunosuppression in young transgenic mice was able to mitigate tau pathology and increase lifespan, suggesting that neuroinflammation may contribute to/or even mediate tau-induced neurotoxicity (Yoshiyama et al., 2007).

A more recent study assessed behavioural dysfunctions in PS19 transgenic mice, including increased hyperactivity in the open field paradigm, decreased anxiety-levels in the elevated plus maze as well as age-related deficits in spatial and memory learning. Interestingly, these behavioural abnormalities seemed to occur in prodromal stages of disease, indicating neurofunctional abnormalities in PS19 transgenic mice (Takeuchi et al., 2011). Some behavioural assessments could not be conducted at later disease stages (Takeuchi et al., 2011), due to the aggressive progression of motor impairments in these mice, which culminate in paralysis at around seven months of age, and result in a median survival rate of about 9 months of age. (Yoshiyama et al., 2007). Notably, due to the early-onset progressive phenotype, PS19 transgenic mice may be suitable to asses early disease manifestations but are rather limited when trying to uncover diseases-specific changes over time. Additionally, some studies report a phenotypic drift in PS19 transgenic mice on mixed backgrounds (Zhang et al., 2012), which may result in misinterpretation of research outcomes, for instance when looking at phenotypical and histopathological changes as readouts of therapeutic interventions.

Another P301S mutant tau transgenic mouse model, known as TAU58/2, was generated more recently (van Eersel et al., 2015). These mice express the shortest

human tau isoform (0N4R) under the control of the neuron specific Thy1.2 promoter. In these mice, tau was found to be hyperphosphorylated at multiple disease-associated sites, resulting in progressively increased tau deposition in several brain regions as mice aged. Interestingly, TAU58/2 mice present with early-onset axonal pathology prior to NFT formation, suggesting that axonal transport dysfunctions may underline tau-mediated neurotoxicity. Furthermore, axonal lesions found in TAU58/2 mice were reminiscent of those observed in human FTLD-tau and AD (van Eersel et al., 2015). Behavioural dysfunctions in TAU58/2 mice were of early onset, starting from 2 months of age and progressively affecting several functional domains, including strength, sensorimotor and spatial motor coordination. However, although motor abilities were reported to be significantly affected in TAU58/2, motor performance of aged transgenic mice suggested overall motor competency even at late disease stages. Finally, throughout the whole study, significant gender specific differences were observed, with male transgenic mice showing higher levels of insoluble tau and more pronounced motor abnormalities, compared to female TAU58/2. In conclusion, TAU58/2 mice recapitulate essential aspects of human FTLD-tau and AD pathology and may thereby enable a better understanding of mechanisms underlying the pathogenesis of FTD and related tauopathies.

1.6 Aims of the current studies

Hyperphosphorylation and deposition of tau in the brain is a characteristic feature of several neurodegenerative diseases, including FTD and AD. Furthermore, tau but not Aβ pathology correlates with cognitive decline and neurodegeneration in these diseases. However, limited knowledge is available about the exact mechanisms underlying tau-induced behavioural and cognitive dysfunctions in disease. Therefore, the primary objective of this thesis is to determine the role of P301S mutant human tau in the development of behavioural, functional and cognitive deficits in the TAU58/2 transgenic mouse line.

Specific aims:

I. Clinically, FTD is characterized by a wide range of profound behavioural abnormalities, including apathy, disinhibition, hyperactivity and aphasia. To better understand the causality between behavioural changes and tau-mediated neurotoxicity **Paper I** aims to elucidate whether the expression of P301S mutant human tau may impact on behavioural outcomes in TAU58/2.

II. Neuronal network dysfunctions and cognitive decline are characteristic features of several tauopathies, including AD and FTD. However, the underlying mechanisms contributing to neuronal vulnerability in tau-mediated diseases is poorly understood.

To get a better understanding of cellular and molecular mechanisms underlying cognitive dysfunction in AD and FTD, the experiments undertaken in **Paper II** aimed to characterize the effects of transgenic P301S mutant human tau expression on neuronal network function in the murine hippocampus.

Paper I

I. Disinhibition-like behavior in a P301S mutant tau transgenic mouse model of frontotemporal dementia

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Published as:

Przybyla, M., Stevens, C. H., van der Hoven, J., Harasta, A., Bi, M., Ittner, A., van Hummel, A., Hodges, J. R., Piguet, O., Karl, T., Kassiou, M., Housley, G. D., Ke, Y. D., Ittner, L. M., van Eersel, J. (2016) Disinhibition-like behavior in a P301S mutant tau transgenic mouse model of frontotemporal dementia, *Neuroscience Letters*, Vol. 631, pp. 24-29, https://doi.org/10.1016/j.neulet.2016.08.007.

2.1 Abstract

Frontotemporal dementia (FTD) presents clinically with behavioral changes including disinhibition. Mutations in the tau-encoding *MAPT* gene identified in familial cases of FTD have been used to generate transgenic mouse models of the human condition. Here, we report behavioral changes in a recently developed P301S mutant tau transgenic mouse, including disinhibition-like behavior in the elevated plus maze and hyperactivity in the open field arena. Furthermore, histological analysis revealed the amygdala as a primary and early site of pathological tau deposition in these mice. Taken together, neuropathological and behavioral changes in P301S tau transgenic mice resembles features of human FTD.

2.2 Introduction

Frontotemporal dementia (FTD) is a term collectively used to describe a clinically, pathologically and genetically diverse group of neurodegenerative disorders. Clinically, FTD patients may show changes in behavior, language, cognition and motor function. The most common clinical phenotype is the behavioral variant of FTD (bvFTD), characterized clinically by changes in behavior and personality, including apathy and disinhibition (Powers et al., 2014). Early disinhibition in bvFTD may present as socially inappropriate behaviors, loss of manners, and impulsive, rash and careless actions (Miki et al., 2016), with failure of inhibitory control (O'Callaghan et al., 2013). Neuropathologically, FTD is characterized by the abnormal deposition of proteins in neurons and glia, including the microtubule associated binding protein tau, the transactive response DNA binding protein 43 (TDP-43), or less frequently, other proteins such as fused in sarcoma (FUS). Approximately one third of FTD cases are familial with the most frequent mutations found in the tau-encoding *MAPT* gene with tau pathology, and in *GRN* and *C9ORF72* associated with TDP-43 deposition (Ferrari et al., 2014). Interestingly, behavioral disinhibition is more frequent amongst *MAPT*, than *GRN* and *C9ORF72* mutation carriers with familial FTD (Snowden et al., 2015).

Tau is an unstructured multi-domain protein that binds to microtubules to regulate their dynamics and intracellular transport processes (Ballatore et al., 2007). Tau is predominantly found in the axon of neurons, although small amounts localize to the post-synapse to regulate excitatory signaling (Ittner et al., 2010). Tau harbors over 80 potential phosphorylation sites, and in disease, it becomes aberrantly phosphorylated at many sites, which coined the term ‘hyperphosphorylated tau’ (Goedert et al., 1992). Hyperphosphorylation of tau compromises its binding to microtubules, resulting in accumulation of tau in the soma and dendrites of neurons (Ittner et al., 2011). Hyperphosphorylated tau is prone to oligomerize and form insoluble fibrillar aggregates that present as neurofibrillary tangles (NFTs), a common feature of FTD with tau pathology (Arriagada et al., 1992).

The identification of *MAPT* mutations in familial FTD has been instrumental in the generation of a significant number of transgenic mouse lines that develop functional deficits and NFT pathology (reviewed by (Gotz et al., 2007)). We have recently

reported TAU58/2 mice with neuronal expression of P301S mutant tau (van Eersel et al., 2015). TAU58/2 mice present with motor deficits, tau and neurofilament pathology reminiscent of human FTD with tau pathology. In the present study, we show that TAU58/2 mice develop early-onset disinhibition-like behavior and increased motor activity together with early NFT pathology in the amygdala, reminiscent of bvFTD.

2.3 Methods

2.3.1 Mice

TAU58/2 mice express the human 0N4R tau isoform with the P301S mutation under the control of the mouse Thy1.2 promoter, as previously described by us (van Eersel et al., 2015). All animal experiments were conducted with male TAU58/2 mice and control littermates only, due to their more pronounced phenotype compared to female mice (van Eersel et al., 2015). All animal experiments were approved by the Animal Ethics Committees of the University of New South Wales. All procedures complied with the statement on animal experimentation issued by the National Health and Medical Research Council of Australia.

2.3.2 Histology

Three, 6 and 12-month-old male TAU58/2 transgenic mice (n=4-9) and non-transgenic littermates (n=3-6) were used for histological analysis. At the desired age, mice were anesthetized and transcardially perfused with phosphate buffered saline (pH 7.4) to remove blood. Brains were removed and immersion fixed in 4% paraformaldehyde, processed in an Excelsior tissue processor (ThermoFisher, Waltham, MA, USA), embedded in paraffin and sectioned in the coronal plane at 3-8 μm. One-2 sections per mouse corresponding to approximately -2.54 mm relative to Bregma were stained with antibodies against tau phosphorylated at Ser214 (pS214, Abcam) and Ser422 (pS422, Abcam), and with Gallyas silver impregnation to reveal NFTs composed of insoluble tau aggregates, using previously published procedures (Ittner et al., 2008). Brain sections stained with pS422 and pS214 were scanned on a Scanscope Aperio

FL (Leica Biosystems). One fluorescent channel Alexa Fluor 488 (Excitation 485/20-25, Emission 521/25) was imaged with an exposure time of 320 ms, which was kept consistent across all slide imaging. The number of pS422 and pS214-positive neurons were counted throughout the amygdala, whilst only neurons that colocalized with a nuclear marker (DAPI,) were included for analysis Neurons with bright (high) staining signal for pS214 were counted separately of those neurons with less intensive (low) staining. Brightfield microscopy was performed with an Olympus BX51 (USA) microscope equipped with a DP70 color camera. The number of Gallyas silver positive NFTs, identified by black colour, neuronal size (<3 µm) and their typical flame- shaped cell morphology, were counted throughout the amygdala. Olympus CellSens software was used to delineate the regions of interest, and area measurements automatically calculated. All cell counts were converted to a density value (cells/mm^2). For all quantitation, repeated measures on different days gave an inter- and intra-rater variability of <5%. All Cell counts were conducted without knowledge of genotypes and quantification was done before de-blinding experimenters.

2.3.3 Behavioral testing

2.3.3.1 Elevated Plus Maze

Anxiety and disinhibition-like behavior were tested in 3, 6 and 10 month-old male TAU58/2 mice (n=10-18) and non-transgenic littermates (n=7-17) in an elevated plus maze (Ugo Basile), consisting of two open and two closed arms (each 35 cm x 5.5 cm), as well as a central platform (5.5 cm x 5.5 cm), arranged in a plus shape and elevated 60cm above the ground. Mice were acclimatized to the room for 1 hour prior to testing, then placed on the central platform facing an open arm and recorded for 5 minutes. Recordings were done at 210-215lx (open arm). 130-140lx (closed arm). Videos were analyzed using the AnyMaze software (Stoelting) by a person blinded to the genotypes.

2.3.3.2 Open Field

Activity, anxiety and exploration pattern were tested in 3, 6 and 10-month-old male TAU58/2 mice (n=6-17) and non-transgenic littermates (n=6-13) in an open field arena. Mice were individually placed at the periphery of a box (40 cm x 40 cm) in an enclosed cupboard and their movement recorded for 10 minutes. Recordings were done at 200lx. Videos were analyzed using the AnyMaze software by a person blinded to their genotypes. For analysis, the box was divided into an outer and inner zone, where the inner zone was represented by a 22.5 cm x 22.5 cm square in the center of the box.

2.3.3.3 Statistical analysis

All statistical analysis was done using the Graphpad Prism 6.0 software (GraphPad, La Jolla, CA, USA) using either Student's *t*-tests for comparison of two data sets, analysis of variance (ANOVA) for comparison of more than two data sets or two-way ANOVA for comparison across time. *P* vales of below 0.05 were considered significant. All values are presented as mean ± standard error of the mean.

2.4 Results

2.4.1 Amygdala is an early site of tau pathology in TAU58/2 mice

TAU58/2 mice express P301S mutant human 0N4R tau under the control of the murine mThy1.2 promoter in CNS neurons, and we have previously reported motor deficits in this line when challenged in different test paradigms (van Eersel et al., 2015) (van Eersel et al., 2015). In the present study, we determined whether TAU58/2 recapitulate features of bvFTD, focusing on disinhibition and risk-taking behavior.

Atrophy of the amygdala is observed at even the earliest stages of FTD (Kril and Halliday, 2004), and considerable tau pathology has been reported in the amygdala of patients with FTD and related neurodegenerative disorders (Cook et al., 2014). While we have previously assessed tau pathology in TAU58/2 mice (van Eersel et al., 2015), this analysis has not included the amygdala. Therefore, we determined

tau pathology in the amygdala of young (3 months), adult (6 months) and aged (12 months) TAU58/2 mice, using both immunohistochemical staining for phosphorylated tau and Gallays silver staining of NFTs (**Figure 8**). PS214 is known to be a marker of early tau involvement, while pS422 is known to be affected later in disease course (Deters et al., 2008). In line with this, abundant pS214-positive neurons were already seen in young TAU58/2, with the number of intensely stained neurons significantly increasing in adult and aged transgenic mice (**Figure 8A and 8B**). In contrast, only a few pS422-positive neurons were seen in the amygdala of young Tau58/2 mice, but their numbers significantly increased with age (**Figure 8C and 8D**). Similarly, NFTs were present in small numbers in young TAU58/2 mice, and significantly increased over time resulting in extensive pathology by 12 months of age (**Figure 8E and 8F**).

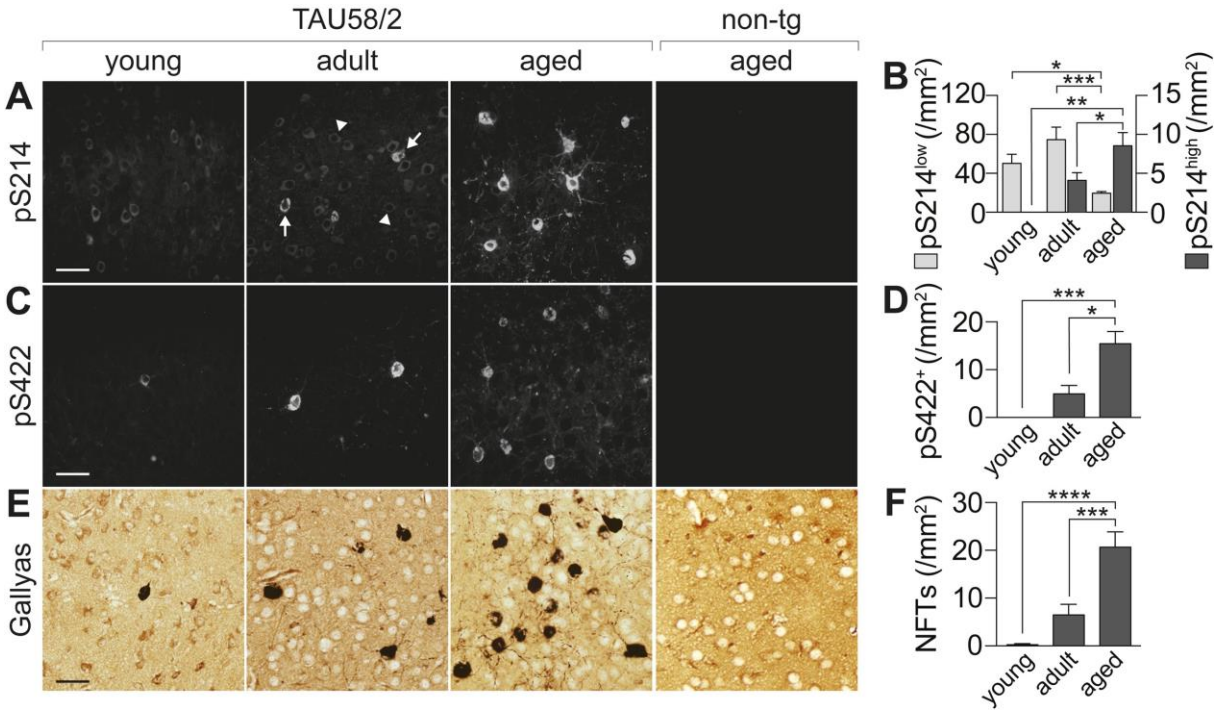

Figure 8: Progressive tau pathology in the amygdala of TAU58/2 mice.

(A) Representative immunofluorescence (IF) staining of the amygdala of young, adult and aged TAU58/2 mice, using an antibody specific for tau phosphorylated at serine 214 (pS214). Arrows indicate neurons with 'high' staining intensity, while arrow heads indicate cells with 'low' staining intensity. No staining was observed in non-transgenic brain tissue. Scale bar, 50µm. (B) Quantification of numbers of pS214-positive cells with low ($pS214^{low}$) and high ($pS214^{high}$) staining intensity per mm^2 (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n=4–9).(C) Representative IF staining of the amygdala of young, adult and aged TAU58/2 mice, using an antibody specific for tau phosphorylated at serine 422 (pS422). No staining was observed in non-transgenic brain tissue. Scale bar, 50µm. (D) Quantification of numbers of pS422-positive cells per mm^2 (*$p < 0.05$; ***$p < 0.001$; n=4–9). (E) Representative Gallyas silver staining of NFTs (black) in the amygdala of young, adult and aged TAU58/2 mice. No staining was observed in non-transgenic brain tissue. Scale bar, 50µm. (F) Quantification of numbers of NFTs per mm^2 (***$p < 0.001$; ****$p < 0.0001$; n=6–9). Error bars represent the standard error.

Non-transgenic littermates were negative for phosphorylated tau and NFT pathology. Taken together, progressive hyperphosphorylated tau and NFT pathology manifests early in the amygdala of TAU58/2 mice.

2.4.2 Behavioural changes in TAU58/2 mice

The amygdala is an important area for behavior control (Seymour and Dolan, 2008). To determine the impact of transgenic P301S mutant tau expression on the behavior in TAU58/2 mice, we subjected transgenic mice and non-transgenic littermates to elevated plus maze (EPM) and open field (OF) testing (Figure 6, Figure 7). Young (3 months), adult (6 months) and aged (10 months) TAU58/2 and non-transgenic control littermate mice were tested. EPM testing revealed that already young TAU58/2 mice spent significantly more time in the open arms than their non-transgenic littermates (**Figure 9A and 9B**). Similarly, both adult and aged TAU58/2 mice spent more time in the open arms than their respective non-transgenic littermates. When comparing TAU58/2 mice across ages, we found that aged spent significantly more time in the open arms than young and adult mice (**Figure 9B**). The time when mice emerged for the first time from the closed into the open arms was significantly reduced in TAU58/2 mice compared to non-transgenic littermates at all ages (Figure 9C). In all age groups, the time animals were mobile, and distance travelled during testing were comparable in TAU58/2 and non-transgenic mice, showing motor competency (**Figure 9D**).

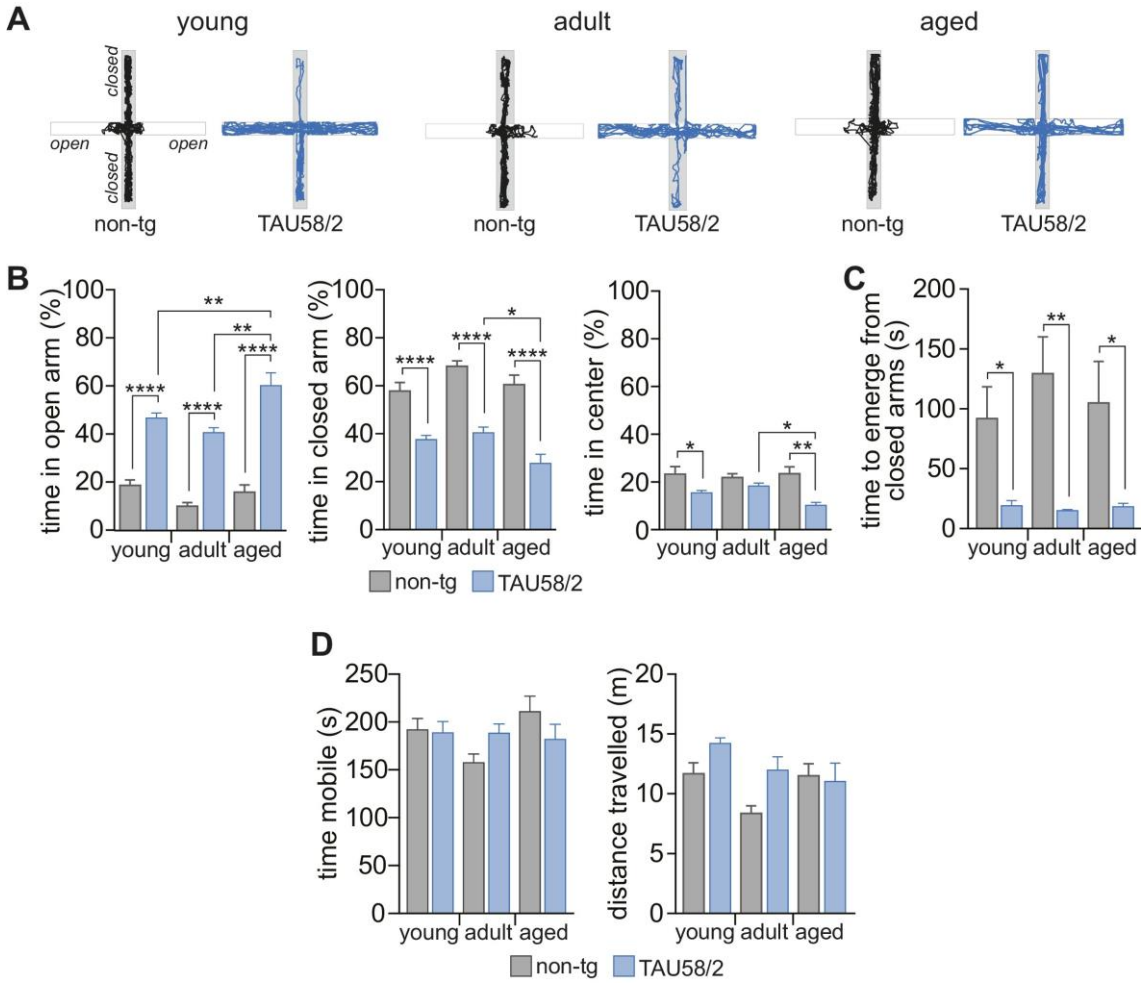

Figure 9: Early-onset disinhibition-like behavior in TAU58/2 mice.

(A) Example traces of young, adult and aged TAU58/2 mice and non-transgenic littermate controls in the EPM, comprising two open and two closed (grey) arms. (B) Quantification of relative time spent in the open arms, closed arms and center in the EPM (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; n=10–18). (C) Quantification of first time to emerge from the closed arms of the EPM into the open (*p < 0.05; **p < 0.01; n=10–18). (D) Quantification of time mice were mobile and total distance travelled during EPM testing (n=10–18). No significant differences were found. Error bars represent the standard error.

Next, we exposed young, adult and aged TAU58/2 mice, and the respective non-transgenic littermate controls, to a novel OF arena and recorded their exploration pattern and locomotion activity continuously over 10 minutes (**Figure 10**). Both young and adult TAU58/2 and their non-transgenic littermates travelled comparable distances during testing (**Figure 10A and 10B**). However, distance travelled and time

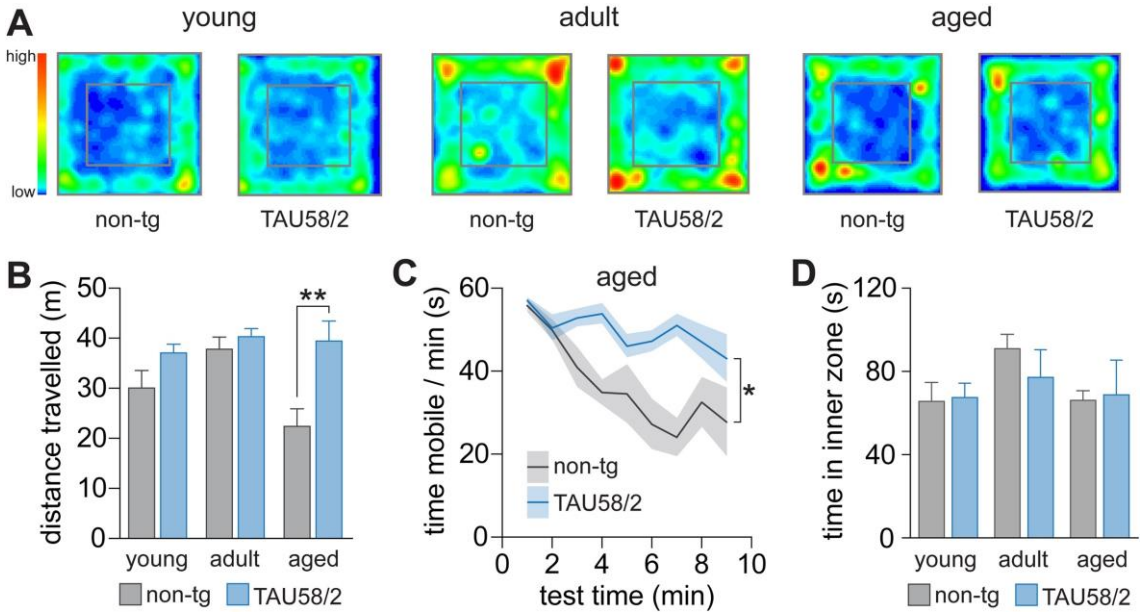

Figure 10: Increased activity in aged TAU58/2 mice.

(A) Heat map tracing of young, adult and aged TAU58/2 mice and non-transgenic littermate controls in the OF. (B) Quantification of distance travelled in the OF over 10 min (**p < 0.01; n=6–10). (C) Time aged mice were mobile each min in the OF over 10 min (*p < 0.05; n=6–10). SEM are depicted as shaded bars. (D) Quantification of time spent in the inner zone in the OF over 10 min (n=6–10). No significant differences were found. Error bars represent the standard error.

mobile during testing were significantly higher in aged TAU58/2 mice compared to non-transgenic littermate controls, which presented reduced activity compared to younger mice (**Figure 10B and 10C**). The exploration paths in the OF arena were comparable between all age groups tested (**Figure 10A**), with similar frequent entries into the inner zone of the arena (**Figure 10D**). Taken together, TAU58/2 present with progressive early-onset deficits in the EPM, while changes in the OF are of late onset.

2.5 Discussion

In the present study, we showed that tau pathology in the amygdala of TAU58/2 mice is of early onset and progressive. EPM testing revealed disinhibition/reduced anxiety

already in young TAU58/2 mice, while deficits in the exploration and hyperactivity in the OF test were of later onset. Previous studies have not assessed tau pathology in the amygdala of TAU58/2 mice (van Eersel et al., 2015, Van der Jeugd et al., 2016). While we reported earlier that tau pathology presents in the hippocampus, cortex and brain stem of TAU58/2 mice (van Eersel et al., 2015), the present study would suggest that the amygdala is a primary site of NFT pathology in this tau transgenic line. Accordingly, numbers of NFTs in the amygdala were already proportionally higher in TAU58/2 mice at 3 months of age compared to other brain regions, including the cortex, and further increased as mice aged. Consequently, at 12 months of age NFT pathology was approximately 4-fold those we reported earlier for the hippocampus and 2-fold higher compared to numbers in the brain stem (van Eersel et al., 2015). Interestingly, significant atrophy of the amygdala has been found in the brains of FTD patients (Barnes et al., 2006, Cerami et al., 2014). Considering the importance of the amygdala in controlling behavior (Seymour and Dolan, 2008), early onset of pathology and behavioural changes in the EPM in TAU58/2 mice support a contributing role to the clinical presentation of FTD.

In parallel to the early tau pathology in the amygdala, we show here already in young TAU58/2 mice decreased anxiety behaviour consistent with disinhibition during EPM testing. Increased impulsivity/disinhibition-like behaviour of TAU58/2 mice is further supported by a reduced latency to leave the closed arms of the EPM. For comparison, abnormal and progressive behaviour in the EPM, with increased time spent in the open arm has been reported in other tau transgenic mouse models of FTD, some associated with widespread tau pathology (including in amygdala and cortex (Dumont et al., 2011, Takeuchi et al., 2011, Cook et al., 2014, Warmus et al., 2014, Cook et al., 2015). Although the youngest group of TAU58/2 mice tested already showed marked EPM deficits, this further progressed as mice aged. In contrast to early EPM deficits in young TAU58/2 mice, exploration and activity in the OF were not altered at 3 and 6 months of age, but only became apparent in aged mice. TAU58/2 and non-transgenic littermates similarly avoided the inner zone of the OF indicating similar anxiety levels. And further supporting that deficits in the EPM rather result from disinhibition than reduced anxiety. Similar behaviour in the OF has been reported for other tau transgenic lines (Cook et al., 2014). Disinhibition and lack of emotional control with

inappropriate social behaviour is a lead symptom of bvFTD (O'Callaghan et al., 2013, Miki et al., 2016), resembled by TAU58/2 mice.

A very recent publication reported early increases in risk-taking behavior in young TAU58/2 when tested in the light/dark paradigm, but no changes to exploration and activity in the OF arena, including in aged mice (Van der Jeugd et al., 2016). Our study found similar behavior in young TAU58/2 mice using the EPM paradigm, as well as hyperactivity in the OF in aged mice, consistent with findings in other tau transgenic lines (Cook et al., 2014, Warmus et al., 2014). The differences in OF behavior of TAU58/2 mice between studies may be due to alternative testing equipment and procedures, subtle differences in the genetic backgrounds or distinctive husbandry conditions.

2.6 Conclusion

Taken together, we found early-onset and progressive disinhibition-like behavior in TAU58/2 mice, together with early NFT pathology in the amygdala, resembling clinical presentations and neuropathology of bvFTD with tau pathology. Moreover, our data suggests a direct causality between behavioural changes and tau-mediated neurotoxicity, given by a positive correlation between the number of NFT-positive neurons and the onset and progressive increase of disinhibition-like behavior as mice age.

We further propose that EPM testing correlates with early tau pathology in the amygdala and is a reliable and quick behavioral testing method to determine functional deficits in TAU58/2, which should be included in studies aiming at modifying disease progression, for example by crossing with other genetically modified mouse lines or by administering therapeutic compounds.

2.7 Acknowledgements

This work has been supported by the National Health and Medical Research Centre (#1037746 ,#1020562, #1069173), the Australian Research Council (#DP130102027,

#CE11000102) and UNSW Australia. L.M.I. and O.P. are NHMRC Senior Research Fellows (#1003083, #1103258).

Paper II

II. Onset of hippocampal network aberration and memory deficits in P301S tau transgenic mice is linked to an immediate early gene signature

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Published as:

Przybyla, M., van Eersel, J., van Hummel, A., van der Hoven, J., Sabale, M., Harasta, A., Müller, J., Gajwani, M., Prikas, E., Mueller, T., Stevens, C. H., Power, J., Housley, G. D., Karl, T., Kassiou, M., Ke, Y. D., Ittner, A., Ittner, L. M., (2020) Onset of hippocampal network aberration and memory deficits in P301S tau mice are associated with an early gene signature, *Brain*, Vol. 143, Iss. 6, pp. 1889–1904, https://doi.org/10.1093/brain/awaa133

3.1 Abstract

Hyperphosphorylation and deposition of tau in the brain characterizes frontotemporal dementia and Alzheimer's disease. Disease-associated mutations in the tau-encoding *MAPT* gene have enabled the generation of transgenic mouse models that recapitulate aspects of the human neurodegenerative diseases, including tau hyperphosphorylation and neurofibrillary tangle (NFT) formation. Here, we characterized the effects of transgenic P301S mutant human tau expression on neuronal network function in the murine hippocampus. Onset of progressive spatial memory deficits in P301S tau transgenic TAU58/2 mice were paralleled by long-term potentiation (LTP) deficits and neuronal network aberrations during electrophysiological and electroencephalography (EEG) recordings. Gene-expression profiling at onset of deficits in TAU58/2 mice revealed a signature of immediate early genes (IEG) that is consistent with neuronal network hypersynchronicity. We found that the increased IEG activity was confined to neurons harboring tau pathology, providing a cellular link between aberrant tau and network dysfunction. Taken together, our data suggests that tau pathology drives neuronal network dysfunction through hyperexcitation of individual, pathology-harboring neurons, contributing to memory deficits.

3.2 Introduction

Alzheimer's disease (AD) and frontotemporal dementia (FTD) are two of the most prevalent forms of dementia. Memory decline is the lead symptom of AD (Gotz et al., 2012), and is associated with behavioral, personality (including disinhibition and apathy) and/or language changes in FTD (Weder et al., 2007, Piguet et al., 2011b). Both AD and FTD are histopathologically characterized by progressive neuronal loss and the deposition of hyperphosphorylated tau in the brain (Ittner and Gotz, 2011, Ballatore et al., 2007). Tau belongs to the family of microtubule-associated proteins and is encoded by the *MAPT* gene (Gendron and Petrucelli, 2009). Under physiological conditions, tau binds to microtubules (MTs) and regulates their dynamics and contributes to maintenance neuronal functions (Ballatore et al., 2007). In the central nervous system, tau is predominately enriched in neuronal axons, but has also physiological functions in other compartments of neurons, including the post-synapse (Ittner et al., 2010, Ittner et al., 2016). The localization and physiological function of tau is determined by a large number of post-translational modifications (Mandelkow and Mandelkow, 2012). In disease, tau becomes increasingly phosphorylated at both physiological and pathological sites (=hyperphosphorylation), compromising tau-MT interactions and eventually causing the detachment of tau from microtubules and its mislocalization from the axon to the somatodendritic compartment (Ittner et al., 2010). Eventually, hyperphosphorylated tau accumulates into insoluble neurofibrillary tangles (NFTs) - the hallmark lesions of AD and FTD.

The identification of *MAPT* mutations in familial FTD established tau dysfunction is sufficient to cause neurodegeneration and cognitive decline (Spillantini et al., 2000, Wszolek et al., 2005). Since then, transgenic mice carrying various *MAPT* mutations have been generated by others and us recapitulating aspects of human neurodegenerative diseases, including tau hyperphosphorylation and NFT-formation. Although behavioral dysfunctions have been studied in several tau transgenic mouse models of AD and FTD (Gotz and Ittner, 2008), underlying mechanisms for hippocampal-dependent learning and neuronal network dysfunctions, in particular in FTD, remain unclear. This may also be due to the assumption that memory dysfunctions are relatively spared, or at least of late onset, in FTD (Ahmed et al., 2017). However, recent studies revealed significant deficits in hippocampal-driven episodic memory function in FTD patients (Hornberger and Piguet, 2012) and further provide

evidence that underlying memory impairments in FTD might even be comparable to those found in AD (Hornberger et al., 2010).

In AD, tau pathology and synaptic failure correlate with cognitive decline (Serrano-Pozo et al., 2011). Similarly, post-mortem studies in FTD with tau pathology reported reduction of key synaptic proteins positively correlating with cognitive dysfunction (Clare et al., 2010, Goetzl et al., 2016), further indicating that tau may compromise neuronal network integrity in FTD. We have previously shown that tau mediates Aβ-induced neuronal network dysfunctions AD mouse models, (Roberson et al., 2007, Ittner et al., 2010). Although, both *in vitro* and *in vivo* studies have demonstrated that tau contributes to neuronal network aberration in disease (Ittner et al., 2010, Fa et al., 2016, Busche et al., 2019), these mechanisms were described in conjunction with Aβ. Whether aberrant tau *per se* is sufficient to drive neuronal network deficits needs to be elucidated.

Here, we show that memory deficits in the P301S mutant human tau transgenic TAU58/2 mouse strain are associated with progressive neuronal network dysfunction in the hippocampus. Interestingly, markers of neuronal hyperexcitation were confined to neurons harboring tau pathology.

3.3 Results

3.3.1 Spatial memory deficits in TAU58/2 mice

TAU58/2 mice present with a progressive tau neuropathology (van Eersel et al., 2015), with expression of P301S mutant human tau (including in the hippocampus) and accumulation of hyperphosphorylated tau. We have previously shown that progressive behavioral abnormalities accompanied by tau pathology are of early onset in TAU58/2 mice (van Eersel et al., 2015, Przybyla et al., 2016), but did not assess their cognitive performance. To determine whether P301S mutant human tau expression causes spatial memory deficits in TAU58/2 mice prior to, or at the onset of, behavioral and pathological changes described previously at 3 months of age, we tested them in the Morris Water Maze (MWM) at 2, 4 and 6 months of age. At 2 months of age, prior to the onset of abundant tau pathology, TAU58/2 mice and non-transgenic littermate

controls presented with similarly learning during 5 days of acquisition trials, as suggested by comparable swim paths and progressive reduction in escape latency over subsequent test days in the MWM (**Figure 11A-C**). Accordingly, escape strategies similarly changed from non-spatial to spatial learning in TAU58/2 mice and non-transgenic controls, indicating normal memory function prior to abundant tau deposition (**Figure 11D** and **11E**). In contrast, 4 and 6 months-old TAU58/2 mice showed delayed learning when compared to non-transgenic littermate controls, as suggested by swim paths comparison and significantly increased escape latencies, as well as delayed and incomplete conversion from non-spatial to spatial escape strategies (**Figure 11F-O**). For comparison, TAU58/2 mice of all ages spent similar time in the target quadrants during probe trials as non-transgenic controls, indicative of intact memory consolidation. Differences in time spend in the opposite quadrant (Q2) between 2 months old TAU58/2 mice and non-tg littermate controls, is possibly due to slightly slower starts of video recordings, since Q2 marks the area of entry into maze (**Figure S1**). At all ages tested, average swimming speeds were comparable between TAU58/2 mice and non-transgenic controls at four months of age and even increased at 2 and 6 months of age. Indicating motor competency controls (**Figure S1**). In summary, TAU58/2 mice presented with spatial learning deficits already at 4 months of age.

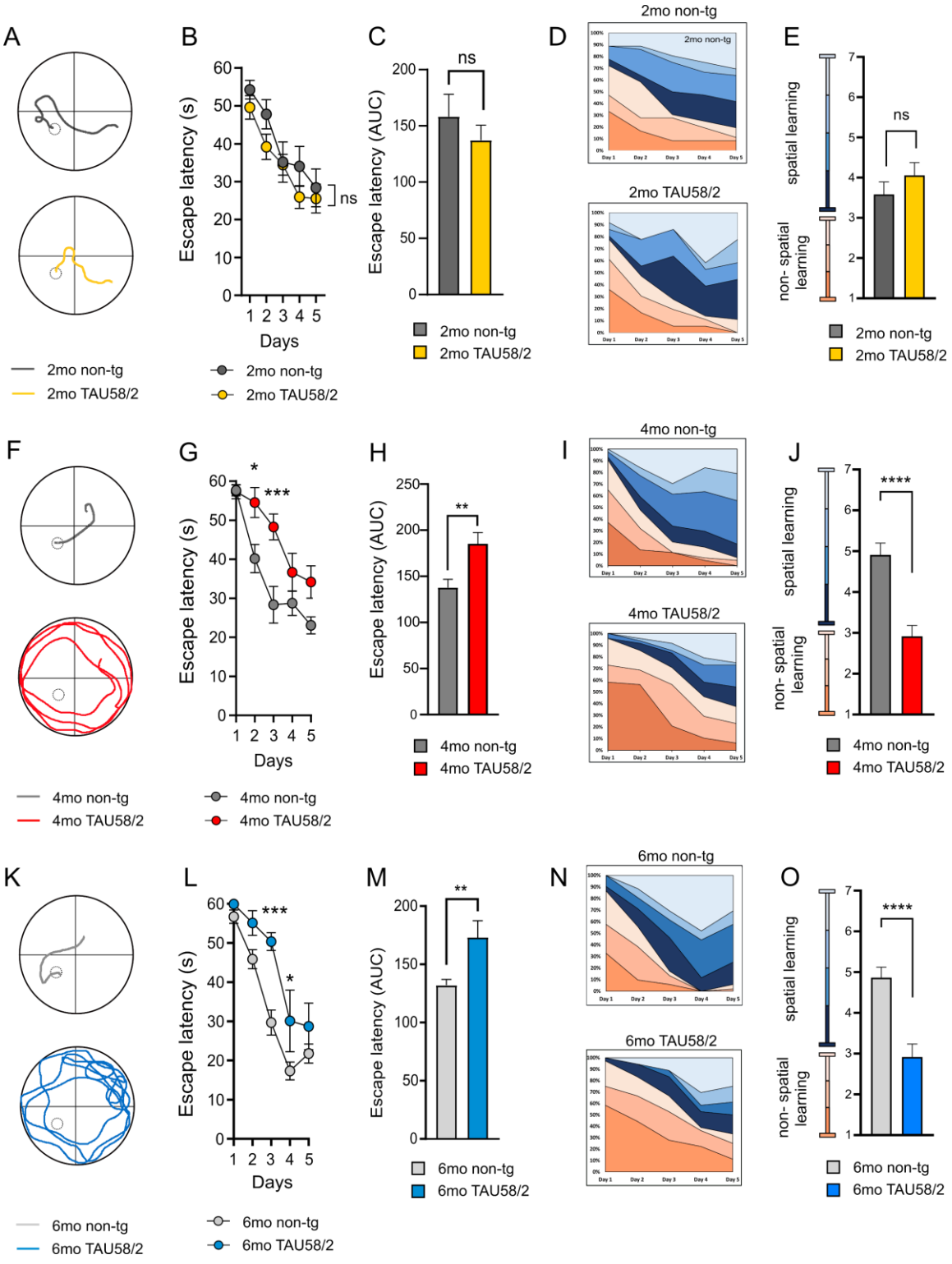

Figure 11: Progressive spatial learning deficits in TAU58/2 mice.

(**A**) Representative swim traces of 2 months old TAU58/2 (yellow) and non-transgenic (non-tg; grey) on day 3 of Morris water maze (MWM) testing. Black lines, quadrant borders; small broken circle, position of submerged escape platform. (**B**) Averaged escape latency over 5 MWM test days (ns, not significant; n=9 TAU58/2,

n=9 non-tg). (**C**) Area under the curve (AUC) analysis of escape latency shown in (B) (ns, not significant). (**D**) Relative distribution of different search strategies used by non-tg (top) and TAU58/2 (bottom) over 5 MWM test days (n=9 TAU58/2, n=9 non-tg). (**E**) Relative contribution of non-spatial and spatial learning to MWM performance of mice on day 3 of testing (ns, not significant; n=9 TAU58/2, n=9 non-tg). (**F**) Representative swim traces of 4 months old TAU58/2 (red) and non-tg (grey) on day 3 of MWM testing. (**G**) Averaged escape latency over 5 MWM test days (*, $p<0.05$; ***, $p<0.001$; n=12 TAU58/2, n=12 non-tg). (**H**) AUC analysis of escape latency shown in (G) (**, $p<0.01$). (**I**) Relative distribution of different search strategies used by non-tg (top) and TAU58/2 (bottom) over 5 MWM test days (n=12 TAU58/2, n=12 non-tg). (**J**) Relative contribution of non-spatial and spatial learning to MWM performance of mice on day 3 of testing (****, $p<0.0001$; n=12 TAU58/2, n=12 non-tg). (**K**) Representative swim traces of 6 months old TAU58/2 (blue) and non-tg (grey) on day 3 of MWM testing. (**L**) Averaged escape latency over 5 MWM test days (*, $p<0.05$; ***, $p<0.001$; n=8 TAU58/2, n=13 non-tg). (**M**) AUC analysis of escape latency shown in (L) (**, $p<0.01$). (**N**) Relative distribution of different search strategies used by non-tg (top) and TAU58/2 (bottom) over 5 MWM test days (n=8 TAU58/2, n=13 non-tg). (**O**) Relative contribution of non-spatial and spatial learning to MWM performance of mice on day 3 of testing (****, $p<0.0001$; n=8 TAU58/2, n=13 non-tg). Error bars represent the standard error.

3.3.2 Reduced synaptic plasticity in TAU58/2 mice

Impaired synaptic plasticity has been linked to memory deficits in the MWM paradigm in mice (Barnhart et al., 2015). Given the onset of spatial memory deficits in TAU58/2 mice at 4 months, and normal memory formation at 2 months of age (**Figure 11**), we next performed electrophysiological recordings in the hippocampal CA1 area of acute brain slices from 2 and 4 months old TAU58/2 mice and non-transgenic littermates to probe plasticity of field excitatory post-synaptic potentials (fEPSPs). Basal synaptic transmission (BST) was determined by measuring the relationship of the slope of the fEPSP evoked through stimuli of increasing intensity (input/output-relationship). fEPSP recordings showed that the input/output properties of Schäffer collateral /commissural fibers were not significantly affected in any of the groups tested (two-way ANOVA genotype, slices from 2 months old mice $F(1,14) = 0.2729$, $p=0.6096$; two-way ANOVA genotype, slices from 4 months old mice [$F(1,11) = 0.6063$ $p = 0.4526$), **Figure S2**), and hence, comparable stimulus intensities were used to induce Long-term-

potentiation (LTP). After a stable fEPSPs recording was established (>30 min, **Figure 12A and D**), LTP was induced utilizing three θ burst stimuli, which resulted in a persistent potentiation of fEPSPs in slices of all groups by the end of the observation period (**Figure 12A and D**). However, whilst LTP induction caused robust potentiation of fEPSPs in 2 months old non-transgenic control slices, synaptic transmission was slightly reduced in brain slices from 2 months old TAU58/2 mice (two-way ANOVA genotype $F(1,14)= 1.862$, $p=0.1940$), but only linear regression analysis of fEPSP was significantly changed (t-test, $p=0.008$), (**Figure 12A-C**). At 4 months of age, however, synaptic transmission was consistently and significantly reduced in brain slices from 4 months old TAU58/2 mice, compared to non-transgenic littermate controls shown by a significant decrease in LTP induction rate (two-way ANOVA genotype $F(1,11)= 13.87$, $p=0.0034$), reduced linear regression slope (t-test, $p=0.001$) and decreased area under the curve analysis (t-test, $p=0.035$) (**Figure 12D-F**). Taken together, these data suggest concomitant onset of reduced synaptic plasticity and spatial memory deficits in TAU58/2 mice.

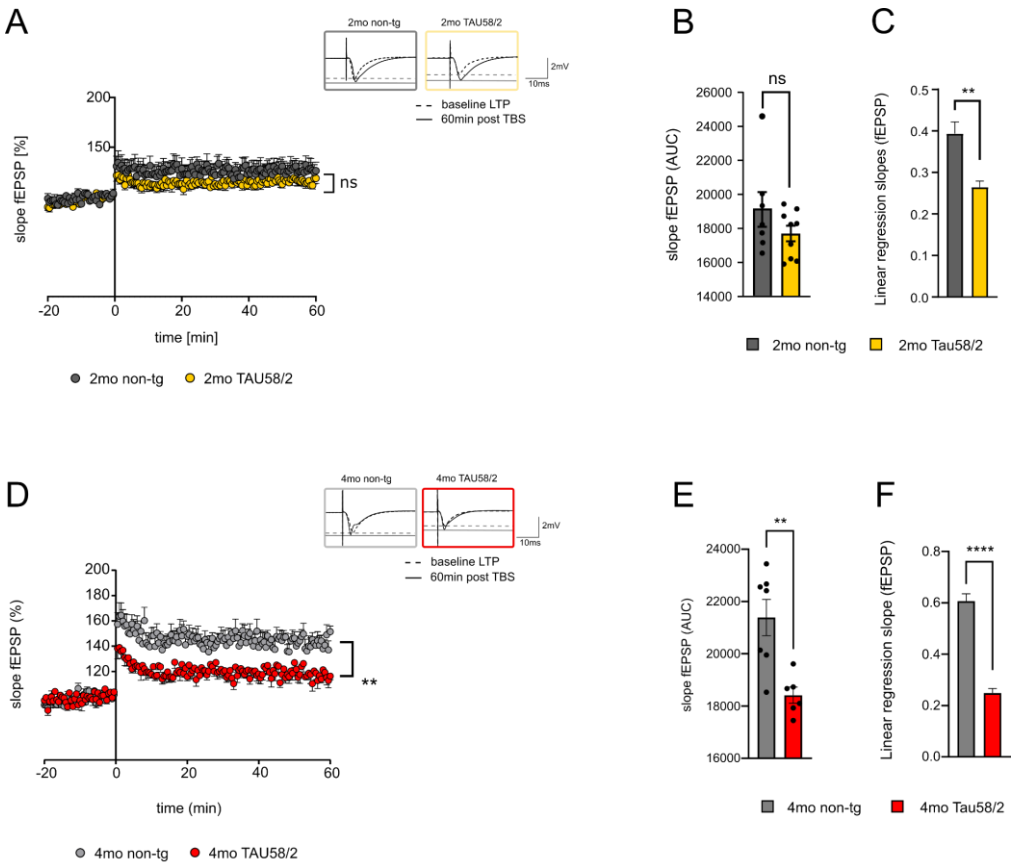

Figure 12: Impaired synaptic plasticity in TAU58/2 mice.

(**A**) Long-term potentiation (LTP) formation in 2 months old TAU58/2 (yellow) and non-transgenic (non-tg; grey) acute brain slices (ns, not significant; n=9 TAU58/2, n=7 non-tg). Sample traces at baseline (dashed lines) and 60 minutes after stimulation (solid lines) are shown for TAU58/2 (yellow box) and non-tg slices (grey box). (**B**) Area under the curve (AUC) analysis of fEPSP slopes after stimulation shown in (A) (ns, not significant). (**C**) Linear regression analysis of fEPSP slopes shown in (A) (**, $p<0.01$; n=9 TAU58/2, n=7 non-tg). (**D**) LTP formation in 4 months old TAU58/2 (red) and non-tg (grey) acute brain slices (****, $p<0.0001$; n=6 TAU58/2, n=7 non-tg). Sample traces at baseline (dashed lines) and 60 minutes after stimulation (solid lines) are shown for TAU58/2 (red box) and non-tg slices (grey box). (**E**) AUC analysis of fEPSP slopes after stimulation shown in (D) (**, $p<0.01$). (**F**) Linear regression analysis of fEPSP slopes shown in (D) (****, $p<0.0001$; n=6 TAU58/2, n=7 non-tg). Error bars represent the standard error.

3.3.3 Aberrant neuronal network activity in TAU58/2 mice

To determine neuronal network activity in TAU58/2 mice *in vivo*, we implanted telemetric electroencephalography (EEG) transmitter into the hippocampus of 4 and

6months old mice to record hippocampal EEGs. Implantation of electrodes in younger mice is limited by their body size. EEG recordings were analyzed for presence of spontaneous hypersynchronisity and epileptiform discharges (hyperactivity), spectral power across wave frequencies and cross frequency coupling (CFC), using our established algorithms (Ittner et al., 2014, Ittner et al., 2016). Average numbers of spikes per hour in 24-hour recordings were increased in 4 months old TAU58/2 mice compared to non-transgenic controls (**Figure 13A** and **13B**). At this age, spectral power of θ waves were significantly reduced at 9-12 Hz in TAU58/2 mice as compared to non-transgenic littermate controls, while low frequency θ power (4-8 Hz) was similar during no-spike episodes of EEG recordings (**Figure 13C**). In contrast, spectral power of low and high frequency γ waves were significantly increased (**Figure 13D**). Spike numbers were further increased at 6 months of age in TAU58/2 mice (**Figure 13E** and **13F**). In parallel, both low and high frequency θ power were reduced in TAU58/2 compared to non-transgenic controls at 6 months of age (**Figure 13G**), while high frequency γ power remained increased (**Figure 3H**). Next, we determined cross frequency coupling (CFC) of θ phase modulation of γ power during no-spike episodes of EEG recordings, a modality linked to memory formation including in humans (Canolty et al., 2006, Tort et al., 2009, Goutagny et al., 2009, Buzsaki and Moser, 2013). Four months old non-transgenic mice showed strong CFC in contrast to TAU58/2 mice with disrupted CFC (**Figure 13I**). While θ phase amplitude were similar in TAU58/2 mice and non-transgenic controls (**Figure 13J**), the modulation index was significantly reduced in TAU58/2 mice (**Figure 13K**). Six months old TAU58/2 mice also showed disrupted CFC of θ phase and γ amplitude (**Figure 13L**). Furthermore, θ phase was reduced (**Figure 13M**) and modulation index was further reduced in TAU58/2 mice when compared to non-transgenic controls (**Figure 13N**). Taken together, TAU58/2 mice showed neuronal network aberrations already at 4 months of age, with further progression of changes as mice age.

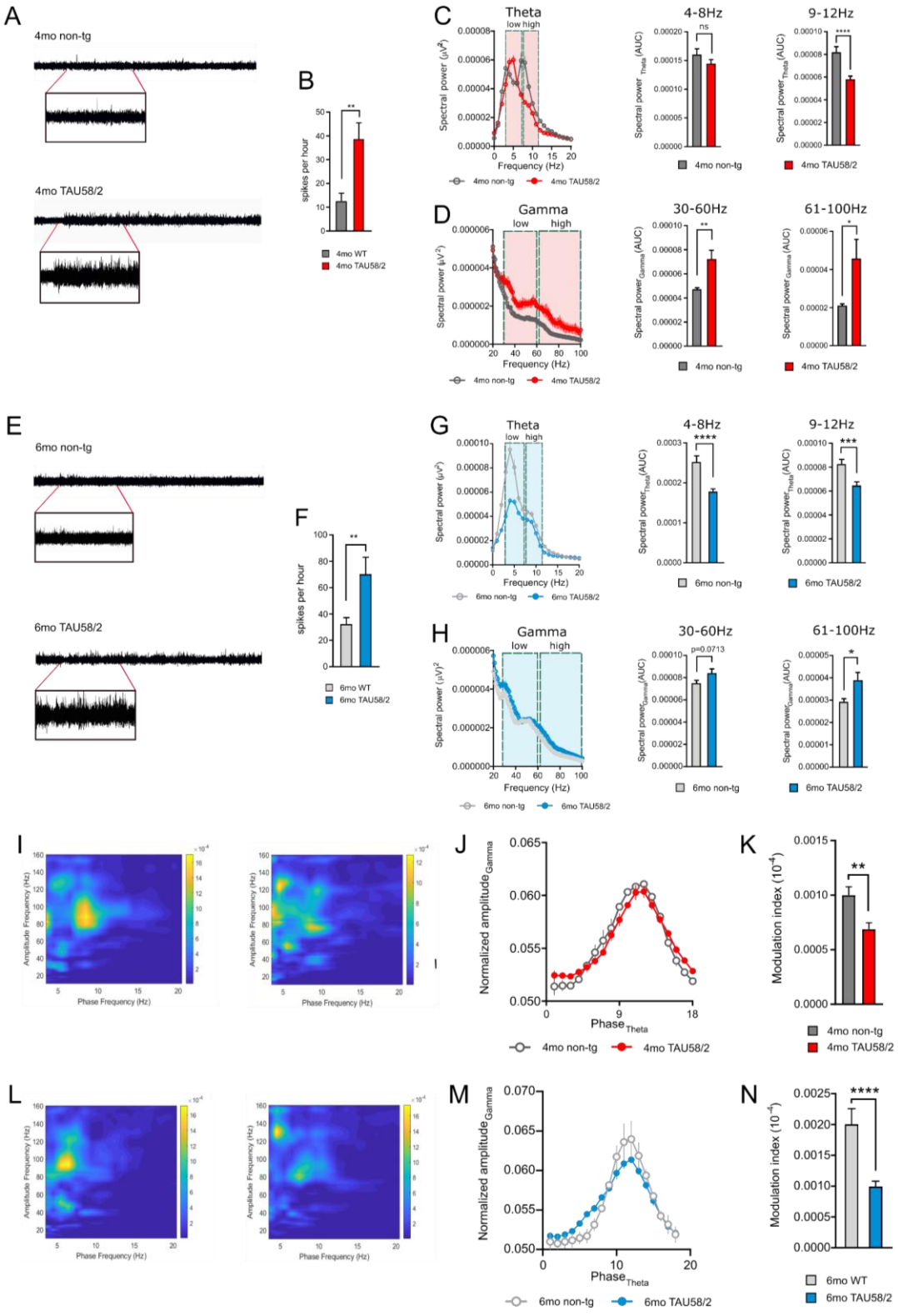

Figure 13: Neuronal network aberrations in TAU58/2 mice.

(**A**) Examples of 3 minutes electroencephalography (EEG) traces from 4 months old non-transgenic (non-tg; top) and TAU58/2 (bottom) mice. Insets, magnification of traces to

demonstrate spike activity in TAU58/2 mice. (**B**) Number of spikes per hours in TAU58/2 (red) and non-tg (grey) mice during 24 hours of EEG recording (**, p<0.01; n=4 TAU58/2, n=4 non-tg). (**C**) Spectral power at low and high theta (θ) frequencies in TAU58/2 and non-tg mice (left). Area under the curve (AUC) analysis of low θ (4-8Hz) (middle) and high θ (9-12Hz) (right) (****, p<0.0001; ns, not significant; n=4 TAU58/2, n=4 non-tg). (**D**) Spectral power at low and high gamma (γ) frequencies in TAU58/2 and non-tg mice (left). AUC analysis of low γ (30-60Hz) (middle) and high γ (61-100Hz) (right) (*, p<0.05; **, p<0.01; n=4 TAU58/2, n=4 non-tg). (**E**) Examples of 3 minutes EEG traces from 6 months old non-tg (top) and TAU58/2 (bottom) mice. Insets, magnification of traces to demonstrate spike activity in TAU58/2 mice. (**F**) Number of spikes per hours in TAU58/2 (blue) and non-tg (grey) mice during 24 hours of EEG recording (**, p<0.01; n=7 TAU58/2, n=7 non-tg). (**G**) Spectral power at low and high θ in TAU58/2 and non-tg mice (left). AUC analysis of low θ (4-8Hz) (middle) and high θ (9-12Hz) (right) (***, p<0.001; ****, p<0.0001; n=7 TAU58/2, n=7 non-tg). (**H**) Spectral power at low and high γ in TAU58/2 and non-tg mice (left). AUC analysis of low γ (30-60Hz) (middle) and high γ (61-100Hz) (right) (*, p<0.05; n=7 TAU58/2, n=7 non-tg). (**I**) Representative phase-amplitude comodulograms of interictal hippocampal EEG recordings showed cross frequency coupling (CFC) at ~8Hz in 4 months old non-tg (left) but not TAU58/2 (right) mice. (**J**) Phase-amplitude plot computed for interictal hippocampal EEG recordings (n=4 TAU58/2, n=4 non-tg). (**K**) Reduced modulation index in TAU58/2 mice compared with non-tg controls (**, p<0.01; n=4 TAU58/2, n=4 non-tg). (**L**) Representative phase-amplitude comodulograms of interictal hippocampal EEG recordings showed CFC at ~7Hz in 6 months old non-tg (left) but not TAU58/2 (right) mice. (**M**) Phase-amplitude plot computed for interictal hippocampal EEG recordings (n=7 TAU58/2, n=7 non-tg). (**N**) Reduced modulation index in TAU58/2 mice compared with non-tg controls (****, p<0.0001; n=7 TAU58/2, n=7 non-tg). Error bars represent the standard error.

3.3.4 Immediate early gene response in TAU58/2 mice

To determine molecular changes in hippocampal neurons at the age of onset of memory deficits in TAU58/2 mice, we next performed gene expression profiling by RNA sequencing of hippocampal extracts at 10 weeks of age. Comparable RNA quality between samples was confirmed by total RNA integrity numbers of 8 or higher (data not shown). DESeq2 analysis with a cut-off of >30% FDR revealed 44 differentially regulated genes (31 down- and 13 upregulated genes) (**Figure 14A** and **Table S1**). Note the marked up-regulation of *MAPT* and *Thy1* mRNA reflective of the transgenic construct used to generate TAU58/2 mice (van Eersel et al., 2015). Importantly, differentially regulated genes from TAU58/2 mice and non-transgenic controls

clustered according to the genotypes, suggesting changes to gene expression are due to transgenic tau expression and/or tau pathology (**Figure 14B**). When subjecting the list of differentially regulated genes to STRING analysis, we found only 2 significant clusters; The major cluster, IEGs, included 7 of the 13 upregulated genes, while none of the down-regulated genes mapped to this cluster, indicating increased overall activity (**Figure 14C**). Additional genes not part of the differentially regulated genes detected by RNA sequencing were predicted to be associated with this cluster, including *Arc* and *Fos*. One of the remaining upregulated genes, *Nr1d1*, mapped to an associated cluster of circadian rhythm genes. Notably, none of the downregulated genes was annotated to gene clusters. Quantitative PCR validated differential expression of selected IEGs *Npy* and *Egr2* in TAU58/2 mice at 3 months of age (**Figure 14D**). Furthermore, *Arc* mRNA was significantly increased and both *FosB* and *cFos* showed a trend to increased mRNA levels in TAU58/2 mice compared to non-transgenic controls. Staining of brain sections from TAU58/2 mice and non-transgenic controls at 3, 6 and 12 months of age showed significantly increased Npy staining in the TAU58/2 hippocampus (**Figure 14E**). Numbers of Npy-positive neurons were significantly increased in 3 and 6 months old TAU58/2 mice compared to non-transgenic controls (**Figure 14F**), while changes were not present after 12 months. Similarly, Npy staining of the hippocampal CA3 region were increased in TAU58/2 mice at all ages, although these changes were only significant in the 6 months group (**Figure 14G**). Taken together, TAU58/2 mice presented with a gene signature consistent with increased neuronal activity.

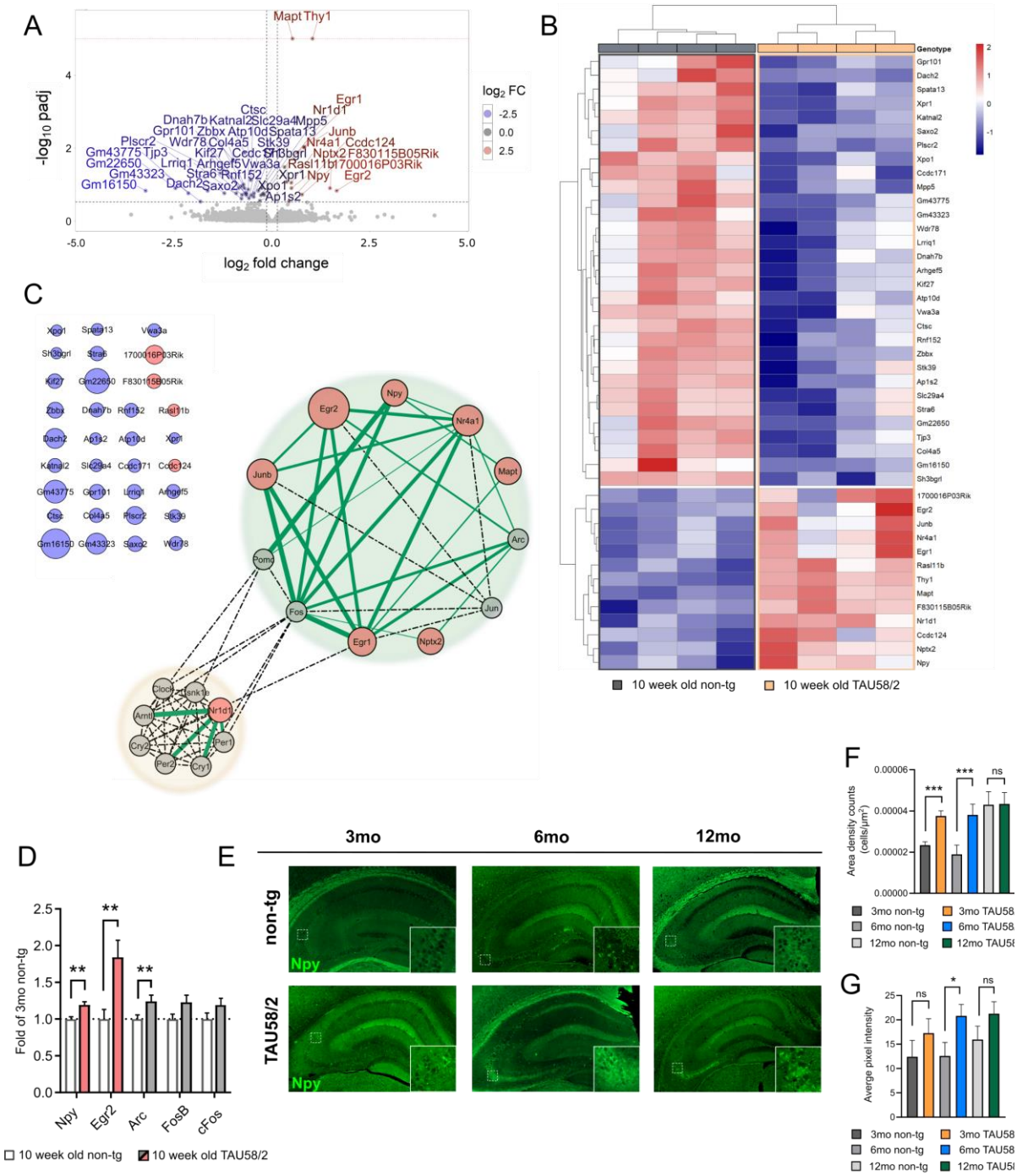

Figure 14: Immediate early gene signature in TAU58/2 mice.

(**A**) RNA sequencing (RNAseq): Volcano blot of differentially up- (red) and down-regulated (blue) mRNAs in the hippocampus of 10 weeks old TAU58/2 compared to non-transgenic (non-tg) littermates (n=4). (**B**) Heatmap shows clustering of differentially up- (red) and down-regulated (blue) gene expression in non-tg (n=4; grey) and TAU58/2 (n=4; orange) samples. (**C**) STRING analysis reveals two

clusters for immediate early genes (green) and circadian rhythm genes (yellow). Genes not annotated to clusters are presented individually (left). Circle size, level of differential regulation; red, up-regulation; blue, down-regulation; grey, genes annotated to cluster by STRING; lines, gene interaction. (**D**) Hippocampal gene expression at 3 months of age in TAU58/2 (closed bars) relative to non-tg (open bars) mRNA levels as determined by quantitative PCR (**, p<0.01; n=7 TAU58/2, n=5 non-tg). (**E**) Representative immunofluorescence staining of Npy (green) in hippocampal sections of TAU58/2 and non-tg mice at indicated ages. Insets, magnification of areas indicated by broken boxes. (**F**) Numbers of Npy-positive neurons in the hippocampal CA1 region of TAU58/2 and non-tg mice at indicated ages (***, p<0.001; ns, not significant; n=9 TAU58/2, n=6 non-tg). (**G**) Npy staining intensity in the hippocampal CA3 area of TAU58/2 and non-tg mice at indicated ages (*, p<0.05; ns, not significant; n=13-17 TAU58/2, n=10-12non-tg). Error bars represent the standard error.

3.3.5 IEG induction locates to tau pathology harboring neurons in TAU58/2 mice

Next, we determined whether IEG activation was a generalized neuronal event in TAU58/2 mice or if it was directly linked to tau expression and/or pathology. Since Arc has previously been implicated in synaptic transmission and plasticity (Nikolaienko et al., 2017) and deregulation of Arc mRNA was found in TAU58/2 at 10 weeks of age, we decided to stain brain sections from 3, 6 and 12 months old TAU58/2 mice and non-transgenic littermates for Arc in combination with tau antibodies.

Low but significantly increased numbers of Arc-positive neurons were detected in the hippocampus of TAU58/2 mice at 3 months of age compared to non-transgenic littermate controls (**Figure 15A** and **15B**), consistent with differentially regulated *Arc* mRNA at this age (**Figure 14D**). Cells staining intensively for Arc (=Arc^{high}) were only detected in TAU58/2 mice (**Figure 15B**). Arc^{high} cells co-labelled in over 80% with the staining for transgenic human tau (Tau13) and for tau phosphorylated at the late-stage sites S396 and S404 (PHF-1), while cells that labeled less intensive for Arc (Arc^{low}) only showed co-labelling with Tau13 and PHF-1 in approximately 40% of cells (**Figure 15A** and **15C**). Arc^{low} cells were also occasionally found in the hippocampus of non-transgenic littermate controls (**Figure 15B**). Numbers of both Arc^{low} and Arc^{high} cells further increased in 6 months old TAU58/2 mice compared to non-transgenic controls

(**Figure 15D** and **15E**), with high co-labelling of Arc with Tau13 and PHF-1 (**Figure 15F**). Similarly, 12 months old TAU58/2 mice presented with higher numbers of Arc-positive cells as compared to aged non-transgenic controls (**Figure 15G** and **15H**). Virtually all Arc^{high} cells co-labelled with Tau13 and to more than 90% with PHF-1 at this age (**Figure 15I**). Taken together, progressively increased labelling of neurons with the IEG maker protein Arc was largely confined to hippocampal neurons that harbored tau pathology.

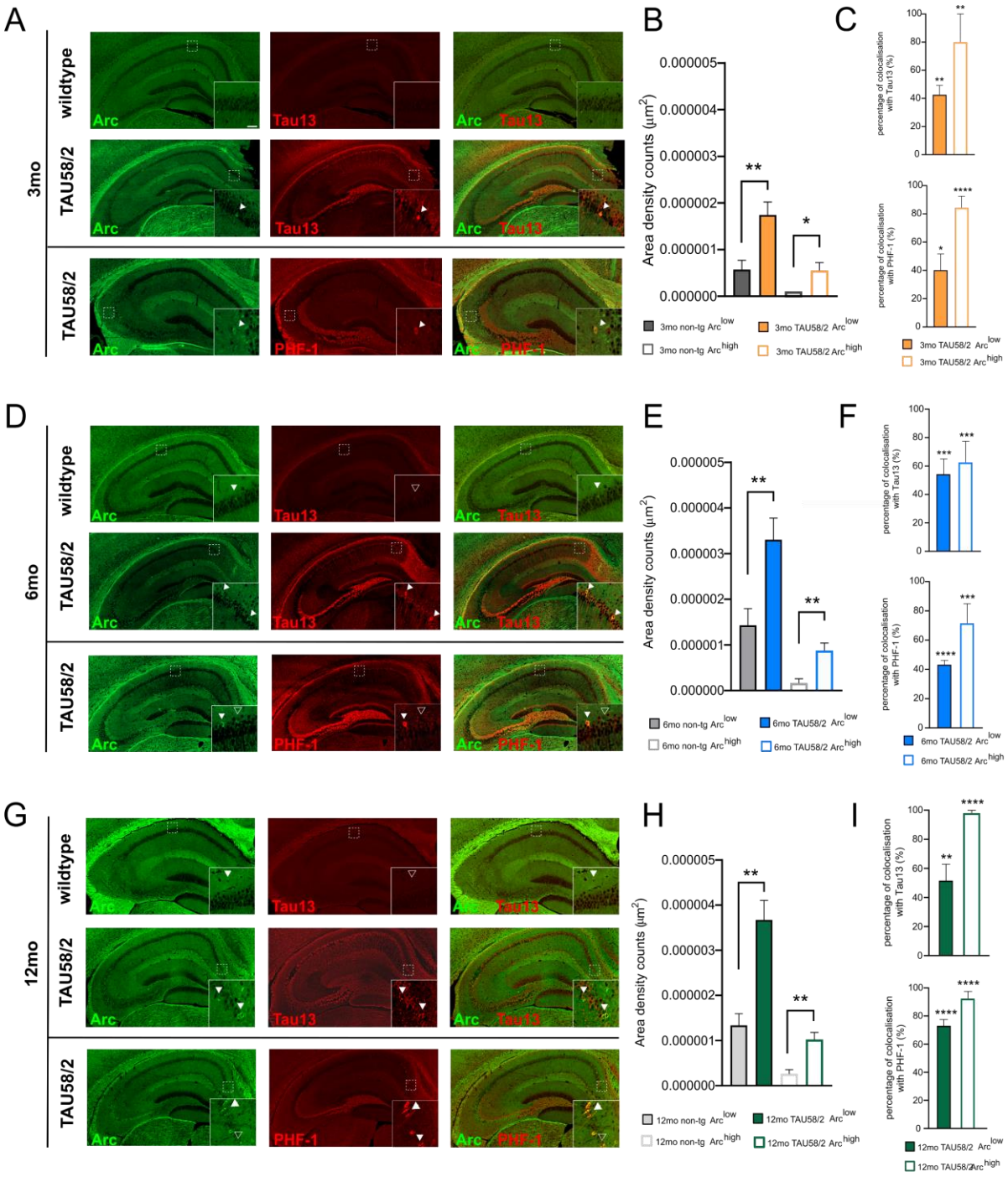

Figure 15: Progressive Arc accumulation in transgenic tau harboring neurons in TAU58/2 mice.

(**A**) Representative immunofluorescence (IF) co-staining of Arc (green) and Tau13 (red; top) or PHF-1 (red; bottom) in hippocampal sections of TAU58/2 and non-transgenic (non-tg) mice at 3 months of age. Insets, magnification of areas indicated by broken boxes. Arrowheads indicate cells with Arc/Tau13 or Arc/PHF-1 co-labelling. (**B**) Numbers of cells with Arc staining (closed bars) and of those with intensive Arc staining (Arc^{high}; open bars) in non-tg (grey) and TAU58/2 (yellow) hippocampi 3

months of age (*, p<0.05; **, p<0.01; n=9 TAU58/2, n=6 non-tg). (**C**) Percentage of Arc-positive (closed bars) and Arc^{high} (open bars) cells that co-label with Tau13 (top) and PHF-1 (bottom) in TAU58/2 hippocampi at 3 months of age (*, p<0.05; **, p<0.01; ****, p<0.0001; n= 9 TAU58/2, n=6 non-tg). (**D**) Representative IF co-staining of Arc (green) and Tau13 (red; top) or PHF-1 (red; bottom) in hippocampal sections of TAU58/2 and non-transgenic (non-tg) mice at 6 months of age. Insets, magnification of areas indicated by broken boxes. Arrowheads indicate cells with Arc/Tau13 or Arc/PHF-1 co-labelling. Open arrowhead indicates Arc-positive cell that does not co-label with PHF-1. (**E**) Numbers of cells with Arc staining (closed bars) and of those with intensive Arc staining (Arc^{high}; open bars) in non-tg (grey) and TAU58/2 (blue) hippocampi at 6 months of age (**, p<0.01; n=11 TAU58/2, n=8 non-tg). (**F**) Percentage of Arc-positive (closed bars) and Arc^{high} (open bars) cells that co-label with Tau13 (top) and PHF-1 (bottom) in TAU58/2 hippocampi at 6months of age (***, p<0.001; ****, p<0.0001; n=11 TAU58/2, n=8 non-tg). (**G**) Representative IF co-staining of Arc (green) and Tau13 (red; top) or PHF-1 (red; bottom) in hippocampal sections of TAU58/2 and non-transgenic (non-tg) mice at 12 months of age. Insets, magnification of areas indicated by broken boxes. Arrowheads indicate cells with Arc/Tau13 or Arc/PHF-1 co-labelling. (**H**) Numbers of cells with Arc staining (closed bars) and of those with intensive Arc staining (Arc^{high}; open bars) in non-tg (grey) and TAU58/2 (green) hippocampus (**, p<0.01; n=9 TAU58/2, n=6 non-tg). (**I**) Percentage of Arc-positive (closed bars) and Arc^{high} (open bars) cells that co-label with Tau13 (top) and PHF-1 (bottom) in TAU58/2 hippocampus (**, p<0.01; ****, p<0.0001; n=9 TAU58/2, n=6 non-tg). Error bars represent the standard error.

3.4 Discussion

In the present study, we showed that TAU58/2 mice develop progressive spatial learning deficits that were associated with impaired LTP, neuronal network aberrations and an IEG expression signature that was indicative of neuronal hyperexcitation. Importantly, staining with excitation marker protein Arc was largely confined to neurons harboring hyperphosphorylated tau, suggesting that tau drives neuronal hyperexcitation via cell intrinsic processes.

We have previously reported an array of behavioral and motor deficits in TAU58/2 mice (van Eersel et al., 2015, Przybyla et al., 2016); TAU58/2 mice present with early onset disinhibition and moderate motor deficits. Revealing now progressive learning deficits in this strain extend its value for studying molecular and cellular processes driven by tau pathology in cognitive decline. To which degree the different

functional changes impact during testing remains to be fully examined. Yet, motor deficits had no impact on disinhibition testing in previous studies (Van der Jeugd et al., 2016, Przybyla et al., 2016). Similarly, swim speed and performance during MWM testing were comparable between TAU58/2 mice and non-transgenic littermates, suggesting that motor deficits were not contributing to progressively reduced performance as mice aged. It is possible that deficits in both behavioral and memory tasks reflect similar underlying disease mechanisms affecting different neuronal networks.

Gene expression profiling in TAU58/2 mice at the onset of LTP, memory and neuronal network deficits revealed only one significant cluster of differentially regulated genes, IEGs. IEGs are expressed in response to neuronal hyperexcitation, including in mouse models of acute brain damage (e.g. epilepsy or stroke) (Bi et al., 2017) and models of AD (Diez et al., 2003, Palop et al., 2007, Krezymon et al., 2013, Gatta et al., 2014). In line with these studies we showed elevated levels of NPY-expression in the hippocampus of 3 and 6 months old TAU58/2 mice. Considering that an alteration to NPY-expression implicates perturbations to inhibitory and excitatory circuits, progressive, tau-mediated increase in NPY immunoreactivity and intensity with age, suggests coordinated neuronal network hyperactivity. Furthermore, the prominent IEG cluster indicates a coordinated hyperactivation of neuronal circuits in TAU58/2, while pathways that lead to down-regulation of genes were diverse and mechanistically unrelated. Together, these findings imply that neuronal dysfunction due to hyperexcitation is a major contributor to the onset of functional deficits in TAU58/2. To this end, it remains to be shown whether the second associated cluster of circadian rhythm genes is a result of neuronal network hyperactivity or independently contributes to functional deficits in TAU58/2 mice. Furthermore, we formally cannot rule out that other processes that contribute to the onset of functional deficits are underrepresented in the gene profiling due to linear signaling pathways (i.e. pathway activity is reflected by differential regulation of a single gene rather than clusters).

IEG marker protein Arc, which has previously been implicated in mediating neuronal morphology (Messaoudi et al., 2007, Peebles et al., 2010), synaptic plasticity (Carmichael and Henley, 2018) and memory function (Bramham et al., 2010, Nikolaienko et al., 2017), co-localized with hyperphosphorylated tau in hippocampal neurons. These findings suggest that tau pathology renders neurons prone to responding by hyperexcitation to network activity, in turn compromising dysfunction of

entire networks resulting in functional deficits including progressive memory decline. Interestingly, a SNP in *ARC* has been associated with a reduced risk for AD (Landgren et al., 2012). Furthermore, studies utilizing both, Arc-knock-out mice and neurons overexpressing Arc showed deficits in synaptic scaling, a key mechanisms required for synaptic plasticity (Shepherd et al., 2006). Hence, progressively increased Arc in tau pathology-harboring neurons may therefore not only be a marker of hyperexcitation but possibly directly involved in pathways that contribute to neuronal dysfunction. A previous study has reported reduced hippocampal Arc activity in P301L mutant tau transgenic mice (Fox et al., 2011). However, Arc activation was only assessed in the context of targeted stimulation (Fox et al., 2011), different from our study that found increased numbers of Arc positive neurons in non-stimulated, naïve TAU58/2 mice. Accordingly, it has previously been shown that network-wide activity-driven Arc expression was not affected by tau pathology (Rudinskiy et al., 2014).

We have previously shown that tau is a mediator of neuronal network hypersynchronisity in Aβ-based mouse models of AD (Koller et al., 2004, Ittner et al., 2010, Ittner and Gotz, 2011, Ittner et al., 2016, Ittner and Ittner, 2018). In line with work by others (Hoover et al., 2010), the present study suggests that pathological tau by its own is sufficient to drive neuronal network deficits in neurodegenerative diseases. Therefore, the idea of targeting neuronal network hypersynchronisity in AD may be translatable to tau-only neurodegenerative conditions such as FTD.

3.5 Methods

3.5.1 Mice

TAU58/2 mice express the human 0N4R tau isoform with the P301S mutation under the control of the mouse Thy1.2 promoter, as previously described by us (van Eersel et al., 2015). Mice were maintained heterozygous on a C57BL/6 background and non-transgenic (non-tg) littermates were used as controls. Mice were housed in filter top cages containing nesting material, a wooden stick and a transparent red dome and maintained on a 12-hour light/dark cycle with food and water *ad libidum*. All animal experiments were conducted with male mice only due to more pronounced phenotypes in male TAU58/2 mice compared to females. All animal experiments were approved

by the Animal Ethics Committees of Macquarie University and the University of New South Wales. All procedures complied with the statement on animal experimentation issued by the National Health and Medical Research Council of Australia.

3.5.2 Memory testing

In order to assess spatial learning and memory, the Morris water maze was conducted on 2, 4 and 6 months old TAU58/2 transgenic mice (n=9-13 males) and non-transgenic littermate controls (n=9-12 males). The Morris water maze apparatus consisted of a 1.2 m diameter tank with a 40 cm high Perspex platform (diameter 10 cm), which was placed approximately 20 cm from the edge of the wall. The tank was filled 0.5-1 cm above the surface of the platform and a non-toxic acrylic-based paint added to the water to obscure the platform. Four signposts with different shapes with placed equidistant around the pool as visual cues. Mice were acclimatized to the room for 1 hour prior to testing each day. Days 1-5 consisted of an acquisition phase, in which mice were placed in the quadrant opposite the platform at one of four starting positions and given 60 seconds to locate the hidden platform. Mice that failed to find the hidden platform were guided to the platform and all mice remained on the platform for an additional 60 seconds before being removed from the maze. Mice had four trials per day, each starting from a different position, and the order of starting positions was altered each day. On the sixth day, the platform was removed, and the mice were given 30 seconds to explore the pool (probe trial). On the seventh day, the platform was placed back in the pool with a flag attached, and visual cues were removed from the outside of the pool, to ensure that all mice had normal vision.
Mice were tested in littermate groups, without knowledge of genotypes and video analysis was done before de-blinding experimenters. Videos were analyzed using the ANY-maze® software (Stoelting Co., IL, USA).

3.5.2.1 MWM path analysis

For all learning days, trace plots were obtained for each swim after video analysis using ANY-maze® (Stoetling Co., IL, USA). Trace plots that most accurately represented an

average swim trace of the cohort tested were chosen as representative examples (Figure 11A, F and K). Swim traces were classified visually using a paradigm based off Garthe et al. (Garthe and Kempermann, 2013, Tan et al., 2018). Briefly, swim patterns were scored as follows: 1, thigmotaxis; 2, random swim; 3, scanning; 4, chaining; 5, directed search; 6, focal search; and 7, direct swim. Based on this scoring scheme category 1, 2 and 3 reflect non-spatial hippocampal learning, whilst search strategy 4, 5, 6 and 7 are considered to reflect spatial hippocampal learning.

3.5.3 Electrophysiology

Horizontal brain slices (350 µm) were prepared from 2 and 4 months old TAU58/2 transgenic mice (n=6-9) and non-transgenic littermates (n=7) using a VT1200 vibratome (Leica) according to standard techniques. Briefly, mice were anesthetized with isoflurane (5%) and decapitated, and brains were removed and sectioned in oxygenated (95% O2, 5% CO2) ice-cold, modified artificial cerebrospinal fluid (ACSF) containing the following (in mM): 125 NaCl, 3 KCl, 1.25 NaHsPO4, 25 NaHCO3, 6 MgCL2, 1 CaCl2, and 10 glucose. Slices were maintained at 31 ± 1 °C thereafter in ACSF solution (in mM): 125 NaCl, 3 KCl, 1.25 NaH2HPO4, 25 NaHCO3, 1 MgCl2, 2 CaCl2, and 25 glucose (95% O2, 5% CO2). After equilibration of at least 60min, slices were transferred into a commercial brain slice recording chamber (Kerr Scientific Instruments Ltd., Christchurch, NZ), constantly superfused at 2-2.5 ml/min with oxygenated ACSF at 31 ± 1 °C. Synaptic potentials were evoked in CA1 by stimulating the Schaffer collateral pathway with a 0.1-ms pulse through a bipolar stimulating electrode (Kerr Scientific Instruments Ltd.). Field excitatory post-synaptic potentials (fEPSPs) were recorded with a recording electrode (Kerr Scientific Instruments Ltd.) placed in the stratum radiatum approximately 300 – 400 microns from the stimulating electrode. Potentials were amplified using an AC amplifier (Kerr Scientific Instruments Ltd) and sampling speed was at 20 kHz. fEPSPs were evoked every 30 seconds. Synaptic strength was determined by measuring the slope of the rising phase of the fEPSP between 10% and 90%. A theta-burst stimulation (TBS) was used to induce LTP. The TBS protocol for induction of LTP consisted of 10 trains of 5 pulses (0.1ms pulse width) at 100 Hz with a 200 ms inter-train interval. This protocol was repeated twice with a 20 sec episode interval. The stimulating intensity was set so that the fEPSP

amplitude was 50-60% of maximum amplitude evoked as analysed by establishing an input-output correlation. Slope fEPSPs were normalized to a 20 min baseline and measured as percentage of baseline fEPSP prior to application of TBS.

3.5.4 Electroencephalography

Hippocampal EEG recordings in freely moving mice was conducted as previously described. Briefly, after mice were anaesthetized with ketamine/xylazine, scalp incision along the midline was performed and head was fixed using a stereotaxic frame (Kopf instrument). Bregma was located and bone openings were drilled at previously described positions for hippocampus (x 2.0, y -2.0, z -2 from bregma) using a bone micro- drill (Fine Science Tools, F.S.T). Wire EEG electrode was inserted at this position, whilst the reference electrode was placed above the cerebellum (x 0, y -6.0, z 0 with reference to bregma). Both electrodes were fixed in place using polyacrylate and wound was closed with staples and rehydrated. Ten days after surgery, EEGs were recorded using a DSI wireless receiver setup (DSI) with amplifier matrices using the Dataquest A.R.T. recording software at 500 Hz sampling rate (Weiergraber et al., 2005). After EEG recordings were successfully conducted, animals were perfused with cold phosphatebuffered saline (PBS) and brains extracted and further processed for histological analysis. Correct electrode placement was confirmed by serial sections of paraffin embedded brain tissue stained with hematoxylin-eosin. Only recordings from mice with correct placement were included in further analysis. EEG recordings were analysed using the NeuroScore software v3.0 (DSI) with integrated spike detection tool. The number of spikes were thus detected automatically and statistical data on number and frequency of spiked obtained. All recordings were visually screened for movement artefacts and only artefact-free episodes were used for further analysis. Spectral analysis (i.e. analysis of signal power at individual frequencies expressed as square of the fast Fourier transform (FFT) magnitude) of interictal sequences was conducted using the integrated FFT spectral analysis function of NeuroScore. Frequency bands of theta and gamma wave forms were defined between 4-12 Hz (low theta 4-8 Hz, high theta 9-12 Hz) and 25-100 Hz (low gamma 30-60 Hz, high gamma 61-100 Hz), respectively. Gamma and theta spectral contributions were quantified by area-under-curve (AUC) analysis across the defined frequency band in 8-10 artefact-

and hypersynchronous spike-free sequences per recording (each 1 min in length). Cross-frequency coupling of theta phase and gamma amplitude was performed using MATLAB (Mathworks) as previously described (Tort et al., 2010). Briefly, for cross frequency coupling (CFC) analysis, raw LFP was noise filtered using a powerline noise filter (Neuroscore, DSI). Noise-filtered LFP was filtered at two frequency ranges of interest for gamma (fA) and theta (fp). The phase time series for theta ($\Phi_{fp}(t)$) and the amplitude envelope time series for gamma ($A_{fA}(t)$) were obtained by Hilbert transformation of the filtered LFPs. The combined series [$\Phi_{fp}(t)$, $A_{fA}(t)$] was then generated. After phase binning, the means $\bar{A}_{fA}(j)$ of A_{fA} for each bin j were calculated and normalized using the sum $\sum_{j=1}^{N} \bar{A}_{fA}(j)$ of $\bar{A}_{fA}(j)$ over N bins to generate phase-amplitude distribution P(j). The modulation index, a measure of CFC (Tort et al., 2010, Tort et al., 2009), is based on calculating the Kullback-Leibler distance D_{KL} between the non-uniform (i.e. coupled) phase-amplitude distribution P(j) over all phase bins and the uniform (i.e. uncoupled) distribution U(j).

$$D_{KL}(P,Q) = \sum_{j=1}^{N} P(j) \log[\frac{P(j)}{U(j)}]$$

The modulation index (MI) is defined as $MI = \frac{D_{KL}(P(j),U(j))}{\log N}$. Phase-amplitude distributions and modulation indices were determined from artefact- and hypersynchronous spike-free 8-10 sequences (each 1 min) per recording.

3.5.5 RNA sequencing

To obtain an unbiased insight into the molecular changes associated with transgenic P301S tau expression, we performed quantitative polyadenylated RNA sequencing (RNAseq) of hippocampal brain samples from naïve 10 weeks old TAU58/2 mice (n=4) and their non-transgenic littermates (n=4). The mRNA extraction and mRNA sequencing were performed by Macrogen (Korea). Briefly, Maxwell 16 LEV simplyRNA tissue kits (Promega) was used for mRNA extraction, followed by library construction using TruSeq Stranded Total RNA with Ribo-Zero Gold sample prep kit & TruSeq rapid

SBS kit. Sequencing was performed on the Illumina HiSeq 2500 platform according to the HiSeq 2500 System User Guide Document #15035786 v01 HCS 2.2.70 protocol, using HCS version 2.2 for sequencing control. To quality filter reads and to remove adapter contamination, trimmomatic 0.35 was used. The first 10 and the last 5 bases were removed after visual inspection of the read quality distribution. After trimming, read length was 86Bp. For read mapping, Star aligner v2.5.2b was used on the Gencode M12 transcriptome (mm10) with settings "--outSAMmultNmax 20". Reads were assigned to genes using featureCounts v1.5.2 with settings "-C -B -M -O". To calculate log2 fold change estimates between groups, a negative binomial general linear model was fit using DESeq2 and only genes with a Benjamini-Hochberg corrected Wald test FDR of less than 30% were labeled significant.

3.5.6 STRING annotation

The list of differentially regulated genes obtained through RNAseq analysis was subjected to STRING v10.5 to identify possible gene clusters (Szklarczyk et al., 2017). Data source settings for nodal associations were set to default (*'on'* for text-mining, databases, experiments, co-expression, neighborhood, gene-fusion, co-occurrence) with 'medium confidence' (≥0.400) interaction score minimums. Gene-networks were visualized using Cytoscape v3.6.1 software (Shannon et al., 2003). Each node represents a protein, while each edge corresponds to a STRING-based physical and/or functional interaction. The node size reflects the fold change (upregulated genes appear bigger than downregulated genes) and is proportional to the FPKM fold-change in transgenic relative to non-transgenic samples. The node colour reflects whether the gene is up (red) or downregulated (blue) or indicates whether the gene was found through RNAseq or was proposed by string as being involved in similar pathways (grey). The thickness of each edge is proportional to the STRING based physical and/ or functional interaction between two genes (the thicker the edge, the stronger is their STRING based association), whilst the edge color indicates interactions between genes obtained through RNAseq (green) vs genes that were proposed by STRING (dotted black lines).

3.5.7 Quantitative PCR

Isolated hippocampal tissue was homogenised in Trizol, according to the manufacturer's instructions. The aqueous phase was then processed further using a RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. To remove contaminating genomic DNA, an on-column DNA-digest was performed with RNase-free DNaseI (Qiagen). cDNA was synthesized from 1µg total RNA using the SuperScript VILO cDNA-synthesis kit (ThermoFisher Scientific). mRNA levels were determined by quantitative PCR using Fast SYBR Green (ThermoFisher Scientific) and gene-specific primer pairs listed in supplementary table (**Table S2**), using an Applied Biosystems ViiA 7 Real-Time PCR System (ThemoFisher Scientific). CT values of genes determined by quantitative PCR were normalized to actin and values displayed as fold changes of non-transgenic.

3.5.8 Staining

Three, 6 and 12-month-old TAU58/2 transgenic males (n= 9-13) and non-transgenic-male littermates (n=6-8) were used for histological analysis. At the specific age, mice were anesthetized and transcardially perfused with phosphate buffered saline (pH 7.4) to remove blood. Brains were removed, hemispheres separated and fixed in 4% paraformaldehyde. Immersion-fixed brains were further processed utilizing an Excelsior tissue processor (ThermoFisher, Waltham, MA, USA), embedded in paraffin and coronally sectioned at 3 µm for immunohistochemistry (IHC). One to two sections per mouse were stained with antibodies against the immediate early gene markers NPY and Arc and antibodies against human tau (Tau13, 1:500, Santa Cruz) and tau phosphorylated at 396/404 (PHF-1, 1:250, kind gift by P. Davies) as described previously (van Eersel et al., 2015). All brain sections were scanned using (Axio scan Z1). Arc (1:200, Santa Cruz) and NPY (1:200, Sigma) positive neurons were counted throughout the hippocampus, whilst neurons with bright staining signal for Arc were counted separately to those with less immunoreactivitylimitation. Once NPY and Arc immuno-positive neurons were identified and marked, each neuron was checked for double labelling with either human tau or tau phosphorylated at Ser396/404. Total and double labelled neurons were counted and the total number of positive and double

labelled neurons per slide and mouse (n=2) calculated. Zen software 2.6 was used to delineate and automatically calculate the hippocampal area. All cell counts were converted to a density value (cells/mm^2). ImageJ software was used to determine and analyse NPY expression in the CA3 area of the hippocampus. Between 3-4 small regions per slide were chosen within the CA3 region, contoured using ring-shaped ROIs and mean pixel intensity was measured by averaging all pixels within the ROI. Mean pixel intensity was then corrected for baseline fluorescence, which was determined using three mean pixel intensity measures. For all quantitation, repeated measures on different days gave an inter- and intra-rater variability of <5%.

3.5.9 Statistical analysis

All statistical analysis was done using the Graphpad Prism 6.0 software (GraphPad, La Jolla, CA, USA) using either Student's *t*-tests for comparison of two data sets, analysis of variance (ANOVA) for comparison of more than two data sets or two-way ANOVA for comparison across time. *P* vales of below 0.05 were considered significant. All values are presented as mean ± standard error of the mean.

3.6 Acknowledgements

The authors would like to thank the staff of the animal facilities at Macquarie University for their help with animal husbandry. The authors received funding from the National Health and Medical Research Council (1081916, 1123564, 1132524, 1136241, 1143848, 1143978), the Australian Research Council (DP150104321, DP170100781, DP170100843) and CurePSP (609-2015-07). Y.K. is a NHMRC Career Development Fellow. L.I. is a NHMRC Principal Research Fellow.

3.7 Conflict of interest

The authors have no conflicts of interests related to this study.

3.8 Supplementary Materials

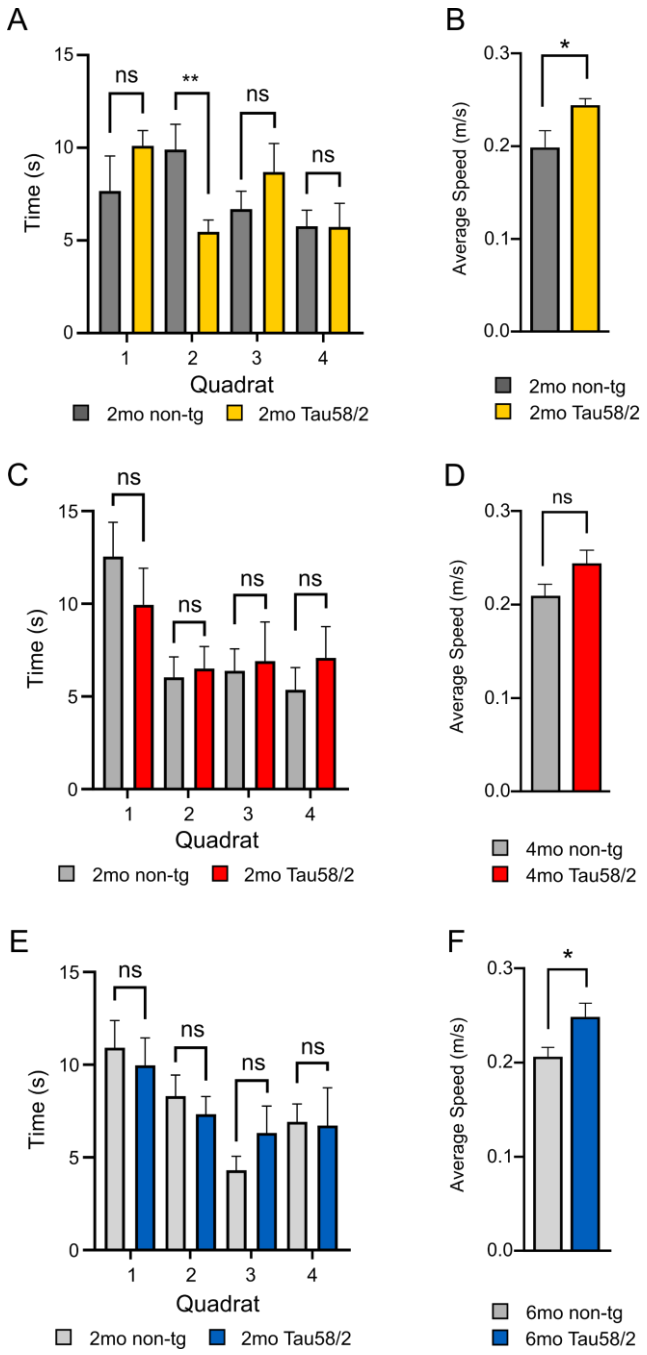

Figure S1: Normal memory consolidation and motor performance in TAU58/2 mice during MWM testing.

(**A**) Time spend (s) in each quadrat as outlined in Figure 11A, of 2 months old TAU58/2 mice (yellow) and non-tg littermate controls (non-tg; grey) in the Morris Water Maze apparatus (ns, not significant, *, $p<0.05$, Q1: target quadrat containing submerged escape platform, Q2: located opposite to Q1 and contains the four positions of entry, Q3: located left to Q1, Q4: right with regards to Q1, n=9 TAU58/2, n=9 non-tg). (**B**) Average swimming speeds (m/s) of 2months old TAU58/2 mice

(yellow) and non-tg littermate controls (non-tg; grey) during spatial memory testing in the Morris Water Maze apparatus (*, $p<0.05$, n=9 TAU58/2, n=9 non-tg). (**C**) Time spend (s) in each quadrat as outlined in Figure 11F, of 4months old TAU58/2 mice (red) and non-tg littermate controls (non-tg; grey) in the Morris Water Maze apparatus (ns, not significant; n=12 TAU58/2, n=12 non-tg, Q1: target quadrat containing submerged escape platform, Q2: located opposite to Q1 and contains the four positions of entry, Q3: located left to Q1, Q4: right with regards to Q1, n=12 TAU58/2, 12 non-tg). (**D**) Average swimming speeds (m/) of 4months old TAU58/2 mice (red) and non-tg littermate controls (non-tg; grey) during spatial memory testing in the Morris Water Maze apparatus (ns, not significant; n=12 TAU58/2, n=12 non-tg). (E) Time spend (s) in each quadrat as outlined in Figure 11K, of 6months old TAU58/2 mice (blue) and non-tg littermate controls (non-tg; grey) in the Morris Water Maze apparatus (ns, not significant, Q1: target quadrat containing submerged escape platform, Q2: located opposite to Q1 and contains the four positions of entry, Q3: located left to Q1, Q4: right with regards to Q1, n=8 TAU58/2, n=13 non-tg). (F) Average swimming speeds (m/s) of 6months old TAU58/2 mice (blue) and non-tg littermate controls (non-tg; grey) during spatial memory testing in the Morris Water Maze apparatus (*, $p<0.05$; n=12 TAU58/2, 12 non-tg).

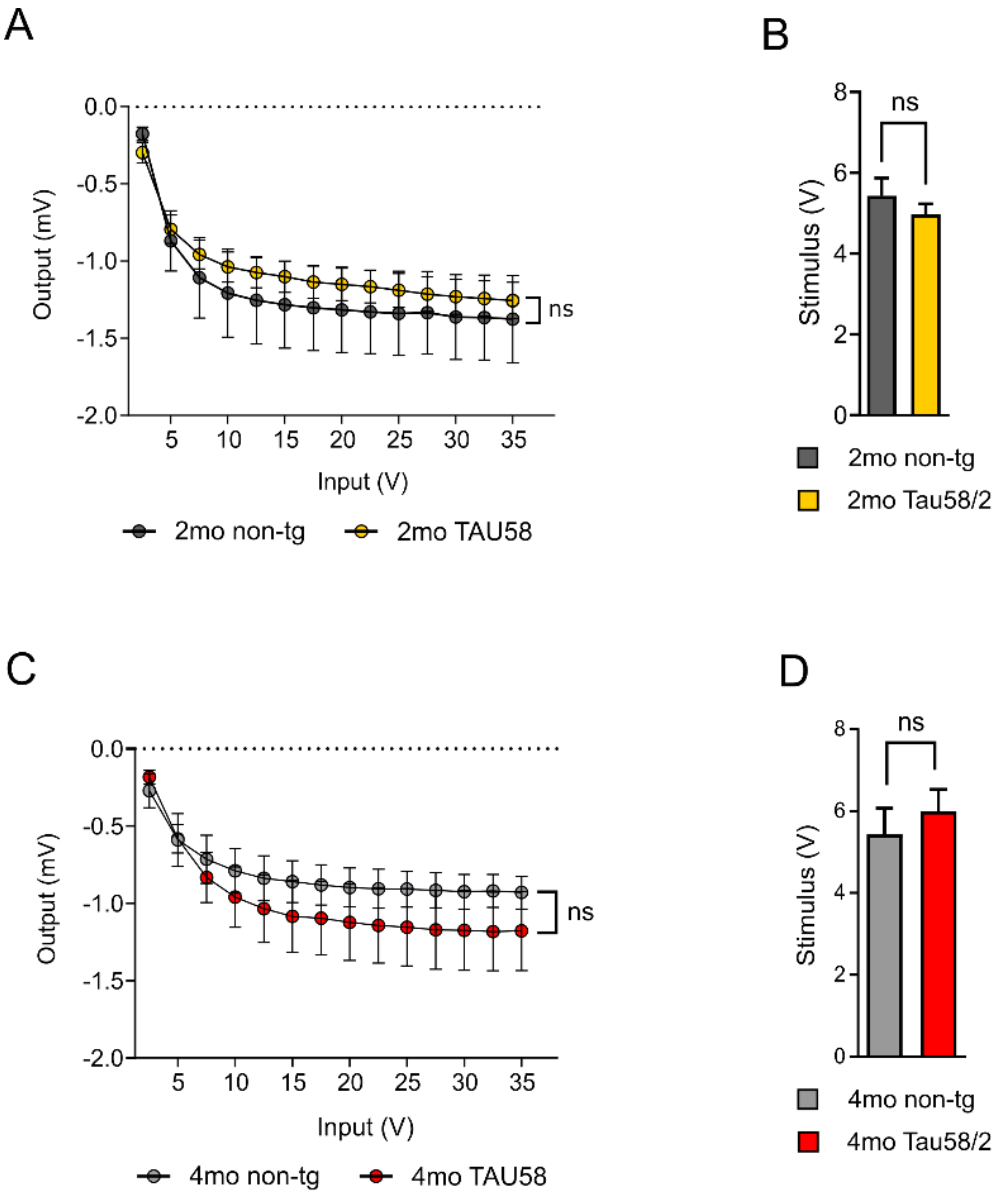

Figure S 2: Input-output functions and stimulus intensity for LTP generation in TAU58/2 mice.

(**A**) Input-output (I/O) functions of stimulus intensity versus EPSP amplitude of 2 months old TAU58/2 (yellow) and aged matched non-transgenic controls (non-tg; grey) (ns; not significant; n=9 TAU58/2, n=7 non-tg). (**B**) Stimulus intensity used to induce the half-max response in 2moths old TAU58/2 (yellow) and aged matched non-tg control slices (non-tg; grey) (ns; not significant; n=9 TAU58/2, n=7 non-tg). (**C**) Input-output (I/O) functions of stimulus intensity versus EPSP amplitude of 4 months old TAU58/2 (red) and aged matched non-tg controls (non-tg; grey) (ns; not significant; n=6 TAU58/2, n=7 non-tg). (**D**) Stimulus intensity used to induce the half-max response in 4 months old TAU58/2 (red) and aged matched non-tg control slices (non-tg; grey) (ns; not significant; n=6 TAU58/2, n=7 non-tg).

Table S 1: List of differentially regulated hippocampal genes of TAU58/2 mice at 10 weeks of age.
Gene expression profiling by RNA sequencing and DESeq2 analysis with a cut-off of >30% FDR revealed 44 differentially regulated genes (31 down- and 13 upregulated genes). Values are given as log2 fold changes of non- transgenic.

log2 Fold change	Base mean	lfcSE	padj	Gene ID	Gene name	Gene/protein function	Reference	Gene type	source	Locus
-3.2410662	6.5898	0.8616	0.1483	ENSMUSG00000085391.1	Gm16150	n/a		antisense_RNA	HAVANA	chr1:38235420-38246666:+
-2.4743729	10.927	0.6372	0.1254	ENSMUSG00000092879.1	Gm22650	n/a		miRNA	ENSEMBL	chrX:146999405-146999509:+
-2.1454411	14.093	0.5852	0.1744	ENSMUSG00000107304.1	Gm43775	n/a		TEC	HAVANA	chr5:66069501-66072895:-
-1.8331205	21.495	0.5348	0.2942	ENSMUSG00000107390.1	Gm43323	n/a		TEC	HAVANA	chr5:66080209-66084466:-
-1.6507019	16.443	0.4398	0.1483	ENSMUSG00000025592.17	Dach2	• *Dachs2* encodes for the Dachshund2 protein • expressed in the brain during development • involved in the regulation of organogenesis and myogenesis • may further play a role in premature ovarian failure	(Davis et al., 2001)	protein_coding	HAVANA	chrX:113297510-113836386:+
-1.326272	30.105	0.3115	0.0487	ENSMUSG00000032372.14	Plscr2	• *Plscr2* encodes for Phospholipid scramblase2 • reported to be involved in Ca2+ dependent migration of phospholipids through the plasma membrane • may play a central role in the recognition of apoptotic and injured cells	(Williamson et al., 1992, Williamson and Schlegel, 1994)	protein_coding	HAVANA	chr9:92275602-92297752:+
-1.222662	67.024	0.3319	0.173	ENSMUSG00000034917.8	Tjp3	• *Tjp3* encodes for the tight junction protein ZO-3 which comprises of 3 domains, a PDZ, SH3, and guanylate kinase-like domain • Tjp3 was reported to interact directly or indirectly with actin filaments • Recruits factors that are involved in signal transduction • Regulates proliferation and cell differentiation	(Wittchen et al., 1999, Matter and Balda, 2003, Kiener et al., 2007)	protein_coding	HAVANA	chr10:81273207-81291581:-
-1.0048355	71.369	0.2236	0.0212	ENSMUSG00000034151.13	Zbbx	• *Zbbx* encodes for the zinc finger B-box domain-containing protein • Binds to zinc ions • Exact function unknown	UniProtKB:D3Z0P7	protein_coding	HAVANA	chr3:75037907-75165034:-
-0.9171207	63.686	0.2486	0.173	ENSMUSG00000038570.15	Saxo2	• *Saxo2* encodes for the stabilizer of axonemal microtubules 2, which seems to be involved in microtubule binding	UniProtKB:Q8IYX7	protein_coding	HAVANA	chr7:82632960-82648528:-

-0.857018	95.944	0.2226	0.1254	ENSMUSG00000036357.5	Gpr101	• *Gpr101* encodes for the Probable G-protein coupled receptor 101 • Mainly expressed in the brain • May contribute to several pathways in the CNS via modulation of cAMP levels	(Bates et al., 2006)	protein_coding	HAVANA	chrX:57496668-57503757:-
-0.7858583	155.08	0.1983	0.1118	ENSMUSG00000060176.4	Kif27	• Kif27 activity is crucial during embryonal development • Kinesins have an important role in neuronal transport • In humans, mutations in Kif27 have been associated with several neurodegenerative disease	(Mandelkow and Mandelkow, 2002, Hirokawa et al., 2009)	protein_coding	HAVANA	chr13:58287502-58359122:-
-0.7830834	172.37	0.2239	0.2437	ENSMUSG00000019892.13	Lrriq1	• *Lrriq1* encodes for Leucine Rich Repeats and IQ Motif Containing 1 • Exact function unknown	UniProtKB - Q96JM4	protein_coding	HAVANA	chr10:103046031-103236322:-
-0.7686685	194.51	0.213	0.1919	ENSMUSG00000032327.14	Stra6	• *Stra6* encodes for the Stra6 protein which is thought to facilitate retinol translocation across the cell membrane • Important regulator of Vitamin A homeostasis in the eye	(Chen et al., 2016)	protein_coding	HAVANA	chr9:58063788-58153996:+
-0.7292378	190.42	0.1819	0.1	ENSMUSG00000031274.16	Col4a5	• *Col4a5* encodes for the protein Collagen alpha-5(IV) • Together with six other subunits, collagen alpha-5(IV) is considered to be a major constituents of basement membranes, forming a physical barrier between different tissues.	(Lemmink et al., 1997)	protein_coding	HAVANA	chrX:141475385-141689234:+
-0.7110485	477.02	0.1845	0.1254	ENSMUSG00000041144.10	Dnah7b	• *Dnah7b* encodes for the dynein, axonemal, heavy chain 7B protein • Dynein, axonemal, heavy chain 7B is thought to be a component of the inner dynein arm of ciliary axonemes	(Zhang et al., 2002)	protein_coding	HAVANA	chr1:46066315-46373546:+
-0.7022476	121.88	0.1519	0.0134	ENSMUSG00000025420.13	Katnal2	• *Katnal2* encodes for the Katanin catalytic subunit A1 like 2, which is thought to be an important regulator of microtubule dynamics, cytokinesis and cell cycle progression.	(Ververis et al., 2016, Dunleavy et al., 2017)	protein_coding	HAVANA	chr18:76977148-77047308:-
-0.6920933	117.57	0.1871	0.173	ENSMUSG00000033542.13	Arhgef5	• *Arhgef5* encodes for the Rho guanine nucleotide exchange factor ARHGEF5. • ARHGEF5 has been described as a regulator of Rho GTPases that are involved in actin organization, cell motility, polarity, growth, survival and gene transcription.	(Hall, 2012)	protein_coding	HAVANA	chr6:43265582-43289320:+
-0.6570988	432.76	0.1839	0.1968	ENSMUSG00000035126.19	Wdr78	• *Wdr78* encodes for the WD repeat domain 78. • WD- Repeat proteins are thought to play a critical role in the formation of protein-protein complexes in nearly all major pathways, suggesting that a deregulation of these proteins may	(Smith, 2008)	protein_coding	HAVANA	chr4:103038065-103114555:-

						have implication in many genetic diseases.				
-0.6529206	202.07	0.1776	0.173	ENSMUSG00000047496.6	Rnf152	• *Rnf152* encodes for the E3 ubiquitin-protein ligase RNF152 • The E3 ubiquitin-protein ligase RNF152 was reported to negatively regulate the mTORC1 pathway by targeting RagA for K63-linked ubiquitination. • Interestingly, deregulation of mTOR1 signaling has been associated with a number of diseases including metabolic and developmental diseases	(Laplante and Sabatini, 2012, Deng et al., 2015)	protein_coding	HAVANA	chr1:105276914-105356710:-
-0.6117743	160.77	0.1603	0.1372	ENSMUSG00000046808.17	Atp10d	• *Atp10d* encodes for the Probable phospholipid-transporting ATPase VD • Has been implicated in the maintenance of phospholipid distribution	UniOrotKB:Q9P241	polymorphic_pseudogene	HAVANA	chr5:72203329-72298775:+
-0.5911376	173.53	0.1684	0.2375	ENSMUSG00000052407.16	Ccdc171	• *Ccdc171* encodes for the Coiled-coil domain-containing protein 171 • Exact function is not known	UniProtKB: E9Q1U1	protein_coding	HAVANA	chr4:83525545-83864670:+
-0.5475075	214.24	0.1324	0.0752	ENSMUSG00000030560.17	Ctsc	• *Ctsc* encodes for cathepsin C • Cathepsin C has been discussed as a regulator of neuroinflammation via the activation of serine proteases as a defense mechanism of immune and inflammatory cells	(Turk et al., 2001, Fan et al., 2012)	protein_coding	HAVANA	chr7:88278085-88310888:+
-0.5328212	355.02	0.1491	0.1968	ENSMUSG00000030889.14	Vwa3a	• *Vwa3a* encodes for the von Willebrand factor A domain containing 3A • The van Willebrand factor has been reported to play an important role in blood coagulation • Deregulations of the von Willebrand factor have been implicated in AD and PD	(Gu et al., 2010, Wolters et al., 2018)	protein_coding	HAVANA	chr7:120739318-120805742:+
-0.4691424	1570.3	0.1326	0.2183	ENSMUSG00000027030.15	Stk39	• *Stk39* encodes for the STE20/SPS1-related proline-alanine-rich protein kinase, which is thought to act as a mediator of stress related responses • A significant association between Stk39 and Parkinson's disease have been found more recently, suggesting Stk30 as possible risk factor of PD	(Johnston et al., 2000, International Parkinson Disease Genomics et al., 2011)	protein_coding	HAVANA	chr2:68210445-68472268:-
-0.4662186	545.06	0.1275	0.1748	ENSMUSG00000050822.11	Slc29a4	• *Slc29a4* encodes the plasma membrane monoamine transporter (PMAT) • PMAT is an integral membrane protein implicated in the transport of monoamine neurotransmitters, including serotonin, dopamine and norepinephrine	(Xia et al., 2009, Lohr et al., 2014)	protein_coding	HAVANA	chr5:142692512-142722490:+

						• Deregulations of PMAT have been implicated in the pathogenesis of neurodegenerative diseases.				
-0.3514569	719.4	0.0933	0.1483	ENSMUSG00000021990.15	Spata13	• *Spata13* encodes for the Spermatogenesis-associated protein 13, which has been implicated in spermatogenesis.	(Huang et al., 2016)	protein_coding	HAVANA	chr14:60634001-60764556:+
-0.3137515	490.43	0.0908	0.2787	ENSMUSG00000031367.15	Ap1s2	• Ap1s2 encodes for the adaptor related protein complex 1 subunit sigma 2, which is thought to play a crucial role in clathrin-mediated endocytosis of G-protein-coupled receptors • Loss of Amp1 has been involved in calcineurin deficiency, which is a regulator of Ca2+ signalling	(Collins et al., 2002, Kita et al., 2004)	protein_coding	HAVANA	chrX:163909017-163933666:+
-0.2845907	791.31	0.0795	0.1968	ENSMUSG00000021112.9	Mpp5	• *Mpp5* encodes for the MAGUK p55 subfamily member 5 (MP55), which belongs to the family of membrane-associated guanylate kinases (MAGUKs) • As a scaffold protein MPP5 has been implicated in the biogenesis of tight junctions and cell polarity	(Fanning et al., 1998, Ozcelik et al., 2010)	protein_coding	HAVANA	chr12:78748907-78840714:+
-0.2670289	1075.7	0.0689	0.1254	ENSMUSG00000031246.14	Sh3bgrl	• *Sh3bgrl* encodes the SH3 domain-binding glutamic acid-rich-like protein, which has been implicates as a mediator of redox activity	(Egeo et al., 1998, Mazzocco et al., 2002)	protein_coding	HAVANA	chrX:109095365-109197873:+
-0.2325035	1444.8	0.0638	0.1764	ENSMUSG00000020290.14	Xpo1	• *Xpo1* encodes the protein Exportin 1, also known as CRM1, which has been implicated in the nuclear export of proteins, rRNA and mRNA • Recently deregulation of CMR1 has been implicated in the development of neurodegenerative diseases including ALS	(Fornerod et al., 1997, Kohler and Hurt, 2007, Fahrenkrog and Harel, 2018, Archbold et al., 2018)	protein_coding	HAVANA	chr11:23256041-23298249:+
-0.203256	2633.6	0.0565	0.1926	ENSMUSG00000026469.14	Xpr1	• *Xpr1* encodes for the xenotropic and polytropic retrovirus receptor 1, which has been described as a mediator of phosphate export from the cell • Recent studies suggest a link between Xpr1 mutations and neurological diseases	(Giovannini et al., 2013, Legati et al., 2015)	protein_coding	HAVANA	chr1:155275701-155417415:-
0.31953528	1137.9	0.0732	0.0333	ENSMUSG00000020889.11	Nr1d1	• *Nr1d1* encodes for the nuclear receptor subfamily 1, group D, member 1, which has been implicated in the regulation of the circadian rhythm • Recently Nr11 has been shown to be involved in corticogenesis via the regulation of excitatory neuron migration and synaptic network formation	(Valnegri et al., 2011, Goto et al., 2017)	protein_coding	HAVANA	chr11:98767932-98775333:-

0.37259887	468.31	0.0912	0.0848	ENSMUSG00000049907.8	Rasl11b	• *Rasl11b* encodes for the Ras-Like Protein Family Member 11B • Ras proteins have been involved in a wide range of cellular processes including cell proliferation, differentiation, intracellular trafficking, cell survival and apoptosis	(Stolle et al., 2007, Fernandez-Medarde and Santos, 2011)	protein_coding	HAVANA	chr5:74195286-74199481:+
0.43068667	550.58	0.1257	0.2942	ENSMUSG00000007721.6	Ccdc124	• *Ccdc124* encodes for the Coiled-coin domain containing 124 • Recent findings suggest that Ccdc 124 may act as functional link between cytokinesis and the activation of Rap signaling, which is crucial for the formation of cell- cell junctions and the establishment of cell polarity	(Gloerich and Bos, 2011, Telkoparan et al., 2013)	protein_coding	HAVANA	chr8:70868227-70873935:-
0.49202115	365.05	0.1214	0.0897	ENSMUSG00000059991.7	Nptx2	• Nptx2 encodes for the protein Neuronal pentraxin-2 • In the adult brain Nptx2 has been reported to localize specifically to excitatory synapses where it is thought to induce the aggregation of AMPA-receptors, suggesting an important role of Nptx2 in maintaining the balance between excitation/inhibition. • More recently Nptx2 has been implicated in the regulation of emotional states including anxiety • In a mouse model of AD deregulation of Nptx2 has been implicated in cognitive failure.	(O'Brien et al., 1999, Pelkey et al., 2015, Xiao et al., 2017, Chang et al., 2018)	protein_coding	HAVANA	chr5:144545902-144557478:+
0.49696843	464.58	0.1287	0.1254	ENSMUSG00000029819.6	Npy	• Npy encodes for the neuropeptide y, which has been implicated in several in cortical excitability, stress response, emotional states and the circadian rhythms • The majority of NPY is expressed in GABAergic interneurons and exerts its effects through specific G-protein coupled receptors • Several studies indicate a link between the alteration of NPY-expression and neurodegenerative diseases	(Tatemoto, 1990, Palop et al., 2007, Global Burden of Disease Study, 2015, Duarte-Neves et al., 2016)	protein_coding	HAVANA	chr6:49822710-49829507:+
0.52452195	7743.7	0.0659	2E-11	ENSMUSG00000018411.17	Mapt	• Mapt encodes for the tau protein. • upregulation of Mapt mRNAs is reflective of transgenic construct used to generate TAU58/2 mice		protein_coding	HAVANA	chr11:104231390-104332090:+
0.6508291	1055.1	0.1405	0.0134	ENSMUSG00000038418.7	Egr1	• Egr1 encodes for the Early growth response protein 1 also known as Zif268 • The immediate-early gene Egr1 has been described as major regulator of higher order processes including synaptic plasticity, learning and	(MacGibbon et al., 1997, Knapska and Kaczmarek, 2004, Gatta et al., 2014, Duclot and Kabbaj, 2017)	protein_coding	HAVANA	chr18:34859823-34864984:+

						memory and response to emotional states • Deregulation of EGr1 expression has been described in human AD brains and mouse models of AD				
0.76866211	58.855	0.2123	0.1884	ENSMUSG00000104576.1	F830115 B05Rik	n/a		TEC	HAVANA	chr5:113117571-113119464:-
0.81802292	707.24	0.1704	0.0095	ENSMUSG00000052837.6	Junb	• Junb encodes for the Transcription factor junB, which was shown to be involved in regulating gene activity following the primary growth factor response • Deregulation of the immediate early gene Junb has been associated with neurocognitive deficits including Alzheimer's disease	(Anderson et al., 1994, Bahrami and Drablos, 2016, Han et al., 2017)	protein_coding	HAVANA	chr8:84974484-84978718:-
0.82792874	711.9	0.1733	0.0095	ENSMUSG00000023034.6	Nr4a1	• Nr4a1 encodes for the Nuclear Receptor Subfamily 4 Group A Member 1, also known as nerve growth factor IB (NGFIB) • NGFIB has been implicated in cell cycle mediation, apoptosis and in mediating inflammatory processes. • Recently a role of Nr4a1 in long-term memory and the development of seizures has been described	(Chang et al., 1989, Pei et al., 2006, Zhang, 2007, McNulty et al., 2012, Zhang et al., 2016)	protein_coding	ENSEMBL	chr15:101266846-101274792:+
1.03679851	6927.2	0.0922	5E-25	ENSMUSG00000032011.5	Thy1	• upregulation of mRNAs is reflective of transgenic construct used to generate TAU58/2 mice		protein_coding	HAVANA	chr9:44043384-44048579:+
1.48503642	38.438	0.3857	0.1254	ENSMUSG00000085609.1	1700016 P03Rik	n/a		processed_transcript	HAVANA	chr11:75172560-75177633:+
1.65624172	30.242	0.4379	0.1483	ENSMUSG00000037868.15	Egr2	• Egr2 encodes for the Early growth response protein 2 • The immediate early gene Egr2 was shown to facilitate certain forms of learning and memory (in particular attention-set shifting task) • Induction of Egr2 mRNA was shown to correlate with seizure activity	(Bhat et al., 1992, Poirier et al., 2008)	protein_coding	HAVANA	chr10:67535475-67542188:+

Table S 2: Gene-specific primer details used for quantitative PCR analysis.

Target Gene	Forward Primer (5'→ 3')	Reverse Primer (5'→ 3')	Reference	Cycling temperature used according to distributor instructions for SybrGreen	Verification of primer specificity
Actin	CCCCCTGAACCCCAAAGC	CCACGTACATGGCTGGGGT	(Bi et al., 2017)	95°C for 10min (activation of polymerase) **40x cycles** 95°C for 5 sec (denaturation) 60°C for 10min (annealing)	Standard- and Meltingcurves were conducted for all primers used to verify primer specificity
Arc	GTGTGGAGGGAGGTCTTCT	CTGCCCACTGGGTATTTGC	(Bi et al., 2017)		
cFos	TACTACCATTCCCCAGCCGAC	TTGGCACTAGAGACGGACAGA	(Bi et al., 2017)		
FosB	TCCAGCCAGAGCCAGGC	CTCTGCGAACCCTTCGCTT	(Bi et al., 2017)		
Egr2	AGCCGTTTCCCTGTCCTCTG	GTCCCTCACCACCTCCACTT	(Morita et al., 2016)		
Npy	CAGAGGACATGGCCAGATA	GGTCTGAAATCAGTGTCTCA	This study		

Conclusion

With rapidly increasing numbers of dementia patients worldwide and no disease-halting treatment available to date, the development of accurate model organisms to study and better understand disease mechanisms is becoming increasingly important. The past decades of dementia research have shown that mouse models are valuable surrogate systems of the human disease and have facilitated new insights into the pathological and physiological roles of disease-associated proteins, including tau (Santacruz et al., 2005, Ittner et al., 2010, Ittner et al., 2016).

In this thesis the TAU58/2 tau transgenic mouse model was utilized to determine the role of P301S mutant human tau in the development of behavioural, functional and cognitive deficits. FTD clinically presents with profound behavioural abnormalities ranging from personality changes to apathy, disinhibition and hyperactivity. To get a better understanding of how P301S mutant human tau impacts on behavioural outcomes, in particular those present in FTD patients, we assessed anxiety and risk-taking behavior together with neuropathological changes in young, adult and old TAU58/2 males and aged matched non-transgenic littermates (**Paper I.**). We found that TAU58/2 mice present with early onset disinhibition-like behaviour and increased motor activity when subjected to the elevated plus maze (EPM) and the Open field apparatus, respectively. Interestingly, these behavioral abnormalities were paralleled by the early and progressive development of tau pathology in the amygdala, a neuropathological feature also reported in early stages of FTD and related tauopathies (Cook et al., 2014). Considering the importance of the amygdala in controlling behaviour, early onset tau pathology and behavioural changes in the EPM in TAU58/2 mice suggests a contributing role to the clinical presentation of FTD (Przybyla et al., 2016). However, it cannot be ruled out that other brain regions, for instance cortical areas, may also have a contributing effect on the behavioural outcomes observed in this study, despite lower levels of cortical pathological tau compared to the levels detected in the amygdala of TAU58/2 mice.

Neuronal network aberrations and cognitive decline are characteristic features of several tauopathies, including AD and FTD. Furthermore, tau - but not Aβ pathology - correlates with cognitive decline and neurodegeneration in these diseases. However,

very little is known about how tau pathology drives cellular and molecular mechanisms involved in memory and cognitive decline. We therefore characterized the effects of transgenic P301S mutant human tau expression on neuronal network function in the murine hippocampus utilizing the TAU58/2 transgenic mouse model (**Paper II**).

In the present study, we showed that the early onset of progressive spatial learning deficits in TAU58/2 mice was associated with impaired LTP, neuronal network aberrations and a prominent IEG expression signature, indicating hyperactivation of neuronal circuits in TAU58/2 mice. Moreover, staining for the hyperactivity marker NPY showed a progressive increase in immunoreactivity and intensity with age, further suggesting coordinated neuronal network hyperactivity. Importantly, staining with the synaptic plasticity marker Arc showed that neuronal labelling was largely confined to neurons harbouring hyperphosphorylated tau, indicating that tau pathology renders neurons prone to hyperexcitation in response to network activity, in turn compromising the entire network resulting in functional deficits including progressive memory decline. Therefore, our data indicates that tau pathology drives neuronal network dysfunction through hyperexcitation of individual, pathology-harbouring neurons as a major contributor to memory and learning deficits.

Taken together, both studies presented in this thesis provide new insights into the pathophysiological role of tau in disease, by demonstrating the impact of P301S mutant human tau on behavioural, functional and cognitive outcomes. This work builds the foundation for future studies of specific pathways and factors in FTD and AD. For example, by crossing TAU58/2 mice with other transgenic or knockout lines to understand the impact of genes on tau pathology and associates behavioural changes. Furthermore, the in-depth characterization of the TAU58/2 mouse models in this thesis is fundamental for using the mice for testing new treatments for AD and FTD. Accordingly, having established the onset and progression of disinhibition and learning deficits in TAU58/2 mice, these readouts can be used to determine preventive and therapeutic effects of novel compounds during pre-clinical testing.

While my study has shed new light on the role of tau pathology in neuronal network and synaptic plasticity failure, further studies are needed to understand the exact role of mutant human tau in the development of neurodegenerative diseases. To this end, both studies showed that the TAU58/2 mouse model recapitulates prominent features of AD and FTD, including early-onset of disinhibition-like behaviour, hyperactivity, neuronal network aberrations and cognitive decline, making them an

exceptionally suitable model for furthering our understanding of disease pathogenesis and/or enabling the identification of new targets for future translations into therapy.

Limitations

The first publication (**Paper I**) aimed to address the question whether mutant P301S human tau may impact on behavioural outcomes in TAU58/2 transgenic mice. The data presented in the first study (**Paper I**) showed that tau pathology in the amygdala of TAU58/2 mice is of early onset and progresses with age. Furthermore, EPM testing revealed disinhibition/reduced anxiety already in young TAU58/2 mice, while deficits in the exploration and hyperactivity in the OF test were of later onset. Moreover, both behavioural abnormalities and the occurrence of pathological tau markedly progressed with age, suggesting a positive correlation between pathological tau in the amygdala and behavioural outcomes. However, there are some limitations of this study that need to be discussed.

First, even though pathological and behavioural changes were significantly different in TAU58/2 mice compared to non-transgenic control mice at all ages tested, the group sizes used to investigate these were not consistent within the age groups tested and methods used. An inconsistent number of subjects within test groups may underpower the dataset or may increase the effect of intervening variables which could negatively affect or mask the results and therefore have to be acknowledged as limitations of the study.

Second, since disinhibition and a lack of emotional control are lead symptoms of bvFTD, and given that the amygdala is an important area for behavioural control (Seymour and Dolan, 2008) young, adult and aged TAU58/2 mice were subjected to the EPM, which is a well-established measure to determine levels of anxiety and disinhibition-like behaviour in mice (Wei et al., 2010, Johnson et al., 2018). Even though the results presented in this publication revealed an early-onset of disinhibition-like behaviour in TAU58/2 transgenic mice, demonstrated by significantly increased time spent in the open arms of the EPM apparatus, it cannot be ruled out that brain areas other than the amygdala may have contributed to these observed deficits (Andersen and Teicher, 1999). In order to specifically assess amygdala dysfunction in TAU58/2 mice, additional tests could be applied in the future (i.e. the auditory fear conditioning paradigm or the Light/Dark chamber), which may corroborate our findings

and/or may give more insights into the extent of the amygdala dysfunction observed in TAU58/2 mice.

Finally, although the study showed increased phosphorylation and deposition of tau in the amygdala, which progressively increased as mice aged (young, adult, old), human tau expression (i.e. by using a human tau specific antibody) was not assessed, nor discussed in the result section. Given the importance of evaluating exogenous tau expression in order to put the amount of tau phosphorylation and deposition in the amygdala into context (i.e. does high human tau expression in neurons result in increased amounts of phosporylated tau or NFT formation compared to neurons with lower human tau expression?), human tau expression should also be assessed in a future experiment to further validate the findings presented in this publication. This could either be accomplished through immunofluorescent staining against human tau (i.e. by utilizing the Tau13 antibody), similar to what is presented for phospho-tau in the results section of the current publication, or via western blots, which would allow for sequential extraction of the amygdala tissue into soluble and insoluble tau fractions as was done previously for other brain regions (van Eersel et al., 2015).

Future directions

Overall, both studies presented in this thesis provide new insights into the pathophysiological role of tau in disease, by demonstrating the impact of P301S mutant human tau on behavioural, functional and cognitive outcomes. This work builds the foundation for future studies of specific pathways and/or factors that may contribute to the pathogenisis of FTD and AD.

One such future study could for example further address the causality between the onset of disinhibition-like behaviour and pathological changes in the amygdala described in the TAU58/2 mice. In this regard it would be interesting to investigate whether an increase in tau burden directly translates into increased disinhibition (i.e. abundant tau pathology leads to increased time spent in the open arms of the elevated plus maze) in these mice, or whether this effect is rather resulting indirectly through other factors or downstream mechanisms. To evaluate this question, one could directly correlate pathological changes with behavioural outcomes for each mouse tested. Tau burden could then be evaluated using biochemical approaches like western blotting and/or immunohistochemical stainings, which may provide new insights into tau-mediated neurotoxicity.

Another way to address this question would be to evaluate whether these behavioral changes are underlined by abnormalities on a cellular level, i.e. whether they result from changes in certain neuronal subtypes (i.e. interneurons) or are due to a deregulation of neurotransmitters (GABA/ Glutamate). In fact, previous studies utilizing tau transgenic mice have shown tau-mediated changes in GABAergic interneurons (Levenga et al., 2013) and altered neurotransmission has been reported in several mouse models of AD (Palop and Mucke, 2010, Klein et al., 2014, Ambrad Giovannetti and Fuhrmann, 2019) as well as in human AD patients (Palop and Mucke, 2016, Frere and Slutsky, 2018). Together, these studies corroborate the importance of balanced neuronal networks (i.e. maintaining a balance between inhibitory and excitatory circuits) for “normal” cognitive functioning and outline that perturbations to this balance may play a critical role in the development of neurodegenerative diseases (Palop and Mucke, 2010). Further, given that information processing in the amygdala strongly depends on inhibitory control, and a deregulation of inhibitory networks have been associated with anxiety disorders in humans (Marin, 2012, Prager et al., 2016),

future work could evaluate whether an imbalance in inhibitory neurotransmission may also be an underlying phenomenon in TAU58/2 mice by which abnormal tau impacts on behavioral and network changes. Additionally, recent work shows that Neuropeptide Y (NPY), which has been implicated in the regulation of pro- and anti-anxiety signaling within the limbic system (Sajdyk et al., 2004) may also play a crucial role in the amygdala, where it is thought to mediate fear responses. Accordingly, NPY over-expression in the amygdala of rodents resulted in decreased anxiety-related behaviours in the elevated plus maze (Primeaux et al., 2005), whilst NPY-knockout mice showed increased anxiogenic related behaviour, suggesting that changes in NPY expression levels may have relevant physiological consequences. In this regard, and considering the results of the second publication, it would be relevant to asses NPY expression levels in the amygdala of TAU58/2 mice, to evaluate whether behavioural changes, in particular their characteristic disinhibition-like behaviour, may result from perturbations in NPY expression.

An additional aspect which should be acknowledged is the previously described axonopathy, in the form of axonal swellings and spheroids found in cortical regions of TAU58/2 mice (van Eersel et al., 2015). Considering that axonal abnormalities are indicative of axonal transport deficits and impairments in axonal transport of vesicles and cargos have been implicated in early stages of AD in humans (Stokin et al., 2005) and mouse models of AD and FTD (Ittner et al., 2008), it would be interesting to evaluate whether P301S mutant human tau may also impact on axonal changes in the amygdala of TAU58/2 mice. Given that perturbations to microtubule-dependent transport are thought to result in the loss of neuronal connectivity via the degeneration of axonal structures, synapses and neurites (Gotz et al., 2006), one could assume that axonopathy in the amygdala of TAU58/2 may have similar consequences, including the deregulation of neuronal transmission and abnormalities to the structure and function neurons. Investigating these changes would be particularly intriguing, since axonopathy in the cortex of TAU58/2 mice has been described prior to abundant tau pathology, suggesting underlying mechanisms other than the deposition of tau *per se* are involved.

Finally, to complement these future studies one could assess whether neuronal loss in the amygdala may have contributed to the underlying phenotype in TAU58/2 mice, described in the first publication. Considering that atrophy of the amygdala has been observed at very early stages in the development of FTD (Kril and Halliday, 2004)

and given the important role of the amygdala in controlling emotional states, including anxiety and fear responses (Seymour and Dolan, 2008), one could assume that abundant pathology and tau deposition in the amygdala may also impact on neuronal viability, consequently resulting in neuronal loss/atrophy.

In the second manuscript, which aims to address whether P301S mutant human tau may impact on cognitive outcomes in TAU58/2 mice, we showed a cellular link between tau expression and IEG activation in the hippocampus of young TAU58/2 mice, that is consistent with neuronal network hypersynchronicity. Further, IEG activation correlated with the onset of memory deficits and neuronal network aberrations in TAU58/2 mice. Together, our data suggests that tau pathology drives neuronal network dysfunction through hyperexcitation of individual, pathology-harbouring neurons, contributing to memory deficits.

However, future studies are needed to further elaborate how P301S mutant human tau impacts on IEG expression in TAU58/2 mice since the exact mechanisms are yet to be established.

Some studies report a decrease in IEG expression associated with memory decline and aging (Rowe et al., 2007), whilst we and others (Fox et al., 2011) showed elevated IEG expression levels in tau transgenic mice. Similar findings were reported in mouse models of AD (Gatta et al., 2014, Bakalash et al., 2011, Killick et al., 2014) and in AD brains (MacGibbon et al., 1997, Gomez Ravetti et al., 2010), where Egr1 expression was shown to correlate with disease progression (Gomez Ravetti et al., 2010). Interestingly, inhibition of IEG Egr1 was shown to reduce tau-pathology and alleviate cognitive deficits in a mouse model of AD, suggesting a direct link between aberrant tau and IEG expression levels (Qin et al., 2017). Further, decreased synaptic plasticity was described in primary neurons overexpressing Arc (Shepherd et al., 2006), whilst markedly increased levels of Arc in a small fraction of hippocampal neurons, was suggested to result from severe neuronal overexcitation in a mouse model of AD (Palop et al., 2005). Moreover, it is commonly believed that IEGs are expressed in response to neuronal hyperexcitation, which is an underlying phenomenon also found in TAU58/2 mice. Additionally, more recently we found pronounced alterations to glutamate receptor signaling in cortical tissue of young (3 months), but not old (12 months) TAU58/2 mice (Ke et al., 2019), which may indicate a contribution of aberrant glutamatergic activation to early deficits found in TAU58/2

mice. In line with our findings, hyperexcitability has been described in other mouse models of tau pathology (Garcia-Cabrero et al., 2013, Liu et al., 2017).

However, in the present study hippocampal gene profiling was conducted in 10-week-old TAU58/mice to determine whether onset of deficits in synaptic plasticity may be reflected by changes in gene profiles. Our data demonstrates that reduced synaptic transmission starts to manifest in TAU58/2 mice at 2 months of age. Given that pronounced spatial memory and learning deficits occur at 4 months of age it would also be interesting to assess how the gene profiling changes with increasing pathology and deficits in TAU58/2 mice, which may provide critical insights into how neuronal networks change prior to and during the development of tau pathology. To complement these experiments and given the pronounced neuronal network aberrations in TAU58/2 mice at 6 months, it would be relevant to asses synaptic plasticity also at a later stage in TAU58/2. These experiments would help to further our understanding of how neuronal networks are affected and altered in the course of the disease and may thereby provide critical insights into tau-mediated neurotoxicity.

Another aspect that needs to be further addressed in the future is the role of NPY-expression levels in the hippocampus of TAU58/2 mice. In the present study we found a pronounced IEG signature together with elevated gene expression levels of NPY in hippocampal tissue of TAU58/2 mice at 10 weeks of age. Moreover, staining for the hyperactivity marker NPY showed progressively increased immunoreactivity and intensity with age, suggesting coordinated neuronal network hyperactivity. These findings are in line with other studies (Diez et al., 2003, Palop et al., 2007, Krezymon et al., 2013), implicating elevated NPY expression, induced by hyperexcitation of hippocampal neuronal networks, in the pathogenesis of neurodegenerative diseases (Palop et al., 2007, Palop and Mucke, 2010). Furthermore, increased levels of NPY have also been described in rodent seizure models (Wahlestedt et al., 1990, Vezzani et al., 1999). In fact, *in vitro* studies showed that hippocampal NPY expression resulted in decreased calcium influx and reduced glutamatergic signaling, most likely mediated by presynaptic Y_2-receptors (Klapstein and Colmers, 1993, Silva et al., 2003) However, in contrast to these and our findings, other groups have reported decreased levels of NPY in AD brain (Chan-Palay et al., 1986, Davies et al., 1990) and mouse models of neurodegenerative diseases (Ramos et al., 2006, Mahar et al., 2016). The discrepancy in NPY expression levels found between mouse strains and also in comparison to

human AD, may have several reasons. First, it needs to be acknowledged that transgenic mouse lines usually mimic some, but not all pathological feature of human AD. For example, certain mouse strains may develop pronounced tau pathology in the hippocampus, but may lack brain atrophy or cognitive decline, which are characteristic features of the human disease. Second, each of the studies mentioned above, utilized different techniques to evaluate NPY expression levels and also evaluated these changes using diverse AD mouse models, which most likely mimic different stages of the disease. For example, PS1 x APP transgenic mice, which rather mimic later stages of the disease, showed pronounced Aβ-burden and decreased levels of NPY expression (Ramos et al., 2006). On the other hand, studies using less progressive AD mouse models, detected rather elevated levels of NPY-expression (Diez et al., 2000, Diez et al., 2003). Accordingly, one could assume, that NPY expression is rather elevated in early stages of AD, as a neuronal mechanism to counterbalance underlying neuronal hyperexcitation (Palop et al., 2007), whilst reduced NPY levels may rather be seen as a late stage manifestation possibly resulting in neurotoxicity (Duarte-Neves et al., 2016). However, future studies are needed to further elaborate on the role of NPY in the pathogenesis of AD and related neurodegenerative diseases.

Although future studies are needed to uncover the exact mechanisms by which P301S mutant human tau may impact on IEG expression and cognitive outcomes in TAU58/2 mice, the data presented in this manuscript outlines some new insights.

Taken together our results suggest that cognitive decline in TAU58/2 may result from both, the combination of neuronal hyperexcitation of individual tau-harbouring neurons (represented by an increase in IEG signature) and the subsequent compensatory inhibition by NPY-associated interneurons (reflected by elevated NPY levels), which at first reduces overexcitation however in the end confines functional agility of specific excitatory networks- an underlying mechanism that has been previously suggested in a mouse model of Aβ-induced neuronal overexcitation (Palop et al., 2007).

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Animal ethics Approval

MACQUARIE University

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2017/053-7 **Date of Expiry:** 31 December 2019

Full Approval Duration: *01 January 2018 to 31 December 2020 (36 months)*

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

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In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above
or Manager, CAF: 9850 7780 / 0428 861 163 and **Animal Welfare Officer**: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: **Breeding and Maintenance of Dementia Research Centre Mouse Colony**

Purpose: **5 - Research: Human or Animal Health and Welfare**

Aims:
1. **To transfer mice from the current breeding colony of the Dementia Research Unit at the University of New South Wales to the MARS Central Animal Facility to establish these lines for the new Dementia Research Centre at Macquarie University**
2. **To expand the colonies and age mice for subsequent studies of the new Dementia Research Centre at Macquarie University**
3. **To collect tissues from mice that have reached specific ages for biochemical and histological analysis.**

Surgical Procedures category: 2 - Animal Unconscious without Recovery

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Weight/Sex	Total	Supplier/Source
01 – Mouse	As per Attachment A of Application	Any	46,560	Dementia Research Unit Colony, University of NSW
			46,560	

Location of research:

Location	*Full street address*
Central Animal Facility	Building F9A, Research Park Drive, Macquarie University, NSW 2109

Amendments approved by the AEC since initial approval:

1. ***Amendment*** *- 28/08/2018 - Add Nicolle Morey and Jessica Spathos to protocol (Executive approved. Ratified by AEC 18 October 2018).*
2. ***Amendment*** *– 17/09/2018 - Add Troy Butler and Dr Annika van Hummel to protocol (Executive approved. Ratified by AEC 18 October 2018).*

AEC Reference No.: 2017/053-7 **Date of Expiry:** 31 December 2019

3. ***Amendment*** *– 11/02/2019 – Add Magdalena Przybyla, Annika Van Hummel, Arne Ittner, Astrid Feiten, Carol Au, Danial Tan, Emmanuel Prikas, Esmerelda Paric, Holly Stefen, Janet van Eersel, Josefine Bertz, Julia van der Hoven, Kristie Stefanoska, Liming Hou, Lucy da Silva, Troy Butler, Wei Lee, Yuanyuan Deng, Prita Riana Asih, Nicolle Morey and Jessica Spathos to protocol (Executive approved. To be ratified by AEC 14 March 2019).*

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

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A/Professor Nathan Hart (Chair, Animal Ethics Committee) **Approval Date:** 19 February 2019

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2018/019-12 **Date of Expiry:** 18 July 2020

Full Approval Duration *19 July 2018 to 18 July 2021 (36 months)*

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

Principal Investigator:
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Associate Investigators:

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Esmeralda Paric	0435 059 704
Josefine Bertz	0452 030 681
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Wei Lee	0421 924 459
Prita Riana Asih	0409 391 983
Nicolle Morey	0416 675 038
Jessica Spathos	0433 682 767
Fabien Delerue	0411 351 446
Yazi Ke	0423 237 287

Associate Investigators:

Arne Ittner	0402 078 166
Thomas Fath	0424 578 120
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Liming Hou	0433 758 578
Magdalene Przybyla	0478 800 313
Yuanyuan Deng	0431 710 802
Kristie Stefanoska	0427 527 797
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Holly Stefen	0401 817 955
Gabbriella Chan	0420 212 744
Neda Assareh	0422 881 674
Stefan Guerra	0425 722 953
Miheer Sabale	0450 774 146

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above
or Manager, CAF: 9850 7780 / 0428 861 163 and **Animal Welfare Officer**: 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: **Dementia Research Centre – Neurodegeneration protocol: investigating disease mechanisms and development of new therapies**

Purpose: 5 - Research: Human or Animal Health and Welfare

Aims: to determine how neuropathological changes and genetic modifications in mouse models of neurodegenerative diseases affect different modes of brain functions and how to intervene in these processes with novel therapeutic approaches.

Surgical Procedures category: 5 - Major Surgery with Recovery

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Weight/Sex	Total	Supplier/Source
01 – Mouse	Any	Weaning to 24 months/Any/Any	99,970	ABR/ACR/DRC breeding and generation
01 – Mouse	Any	Embryos	1,560	ABR/ACR/DRC breeding and generation
			101,530	

Location of research:

Location	*Full street address*
Central Animal Facility	Building F9A, Research Park Drive, Macquarie University, NSW 2109

Amendments approved by the AEC since initial approval:

1. **Amendment –** 10/12/2018 – Add Yazi Ke, Arne Ittner, Thomas Fath, Janet van Eersel, Liming Hou, Magdalene Przybyla, Yuanyuan Deng, Kristie Stefanoska, Julia van der Hoven, Astrid Feiten, Carol Au, Daniel Tan, Adam Martin and Emmanuel Prikas, Holly Stefen and Gabbriella Chan to protocol (Executive approved. Ratified by AEC 14 February 2019).
2. **Amendment –** 08/12/2018 – Add experimental procedure to include the use of foetal neurons (Executive approved. To be ratified by AEC 14 February 2019).
3. **Amendment –** 08/12/2018 – Additional animals requested (1560) (Executive approved. Ratified by AEC 14 February 2019).
4. **Amendment –** 12/02/2019 – Add Annika van Hummel, Esmeralda Paric, Josefine Bertz, Lucy da Silva, Troy Butler, Wei Lee, Prita Riana Asih, Nicolle Morey and Jessica Spathos to protocol (Executive approved. Ratified by AEC 14 March 2019).
5. **Amendment –** 23/02/2019 – Change of procedure to include newly approved SOPs (AEC approved 11 April 2019).

6. **Amendment –** 05/04/2019 – Remove Lucy Da Silva from project (Executive approved. Ratified by AEC 16 May 2019).
7. **Amendment –** 17/06/2019 – New experimental procedure (to further refine the brain delivery of AAV and peptides, in order to reduce doses used and off target effects) (AEC approved 18 July 2019).
8. **Amendment –** 04/07/2019 – Add Neda Assareh to project (Executive approved. To be ratified by AEC 15 August 2019).
9. **Amendment –** 18/07/2019 – Add Stefan Guerra to project (Executive approved. To be ratified by AEC 15 August 2019).
10. **Amendment –** 18/07/2019 – Add Miheer Sabale to project (Executive approved. To be ratified by AEC 15 August 2019).

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Conditions of Approval: N/A

A/Prof. Nathan Hart (Chair, Animal Ethics Committee) **Approval Date:** 18 July 2019