

1 **Characterization of preclinical transgenic rat**
2 **models for Alzheimer's disease-like**
3 **pathology**
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19 Submitted, May 15, 2023
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23 A thesis submission to McGill University in partial fulfillment
24 of the requirements of the degree of
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27 Doctor of Philosophy, Pharmacology & Therapeutics
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*'You step into the Road, and if you don't keep your feet, there is no telling
where you might be swept off to.'*

J.R.R. Tolkein

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232 **LIST OF ABBREVIATIONS**

233	α 7-nAChR	alpha 7 nicotinic acetylcholine receptor
234	A β	Amyloid beta
235	ACE-R	Addenbrookes cognitive examination-revised
236	aCSF	Artificial cerebral spinal fluid
237	AD	Alzheimer's disease
238	ADAM 10/17	A disintegrin and metalloproteinase domain-containing protein
239	ADME	Absorption, distribution, metabolism and excretion
240	AICD	Amyloid intracellular C-terminal domain (product of γ -secretase cleaving)
241	APH1	Anterior pharynx-defective 1
242	ApoE	Apolipoprotein E
243	APP	Amyloid precursor protein
244	AQP4	Aquaporin 4
245	ARIA	Amyloid-related imaging abnormalities
246	ATP	Adenosine triphosphate
247	AU	Arbitrary fluorescence units
248	BACE1	Beta amyloid convertase enzyme 1
249	BBB	Blood-brain-barrier
250	BDNF	Brain-derived neurotrophic factor
251	CAA	Cerebral amyloid angiopathy
252	CaMKII	Ca ²⁺ /calmodulin-dependent protein kinase II
253	CCAC	Canadian Council on Animal Care
254	CCNA	Canadian Consortium on Neurodegeneration in Aging
255	CDK5	Cyclin dependent kinase 5
256	CDR	Clinical dementia rating
257	ColIV	Collagen IV
258	COX-2	Cyclooxygenase 2
259	CNS	Central nervous system
260	CSF	Cerebrospinal fluid
261	CRTC1	CREB-regulated transcription coactivator 1
262	CX3CL1	Fractalkine
263	DAB	3',3'- diaminobenzidine
264	DNA	Deoxyribonucleic acid
265	DS	Down's Syndrome
266	EB	Evan's blue dye
267	EC	Entorhinal cortex
268	ECLIA	Electrochemiluminescence assay
269	EOAD	Early Onset Alzheimer's disease
270	EPSP	Excitatory postsynaptic potential
271	ERK	extracellular signal-regulated kinase
272	FAD	Familial Alzheimer's disease
273	FAM	Familiarization phase
274	FC	Fear conditioning
275	FDG	Fluorodeoxyglucose
276	FTD	Frontotemporal dementia
277	FTDP-17	Frontotemporal dementia and Parkinsonism linked to chromosome 17

278	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
279	GFAP	Glial fibrillary acidic protein
280	GSK-3 β	Glycogen synthase kinase 3 beta
281	GWAS	Genome Wide Association Studies
282	HFS	High frequency stimulation
283	HRP	Horseradish peroxidase
284	htau	Human tau
285	iA β	Intracellular amyloid beta
286	Iba1	Ionized calcium binding adaptor molecule 1
287	ICV	Intracerebroventricular
288	IDE	Insulin degrading enzyme
289	IF	Immunofluorescence
290	IHC	Immunohistochemistry
291	INSIGHT	Investigation of Alzheimer's Predictors-Pre Alzheimers
292	IL-1B	Interleukin 1 beta
293	IR	Immunoreactivity
294	JNK-1	c-Jun N-terminal Kinase
295	LOAD	Late-onset Alzheimer's disease
296	LC	Locus Coeruleus
297	LDL	Low density lipoprotein
298	LRP-1	LDL receptor-related protein-1
299	LTD	Long-term depression
300	LTP	Long-term potentiation
301	MAP	Mouse anti-horseradish peroxidase
302	MAP2	Microtubule-associated protein 2
303	MAPT	Microtubule associated protein tau
304	MCI	Mild cognitive impairment
305	MMP-2/9	Matrix metalloprotease
306	MMSE	Mini-mental state exam
307	MRI	Magnetic resonance imaging
308	MWM	Morris water maze
309	NFT	Neurofibrillary tangle
310	Nf-L	Neurofilament light chain
311	NGS	Normal goat serum
312	NMDA	N-methyl-D-Aspartic acid
313	NMDAR	N-methyl-D-Aspartic acid receptor
314	NOL	Novel object location
315	NOR	Novel object recognition
316	NSAID	Non-steroidal anti-inflammatory drugs
317	NVU	Neurovascular unit
318	OF	Open field
319	PBS	Phosphate-buffered saline
320	PCR	Polymerase chain reaction
321	PET	Positron emission tomography
322	PEN-2	Presenilin 2
323	PFA	Paraformaldehyde

324	PHF	Paired helical filament
325	PiB	Pittsburgh Compound B
326	PiD	Pick's disease
327	PP2A	Protein phosphatase 2A
328	PSD95	Postsynaptic density protein
329	PSEN1	Presenilin-1
330	PSP	Progressive supranuclear palsy
331	ptau	Phosphorylated tau
332	p25/p35	CDK5 gene; neuron specific activator of CDK5
333	RAGE	Receptor for advanced glycation end products
334	RNA	Ribonucleic acid
335	SAD	Sporadic Alzheimer's disease
336	sAPP α	Soluble alpha-secretase cleaved amyloid precursor protein fragment
337	sAPP β	Soluble beta-secretase cleaved amyloid precursor protein fragment
338	SEM	Standard error of the mean
339	SHR	Spontaneously hypertensive rat
340	SI	Social interaction task
341	SLM	Stratum lacunosum moleculare
342	SSCtx	Somatosensory cortex
343	Sub	Subiculum
344	TBI	Traumatic brain injury
345	TBS	Tris-buffered saline
346	Tg	Transgenic
347	TNF- α	Tumor necrosis factor alpha

ABSTRACT

Alzheimer's disease (AD) is a devastating neurodegenerative disease and is the most common cause of dementias. People with AD have two pathological hallmarks in the central nervous system: accumulation of extracellular amyloid beta (A β) plaques and intraneuronal hyperphosphorylated tau tangles. No definitive disease modifying treatment for AD exists despite decades of research efforts and clinical trials. Existing animal models of AD have been useful in elucidating advanced stages of disease, however, these models do not recapitulate pathological stages as seen in the human pathology. These failures suggest that 1) the models applied thus far may not accurately reproduce the human-like AD pathology at stages more amenable to therapeutic intervention, and 2) the initiating stages of AD are not well understood. Rat models, as compared to mice, present significant genetic, behavioural, neurophysiological, and anatomical advantages for translational studies given their closer proximity to humans.

Taking advantage of the established McGill-R-Thy1-APP model for human amyloid pathology and the recently generated McGill-R955-hTau transgenic model of human tauopathy, respectively, heterozygous transgenes were crossed to generate a novel bigenic rat model named McGill-R-APPxhTau which possesses both human amyloid and tau-like pathologies in an aged rat. **The purpose of this thesis was to investigate the characteristics of brain tauopathy as well as the combined role of amyloid and tau in transgenic rat models of Alzheimer's disease-like pathology.**

Characterization of these rat models was accomplished primarily using immunohistochemical and biochemical approaches. First, the unknown phenotype of R955-hTau rats in both their homozygous and heterozygous forms was characterized to determine the capability of the R955-hTau rat model to recapitulate key features of human tauopathy. Over the life span, homozygous R955-hTau rats developed such features including a progressive accumulation and hyperphosphorylation of human tau, neurodegeneration, and cognitive impairments as seen in humans with AD and frontotemporal dementia (FTD). Alterations to the neurovascular unit and blood-brain barrier function were discovered, demonstrating a novel tau-mediated mechanism of compromising brain neurovasculature.

The phenotype of heterozygous R955-hTau rats developed a gradual, slow-developing tau pathology with tau hyperphosphorylation reminiscent of the earliest stages of human tauopathy. At such a stage, new evidence showed that tau hyperphosphorylation and morphological

abnormalities, inflammation, and neuronal loss could occur in the absence of neurofibrillary lesions. Together, the R955-hTau rat model provided two phenotypes of great value in aged rats: heterozygous rats for the investigation of early tauopathy, and homozygous rats for elucidating a full human-like tauopathy without A β .

Toward the pursuit on the converging effects between A β and tau, we launched a longitudinal characterization of the bigenic McGill-R-APP $\times$ hTau rat model. Here, we report a novel an unexpected compensatory mechanism of the human-like tau accumulation on the A β -induced effects on cognition preceding the initial amyloid plaque formation at 20 months of age. On the other hand, during more advanced plaque pathology at 24 months, bigenic rats developed exacerbated disease pathologies, cognitive impairments and neuronal loss. Here, findings support the role of human tau in facilitating CNS degeneration stretching beyond the neuron as well as a new, protective role of tau in early AD.

The generation and characterization of these novel transgenic rat models offer novel lines of investigation for the exploration of mechanisms of disease, therapeutics, biomarker diagnostics as well as inviting further investigations regarding the perplexing earliest amyloid-tau relationships, which might have significant implications for the early stages of the AD pathology.

Key words: Alzheimer's Disease, dementia, amyloid pathology, tauopathy, transgenic rat model

RÉSUMÉ

La maladie d'Alzheimer (MA) est une maladie neurodégénérative et est la cause la plus fréquente de démence. Les personnes avec la MA ont deux caractéristiques pathologiques dans le système nerveux central: l'accumulation de plaques extracellulaires d'amyloïde bêta (A β) et d'enchevêtrements tau hyperphosphorylés intraneuronaux (ptau). Aucun traitement modificateur n'existe pour la MA malgré des efforts de recherche et d'essais cliniques. Les modèles animaux existants de la MA ont été utiles pour élucider les stades avancés de la maladie, mais ces modèles ne récapitulent pas les stades pathologiques observés chez les humains âgés. Ces échecs suggèrent que 1) les modèles appliqués peuvent ne pas reproduire avec précision la pathologie de la MA à des stades plus propices à une intervention thérapeutique, et 2) les stades d'initiation de la MA ne sont pas bien compris. Les modèles de rats, par rapport aux souris, présentent des avantages génétiques, comportementaux, neurophysiologiques et anatomiques significatifs pour les études translationnelles compte tenu de leur plus grande proximité avec les humains.

Tirant parti du modèle McGill-R-Thy1-APP établi pour la pathologie amyloïde humaine et du modèle transgénique McGill-R955-hTau récemment généré de la tauopathie, respectivement, des transgènes hétérozygotes ont été croisés pour générer un nouveau modèle de rat bigénique nommé McGill-R-APPxhTau qui possède les deux pathologies chez un rat âgé. **Le but de cette thèse était d'étudier les caractéristiques de la tauopathie cérébrale ainsi que le rôle combiné de l'amyloïde et du tau dans des modèles de rats transgéniques de la pathologie de type MA.**

La caractérisation de ces modèles de rats a été réalisée principalement avec des approches immunohistochimiques et biochimiques. Le phénotype des rats R955-hTau dans leurs formes homozygotes et hétérozygotes a été caractérisé pour déterminer la capacité du modèle de R955-hTau à récapituler les caractéristiques de la tauopathie humaine. Les rats homozygotes R955-hTau ont développé les caractéristiques, notamment une accumulation progressive de la tau et ptau humaines, une neurodégénérescence et des troubles cognitifs comme les humains atteints de MA et de FTD. Des altérations de l'unité neurovasculaire et de la fonction de barrière hémato-encéphalique ont été découvertes, démontrant un nouveau mécanisme médié par tau compromettant la microcirculation cérébrale. Le phénotype des rats R955-hTau hétérozygotes a montré une pathologie tau progressive à développement lent de ptau. À un tel stade, de nouvelles preuves ont montré que ptau et les anomalies morphologiques, l'inflammation et la perte neuronale se produisaient en l'absence de lésions neurofibrillaires. Ensemble, le modèle de rat R955-hTau

offrent deux phénotypes: des rats hétérozygotes pour l'étude de la tauopathie précoce et des rats homozygotes pour élucider une tauopathie humaine complète sans A β .

Vers la poursuite des effets convergents entre A β et tau, la caractérisation longitudinale de rats McGill-R-APP $\times$ hTau a révélé un nouveau mécanisme compensatoire de la tauopathie humaine sur les effets induits par A β sur la cognition qui précédé l'apparition des plaques d'A β à 20 mois de l'âge. D'autre part, des rats bigéniques âgés de 24 mois avec des plaques d'A β bien établies ont développé des pathologies pathologiques exacerbées, des troubles cognitifs et une perte neuronale. Nos résultats soutiennent un rôle du tau humain dans la facilitation de la dégénérescence du SNC s'étendant au-delà du neurone ainsi qu'un nouveau rôle protecteur du tau au début de la MA. La génération et la caractérisation de ces nouveaux modèles de rats transgéniques offrent de nouvelles pistes d'investigation pour les mécanismes de la MA, son traitement, l'identification de biomarqueurs, et permettent la découverte de relations A β -tau déconcertantes avec des implications significatives pour les premiers stades de la maladie humaine.

ACKNOWLEDGEMENTS

First, I must sincerely and give undying gratitude to Professor A. Claudio Cuello and Dr. Sonia Do Carmo as they have been instrumental in providing professional guidance and mentorship throughout the duration of my degree and with the projects within this thesis. Their support and passion helped me to become a better, independent scientist with confidence.

To Janice Malcolm, H  l  ne Hall and Sonia Do Carmo: you provided opportunities to learn many technical skills at different stages of my PhD. To the very helpful laboratory technicians and colleagues, Adriana, Tina, Chunwei, Johanne and Manon, your assistance, methods and coordination have made my PhD and lab environment easier to work in and be more efficient.

To my thesis advisory committee: Professors Bernard Robaire (advisor), Lisa Munter and Dusica Maysinger, as well as Professors Paul Clarke and Alfredo Ribeiro-da-Silva for fruitful discussions. You kept me on my toes scientifically and mentally by bringing multiple angles and perspectives related to my thesis work. This voluntary service shall never be forgotten.

To my past supervisors and mentors Drs Nafisa Jadavji, Patrice Smith and Kathleen Gilmour, as you have initially showed me the academic path to tread and have sculpted me for a PhD, I would not have made it this far without your teaching and mentorship.

To my family: brother Richard J. Emmerson, mother Mary-Ann Emmerson, father Richard K. Emmerson and step-mother Doris Lenhart - you have always been a source of personal support leading up to this point and have provided means to give me a second wind when I needed it most. As a first-generation scientist, it is just as much a family victory as it is a personal one. To my grandfather Joshua John, you have been a source of inspiration and willpower in my pursuit of higher education such as this PhD and your support has been unwavering. To my grandmother Una John, dementia took you and its impact echoes in our family. I only hope is that the enclosed works provides a meaningful contribution as to help stop others from suffering it in the future. Rest in peace to both of you.

To the many friends, colleagues and connections forged over these years (M.F., C.O., N.Y., R.P., B.K., A.L., Q.B., E.R. & G.R., K.C. & N.W., T.Y., N.M., & A.P., C.S., L.T., C.K., J.T., J.C., T.N., S.M., R.M. *et. al*), thank you for the relationships we shared and the camaraderie. A special thanks to McGill's PGSS Thomson House for being a 'sanctuary' in times of need, and to Spotify (un-sponsored) for the stimulating music during the many, long, experimental hours.

CONTRIBUTIONS TO ORIGINAL KNOWLEDGE

To investigate the consequences of tau pathology during the earliest and advanced stages, we established the McGill-R955-hTau transgenic rat model and characterized rats in their homozygous and heterozygous forms. Secondly, to investigate the relationship between amyloid and tau in early stages, we generated another transgenic rat model named McGill-R-APPxhTau. Through these investigations, the following contributions to original knowledge are:

- I.** The homozygous R955-hTau transgenic rat model recapitulates several aspects of human tauopathy suitable for experimental therapeutics. This transgenic rat provides a higher degree of translation to human disease compared to other existing mouse models as demonstrated by the occurrence of progressive tau accumulation and hyperphosphorylation, genuine neurofibrillary inclusions, neuronal loss, and prion-like recruitment of endogenous murine tau (Chapter II).
- II.** During advanced stages of human-like tauopathy in the homozygous R955-hTau transgenic rat model, I discovered and demonstrated that progressive tauopathy induces neurovascular compromise as demonstrated by the occurrence of multiple structural and cellular abnormalities attributed to misfolded tau (Chapter II).
- III.** Heterozygous R955-hTau rats recapitulate aspects resembling the earliest stages of tauopathy and incorporate the factor of aging unlike other available models. I discovered the occurrence of cognitive impairment, neuronal loss, myelin degeneration, and tau hyperphosphorylation at sites Ser202-Thr205 and Thr231, in the absence of neurofibrillary inclusions (Chapter III).
- IV.** By combining human-like amyloid and tau pathologies in the APPxhTau bigenic rat, I have produced a new transgenic rat model unlike other available models. I have discovered that tau produces an early compensatory on the CNS during the initial amyloid plaque pathology, thereby rescuing cognition and synaptic plasticity (Chapter IV). This compensatory mechanism is tentatively also attributed to the protein Fyn.
- V.** During the advanced stages of amyloid plaque pathology preceding neurofibrillary degeneration, I discovered that the combination of human amyloid beta and tau exacerbates each others' pathologies, neuronal loss, and deficits in cognition and CNS inflammation (Chapter IV).

CONTRIBUTION OF AUTHORS

Chapters II, III and IV of this doctoral thesis entail works for three respective publications, of which Joshua T. Emmerson was the lead investigator responsible for study design, data generation, analysis and drafting of the manuscripts unless otherwise stated below. Professor A. Claudio Cuello provided project direction, guidance, mentorship, and editorial support on the manuscripts.

Chapter II

“Progressive human-like tauopathy with downstream neurodegeneration and neurovascular compromise in a transgenic rat model”

Joshua T. Emmerson, Sonia Do Carmo, Yingying Liu, Ali Shalhoub, Ai Liu, Quentin Bonomo, Janice C. Malcolm, Lionel Breuillaud and A. Claudio Cuello
Submitted to Neurobiology of Disease

Sonia Do Carmo provided contributions to the design and creation of the R955-hTau transgene construct and editorial support for the manuscript.

Yingying Liu assisted with neuron staining and cell counting, and troubleshooting for MAP2, Synaptophysin immunofluorescence and initial Western blotting experiments.

Ali Shalhoub assisted with Western blot experiments including APOE, Nf-L, and NMDAR

Ai Liu and **Quentin Bonomo** assisted with Sarkosyl sample preparation for the pTau231/total Tau assays.

Janice C. Malcolm Provided preliminary data and insights for study conceptualization.

Lionel Breuillaud provided contributions to the design and creation of the R955-hTau transgene construct with SDC.

Chapter III

“Neuronal loss and inflammation preceding fibrillary tau pathology in a rat model with early human-like tauopathy”

Joshua T Emmerson¹, Janice C Malcolm¹, Sonia Do Carmo, Lionel Breuillaud and A. Claudio Cuello

¹ denotes equal contribution to the work

Submitted to Neurobiology of Disease

Janice C. Malcolm performed behavioral experiments and initial tau immunohistochemical staining on 10 and 18-month-old rats, assisted with tissue processing and imaging for electron microscopy experiments. JCM also provided insights for study conceptualization.

Sonia Do Carmo provided contributions to the design and creation of the R955-hTau transgene construct and editorial support for the manuscript.

Lional Breuillaud provided contributions to the design and creation of the R955-hTau transgene construct with SDC.

Chapter IV

“Tau counteracts the initial amyloid beta-derived impairments in cognition in a transgenic rat model”

Joshua T. Emmerson, Sonia Do Carmo, Agustina Lavagna, Chunwei Huang, Tak Pan Wong, A. Claudio Cuello

In preparation for submission

Sonia Do Carmo performed amyloid beta and inflammation electrochemiluminescence assays, assisted with qPCR experiments and provided editorial support for the manuscript.

Agustina Lavagna assisted with qPCR experiments.

Chunwei Huang oversaw the initial breeding and management of rat colonies and assisted with genotyping.

Tak Pan Wong provided collaborative support, equipment and oversight on the acute brain slice electrophysiology for the assessment of synaptic plasticity by LTP.

OTHER CONTRIBUTIONS

The following articles were published or are in preparation to be published but are not part of this thesis:

1. Wilson EN, Do Carmo S, Welikovitch LA, Hall H, Aguilar LF, Foret MK, Iulita MF, Jia DT, Marks AR, Allard S, **Emmerson JT**, Ducatenzeiler A, Cuello AC. NP03, a Microdose Lithium Formulation, Blunts Early Amyloid Post-Plaque Neuropathology in McGill-R-Thy1-APP Alzheimer-Like Transgenic Rats. *J Alzheimers Dis.* 2020;73(2):723-739. doi: 10.3233/JAD-190862.
2. Do Carmo S, **Emmerson JT**, Foret MK, Welikovitch L, Cuello AC. Combined Treatment with the Microdose Lithium Formulation NP03 and S-adenosylmethionine Reduces Cognitive Decline and Alzheimer's-like Amyloid Pathology in McGill-R-Thy1-APP rats. *In preparation.*

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CHAPTER I: GENERAL INTRODUCTION

1.1 Alzheimer's disease and dementia: definition, diagnosis and risk factors

Alzheimer's disease (AD) is a devastating neurodegenerative disease in the world today, remaining as a perplexing research challenge to address and to effectively provide disease-modifying therapeutic intervention. AD was originally discovered in 1906 from a demented patient of Dr. Alois Alzheimer, named Auguste Deter (Maurer et al., 1997). The patient was experiencing delusions, loss of memory and loss of reasoning including a loss of self. An autopsy and a Nissl silver stain of Deter's brain revealed protein aggregates encroaching around and within neurons. Today these structures are extracellular amyloid beta ($A\beta$) plaques and intracellular neurofibrillary hyperphosphorylated tau tangles (NFTs), or plaques and tangles, respectively. Accompanying CNS $A\beta$ and tau deposition in AD are a broad set of clinical symptoms of dementia including memory loss, loss of executive functions, trouble recognizing words or visual stimuli, problems speaking, changes in mood or personality and social withdrawal (Alzheimer's Association, 2017; Alzheimer Association, 2022). These symptoms can be clinically detected when the disease has already been established. Because of the distinct presence of $A\beta$ and tau deposits in the diseased central nervous system (CNS) alongside inflammation and brain degeneration, these two proteins and their histories are the basis of the major driving hypotheses for the cause of AD. The Amyloid Hypothesis states that amyloid beta ($A\beta$) causes Alzheimer's disease (D'Andrea, 2016; Kaye and Lasagna-Reeves, 2013; Selkoe and Hardy, 2016), whereas the Tau Hypothesis implicates tau as the leading toxic peptide driving brain degeneration (Goedert et al., 1989; Kametani and Hasegawa, 2018; Kosik et al., 1986). The Trigger and Bullet hypothesis combines these two, stating that $A\beta$ supports tau-induced neurodegeneration (Bloom, 2014). AD can only be confirmed post-mortem using histological approaches, requiring both $A\beta$ and tau deposits in the CNS. Genetic screening enables the detection of Early Onset Alzheimer's disease (EOAD), also known as Familial Alzheimer's disease (FAD), and these individuals are expected to develop AD before the age of 65 years. EOAD genetic mutations are found in scarce subpopulations (Cacace et al., 2016; Hatami et al., 2017; Shea et al., 2016), however, the majority of AD cases develop sporadically. Sporadic AD (SAD), also referred to as late-onset Alzheimer's Disease (LOAD), shares a clinical phenotype with FAD, however, LOAD occurs after an onset of up to *two decades* (Bateman et al., 2012; Villemagne et al., 2013), typically in old age and with several comorbidities.

There are several risk factors for LOAD with age as the most significant risk factor. In fact, age plays a critical role in overall physiological function and especially in the nervous system. For

example, cellular senescence (Liu, 2022; Martínez-Cué and Rueda, 2020), proteosome function (Cuervo and Dice, 2000; Kelmer Sacramento et al., 2020; Vilchez et al., 2014), DNA repair and DNA methylation (Gorbunova et al., 2007a, 2007b; Jones et al., 2015; Kametani and Hasegawa, 2018) are affected. In addition, neurogenesis (Kempermann, 2015) and brain neuroplasticity are reduced in AD pathology (Burke and Barnes, 2006; Foster and Norris, 1997; Thibault and Landfield, 1996). Evidence also implicates sensitized immune responses (Chee and Solito, 2021) and increased systemic inflammation with aging, a term called inflammaging. These disease-relevant aspects affected by aging become compromised in AD (Ferrucci and Fabbri, 2018; Finger et al., 2022; Hosokawa et al., 2000; Jin et al., 2022; Lazarov and Marr, 2013; Xia et al., 2018). Pharmacologically, metabolism of nutrients and drugs are reduced with aging, hence, shifting pharmacodynamic profiles in the body and brain (Kinirons and O'Mahony, 2004; Mangoni and Jackson, 2004; Pannérec et al., 2018; Sikora et al., 2021; Sowell et al., 2003). The second most impactful risk factor for AD is the presence of allele variants for the lipid transporter Apolipoprotein E gene (*ApoE*) (Lin et al., 2018), which elevates a person's risk to develop AD by up to 13-fold with two E4 alleles (*ApoE*^{4/4}) and 3-fold when with one E4 allele (*ApoE*^{3/4}) (Bertram et al., 2007). Other factors implicated in AD are diet (Morris, 2009), exercise (Ahlskog et al., 2011; Meng et al., 2020), level of education (Sharp and Gatz, 2011), diabetes (Huang et al., 2014; Jayaraj et al., 2020), social activity (Wilson et al., 2007), smoking (Crous-Bou et al., 2017), physical activity, vascular health (De La Torre, 2012; Morris, 2003), blood-brain-barrier dysfunction (Montagne et al., 2015) and traumatic brain injury (Ramos-Cejudo et al., 2018). Other risk factors that have been identified from genome-wide association studies (GWAS) are polymorphisms in inflammation, lipid metabolism and endocytosis-related genes such as *TREM2*, *CLU*, *ABCA7*, *SORL1* among others (Karch and Goate, 2015). Ultimately, understanding the cause of AD rapidly becomes complicated because of the presence of several co-morbidities. Thus, reliable measures like A β and tau deposition and assessment of cognition are utilized.

1.2 Diagnosing AD before death: a grand movement toward characterizing earlier stages

When AD and its complexity became rapidly known, there was no reliable way to track early and asymptomatic stages of AD in living humans; the earliest stage of clinical AD that could be identified was the onset of significant changes in cognition, termed mild cognitive impairment (MCI). However, MCI is not a specific predictor of AD and importantly does not completely

predict which patients will transition from MCI to AD. Neurological testing for MCI and AD is used to quantify and evaluate cognition. Such tests employ an array of standardized tasks to examine cognitive deficits in performance for tasks pertaining to learning, memory, coordination, speech and everyday function from multiple facets (Khan, 2016a; Tsoi et al., 2015). Tasks such as the Mini-mental state exam (MMSE) (McHugh, 1975), Mini-cog (Borson et al., 2000) and Addenbrookes Cognitive Examination-Revised (ACE-R) (Mioshi et al., 2006) are typically conducted with a semi-structured interview to determine a Clinical Dementia Rating (CDR) score. Generally, decline in short-term working and recognition memory precede the drastic spatial learning and long-term memory. However, some types of memories remain preserved well into advanced stages of disease (Jahn, 2013) for reason(s) unexplained.

Complementary to neurological testing, brain imaging can be requested to detect deposits of A β and tau in the brain (Alzheimer's Association, 2017; Alzheimer Association, 2022). The density of A β plaques and NFTs can be identified and crudely estimated with positron emission tomography (PET) (Dubois et al., 2016; Khan, 2016b). PET imaging detects radioactive tracers that bind non-specifically to proteinaceous aggregates in a β -pleated sheet conformation. For amyloid, [^{18}F]Florbetapir or Pittsburgh Compound B (PiB) (Klunk et al., 2004) tracers are primarily used. For tau, [^{18}F]Flortaucipir (18F-AV1451) (Khan, 2016b; Okamura et al., 2018; Zhao et al., 2019) was initially described, and with next generation tau tracers such as PI2620, and MK6240 in development (Yap et al., 2021). Unfortunately, these tracers have limited application due to background signal noise and, consequentially, they are unable to distinguish low sub-threshold peptides accumulating in preclinical stages of AD. In addition, the use of 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG)-based PET can be used to identify glucose hypometabolism, another consequence of AD (Mosconi, 2013; Mosconi et al., 2009; Sokoloff et al., 1977; Wienhard et al., 1985). Other structural brain imaging such as magnetic resonance imaging (MRI) can also be utilized to detect regional CNS structural changes such as brain atrophy (Frisoni et al., 2010) at-cost. Combining post-mortem histology, brain imaging and cognition allowed for the initial evaluation of disease progression in the brain.

By combining data from post-mortem and brain imaging studies, A β pathology is typically followed by tau pathology in an age-dependant manner and these two pathologies manifest several years before the initial onset of clinical symptoms (Barthélemy et al., 2020; Jack et al., 2013, 2011, 2010). A β and tau deposition follow a pattern of disease progression called Braak staging (Braak

et al., 2006; Braak and Braak, 1991; Pascoal et al., 2021, 2020; Thal et al., 2002), occurring in cortical brain regions, extending into the limbic system through connected neuronal circuits then spreading to the thalamus and then ultimately reaching the rest of the brain. Post-mortem analysis of AD-affected brain tissue suggests synaptic dysfunction and loss (Dekosky and Scheff, 1990; Masliah et al., 1994) as shown by a decrease in synaptophysin (Masliah et al., 1989; Mufson et al., 2003, 2000), endosomal dysfunction (Cataldo et al., 2000) or loss of forebrain cholinergic nuclei (Coyle et al., 1983; Davies and Maloney, 1976; J. Whitehouse et al., 2004; Pearson et al., 1983; Perry et al., 1978), chronic inflammatory processes such as microgliosis and astrogliosis (Griffin et al., 1995; Kinney et al., 2018; McGeer et al., 1989; McGeer and McGeer, 1995; Walters et al., 2016), cortical atrophy and neurodegeneration (Bateman et al., 2012; Buchhave et al., 2012; Jack et al., 2011, 2010; Jansen et al., 2015; Sperling et al., 2011). Only recently the accumulation of intraneuronal A β in an age-dependant manner occurring prior to tau pathology has been demonstrated using optimally preserved brain tissue from non-demented subjects (Welikovitch et al., 2018). Convincing evidence suggests that such an accumulation of intraneuronal amyloid beta (iA β) plays an important role in early stages of AD because of its appearance prior to the onset of MCI and extracellular deposits (Cuello et al., 2012; Echeverria and Cuello, 2002; Gouras et al., 2000). However, while detection of amyloid accumulation is an important event in the earliest stages of AD, amyloid content remains a poor correlate of cognitive decline unlike tau, which is a dominating correlate (Arriagada et al., 1992; Bejanin et al., 2017; Bennett et al., 2004; Biel et al., 2021; Giannakopoulos et al., 2003; Guillozet et al., 2003; Hanseeuw et al., 2019; Schöll et al., 2016). Yet, despite this knowledge, early stages of AD that are gradually occurring over 20 years remain poorly characterized, emphasizing that better methods of understanding and tracking earlier stages of AD are required.

The possibility of using biomarkers, defined as any kind of biological substance that is altered in disease and associated with disease progression or status, has recently emerged as an effort to diagnose early stages of AD (Dubois et al., 2016; Molinuevo et al., 2018) because A β , tau and phosphorylated tau (ptau) are cleared from the brain into cerebral spinal fluid (CSF) and blood in miniscule amounts (Maness et al., 1994; Roberts et al., 2014; Wang et al., 2018; Xiang et al., 2015). A combination of biomarkers including A β _{42/40} ratio (Baldeiras et al., 2018), total tau and phosphorylated tau at residues Thr231 (Ashton et al., 2021; Glodzik et al., 2011; Santos et al., 2019; Spiegel et al., 2015; Suárez-Calvet et al., 2020), Thr217 (Hanes et al., 2020; Milà-Alomà et

al., 2022) and Thr181 (Olsson et al., 2005) are able to distinguish probable AD cases from cognitively normal individuals. The use of these biomarkers are an ongoing experimental investigation for clinical use (Blennow and Zetterberg, 2018; Hansson et al., 2022). Thus far, a decrease in $A\beta_{42/40}$ ratio in CSF is associated with increased plaque load and amyloid pathology in the brain (Fagan et al., 2014; Strozyk et al., 2003) and an increase in phosphorylated tau in CSF is associated with an increased tangle burden and neuronal death (Fagan et al., 2014). While $A\beta_{42/40}$ ratio, tau and ptau explain over 70% of the variation in regression models, the addition of other biomarkers including the axonal protein neurofilament light chain (Nf-L) (Baldacci et al., 2020; de Wolf et al., 2020; Gaetani et al., 2019; Preische et al., 2019) and glial fibrillary acidic protein (GFAP) (Abdelhak et al., 2022; Benedet et al., 2021; Fukuyama et al., 2001) explain over 80% of the variation, however, changes in Nf-L and GFAP alone are nonspecific markers of degeneration and inflammation, respectively. As such, these recent advancements in AD diagnostics are shifting away from costly brain imaging and invasive CSF lumbar punctures towards non-invasive blood sampling of amyloid, tau, and Nf-L (Preische et al., 2019).

1.3 Therapeutic approaches to AD

Over the past 40 years, focus has primarily been directed to categorizing disease progression and finding therapeutic solutions for deposits of aggregated $A\beta$ given its early appearance in disease. Currently, there are no disease-modifying treatments for AD and there is an immense struggle to find a promising therapeutic option to a growing socioeconomic problem (Alzheimer's Association, 2017; Karlawish et al., 2017). There have been several hundred clinical trials to treat AD with approximately 172 ongoing trials as of 2022 (Cummings et al., 2022). Historically, the initial therapeutic agents relieved AD symptoms and include acetylcholinesterase inhibitors (Donepezil, Rivastigmine, Galantamine) to improve memory and attention, N-methyl-D-aspartic acid receptor (NMDAR) antagonists (Memantine) to improve memory and prevent excitotoxicity (Jouanne et al., 2017), antipsychotics and mood stabilizing agents (Respiridone, Olanzapine, Lithium) and Selective Serotonin Re-uptake Inhibitors (SSRIs; Fluoxetine, Citalopram) to combat depressive symptoms. The use of non-steroidal anti-inflammatory drugs (NSAIDS) (Aisen et al., 2003; Gasparini et al., 2004; Lyketsos et al., 2007) and corticosteroids (Aisen et al., 2000; Beeri et al., 2012) were found to be associated with less incidence of AD, but no significant reduction in disease pathology was shown. While these drugs addressed certain

symptoms of AD, they lacked disease-modifying properties and therefore have failed to reverse, stop or slow the progression of disease (Cuello, 2007; Cuello et al., 2019a).

The first set of attempts at disease-modifying therapeutic compounds were rationalized by the amyloid hypothesis. These compounds directly targeted enzymes involved with the processing of A β 's precursor, amyloid precursor protein (APP), in hopes to reduce A β production. Inhibitors of the two major proteolytic enzymes were developed: beta secretase (β -secretase) inhibitors (Lanabesestat, Rosiglitazone) (Coimbra et al., 2018; Das and Yan, 2019) and gamma secretase (γ -secretase) inhibitors (Semagacestat) (Doody et al., 2013; Henley et al., 2009). Unfortunately, β -secretase inhibitors showed no significant effect on cognition in mild-to-moderate AD and in cognitively normal patients at risk for AD. Inhibitors of γ -secretase ended poorly; clinical outcomes instead worsened (Doody et al., 2013) and dozens of participants developed adverse effects attributed to a non-specific inhibition of Notch signalling, a protein essential for neurodevelopment and growth. The first line of A β immunotherapy approaches were developed with the goal of utilizing monoclonal antibodies to directly target A β in different conformations while taking advantage of the patient's own immune system. For example, Antibodies targeting A β plaques (Aducanumab) (Sevigny et al., 2016), fibrillar A β (Bapineuzumab) (Salloway et al., 2014), monomeric A β species (Solanezumab) (Honig et al., 2018) and pan-specific antibodies for plaques and fibrils (Crenezumab, Gantenerumab) were developed. Aducanumab was successful in reducing plaque burden, but unexpectedly did not initially rescue cognition in a similar magnitude for all AD patients. The trial for Solanezumab was halted due to its failure to demonstrate any benefit in both the reduction of soluble A β nor cognition. While immunotherapies in AD seemed promising, these attempts have led to disappointing and financially draining outcomes for the industry and have cast shadows of doubt for AD therapeutics. Explanations for these outcomes may be attributed to an insufficient patient sample size, insufficient dosage, misalignment of diagnostic and exclusion criteria in the target population, there is also a gap in translation in therapeutic success in mice versus humans (Franco and Cedazo-Minguez, 2014; Windisch, 2014). These failures thus far suggest that treatments were applied too late and possibly after irreversible damage in the brain has occurred and that current animal models do not completely reflect the disease in humans.

The second line of amyloid therapeutics is ongoing. Trials including Aducanumab, Lecanemab, Donanemab, and Solunazemab are being pursued into phase III with improved study

design regarding dosing, length of study, sample size and inclusion of more appropriate cohorts of preclinical AD patients or those at-risk. An important limitation with such immunotherapy-based approaches have been realized from recent reports have highlighted significantly adverse effects regarding Amyloid-related imaging abnormalities (ARIA) including headache, confusion, nausea, tremor and subsequent seizures or brain bleeding in 26.4% of the amyloid-treated patients (Riederer, 2021).

The several years of unsatisfactory amyloid therapeutics has led to a shift toward finding alternative solutions. Several other targets in addition to amyloid-centered therapies are now being pursued, including targeting tau, inflammation, synaptic plasticity and neurogenesis among others (Cummings et al., 2022, 2021) and have led to a new hope for AD therapeutics. Indeed, it is likely that that a multi-targeted or combination therapeutic approach directed towards asymptomatic and probable AD cohorts will be more amenable for therapeutic intervention. However, having a better understanding of early stages is paramount for the advancement of disease-modifying therapies that will ideally prevent AD from developing or significantly slow the progression before irreversible damage occurs.

1.4 Fundamentals of amyloid beta in Alzheimer's disease and the amyloid hypothesis

George Glenner and Caine Wong proposed that accumulation of A β into aggregates and then into A β plaques are the causative component of Alzheimer's disease (AD) (Dickson, 1997; Glenner and Wong, 1984; Masters et al., 1985a; Selkoe, 2001; Selkoe and Hardy, 2016). This hypothesis is based on findings in AD patients, whether SAD or FAD, possessing a significant increase in amyloid precursor protein (APP) products, particularly the A β ₁₋₄₂ peptide. Further, rare familial mutations in the APP gene (*APP*) or gamma secretase (γ -secretase) mutations lead to an overproduction of A β peptides and deposition plaques and development of EOAD. γ -Secretase is a large protein complex, composing of four subunits: presenilin-1 (PSEN1), nicastrin, anterior pharynx-defective 1 (APH1) and presenilin enhancer 2 (PEN-2). Thus far, 58 mutations for APP, 267 mutations for Presenilin 1, 48 mutations for Presenilin 2 have been identified and are associated with AD. These rare mutations such as the Dutch (E693Q) (Levy et al., 1990; Van Broeckhoven et al., 1990), Indiana (V717F) (Murrell et al., 1991), Swedish (K670N/M671L) (Mullan et al., 1992), London (V717I) (Goate et al., 1991), among others, are causal for EOAD. In contrast, the Icelandic (A673T) mutation is protective; people with the mutation do not develop

AD (Jonsson et al., 2012; Warren et al., 1993). Aside from APP mutations, individuals with Down's Syndrome (DS) have a trisomy of chromosome 21 resulting in overproduction of APP and consequently are guaranteed to develop AD by the fifth decade of life (Caraci et al., 2017; Flores-Aguilar et al., 2020; Glenner and Wong, 1984; Masters et al., 1985b). The amyloid hypothesis which implicates A β as a causative agent has not yet been disproven, as both A β and tau are also required for AD. In contradiction to the amyloid hypothesis, there are elderly individuals that have high A β burden or A β plaques but are not cognitively impaired (Ch  telat et al., 2013; Fagan et al., 2009; Price et al., 2009) including the recent discovery of the Christchurch mutation case study (Arboleda-Velasquez et al., 2019). While amyloid deposition is one of the first signs of AD, there is also a poor correlation between plaque burden and cognitive decline and brain degeneration (McLean et al., 1999; Selkoe and Hardy, 2016). While A β likely plays an important role in AD and its early accumulation in the CNS as a disease-aggravating engine cannot be ignored, it is likely that other components of AD such as tau, inflammation or APP and its processing have their own distinct roles in neurodegeneration and dementia.

1.5 Cleavage of APP and formation of amyloid beta

Amyloid precursor protein (APP) is the protein responsible for the generation of A β via proteolysis. Physiologically, APP is a type I transmembrane protein with a larger ectodomain relative to its small transmembrane domain and C-terminal domain. While not completely understood, APP is associated with neuronal growth, neurotransmission, immune response and transition metal homeostasis (D'Andrea, 2016; Farooqui, 2017; Gulisano et al., 2018; Multhaup et al., 1996; van der Kant and Goldstein, 2015). APP can be cleaved in an amyloidogenic or nonamyloidogenic fashion (Patterson et al., 2008). Nonamyloidogenic proteolysis of APP is executed by α -secretases such as the disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) or 17 (ADAM17) which cleave APP at residue Leu17 of the A β domain and produce a neuroprotective soluble APP alpha fragment (sAPP α) (Chasseigneaux and Allinquant, 2012; Furukawa et al., 1996). Amyloidogenic proteolysis of APP is achieved by its cleavage by the β -secretase Beta amyloid convertase enzyme1 (BACE-1). For both the nonamyloidogenic and amyloidogenic cleavage of APP, APP is subsequently cleaved by γ -secretase in a stepwise fashion at multiple potential sites including Val40 or Ala42 (Ch  vez-Guti  rrez et al., 2012). In AD, enhanced amyloidogenic processing of APP by β -secretase leads to an accumulation of

intraneuronal A β (iA β) (Bayer and Wirths, 2010; Cuellar, 2005; Echeverria and Cuellar, 2002; LaFerla et al., 2007) with A β peptides of 40 (A β ₁₋₄₀) or 42 (A β ₁₋₄₂) amino acids in length. A β is physiologically localized in the plasma membrane, trans-Golgi vesicles, endoplasmic reticulum, endolytic vesicles, endosomal and lysosomal compartments (Cuellar et al., 2012; Zhang and Song, 2013). A β is principally degraded by insulin degrading enzyme (IDE) (Farris et al., 2003) and neprilysin (Marr et al., 2004; Shirotani et al., 2001) and proteases including matrix metalloproteases MMP-2 and MMP-9, aspartyl proteases BACE1/2, the serine proteases plasmin, and by microglial uptake (Saido and Leissring, 2012). Removal of A β from the brain into the periphery can be facilitated by transport through the LDL receptor-related protein-1 (LRP-1) and the receptor for advanced glycation end products (RAGE) (Deane et al., 2008; Kanekiyo and Bu, 2014; Shibata et al., 2000). Taken together, it is generally perceived that the accumulation of iA β in AD occurs from an imbalance of A β overproduction, clearance and processing in the CNS.

In AD, soluble A β aggregates with itself to form soluble oligomeric species with distinct sequential intermediates of 3-50 A β monomers, granular oligomers, and protofibrils ultimately leading to insoluble deposits of fibrils and plaques (Bernstein et al., 2009; Ferreira et al., 2015, 2007; Glabe, 2008; Novo et al., 2018; Selkoe and Hardy, 2016; Serra-Batiste et al., 2016; Walsh et al., 2000). Soluble oligomeric A β species in the extracellular space are thought to be the most toxic because they correlate with cognitive impairment more than plaques (Koffie et al., 2009; Lue et al., 1999; McLean et al., 1999) and induce impairments in neuroplasticity by long-term potentiation (LTP) and promote cell death (Lambert et al., 1998; Li et al., 2011; Walsh et al., 2002; Wang et al., 2002). The initial A β plaques are diffuse and lack inflammatory cells whereas, in advanced stages of AD, compact (dense) plaques with spheroid clouds and with recruited inflammatory cells (D'Andrea and Nagele, 2010). Amyloid beta is but one protein, and is not the only pathological hallmark implicated in the AD continuum.

1.6 Tau pathology and neurofibrillary tangles in Alzheimer's disease

In 1986, the hyperphosphorylated protein tau was identified as the same protein constituent of the neurofibrillary tangles (NFTs) within the neurons of AD patients (Goedert et al., 1992; Grundke-Iqbal et al., 1986a, 1986b; Iqbal et al., 1986; Kosik et al., 1988). Because changes in tau correlate more with neuronal loss, degeneration and cognitive decline as compared to A β as mentioned above, tau is thought to cause neuronal damage and neuronal cell loss. There are three

types of neurofibrillary lesions: NFTs, neuropil threads and dystrophic neurites (Probst et al., 1991), with neuropil threads localized to the axon initiating segment and axon hillock of pyramidal neurons. In addition to neurofibrillary lesions, there are also intracellular and extracellular axonal spheroid deposits and these are thought to facilitate neurodegeneration and synaptic loss due to their co-localization with dying neurons in post-mortem tissue (Ballatore et al., 2007; Brandt et al., 2005). According to Braak staging of the AD tau pathology mentioned above (Braak et al., 2006; Braak and Braak, 1995), the earliest signs of NFTs in the CNS was identified in the transentorhinal cortex however the locus coeruleus also appears to be affected around this stage. Then, tau pathology progressively spreads topographically into subregions within the hippocampus and cerebral cortex over time. These observations lead to further investigations of the progressive changes of tau in AD.

It is thought that tau accumulates, becomes hyperphosphorylated and then aggregates before developing into NFTs. In AD, tau is notably missorted from the axon to the neuronal cell soma, dendritic compartment and synaptic terminals (Fein et al., 2008; Tai et al., 2012; Zhou et al., 2017). In addition, aggregation of hyperphosphorylated tau and formation of paired helical filament (PHF) structures appears to be a sequential event preceding NFTs (Friedhoff et al., 1998; Shamas et al., 2015) based on observations that hyperphosphorylation precedes the occurrence of tau aggregates (Friedhoff et al., 2000; Mandelkow and Mandelkow, 2012, 1998; Mandelkow et al., 2007) and that ptau must undergo a particular conformational change between the N- and C-terminus into a “hairpin” structure necessary for PHF formation (Combs et al., 2016; Jeganathan et al., 2008). PHFs are detected in advanced stages of AD and are thus perceived to be one of the first steps toward formation of pathological tau inclusions (Cripps et al., 2006; Weaver et al., 2000). PHF fibrils likely aggregate into neurofibrillary tangles (NFTs). While this proposed sequence of tau has been supported both in post-mortem and in animal models, the early pathological events preceding PHF formation and tau-mediated degeneration are not fully understood.

However, it is important to recognize that the abnormal accumulation and hyperphosphorylation of tau have their own distinct pathology, termed tauopathies, and play an important role in AD and dementias. Tauopathies can occur independently of A β pathology and are categorized as primary tauopathies (Höglinger et al., 2018; Lee et al., 2005). Such tauopathies are a spectrum of conditions including Frontotemporal Dementia (FTD), FTD and Parkinsonism

Linked to Chromosome 17 (FTDP-17), Pick's disease (PiD), traumatic brain injury (TBI), and Progressive Supranuclear Palsy (PSP) among others. Neuropathological investigations of these tauopathies revealed distinct patterns in cellular morphologies, involvement of specific tau isoforms and affected cell types (Kovacs, 2015). Interestingly, Goedert and colleagues elegantly discovered the tau fibrillary structure in these different tauopathies possessed unique conformational changes (Fitzpatrick et al., 2017). FTD has over 50 pathogenic mutations in *MAPT* (N279K, ΔK280, P301L, P301S, V337M and R406W) causal for FTD, but currently no *MAPT* mutations cause AD (Goedert and Spillantini, 2000).

Recently, it was discovered that extracellular soluble tau species can propagate and “seed” non-pathological tau to aggregate and become pathological in a prion-like manner (Clavaguera et al., 2015, 2014, 2009) which has been confirmed independently by other groups (Dujardin and Hyman, 2019; Goedert and Spillantini, 2017; Kaufman et al., 2018; Mudher et al., 2017). In AD, it was initially conceived that tau-induced neuronal death consequentially released aggregated tau. However, tau can be secreted to the extracellular space and released between neurons through synapses. While aggregated tau appears to be the most toxic, smaller aggregates such as oligomers (Castillo-Carranza et al., 2017; Fá et al., 2016; Lasagna-Reeves et al., 2012; Shammass et al., 2015), make up approximately 40% of total tau in AD (Iqbal et al., 2016) and also play a toxic role (Sengupta et al., 2017). However, since oligomeric tau is heterogeneous and difficult to define, it is currently challenging to investigate experimentally unlike native tau. Nonetheless, established molecular mechanisms for tau toxicity include an impairment of axonal transport (Kim et al., 2011; Kneynsberg et al., 2017; Majid et al., 2014; Morfini et al., 2009), synaptic dysfunction and loss (Hoover et al., 2010; Ittner et al., 2010), and calcium dysregulation (Datta et al., 2021; Gómez-Ramos et al., 2008, 2006).

1.7 Role of the dynamic microtubule associated protein tau in physiology and AD tauopathy

Originally discovered in 1975, the microtubule associated protein tau (MAPT) is predominantly an axonal protein that helps to assemble and stabilize microtubules of the cell in a phosphorylation-dependant manner (Goedert, 2018; Kadavath et al., 2015; Weingarten et al., 1975; Zempel and Mandelkow, 2019) such that phosphorylation of tau (ptau) discourages attachment to microtubules. Tau and ptau at physiologically normal concentrations (2-3 mol phosphate/mol protein) are insufficient for aggregation as observed in tauopathies (Iqbal et al.,

2016). Tau is a dynamic and flexible protein (Mandelkow et al., 2007) that interacts with several families of proteins including caspases (Gamblin et al., 2003), kinases (Avila et al., 2004), lipid membranes and lipoproteins (Rauch et al., 2020) and RNA-binding proteins and DNA (Brandt et al., 2005; Guo et al., 2017; Violet et al., 2014; Wang and Mandelkow, 2015; Zheng-Fischhöfer et al., 1998). Hence, the physiological role of tau includes the maintenance synaptic connections, transport of cargos and the maintenance of DNA. Encoded by *MAPT* on chromosome 17, tau contains 16 exons within four distinct regions including an acidic N-terminus (exon 2 and 3), a proline-rich region (exon 7 and 9), microtubule repeat binding domains (exons 9-12), and a C-terminus (exon 13). Human tau is alternatively spliced in the N-terminus (0, 1 or 2N) and the microtubule repeat binding domains (3 or 4R), resulting in six isoforms (Goedert et al., 1989). In AD tauopathy, both 3R and 4R tau isoforms are found in NFTs (Espinoza et al., 2008). Tau isoforms likely have unique roles; 0N3R tau is only in the fetal brain, and 4R isoforms are more prone to aggregation.

As a testament to the dynamic nature of tau, tau is immensely post-translationally modified (Chaiyawat et al., 2014; Mair et al., 2016; Marcus and Schachter, 2011; Martin et al., 2011; Schedin-Weiss et al., 2014), containing approximately 85 phosphorylation (80 Ser/Thr, 5 Tyr), 12 glycation, 11 glycosylation, 8 polyamination, 4 nitration and 3 ubiquitination sites (Martin et al., 2011). Of these, 17 proline-directed serine/threonine kinase (Thr-Pro, Ser-Pro) motifs are abnormally phosphorylated in the AD brain, mostly within the proline-rich middle region but over half of these epitopes are actively phosphorylated in normal physiology (Wang and Mandelkow, 2015). It is not fully understood how or why tau becomes abnormally phosphorylated in the AD pathology. Adding to the complexity of tauopathies, ptau is highly regulated by both kinases and phosphatases to allow for its dynamic function (Iqbal et al., 2016; Wang and Mandelkow, 2015). Cyclin-dependant kinase 5 (Cdk5) (Dhavan and Tsai, 2001; Town et al., 2002) and glycogen synthase kinase (GSK-3 β) (Cho and Johnson, 2004; Hooper et al., 2008) are the most established kinases, whereas Protein phosphatase 2A (PP2A) is the major phosphatase (Liu et al., 2005; Ponce-Lopez et al., 2017; Vogelsberg-Ragaglia et al., 2001). The phosphorylation-independent post-translational modifications may influence tauopathy but are not fully understood. In brief, glycation is an enzyme-independent glycosylation observed in aging tissue (Martin et al., 2011) and was found to enhance p-tau aggregation *in vitro* (Necula and Kuret, 2004). Acetylation of lysine residues, Lys-280 in particular, can hinder microtubule-assembly and encourage tau

pathology (Cohen et al., 2011; Min et al., 2010). Other modifications, such as polyubiquitination in PHF tau (Cripps et al., 2006), O- or N-glycosylation (Graham et al., 2014; Takahashi et al., 1999; Wang et al., 1996). Therefore, modulating disease-relevant post-translational modifications of tau may help to minimize or prevent pathology. Importantly, the changes associated with tau and ptau dynamics in the context of AD in the initial stages of AD are not well understood.

1.8 Animal models of AD-like pathology and tauopathies

Animal models provide a pivotal role in understanding mechanistic aspects of health and disease. There are an abundance of animal models for AD, mainly in mice and rats, that been independently characterized to reproduce aspects as reviewed elsewhere (Cavanaugh et al., 2014; Do Carmo and Cuello, 2013; Drummond and Wisniewski, 2017; Ittner and Götz, 2011; McKean et al., 2021; Vitek et al., 2020) and summarized on the public database Alzforum.org. In rare cases, canines, felines (Fiock et al., 2019), rabbits (Jaya Prasanthi et al., 2008), guinea pigs (Sharman et al., 2013) and non-human primates (Latimer et al., 2019) have been used. In order for an animal model to be useful for AD therapeutics, they must recapitulate all the sequential primary and secondary aspects of the human disease including A β and tau pathologies, CNS inflammation, neuronal loss, brain atrophy and synaptic loss. Unfortunately, none of the existing models completely recapitulate human AD because models either do not develop genuine NFTs, neuronal loss, or they exhibit only one of the two hallmark pathologies. These models are primarily based on genetic mutations causative of FAD or FTD and harbor mutations for either APP (Cohen et al., 2013; Javonillo et al., 2022; Leon et al., 2010; Oakley et al., 2006; Pang et al., 2022; Wirths et al., 2006) or tau (Dujardin et al., 2015; Kopeikina et al., 2013; Malcolm et al., 2019; Yoshiyama et al., 2007) or both (Bolmont et al., 2007; Javonillo et al., 2022; Lewis et al., 2001; Oddo et al., 2003; Sterniczuk et al., 2010; Terwel et al., 2008). A limitation is that their respective pathologies, whether possessing A β and/or tau, possess high transgene copy numbers as to produce a overaggressive and possibly artifactual phenotype (Gamache et al., 2019; Saito et al., 2016). Further, such phenotypes saturating in early adulthood ignores the risk factor of aging in AD as mentioned above. These animal models also have varying pathological phenotypes which are likely attributable to different parameters of their transgenesis such as the method of DNA insertion, transgene promoter as to direct cell and region-specific expression profiles, number of transgene insertions, number and locations of mutations, selection of protein isoform, genetic

background of the animal and zygosity. Despite these limitations and sources of variation, such models have enabled for the investigation of mechanistic aspects of amyloid and tau pathology *in vivo*.

Commonly used mouse models for the investigation of amyloid pathology include the 5XFAD (APP_{Swe}, Flor, Lon, PS1_{M146L, L286V}; Thy1 promoter) mouse model (Jawhar et al., 2012), APP23 (hAPP751_{Swe}; Thy1 promoter) (Sturchler-Pierrat et al., 1997) and variations of APP and PS1 mutations in transgenic mouse models including APP/PS1dE9 (APP_{Swe}, PSEN1_{dE9}; murine prion promoter) (Jankowsky et al., 2004; Volianskis et al., 2010), APPPS1 (APP_{Swe}PSEN1_{L166P}, Thy1 promoter) mouse model (Radde et al., 2006), APPxPS1 (APP_{Lon}PSEN1_{A246E}; Thy1 promoter) bigenic mouse (Dewachter et al., 2018) and the McGill-R-Thy1-APP Tg (hAPP_{Swe}, Ind; Thy1 promoter) mouse (Ferretti et al., 2012, 2011). These models demonstrated the important role of A β on inflammation, synaptic plasticity, and cognition.

Mouse models for the investigation of tauopathy stemmed from the introduction of different human tau isoforms of wild type tau (hTau) and tau with FTD mutations (Dujardin et al., 2015; Frank et al., 2008; Götz and Götz, 2019) including the commonly used PS19 (P301S) (Lasagna-Reeves et al., 2016; Yoshiyama et al., 2007), rTg4510 (P301L) (Ramsden et al., 2005), and Tau22 (G272V, P301S) models (Schindowski et al., 2006). A limitation to the currently existing P301S mouse models including the PS19 and hTau.P301S (Allen et al., 2002) is the development of tau pathology in the brainstem, convoluting the evaluation of exploration-dependent evaluation of learning and memory. In addition, there are transgenic mice overexpressing wild type human tau to produce a robust tauopathy phenotype (Andorfer et al., 2003) but this was not the case for human tau knock-in mice (Saito et al., 2014). Indeed, some overexpression of tau and/or the introduction of *MAPT* mutations causing FTD are required to induce tauopathy in rodents and to recapitulate aspects of neurodegeneration such as synaptic loss and brain atrophy.

Some research groups have developed models with both amyloid and tau in mice; a phenotype that would possess both pathological hallmarks. The triple transgenic 3xTg mouse (APP_{Swe}, MAPT_{P301L}, PSEN1_{M146V}; Thy1 promoter) (Oddo et al., 2003) and the ^{Triple}AD mouse (APP_{Swe}; Thy1 promoter, PS2_{N141I}; murine prion promoter; MAPT_{P301L}; Thy1 promoter) (Rhein et al., 2009), each with three separate transgenes, have both amyloid pathologies by six months and tau pathologies by one year of age but, still, no record of neuronal loss. While the mouse models

highlighted above provide important insights and history to our current understanding of AD, the gaps in translation and incomplete human-like disease phenotype has beckoned for improved models of higher translational value.

1.9 Rat models for AD-like pathology

Conceptually, rats provide several disease-relevant advantages for AD and FTD over the use of mice when comparing with humans (Do Carmo and Cuello, 2013). Their larger brain and body size allows for easier dissection of tissue, injection of drugs, stereotaxic surgeries, electrophysiology, and neuroimaging (Iulita et al., 2014; Parent et al., 2017; Qi et al., 2019; Zimmer et al., 2014). In addition, rats show an enriched and dynamic behavioural display such as social play and courtship, and learn tasks more efficiently than mice (Ellenbroek and Yoon, 2016), together allowing for the evaluation and characterization of complex behavioural tasks. Unlike mice, rats and humans possess both 3R and 4R tau isoforms endogenously (Hanes et al., 2009) and a C-terminal region, implicated in tau misfolding, highly homologous to human tau (Hernández et al., 2020). Rats also possess an ApoE protein with similar functional properties akin to the human APOE4 (LaDu et al., 1997; Saroja et al., 2022; Tran et al., 2013) and APOE4 has been shown to accelerate tau-mediated degeneration in transgenic tau mice (Shi et al., 2017) as observed in APOE4 carriers (Baek et al., 2020; Montagne et al., 2020; Therriault et al., 2020). Furthermore, a prion-like spread of tau pathology is another observed feature in rats as has been demonstrated by the endogenous recruitment of rodent tau in Sarkosyl-insoluble fractions of McGill-R962-hTau rats but was undetected in similar P301S transgenic mice (Malcolm et al., 2019). It has also been demonstrated that brain chemokine/cytokine profiles are closer between rats and humans than mice (Du et al., 2017; Zhou et al., 2020). Likewise, the peripheral immune system and other types of immune responses are analogous to humans and such responses can modulate AD progression (Cao and Zheng, 2018), including the expression of major histocompatibility complex (MHC) class II molecules on activated T cells and the presence of CD4⁺/CD8⁺ macrophages (Wildner, 2019). One advantage of using mice, however, is their genetic manipulability and slightly lower cost.

Traditionally, transgenesis was difficult to perform in rats because of a less visible pronucleus, and a more flexible and thus puncture-resistant pronuclear membrane as compared with mice. Hence, AD-related investigations in rats historically started with

intracerebroventricular (ICV) injections of A β peptides or insoluble extracts (Faucher et al., 2016; Frautschy et al., 1992; Malin et al., 2001; Moreno et al., 2009) which were sufficient to induce acute AD-like phenotypes such as cholinergic dysfunction, increased A β deposition and behavioural deficits in learning and memory but did not show a progression of pathology. A limitation of injection models is that they do not reflect the endogenous accumulation and progression of amyloid pathology as it occurs in human AD. The initial generation of transgenic rat models, the APP21(APP695^{Swe, Ind}; Ubiquitin C promoter) rat model, for example, did not produce robust human amyloid pathology (Agca et al., 2008) without the additional introduction of the PSEN1^{L166P} mutation (Agca et al., 2016). Models including the UKUR25 (APP751^{Swe, Ind}; PDGF- β promoter) (Lopez et al., 2004), TgAPP^{Swe}(APP751^{Swe}; PDGF- β promoter) (Ruiz-Opazo et al., 2004) and PSAPPTg478/Tg116/Tg11587 (synapsin I promoter, APP695^{Swe, Lon}; PDGF- β promoter, PSEN1^{M146V}; rat synapsin I promoter) (Flood et al., 2009) transgenic animals highlighted the presence and importance of iA β in early AD-like pathology due to findings of cognitive impairments in the absence of plaque deposition. Intriguingly, the TgF344 (APP695^{Swe}, PSEN1 ^{Δ E9}; murine prion promoter) transgenic rat with amyloid-targeted mutations was one of the first models to produce both progressive amyloid and tau pathologies. Gliosis, plaques and cognitive impairments occurred by six months, followed by neuronal loss and tangles much later by 15 months (Cohen et al., 2013). Arguably, the TgF344 rat was the closest to recapitulating human AD-like pathology. On the other hand, transgenic rat models for human tauopathies are scarce. The first transgenic rat models of tauopathy harbored truncated versions of human tau, including the SHR24 rat (3R tau; Thy1 promoter), which generated neurofibrillary degeneration however no neuronal loss was observed in this model (Filipcik et al., 2012). Others have performed injections of tau fibrils into rats and have found similar properties of tau spreading and propagation previously identified in mice (Smolek et al., 2019).

Overall, there have been significant improvements made in the quality of models for AD-like pathology over the past 20 years. Due to the potential artifactual phenotypes attributed to overexpression-based transgenic animals, recent advances have generated knock-in mice (Huang et al., 2022; Saito et al., 2019, 2014) and rats (Pang et al., 2022). However, humanization of the wild type proteins did not produce a disease phenotype and instead required mutated forms of the protein to generate a modest phenotype, emphasizing *some* increased expression of tau or introduction of *MAPT* mutations is required to produce tauopathy in rodents.

1.10 The McGill-R-Thy1-APP and R955-hTau rat models: potential to recapitulate human AD-like pathology

The McGill-R-Thy1-APP transgenic rat model (APP751^{Swe, Ind}; Thy1 promoter)(Leon et al., 2010), developed by the Cuello lab, produced AD-like amyloid pathology with a single transgene in both homozygous and heterozygous animals and is arguably the most extensively characterized transgenic rat model for AD-like pathology to date. Homozygous McGill-APP rats accumulate iA β commencing one week after birth, develop plaques by six to nine months starting in the subiculum, spreading to the hippocampus and cortex by 12 months and then extend into the thalamus and amygdala by 18 months (Hegglund et al., 2015; Leon et al., 2010). Cognitive deficits in learning and memory occur at three months of age and prior to the onset of detectable extracellular A β deposits and mature plaques. Additionally, LTP impairments, rescuable by antibodies targeted at A β , BACE1 inhibition, and γ -secretase inhibition, were also detected (Qi et al., 2014a). Further, an amyloid-associated inflammatory response derived from A β -burdened neurons was found prior to amyloid plaque deposition (Hanzel et al., 2014). Furthermore an upregulation of several classical inflammatory markers were reported in transgenic rats including COX-2, IL-1 β , TNF- α and CX3CL1 in both cortex and hippocampus in addition to increased number of microglia and astrocytes and altered glial morphology to A β -burdened neurons (Hanzel et al., 2014; Iulita et al., 2014; Leon et al., 2010). Additionally, impaired CREB-regulated transcription coactivator 1 (CRTC1) signalling (Wilson et al., 2016) as well as resting brain connectivity abnormalities, brain hypometabolism, reduction in A β ₁₋₄₂ in CSF and hippocampal atrophy (Parent et al., 2017) were shown, demonstrating further comparable characteristics to human AD. Homozygous McGill-APP transgenic rats show a remarkable breadth of amyloid pathology over the life span despite its somewhat aggressive phenotype and is an excellent model in comparison to other models.

In comparison to homozygous McGill-APP rats, heterozygous animals display a milder phenotype such that, despite accumulation of iA β , behavioural deficits in learning and memory have been reported as early as 3 months and, intriguingly, plaques appearing in an age-dependant manner only in very late life stages after 20 months (Adami et al., 2015; Galeano et al., 2014; Iulita et al., 2014; Leon et al., 2010). Heterozygous rats could provide a phenotype of amyloid pathology that takes age into consideration. While heterozygous rats have not been as extensively

characterized as their homozygous kin, dysfunction in pre-synaptic mitochondrial respiratory capacity of Complex I, decreased ATP synthesis, enhanced H₂O₂ generation in hippocampal neurons of six-month-old rats was reported (Adami et al., 2015).

For the investigation of human tauopathies, the Cuello lab has most recently produced two transgenic rat models named McGill-R962-hTau (Malcolm et al., 2019) and McGill-R955-hTau, a weaker expressing line which had not been thoroughly characterized at the time of these studies. These lines express the longest isoform (2N4R) of human tau with the P301S mutation causing FTD and under the control of the Calcium and Calmodulin kinase II alpha (CaMKIIa) promoter. Unfortunately, the R962 line was lost due to a fatal breeding error. In brief, R962-hTau rats possessed approximately 30 copies of the transgene and recapitulated several features of human tauopathy (Malcolm et al., 2019). For example, transgenic rats produced an age-dependent aggressive accumulation of tau and ptau in AD-affected regions followed by intracellular fibrillary inclusions, immense hippocampal atrophy, ventricular dilation, neuronal loss, myelin loss as well as alterations in astrocytic and microglial morphology in later life stages. Intriguingly, cognitive deficits in learning and memory occurs only at later stages, correlating with neuronal loss (Malcolm et al., 2019). In contrast, the R955-hTau weaker expressing line possessing approximately 4 copies of the transgene (see Chapter III, Supplemental Figure 8), and presented an opportunity to model the tauopathy described in R962-hTau rats in a more progressive manner as reflected in human AD and FTD.

Both the McGill-R-Thy1-APP and McGill-R955-hTau transgenic rats could be excellent models to investigate singular amyloid and tau pathologies, respectively. Of greater interest will be the crossing of these two transgenic lines to generate a model that will allow for the investigation of both the amyloid and tau components of AD-like pathology in the aged rodent and should thus provide a superior recapitulation of the human AD phenotype. Establishing such a model would enable for multiple future investigations of important unanswered research questions such as whether and how amyloid and tau pathologies converge on one another. Such a new transgenic rat model would also allow for the investigation of biomarker abnormalities as well as early-stage pathological changes to a degree of higher translation to humans.

1.11 Amyloid and tau: a tale of two intersecting pathologies

As defined and described above, AD requires the presence of both amyloid and tau pathologies in the CNS. The concept that amyloid beta and tau affect each other to aggravate disease pathology in the AD continuum, as would be in accordance with the Trigger and Bullet hypothesis, remains a mystery (Bloom, 2014; Gulisano et al., 2018; Nisbet et al., 2015). Elucidating the relationship between A β and tau is complicated by the fact that they each have their own established, independent pathologies on the CNS that overlap in several aspects including proteinaceous aggregation, cognitive impairments in learning and memory, activation of kinases and phosphatases, CNS inflammation, synaptic function and neurodegeneration by cytotoxicity (Bloom, 2014; Ittner and Götz, 2011). In addition, both amyloid and tau pathologies affect similar regions of the brain during the initial and advanced stages of AD including the entorhinal cortex and hippocampus, as demonstrated by Braak and colleagues (Braak and Braak, 1995, 1991).

The initial investigations leading to this hypothesis demonstrated that adding A β *in vivo*, whether by the injection of fibrils or by the introduction of an amyloidogenic mutated APP transgene, was sufficient to enhance the deposition of aggregated tau filamentous material in transgenic P301L mice (Götz et al., 2001; Lewis et al., 2001). Since this discovery, there have been several independent investigations demonstrating significant impacts of A β on enhancing the propagation of NFT tau during the advanced stages of AD pathology (Bennett et al., 2017; Bloom, 2014; Hurtado et al., 2010; Stancu et al., 2014). This amyloid-tau relationship suggests A β affected tau unidirectionally because of these initial studies as well as the observed clinical progression of A β abnormalities preceding tau in AD. These findings corroborated with clinical investigations associating the detrimental effects of A β including brain volume loss, cortical atrophy and cognitive decline with a required abnormal elevation of ptau in cognitively impaired individuals (Desikan et al., 2012, 2011; Fortea et al., 2014). Additionally, a significant amount of A β deposition was associated with abnormally increased neocortical tau (Doré et al., 2021), metabolic decline and accelerated progression to AD as compared to abnormalities of one of the markers over a two years (Pascoal et al., 2017b, 2017a). In a APP/PS1xrTgTauEC transgenic mouse model, deposition and spread of tau was enhanced in cortical A β burdened neurons (Pooler et al., 2015). Similarly, enhanced propagation of tau was achieved from an injection of PHF fibrils in 5XFAD transgenic mice, but not in Tau KO nor in APP KO mice (Vergara et al., 2019). The above

suggesting that the presence of amyloid pathology may also be enhancing the spreading of pathological tau.

Most interestingly, the A β /tau relationship has since become rather convoluted but specific, as demonstrated by multiple reports of mitigated degeneration after genetic ablation of tau in transgenic APP mice (DeVos et al., 2018; Rapoport et al., 2002; Roberson et al., 2007; Vossel et al., 2010). In addition, site-directed phosphorylation conferred protection against amyloid-induced excitotoxicity at certain ptau sites, demonstrating that some tau species may confer a protective role in the context of AD (Ittner et al., 2016). Ittner and colleagues demonstrated that preventing the somatodendritic accumulation of tau by genetic ablation or mutation is sufficient to disrupt A β -mediated excitotoxicity. In contrast, knocking out APP in APPxTg30hTau mice instead enhanced cognitive deficits, ptau deposition and NFT load (Vanden Dries et al., 2017), but blocked extracellular oligomeric A β or tau induced LTP impairments (Puzzo et al., 2017), demonstrating that the additional roles of APP-derived fragments may also contribute to the overall status of disease and tau pathology.

The effects of A β on tau pathology during the advanced, post-plaque stages have been reported in several transgenic mouse models (Bennett et al., 2017; Busche et al., 2019; Chabrier et al., 2014; Ghosal et al., 2016; Hurtado et al., 2010; Ittner et al., 2010; Pickett et al., 2019; Rhein et al., 2009; Vanden Dries et al., 2017). In brief, infusion of A β into the brain or crossing APP transgenic mice with P301L tau transgenic mice increased ptau in close proximity to plaques (Bolmont et al., 2007). Additionally, increases in tau misfolding have been observed in 5XFADxTg30-tau mice (Héraud et al., 2014). Intriguingly, Terwel *et al.* (2008) found parallel effects on increased cognitive dysfunction and tau pathology in APP_{Ind}xTauP_{301L} and GSK3 β xTauP_{301L} transgenic mice (Terwel et al., 2008). Rhein et al. (2009) showed altered protein expression of Cytochrome C and Complex I as well as impaired ATP synthesis, decreased mitochondrial membrane potential and increased reactive oxygen species (ROS) in APP_{Swe}xPS2_{N141I}xTauP_{301S} triple transgenic mice (Eckert et al., 2010; Rhein et al., 2009). In a floxed APP_{Swe, Lon} x hTau mouse model, increased ptau and reduced dendritic spines compared to single transgenes were detected (Chabrier et al., 2014) whereas others have shown APP_{Swe}xTauP_{301L} mice develop enhanced impairments in axonal transport as compared to P301L single transgenics (Adalbert et al., 2018). These A β -mediated effects on tau are, at least in part, dependent on A β binding to nicotinic receptors (α 7nAChRs) and activating the extracellular

signal-regulated kinase (ERK) and c-Jun N-terminal Kinase (JNK-1) (Wang et al., 2003). Importantly, A β has a clear effect in disinhibiting the tau kinase, GSK3 β (Hernandez et al., 2012; Hernández et al., 2010; Hooper et al., 2008; Leroy et al., 2007), p35/Cdk5 (Town et al., 2002), as well as altering cell cycle re-entry via Fyn and Calcium and calmodulin dependent kinase II (CaMKII) (Seward et al., 2013), and activation of apoptosis by Caspase-3 cleavage (Gamblin et al., 2003).

The Src kinase Fyn is an emerging binding partner of tau located at the dendritic compartment. Fyn is important for synaptic plasticity and in modulating A β -mediated synaptic dysfunction through an overlapping effect on the N-methyl-D-aspartate receptor (NMDAR) in the postsynaptic density (Briner et al., 2020; Chin et al., 2005; Ittner et al., 2010; Kojima et al., 1997; Liu et al., 2020; Nygaard et al., 2014; Padmanabhan et al., 2019). Mice expressing truncated tau or mice deficient in tau, as to prevent the interaction between tau and Fyn, abolished Fyn-mediated coupling of postsynaptic density (PSD95) and NMDARs as well as abolished A β -related excitotoxicity in APP23 transgenic mice (Ittner et al., 2010).

Few studies have described the possibility of amyloid and tau physically interacting or binding to one another to form soluble complexes in neurons and around dystrophic neuritic plaques (Guo et al., 2006; Manczak and Reddy, 2013; Miller et al., 2011; Raz and Miller, 2013). In parallel, binding of amyloid or tau, secreted at the synapse, to APP has been described (Takahashi et al., 2015) but these aspects have remained elusive for years. Overall, there are many possible ways in which A β and tau may affect each other. Their combined effects on one another have been investigated during the advanced stages of disease. It would be of great interest to investigate such effects in early stages of disease at the synapse, on neurodegeneration, on tau-associated kinases, and/or on inflammatory processes. Such investigations should provide a needed understanding of the initiating disease process.

OBJECTIVES AND RATIONALE

Summarized in the first chapter, neurodegenerative diseases such as AD and FTD are complicated CNS disorders without effective disease-modifying therapies. Past research has been focused on the advanced stages, neglecting the early stages of disease. Therefore, the primary objective of this thesis is to investigate the sequential and consequential effects of human-like tau pathology in a novel transgenic rat model throughout the course of the human-like neuropathology. Building on these investigations, the secondary objective was to elucidate the relationship between amyloid and tau in the context of AD in another novel transgenic rat model.

Aim 1: To characterize the spatiotemporal progression of CNS tauopathy in the homozygous R955-hTau transgenic rat model.

Hypothesis 1.1 Overexpression of mutated human tau will lead to tau hyperphosphorylation, aggregation and development of neurofibrillary lesions.

Hypothesis 1.2 Transgenic rats will develop memory impairments, aspects of neurodegeneration and CNS inflammation consequential to the advanced stages of human-like tauopathy.

Aim 2: To determine and identify the earliest aspects of CNS tauopathy in the heterozygous R955-hTau transgenic rat model.

Hypothesis 2.1 Transgenic rats will develop a sequential and age-dependent accumulation and hyperphosphorylation of tau concomitant with impairments in cognition.

Hypothesis 2.2 Tau species preceding fibrillary inclusions induce neurodegeneration.

Aim 3: To determine how and to which extent the combined effects of amyloid and tau pathologies together as compared to single pathologies, starting at early stages.

Hypothesis 3.1 APPxhTau rats will have accelerated and amplified behavioral deficits in learning and memory starting in early stages and worsening in later stages.

Hypothesis 3.2 APPxhTau rats will have accelerated accumulation of iA β and tau starting in early stages, resulting in a more severe deposition of amyloid plaques and neurofibrillary inclusions in later life stages.

Hypothesis 3.3 APPxhTau rats will have an exacerbated neuroinflammatory response, synaptic dysfunction and neurodegeneration.

Connecting text: Chapter I and II

Taking the pertinent background information highlighted in Chapter I together, it is increasingly clear that investigating CNS tauopathies in the context of FTD and AD are required to elucidate the role of tau and degeneration before pursuing investigations on the effects between amyloid and tau. Chapter II details the investigation of CNS tauopathy in the homozygous R955-hTau transgenic rat model. Herein, an analysis of tau species in the context of FTD tauopathy and AD was conducted using a combination of behavioral, immunohistochemical and biochemical approaches with translational relevance to human disease. Such investigations took place in independent cohorts of rats at the ages of 9, 14 and 20 months and describe novel insights into the advanced stages of CNS tauopathy such as vasculopathy.

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CHAPTER II:
**“Progressive human-like tauopathy with downstream
neurodegeneration and neurovascular compromise in a
transgenic rat model”**

Title: Progressive human-like tauopathy with downstream neurodegeneration and neurovascular compromise in a transgenic rat model

Running title: Progressive brain degeneration in a rat model of tauopathy

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Keywords: human tauopathy, transgenic rat model, neurofibrillary tangles, neurovascular compromise

Abstract

Tauopathies, including frontotemporal dementia (FTD) and Alzheimer's disease (AD), clinically present with progressive cognitive decline and the deposition of neurofibrillary tangles (NFTs) in the brain. Neurovascular compromise is also prevalent in AD and FTD, however, the relationship between tau and the neurovascular unit is less understood relative to other degenerative phenotypes. Current animal models confer the ability to recapitulate aspects of the CNS tauopathies. However, existing models either display overaggressive phenotypes, or do not develop neuronal loss or genuine neurofibrillary lesions. In this report, we communicate the longitudinal characterization of brain tauopathy in a novel transgenic rat model, coded McGill-R955-hTau. The model expresses the longest isoform of human P301S tau. Homozygous R955-hTau rats displayed a robust, progressive accumulation of mutated human tau leading to the detection of tau hyperphosphorylation and cognitive deficits accelerating from 14 months of age. This model features extensive tau hyperphosphorylation with endogenous tau recruitment, authentic neurofibrillary lesions, and tau-associated neuronal loss, ventricular dilation, decreased brain volume, and gliosis. Further, we demonstrate how neurovascular integrity becomes compromised at aged life stages using a combination of electron microscopy, injection of the tracer horseradish peroxidase and immunohistochemical approaches.

1. Introduction

Hyperphosphorylation and aggregation of the microtubule associated protein tau (MAPT) in tauopathies is associated with a myriad of degenerative phenotypes in the CNS including frontotemporal dementia (FTD) and Alzheimer's disease (AD). Positron emission tomography (PET) and post-mortem studies link tau with neurodegeneration, neuroinflammation (Nilson et al., 2017; Serrano-Pozo et al., 2011) neuronal loss (Di et al., 2016; Furcila et al., 2019; Padurariu et al., 2012; West et al., 2004), synaptic loss (Buchanan et al., 2020; Holland et al., 2020), and cortical thinning (Joie et al., 2020; Knopman et al., 2019; Terry et al., 1981). Importantly, tau pathology emerges much closer to cognitive symptoms onset, and it is associated with grey matter loss and cognitive decline more robustly than cerebral amyloid beta deposition (Arriagada et al., 1992; Bejanin et al., 2017; Bennett et al., 2004; Biel et al., 2021; Giannakopoulos et al., 2003; Guillozet et al., 2003; Hanseeuw et al., 2019; Schöll et al., 2016). Additionally, evidence points to a contribution of tau to neurovascular dysfunction independent from amyloid-associated vasculopathy and inflammation (Arvanitakis et al., 2016; Carrano et al., 2012; Kalaria and Deramecourt, 2012; Wisniewski et al., 1997; Wisniewski and Kozlowski, 1982). Such tau-associated contribution(s) to vasculopathy has not yet been thoroughly investigated. Altogether, this evidence further supports the tau hypothesis which proposes tau as the major toxic factor driving disease progression, cognitive decline, neurodegeneration and ultimately dementia.

Investigating tauopathy, including the factors participating in tau-driven pathways and the chain of events leading to adverse outcomes, calls for models faithfully recapitulating aspects of human tauopathy. Given that knock-in mouse models possessing wild-type human tau do not produce a disease phenotype (Saito et al., 2019), most models are based on the overexpression of mutated human tau. Hence, the insertion of tau mutations or increased expression of tau is necessary to produce tauopathy in rodents. There are several mouse models of tauopathy with a variety of gene promoters and isoform(s) of tau (Dujardin et al., 2015; Frank et al., 2008; Götz and Götz, 2019). Examples of mouse models include the commonly used PS19 (P301S)(Yoshiyama et al., 2007), rTg4510 (P301L)(Ramsden et al., 2005), and Tau22 (G272V, P301S)(Schindowski et al., 2006) models. While such models have been useful in understanding tauopathies, several models fall short of producing genuine neurofibrillary lesions, neuronal loss or brain degeneration - limitations which could be overcome by the transgenic addition of the human APOE4 gene (Shi et al., 2017). Further, other models do not show a recruitment of endogenous murine tau after

introducing the human protein as to demonstrate the prion-like nature of human tau (Strang et al., 2018; Xia et al., 2022). Additionally, the ectopic and pathological expression of transgenic tau in the brain stem resulted motor deficits limiting the interpretation of behavioural tests requiring animal movement and exploration. Other mouse models exhibit high transgene expression resulting in a saturated phenotype equivalent to Braak stage V-VI with ~40% loss of brain weight and over 65% CA1 neuronal loss by their early adulthood (Kopeikina et al., 2013; Ramalho et al., 2008; Spires et al., 2006). Such models bypass the aging process which is the most significant risk factor in AD and FTD (Guerreiro and Bras, 2015; Nilsson et al., 2014). The contribution of aging in accelerating tau pathology is further exemplified in senescence-accelerated phospho-tau (SAPt) mice (Bodea et al., 2017).

Rat models provide an advantageous degree of translation to human tauopathy compared to mice. Their larger brain, advanced behavioral aptitude and CNS make them more suitable to study cognitive, biochemical, electrophysiological and imaging-related features of tauopathies (Do Carmo and Cuello, 2013; Iulita et al., 2014; Parent et al., 2017; Qi et al., 2019; Zimmer et al., 2014). Of relevance to tau pathology, rats, like humans, display endogenous expression of all six tau isoforms (Hanes et al., 2009), and the C-terminal half of their tau protein, implicated in tau misfolding, is highly homologous to human tau (Hernández et al., 2020). Additionally, rat ApoE possesses functional properties resembling human APOE4 which has shown to exacerbate tau accumulation and hyperphosphorylation and to accelerate tau-mediated neurodegeneration (LaDu et al., 1997; Saroja et al., 2022; Shi et al., 2017; Tran et al., 2013), findings corroborated by human tau PET studies in APOE4 carriers (Baek et al., 2020; Montagne et al., 2020; Therriault et al., 2020). Furthermore, a prion-like spread of tau pathology, which plays a central role in tau-mediated neurodegeneration, was demonstrated through the endogenous recruitment of rodent tau in Sarkosyl-insoluble fractions of McGill-R962-hTau (P301S) rats but was undetected in P301S mice (Malcolm et al., 2019). It has also been demonstrated that brain chemokine/cytokine profiles are closer between rats and humans than mice (Du et al., 2017; Zhou et al., 2020). Likewise, the peripheral immune system and other types of immune responses in rats, which can modulate AD progression (Cao and Zheng, 2018) including the expression of major histocompatibility complex (MHC) class II molecules on activated T cells and the presence of CD4⁺/CD8⁺ macrophages (Wildner, 2019) are analogous to humans.

Our lab has previously developed a rat model of human-like tauopathy (Malcolm et al., 2019) which was regrettably lost. In order to recreate a transgenic rat model closer to human tauopathy we have generated a new transgenic rat line named McGill-R955-hTau. We investigated the capacity of homozygous R955-hTau rats to recapitulate a robust progression of human tauopathy including tau accumulation, hyperphosphorylation and aggregation into neurofibrillary tangles while preserving the aging facet of the disease. Because of such pathology, we investigated secondary aspects such as synaptic loss, brain atrophy, neuronal loss, and cognitive deficits in aged rodents at fibrillary stages. At such a neurofibrillary stage we also explored the role of tau as a contributor to neurovascular impairment and compromise of the blood-brain-barrier (BBB) *in vivo*.

2. Materials and methods

2.1 Animals

Generation of the McGill-R955-hTau transgenic rat was achieved as previously described (Malcolm et al., 2019). McGill-R955-hTau transgenic rats overexpress the coding region of the 2N4R isoform of human tau bearing the P301S missense mutation causative of FTDP-17 (Bugiani et al., 1999). The Calcium-calmodulin kinase II alpha subunit (CaMKII- α) promoter directs expression to excitatory neurons in the forebrain, avoiding overexpression of tau in motor regions. Rats were housed in groups of 3-4 in a GR1800 double-decker cage system (Techniplast, Int.) under a 12:12 light:dark cycle and were provided standard chow and water *ad libitum*. The use of animals for this study was approved by the McGill Animal Care Committee under the guidelines of the Canadian Council on Animal Care (CCAC).

2.2 Experimental design

Male and female homozygous rats were bred alongside wild-type littermates and raised in three independent sets of cohorts to one of three endpoints at 9, 14 and 20 months of age. A sample size of at least 10 rats per group per time point was selected for characterization (**Supplemental Table 1**). At each endpoint, rats underwent behavioral testing for cognition followed by brain perfusion and tissue collection for the subsequent examination of tau-related pathologies in the brain. A subset of additional rats (n=3-4) was sent for brain magnetic resonance imaging (MRI) imaging. Other subsets of rats were used for electron microscopy (n=2) and blood-brain-barrier studies (n=4-5).

1451

1452 *2.3 Animal behavior*

1453 Animals were habituated to handling at least one week prior to behavioral testing and all
 1454 tests commenced at the beginning of the light phase of the circadian cycle. Assessment of cognition
 1455 was performed using a battery of behavioral tests including novel object location (NOL), novel
 1456 object recognition (NOR), Morris water maze (MWM) and fear conditioning (FC) tasks using
 1457 protocols previously described (Hall et al., 2018; Malcolm et al., 2019) with minor modification
 1458 as well as the social interaction (SI) test for social withdrawal. Animal tracking data was recorded
 1459 with the assistance of Ethovision XT (Noldus, The Netherlands) and Freezing software for
 1460 automated fear conditioning. (v1.3.01, Panlab, Spain). Behavior scores were converted into a
 1461 fraction out of 1 and then averaged as a global cognitive index (CI) composite score. Experimenters
 1462 were blinded to experimental groups.

1463 *2.3.1 Novel object location and recognition*

1464 Twenty-four hours after the open field test, rats were placed in the same 80x80 cm arena
 1465 containing visual cues. In the familiarization phase, five objects of equal bias were placed in the
 1466 arena and secured to the floor in distinct locations. Rats were placed in the arena and given two
 1467 minutes to explore and then were placed back into their home cages. For NOL, one object was
 1468 moved to another location and the rats were given two minutes to explore. For NOR, a familiar
 1469 object was replaced with a novel object and the rats were given two minutes to explore. An inter-
 1470 trial interval of 15 minutes separated each phase of the test. Time spent exploring each object,
 1471 judged by the rats' sniffing and whisking, close proximity to an object or touching, was recorded.
 1472 The percentage of time spent with the novel object and discrimination ratios were calculated as
 1473 described previously (Antunes and Biala, 2012).

1474 *2.3.2 Morris water maze*

1475 A 170 cm diameter pool was filled with water mixed with opaque, non-toxic white paint
 1476 and was maintained at 22°C. During pre-training habituation rats were given 30 s to swim freely
 1477 in the water. Five days of training ensued. For these days, three extra-maze cues were placed on
 1478 the walls of the room and a transparent platform was submerged 2 cm below the surface in a fixed
 1479 location. Each training day included four training sessions where the rats were given 2 min to find
 1480 the platform separated by an intertrial interval of 20 min. The release position (north, south, east,
 1481 west) for each training session was randomized such that every direction was tested each day with

the sequence being unique between days. If the rat failed to find the platform, it was gently guided to the platform and left for 20 s before being removed from the pool. Twenty-four hours after the last day of training, a retention trial (probe test) was performed where the platform was removed, and the rats were given one minute to explore the pool. Latency to reach the platform, swim speed and swim distance as well as the number of platform area crossings during the probe test were recorded using an overhead camera connected to Ethovision XT software. After the probe trial, a visual platform test was performed to confirm motivation to exit the pool and absence of vision impairment.

2.3.3 Contextual and auditory cued fear conditioning

This task was split into four days. On the first day: Habituation, rats were pre-exposed to the fear conditioning chamber system (Panlab, Spain) and environmental context for 5 min and baseline activity was measured using the Freezing software (v1.3.01, Panlab, Spain). On day 2: Conditioning, rats were placed in the chamber and left to explore for 120 s (baseline), before being subjected to an auditory stimulus tone (75 dB, 5 kHz, 28s) coupled to a foot shock (0.75 mA, 2 s). Rats recovered for 90 sec (post-shock phase) and were then returned to their home cages. On day 3: Contextual fear-conditioning, contextual memory was assessed by placing rats in the same context as day 1 and 2, but without the tone or shock, and their freezing behavior was recorded over 5 min. During the habituation, conditioning, and contextual fear-conditioning test (days 1 to 3), the chamber was scented with coconut extract and cleaned with 70% ethanol between rats (Context A). On day 4: Cued recall, cued memory was assessed by placing the rat in the chamber with an entirely different scent (peppermint extract and cleaned with 1% acetic acid between rats) and environment, allowing it to explore, and then playing the auditory stimulus three times (30 s each), separated by 15 s pauses. Fear was measured by the time spent freezing during all phases of the test. Finally, 48 h after auditory conditioning, rats explored the new, modified chamber (Context B) for 120 sec (baseline). This was followed by three consecutive tone presentations (30 sec, 75dB, 5 kHz), each separated by a 30 sec pause.

2.3.4 Social Interaction

The Social Interaction (SI) test done as described previously (Yang et al., 2011) with minor modification. In brief, animals were habituated to an 80 x 80 cm arena divided equally into three parts: chamber 1, center and chamber 2 with a single passage between each. Each chamber contained a rectangular prism-shaped barred 'jail' anchored by a brick. Rats were first given two

minutes to habituate to the arena. Following a 15-minute inter-trial interval, a social withdrawal paradigm was employed such that an age- and sex-matched wild-type rat was secured inside one of the two jails with a rat-shaped object containing soiled bedding from the jail rat(s) secured to the other. Rats were given 5 minutes to interact, with the total number of interactions and total interaction time, scored during whisking, sniffing, nose pokes and paws on the cage, was recorded.

2.4 Tissue collection

Rats were deeply anesthetized with 1% pentobarbital (Equithesin). After brain perfusion with ice-cold saline, one brain hemisphere was fixed in 4% paraformaldehyde (PFA) for 24 h followed by saturation in 30% sucrose for at least one week. The other hemisphere was further dissected to isolate the hippocampus and cerebral cortex, and then tissues were snap-frozen on dry ice and stored at -80°C.

2.5 Immunohistochemistry

Immunohistochemistry (IHC) procedures were carried out as previously described (Malcolm et al., 2019). In brief, after heat-mediated antigen retrieval (80°C) in sodium citrate buffer (10 mM, pH 6) free-floating brain tissue sections (40 µm) were incubated in 3% hydrogen peroxide and 10% methanol to quench endogenous peroxides, washed, then blocked in 10% normal goat serum (NGS) in Tris-buffered saline (TBS, 50 mM Tris, 150 mM NaCl) containing 0.1% Triton X-100 (TBS-T) for 1 hr at room temperature. After blocking, sections were incubated with primary antibodies (listed in **Supplemental Table 2**) with 5% NGS in TBS-T gently shaking overnight at 4°C. The next day, following washes the sections were incubated with species-specific secondary antibodies with 5% NGS 1 hr at room temperature. Sections were then incubated for 1 hr at room temperature in mouse anti-horseradish peroxidase (1:200) and horseradish peroxidase (1:30) (MAP/HRP, Medimabs, Canada) or Vectastain Elite ABC-HRP (Vector labs, USA). After developing the reaction with 3',3'-diaminobenzidine (DAB, Sigma, USA), mounted sections were dried overnight at room temperature. On the following day, sections were dehydrated in increasing concentrations of ethanol, ending with xylenes and coverslipped using Entellan mounting media (EM Science, USA).

2.6 Immunofluorescence

Immunofluorescence (IF) procedures were carried out as previously described (Malcolm et al., 2019). After heat-mediated antigen retrieval in either citrate buffer (10mM, pH 6) or Tris-EDTA (10mM Tris, 1mM EDTA, pH 9), free-floating tissue sections were quenched in 50% ethanol for 20 min at room temperature, followed by blocking in 10% NGS in phosphate-buffered saline with 0.2% Triton for 1 hr at room temperature and then incubated with primary antibodies with 5% NGS overnight at 4°C. The next day, sections were incubated with species-specific fluorescent-conjugated secondary antibodies (1:400) with 5% NGS in phosphate-buffered saline with 0.2% Triton for 2 hours at room temperature in the dark. Autofluorescence was quenched with 0.3% Sudan Black for 5 min at room temperature. Following washes in PBS, sections were incubated in 4',6-diamidino-2-phenylindole (DAPI, 0.2 mg/mL) before being coverslipped using Aqua polymount (Polysciences, USA). For all staining of tau species, TBS was used instead of PBS.

2.7 Brightfield and Fluorescence Microscopy

Brightfield microscopy was performed using an Axio Imager M2 with an AxioCam506 color digital camera and ZEN v2.3 Blue imaging software (Zeiss, Germany) on regions of interest (ROI). Z-stacks were acquired and merged using the extended depth of focus function. Microscopy of immunofluorescent tissue was performed using an LSM800 confocal microscope AxioObserver and ZEN x2.3 Black imaging software (Zeiss, Germany). Three 1 µm Z-stacks were acquired and merged into maximum intensity projections. Analysis of total fluorescence intensity, cell counts, percent area and colocalization with consistent mask thresholds per experiment was accomplished using ImageJ (NIH, USA) using at least two slices and one to two images per region of interest per slice. Fluorescence intensity is expressed in arbitrary units (AU).

2.8 Modified Bielschowsky Silver stain

Silver staining was accomplished using a modified commercial kit (American Mastertech, USA) following manufacturer's instructions. Briefly, 10 µm slices were mounted onto gelatin coated slides and dried overnight. The next day, slices underwent rehydration followed by incubation in silver nitrate solution for 15 min at 40°C using a water bath. Then, after rinsing in distilled water, slices were incubated in ammoniacal silver solution for 10 min at 40°C before immersion in developer solution for 35 s. The reaction was stopped using ammonia water and

slices were washed with distilled water and 5% sodium thiosulfate prior to dehydrations and coverslipping using Entellan mounting media (EM Science, USA).

2.9 Electron microscopy

The hippocampus and cerebral cortex of 9- and 20-month-old R955-hTau rats (n=2) were processed for electron microscopy using the protocol previously described with minor modification (Malcolm et al., 2019; Ribeiro-da-Silva et al., 1993). Rats were anesthetized with 1% sodium pentobarbital and then perfused with cold saline for 45 s followed by perfusion with 0.1 % glutaraldehyde for 1 min, slowed to dropwise for 30 min then perfused with 4% PFA dropwise for 30 min. The brain was then dissected and fixed in 4% PFA for 24 hours. 50 μ m brain sections were cut using a Vibratome 1000 Plus. Sections were cryopreserved in 30% sucrose and 20% glycerol, then subjected to three permeabilization washes using isopentane cooled with liquid nitrogen and left in 0.1M phosphate buffer until the next day. Sections were incubated in 1% glutaraldehyde for 10 min and then underwent silver intensification (Nanoprobes, HQ Silver, USA) for 8 min in a dark room. Sections were washed with water before treatment with osmium tetroxide for 1 hr at 4°C. Sections then underwent stepwise dehydrations using ethanol, then propylene oxide and finally infiltrated with increasing concentrations of Epon in propylene oxide. Sections were flat embedded on acetate foil and affixed to Epon blocks via incubation for 24 hr at 60°C. Blocks were ultrasectioned into 70 nm slices using an ultramicrotome (Ultracut III, Reichert-Jung, Leica, Germany). Ultrasections were mounted onto 200-mesh copper grids with carbon film and contrast stained with uranyl acetate and lead citrate. Images were acquired using a FEI Tecnai G2 Spirit Twin 120 kV Cryo-TEM equipped with a Gatan Ultrascan 4000 4k x 4k CCD Camera System Model 895.

2.10 Extraction of Sarkosyl-insoluble tau species

Sarkosyl-soluble and -insoluble tau was extracted following the protocol previously described for human AD brain tissue as previously described (Allen et al., 2002; Hasegawa et al., 1992; Malcolm et al., 2019). In brief, cortical brain tissue was homogenized in ‘A68 Buffer’ (10 mM Tris pH 7.4, 0.8 M NaCl, 1 mM EGTA, 5 mM EDTA, 10% sucrose, 1 mM PMSF) containing protease and phosphatase inhibitors (Complete mini; PhosSTOP, Roche, Switzerland). Homogenates were centrifuged at 21, 000 x g for 20 min at 4°C. Pellets were resuspended in A68

buffer. Supernatants were then incubated in 1% Sarkosyl (Sigma, Germany) for 1 hr at room temperature and then ultracentrifuged at 100,000 x g for 1 hr at 4°C. The supernatant containing the Sarkosyl-soluble fraction was immediately removed and stored at -20°C, while the pellet was resuspended in 30 uL of 50 mM Tris-HCl, pH 7.4, labelled as the ‘Sarkosyl-insoluble fraction’ and also stored at -20°C. Detection of tau aggregates and neurofibrillary tangles was achieved by blotting Sarkosyl-insoluble extracts onto EM grids as previously described (Malcolm et al., 2019) as well as by Western blotting (see below).

2.11 Quantification of tau by electrochemiluminescence (ECLIA)

Twenty-five microlitres of cortical brain homogenate from Sarkosyl-soluble and insoluble fractions of wild-type and transgenic rats were added to the Phospho(Thr231)/Total Tau kit (#K15121D, Mesoscale Discovery, USA) and the assay was run according to the manufacturer’s instructions. Protein concentration of samples were determined and normalized using Lowry’s method.

2.12 Western blot

Twenty to forty milligrams of rat brain tissue were homogenized using Cell Lysis Buffer (Cell Signaling Technologies, USA) mixed with protease and phosphatase inhibitors (Complete mini and PhosSTOP, Roche, Switzerland). Cortical and hippocampal homogenates were then centrifuged at 13,000 rpm for 45 min and supernatants were stored at -20°C until blotting. Supernatant protein content was quantified using Lowry’s method and then normalized to equal concentrations. Supernatants and Sarkosyl-insoluble tau fractions were loaded and ran on polyacrylamide gels, then transferred onto polyvinylidene difluoride membranes activated by methanol. Blots were then blocked in 5% skim milk or bovine serum albumin in Tris-buffered saline with 0.1% Tween 20 (TBS-Tween) for one hour before primary antibody diluted in 5% skim milk or 5% BSA in TBS-Tween were added and incubated overnight at 4°C. Blots were washed with TBS-Tween followed by incubation with a species-specific secondary antibody conjugated to horseradish peroxidase (1:5000) in 5% skim milk in TBS-Tween for 1 hour at room temperature. Blots were developed using the Western Lightning Plus chemiluminescence substrate (PerkinElmer, USA) and imaged using an Amersham 600 Imager (GE Healthcare, Canada). Optical densities of protein bands of interest were obtained using ImageLab 6.0 (Bio-Rad, USA),

normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as fold-change of wild type littermates.

2.13 Functionality of the blood-brain-barrier: Horseradish peroxidase and Evan's Blue dye

Injection of tracer was done as previously described (Reese and Karnovsky, 1967) with modification. A subset of rats (n=4) was deeply anesthetized in 1% pentobarbital (Equithesin). A single bolus of 20 mg/kg body weight of horseradish peroxidase (HRP IV, Sigma) mixed with 1% Evan's Blue (EB, Sigma) dissolved in 0.9% sterile saline was injected into the lateral tail vein followed by a 1 mL saline flush. After 45 minutes of circulation, rats were decapitated, and brains were extracted without perfusion and processed as described above. For detection of HRP content, coronal brain sections (40 μ m) were washed with phosphate buffered saline followed by incubation with 3',3'-diaminobenzidine (DAB) as described below. For detection of EB leakage, sections were placed in phosphate-buffered saline before mounting onto gelatin-coated slides and cover slipped with Entellan mounting media. Both HRP and EB were used as proxies for BBB leakage as HRP and EB both are readily detectable in BBB-compromised animals but with EB reversibly binding to albumin.

2.14 Prussian Blue staining for the detection cerebral microbleeds

The Prussian Blue staining depends on the sensitive reaction of acids with ferric ions from ferrocyanide resulting in blue pigmentation. Briefly, 40 μ m sections were mounted onto gelatin coated slides and mounted overnight at room temperature. Immediately before use, a ferric solution of 20% hydrochloric acid (10N) and 10% potassium ferrocyanide in distilled water was mixed. Sections were rehydrated in distilled water before incubation in ferric solution for 20 minutes at room temperature. Sections were then rinsed with distilled water three times for 5 minutes, then underwent gradual dehydrations in ethanol for 30 seconds each ending with xylenes for 5 minutes. Sections were coverslipped with Entellan mounting media (EM Science, USA). No counterstain was used.

2.15 Magnetic resonance imaging

Magnetic resonance imaging (MRI) procedures were performed as previously described (Malcolm et al., 2019) with minor modification. In brief, rats at 9 and 20 months (n=3-4) were

anesthetized by isoflurane before being placed in a Bruker Biospec 70/30 MRI scanner equipped with a rat volume surface coil. Total brain volume and ventricular volume was estimated using Dragonfly (Montreal, Canada) software.

2.16 Statistical analysis

Statistical analysis was performed with the assistance of GraphPad Prism 8 software (San Diego, USA) using a global alpha of 0.05. Data are presented as mean +/- standard error of the mean (SEM). Each time point and each brain region was analyzed independently. Two-tailed unpaired t-tests were performed on single factor, single comparisons. One-way and two-way ANOVA, as appropriate, were followed up with Bonferroni's adjustment for three or less comparisons, and Tukey's for more than three comparisons. One-way and two-way repeated measures ANOVA (RM-ANOVA) using a mixed design were followed up with Holm-Sidak's pairwise comparisons. If a significant interaction was found, only simple main effects of genotype were examined. Statistical outliers were identified using Grubbs test and removed from datasets and analysis.

3. Results

3.1 Progressive and robust accumulation and hyperphosphorylation of tau accelerates in aged R955-hTau^{+/+} rats.

We first examined tau pathology in R955-hTau^{+/+} rats at the time points of 9, 14 and 20 months by probing total human tau immunoreactivity (IR) (HT7) (**Figure 1**) and phosphorylated tau for disease-relevant epitopes: AT8 (pS202-T205) for hyperphosphorylated tau as used in Braak staging, MC1 for conformational-specific misfolded tau (Jicha et al., 1997) and late-stage hyperphosphorylated tau via PHF1 (pS396-S404) (**Figure 2-3**). Notably, expression of the transgene was limited to the forebrain, with no detectable expression of human tau in regions affecting motor function such as the cerebellum and the spinal cord (**Figure 1B, red arrow**). At 9 months, tau HT7-IR was initially restricted to the soma of pyramidal neurons throughout the hippocampal CA1-3 (**Figure 1H**), subiculum and dentate gyrus as well as the layers II-III and V of the cerebral cortex (**Figure 1L**) especially in the entorhinal, parietal, and somatosensory regions. The initial up-regulation of phosphorylated tau (ptau) was limited to intraneuronal AT8 IR in the subiculum region of the hippocampus (**Figure 2F**).

1698 At 14 months, a noticeable overall increase in HT7-IR (**Figure 1E, I, M**) was met with
1699 ptau via AT8 (**Figure 2C, G, K**) and MC1-IR that became abundant in the cerebral cortex (**Figure**



Figure 1. Progressive intracellular accumulation of the human tau protein in McGill-R955-hTau^{+/+} transgenic rats as revealed by the antibody HT7. (A-B) Representative micrographs of sagittal sections of HT7 immunoreactivity at 9 and 20 months, respectively. Note the ventricular dilation (**B**, **black arrow**), and lack of human tau expression in the cerebellum (**B**, **red arrow**) and brainstem. (**C-F**) Representative low magnification micrographs of coronal sections of the somatosensory cortex and dorsal hippocampus (**C**) wild-type and (**D-F**) R955-hTau rats at 9, 14 and 20 months of age. (**G-N**) Representative high magnification micrographs of the CA1 region of the hippocampus (**G-J**) and layers II-V of the somatosensory cortex (**K-N**). Note the progressive HT7 immunoreactivity in neuronal cell bodies and processes in the cerebral cortex and hippocampus, with aggregated material appearing in the cerebral cortex after 14 months. **A-B** scale bar = 5000 μm; **C-F** scale bar = 1000 μm; **G-N** scale bar = 100 μm.

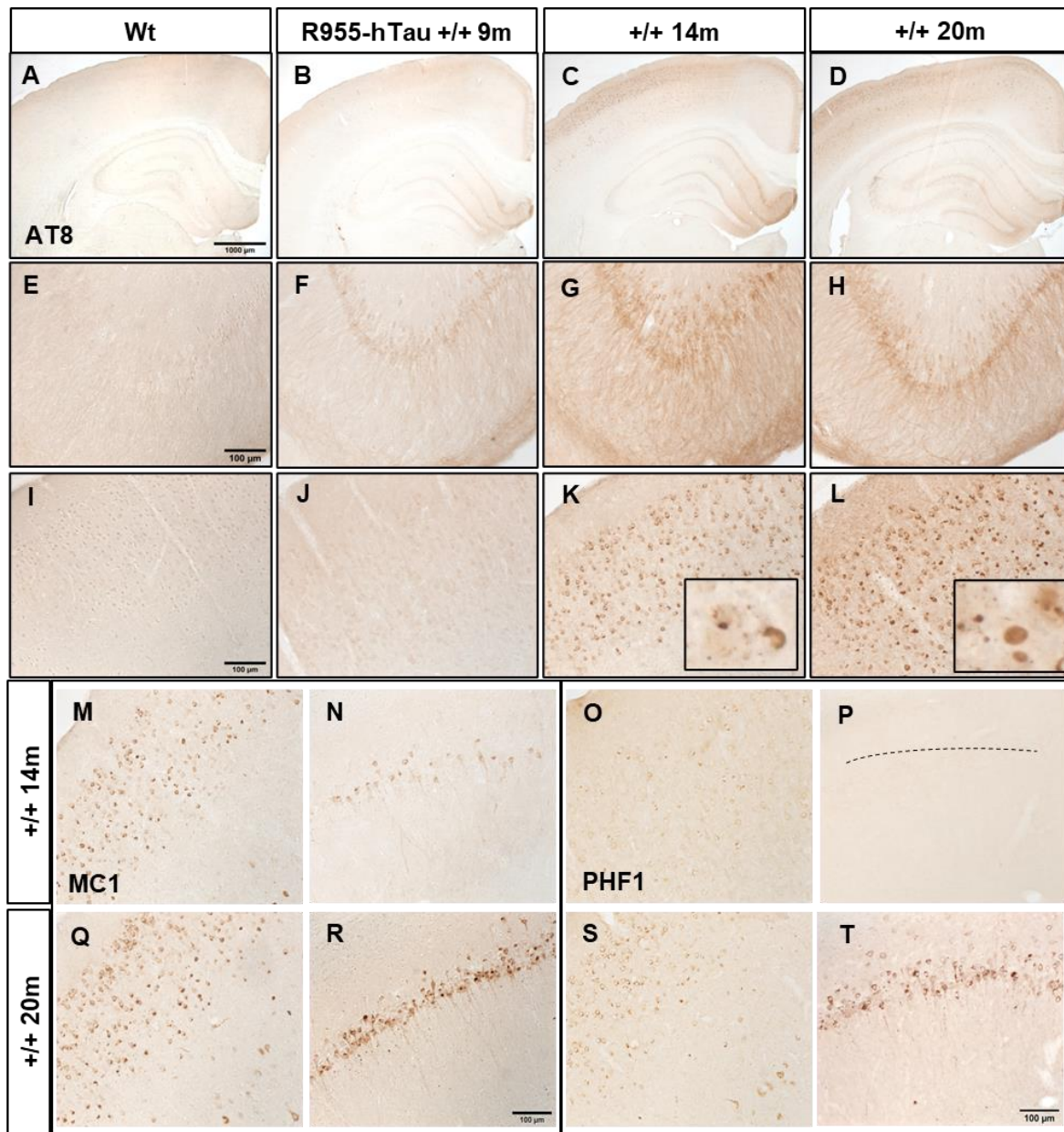


Figure 2. Progressive hyperphosphorylation and misfolding of tau proteins in McGill-R955-hTau^{+/+} transgenic rats accelerate after 14 months. (A-D) Representative low magnification micrographs of coronal sections immunostained with the AT8 monoclonal antibody for hyperphosphorylation of tau proteins at sites pS202 and pT205, in neurons of the cerebral cortex, hippocampus and subiculum. (E-L) Representative high magnification micrographs of the subiculum region of the hippocampus (E-H) and layers I-III of the somatosensory cortex (I-L). Note that AT8 immunoreactivity in the subiculum peaks after 14 months while the cortical tau burden continues to aggregate. The lower panels illustrate MC1 immunoreactivity signaling misfolded tau proteins at a pre-tangle stage in the cerebral cortex (M, Q) and the CA1 region of the hippocampus (N, R). PHF1 (pS396-pS404) tau immunoreactivity suggesting paired-helical tau formation is revealed in the cerebral cortex (O-S) and CA1 region of the hippocampus (P-T) regions, respectively. A-D scale bar = 1000 μ m; E-T scale bar = 100 μ m.

2M) and detectable in the CA1 (**Figure 2N**). These regions presented with tau debris, bodies, and axonal spheroids (**Figure 3A-C**) whereas minimal PHF1-IR (**Figure 2O-P**) was detected suggesting an accelerated tau pathology and toxicity in the absence of fibrillary tau. Interestingly, in the subiculum, ptau (AT8-IR) culminated at 14 months without further developing misfolded (MC1-IR) or aggregated (PHF1-IR) tau. Advanced tau pathology was observed at 20 months as shown by stronger ptau IR via AT8 in the cerebral cortex (**Figure 2D, H, L**) compared to earlier time-points. Additionally, pronounced IR for both MC1 and PHF1 was detected in the hippocampus and cerebral cortex (**Figure 2Q-T**), more so than at 14 months (**Figure 2M-P**). The R955-hTau^{+/+} brain overall showed signs of degeneration, indicated by dilated ventricles and a shrunken appearance (**Figure 1B, Figure 2D**). Interestingly, overall total human tau IR plateaued as early as 9 months (**Figure 1E-F, I-J, M-N**).

3.2 Progressive tau accumulation and phosphorylation leads to late-stage aggregation and neurofibrillary tau.

With the incremental tau accumulation, phosphorylation, and conformational changes, R955-hTau^{+/+} rats developed morphologies resembling tau aggregates and tau fibrils which was detectable from 14 months of age and to a more severe extent at 20 months (**Figure 3**). Using a combination of AT8, MC1, and PHF1 antibodies, we observed morphologies including neuron balloons (**Figure 3A**) and clumps of tau immunoreactive material resembling aggregates and fibrillary tau first appearing in pyramidal neurons of cortical regions at 14 months (**Figure 3A-C**) then by 20 months progressing into the hippocampus such as the CA1 (**Figure 3G-H**). At 20 months, neurons bore severe ptau-IR and possessed a tortuous dendritic morphology in the CA1 (**Figure 3D**) and CA2 (**Figure 3E**) regions of the hippocampus. Silver staining confirmed the presence of aggregated tau in pyramidal neurons of these regions (**Figure 3I**). Neurofibrillary ultrastructure was also confirmed by electron microscopy of Sarkosyl-insoluble extracts labeled with nanogold-conjugated antibodies bound to ptau (**Figure 3J-L**), demonstrating the R955-hTau lineage is capable of developing twisted ribbon-like tau filaments in aged, homozygous transgenic rats.

The quantitative examination of Sarkosyl-insoluble extracts by ECLIA revealed that R955-hTau^{+/+} rats had approximately three-fold more total tau compared to wild-types as early as 9

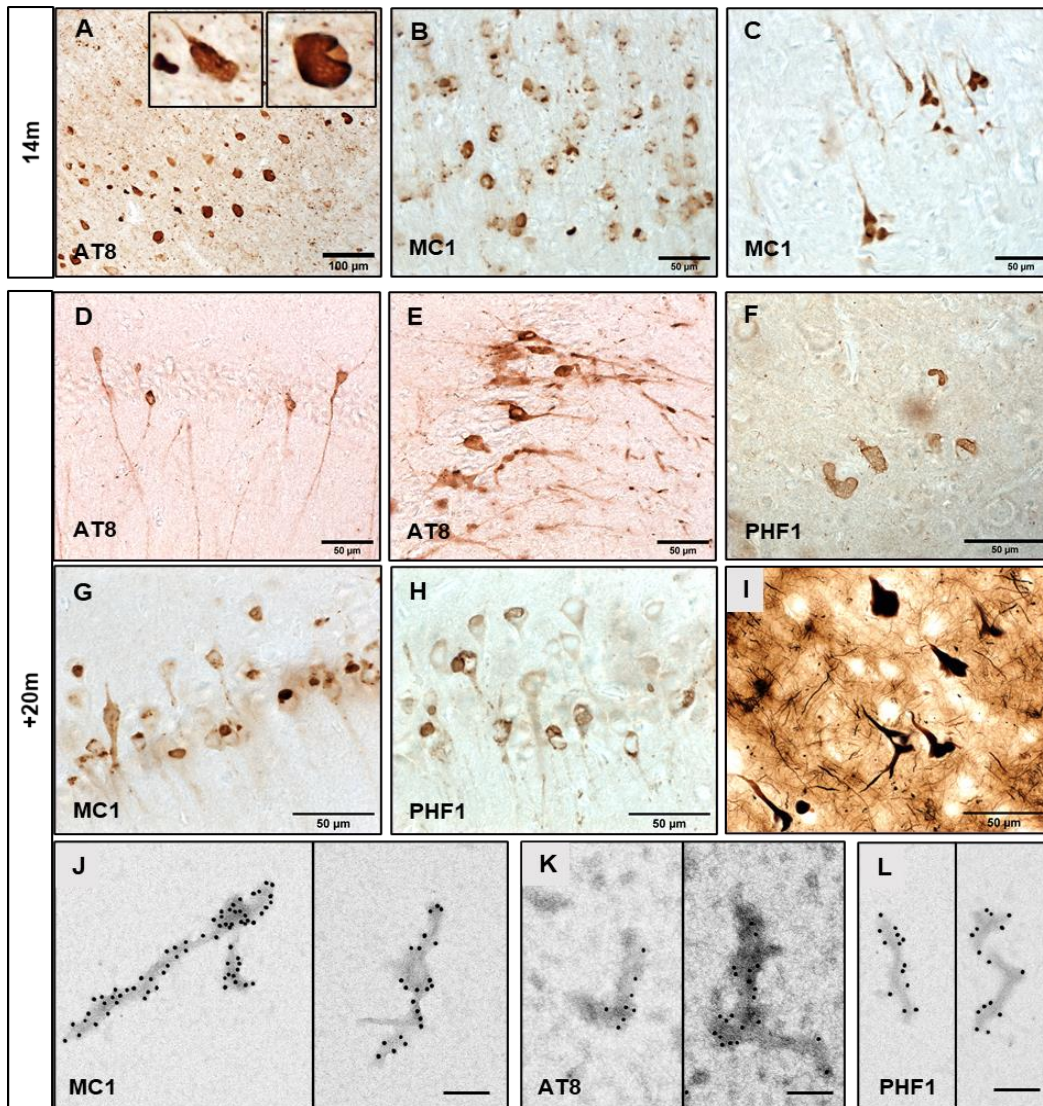


Figure 3. Homozygous R955-hTau rats develop well-established intraneuronal hyperphosphorylated tau aggregates and neurofibrillary pathology starting at 14 months of age. Representative high magnification micrographs depicting AT8-immunoreactive neurons in the somatosensory cortex, many with tangle-like and neuron balloon morphologies (A). Note MC1-immunoreactive pre-tangle tau material in the cerebral cortex (B) and in the CA1 region of the hippocampus (C) in 14-month-old R955-hTau rats. Representative high magnification micrographs illustrating varied pathological tau species in 20-month-old R955-hTau rats: CA1 neurons (D) and CA2 neurons (E) heavily burdened with AT8 immunoreactivity, as well as the ghost neurons in layer V of the cerebral cortex (F). At this stage, numerous neurons display misfolded tau (MC1) (G) as well as paired helical filament-IR material (PHF1) (H) in the CA1 region. Representative Bielschowsky silver stain revealing the presence of aggregated neurofibrillary material in layer V of the somatosensory cortex (I). Representative electron micrographs of Sarkosyl-insoluble tau species confirming the presence of diverse human like tau fibrillary material in 20-month-old R955-hTau rats using multiple tau epitopes of MC1, AT8 and PHF1 (J-L). A scale bar = 100 μm; B-I scale bar = 50 μm; J-L scale bar = 100 nm.

months of age (**Figure 4C**; $t(34)=8.822$, $p<0.0001$). In contrast, 20-month-old transgenic rats had lower total tau levels compared to younger transgenics (**Figure 4C**; $F(2, 34) = 6.751$, $p=0.0034$; 20 vs 14 months $t(34)=10.57$, $p=0.0009$; 20 vs 9 months $t(34)=4.552$, $p=0.0310$). In contrast, Sarkosyl-insoluble phosphorylated tau at Thr231, an early stages marker of tauopathy in FTD and AD, showed an age-dependent increase (**Figure 4D**). By 20 months, R955-hTau^{+/+} rats had approximately 10-fold higher ptau Thr231 levels compared to age-matched wild-types (**Figure 4D**; $t(33)=9.701$, $p<0.0001$) and these levels were also significantly higher than younger transgenics (**Figure 4D**; 20 vs 14 months $t(33)=6.122$, $p=0.0017$; 20 vs 9 months $t(33)=8.067$, $p<0.0001$). These results indicated a shift from soluble to insoluble tau as the pathology progresses, as evidenced by a decrease in total tau (**Figure 4C**) but increase in ptau at Thr231 (**Figure 4D**), suggesting an altered profile of tau species in the insoluble fraction.

Likewise, Sarkosyl-soluble total tau was approximately three-fold higher in transgenics versus wild-types (**Figure 4A**; 9 months $t(34)=11.89$; 14 months $t(34)=7.967$; 20 months $t(34)=6.605$, $p<0.0001$) and the levels of soluble total tau decreased significantly in 20 month-old R955-hTau^{+/+} rats compared to their younger 9 month-old transgenic counterparts (**Figure 4A**; $t(34)=7.723$, $p<0.0001$). Similar to total tau, Sarkosyl-soluble ptau Thr231 levels were increased (**Figure 4B**; 9 months $t(34)=5.721$, $p=0.0036$; 14 months $t(34)=5.578$, $p=0.0047$; 20 months $t(34)=4.867$, $p=0.0178$). Unexpectedly, however, unlike the observations in the insoluble fraction, soluble ptau Thr231 levels plateaued at 9 months onwards.

Importantly, in R955-hTau^{+/+} rats, human P301S tau recruited endogenous rodent tau which was incorporated in tau aggregates as the pathology progressed, as evidenced by the decrease in T49-IR in the soluble fraction accompanied by an increase in T49-IR in the Sarkosyl-insoluble fraction as examined by Western blotting (**Figure 4E**). Such observations demonstrate the essential prion-like nature of tau pathology occurring in a consistent manner in this model. Together, these findings demonstrate an accelerative nature of tau pathology at late stages in this model, similar to that occurring in the human condition.

3.3 R955-hTau^{+/+} develop severe cognitive deficits in late life stages, paralleling a human-like tau-associated degeneration.

Utilizing a comprehensive selection of behavioral tests to assess different facets of cognition, we observed that R955-hTau^{+/+} rats progressively develop cognitive deficits in a tau

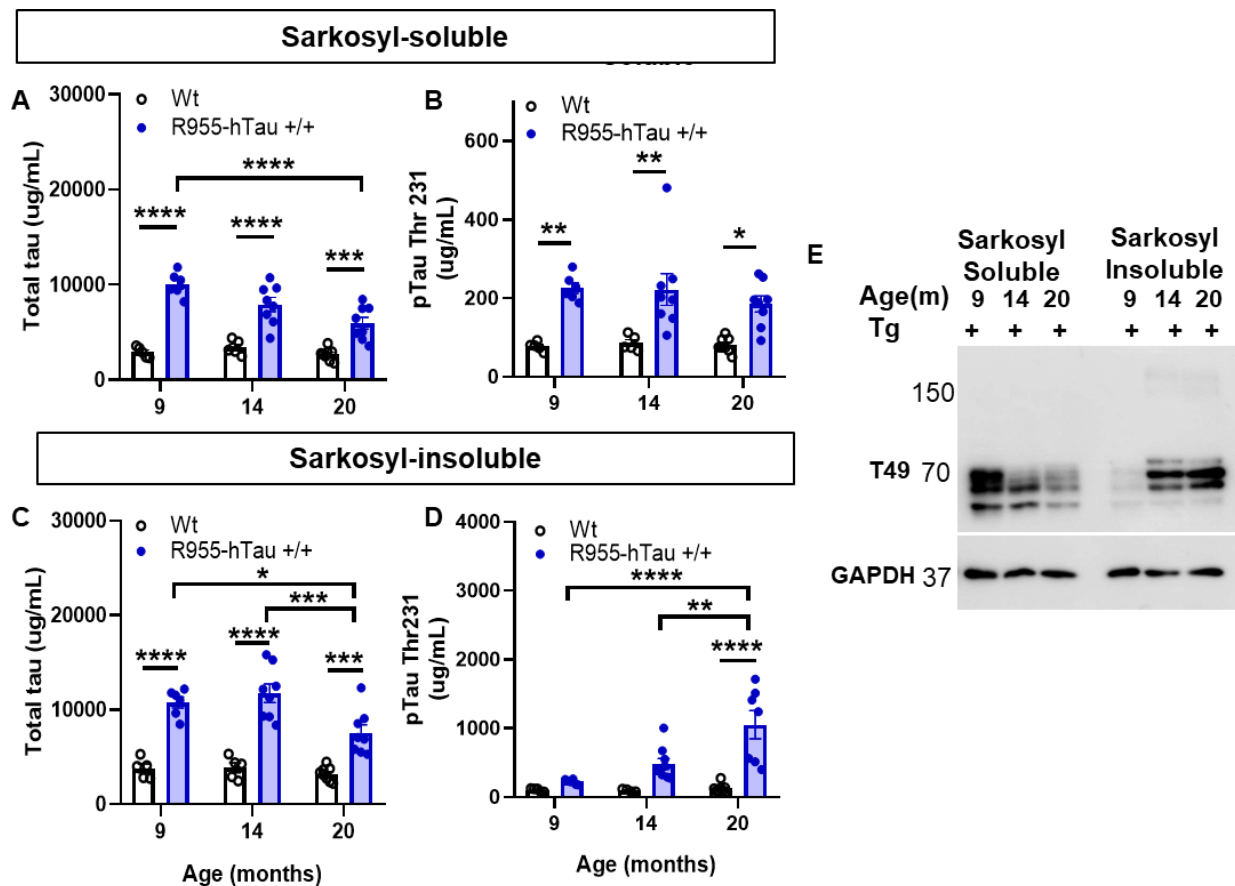


Figure 4. The progressive tau pathology in R955-hTau^{+/+} eventually recruits endogenous rodent tau at 20 months as revealed by the accumulation of pT231 immunoreactive material in the Sarkosyl-insoluble fraction. (A-D) Quantification of **(A, C)** total tau and **(B, D)** phosphorylated tau at Thr231 in Sarkosyl-soluble **(A-B)** and insoluble **(C-D)** cortical extracts demonstrating the nearly three-fold higher total tau protein levels in R955-hTau rats compared to wild-type controls with a progressive accumulation of Thr231 phosphorylated tau in the insoluble fraction. Interestingly, when ptau Thr231 is significantly increased at 20 months, the total tau levels are decreased. **(E)** Representative Western blot probing rodent tau using the T49 antibody on equally loaded cortical homogenates. **Lanes 1-3:** Sarkosyl-soluble extracts at 9, 14 and 20 months of age, respectively, **Lanes 5-7:** Sarkosyl-insoluble extracts at 9, 14 and 20 months, respectively. Note the presence of multiple bands indicating both 3R and 4R tau isoforms as well as a shift in signal strength from soluble to insoluble in R955-hTau^{+/+} rats with age. Faint high-molecular weight bands can also be observed in 14 and 20 months in Sarkosyl insoluble (Lanes 8-9), suggestive of tau aggregation (n=3). Error bars represent the mean ± SEM (n=6-8). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

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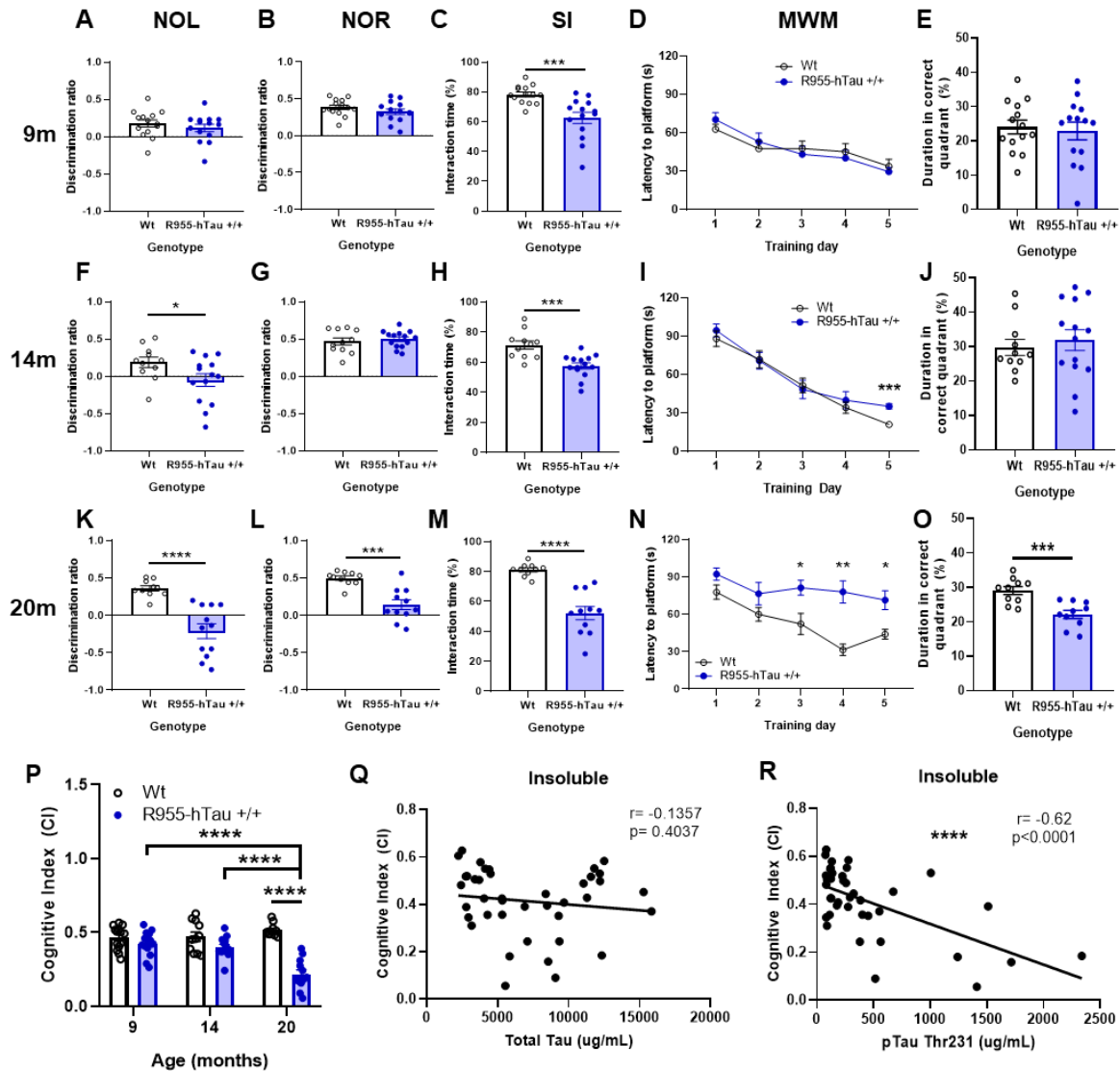


Figure 5. Homozygous R955-hTau rats display cognitive deficits in learning and memory in an age-dependent manner, with an acceleration of cognitive deficits as from 14 months of age. (A, F, K) Discrimination ratio is compromised in transgenic rats during the novel object location (NOL) task at 14 months (F) and observable at 20 months of age (K). Note compromised discrimination ratio (B, G, L) for the novel object recognition task (NOR) at 20 months (L). Transgenic rats displayed a sustained reduction in social interaction time (C, H, M) with a novel rat and is compromised as early as 9 months (C) and more markedly at 14 (I) and 20 (M) months of age. Spatial learning and memory (D, I, N) in transgenic rats is severely impaired for the Morris water maze (MWM) task at (O) 20 months of age. Similarly, the probe trial of the MWM (E, J, O) revealed impairments only at 20 months of age (O). Compilation of all behavioral tests into a global Cognitive Index (CI) (P) shows a strong decline in 20-month-old R955-hTau rats. Correlative analysis (Q-R) between Sarkosyl-insoluble total tau and CI (Q) were not correlated whereas ptau Thr231 negatively correlates with CI (R). Error bars represent the mean $\pm$ SEM (n=10-14). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

pathology-associated manner. At 9 months R955-hTau^{+/+} rats performed similarly to wild-types in almost all behavioral tests including the novel object location (NOL) (**Figure 5A**, $p=0.4005$), novel object recognition (NOR) (**Figure 5B**, $p=0.2290$), Morris water maze (MWM) platform latency during five days of training (**Figure 5D**; $F(1,26)=0.001$, $p=0.9750$) and latency in the correct quadrant during the probe trial (**Figure 5E**, $p=0.7215$).

Interestingly, the first cognitive presentation was related to social behavior as there was a reduced social interaction time in transgenic rats (**Figure 5C**; $t(26)=3.857$, $p=0.0007$). During this stage of tau accumulation, human tau (HT7)-IR was elevated in the CA2 as well as pS202-T205 IR and pT231 p-tau in the entorhinal cortex, two regions linked to social memory (Leung et al., 2018a, 2018b).

At 14 months, an age when cortical tau hyperphosphorylation and misfolded tau is established, cognitive impairment was evidenced by a loss of discrimination during the NOL (**Figure 5F**; $t(23)=2.110$, $p=0.0459$) but not NOR (**Figure 5G**; $p=0.5676$), as well as in an increased latency in reaching the platform in the last day of the acquisition phase of the MWM test (**Figure 5I**; $t(22.98)=5.148$, $p=0.002$). Social interaction remained impaired as observed at 9 months (**Figure 5H**; $t(23)=4.165$, $p=0.0004$).

Alongside the neurofibrillary tau and otherwise more severe tau pathology reminiscent of Braak stage IV, at 20 months cognition was severely compromised in several cognitive domains as indicated by impairments in all memory tasks including NOL (**Figure 5K**; $t(20)=5.124$, $p<0.0001$), NOR (**Figure 5L**; $t(19)=4.755$, $p=0.0001$), MWM training latency (**Figure 5N**; $F(1, 19)=22.89$, $p=0.0001$) on day 3 ($t(16.41)=2.749$, $p=0.0378$), day 4 ($t(14.85)=4.662$, $p=0.0016$) and day 5 ($t(14.99)=3.207$, $p=0.0233$) as well as the latency in the correct quadrant during the probe trial (**Figure 5O**; $t(18)=4.162$, $p=0.0006$). Notably, such spatial learning and memory impairments during the MWM appeared during advanced hippocampal ptau pathology and fibrillary tau as assessed with AT8, MC1 misfolded tau and PHF1 (**Figure 3D-L**). Social interaction was further impaired at 20 months (**Figure 5M**; $t(19)=5.996$, $p<0.0001$). In R955-hTau^{+/+} rats, an impairment during the cued recall of the fear conditioning task (**Supplemental Figure 1L**; $t(19)=2.936$, $p=0.0085$) was observed only at 20 months.

Compiling all behavioral tests into a global cognitive index (CI), which comprises an average score of all performed cognitive tests for each animal, revealed a significantly lower CI in 20-month-old R955-hTau^{+/+} rats compared to age-matched wild-type littermates (**Figure 5P**;

t(69)=12, $p<0.0001$). Taken together with younger transgenic rats, CI declined over age however differences at 9 months (**Figure 5P**, $p=0.7848$) and 14 months (**Figure 5P**, $p=0.2827$) did not reach statistical significance compared to wild types. After pooling all tested animals in all groups, cortical ptau Thr231 levels in the Sarkosyl-insoluble fraction negatively correlated with CI showed a clear negative correlation (**Figure 5R**; $r = -0.62$, $p<0.0001$), while total tau levels in the Sarkosyl-insoluble fraction showed no such correlation (**Figure 5Q**; $r = -0.1357$, $p=0.4037$). Altogether, these findings demonstrate a progressive impairment of cognition associated with tau pathology.

3.4 Neuronal loss and brain atrophy as a consequence of progressive human tauopathy.

Utilizing MRI, a more clinically relevant approach for the investigation of brain degeneration, a decrease in brain volume was observed in 20-month-old R955-hTau^{+/+} rats (**Figure 6E**; $t(4)=3.471$, $p=0.0256$) compared to age-matched controls, which is concurrent with the fibrillary stage of tau pathology in this model. In contrast, 9-month-old rats, at a stage of tau accumulation, no differences in brain volume were detected (**Figure 6E**, $p=0.5660$). Additionally, an increase in ventricular volume was detected at 20 months by approximately two-fold (**Figure 6F**; $t(4)=3.055$, $p=0.0378$) but not at 9 months (**Figure 6F**, $p=0.8701$). In addition to the brain atrophy and ventricular expansion, neuronal loss was also only detected at 20 months as shown by a decrease in NeuN-positive neuronal cells in the CA1 region by approximately 17-20% (**Figure 6J**; $t(11)=3.265$, $p=0.0075$). However, neuronal loss was not detected at this stage in the somatosensory cortex (**Supplemental Figure 2K**, $p=0.1151$) although this region already presented elevated levels of tau hyperphosphorylation and abnormal neuronal morphology changes (**Figure 1N, 2L, 2S, 4F**).

3.5 Synaptic alterations associated with tau pathology precede neuronal loss.

The investigation of overall synaptic markers in hippocampal homogenates revealed altered protein expression in R955-hTau^{+/+} rats suggestive of synaptic loss (**Figure 6, Supplemental Figure 2**). As such, the axonal cytoskeleton and neuronal integrity marker, neurofilament light (Nf-L), was severely reduced by four-fold compared to wild-type littermates (**Figure 6L**; $t(10)=3.003$, $p=0.0133$). Furthermore, the glutamate N-methyl-D-aspartate receptor (NMDAR1), an important protein for learning and memory, was reduced by half as early as 9

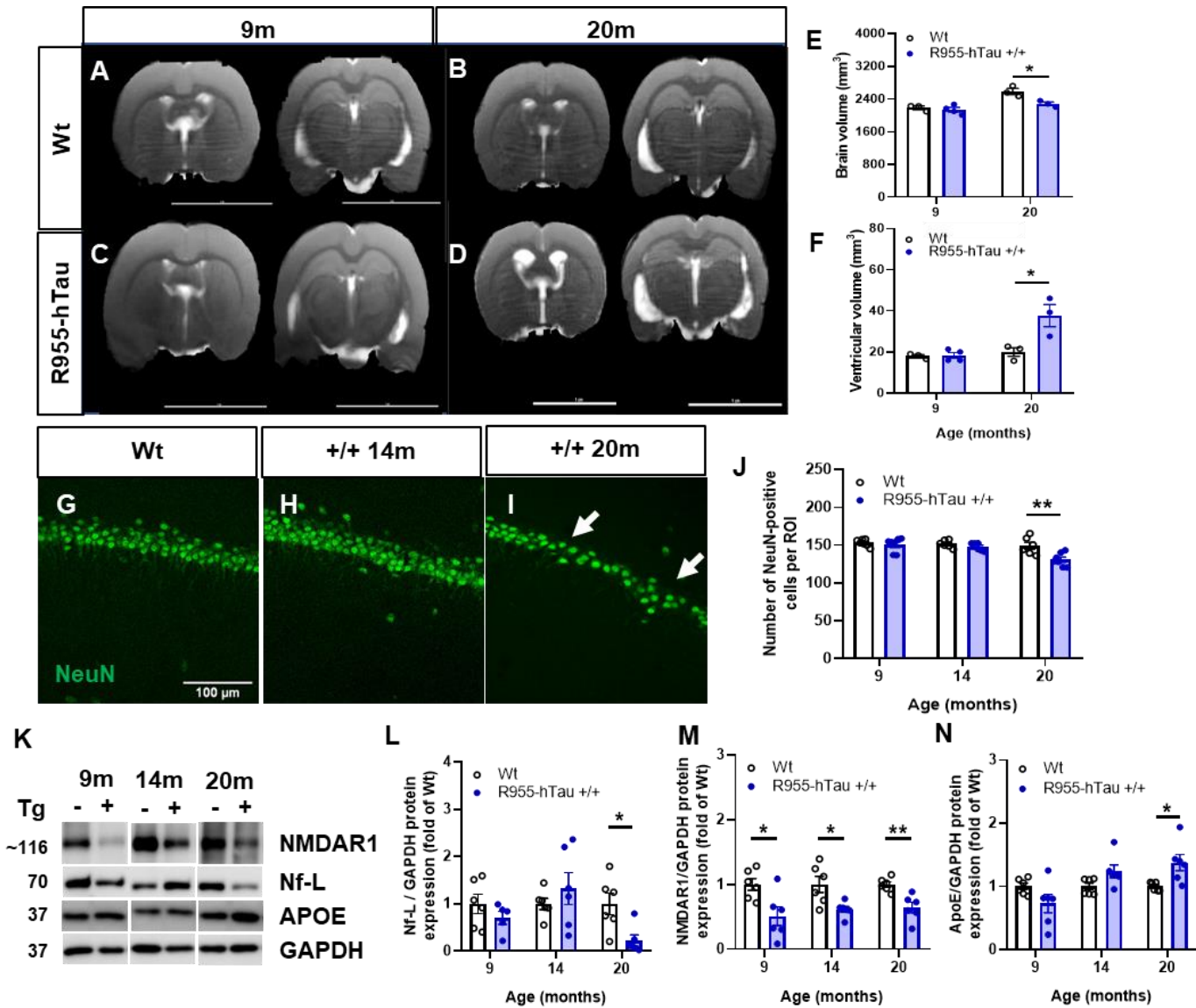


Figure 6. Progressive human tauopathy leads to neurodegeneration in the R955-hTau transgenic rat model.

Notably, several indicators of neurodegeneration were detected after 20 months. (A, D) Representative 2D coronal images from brain-wide MRI scans illustrating brain atrophy and ventricular dilation at 20 months of age. (E) Quantification of total brain volume and of (F) expansion of the ventricular space at 20 months of age (n=3-4). (G-I) Representative confocal images illustrating loss of CA1 NeuN-immunoreactive neurons in 20-month-old R955-hTau rats but not at 14 months. (J) Quantification of total NeuN-positive cells per region of interest (ROI) (n=6-10) in the (J) CA1 region of the hippocampus. (K) Representative Western blots of hippocampal homogenates probing the synaptic marker NMDAR1, and the axonal marker neurofilament light chain (Nf-L) and ApoE. (L-N) Quantification of protein expression normalized to the loading control GAPDH illustrating altered levels of neurofilament light (Nf-L) (L), NMDAR1 (M) and ApoE (N). Error bars represent the mean $\pm$ SEM (n=6-7). A, D scale bar = 1 cm; G-I scale bar = 100 μ m. * = $p < 0.05$, ** = $p < 0.01$.

months, remaining reduced at 14 and 20 months of age (**Figure 6M**; 9 mo $t(10)=2.867$, $p=0.0167$; 14 mo $t(10)=2.906$, $p=0.0157$; 20 mo $t(10)=3.907$, $p=0.0029$). Additionally, a drastic reduction in immunofluorescence (IF) for the dendritic marker, MAP2, was observed at 20 months of age (**Supplemental Figure 2G**; EC $t(11)=7.471$, $p<0.0001$), SSCtx $t(12)=6.458$, $p<0.0001$) and CA1 $t(12)=5.209$, $p=0.0002$).

Probing synaptophysin, a marker for overall presynaptic terminals, revealed reduced IF intensity at 14 months of age initially in the entorhinal cortex (EC) (**Supplemental Figure 2I**; $t(12)=2.938$, $p=0.0124$) and somatosensory cortex (SSCtx) ($t(12)=4.309$, $p=0.0010$). At 20-months, such a decrease in IF intensity was observed throughout multiple investigated brain regions, indirectly indicating synaptic loss consequential to the spreading of the tauopathy and prior to neuronal loss (**Supplemental Figure 2J**; EC $t(11)=4.175$, $p=0.0015$; SSCtx $t(12)=5.577$, $p=0.0001$; CA1 $t(12)=3.622$, $p=0.0035$; CA2 $t(12)=2.701$, $p=0.0193$; CA3 $t(12)=3.438$, $p=0.0049$).

3.6 Microglial response and reactive astrocytes trails neuronal tau pathology.

We examined the overall presence and morphology of Iba1-IR microglia (**Figure 7**) as well as total Iba1-IF, a proxy of reactivity and activation (**Supplemental Figure 3**) in response to tau pathology in R955-hTau^{+/+} rats. When misfolded, MC1 immuno-positive material is established at 14 months, an increase in the number of microglia (**Figure 7E**; EC $t(12)=2.780$, $p=0.0167$; SSCtx $t(12)=3.237$, $p=0.0071$; CA1 $t(12)=2.740$, $p=0.0179$) and increased total Iba1 IF was observed in all brain regions burdened with tau pathology (**Supplemental Figure 3G**). At 20 months, R955-hTau^{+/+} rats developed a more marked increase in the total number of microglia (**Figure 7F**; EC $t(13)=5.254$, $p=0.002$; SSCtx $t(14)=5.287$, $p=0.0001$; CA1 $t(14)=6.013$, $p<0.0001$) and increased Iba1 IF intensity (**Supplemental Figure 3H**; EC $t(11)=7.082$, $p<0.0001$; SSCtx $t(12)=2.245$, $p=0.0444$; CA1 $t(12)=2.453$, $p=0.0304$). Consistent with the absence of frank tau pathology (MC1- and PHF-1-IR), at 9 months, the total number of Iba1-IR microglial cells (**Supplemental Figure 3E**) and Iba1 IF intensity (**Supplemental Figure 3F**) was unaltered in any investigated regions. To further ascertain the phagocytic activity of these microglia, co-staining with the phagocytic marker cluster of differentiation 68 (CD68) was detected in the EC (**Figure 7H**) of 20-month-old transgenic rats, emphasizing the likelihood of microglial phagocytosis of tau-burdened neuronal processes.

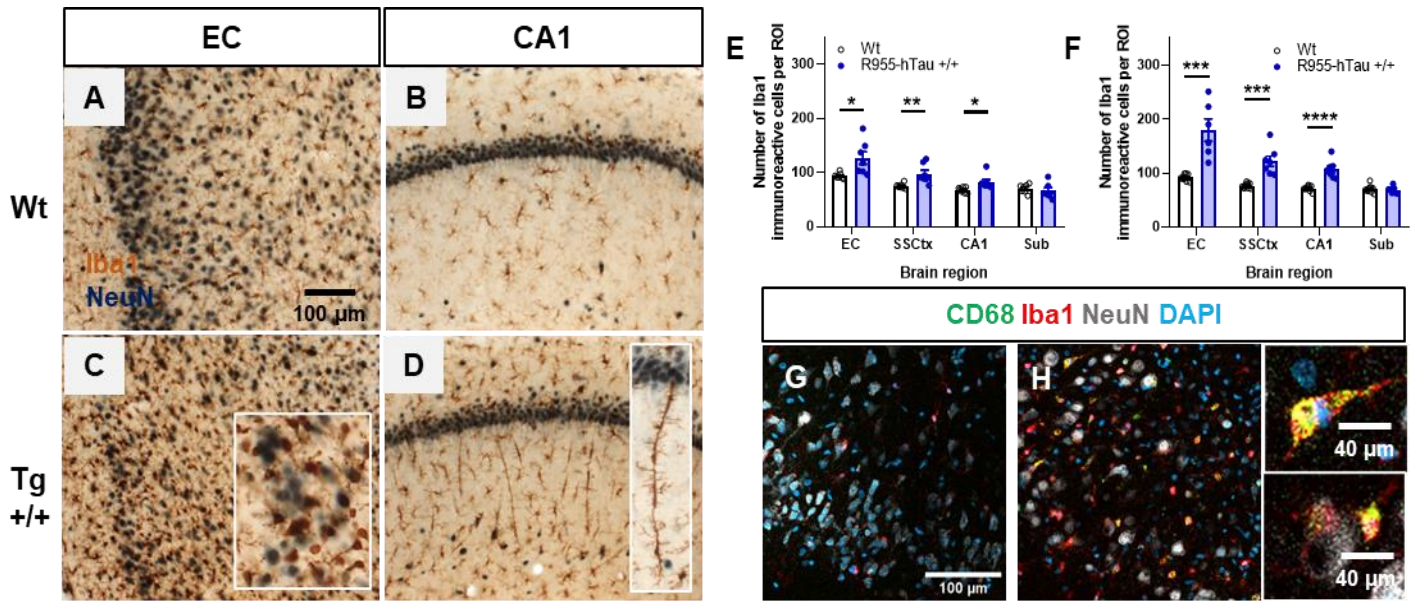


Figure 7. Microgliosis in R955-hTau^{+/+} rats is associated with tau pathology. (A-D) Representative micrographs of 20-month-old rats illustrating abnormal microglial morphologies and enhanced recruitment in regions with pathological tau defined as the presence of microglia with retracted processes and swollen cell somas the entorhinal cortex (C, **insert**) as well as occurrence of rod-shaped microglia trailing the apical dendrites (D, **insert**). (E-F) Quantification of the total number of Iba1-immunoreactive cells per region of interest (ROI) highlighting a significant increase in R955-hTau^{+/+} at 14 months (E) and 20 months of age (F). (G-H) Representative micrographs illustrating microglia in close proximity to tau-burdened neurons in the entorhinal cortex in 20-month-old R955-hTau^{+/+} rats with CD68-immunopositive microglia (H) in close proximity to neurons. Error bars represent the mean $\pm$ SEM (n=6-7). A-D, G-H scale bar = 100 μ m; H scale bar = 40 μ m; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

In addition, investigation of astrocytes as indicated by glial fibrillary acidic protein (GFAP) revealed an evolving phenotype encompassing different cells and structures as the tauopathy progresses, particularly at sites rich in neurovasculature. In addition to reacting to regions with neuronal tau pathology, astrocytes became morphologically aberrant in vascular dense regions such as the *stratum lacunosum moleculare* (SLM) (Figure 8A-B) after misfolded tau became established in that region. Similar to microglia, no alterations in overall GFAP IF intensity, a well-established measure, were detected at 9 months during the stages of tau accumulation (Supplemental Figure 4A). An increase in GFAP IF was detected in the SLM and EC at 14 months, but not CA1 nor SSCtx however this was less severe relative to 20-month-old transgenic rats (Supplemental Figure 4D; CA1 $p = 0.2354$; SLM $t(12) = 2.540$, $p = 0.0259$; SSCtx $p = 0.4693$; EC $t(12) = 2.278$, $p = 0.0418$). At 20 months, GFAP IF was markedly increased in multiple tau-

burdened regions including the CA1, SLM, SSCtx and EC (**Figure 8G**; CA1 $t(11)=2.509$, $p=0.0291$; SLM $t(11)=3.816$, $p=0.0029$; SSCtx $t(11)=4.568$, $p=0.0008$; EC $t(10)=5.356$, $p=0.0003$), indicating that a progressive astrocytosis became detectable as from the appearance of hyperphosphorylated and conformationally altered tau. In addition to the tau-associated astrocytosis in the forebrain, tau IF material for human tau and misfolded tau was detected in the SLM (**Supplemental Figure 5**). Furthermore, MC1-immunoreactive misfolded tau was notably abundant in the perivascular space (**Figure 8E**) and co-localized with GFAP-positive perivascular astrocytes (**Figure 8F**) but not in microglia (**Supplemental Figure 5A-F**) nor in astrocytes rooted in other brain areas beyond vessels (**Supplemental Figure 5G-I, H-L**). These observations implicated a possible role of pathological tau at the neurovascular unit.

3.7 Aged R955-hTau^{+/+} rats develop tauopathy-related neurovascular abnormalities

To further investigate the consequences of tau pathology in the perivascular space, we probed the astrocytic water channel aquaporin 4 (AQP4), which is mainly expressed on the astrocytic end-feet surrounding the blood vessels. In 20-month-old R955-hTau^{+/+} rats, AQP4 IF intensity was increased in astrocyte-abundant regions including the SLM (**Figure 8H**; SSCtx $p=0.8288$; SLM $t(12)=2.722$, $p=0.0185$). A similar increase was found at 14 months to a lesser extent than at 20 months (**Supplemental Figure 4E**; SLM $t(12)=2.236$, $p=0.0451$). At 9 months of age there were no significant changes in IF intensity (**Supplemental Figure 4B**) which was expected given the absence of misfolded and hyperphosphorylated tau and brain degeneration at this age. AQP4 was also found to be abnormally localized in 20-month-old R955-hTau^{+/+} rats indicating a loss of astrocyte polarization. This altered polarization was revealed by an increased co-localization between GFAP and AQP4 in the SLM but not SSCtx (**Figure 8I**; SSCtx $p=0.1626$; SLM $t(12)=2.355$, $p=0.0364$). No such increases of colocalization were observed at 9 (**Supplemental Figure 4C**) or 14 months of age (**Supplemental Figure 4F**). In parallel with the astrocytosis, we next investigated the other components of the neurovascular unit (NVU), a complex structure surrounding the blood vessels in the brain that comprises of several cell types (**Figure 9**). Pericyte coverage (**Figure 9A-C**), as determined by the percent area of co-localization between a pericyte marker, CD13, and marker of the basement membrane, Collagen IV (ColIV),

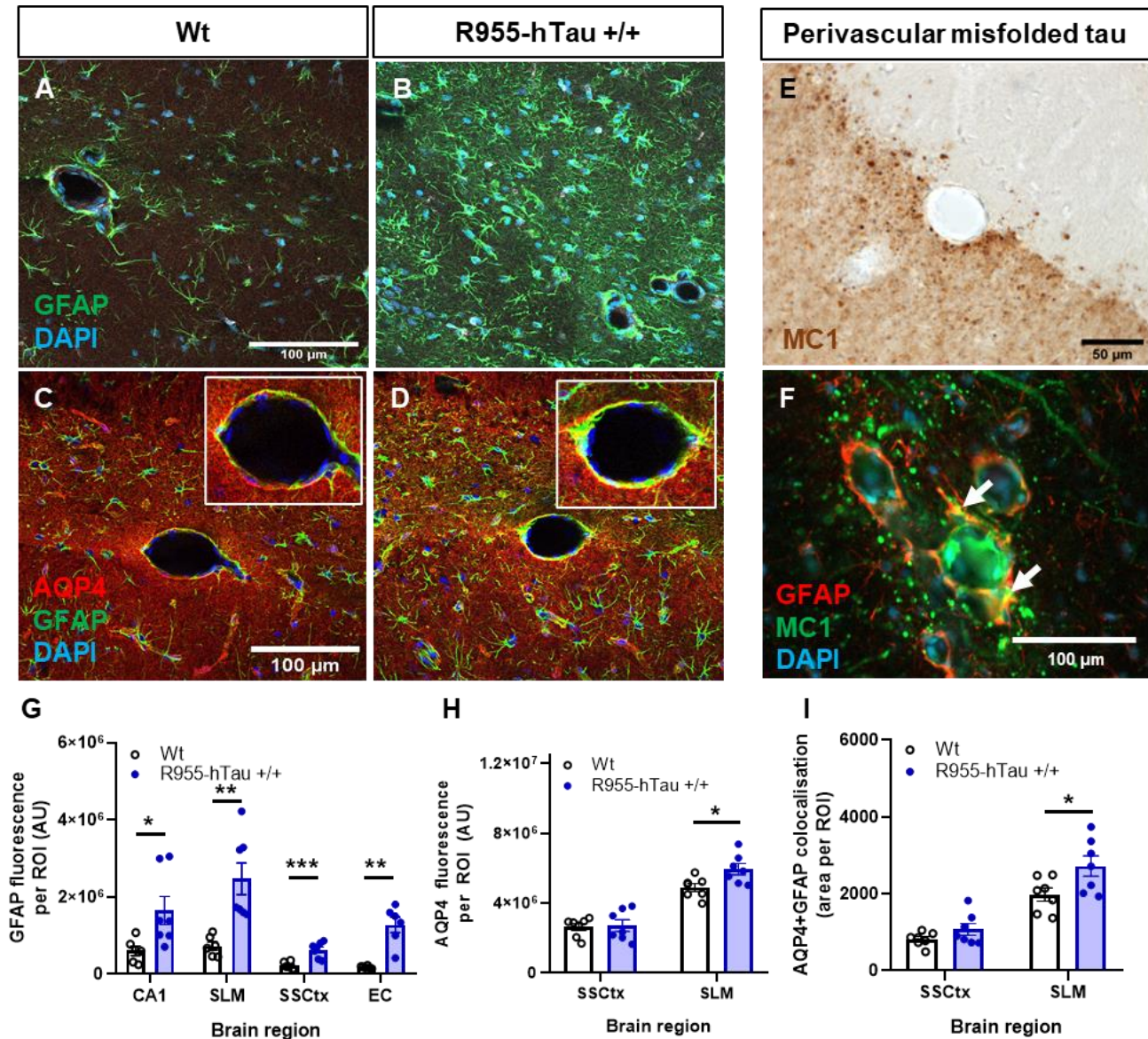


Figure 8. Astrocytosis with altered polarization of aquaporin water channels in 20-month-old R955-hTau^{+/+} rats associated with perivascular pathological tau. (A-B) Representative micrographs of the *stratum lacunosum* (SLM) in the hippocampus, a vascular rich region, illustrating elevated GFAP immunofluorescence as an indication of astrocytosis. (C-D) Representative micrographs of the neurovascular unit illustrating an abnormally increased co-localization of aquaporin4 (AQP4) with astrocytic GFAP. MC1 immunoreactivity, indicating misfolded tau, was observed near blood vessels (E) and co-localized with astrocytes in the SLM (F). (G) Quantification of total GFAP IF, (H) AQP4 IF and (I) AQP4+GFAP colocalized area per region of interest (ROI) are significantly increased in 20-month-old R955-hTau^{+/+} rats. Error bars represent the mean ± SEM (n=6-7). A-D, F scale bar = 100 µm; E scale bar = 50 µm. * = p<0.05, ** = p<0.01, *** = p<0.001

was reduced in the SLM and SSCtx (**Figure 9C**; CA1 $t(4)=5.193$, $p=0.0065$; SLM $t(4)=3.613$, $p=0.0225$; SSCtx $t(6)=3.544$, $p=0.0122$). Here, CD13 immunoreactive pericytes had a reduced vessel area (**Supplemental Figure 6A**; CA1 $t(4)=7.297$, $p=0.0019$; SLM $t(4)=5.768$, $p=0.0045$; SSCtx $t(6)=2.933$, $p=0.0262$; EC $p=0.8549$; fCtx $p=0.7278$) whereas the blood vessels were more abundant as determined by increased ColIV area (**Figure 9F**; CA1 $t(4)=4.685$, $p=0.0094$; SLM $t(4)=5.768$, $p=0.0045$; SSCtx $t(6)=2.772$, $p=0.0324$; EC $t(4)=2.820$, $p=0.0478$; fCtx $p=0.5766$) and increased ColIV IF intensity (**Supplemental Figure 6B**; CA1 $p=0.8558$; SLM $t(4)=4.506$, $p=0.0108$; SSCtx $p=0.0863$; EC $t(4)=3.263$, $p=0.0310$; fCtx $p=0.3816$). Additionally, a subset of the smaller ColIV immunofluorescent vessels bore a tortuous, spiraled, and chaotic morphology compared to wild-type littermates reminiscent of small vessel disease (**Figure 9E**). Together, these findings suggest a type of vascular remodeling that is occurring as a consequence of perivascular misfolded tau.

Using an ultrastructural approach, transmission electron microscopy revealed an alteration of vascular morphologies indicating neurovascular damage in aged, 20-month-old R955-hTau^{+/+} rats. Compared to age-matched wild-types (**Figure 9G**), endothelial blebbing and dilation of the perivascular space (**Figure 9H**), swollen astrocytic end-feet (**Figure 9I**) and in electron-dense nuclei of pericyte cells of the neurovascular unit indicative of degeneration (**Figure 9J**).

3.7 Evidence of a tau-associated compromised blood-brain barrier in vivo

Given the ultrastructural evidence of a damaged NVU in 20-month-old R955-hTau^{+/+} rats, and alterations at the NVU can lead to a disruption of the blood-brain-barrier (BBB), we then assessed the BBB function by a co-injection of the classical Evan's blue (EB) dye as well as horseradish peroxidase (HRP) into a lateral tail vein (**Figure 10**). In regions possessing advanced tau pathology, a vascular leakage of HRP into the brain was detected in the SSCtx and EC in transgenic rats (**Figure 10F-G**) whereas HRP reactivity was limited to the vessel interior in wild-type rats (**Figure 10B-D**). In regions of HRP leakage, we also observed morphologies resembling string vessels as remnants of dead capillaries (**Figure 10F-G**). In addition to HRP leakage, an extravasation of endogenous immunoglobulins (IgG) (**Figure 11A-D**), as detected by an increase in overall rat IgG IF intensity in the SSCtx, EC and SLM of aged R955-hTau^{+/+} rats (**Figure 12D**; SSCtx $t(14)=2.314$, $p=0.0364$; EC $t(13)=2.795$, $p=0.0152$; SLM $t(14)=2.244$, $p=0.0415$). As expected, extravasation of IgGs was not observed at 9 (**Supplemental Figure 6C**) nor 14 months

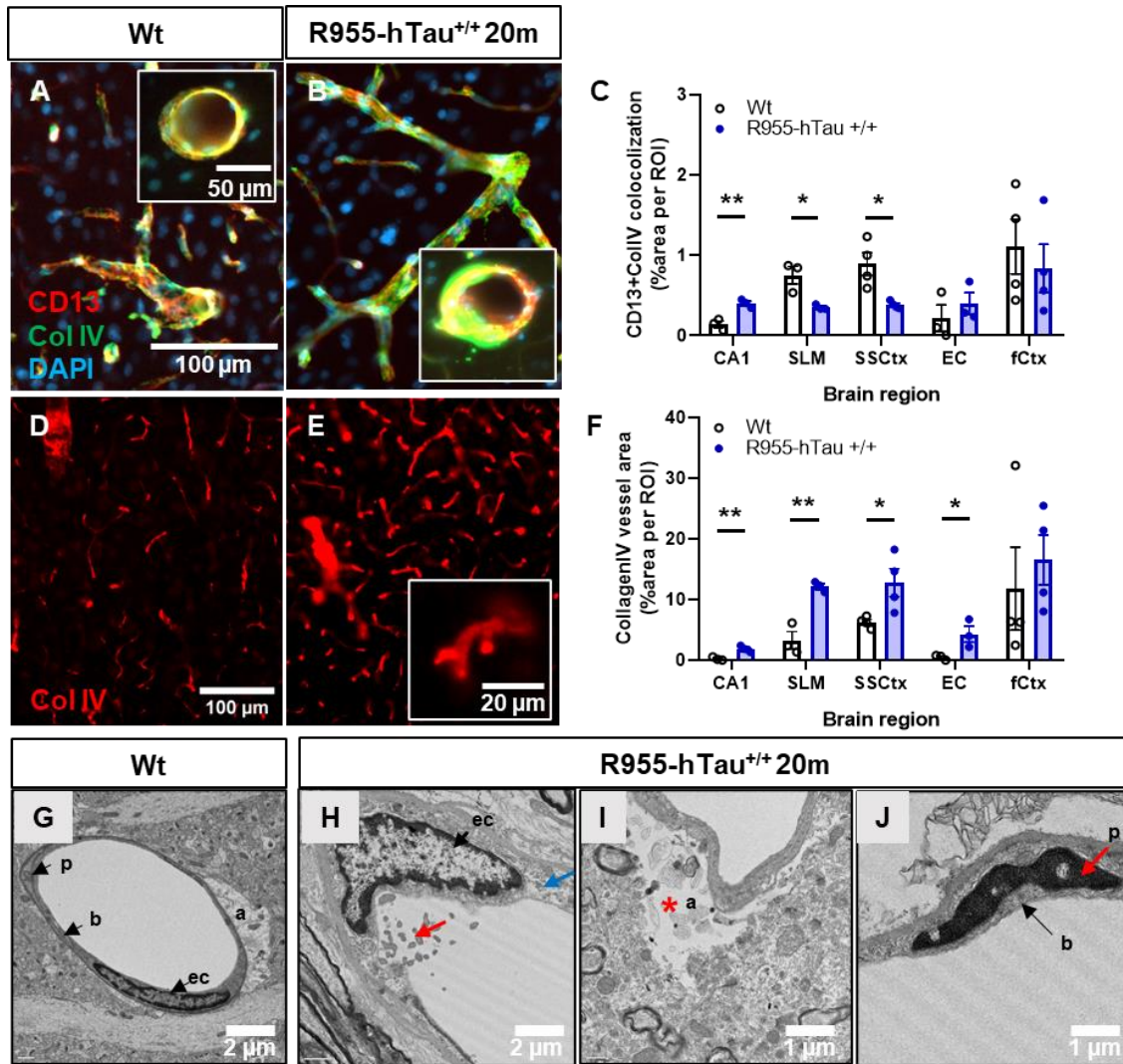


Figure 9. Tau-associated abnormal neurovascular features in aged R955-hTau^{+/+} rats. (A-D) Representative immunofluorescence micrographs illustrating the CD13-immunoreactive pericytes and ColIV-immunoreactive basement membrane dynamically shifted in the somatosensory cortex (SSCtX) of R955-hTau (B) as compared with wild-type (A). (C) Quantification of colocalization area between CD13 and ColIV illustrated by reduced pericyte vessel coverage per region of interest (ROI) in transgenic rats. Using ColIV to label vessels, compared to wild-type (D), R955-hTau rats (E) displayed an increase in vascularization with the appearance of tortuous vessels and string vessels (insert). (F) Quantification of ColIV area demonstrating an increase in basal lamina content in the stratum lacunosum (SLM), SSCtX and entorhinal cortex (EC). (G-J) Representative electron micrographs illustrating normal vascular morphology in 20-month-old wild-types (G) whereas transgenics rats exhibit endothelial blebbing (H, red arrow) and expansion of the perivascular space (H, blue arrow), swelling of astrocytic end-feet (I, red asterisk), and electron-dense pycnotic nuclei of pericytes (J, red arrow) indicating degeneration (a, astrocyte; p, pericyte; b, basal lamina; ec, endothelial cells) (n=2-3). Error bars represent the mean ± SEM (n=4-5). A-B, D-E scale bar = 100 µm; G-H scale bar = 2 µm, I-J scale bar = 1 µm. * = p<0.05, ** = p<0.01.

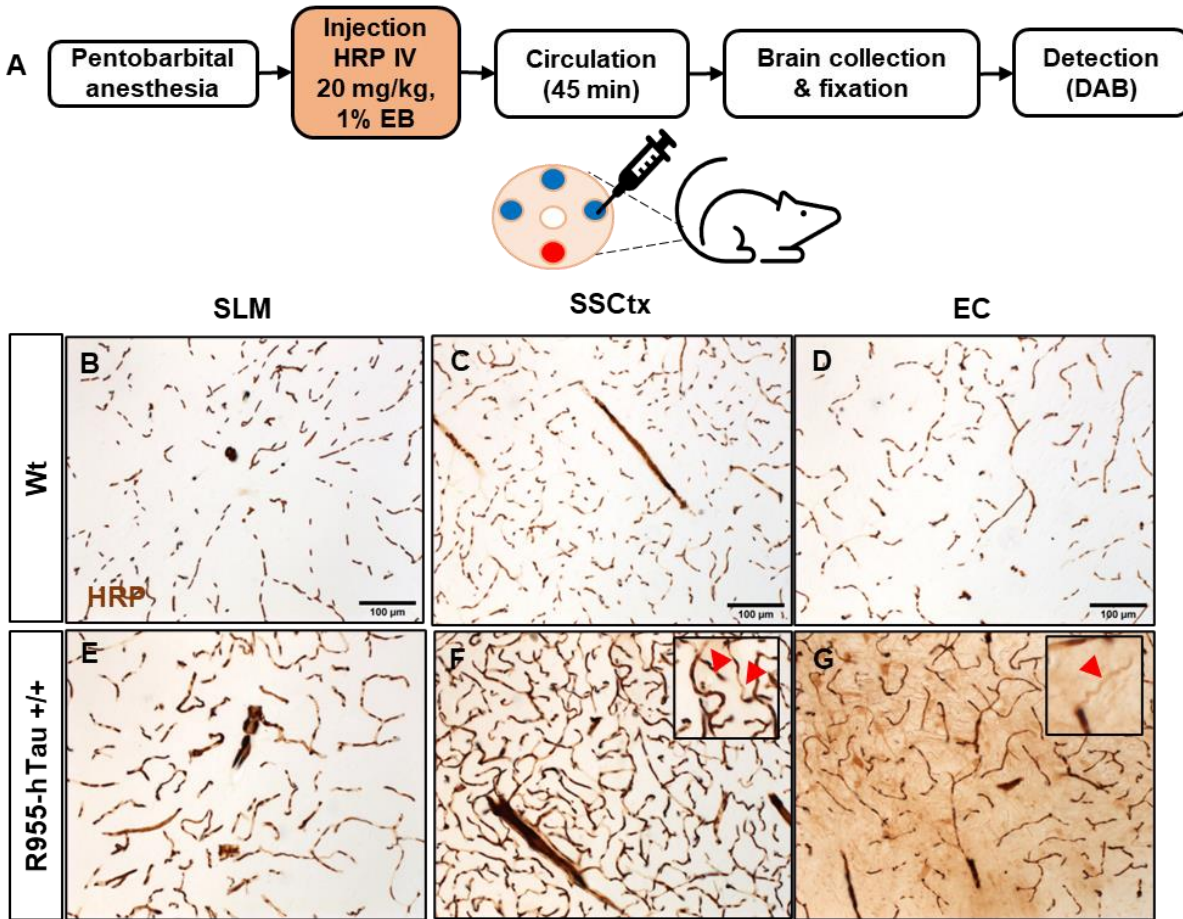


Figure 10. Enhanced peripheral penetration and leakage of horseradish peroxidase to the neurovasculature of aged R955-hTau^{+/+} rats. (A) Illustration of experimental paradigm by which the blood-brain-barrier tracers horseradish peroxidase (HRP) and Evan's Blue dye (EB) were co-injected into the lateral tail vein in 20-month-old rats. (B-G) Representative micrographs illustrating overall HRP reactivity is enhanced in the (E) *stratum lacunosum* (SLM), (F) somatosensory cortex (SSCtX) and (G) entorhinal cortex (EC) of R955-hTau^{+/+} rats compared to (B-D) age-matched wild-type littermates. Note the presence of string vessels (F-G, red arrow) and substantial HRP leakage outside of the vessels in the EC (G). B-G scale bar = 100 μ m.

(Supplemental Figure 6D). Notably, extravasations were observed predominantly from larger vessels (Figure 12C) in these regions, indicated by an emergence of IgG IF particles from the vessel walls.

Cerebral microbleeds are a common feature of a dysfunctional or damaged BBB. Furthermore, taking advantage of the Prussian Blue staining method which develops reactivity with iron to visualize bleeding into the CNS, 5 out of 11 (45%) of 20-month-old R955-hTau^{+/+} rats exhibited sparse cerebral microbleeds throughout the cerebral cortex (Figure 11E-F) in a seemingly sporadic spatiotemporal nature. Unexpectedly, during the examination for microbleeds

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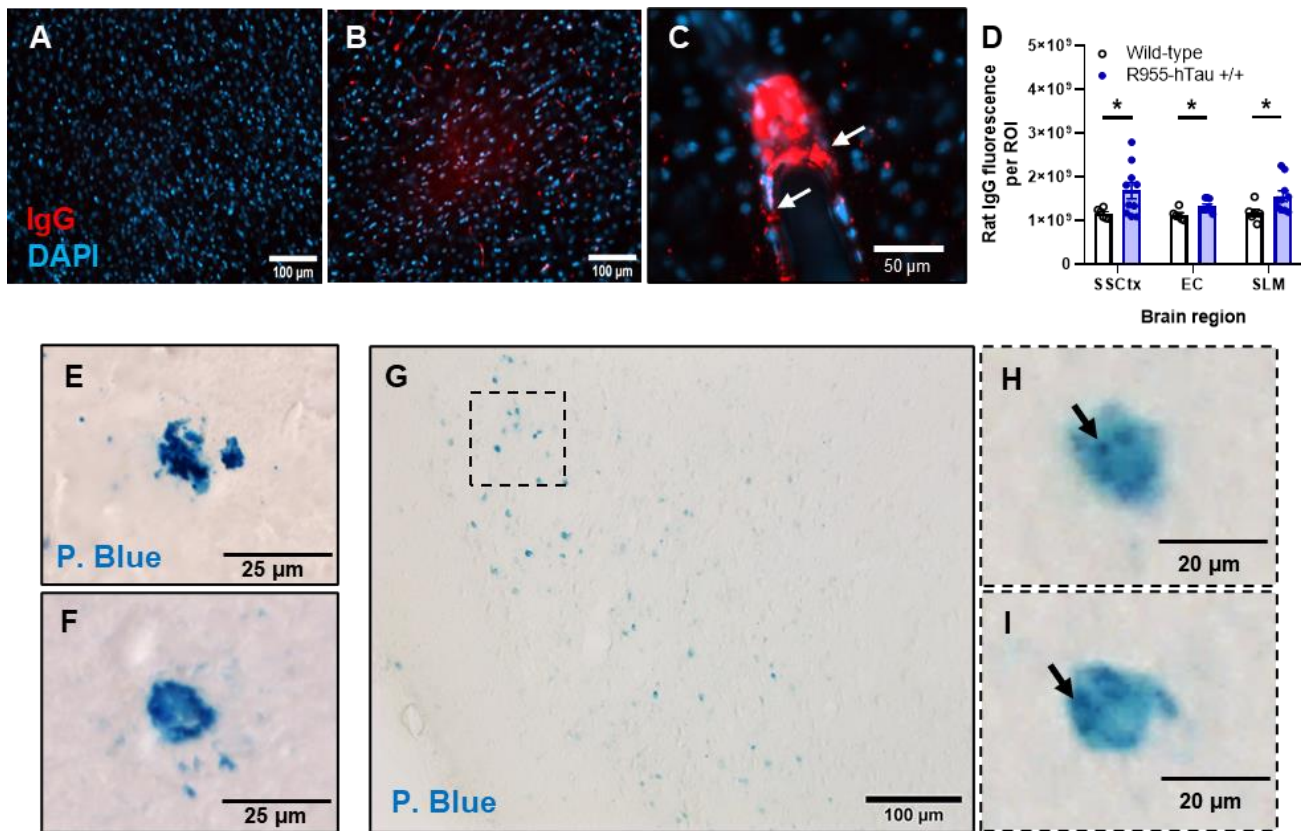


Figure 11. Aged R955-hTau^{+/+} rats develop extravasation of endogenous immunoglobulins (IgG), cerebral microbleeds and intraneuronal iron bodies in tau-burdened cortical regions. (A-C) Representative immunofluorescent micrographs illustrating substantial leakage of rat IgGs between wild-type (A) and R955-hTau^{+/+} (B) rats predominantly from damaged large cortical blood vessels (C). (D) Quantification of IgG fluorescence in 20-month-old rats, demonstrating extravasation in regions of compromised vasculature and severe tau pathology. (E-I) Representative Prussian blue (P. Blue) staining illustrating the sporadic appearance of iron-reactive cerebral microbleeds in the cerebral cortex (E-F). Unexpectedly, P. Blue staining also stained the entorhinal cortex of 20-month-old R955-hTau^{+/+} rats (G, high magnification in H-I), indicating intraneuronal iron deposits in a region harbouring severe tau pathology. Error bars represent the mean $\pm$ SEM (n=6-7). A-B, G scale bar = 100 μ m; C scale bar = 50 μ m; E-F scale bar = 25 μ m; H-I scale bar = 20 μ m * = p<0.05

1942 we had discovered positively stained neurons in the EC (**Figure 11G-I**), suggesting accumulation
 1943 of iron deposits within neurons in a region which is highly affected by the advanced tau pathology.

4. Discussion

The homozygous McGill-R955-hTau rat model displays a phenotype recapitulating several aspects of a progressive human-like tauopathy. This model develops a sequential progression of tau accumulation, hyperphosphorylation and aggregation akin to the regional progression of tauopathy in AD described by Braak and Braak (Braak et al., 2006; Braak and Braak, 1995, 1991) whereby pathology is first detected in the entorhinal area, then later occurring in other cortical regions and connected hippocampal regions, and ultimately resulting in multiple aspects of neurodegeneration. Notably this model displays significant aspects of brain tauopathy such as the evolution of the tau pathology with the aging process, the development of human-like neurofibrillary tangles (**Figure 3J-L**), the recruitment of endogenous rodent tau (**Figure 4**) and exhibition of neuronal loss and brain atrophy (**Figure 6**). Most, but not all of these aspects, are described in the majority of the currently available transgenic models of tauopathy (Allen et al., 2002; Gotz et al., 2001; Götz et al., 2001; Yoshiyama et al., 2007). Having combined these features of tauopathy with the advantages of using rats, we were for example able to detect a social withdrawal behaviour preceding inflammation and misfolded MC1-positive tau, the appearance of rod-shaped microglia around the onset of misfolded tau, as well as a clear, yet underestimated role of tau at the neurovascular unit at late-stage tauopathy.

As observed in R955-hTau^{+/+} rats and consistent with other rodent models of brain tauopathy, drastic cognitive deficits are observed at advanced and fibrillary pathological stages (Malcolm et al., 2019; Shi et al., 2017; Yoshiyama et al., 2007). Several existing animal models for tauopathy (Cohen et al., 2013; Do Carmo and Cuellar, 2013; Dujardin et al., 2015) clearly model tau pathology and associated neurodegeneration (Bugiani et al., 1999; Goedert and Spillantini, 2000). However, an advantage of the R955-hTau^{+/+} rat is its milder, more progressive human-like tau pathology along with the aging process and genuine neurofibrillary lesions.

Synaptic alterations appeared in a sequential nature. First we observed a decrease of synaptophysin IF in R955-hTau^{+/+} rats at 14 months, then this was followed by a loss of MAP2 IF and Nf-L at 20 months of age. These findings are consistent with the relatively early synaptophysin loss in AD (Buchanan et al., 2020; Goetzl et al., 2016; Masliah et al., 1989) followed by more prominent axodendritic degeneration in more advanced disease stages. Additionally, the occurrence of rod-shaped microglia was notable as this morphology has been detected in traumatic brain injury (Jørgensen et al., 1993), tauopathies (Bachstetter et al., 2015) and in Down's

Syndrome (Flores-Aguilar et al., 2020) but not in models with amyloid beta pathology (Guo et al., 2013; Leon et al., 2010). We also find it particularly intriguing that APOE protein levels are increased during the advanced stages of tauopathy in R955-hTau^{+/+} rats (**Figure 6M**), suggesting a disruption in fatty acid metabolism and transport that has been recently associated with neurodegeneration (Jablonski et al., 2021; Jack et al., 2020; Qi et al., 2021; Shi et al., 2017) and such an imbalance of APOE may be a cause of sporadic AD (Lozupone et al., 2023). Altogether, findings suggest that tau aggregation and misfolding are required to generate the unique, human-like features.

In AD, aggregation of tau correlates more closely with cognitive decline and ultimately dementia in comparison to amyloid beta (A β) (Bateman et al., 2012; Fleisher et al., 2015; Jack et al., 2018) as revealed by positron emission topography (PET) imaging (Ossenkoppele et al., 2016; L. Wang et al., 2016), fluid biomarkers (Jack et al., 2013), and post-mortem brain examinations (Bennett et al., 2004; Serrano-Pozo et al., 2016). The clinical impact of tau pathology is well reflected by the sudden rise in tau biomarkers in cerebrospinal fluid (CSF), coinciding with the onset of clinical presentation of AD (McDade et al., 2018) as well as PET tau deposition (Arboleda-Velasquez et al., 2019; Aschenbrenner et al., 2018; Biel et al., 2021; Brier et al., 2016; Chiotis et al., 2020; Jack et al., 2020; Pontecorvo et al., 2019). Further, in line with CSF and plasma biomarker data for early tau pathology reported in AD and FTD (Ashton et al., 2021; Foidl and Humpel, 2018; Ossenkoppele et al., 2022; Suárez-Calvet et al., 2020), we found that rising ptau Thr231, but not total tau, in the brains of R955-hTau^{+/+} rats was associated with a decrease in cognitive scores during stages of tau pathology positive for misfolded and aggregated tau. Therefore, diagnosis and intervention of tauopathy with a more preventative approach, such as before the occurrence of misfolded and ptau Thr231, should have the greatest likelihood of altering AD's trajectory. Overall, this transgenic rat model may be useful for future tau experimental therapeutics. While this model may present a phenotype of greater translational value, one limitation may be its overexpression of multiple copies of the transgene which encodes the longest isoform (2N4R) of human P301S mutated tau rather than unmutated human tau and therefore may not be reflective of all aspects of FTD and AD tauopathies.

Unexpectedly, we have found clear signs of iron reactivity in cells of the EC in R955-hTau^{+/+} rats suggesting the presence of oxidative damage, ferroptotic processes and abnormal lipid metabolism (manifested by ferroptosis) (Rao and Adlard, 2018; Tran et al., 2022). To our

knowledge, such abnormal iron accumulation has not been reported related to tau pathology. Such a relationship between cellular iron accumulation and tau aggregation is consistent with recent neuroimaging data (Spotorno et al., 2020) yet an aspect poorly understood in AD pathology (Goodman, 1953; Smith et al., 1997; Zhou et al., 2022). Iron becomes particularly abundant in brain regions which accumulate PHFs and NFTs (Andrási et al., 1995) among other metals. For instance, tau aggregation into PHFs can be encouraged *in vitro* by the introduction of Fe^{3+} but reversed when reduced to Fe^{2+} (Yamamoto et al., 2002). In AD, iron can also cross a compromised BBB without endocytosis (van de Haar et al., 2016). Additionally, administration of iron *in vitro* (Chen et al., 2018) or *in vivo* (Peters et al., 2018) worsened amyloidogenic processing and altered microglial morphology. Taken together, iron accumulation as a downstream consequence of tauopathy may further exacerbate amyloid beta in AD.

Of particular interest was the evidence of a clear relationship between tau and the downstream neurovascular damage in the R955-hTau^{+/+} rat model. Neurovascular impairments in the CNS are multifactorial and can be caused by a dysfunctional BBB and/or a damaged NVU. Observations of neurovascular impairments reported in AD are generally associated with soluble amyloid beta species inducing cerebral atherosclerosis and arteriolosclerosis. (Arvanitakis et al., 2016; Carrano et al., 2012; Kalaria and Deramecourt, 2012; Wisniewski et al., 1997; Wisniewski and Kozlowski, 1982). Otherwise, amyloid deposits in the microvasculature, also known as cerebral amyloid angiopathy (CAA), has been described. Otherwise, amyloid deposits in the microvasculature, also known as cerebral amyloid angiopathy (CAA), has been described. As tau pathology is typically presented with post-mortem comorbidities such as neurovascular impairment, the fibrillary tau pathology may be an important contributor (Canepa and Fossati, 2021; Michalicova et al., 2020). In agreement with the neurovascular damage observed in R955-hTau^{+/+} rats, tau has been shown to correlate with cognition in small vessel disease (SVD) independent of amyloid beta in familial AD (Littau et al., 2022). In addition, cerebral microbleeds were detected in patients with frontotemporal lobar degeneration (De Reuck et al., 2012) and Lewy Body dementia (De Reuck et al., 2013) which are also conditions devoid of overt amyloid pathology. Furthermore, the typical indicators of NVU damage such as dilation of the perivascular space and cell loss (Merlini et al., 2016; Sengillo et al., 2013; Vilor-Tejedor et al., 2021; Wardlaw et al., 2013), swollen astrocytic end-feet (Hoshi et al., 2012; Moftakhar et al., 2010; Wilcock et al., 2009; Zeppenfeld et al., 2017), as well as damaged endothelial cell morphologies (Hohman et

al., 2015; Tang et al., 2013; Thomas et al., 2015), and SVD (Liu et al., 2018; Wardlaw et al., 2017) were similarly revealed in the R955-hTau^{+/+} rat model of human tauopathy.

The present report illustrates NVU alterations including the extravasation of immunoglobulins (IgG), leakage of peripherally injected HRP tracer into the CNS, reduced pericyte coverage and thickening of the basement membrane, which are findings consistent with a report of pericyte-deficient mice displaying reduced cerebral blood flow, and increased leakage of IgG's (Bell et al., 2010). An increase of Collagen IV content in the brains of R955-hTau^{+/+} rats suggests a tau-dependent neurovascular remodeling in the absence of CAA, which has similarly been observed in a mouse model of tauopathy (Bennett et al., 2020, 2018). Interestingly, halting tau expression in a transgenic model of tauopathy, as done by Blair and colleagues, reduced IgG's, further emphasizing how tau pathology influences the status of the BBB (Blair et al., 2015).

Importantly, the initial, overt, tau hyperphosphorylation and astrogliosis was first identified in the vascular rich SLM after 14 months of age, which likely had a downstream implication on the neurovascular unit. Tau pathology in astrocytes has recently been reported in FTD (Garwood et al., 2017). It has been shown that tau-burdened astrocytes in P301S mice were unable to provide support to neurons *in vitro* (Sidoryk-Wegrzynowicz et al., 2017; Yousef et al., 2017). In the CNS of R955-hTau^{+/+} rats, astrocytes were also observed with an altered polarization and upregulation of AQP4 water channels. AQP4 plays a pivotal role in fluid movement between perivascular spaces and interstitium (Lan et al., 2016; Silva et al., 2021) and its altered polarization has already been described in AD (Chandra et al., 2021; Lan et al., 2016; Valenza et al., 2020; Zeppenfeld et al., 2017) and in hydrocephalus (Skjolding et al., 2010). Therefore, the altered polarization of AQP4 in the R955-hTau^{+/+} may serve to exacerbate tau accumulation and the ventricular dilation by a mechanism related to fluid exchange in the CNS. Taken together, the occurrence of misfolded tau at the neurovascular unit would suggest that astrocytes play a primary role in tau-associated neurovascular compromise.

In summary, the R955-hTau transgenic rat model has considerable translational value for experimental pharmacology studies with a phenotype reminiscent of several key aspects of human tauopathy. Using the R955-hTau transgenic rat enabled the discovery of novel aspects of tauopathy at early pre- and post-fibrillary stages such as a clear downstream consequence on the neurovascular unit.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JTE contributed to study conceptualization and design, formal data analysis and visualization, optimized methodologies, undertook the bulk of the investigation, drafting the original manuscript. YL assisted with IHC experiments, cell counts and Western blots. AS assisted with Western blot experiments. QB and AL assisted with tissue preparation of Sarkosyl extracts. JCM contributed to conceptualization and methodologies. SDC and LB designed the R955-hTau transgene construct. SDC and ACC supervised, acquired funding, contributed to study conceptualization, review & editing of the manuscript.

Funding

JTE received support from the Fonds de recherche Quebec - Santé (FRQS) doctoral training fellowship 2020-21, Hugh E. Burke fellowship 2019-20 and F.S.B Miller Memorial fellowship 2017-18. JCM was the recipient of the Rotary Foundation's Global Grant Scholarship. SDC is the holder of the Charles E. Frosst/Merck Research Associate position. This study was supported by the Canadian Institute of Health Research, the Canadian Consortium of Neurodegeneration and Aging (CCNA), the Healthy Brains Healthy Lives and BrainsCAN McGill - Western Collaboration Grant (MWCG) and the Morris and Rosalind Goodman Family Foundation and a donation from Dr. Moussa Youdin to ACC's Laboratory. ACC is the holder of the Charles E. Frosst/Merck-endowed Chair in Pharmacology and a member of the Canadian Consortium on Neurodegeneration in Aging.

Acknowledgements

The cDNA of the transgene construct was obtained with the advice of Dr. Michel Goedert and the MRC lab of molecular biology, Cambridge, UK. Confocal image collection was performed in the McGill University Life Sciences Complex Advanced BioImaging Facility. We thank and acknowledge Johanne Oulette for the technical assistance provided for some of the sample preparation for electron microscopy. MRI procedures were facilitated by the Small Animals Imaging Labs located at the Research Institute of the McGill University Health Center (RI-MUHC).

5. References

- Allen, B., Ingram, E., Takao, M., Smith, M.J., Jakes, R., Virdee, K., Yoshida, H., Holzer, M., Craxton, M., Emson, P.C., Atzori, C., Migheli, A., Crowther, R.A., Ghetti, B., Spillantini, M.G., Goedert, M., 2002. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J. Neurosci.* 22, 9340–9351. <https://doi.org/22/21/9340> [pii]
- Andrási, E., Farkas, É., Scheibler, H., Réffy, A., Bezúr, L., 1995. Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. *Arch. Gerontol. Geriatr.* 21, 89–97. [https://doi.org/10.1016/0167-4943\(95\)00643-Y](https://doi.org/10.1016/0167-4943(95)00643-Y)
- Antunes, M., Biala, G., 2012. The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cogn. Process.* 13, 93–110. <https://doi.org/10.1007/s10339-011-0430-z>
- Arboleda-Velasquez, J.F., Lopera, F., O'Hare, M., Delgado-Tirado, S., Marino, C., Chmielewska, N., Saez-Torres, K.L., Amarnani, D., Schultz, A.P., Sperling, R.A., Leyton-Cifuentes, D., Chen, K., Baena, A., Aguillon, D., Rios-Romenets, S., Giraldo, M., Guzmán-Vélez, E., Norton, D.J., Pardilla-Delgado, E., Artola, A., Sanchez, J.S., Acosta-Uribe, J., Lalli, M., Kosik, K.S., Huentelman, M.J., Zetterberg, H., Blennow, K., Reiman, R.A., Luo, J., Chen, Y., Thiyyagura, P., Su, Y., Jun, G.R., Naymik, M., Gai, X., Bootwalla, M., Ji, J., Shen, L., Miller, J.B., Kim, L.A., Tariot, P.N., Johnson, K.A., Reiman, E.M., Quiroz, Y.T., 2019. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nat. Med.* 25, 1680–1683. <https://doi.org/10.1038/s41591-019-0611-3>
- Arriagada, P. V, Growdon, J.H., Hedleywhyte, E.T., Hyman, B.T., Hedley-Whyte, E.T., Hyman, B.T., 1992. Neurofibrillary Tangles But Not Senile Plaques Parallel Duration and Severity of Alzheimers-Disease. *Neurology* 42, 631–639. <https://doi.org/10.1212/WNL.42.3.631>
- Arvanitakis, Z., Capuano, A.W., Leurgans, S.E., Bennett, D.A., Schneider, J.A., 2016. Relation of cerebral vessel disease to Alzheimer's disease dementia and cognitive function in elderly people: a cross-sectional study. *Lancet Neurol.* 15, 934–943. [https://doi.org/10.1016/S1474-4422\(16\)30029-1](https://doi.org/10.1016/S1474-4422(16)30029-1)
- Aschenbrenner, A.J., Gordon, B.A., Benzinger, T.L.S., Morris, J.C., Hassenstab, J.J., 2018. Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology* 91, e859–e866. <https://doi.org/10.1212/WNL.00000000000006075>
- Ashton, N.J., Pascoal, T.A., Karikari, T.K., Benedet, A.L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., Blennow, K., 2021. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* 141, 709–724. <https://doi.org/10.1007/s00401-021-02275-6>
- Bachstetter, A.D., Van Eldik, L.J., Schmitt, F.A., Neltner, J.H., Ighodaro, E.T., Webster, S.J., Patel, E., Abner, E.L., Kryscio, R.J., Nelson, P.T., 2015. Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging. *Acta Neuropathol. Commun.* 3, 32. <https://doi.org/10.1186/s40478-015-0209-z>

- 2141 Baek, M.S., Cho, H., Lee, H.S., Lee, J.H., Ryu, Y.H., Lyoo, C.H., 2020. Effect of APOE ϵ 4
2142 genotype on amyloid- β and tau accumulation in Alzheimer's disease. *Alzheimer's Res. Ther.*
2143 12, 1–12. <https://doi.org/10.1186/s13195-020-00710-6>
- 2144 Bateman, R.J., Xiong, C., Benzinger, T.L.S., Fagan, A.M., Goate, A., Fox, N.C., Marcus, D.S.,
2145 Cairns, N.J., Xie, X., Blazey, T.M., Holtzman, D.M., Santacruz, A., Buckles, V., Oliver, A.,
2146 Moulder, K., Aisen, P.S., Ghetti, B., Klunk, W.E., McDade, E., Martins, R.N., Masters, C.L.,
2147 Mayeux, R., Ringman, J.M., Rossor, M.N., Schofield, P.R., Sperling, R.A., Salloway, S.,
2148 Morris, J.C., Dominantly Inherited Alzheimer Network, 2012. Clinical and biomarker
2149 changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* 367, 795–804.
2150 <https://doi.org/10.1056/NEJMoa1202753>
- 2151 Bejanin, A., Schonhaut, D.R., La Joie, R., Kramer, J.H., Baker, S.L., Sosa, N., Ayakta, N.,
2152 Cantwell, A., Janabi, M., Lauriola, M., O'Neil, J.P., Gorno-Tempini, M.L., Miller, Z.A.,
2153 Rosen, H.J., Miller, B.L., Jagust, W.J., Rabinovici, G.D., 2017. Tau pathology and
2154 neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain* 140,
2155 3286–3300. <https://doi.org/10.1093/brain/awx243>
- 2156 Bell, R.D., Winkler, E.A., Sagare, A.P., Singh, I., LaRue, B., Deane, R., Zlokovic, B. V., 2010.
2157 Pericytes Control Key Neurovascular Functions and Neuronal Phenotype in the Adult Brain
2158 and during Brain Aging. *Neuron* 68, 409–427. <https://doi.org/10.1016/j.neuron.2010.09.043>
- 2159 Bennett, D.A., Schneider, J.A., Wilson, R.S., Bienias, J.L., Arnold, S.E., 2004. Neurofibrillary
2160 Tangles Mediate the Association of Amyloid Load with Clinical Alzheimer Disease and
2161 Level of Cognitive Function. *Arch. Neurol.* 61, 378–384.
2162 <https://doi.org/10.1001/archneur.61.3.378>
- 2163 Bennett, R.E., Hu, M., Fernandes, A., Perez-Rando, M., Robbins, A., Kamath, T., Dujardin, S.,
2164 Hyman, B.T., 2020. Tau reduction in aged mice does not impact Microangiopathy. *Acta*
2165 *Neuropathol. Commun.* 8, 1–16. <https://doi.org/10.1186/s40478-020-01014-4>
- 2166 Bennett, R.E., Robbins, A.B., Hu, M., Cao, X., Betensky, R.A., Clark, T., Das, S., Hyman, B.T.,
2167 2018. Tau induces blood vessel abnormalities and angiogenesis-related gene expression in
2168 P301L transgenic mice and human Alzheimer's disease. *Proc. Natl. Acad. Sci.* 115, 1–10.
2169 <https://doi.org/10.1073/pnas.1710329115>
- 2170 Biel, D., Brendel, M., Rubinski, A., Buerger, K., Janowitz, D., Dichgans, M., Franzmeier, N.,
2171 2021. Tau-PET and in vivo Braak-staging as prognostic markers of future cognitive decline
2172 in cognitively normal to demented individuals. *Alzheimer's Res. Ther.* 13, 1–13.
2173 <https://doi.org/10.1186/s13195-021-00880-x>
- 2174 Blair, L.J., Frauen, H.D., Zhang, B., Nordhues, B.A., Bijan, S., Lin, Y.C., Zamudio, F., Hernandez,
2175 L.D., Sabbagh, J.J., Selenica, M.L.B., Dickey, C.A., 2015. Tau depletion prevents progressive
2176 blood-brain barrier damage in a mouse model of tauopathy. *Acta Neuropathol. Commun.* 3,
2177 8. <https://doi.org/10.1186/s40478-015-0186-2>
- 2178 Bodea, L.G., Evans, H.T., Van der Jeugd, A., Ittner, L.M., Delerue, F., Kril, J., Halliday, G.,
2179 Hodges, J., Kiernan, M.C., Götz, J., 2017. Accelerated aging exacerbates a pre-existing
2180 pathology in a tau transgenic mouse model. *Aging Cell* 16, 377–386.
2181 <https://doi.org/10.1111/accel.12565>
- 2182 Braak, H., Alafuzoff, I., Arzberger, T., Kretschmar, H., Tredici, K., 2006. Staging of Alzheimer

- 2183 disease-associated neurofibrillary pathology using paraffin sections and
 2184 immunocytochemistry. *Acta Neuropathol.* 112, 389–404. [https://doi.org/10.1007/s00401-](https://doi.org/10.1007/s00401-006-0127-z)
 2185 006-0127-z
- 2186 Braak, H., Braak, E., 1995. Staging of Alzheimer's Disease-Related Neurofibrillary Changes.
 2187 *Neurobiol. Aging* 16, 271–278.
- 2188 Braak, H., Braak, E., 1991. Neuropathological staging of Alzheimer-related changes. *Acta*
 2189 *Neuropathol.* 82, 239–259. <https://doi.org/10.1007/BF00308809>
- 2190 Brier, M.R., Gordon, B., Friedrichsen, K., McCarthy, J., Stern, A., Christensen, J., Owen, C.,
 2191 Aldea, P., Su, Y., Hassenstab, J., Cairns, N.J., Holtzman, D.M., Fagan, A.M., Morris, J.C.,
 2192 Benzinger, T.L.S., Ances, B.M., 2016. Tau and Ab imaging, CSF measures, and cognition in
 2193 Alzheimer's disease. *Sci. Transl. Med.* 8, 1–10. <https://doi.org/10.1126/scitranslmed.aaf2362>
- 2194 Buchanan, H., Mackay, M., Palmer, K., Tothová, K., Katsur, M., Platt, B., Koss, D.J., 2020.
 2195 Synaptic Loss, ER Stress and Neuro-Inflammation Emerge Late in the Lateral Temporal
 2196 Cortex and Associate with Progressive Tau Pathology in Alzheimer's Disease. *Mol.*
 2197 *Neurobiol.* 3258–3272. <https://doi.org/10.1007/s12035-020-01950-1>
- 2198 Bugiani, O., Murrell, J.R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., Morbin, M.,
 2199 Primavera, A., Carella, F., Solaro, C., Grisoli, M., Savoirdo, M., Spillantini, M.G.,
 2200 Tagliavini, F., Goedert, M., Ghetti, B., 1999. Frontotemporal dementia and corticobasal
 2201 degeneration in a family with a P301S mutation in Tau. *J. Neuropathol. Exp. Neurol.* 58, 667–
 2202 677.
- 2203 Canepa, E., Fossati, S., 2021. Impact of Tau on Neurovascular Pathology in Alzheimer's Disease.
 2204 *Front. Neurol.* 11, 1–19. <https://doi.org/10.3389/fneur.2020.573324>
- 2205 Cao, W., Zheng, H., 2018. Correction to: Peripheral immune system in aging and Alzheimer's
 2206 disease. *Mol. Neurodegener.* 13, 1–17. <https://doi.org/10.1186/s13024-018-0290-4>
- 2207 Carrano, A., Hoozemans, J.J.M., Van Der Vies, S.M., Van Horssen, J., De Vries, H.E.,
 2208 Rozemuller, A.J.M., 2012. Neuroinflammation and blood-brain barrier changes in capillary
 2209 amyloid angiopathy. *Neurodegener. Dis.* 10, 329–331. <https://doi.org/10.1159/000334916>
- 2210 Chandra, A., Farrell, C., Wilson, H., Dervenoulas, G., De Natale, E.R., Politis, M., 2021.
 2211 Aquaporin-4 polymorphisms predict amyloid burden and clinical outcome in the Alzheimer's
 2212 disease spectrum. *Neurobiol. Aging* 97, 1–9.
 2213 <https://doi.org/10.1016/j.neurobiolaging.2020.06.007>
- 2214 Chen, Y.T., Chen, W.Y., Huang, X.T., Xu, Y.C., Zhang, H.Y., 2018. Iron dysregulates APP
 2215 processing accompanying with sAPP α cellular retention and β -secretase inhibition in rat
 2216 cortical neurons. *Acta Pharmacol. Sin.* 39, 177–183. <https://doi.org/10.1038/aps.2017.113>
- 2217 Chiotis, K., Savitcheva, I., Poulakis, K., Saint-Aubert, L., Wall, A., Antoni, G., Nordberg, A.,
 2218 2020. [18F]THK5317 imaging as a tool for predicting prospective cognitive decline in
 2219 Alzheimer's disease. *Mol. Psychiatry.* <https://doi.org/10.1038/s41380-020-0815-4>
- 2220 Cohen, R.M., Rezai-zadeh, K., Weitz, T.M., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y.,
 2221 Vasilevko, V., Glabe, C.G., Breunig, J.J., Rakic, P., Davtayan, H., Agadjanyan, M.G., 2013.
 2222 A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric
 2223 A β and frank neuronal loss. *J. Neurosci* 33, 6245–6256.

- 2224 <https://doi.org/10.1523/JNEUROSCI.3672-12.2013.A>
- 2225 De Reuck, J., Deramecourt, V., Cordonnier, C., Auger, F., Durieux, N., Bordet, R., Maurage, C.A.,
 2226 Leys, D., Pasquier, F., 2012. Detection of microbleeds in post-mortem brains of patients with
 2227 frontotemporal lobar degeneration: A 7.0-Tesla magnetic resonance imaging study with
 2228 neuropathological correlates. *Eur. J. Neurol.* 19, 1355–1360. [https://doi.org/10.1111/j.1468-](https://doi.org/10.1111/j.1468-1331.2012.03776.x)
 2229 1331.2012.03776.x
- 2230 De Reuck, J., Deramecourt, V., Cordonnier, C., Leys, D., Pasquier, F., Maurage, C.A., 2013.
 2231 Prevalence of cerebrovascular lesions in patients with Lewy body dementia: A
 2232 neuropathological study. *Clin. Neurol. Neurosurg.* 115, 1094–1097.
 2233 <https://doi.org/10.1016/j.clineuro.2012.11.005>
- 2234 Di, J., Cohen, L.S., Corbo, C.P., Phillips, G.R., El Idrissi, A., Alonso, A.D., 2016. Abnormal tau
 2235 induces cognitive impairment through two different mechanisms: Synaptic dysfunction and
 2236 neuronal loss. *Sci. Rep.* 6, 1–12. <https://doi.org/10.1038/srep20833>
- 2237 Do Carmo, S., Cuello, A.C., 2013. Modeling Alzheimer's disease in transgenic rats. *Mol.*
 2238 *Neurodegener.* 8, 37. <https://doi.org/10.1186/1750-1326-8-37>
- 2239 Du, Y., Deng, W., Wang, Z., Ning, M., Zhang, W., Zhou, Y., Lo, E.H., Xing, C., 2017. Differential
 2240 subnetwork of chemokines/ cytokines in human, mouse, and rat brain cells after oxygen-
 2241 glucose deprivation. *J. Cereb. Blood Flow Metab.* 37, 1425–1434.
 2242 <https://doi.org/10.1177/0271678X16656199>
- 2243 Dujardin, S., Colin, M., Buée, L., 2015. Invited review: Animal models of tauopathies and their
 2244 implications for research/translation into the clinic. *Neuropathol. Appl. Neurobiol.* 41, 59–
 2245 80. <https://doi.org/10.1111/nan.12200>
- 2246 Fleisher, A.S., Chen, K., Quiroz, Y.T., Jakimovich, L.J., Gutierrez Gomez, M., Langois, C.M.,
 2247 Langbaum, J.B.S., Roontiva, A., Thiyyagura, P., Lee, W., Ayutyanont, N., Lopez, L.,
 2248 Moreno, S., Muñoz, C., Tirado, V., Acosta-Baena, N., Fagan, A.M., Giraldo, M., Garcia, G.,
 2249 Huentelman, M.J., Tariot, P.N., Lopera, F., Reiman, E.M., 2015. Associations between
 2250 biomarkers and age in the presenilin 1 E280A autosomal dominant Alzheimer disease
 2251 kindred: A cross-sectional study. *JAMA Neurol.* 72, 316–324.
 2252 <https://doi.org/10.1001/jamaneurol.2014.3314>
- 2253 Flores-Aguilar, L., Iulita, M.F., Kovacs, O., Torres, M.D., Levi, S.M., Zhang, Y., Askenazi, M.,
 2254 Wisniewski, T., Busciglio, J., Cuello, A.C., 2020. Evolution of neuroinflammation across the
 2255 lifespan of individuals with down syndrome. *Brain* 143, 3653–3671.
 2256 <https://doi.org/10.1093/brain/awaa326>
- 2257 Foidl, B.M., Humpel, C., 2018. Differential Hyperphosphorylation of Tau-S199, -T231 and -S396
 2258 in Organotypic Brain Slices of Alzheimer Mice. A Model to Study Early Tau
 2259 Hyperphosphorylation Using Okadaic Acid. *Front. Aging Neurosci.* 10, 113.
 2260 <https://doi.org/10.3389/fnagi.2018.00113>
- 2261 Frank, S., Clavaguera, F., Tolnay, M., 2008. Tauopathy models and human neuropathology:
 2262 Similarities and differences. *Acta Neuropathol.* 115, 39–53. [https://doi.org/10.1007/s00401-](https://doi.org/10.1007/s00401-007-0291-9)
 2263 007-0291-9
- 2264 Furcila, D., Domínguez-Álvaro, M., DeFelipe, J., Alonso-Nanclares, L., 2019. Subregional
 2265 Density of Neurons, Neurofibrillary Tangles and Amyloid Plaques in the Hippocampus of

- 2266 Patients With Alzheimer's Disease. *Front. Neuroanat.* 13, 1–11.
 2267 <https://doi.org/10.3389/fnana.2019.00099>
- 2268 Garwood, C.J., Ratcliffe, L.E., Simpson, J.E., Heath, P.R., Ince, P.G., Wharton, S.B., 2017.
 2269 Review: Astrocytes in Alzheimer's disease and other age-associated dementias: a supporting
 2270 player with a central role. *Neuropathol. Appl. Neurobiol.* <https://doi.org/10.1111/nan.12338>
- 2271 Giannakopoulos, P., Herrmann, F.R., Bussière, T., Bouras, C., Kövari, E., Perl, D.P., Morrison,
 2272 J.H., Gold, G., Hof, P.R., 2003. Tangle and neuron numbers, but not amyloid load, predict
 2273 cognitive status in Alzheimer's disease. *Neurology* 60, 1495–1500.
 2274 <https://doi.org/10.1212/01.WNL.0000063311.58879.01>
- 2275 Goedert, M., Spillantini, M.G., 2000. Tau mutations in frontotemporal dementia FTDP-17 and
 2276 their relevance for Alzheimer's disease. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1502, 110–
 2277 121. [https://doi.org/10.1016/S0925-4439\(00\)00037-5](https://doi.org/10.1016/S0925-4439(00)00037-5)
- 2278 Goetzl, E.J., Kapogiannis, D., Schwartz, J.B., Lobach, I. V., Goetzl, L., Abner, E.L., Jicha, G.A.,
 2279 Karydas, A.M., Boxer, A., Miller, B.L., 2016. Decreased synaptic proteins in neuronal
 2280 exosomes of frontotemporal dementia and Alzheimer's disease. *FASEB J.* 30, 4141–4148.
 2281 <https://doi.org/10.1096/fj.201600816R>
- 2282 Goodman, L., 1953. Alzheimer's disease; a clinico-pathologic analysis of twenty-three cases with
 2283 a theory on pathogenesis. *J. Nerv. Ment. Dis.* 118, 97–130.
- 2284 Götz, J., Chen, F., Barmettler, R., Nitsch, R.M., 2001. Tau filament formation in transgenic mice
 2285 expressing P301L tau. *J. Biol. Chem.* 276, 529–534. <https://doi.org/10.1074/jbc.M006531200>
- 2286 Gotz, J., Chen, F., van Dorpe, J., Nitsch, R.M., 2001. Formation of Neurofibrillary Tangles in
 2287 P301L Tau Transgenic Mice Induced by Abeta 42 Fibrils. *Science (80-.)*. 293, 1491–1495.
 2288 <https://doi.org/10.1126/science.1062097>
- 2289 Götz, J.J., Götz, J., 2019. Experimental Models of Tauopathy – From Mechanisms to Therapies,
 2290 in: Takashima, A., Wolozin, B., Buee, L. (Eds.), *Advances in Experimental Medicine and*
 2291 *Biology*. Springer Singapore, Singapore, pp. 381–391. [https://doi.org/10.1007/978-981-32-](https://doi.org/10.1007/978-981-32-9358-8_28)
 2292 [9358-8_28](https://doi.org/10.1007/978-981-32-9358-8_28)
- 2293 Guerreiro, R., Bras, J., 2015. The age factor in Alzheimer's disease. *Genome Med.* 7, 1–3.
 2294 <https://doi.org/10.1186/s13073-015-0232-5>
- 2295 Guillozet, A.L., Weintraub, S., Mash, D.C., Marsel Mesulam, M., 2003. Neurofibrillary tangles,
 2296 amyloid, and memory in aging and mild cognitive impairment. *Arch. Neurol.* 60, 729–736.
 2297 <https://doi.org/10.1001/archneur.60.5.729>
- 2298 Guo, Q., Li, H., Cole, A.L., Hur, J.Y., Li, Y., Zheng, H., 2013. Modeling alzheimer's disease in
 2299 mouse without mutant protein overexpression: Cooperative and independent effects of A β
 2300 and tau. *PLoS One* 8, 1–14. <https://doi.org/10.1371/journal.pone.0080706>
- 2301 Hall, H., Iulita, M.F., Gubert, P., Flores Aguilar, L., Ducatzenzeiler, A., Fisher, A., Cuellar, A.C.,
 2302 2018. AF710B, an M1/sigma-1 receptor agonist with long-lasting disease-modifying
 2303 properties in a transgenic rat model of Alzheimer's disease. *Alzheimer's Dement.* 14, 811–
 2304 823. <https://doi.org/10.1016/j.jalz.2017.11.009>
- 2305 Hanes, J., Zilka, N., Bartkova, M., Caletkova, M., Dobrota, D., Novak, M., 2009. Rat tau proteome
 2306 consists of six tau isoforms: Implication for animal models of human tauopathies. *J.*

- 2307 Neurochem. 108, 1167–1176. <https://doi.org/10.1111/j.1471-4159.2009.05869.x>
- 2308 Hanseeuw, B.J., Betensky, R.A., Jacobs, H.I.L., Schultz, A.P., Sepulcre, J., Becker, J.A., Cosio,
2309 D.M.O., Farrell, M., Quiroz, Y.T., Mormino, E.C., Buckley, R.F., Papp, K. V., Amariglio,
2310 R.A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G.A., Chhatwal, J.P.,
2311 Rentz, D.M., Sperling, R.A., Johnson, K., 2019. Association of Amyloid and Tau with
2312 Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. *JAMA Neurol.* 76, 915–
2313 924. <https://doi.org/10.1001/jamaneurol.2019.1424>
- 2314 Hasegawa, M., Morishima-Kawashima, M., Takio, K., Suzuki, M., Titani, K., Ihara, Y., 1992.
2315 Protein sequence and mass spectrometric analyses of tau in the Alzheimer's disease brain. *J.*
2316 *Biol. Chem.* 267, 17047–17054.
- 2317 Hernández, F., Merchán-Rubira, J., Vallés-Saiz, L., Rodríguez-Matellán, A., Avila, J., 2020.
2318 Differences Between Human and Murine Tau at the N-terminal End. *Front. Aging Neurosci.*
2319 12, 1–6. <https://doi.org/10.3389/fnagi.2020.00011>
- 2320 Hohman, T.J., Bell, S.P., Jefferson, A.L., 2015. The role of vascular endothelial growth factor in
2321 neurodegeneration and cognitive decline: Exploring interactions with biomarkers of
2322 Alzheimer disease. *JAMA Neurol.* 72, 520–529.
2323 <https://doi.org/10.1001/jamaneurol.2014.4761>
- 2324 Holland, N., Jones, P.S., Savulich, G., Wiggins, J.K., Hong, Y.T., Fryer, T.D., Manavaki, R.,
2325 Sephton, S.M., Boros, I., Malpetti, M., Hezemans, F.H., Aigbirhio, F.I., Coles, J.P., O'Brien,
2326 J., Rowe, J.B., 2020. Synaptic Loss in Primary Tauopathies Revealed by [11C]UCB-J
2327 Positron Emission Tomography. *Mov. Disord.* mds.28188.
2328 <https://doi.org/10.1002/mds.28188>
- 2329 Hoshi, A., Yamamoto, T., Shimizu, K., Ugawa, Y., Nishizawa, M., Takahashi, H., Kakita, A.,
2330 2012. Characteristics of aquaporin expression surrounding senile plaques and cerebral
2331 amyloid angiopathy in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 71, 750–759.
2332 <https://doi.org/10.1097/NEN.0b013e3182632566>
- 2333 Iulita, M.F., Allard, S., Richter, L., Munter, L.-M., Ducatenzeiler, A., Weise, C., Do Carmo, S.,
2334 Klein, W.L., Multhaup, G., Cuellar, A.C., 2014. Intracellular A β pathology and early cognitive
2335 impairments in a transgenic rat overexpressing human amyloid precursor protein: a
2336 multidimensional study. *Acta Neuropathol. Commun.* 2, 61. <https://doi.org/10.1186/2051-5960-2-61>
- 2338 Jablonski, A.M., Warren, L., Usenovic, M., Zhou, H., Sugam, J., Parmentier-Batteur, S., Voleti,
2339 B., 2021. Astrocytic expression of the Alzheimer's disease risk allele, ApoE ϵ 4, potentiates
2340 neuronal tau pathology in multiple preclinical models. *Sci. Rep.* 11, 3438.
2341 <https://doi.org/10.1038/s41598-021-82901-1>
- 2342 Jack, C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman,
2343 D.M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J.L., Montine, T., Phelps, C.,
2344 Rankin, K.P., Rowe, C.C., Scheltens, P., Siemers, E., Snyder, H.M., Sperling, R., Elliott, C.,
2345 Masliah, E., Ryan, L., Silverberg, N., 2018. NIA-AA Research Framework: Toward a
2346 biological definition of Alzheimer's disease. *Alzheimer's Dement.*
2347 <https://doi.org/10.1016/j.jalz.2018.02.018>
- 2348 Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M.,

- 2349 Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C.,
 2350 Trojanowski, J.Q., 2013. Tracking pathophysiological processes in Alzheimer's disease: An
 2351 updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12, 207–216.
 2352 [https://doi.org/10.1016/S1474-4422\(12\)70291-0](https://doi.org/10.1016/S1474-4422(12)70291-0)
- 2353 Jack, C.R., Wiste, H.J., Weigand, S.D., Therneau, T.M., Lowe, V.J., Knopman, D.S., Botha, H.,
 2354 Graff-Radford, J., Jones, D.T., Ferman, T.J., Boeve, B.F., Kantarci, K., Vemuri, P., Mielke,
 2355 M.M., Whitwell, J., Josephs, K., Schwarz, C.G., Senjem, M.L., Gunter, J.L., Petersen, R.C.,
 2356 2020. Predicting future rates of tau accumulation on PET. *Brain* 143, 3136–3150.
 2357 <https://doi.org/10.1093/brain/awaa248>
- 2358 Jicha, G.A., Bowser, R., Kazam, I.G., Davies, P., 1997. Alz-50 and MC-1, a new monoclonal
 2359 antibody raised to paired helical filaments, recognize conformational epitopes on
 2360 recombinant tau. *J. Neurosci. Res.* 48, 128–132. [https://doi.org/10.1002/\(SICI\)1097-4547\(19970415\)48:2<128::AID-JNR5>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-4547(19970415)48:2<128::AID-JNR5>3.0.CO;2-E)
- 2362 Joie, R. La, Visani, A. V., Baker, S.L., Brown, J.A., Bourakova, V., Cha, J., Chaudhary, K.,
 2363 Edwards, L., Iaccarino, L., Janabi, M., Lesman-Segev, O.H., Miller, Z.A., Perry, D.C.,
 2364 O'Neil, J.P., Pham, J., Rojas, J.C., Rosen, H.J., Seeley, W.W., Tsai, R.M., Miller, B.L.,
 2365 Jagust, W.J., Rabinovici, G.D., 2020. Prospective longitudinal atrophy in Alzheimer's disease
 2366 correlates with the intensity and topography of baseline tau-PET. *Sci. Transl. Med.* 12, 1–13.
 2367 <https://doi.org/10.1126/scitranslmed.aau5732>
- 2368 Jørgensen, M.B., Finsen, B.R., Jensen, M.B., Castellano, B., Diemer, N.H., Zimmer, J., 1993.
 2369 Microglial and Astroglial Reactions to Ischemic and Kainic Acid-Induced Lesions of the
 2370 Adult Rat Hippocampus. *Exp. Neurol.* <https://doi.org/10.1006/exnr.1993.1041>
- 2371 Kalaria, R.N., Deramecourt, V., 2012. Staging and natural history of cerebrovascular pathology in
 2372 dementia. *Neurology* 79, 107.
- 2373 Knopman, D.S., Lundt, E.S., Therneau, T.M., Vemuri, P., Lowe, V.J., Kantarci, K., Gunter, J.L.,
 2374 Senjem, M.L., Mielke, M.M., Machulda, M.M., Boeve, B.F., Jones, D.T., Graff-Radford, J.,
 2375 Albertson, S.M., Schwarz, C.G., Petersen, R.C., Jack, C.R., 2019. Entorhinal cortex tau,
 2376 amyloid- β , cortical thickness and memory performance in non-demented subjects. *Brain* 1–
 2377 13. <https://doi.org/10.1093/brain/awz025>
- 2378 Kopeikina, K.J., Polydoro, M., Tai, H.C., Yaeger, E., Carlson, G.A., Pitstick, R., Hyman, B.T.,
 2379 Spires-Jones, T.L., 2013. Synaptic alterations in the rTg4510 mouse model of tauopathy. *J.*
 2380 *Comp. Neurol.* 521, 1334–1353. <https://doi.org/10.1002/cne.23234>
- 2381 LaDu, M.J., Lukens, J.R., Reardon, C.A., Getz, G.S., 1997. Association of human, rat, and rabbit
 2382 apolipoprotein E with β -amyloid. *J. Neurosci. Res.* 49, 9–18.
 2383 [https://doi.org/10.1002/\(SICI\)1097-4547\(19970701\)49:1<9::AID-JNR2>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-4547(19970701)49:1<9::AID-JNR2>3.0.CO;2-H)
- 2384 Lan, Y.L., Zhao, J., Ma, T., Li, S., 2016. The Potential Roles of Aquaporin 4 in Alzheimer's
 2385 Disease. *Mol. Neurobiol.* 53, 5300–5309. <https://doi.org/10.1007/s12035-015-9446-1>
- 2386 Leon, W.C., Canneva, F., Partridge, V., Allard, S., Ferretti, M.T., Dewilde, A., Vercauteren, F.,
 2387 Atifeh, R., Ducatzenzeiler, A., Klein, W., Szyf, M., Alhonen, L., Cuellar, A.C., 2010. A novel
 2388 transgenic rat model with a full Alzheimer's-Like amyloid pathology displays pre-plaque
 2389 intracellular amyloid-B-Associated cognitive impairment. *J. Alzheimer's Dis.* 20, 113–126.
 2390 <https://doi.org/10.3233/JAD-2010-1349>

- 2391 Leung, C., Cao, F., Nguyen, R., Joshi, K., Aqrabawi, A.J., Xia, S., Cortez, M.A., Snead, O.C.,
 2392 Kim, J.C., Jia, Z., 2018a. Activation of Entorhinal Cortical Projections to the Dentate Gyrus
 2393 Underlies Social Memory Retrieval. *Cell Rep.* 23, 2379–2391.
 2394 <https://doi.org/10.1016/j.celrep.2018.04.073>
- 2395 Leung, C., Kim, J., Jia, Z., 2018b. Three-chamber Social Approach Task with Optogenetic
 2396 Stimulation (Mice). *Bio-Protocol* 8, 1–12. <https://doi.org/10.21769/bioprotoc.3120>
- 2397 Littau, J.L., Velilla, L., Hase, Y., Villalba-Moreno, N.D., Hagel, C., Drexler, D., Osorio Restrepo,
 2398 S., Villegas, A., Lopera, F., Vargas, S., Glatzel, M., Krasemann, S., Quiroz, Y.T., Arboleda-
 2399 Velasquez, J.F., Kalara, R., Sepulveda-Falla, D., Villalba-Moreno, N.D., Hagel, C., Drexler,
 2400 D., Osorio Restrepo, S., Villegas, A., Lopera, F., Vargas, S., Glatzel, M., Krasemann, S.,
 2401 Quiroz, Y.T., Arboleda-Velasquez, J.F., Kalara, R., Sepulveda-Falla, D., 2022. Evidence of
 2402 beta amyloid independent small vessel disease in familial Alzheimer's disease. *Brain Pathol.*
 2403 1–16. <https://doi.org/10.1111/bpa.13097>
- 2404 Liu, Y., Braidy, N., Poljak, A., Chan, D.K.Y., Sachdev, P., 2018. Cerebral small vessel disease
 2405 and the risk of Alzheimer's disease: A systematic review. *Ageing Res. Rev.* 47, 41–48.
 2406 <https://doi.org/10.1016/j.arr.2018.06.002>
- 2407 Lozupone, M., Imbimbo, B. Pietro, Balducci, C., Lo Vecchio, F., Bisceglia, P., Latino, R.R.,
 2408 Leone, M., Dibello, V., Solfrizzi, V., Greco, A., Daniele, A., Watling, M., Seripa, D., Panza,
 2409 F., 2023. Does the imbalance in the apolipoprotein E isoforms underlie the pathophysiological
 2410 process of sporadic Alzheimer's disease? *Alzheimer's Dement.* 19, 353–368.
 2411 <https://doi.org/https://doi.org/10.1002/alz.12728>
- 2412 Malcolm, J.C., Breuillaud, L., Do Carmo, S., Hall, H., Welikovich, L.A., Macdonald, J.A.,
 2413 Goedert, M., Cuellar, A.C., 2019. Neuropathological changes and cognitive deficits in rats
 2414 transgenic for human mutant tau recapitulate human tauopathy. *Neurobiol. Dis.* 127, 323–
 2415 338. <https://doi.org/10.1016/j.nbd.2019.03.018>
- 2416 Masliah, E., Terry, R.D., DeTeresa, R.M., Hansen, L.A., 1989. Immunohistochemical
 2417 quantification of the synapse-related protein synaptophysin in Alzheimer disease. *Neurosci.*
 2418 *Lett.* 103, 234–239. [https://doi.org/10.1016/0304-3940\(89\)90582-X](https://doi.org/10.1016/0304-3940(89)90582-X)
- 2419 McDade, E., Wang, G., Gordon, B.A., Hassenstab, J., Benzinger, T.L.S., Buckles, V., Fagan,
 2420 A.M., Holtzman, D.M., Cairns, N.J., Goate, A.M., Marcus, D.S., Morris, J.C., Paumier, K.,
 2421 Xiong, C., Allegri, R., Berman, S.B., Klunk, W., Noble, J., Ringman, J., Ghetti, B., Farlow,
 2422 M., Sperling, R.A., Chhatwal, J., Salloway, S., Graff-Radford, N.R., Schofield, P.R., Masters,
 2423 C., Rossor, M.N., Fox, N.C., Levin, J., Jucker, M., Bateman, R.J., 2018. Longitudinal
 2424 cognitive and biomarker changes in dominantly inherited Alzheimer disease. *Neurology* 91,
 2425 E1295–E1306. <https://doi.org/10.1212/WNL.0000000000006277>
- 2426 Merlini, M., Wanner, D., Nitsch, R.M., 2016. Tau pathology-dependent remodelling of cerebral
 2427 arteries precedes Alzheimer's disease-related microvascular cerebral amyloid angiopathy.
 2428 *Acta Neuropathol.* 131, 737–752. <https://doi.org/10.1007/s00401-016-1560-2>
- 2429 Michaliova, A., Majerova, P., Kovac, A., 2020. Tau Protein and Its Role in Blood–Brain Barrier
 2430 Dysfunction. *Front. Mol. Neurosci.* 13, 1–13. <https://doi.org/10.3389/fnmol.2020.570045>
- 2431 Moftakhar, P., Lynch, M.D., Pomakian, J.L., Vinters, H. V., 2010. Aquaporin expression in the
 2432 brains of patients with or without cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol.*

- 2433 69, 1201–1209. <https://doi.org/10.1097/NEN.0b013e3181fd252c>
- 2434 Montagne, A., Nation, D.A., Sagare, A.P., Barisano, G., Sweeney, M.D., Chakhoyan, A.,
 2435 Pachicano, M., Joe, E., Nelson, A.R., D’Orazio, L.M., Buennagel, D.P., Harrington, M.G.,
 2436 Benzinger, T.L.S., Fagan, A.M., Ringman, J.M., Schneider, L.S., Morris, J.C., Reiman, E.M.,
 2437 Caselli, R.J., Chui, H.C., Tcw, J., Chen, Y., Pa, J., Conti, P.S., Law, M., Toga, A.W.,
 2438 Zlokovic, B. V., 2020. APOE4 leads to blood–brain barrier dysfunction predicting cognitive
 2439 decline. *Nature* 581, 71–76. <https://doi.org/10.1038/s41586-020-2247-3>
- 2440 Nilson, A.N., English, K.C., Gerson, J.E., Barton Whittle, T., Nicolas Crain, C., Xue, J., Sengupta,
 2441 U., Castillo-Carranza, D.L., Zhang, W., Gupta, P., Kaye, R., 2017. Tau oligomers associate
 2442 with inflammation in the brain and retina of tauopathy mice and in neurodegenerative
 2443 diseases. *J. Alzheimer’s Dis.* 55, 1083–1099. <https://doi.org/10.3233/JAD-160912>
- 2444 Nilsson, C., Waldö, M.L., Nilsson, K., Santillo, A., Vestberg, S., 2014. Age-related incidence and
 2445 family history in Frontotemporal dementia: Data from the Swedish Dementia Registry. *PLoS*
 2446 *One* 9, 4–9. <https://doi.org/10.1371/journal.pone.0094901>
- 2447 Ossenkoppele, R., Schonhaut, D.R., Schöll, M., Lockhart, S.N., Ayakta, N., Baker, S.L., O’Neil,
 2448 J.P., Janabi, M., Lazaris, A., Cantwell, A., Vogel, J., Santos, M., Miller, Z.A., Bettcher, B.M.,
 2449 Vossel, K.A., Kramer, J.H., Gorno-Tempini, M.L., Miller, B.L., Jagust, W.J., Rabinovici,
 2450 G.D., 2016. Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer’s
 2451 disease. *Brain* 139, 1551–1567. <https://doi.org/10.1093/brain/aww027>
- 2452 Ossenkoppele, R., van der Kant, R., Hansson, O., 2022. Tau biomarkers in Alzheimer’s disease:
 2453 towards implementation in clinical practice and trials. *Lancet Neurol.* 21, 726–734.
 2454 [https://doi.org/10.1016/S1474-4422\(22\)00168-5](https://doi.org/10.1016/S1474-4422(22)00168-5)
- 2455 Padurariu, M., Ciobica, A., Mavroudis, I., Fotiou, D., Baloyannis, S., 2012. Hippocampal neuronal
 2456 loss in the CA1 and CA3 areas of Alzheimer’s disease patients. *Psychiatr Danub* 24, 152–
 2457 158.
- 2458 Parent, M.J., Zimmer, E.R., Shin, M., Kang, M.S., Fonov, V.S., Mathieu, A., Aliaga, A., Kostikov,
 2459 A., Do Carmo, S., Dea, D., Poirier, J., Soucy, J.-P., Gauthier, S., Cuello, A.C., Rosa-Neto, P.,
 2460 2017. Multimodal Imaging in Rat Model Recapitulates Alzheimer’s Disease Biomarkers
 2461 Abnormalities. *J. Neurosci.* 37, 12263–12271. <https://doi.org/10.1523/JNEUROSCI.1346-17.2017>
- 2463 Peters, D.G., Pollack, A.N., Cheng, K.C., Sun, D., Saido, T., Haaf, M.P., Yang, Q.X., Connor,
 2464 J.R., Meadowcroft, M.D., 2018. Dietary lipophilic iron alters amyloidogenesis and microglial
 2465 morphology in Alzheimer’s disease knock-in APP mice. *Metallomics* 10, 426–443.
 2466 <https://doi.org/10.1039/c8mt00004b>
- 2467 Pontecorvo, M.J., Devous, M.D., Kennedy, I., Navitsky, M., Lu, M., Galante, N., Salloway, S.,
 2468 Murali Doraiswamy, P., Southekal, S., Arora, A.K., McGeehan, A., Lim, N.C., Xiong, H.,
 2469 Truocchio, S.P., Joshi, A.D., Shcherbinin, S., Teske, B., Fleisher, A.S., Mintun, M.A., 2019.
 2470 A multicentre longitudinal study of flortaucipir (18F) in normal ageing, mild cognitive
 2471 impairment and Alzheimer’s disease dementia. *Brain* 142, 1723–1735.
 2472 <https://doi.org/10.1093/brain/awz090>
- 2473 Qi, G., Mi, Y., Shi, X., Gu, H., Brinton, R.D., Yin, F., 2021. ApoE4 Impairs Neuron-Astrocyte
 2474 Coupling of Fatty Acid Metabolism. *Cell Rep.* 34, 108572.

- 2475 <https://doi.org/10.1016/j.celrep.2020.108572>
- 2476 Qi, Y., Klyubin, I., Hu, N.W., Ondrejcek, T., Rowan, M.J., 2019. Pre-plaque A β -Mediated
2477 Impairment of Synaptic Depotentiation in a Transgenic Rat Model of Alzheimer's Disease
2478 Amyloidosis. *Front. Neurosci.* 13, 1–13. <https://doi.org/10.3389/fnins.2019.00861>
- 2479 Ramalho, R.M., Viana, R.J.S., Castro, R.E., Steer, C.J., Low, W.C., Rodrigues, C.M.P., 2008.
2480 Apoptosis in transgenic mice expressing the P301L mutated form of human tau. *Mol. Med.*
2481 14, 309–317. <https://doi.org/10.2119/2007-00133.Ramalho>
- 2482 Ramsden, M., Kotilinek, L., Forster, C., Paulson, J., McGowan, E., SantaCruz, K., Guimaraes, A.,
2483 Yue, M., Lewis, J., Carlson, G., Hutton, M., Ashe, K.H., 2005. Age-dependent neurofibrillary
2484 tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy
2485 (P301L). *J. Neurosci.* 25, 10637–10647. <https://doi.org/10.1523/JNEUROSCI.3279-05.2005>
- 2486 Rao, S.S., Adlard, P.A., 2018. Untangling Tau and Iron: Exploring the Interaction Between Iron
2487 and Tau in Neurodegeneration. *Front. Mol. Neurosci.* 11, 1–13.
2488 <https://doi.org/10.3389/fnmol.2018.00276>
- 2489 Reese, T.S., Karnovsky, M.J., 1967. Fine structural localization of a blood-brain barrier to
2490 exogenous peroxidase. *J. Cell Biol.* 34, 207–217. <https://doi.org/10.1083/jcb.34.1.207>
- 2491 Ribeiro-da-Silva, A., Priestley, J. V., Cuellar, A.C., 1993. Pre-embedding ultrastructural
2492 immunocytochemistry, in: Cuellar, A.C. (Ed.), *Immunocytochemistry II*. John Wiley & Sons,
2493 Chichester, pp. 181–227.
- 2494 Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., Zhang, B.,
2495 Murayama, S., Higuchi, M., Lee, V.M.Y., Trojanowski, J.Q., Saido, T.C., 2019.
2496 Humanization of the entire murine Mapt gene provides a murine model of pathological human
2497 tau propagation. *J. Biol. Chem.* 294, 12754–12765.
2498 <https://doi.org/10.1074/jbc.RA119.009487>
- 2499 Saroja, S.R., Gorbachev, K., Julia, T., Goate, A.M., Pereira, A.C., 2022. Astrocyte-secreted
2500 glypican-4 drives APOE4-dependent tau hyperphosphorylation. *Proc. Natl. Acad. Sci.* 119,
2501 1–12. <https://doi.org/10.1073/pnas.2108870119>
- 2502 Schindowski, K., Bretteville, A., Leroy, K., Bégar, S., Brion, J.P., Hamdane, M., Buée, L., 2006.
2503 Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional
2504 synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am. J. Pathol.*
2505 169, 599–616. <https://doi.org/10.2353/ajpath.2006.060002>
- 2506 Schöll, M., Lockhart, S.N., Schonhaut, D.R., O'Neil, J.P., Janabi, M., Ossenkoppele, R., Baker,
2507 S.L., Vogel, J.W., Faria, J., Schwimmer, H.D., Rabinovici, G.D., Jagust, W.J., 2016. PET
2508 Imaging of Tau Deposition in the Aging Human Brain. *Neuron* 89, 971–982.
2509 <https://doi.org/10.1016/j.neuron.2016.01.028>
- 2510 Sengillo, J.D., Winkler, E.A., Walker, C.T., Sullivan, J.S., Johnson, M., Zlokovic, B. V., 2013.
2511 Deficiency in mural vascular cells coincides with blood-brain barrier disruption in
2512 Alzheimer's disease. *Brain Pathol.* 23, 303–310. <https://doi.org/10.1111/bpa.12004>
- 2513 Serrano-Pozo, A., Mielke, M.L., Gómez-Isla, T., Betensky, R.A., Growdon, J.H., Frosch, M.P.,
2514 Hyman, B.T., 2011. Reactive glia not only associates with plaques but also parallels tangles
2515 in Alzheimer's disease. *Am. J. Pathol.* 179, 1373–1384.

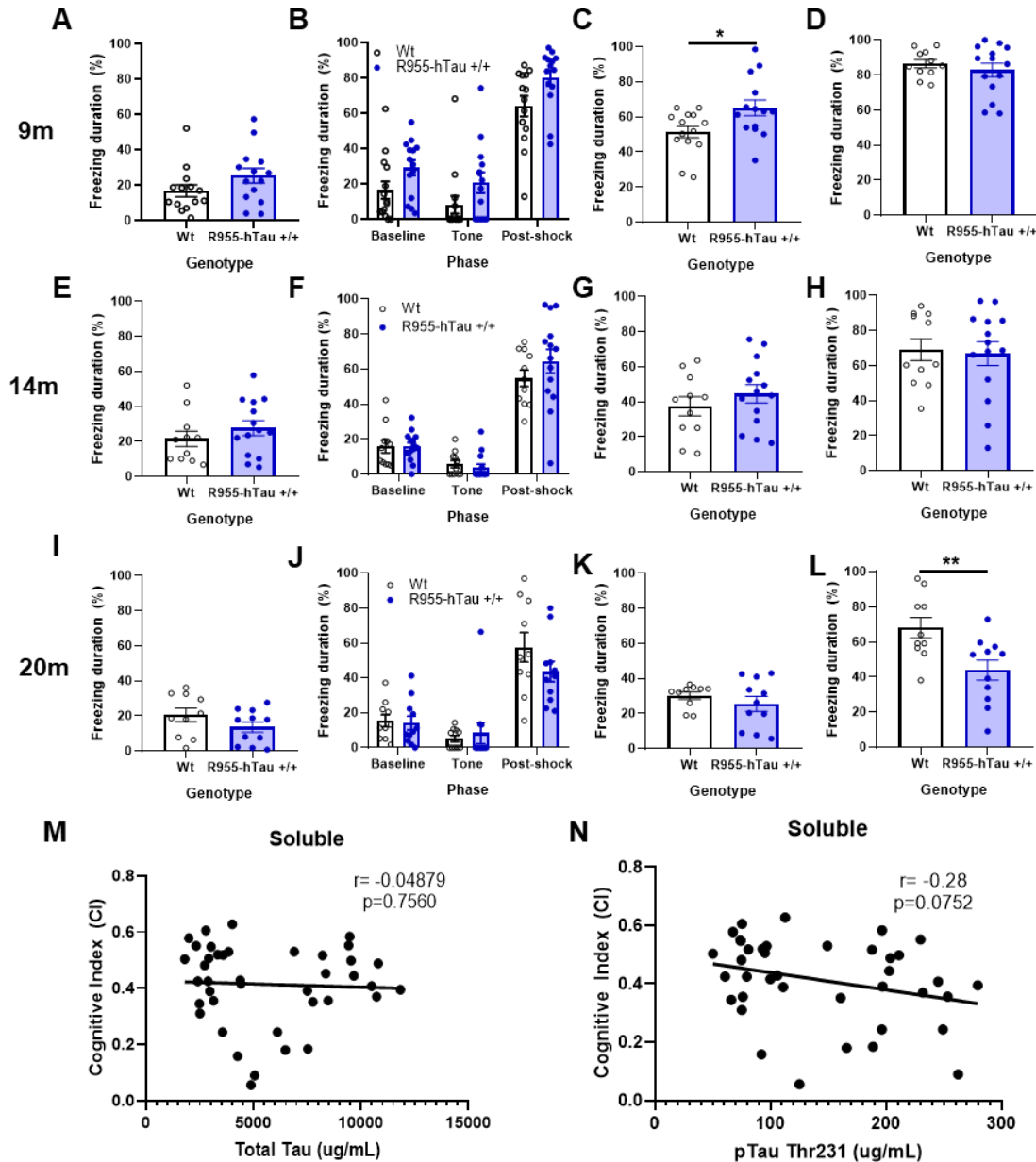
- 2516 <https://doi.org/10.1016/j.ajpath.2011.05.047>
- 2517 Serrano-Pozo, A., Qian, J., Muzikansky, A., Monsell, S.E., Montine, T.J., Frosch, M.P., Betensky,
2518 R.A., Hyman, B.T., 2016. Thal amyloid stages do not significantly impact the correlation
2519 between neuropathological change and cognition in the Alzheimer disease continuum. *J.*
2520 *Neuropathol. Exp. Neurol.* 75, 516–526. <https://doi.org/10.1093/jnen/nlw026>
- 2521 Shi, Y., Yamada, K., Liddelow, S.A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S.,
2522 Grinberg, L.T., Rojas, J.C., Gallardo, G., Wang, K., Roh, J., Robinson, G., Finn, M.B., Jiang,
2523 H., Sullivan, P.M., Baufeld, C., Wood, M.W., Sutphen, C., McCue, L., Xiong, C., Del-Aguila,
2524 J.L., Morris, J.C., Cruchaga, C., Fagan, A.M., Miller, B.L., Boxer, A.L., Seeley, W.W.,
2525 Butovsky, O., Barres, B.A., Paul, S.M., Holtzman, D.M., 2017. ApoE4 markedly exacerbates
2526 tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* 549, 523–527.
2527 <https://doi.org/10.1038/nature24016>
- 2528 Sidoryk-Wegrzynowicz, M., Gerber, Y.N., Ries, M., Sastre, M., Tolkovsky, A.M., Spillantini,
2529 M.G., 2017. Astrocytes in mouse models of tauopathies acquire early deficits and lose
2530 neurosupportive functions. *Acta Neuropathol. Commun.* 5, 89.
2531 <https://doi.org/10.1186/s40478-017-0478-9>
- 2532 Silva, I., Silva, J., Ferreira, R., Trigo, D., 2021. Glymphatic system, AQP4, and their implications
2533 in Alzheimer’s disease. *Neurol. Res. Pract.* 3. <https://doi.org/10.1186/s42466-021-00102-7>
- 2534 Skjolding, A.D., Rowland, I.J., Søgaaard, L. V., Praetorius, J., Penkowa, M., Juhler, M., 2010.
2535 Hydrocephalus induces dynamic spatiotemporal regulation of aquaporin-4 expression in the
2536 rat brain. *Cerebrospinal Fluid Res.* 7, 1–13. <https://doi.org/10.1186/1743-8454-7-20>
- 2537 Smith, M.A., Harris, P.L.R., Sayre, L.M., Perry, G., 1997. Iron accumulation in Alzheimer disease
2538 is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9866–9868.
2539 <https://doi.org/10.1073/pnas.94.18.9866>
- 2540 Spires, T.L., Orne, J.D., SantaCruz, K., Pitstick, R., Carlson, G.A., Ashe, K.H., Hyman, B.T.,
2541 2006. Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse
2542 model of tauopathy. *Am. J. Pathol.* 168, 1598–1607.
2543 <https://doi.org/10.2353/ajpath.2006.050840>
- 2544 Spotorno, N., Acosta-Cabronero, J., Stomrud, E., Lampinen, B., Strandberg, O.T., van Westen,
2545 D., Hansson, O., 2020. Relationship between cortical iron and tau aggregation in Alzheimer’s
2546 disease. *Brain* 143, 1341–1349. <https://doi.org/10.1093/brain/awaa089>
- 2547 Strang, K.H., Croft, C.L., Sorrentino, Z.A., Chakrabarty, P., Golde, T.E., Giasson, B.I., 2018.
2548 Distinct differences in prion-like seeding and aggregation between Tau protein variants
2549 provide mechanistic insights into tauopathies. *J. Biol. Chem.* 293, 2408–2421.
2550 <https://doi.org/10.1074/jbc.M117.815357>
- 2551 Suárez-Calvet, M., Karikari, T.K., Ashton, N.J., Lantero Rodríguez, J., Milà-Alomà, M., Gispert,
2552 J.D., Salvadó, G., Minguillon, C., Fauria, K., Shekari, M., Grau-Rivera, O., Arenaza-Urquijo,
2553 E.M., Sala-Vila, A., Sánchez-Benavides, G., González-de-Echávarri, J.M., Kollmorgen, G.,
2554 Stoops, E., Vanmechelen, E., Zetterberg, H., Blennow, K., Molinuevo, J.L., Beteta, A.,
2555 Cacciaglia, R., Cañas, A., Deulofeu, C., Cumplido, I., Dominguez, R., Emilio, M., Falcon,
2556 C., Fuentes, S., Hernandez, L., Huesa, G., Huguet, J., Marne, P., Menchón, T., Operto, G.,
2557 Polo, A., Pradas, S., Soteras, A., Vilanova, M., Vilor-Tejedor, N., 2020. Novel tau biomarkers

- phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in A β pathology are detected. *EMBO Mol. Med.* 12, 1–19. <https://doi.org/10.15252/emmm.202012921>
- Tang, H., Mao, X.O., Xie, L., Greenberg, D.A., Jin, K., 2013. Expression level of vascular endothelial growth factor in hippocampus is associated with cognitive impairment in patients with Alzheimer's disease. *Neurobiol. Aging* 34, 1412–1415. <https://doi.org/10.1016/j.neurobiolaging.2012.10.029>
- Terry, R.D., Peck, A., DeTeresa, R., Schechter, R., Horoupian, D.S., 1981. Some morphometric aspects of the brain in senile dementia of the alzheimer type. *Ann. Neurol.* 10, 184–192. <https://doi.org/10.1002/ana.410100209>
- Therriault, J., Benedet, A.L., Pascoal, T.A., Mathotaarachchi, S., Chamoun, M., Savard, M., Thomas, E., Kang, M.S., Lussier, F., Tissot, C., Parsons, M., Qureshi, M.N.I., Vitali, P., Massarweh, G., Soucy, J.P., Rej, S., Saha-Chaudhuri, P., Gauthier, S., Rosa-Neto, P., 2020. Association of Apolipoprotein e ϵ 4 with Medial Temporal Tau Independent of Amyloid- β . *JAMA Neurol.* 77, 470–479. <https://doi.org/10.1001/jamaneurol.2019.4421>
- Thomas, T., Miners, S., Love, S., 2015. Post-mortem assessment of hypoperfusion of cerebral cortex in Alzheimer's disease and vascular dementia. *Brain* 138, 1059–1069. <https://doi.org/10.1093/brain/awv025>
- Tran, D., DiGiacomo, P., Born, D.E., Georgiadis, M., Zeineh, M., 2022. Iron and Alzheimer's Disease: From Pathology to Imaging. *Front. Hum. Neurosci.* 16, 1–19. <https://doi.org/10.3389/fnhum.2022.838692>
- Tran, T.N., Kim, S.H., Gallo, C., Amaya, M., Kyees, J., Narayanaswami, V., 2013. Biochemical and biophysical characterization of recombinant rat apolipoprotein E: Similarities to human apolipoprotein E3. *Arch. Biochem. Biophys.* 529, 18–25. <https://doi.org/10.1016/j.abb.2012.10.007>
- Valenza, M., Facchinetti, R., Steardo, L., Scuderi, C., 2020. Altered waste disposal system in aging and Alzheimer's disease: Focus on astrocytic aquaporin-4. *Front. Pharmacol.* 10, 1–7. <https://doi.org/10.3389/fphar.2019.01656>
- van de Haar, H.J., Jansen, J.F.A., van Osch, M.J.P., van Buchem, M.A., Muller, M., Wong, S.M., Hofman, P.A.M., Burgmans, S., Verhey, F.R.J., Backes, W.H., 2016. Neurovascular unit impairment in early Alzheimer's disease measured with magnetic resonance imaging. *Neurobiol. Aging* 45, 190–196. <https://doi.org/10.1016/j.neurobiolaging.2016.06.006>
- Vilor-Tejedor, N., Ciampa, I., Operto, G., Falcón, C., Suárez-Calvet, M., Crous-Bou, M., Shekari, M., Arenaza-Urquijo, E.M., Milà-Alomà, M., Grau-Rivera, O., Minguillon, C., Kollmorgen, G., Zetterberg, H., Blennow, K., Guigo, R., Molinuevo, J.L., Gispert, J.D., Beteta, A., Brugnulat, A., Cacciaglia, R., Cañas, A., Deulofeu, C., Cumplido, I., Dominguez, R., Emilio, M., Fauria, K., Fuentes, S., Hernandez, L., Huesa, G., Huguet, J., Marne, P., Menchón, T., Polo, A., Pradas, S., Rodriguez-Fernandez, B., Sala-Vila, A., Sánchez-Benavides, G., Salvadó, G., Soteras, A., Vilanova, M., 2021. Perivascular spaces are associated with tau pathophysiology and synaptic dysfunction in early Alzheimer's continuum. *Alzheimers. Res. Ther.* 13, 135. <https://doi.org/10.1186/s13195-021-00878-5>
- Wang, L., Benzinger, T.L., Su, Y., Christensen, J., Friedrichsen, K., Aldea, P., McConathy, J.,

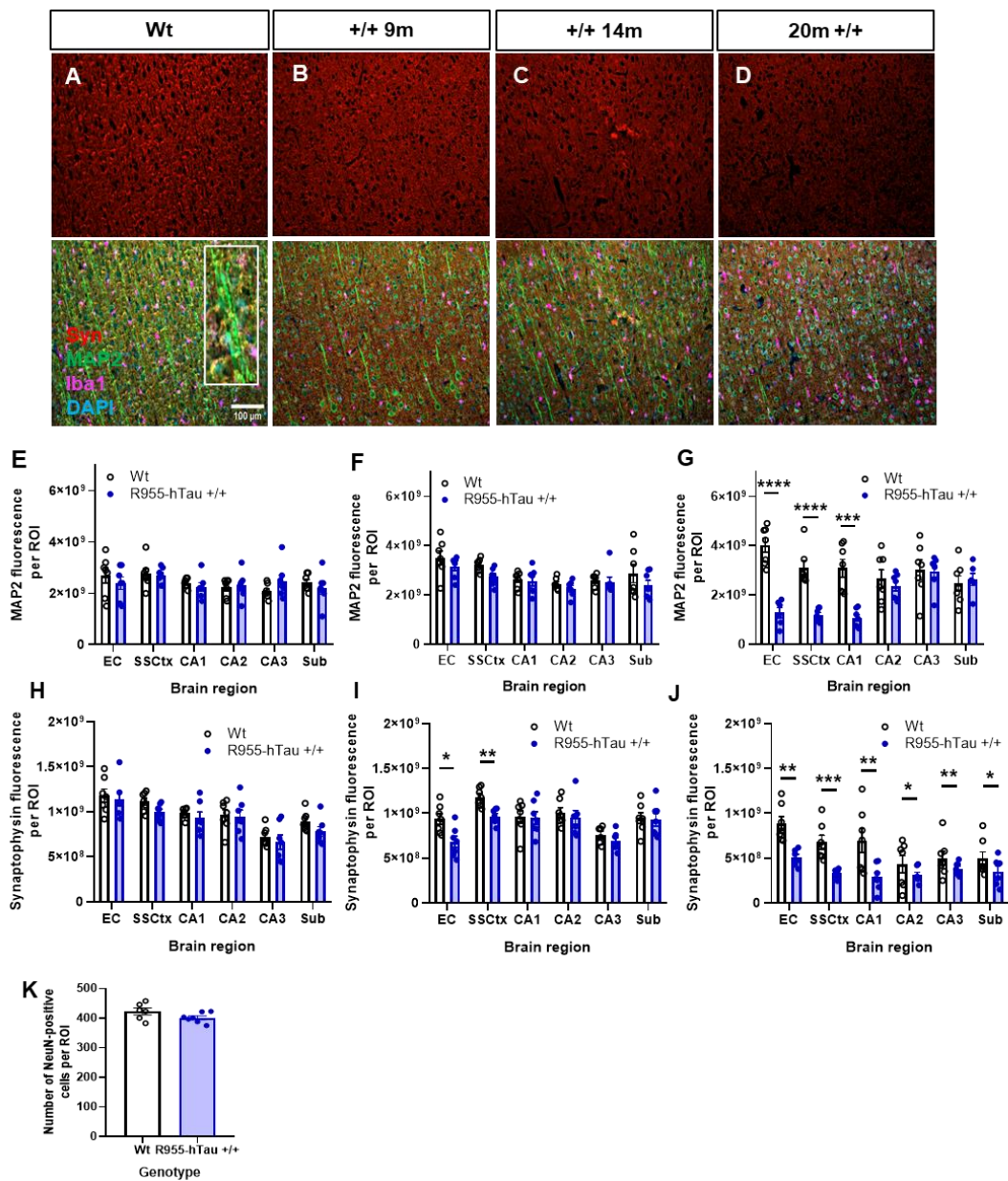
- 2600 Cairns, N.J., Fagan, A.M., Morris, J.C., Ances, B.M., Louis, S., Wang, M., Charles, T.F.,
 2601 Knight Alzheimer, J., 2016. Evaluation of Tau Imaging in Staging Alzheimer Disease and
 2602 Revealing Interactions Between β -Amyloid and Tauopathy. *JAMA Neurol* 73, 1070–1077.
 2603 <https://doi.org/10.1001/jamaneurol.2016.2078>
- 2604 Wardlaw, J.M., Makin, S.J., Valdés Hernández, M.C., Armitage, P.A., Heye, A.K., Chappell,
 2605 F.M., Muñoz-Maniega, S., Sakka, E., Shuler, K., Dennis, M.S., Thrippleton, M.J., 2017.
 2606 Blood-brain barrier failure as a core mechanism in cerebral small vessel disease and dementia:
 2607 evidence from a cohort study. *Alzheimer's Dement.* 13, 634–643.
 2608 <https://doi.org/10.1016/j.jalz.2016.09.006>
- 2609 Wardlaw, J.M., Smith, E.E., Biessels, G.J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R.I.,
 2610 O'Brien, J.T., Barkhof, F., Benavente, O.R., Black, S.E., Brayne, C., Breteler, M., Chabriot,
 2611 H., DeCarli, C., de Leeuw, F.E., Doubal, F., Duering, M., Fox, N.C., Greenberg, S.,
 2612 Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R. van, Pantoni, L., Speck, O., Stephan,
 2613 B.C.M., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van Buchem, M.,
 2614 Norrving, B., Gorelick, P.B., Dichgans, M., 2013. Neuroimaging standards for research into
 2615 small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* 12,
 2616 822–838. [https://doi.org/10.1016/S1474-4422\(13\)70124-8](https://doi.org/10.1016/S1474-4422(13)70124-8)
- 2617 West, M.J., Kawas, C.H., Stewart, W.F., Rudow, G.L., Troncoso, J.C., 2004. Hippocampal
 2618 neurons in pre-clinical Alzheimer's disease. *Neurobiol. Aging* 25, 1205–1212.
 2619 <https://doi.org/10.1016/j.neurobiolaging.2003.12.005>
- 2620 Wilcock, D.M., Vitek, M.P., Colton, C.A., 2009. Vascular amyloid alters astrocytic water and
 2621 potassium channels in mouse models and humans with Alzheimer's disease. *Neuroscience*
 2622 159, 1055–1069. <https://doi.org/10.1016/j.neuroscience.2009.01.023>
- 2623 Wildner, G., 2019. Are rats more human than mice? *Immunobiology* 224, 172–176.
 2624 <https://doi.org/10.1016/j.imbio.2018.09.002>
- 2625 Wisniewski, H.M., Kozlowski, P.B., 1982. Evidence for Blood-Brain Barrier Changes in Senile
 2626 Dementia of the Alzheimer Type (SDAT). *Ann. N. Y. Acad. Sci.* 396, 119–129.
 2627 <https://doi.org/10.1111/j.1749-6632.1982.tb26848.x>
- 2628 Wisniewski, H.M., Vorbrodt, A.W., Wegiel, J., 1997. Amyloid angiopathy and blood-brain barrier
 2629 changes in Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 826, 161–172.
 2630 <https://doi.org/10.1111/j.1749-6632.1997.tb48468.x>
- 2631 Xia, Y., Prokop, S., Bell, B.M., Gorion, K.M.M., Croft, C.L., Nasif, L., Xu, G., Riffe, C.J.,
 2632 Manaois, A.N., Strang, K.H., Quintin, S.S., Paterno, G., Tansey, M.G., Borchelt, D.R., Golde,
 2633 T.E., Giasson, B.I., 2022. Pathogenic tau recruits wild-type tau into brain inclusions and
 2634 induces gut degeneration in transgenic SPAM mice. *Commun. Biol.* 5, 1–18.
 2635 <https://doi.org/10.1038/s42003-022-03373-1>
- 2636 Yamamoto, A., Shin, R.W., Hasegawa, K., Naiki, H., Sato, H., Yoshimasu, F., Kitamoto, T., 2002.
 2637 Iron (III) induces aggregation of hyperphosphorylated τ and its reduction to iron (II) reverses
 2638 the aggregation: Implications in the formation of neurofibrillary tangles of Alzheimer's
 2639 disease. *J. Neurochem.* 82, 1137–1147. <https://doi.org/10.1046/j.1471-4159.2002.01061.x>
- 2640 Yang, M., Silverman, J.L., Crawley, J.N., 2011. Automated Three-Chambered Social Approach
 2641 Task for Mice, in: *Current Protocols in Neuroscience*. John Wiley & Sons, Inc., Hoboken,

- 2642 NJ, USA, pp. 1–16. <https://doi.org/10.1002/0471142301.ns0826s56>
- 2643 Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.M., Iwata, N., Saido, T.C., Maeda, J., Suhara,
2644 T., Trojanowski, J.Q., Lee, V.M.Y., 2007. Synapse Loss and Microglial Activation Precede
2645 Tangles in a P301S Tauopathy Mouse Model. *Neuron* 53, 337–351.
2646 <https://doi.org/10.1016/j.neuron.2007.01.010>
- 2647 Yousef, A., Robinson, J.L., Irwin, D.J., Byrne, M.D., Kwong, L.K., Lee, E.B., Xu, Y., Xie, S.X.,
2648 Rennert, L., Suh, E.R., Van Deerlin, V.M., Grossman, M., Lee, V.M.Y., Trojanowski, J.Q.,
2649 2017. Neuron loss and degeneration in the progression of TDP-43 in frontotemporal lobar
2650 degeneration. *Acta Neuropathol. Commun.* 5, 68. [https://doi.org/10.1186/s40478-017-0471-](https://doi.org/10.1186/s40478-017-0471-3)
2651 3
- 2652 Zeppenfeld, D.M., Simon, M., Haswell, J.D., D’Abreo, D., Murchison, C., Quinn, J.F., Grafe,
2653 M.R., Woltjer, R.L., Kaye, J., Iliff, J.J., 2017. Association of perivascular localization of
2654 aquaporin-4 with cognition and Alzheimer disease in aging brains. *JAMA Neurol.* 74, 91–99.
2655 <https://doi.org/10.1001/jamaneurol.2016.4370>
- 2656 Zhou, Y., Lin, W., Rao, T., Zheng, J., Zhang, T., Zhang, M., Lin, Z., 2022. Ferroptosis and Its
2657 Potential Role in the Nervous System Diseases. *J. Inflamm. Res.* 15, 1555–1574.
2658 <https://doi.org/10.2147/JIR.S351799>
- 2659 Zhou, Y., Song, W.M., Andhey, P.S., Swain, A., Levy, T., Miller, K.R., Poliani, P.L., Cominelli,
2660 M., Grover, S., Gilfillan, S., Cella, M., Ulland, T.K., Zaitsev, K., Miyashita, A., Ikeuchi, T.,
2661 Sainouchi, M., Kakita, A., Bennett, D.A., Schneider, J.A., Nichols, M.R., Beausoleil, S.A.,
2662 Ulrich, J.D., Holtzman, D.M., Artyomov, M.N., Colonna, M., 2020. Human and mouse
2663 single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular
2664 responses in Alzheimer’s disease. *Nat. Med.* 26, 131–142. [https://doi.org/10.1038/s41591-](https://doi.org/10.1038/s41591-019-0695-9)
2665 019-0695-9
- 2666 Zimmer, E.R., Parent, M.J., Cuello, A.C., Gauthier, S., Rosa-Neto, P., 2014. MicroPET imaging
2667 and transgenic models: a blueprint for Alzheimer’s disease clinical research. *Trends Neurosci.*
2668 37, 629–641. <https://doi.org/10.1016/j.tins.2014.07.002>
- 2669

2670 6. Supplemental Figures

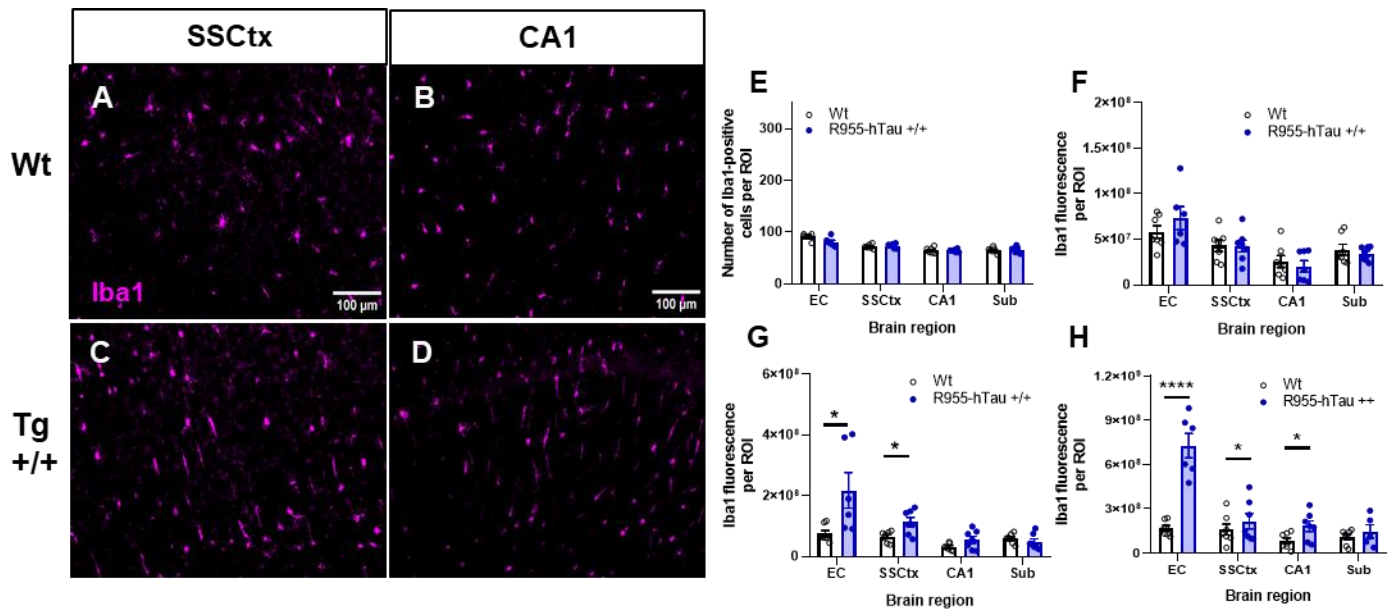


Supplemental Figure 1. In aged R955-hTau^{+/±} rats, impairment in the cued recall during the fear conditioning task is detectable after severe tau pathology. Quantification of freezing behavior over four days: (A, E, I) baseline habituation, (B, F, J) Conditioning of an auditory tone-shock paired stimulus, (C, G, K) contextual recall and (D, H, L) cued recall with a different environment. Impaired freezing is not detected at (A-D) 9 months nor (E-H) 14 months. (M-N) Correlation between (M) total cortical Sarkosyl-soluble tau and cognitive index (CI) shows no clear correlation however (N) Sarkosyl-soluble ptau Thr231 trends toward a negative correlation with CI. Error bars represent the mean ± SEM (n=10-14 per group per time point). **p<0.01



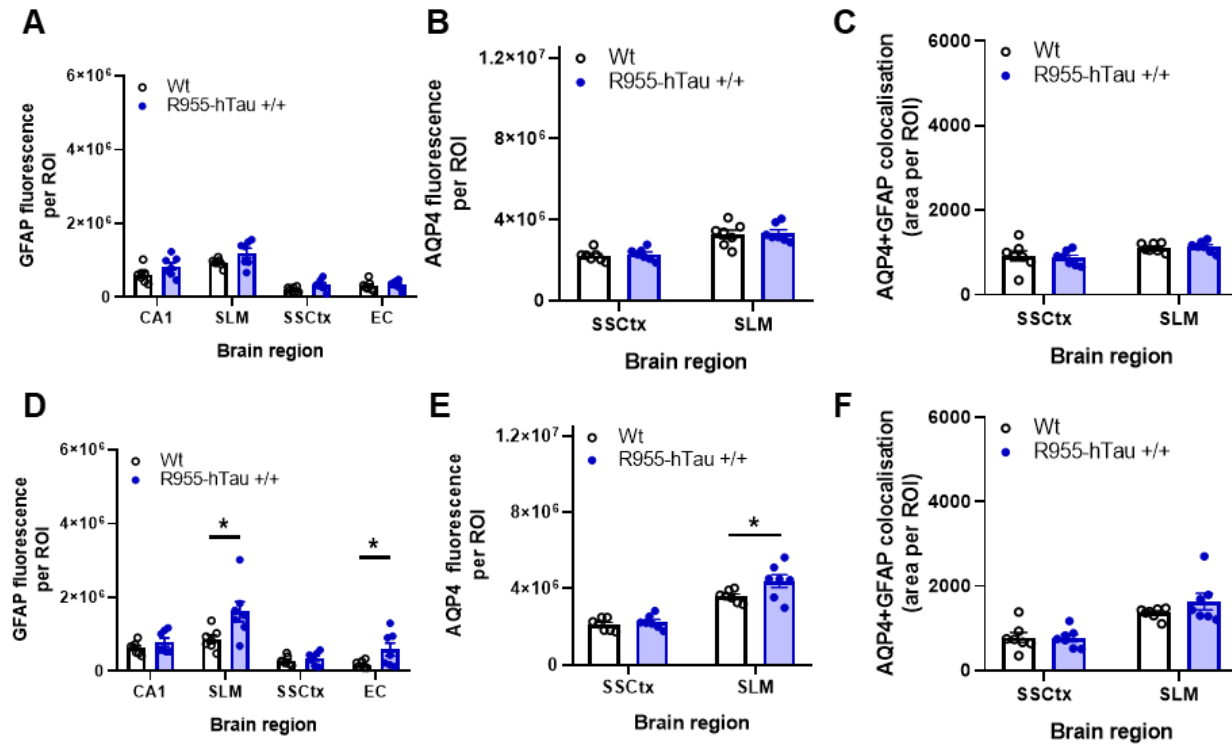
2671 **Supplemental Figure 2. Tau-associated reduction in synaptic markers following tau hyperphosphorylation and misfolding precede neuronal loss in R955-hTau rats. (A-D)** Representative coronal micrographs illustrating a progressive and eventual global loss in the presynaptic marker, synaptophysin, and dendritic marker, MAP2. **(E-G)** Quantification of overall MAP2 fluorescence intensity at **(E)** 9, **(F)** 14, and **(G)** 20 months of age, showing no significant differences in multiple brain regions before 20 months. At 20 months, MAP2 is decreased in regions with severe tau pathology: the entorhinal cortex (EC), somatosensory cortex (SSCtx), hippocampal CA1 and subiculum (Sub). **(H-J)** Quantification of synaptophysin (Syn) fluorescence at **(H)** 9, **(I)** 14 and **(J)** 20 months demonstrating a reduction commencing at **(I)** 14 months of age in the EC and SSCtx, then more so at **(H)** 20 months occurring in multiple cortical and hippocampal regions. At 20 months, neuronal loss was not detected in the somatosensory region of the cerebral cortex **(K)**. Error bars represent the mean ± SEM (n=6-7 per group per time point). * = p<0.05, ** = p<0.01, *** = p<0.001. **A-D** Scale bar = 100µm.

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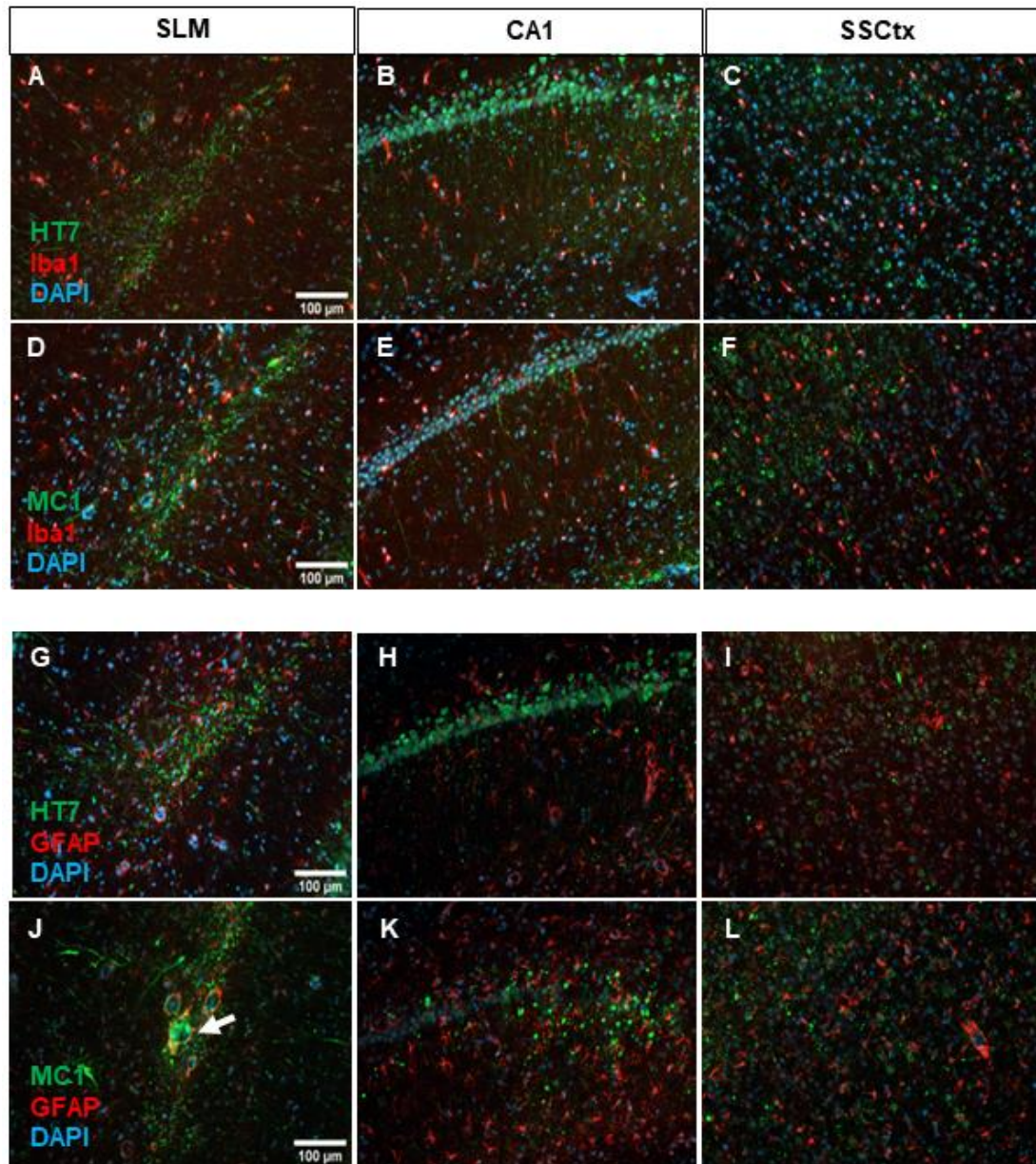


Supplemental Figure 3. Microgliosis assessed by immunofluorescence in R955-hTau^{+/+} rats is absent in younger rats and parallels the number of microglial cells. (A-D) Representative immunofluorescence micrographs of the (E, G) somatosensory cortex and (F, H) CA1 of the hippocampus of 20-month-old rats. (F-H) Quantification of (E) Iba1-positive cells and (F) Iba1 fluorescence at 9 months showed no differences in multiple brain regions. (G-H) Quantification of Iba1 immunofluorescence parallels the increase in microglial cells at (G) 14 and (H) 20 months showed elevated fluorescence in tau-burdened regions such as the entorhinal cortex (EC), somatosensory cortex (SSCtx) and hippocampal CA1. Error bars represent the mean $\pm$ SEM (n=6-7 per group per time point). * p<0.05 ** p<0.01 *** p<0.001.

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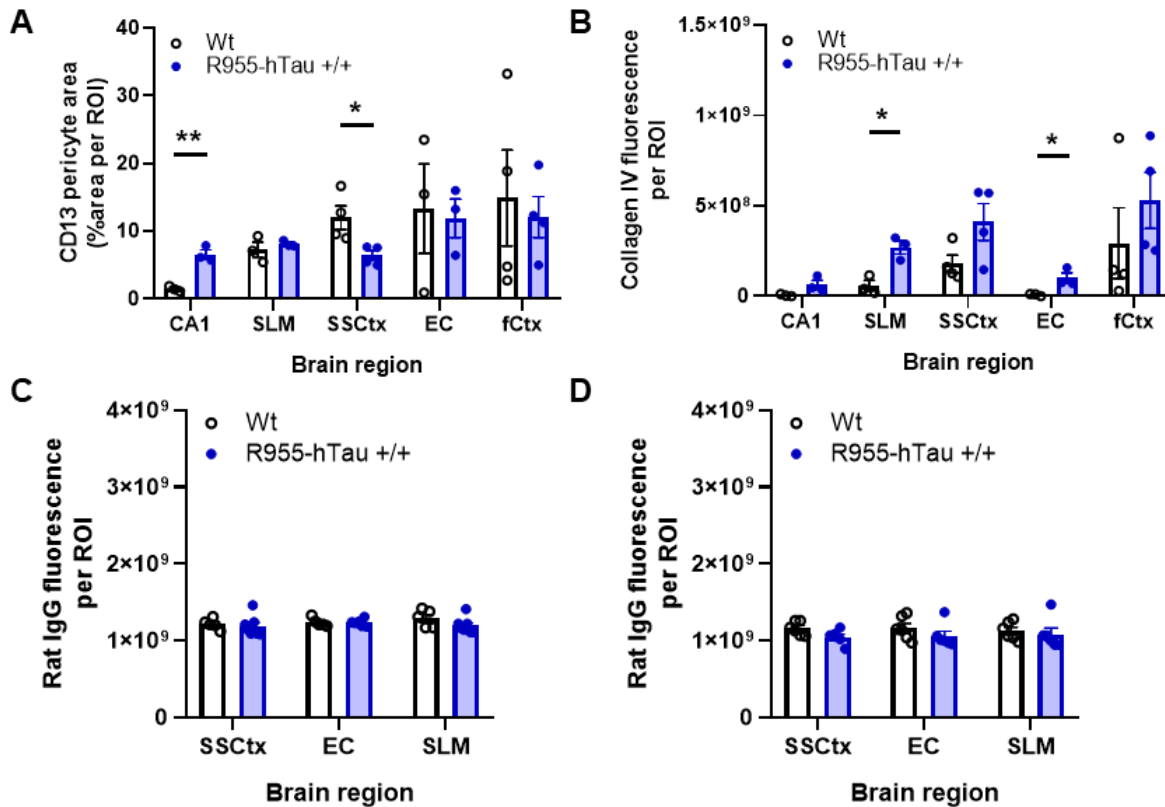
Supplemental Figure 4. Progressive astrocytosis in younger R955-hTau^{+/+} rats culminates in aged R955-hTau rats. Quantification of fluorescence in (A-C) 9-month-old rats clarifying no differences in multiple brain regions for (A) GFAP, (B) Aquaporin 4 (AQP4) and the (C) area of colocalization. (D-F) At 14 months old, (D) GFAP fluorescence intensity was increased in the *stratum lacunosum moleculare* (SLM) and entorhinal cortex (EC) (D) whereas AQP4 fluorescence was elevated in the SLM (E) while the overall area of colocalization remained at similar (F). Error bars represent the mean $\pm$ SEM (n=6-7 per group per time point). * $p < 0.05$, *** $p < 0.001$.



Supplemental Figure 5. Lack of tau colocalization in glial cells and non-perivascular astrocytes. (A-F) Representative micrographs illustrating a co-staining of Iba1-positive microglia and (A-C) total human tau and (D-F) pre-tangle tau as assessed by MC1 in the (A, D) *stratum lacunosum moleculare* (SLM), (B, E) CA1 and (C, F) Somatosensory cortex (SSCtx) of R955-hTau^{+/+} rats. No colocalization was detected in microglia. (G-L) Representative micrographs illustrating the co-staining of GFAP-positive astrocytes and (G-I) total human tau and (J-L) pre-tangle tau in the (G, J) SLM, (H, K) CA1 and (I, L) SSCtx. Note that only co-localization occurred in perivascular astrocytes (J). A-L Scale bar = 100µm.

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Supplemental Figure 6. Neurovascular alterations in R955-hTau rats and absence of endogenous IgG extravasation in younger R955-hTau^{+/+} rats. (A-B) Quantification of overall (A) CD13 and (B) Collagen IV vessel area was differentially altered in regions of severe tau pathology including the hippocampal CA1, *Stratum lacunosum moleculare* (SLM), somatosensory cortex (SSCtx) and entorhinal cortex (EC) (n=3-4). (C-D) Quantification of rat immunoglobulin (IgG) fluorescence intensity at (C) 9-month-old and (D) 14-month-old rats were unaffected in multiple brain regions unlike the later affected 20-month-old transgenic rats. Error bars represent the mean $\pm$ SEM (n=6-7 per group per time point). * p<0.05, ** p<0.01

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7. Supplemental Tables

Supplemental Table 1. Demographics of R955-hTau rat cohorts for this study. Both males and females were used. Some age-related loss occurred for both wild-type littermates and transgenics. Experiments C and D were limited to one sex for data accuracy.

A. Main Characterization (IHC and Behavior)

Age (months)	<u>Wild type (Wt)</u>		<u>R955-hTau^{+/+}</u>		<u>Total (Wt / R955)</u>
	Female	Male	Female	Male	
9	6	8	4	10	28 (14/14)
14-15	4	7	11	3	25 (11/14)
19-20	6	4	6	5	21 (10/11)
Total	16	19	21	18	74

B. Electron Microscopy (EM)

Age (months)	<u>Wild type (Wt)</u>		<u>R955-hTau^{+/+}</u>		<u>Total (Wt / R955)</u>
	Female	Male	Female	Male	
9	1	1	1	1	4 (2/2)
20	1	1	1	1	4 (2/2)
Total	2	2	2	2	4

C. Magnetic resonance imaging (MRI)

Age (months)	<u>Wild type (Wt)</u>		<u>R955-hTau^{+/+}</u>		<u>Total (Wt / R955)</u>
	Female	Male	Female	Male	
9	0	3	0	3	6 (3/3)
20	0	3	0	3	6 (3/3)
Total	0	6	0	6	12

D. Blood-brain-barrier (BBB) dysfunction

Age (months)	<u>Wild type (Wt)</u>		<u>R955-hTau^{+/+}</u>		<u>Total (Wt / R955)</u>
	Female	Male	Female	Male	
20	4	0	4	0	8 (4/4)

2684 **Supplemental Table 2. List of primary and secondary antibodies.**

Primary Antibody	Company/Source	Epitope	Use	Concentration
Tau species				
HT7	ThermoFisher	aa 159-163, hTau	IHC, IF	1:1200, 1:700
AT8	ThermoFisher	pSer202-pThr205	IHC, IF	1:500, 1:200
MC1	Peter Davies	aa 7-9, 312-322	IHC, IF	1:500, 1:200
PHF1	Peter Davies	pSer396/404	IHC	1:4000
T49	Millipore	-	WB	1:1500
Cell Type markers				
Iba1 (Microglia)	Wako	-	IHC, IF	1:500
CD68 (Macrophages)	Bio-Rad		IF	1:100
GFAP (Astrocyte)	Novus	-	IF	1:2000
NeuN (Neuron)	Abcam	-	IHC, IF	1:4000
Synaptic				
Synaptophysin	Abcam	-	IF	1:1500
MAP2	Abcam	-	IF	1:2500
Nf-L	Abcam	-	WB	1:1000
NMDAR1	Sigma	-	WB	1:1000
Neurovascular				
CD13	Cell Signaling	-	IF	1:200
Collagen IV	Hybridoma Bank	-	IF	1:300
Aquaporin4 (AQP4)	Alamone	-	IF	1:1500
Other				
APOE	Cell Signalling		WB	1:2000
GAPDH	Millipore		WB	1:5000
Secondary Antibody				
Rb anti-Fc Ms	Cuello Lab	-	IHC	1:100
Gt anti-Rb (Biotinyl.)	MP Biomedicals	-	IHC	1:200
Gt anti-Rb AF-568	Abcam	-	IF	1:400
Gt anti-Gp AF-647	Abcam	-	IF	1:400
Gt anti-Ms AF-488	Abcam	-	IF	1:400
Gt anti-Rt AF-488	Abcam	-	IF	1:400
Gt anti-Ms 12nm colloidal gold	Jackson Immunoresearch	-	EM	1:20

2685 **IF** = Immunofluorescence; **IHC** = Immunohistochemistry; **WB** = Western blot, **EM** = Electron microscopy,

Connecting text: Chapter II and III

In line with our aims and hypotheses, the characterization of the homozygous R955-hTau transgenic rat revealed a phenotype of a progressive human-like tauopathy that was independent of concurrent amyloid pathology. Such features included a progressive accumulation and hyperphosphorylation of tau resulting in aggregation and development of neurofibrillary inclusions by 20 months of age in this transgenic model. Increasing severity of tau pathology led to brain atrophy, ventricular dilation, neuronal loss, and CNS inflammation involving an early involvement of microglia and downstream astrogliosis, as observed in the human species.

We found that the homozygous R955-hTau rat model also provides a phenotype akin to that described for the lost R962-hTau line. Additionally, we found its slower development of the tauopathy was an advantage as it is more reminiscent of the stepwise progression of the CNS tauopathy in the human condition. This is supported by the occurrence of key aspects of human tauopathy which they are not present on their totality in other available transgenic animal models. These include a pathological recruitment of endogenous tau, neuronal loss and genuine neurofibrillary lesions. During the characterization of this rat model, we discovered multiple abnormalities in the neurovasculature, which warranted further investigation.

While the homozygous R955-hTau rat is an excellent model for the investigation of the full and end point human-like tauopathy as well as experimental therapeutics, in Chapter III we importantly investigated the earliest stages of the evolution of the CNS tauopathy applying the heterozygous R955-hTau rats, displaying a slowly progressive pathology - characteristics which allowed us to elucidate the early-stage alterations in CNS tauopathy in the absence of amyloid beta pathology.

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CHAPTER III:
“Neuronal loss and inflammation preceding fibrillary tau pathology
in a rat model with early human-like tauopathy”

Title: Neuronal loss and inflammation preceding fibrillary tau pathology in a rat model with early human-like tauopathy

Running title: Tau-associated degeneration during *in vivo* pre-fibrillary stages

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Keywords: Alzheimer's disease, human tauopathy, transgenic rat model, soluble tau, neuronal loss

Abstract

In tauopathies such as Alzheimer's disease (AD) and frontotemporal dementia (FTD), the microtubule associated protein tau undergoes conformational and posttranslational modifications in a gradual, staged pathological process. While brain atrophy and cognitive decline are well-established in the advanced stages of tauopathy, it is unclear how the early pathological processes manifest, prior to extensive neurodegeneration. For these studies we have applied a transgenic rat model of human-like tauopathy in its heterozygous form, named McGill-R955-hTau. The goal of the present study was to investigate whether lifelong accumulation of mutated human tau could reveal the earliest tau pathological processes in a context of advanced aging, and, at stages before the overt aggregated or fibrillary tau deposition. We characterized the phenotype of heterozygous R955-hTau rats at three endpoints, 10, 18 and 24-26 months of age, focusing on markers of cognitive capabilities, progressive tau pathology, neuronal health, neuroinflammation and brain ultrastructural integrity, using immunohistochemistry and electron microscopy. Heterozygous R955-hTau transgenic rats feature a modest, life-long accumulation of mutated human tau that led to tau hyperphosphorylation and produced deficits in learning and memory tasks after 24 months of age. Such impairments coincided with more extensive tau hyperphosphorylation in the brain at residues pThr231 and with indirect evidence of aggregation. Importantly, aged R955-hTau rats presented evidence of neuroinflammation, detriments to myelin morphology and detectable hippocampal neuronal loss in the absence of overt neurofibrillary lesions and brain atrophy. The slow-progressing tauopathy of R955-hTau rats should allow to better delineate the temporal progression of tau pathological events and therefore to distinguish early indicators of tauopathy as having the capability to induce degenerative events in the aged CNS.

1. Introduction

Abnormal accumulation, misfolding and aggregation of the microtubule associated protein tau (MAPT) play an important role in neurodegenerative diseases, termed tauopathies (Lee et al., 2001; Spillantini and Goedert, 1998) such as frontotemporal dementia (FTD) and Alzheimer's disease (AD), the most common causes of dementia. Tau is primarily localized in axonal processes with the main function of stabilizing microtubules (Avila et al., 2004; Kadavath et al., 2015; Mandelkow and Mandelkow, 1998). Tau binding properties are influenced by its phosphorylation (Kadavath et al., 2015; Weingarten et al., 1975). The tau molecule is dynamic with approximately 85 putative phosphorylation sites, most of which are serine-threonine dependent and active under normal physiological conditions (Mandelkow and Mandelkow, 1998; Wang and Mandelkow, 2015). In tauopathy during AD, tau is mislocalized to the somatodendritic compartments of neurons and becomes aberrantly phosphorylated at key residues. In advanced stages, these tau species are misfolded and found in aggregates constituting of paired helical filaments (PHF) that constitute the core of the microscopically visible intraneuronal neurofibrillary tangles (NFT) and neuropil threads observed in the AD brain (Goedert et al., 1996, 1992). Pathologic aggregated tau species are deeply associated with neuroinflammation (Nilson et al., 2017; Serrano-Pozo et al., 2011), neurodegeneration (Joie et al., 2020; Knopman et al., 2019; Terry et al., 1981) and cognitive impairments (Arriagada et al., 1992; Bejanin et al., 2017; Berron et al., 2021; Hanseeuw et al., 2019; Schöll et al., 2016). However, the sequence of event(s) that tau undergoes during an early disease state of tau accumulation and prior to tau misfolding requires further investigation.

Defined by the widely accepted Braak staging as a model of the progressive tauopathy in AD, elevated phosphorylated tau (ptau) at Ser202-Thr205, guided by the monoclonal antibody AT8, first occurs in the transentorhinal cortex (Braak et al., 2006; Braak and Braak, 1995, 1991; Braak and Del Tredici, 2020) but also the locus coeruleus (Grudzien et al., 2007). Similar patterning approaches of tau evolution may be accomplished in other tauopathies including Argrophilic Grain disease (AGD) (Rodriguez and Grinberg, 2015; Saito et al., 2004) and Progressive Supranuclear Palsy (PSP) (Kovacs et al., 2020), emphasizing the plausibility that some ptau species, such as ptau at Thr231, appear before others as a predecessor to abnormal tau processing (Luna-Muñoz et al., 2007, 2005). However, investigating the early consequences of tau accumulation and hyperphosphorylation *in vivo* is challenging due to ligand detection threshold

limitations during neuroimaging and the heterogeneity of post-mortem tissues. Hence, valuable animal models allow for the investigation of such initial stages of the CNS tauopathy.

Transgenic mouse models effectively display components of human brain tauopathy (Dujardin et al., 2015) and have demonstrated that powerful transgenic overexpression of mutated forms of tau is sufficient to generate tau pathology in the CNS (Goedert and Spillantini, 2000; Spillantini and Goedert, 1998). The majority of these models overexpress tau bearing one or more *MAPT* mutations present in FTD (Bugiani et al., 1999; Goedert and Spillantini, 2000). Recent advances with *MAPT* knock-in mice revealed an absence of an overt phenotype of tauopathy (Saito et al., 2019), emphasizing that *some* increased expression and/or introduction of mutated tau is required for tauopathy in rodents. Rats offer considerable advantages over mice for the investigation of tauopathies given they express all six isoforms of tau as seen in humans (Hanes et al., 2009) and have an apolipoprotein E (APOE) protein functionally akin to the human APOE protein which accelerates tau-mediated neurodegeneration *in vivo* (LaDu et al., 1997; Maloney et al., 2007; Shi et al., 2017; Tran et al., 2013). Rats also exhibit an enriched behavioral display and possess a larger brain more amenable for surgical and imaging procedures (Do Carmo and Cuellar, 2013; Zimmer et al., 2014).

Consequently, we investigated the stages of tau accumulation and hyperphosphorylation in a transgenic rat model, coded McGill-R955-hTau along with the brain aging process. R955-hTau rats overexpress the longest isoform of human tau (2N4R) with the P301S mutation causative of FTDP-17 (Bugiani et al., 1999). In this report we illustrate the characteristics of this transgenic rat model in its heterozygous form in contrast to homozygous R955-hTau (Chapter II) and R962-hTau transgenic rats (Malcolm et al., 2019). Here we report that heterozygous McGill-R955-hTau rats display a slow progressing accumulation of mutated human tau over the lifespan. Such a relatively mild phenotype was sufficient to reproduce some key features of the early tauopathy during their lifespan. These features including the hyperphosphorylation of tau at pathology-associated residues, impairments in learning and memory-based tasks in the absence of fibrillary lesions. Further, mild tau pathology in aged rats was sufficient to induce alterations to the CNS myelin, microglial and astrocytic inflammatory responses and hippocampal neuronal loss.

2. Materials and methods

2.1 Animals

Generation of the tau transgenic line coded McGill-R955-hTau line was derived in parallel with R962-hTau as previously described (Malcolm et al., 2019). Expression of human P301S tau is driven by the calcium-calmodulin kinase II alpha (CaMKII- α) promoter. Rats were housed as described in Chapter II. The use of animals for this study was approved by the McGill Animal Care Committee under the guidelines of the Canadian Council on Animal Care (CCAC).

2.2 Experimental Design

For characterization, a similar approach to that of Chapter II was utilized with modification to maintain consistency between studies. Male and female heterozygous R955-hTau rats were bred alongside wild-type (Wt) littermates and raised in three separate cohorts to one of three endpoints at 10, 18 and 24-26 months of age (**Supplemental Table 1**). A minimum sample size of 10 per group per time was initially chosen however this was reduced at 24-26 months-old from age-related losses. These three endpoints were selected based on preliminary R955-hTau data prior to the full investigation. At each endpoint, behavioral testing was performed followed by brain perfusion and tissue collection.

2.3 Animal behavior

Assessment of cognition was performed using a battery of behavioral tests including, in this order, the open field (OF), novel object location (NOL), novel object recognition (NOR), Morris water maze (MWM) and fear conditioning (FC) tasks using protocols previously established (Hall et al., 2018; Malcolm et al., 2019). For the OF task, rats were placed in an 80x80 cm arena coated with a mix of fresh bedding and bedding soiled from all experimental animal cages and containing distinct visual cues on three of four walls. The arena was divided equally into center and peripheral zones. Rats were left to freely explore the arena for three minutes and the percentage of time spent in the center of the arena was recorded. The NOL, NOLR, MWM and FC tasks were done as described in Chapter II. At the end of behavioral testing, a Cognitive Index score was calculated by converting each behavior test performance output into a score out of 1 and then averaged into a composite score across all tests for each time point. If a particular test showed no differences across any groups and time points, its test output was excluded from the CI

calculation. Animals were accustomed to handling before behavioral testing and were allotted two days of rest between tests. Tracking data was recorded with the assistance of Ethovision XT (Noldus, The Netherlands).

2.4 Tissue collection

Tissue collection of paraformaldehyde (PFA)-fixed and snap frozen brain tissues was performed as previously described (Chapter II). Briefly, rats were deeply anesthetized in 1% sodium pentobarbital (Equithesin), then transcardially perfused with chilled phosphate-buffered saline (PBS). One hemisphere was macrodissected then snap frozen in dry ice and stored at -80°C and the other hemisphere was fixed in 4% paraformaldehyde (PFA) for 24 hours followed by incubation in 30% sucrose for at least one week.

2.5 Immunohistochemistry and immunofluorescence

Immunoenzymatic procedures were carried out as previously described (Malcolm et al., 2019) (Chapter II) with minor modification. Free-floating brain tissue sections (40 µm) were quenched in a solution containing hydrogen peroxide and methanol. Slices were blocked in 10% normal goat serum (NGS) containing 0.1% TritonX-100 for 1 hr at room temperature. Sections were incubated with primary antibodies in 10% NGS in Tris-buffered saline (TBS, 50 mM Tris, 150 mM NaCl) containing 0.1% Triton X-100 (TBS-T) overnight at 4°C (antibodies listed in **Supplemental Table 2**). The next day, sections were incubated with species-specific secondary antibodies with 10% NGS for 1 hr at room temperature. Secondary antibodies were linked to HRP by adding mouse anti-horseradish peroxidase (1:200) and horseradish peroxidase (1:30) (MAP/HRP) (MediMabs, Canada) or Vectastain Elite ABC-HRP kit (Vector labs, USA) to slices, prepared 30 min prior to incubation for 1 hour at room temperature. Sections were then mounted onto gelatin coated slides coverslipped using Entellan mounting media after gradual dehydrations (EM Science, USA). Immunofluorescence procedures were carried out as previously described (Chapter II). Acquisition and analyses of microscopy images were performed as previously described (Chapter II) on regions of interest (ROI).

2.6 Electron microscopy

The corpus collosum, hippocampus and frontal cortex of 26-month-old R955-hTau^{+/-} rats (n=2) were examined using a tissue processing protocol over seven days as previously described (Malcolm et al., 2019; Ribeiro-da-Silva et al., 1993) (Chapter II) with minor modification. Following anesthesia, rats were perfused with cold saline perfusion buffer, then perfused with 0.1 % glutaraldehyde dropwise for 30 min and then perfused with 4% PFA dropwise for 30 min. The brain was then dissected and fixed in 4% PFA for 2 hours. 50 µm brain sections were cut using a Vibratome 1000 Plus. Sections cryopreserved in 30% sucrose and 20% glycerol were then subjected permeabilization using isopentane and liquid nitrogen freeze-thaw cycles and left in 0.1M phosphate buffer until immunohistochemistry. A subset of sections was allocated to examine morphology and another to probe total human tau (HT7) by immunogold. Sections were washed with 1% sodium borohydride for 30 min at room temperature, followed by washing with PBS and then blocking with 10% NGS and 3% bovine serum albumin (BSA) for 1 hr at room temperature before incubation with primary antibody (HT7 1:400, Thermofisher Scientific) overnight at 4°C in PBS. The following day, sections were washed in 0.5% fish gelatin, 0.8% BSA and 1% non-fat milk in PBS for 10 minutes followed by incubation with nanogold anti-mouse conjugated to gold (1.4nm, 1:200 Nanoprobes, USA) in washing buffer overnight at 4°C. Sections from both immunogold and morphology were incubated in 1% glutaraldehyde for 10 min and then underwent silver intensification (Nanoprobes, HQ Silver, USA) for 8 min in a dark room. Sections were washed with water before treatment with osmium tetroxide for 1 hr at 4°C, then underwent stepwise dehydrations using ethanol, propylene oxide and increasing concentrations of Epon in propylene oxide. Sections underwent flat embedding onto acetate foil and affixed to Epon blocks. Blocks were ultrasectioned into 70 nm slices using a ultramicrotome (Ultracut III, Reichert-Jung, Leica, Germany) and then slices were mounted onto 200 mesh copper grids with carbon film and contrast stained with uranyl acetate and lead citrate. Images were acquired using a FEI Tecnai G2 Spirit Twin 120 kV Cryo-TEM equipped with a Gatan Ultrascan 4000 4k x 4k CCD Camera System Model 895.

2.7 Extraction of Sarkosyl-insoluble tau and quantification of human tau

Sarkosyl-soluble and -insoluble tau was extracted following the protocol previously described (Chapter II) to isolate human PHF material in AD brain tissue (Allen et al., 2002; Hasegawa et al., 1992; Malcolm et al., 2019) with minor modification. In brief, cortical brain tissue

homogenized in A68 Buffer (10 mM Tris pH 7.4, 0.8 M NaCl, 1 mM EGTA, 5 mM EDTA, 10% sucrose, 1 mM PMSF) containing protease and phosphatase inhibitors (Complete mini and PhosSTOP, Roche, Switzerland) was used. Supernatants were then incubated in 1% Sarkosyl (Sigma, Germany) then ultracentrifuged to separate the Sarkosyl-soluble supernatant and insoluble pellet fractions. The supernatant containing the Sarkosyl-soluble fraction was immediately removed and stored at -20°C, while the pellet was resuspended in 200 µL 50 mM Tris-HCl, pH 7.4 and labelled as the Sarkosyl-insoluble fraction and also stored at -20°C.

Concentration of human tau in Sarkosyl-soluble and Sarkosyl-insoluble fractions were assessed using a commercial total human tau enzyme-linked immunosorbent assay (ELISA) kit (KHB0041, Thermofisher Scientific) following manufacturer's instructions. Samples with a normalized protein concentration from Lowry's method of protein quantification, and then optimized dilutions were used for soluble and Sarkosyl-insoluble fractions, respectively.

2.8 Western blot

Blotting procedures were performed as described previously (Chapter II) with minor modification. Twenty milligrams of rat brain tissue, homogenized using Cell Lysis Buffer (Cell Signaling Technologies, USA) mixed with protease and phosphatase inhibitors (Complete mini and PhosSTOP, Roche, Switzerland), was used. Supernatants and Sarkosyl-insoluble fractions were loaded and ran on 10% polyacrylamide gels, then transferred onto Polyvinylidene difluoride membranes activated by methanol. Blots were developed using the Western Lightning Plus chemiluminescence substrate (Perkin Elmer, USA) and imaged using an Amersham 600 (GE Healthcare, Canada).

2.9 Statistical analysis

Statistical analysis was performed as described previously (Chapter II) using GraphPad Prism 8 software (San Diego, USA). Data are expressed as mean +/- standard error of the mean (SEM). Two-tailed unpaired t-tests were performed on single factor, single comparisons. Each time point was analyzed independently. As appropriate, one-way ANOVA was followed up with Bonferroni's adjustment for three or less comparisons and Tukey's for more than three comparisons. Two-way repeated measures ANOVA (RM-ANOVA) using a mixed design was followed up with Holm-Sidak's pairwise comparisons. If a significant interaction was found, only

simple main effects of genotype were examined. A standard global alpha of 0.05 was used. Statistical outliers, as assessed using Grubbs test, were removed from datasets and analysis.

3. Results

3.1 Intraneuronal accumulation and distribution of human tau in R955-hTau^{+/-} rats.

As expected, McGill-R955-hTau^{+/-} rats showed an age-dependent accumulation of total human tau in the brain as examined by HT7 immunoreactivity (IR) (**Figure 1**) which was restricted to the forebrain (**Figure 1A-D**) without detectable IR in the cerebellum, brain stem or spinal cord. Given the CaMKII- α promoter driving human tau expression, the transgene was predominantly expressed in telencephalic structures within the neuronal soma and the apical dendrites of pyramidal neurons. Similar to young adult 3–6-month-old rats (**Supplemental Figure 1A-G**), by 10 months (**Figure 1E-G**), human tau-immunoreactive material was detected in brain regions dominated by excitatory neurons such as the subiculum (**Figure 1E, H, K**), CA1 region of the hippocampus (**Figure 1F, I, L**) and layers II, III of the cerebral cortex (**Figure 1G, J, M**). Additionally, HT7 IR was detected in other key areas including the CA2 and CA3 region of the hippocampus (**Supplemental Figure 1H-I, K-L, N-O**) and in the amygdala (**Supplemental Figure 3B**). Human tau IR was more intense at 18 months of age and widely observed across the hippocampus and cerebral cortical (**Figure 1H-J**), suggestive of human tau accumulation. At the oldest age and latest time point of 24-26 months, the distribution of HT7 IR, while regionally like the other time points, was noticeably more intraneuronal and somatodendritic (**Figure 1K-M**).

3.2 Tortuous dendrites, neuritic beading and somatodendritic mislocalisation of tau are early morphological indicators of tauopathy

At 24-26-months, aged R955-hTau^{+/-} rats exhibited abnormal morphologies from HT7 IR signifying an acceleration of the tau pathology - features which were not present at younger ages. Hippocampal neurons were lost or otherwise displaced from the orderly pyramidal neuronal layers of the CA1 and CA2 regions in a disorganized fashion (**Figure 1N**). Additionally, HT7 immunoreactive pyramidal neurons in layer V of the cerebral cortex adopted a twisted dendritic

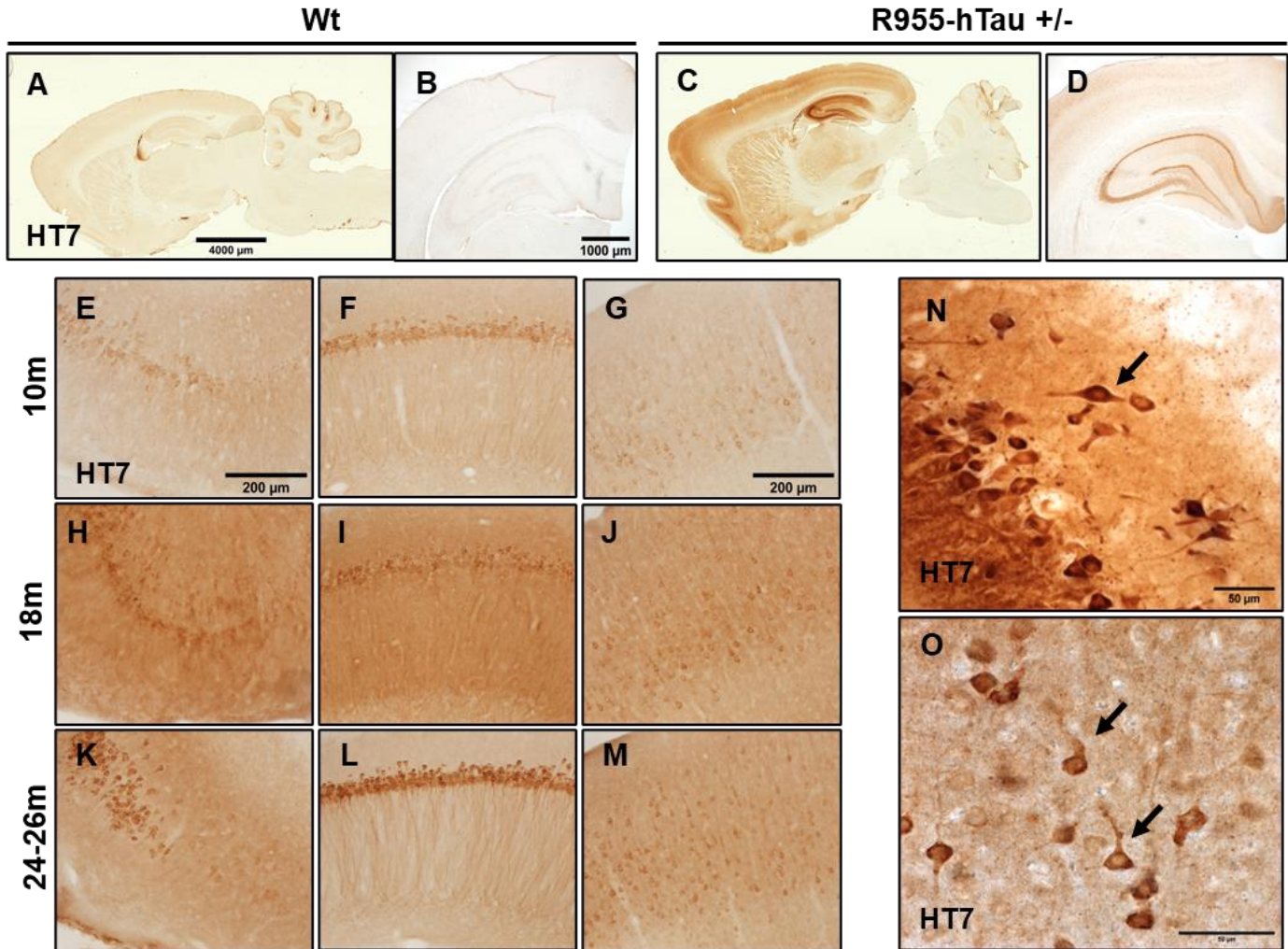


Figure 1. R955-hTau^{+/-} rats accumulate intraneuronal human tau in an age-dependent fashion. (A-D) Representative low-magnification micrographs illustrating the distribution of human tau by HT7-immunoreactivity in the forebrain and telencephalic regions of the CNS from sagittal (A, C) and coronal (B, D) brain sections of 18-month-old wild-type (A) and 18-20-month-old R955-hTau^{+/-} transgenic rats (C). (E-M) Representative images illustrating the time-dependent distribution of neuronal expression of transgenic human tau in the subiculum (E, H, K), CA1 region of the hippocampus (F, I, L), layers II-III of the cerebral cortex (G, J, M) at the three investigated time-points. At 24 months of age, note the separation and disorganization of hippocampal neurons from the CA1 and CA2 region of the hippocampus (N, **black arrow**) and crooked apical dendrites in cortical pyramidal neurons (O, **black arrows**). A, C: scale bar = 4000 μ m; B, D: scale bar = 1000 μ m; E-M: scale bar = 200 μ m; N-O: scale bar = 50 μ m.

morphology (**Figure 1O**). Notably, intense HT7-IR was observed in the somatodendritic compartment of pyramidal neurons in the cerebral cortex (**Figure 1O**) and hippocampus (**Figure 2I**). Unexpectedly, HT7-IR also detected a small speck-like morphology in the CA1/CA2 neurites (**Figure 2J**) and corpus callosum myelin (**Figure 2K**) suggestive of tau accumulation in the synaptic compartment. The intense HT7 IR, denoting tau accumulation, coupled with the identified morphological abnormalities of the somatodendritic compartments of pyramidal neurons in the absence of obvious fibrillary lesions.

3.3 Aged R955-hTau^{+/-} rats develop tau hyperphosphorylation at pSer202-Thr205 and pThr231.

Probing phosphorylated tau (ptau) in R955-hTau^{+/-} rats revealed a notable increase in tau hyperphosphorylation after 24 months, as compared to 18 months (**Figure 2A-H**). After 24 months, remarkable surge of pSer202-pThr205 immunoreactive material, as probed with the AT8 monoclonal antibody, was observed in the subiculum (**Figure 2E**), CA1 (**Figure 2F**), CA2/CA3 (**Figure 2G**) and cerebral cortex (**Figure 2H**). While absent at earlier time-points, faint AT8 IR was evident as from 18 months of age in the subiculum (**Figure 2A**), the CA1 (**Figure 2B**) and in layers II-III of the cerebral cortex (**Figure 2D**), but less conspicuously in the CA3 region (**Figure 2C**). At the time of the surge of AT8 IR detected in 24-26-month-old R955-hTau^{+/-} rats, we observed a minute subset of neurons which were heavily AT8 immunoreactive in the CA2 and CA3 compared to other neighboring neurons (**Figure 2G**). Overlapping morphological abnormalities as those revealed with HT7 were also observed with ptau-immunoreactive epitopes such as pSer202-Thr205.

Under high magnification, in addition to the CA1 region of the hippocampus, pyramidal neurons located in layers II-III and V of the cerebral cortex bore small beads of AT8-immunoreactive material (**Figure 2L**) as well as numerous dendritic elements with a tortuous morphology (**Figure 2M**). Such neurons intensely immunostained with AT8 displayed clumped immunoreactive material at their processes suggestive of aggregation.

In contrast to AT8, ptau at pThr231 labelled by the monoclonal antibody AT180 was restricted to the pyramidal neurons of the CA1 region (**Figure 2F**) whereas ptau epitopes associated with advanced fibrillary stages of tauopathy including pSer396-pSer404 using the C-terminal antibody PHF1 (**Supplemental Figure 2Q-R**) and tau IR to misfolded tau with the antibody MC1 (**Supplemental Figure 2S-T**) proved absent. This demonstrated that the observed

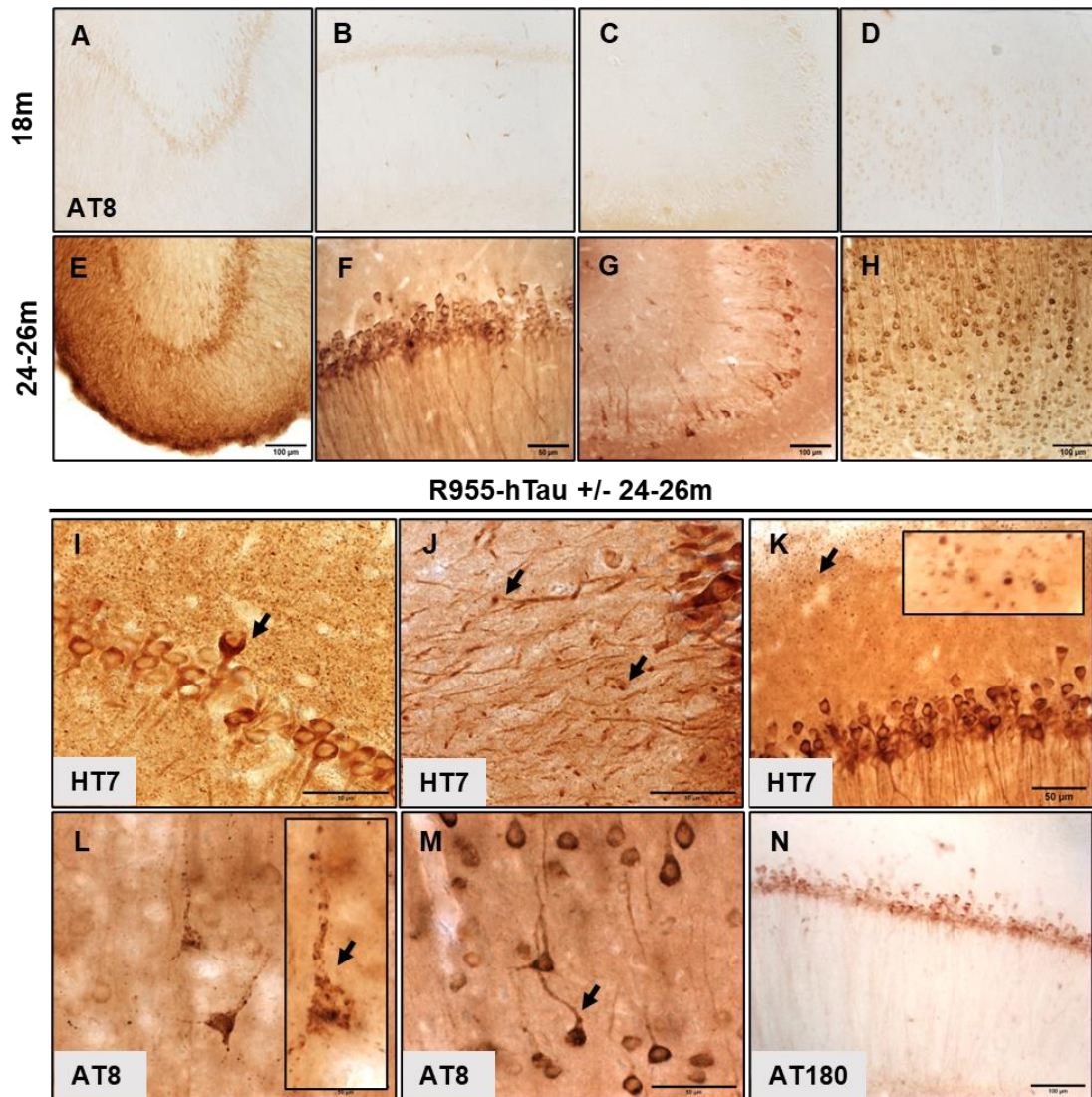


Figure 2. Intraneuronal human tau (HT7) accumulation and hyperphosphorylation (AT8) produce morphological abnormalities indicative of tau pathology in aged R955-hTau^{+/-} rats. (A-H) Representative micrographs of coronal sections of 18-month-old (A-D) and 24-26-month-old R955-hTau^{+/-} rats (E-H) illustrating a surge of ptau (AT8) immunoreactivity at Ser202-Thr205. Barely detectable AT8 IR at 18 months in the subiculum (A, D), CA1 (B, F), and CA3 region of the hippocampus (C, G), and layers II-III and V of the cerebral cortex (D, H). Note the selective AT8 IR in neurons of the CA3 (G) and (H) layers II-V of the cerebral cortex. (I-M) Representative high-magnification micrographs of 24-26-month-old R955-hTau^{+/-} rat brain illustrating human tau IR predominantly restricted to the intraneuronal soma and apical dendrites (I-J). Unexpectedly, dense HT7 IR material was observed in the neurites of hippocampal pyramidal neurons (J, **black arrow**) and in the corpus callosum white matter (K, **black arrow**). Additionally, accumulation of ptau by AT8 IR in the somatodendritic compartment of pyramidal neurons in the cerebral cortex indicating reversal of tau polarization and suggestive of protein aggregation (L). Further, cortical neurons bearing dense AT8 IR were misshapen (M). Notably, pyramidal neurons of the CA1 region in the hippocampus also developed ptau Thr231 (AT180) IR at 24-26 months of age (N). A, C-D, E, G-H, N: scale bar = 100 μm; B, F, I-M: scale bar = 50 μm.

epitope-specific tau alterations occurred in the absence of misfolded or fibrillary tau.

During the accelerated tau pathology depicted by HT7, AT8 and AT180 IR in 24–26-month-old R955-hTau^{+/-} rats, other ptau epitopes such as pThr181 (**Supplemental Figure 2A-H**) and pS422 (**Supplemental Figure 2I-P**) did not display detectable differential IR at 18 or 24-26 months, as compared to wild types. Further, epitopes such as pTyr18, pThr175, Ser208/210, and fibril-associated tau cleaved at Asp421 (TauC3), showed no detectable IR or no differences from wild-type rats (data not shown). Altogether, these observations implicate the ptau epitopes of pSer202, pThr205 and pThr231 as the earliest indicators of tau pathology in the R955-hTau^{+/-} rat model, as assessed by immunohistochemical approaches.

3.4 Age-related behavioral impairments in learning and memory parallel the tau pathology.

Notwithstanding the slow developing tauopathy of the heterozygous R955-hTau transgenic model, some deficits in cognition in transgenic rats were detected already at 18 months at a time point with observable human tau accumulation as well as detectable ptau in the cerebral cortex (**Figure 3A-F**). As expected, 10-month-old transgenic rats performed comparable to wild-type littermates in all investigated learning and memory tasks (**Supplemental Figure 3**).

At 18 months, wild-type and R955-hTau^{+/-} rats spent a similar amount of time exploring the center of the open field (**Figure 3A**; $p=0.6772$). During the novel object location (NOL) and recognition (NOR) tasks, animals explored the five objects equally during the familiarization phase (**Supplemental Figure 3F-H**) indicating no baseline difference in explorative behavior. Transgenic R955-hTau^{+/-} rats showed an impaired ability to discriminate novelty during the NOL (**Figure 3B**; $t(26)=2.645$, $p=0.0137$, unpaired t-test) and NOR tasks (**Figure 3C**; $t(27)=4.806$, $p<0.0001$, unpaired t-test) whereas, during the Morris water maze (MWM), R955-hTau^{+/-} rats at 18 months of age performed similar to wild-type littermates over the five days of training (**Figure 3D**; $p=0.1232$). Further, during the probe trial, 18-month-old R955-hTau^{+/-} rats spent a similar percentage of time in the correct quadrant (**Figure 3E**; $p=0.7936$), together showing no initial impairment in spatial learning and memory.

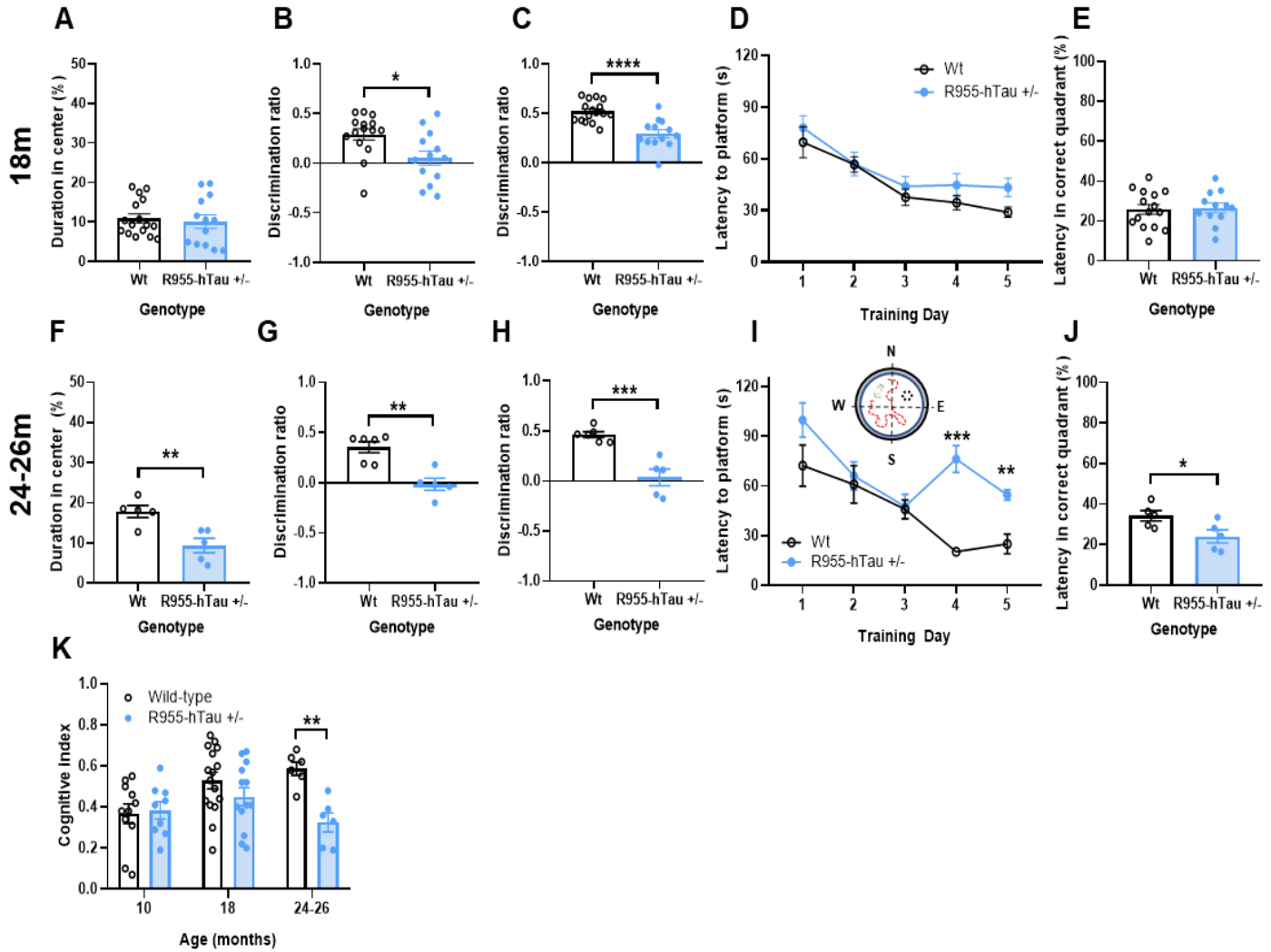


Figure 3. Cognitive impairments in R955-hTau^{+/-} rats were detected after 18 months of age, paralleling the tau pathology. Behavioral assessment of cognition at 18 months (A-E) and 24-26 months (F-J). (A, F) Time spent in the exposed arena center during the open field (OF) task. Impaired discrimination ratio between novel and familiar objects during the novel object location (NOL) (B, G) and recognition (NOR) tasks (C, H) in transgenic R955-hTau^{+/-} rats as compared to wild-types (Wt). (D-E, I-J) Spatial learning and memory, assessed by the Morris water maze (MWM) task, was impaired in 24-26-month-old R955-hTau^{+/-} rats as shown by an impaired ability to learn the location of the platform over five days of training (I) and to correctly locate the platform during the probe trial (J). (K) Lower global cognitive index in transgenic rats only at the 24-26-month-old time point. *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ (n= 4-16).

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At 24-26 months, alongside the surge of pSer202-Thr205 and hippocampal pThr231 ptau, aged R955-hTau^{+/-} rats displayed cognitive deficits in learning and memory tasks as compared to age-matched wild-type littermates which was in the absence of motor impairments, as determined by total distance travelled during the OF task (**Supplemental Figure 3I**) and swim velocity during the MWM (**Supplemental Figure 3J**). As such, transgenic rats spent half as much time exploring the exposed center during the open-field task (**Figure 3F**; $t(8)=3.633$, $p=0.0067$, unpaired t-test), and completely lost the ability to discriminate the NOL (**Figure 3G**; $t(9)=4.555$, $p=0.014$, unpaired t-test) and novel object in the NOR task (**Figure 3H**; $t(9)=5.211$, $p=0.006$, unpaired t-test). During the MWM task, R955-hTau^{+/-} rats required a significantly longer duration to find the platform during days 4 and 5 of training (**Figure 3I**; Day 4 $t(8)=6.662$, $p=0.0002$; Day 5 $t(8)=4.397$, $p=0.0023$, pairwise comparison). Further, after the last day of training, the probe test revealed that R955-hTau^{+/-} rats spent a lower percentage of time in the correct quadrant compared to wild-type littermates (**Figure 3J**; $t(8)=2.490$, $p=0.0375$, unpaired t-test), the above demonstrating impairments in spatial learning and memory. In contrast to the OF, NOLR and MWM tasks, the fear conditioning (FC) task revealed no impairments in freezing behaviors (**Supplemental Figure 4**).

Combining the outcomes of the behavioral tasks into a global cognitive index score calculating the combined performance of all behavioral tasks performed for each rat revealed a drastic reduction in cognitive index by two-fold only in 24-26 month-old R955-hTau^{+/-} rats compared to wild-type littermates, while not at the 18-month-old time point (**Figure 3K**; $F(1, 54)=16.25$, $p=0.0002$, 2-way ANOVA; $t(54)=4.503$, 18m $p=0.3104$; 24-26m $p=0.0001$). Taken together, the sequential changes in the aforementioned tau accumulation and phosphorylation parallels the impairments in cognition in aged transgenic rats.

3.5 Tau accumulation and phosphorylation are followed by the occurrence of modified tau protein species

HT7-IR indicated that total human tau accumulated gradually with age in R955-hTau^{+/-} rats across multiple brain regions. To best determine quantitatively the brain concentrations of accumulated human tau protein, we applied a total human tau ELISA assay on Sarkosyl-soluble (**Figure 4A**) and Sarkosyl-insoluble tau (**Figure 4B**) on cortical brain homogenates. As expected, Sarkosyl-soluble and -insoluble tau levels increased between 10 and 18 months of age with

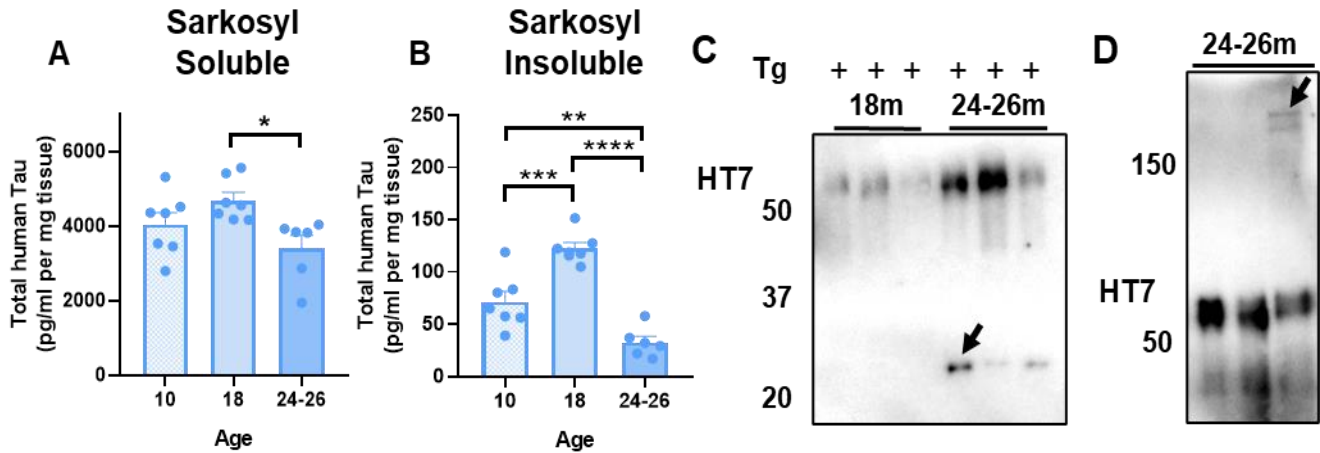


Figure 4. Differential human tau protein levels in R955-hTau^{+/-} rats foreshadows degeneration at 24-26 months. Quantification of human tau levels in (A) Sarkosyl-soluble and (B) Sarkosyl-insoluble fractions of cortical brain tissue across age and (C-D) Representative Western blots of Sarkosyl-insoluble brain extracts demonstrating the presence of low molecular weight (C, black arrow) and high molecular weight tau bands over 150 kDa in 24-26-month-old R955-hTau^{+/-} rats (D, black arrow). *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ (n=5-7).

significance being reached only in the Sarkosyl-insoluble fraction (Figure 4A; $p=0.3769$; Figure 4B; $t(17)=4.982$, $p=0.003$, unpaired t-test). On the other hand, tau levels decreased significantly at 24-26 months of age compared to 18-month-old rats (Figure 4A; $t(17)=3.075$, $p=0.0206$; Figure 4B $t(17)=8.434$, $p<0.0001$, unpaired t-test).

To elucidate the Sarkosyl-insoluble tau species present in aged R955-hTau^{+/-} rats, probing total human tau (HT7) by Western blotting revealed the presence of lower ~29 kDa molecular weight bands in 24–26-month-old transgenic rats which were absent at 18 months (Figure 4C) suggestive of degradation or truncation events. Furthermore, in 24–26-month-old transgenic R955-hTau^{+/-} high molecular weight bands over ~150 kDa were also partially detected (Figure 4D) conversely suggesting the occurrence of tau oligomerization.

3.6 Hippocampal neuronal loss in aged McGill-R955-hTau^{+/-} rats coincides with a CNS inflammatory response.

As gliosis and neuronal loss are evident in individuals with AD and in animal models of tauopathy (Brandt et al., 2005; Eikelenboom et al., 2002; Laurent et al., 2018; Malcolm et al., 2019; Padurariu et al., 2012; Shi et al., 2017; Yoshiyama et al., 2007), we therefore investigated these aspects using quantitative immunofluorescent studies of total neuronal and glia cell numbers,

total fluorescence intensity (**Figures 5-6**). Studies focused on the hippocampal CA1 and subiculum regions as these areas displayed the most intense AT8 IR and given the unique detection of AT180 IR in the CA1 region of 24-26-month-old transgenic rats. At 24-26 months, there was approximately a 15% loss of NeuN-positive neurons in the CA1 pyramidal layer of the hippocampus in R955-hTau^{+/-} rats compared to age-matched wild-types (**Figure 5D**; $t(13)=3.281$, $p=0.0060$, unpaired t-test) whereas no detectable neuronal loss was found at 18 months (**Figure 8D**; $p=0.1476$). In contrast, neuronal loss was not detected in other ptau burdened brain regions such as the subiculum (**Supplemental Figure 5F**; 18m $p=0.3846$; 24-26m $p=0.8066$) nor somatosensory cortex (**Supplemental Figure 6E**; 18m $p=0.4515$; 24-26m $p=0.2608$) regardless of the age.

Signs of astrogliosis were also identified in 24-26-month-old R955-hTau^{+/-} rats as determined by the presence of scattered astrocytes displaying a subtle swollen morphology (**Figure 5G**). GFAP immunofluorescence (IF) intensity in the CA1 region of the hippocampus was approximately two-fold higher in 24-26-month-old transgenic R955-hTau^{+/-} rats. Intriguingly, the increase in GFAP IF at 24-26 months was preceded by a decreased IF detected at 18 months (**Figure 5H**; 18m $t(12)=2.983$, $p=0.0114$; 24-26m $t(12)=3.035$, $p=0.0104$, unpaired t-test). In the subiculum, an initial reduction in GFAP IF at 18 months was also observed, however, no differences in GFAP IF were detected in this brain area at 24-26 months of age (**Supplemental Figure 5E**; 18m $t(13)=2.904$, $p=0.0123$, unpaired t-test; 24-26m $p=0.3256$). This loss of GFAP fluorescence in R955-hTau^{+/-} rats at the age of 18 months may be the consequence of an early loss of astrocytic support and reduced neuronal health as it has been suggested to occur in FTDP [46].

During the examination of microglia, at 24-26 months, R955-hTau^{+/-} rats developed an increase in the total number of Iba1-immunoreactive microglial cells in the CA1 (**Figure 6E**, $t(13)=2.606$, $p=0.0217$, unpaired t-test). As expected, no differences in total number of Iba1-immunoreactive cells or overall morphology in the CA1 were present at 18 months of age or earlier (**Figure 6E**; $p=0.9306$). A subset of microglia displayed abnormal morphologies including a swollen cell soma and retracted processes whereas other morphologies were reminiscent of rod-shaped microglia that trailed alongside the dendritic processes of CA1 pyramidal neurons (**Figure**

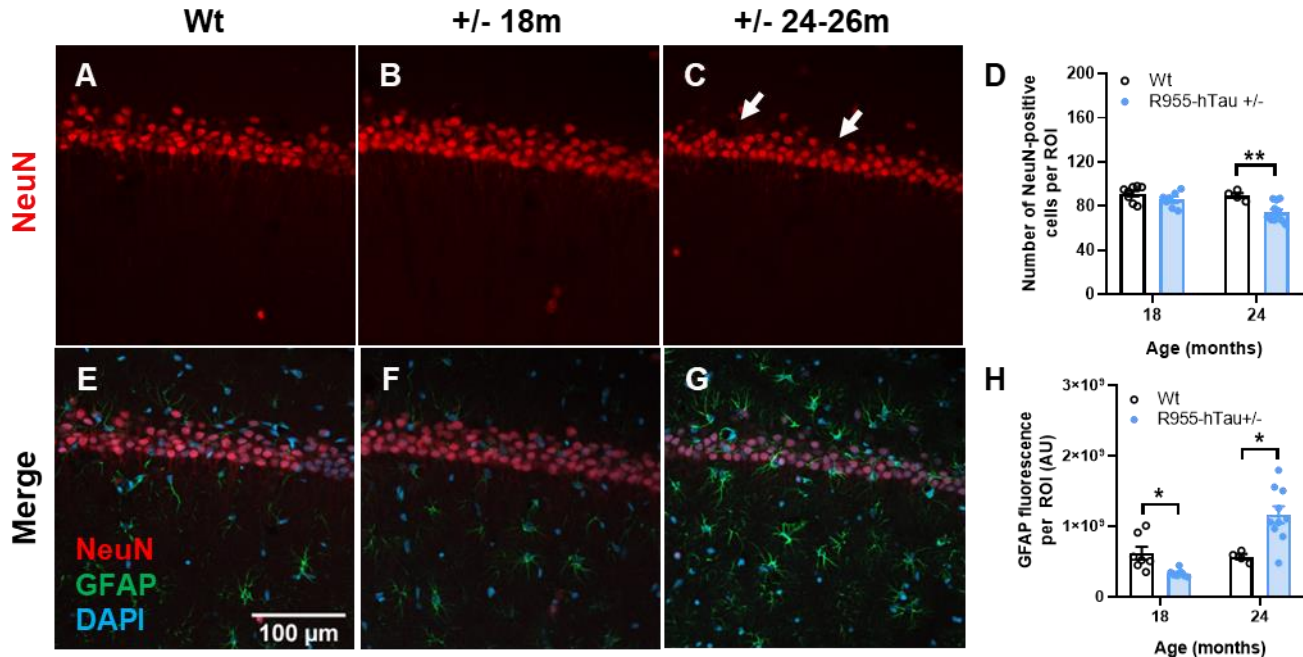


Figure 5. Aged R955-hTau^{+/-} rats develop CA1 neuronal loss and display a shift in astrocytic activity. (A-C) Representative confocal images of the hippocampal CA1 neuronal layer illustrating neuronal loss in 24-26-month-old transgenic R955-hTau^{+/-} rats (C, **white arrow**) as compared with wild-types (Wt) (A) and 18-month-old rats (B). (D) Quantification of the total number of NeuN-immunofluorescent cells in the CA1, the region of interest (ROI). (E-G) Merged confocal images illustrating astrocytosis in 24-26-month-old transgenic R955-hTau^{+/-} rats, noting the subtle swelling of astrocytes (H) Quantification of GFAP fluorescence intensity of the CA1 region (n=4-11). *= $p < 0.05$, **= $p < 0.01$.

3132 **6F)**. These atypical microglia morphologies have been described in homozygous R955-hTau (Chapter II) and R962-hTau (Malcolm et al., 2019) rats where they appeared to a more severe and defined extent. In contrast, in the subiculum region of the hippocampus there were no such differences in morphology and number regardless of age (**Supplemental Figure 5K**; 18m, $p=0.8425$; 24-26m $t(13)=1.085$, $p=0.2978$). Altogether, these observations in both astrocytes and microglia implicate an important glial response on the CA1 region related to ptau Thr231 IR in the absence of fibrillar tau.

3.7 Evidence of myelin degeneration in the absence of fibrillary tau.

To further explore degenerative consequences of the tau-associated pathology, transmission electron microscopy was performed on aged R955-hTau^{+/-} rats to examine ultrastructural cellular morphology of cells of the cerebral cortex and the corpus callosum white matter in comparison with wild-type controls (**Figure 7**).

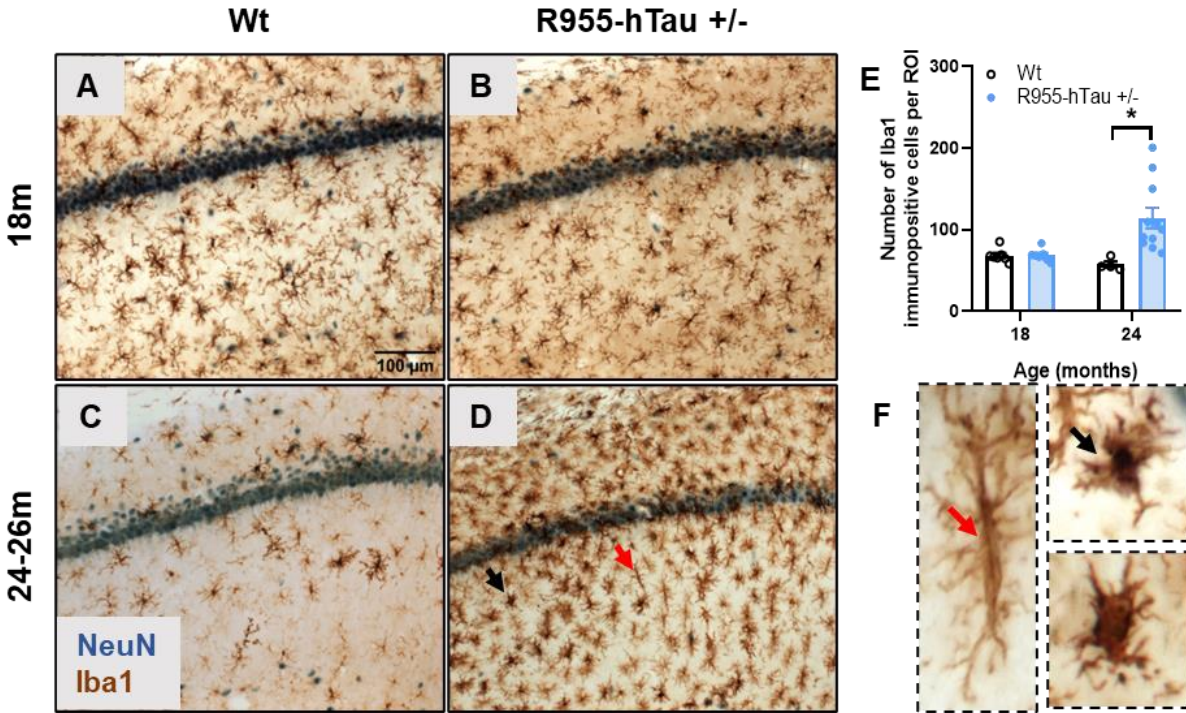


Figure 6. Hippocampal microgliosis and altered morphology was detected in 24-month-old R955-hTau^{+/-} rats. (A-B) Representative images of 20-month-old rat CA1 neurons probed by NeuN IR (blue) and microglia probed by Iba1 IR (brown). (C-D) Representative images of 24-month-old rats illustrating an increase in microglial cells in the CA1 region of the hippocampus in R955-hTau rats compared to age-matched wild-type littermates. (E) Quantification of total number of Iba1 immunoreactive cells in the CA1 region (n=4-11). (F) Higher magnification images of 24-month-old rat CA1. Note the presence of rod-shaped microglia (F, red arrow) and microglia with a swollen, disease-associated morphology (F, black arrow). Scale bar = 100 μ m. * p<0.05.

Multiple abnormalities indicative of degeneration was found in the myelin in 26-month-old R955-hTau^{+/-} rats (Figure 7C) as compared to wild-type age-matched controls (Figure 7A) and 21-month-old R955-hTau rats^{+/-} (Figure 7B). Some of the myelin strands of aged transgenic rats possessed a twisted and thinned appearance (Figure 7C), formed into myelin balloons (Figure 7D) or axonal distortions (Figure 7E) indicating a loss of myelin integrity and axonal degeneration. Probing total human tau (HT7) with immunogold-conjugated nanoparticles revealed clear HT7 IR in close proximity to the highlighted abnormalities observed in the myelin (Figure 7F). Additionally, HT7-IR was observed inside and around the neighbouring lysosomes (Figure 7G-H) as well as near pronounced vacuolization in the corpus callosum (Figure 7I). These observations demonstrated subtle, ultrastructural degenerative phenomena attributed to the human tau pathology in aged R955-hTau^{+/-} rats.

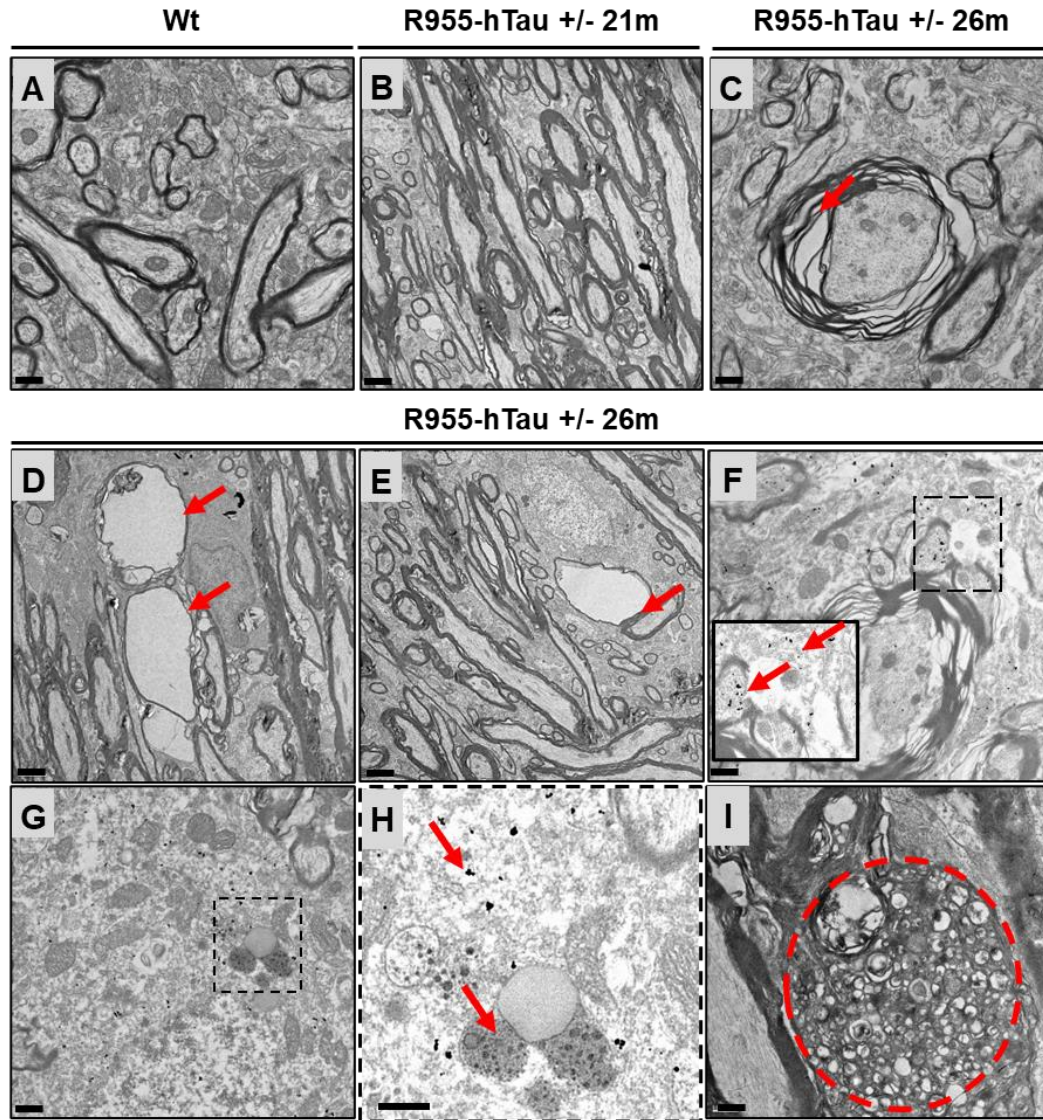


Figure 7. Ultrastructural alterations to myelin indicate a progression of underlying subtle degenerative processes in the brains of 26-month-old R955-hTau^{+/-} rats. (A-C) Representative high magnification electron microscopy micrographs, with lipids like myelin counterstained in black, illustrating the status of myelinated structures of the corpus callosum and dendrites of age-matched wild-types (Wt) (A), 21-month-old (B), and 26-month-old transgenic R955-hTau^{+/-} rats (C-I). At 26 months, myelin strands of the corpus callosum and frontal cortex of R955-hTau rats were notably thinned and displayed a tortuous appearance (C, red arrow). Loss of the integrity of the myelin sheath and myelin ballooning (D red arrows) accompanied by degenerating axonal processes as identified from axonal distortions, were observed (E, red arrow). These degenerative morphologies of the myelin were in close proximity to human tau (HT7) immunoreactive material (F, red arrows with black immunoreactive specks). Clusters of HT7 immunogold material were also in close proximity to enlarged lysosomes of oligodendroglial cells (G-H, red arrow). Intense vacuolization in close proximity to the myelin of the corpus callosum (I, red dashed circle). All these observations are indicative of degenerative processes. B, D-E scale bar = 2 μ m; A, C, F-I scale bar = 0.5 μ m.

4. Discussion

In the present study, we provide evidence that the heterozygous McGill-R955-hTau rat model displays a slow progressing human-like tauopathy which is sufficient to induce hippocampal neuronal loss at an advanced life stage. In this transgenic model, signs of neuronal damage are observed with oligomeric tau and preceding the overt fibrillary tau formation or aberrant changes in tau conformation and coinciding with the initial acceleration of the CNS tau pathology. Importantly, indications of myelin degeneration that would not have been readily detected by conventional IHC or IF were well-defined by electron microscopy. Therefore, the R955-hTau rat model in its heterozygous form may be a suitable model of the early stages of tauopathy, allowing to unravel pathological processes while preserving the aging facet of the disease. However, this model may not be suitable to evaluate experimental therapeutics.

The findings of this study are consistent with observations reported in other transgenic mouse models, showing that tau accumulation precedes the progressive hyperphosphorylation and the sequential toxic tau aggregation (Avila, 2006; Ballatore et al., 2007; Goedert et al., 1991; Hernández and Avila, 2007; Iqbal et al., 2016, 2010, 2005; Lee et al., 2001; Wang and Mandelkow, 2015). However, the sequential changes of ptau species (**Supplemental Table 3**) that occurred in the R955-hTau^{+/-} transgenic rat model at the age of 24-26 months provided new evidence for neuronal degenerative alterations coincident with tau hyperphosphorylation at pThr231 (AT180). Further, such neurodegenerative events, including neuronal loss and myelin degeneration occurred in the absence of advanced tau pathology as indicated by an absence of misfolded tau (MC1) (**Supplemental Figure 2S-T**) (Weaver et al., 2000) or neurofibrillary tangles (NFTs) as assessed by a combination of ptau Ser396-Ser404 (PHF1) (**Supplemental Figure 2Q-R**), pThr212-pSer214 (AT100) or the ptau at the PHF core at Glu391 (NOAL/MN423) (Novak et al., 1989). While heterozygous McGill-R955-hTau transgenic rats did not produce overt NFT's by 24 months, the advanced tau pathology develops earlier in homozygous R955-hTau rats (Chapter II) and heterozygous R962-hTau rats (Malcolm et al., 2019), demonstrating the capacity of the tau transgene to readily develop NFTs in these transgenic rat models.

Yet, despite the absence of advanced tau pathology, we observed dendritic morphologies reminiscent of neuritic beading and immunoreactive specks containing human tau and AT8- ptau, at this same time point of 24-26 months, as illustrated in **Figure 2**. Further, these changes occurred with a lack of immunoreactivity at other ptau epitopes including pThr181 (AT270), pSer422 p-

Tyr18, pThr175, Ser208/210, and cleaved tau at Asp421 (TauC3) (**Supplemental Figure 2**). Neuritic beading has been described in axonal damage and degeneration involving microglia (Tseng et al., 2017). Importantly, the clumps of intraneuronal AT8-IR and HT7-IR bear remarkable morphological resemblance to granular oligomeric tau in human AD cortical neurons (Merino-Serrais et al., 2013; Takashima, 2016). The small speck-like structures are reminiscent of tau “excrescences” described previously in early Braak stages (Braak and Del Tredici, 2020; Tai et al., 2012). Consistent with the altered tau species detected in Sarkosyl-insoluble brain homogenates of 24-26-month-old R955-hTau^{+/-} rats, revealed truncated and high molecular weight tau. Truncated human tau previously showed to produce robust fibrillary degeneration, yet no reported neuronal loss *in vivo* (Filipcik et al., 2012; Hrnkova et al., 2007; Zilka et al., 2006). Consistent with the R955-hTau transgenic rat model, truncated tau species may be drivers of furthering the tau pathology. Furthermore, in line with the tau oligomer hypothesis that suggests oligomeric tau as the most bioactive and pathogenic species, the high molecular weight tau may be contributing to neuronal loss in this model.

In the hippocampus of aged McGill-R955-hTau^{+/-} transgenic rats, the CA1 pyramidal neurons appeared as the most vulnerable to tau pathology, as approximately 15% of neurons were lost while no obvious loss was detected in other brain regions. Given that CA1 neurons become compromised at prodromal stages (Li et al., 2018; Scheff et al., 2007, 2006) and that this region becomes atrophic at clinical AD stages by up to 40% (West et al., 2004), the observed CA1 neuronal loss may represent an earlier or preclinical aspect of the CNS tauopathy in this model. In mouse models, neuronal loss is typically described in post-fibrillary stages of tauopathy, however reports suggest that neuronal loss precedes or is independent of NFT formation (Gómez-Isla et al., 1997), with NFT-positive neurons being claimed to survive for an extended period of time (Morsch et al., 1999). Established mouse models of brain’s tauopathy such as the PS19 and Tau22 mice (Dujardin et al., 2015; Götz and Götz, 2019) exhibit advanced tau pathology before one year of age, however, curiously, most fail to display neuronal loss (Allen et al., 2002; Gotz et al., 2001; Götz et al., 2001; Yoshiyama et al., 2007). Further, transgenic P301L rats with a mild transgene expression under a murine prion promoter model failed to produce a tauopathy phenotype, behavioral impairments, neuronal loss and inflammation (Korhonen et al., 2011). Possible explanations for these differences are likely due to a combination of the aging process, gene

promoter, transgene strength and the functional similarity between rat ApoE and human APOE4, which accelerates tau-mediated neurodegeneration (Shi et al., 2017).

Apoptosis has been described in the AD brain (Cotman et al., 2005; Rohn et al., 2001; Su et al., 2001; Yoshida, 2014), and precedes early NFT pathology (De Calignon et al., 2010), resulting in the occurrence of caspase-cleaved tau products. However, the mechanism of degeneration in tauopathies such as FTD and AD are not fully understood. Consistent with this work, we were unable to detect indicators of apoptosis such as cleaved Caspase-3 at Asp175 (**Supplemental Figure 7A-B**) nor caspase-cleaved tau. Tau hyperphosphorylation has been shown to circumvent apoptosis by altering cell-cycle re-entry pathways (Andorfer et al., 2005; Ramalho et al., 2008; Wang et al., 2014). Cleavage of Caspase-3 and TUNEL activation can be induced *in vitro* when tau is phosphorylated at pThr231 and pSer262 (Alonso et al., 2010) of which only elevated pThr231 was accelerated in aged R955-hTau^{+/-} rats.

An inflammatory response (Streit et al., 2014) to tau was observed in aged R955-hTau^{+/-} rats as determined by increased total microglia cell number and altered morphology as well as increased GFAP fluorescence as originally reported in the brainstem and spinal cord of P301L mice by Lewis and colleagues (Lewis et al., 2000). Notably, the puzzling occurrence of rod-shaped bipolar microglia has been described in homozygous R955-hTau (Chapter II) and R962-hTau transgenic rats (Malcolm et al., 2019) in a more severe and defined state, but also in the aftermath of CNS brain injury (Jørgensen et al., 1993; Tam and Ma, 2014; Taylor et al., 2014) as well as in human tauopathies and dementia (Bachstetter et al., 2015) and Down's Syndrome (Flores-Aguilar et al., 2020). The functional significance of this microglia phenotype remains unresolved, however, it has been suggested to be an age-associated phenomenon to cellular damage (Bachstetter et al., 2017) or having a protective role in the CNS (Holloway et al., 2019). Due to their localization to tau-burdened neurons, we propose that the rod-shaped glial cell phenotype could be in response to the release of the tau-immunoreactive specks or altered dendritic processes, internalizing them and rendering glia dysfunctional (Bolós et al., 2015; Hassan-Abdi et al., 2019).

In summary, the present report provides supporting evidence that pThr231 (AT180) and a surge of pSer202-pThr205 (AT8) occur during the initiating stages of tauopathy in this model. Further, we show how morphological changes to neurons, myelin degeneration and underlying neuronal loss occurred in the absence of fibrillary tau at the most vulnerable neuronal sites. Because the heterozygous R955-hTau rat model confers a relatively milder phenotype and more

3249 biologically and clinically relevant age, this model as such should be suitable for the investigation
3250 of earlier stages of human-like tauopathy in the context of AD and FTD as well as for the
3251 elucidation of factors exacerbating the earliest stages of the tauopathy.

5. References

- Allen, B., Ingram, E., Takao, M., Smith, M.J., Jakes, R., Virdee, K., Yoshida, H., Holzer, M., Craxton, M., Emson, P.C., Atzori, C., Migheli, A., Crowther, R.A., Ghetti, B., Spillantini, M.G., Goedert, M., 2002. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J. Neurosci.* 22, 9340–9351. <https://doi.org/22/21/9340> [pii]
- Alonso, A.D., Di Clerico, J., Li, B., Corbo, C.P., Alaniz, M.E., Grundke-Iqbal, I., Iqbal, K., 2010. Phosphorylation of Tau at Thr212, Thr231, and Ser262 combined causes neurodegeneration. *J. Biol. Chem.* 285, 30851–30860. <https://doi.org/10.1074/jbc.M110.110957>
- Andorfer, C., Acker, C.M., Kress, Y., Hof, P.R., Duff, K., Davies, P., 2005. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J. Neurosci.* 25, 5446–5454. <https://doi.org/10.1523/JNEUROSCI.4637-04.2005>
- Arriagada, P. V, Growdon, J.H., Hedleywhyte, E.T., Hyman, B.T., Hedley-Whyte, E.T., Hyman, B.T., 1992. Neurofibrillary Tangles But Not Senile Plaques Parallel Duration and Severity of Alzheimers-Disease. *Neurology* 42, 631–639. <https://doi.org/10.1212/WNL.42.3.631>
- Avila, J., 2006. Tau phosphorylation and aggregation in Alzheimer's disease pathology. *FEBS Lett.* 580, 2922–2927. <https://doi.org/10.1016/j.febslet.2006.02.067>
- Avila, J., Lucas, J.J., Pérez, M., Hernández, F., 2004. Role of Tau Protein in Both Physiological and Pathological Conditions. *Physiol. Rev.* 84, 361–384. <https://doi.org/10.1152/physrev.00024.2003>
- Bachstetter, A.D., Ighodaro, E.T., Hassoun, Y., Aldeiri, D., Neltner, J.H., Patel, E., Abner, E.L., Nelson, P.T., 2017. Rod-shaped microglia morphology is associated with aging in 2 human autopsy series. *Neurobiol. Aging* 52, 98–105. <https://doi.org/10.1016/j.neurobiolaging.2016.12.028>
- Bachstetter, A.D., Van Eldik, L.J., Schmitt, F.A., Neltner, J.H., Ighodaro, E.T., Webster, S.J., Patel, E., Abner, E.L., Kryscio, R.J., Nelson, P.T., 2015. Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging. *Acta Neuropathol. Commun.* 3, 32. <https://doi.org/10.1186/s40478-015-0209-z>
- Ballatore, C., Lee, V.M.Y., Trojanowski, J.Q., 2007. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* 8, 663–672. <https://doi.org/10.1038/nrn2194>
- Bejanin, A., Schonhaut, D.R., La Joie, R., Kramer, J.H., Baker, S.L., Sosa, N., Ayakta, N., Cantwell, A., Janabi, M., Lauriola, M., O'Neil, J.P., Gorno-Tempini, M.L., Miller, Z.A., Rosen, H.J., Miller, B.L., Jagust, W.J., Rabinovici, G.D., 2017. Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain* 140, 3286–3300. <https://doi.org/10.1093/brain/awx243>
- Berron, D., Vogel, J.W., Insel, P.S., Pereira, J.B., Xie, L., Wisse, L.E.M., Yushkevich, P.A., Palmqvist, S., Mattsson-Carlgrén, N., Stomrud, E., Smith, R., Strandberg, O., Hansson, O., 2021. Early stages of tau pathology and its associations with functional connectivity, atrophy and memory. *Brain* 144, 2771–2783. <https://doi.org/10.1093/brain/awab114>

- 3293 Bolós, M., Llorens-Martín, M., Jurado-Arjona, J., Hernández, F., Rábano, A., Avila, J., 2015.
3294 Direct Evidence of Internalization of Tau by Microglia In Vitro and In Vivo. *J. Alzheimer's*
3295 *Dis.* 50, 77–87. <https://doi.org/10.3233/JAD-150704>
- 3296 Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., Tredici, K., 2006. Staging of Alzheimer
3297 disease-associated neurofibrillary pathology using paraffin sections and
3298 immunocytochemistry. *Acta Neuropathol.* 112, 389–404. [https://doi.org/10.1007/s00401-](https://doi.org/10.1007/s00401-006-0127-z)
3299 006-0127-z
- 3300 Braak, H., Braak, E., 1995. Staging of Alzheimer's Disease-Related Neurofibrillary Changes.
3301 *Neurobiol. Aging* 16, 271–278.
- 3302 Braak, H., Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. *Acta*
3303 *Neuropathol.* 82, 239–259. <https://doi.org/10.1007/BF00308809>
- 3304 Braak, H., Del Tredici, K., 2020. From the Entorhinal Region via the Prosubiculum to the Dentate
3305 Fascia: Alzheimer Disease-Related Neurofibrillary Changes in the Temporal Allocortex. *J.*
3306 *Neuropathol. Exp. Neurol.* 79, 163–175. <https://doi.org/10.1093/jnen/nlz123>
- 3307 Brandt, R., Hundelt, M., Shahani, N., 2005. Tau alteration and neuronal degeneration in
3308 tauopathies: Mechanisms and models. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1739, 331–
3309 354. <https://doi.org/10.1016/j.bbadis.2004.06.018>
- 3310 Bugiani, O., Murrell, J.R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., Morbin, M.,
3311 Primavera, A., Carella, F., Solaro, C., Grisoli, M., Savoirdo, M., Spillantini, M.G.,
3312 Tagliavini, F., Goedert, M., Ghetti, B., 1999. Frontotemporal dementia and corticobasal
3313 degeneration in a family with a P301S mutation in Tau. *J. Neuropathol. Exp. Neurol.* 58, 667–
3314 677.
- 3315 Cotman, C.W., Poon, W.W., Rissman, R.A., Blurton-Jones, M., 2005. The role of caspase cleavage
3316 of tau in Alzheimer disease neuropathology. *J. Neuropathol. Exp. Neurol.* 64, 104–112.
3317 <https://doi.org/10.1093/jnen/64.2.104>
- 3318 De Calignon, A., Fox, L.M., Pitstick, R., Carlson, G.A., Bacskai, B.J., Spires-Jones, T.L., Hyman,
3319 B.T., 2010. Caspase activation precedes and leads to tangles. *Nature* 464, 1201–1204.
3320 <https://doi.org/10.1038/nature08890>
- 3321 Do Carmo, S., Cuellar, A.C., 2013. Modeling Alzheimer's disease in transgenic rats. *Mol.*
3322 *Neurodegener.* 8, 37. <https://doi.org/10.1186/1750-1326-8-37>
- 3323 Dujardin, S., Colin, M., Buée, L., 2015. Invited review: Animal models of tauopathies and their
3324 implications for research/translation into the clinic. *Neuropathol. Appl. Neurobiol.* 41, 59–
3325 80. <https://doi.org/10.1111/nan.12200>
- 3326 Eikelenboom, P., Bate, C., Van Gool, W.A., Hoozemans, J.J.M., Rozemuller, J.M., Veerhuis, R.,
3327 Williams, A., 2002. Neuroinflammation in Alzheimer's disease and prion disease. *Glia* 40,
3328 232–239. <https://doi.org/10.1002/glia.10146>
- 3329 Filipcik, P., Zilka, N., Bugos, O., Kucerak, J., Koson, P., Novak, P., Novak, M., 2012. First
3330 transgenic rat model developing progressive cortical neurofibrillary tangles. *Neurobiol.*
3331 *Aging* 33, 1448–1456. <https://doi.org/10.1016/j.neurobiolaging.2010.10.015>
- 3332 Flores-Aguilar, L., Iulita, M.F., Kovacs, O., Torres, M.D., Levi, S.M., Zhang, Y., Askenazi, M.,
3333 Wisniewski, T., Busciglio, J., Cuellar, A.C., 2020. Evolution of neuroinflammation across the

- 3334 lifespan of individuals with down syndrome. *Brain* 143, 3653–3671.
 3335 <https://doi.org/10.1093/brain/awaa326>
- 3336 Goedert, M., Jakes, R., Spillantini, M.G., Hasegawa, M., Smith, M.J., Crowther, R.A., 1996.
 3337 Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by
 3338 sulphated glycosaminoglycans. *Nature*. <https://doi.org/10.1038/383550a0>
- 3339 Goedert, M., Spillantini, M.G., 2000. Tau mutations in frontotemporal dementia FTDP-17 and
 3340 their relevance for Alzheimer's disease. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1502, 110–
 3341 121. [https://doi.org/10.1016/S0925-4439\(00\)00037-5](https://doi.org/10.1016/S0925-4439(00)00037-5)
- 3342 Goedert, M., Spillantini, M.G., Cairns, N.J., Crowther, R.A., 1992. Tau proteins of alzheimer
 3343 paired helical filaments: Abnormal phosphorylation of all six brain isoforms. *Neuron* 8, 159–
 3344 168. [https://doi.org/10.1016/0896-6273\(92\)90117-V](https://doi.org/10.1016/0896-6273(92)90117-V)
- 3345 Goedert, M., Spillantini, M.G., Crowther, R.A., 1991. Tau Proteins and Neurofibrillary
 3346 Degeneration. *Brain Pathol.* 1, 279–286. <https://doi.org/10.1111/j.1750-3639.1991.tb00671.x>
- 3347 Gómez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J.H., Petersen, R.C., Parisi, J.E.,
 3348 Hyman, B.T., 1997. Neuronal loss correlates with but exceeds neurofibrillary tangles in
 3349 Alzheimer's disease. *Ann. Neurol.* 41, 17–24. <https://doi.org/10.1002/ana.410410106>
- 3350 Götz, J., Chen, F., Barmettler, R., Nitsch, R.M., 2001. Tau filament formation in transgenic mice
 3351 expressing P301L tau. *J. Biol. Chem.* 276, 529–534. <https://doi.org/10.1074/jbc.M006531200>
- 3352 Gotz, J., Chen, F., van Dorpe, J., Nitsch, R.M., 2001. Formation of Neurofibrillary Tangles in
 3353 P301L Tau Transgenic Mice Induced by Abeta 42 Fibrils. *Science (80-.)*. 293, 1491–1495.
 3354 <https://doi.org/10.1126/science.1062097>
- 3355 Götz, J.J., Götz, J., 2019. Experimental Models of Tauopathy – From Mechanisms to Therapies,
 3356 in: Takashima, A., Wolozin, B., Buee, L. (Eds.), *Advances in Experimental Medicine and*
 3357 *Biology*. Springer Singapore, Singapore, pp. 381–391. https://doi.org/10.1007/978-981-32-9358-8_28
- 3359 Grudzien, A., Shaw, P., Weintraub, S., Bigio, E., Mash, D.C., Mesulam, M.M., 2007. Locus
 3360 coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early
 3361 Alzheimer's disease. *Neurobiol. Aging* 28, 327–335.
 3362 <https://doi.org/10.1016/j.neurobiolaging.2006.02.007>
- 3363 Hall, H., Iulita, M.F., Gubert, P., Flores Aguilar, L., Ducatzenzeiler, A., Fisher, A., Cuellar, A.C.,
 3364 2018. AF710B, an M1/sigma-1 receptor agonist with long-lasting disease-modifying
 3365 properties in a transgenic rat model of Alzheimer's disease. *Alzheimer's Dement.* 14, 811–
 3366 823. <https://doi.org/10.1016/j.jalz.2017.11.009>
- 3367 Hanes, J., Zilka, N., Bartkova, M., Caletkova, M., Dobrota, D., Novak, M., 2009. Rat tau proteome
 3368 consists of six tau isoforms: Implication for animal models of human tauopathies. *J.*
 3369 *Neurochem.* 108, 1167–1176. <https://doi.org/10.1111/j.1471-4159.2009.05869.x>
- 3370 Hanseeuw, B.J., Betensky, R.A., Jacobs, H.I.L., Schultz, A.P., Sepulcre, J., Becker, J.A., Cosio,
 3371 D.M.O., Farrell, M., Quiroz, Y.T., Mormino, E.C., Buckley, R.F., Papp, K. V., Amariglio,
 3372 R.A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G.A., Chhatwal, J.P.,
 3373 Rentz, D.M., Sperling, R.A., Johnson, K., 2019. Association of Amyloid and Tau with
 3374 Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. *JAMA Neurol.* 76, 915–

- 3375 924. <https://doi.org/10.1001/jamaneurol.2019.1424>
- 3376 Hasegawa, M., Morishima-Kawashima, M., Takio, K., Suzuki, M., Titani, K., Ihara, Y., 1992.
3377 Protein sequence and mass spectrometric analyses of tau in the Alzheimer's disease brain. *J.*
3378 *Biol. Chem.* 267, 17047–17054.
- 3379 Hassan-Abdi, R., Brenet, A., Bennis, M., Yanicostas, C., Soussi-Yanicostas, N., 2019. Neurons
3380 Expressing Pathological Tau Protein Trigger Dramatic Changes in Microglial Morphology
3381 and Dynamics. *Front. Neurosci.* 13, 1–11. <https://doi.org/10.3389/fnins.2019.01199>
- 3382 Hernández, F., Avila, J., 2007. Tauopathies. *Cell. Mol. Life Sci.* 64, 2219–2233.
3383 <https://doi.org/10.1007/s00018-007-7220-x>
- 3384 Holloway, O.G., Canty, A.J., King, A.E., Ziebell, J.M., 2019. Rod microglia and their role in
3385 neurological diseases. *Semin. Cell Dev. Biol.* 94, 96–103.
3386 <https://doi.org/10.1016/j.semcdb.2019.02.005>
- 3387 Hrnkova, M., Zilka, N., Minichova, Z., Koson, P., Novak, M., 2007. Neurodegeneration caused
3388 by expression of human truncated tau leads to progressive neurobehavioural impairment in
3389 transgenic rats. *Brain Res.* 1130, 206–213. <https://doi.org/10.1016/j.brainres.2006.10.085>
- 3390 Iqbal, K., Del C. Alonso, A., Chen, S., Chohan, M.O., El-Akkad, E., Gong, C.X., Khatoon, S., Li,
3391 B., Liu, F., Rahman, A., Tanimukai, H., Grundke-Iqbal, I., 2005. Tau pathology in Alzheimer
3392 disease and other tauopathies. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1739, 198–210.
3393 <https://doi.org/10.1016/j.bbadis.2004.09.008>
- 3394 Iqbal, K., Liu, F., Gong, C.-X., Grundke-Iqbal, I., 2010. Tau in Alzheimer Disease and Related
3395 Tauopathies. *Curr. Alzheimer Res.* 7, 656–664.
3396 <https://doi.org/10.2174/156720510793611592>
- 3397 Iqbal, K., Liu, F., Gong, C.X., 2016. Tau and neurodegenerative disease: The story so far. *Nat.*
3398 *Rev. Neurol.* 12, 15–27. <https://doi.org/10.1038/nrneurol.2015.225>
- 3399 Joie, R. La, Visani, A. V., Baker, S.L., Brown, J.A., Bourakova, V., Cha, J., Chaudhary, K.,
3400 Edwards, L., Iaccarino, L., Janabi, M., Lesman-Segev, O.H., Miller, Z.A., Perry, D.C.,
3401 O'Neil, J.P., Pham, J., Rojas, J.C., Rosen, H.J., Seeley, W.W., Tsai, R.M., Miller, B.L.,
3402 Jagust, W.J., Rabinovici, G.D., 2020. Prospective longitudinal atrophy in Alzheimer's disease
3403 correlates with the intensity and topography of baseline tau-PET. *Sci. Transl. Med.* 12, 1–13.
3404 <https://doi.org/10.1126/scitranslmed.aau5732>
- 3405 Jørgensen, M.B., Finsen, B.R., Jensen, M.B., Castellano, B., Diemer, N.H., Zimmer, J., 1993.
3406 Microglial and Astroglial Reactions to Ischemic and Kainic Acid-Induced Lesions of the
3407 Adult Rat Hippocampus. *Exp. Neurol.* <https://doi.org/10.1006/exnr.1993.1041>
- 3408 Kadavath, H., Hofele, R. V., Biernat, J., Kumar, S., Tepper, K., Urlaub, H., Mandelkow, E.,
3409 Zweckstetter, M., 2015. Tau stabilizes microtubules by binding at the interface between
3410 tubulin heterodimers. *Proc. Natl. Acad. Sci.* 112, 7501–7506.
3411 <https://doi.org/10.1073/pnas.1504081112>
- 3412 Knopman, D.S., Lundt, E.S., Therneau, T.M., Vemuri, P., Lowe, V.J., Kantarci, K., Gunter, J.L.,
3413 Senjem, M.L., Mielke, M.M., Machulda, M.M., Boeve, B.F., Jones, D.T., Graff-Radford, J.,
3414 Albertson, S.M., Schwarz, C.G., Petersen, R.C., Jack, C.R., 2019. Entorhinal cortex tau,
3415 amyloid- β , cortical thickness and memory performance in non-demented subjects. *Brain* 1–

- 3416 13. <https://doi.org/10.1093/brain/awz025>
- 3417 Korhonen, P., van Groen, T., Thornell, A., Kyrylenko, S., Soininen, M.L., Ojala, J., Peltomaa, E.,
 3418 Tanila, H., Salminen, A., Mandelkow, E.M., Soininen, H., 2011. Characterization of a novel
 3419 transgenic rat carrying human tau with mutation P301L. *Neurobiol. Aging* 32, 2314–2315.
 3420 <https://doi.org/10.1016/j.neurobiolaging.2009.12.022>
- 3421 Kovacs, G.G., Lukic, M.J., Irwin, D.J., Arzberger, T., Respondek, G., Lee, E.B., Coughlin, D.,
 3422 Giese, A., Grossman, M., Kurz, C., McMillan, C.T., Gelpi, E., Compta, Y., van Swieten, J.C.,
 3423 Laat, L.D., Troakes, C., Al-Sarraj, S., Robinson, J.L., Roeber, S., Xie, S.X., Lee, V.M.Y.,
 3424 Trojanowski, J.Q., Höglinger, G.U., 2020. Distribution patterns of tau pathology in
 3425 progressive supranuclear palsy. *Acta Neuropathol.* 140, 99–119.
 3426 <https://doi.org/10.1007/s00401-020-02158-2>
- 3427 LaDu, M.J., Lukens, J.R., Reardon, C.A., Getz, G.S., 1997. Association of human, rat, and rabbit
 3428 apolipoprotein E with β -amyloid. *J. Neurosci. Res.* 49, 9–18.
 3429 [https://doi.org/10.1002/\(SICI\)1097-4547\(19970701\)49:1<9::AID-JNR2>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-4547(19970701)49:1<9::AID-JNR2>3.0.CO;2-H)
- 3430 Laurent, C., Buée, L., Blum, D., 2018. Tau and neuroinflammation: What impact for Alzheimer's
 3431 Disease and Tauopathies? *Biomed. J.* 41, 21–33. <https://doi.org/10.1016/j.bj.2018.01.003>
- 3432 Lee, V.M.-Y., Goedert, M., Trojanowski, J.Q., 2001. Neurodegenerative Tauopathies. *Annu. Rev.*
 3433 *Neurosci.* 24, 1121–1159. <https://doi.org/10.1146/annurev.neuro.24.1.1121>
- 3434 Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Van Slegtenhorst, M., Gwinn-
 3435 Hardy, K., Murphy, M.P., Baker, M., Yu, X., Duff, K., Hardy, J., Corral, A., Lin, W.L., Yen,
 3436 S.H., Dickson, D.W., Davies, P., Hutton, M., 2000. Neurofibrillary tangles, amyotrophy and
 3437 progressive motor disturbance in mice expressing mutant (P301L)tau protein. *Nat. Genet.* 25,
 3438 402–405. <https://doi.org/10.1038/78078>
- 3439 Li, H., Jia, X., Qi, Z., Fan, X., Ma, T., Pang, R., Ni, H., Li, C. shan R., Lu, J., Li, K., 2018.
 3440 Disrupted functional connectivity of cornu ammonis subregions in amnesic mild cognitive
 3441 impairment: A longitudinal resting-state fMRI study. *Front. Hum. Neurosci.* 12, 1–9.
 3442 <https://doi.org/10.3389/fnhum.2018.00413>
- 3443 Luna-Muñoz, J., Chávez-Macías, L., García-Sierra, F., Mena, R., 2007. Earliest stages of tau
 3444 conformational changes are related to the appearance of a sequence of specific phospho-
 3445 dependent tau epitopes in Alzheimer's disease. *J. Alzheimer's Dis.* 12, 365–375.
 3446 <https://doi.org/10.3233/JAD-2007-12410>
- 3447 Luna-Muñoz, J., García-Sierra, F., Falcón, V., Menéndez, I., Chávez-Macías, L., Mena, R., 2005.
 3448 Regional conformational change involving phosphorylation of tau protein at the Thr231,
 3449 precedes the structural change detected by Alz-50 antibody in Alzheimer's disease. *J.*
 3450 *Alzheimer's Dis.* 8, 29–41. <https://doi.org/10.3233/JAD-2005-8104>
- 3451 Malcolm, J.C., Breuillaud, L., Do Carmo, S., Hall, H., Welikovitch, L.A., Macdonald, J.A.,
 3452 Goedert, M., Cuello, A.C., 2019. Neuropathological changes and cognitive deficits in rats
 3453 transgenic for human mutant tau recapitulate human tauopathy. *Neurobiol. Dis.* 127, 323–
 3454 338. <https://doi.org/10.1016/j.nbd.2019.03.018>
- 3455 Maloney, B., Ge, Y.W., Alley, G.M., Lahiri, D.K., 2007. Important differences between human
 3456 and mouse APOE gene promoters: Limitation of mouse APOE model in studying
 3457 Alzheimer's disease. *J. Neurochem.* 103, 1237–1257. <https://doi.org/10.1111/j.1471->

- 3458 4159.2007.04831.x
- 3459 Mandelkow, E.M., Mandelkow, E., 1998. Tau in Alzheimer's disease. *Trends Cell Biol.* 8, 425–
3460 427. [https://doi.org/10.1016/S0962-8924\(98\)01368-3](https://doi.org/10.1016/S0962-8924(98)01368-3)
- 3461 Merino-Serrais, P., Benavides-Piccione, R., Blazquez-Llorca, L., Kastanauskaite, A., Rábano, A.,
3462 Avila, J., Defelipe, J., 2013. The influence of phospho-tau on dendritic spines of cortical
3463 pyramidal neurons in patients with Alzheimer's disease. *Brain* 136, 1913–1928.
3464 <https://doi.org/10.1093/brain/awt088>
- 3465 Morsch, R., Simon, W., Coleman, P.D., 1999. Neurons may live for decades with neurofibrillary
3466 tangles. *J. Neuropathol. Exp. Neurol.* 58, 188–197. [https://doi.org/10.1097/00005072-
3467 199902000-00008](https://doi.org/10.1097/00005072-199902000-00008)
- 3468 Nilson, A.N., English, K.C., Gerson, J.E., Barton Whittle, T., Nicolas Crain, C., Xue, J., Sengupta,
3469 U., Castillo-Carranza, D.L., Zhang, W., Gupta, P., Kayed, R., 2017. Tau oligomers associate
3470 with inflammation in the brain and retina of tauopathy mice and in neurodegenerative
3471 diseases. *J. Alzheimer's Dis.* 55, 1083–1099. <https://doi.org/10.3233/JAD-160912>
- 3472 Novak, M., Wischik, C.M., Edwards, P., Pannell, R., Milstein, C., 1989. Characterisation of the
3473 first monoclonal antibody against the pronase resistant core of the Alzheimer PHF. *Prog.*
3474 *Clin. Biol. Res.* 317, 755–761.
- 3475 Padurariu, M., Ciobica, A., Mavroudis, I., Fotiou, D., Baloyannis, S., 2012. Hippocampal neuronal
3476 loss in the CA1 and CA3 areas of Alzheimer's disease patients. *Psychiatr Danub* 24, 152–
3477 158.
- 3478 Ramalho, R.M., Viana, R.J.S., Castro, R.E., Steer, C.J., Low, W.C., Rodrigues, C.M.P., 2008.
3479 Apoptosis in transgenic mice expressing the P301L mutated form of human tau. *Mol. Med.*
3480 14, 309–317. <https://doi.org/10.2119/2007-00133.Ramalho>
- 3481 Ribeiro-da-Silva, A., Priestley, J. V., Cuellar, A.C., 1993. Pre-embedding ultrastructural
3482 immunocytochemistry, in: Cuellar, A.C. (Ed.), *Immunocytochemistry II*. John Wiley & Sons,
3483 Chichester, pp. 181–227.
- 3484 Rodriguez, R.D., Grinberg, L.T., 2015. Argyrophilic grain disease: An underestimated tauopathy.
3485 *Dement. Neuropsychol.* 9, 2–8. <https://doi.org/10.1590/S1980-57642015DN91000002>
- 3486 Rohn, T.T., Head, E., Su, J.H., Anderson, A.J., Bahr, B.A., Cotman, C.W., Cribbs, D.H., 2001.
3487 Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's
3488 disease. *Am. J. Pathol.* 158, 189–198. [https://doi.org/10.1016/S0002-9440\(10\)63957-0](https://doi.org/10.1016/S0002-9440(10)63957-0)
- 3489 Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., Zhang, B.,
3490 Murayama, S., Higuchi, M., Lee, V.M.Y., Trojanowski, J.Q., Saido, T.C., 2019.
3491 Humanization of the entire murine Mapt gene provides a murine model of pathological human
3492 tau propagation. *J. Biol. Chem.* 294, 12754–12765.
3493 <https://doi.org/10.1074/jbc.RA119.009487>
- 3494 Saito, Y., Ruberu, N.N., Sawabe, M., Arai, T., Tanaka, N., Kakuta, Y., Yamanouchi, H.,
3495 Murayama, S., 2004. Staging of argyrophilic grains: An age-associated tauopathy. *J.*
3496 *Neuropathol. Exp. Neurol.* 63, 911–918. <https://doi.org/10.1093/jnen/63.9.911>
- 3497 Scheff, S.W., Price, D.A., Schmitt, F.A., Dekosky, S.T., Mufson, E.J., 2007. Synaptic alterations
3498 in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* 68, 1501–1508.

- 3499 <https://doi.org/10.1212/01.wnl.0000260698.46517.8f>
- 3500 Scheff, S.W., Price, D.A., Schmitt, F.A., Mufson, E.J., 2006. Hippocampal synaptic loss in early
3501 Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging* 27, 1372–1384.
3502 <https://doi.org/10.1016/j.neurobiolaging.2005.09.012>
- 3503 Schöll, M., Lockhart, S.N., Schonhaut, D.R., O'Neil, J.P., Janabi, M., Ossenkoppele, R., Baker,
3504 S.L., Vogel, J.W., Faria, J., Schwimmer, H.D., Rabinovici, G.D., Jagust, W.J., 2016. PET
3505 Imaging of Tau Deposition in the Aging Human Brain. *Neuron* 89, 971–982.
3506 <https://doi.org/10.1016/j.neuron.2016.01.028>
- 3507 Serrano-Pozo, A., Mielke, M.L., Gómez-Isla, T., Betensky, R.A., Growdon, J.H., Frosch, M.P.,
3508 Hyman, B.T., 2011. Reactive glia not only associates with plaques but also parallels tangles
3509 in Alzheimer's disease. *Am. J. Pathol.* 179, 1373–1384.
3510 <https://doi.org/10.1016/j.ajpath.2011.05.047>
- 3511 Shi, Y., Yamada, K., Liddelow, S.A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S.,
3512 Grinberg, L.T., Rojas, J.C., Gallardo, G., Wang, K., Roh, J., Robinson, G., Finn, M.B., Jiang,
3513 H., Sullivan, P.M., Baufeld, C., Wood, M.W., Sutphen, C., McCue, L., Xiong, C., Del-Aguila,
3514 J.L., Morris, J.C., Cruchaga, C., Fagan, A.M., Miller, B.L., Boxer, A.L., Seeley, W.W.,
3515 Butovsky, O., Barres, B.A., Paul, S.M., Holtzman, D.M., 2017. ApoE4 markedly exacerbates
3516 tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* 549, 523–527.
3517 <https://doi.org/10.1038/nature24016>
- 3518 Spillantini, M.G., Goedert, M., 1998. Tau protein pathology in neurodegenerative diseases. *Trends*
3519 *Neurosci.* 21, 428–433. [https://doi.org/10.1016/S0166-2236\(98\)01337-X](https://doi.org/10.1016/S0166-2236(98)01337-X)
- 3520 Streit, W.J., Xue, Q.S., Tischer, J., Bechmann, I., 2014. Microglial pathology. *Acta Neuropathol.*
3521 *Commun.* 2, 1–17. <https://doi.org/10.1186/s40478-014-0142-6>
- 3522 Su, J.H., Zhao, M., Anderson, A.J., Srinivasan, A., Cotman, C.W., 2001. Activated caspase-3
3523 expression in Alzheimer's and aged control brain: Correlation with Alzheimer pathology.
3524 *Brain Res.* 898, 350–357. [https://doi.org/10.1016/S0006-8993\(01\)02018-2](https://doi.org/10.1016/S0006-8993(01)02018-2)
- 3525 Tai, H.C., Serrano-Pozo, A., Hashimoto, T., Frosch, M.P., Spires-Jones, T.L., Hyman, B.T., 2012.
3526 The synaptic accumulation of hyperphosphorylated tau oligomers in alzheimer disease is
3527 associated with dysfunction of the ubiquitin-proteasome system. *Am. J. Pathol.* 181, 1426–
3528 1435. <https://doi.org/10.1016/j.ajpath.2012.06.033>
- 3529 Takashima, A., 2016. Mechanism of neurodegeneration through tau and therapy for Alzheimer's
3530 disease. *J. Sport Heal. Sci.* 5, 391–392. <https://doi.org/10.1016/j.jshs.2016.08.009>
- 3531 Tam, W.Y., Ma, C.H.E., 2014. Bipolar/rod-shaped microglia are proliferating microglia with
3532 distinct M1/M2 phenotypes. *Sci. Rep.* 4, 1–7. <https://doi.org/10.1038/srep07279>
- 3533 Taylor, S.E., Morganti-Kossmann, C., Lifshitz, J., Ziebell, J.M., 2014. Rod microglia: A
3534 morphological definition. *PLoS One* 9. <https://doi.org/10.1371/journal.pone.0097096>
- 3535 Terry, R.D., Peck, A., DeTeresa, R., Schechter, R., Horoupian, D.S., 1981. Some morphometric
3536 aspects of the brain in senile dementia of the alzheimer type. *Ann. Neurol.* 10, 184–192.
3537 <https://doi.org/10.1002/ana.410100209>
- 3538 Tran, T.N., Kim, S.H., Gallo, C., Amaya, M., Kyees, J., Narayanaswami, V., 2013. Biochemical
3539 and biophysical characterization of recombinant rat apolipoprotein E: Similarities to human

- 3540 apolipoprotein E3. Arch. Biochem. Biophys. 529, 18–25.
 3541 <https://doi.org/10.1016/j.abb.2012.10.007>
- 3542 Tseng, J.H., Xie, L., Song, S., Xie, Y., Allen, L., Ajit, D., Hong, J.S., Chen, X., Meeker, R.B.,
 3543 Cohen, T.J., 2017. The Deacetylase HDAC6 Mediates Endogenous Neuritic Tau Pathology.
 3544 Cell Rep. 20, 2169–2183. <https://doi.org/10.1016/j.celrep.2017.07.082>
- 3545 Wang, J.Z., Wang, Z.H., Tian, Q., 2014. Tau hyperphosphorylation induces apoptotic escape and
 3546 triggers neurodegeneration in Alzheimer's disease. Neurosci. Bull. 30, 359–366.
 3547 <https://doi.org/10.1007/s12264-013-1415-y>
- 3548 Wang, Y., Mandelkow, E., 2015. Tau in physiology and pathology. Nat. Rev. Neurosci. 17, 22–
 3549 35. <https://doi.org/10.1038/nrn.2015.1>
- 3550 Weaver, C.L., Espinoza, M., Kress, Y., Davies, P., 2000. Conformational change as one of the
 3551 earliest alterations of tau in Alzheimer's disease. Neurobiol. Aging 21, 719–727.
 3552 [https://doi.org/10.1016/S0197-4580\(00\)00157-3](https://doi.org/10.1016/S0197-4580(00)00157-3)
- 3553 Weingarten, M.D., Lockwood, A.H., Hwo, S., Kirschner, M.W., 1975. A Protein factor essential
 3554 for microtubule assembly. Proc Nat Acad Sci USA 72, 1858–1862.
- 3555 West, M.J., Kawas, C.H., Stewart, W.F., Rudow, G.L., Troncoso, J.C., 2004. Hippocampal
 3556 neurons in pre-clinical Alzheimer's disease. Neurobiol. Aging 25, 1205–1212.
 3557 <https://doi.org/10.1016/j.neurobiolaging.2003.12.005>
- 3558 Yoshida, M., 2014. Astrocytic inclusions in progressive supranuclear palsy and corticobasal
 3559 degeneration. Neuropathology 34, 555–570. <https://doi.org/10.1111/neup.12143>
- 3560 Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.M., Iwata, N., Saido, T.C., Maeda, J., Suhara,
 3561 T., Trojanowski, J.Q., Lee, V.M.Y., 2007. Synapse Loss and Microglial Activation Precede
 3562 Tangles in a P301S Tauopathy Mouse Model. Neuron 53, 337–351.
 3563 <https://doi.org/10.1016/j.neuron.2007.01.010>
- 3564 Zilka, N., Filipcik, P., Koson, P., Fialova, L., Skrabana, R., Zilkova, M., Rolkova, G., Kontsekkova,
 3565 E., Novak, M., 2006. Truncated tau from sporadic Alzheimer's disease suffices to drive
 3566 neurofibrillary degeneration in vivo. FEBS Lett. 580, 3582–3588.
 3567 <https://doi.org/10.1016/j.febslet.2006.05.029>
- 3568 Zimmer, E.R., Parent, M.J., Cuellar, A.C., Gauthier, S., Rosa-Neto, P., 2014. MicroPET imaging
 3569 and transgenic models: a blueprint for Alzheimer's disease clinical research. Trends Neurosci.
 3570 37, 629–641. <https://doi.org/10.1016/j.tins.2014.07.002>
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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JTE contributed to study conceptualization, data curation, consolidation and formal analysis, methodology, conducted most experiments including behavior, IHC and EM, writing of the original manuscript. JCM conducted some of the behavioral experiments and contributed to study conceptualization. SDC and LB designed the R955-hTau transgene construct. SDC and ACC contributed to study conceptualization, supervision and writing – review & editing of the manuscript.

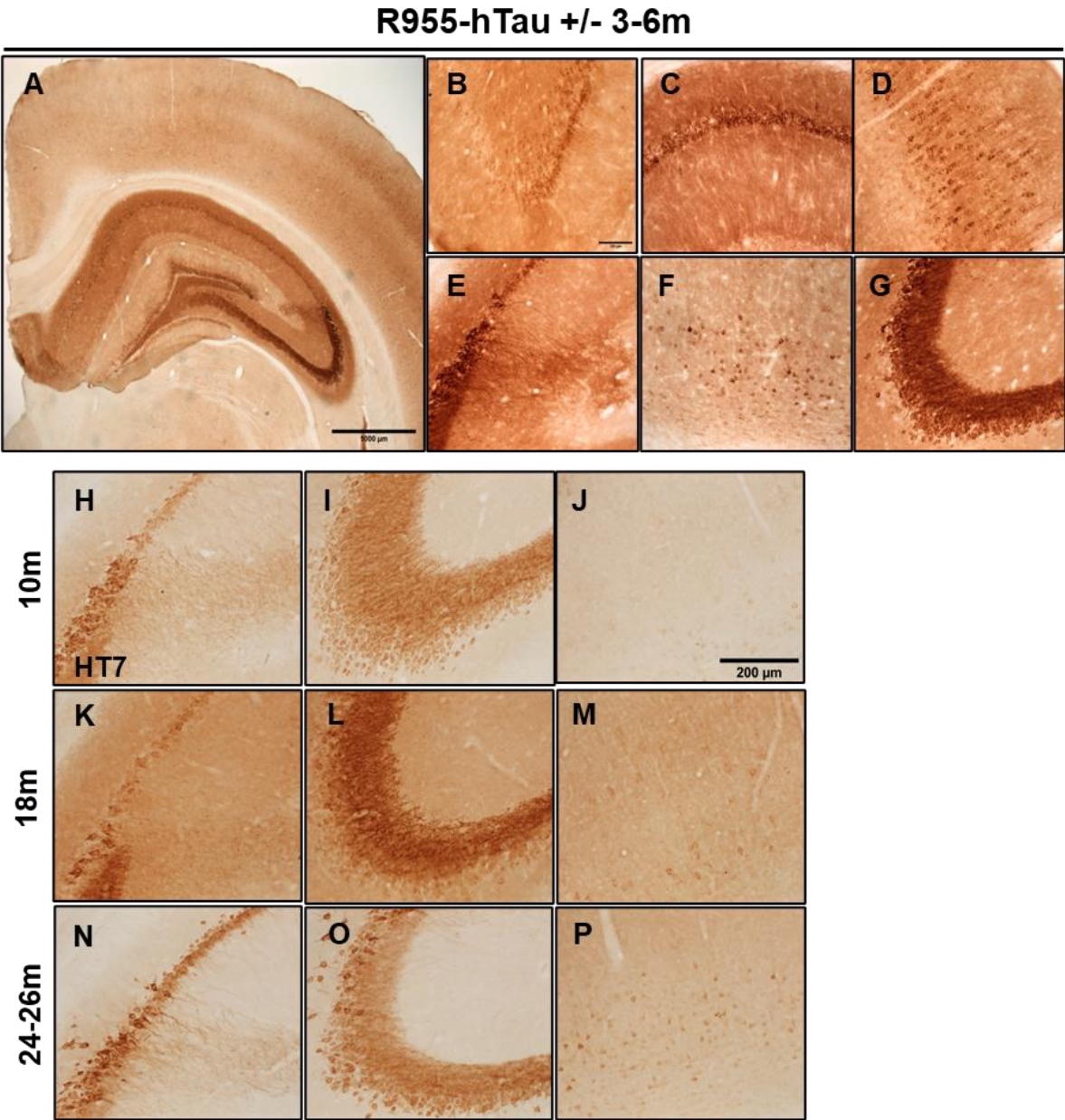
Funding

JTE received support from the Fonds de recherche Quebec – Santé (FRQS) doctoral training fellowship 2020-21, Hugh E. Burke fellowship 2019-20 and F.S.B Miller Memorial fellowship 2017-18. JCM was the recipient of the Rotary Foundation’s Global Grant Scholarship. SDC is the holder of the Charles E. Frosst/Merck Research Associate position. This study was supported by the Canadian Institute of Health Research, the Canadian Consortium of Neurodegeneration and Aging (CCNA), the Healthy Brains Healthy Lives and BrainsCAN McGill – Western Collaboration Grant (MWCG) and the Morris and Rosalind Goodman Family Foundation to ACC’s Laboratory. ACC is the holder of the Charles E. Frosst/Merck-endowed Chair in Pharmacology and a member of the Canadian Consortium on Neurodegeneration in Aging.

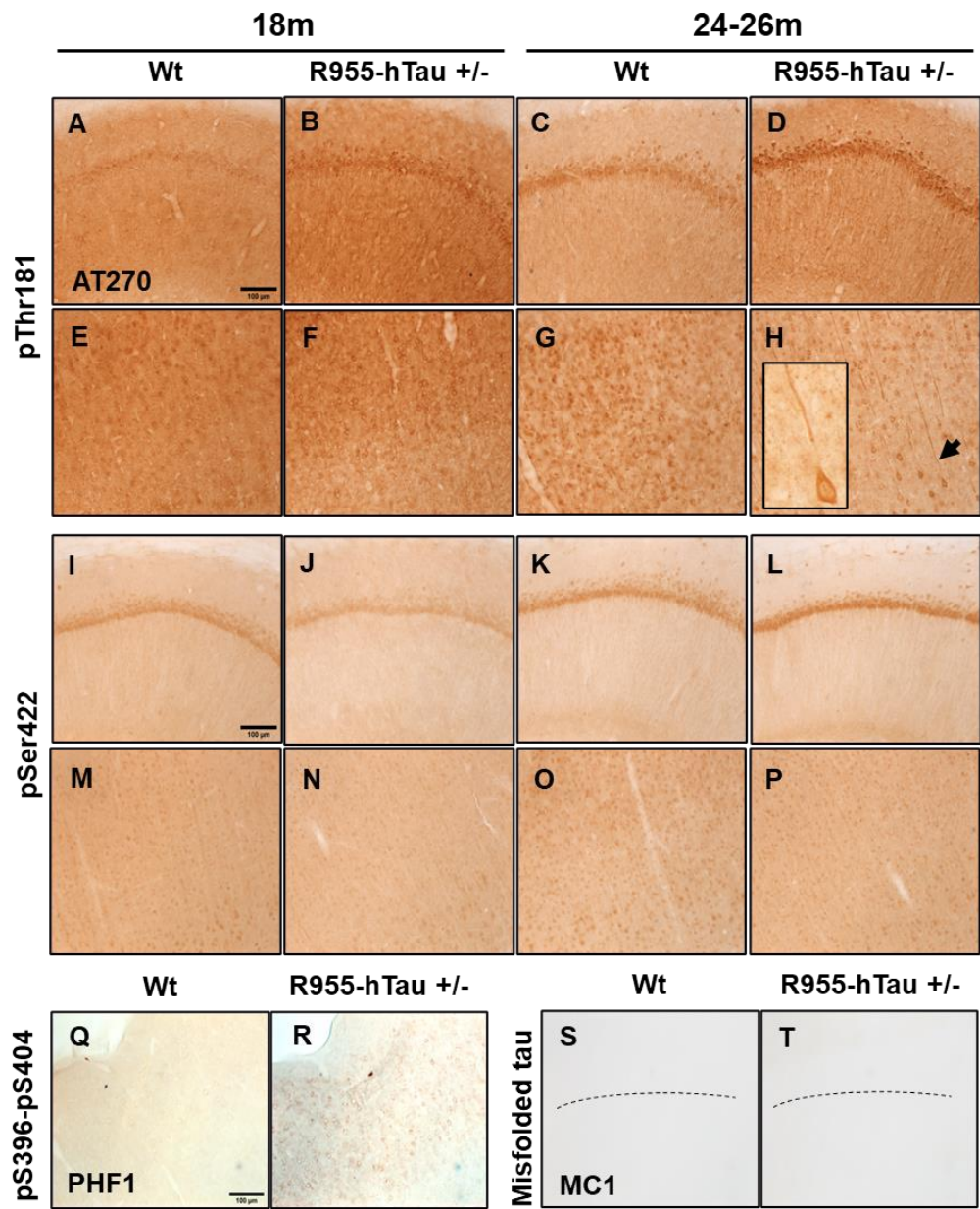
Acknowledgments

The cDNA of the transgene construct was obtained with the advice of Dr. Michel Goedert and the MRC lab of molecular biology, Cambridge, UK. Confocal images were obtained with the assistance of and access to the Advanced BioImaging Facility of the McGill University Life Sciences Complex. We thank Johanne Oulette for the outstanding technical assistance in the preparation of samples for electron microscopy.

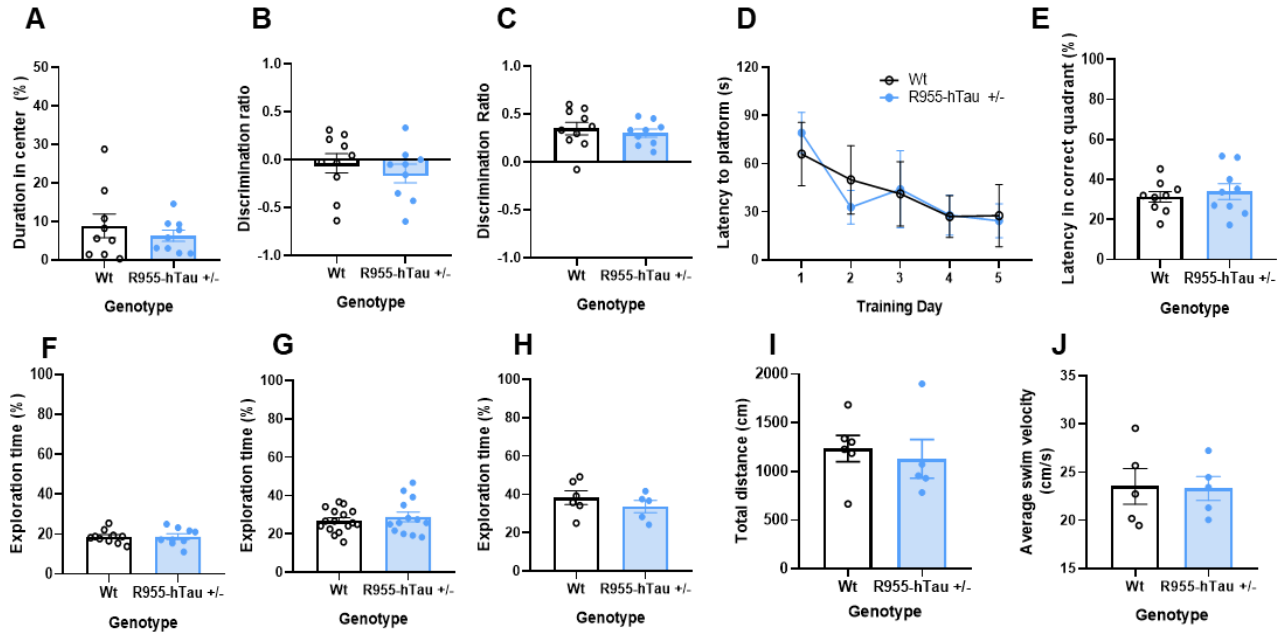
3602 6. Supplemental Figures



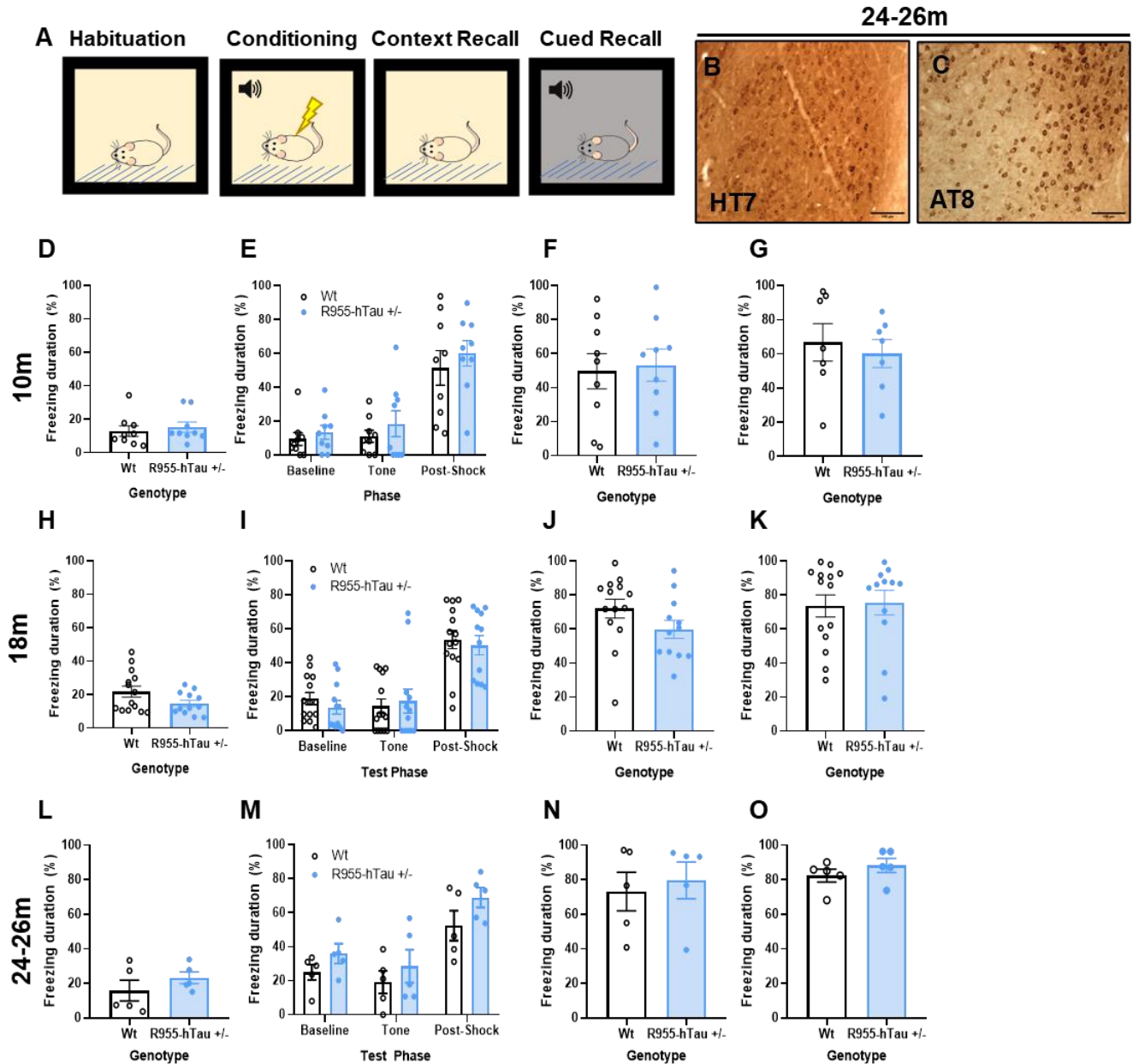
Supplemental Figure 1. Human tau immunoreactivity in 3-6-month-old R955-hTau rats and other regions of interest. Representative low- and high-resolution micrographs of HT7 immunoreactive neurons in (A-G) 3-month-old and (H-N) 6-month-old R955-hTau rats throughout the hippocampus and cortex are comparable to that of 10-month-old rats. (O-Q) Representative images of the hilus of the dentate gyrus in 10, 18-20- and 24-26-month-old rats. (R) HT7- and (S) AT8-immunoreactive neurons are present in the amygdala of 24-26-month-old R955-hTau rats but do not display obvious morphological abnormalities.



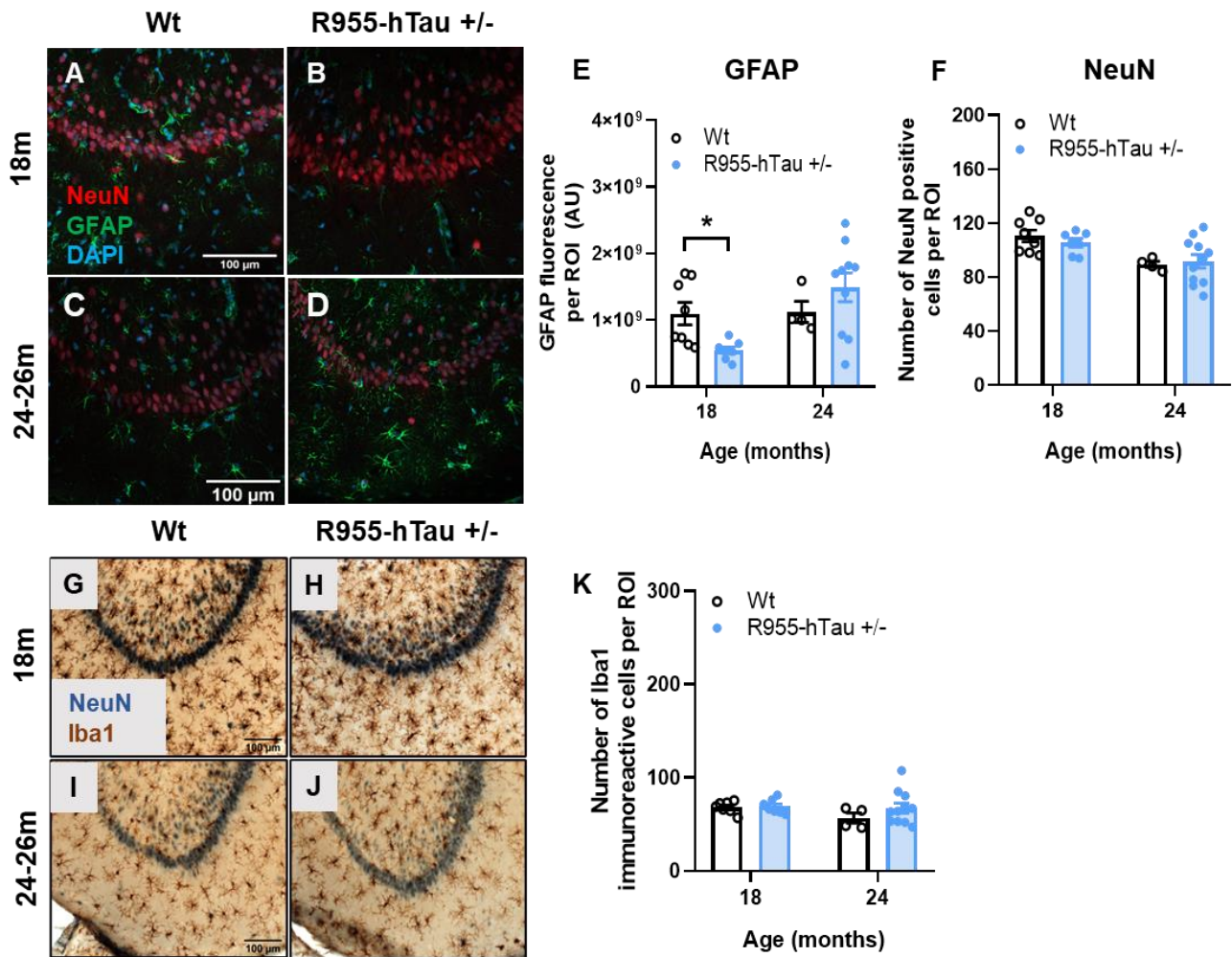
Supplemental Figure 2. Other epitopes of phosphorylated tau at Thr181 and Ser422 show no differences in immunoreactivity after 18 months of age. Representative low- resolution micrographs of (A-H) pThr181 and (I-P) pSer422 immunoreactive neurons in (A-D, I-L) the CA1 region of the hippocampus and (E-H, M-P) the cerebral cortex in R955-hTau rats are comparable to that of wild-types. (H, black arrow) Note the increased localization of pThr181 to the somatodendritic compartment of layer V pyramidal neurons.



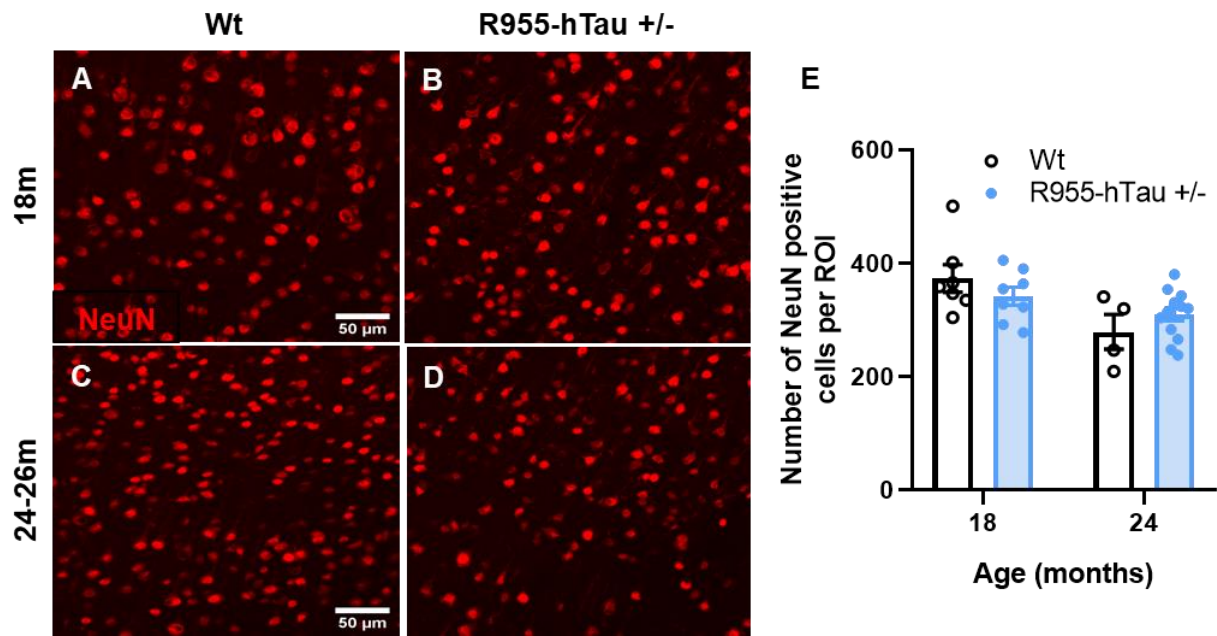
Supplemental Figure 3. Behavioral phenotype at 10 months of age shows no cognitive deficits in R955-hTau rats. (A) Time spent in the center of the open field. Discrimination ratio of the NOL (B) and NOR (C) tasks. (E) Training phase of the MWM followed by (F) percentage of time spent in the correct quadrant for the probe trial. (F-H) Time spent with all objects during familiarization (FAM) of the NOLR task indicating no differences in exploratory behaviors between transgenic R955-hTau^{+/-} and wild-type (Wt) rats at 10 (F), 18 (G) and 24–26-month-old rats (H). In 24–26-month-old rats, total movement during the OF test (I) and swim velocity averaged over the five days of training during the MWM (J) are unaffected, indicating an absence of motor impairments in R955-hTau^{+/-} rats (n=9-10). $p>0.05$.



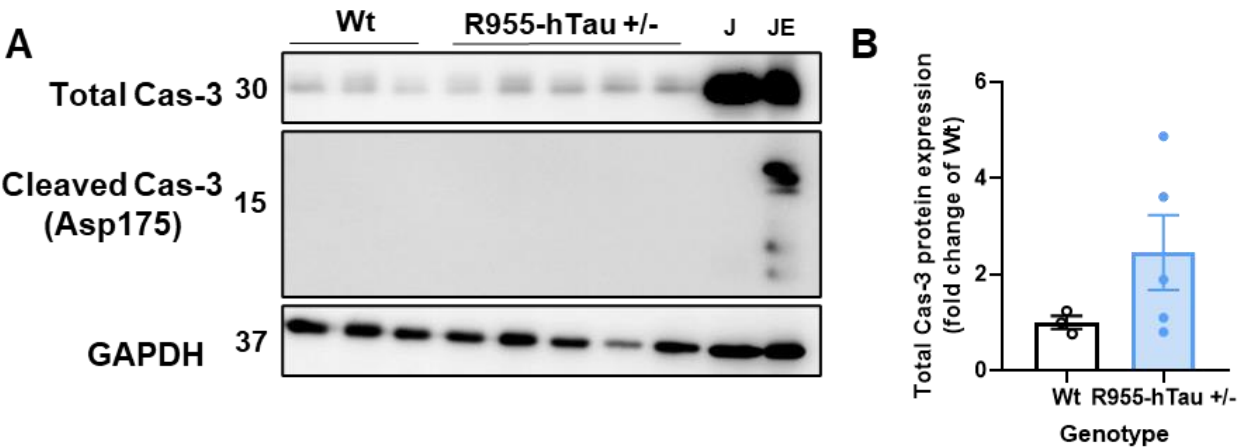
Supplemental Figure 4. Behavioral phenotype of the Fear Conditioning task (FC) remained unchanged in heterozygous R955-hTau rats. (A) Illustration of the four days of the auditory fear conditioning task. (D-O) Quantification of the freezing response in R955-hTau^{+/-} rats was not significantly different than wild-type (Wt) controls during the (B, F, J) habituation to the chamber, (C, G, K) conditioning to the tone-shock stimulus, (D, H, L) contextual recall and (E, I, M) cued recall. (B-C) Representative micrographs illustrating the amygdala immunoreactive for human tau (HT7) and ptau at Ser202-Thr205 (AT8) in the CNS of 24-26-month-old R955-hTau^{+/-} rats. $p > 0.05$.



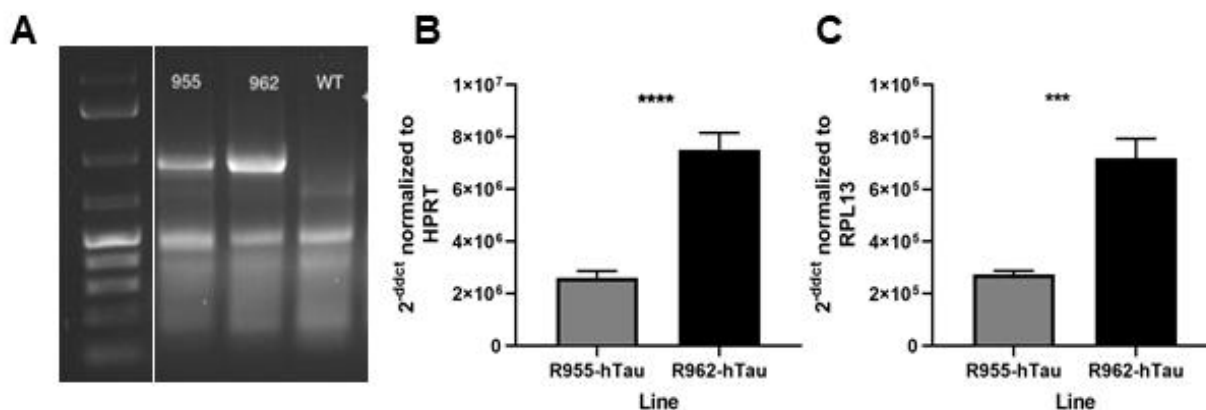
Supplemental Figure 5. Microglial and astrocytic inflammatory-like processes and loss of neurons did not occur at 18-20 months of age nor in the AT8-burdened subiculum of 24-26-month-old R955-hTau rats. (A-B, C-D) Representative confocal images illustrating the subiculum in 18-20 months and 24-26 months-old, respectively. Quantification of **(E)** the GFAP fluorescence intensity, **(G)** Percent area covered by GFAP, **(F)** Number of NeuN-positive cells. **(H-I, J-K)** Representative images of the subiculum probing Iba1 (brown) and NeuN (blue) with the quantification of the number of Iba1 positive cells at **(L)** 18-20 and 24-26 months (n=4-11). * $p < 0.05$.



Supplemental Figure 6. Detectable neuronal loss does not occur in the cerebral cortex in heterozygous R955-hTau. (A-D) Representative images of the Layer V pyramidal neurons of the cerebral cortex remaining intact using sections probed with NeuN (Red). Quantification of the number of NeuN positive cells at (E) 18-20 months and 24-26 months (n=4-11), $p>0.05$.



Supplemental Figure 7. Hippocampal neuronal loss occurs in the absence of detectable apoptosis via Caspase-3 activation and cleavage. (A) Representative Western Blot probing total and cleaved caspase-3 (Asp175) for apoptotic signalling in 24-26-month-old R955-hTau ^{+/-} rats. Lanes 1-3 wild type (Wt), Lanes 4-8 R955-hTau, J=Jurkat cell extract and JE=Jurkat+Etoposide as apoptosis positive controls. (B) Quantification of total Cas-3 protein expression. n=3-5, p>0.05.



Supplemental Figure 8. Expression of the R955-hTau transgene relative to a previously characterized but lost R962-hTau rat model is approximately four-fold lower in heterozygous rats. (A) Representative agarose gel (1kb ladder on left) using equally loaded DNA samples. Quantitative real-time PCR (qPCR) was also performed, reporting the cycle threshold (ddCT) normalized separately to two stable housekeeping genes (B) HPRT and (C) RPL13 (n=6, samples ran in duplicate). ***=p<0.001, ****=p<0.0001.

3613 **7. Supplemental Tables**

3614 **Supplemental Table 1. Demographics of rat cohorts for this study.** Males and females were
 3615 used. Some age-related loss occurred for both wild-types and transgenics.

	Wild-type (Wt)		R955-hTau +/- (Tg)		
Age (months)	Female	Male	Female	Male	Total (Wt/Tg)
10	6	4	2	7	19 (10/9)
18-20	10	6	6	7	29 (16/13)
24-26	3	3	7	4	17 (6/11)
Total	19	13	15	18	65

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3617 **Supplemental Table 2. List of primary and secondary antibodies.**

Antibody	Company	Epitope	Use	Concentration
HT7	ThermoFisher	aa 159-163	IHC	1:1000
AT8	ThermoFisher	pSer202-pThr205	IHC	1:500
CP13	Peter Davies	pSer202	IHC	1:1000-1:2000
MC1	Peter Davies	aa 7-9, 312-322	IHC	1:500
PHF1	Peter Davies	pSer396/404	IHC	1:2000-4000
AT180	ThermoFisher	pThr231	IHC	1:250-1:500
pS422	Medimabs	pSer422	IHC	1:2000
pThr175	Medimabs	pThr175	IHC	1:1000
pSer208/210	Medimabs	pSer208/210	IHC	1:1000
pThr181	ThermoFisher	pThr181	IHC	1:1000
pTyr18	Medimabs	pTyr18	IHC	1:1000
TauC3	Invitrogen	Asp421-422	IHC	1:1000
Iba1	Wako	-	IF	1:1000
GFAP	Novus	-	IF	1:2000
NeuN	Abcam	-	IF	1:2000
Goat anti-mouse	MP Biomedicals	-	IHC	1:100
Biotinylated goat anti-rabbit	MP Biomedicals	-	IHC	1:200
Goat anti-rabbit Alexa Fluor 568	Abcam	-	IF	1:400
Goat anti-mouse Alexa Fluor 488	Abcam	-	IF	1:400
Caspase-3	Cell Signaling Technologies	-	WB	1:1000
Cleaved Cas-3 (Asp175)	Cell Signaling Technologies	N-term to Asp175	WB	1:1000

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3619 **Supplemental Table 3. Qualitative scoring of changes in tau and tau-related pathology across**
 3620 **the heterozygous R955-hTau rat lifespan relative to wild-type rats.**

		Cerebral Cortex				Hippocampus			
		Time point (age, in months)							
Cell Type	Tau Species	3	10	18	24-26	3	10	18	24-26
Neurons	<u>Total Tau</u>								
	hTau	+	+	++	+++ ^{T,D}	-	+	++	+++ ^{B, D}
	<u>Phosphorylated Tau</u>								
	pSer202-pThr205	-	-	+	++ ^{T,D}	-	-	+ ^{1, S}	++ ^{S,C, B}
	pThr231	-	-	-	-	-	-	-	+ ^C
	pThr181	-	-	-	-	-	-	-	-
	pSer396-Ser404	-	-	-	+	-	-	-	-
	pSer202	-	-	-	+	-	-	-	+ ^{S,C}
	pSer422	-	-	-	-	-	-	-	-
	pThr175	-	-	-	-	-	-	-	-
	pSer208/210	-	-	-	-	-	-	-	-
	pTyr 18	-	-	-	-	-	-	-	-
	<u>Misfolded tau</u>								
	MC1 (aa. 7-9, 312-322)	-	-	-	-	-	-	-	-
	Cas-cleaved Tau Asp421-422	-	-	-	-	-	-	-	-
Glial marker									
Microglia ²	Iba1	-	-	-	-	-	-	-	++ ^{C1}
Astrocytes ²	GFAP	-	-	-	-	-	-	-	+

3621 ¹ Distribution was initially observed in the entorhinal cortices and found across somatosensory,
 3622 parietal, temporal cortices in pyramidal neurons at layers II-III and V.

3623 ² No human tau nor pathological tau was detected in this cell type.

3624 ^S Subiculum

3625 ^C Cornu Ammonis (CA1)

3626 ^T Tortuous dendrites

3627 ^B Neuritic beading

3628 ^D Immunoreactive Speck debris

Connecting Text: Chapter III and IV

In line with our hypothesis, heterozygous R955-hTau transgenic rats rendered a milder phenotype with a slow-progressing tauopathy in comparison to their homozygous kindred, as detailed in Chapter II. In Chapter III we demonstrated that heterozygous R955-hTau rats produced a modest accumulation human tau over the lifespan, seemingly without consequence until the occurrence of marked tau hyperphosphorylation as from 24 months of age, at which time points we detected multiple morphological abnormalities in tau-burdened neurons.

Interestingly, such tau hyperphosphorylation was sufficient to induce cognitive impairments, a CNS microglial and astrocytic inflammatory response and CA1 hippocampal neuron loss, albeit to a milder extent than that of homozygous R955-hTau rats. This, without developing detectable neurofibrillary inclusions. While neurofibrillary inclusions were not detected by 24-26 months of age, we provided evidence of tau oligomerization and possible aggregation, but not misfolding, demonstrating an early-stage state of tau pathology. Notably, these initial molecular species of tau pathology were capable of inducing some neurodegenerative events currently associated with advanced tauopathy.

One of the significant issues remaining is how the stage-wise brain tauopathy and amyloidosis impact each other. This aspect was investigated in Chapter IV by crossing the McGill-R-Thy1-APP and McGill-R955-hTau transgenic rat models in their slowly progressing heterozygous forms. The generation of this novel bigenic rat model possessing both amyloid and tau pathologies enabled such investigation with predictable as well as totally unexpected outcomes.

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CHAPTER IV:
**“Tau counteracts the initial amyloid beta-derived impairments in
cognition in a transgenic rat model”**

Title: Tau counteracts the initial amyloid beta-derived impairments in cognition in a transgenic rat model

Running title: Combined effects of amyloid beta and tau *in vivo*

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Key words: Alzheimer's disease, transgenic rat model, animal behavior, human tauopathy, synaptic plasticity

Abstract:

The increased deposition of amyloid beta (A β) and microtubule-associated protein tau (MAPT) in a hyperphosphorylated state are hallmarks of AD. As AD progresses over decades, A β and tau contribute to the progressive brain pathology. However, how these pathologies impact one another remains unclear especially during the early disease stages. To clarify such interactions, we generated a novel bigenic rat, coded McGill-R-APPxhTau, possessing both human-like amyloid and tau pathologies which was achieved by crossing the McGill-R-Thy1-APP transgenic rat with the recently established R955-hTau transgenic rat model in their heterozygous forms. Using this bigenic APPxhTau rat, we sought to investigate the neurochemical, neuropathological and cognitive interactions of these two pathognomonic AD-like pathologies. An extensive longitudinal and multifaceted behavioral testing paradigm at three time points over the life span was performed and was followed up by post-mortem examination of brain pathology, CNS inflammation, and neuronal loss. The APPxhTau rats were evaluated alongside single transgenics and wild type littermate controls. In this report, we show that APPxhTau rats possess features of early human AD-like pathology including progressive accumulation of human A β and tau, tau hyperphosphorylation, and deposition of plaques in aged rats. Unexpectedly, we discovered an early compensatory effect of the tau transgene manifested as a rescue of the amyloid-induced cognitive impairments and deficits in synaptic plasticity at the onset of plaque formation. On the other hand, after plaque pathology was well-established, this APPxhTau bigenic rat model displayed exacerbated amyloid AD-like pathology, brain inflammation and tau-driven neuronal loss as compared to the single APP^{+/-} or R955-hTau^{+/-} transgenic rats. Our findings describing this A β -tau relationship may have implications during the earliest stages of human AD-pathology.

1. Introduction

The two neuropathological hallmarks required for a diagnosis of Alzheimer's disease (AD) are the extracellular deposition of amyloid beta (A β) plaques and intracellular neurofibrillary tangles (NFTs) composed of abnormally phosphorylated tau proteins and paired helical filaments (PHFs). The neurotoxic effects of A β (Mucke and Selkoe, 2012a; Yankner et al., 1990) and tau proteins (Krüger and Mandelkow, 2016; Pampuscenko et al., 2021; Sebastián-Serrano et al., 2018; Ward et al., 2012) have been extensively studied separately. However, interventions that individually target A β (Cummings et al., 2022; Mintun et al., 2021; Sevigny et al., 2016) or tau (Florian et al., 2023; Novak et al., 2021; Wilcock et al., 2018) have not yielded substantial outcomes on cognition, emphasizing the complex and multifaceted nature of AD that involves multiple simultaneous and underlying pathological processes.

In line with the Trigger and Bullet hypothesis proposing A β as an instigator of tau-mediated degeneration (Bloom, 2014), tau accumulation correlates more highly with cognitive decline and degeneration than A β (Arriagada et al., 1992; Bejanin et al., 2017; Bennett et al., 2004; Biel et al., 2021; Giannakopoulos et al., 2003; Guillozet et al., 2003; Hanseeuw et al., 2019; Schöll et al., 2016). The initial investigations demonstrating a significant relationship between A β and tau stemmed from exacerbated fibrillary tau pathology by the combination of both amyloid and tau pathologies *in vivo* (Chabrier et al., 2014; Gotz et al., 2001; Lewis et al., 2001; Vergara et al., 2019). In addition, it is well-established that A β impairs synaptic plasticity and learning and memory by affecting long term potentiation (LTP) (Hu et al., 2008; Klyubin et al., 2004; Koffie et al., 2009; Qi et al., 2014b; Walsh et al., 2002). Yet, genetic removal of tau demonstrated its necessity in mediating A β -induced LTP impairments *in vivo* (Ittner et al., 2010; Shipton et al., 2011) further emphasizing a clear, tightly woven relationship and possible synergies between A β and tau (Ittner and Götz, 2011) during the more advanced stages of disease. However, the role of both A β and tau and their interconnection during the early stages require a deeper understanding.

It is well established that patients develop Alzheimer's pathology for approximately 20 years before clinical symptoms appear (Buchhave et al., 2012; Fagan et al., 2014). Early stages which can be categorized by the onset of A β plaque deposition (Dubois et al., 2014) or individuals that are amyloid positive and tau negative in accordance to the amyloid, tau and neurodegeneration (A/T/N) framework (Jack et al., 2016; Pascoal et al., 2023), remain poorly characterized. In these populations, it is impossible thus far to predict in cognitively normal individuals which ones will

progress to AD. Identifying the underlying mechanism(s) and early indicators of disease during such early and slowly progressing disease stages related to A β and tau in the aged brain should therefore provide novel insights into the initial disease-aggravating events. To investigate such early stages that are otherwise impossible to achieve in humans, we rely on animal models that faithfully reproduce key aspects observed in the human brain.

Rodent models for AD-like pathology have enabled the investigation of mechanistic changes occurring in the CNS and subsequent therapeutics. Such rodent models, mainly in mice, are primarily based on genetic mutations causative of familial AD or frontotemporal dementia (FTD) and such models harbor either A β (Cohen et al., 2013; Javonillo et al., 2022; Leon et al., 2010; Oakley et al., 2006; Pang et al., 2022; Wirths et al., 2006) or tau (Dujardin et al., 2015; Kopeikina et al., 2013; Malcolm et al., 2019; Yoshiyama et al., 2007) pathologies or both (Bolmont et al., 2007; Javonillo et al., 2022; Lewis et al., 2001; Oddo et al., 2003; Sterniczuk et al., 2010; Terwel et al., 2008). However, these existing models do not fully recapitulate human AD. A limitation is that their respective pathologies, whether featuring amyloid and/or tau, possess high transgene copy numbers and produce an aggressive phenotype commencing in early adulthood, thus foregoing the factor of aging. Aging is a principal component and risk factor in neurodegenerative diseases such as AD that significantly alters disease relevant aspects including the inflammatory response or inflammaging (Ferrucci and Fabbri, 2018; Finger et al., 2022; Jin et al., 2022), cellular senescence (Liu, 2022; Martínez-Cué and Rueda, 2020), neuroplasticity (Burke and Barnes, 2006; Foster and Norris, 1997; Thibault and Landfield, 1996), proteasome function (Cuervo and Dice, 2000; Kelmer Sacramento et al., 2020; Vilchez et al., 2014), metabolism of drugs and absorption of nutrients (Kinirons and O'Mahony, 2004; Mangoni and Jackson, 2004; Pannérec et al., 2018; Sikora et al., 2021; Sowell et al., 2003). Additionally, the use of rats presents several disease-relevant advantages over mice for the study of AD and related tauopathies in humans including endogenous expression of all tau isoforms (Hanes et al., 2009), peripheral immune response (Cao and Zheng, 2018; Wildner, 2019), superior display of behavior (Do Carmo and Cuello, 2013; Kummer et al., 2014; Whishaw and Tomie, 1996) and APOE protein akin to the human APOE4 which accelerates tau-mediated neurodegeneration (Shi et al., 2017; Tran et al., 2013).

To explore and to better understand the combined and exacerbating effects of both A β and tau in aged animals with a focus on the early disease stages, we crossed the well-established

3787 McGill-R-Thy1-APP (Hanzel et al., 2014; Iulita et al., 2014; Leon et al., 2010) and recently
 3788 established McGill-R955-hTau transgenic (Chapters II and III) rat models for human amyloid
 3789 pathology and tauopathy, in their respective heterozygous forms, to produce a bigenic rat model
 3790 with both human-like amyloid and tau pathologies. We investigated whether combining these two
 3791 Transgenic (Tg) rat models was sufficient to spur each others' initial pathologies in comparison to
 3792 single Tg rats. Over the life span, cohorts of rats underwent an extensive and multidimensional
 3793 battery of cognitive testing and a post-mortem examination of AD-like pathology including A β ,
 3794 tau, inflammation, neuronal loss, and synaptic status. We show how McGill-R-APPxhTau rats
 3795 (referred to as APPxhTau) unexpectedly produced an initial protective and dominating effects
 3796 against the amyloid pathology that was attributed to human tau in hippocampal dependant
 3797 cognition and synaptic plasticity. We then show how APPxhTau rats ultimately developed
 3798 exacerbated disease pathologies as compared to single Tg rats during stages of advanced amyloid
 3799 plaque pathology.

2. Materials & Methods

2.1 Animals

Generation of the McGill-R-Thy1-APP(Leon et al., 2010) and McGill-R955-hTau(Malcolm et al., 2019) transgenic rat models was achieved as previously described without modification. For simplification, McGill-R-Thy1-APP rats are referred to as “APP”, McGill-R955-hTau is “R955-hTau” and the bigenic McGill-R-APPxhTau is “APPxhTau” throughout this report. Only heterozygous animals were used as heterozygous animals produce relatively milder pathologies compared to their homozygous kin, enabling a greater sensitivity to observe early effects and incorporates the factor of aging. Transgenic (Tg) APP rats (Leon et al., 2010) expressing human APP⁷⁵¹_{Swe, Ind} under the murine Thy1.2 promoter and transgenic R955-hTau rats (Malcolm et al., 2019) expressing the longest isoform (2N4R) of human P301S mutated tau were genetically crossed to produce the bigenic APPxhTau rats with a Wistar background. Transgenic rats and their wild-type littermates were housed in groups of 3-4 in a GR1800 double-decker cage system (Techniplast, Int.) under a 12:12 light:dark cycle and were provided standard chow and water ad libitum. The use of animals for this study was approved by the McGill Animal Care Committee under the guidelines of the Canadian Council on Animal Care (CCAC).

2.2 Experimental design

Male and female transgenic rats were bred alongside wild-type littermate controls and raised in three independent sets of cohorts to one of three endpoints at 12, 20 and 24 months of age with a sample size of at least 10 per group per time point (**Supplemental Table 1**). At each endpoint, rats underwent behavioral testing for cognition, followed by brain perfusion and tissue collection for the subsequent examination of amyloid and tau pathologies in the brain.

2.3 Animal behavior

Animals were habituated to handling at least two weeks prior to behavioral testing. Assessment of cognition was achieved using a battery of behavioral tests during the beginning of the light cycle including the novel object location (NOL), novel object recognition (NOR) and Morris water maze (MWM) tasks using previously established protocols (Hall et al., 2018; Malcolm et al., 2019) as well as the Y-maze for exploration and working memory (Galeano et al., 2014) and the social interaction (SI) test for social withdrawal. The NOL, NOR, SI, and MWM were performed as

previously described (Chapter II). Animal data was recorded and scored with the assistance of Ethovision XT (Noldus, The Netherlands) and Freezing software for automated fear conditioning. (v1.3.01, Panlab, Spain). Experimenters were blinded to genotype. A global cognitive index (CI) was calculated by converting the raw behavioral performance score into a fraction of the maximum achievable value for each task ranging from 0 to 1 and then values were averaged across tasks for each animal into a composite score.

2.3.1 *Y-maze*

The three-armed Y shaped maze (Y-maze) task to evaluate short term working memory and general exploratory behavior was achieved as previously described (Galeano et al., 2014) with minor modification. In brief, rats were placed in a three-armed plexiglass apparatus (45 x 12 x 35 cm) diverging at 120° coated in fresh bedding mixed with soiled bedding of experimental animals, in one arm facing the end of the arm and were given 8 min to explore freely. Entries into each arm were scored when the animal placed all four limbs inside the arm. Total number of entries and number of alternations or ‘triads’ was recorded.

2.4 *Tissue collection*

Rat brain tissues were collected as previously described (Chapter II). In brief, rats were heavily anesthetized in 1% sodium pentobarbital (Equithesin) prior to transcardial brain perfusion with chilled phosphate-buffered saline. One brain hemisphere was fixed in 4% paraformaldehyde for 24 hours and the other hemisphere was further dissected to separate the cerebral cortex and hippocampus before being snap frozen in dry ice and stored at -80°C until use for biochemistry.

2.5 *Immunohistochemistry and Immunofluorescence*

Immunohistochemical procedures on 40 µm coronal sections obtained from a freezing sledge microtome (Leica, Germany) were done as previously described with modification (Chapter II). Immediately after blocking in 10% normal goat serum, primary antibodies (**Supplemental Table 3**) were incubated overnight at 4°C on a shaker. Co-localization and cell counting experiments was achieved by examining images containing three 1 µm Z-stacks merged into a Z-projection. Brightfield images were obtained using an Axio Imager M2 with an AxioCam506 color digital camera and ZEN v2.3 Blue imaging software (Zeiss, Germany). Fluorescence images were acquired using an LSM800 confocal laser scanning microscope (Zeiss, Germany). Image analysis

of total fluorescence intensity, cell counts, and percent area was accomplished using ImageJ (NIH, USA) with consistent mask thresholds per experiment using at least two slices per animal and one to two images per region of interest per slice. Fluorescence intensity is expressed in arbitrary fluorescence units (AU).

2.6 Quantification of amyloid beta and tau species by electrochemiluminescence immunoassay (ECLIA)

Thirty milligrams of cortical brain tissue were homogenized in Tris-buffered saline (TBS) buffer (150 mM NaCl, 50 mM Tris-HCl, 5 mM EDTA, pH 7.6) with protease inhibitors (Complete mini, Roche). Ultracentrifugation (Beckman-Coulter Optima MAX-XP) at 100,000 g for 1 hour at 4°C, then supernatants were stored as soluble fraction. Pellets were resuspended in a Tris-guanidine buffer (5M guanidine HCl, 50mM Tris HCl, pH 8.0), spun again at 100,000 g to obtain the insoluble fraction. Supernatants were stored at -80°C. Amyloid beta (A β) peptides 1-42, 1-40 and 1-38 were quantified using the V-Plex A β peptide panel (6e10) kit (K15200E-1, Mesoscale Discovery, USA) according to manufacturer's instructions.

Procedures for Sarkosyl-based separation of tau species, as is done in human brain to isolate paired helical filaments (Allen et al., 2002) were done as previously described without modification (Chapter II). Total tau and total ptau Thr231 was quantified using the Phospho(Thr231)/Total Tau kit (#K15121D, Mesoscale Discovery, USA) and the assay was run according to manufacturer's instructions.

2.7 Western blot

Twenty to fifty micrograms of snap frozen brain homogenates were processed as previously described without modification (Chapter II). After blocking in 5% non-fat milk or bovine serum albumin (BSA), primary antibodies (**Supplemental Table 3**) were incubated overnight at 4°C on a shaker. The following day, blots were incubated in a species-specific HRP-conjugated secondary antibody and bands were activated using Western lightning plus chemiluminescence substrate (Perkin Elmer, USA) and imaged using an Amersham 600 Imager (GE Healthcare, Canada). Optical densities of protein bands of interest were obtained using ImageLab 6.0 (Bio-Rad, USA), normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as fold-change of wild-type littermates.

2.8 Semi-quantitative real-time polymerase chain reaction (rt-qPCR)

Total RNA content was extracted from ten milligrams of cortical and hippocampal brain homogenates using an RNEasy mini prep kit (Qiagen #74106, Germany) according to manufacturer's instructions. RNA underwent reverse transcription into cDNA using the IScript rt-qPCR Supermix (Bio-Rad #170-8841, USA) and a standardized PCR protocol (priming at 25°C for 5 min, reverse transcription at 46°C for 20 min, inactivation at 95°C for 1 min). cDNA was stored at -20°C until use. Optimized dilutions of cDNA underwent PCR to detect the respective transgenes (hAPP: For 5' CAG ATC CAT CAG GGA CCA AA 3', Rev 5' ACT GGT TGG TTG GCT TCT AC 3'; hTau: For 5' GAA GAT GTG ACA GCA CCC TTA G 3', Rev 5' GTC TCC AAT GCC TGC TTC TT 3') and was normalized against to two housekeeping genes (GAPDH: For 5' TGA TGG GTG TGA ACC ACG AG 3', Rev 5' TCA TGA GCC CTT CCA CGA TG 3'; HPRT1: For 5' CAG GCC AGA CTT TGT TGG AT 3', Rev 5' TCC ACT TTC GCT GAT GAC AC 3'). Relative gene expression was quantified and expressed as 2^{ddCT} .

2.9 Acute brain slice electrophysiology and induction of long-term potentiation

Twenty-month-old rats (n=4-6) were deeply anesthetized using CO₂. After confirming unresponsiveness, rats were immediately decapitated, and forebrains were carefully extracted and mounted onto a VT1200S vibratome (Leica, Germany) with a basin containing fresh ice-cold cutting solution containing (2.5 mM KCl, 0.1 mM CaCl₂, 4 mM MgCl, 1.25 mM KH₂PO₄, 26 mM NaHCO₃, 1.6 mM glucose and 0.25 M sucrose, osmolarity of 350-360 and pH 7.35). Coronal slices 350 µm thick were then transferred into artificial cerebral spinal fluid (aCSF, 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, 25 mM glucose, osmolarity 310-320, pH 7.35) warmed to 32°C and allowed to stabilize for 1 hr followed by further resting at room temperature for 30 minutes. Slices were transferred to a Faraday cabinet equipped with a slice recording chamber containing aCSF and 10 µM bicuculline methobromide to block GABAergic inputs and allowed to rest for 30 minutes before recording baseline field excitatory post-synaptic potentials (EPSPs). A tungsten stimulating electrode was gently positioned at the CA1 *stratum radiatum* with a glass micropipette on the CA1 Schaeffer collateral downstream of the stimulating electrode. EPSPs were obtained and recorded using Clampex, Multiclamp and Clampfit software (Molecular Devices, USA). After a stable baseline recording of 30 minutes was

achieved, high frequency stimulation (HFS, 100 pulses over 10 milliseconds) was invoked to induce long-term potentiation (LTP). EPSPs were recorded for at least one hour after HFS.

2.10 Statistical analysis

Statistical analysis was performed with the assistance of GraphPad Prism 8 software (San Diego, USA) using a global alpha of 0.05. Data are presented as mean +/- standard error of the mean (SEM). Each time point and each brain region were analyzed independently. Two-tailed unpaired t-tests were performed on single factor, single comparisons. One-way and Two-way ANOVA, as appropriate, were followed up with Bonferroni's adjustment for three or less comparisons, and Holm-Sidak's otherwise. One-way and two-way repeated measures ANOVA (RM-ANOVA) were followed up with Holm-Sidak's pairwise comparisons using a mixed design. If a significant interaction was found, only simple main effects of genotype were examined. Statistical outliers were removed from datasets using Grubbs test.

3. Results

The slow progressing human amyloid pathology in APP^{+/-} transgenic (Tg) rats was examined and compared to APPxhTau rats at 12, 20 and 24 months of age. At 12 months, both APP^{+/-} and APPxhTau Tg rats exhibited similar magnitudes of human A β immunoreactivity (IR) in the hippocampus and cerebral cortex as revealed by the human A β specific monoclonal antibody McSA1 (Grant et al., 2000) (**Figure S1B-C**), which was restricted to the intracellular compartment. By 20 months of age, the initial plaque pathology commenced in the entorhinal area and, more prominently in the subiculum of the hippocampus shown by the appearance of small, diffuse plaques (**Figure 1A-B, D-E**). Approximately one third of the rats possessing the APP^{+/-} transgene developed plaques at this age (**Supplemental Table 2**). In rats that developed plaques, no differences in the number of plaques between APP^{+/-} and APPxhTau rats was observed in the subiculum region (**Figure 1M**; $p=0.6912$), nor in other regions of the hippocampus (**Figure S1D**; $p>0.9$) and cerebral cortex (**Figure S1E**; $p>0.9$). Few plaques in the subiculum showed dense core properties as shown by ThioflavinS-immunoreactive particles colocalized with A β (**Figure 1C, F**).

At 24 months, APP^{+/-} and APPxhTau Tg rats developed a more advanced plaque pathology as shown by an overall increased abundance and spread of plaques into other cortical and caudal hippocampal regions (**Figure 1G, J**). Further, half of the APPxhTau rats developed cortical plaques whereas only one third in APP^{+/-} Tg rats, suggestive of elevated cortical amyloid pathology (**Supplemental Table 2**). In contrast, half of the rats in both APPxhTau and APP^{+/-} Tg groups developed hippocampal plaques. Notably, in the subiculum region of the hippocampus possessing the initial amyloid plaque pathology in Tg rats, APPxhTau rats developed approximately two-fold more plaques than APP^{+/-} Tg rats (**Figure 1N**; $t(13)=2.367$, $p=0.0341$) with an overall elevation in ThioflavinS immunofluorescent material indicating elevated density of mature plaques (**Figure 1L**). However, while the overall distribution of plaques had become more widespread at 24 months, other brain regions within the hippocampus (**Figure S1F**) and cerebral cortex (**Figure S1G**) showed no significant differences in the total number of plaques.

In parallel with the analysis of plaque pathology, quantification of A β peptides at 12, 20 and 24 months revealed a marked increase in the A β _{42/40} ratio in APPxhTau rats as compared to APP^{+/-} at 24 months of age for both the soluble (**Figure 1O**; $t(59) = 2.616$, $p=0.0334$) and insoluble

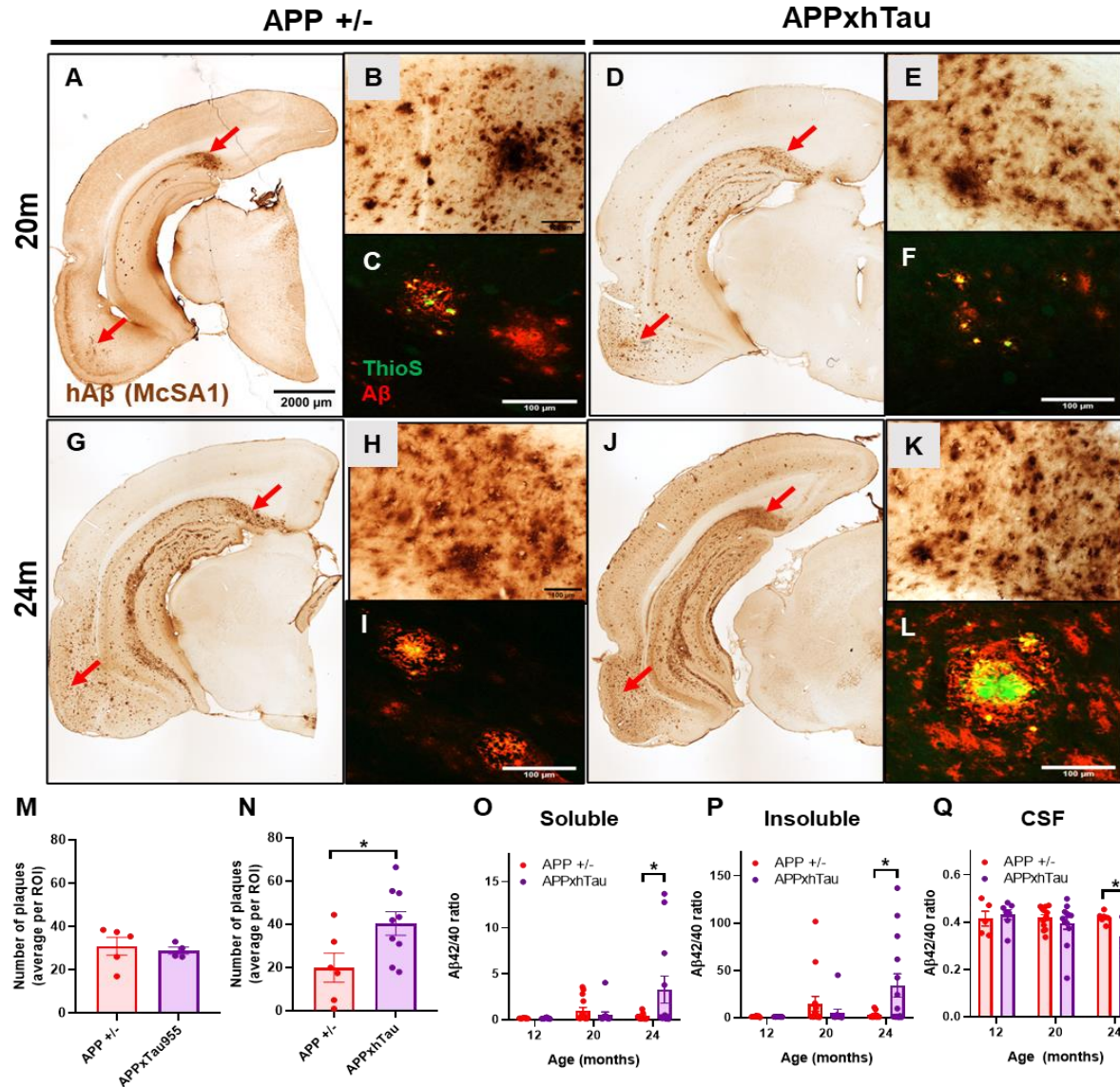


Figure 1. Amyloid plaque pathology in heterozygous APP and APPxhTau transgenic rats. (A, D, G, J) Representative micrographs of coronal brain sections illustrating human amyloid beta (Aβ) immunoreactivity the caudal hippocampus and cerebral cortex at 20 (A-F) and 24 months of age (G, L). Note the plaque deposition initially appears in the subiculum and entorhinal area (red arrows). (B, E, H, K) Representative micrographs of the subiculum region of the hippocampus illustrating the appearance of diffuse and dense core plaques, noting the abundance of dense core plaques in 24-month-old bigenic rats. (C, F, I, L) Representative immunofluorescence micrographs illustrating aggregated amyloid material as shown by the colocalization between ThioflavinS (ThioS) and Aβ in the subiculum. Quantification of the number of plaques in the subiculum at 20 (M) and 24 months of age (N) per region of interest (ROI). Quantification of the Aβ42/40 ratio in the soluble (O) and insoluble brain fractions (P) as well as in cerebrospinal fluid (CSF) (Q) showing an acceleration of Aβ peptides in 24-month-old APPxhTau rats as compared to APP^{+/-} single transgenics (n=5-9). Scale bar A, D, G, J = 2000 μm; B-C, E-F, H-I, K-L = 100 μm. * p < 0.05; ** p < 0.01.

fractions (**Figure 1P**; $t(63)=2.942$, $p=0.0136$). Further, a decrease in the A β 42/40 ratio in the CSF was detected in APPxhTau compared to APP^{+/-} Tg rats (**Figure 1Q**; $t(49)=2.511$, $p=0.0455$), which parallels the increased plaque pathology, further emphasizing that a mild addition of human tau exacerbated the advanced, but not initial, amyloid pathology in this rat model.

To determine whether the increased amyloid pathology in APPxhTau compared to APP^{+/-} Tg rats is related to differences in APP processing we investigated the transcript and protein levels of APP as well as the protein levels of the soluble APP alpha (sAPP α) fragment. As expected, total APP protein levels (22C11) were elevated by approximately three to four fold in APP^{+/-} and APPxhTau Tg rats compared to wild type rats due to the addition of the human APP transgene at 20 (Figure S2B; APPxhTau vs. Wt $t(25)=2.8$, $p=0.0476$; APP^{+/-} vs. Wt $t(25)=3.259$, $p=0.0191$) and 24 months of age (**Figure S2C**; APPxhTau vs Wt $t(25)=3.609$, $p=0.0080$; APP^{+/-} vs. Wt $t(25)=2.756$, $p=0.0423$) however APP levels in APPxhTau was similar to that of APP^{+/-} Tg rats at both 20 (**Figure S2B**; $p=0.8097$) and 24 months of age (**Figure S2C**; $p=0.6431$). Accordingly, gene expression of the human APP transgene was similar between APP^{+/-} and APPxhTau Tg rats as assessed by qPCR (**Figure S2G**; $p>0.6541$). Further, the levels of the sAPP α protein fragment derived from nonamyloidogenic human APP processing was not significantly different between APP^{+/-} and APPxhTau Tg rats at 20 (**Figure S2E**; $p=0.5597$) and 24 months of age (**Figure S2F**; $p=0.5283$).

3.2 Amyloid encourages tau hyperphosphorylation during advanced plaque deposition.

The initial human-like tauopathy in R955-hTau^{+/-} Tg rats commences with detectable increases in ptau Ser202-Thr205 IR after 18 months, shown by the monoclonal antibody AT8, and leads to a surge of ptau at Ser202-Thr205 and pThr231 after 24 months of age (Chapter III). In this report, 20-month-old R955-hTau^{+/-} and APPxhTau Tg rats exhibited detectable AT8 IR in neurons of the cerebral cortex and hippocampus (**Figure 2A, C**), particularly in the entorhinal cortex (**Figure 2B, D**) and subiculum (**Figure S3A-B**), however, ptau Ser202-Thr205 protein levels as examined by Western blot were not significantly different in the Sarkosyl-soluble fraction (**Figure 2L**; $p>0.2821$). Interestingly, Sarkosyl-insoluble AT8 was elevated by approximately 3-4-fold in APPxhTau rats and 2-fold in R955-hTau^{+/-} rats as compared to wild type rats (**Figure 2M**; APPxhTau vs. Wt $t(19)=2.651$, $p=0.0473$; R955-hTau vs. Wt $p>0.999$).

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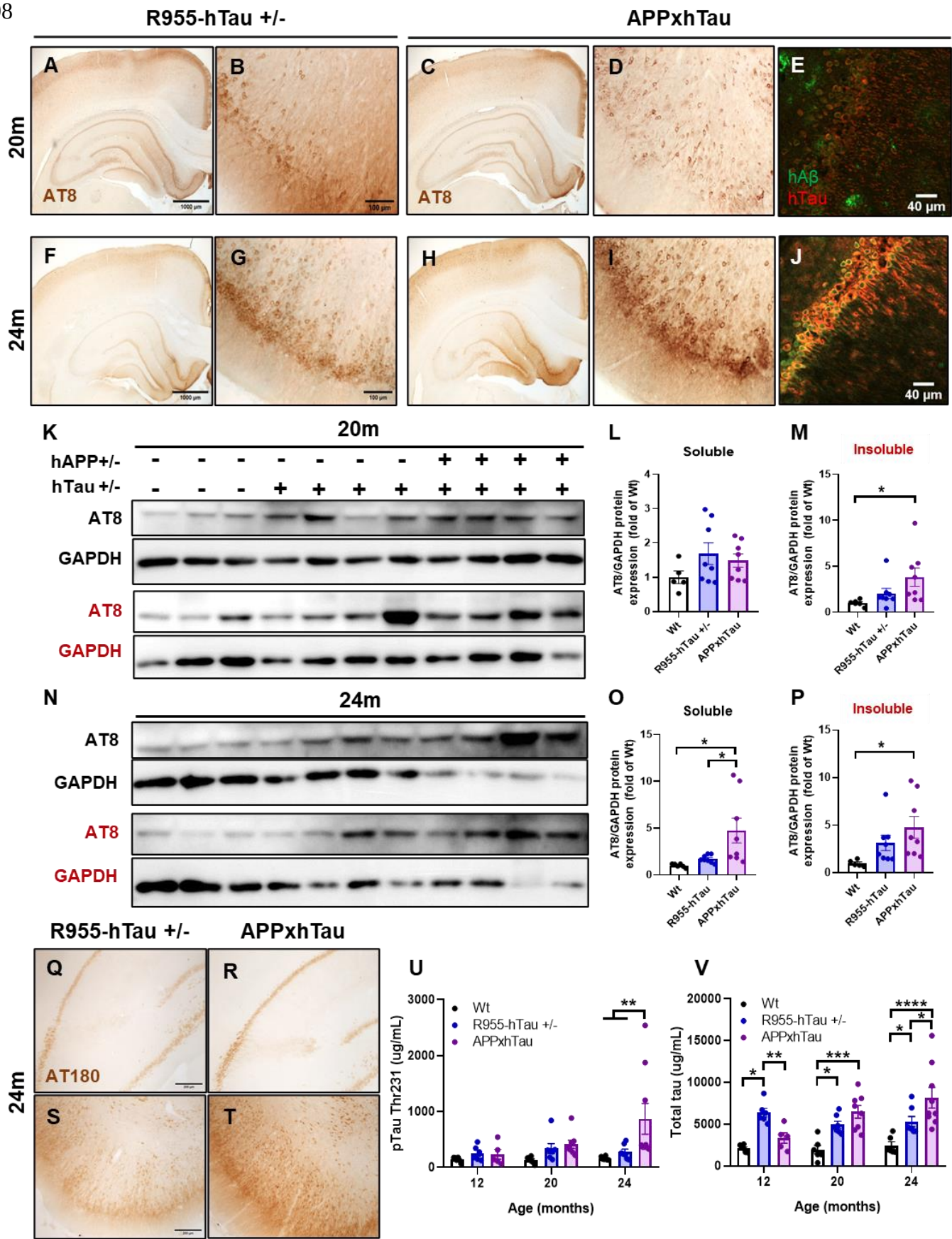


Figure 2. Acceleration of tau hyperphosphorylation at pSer202-Thr205 and pThr231 in 24-month-old APPxhTau rats. (A, C, F, H) Representative micrographs illustrating pSer202-Thr205 immunoreactivity in the cerebral cortex and hippocampus as probed using the monoclonal antibody AT8 and in the entorhinal area (B, D, G, I), in neurons at 20 (B-D) and 24 months of age (G-I). In APPxhTau rats, colocalization of human amyloid beta and tau was detected in the most severe cases as shown by representative confocal micrographs at 20 (E) and 24 months of age (J). (K, N) Representative Western blots of cortical brain homogenates from the Sarkosyl-soluble (upper panels) and Sarkosyl-insoluble (lower panels labelled in red) fractions in 20 (K) and 24-month-old rats (N). Quantification of ptau at Ser202-Thr205 revealed increased Sarkosyl-insoluble tau protein in APPxhTau rats at 20 months (M) as well as at 24 months in the Sarkosyl-soluble (O) and insoluble (P). (Q-T) Representative micrographs of the CA1-CA2 region of the hippocampus (Q-R) and the entorhinal cortex (S-T) at 24 months of age illustrating elevated ptau Thr231 immunoreactivity in APPxhTau rats as compared to R955-hTau^{+/-} single transgenics. Quantification of Sarkosyl-insoluble ptau Thr231 (U) and total tau (V) by electrochemiluminescence assays revealed increased accumulation of tau proteins in 24-month-old APPxhTau rats as compared to wild type and R955-hTau^{+/-} rats (n=6-9). Scale bar A, C, F, H 1000 μ m; B, D, G, I 100 μ m Q-T 200 μ m. * p<0.05; ** p<0.01; ***p<0.001; ****p<0.0001.

3999

4000 In contrast, at 24 months of age, ptau Ser202-Thr205 was elevated in APPxhTau rats as
 4001 shown by an increased AT8 IR (**Figure 2G-I**) and by Western blot in both the Sarkosyl-soluble
 4002 (**Figure 2O**; $t(19)=2.627$, $p=0.0498$) and Sarkosyl-insoluble fractions (**Figure 2P**; APPxhTau vs.
 4003 Wt $t(18)=2.666$, $p=0.0472$; APPxhTau vs. R955-hTau^{+/-} $p>0.6230$). Interestingly, a colocalization
 4004 event between human A β and human tau was observed in 24-month-old APPxhTau rats in severe
 4005 cases (**Figure 2E, J**) suggestive of a downstream co-accumulation in neurons which was not
 4006 detected at in milder cases of deposition or at 20 months of age.

4007 Immunoreactivity for phosphorylated tau at Thr231, as assessed with the monoclonal
 4008 antibody AT180, was elevated in the CA1-CA2 region of the hippocampus (**Figure 2Q-R**) and
 4009 the entorhinal cortex (**Figure 2S-T**) in APPxhTau rats as compared to R955-hTau^{+/-} Tg rats.
 4010 Ultrasensitive electrochemiluminescence assays revealed ptau Thr231 was two- to three-fold
 4011 higher in the Sarkosyl-insoluble fraction of APPxhTau rats as compared to R955-hTau^{+/-} rats at 24
 4012 months of age, whereas no differences were found at younger ages (**Figure 2U**; APPxhTau vs. Wt
 4013 $t(51)=3.786$, $p=0.0012$; APPxhTau vs. R955-hTau^{+/-} $t(51)=3.325$, $p=0.0033$). At 12 months of age,
 4014 an approximately two to three-fold increase of Sarkosyl-insoluble total tau was observed in R955-
 4015 hTau^{+/-} Tg rats as compared to wild type rats, which was unexpectedly abolished in APPxhTau rats
 4016 (**Figure 2V**; R955-hTau vs. Wt $t(51)=3.775$, $p=0.0013$; R955-hTau^{+/-} vs. APPxhTau $t(51)=2.545$,
 4017 $p=0.0278$; APPxhTau vs. Wt $p=0.2968$). By 20 months of age, Sarkosyl-insoluble total tau protein
 4018 was elevated in both APPxhTau and R955-hTau^{+/-} Tg rats (**Figure 2V**; APPxhTau vs. Wt

t(51)=4.279, $p=0.0002$; R955-hTau^{+/-} vs. Wt t(51)=2.743, $p=0.0167$). Interestingly, at 24 months of age, Sarkosyl-insoluble total tau was two-fold higher in APPxhTau rats as compared to R955-hTau^{+/-} Tg (**Figure 2V**; APPxhTau vs. R955-hTau t(51)=2.894, $p=0.0111$) signifying an acceleration of total tau accumulation. In contrast, Sarkosyl-soluble fractions yielded increased levels in R955-hTau^{+/-} and APPxhTau Tg rats starting at 12 months of age as compared to wild type rats by approximately two to three-fold for both ptau Thr231 (**Figure S3E**; APPxhTau vs. Wt t(51)=4.168, $p=0.0002$; R955-hTau^{+/-} vs. Wt t(51)=5.032, $p<0.0001$) and total tau (**Figure S3F**; APPxhTau vs. Wt t(51)=6.489, $p<0.0001$; R955-hTau^{+/-} vs. Wt t(51)=7.326, $p<0.0001$) with no detectable differences between R955-hTau^{+/-} and APPxhTau rats regardless of age. Unexpectedly, tau pathology in APPxhTau rats did not sufficiently accelerate into the advanced stages as to produce overt neurofibrillary tau inclusions, highlighting that the amyloid pathology was sufficient to encourage the initial tau hyperphosphorylation at sites Ser202-Thr205 and Thr231 in this model.

We also investigated the levels of the major tau kinases including glycogen synthase kinase 3 Beta (GSK3 β) as well as the p38 mitogen-activated protein kinase (p38MAPK). GSK3 β protein was significantly elevated in 20-month-old APPxhTau rats as compared to APP^{+/-} Tg rats (**Figure S3H**; t(24)=3.319, $p=0.0165$). In contrast, p38MAPK was unaltered at this age (**Figure S3I**, $p>0.9999$). At 24 months, GSK3 β was significantly increased by two to three-fold in APPxhTau Tg rats as compared to APP^{+/-} Tg and wild type rats (**Figure S3K**; APPxhTau vs. APP^{+/-} t(25)=4.049, $p=0.0026$; APPxhTau vs. Wt t(25)=3.040, $p=0.0271$). p38MAPK was elevated in a similar manner as GSK3 β (**Figure S3L**; APPxhTau vs. APP^{+/-} t(25)=4.271, $p=0.0015$; APPxhTau vs. Wt t(25)=3.484, $p=0.0091$) emphasizing that the combination of amyloid tau spurred cellular responses that may recruit tau kinases and aggravate tau phosphorylation.

3.3 A β -induced impairments in cognition are initially counteracted by tau

During the examination of cognition, there was a clear and early effect attributed to amyloid beta observed in APP^{+/-} Tg rats. At 12 months, an age where APP^{+/-} Tg rats display iA β and no amyloid plaque deposition, APP^{+/-} Tg rats developed an impaired discriminatory ability for the relocated object during the novel object location (NOL) task (**Figure S4A**; t(50)=5.148, $p<0.0001$). However, R955-hTau^{+/-} Tg, which are accumulating human tau but do not yet present hyperphosphorylated tau (AT8-IR), and APPxhTau rats showed no significant impairments during the task. All Tg rat groups showed no impairments in the other investigated behavioral tasks as

compared to wild type rats at 12 months of age. This includes the discrimination ratio during the novel object recognition (NOR) (**Figure 3A**; APP^{+/-} $p>0.0748$), interaction time with an age- and sex-matched nonfamiliar wild type rat during the social interaction (SI) (**Figure 3B**; $p>0.8839$), latency to find the hidden platform during the training (**Figure 3C**; $p>0.05$) and probe trials during Morris water maze (MWM) (**Figure 3D**; $p>0.9930$) as well as the percentage of alternations during the y-maze (**Figure S4B**; $p>0.4565$). Compiling the scores from these behavioral tasks into a global cognitive index translated into a 20% reduction in overall cognitive performance in APP^{+/-} Tg rats compared to wild type rats (**Figure S4C**; $t(51)=4.775$, $p<0.0001$). These minor and early amyloid-associated impairments did not carry over in 12-month-old APPxhTau bigenic rats. Instead, APPxhTau rats showed a cognitive index similar to that of wild type and R955-hTau^{+/-} Tg rats.

At 20 months of age, a time point when the initial amyloid plaque pathology typically develops in APP^{+/-} Tg rats, and human-like tau hyperphosphorylation at Ser202-Thr205 appears in R955-hTau^{+/-} Tg rats, notable impairments in cognition were observed. A reduction in discrimination ratio during the NOL task was observed in all transgenic groups as compared to wild type rats (**Figure S4D**; APPxhTau $t(54)=5.726$, APP^{+/-} $t(54)=5.891$; R955-hTau^{+/-} $t(54)=5.241$, $p<0.0001$). During the NOR task, APP^{+/-} Tg rats lost the ability to discriminate the novel object compared to wild type rats while APPxhTau rats performed similarly to wild type and R955-hTau rats (**Figure 3E**; APP^{+/-} vs. Wt $t(54)=5.237$, $p<0.0001$; APPxhTau vs. Wt $p=0.1539$). This apparent rescue of A β -driven cognitive deficits by tau was also observed during the y-maze as shown by a reduction in the percentage of alternations in APP^{+/-} Tg rats but not in APPxhTau rats (**Figure S4E**; APP^{+/-} vs. Wt $t(53)=4.713$, $p=0.0001$; APPxhTau vs. Wt $t(53)=3.140$, $p=0.0166$). Intriguingly, the SI task revealed social withdrawal behaviors attributed to the tau transgene as shown by a significant reduction in the interaction time with an unfamiliar rat in R955-hTau^{+/-} Tg and APPxhTau bigenic rats (**Figure 3F**; APPxhTau vs. Wt $t(54)=3.846$, $p=0.0019$; R955-hTau^{+/-} vs. Wt $t(54)=2.777$, $p=0.0451$; APP^{+/-} vs. Wt $p=0.2157$). Additionally, deficits in spatial learning and memory developed in both APP^{+/-} and R955-hTau^{+/-} Tg rats as shown by an increased time required to reach the hidden platform during the MWM task on days 3 to 4 (APP^{+/-} only) and on day 5 as compared to wild type rats (**Figure 3G**; Day 3 $p=0.0115$, Day 4 $p=0.0139$; Day 5 APP^{+/-} $p=0.0495$, R955-hTau^{+/-} $p=0.0338$). Unexpectedly, APPxhTau rats

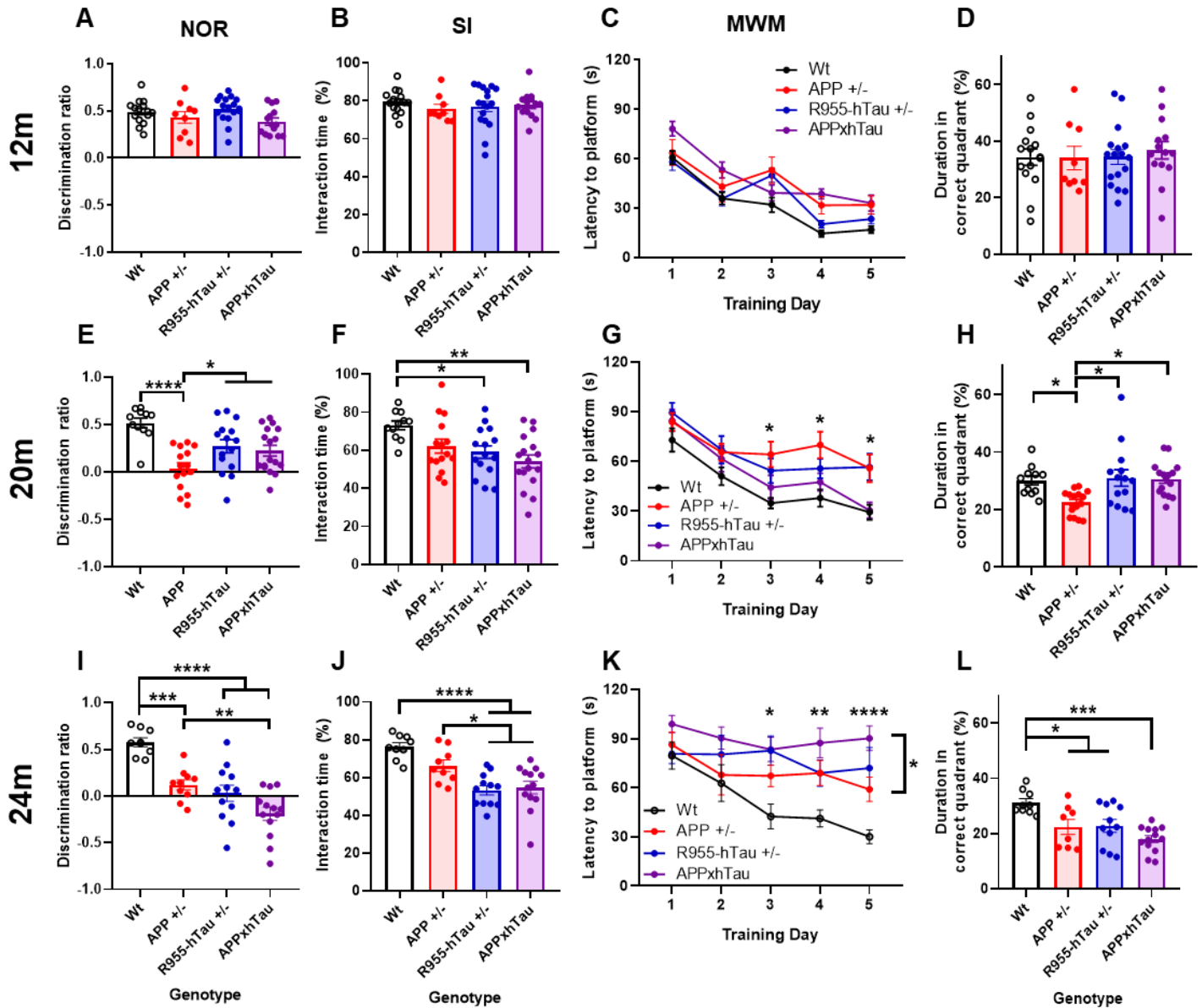


Figure 3. Tau dictates cognitive status with an initial compensatory effect followed by exacerbation of impairments in APPxhTau rats. Evaluation of cognition using a panel of behavioral tests conducted in cohorts of rats at 12 (A-D), 20 (E-H) and 24 months of age (I-L) including discrimination of a novel object during the novel object recognition (NOR) (A, E, I), interaction time with an age- and sex-matched unfamiliar conspecific during the three-chamber social interaction (B, F, J), and time required to find the hidden platform during the training phase of the Morris water maze (C, G, K) and the probe trial (D, H, L). At 20 months of age, the absence of amyloid-derived impairments in APPxhTau rats during the NOR (E) and MWM tasks (G) are compensated by tau. Note that performance of APPxhTau rats during the MWM is similar to that of Wt rats whereas single transgenic APP^{+/-} and R955-hTau^{+/-} rats show impairments. At 24 months, the NOR (I) and MWM (K) tasks show significantly lower scores in APPxhTau rats as compared to APP^{+/-} rats (n=8-15). * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

performed similar to the cognitively unimpaired wild type rats during the training phase (**Figure 3G**; $p=0.9981$). A similar unexpected rescue of cognition was observed during probe trial of the MWM as shown by the percentage of time in the correct quadrant (**Figure 3H**; APPxhTau vs. Wt $p=0.9867$; APP^{+/-} vs. Wt $t(52)=2.770$ $p=0.0307$; R955-hTau^{+/-} vs. Wt $p=0.9867$). The cognitive index at 20 months indicated an overall reduced performance in all transgenic animals by approximately 40% compared to wild type rats (**Figure S4F**; APPxhTau $t(54)=6.505$, APP^{+/-} $t(54)=7.656$; R955-hTau^{+/-} $t(54)=5.235$, $p<0.0001$). These findings together demonstrated that in APPxhTau rats the negative effects of iA β on cognitive performance are counteracted by tau until 20 months of age, a time point coinciding with the initial A β plaque deposition.

As expected, at the latest time point of 24 months when plaque pathology was well-established, multiple and more advanced deficits in cognition were detected. During the NOL task, APPxhTau rats presented significantly lower discrimination ratio scores compared to wild type rats as well as single APP^{+/-} rats, suggesting an exacerbation of cognitive impairment and increasing preference for familiar objects (**Figure S4G**; APPxhTau vs. Wt $t(40)=7.40$, $p<0.0001$; APPxhTau vs. APP^{+/-} $t(40)=3.320$, $p=0.0058$). The NOR task revealed similar observations as the NOL showing significantly reduced scores in APPxhTau rats as compared to APP^{+/-} and R955-hTau^{+/-} Tg rats (**Figure 3I**; APPxhTau vs. APP^{+/-} $t(40)=3.132$, $p=0.0097$; APPxhTau vs. R955-hTau^{+/-} $t(40)=2.327$, $p=0.0496$). In contrast, R955-hTau^{+/-} Tg and APPxhTau rats displayed a reduction in interaction time during the SI task as compared to wild type rats, however there was no exacerbated social withdrawal behavior in APPxhTau rats (**Figure 3J**; APPxhTau vs. Wt $t(39)=5.242$, $p<0.0001$; APPxhTau vs. APP^{+/-} $t(39)=2.784$, $p=0.0245$; R955-hTau^{+/-} vs. Wt $t(39)=5.506$, $p<0.0001$; R955-hTau^{+/-} vs. APP^{+/-} $t(39)=3.089$, $p=0.0147$). Regarding the MWM task, all transgenic animals showed clear impairments, however, APPxhTau rats performed worse than single APP^{+/-} Tg rats as shown by an increased latency at day 5 of training (**Figure 3K**; $p=0.0410$), with a trended decrease in the time spent in the correct quadrant during the probe trial (**Figure 3L**; $p=0.1907$). Compiled together, the cognitive index scores at 24 months indicated that cognitive status in APPxhTau rats was overall lower than that of the single APP^{+/-} and R955-hTau^{+/-} Tg rats (**Figure S4I**; APPxhTau vs. APP^{+/-} $t(41)=2.363$, $p=0.0454$; APPxhTau vs. R955-hTau^{+/-} $t(41)=2.711$, $p=0.0290$). Further, APP^{+/-} and R955-hTau^{+/-} Tg rats scores were approximately 40% lower compared to wild type rats, with APPxhTau rats having a more marked reduction by approximately 65%.

3.4 *Tau counteracts A β -induced impairments in synaptic plasticity during the initial plaque pathology.*

To confirm and further investigate the unexpected lack of cognitive changes observed in 20-month-old APPxhTau rats, we applied acute brain slice electrophysiology and induced long-term potentiation (LTP) in the CA1 region of the hippocampus. Twenty-month-old APP^{+/-} and R955-hTau^{+/-} Tg rats clearly exhibited an impaired LTP response 60 min after high frequency stimulation (HFS) compared to wild type rats, as shown by a decaying of field excitatory post-synaptic potential (fEPSPs) back to baseline levels as early as 30 min post-HFS (**Figure 4B**; APP^{+/-} vs. Wt $q(52)=7.234$, $p<0.0001$; R955-hTau^{+/-} vs. Wt $q(52)=8.317$, $p<0.0001$). Interestingly, the combination of APP^{+/-} and R955-hTau^{+/-} transgenes produced a significant and partial rescue in synaptic function as shown by a restoration of in fEPSP strength in APPxhTau rats compared to APP^{+/-} and R955-hTau^{+/-} Tg rats at 60 minutes post-HFS (**Figure 4B**; APPxhTau vs. APP^{+/-} $q(52)=4.051$, $p=0.0297$; APPxhTau vs. R955-hTau^{+/-} $q(52)=4.724$, $p=0.0082$; APPxhTau vs. Wt $q(52)=2.984$, $p=0.1634$).

Investigation of synaptic proteins involved in the LTP response including the N-methyl-D-aspartate receptor (NMDAR) as well as the post-synaptic density (PSD95) and the Src kinase Fyn, which are proteins involved in the regulation, trafficking and stabilization of NMDARs at the synapse was conducted using Western blot on hippocampal homogenates. At 20 months, the protein levels of hippocampal PSD95 (**Figure 4D**; APPxhTau vs. Wt $t(25)=3.178$, $p=0.0233$; APPxhTau vs. APP^{+/-} $t(25)=2.810$, $p=0.0466$) and fyn kinase were increased, by 6- and 4-fold respectively, in APPxhTau rats compared to wild type rats and APP^{+/-} Tg rats (**Figure 4E**; APPxhTau vs. Wt $t(25)=4.485$, $p=0.0008$; APPxhTau vs. APP^{+/-} $t(25)=3.308$, $p=0.0142$) while no differences in overall NMDAR1 expression were detected (**Figure 4F**; $p>0.5$). At 24 months of age, unexpectedly, PSD95 levels of APPxhTau were not statistically different from wild type rats and APP^{+/-} Tg rats (**Figure 4H**; APPxhTau vs. Wt $p>0.1110$). In contrast, fyn levels were consistently higher than in APP^{+/-} rats however with diminished magnitude than that observed at 20 months of age (**Figure 4I**; $t(25)=4.886$, $p=0.0003$). Similar to the 20-month-old time point, at 24 months the total NMDAR1 expression was unaffected by amyloid or tau in these heterozygous transgenic rat models (**Figure 4J**; $p>0.4494$).

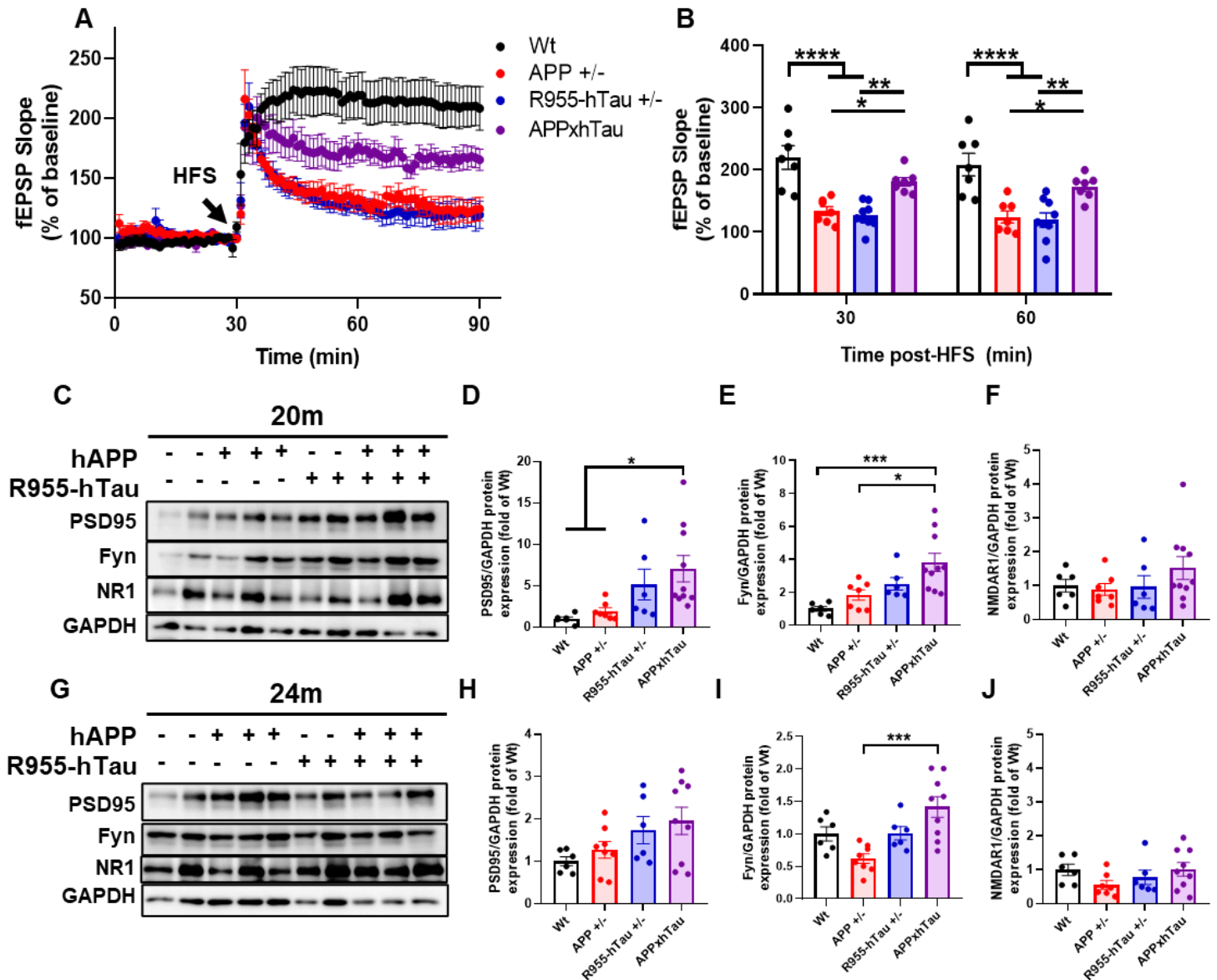


Figure 4. Tau counteracts amyloid-induced deficits in synaptic plasticity and induces alterations to postsynaptic density proteins involved with NMDA receptor activity. Acute brain slice recordings of field excitatory postsynaptic potentials (fEPSP) slope expressed as a percentage of baseline (**A**). (**B**) Quantification of fEPSP 30 or 60 minutes after HFS demonstrating impaired responses after high frequency stimulation (HFS) in 20-month-old APP^{+/-} and R955-hTau^{+/-} rats and a partially abolished response in APPxhTau rats. (**C-G**) Representative Western blots on hippocampal homogenates of 20-month-old (**C**) and 24-month-old rats (**G**) illustrating elevated postsynaptic density 95 (PSD95) (**D**) and fyn (**E**) protein expression in 20-month-old APPxhTau rats as compared to APP^{+/-} Tg and wild type rats, whereas NMDAR1 expression is unaffected (**F**). At 24 months, PSD95 (**H**) and fyn (**I**) are elevated in APPxhTau Tg rats as compared to wild type and APP^{+/-} Tg rats and with NMDAR1 (**J**) remained at levels similar to wild type rats (n=6-9). * p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001.

3.5 Enhanced hippocampal neuronal loss in aged APPxhTau rats

We previously showed that R955-hTau^{+/-} rats develop detectable neuronal loss in the CA1 region of the hippocampus after 24 months (Chapter III). To examine whether such degenerative aspects would occur in APPxhTau rats in an accelerated fashion we examined the number of NeuN-IR cells in the CA1 and subiculum regions of the hippocampus and in the entorhinal cortex. At 20 months of age (**Figure 5A-E**), there was no significant neuronal loss in any of the Tg rat groups (**Figure 5E**; $p>0.78$) in the CA1 or other brain regions including the subiculum (**Figure S5A**; $p>0.28$) and entorhinal cortex (**Figure S5B**; $p>0.07$). However, at 24 months of age, a time point during the more advanced plaque pathology and tau hyperphosphorylation, a marked reduction in the number of NeuN-immunofluorescent labelled neurons was detected in APPxhTau rats as compared to wild type (~25%), APP^{+/-} Tg (~25%) and to R955-hTau^{+/-} Tg (~15%) rats (**Figure 5J**; APPxhTau vs. Wt $t(31)=5.983$, $p<0.0001$; APPxhTau vs. APP^{+/-} $t(31)=3.660$, $p=0.0046$; APPxhTau vs. R955-hTau^{+/-} $t(31)=2.717$, $p=0.0317$). Expectedly, single R955-hTau^{+/-} rats showed some neuronal loss compared to wild type rats, while single APP^{+/-} Tg rats did not present a statistically different number of NeuN-IF cells in the CA1 (**Figure 5J**, R955-hTau^{+/-} vs. Wt $t(31)=3.318$, $p=0.0093$, APP^{+/-} vs. Wt $p>0.05$). In addition to the significant neuronal loss in APPxhTau rats was also evident in the entorhinal cortex with a trend towards loss in APP^{+/-} Tg rats (**Figure S5D**; APPxhTau vs. Wt $t(30)=3.099$, $p=0.0208$; APP^{+/-} vs. Wt $t(30)=2.446$, $p=0.0603$) suggesting a synergy between amyloid and tau-driven degeneration.

3.5 Synergistic effects of amyloid and tau on microglia and astrocytes

As a CNS inflammatory response plays a principal component in amyloid and tau pathologies, we examined whether such a combination would amplify the inflammatory response in APPxhTau rats. Using classical quantitative immunohistochemical approaches, at 20 months of age, there were no differences between genotypes in the total number of microglial cells in the CA1 region of the hippocampus (**Figure 6E**; $p>0.4560$) nor the other brain regions such as the subiculum (**Figure S5E**; $p>0.2299$) and entorhinal cortex (**Figure S5F**; $p>0.1815$). Interestingly, at 24 months of age, all transgenic rats whether APP^{+/-}, R955-hTau^{+/-} or APPxhTau rats, showed neuronal loss in the CA1 region, and the neuronal layers adopted a disorganized appearance (**Figure 5I**). In the subiculum, neuronal loss was not observed (**Figure S5C**; $p>0.8$). Interestingly,

4173 significantly elevated microglial cell abundance was detected in the CA1 as compared to wild type
 4174 rats (**Figure**

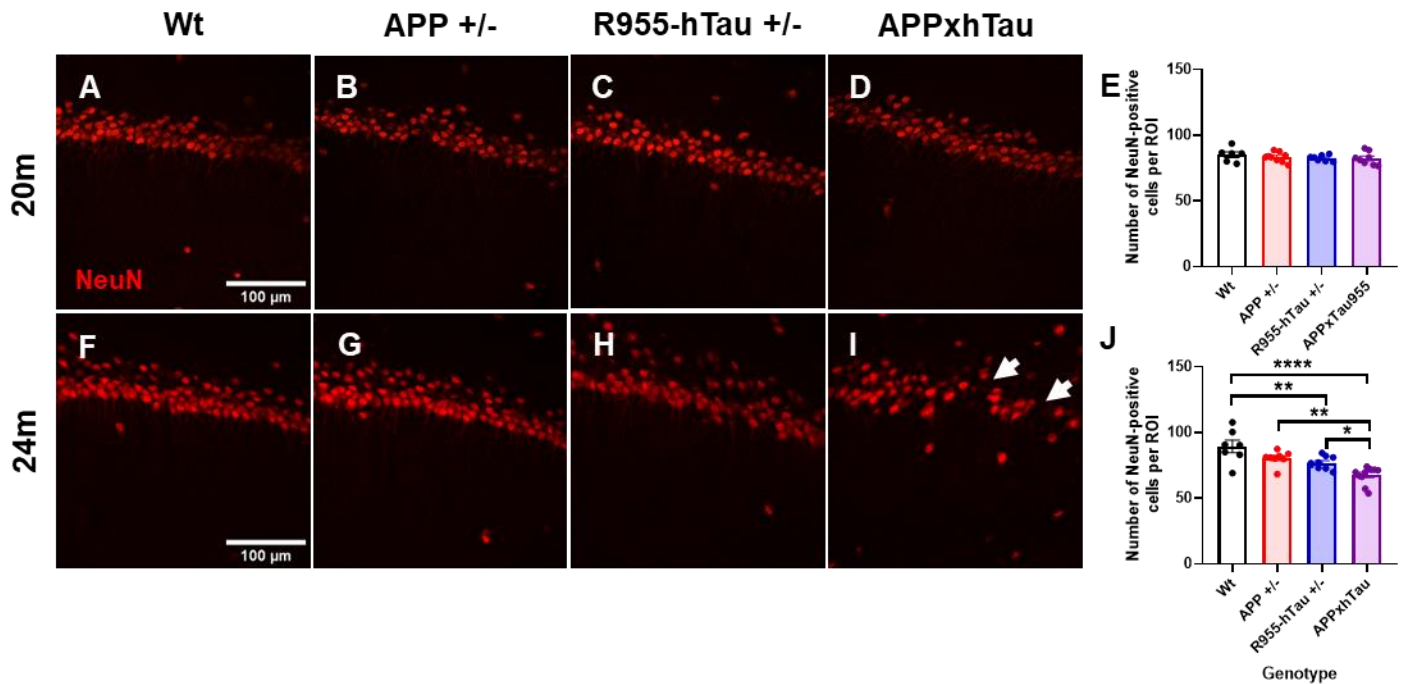
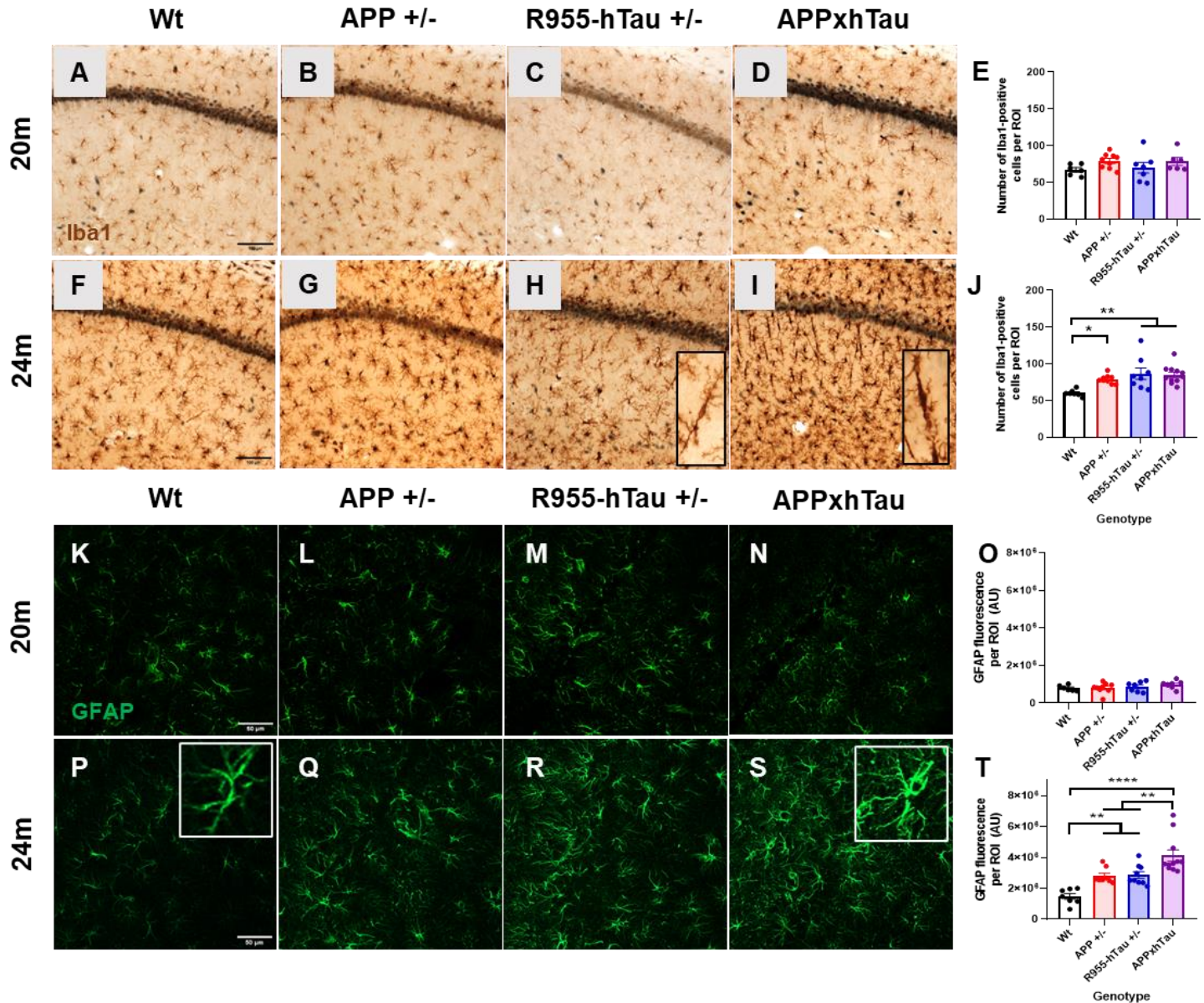


Figure 5. Accelerated neuronal loss in 24-month-old APPxhTau rats. Representative immunofluorescence micrographs of the CA1 region of the hippocampus at 20 (A-D) and 24 months of age (F-I) illustrating the disrupted organization of neurons in R955-hTau^{+/-} (H) and APPxhTau rats at 24 months (I, white arrows). Quantification of the total number of NeuN-immunolabelled neurons per region of interest (ROI) in the CA1 at 20 (E) and 24 (J) months of age demonstrating a significant reduction of neurons in R955-hTau^{+/-} Tg rats, with greater losses in APPxhTau rats (n=6-9). Scale bar: A-D, F-I 100 μm. * p < 0.05, ** p<0.01, **** p<0.0001.

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 4176
 4177 **6J**; APPxhTau vs. Wt t(31)=3.940, p=0.0026; APP^{+/-} vs. Wt t(31)=2.860, p=0.0297; R955-
 4178 hTau^{+/-} vs. Wt t(31)=3.905, p=0.0026). In contrast, APPxhTau rats were no different from single
 4179 transgenics (**Figure 6J**; p>0.5640). Notably, microglia appeared to be recruited to the Aβ-
 4180 burdened neuronal layers of the hippocampus in APP^{+/-} Tg rats whereas, on the other hand, R955-
 4181 hTau^{+/-} rats uniquely developed the appearance of rod-shaped microglia trailing the apical
 4182 dendrites of CA1 pyramidal neurons. Most intriguingly, both these transgene-specific observations
 4183 were detected in combination in aged APPxhTau rats.



Regarding astrocytes, there were no initial differences in astrocytic activities at 20 months as demonstrated by glial fibrillary acidic protein (GFAP) immunofluorescence intensity in the CA1 region of the hippocampus (**Figure 6O**; $p>0.5691$) as well as in other brain regions including the subiculum (**Figure S5I**; $p>0.8050$) and entorhinal cortex (**Figure S5J**; $p>0.7562$). In contrast, at 24 months of age signs of astrogliosis emerged as shown by a two-fold increase in GFAP fluorescence in APP^{+/-} and R955-hTau^{+/-} Tg rats as compared to wild type rats (**Figure 6T**; APP^{+/-} vs. Wt $t(31)=3.082$, $p=0.0091$; R955-hTau^{+/-} vs. Wt $t(31)=3.321$, $p=0.0091$). Interestingly, in APPxhTau rats, GFAP fluorescence intensity in the CA1 region was greater than that of the single transgenics and wild type littermates (**Figure 6T**; APPxhTau vs. APP^{+/-} $t(31)=3.410$, $p=0.0091$; APPxhTau vs. R955-hTau^{+/-} $t(31)=3.351$, $p=0.0091$; APPxhTau vs. Wt $t(31)=6.577$, $p<0.0001$). In addition, increased GFAP fluorescence was detected in other investigated brain regions including the subiculum in 24-month-old R955-hTau^{+/-} and APPxhTau rats as compared to wild type rats (**Figure S5K**; APPxhTau vs Wt $t(31)=3.879$, $p=0.0031$; R955-hTau^{+/-} vs. Wt $t(31)=3.107$, $p=0.0241$; APP^{+/-} vs. Wt $p=0.4360$). Together, these findings demonstrate a robust and synergistic CNS inflammatory response to the combination of amyloid and tau pathology during the advanced plaque pathology.

4. Discussion

The present report compares the combined effects of progressive accumulation of human A β and tau in the McGill-R-APPxR955-hTau transgenic rat model to their single transgenic counterparts and wild type littermates to shed light into the interaction between the two hallmark pathologies of AD at their earliest and latest stages. Given the heterozygous nature of the crossed mutated human APP (K670N/M671L, V717F) and human tau (P301S) transgenes, the APPxR955-hTau bigenic rat model possesses features of the earliest disease stages, including the initial A β accumulation, formation of amyloid plaques and hyperphosphorylation of tau. APPxhTau rats accumulated Sarkosyl-insoluble ptau at Thr231, a ptau site which has been implicated in early stages of AD (Ashton et al., 2022, 2021; Glodzik et al., 2011), in the absence of overt tau fibrillary inclusions. At later stages, we demonstrated the existence of significant converging effects between A β and tau on each others' pathologies, on cognition, inflammation, neuronal loss as well as an elevated expression of GSK3 β and p38MAPK, two major tau-phosphorylating kinases involved in the human AD pathology. Importantly, such additive and synergistic effects between

A β and tau were preceded at the earliest stages by an unexpected compensatory effect of the overexpression of human mutated tau on the amyloid-driven impairments in cognition.

The compensatory effect of tau on A β -induced impairments extended to an improvement on the *ex vivo* LTP in the hippocampus. This improvement of synaptic plasticity provided a mechanistic explanation for the absence of cognitive impairments in hippocampal-dependent spatial learning and memory, as shown by the outcome of the MWM task in 20-month-old APPxhTau rats. The counteractive effect of tau on cognition may explain, in part, the decades-long asymptomatic phase during prodromal stages of AD despite the unrelenting accumulation of A β peptides. Interestingly, a counteractive-like mechanism of tau on A β in synaptic function has been previously described in different forms. For example, tau produced a dominating effect, suppressing Ca²⁺ currents over amyloid-induced hyperactivity in APP/PS1xrTg4510 mice (Busche et al., 2019) and a diminishing of entorhinal circuit excitability in APPxP301L Tg mice (Angulo et al., 2017). In addition, the introduction of oligomeric A β has been found to induce dendritic mislocalization and hyperphosphorylation of tau (Zempel et al., 2013, 2010). Previous findings showed that dendritic tau can mediate A β toxicity (Ittner et al., 2010) and that oligomeric A β can induce tau phosphorylation at Ser396, a site essential for long term depression (LTD) (Kimura et al., 2013; Regan et al., 2015). Taken together with the findings in this report it could be hypothesized that the physiological activities of tau on synaptic plasticity at the postsynaptic density could initially counteract the hyperexcitability induced by CNS A β pathology. While significant interactions between A β - and tau-driven functions at the synaptic compartment are undeniable, it is equally undeniable that a balance of trophic support, clearance of toxic molecular species such as free radicals, and inflammatory processes are also likely to contribute to the overall synaptic status.

In support of our LTP observations in 20-month-old APPxhTau rats, the increased Fyn protein expression in 20-month-old APPxhTau Tg rats is likely a key contributor to the protective effects of early tau overexpression as observed in this study. Other reports, have shown that an overexpression of tau protein can rescue LTP impairments *in vivo* (Briner et al., 2020; Chin et al., 2005; Iannuzzi et al., 2020; Kojima et al., 1997; Nygaard et al., 2014). In addition, Ittner and colleagues demonstrated an essential role of tau through its interaction with Fyn and PSD95 to stabilize the NMDAR receptor to mediate excitotoxicity (Ittner et al., 2010), which would be in support of the present findings. Taken together, a dysregulation of Fyn is likely detrimental to

synaptic plasticity through NMDA receptors. The increased Fyn kinase and PSD95 protein expression in APPxhTau bigenic rats at 20 months of age would be in agreement with our LTP results, further implicating a postsynaptic role of tau in the presence of A β pathology. In addition, this compensatory effect of tau may be phosphorylation dependent, as our investigations illustrating that the rise of ptau at Ser202-Thr205 by the antibody AT8 at 20 months of age would be consistent with reports of protection from A β -mediated toxicity by ptau at Thr205 in a fyn-dependent manner (Ittner et al., 2016).

On the other hand, as tau, fyn, PSD95 and the NMDAR interactions might also enable glutamatergic excitotoxicity at more advance stages of pathology, the continuous abnormal activation of the NMDAR over time may also facilitate neurodegeneration. If a pronounced synaptic disbalance of the interaction of tau/fyn/NMDA receptor results in a chronic overactivation of this pathway, this may explain the exacerbated CA1 neuronal loss in a tau-dependent manner as observed at 24 months of age. Astrocytes are the main cells responsible for removing excess glutamate during toxicity (Mahmoud et al., 2019; Maragakis et al., 2004). As such, the exacerbated intensity of GFAP immunofluorescence in the CA1 in the APPxhTau rats may further indicate excitotoxicity. We also detected an intraneuronal colocalization of A β and tau immunoreactive material in the CA1 in 24-month-old APPxhTau rats exhibiting the most severe of pathologies (**Figure 1J**), suggesting that such neuronal loss may be driven by the combination of amyloid and tau pathologies *in vivo*.

At the latest life stage, APPxhTau rats exhibited multiple indications of a worsened disease condition and cognitive status driven by tau. It has been well established that the combined presence of A β and tau abnormalities is associated with a steeper cognitive decline compared to A β -negative individuals (Desikan et al., 2012, 2011; Hanseeuw et al., 2019; Pascoal et al., 2017a, 2017b; Quiroz et al., 2018) and that tau is a more powerful correlate of cognitive decline and neurodegeneration. Pooler and colleagues reported elevated cortical amyloid deposition in tau-positive individuals (Pooler et al., 2015) and this finding is consistent with the observed accelerated tau and A β pathologies in bigenic APPxhTau rats. In agreement with the above findings in 24 month-old APPxhTau rats, several independent investigations using a variety of mouse models have demonstrated that combining mutated human APP transgenes or APP knock-ins with tau can produce phenotypes leading to an exacerbation of amyloid and/or tau pathologies (Bennett et al., 2017; Bolmont et al., 2007; Gotz et al., 2001; Huang et al., 2022; Lewis et al., 2001;

Ribé et al., 2005; Terwel et al., 2008; Vergara et al., 2019). These observations are in line with the earliest reports demonstrating that the introduction of A β fibrils in P301L mice, or the crossing of APP^{Sw} and tau P301L transgenic mice, enhanced the production of aggregated tau material (Gotz et al., 2001; Lewis et al., 2001).

A limitation to the present study is that not all transgenic APP heterozygous animals developed amyloid plaques at the selected time-points. Another limitation is that while A β and tau showed clear exacerbating effects on the CNS, this did not accelerate the tau pathology severely enough as to provoke the appearance of fibrillary inclusions by 24 months of age. Our observations and those of Huang and collaborators (Huang et al., 2022) indicated that while A β amyloid peptides provide an environment encouraging tau pathology, they do not necessarily cause the *de novo* assembly of neurofibrillary tangles (NFTs) inclusions. Given that NFTs are a requirement for a post-mortem diagnosis of AD, this present APPxhTau heterozygous bigenic model can not be considered as a complete model for AD pathology. Instead, the heterozygous bigenic APPxhTau rat characterized in the present report would be more suitable for investigating the early stages of disease, that is, at early or preclinical time points of convergence between amyloid and tau pathologies. These time points are likely more amenable to therapeutic intervention as they may occur before irreversible and untreatable pathology and, as such, may be of potential translational value.

5. References

- Allen, B., Ingram, E., Takao, M., Smith, M.J., Jakes, R., Virdee, K., Yoshida, H., Holzer, M., Craxton, M., Emson, P.C., Atzori, C., Migheli, A., Crowther, R.A., Ghetti, B., Spillantini, M.G., Goedert, M., 2002. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J. Neurosci.* 22, 9340–9351. <https://doi.org/22/21/9340> [pii]
- Angulo, S.L., Orman, R., Neymotin, S.A., Liu, L., Buitrago, L., Cepeda-Prado, E., Stefanov, D., Lytton, W.W., Stewart, M., Small, S.A., Duff, K.E., Moreno, H., 2017. Tau and amyloid-related pathologies in the entorhinal cortex have divergent effects in the hippocampal circuit. *Neurobiol. Dis.* 108, 261–276. <https://doi.org/10.1016/j.nbd.2017.08.015>
- Arriagada, P. V, Growdon, J.H., Hedleywhyte, E.T., Hyman, B.T., Hedley-Whyte, E.T., Hyman, B.T., 1992. Neurofibrillary Tangles But Not Senile Plaques Parallel Duration and Severity of Alzheimers-Disease. *Neurology* 42, 631–639. <https://doi.org/10.1212/WNL.42.3.631>
- Ashton, N.J., Benedet, A.L., Pascoal, T.A., Karikari, T.K., Lantero-Rodriguez, J., Brum, W.S., Mathotaarachchi, S., Therriault, J., Savard, M., Chamoun, M., Stoops, E., Francois, C., Vanmechelen, E., Gauthier, S., Zimmer, E.R., Zetterberg, H., Blennow, K., Rosa-Neto, P., 2022. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer’s disease. *eBioMedicine* 76, 1–13. <https://doi.org/10.1016/j.ebiom.2022.103836>
- Ashton, N.J., Pascoal, T.A., Karikari, T.K., Benedet, A.L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., Blennow, K., 2021. Plasma p-tau231: a new biomarker for incipient Alzheimer’s disease pathology. *Acta Neuropathol.* 141, 709–724. <https://doi.org/10.1007/s00401-021-02275-6>
- Bejanin, A., Schonhaut, D.R., La Joie, R., Kramer, J.H., Baker, S.L., Sosa, N., Ayakta, N., Cantwell, A., Janabi, M., Lauriola, M., O’Neil, J.P., Gorno-Tempini, M.L., Miller, Z.A., Rosen, H.J., Miller, B.L., Jagust, W.J., Rabinovici, G.D., 2017. Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer’s disease. *Brain* 140, 3286–3300. <https://doi.org/10.1093/brain/awx243>
- Bennett, D.A., Schneider, J.A., Wilson, R.S., Bienias, J.L., Arnold, S.E., 2004. Neurofibrillary Tangles Mediate the Association of Amyloid Load with Clinical Alzheimer Disease and Level of Cognitive Function. *Arch. Neurol.* 61, 378–384. <https://doi.org/10.1001/archneur.61.3.378>
- Bennett, R.E., DeVos, S.L., Dujardin, S., Corjuc, B., Gor, R., Gonzalez, J., Roe, A.D., Frosch, M.P., Pitstick, R., Carlson, G.A., Hyman, B.T., 2017. Enhanced Tau Aggregation in the Presence of Amyloid β . *Am. J. Pathol.* 187, 1601–1612. <https://doi.org/10.1016/j.ajpath.2017.03.011>
- Biel, D., Brendel, M., Rubinski, A., Buerger, K., Janowitz, D., Dichgans, M., Franzmeier, N., 2021. Tau-PET and in vivo Braak-staging as prognostic markers of future cognitive decline in cognitively normal to demented individuals. *Alzheimer’s Res. Ther.* 13, 1–13. <https://doi.org/10.1186/s13195-021-00880-x>
- Bloom, G.S., 2014. Amyloid-B and tau: The trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurol.* 71, 505–508. <https://doi.org/10.1001/jamaneurol.2013.5847>

- 4340 Bolmont, T., Clavaguera, F., Meyer-Luehmann, M., Herzig, M.C., Radde, R., Staufenbiel, M.,
 4341 Lewis, J., Hutton, M., Tolnay, M., Jucker, M., 2007. Induction of tau pathology by
 4342 intracerebral infusion of amyloid- β -containing brain extract and by amyloid- β deposition in
 4343 APP x tau transgenic mice. *Am. J. Pathol.* 171, 2012–2020.
 4344 <https://doi.org/10.2353/ajpath.2007.070403>
- 4345 Briner, A., Götz, J., Polanco, J.C., 2020. Fyn Kinase Controls Tau Aggregation In Vivo. *Cell Rep.*
 4346 32. <https://doi.org/10.1016/j.celrep.2020.108045>
- 4347 Buchhave, P., Minthon, L., Zetterberg, H., Wallin, A.K., Blennow, K., Hansson, O., 2012.
 4348 Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to
 4349 10 years before the onset of Alzheimer dementia. *Arch. Gen. Psychiatry* 69, 98–106.
 4350 <https://doi.org/10.1001/archgenpsychiatry.2011.155>
- 4351 Burke, S.N., Barnes, C.A., 2006. Neural plasticity in the ageing brain. *Nat. Rev. Neurosci.* 7, 30–
 4352 40. <https://doi.org/10.1038/nrn1809>
- 4353 Busche, M.A., Wegmann, S., Dujardin, S., Commings, C., Schiantarelli, J., Klickstein, N., Kamath,
 4354 T. V., Carlson, G.A., Nelken, I., Hyman, B.T., 2019. Tau impairs neural circuits, dominating
 4355 amyloid- β effects, in Alzheimer models in vivo. *Nat. Neurosci.* 22, 57–64.
 4356 <https://doi.org/10.1038/s41593-018-0289-8>
- 4357 Cao, W., Zheng, H., 2018. Correction to: Peripheral immune system in aging and Alzheimer's
 4358 disease. *Mol. Neurodegener.* 13, 1–17. <https://doi.org/10.1186/s13024-018-0290-4>
- 4359 Chabrier, M.A., Cheng, D., Castello, N.A., Green, K.N., LaFerla, F.M., 2014. Synergistic effects
 4360 of amyloid-beta and wild-type human tau on dendritic spine loss in a floxed double transgenic
 4361 model of Alzheimer's disease. *Neurobiol. Dis.* 64, 107–117.
 4362 <https://doi.org/10.1016/j.nbd.2014.01.007>
- 4363 Chin, J., Palop, J.J., Puoliväli, J., Massaro, C., Bien-Ly, N., Gerstein, H., Scarce-Levie, K.,
 4364 Masliah, E., Mucke, L., 2005. Fyn kinase induces synaptic and cognitive impairments in a
 4365 transgenic mouse model of Alzheimer's disease. *J. Neurosci.* 25, 9694–9703.
 4366 <https://doi.org/10.1523/JNEUROSCI.2980-05.2005>
- 4367 Cohen, R.M., Rezai-zadeh, K., Weitz, T.M., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y.,
 4368 Vasilevko, V., Glabe, C.G., Breunig, J.J., Rakic, P., Davtyan, H., Agadjanyan, M.G., 2013.
 4369 A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric
 4370 A β and frank neuronal loss. *J. Neurosci.* 33, 6245–6256.
 4371 <https://doi.org/10.1523/JNEUROSCI.3672-12.2013.A>
- 4372 Cuervo, A.M., Dice, J.F., 2000. Age-related decline in chaperone-mediated autophagy. *J. Biol.*
 4373 *Chem.* 275, 31505–31513. <https://doi.org/10.1074/jbc.M002102200>
- 4374 Cummings, J., Lee, G., Nahed, P., Kambar, M.E.Z.N., Zhong, K., Fonseca, J., Taghva, K., 2022.
 4375 Alzheimer's disease drug development pipeline: 2022. *Alzheimer's Dement. Transl. Res.*
 4376 *Clin. Interv.* 8. <https://doi.org/10.1002/trc2.12295>
- 4377 Desikan, R.S., Mcevoy, L.K., Thompson, W., Holland, D., Roddey, J.C., Blennow, K., Aisen, P.S.,
 4378 Brewer, J.B., Hyman, B.T., Dale, A.M., Azleheimers Disease Neuroimaging Initiative, 2011.
 4379 Amyloid-B associated volume loss occurs only in the presence of phospho-tau. *Ann Neurol*
 4380 70, 657–661. <https://doi.org/10.1097/MCA.000000000000178>.Endothelial

- 4381 Desikan, R.S., McEvoy, L.K., Thompson, W.K., Holland, D., Brewer, J.B., Aisen, P.S., Sperling,
4382 R.A., Dale, A.M., Alzheimers Disease Neuroimaging Initiative, 2012. Amyloid- β associated
4383 clinical decline occurs only in the presence of elevated p-tau. *Arch Neurol* 69, 709–713.
4384 <https://doi.org/10.1001/archneurol.2011.3354>. Amyloid-
- 4385 Do Carmo, S., Cuellar, A.C., 2013. Modeling Alzheimer's disease in transgenic rats. *Mol.*
4386 *Neurodegener.* 8, 37. <https://doi.org/10.1186/1750-1326-8-37>
- 4387 Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., Dekosky, S.T.,
4388 Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B.,
4389 Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici,
4390 G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B.,
4391 Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014. Advancing
4392 research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *Lancet Neurol.* 13,
4393 614–629. [https://doi.org/10.1016/S1474-4422\(14\)70090-0](https://doi.org/10.1016/S1474-4422(14)70090-0)
- 4394 Dujardin, S., Colin, M., Buée, L., 2015. Invited review: Animal models of tauopathies and their
4395 implications for research/translation into the clinic. *Neuropathol. Appl. Neurobiol.* 41, 59–
4396 80. <https://doi.org/10.1111/nan.12200>
- 4397 Fagan, A.M., Xiong, C., Jasielec, M.S., Bateman, R.J., Goate, A.M., Benzinger, T.L.S., Ghetti, B.,
4398 Martins, R.N., Masters, C.L., Mayeux, R., Ringman, J.M., Rossor, M.N., Salloway, S.,
4399 Schofield, P.R., Sperling, R.A., Marcus, D., Cairns, N.J., Buckles, V.D., Ladenson, J.H.,
4400 Morris, J.C., Holtzman, D.M., 2014. Longitudinal Change in CSF Biomarkers in Autosomal-
4401 Dominant Alzheimer's Disease. *Sci. Transl. Med.* 6, 226ra30-226ra30.
4402 <https://doi.org/10.1126/scitranslmed.3007901>
- 4403 Ferrucci, L., Fabbri, E., 2018. Inflammageing: chronic inflammation in ageing, cardiovascular
4404 disease, and frailty. *Nat. Rev. Cardiol.* 15, 505–522. [https://doi.org/10.1038/s41569-018-](https://doi.org/10.1038/s41569-018-0064-2)
4405 0064-2
- 4406 Finger, C.E., Moreno-Gonzalez, I., Gutierrez, A., Moruno-Manchon, J.F., McCullough, L.D.,
4407 2022. Age-related immune alterations and cerebrovascular inflammation. *Mol. Psychiatry* 27,
4408 803–818. <https://doi.org/10.1038/s41380-021-01361-1>
- 4409 Florian, H., Wang, D., Arnold, S.E., Boada, M., Guo, Q., Jin, Z., Zheng, H., Fisseha, N., Kalluri,
4410 H.V., Rendenbach-Mueller, B., Budur, K., Gold, M., 2023. Tilavonemab in early Alzheimer's
4411 disease: results from a phase 2, randomized, double-blind study. *Brain.*
4412 <https://doi.org/10.1093/brain/awad024>
- 4413 Foster, T.C., Norris, C.M., 1997. Age-associated changes in Ca²⁺-dependent processes: Relation
4414 to hippocampal synaptic plasticity. *Hippocampus* 7, 602–612.
4415 [https://doi.org/10.1002/\(SICI\)1098-1063\(1997\)7:6<602::AID-HIPO3>3.0.CO;2-G](https://doi.org/10.1002/(SICI)1098-1063(1997)7:6<602::AID-HIPO3>3.0.CO;2-G)
- 4416 Galeano, P., Martino Adami, P. V., Do Carmo, S., Blanco, E., Rotondaro, C., Capani, F., Castaño,
4417 E.M., Cuellar, A.C., Morelli, L., Castano, E.M., Cuellar, A.C., Morelli, L., Castaño, E.M.,
4418 Cuellar, A.C., Morelli, L., 2014. Longitudinal analysis of the behavioral phenotype in a novel
4419 transgenic rat model of early stages of Alzheimer's disease. *Front. Behav. Neurosci.* 8, 1–15.
4420 <https://doi.org/10.3389/fnbeh.2014.00321>
- 4421 Giannakopoulos, P., Herrmann, F.R., Bussi re, T., Bouras, C., Kovari, E., Perl, D.P., Morrison,
4422 J.H., Gold, G., Hof, P.R., 2003. Tangle and neuron numbers, but not amyloid load, predict

- cognitive status in Alzheimer's disease. *Neurology* 60, 1495–1500.
<https://doi.org/10.1212/01.WNL.0000063311.58879.01>
- Glodzik, L., de Santi, S., Tsui, W.H., Mosconi, L., Zinkowski, R., Pirraglia, E., Wang, H.Y., Li, Y., Rich, K.E., Zetterberg, H., Blennow, K., Mehta, P., de Leon, M.J., 2011. Phosphorylated tau 231, memory decline and medial temporal atrophy in normal elders. *Neurobiol. Aging* 32, 2131–2141. <https://doi.org/10.1016/j.neurobiolaging.2009.12.026>
- Gotz, J., Chen, F., van Dorpe, J., Nitsch, R.M., 2001. Formation of Neurofibrillary Tangles in P301L Tau Transgenic Mice Induced by Abeta 42 Fibrils. *Science* (80-.). 293, 1491–1495.
<https://doi.org/10.1126/science.1062097>
- Grant, S.M., Ducatenzeiler, A., Szyf, M., Cuellar, A.C., 2000. Abeta immunoreactive material is present in several intracellular compartments in transfected, neuronally differentiated, P19 cells expressing the human amyloid beta-protein precursor. *J Alzheimers Dis* 2, 207–222.
- Guillozet, A.L., Weintraub, S., Mash, D.C., Marsel Mesulam, M., 2003. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch. Neurol.* 60, 729–736.
<https://doi.org/10.1001/archneur.60.5.729>
- Hall, H., Iulita, M.F., Gubert, P., Flores Aguilar, L., Ducatenzeiler, A., Fisher, A., Cuellar, A.C., 2018. AF710B, an M1/sigma-1 receptor agonist with long-lasting disease-modifying properties in a transgenic rat model of Alzheimer's disease. *Alzheimer's Dement.* 14, 811–823. <https://doi.org/10.1016/j.jalz.2017.11.009>
- Hanes, J., Zilka, N., Bartkova, M., Caletkova, M., Dobrota, D., Novak, M., 2009. Rat tau proteome consists of six tau isoforms: Implication for animal models of human tauopathies. *J. Neurochem.* 108, 1167–1176. <https://doi.org/10.1111/j.1471-4159.2009.05869.x>
- Hanseeuw, B.J., Betensky, R.A., Jacobs, H.I.L., Schultz, A.P., Sepulcre, J., Becker, J.A., Cosio, D.M.O., Farrell, M., Quiroz, Y.T., Mormino, E.C., Buckley, R.F., Papp, K. V., Amariglio, R.A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G.A., Chhatwal, J.P., Rentz, D.M., Sperling, R.A., Johnson, K., 2019. Association of Amyloid and Tau with Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. *JAMA Neurol.* 76, 915–924. <https://doi.org/10.1001/jamaneurol.2019.1424>
- Hanzel, C.E., Pichet-Binette, A., Pimentel, L.S.B., Iulita, M.F., Allard, S., Ducatenzeiler, A., Do Carmo, S., Cuellar, A.C., 2014. Neuronal driven pre-plaque inflammation in a transgenic rat model of Alzheimer's disease. *Neurobiol. Aging* 35, 2249–2262.
<https://doi.org/10.1016/j.neurobiolaging.2014.03.026>
- Hu, N.W., Smith, I.M., Walsh, D.M., Rowan, M.J., 2008. Soluble amyloid- β peptides potently disrupt hippocampal synaptic plasticity in the absence of cerebrovascular dysfunction in vivo. *Brain* 131, 2414–2424. <https://doi.org/10.1093/brain/awn174>
- Huang, M., Macdonald, J., Lavenir, I., Chen, R., Craxton, M., Slavik-Smith, E., Davies, S.W., Goedert, M., 2022. Increase in Tau Pathology in P290S Mapt Knock-In Mice Crossed with App NL-G-F Mice. *eneuro* 9, ENEURO.0247-22.2022.
<https://doi.org/10.1523/ENEURO.0247-22.2022>
- Iannuzzi, F., Sirabella, R., Canu, N., Maier, T.J., Annunziato, L., Matrone, C., 2020. Fyn Tyrosine Kinase Elicits Amyloid Precursor Protein Tyr682 Phosphorylation in Neurons from Alzheimer's Disease Patients. *Cells* 9, 1–21. <https://doi.org/10.3390/cells9081807>

- 4465 Ittner, A., Chua, S.W., Bertz, J., Volkerling, A., van der Hoven, J., Gladbach, A., Przybyla, M.,
 4466 Bi, M., van Hummel, A., Stevens, C.H., Ippati, S., Suh, L.S., Macmillan, A., Sutherland, G.,
 4467 Kril, J.J., Silva, A.P.G., Mackay, J.P., Poljak, A., Delerue, F., Ke, Y.D., Ittner, L.M., 2016.
 4468 Site-specific phosphorylation of tau inhibits amyloid- β toxicity in Alzheimer's mice. *Science*
 4469 (80-). 354, 904–908. <https://doi.org/10.1126/science.aah6205>
- 4470 Ittner, L.M., Götz, J., 2011. Amyloid- β and tau - A toxic pas de deux in Alzheimer's disease. *Nat.*
 4471 *Rev. Neurosci.* 12, 67–72. <https://doi.org/10.1038/nrn2967>
- 4472 Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wölfling, H., Chieng, B.C.,
 4473 Christie, M.J., Napier, I.A., Eckert, A., Staufenbiel, M., Hardeman, E., Götz, J., 2010.
 4474 Dendritic function of tau mediates amyloid- β toxicity in alzheimer's disease mouse models.
 4475 *Cell* 142, 387–397. <https://doi.org/10.1016/j.cell.2010.06.036>
- 4476 Iulita, M.F., Allard, S., Richter, L., Munter, L.-M., Ducatenzeiler, A., Weise, C., Do Carmo, S.,
 4477 Klein, W.L., Multhaup, G., Cuellar, A.C., 2014. Intracellular A β pathology and early cognitive
 4478 impairments in a transgenic rat overexpressing human amyloid precursor protein: a
 4479 multidimensional study. *Acta Neuropathol. Commun.* 2, 61. [https://doi.org/10.1186/2051-](https://doi.org/10.1186/2051-5960-2-61)
 4480 5960-2-61
- 4481 Jack, C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Feldman, H.H., Frisoni, G.B., Hampel, H.,
 4482 Jagust, W.J., Johnson, K.A., Knopman, D.S., Petersen, R.C., Scheltens, P., Sperling, R.A.,
 4483 Dubois, B., 2016. A/T/N: An unbiased descriptive classification scheme for Alzheimer
 4484 disease biomarkers. *Neurology* 87, 539–547.
 4485 <https://doi.org/10.1212/WNL.0000000000002923>
- 4486 Javonillo, D.I., Tran, K.M., Phan, J., Hingco, E., Kramár, E.A., da Cunha, C., Forner, S.,
 4487 Kawauchi, S., Milinkeviciute, G., Gomez-Arboledas, A., Neumann, J., Banh, C.E., Huynh,
 4488 M., Matheos, D.P., Rezaie, N., Alcantara, J.A., Mortazavi, A., Wood, M.A., Tenner, A.J.,
 4489 MacGregor, G.R., Green, K.N., LaFerla, F.M., 2022. Systematic Phenotyping and
 4490 Characterization of the 3xTg-AD Mouse Model of Alzheimer's Disease. *Front. Neurosci.* 15.
 4491 <https://doi.org/10.3389/fnins.2021.785276>
- 4492 Jin, R., Chan, A.K.Y., Wu, J., Lee, T.M.C., 2022. Relationships between Inflammation and Age-
 4493 Related Neurocognitive Changes. *Int. J. Mol. Sci.* 23. <https://doi.org/10.3390/ijms232012573>
- 4494 Kelmer Sacramento, E., Kirkpatrick, J.M., Mazzetto, M., Baumgart, M., Bartolome, A., Di Sanzo,
 4495 S., Caterino, C., Sanguanini, M., Papaevgeniou, N., Lefaki, M., Childs, D., Bagnoli, S.,
 4496 Terzibasi Tozzini, E., Di Fraia, D., Romanov, N., Sudmant, P.H., Huber, W., Chondrogianni,
 4497 N., Vendruscolo, M., Cellerino, A., Ori, A., 2020. Reduced proteasome activity in the aging
 4498 brain results in ribosome stoichiometry loss and aggregation. *Mol. Syst. Biol.* 16, 1–22.
 4499 <https://doi.org/10.15252/msb.20209596>
- 4500 Kimura, T., Whitcomb, D.J., Jo, J., Regan, P., Piers, T., Heo, S., Brown, C., Hashikawa, T.,
 4501 Murayama, M., Seok, H., Sotiropoulos, I., Kim, E., Collingridge, G.L., Takashima, A., Cho,
 4502 K., 2013. Microtubule-associated protein tau is essential for long-term depression in the
 4503 hippocampus. *Philos. Trans. R. Soc. B Biol. Sci.* 369, 20130144–20130144.
 4504 <https://doi.org/10.1098/rstb.2013.0144>
- 4505 Kinirons, M.T., O'Mahony, M.S., 2004. Drug metabolism and ageing. *Br. J. Clin. Pharmacol.* 57,
 4506 540–544. <https://doi.org/10.1111/j.1365-2125.2004.02096.x>

- 4507 Klyubin, I., Walsh, D.M., Cullen, W.K., Fadeeva, J. V., Anwyl, R., Selkoe, D.J., Rowan, M.J.,
 4508 2004. Soluble Arctic amyloid β protein inhibits hippocampal long-term potentiation in vivo.
 4509 Eur. J. Neurosci. 19, 2839–2846. <https://doi.org/10.1111/j.1460-9568.2004.03389.x>
- 4510 Koffie, R.M., Meyer-Luehmann, M., Hashimoto, T., Adams, K.W., Mielke, M.L., Garcia-Alloza,
 4511 M., Micheva, K.D., Smith, S.J., Kim, M.L., Lee, V.M., Hyman, B.T., Spires-Jones, T.L.,
 4512 2009. Oligomeric amyloid beta associates with postsynaptic densities and correlates with
 4513 excitatory synapse loss near senile plaques. Proc. Natl. Acad. Sci. U. S. A. 106, 4012–7.
 4514 <https://doi.org/10.1073/pnas.0811698106>
- 4515 Kojima, N., Wang, J., Mansu, I.M., Grant, S.G.N., Mayford, M., Kandel, E.R., 1997. Rescuing
 4516 impairment of long-term potentiation in fyn-deficient mice by introducing Fyn transgene.
 4517 Proc. Natl. Acad. Sci. U. S. A. 94, 4761–4765. <https://doi.org/10.1073/pnas.94.9.4761>
- 4518 Kopeikina, K.J., Polydoro, M., Tai, H.C., Yaeger, E., Carlson, G.A., Pitstick, R., Hyman, B.T.,
 4519 Spires-Jones, T.L., 2013. Synaptic alterations in the rTg4510 mouse model of tauopathy. J.
 4520 Comp. Neurol. 521, 1334–1353. <https://doi.org/10.1002/cne.23234>
- 4521 Krüger, L., Mandelkow, E.M., 2016. Tau neurotoxicity and rescue in animal models of human
 4522 Tauopathies. Curr. Opin. Neurobiol. 36, 52–58. <https://doi.org/10.1016/j.conb.2015.09.004>
- 4523 Kummer, K.K., Hofhansel, L., Barwitz, C.M., Schardl, A., Prast, J.M., Salti, A., El Rawas, R.,
 4524 Zernig, G., 2014. Differences in social interaction- vs. cocaine reward in mouse vs. rat. Front.
 4525 Behav. Neurosci. 8, 1–7. <https://doi.org/10.3389/fnbeh.2014.00363>
- 4526 Leon, W.C., Canneva, F., Partridge, V., Allard, S., Ferretti, M.T., Dewilde, A., Vercauteren, F.,
 4527 Atifeh, R., Ducatenzeiler, A., Klein, W., Szyf, M., Alhonen, L., Cuellar, A.C., 2010. A novel
 4528 transgenic rat model with a full Alzheimer's-Like amyloid pathology displays pre-plaque
 4529 intracellular amyloid-B-Associated cognitive impairment. J. Alzheimer's Dis. 20, 113–126.
 4530 <https://doi.org/10.3233/JAD-2010-1349>
- 4531 Lewis, J., Dickson, D.W., Lin, W., Chisholm, L., Corral, A., Jones, G., Yen, S., Sahara, N.,
 4532 Skipper, L., Yager, D., Eckman, C., Hutton, M., McGowan, E., Hardy, J., 2001.
 4533 Neurofibrillary Degeneration in Transgenic Mice Expressing Mutant tau and APP. Science
 4534 (80-.). 293, 1487–1491.
- 4535 Liu, R., 2022. Aging, Cellular Senescence, and Alzheimer's Disease. Int. J. Mol. Sci. 23, 1989.
 4536 <https://doi.org/10.3390/ijms23041989>
- 4537 Mahmoud, S., Gharagozloo, M., Simard, C., Gris, D., 2019. Astrocytes maintain glutamate
 4538 homeostasis in the CNS by controlling the balance between glutamate uptake and release. Cells
 4539 8, 1–27. <https://doi.org/10.3390/cells8020184>
- 4540 Malcolm, J.C., Breuillaud, L., Do Carmo, S., Hall, H., Welikovich, L.A., Macdonald, J.A.,
 4541 Goedert, M., Cuellar, A.C., 2019. Neuropathological changes and cognitive deficits in rats
 4542 transgenic for human mutant tau recapitulate human tauopathy. Neurobiol. Dis. 127, 323–
 4543 338. <https://doi.org/10.1016/j.nbd.2019.03.018>
- 4544 Mangoni, A.A., Jackson, S.H.D., 2004. Age-related changes in pharmacokinetics and
 4545 pharmacodynamics: Basic principles and practical applications. Br. J. Clin. Pharmacol. 57,
 4546 6–14. <https://doi.org/10.1046/j.1365-2125.2003.02007.x>
- 4547 Maragakis, N.J., Dykes-Hoberg, M., Rothstein, J.D., 2004. Altered Expression of the Glutamate

- 4548 Transporter EAAT2b in Neurological Disease. *Ann. Neurol.* 55, 469–477.
4549 <https://doi.org/10.1002/ana.20003>
- 4550 Martínez-Cué, C., Rueda, N., 2020. Cellular Senescence in Neurodegenerative Diseases. *Front.*
4551 *Cell. Neurosci.* 14. <https://doi.org/10.3389/fncel.2020.00016>
- 4552 Mintun, M.A., Lo, A.C., Duggan Evans, C., Wessels, A.M., Ardayfio, P.A., Andersen, S.W.,
4553 Shcherbinin, S., Sparks, J., Sims, J.R., Brys, M., Apostolova, L.G., Salloway, S.P.,
4554 Skovronsky, D.M., 2021. Donanemab in Early Alzheimer’s Disease. *N. Engl. J. Med.* 384,
4555 1691–1704. <https://doi.org/10.1056/NEJMoa2100708>
- 4556 Mucke, L., Selkoe, D.J., 2012. Neurotoxicity of Amyloid β -Protein: Synaptic and Network
4557 Dysfunction. *Cold Spring Harb. Perspect. Med.* 2, a006338–a006338.
4558 <https://doi.org/10.1101/cshperspect.a006338>
- 4559 Novak, P., Kovacech, B., Katina, S., Schmidt, R., Scheltens, P., Kontsekova, E., Ropele, S.,
4560 Fialova, L., Kramberger, M., Paulenka-Ivanovova, N., Smisek, M., Hanes, J., Stevens, E.,
4561 Kovac, A., Sutovsky, S., Parrak, V., Koson, P., Prcina, M., Galba, J., Cente, M., Hromadka,
4562 T., Filipcik, P., Piestansky, J., Samcova, M., Prenn-Gologranc, C., Sivak, R., Froelich, L.,
4563 Fresser, M., Rakusa, M., Harrison, J., Hort, J., Otto, M., Tosun, D., Ondrus, M., Winblad, B.,
4564 Novak, M., Zilka, N., 2021. ADAMANT: a placebo-controlled randomized phase 2 study of
4565 AADvac1, an active immunotherapy against pathological tau in Alzheimer’s disease. *Nat.*
4566 *Aging* 1, 521–534. <https://doi.org/10.1038/s43587-021-00070-2>
- 4567 Nygaard, H.B., Dyck, C.H. Van, Strittmatter, S.M., 2014. Fyn Kinase Inhibition as a novel therapy
4568 for AD. *Alzheimers. Res. Ther.* 6, 1–8.
- 4569 Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M.,
4570 Disterhoft, J., Van Eldik, L., Berry, R., Vassar, R., 2006. Intraneuronal β -amyloid aggregates,
4571 neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer’s disease
4572 mutations: Potential factors in amyloid plaque formation. *J. Neurosci.* 26, 10129–10140.
4573 <https://doi.org/10.1523/JNEUROSCI.1202-06.2006>
- 4574 Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kaye, R., Metherate, R.,
4575 Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003. Triple-transgenic model of Alzheimer’s
4576 Disease with plaques and tangles: Intracellular A β and synaptic dysfunction. *Neuron* 39, 409–
4577 421. [https://doi.org/10.1016/S0896-6273\(03\)00434-3](https://doi.org/10.1016/S0896-6273(03)00434-3)
- 4578 Pampuscenko, K., Morkuniene, R., Krasauskas, L., Smirnovas, V., Tomita, T., Borutaite, V., 2021.
4579 Distinct Neurotoxic Effects of Extracellular Tau Species in Primary Neuronal-Glial Cultures.
4580 *Mol. Neurobiol.* 58, 658–667. <https://doi.org/10.1007/s12035-020-02150-7>
- 4581 Pang, K., Jiang, R., Zhang, W., Yang, Z., Li, L.L., Shimozaawa, M., Tambaro, S., Mayer, J., Zhang,
4582 B., Li, M., Wang, J., Liu, H., Yang, A., Chen, X., Liu, J., Winblad, B., Han, H., Jiang, T.,
4583 Wang, W., Nilsson, P., Guo, W., Lu, B., 2022. An App knock-in rat model for Alzheimer’s
4584 disease exhibiting A β and tau pathologies, neuronal death and cognitive impairments. *Cell*
4585 *Res.* 32, 157–175. <https://doi.org/10.1038/s41422-021-00582-x>
- 4586 Pannérec, A., Migliavacca, E., De Castro, A., Michaud, J., Karaz, S., Goulet, L., Rezzi, S., Ng,
4587 T.P., Bosco, N., Larbi, A., Feige, J.N., 2018. Vitamin B12 deficiency and impaired expression
4588 of amnionless during aging. *J. Cachexia. Sarcopenia Muscle* 9, 41–52.
4589 <https://doi.org/10.1002/jcsm.12260>

- 4590 Pascoal, T.A., Leuzy, A., Therriault, J., Chamoun, M., Lussier, F., Tissot, C., Strandberg, O.,
 4591 Palmqvist, S., Stomrud, E., Ferreira, P.C.L., Ferrari-Souza, J.P., Smith, R., Benedet, A.L.,
 4592 Gauthier, S., Hansson, O., Rosa-Neto, P., 2023. Discriminative accuracy of the A/T/N scheme
 4593 to identify cognitive impairment due to Alzheimer's disease. *Alzheimer's Dement.*
 4594 *Diagnosis, Assess. Dis. Monit.* 15, 1–9. <https://doi.org/10.1002/dad2.12390>
- 4595 Pascoal, T.A., Mathotaarachchi, S., Mohades, S., Benedet, A.L., Chung, C.-O., Shin, M., Wang,
 4596 S., Beaudry, T., Kang, M.S., Soucy, J.-P., Labbe, A., Gauthier, S., Rosa-Neto, P., 2017a.
 4597 Amyloid- β and hyperphosphorylated tau synergy drives metabolic decline in preclinical
 4598 Alzheimer's disease. *Mol. Psychiatry* 22, 306–311. <https://doi.org/10.1038/mp.2016.37>
- 4599 Pascoal, T.A., Mathotaarachchi, S., Shin, M., Benedet, A.L., Mohades, S., Wang, S., Beaudry, T.,
 4600 Kang, M.S., Soucy, J., Labbe, A., Gauthier, S., Rosa-Neto, P., 2017b. Synergistic interaction
 4601 between amyloid and tau predicts the progression to dementia. *Alzheimer's Dement.* 13, 644–
 4602 653. <https://doi.org/10.1016/j.jalz.2016.11.005>
- 4603 Pooler, A.M., Polydoro, M., Maury, E.A., Nicholls, S.B., Reddy, S.M., Wegmann, S., William,
 4604 C., Saqran, L., Cagsal-Getkin, O., Pitstick, R., Beier, D.R., Carlson, G.A., Spire-Jones, T.L.,
 4605 Hyman, B.T., 2015. Amyloid accelerates tau propagation and toxicity in a model of early
 4606 Alzheimer's disease. *Acta Neuropathol. Commun.* 3, 14. <https://doi.org/10.1186/s40478-015-0199-x>
- 4608 Qi, Y., Klyubin, I., Harney, S.C., Hu, N., Cullen, W.K., Grant, M.K., Steffen, J., Wilson, E.N., Do
 4609 Carmo, S., Remy, S., Fuhrmann, M., Ashe, K.H., Cuellar, A.C., Rowan, M.J., 2014.
 4610 Longitudinal testing of hippocampal plasticity reveals the onset and maintenance of
 4611 endogenous human A β -induced synaptic dysfunction in individual freely behaving pre-
 4612 plaque transgenic rats: rapid reversal by anti-A β agents. *Acta Neuropathol. Commun.* 2, 175.
 4613 <https://doi.org/10.1186/s40478-014-0175-x>
- 4614 Quiroz, Y.T., Sperling, R.A., Norton, D.J., Baena, A., Arboleda-Velasquez, J.F., Cosio, D.,
 4615 Schultz, A., Lapoint, M., Guzman-Velez, E., Miller, J.B., Kim, L.A., Chen, K., Tariot, P.N.,
 4616 Lopera, F., Reiman, E.M., Johnson, K.A., 2018. Association Between Amyloid and Tau
 4617 Accumulation in Young Adults With Autosomal Dominant Alzheimer Disease. *JAMA*
 4618 *Neurol.* 02129, 1–9. <https://doi.org/10.1001/jamaneurol.2017.4907>
- 4619 Regan, P., Piers, T., Yi, J.-H., Kim, D.-H., Huh, S., Park, S.J., Ryu, J.H., Whitcomb, D.J., Cho,
 4620 K., 2015. Tau Phosphorylation at Serine 396 Residue Is Required for Hippocampal LTD. *J.*
 4621 *Neurosci.* 35, 4804–4812. <https://doi.org/10.1523/JNEUROSCI.2842-14.2015>
- 4622 Ribé, E.M., Pérez, M., Puig, B., Gich, I., Lim, F., Cuadrado, M., Sesma, T., Catena, S., Sánchez,
 4623 B., Nieto, M., Gómez-Ramos, P., Morán, M.A., Cabodevilla, F., Samaranch, L., Ortiz, L.,
 4624 Pérez, A., Ferrer, I., Avila, J., Gómez-Isla, T., 2005. Accelerated amyloid deposition,
 4625 neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice.
 4626 *Neurobiol. Dis.* 20, 814–822. <https://doi.org/10.1016/j.nbd.2005.05.027>
- 4627 Schöll, M., Lockhart, S.N., Schonhaut, D.R., O'Neil, J.P., Janabi, M., Ossenkoppele, R., Baker,
 4628 S.L., Vogel, J.W., Faria, J., Schwimmer, H.D., Rabinovici, G.D., Jagust, W.J., 2016. PET
 4629 Imaging of Tau Deposition in the Aging Human Brain. *Neuron* 89, 971–982.
 4630 <https://doi.org/10.1016/j.neuron.2016.01.028>
- 4631 Sebastián-Serrano, Á., de Diego-García, L., Díaz-Hernández, M., 2018. The Neurotoxic Role of
 4632 Extracellular Tau Protein. *Int. J. Mol. Sci.* 19, 998. <https://doi.org/10.3390/ijms19040998>

- Sevigny, J., Chiao, P., Bussière, T., Weinreb, P.H., Williams, L., Maier, M., Dunstan, R., Salloway, S., Chen, T., Ling, Y., O’Gorman, J., Qian, F., Arastu, M., Li, M., Chollate, S., Brennan, M.S., Quintero-Monzon, O., Scannevin, R.H., Arnold, H.M., Engber, T., Rhodes, K., Ferrero, J., Hang, Y., Mikulskis, A., Grimm, J., Hock, C., Nitsch, R.M., Sandrock, A., 2016. The antibody aducanumab reduces A β plaques in Alzheimer’s disease. *Nature* 537, 50–6. <https://doi.org/10.1038/nature19323>
- Shi, Y., Yamada, K., Liddelow, S.A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S., Grinberg, L.T., Rojas, J.C., Gallardo, G., Wang, K., Roh, J., Robinson, G., Finn, M.B., Jiang, H., Sullivan, P.M., Baufeld, C., Wood, M.W., Sutphen, C., McCue, L., Xiong, C., Del-Aguila, J.L., Morris, J.C., Cruchaga, C., Fagan, A.M., Miller, B.L., Boxer, A.L., Seeley, W.W., Butovsky, O., Barres, B.A., Paul, S.M., Holtzman, D.M., 2017. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* 549, 523–527. <https://doi.org/10.1038/nature24016>
- Shipton, O.A., Leitz, J.R., Dworzak, J., Acton, C.E.J., Tunbridge, E.M., Denk, F., Dawson, H.N., Vitek, M.P., Wade-Martins, R., Paulsen, O., Vargas-Caballero, M., 2011. Tau Protein Is Required for Amyloid -Induced Impairment of Hippocampal Long-Term Potentiation. *J. Neurosci.* 31, 1688–1692. <https://doi.org/10.1523/JNEUROSCI.2610-10.2011>
- Sikora, E., Bielak-Zmijewska, A., Dudkowska, M., Krzystyniak, A., Mosieniak, G., Wesierska, M., Włodarczyk, J., 2021. Cellular Senescence in Brain Aging. *Front. Aging Neurosci.* 13, 1–23. <https://doi.org/10.3389/fnagi.2021.646924>
- Sowell, E.R., Peterson, B.S., Thompson, P.M., Welcome, S.E., Henkenius, A.L., Toga, A.W., 2003. Mapping cortical change across the human life span. *Nat. Neurosci.* 6, 309–315. <https://doi.org/10.1038/nn1008>
- Sterniczuk, R., Antle, M.C., Laferla, F.M., Dyck, R.H., 2010. Characterization of the 3xTg-AD mouse model of Alzheimer’s disease: Part 2. Behavioral and cognitive changes. *Brain Res.* 1348, 149–155. <https://doi.org/10.1016/j.brainres.2010.06.011>
- Terwel, D., Muyllaert, D., Dewachter, I., Borghgraef, P., Croes, S., Devijver, H., Van Leuven, F., 2008. Amyloid activates GSK-3 β to aggravate neuronal tauopathy in bigenic mice. *Am. J. Pathol.* 172, 786–798. <https://doi.org/10.2353/ajpath.2008.070904>
- Thibault, O., Landfield, P.W., 1996. Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* (80-.). 272, 1017–1020. <https://doi.org/10.1126/science.272.5264.1017>
- Tran, T.N., Kim, S.H., Gallo, C., Amaya, M., Kyees, J., Narayanaswami, V., 2013. Biochemical and biophysical characterization of recombinant rat apolipoprotein E: Similarities to human apolipoprotein E3. *Arch. Biochem. Biophys.* 529, 18–25. <https://doi.org/10.1016/j.abb.2012.10.007>
- Vergara, C., Houben, S., Suain, V., Yilmaz, Z., De Decker, R., Vanden Dries, V., Boom, A., Mansour, S., Leroy, K., Ando, K., Brion, J.-P., 2019. Amyloid- β pathology enhances pathological fibrillary tau seeding induced by Alzheimer PHF in vivo. *Acta Neuropathol.* 137, 397–412. <https://doi.org/10.1007/s00401-018-1953-5>
- Vilchez, D., Saez, I., Dillin, A., 2014. The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nat. Commun.* 5. <https://doi.org/10.1038/ncomms6659>

- Walsh, D.M., Klyubin, I., Fadeeva, J. V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416, 535–539. <https://doi.org/10.1038/416535a>
- Ward, S.M., Himmelstein, D.S., Lancia, J.K., Binder, L.I., 2012. Tau oligomers and tau toxicity in neurodegenerative disease. *Biochem. Soc. Trans.* 40, 667–671. <https://doi.org/10.1042/BST20120134>
- Whishaw, I.Q., Tomie, J., 1996. Of Mice and Mazes: Similarities Between Mice and Rats on Dry Land But Not Water Mazes. *Physiol. Behav.* 60, 1191–1197. [https://doi.org/10.1016/S0031-9384\(96\)00176-X](https://doi.org/10.1016/S0031-9384(96)00176-X)
- Wilcock, G.K., Gauthier, S., Frisoni, G.B., Jia, J., Hardlund, J.H., Moebius, H.J., Bentham, P., Kook, K.A., Schelter, B.O., Wischik, D.J., Davis, C.S., Staff, R.T., Vuksanovic, V., Ahearn, T., Bracoud, L., Shamsi, K., Marek, K., Seibyl, J., Riedel, G., Storey, J.M.D., Harrington, C.R., Wischik, C.M., 2018. Potential of Low Dose Leuco-Methylthioninium Bis(Hydromethanesulphonate) (LMTM) Monotherapy for Treatment of Mild Alzheimer's Disease: Cohort Analysis as Modified Primary Outcome in a Phase III Clinical Trial. *J. Alzheimer's Dis.* 61, 435–457. <https://doi.org/10.3233/JAD-170560>
- Wildner, G., 2019. Are rats more human than mice? *Immunobiology* 224, 172–176. <https://doi.org/10.1016/j.imbio.2018.09.002>
- Wirths, O., Weis, J., Szczygielski, J., Multhaup, G., Bayer, T.A., 2006. Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathol.* 111, 312–319. <https://doi.org/10.1007/s00401-006-0041-4>
- Yankner, B.A., Duffy, L.K., Kirschner, D.A., 1990. Neurotrophic and Neurotoxic Effects of Amyloid β Protein: Reversal by Tachykinin Neuropeptides. *Science* (80-.). 250, 279–282. <https://doi.org/10.1126/science.2218531>
- Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.M., Iwata, N., Saido, T.C., Maeda, J., Suhara, T., Trojanowski, J.Q., Lee, V.M.Y., 2007. Synapse Loss and Microglial Activation Precede Tangles in a P301S Tauopathy Mouse Model. *Neuron* 53, 337–351. <https://doi.org/10.1016/j.neuron.2007.01.010>
- Zempel, H., Luedtke, J., Kumar, Y., Biernat, J., Dawson, H., Mandelkow, E., Mandelkow, E.M., 2013. Amyloid- β oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin. *EMBO J.* 32, 2920–2937. <https://doi.org/10.1038/emboj.2013.207>
- Zempel, H., Thies, E., Mandelkow, E.-M., Mandelkow, E.-M., 2010. AB Oligomers Cause Localized Ca^{2+} Elevation, Missorting of Endogenous Tau into Dendrites, Tau Phosphorylation, and Destruction of Microtubules and Spines. *J. Neurosci.* 30, 11938–11950. <https://doi.org/10.1523/JNEUROSCI.2357-10.2010>

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

JTE received support from the Fonds de recherche Quebec - Santé (FRQS) doctoral training fellowship 2020-21, Hugh E. Burke fellowship 2019-20 and F.S.B Miller Memorial fellowship 2017-18. SDC is the holder of the Charles E. Frosst/Merck Research Associate position. This study was supported by the Canadian Institute of Health Research, the Canadian Consortium of Neurodegeneration and Aging (CCNA), the Healthy Brains Healthy Lives and BrainsCAN McGill - Western Collaboration Grant (MWCG) and the Morris and Rosalind Goodman Family Foundation to ACC's Laboratory. ACC is the holder of the Charles E. Frosst/Merck-endowed Chair in Pharmacology and a member of the Canadian Consortium on Neurodegeneration in Aging.

Acknowledgements

The cDNA of the transgene construct was obtained with the advice of Dr. Michel Goedert and the MRC Lab of Molecular Biology, Cambridge, UK. Confocal images were obtained with the assistance of and access to the Advanced BioImaging Facility of the McGill University Life Sciences Complex.

Contributions of authors

JTE conceptualized and lead the study and was primarily responsible for execution of experiments, primary data collection, analysis and drafting the manuscript. SDC performed A β and Inflammation ECLIA assays. AL assisted with qPCR experiments. CH oversaw the initial rat colonies, breeding and assisted with genotyping. TPW provided collaborative support with training and oversight on synaptic plasticity and LTP experiments. SDC and ACC provided editorial support and contributed to study conceptualization.

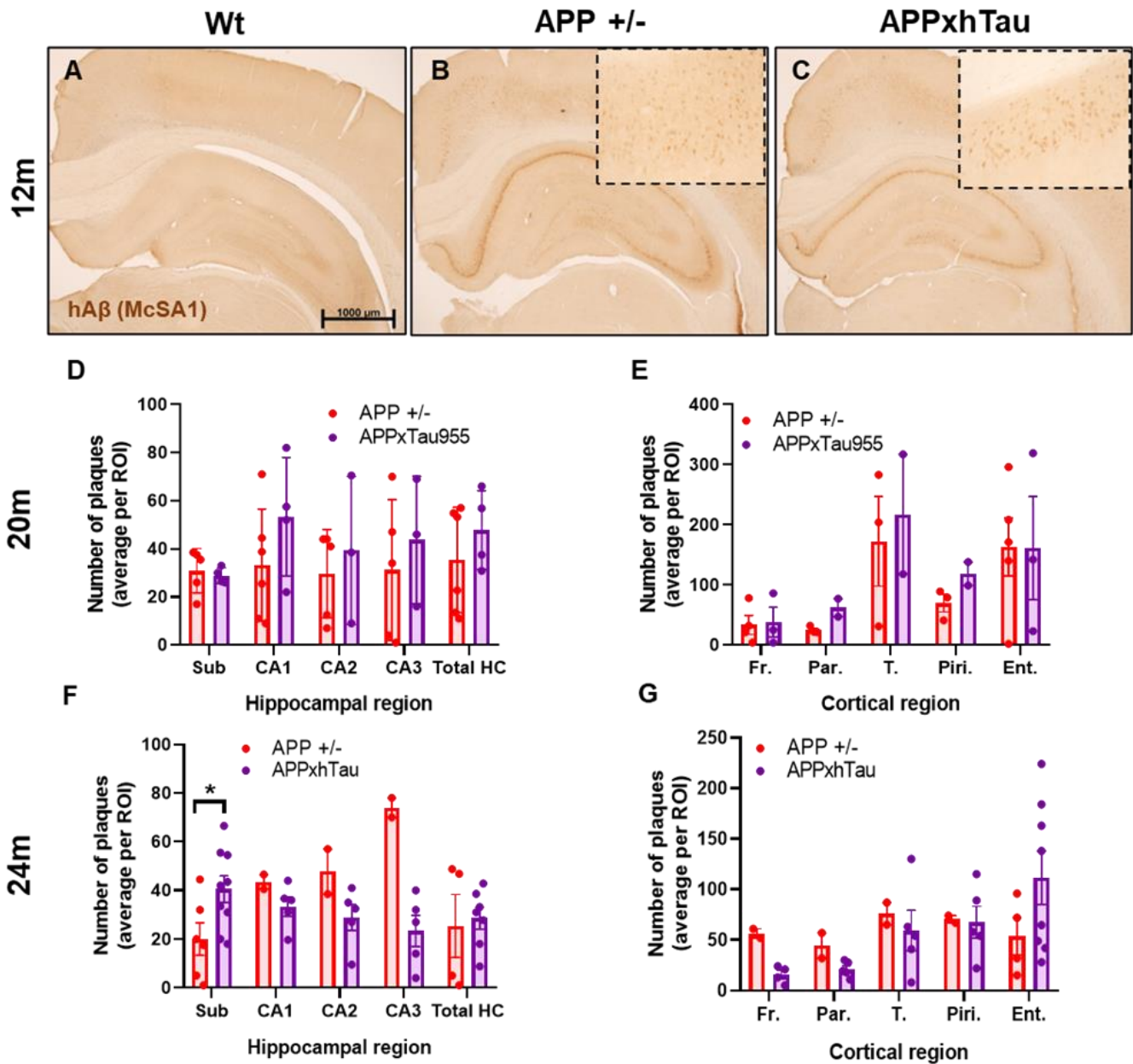
4738 **6. Supplemental Figures**

Figure S1. Additional aspects of the human amyloid plaque pathology. (A-C) Representative micrographs illustrating intraneuronal human amyloid beta accumulation in 12-month-old APP^{+/-} and APPxhTau transgenic rats (C) and an absence of immunoreactivity in wild type rats (Wt) (A). Quantification of the number of amyloid plaques in rats that developed at least one plaque at 20 (D-E) and 24 months of age (F-G) in other brain regions of interest (ROI) in the hippocampus including the subiculum (Sub), and CA1, CA2 and CA3 (D, F) as well as regions in the cerebral cortex including the frontal (Fr.), Parietal (Par.), Temporal (T.), Piriform (Piri.) and Entorhinal cortices (Ent.) (E-G) (n=2-10). Scale bar A-C 1000 μm. * p<0.05.

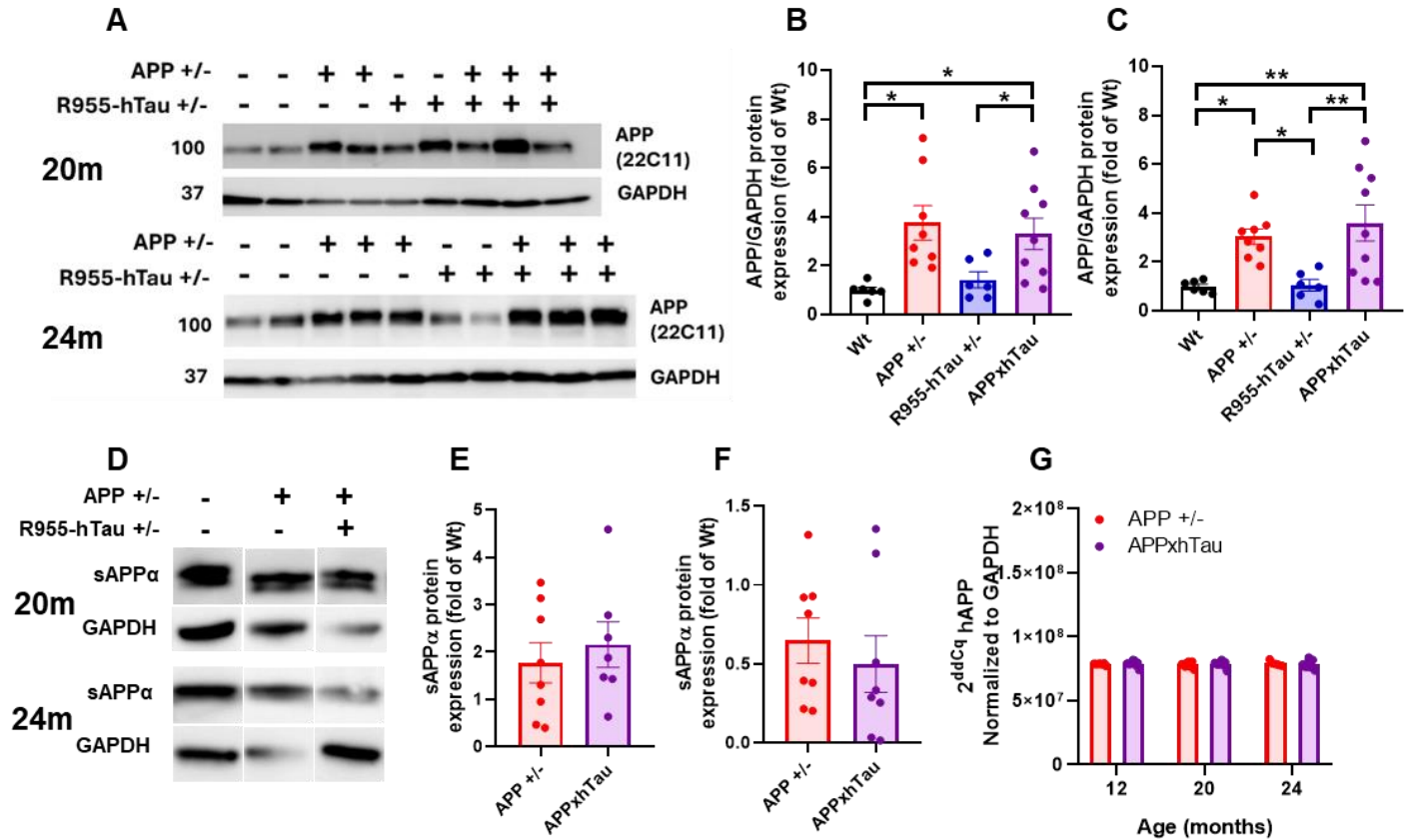
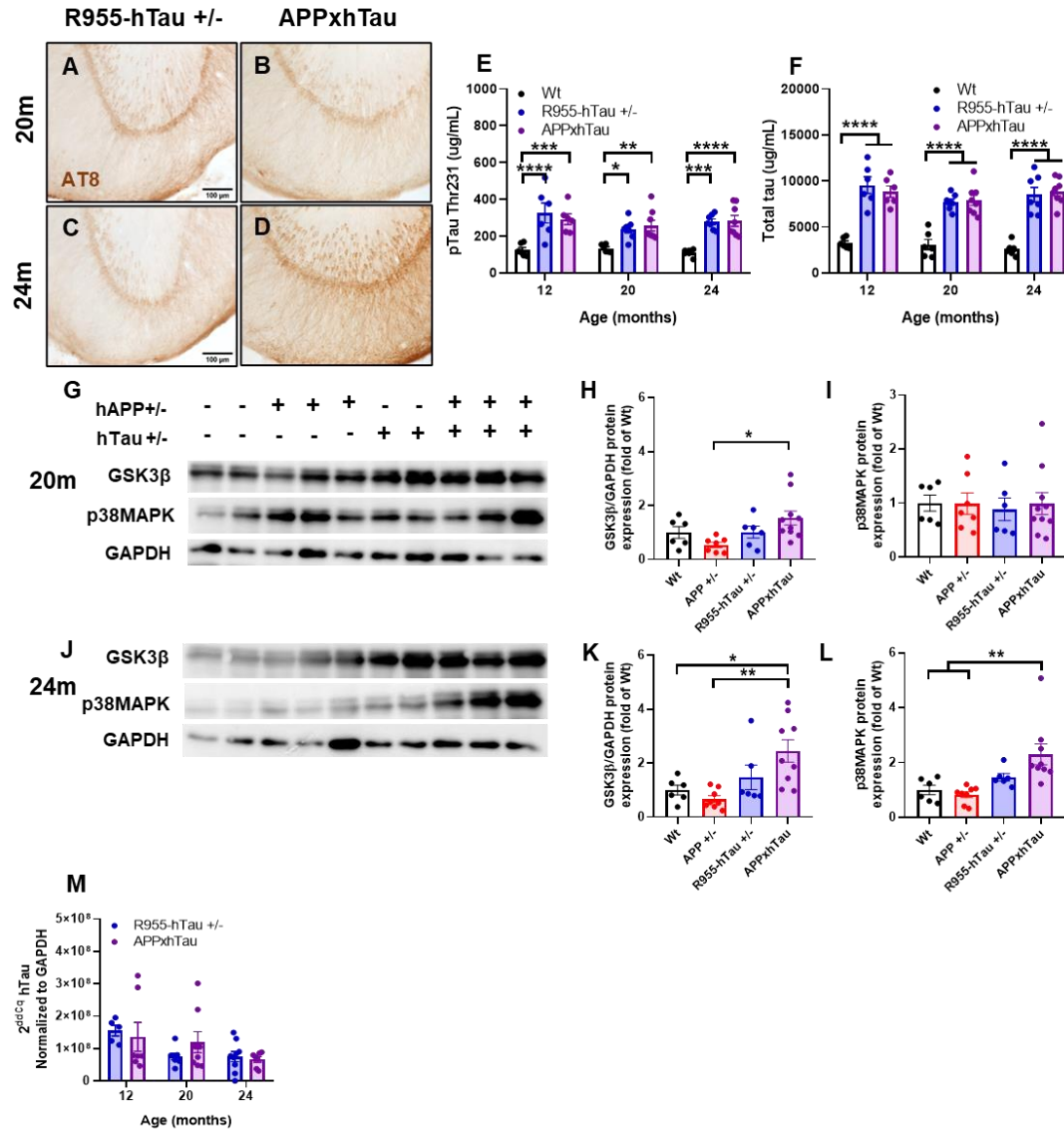


Figure S2. Additional aspects of amyloid beta processing are unaltered in APPxhTau rats. (A) Representative Western blots illustrating elevated total APP (clone 22C11) protein expression in hippocampal homogenates of APP^{+/-} and APPxhTau transgenic rats at 20 and 24 months of age. Quantification of total APP expression depicting an approximately 3-5-fold increase in APP^{+/-} and APPxhTau rats as compared to wild type rats (Wt) at 20 (B) and 24 months of age (C) likely attributed to the addition of the human APP transgene. (D) Representative Western blots illustrating soluble APP alpha (sAPPα) fragment of APP with the quantification at 20 (E) and 24 months of age (F) demonstrating no significant differences between APP^{+/-} and APPxhTau rats (n=7-8). Quantification of human APP transgene expression across age and genotype showing no differences in gene expression (G). *p<0.05, ** p<0.01, *** p<0.001.



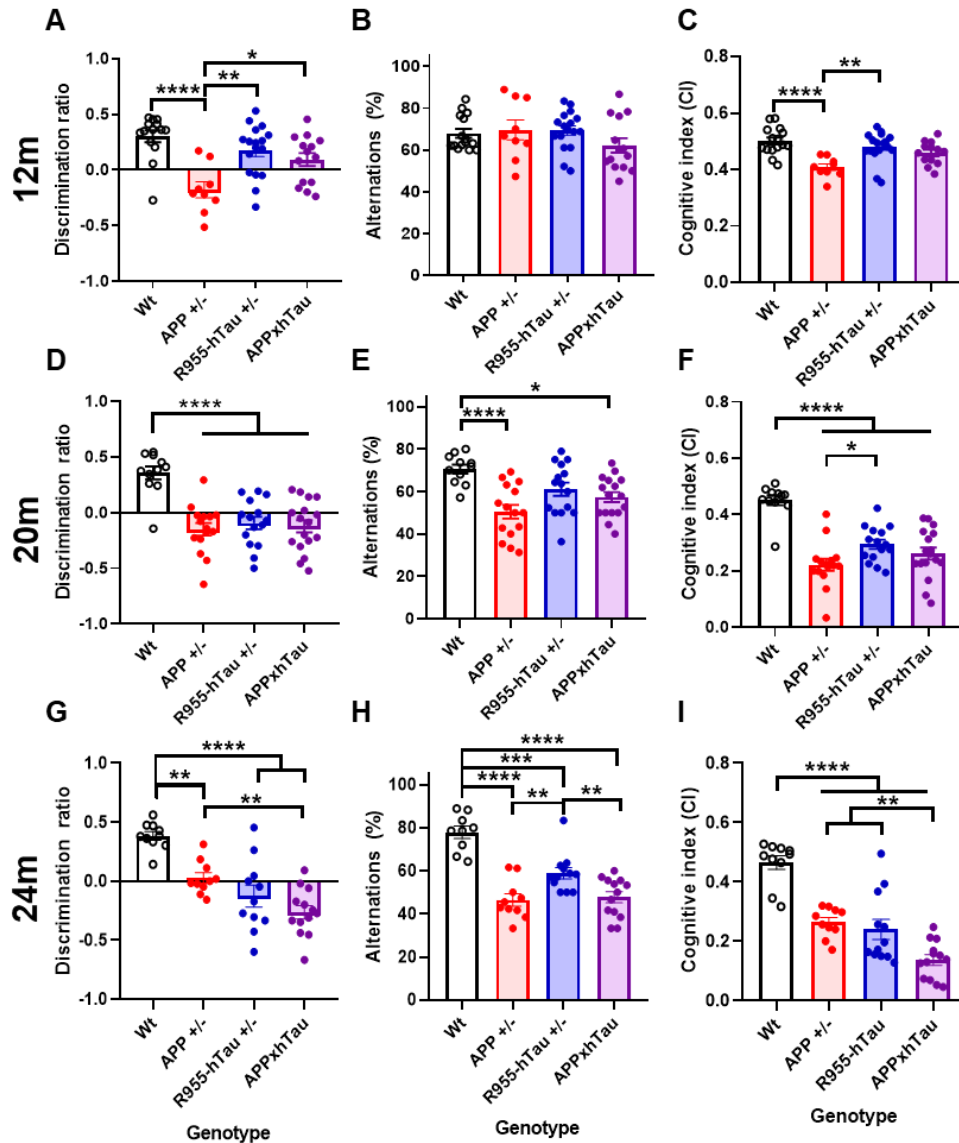


Figure S4. Tau-mediated progressive impairments in cognition in APPxhTau rats. Cognitive evaluation from in the novel object location (NOL) (A, E, I), probe trial of the Morris water maze (MWM) (B, F, J), y-maze (C, G, K) and cognitive index (C, F, I) at 12 (A-C), 20 (D-F) and 24 months of age (G-I). Note the abolishment of amyloid-derived impairments in APPxhTau rats as compared to wild type rats (Wt) for the discrimination ratio during the NOL task at 12 months (A) and this abolishment is also reflected in the overall cognitive index (C). At 20 months, this abolishment is also observed during the y-maze task, as shown by the percentage of alternations, while impaired in APP^{+/-} rats is partially restored in APPxhTau rats as compared to Wt rats (E). At 24 months of age, APPxhTau rats exhibited exacerbated cognitive impairments as shown by the discrimination ratio during the NOL (G), and amyloid-derived impairments persisting into APPxhTau rats during the y-maze (H) and an overall depleted cognitive performance indicated by the cognitive index (I) (n=8-15). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

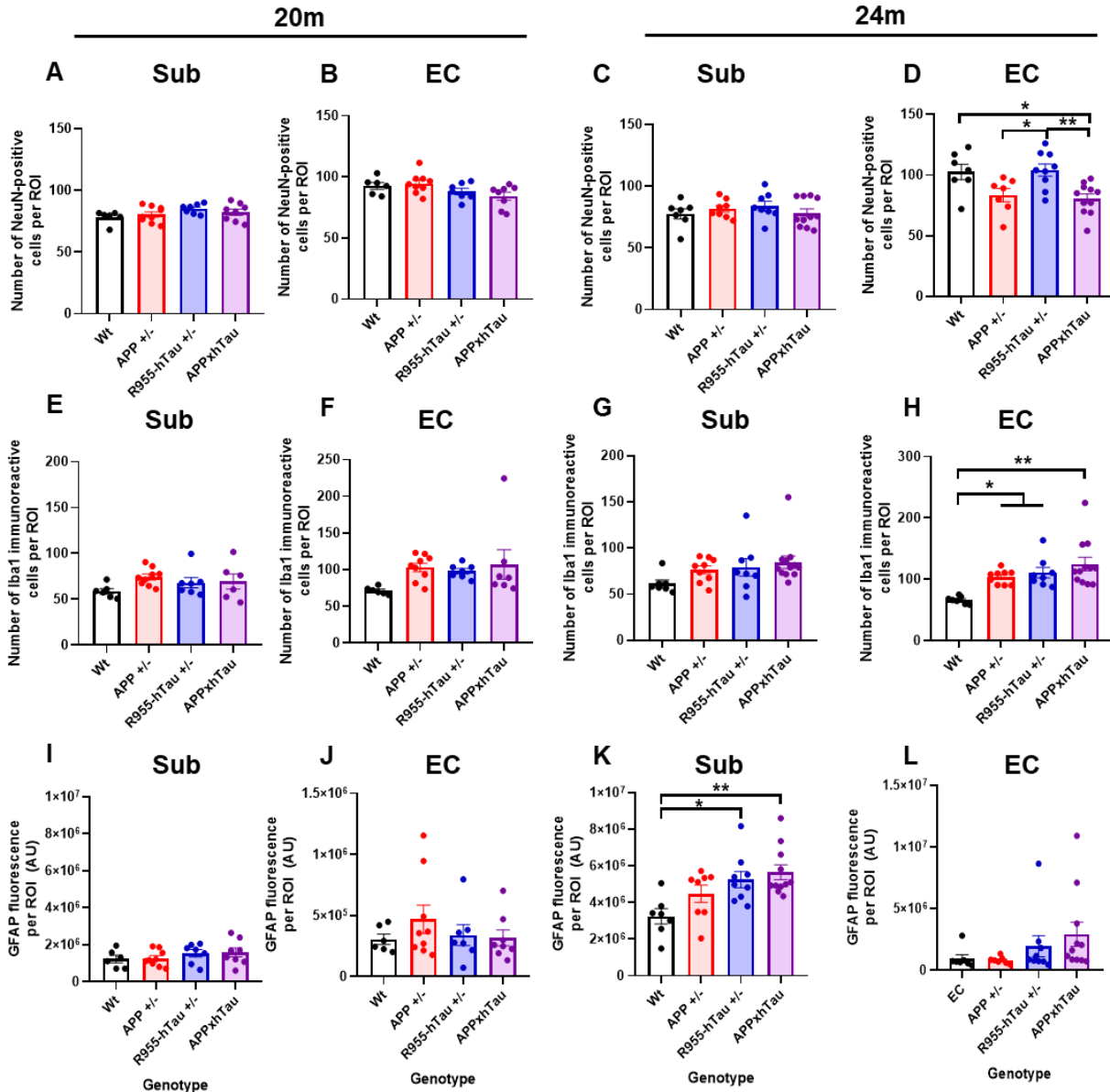


Figure S5. Assessment of neuronal loss and inflammatory responses in other brain regions. Quantification of neuronal loss per region of interest (ROI) at 20 (A-B) and 24 months of age (C-D) in the CA2 region of the hippocampus (A, C) and entorhinal cortex (EC) (B, D) demonstrating an absence of neuronal loss at 20 months of age as well as accelerated neuronal loss in the CA2 region (C). Note that in the entorhinal cortex, amyloid-derived neuronal loss during advanced stages of amyloid plaque pathology persists in APPxhTau^{+/-} rats. Quantification of the number of Iba1-immunoreactive microglial cells in the subiculum region of the hippocampus (Sub) (E, G) and EC (F, H) at 20 (E-F) and 24 months of age (G-H). The number of microglia in APPxhTau rats trended towards exacerbation in the EC as compared to single APP^{+/-} and R955-hTau^{+/-} rats (H). Quantification of the glial astrocytic fibrillary protein (GFAP) immunofluorescence in the subiculum (I, K) and EC (J, L) at 20 (I-J) and 24 months of age (K-L) depicting that, at 24 months, GFAP fluorescence was elevated in a tau-dependent manner in the subiculum (K) with a trended increase in the EC (L) (n=6-9) * p < 0.05, ** p < 0.01.

7. Supplemental Tables

Supplemental Table 1. Animal groups used throughout the study

Age (mo)	Wild type	McGill-APP +/-	McGill-R955- hTau +/-	APPxhTau	Total
12	15 (9/6)	10 (7/3)	17 (12/5)	14 (8/6)	56
20	11 (4/7)	15 (7/8)	14 (7/7)	20 (7/13)	60
24	11 (4/7)	11 (5/6)	12 (4/8)	14 (6/8)	48
Total (M/F)	37 (17/20)	36 (19/17)	43 (23/20)	48 (21/27)	164

Supplemental Table 2. Frequency of plaque-positive animals.

Age (mo)	Genotype	Hippocampus	Chi square	Cerebral Cortex	Chi square
20	APP +/-	6/15 (40 %)	p=0.6112	5/15 (33.3 %)	p=0.5491
	APPxhTau	4/13 (30 %)		3/13 (23.1 %)	
24	APP +/-	6/12 (50.0 %)	p=0.5083	4/12 (33.3 %)	p=0.2289
	APPxhTau	10/16 (62.5 %)		9/16 (56.3 %)	

4750 **Supplemental Table 3. List of antibodies used in the study.**

Primary Antibody	Company/Source	Epitope	Application	Concentration
Amyloid beta				
APP (6e10)	Biolegend	aa 3-8	IF, WB	1:1000
APP (22C11)	EMD Millipore	aa 66-81	WB	1:1000
McSA1	Medimabs	hA β aa 1-12	IHC, IF	1:1000
Anti β -Amyloid	Cell Signalling Tech	-	IF	1:500
sAPP α (2B3)	IBL America	-	WB	1:500
Tau, ptau				
HT7	Thermofisher	aa 159-163, hTau	IHC	1:1200
AT8	Thermofisher	pSer202-pThr205	IHC, WB	1:500, 1:1000
MC1	Peter Davies	aa 7-9, 312-322	IHC	1:500
PHF1	Peter Davies	pSer396/404	IHC	1:4000
AT180	Thermofisher	pThr231	IHC	1:500
Cell Markers				
Iba1 (Microglia)	Wako	-	IHC, IF	1:500
GFAP (Astrocyte)	Novus	-	IF	1:2000
NeuN (Neuron)	Abcam	-	IHC, IF	1:4000
Synaptic				
NMDAR1	Sigma	-	WB	1:1000
PSD95	Cell Signalling Tech	-	WB	1:1000
Fyn	Abcam	-	WB	1:1000
Other				
GSK3B (27C10)	Cell Signalling Tech	-	WB	1:1000
P38MAPK	Cell Signalling Tech	-	WB	1:1000
GAPDH	Millipore	-	WB	1:5000
Secondary Antibody	Company/Source	Epitope	Application	Concentration
Rb anti-Fc Ms	Cuello Lab	-	IHC	1:100
Gt anti-Rb (Biotinyl.)	MP Biomedicals	-	IHC	1:200
Gt anti-Rb AF-568	Abcam	-	IF	1:400
Gt anti-Gp AF-647	Abcam	-	IF	1:400
Gt anti-Ms AF-488	Abcam	-	IF	1:400

4751 **IF** = Immunofluorescence; **IHC** = Immunohistochemistry; **WB** = Western blot

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CHAPTER V: GENERAL DISCUSSION

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5.0 Summary of thesis findings

Tauopathies, as investigated in the past, converge on the concept that fibrillar tau mediates multiple aspects of neurodegeneration and cognitive decline in the CNS (Buchanan et al., 2020; Goedert et al., 1991; Hernández and Avila, 2007; Holland et al., 2020; Joie et al., 2020; Knopman et al., 2019; Lee et al., 2001; Spillantini and Goedert, 1998; Terry et al., 1981). As investigated in this thesis and from others (Acquarone et al., 2019; Niewiadomska et al., 2021; Shafiei et al., 2017; Usenovic et al., 2015; Ward et al., 2012), it is becoming increasingly clear that the abnormal accumulation and hyperphosphorylation of tau leading to the aggregation of soluble, bioactive tau is more important for the induction of disease.

Our initial investigations of tauopathy in the newly generated homozygous McGill-R955-hTau transgenic rat model revealed a sequential progression of tauopathy over the life span: an accumulation of mutated human tau, tau hyperphosphorylation, then misfolding and aggregation into neurofibrillary inclusions reaching terminal stages after 20 months. Along this continuum, the majority of downstream abnormalities including CNS inflammation, synaptic loss and neurodegeneration occurred during more advanced stages – findings similar to that of the lost R962-hTau line (Malcolm et al., 2019) and other established transgenic mouse models (Allen et al., 2002; Ramsden et al., 2005; Schindowski et al., 2006; Yoshiyama et al., 2007). What sets the homozygous R955-hTau transgenic rat model apart from other existing models, notably, is the faithful recapitulation of several key aspects of human tauopathy in their totality including a prion-like recruitment of endogenous tau, robust synaptic and brain-wide neurodegeneration including neuronal loss, and genuine neurofibrillary inclusions. Further, the abnormalities to the brain vasculature identified in this thesis, including the involvement of astrocytes bearing misfolded tau species, brain vessel remodelling of the basement membrane and pericytes, and compromise of the blood-brain-barrier, presented a novel avenue by which tau elicits damage to the CNS to which we explored in detail.

Emphasized in Chapter I, there is a current need to better understand the early stages of tauopathy as to be better positioned for the successful development of tau therapeutics in the future. Further, it is unlikely that tau therapies could undo the degenerative consequences of advanced fibrillary tau pathology. In pursuit of our aims and hypotheses, we investigated the effect of a milder, more progressive tau accumulation as reported in the heterozygous R955-hTau transgenic rat model (Chapter III). While we initially suspected that the line would produce neurofibrillary

lesions at terminal life stages, we unexpectedly discovered that aspects of neurodegeneration to the myelin and neuronal populations developed in their absence at the investigated time points. These findings emphasize that such aspects of tau-mediated degeneration can occur during the early stages of human tauopathy which has been difficult to demonstrate in other, more aggressively expressing, tau transgenic models that simultaneously possess fibrillary and oligomeric tau species. These differences may also be further exemplified by the added translational value of the rat compared to mice. Furthermore, our observations provided evidence that the hyperphosphorylated and oligomeric species in aged rats were responsible for such degeneration which has been difficult to investigate in other, more aggressive transgenic mouse models.

Lastly, as described in Chapter IV, we took advantage of the milder phenotype of tauopathy produced in the heterozygous R955-hTau rat and combined it with the well-established McGill-R-Thy1-APP transgenic rat model for human amyloid-like pathology. Our initial objectives were to produce a transgenic rat model with a complete AD-like phenotype as to pursue experimental therapeutics and explore mechanisms of disease. Indeed, the APPxhTau bigenic rat model produced a more complete AD-like pathology and enabled for investigations into the relationship between amyloid and tau pathologies, at least for the context of early stages as demonstrated by the absence of fibrillary tau. Aspects by which amyloid and tau converge on one another, such as CNS inflammation, neurodegeneration, synaptic plasticity and cognition, were primary lines of investigation as they were reportedly affected during advanced stages of AD-like pathology (Bloom, 2014; Gulisano et al., 2018; Nisbet et al., 2015). Our findings revealed an unexpected compensatory effect of human tau on cognitive impairments elicited by the early amyloidosis. Contrary to it, the evolution of the AD-like pathology was later exacerbated by the combined tauopathy and amyloidosis.

5.1 Modeling AD and tauopathies in animals: advantages and limitations

As demonstrated in this thesis and from the plethora of available mouse models for AD-like pathology highlighted in Chapters II and III including the commonly used PS19, Tau22, rTg4510 mouse models among others (Cavanaugh et al., 2014; Cohen et al., 2013; Javonillo et al., 2022; Oddo et al., 2003) as well as models of human tauopathies in general as reviewed elsewhere (Dujardin et al., 2015; Lee et al., 2005), animal models confer the ability to investigate mechanistic

aspects of disease not achievable in humans. In AD and FTD dementias, the many clinical investigations over the past 25 years have utilized a combination of cognitive evaluations, brain imaging by PET and, recently, implementing the use of biomarkers to detect, track and diagnosis disease (Jack et al., 2013, 2010; Jack and Holtzman, 2013). While detecting and tracking disease are essential components of disease characterization, such investigations in AD and FTD are limited by technical shortcomings in their ability to detect changes in the brain during early stages of disease. An additional complication to investigating dementias in humans is that they are contained in the heavily protected, therapeutically less accessible, and critical part of the human body – the brain. One of the most challenging aspects of gaining a better understanding of AD and FTD dementias is that they progress over decades before rendering a clinical presentation. As such, longitudinal investigations spanning several years are required to achieve clinically meaningful information for the pursuit of any novel therapeutics before irreversible pathological changes. Hence, using animal model systems present one of the most feasible ways to investigate the underlying pathology of dementias, *in vivo*.

Typically, mechanistic studies are first conducted in cell culture models, however, in dementias, cellular models are highly limited in its translational value given that AD and FTD are extraordinarily complex diseases that recruit and involve multiple cell types and systems with an aggravation of individual responses. While AD is primarily neuronal, multiple neuron populations and their complex circuitry within the adult brain architecture are affected. The involvement of the basal forebrain cholinergic system (Counts and Mufson, 2005; Coyle et al., 1983; Cuello et al., 2019b; Fortress et al., 2011; Francis et al., 1999; Giacobini et al., 2022; Hampel et al., 2018; J. Whitehouse et al., 2004) has occupied significant amounts of attention since the early detection of deficits of cholinergic markers in AD post-mortem brain samples (BOWEN et al., 1976; Davies and Maloney, 1976). Furthermore, there is a well-established recruitment of other cell types in the CNS including microglia, astrocytes and oligodendrocytes as has been demonstrated in the recently achievable large scale proteomic and transcriptomic analyses (Bai et al., 2021; Grubman et al., 2019; Johnson et al., 2022; Papassotiropoulos et al., 2006; Wang and Li, 2021). In addition, there are multiple mechanisms in place to regulate the abundance and clearance of pathological proteins in the CNS and it is thought that any imbalance may induce disease in the sporadic condition. Together, this rapidly limits the ability to interpret or extrapolate findings to human disease. These are the main reasons why our lab and others place reliance on animal models.

Animal models in mice and rats enables for the discovery of translatable aspects of brain disease based on similarities of brain anatomy and genetic similarity. However, despite their high DNA sequence homology, mice and rats are not humans and one must be cautious in evaluating the translatability of such models for the human condition (Bryda, 2013; Perlman, 2016; Vandamme, 2014) as demonstrated by the poor translatability in CNS inflammatory signalling pathways (Mestas and Hughes, 2004; Seok et al., 2013). In AD and FTD tauopathies, the use of viral vectors was paramount in identifying the prion-like spreading of tau in the CNS. However, viral vectors do not reflect the endogenous development of disease unlike transgenic models and in human disease (Clavaguera et al., 2013; Goedert et al., 2017; Mudher et al., 2017; Strang et al., 2018; Xia et al., 2022). Unfortunately, even with a multitude of available animal models, there has been a large disconnect in therapeutic success between mice and humans (Franco and Cedazo-Minguez, 2014; Windisch, 2014) and this may partially be explained by the absence of a complete AD phenotype in such models as mentioned in Chapter I. Providing a model that recapitulates all aspects of human disease will assist in the clinical translation of preclinical therapeutic studies.

The development and characterization of transgenic rat models illustrated in this thesis are a step forward in the search for models with more translational value to human disease. As described in Chapters I-IV and reviewed elsewhere (Do Carmo and Cuello, 2013; Zimmer et al., 2014), rats are in many aspects more suitable than mice for the investigation of AD and FTD tauopathy. This concept is reinforced by the observations made in this thesis and is exemplified by the increased translational value of possessing all tau isoforms and the expression of an APOE protein akin to human APOE4. Furthermore, our findings highlight the advantage of rats for behavioral studies. The rat is more apt for the evaluation of complicated behavioral tasks, including the Morris water maze (MWM) and the social interaction - tasks in which mice perform poorly, or otherwise inconsistently due to anxiety and stress. The larger size of rats also offers an experimental advantage for surgical interventions or, as in our case, for the injection of the tracer horseradish peroxidase (Chapter II) into the lateral tail vein, a procedure more challenging in mice. Their larger brain size not only provided an increased tissue mass and tissue availability to explore the multiple biochemical aspects of disease described in this thesis, but also eased the examination of synaptic plasticity by acute brain slice electrophysiology (Chapter IV). Additional advantages of rat models such as those described in this thesis is their greater resemblance to human physiology regarding glucose and fatty acid metabolism as well as its historical use in toxicology

as a ‘higher’ species (Arias-Reyes et al., 2021; Bryda, 2013; Fuller and Thyfault, 2021; Jacob, 1999; Kowalski and Bruce, 2014).

There are some limitations to the R955-hTau and APPxhTau transgenic rat models: The R955-hTau transgene expresses a mutated form of human tau that causes FTD and thus is explicitly not a model for AD despite the significant overlap and Braak-like evolution of its disease pathology in the brain. In addition, the transgene promoter of CamKII α directs expression predominantly into the forebrain and telencephalic regions of the brain in excitatory neurons (Coultrap and Bayer, 2012; Juárez-Muñoz et al., 2017; Wang et al., 2013). As such, it can not be assumed that the transgene expressed in this rat model is entirely representative of human disease. However, the striking overlap in the progression tau pathology, localisation of tau and downstream consequences makes it an excellent model to make significant inferences regarding pathology and experimental therapeutics without the confound of motor impairments. Lastly, one limitation may be the trade-off between increasing translatability by incorporated aging in exchange for increased animal costs and more extended duration of studies.

While the heterozygous transgenic rats described in this thesis are excellent for modeling the longitudinal and earliest stages of tauopathy, as is observed in human disease, the long experimental incubation period of 20 or more months to observe detectable tau pathology presents its own logistical challenges including age-related losses. However, these extended experimental timeframes are valuable for the investigation of age-related conditions such as AD and dementias. Future experimental plans incorporating short-term experiments in parallel to the longitudinal works such as for example, including rapidly obtainable large-scale proteomics or RNA sequencing could increase the value of future studies when addressing experimental pharmacology issues. Such additional experiments should also provide insights into factors and proteins implicated in early stages of human tauopathy that incorporates the factor of aging, provided they can be validated with human tissues.

Regarding the APPxhTau transgenic rat as a model for AD, this model possesses two mutations: one that causes familial AD and the other a rare case of FTD. Each mutation comprises a small fraction of the total AD population. While there is marked similarity in the phenotype and progression of pathology observed in humans, we must acknowledge and assume that there may be other disease-relevant factors affiliated to the sporadic condition that are not necessarily incorporated into this model. However, as there is currently no way to reproduce human AD in

rodents without the expression of mutated APP, human tau or human mutated tau, this remains as a technical limitation of the *in vivo* investigations of AD and related tauopathies. One overarching goal of generating the APPxhTau transgenic rat model was to produce a complete AD-like phenotype that contained both early and late-stage phases within its lifespan. Contrary to our hypotheses, R955-hTau and APPxhTau rats did not develop neurofibrillary inclusions, and consequential to this, indicated that APPxhTau rats did not accelerate to the expected magnitude. This occurred despite convincing evidence of enhanced tau hyperphosphorylation and downstream neuronal loss. However, future investigations crossing homozygous versions of both the McGill-R-Thy1-APP and R955-hTau rat models or simultaneous pronuclear injection of both transgenes with sufficient expression could accomplish this goal.

5.2 Tau pathology and the neurovascular unit

As demonstrated in Chapter II, there was a clear role of misfolded tau in downstream neurovascular damage in the homozygous R955-hTau transgenic rat. Interestingly, AD-related studies have attributed neurovascular abnormalities to cerebral atherosclerosis and arteriolosclerosis as well as cerebral amyloid angiopathy (CAA), a condition where amyloid beta deposits are found in the vascular space, and inflammation (Arvanitakis et al., 2016; Carrano et al., 2012; Kalaria and Deramecourt, 2012; Wisniewski et al., 1997; Wisniewski and Kozlowski, 1982). Our findings emphasize that pathological tau, in the absence of amyloid beta, induces astrocytosis, astrocytic swelling of end-feet and altered localisation of the aquaporin4 water channel (AQP4). The initial increase in glial-fibrillary acidic protein (GFAP) fluorescence intensity preceded the tau-mediated effect on astrocytes and may have spurred multiple neurovascular abnormalities including altered Collagen IV, CD13 labelled pericytes, cerebral microbleeds, extravasation of IgG's, and penetration of a peripheral tracer into tau-burdened brain regions. Astrocytes play a principal component in mediating glutamate excitotoxicity (Gonzalez-Reyes et al., 2017; Mahmoud et al., 2019; Rodríguez-Arellano et al., 2016) and in the neurovascular unit. Given the established role of tau to induce excitotoxicity and synaptic loss, we suspect that tau pathology in a progressive setting eventually leads to glutamate toxicity, a loss of support to neurons and then a spreading of pathological tau from dying neurons to the perivascular astrocytes. Given that we have observed that tau pathology affects polarization of the vascular astrocytic AQP4 channel, induces ventricular dilation, alters the neurovascular unit morphology

and induces neurovascular remodelling of tau-burdened brain regions, we suspect that these changes are likely a failed response to clear tau pathology primarily through the glymphatic system. It can also be speculated that these altered mechanisms consequentially damage the blood-brain-barrier. Of future interest could be to mechanistically remove misfolded tau, either by an antisense oligonucleotide or small molecule targeting this conformational-dependent tau species to determine whether such neurovascular abnormalities could be prevented or rescued.

Of additional interest was the occurrence of elevated iron reactivity in the entorhinal cortex, a brain region that suffers the initial amyloid and tau pathologies and is thought to be more susceptible to damage. This finding was particularly novel given that iron accumulation has not been reported related to tau and possibly linked with a ferroptosis mechanism of inducing cell death by oxidative damage (Rao and Adlard, 2018; Tran et al., 2022). The mechanism of cell death in AD is not fully understood despite several reports of amyloid-induced apoptotic signalling by activation of Caspases. Adding to the controversy of cell death mechanisms are the recent reports of necroptosis in tauopathy (Caccamo et al., 2017; De Calignon et al., 2010; Dong et al., 2022; Gamblin et al., 2003; Wang et al., 2015) as well as the ability of tau to initiate pathways that circumvent apoptosis (Wang et al., 2014). Future investigations genetically silencing genes encoding proteins involved with different cell death pathways, such as p53, Caspase-3 or 6, MLKL, may provide better insight for the mechanism of cell death in tauopathy, however, such investigations might not necessarily yield fruitful therapeutic avenues. Alternatively, sequestering excess iron with a dietary administration of antioxidants may reduce ferroptotic signalling and may mitigate cellular damage downstream of tauopathy, but may adversely produce gastrointestinal complications.

Taking the findings from the R955-hTau transgenic rat model as described in their homozygous (Chapter II) and heterozygous (Chapter III) forms, we were able to analyze and gain insight into the sequential tau phosphorylation in human FTD tauopathy. A significant research gap and point of interest are the post-translational modifications of tau, as they appear to play an important role in regulating tau function with microtubules and with other binding partners. In line with human post-mortem evaluations (Braak et al., 2006; Braak and Braak, 1991; Braak and Del Tredici, 2020) and other transgenic animal models, the ptau epitopes located at Ser 202-Thr205 and Thr231 were associated with the initial acceleration of tau pathology, whereas misfolded tau and c-terminal hyperphosphorylation at Ser396-Ser404 occur during more advanced stages. However, hyperphosphorylation of other epitopes remains controversial, with the ptau epitopes of

pThr181, pThr217 and pThr231 being implicated in AD and FTD tauopathies (Ashton et al., 2022, 2021; de Calignon et al., 2012; Mielke et al., 2022; Milà-Alomà et al., 2022; Thijssen et al., 2020; Zheng et al., 2020). Of these epitopes, pThr181 appears to be specifically altered in AD whereas pThr217 and pThr231 are indicators of tauopathies including AD. Because the pThr231 epitope was more regionally specific to sites of damage and neuronal loss, this epitope likely represents one of the initial indications of brain tau pathology. However, it is important to acknowledge the technical limitations of studying tau post-translational modifications as it was not feasible to investigate every possible epitope in this model. Therefore, there may be other sites that are contributing to the tauopathy in this model.

5.3 Mechanistic insights on the link between amyloid and tau in the APP^{xh}Tau rat model

As described in Chapter IV, one of the major findings implicated a compensatory effect of human tau on A β -induced impairments in cognition leading up to the initial plaque pathology. Such A β -induced impairments on cognition observed in the McGill-R-Thy1-APP rat model have been well established in transgenic rodent models and can be explained by A β -induced impairments in synaptic plasticity in the brain.

Synaptic plasticity is a fundamental mechanism that allows neurons to ‘learn’ by adjusting the strength of their electrical signals in accordance with the frequency and strength of the inputs they receive from its connections (Bliss and Collingridge, 1993; Foster and Norris, 1997). In essence, LTP can be induced endogenously or artificially by a high frequency of electrical stimulation and, subsequently, this stimulation releases the neurotransmitter glutamate into the synaptic cleft, activates the NMDAR by removal of a magnesium ion blockade and its influx of calcium induces gene transcriptional changes to improve synaptic signalling at the postsynaptic density (Baltaci et al., 2019; Grover and Teyler, 1990; Nicoll and Schmitz, 2005; H. Wang et al., 2016). Prolonged LTP or overstimulation of neurons results in an excess of synaptic glutamate and can induce excitotoxicity and neuronal cell death (Armada-Moreira et al., 2020; Dong et al., 2009; Gonçalves-Ribeiro et al., 2019). As A β -induced impairments in LTP are well-established (Hu et al., 2008; Klyubin et al., 2004; Koffie et al., 2009; Qi et al., 2014b; Walsh et al., 2002), it was thought that this impairment caused the neuronal loss as a result of chronic synaptic dysfunction (Ferreira et al., 2015; Mucke and Selkoe, 2012b).

A puzzling feature of A β -induced impairments in cognition was a seemingly quicker onset in APP transgenic rodent models (highlighted in Chapter I) in contrast to the lack of initial cognitive changes in A β -positive, tau-negative human individuals. One explanation for this discrepancy could be due to a higher degree of cognitive reserve in humans, as defined by an elevated resilience to cellular damage and age-related decline. While the underlying basis of cognitive reserve is not absolutely defined, it appears that lifetime exposures to stimulating environments through educational, occupational and leisure activities as well as the brain's ability to form new and/or healthy neurons mediated by brain-derived neurotrophic factor (BDNF) confers such resilience in humans (Kremen et al., 2022; Steffener and Stern, 2012; Stern, 2013). As shown in this thesis work and the previously described role of tau in maintaining synaptic connections, perhaps the tau accumulation in human AD might also confer effects that mitigate A β -induced impairments in cognition and delay the onset of symptoms in AD.

Recently, tau has also shown to play an important role at the synapse in addition to its physiological function of stabilizing microtubules. Briefly discussed in Chapters I and IV, tau plays an essential role in mediating synaptic plasticity and A β -induced impairments as shown by tau knock-out mouse models. Additionally, other intriguing reports of ptau at Ser396, a similar location as the later-stage c-terminal hyperphosphorylation at Ser396-Ser404, is essential to mediate long-term depression (LTD) (Kimura et al., 2013). LTD is a complementary mechanism to LTP that results in a reduction of signal strength and is thought to mediate processes related to forgetting and fine motor control. Together, LTP and LTD work to encode memories in the brain. Another role of tau at the synapse is its interaction with the Src kinase Fyn and the post synaptic density (PSD95) of dendrites (Ittner et al., 2010; Matrone et al., 2020). In consolidation with the findings in this thesis, we suspect that these physiological mechanisms of learning and memory affected by A β and tau pathology are counteracting one another.

Fyn is the only Src kinase of that is associated with impairments in LTP and spatial learning and memory, implicating this protein in synaptic function as has been shown in Fyn-deficient and Fyn transgenic mice (Chin et al., 2005; Kojima et al., 1997). Fyn has gained significant attention as it appears to facilitate a relationship between A β and tau (Ittner et al., 2010) and has been found to be elevated in ptau burdened neurons in AD (Shirazi and Wood, 1993). It is no surprise that inhibition of Fyn is actively being pursued therapeutically (Nygaard et al., 2014). However, current evidence suggests that lack of Fyn (Kojima et al., 1997), or overexpression of Fyn (Chin et al.,

2005) can be detrimental, emphasizing that Fyn has an important, dynamic, physiological role in synaptic plasticity. Such a dynamic role of Fyn makes this protein a non druggable target. Evidence converges on the concept that both tau and Fyn are essential components of synaptic function as shown by transgenic mouse models mutating tau-Fyn interaction sites, knocking out tau or knocking out Fyn (Briner et al., 2020; Ittner et al., 2016, 2010). Notably, ptau at Thr205 was shown to prevent its interaction with Fyn (Ittner et al., 2016). Intriguingly, the Thr205 epitope is abnormally elevated in the initial stages in human brain tauopathy (Braak et al., 2006) as well as in the R955-hTau transgenic rat model described in this thesis. In P301S cell cultures, Fyn is immobilized to the dendritic space with tau and the genetic ablation of Fyn rescues tauopathy in P301L transgenic mice (Briner et al., 2020; Padmanabhan et al., 2019). Therefore, it could be hypothesized that these tau-Fyn interactions in the dendritic compartment are critical for the A β -induced toxicity in a phosphorylation dependent manner. In addition, our findings in 20-month-old APPxhTau rats, as reported in Chapter IV, strongly implicate a significant relationship between A β and tau at the dendritic compartment during the early stages of disease and this relationship involves elevated Fyn and PSD95 in relation to synaptic plasticity.

The findings in this Thesis and the cited literature would be in line with the Trigger and Bullet hypothesis (Bloom, 2014) and they may explain the key initial mechanisms by which amyloid triggers tauopathy at the early AD pathology stages. This, amongst the multiple factors linked to the initial intra- extracellular A β accumulation. Many of these aspects are still unresolved in sporadic AD. Excess A β could then trigger responses impairing proper synaptic function, thereby hindering the ability to form, process, and store memories in the brain. However, in response, the established Tau-PSD95-Fyn complex gets recruited and localized to NMDARs in the dendritic compartment of the postsynaptic density. This, in principle, could initially counteract the effect of extracellular A β . However, such persistent and sustained synaptic impairments induced by extracellular A β could lead to a progressive recruitment and mis-localisation of tau from axons to the somatodendritic compartment. As such, the induction of Fyn-mediated excitotoxic neuronal cell death is enabled and possibly elevated. Ultimately, this could recruit astrocytes to quench the excess of glutamate and recruit microglia to sites of plaque containing pathology. Over time, ptau consequentially becomes hyperphosphorylated and tau pathology ensues. If this sequence of events is true, addressing this mechanism therapeutically in the future, could help to halt or slow down disease.

5.4 Concluding remarks

Alzheimer's disease (AD) and related tauopathies like frontotemporal dementia (FTD) present as a major challenge in the field of neuroscience, medicine and pharmacology. The complex nature of such neurodegenerative diseases stems from the gradual, decade-long progression and combined occurrence of multiple CNS pathologies, making it difficult to explore the relationships driving disease in humans.

In this thesis, we present and discuss multiple translatable aspects and discoveries regarding the early stages of amyloid and tau pathologies through the generation and characterization of novel transgenic rat models. As described in this thesis, we provided evidence for the occurrence of additional neurodegenerative aspects of the CNS tauopathy, such as a neurovascular compromise and blood-brain-barrier dysfunction which are aspects that can be attributed to the impact of misfolded tau on astrocytes. Additionally, we have shown that the earliest stages of tau accumulation and hyperphosphorylation are sufficient to induce aspects of neurodegeneration including myelin degeneration and neuronal loss. This thesis also illustrates that, at the earliest stages of the A β amyloidosis, there might be a neuroprotective effect of prefibrillar tau accumulation.

Lastly, in a newly established model for AD/FTD-like tauopathy we provide novel information regarding the continuum of this pathology from the early stages to the late, human-like, stages culminating with neuronal loss, vascular and myelin compromise, ventricular dilation accompanied by an expected impairment of cognition. We propose that this model should be of value for basic science discoveries and experimental therapeutics for a pathology for which there is not currently any effective disease-modifying treatment.

5117 **REFERENCES**

- 5118 Abdelhak, A., Foschi, M., Abu-Rumeileh, S., Yue, J.K., D'Anna, L., Huss, A., Oeckl, P., Ludolph,
5119 A.C., Kuhle, J., Petzold, A., Manley, G.T., Green, A.J., Otto, M., Tumani, H., 2022. Blood
5120 GFAP as an emerging biomarker in brain and spinal cord disorders. *Nat. Rev. Neurol.*
5121 <https://doi.org/10.1038/s41582-021-00616-3>
- 5122 Acquarone, E., Argyrousi, E.K., Van Den Berg, M., Gulisano, W., Fà, M., Staniszewski, A.,
5123 Calcagno, E., Zuccarello, E., D'Adamio, L., Deng, S.X., Puzzo, D., Arancio, O., Fiorito, J.,
5124 2019. Synaptic and memory dysfunction induced by tau oligomers is rescued by up-regulation
5125 of the nitric oxide cascade. *Mol. Neurodegener.* 14, 1–19. [https://doi.org/10.1186/s13024-](https://doi.org/10.1186/s13024-019-0326-4)
5126 [019-0326-4](https://doi.org/10.1186/s13024-019-0326-4)
- 5127 Adalbert, R., Milde, S., Durrant, C., Ando, K., Stygelbout, V., Yilmaz, Z., Gould, S., Brion, J.P.,
5128 Coleman, M.P., 2018. Interaction between a MAPT variant causing frontotemporal dementia
5129 and mutant APP affects axonal transport. *Neurobiol. Aging* 68, 68–75.
5130 <https://doi.org/10.1016/j.neurobiolaging.2018.03.033>
- 5131 Adami, P.M., Quijano, C., Magnani, N., Galeano, P., Evelson, P., Cassina, A., Carmo, S.D., Leal,
5132 M.C.M.C., Castano, E.M., Cuello, A.C., Morelli, L., Martino Adami, P. V, Quijano, C.,
5133 Magnani, N., Galeano, P., Evelson, P., Cassina, A., Do Carmo, S., Leal, M.C.M.C., Castano,
5134 E.M., Cuello, A.C., Morelli, L., Castaño, E.M., Cuello, A.C., Morelli, L., 2015. Synaptosomal
5135 bioenergetic defects are associated with cognitive impairment in a transgenic rat model of
5136 early Alzheimer's disease. *J. Cereb. Blood Flow Metab.* 37, 69–84.
5137 <https://doi.org/10.1177/0271678X15615132>
- 5138 Agca, C., Fritz, J.J., Walker, L.C., Levey, A.I., Chan, A.W.S., Lah, J.J., Agca, Y., 2008.
5139 Development of transgenic rats producing human β -amyloid precursor protein as a model for
5140 Alzheimer's disease: Transgene and endogenous APP genes are regulated tissue-specifically.
5141 *BMC Neurosci.* 9, 28. <https://doi.org/10.1186/1471-2202-9-28>
- 5142 Agca, C., Klakotskaia, D., Schachtman, T.R., Chan, A.W., Lah, J.J., Agca, Y., 2016. Presenilin 1
5143 transgene addition to amyloid precursor protein overexpressing transgenic rats increases
5144 amyloid beta 42 levels and results in loss of memory retention. *BMC Neurosci.* 17, 1–10.
5145 <https://doi.org/10.1186/s12868-016-0281-8>
- 5146 Ahlskog, J.E., Geda, Y.E., Graff-Radford, N.R., Petersen, R.C., 2011. Physical exercise as a
5147 preventive or disease-modifying treatment of dementia and brain aging. *Mayo Clin. Proc.* 86,
5148 876–884. <https://doi.org/10.4065/mcp.2011.0252>
- 5149 Aisen, P.S., Davis, K.L., Berg, J.D., Schafer, K., Campbell, K., Thomas, R.G., Weiner, M.F.,
5150 Farlow, M.R., Sano, M., Grundman, M., Thal, L.J., Alzheimers Disease Cooperative Study,
5151 2000. A randomized controlled trial of prednisone in Alzheimer's disease. *Neurology* 54,
5152 588–593. <https://doi.org/10.1212/WNL.54.3.575>
- 5153 Aisen, P.S., Schafer, K.A., Grundman, M., Pfeiffer, E., Sano, M., Davis, K.L., Farlow, M.R., Jin,
5154 S., Thomas, R.G., Thal, L.J., for the Alzheimer's Disease Cooperative Study, 2003. Effects
5155 of Rofecoxib or Naproxen vs Placebo on Alzheimer Disease Progression. *Jama* 289, 2819.
5156 <https://doi.org/10.1001/jama.289.21.2819>
- 5157 Allen, B., Ingram, E., Takao, M., Smith, M.J., Jakes, R., Virdee, K., Yoshida, H., Holzer, M.,
5158 Craxton, M., Emson, P.C., Atzori, C., Migheli, A., Crowther, R.A., Ghetti, B., Spillantini,
5159 M.G., Goedert, M., 2002. Abundant tau filaments and nonapoptotic neurodegeneration in
5160 transgenic mice expressing human P301S tau protein. *J. Neurosci.* 22, 9340–9351.
5161 <https://doi.org/22/21/9340> [pii]

- Alonso, A.D., Di Clerico, J., Li, B., Corbo, C.P., Alaniz, M.E., Grundke-Iqbal, I., Iqbal, K., 2010. Phosphorylation of Tau at Thr212, Thr231, and Ser262 combined causes neurodegeneration. *J. Biol. Chem.* 285, 30851–30860. <https://doi.org/10.1074/jbc.M110.110957>
- Alzheimer's Association, 2017. 2017 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 13, 325–373. <https://doi.org/10.1016/j.jalz.2017.02.001>
- Alzheimer Association, 2022. 2022 Alzheimer's disease facts and figures. *Alzheimer's Dement.* 18.
- Andorfer, C., Acker, C.M., Kress, Y., Hof, P.R., Duff, K., Davies, P., 2005. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J. Neurosci.* 25, 5446–5454. <https://doi.org/10.1523/JNEUROSCI.4637-04.2005>
- Andorfer, C., Kress, Y., Espinoza, M., De Silva, R., Tucker, K.L., Barde, Y.A., Duff, K., Davies, P., Silva, R. De, Tucker, K.L., Barde, Y.A., Duff, K., Davies, P., De Silva, R., Tucker, K.L., Barde, Y.A., Duff, K., Davies, P., 2003. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J. Neurochem.* 86, 582–590. <https://doi.org/10.1046/j.1471-4159.2003.01879.x>
- Andrási, E., Farkas, É., Scheibler, H., Réffy, A., Bezúr, L., 1995. Al, Zn, Cu, Mn and Fe levels in brain in Alzheimer's disease. *Arch. Gerontol. Geriatr.* 21, 89–97. [https://doi.org/10.1016/0167-4943\(95\)00643-Y](https://doi.org/10.1016/0167-4943(95)00643-Y)
- Angulo, S.L., Orman, R., Neymotin, S.A., Liu, L., Buitrago, L., Cepeda-Prado, E., Stefanov, D., Lytton, W.W., Stewart, M., Small, S.A., Duff, K.E., Moreno, H., 2017. Tau and amyloid-related pathologies in the entorhinal cortex have divergent effects in the hippocampal circuit. *Neurobiol. Dis.* 108, 261–276. <https://doi.org/10.1016/j.nbd.2017.08.015>
- Antunes, M., Biala, G., 2012. The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cogn. Process.* 13, 93–110. <https://doi.org/10.1007/s10339-011-0430-z>
- Arboleda-Velasquez, J.F., Lopera, F., O'Hare, M., Delgado-Tirado, S., Marino, C., Chmielewska, N., Saez-Torres, K.L., Amarnani, D., Schultz, A.P., Sperling, R.A., Leyton-Cifuentes, D., Chen, K., Baena, A., Aguillon, D., Rios-Romenets, S., Giraldo, M., Guzmán-Vélez, E., Norton, D.J., Pardilla-Delgado, E., Artola, A., Sanchez, J.S., Acosta-Urbe, J., Lalli, M., Kosik, K.S., Huentelman, M.J., Zetterberg, H., Blennow, K., Reiman, R.A., Luo, J., Chen, Y., Thiyyagura, P., Su, Y., Jun, G.R., Naymik, M., Gai, X., Bootwalla, M., Ji, J., Shen, L., Miller, J.B., Kim, L.A., Tariot, P.N., Johnson, K.A., Reiman, E.M., Quiroz, Y.T., 2019. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nat. Med.* 25, 1680–1683. <https://doi.org/10.1038/s41591-019-0611-3>
- Arias-Reyes, C., Soliz, J., Joseph, V., 2021. Mice and Rats Display Different Ventilatory, Hematological, and Metabolic Features of Acclimatization to Hypoxia. *Front. Physiol.* 12. <https://doi.org/10.3389/fphys.2021.647822>
- Armada-Moreira, A., Gomes, J.I., Pina, C.C., Savchak, O.K., Gonçalves-Ribeiro, J., Rei, N., Pinto, S., Morais, T.P., Martins, R.S., Ribeiro, F.F., Sebastião, A.M., Crunelli, V., Vaz, S.H., 2020. Going the Extra (Synaptic) Mile: Excitotoxicity as the Road Toward Neurodegenerative Diseases. *Front. Cell. Neurosci.* 14, 1–27. <https://doi.org/10.3389/fncel.2020.00090>
- Arriagada, P. V, Growdon, J.H., Hedleywhyte, E.T., Hyman, B.T., Hedley-Whyte, E.T., Hyman, B.T., 1992. Neurofibrillary Tangles But Not Senile Plaques Parallel Duration and Severity of Alzheimers-Disease. *Neurology* 42, 631–639. <https://doi.org/10.1212/WNL.42.3.631>
- Arvanitakis, Z., Capuano, A.W., Leurgans, S.E., Bennett, D.A., Schneider, J.A., 2016. Relation of

- cerebral vessel disease to Alzheimer's disease dementia and cognitive function in elderly people: a cross-sectional study. *Lancet Neurol.* 15, 934–943. [https://doi.org/10.1016/S1474-4422\(16\)30029-1](https://doi.org/10.1016/S1474-4422(16)30029-1)
- Aschenbrenner, A.J., Gordon, B.A., Benzinger, T.L.S., Morris, J.C., Hassenstab, J.J., 2018. Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology* 91, e859–e866. <https://doi.org/10.1212/WNL.0000000000006075>
- Ashton, N.J., Benedet, A.L., Pascoal, T.A., Karikari, T.K., Lantero-Rodriguez, J., Brum, W.S., Mathotaarachchi, S., Therriault, J., Savard, M., Chamoun, M., Stoops, E., Francois, C., Vanmechelen, E., Gauthier, S., Zimmer, E.R., Zetterberg, H., Blennow, K., Rosa-Neto, P., 2022. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *eBioMedicine* 76, 1–13. <https://doi.org/10.1016/j.ebiom.2022.103836>
- Ashton, N.J., Pascoal, T.A., Karikari, T.K., Benedet, A.L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., Blennow, K., 2021. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* 141, 709–724. <https://doi.org/10.1007/s00401-021-02275-6>
- Avila, J., 2006. Tau phosphorylation and aggregation in Alzheimer's disease pathology. *FEBS Lett.* 580, 2922–2927. <https://doi.org/10.1016/j.febslet.2006.02.067>
- Avila, J., Lucas, J.J., Pérez, M., Hernández, F., 2004. Role of Tau Protein in Both Physiological and Pathological Conditions. *Physiol. Rev.* 84, 361–384. <https://doi.org/10.1152/physrev.00024.2003>
- Bachstetter, A.D., Ighodaro, E.T., Hassoun, Y., Aldeiri, D., Neltner, J.H., Patel, E., Abner, E.L., Nelson, P.T., 2017. Rod-shaped microglia morphology is associated with aging in 2 human autopsy series. *Neurobiol. Aging* 52, 98–105. <https://doi.org/10.1016/j.neurobiolaging.2016.12.028>
- Bachstetter, A.D., Van Eldik, L.J., Schmitt, F.A., Neltner, J.H., Ighodaro, E.T., Webster, S.J., Patel, E., Abner, E.L., Kryscio, R.J., Nelson, P.T., 2015. Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging. *Acta Neuropathol. Commun.* 3, 32. <https://doi.org/10.1186/s40478-015-0209-z>
- Baek, M.S., Cho, H., Lee, H.S., Lee, J.H., Ryu, Y.H., Lyoo, C.H., 2020. Effect of APOE ϵ 4 genotype on amyloid- β and tau accumulation in Alzheimer's disease. *Alzheimer's Res. Ther.* 12, 1–12. <https://doi.org/10.1186/s13195-020-00710-6>
- Bai, B., Vanderwall, D., Li, Y., Wang, X., Poudel, S., Wang, H., Dey, K.K., Chen, P.C., Yang, K., Peng, J., 2021. Proteomic landscape of Alzheimer's Disease: novel insights into pathogenesis and biomarker discovery. *Mol. Neurodegener.* 16, 1–16. <https://doi.org/10.1186/s13024-021-00474-z>
- Baldacci, F., Lista, S., Manca, M.L., Chiesa, P.A., Cavedo, E., Lemercier, P., Zetterberg, H., Blennow, K., Habert, M.-O., Potier, M.C., Dubois, B., Vergallo, A., Hampel, H., 2020. Age and sex impact plasma NFL and t-Tau trajectories in individuals with subjective memory complaints: a 3-year follow-up study. *Alzheimers. Res. Ther.* 12, 147. <https://doi.org/10.1186/s13195-020-00704-4>
- Baldeiras, I., Santana, I., Leitão, M.J., Gens, H., Pascoal, R., Tábuas-Pereira, M., Beato-Coelho, J., Duro, D., Almeida, M.R., Oliveira, C.R., 2018. Addition of the A β 42/40 ratio to the cerebrospinal fluid biomarker profile increases the predictive value for underlying Alzheimer's disease dementia in mild cognitive impairment. *Alzheimer's Res. Ther.* 10, 1–

- 5254 15. <https://doi.org/10.1186/s13195-018-0362-2>
- 5255 Ballatore, C., Lee, V.M.Y., Trojanowski, J.Q., 2007. Tau-mediated neurodegeneration in
5256 Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* 8, 663–672.
5257 <https://doi.org/10.1038/nrn2194>
- 5258 Baltaci, S.B., Mogulkoc, R., Baltaci, A.K., 2019. Molecular Mechanisms of Early and Late LTP.
5259 *Neurochem. Res.* 44, 281–296. <https://doi.org/10.1007/s11064-018-2695-4>
- 5260 Barthélemy, N.R., Li, Y., Joseph-Mathurin, N., Gordon, B.A., Hassenstab, J., Benzinger, T.L.S.,
5261 Buckles, V., Fagan, A.M., Perrin, R.J., Goate, A.M., Morris, J.C., Karch, C.M., Xiong, C.,
5262 Allegri, R., Mendez, P.C., Berman, S.B., Ikeuchi, T., Mori, H., Shimada, H., Shoji, M.,
5263 Suzuki, K., Noble, J., Farlow, M., Chhatwal, J., Graff-Radford, N.R., Salloway, S., Schofield,
5264 P.R., Masters, C.L., Martins, R.N., O'Connor, A., Fox, N.C., Levin, J., Jucker, M., Gabelle,
5265 A., Lehmann, S., Sato, C., Bateman, R.J., McDade, E., Allegri, R., Bateman, R., Bechara, J.,
5266 Benzinger, T., Berman, S., Bodge, C., Brandon, S., Brooks, W. (Bill), Buck, J., Buckles, V.,
5267 Chea, S., Chhatwal, J., Chrem Mendez, P., Chui, H., Cinco, J., Clifford, J., Cruchaga, C.,
5268 Donahue, T., Douglas, J., Edigo, N., Erekin-Taner, N., Fagan, A., Farlow, M., Fitzpatrick, C.,
5269 Flynn, G., Fox, N., Franklin, E., Fujii, H., Gant, C., Gardener, S., Ghetti, B., Goate, A.,
5270 Goldman, J., Gordon, B., Graff-Radford, N., Gray, J., Groves, A., Hassenstab, J., Hoechst-
5271 Swisher, L., Holtzman, D., Hornbeck, R., DiBari, S.H., Ikeuchi, T., Ikononovic, S., Jerome,
5272 G., Jucker, M., Karch, C., Kasuga, K., Kawarabayashi, T., Klunk, W. (Bill), Koeppe, R.,
5273 Kuder-Bulletta, E., Laske, C., Lee, J.H., Levin, J., Martins, R., Mason, N.S., Masters, C.,
5274 Maue-Dreyfus, D., McDade, E., Mori, H., Morris, J., Nagamatsu, A., Neimeyer, K., Noble,
5275 J., Norton, J., Perrin, R., Raichle, M., Renton, A., Ringman, J., Roh, J.H., Salloway, S.,
5276 Schofield, P., Shimada, H., Sigurdson, W., Sohrabi, H., Sparks, P., Suzuki, K., Taddei, K.,
5277 Wang, P., Xiong, C., Xu, X., 2020. A soluble phosphorylated tau signature links tau, amyloid
5278 and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat. Med.* 26, 398–
5279 407. <https://doi.org/10.1038/s41591-020-0781-z>
- 5280 Bateman, R.J., Xiong, C., Benzinger, T.L.S., Fagan, A.M., Goate, A., Fox, N.C., Marcus, D.S.,
5281 Cairns, N.J., Xie, X., Blazey, T.M., Holtzman, D.M., Santacruz, A., Buckles, V., Oliver, A.,
5282 Moulder, K., Aisen, P.S., Ghetti, B., Klunk, W.E., McDade, E., Martins, R.N., Masters, C.L.,
5283 Mayeux, R., Ringman, J.M., Rossor, M.N., Schofield, P.R., Sperling, R.A., Salloway, S.,
5284 Morris, J.C., Dominantly Inherited Alzheimer Network, 2012. Clinical and biomarker
5285 changes in dominantly inherited Alzheimer's disease. *N. Engl. J. Med.* 367, 795–804.
5286 <https://doi.org/10.1056/NEJMoa1202753>
- 5287 Bayer, T.A., Wirths, O., 2010. Intracellular accumulation of amyloid-beta – a predictor for
5288 synaptic dysfunction and neuron loss in Alzheimer's disease. *Front. Aging Neurosci.* 2, 1–
5289 10. <https://doi.org/10.3389/fnagi.2010.00008>
- 5290 Beeri, M.S., Schmeidler, J., Lesser, G.T., Maroukian, M., West, R., Leung, S., Wysocki, M., Perl,
5291 D.P., Purohit, D.P., Haroutunian, V., 2012. Corticosteroids, but not NSAIDs, are associated
5292 with less Alzheimer neuropathology. *Neurobiol. Aging* 33, 1258–1264.
5293 <https://doi.org/10.1016/j.neurobiolaging.2011.02.011>
- 5294 Bejanin, A., Schonhaut, D.R., La Joie, R., Kramer, J.H., Baker, S.L., Sosa, N., Ayakta, N.,
5295 Cantwell, A., Janabi, M., Lauriola, M., O'Neil, J.P., Gorno-Tempini, M.L., Miller, Z.A.,
5296 Rosen, H.J., Miller, B.L., Jagust, W.J., Rabinovici, G.D., 2017. Tau pathology and
5297 neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain* 140,
5298 3286–3300. <https://doi.org/10.1093/brain/awx243>
- 5299 Bell, R.D., Winkler, E.A., Sagare, A.P., Singh, I., LaRue, B., Deane, R., Zlokovic, B. V., 2010.

- Pericytes Control Key Neurovascular Functions and Neuronal Phenotype in the Adult Brain and during Brain Aging. *Neuron* 68, 409–427. <https://doi.org/10.1016/j.neuron.2010.09.043>
- Benedet, A.L., Milà-Alomà, M., Vrillon, A., Ashton, N.J., Pascoal, T.A., Lussier, F., Karikari, T.K., Hourregue, C., Cognat, E., Dumurgier, J., Stevenson, J., Rahmouni, N., Pallen, V., Poltronetti, N.M., Salvadó, G., Shekari, M., Operto, G., Gispert, J.D., Minguillon, C., Fauria, K., Kollmorgen, G., Suridjan, I., Zimmer, E.R., Zetterberg, H., Molinuevo, J.L., Paquet, C., Rosa-Neto, P., Blennow, K., Suárez-Calvet, M., 2021. Differences between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels across the Alzheimer Disease Continuum. *JAMA Neurol.* 78, 1471–1483. <https://doi.org/10.1001/jamaneurol.2021.3671>
- Bennett, D.A., Schneider, J.A., Wilson, R.S., Bienias, J.L., Arnold, S.E., 2004. Neurofibrillary Tangles Mediate the Association of Amyloid Load with Clinical Alzheimer Disease and Level of Cognitive Function. *Arch. Neurol.* 61, 378–384. <https://doi.org/10.1001/archneur.61.3.378>
- Bennett, R.E., DeVos, S.L., Dujardin, S., Corjuc, B., Gor, R., Gonzalez, J., Roe, A.D., Frosch, M.P., Pitstick, R., Carlson, G.A., Hyman, B.T., 2017. Enhanced Tau Aggregation in the Presence of Amyloid β . *Am. J. Pathol.* 187, 1601–1612. <https://doi.org/10.1016/j.ajpath.2017.03.011>
- Bennett, R.E., Hu, M., Fernandes, A., Perez-Rando, M., Robbins, A., Kamath, T., Dujardin, S., Hyman, B.T., 2020. Tau reduction in aged mice does not impact Microangiopathy. *Acta Neuropathol. Commun.* 8, 1–16. <https://doi.org/10.1186/s40478-020-01014-4>
- Bennett, R.E., Robbins, A.B., Hu, M., Cao, X., Betensky, R.A., Clark, T., Das, S., Hyman, B.T., 2018. Tau induces blood vessel abnormalities and angiogenesis-related gene expression in P301L transgenic mice and human Alzheimer's disease. *Proc. Natl. Acad. Sci.* 115, 1–10. <https://doi.org/10.1073/pnas.1710329115>
- Bernstein, S.L., Dupuis, N.F., Lazo, N.D., Wytenbach, T., Condrón, M.M., Bitan, G., Teplow, D.B., Shea, J.E., Ruotolo, B.T., Robinson, C. V., Bowers, M.T., 2009. Amyloid- β 2 protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nat. Chem.* 1, 326–331. <https://doi.org/10.1038/nchem.247>
- Berron, D., Vogel, J.W., Insel, P.S., Pereira, J.B., Xie, L., Wisse, L.E.M., Yushkevich, P.A., Palmqvist, S., Mattsson-Carlén, N., Stomrud, E., Smith, R., Strandberg, O., Hansson, O., 2021. Early stages of tau pathology and its associations with functional connectivity, atrophy and memory. *Brain* 144, 2771–2783. <https://doi.org/10.1093/brain/awab114>
- Bertram, L., McQueen, M.B., Mullin, K., Blacker, D., Tanzi, R.E., 2007. Systematic meta-analyses of Alzheimer disease genetic association studies: The AlzGene database. *Nat. Genet.* 39, 17–23. <https://doi.org/10.1038/ng1934>
- Biel, D., Brendel, M., Rubinski, A., Buerger, K., Janowitz, D., Dichgans, M., Franzmeier, N., 2021. Tau-PET and in vivo Braak-staging as prognostic markers of future cognitive decline in cognitively normal to demented individuals. *Alzheimer's Res. Ther.* 13, 1–13. <https://doi.org/10.1186/s13195-021-00880-x>
- Blair, L.J., Frauen, H.D., Zhang, B., Nordhues, B.A., Bijan, S., Lin, Y.C., Zamudio, F., Hernandez, L.D., Sabbagh, J.J., Selenica, M.L.B., Dickey, C.A., 2015. Tau depletion prevents progressive blood-brain barrier damage in a mouse model of tauopathy. *Acta Neuropathol. Commun.* 3, 8. <https://doi.org/10.1186/s40478-015-0186-2>
- Blennow, K., Zetterberg, H., 2018. Biomarkers for Alzheimer's disease: current status and prospects for the future. *J. Intern. Med.* 284, 643–663. <https://doi.org/10.1111/joim.12816>
- Bliss, T.V.P., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in

- the hippocampus. *Nature* 361, 31–39. <https://doi.org/10.1038/361031a0>
- Bloom, G.S., 2014. Amyloid-B and tau: The trigger and bullet in Alzheimer disease pathogenesis. *JAMA Neurol.* 71, 505–508. <https://doi.org/10.1001/jamaneurol.2013.5847>
- Bodea, L.G., Evans, H.T., Van der Jeugd, A., Ittner, L.M., Delerue, F., Kril, J., Halliday, G., Hodges, J., Kiernan, M.C., Götz, J., 2017. Accelerated aging exacerbates a pre-existing pathology in a tau transgenic mouse model. *Aging Cell* 16, 377–386. <https://doi.org/10.1111/acer.12565>
- Bolmont, T., Clavaguera, F., Meyer-Luehmann, M., Herzig, M.C., Radde, R., Staufenbiel, M., Lewis, J., Hutton, M., Tolnay, M., Jucker, M., 2007. Induction of tau pathology by intracerebral infusion of amyloid- β -containing brain extract and by amyloid- β deposition in APP x tau transgenic mice. *Am. J. Pathol.* 171, 2012–2020. <https://doi.org/10.2353/ajpath.2007.070403>
- Bolós, M., Llorens-Martín, M., Jurado-Arjona, J., Hernández, F., Rábano, A., Avila, J., 2015. Direct Evidence of Internalization of Tau by Microglia In Vitro and In Vivo. *J. Alzheimer's Dis.* 50, 77–87. <https://doi.org/10.3233/JAD-150704>
- Borson, S., Scanlan, J., Brush, M., Vitaliano, P., Dokmak, A., 2000. The mini-cog: a cognitive “vital signs” measure for dementia screening in multi-lingual elderly. *Int. J. Geriatr. Psychiatry* 15, 1021–1027.
- BOWEN, D.M., SMITH, C.B., WHITE, P., DAVISON, A.N., 1976. NEUROTRANSMITTER-RELATED ENZYMES AND INDICES OF HYPOXIA IN SENILE DEMENTIA AND OTHER ABIOTROPHIES. *Brain* 99, 459–496. <https://doi.org/10.1093/brain/99.3.459>
- Braak, H., Alafuzoff, I., Arzberger, T., Kretschmar, H., Tredici, K., 2006. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 112, 389–404. <https://doi.org/10.1007/s00401-006-0127-z>
- Braak, H., Braak, E., 1995. Staging of Alzheimer's Disease-Related Neurofibrillary Changes. *Neurobiol. Aging* 16, 271–278.
- Braak, H., Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 82, 239–259. <https://doi.org/10.1007/BF00308809>
- Braak, H., Del Tredici, K., 2020. From the Entorhinal Region via the Prosubiculum to the Dentate Fascia: Alzheimer Disease-Related Neurofibrillary Changes in the Temporal Allocortex. *J. Neuropathol. Exp. Neurol.* 79, 163–175. <https://doi.org/10.1093/jnen/nlz123>
- Brandt, R., Hundelt, M., Shahani, N., 2005. Tau alteration and neuronal degeneration in tauopathies: Mechanisms and models. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1739, 331–354. <https://doi.org/10.1016/j.bbadis.2004.06.018>
- Brier, M.R., Gordon, B., Friedrichsen, K., McCarthy, J., Stern, A., Christensen, J., Owen, C., Aldea, P., Su, Y., Hassenstab, J., Cairns, N.J., Holtzman, D.M., Fagan, A.M., Morris, J.C., Benzinger, T.L.S., Ances, B.M., 2016. Tau and Ab imaging, CSF measures, and cognition in Alzheimer's disease. *Sci. Transl. Med.* 8, 1–10. <https://doi.org/10.1126/scitranslmed.aaf2362>
- Briner, A., Götz, J., Polanco, J.C., 2020. Fyn Kinase Controls Tau Aggregation In Vivo. *Cell Rep.* 32. <https://doi.org/10.1016/j.celrep.2020.108045>
- Bryda, E.C., 2013. The Mighty Mouse: The Impact of Rodents on Advances in Biomedical Research. *Mo. Med.* 207–211.
- Buchanan, H., Mackay, M., Palmer, K., Tothová, K., Katsur, M., Platt, B., Koss, D.J., 2020. Synaptic Loss, ER Stress and Neuro-Inflammation Emerge Late in the Lateral Temporal Cortex and Associate with Progressive Tau Pathology in Alzheimer's Disease. *Mol.*

- Neurobiol. 3258–3272. <https://doi.org/10.1007/s12035-020-01950-1>
- Buchhave, P., Minthon, L., Zetterberg, H., Wallin, A.K., Blennow, K., Hansson, O., 2012. Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch. Gen. Psychiatry* 69, 98–106. <https://doi.org/10.1001/archgenpsychiatry.2011.155>
- Bugiani, O., Murrell, J.R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., Morbin, M., Primavera, A., Carella, F., Solaro, C., Grisoli, M., Savoirdo, M., Spillantini, M.G., Tagliavini, F., Goedert, M., Ghetti, B., 1999. Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in Tau. *J. Neuropathol. Exp. Neurol.* 58, 667–677.
- Burke, S.N., Barnes, C.A., 2006. Neural plasticity in the ageing brain. *Nat. Rev. Neurosci.* 7, 30–40. <https://doi.org/10.1038/nrn1809>
- Busche, M.A., Wegmann, S., Dujardin, S., Commins, C., Schiantarelli, J., Klickstein, N., Kamath, T. V., Carlson, G.A., Nelken, I., Hyman, B.T., 2019. Tau impairs neural circuits, dominating amyloid- β effects, in Alzheimer models in vivo. *Nat. Neurosci.* 22, 57–64. <https://doi.org/10.1038/s41593-018-0289-8>
- Cacace, R., Slegers, K., Van Broeckhoven, C., 2016. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimer's Dement.* 12, 733–748. <https://doi.org/10.1016/j.jalz.2016.01.012>
- Caccamo, A., Branca, C., Piras, I.S., Ferreira, E., Huentelman, M.J., Liang, W.S., Readhead, B., Dudley, J.T., Spangenberg, E.E., Green, K.N., Belfiore, R., Winslow, W., Oddo, S., 2017. Necroptosis activation in Alzheimer's disease. *Nat Neurosci advance on.*
- Canepa, E., Fossati, S., 2021. Impact of Tau on Neurovascular Pathology in Alzheimer's Disease. *Front. Neurol.* 11, 1–19. <https://doi.org/10.3389/fneur.2020.573324>
- Cao, W., Zheng, H., 2018. Correction to: Peripheral immune system in aging and Alzheimer's disease. *Mol. Neurodegener.* 13, 1–17. <https://doi.org/10.1186/s13024-018-0290-4>
- Caraci, F., Iulita, M.F., Pentz, R., Aguilar, L.F., Orciani, C., Barone, C., Romano, C., Drago, F., Cuello, A.C., 2017. Searching for new pharmacological targets for the treatment of Alzheimer's disease in Down syndrome. *Eur. J. Pharmacol.* 817, 7–19. <https://doi.org/10.1016/j.ejphar.2017.10.004>
- Carrano, A., Hoozemans, J.J.M., Van Der Vies, S.M., Van Horssen, J., De Vries, H.E., Rozemuller, A.J.M., 2012. Neuroinflammation and blood-brain barrier changes in capillary amyloid angiopathy. *Neurodegener. Dis.* 10, 329–331. <https://doi.org/10.1159/000334916>
- Castillo-Carranza, D.L., Nilson, A.N., Van Sike, C.E., Jahrling, J.B., Patel, K., Garach, P., Gerson, J.E., Sengupta, U., Abisambra, J., Nelson, P., Troncoso, J., Ungvari, Z., Galvan, V., Kaye, R., 2017. Cerebral Microvascular Accumulation of Tau Oligomers in Alzheimer's Disease and Related Tauopathies. *Aging Dis.* 8, 257. <https://doi.org/10.14336/AD.2017.0112>
- Cataldo, A.M., Peterhoff, C.M., Troncoso, J.C., Gomez-Isla, T., Hyman, B.T., Nixon, R.A., 2000. Endocytic pathway abnormalities precede amyloid β deposition in sporadic Alzheimer's disease and down syndrome: Differential effects of APOE genotype and presenilin mutations. *Am. J. Pathol.* 157, 277–286. [https://doi.org/10.1016/S0002-9440\(10\)64538-5](https://doi.org/10.1016/S0002-9440(10)64538-5)
- Cavanaugh, S.E., Pippin, J.J., Barnard, N.D., 2014. Animal models of Alzheimer disease: Historical pitfalls and a path forward. *ALTEX* 31, 279–302. <https://doi.org/10.14573/altex.1310071>
- Chabrier, M.A., Cheng, D., Castello, N.A., Green, K.N., LaFerla, F.M., 2014. Synergistic effects of amyloid-beta and wild-type human tau on dendritic spine loss in a floxed double transgenic

- model of Alzheimer's disease. *Neurobiol. Dis.* 64, 107–117.
<https://doi.org/10.1016/j.nbd.2014.01.007>
- Chaiyawat, P., Netsirisawan, P., Svasti, J., Champattanachai, V., 2014. Aberrant O-GlcNAcylated proteins: New perspectives in breast and colorectal cancer. *Front. Endocrinol. (Lausanne)*. 5, 1–10. <https://doi.org/10.3389/fendo.2014.00193>
- Chandra, A., Farrell, C., Wilson, H., Dervenoulas, G., De Natale, E.R., Politis, M., 2021. Aquaporin-4 polymorphisms predict amyloid burden and clinical outcome in the Alzheimer's disease spectrum. *Neurobiol. Aging* 97, 1–9.
<https://doi.org/10.1016/j.neurobiolaging.2020.06.007>
- Chasseigneaux, S., Allinquant, B., 2012. Functions of A β , sAPP α and sAPP β : Similarities and differences. *J. Neurochem.* 120, 99–108. <https://doi.org/10.1111/j.1471-4159.2011.07584.x>
- Chávez-Gutiérrez, L., Bammens, L., Benilova, I., Vandersteen, A., Benurwar, M., Borgers, M., Lismont, S., Zhou, L., Van Cleynebreugel, S., Esselmann, H., Wiltfang, J., Serneels, L., Karran, E., Gijzen, H., Schymkowitz, J., Rousseau, F., Broersen, K., De Strooper, B., 2012. The mechanism of γ -Secretase dysfunction in familial Alzheimer disease. *EMBO J.* 31, 2261–2274. <https://doi.org/10.1038/emboj.2012.79>
- Chee, S.E.J., Solito, E., 2021. The Impact of Ageing on the CNS Immune Response in Alzheimer's Disease. *Front. Immunol.* 12, 1–15. <https://doi.org/10.3389/fimmu.2021.738511>
- Chen, Y.T., Chen, W.Y., Huang, X.T., Xu, Y.C., Zhang, H.Y., 2018. Iron dysregulates APP processing accompanying with sAPP α cellular retention and β -secretase inhibition in rat cortical neurons. *Acta Pharmacol. Sin.* 39, 177–183. <https://doi.org/10.1038/aps.2017.113>
- Chételat, G., La Joie, R., Villain, N., Perrotin, A., De La Sayette, V., Eustache, F., Vandenberghe, R., 2013. Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *NeuroImage Clin.* 2, 356–365.
<https://doi.org/10.1016/j.nicl.2013.02.006>
- Chin, J., Palop, J.J., Puoliväli, J., Massaro, C., Bien-Ly, N., Gerstein, H., Scarce-Levie, K., Masliah, E., Mucke, L., 2005. Fyn kinase induces synaptic and cognitive impairments in a transgenic mouse model of Alzheimer's disease. *J. Neurosci.* 25, 9694–9703.
<https://doi.org/10.1523/JNEUROSCI.2980-05.2005>
- Chiotis, K., Savitcheva, I., Poulakis, K., Saint-Aubert, L., Wall, A., Antoni, G., Nordberg, A., 2020. [18F]THK5317 imaging as a tool for predicting prospective cognitive decline in Alzheimer's disease. *Mol. Psychiatry*. <https://doi.org/10.1038/s41380-020-0815-4>
- Cho, J., Johnson, G.V.W., 2004. Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3B (GSK3B) plays a critical role in regulating tau's ability to bind and stabilize microtubules. *J. Neurochem.* 88, 349–358. <https://doi.org/10.1046/j.1471-4159.2003.02155.x>
- Clavaguera, F., Akatsu, H., Fraser, G., Crowther, R.A., Frank, S., Hench, J., Probst, A., Winkler, D.T., Reichwald, J., Staufenbiel, M., Ghetti, B., Goedert, M., Tolnay, M., 2013. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. *Proc. Natl. Acad. Sci.* 110, 9535–9540. <https://doi.org/10.1073/pnas.1301175110>
- Clavaguera, F., Bolmont, T., Crowther, R.A., Abramowski, D., Frank, S., Probst, A., Fraser, G., Stalder, A.K., Beibel, M., Staufenbiel, M., Jucker, M., Goedert, M., Tolnay, M., 2009. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat. Cell Biol.* 11, 909–913. <https://doi.org/10.1038/ncb1901>
- Clavaguera, F., Grueninger, F., Tolnay, M., 2014. Intercellular transfer of tau aggregates and spreading of tau pathology : Implications for therapeutic strategies. *Neuropharmacology* 76,

- 9–15. <https://doi.org/10.1016/j.neuropharm.2013.08.037>
- Clavaguera, F., Hench, J., Goedert, M., Tolnay, M., 2015. Invited review: Prion-like transmission and spreading of tau pathology. *Neuropathol. Appl. Neurobiol.* 41, 47–58. <https://doi.org/10.1111/nan.12197>
- Cohen, R.M., Rezai-zadeh, K., Weitz, T.M., Rentsendorj, A., Gate, D., Spivak, I., Bholat, Y., Vasilevko, V., Glabe, C.G., Breunig, J.J., Rakic, P., Davtyan, H., Agadjanyan, M.G., 2013. A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric A β and frank neuronal loss. *J Neurosci* 33, 6245–6256. <https://doi.org/10.1523/JNEUROSCI.3672-12.2013.A>
- Cohen, T.J., Guo, J.L., Hurtado, D.E., Kwong, L.K., Mills, I.P., Trojanowsky, J.Q., Lee, V.M.Y., 2011. The acetylation of tau inhibits its function and promotes pathological tau aggregation. *Nat. Commun.* 2, 252. <https://doi.org/10.1038/ncomms1255>
- Coimbra, J.R.M., Marques, D.F.F., Baptista, S.J., Pereira, C.M.F., Moreira, P.I., Dinis, T.C.P., Santos, A.E., Salvador, J.A.R., 2018. Highlights in BACE1 Inhibitors for Alzheimer's Disease Treatment. *Front. Chem.* 6, 1–10. <https://doi.org/10.3389/fchem.2018.00178>
- Combs, B., Hamel, C., Kanaan, N.M., 2016. Pathological conformations involving the amino terminus of tau occur early in Alzheimer's disease and are differentially detected by monoclonal antibodies. *Neurobiol. Dis.* 94, 18–31. <https://doi.org/10.1016/j.nbd.2016.05.016>
- Cotman, C.W., Poon, W.W., Rissman, R.A., Blurton-Jones, M., 2005. The role of caspase cleavage of tau in Alzheimer disease neuropathology. *J. Neuropathol. Exp. Neurol.* 64, 104–112. <https://doi.org/10.1093/jnen/64.2.104>
- Coultrap, S.J., Bayer, K.U., 2012. CaMKII regulation in information processing and storage. *Trends Neurosci.* 35, 607–618. <https://doi.org/10.1016/j.tins.2012.05.003>
- Counts, S.E., Mufson, E.J., 2005. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 64, 263–272. <https://doi.org/10.1093/jnen/64.4.263>
- Coyle, J.T., Price, D.L., DeLong, M.R., 1983. Alzheimer's Disease: A Disorder of Cortical Cholinergic Innervation. *Science* (80-.). 219, 1184–1190.
- Cripps, D., Thomas, S.N., Jeng, Y., Yang, F., Davies, P., Yang, A.J., 2006. Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. *J. Biol. Chem.* 281, 10825–10838. <https://doi.org/10.1074/jbc.M512786200>
- Crous-Bou, M., Minguillón, C., Gramunt, N., Molinuevo, J.L., 2017. Alzheimer's disease prevention: From risk factors to early intervention. *Alzheimer's Res. Ther.* 9, 1–9. <https://doi.org/10.1186/s13195-017-0297-z>
- Cuello, A.C., 2007. *Pharmacological Mechanisms in Alzheimer's Therapeutics*. Springer.
- Cuello, A.C., 2005. Intracellular and extracellular A β , a tale of two neuropathologies. *Brain Pathol.* 15, 66–71. <https://doi.org/10.1111/j.1750-3639.2005.tb00101.x>
- Cuello, A.C., Allard, S., Ferretti, M.T., 2012. Evidence for the accumulation of Abeta immunoreactive material in the human brain and in transgenic animal models. *Life Sci.* 91, 1141–1147. <https://doi.org/10.1016/j.lfs.2012.05.020>
- Cuello, A.C., Hall, H., Do Carmo, S., 2019a. Experimental Pharmacology in Transgenic Rodent Models of Alzheimer's Disease. *Front. Pharmacol.* 10, 1–10. <https://doi.org/10.3389/fphar.2019.00189>
- Cuello, A.C., Pentz, R., Hall, H., 2019b. The Brain NGF Metabolic Pathway in Health and in Alzheimer's Pathology. *Front. Neurosci.* 13, 1–8. <https://doi.org/10.3389/fnins.2019.00062>

- 5530 Cuervo, A.M., Dice, J.F., 2000. Age-related decline in chaperone-mediated autophagy. *J. Biol.*
 5531 *Chem.* 275, 31505–31513. <https://doi.org/10.1074/jbc.M002102200>
- 5532 Cummings, J., Lee, G., Nahed, P., Kambar, M.E.Z.N., Zhong, K., Fonseca, J., Taghva, K., 2022.
 5533 Alzheimer's disease drug development pipeline: 2022. *Alzheimer's Dement. Transl. Res.*
 5534 *Clin. Interv.* 8. <https://doi.org/10.1002/trc2.12295>
- 5535 Cummings, J., Lee, G., Zhong, K., Fonseca, J., Taghva, K., 2021. Alzheimer's disease drug
 5536 development pipeline: 2021. *Alzheimer's Dement. Transl. Res. Clin. Interv.* 7, 1–24.
 5537 <https://doi.org/10.1002/trc2.12179>
- 5538 D'Andrea, M., Nagele, R., 2010. Morphologically distinct types of amyloid plaques point the way
 5539 to a better understanding of Alzheimer's disease pathogenesis. *Biotech. Histochem.* 85, 133–
 5540 147. <https://doi.org/10.3109/10520290903389445>
- 5541 D'Andrea, M.R., 2016. Amyloid Basis of Alzheimer's Disease, in: *Intracellular Consequences of*
 5542 *Amyloid in Alzheimer's Disease.* pp. 1–14. [https://doi.org/10.1016/B978-0-12-804256-](https://doi.org/10.1016/B978-0-12-804256-4.00001-2)
 5543 [4.00001-2](https://doi.org/10.1016/B978-0-12-804256-4.00001-2)
- 5544 Das, B., Yan, R., 2019. A Close Look at BACE1 Inhibitors for Alzheimer's Disease Treatment.
 5545 *CNS Drugs* 33, 251–263. <https://doi.org/10.1007/s40263-019-00613-7>
- 5546 Datta, D., Leslie, S.N., Wang, M., Morozov, Y.M., Yang, S., Mentone, S.A., Zeiss, C., Duque, A.,
 5547 Rakic, P., Horvath, T.L., van Dyck, C.H., Nairn, A.C., Arnsten, A.F.T., 2021. Age-related
 5548 calcium dysregulation linked with tau pathology and impaired cognition in non-human
 5549 primates. *Alzheimer's Dement.* 17, 920–932. <https://doi.org/10.1002/alz.12325>
- 5550 Davies, P., Maloney, A.J.F., 1976. Selective loss of central cholinergic neurons in AD. *Lancet*
 5551 1403-.
- 5552 De Calignon, A., Fox, L.M., Pitstick, R., Carlson, G.A., Bacskai, B.J., Spires-Jones, T.L., Hyman,
 5553 B.T., 2010. Caspase activation precedes and leads to tangles. *Nature* 464, 1201–1204.
 5554 <https://doi.org/10.1038/nature08890>
- 5555 de Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D.H., Kopeikina,
 5556 K.J., Pitstick, R., Sahara, N., Ashe, K.H., Carlson, G.A., Spires-Jones, T.L., Hyman, B.T.,
 5557 2012. Propagation of Tau Pathology in a Model of Early Alzheimer's Disease. *Neuron* 73,
 5558 685–697. <https://doi.org/10.1016/j.neuron.2011.11.033>
- 5559 De La Torre, J.C., 2012. Cardiovascular risk factors promote brain hypoperfusion leading to
 5560 cognitive decline and dementia. *Cardiovasc. Psychiatry Neurol.* 2012.
 5561 <https://doi.org/10.1155/2012/367516>
- 5562 De Reuck, J., Deramecourt, V., Cordonnier, C., Auger, F., Durieux, N., Bordet, R., Maurage, C.A.,
 5563 Leys, D., Pasquier, F., 2012. Detection of microbleeds in post-mortem brains of patients with
 5564 frontotemporal lobar degeneration: A 7.0-Tesla magnetic resonance imaging study with
 5565 neuropathological correlates. *Eur. J. Neurol.* 19, 1355–1360. [https://doi.org/10.1111/j.1468-](https://doi.org/10.1111/j.1468-1331.2012.03776.x)
 5566 [1331.2012.03776.x](https://doi.org/10.1111/j.1468-1331.2012.03776.x)
- 5567 De Reuck, J., Deramecourt, V., Cordonnier, C., Leys, D., Pasquier, F., Maurage, C.A., 2013.
 5568 Prevalence of cerebrovascular lesions in patients with Lewy body dementia: A
 5569 neuropathological study. *Clin. Neurol. Neurosurg.* 115, 1094–1097.
 5570 <https://doi.org/10.1016/j.clineuro.2012.11.005>
- 5571 de Wolf, F., Ghanbari, M., Licher, S., McRae-McKee, K., Gras, L., Weverling, G.J., Wermeling,
 5572 P., Sedaghat, S., Ikram, M.K., Waziry, R., Koudstaal, W., Klap, J., Kostense, S., Hofman, A.,
 5573 Anderson, R., Goudsmit, J., Ikram, M.A., 2020. Plasma tau, neurofilament light chain and
 5574 amyloid- β levels and risk of dementia; a population-based cohort study. *Brain* 143, 1220–
 5575 1232. <https://doi.org/10.1093/brain/awaa054>

- Deane, R., Sagare, A., Zlokovic, B. V, 2008. The role of the cell surface LRP and soluble LRP in blood-brain barrier Abeta clearance in Alzheimer's disease. *Curr. Pharm. Des.* 14, 1601–5.
- Dekosky, S.T., Scheff, S.W., 1990. Synapse Loss in Frontal Cortex Biopsies in Alzheimer's Disease : Correlation with Logmuve 3eventy. *Ann. Neurol.* 457–464.
- Desikan, R.S., Mcevoy, L.K., Thompson, W., Holland, D., Roddey, J.C., Blennow, K., Aisen, P.S., Brewer, J.B., Hyman, B.T., Dale, A.M., Azleheimers Disease Neuroimaging Initiative, 2011. Amyloid-B associated volume loss occurs only in the presence of phospho-tau. *Ann Neurol* 70, 657–661. <https://doi.org/10.1097/MCA.000000000000178>.
- Desikan, R.S., McEvoy, L.K., Thompson, W.K., Holland, D., Brewer, J.B., Aisen, P.S., Sperling, R.A., Dale, A.M., Alzheimers Disease Neuroimaging Initiative, 2012. Amyloid- β associated clinical decline occurs only in the presence of elevated p-tau. *Arch Neurol* 69, 709–713. <https://doi.org/10.1001/archneurol.2011.3354>.
- DeVos, S.L., Corjuc, B.T., Commins, C., Dujardin, S., Bannon, R.N., Corjuc, D., Moore, B.D., Bennett, R.E., Jorfi, M., Gonzales, J.A., Dooley, P.M., Roe, A.D., Pitstick, R., Irimia, D., Frosch, M.P., Carlson, G.A., Hyman, B.T., 2018. Tau reduction in the presence of amyloid- β prevents tau pathology and neuronal death in vivo. *Brain* 141, 2194–2212. <https://doi.org/10.1093/brain/awy117>
- Dewachter, I., Van Dorpe, J., Smeijers, L., Gilis, M., Kuipéri, C., Laenen, I., Caluwaerts, N., Moechars, D., Checler, F., Vanderstichele, H., Van Leuven, F., 2018. Aging Increased Amyloid Peptide and Caused Amyloid Plaques in Brain of Old APP/V717I Transgenic Mice by a Different Mechanism than Mutant Presenilin1. *J. Neurosci.* 20, 6452–6458. <https://doi.org/10.1523/jneurosci.20-17-06452.2000>
- Dhavan, R., Tsai, L.-H., 2001. A decade of CDK5. *Nat. Rev. Mol. Cell Biol.* 2, 749–759. <https://doi.org/10.1038/35096019>
- Di, J., Cohen, L.S., Corbo, C.P., Phillips, G.R., El Idrissi, A., Alonso, A.D., 2016. Abnormal tau induces cognitive impairment through two different mechanisms: Synaptic dysfunction and neuronal loss. *Sci. Rep.* 6, 1–12. <https://doi.org/10.1038/srep20833>
- Dickson, D.W., 1997. The Pathogenesis of Senile Plaques. *J. Neuropathol. Exp. Neurol.* 56, 321–339. <https://doi.org/10.1097/00005072-199704000-00001>
- Do Carmo, S., Cuello, A.C., 2013. Modeling Alzheimer's disease in transgenic rats. *Mol. Neurodegener.* 8, 37. <https://doi.org/10.1186/1750-1326-8-37>
- Dong, X.X., Wang, Y., Qin, Z.H., 2009. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol. Sin.* 30, 379–387. <https://doi.org/10.1038/aps.2009.24>
- Dong, Y., Yu, H., Li, X., Bian, K., Zheng, Y., Dai, M., Feng, X., Sun, Y., He, Y., Yu, B., Zhang, H., Wu, J., Yu, X., Wu, H., Kong, W., 2022. Hyperphosphorylated tau mediates neuronal death by inducing necroptosis and inflammation in Alzheimer's disease. *J. Neuroinflammation* 19, 1–18. <https://doi.org/10.1186/s12974-022-02567-y>
- Doody, R.S., Raman, R., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, S., Kieburtz, K., He, F., Sun, X., Thomas, R.G., Aisen, P.S., Siemers, E., Sethuraman, G., Mohs, R., 2013. A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. *N. Engl. J. Med.* 369, 341–350. <https://doi.org/10.1056/NEJMoa1210951>
- Doré, V., Krishnadas, N., Bourgeat, P., Huang, K., Li, S., Burnham, S., Masters, C.L., Fripp, J., Villemagne, V.L., Rowe, C.C., 2021. Relationship between amyloid and tau levels and its impact on tau spreading. *Eur. J. Nucl. Med. Mol. Imaging* 48, 2225–2232. <https://doi.org/10.1007/s00259-021-05191-9>

- Drummond, E., Wisniewski, T., 2017. Alzheimer's disease: experimental models and reality. *Acta Neuropathol.* 133, 155–175. <https://doi.org/10.1007/s00401-016-1662-x>
- Du, Y., Deng, W., Wang, Z., Ning, M., Zhang, W., Zhou, Y., Lo, E.H., Xing, C., 2017. Differential subnetwork of chemokines/ cytokines in human, mouse, and rat brain cells after oxygen-glucose deprivation. *J. Cereb. Blood Flow Metab.* 37, 1425–1434. <https://doi.org/10.1177/0271678X16656199>
- Dubois, B., Feldman, H.H., Jacova, C., Hampel, H., Molinuevo, J.L., Blennow, K., Dekosky, S.T., Gauthier, S., Selkoe, D., Bateman, R., Cappa, S., Crutch, S., Engelborghs, S., Frisoni, G.B., Fox, N.C., Galasko, D., Habert, M.O., Jicha, G.A., Nordberg, A., Pasquier, F., Rabinovici, G., Robert, P., Rowe, C., Salloway, S., Sarazin, M., Epelbaum, S., de Souza, L.C., Vellas, B., Visser, P.J., Schneider, L., Stern, Y., Scheltens, P., Cummings, J.L., 2014. Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *Lancet Neurol.* 13, 614–629. [https://doi.org/10.1016/S1474-4422\(14\)70090-0](https://doi.org/10.1016/S1474-4422(14)70090-0)
- Dubois, B., Hampel, H., Feldman, H.H., Scheltens, P., Aisen, P., Andrieu, S., Bakardjian, H., Benali, H., Bertram, L., Blennow, K., Broich, K., Cavedo, E., Crutch, S., Dartigues, J.F., Duyckaerts, C., Epelbaum, S., Frisoni, G.B., Gauthier, S., Genthon, R., Gouw, A.A., Habert, M.O., Holtzman, D.M., Kivipelto, M., Lista, S., Molinuevo, J.L., O'Bryant, S.E., Rabinovici, G.D., Rowe, C., Salloway, S., Schneider, L.S., Sperling, R., Teichmann, M., Carrillo, M.C., Cummings, J., Jack, C.R., 2016. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria, Alzheimer's and Dementia. <https://doi.org/10.1016/j.jalz.2016.02.002>
- Dujardin, S., Colin, M., Buée, L., 2015. Invited review: Animal models of tauopathies and their implications for research/translation into the clinic. *Neuropathol. Appl. Neurobiol.* 41, 59–80. <https://doi.org/10.1111/nan.12200>
- Dujardin, S., Hyman, B.T., 2019. Tau Prion-Like Propagation: State of the Art and Current Challenges BT - Tau Biology, in: Takashima, A., Wolozin, B., Buee, L. (Eds.), . Springer Singapore, Singapore, pp. 305–325. https://doi.org/10.1007/978-981-32-9358-8_23
- Echeverria, V., Cuello, A.C., 2002. Intracellular A-beta amyloid, a sign for worse things to come? *Mol. Neurobiol.* 26, 299–316. <https://doi.org/10.1385/MN:26:2-3:299>
- Eckert, A., Schulz, K.L., Rhein, V., Götz, J., 2010. Convergence of amyloid- β and tau pathologies on mitochondria in vivo. *Mol. Neurobiol.* <https://doi.org/10.1007/s12035-010-8109-5>
- Eikelenboom, P., Bate, C., Van Gool, W.A., Hoozemans, J.J.M., Rozemuller, J.M., Veerhuis, R., Williams, A., 2002. Neuroinflammation in Alzheimer's disease and prion disease. *Glia* 40, 232–239. <https://doi.org/10.1002/glia.10146>
- Ellenbroek, B., Youn, J., 2016. Rodent models in neuroscience research: is it a rat race? *Dis. Model. Mech.* 9, 1079–1087. <https://doi.org/10.1242/dmm.026120>
- Espinoza, M., de Silva, R., Dickson, D.W., Davies, P., 2008. Differential incorporation of tau isoforms in Alzheimer's disease. *J. Alzheimers. Dis.* 14, 1–16.
- Fá, M., Puzzo, D., Piacentini, R., Staniszewski, A., Zhang, H., Baltrons, M.A., Li Puma, D.D., Chatterjee, I., Li, J., Saeed, F., Berman, H.L., Ripoli, C., Gulisano, W., Gonzalez, J., Tian, H., Costa, J.A., Lopez, P., Davidowitz, E., Yu, W.H., Haroutunian, V., Brown, L.M., Palmeri, A., Sigurdsson, E.M., Duff, K.E., Teich, A.F., Honig, L.S., Sierks, M., Moe, J.G., D'Adamio, L., Grassi, C., Kanaan, N.M., Fraser, P.E., Arancio, O., 2016. Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory. *Sci. Rep.* 6, 19393. <https://doi.org/10.1038/srep19393>
- Fagan, A.M., Mintun, M.A., Shah, A.R., Aldea, P., Roe, C.M., Mach, R.H., Marcus, D., Morris, J.C., Holtzman, D.M., 2009. Cerebrospinal fluid tau and ptau181 increase with cortical

- 5668 amyloid deposition in cognitively normal individuals: Implications for future clinical trials of
 5669 Alzheimer's disease. *EMBO Mol. Med.* 1, 371–380.
 5670 <https://doi.org/10.1002/emmm.200900048>
- 5671 Fagan, A.M., Xiong, C., Jasielec, M.S., Bateman, R.J., Goate, A.M., Benzinger, T.L.S., Ghetti, B.,
 5672 Martins, R.N., Masters, C.L., Mayeux, R., Ringman, J.M., Rossor, M.N., Salloway, S.,
 5673 Schofield, P.R., Sperling, R.A., Marcus, D., Cairns, N.J., Buckles, V.D., Ladenson, J.H.,
 5674 Morris, J.C., Holtzman, D.M., 2014. Longitudinal Change in CSF Biomarkers in Autosomal-
 5675 Dominant Alzheimer's Disease. *Sci. Transl. Med.* 6, 226ra30-226ra30.
 5676 <https://doi.org/10.1126/scitranslmed.3007901>
- 5677 Farooqui, A.A., 2017. Neurochemical Aspects of β -Amyloid Cascade Hypothesis for Alzheimer's
 5678 Disease: A Critical Evaluation, in: *Neurochemical Aspects of Alzheimer's Disease*. pp. 1–46.
 5679 <https://doi.org/10.1016/B978-0-12-809937-7.00001-X>
- 5680 Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E.A., Frosch, M.P., Eckman, C.B.,
 5681 Tanzi, R.E., Selkoe, D.J., Guenette, S., 2003. Insulin-degrading enzyme regulates the levels
 5682 of insulin, amyloid α -protein, and the α -amyloid precursor protein intracellular domain in vivo.
 5683 *Proc. Natl. Acad. Sci.* 100, 4162–4167. <https://doi.org/10.1073/pnas.0230450100>
- 5684 Faucher, P., Mons, N., Micheau, J., Louis, C., Beracochea, D.J., 2016. Hippocampal injections of
 5685 oligomeric amyloid β -peptide (1-42) induce selective working memory deficits and long-
 5686 lasting alterations of ERK signaling pathway. *Front. Aging Neurosci.* 7, 1–15.
 5687 <https://doi.org/10.3389/fnagi.2015.00245>
- 5688 Fein, J.A., Sokolow, S., Miller, C.A., Vinters, H. V., Yang, F., Cole, G.M., Gylys, K.H., 2008. Co-
 5689 localization of amyloid beta and tau pathology in Alzheimer's disease synaptosomes. *Am. J.*
 5690 *Pathol.* 172, 1683–1692. <https://doi.org/10.2353/ajpath.2008.070829>
- 5691 Ferreira, S.T., Lourenco, M. V., Oliveira, M.M., De Felice, F.G., 2015. Soluble amyloid-B
 5692 oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. *Front.*
 5693 *Cell. Neurosci.* 9, 1–17. <https://doi.org/10.3389/fncel.2015.00191>
- 5694 Ferreira, S.T., Vieira, M.N.N., De Felice, F.G., 2007. Soluble protein oligomers as emerging toxins
 5695 in Alzheimer's and other amyloid diseases, in: *IUBMB Life*. pp. 332–345.
 5696 <https://doi.org/10.1080/15216540701283882>
- 5697 Ferretti, M., Allard, S., Partridge, V., Ducatenzeiler, A., Cuello, a C., 2012. Minocycline corrects
 5698 early, pre-plaque neuroinflammation and inhibits BACE-1 in a transgenic model of
 5699 Alzheimer's disease-like amyloid pathology. *J. Neuroinflammation* 9, 62.
 5700 <https://doi.org/10.1186/1742-2094-9-62>
- 5701 Ferretti, M.T., Partridge, V., C. Leon, W., Canneva, F., Allard, S., N. Arvanitis, D., Vercauteren,
 5702 F., Houle, D., Ducatenzeiler, A., L. Klein, W., G. Glabe, C., Szyf, M., C. Cuello, A., 2011.
 5703 Transgenic Mice as a Model of Pre-Clinical Alzheimers Disease. *Curr. Alzheimer Res.* 8, 4–
 5704 23. <https://doi.org/10.2174/156720511794604561>
- 5705 Ferrucci, L., Fabbri, E., 2018. Inflammageing: chronic inflammation in ageing, cardiovascular
 5706 disease, and frailty. *Nat. Rev. Cardiol.* 15, 505–522. <https://doi.org/10.1038/s41569-018-0064-2>
- 5707
- 5708 Filipcik, P., Zilka, N., Bugos, O., Kucerak, J., Koson, P., Novak, P., Novak, M., 2012. First
 5709 transgenic rat model developing progressive cortical neurofibrillary tangles. *Neurobiol.*
 5710 *Aging* 33, 1448–1456. <https://doi.org/10.1016/j.neurobiolaging.2010.10.015>
- 5711 Finger, C.E., Moreno-Gonzalez, I., Gutierrez, A., Moruno-Manchon, J.F., McCullough, L.D.,
 5712 2022. Age-related immune alterations and cerebrovascular inflammation. *Mol. Psychiatry* 27,
 5713 803–818. <https://doi.org/10.1038/s41380-021-01361-1>

- 5714 Fiock, K.L., Smith, J.D., Crary, J.F., Hefti, M.M., 2019. B-Amyloid and Tau Pathology in the
5715 Aging Feline Brain. *J. Comp. Neurol.* cne.24741. <https://doi.org/10.1002/cne.24741>
- 5716 Fitzpatrick, A.W.P., Falcon, B., He, S., Murzin, A.G., Murshudov, G., Garringer, H.J., Crowther,
5717 R.A., Ghetti, B., Goedert, M., Scheres, S.H.W., 2017. Cryo-EM structures of tau filaments
5718 from Alzheimer's disease. *Nature* 547, 185.
- 5719 Fleisher, A.S., Chen, K., Quiroz, Y.T., Jakimovich, L.J., Gutierrez Gomez, M., Langois, C.M.,
5720 Langbaum, J.B.S., Roontiva, A., Thiyyagura, P., Lee, W., Ayutyanont, N., Lopez, L.,
5721 Moreno, S., Muñoz, C., Tirado, V., Acosta-Baena, N., Fagan, A.M., Giraldo, M., Garcia, G.,
5722 Huentelman, M.J., Tariot, P.N., Lopera, F., Reiman, E.M., 2015. Associations between
5723 biomarkers and age in the presenilin 1 E280A autosomal dominant Alzheimer disease
5724 kindred: A cross-sectional study. *JAMA Neurol.* 72, 316–324.
5725 <https://doi.org/10.1001/jamaneurol.2014.3314>
- 5726 Flood, D.G., Lin, Y.G., Lang, D.M., Trusko, S.P., Hirsch, J.D., Savage, M.J., Scott, R.W.,
5727 Howland, D.S., 2009. A transgenic rat model of Alzheimer's disease with extracellular A β
5728 deposition. *Neurobiol. Aging* 30, 1078–1090.
5729 <https://doi.org/10.1016/j.neurobiolaging.2007.10.006>
- 5730 Flores-Aguilar, L., Iulita, M.F., Kovacs, O., Torres, M.D., Levi, S.M., Zhang, Y., Askenazi, M.,
5731 Wisniewski, T., Busciglio, J., Cuellar, A.C., 2020. Evolution of neuroinflammation across the
5732 lifespan of individuals with down syndrome. *Brain* 143, 3653–3671.
5733 <https://doi.org/10.1093/brain/awaa326>
- 5734 Florian, H., Wang, D., Arnold, S.E., Boada, M., Guo, Q., Jin, Z., Zheng, H., Fisseha, N., Kalluri,
5735 H.V., Rendenbach-Mueller, B., Budur, K., Gold, M., 2023. Tilavonemab in early Alzheimer's
5736 disease: results from a phase 2, randomized, double-blind study. *Brain*.
5737 <https://doi.org/10.1093/brain/awad024>
- 5738 Foidl, B.M., Humpel, C., 2018. Differential Hyperphosphorylation of Tau-S199, -T231 and -S396
5739 in Organotypic Brain Slices of Alzheimer Mice. A Model to Study Early Tau
5740 Hyperphosphorylation Using Okadaic Acid. *Front. Aging Neurosci.* 10, 113.
5741 <https://doi.org/10.3389/fnagi.2018.00113>
- 5742 Fortea, J., Vilaplana, E., Alcolea, D., Carmona-Iragui, M., Sánchez-Saudinos, M.B., Sala, I.,
5743 Antón-Aguirre, S., González, S., Medrano, S., Pegueroles, J., Morenas, E., Clarimón, J.,
5744 Blesa, R., Lleó, A., 2014. Cerebrospinal fluid β -amyloid and phospho-tau biomarker
5745 interactions affecting brain structure in preclinical Alzheimer disease. *Ann. Neurol.* 76, 223–
5746 230. <https://doi.org/10.1002/ana.24186>
- 5747 Fortress, A.M., Buhusi, M., Helke, K.L., Granholm, A.C.E., 2011. Cholinergic degeneration and
5748 alterations in the TrkA and p75NTR balance as a result of pro-NGF injection into aged rats.
5749 *J. Aging Res.* 2011. <https://doi.org/10.4061/2011/460543>
- 5750 Foster, T.C., Norris, C.M., 1997. Age-associated changes in Ca²⁺-dependent processes: Relation
5751 to hippocampal synaptic plasticity. *Hippocampus* 7, 602–612.
5752 [https://doi.org/10.1002/\(SICI\)1098-1063\(1997\)7:6<602::AID-HIPO3>3.0.CO;2-G](https://doi.org/10.1002/(SICI)1098-1063(1997)7:6<602::AID-HIPO3>3.0.CO;2-G)
- 5753 Francis, P.T., Palmer, A.M., Snape, M., Wilcock, G.K., 1999. The cholinergic hypothesis of
5754 Alzheimer's disease: A review of progress. *J. Neurol. Neurosurg. Psychiatry* 66, 137–147.
5755 <https://doi.org/10.1136/jnnp.66.2.137>
- 5756 Franco, R., Cedazo-Minguez, A., 2014. Successful therapies for Alzheimer's disease: Why so
5757 many in animal models and none in humans? *Front. Pharmacol.* 5 JUN, 1–13.
5758 <https://doi.org/10.3389/fphar.2014.00146>
- 5759 Frank, S., Clavaguera, F., Tolnay, M., 2008. Tauopathy models and human neuropathology:

- 5760 Similarities and differences. *Acta Neuropathol.* 115, 39–53. [https://doi.org/10.1007/s00401-](https://doi.org/10.1007/s00401-007-0291-9)
5761 007-0291-9
- 5762 Frautschy, S.A., Cole, G.M., Baird, A., 1992. Phagocytosis and deposition of vascular beta-
5763 amyloid in rat brains injected with Alzheimer beta-amyloid. *Am. J. Pathol.* 140, 1389–99.
- 5764 Friedhoff, P., von Bergen, M., Mandelkow, E.-M., Davies, P., Mandelkow, E., 1998. A nucleated
5765 assembly mechanism of Alzheimer paired helical filaments. *Proc. Natl. Acad. Sci.* 95, 15712–
5766 15717. <https://doi.org/10.1073/pnas.95.26.15712>
- 5767 Friedhoff, P., Von Bergen, M., Mandelkow, E.M., Mandelkow, E., 2000. Structure of tau protein
5768 and assembly into paired helical filaments. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1502,
5769 122–132. [https://doi.org/10.1016/S0925-4439\(00\)00038-7](https://doi.org/10.1016/S0925-4439(00)00038-7)
- 5770 Frisoni, G.B., Fox, N.C., Jack, C.R., Scheltens, P., Thompson, P.M., 2010. The clinical use of
5771 structural MRI in Alzheimer disease. *Nat. Rev. Neurol.* 6, 67–77.
5772 <https://doi.org/10.1038/nrneurol.2009.215>
- 5773 Fukuyama, R., Izumoto, T., Fushiki, S., 2001. The cerebrospinal fluid level of glial fibrillary acidic
5774 protein is increased in cerebrospinal fluid from Alzheimer's disease patients and correlates
5775 with severity of dementia. *Eur. Neurol.* 46, 35–38. <https://doi.org/10.1159/000050753>
- 5776 Fuller, K.N.Z., Thyfault, J.P., 2021. Barriers in translating preclinical rodent exercise metabolism
5777 findings to human health. *J. Appl. Physiol.* 130, 182–192.
5778 <https://doi.org/10.1152/JAPPLPHYSIOL.00683.2020>
- 5779 Furcila, D., Domínguez-Álvaro, M., DeFelipe, J., Alonso-Nanclares, L., 2019. Subregional
5780 Density of Neurons, Neurofibrillary Tangles and Amyloid Plaques in the Hippocampus of
5781 Patients With Alzheimer's Disease. *Front. Neuroanat.* 13, 1–11.
5782 <https://doi.org/10.3389/fnana.2019.00099>
- 5783 Furukawa, K., Sopher, B.L., Rydel, R.E., Begley, J.G., Pham, D.G., Martin, G.M., Fox, M.,
5784 Mattson, M.P., 1996. Increased activity-regulating and neuroprotective efficacy of α -
5785 secretase-derived secreted amyloid precursor protein conferred by a C- terminal heparin-
5786 binding domain. *J. Neurochem.* 67, 1882–1896. [https://doi.org/10.1046/j.1471-](https://doi.org/10.1046/j.1471-4159.1996.67051882.x)
5787 4159.1996.67051882.x
- 5788 Gaetani, L., Blennow, K., Calabresi, P., Di Filippo, M., Parnetti, L., Zetterberg, H., 2019.
5789 Neurofilament light chain as a biomarker in neurological disorders. *J. Neurol. Neurosurg.*
5790 *Psychiatry* 870–881. <https://doi.org/10.1136/jnnp-2018-320106>
- 5791 Galeano, P., Martino Adami, P. V, Do Carmo, S., Blanco, E., Rotondaro, C., Capani, F., Castaño,
5792 E.M., Cuello, A.C., Morelli, L., Castano, E.M., Cuello, A.C., Morelli, L., Castaño, E.M.,
5793 Cuello, A.C., Morelli, L., 2014. Longitudinal analysis of the behavioral phenotype in a novel
5794 transgenic rat model of early stages of Alzheimer's disease. *Front. Behav. Neurosci.* 8, 1–15.
5795 <https://doi.org/10.3389/fnbeh.2014.00321>
- 5796 Gamache, J., Benzow, K., Forster, C., Kemper, L., Hlynialuk, C., Furrow, E., Ashe, K.H., Koob,
5797 M.D., 2019. Factors other than hTau overexpression that contribute to tauopathy-like
5798 phenotype in rTg4510 mice. *Nat. Commun.* 10, 1–12. [https://doi.org/10.1038/s41467-019-](https://doi.org/10.1038/s41467-019-10428-1)
5799 10428-1
- 5800 Gamblin, T.C., Chen, F., Zambrano, A., Abraha, A., Lagalwar, S., Guillozet, A.L., Lu, M., Fu, Y.,
5801 Garcia-Sierra, F., LaPointe, N., Miller, R., Berry, R.W., Binder, L.I., Cryns, V.L., 2003.
5802 Caspase cleavage of tau: Linking amyloid and neurofibrillary tangles in Alzheimer's disease.
5803 *Proc. Natl. Acad. Sci.* 100, 10032–10037. <https://doi.org/10.1073/pnas.1630428100>
- 5804 Garwood, C.J., Ratcliffe, L.E., Simpson, J.E., Heath, P.R., Ince, P.G., Wharton, S.B., 2017.
5805 Review: Astrocytes in Alzheimer's disease and other age-associated dementias: a supporting

- player with a central role. *Neuropathol. Appl. Neurobiol.* <https://doi.org/10.1111/nan.12338>
- Gasparini, L., Ongini, E., Wenk, G., 2004. Non-steroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease: Old and new mechanisms of action. *J. Neurochem.* 91, 521–536. <https://doi.org/10.1111/j.1471-4159.2004.02743.x>
- Ghosal, K., Fan, Q., Dawson, H.N., Pimplikar, S.W., 2016. Tau protein mediates APP Intracellular domain (AICD)-Induced Alzheimer's-Like pathological features in mice. *PLoS One* 11, 1–22. <https://doi.org/10.1371/journal.pone.0159435>
- Giacobini, E., Cuellar, A.C., Fisher, A., 2022. Reimagining cholinergic therapy for Alzheimer's disease. *Brain* 145, 2250–2275. <https://doi.org/10.1093/brain/awac096>
- Giannakopoulos, P., Herrmann, F.R., Bussière, T., Bouras, C., Kovari, E., Perl, D.P., Morrison, J.H., Gold, G., Hof, P.R., 2003. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology* 60, 1495–1500. <https://doi.org/10.1212/01.WNL.0000063311.58879.01>
- Glabe, C.G., 2008. Structural classification of toxic amyloid oligomers. *J. Biol. Chem.* <https://doi.org/10.1074/jbc.R800016200>
- Glennner, G.G., Wong, C.W., 1984. Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochem. Biophys. Res. Commun.* 122, 1131–1135. [https://doi.org/10.1016/0006-291X\(84\)91209-9](https://doi.org/10.1016/0006-291X(84)91209-9)
- Glodzik, L., de Santi, S., Tsui, W.H., Mosconi, L., Zinkowski, R., Pirraglia, E., Wang, H.Y., Li, Y., Rich, K.E., Zetterberg, H., Blennow, K., Mehta, P., de Leon, M.J., 2011. Phosphorylated tau 231, memory decline and medial temporal atrophy in normal elders. *Neurobiol. Aging* 32, 2131–2141. <https://doi.org/10.1016/j.neurobiolaging.2009.12.026>
- Goate, A., Chartier-Harlin, M. et al. M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., Hardy, J., 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349, 704–706. <https://doi.org/10.1038/349704a0>
- Goedert, M., 2018. Neurodegeneration and the Ordered Assembly of Tau. *Mol. Cell. Basis Neurodegener. Dis. Underlying Mech.* 81–98. <https://doi.org/10.1016/B978-0-12-811304-2.00004-3>
- Goedert, M., Jakes, R., Spillantini, M.G., Hasegawa, M., Smith, M.J., Crowther, R.A., 1996. Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans. *Nature*. <https://doi.org/10.1038/383550a0>
- Goedert, M., Masuda-Suzukake, M., Falcon, B., 2017. Like prions: The propagation of aggregated tau and α -synuclein in neurodegeneration. *Brain* 140, 266–278. <https://doi.org/10.1093/brain/aww230>
- Goedert, M., Spillantini, M.G., 2017. Propagation of Tau aggregates. *Mol. Brain* 10. <https://doi.org/10.1186/s13041-017-0298-7>
- Goedert, M., Spillantini, M.G., 2000. Tau mutations in frontotemporal dementia FTDP-17 and their relevance for Alzheimer's disease. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1502, 110–121. [https://doi.org/10.1016/S0925-4439\(00\)00037-5](https://doi.org/10.1016/S0925-4439(00)00037-5)
- Goedert, M., Spillantini, M.G., Cairns, N.J., Crowther, R.A., 1992. Tau proteins of alzheimer paired helical filaments: Abnormal phosphorylation of all six brain isoforms. *Neuron* 8, 159–168. [https://doi.org/10.1016/0896-6273\(92\)90117-V](https://doi.org/10.1016/0896-6273(92)90117-V)
- Goedert, M., Spillantini, M.G., Crowther, R.A., 1991. Tau Proteins and Neurofibrillary Degeneration. *Brain Pathol.* 1, 279–286. <https://doi.org/10.1111/j.1750-3639.1991.tb00671.x>

- 5852 Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D., Crowther, R.A., 1989. Multiple isoforms
5853 of human microtubule-associated protein tau: sequences and localization in neurofibrillary
5854 tangles of Alzheimer's disease. *Neuron* 3, 519–526. [https://doi.org/10.1016/0896-](https://doi.org/10.1016/0896-6273(89)90210-9)
5855 [6273\(89\)90210-9](https://doi.org/10.1016/0896-6273(89)90210-9)
- 5856 Goetzl, E.J., Kapogiannis, D., Schwartz, J.B., Lobach, I. V., Goetzl, L., Abner, E.L., Jicha, G.A.,
5857 Karydas, A.M., Boxer, A., Miller, B.L., 2016. Decreased synaptic proteins in neuronal
5858 exosomes of frontotemporal dementia and Alzheimer's disease. *FASEB J.* 30, 4141–4148.
5859 <https://doi.org/10.1096/fj.201600816R>
- 5860 Gómez-Isla, T., Hollister, R., West, H., Mui, S., Growdon, J.H., Petersen, R.C., Parisi, J.E.,
5861 Hyman, B.T., 1997. Neuronal loss correlates with but exceeds neurofibrillary tangles in
5862 Alzheimer's disease. *Ann. Neurol.* 41, 17–24. <https://doi.org/10.1002/ana.410410106>
- 5863 Gómez-Ramos, A., Díaz-Hernández, M., Cuadros, R., Hernández, F., Avila, J., 2006. Extracellular
5864 tau is toxic to neuronal cells. *FEBS Lett.* 580, 4842–4850.
5865 <https://doi.org/10.1016/j.febslet.2006.07.078>
- 5866 Gómez-Ramos, A., Díaz-Hernández, M., Rubio, A., Miras-Portugal, M.T., Avila, J., 2008.
5867 Extracellular tau promotes intracellular calcium increase through M1 and M3 muscarinic
5868 receptors in neuronal cells. *Mol. Cell. Neurosci.* 37, 673–681.
5869 <https://doi.org/10.1016/j.mcn.2007.12.010>
- 5870 Gonçalves-Ribeiro, J., Pina, C.C., Sebastião, A.M., Vaz, S.H., 2019. Glutamate Transporters in
5871 Hippocampal LTD/LTP: Not Just Prevention of Excitotoxicity. *Front. Cell. Neurosci.* 13, 1–
5872 7. <https://doi.org/10.3389/fncel.2019.00357>
- 5873 Gonzalez-Reyes, R.E., Nava-Mesa, M.O., Vargas-Sanchez, K., Ariza-Salamanca, D., Mora-
5874 Munoz, L., 2017. Involvement of Astrocytes in Alzheimer's Disease from a
5875 Neuroinflammatory and Oxidative Stress Perspective. *Front. Mol. Neurosci.* 10, 427.
5876 <https://doi.org/10.3389/fnmol.2017.00427>
- 5877 Goodman, L., 1953. Alzheimer's disease; a clinico-pathologic analysis of twenty-three cases with
5878 a theory on pathogenesis. *J. Nerv. Ment. Dis.* 118, 97–130.
- 5879 Gorbunova, V., Seluanov, A., Mao, Z., Hine, C., 2007a. Changes in DNA repair during aging.
5880 *Nucleic Acids Res.* <https://doi.org/10.1093/nar/gkm756>
- 5881 Gorbunova, V., Seluanov, A., Mao, Z., Hine, C., 2007b. The Role of DNA Methylation in Aging,
5882 Rejuvenation, and Age-Related Disease. *Nucleic Acids Res.*
5883 <https://doi.org/10.1089/rej.2012.1324>
- 5884 Götz, J., Chen, F., Barmettler, R., Nitsch, R.M., 2001. Tau filament formation in transgenic mice
5885 expressing P301L tau. *J. Biol. Chem.* 276, 529–534. <https://doi.org/10.1074/jbc.M006531200>
- 5886 Gotz, J., Chen, F., van Dorpe, J., Nitsch, R.M., 2001. Formation of Neurofibrillary Tangles in
5887 P301L Tau Transgenic Mice Induced by Abeta 42 Fibrils. *Science (80-.)*. 293, 1491–1495.
5888 <https://doi.org/10.1126/science.1062097>
- 5889 Götz, J.J., Götz, J., 2019. Experimental Models of Tauopathy – From Mechanisms to Therapies,
5890 in: Takashima, A., Wozozin, B., Buee, L. (Eds.), *Advances in Experimental Medicine and*
5891 *Biology*. Springer Singapore, Singapore, pp. 381–391. [https://doi.org/10.1007/978-981-32-](https://doi.org/10.1007/978-981-32-9358-8_28)
5892 [9358-8_28](https://doi.org/10.1007/978-981-32-9358-8_28)
- 5893 Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J.P.,
5894 Haroutunian, V., Buxbaum, J.D., Xu, H., Greengard, P., Relkin, N.R., 2000. Intraneuronal
5895 Aβ42 accumulation in human brain. *Am. J. Pathol.* 156, 15–20.
5896 [https://doi.org/10.1016/S0002-9440\(10\)64700-1](https://doi.org/10.1016/S0002-9440(10)64700-1)
- 5897 Graham, D.L., Gray, A.J., Joyce, J.A., Yu, D., O'Moore, J., Carlson, G.A., Shearman, M.S.,

- 5898 Dellovade, T.L., Hering, H., 2014. Increased O-GlcNAcylation reduces pathological tau
5899 without affecting its normal phosphorylation in a mouse model of tauopathy.
5900 *Neuropharmacology* 79, 307–313. <https://doi.org/10.1016/j.neuropharm.2013.11.025>
- 5901 Grant, S.M., Ducatenzeiler, A., Szyf, M., Cuellar, A.C., 2000. Abeta immunoreactive material is
5902 present in several intracellular compartments in transfected, neuronally differentiated, P19
5903 cells expressing the human amyloid beta-protein precursor. *J Alzheimers Dis* 2, 207–222.
- 5904 Griffin, W.S.T., Sheng, J.G., Roberts, G.W., Mrak, R.E., 1995. Interleukin-1 Expression in
5905 Different Plaque Types in Alzheimer's Disease: Significance in Plaque Evolution. *J.*
5906 *Neuropathol. Exp. Neurol.* 54, 276–281. [https://doi.org/10.1097/00005072-199503000-](https://doi.org/10.1097/00005072-199503000-00014)
5907 00014
- 5908 Grover, L.M., Teyler, T.J., 1990. Two components of long-term potentiation induced by different
5909 patterns of afferent activation. *Nature* 347, 477–479. <https://doi.org/10.1038/347477a0>
- 5910 Grubman, A., Chew, G., Ouyang, J.F., Sun, G., Choo, X.Y., McLean, C., Simmons, R.K.,
5911 Buckberry, S., Vargas-Landin, D.B., Poppe, D., Pflueger, J., Lister, R., Rackham, O.J.L.,
5912 Petretto, E., Polo, J.M., 2019. A single-cell atlas of entorhinal cortex from individuals with
5913 Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat. Neurosci.* 22,
5914 2087–2097. <https://doi.org/10.1038/s41593-019-0539-4>
- 5915 Grudzien, A., Shaw, P., Weintraub, S., Bigio, E., Mash, D.C., Mesulam, M.M., 2007. Locus
5916 coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early
5917 Alzheimer's disease. *Neurobiol. Aging* 28, 327–335.
5918 <https://doi.org/10.1016/j.neurobiolaging.2006.02.007>
- 5919 Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y.C., Zaidi, M.S., Wisniewski, H.M., 1986a.
5920 Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J.*
5921 *Biol. Chem.* 261, 6084–9. <https://doi.org/10.1074/jbc.M111.271320>
- 5922 Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., Quinlan, M., Wisniewski, H., Binder, L., 1986b.
5923 Abnormal phosphorylation of the microtubule-associated protein? (tau) in Alzheimer
5924 cytoskeletal pathology. *Proc Nat Acad Sci USA* 83, 4913–4917.
5925 <https://doi.org/10.1097/00002093-198701030-00020>
- 5926 Guerreiro, R., Bras, J., 2015. The age factor in Alzheimer's disease. *Genome Med.* 7, 1–3.
5927 <https://doi.org/10.1186/s13073-015-0232-5>
- 5928 Guillozet, A.L., Weintraub, S., Mash, D.C., Marsel Mesulam, M., 2003. Neurofibrillary tangles,
5929 amyloid, and memory in aging and mild cognitive impairment. *Arch. Neurol.* 60, 729–736.
5930 <https://doi.org/10.1001/archneur.60.5.729>
- 5931 Gulisano, W., Maugeri, D., Baltrons, M.A., Fà, M., Amato, A., Palmeri, A., D'Adamio, L., Grassi,
5932 C., Devanand, D.P., Honig, L.S., Puzzo, D., Arancio, O., 2018. Role of Amyloid- β and Tau
5933 Proteins in Alzheimer's Disease: Confuting the Amyloid Cascade. *J. Alzheimer's Dis.*
5934 <https://doi.org/10.3233/JAD-179935>
- 5935 Guo, J.-P., Arai, T., Miklossy, J., McGeer, P.L., 2006. Abeta and tau form soluble complexes that
5936 may promote self aggregation of both into the insoluble forms observed in Alzheimer's
5937 disease. *Proc. Natl. Acad. Sci.* 103, 1953–1958. <https://doi.org/10.1073/pnas.0509386103>
- 5938 Guo, Q., Li, H., Cole, A.L., Hur, J.Y., Li, Y., Zheng, H., 2013. Modeling alzheimer's disease in
5939 mouse without mutant protein overexpression: Cooperative and independent effects of A β
5940 and tau. *PLoS One* 8, 1–14. <https://doi.org/10.1371/journal.pone.0080706>
- 5941 Guo, T., Noble, W., Hanger, D.P., 2017. Roles of tau protein in health and disease. *Acta*
5942 *Neuropathol.* 133, 665–704. <https://doi.org/10.1007/s00401-017-1707-9>
- 5943 Hall, H., Iulita, M.F., Gubert, P., Flores Aguilar, L., Ducatenzeiler, A., Fisher, A., Cuellar, A.C.,

2018. AF710B, an M1/sigma-1 receptor agonist with long-lasting disease-modifying properties in a transgenic rat model of Alzheimer's disease. *Alzheimer's Dement.* 14, 811–823. <https://doi.org/10.1016/j.jalz.2017.11.009>
- Hampel, H., Mesulam, M.-M., Cuello, A.C., Farlow, M.R., Giacobini, E., Grossberg, G.T., Khachaturian, A.S., Vergallo, A., Cavedo, E., Snyder, P.J., Khachaturian, Z.S., 2018. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain - a J. Neurol.* 1–17. <https://doi.org/10.1093/brain/awy132>
- Hanes, J., Kovac, A., Kvartsberg, H., Kontseikova, E., Fialova, L., Katina, S., Kovacech, B., Stevens, E., Hort, J., Vyhnalek, M., Boonkamp, L., Novak, M., Zetterberg, H., Hansson, O., Scheltens, P., Blennow, K., Teunissen, C.E., Zilka, N., 2020. Evaluation of a novel immunoassay to detect p-Tau Thr127 in the CSF to distinguish Alzheimer disease from other dementias. *Neurology* 95, e3026–e3035. <https://doi.org/10.1212/WNL.00000000000010814>
- Hanes, J., Zilka, N., Bartkova, M., Caletkova, M., Dobrota, D., Novak, M., 2009. Rat tau proteome consists of six tau isoforms: Implication for animal models of human tauopathies. *J. Neurochem.* 108, 1167–1176. <https://doi.org/10.1111/j.1471-4159.2009.05869.x>
- Hanseeuw, B.J., Betensky, R.A., Jacobs, H.I.L., Schultz, A.P., Sepulcre, J., Becker, J.A., Cosio, D.M.O., Farrell, M., Quiroz, Y.T., Mormino, E.C., Buckley, R.F., Papp, K. V., Amariglio, R.A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G.A., Chhatwal, J.P., Rentz, D.M., Sperling, R.A., Johnson, K., 2019. Association of Amyloid and Tau with Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. *JAMA Neurol.* 76, 915–924. <https://doi.org/10.1001/jamaneurol.2019.1424>
- Hansson, O., Edelmayer, R.M., Boxer, A.L., Carrillo, M.C., Mielke, M.M., Rabinovici, G.D., Salloway, S., Sperling, R., Zetterberg, H., Teunissen, C.E., 2022. The Alzheimer's Association appropriate use recommendations for blood biomarkers in Alzheimer's disease. *Alzheimer's Dement.* 1–18. <https://doi.org/10.1002/alz.12756>
- Hanzel, C.E., Pichet-Binette, A., Pimentel, L.S.B., Iulita, M.F., Allard, S., Ducatenzeiler, A., Do Carmo, S., Cuello, A.C., 2014. Neuronal driven pre-plaque inflammation in a transgenic rat model of Alzheimer's disease. *Neurobiol. Aging* 35, 2249–2262. <https://doi.org/10.1016/j.neurobiolaging.2014.03.026>
- Hasegawa, M., Morishima-Kawashima, M., Takio, K., Suzuki, M., Titani, K., Ihara, Y., 1992. Protein sequence and mass spectrometric analyses of tau in the Alzheimer's disease brain. *J. Biol. Chem.* 267, 17047–17054.
- Hassan-Abdi, R., Brenet, A., Bennis, M., Yanicostas, C., Soussi-Yanicostas, N., 2019. Neurons Expressing Pathological Tau Protein Trigger Dramatic Changes in Microglial Morphology and Dynamics. *Front. Neurosci.* 13, 1–11. <https://doi.org/10.3389/fnins.2019.01199>
- Hatami, A., Monjaze, S., Milton, S., Glabe, C.G., 2017. Familial Alzheimer's disease mutations within the amyloid precursor protein alter the aggregation and conformation of the amyloid- β peptide. *J. Biol. Chem.* 292, 3172–3185. <https://doi.org/10.1074/jbc.M116.755264>
- Heggland, I., Storkaas, I.S., Soligard, H.T., Kobro-Flatmoen, A., Witter, M.P., 2015. Stereological estimation of neuron number and plaque load in the hippocampal region of a transgenic rat model of Alzheimer's disease. *Eur. J. Neurosci.* 41, 1245–1262. <https://doi.org/10.1111/ejn.12876>
- Henley, D.B., May, P.C., Dean, R.A., Siemers, E.R., 2009. Development of semagacestat (LY450139), a functional γ -secretase inhibitor, for the treatment of Alzheimer's disease. *Expert Opin. Pharmacother.* 10, 1657–1664. <https://doi.org/10.1517/14656560903044982>
- Héraud, C., Goufak, D., Ando, K., Leroy, K., Suain, V., Yilmaz, Z., De Decker, R., Authélet, M.,

- 5990 Laporte, V., Octave, J.N., Brion, J.P., 2014. Increased misfolding and truncation of tau in
 5991 APP/PS1/tau transgenic mice compared to mutant tau mice. *Neurobiol. Dis.* 62, 100–112.
 5992 <https://doi.org/10.1016/j.nbd.2013.09.010>
- 5993 Hernández, F., Avila, J., 2007. Tauopathies. *Cell. Mol. Life Sci.* 64, 2219–2233.
 5994 <https://doi.org/10.1007/s00018-007-7220-x>
- 5995 Hernández, F., Gómez de Barreda, E., Fuster-Matanzo, A., Lucas, J.J., Avila, J., 2010. GSK3: A
 5996 possible link between beta amyloid peptide and tau protein. *Exp. Neurol.* 223, 322–325.
 5997 <https://doi.org/10.1016/j.expneurol.2009.09.011>
- 5998 Hernandez, F., Lucas, J.J., Avila, J., 2012. GSK3 and Tau: Two Convergence Points in
 5999 Alzheimer's Disease. *J. Alzheimer's Dis.* 33, S141–S144. [https://doi.org/10.3233/JAD-2012-](https://doi.org/10.3233/JAD-2012-129025)
 6000 129025
- 6001 Hernández, F., Merchán-Rubira, J., Vallés-Saiz, L., Rodríguez-Matellán, A., Avila, J., 2020.
 6002 Differences Between Human and Murine Tau at the N-terminal End. *Front. Aging Neurosci.*
 6003 12, 1–6. <https://doi.org/10.3389/fnagi.2020.00011>
- 6004 Höglinger, G.U., Respondek, G., Kovacs, G.G., 2018. New classification of tauopathies. *Rev.*
 6005 *Neurol. (Paris)*. 174, 664–668. <https://doi.org/10.1016/j.neurol.2018.07.001>
- 6006 Hohman, T.J., Bell, S.P., Jefferson, A.L., 2015. The role of vascular endothelial growth factor in
 6007 neurodegeneration and cognitive decline: Exploring interactions with biomarkers of
 6008 Alzheimer disease. *JAMA Neurol.* 72, 520–529.
 6009 <https://doi.org/10.1001/jamaneurol.2014.4761>
- 6010 Holland, N., Jones, P.S., Savulich, G., Wiggins, J.K., Hong, Y.T., Fryer, T.D., Manavaki, R.,
 6011 Sephton, S.M., Boros, I., Malpetti, M., Hezemans, F.H., Aigbirhio, F.I., Coles, J.P., O'Brien,
 6012 J., Rowe, J.B., 2020. Synaptic Loss in Primary Tauopathies Revealed by [11C]UCB-J
 6013 Positron Emission Tomography. *Mov. Disord.* mds.28188.
 6014 <https://doi.org/10.1002/mds.28188>
- 6015 Holloway, O.G., Canty, A.J., King, A.E., Ziebell, J.M., 2019. Rod microglia and their role in
 6016 neurological diseases. *Semin. Cell Dev. Biol.* 94, 96–103.
 6017 <https://doi.org/10.1016/j.semcdb.2019.02.005>
- 6018 Honig, L.S., Vellas, B., Woodward, M., Boada, M., Bullock, R., Borrie, M., Hager, K., Andreasen,
 6019 N., Scarpini, E., Liu-Seifert, H., Case, M., Dean, R.A., Hake, A., Sundell, K., Poole
 6020 Hoffmann, V., Carlson, C., Khanna, R., Mintun, M., DeMattos, R., Selzler, K.J., Siemers, E.,
 6021 2018. Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease. *N. Engl. J. Med.*
 6022 378, 321–330. <https://doi.org/10.1056/NEJMoa1705971>
- 6023 Hooper, C., Killick, R., Lovestone, S., 2008. The GSK3 hypothesis of Alzheimer's disease. *J.*
 6024 *Neurochem.* 104, 1433–1439. <https://doi.org/10.1111/j.1471-4159.2007.05194.x>
- 6025 Hoover, B.R., Reed, M.N., Su, J., Penrod, R.D., Kotilinek, L.A., Grant, M.K., Pitstick, R., Carlson,
 6026 G.A., Lanier, L.M., Yuan, L.L., Ashe, K.H., Liao, D., 2010. Tau Mislocalization to Dendritic
 6027 Spines Mediates Synaptic Dysfunction Independently of Neurodegeneration. *Neuron* 68,
 6028 1067–1081. <https://doi.org/10.1016/j.neuron.2010.11.030>
- 6029 Hoshi, A., Yamamoto, T., Shimizu, K., Ugawa, Y., Nishizawa, M., Takahashi, H., Kakita, A.,
 6030 2012. Characteristics of aquaporin expression surrounding senile plaques and cerebral
 6031 amyloid angiopathy in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 71, 750–759.
 6032 <https://doi.org/10.1097/NEN.0b013e3182632566>
- 6033 Hosokawa, M., Fujisawa, H., Ax, S., Zahn-Daimler, G., Zahn, R.K., 2000. Age-associated DNA
 6034 damage is accelerated in the senescence-accelerated mice. *Mech. Ageing Dev.* 118, 61–70.
 6035 [https://doi.org/10.1016/S0047-6374\(00\)00158-5](https://doi.org/10.1016/S0047-6374(00)00158-5)

- 6036 Hrnkova, M., Zilka, N., Minichova, Z., Koson, P., Novak, M., 2007. Neurodegeneration caused
6037 by expression of human truncated tau leads to progressive neurobehavioural impairment in
6038 transgenic rats. *Brain Res.* 1130, 206–213. <https://doi.org/10.1016/j.brainres.2006.10.085>
- 6039 Hu, N.W., Smith, I.M., Walsh, D.M., Rowan, M.J., 2008. Soluble amyloid- β peptides potentially
6040 disrupt hippocampal synaptic plasticity in the absence of cerebrovascular dysfunction in vivo.
6041 *Brain* 131, 2414–2424. <https://doi.org/10.1093/brain/awn174>
- 6042 Huang, C.C., Chung, C.M., Leu, H.B., Lin, L.Y., Chiu, C.C., Hsu, C.Y., Chiang, C.H., Huang,
6043 P.H., Chen, T.J., Lin, S.J., Chen, J.W., Chan, W.L., 2014. Diabetes mellitus and the risk of
6044 Alzheimer's disease: A nationwide population-based study. *PLoS One* 9.
6045 <https://doi.org/10.1371/journal.pone.0087095>
- 6046 Huang, M., Macdonald, J., Lavenir, I., Chen, R., Craxton, M., Slavik-Smith, E., Davies, S.W.,
6047 Goedert, M., 2022. Increase in Tau Pathology in P290S Mapt Knock-In Mice Crossed with
6048 App NL-G-F Mice. *eneuro* 9, ENEURO.0247-22.2022.
6049 <https://doi.org/10.1523/ENEURO.0247-22.2022>
- 6050 Hurtado, D.E., Molina-Porcel, L., Iba, M., Aboagye, A.K., Paul, S.M., Trojanowski, J.Q., Lee,
6051 V.M.Y., 2010. A β accelerates the spatiotemporal progression of tau pathology and augments
6052 tau amyloidosis in an Alzheimer mouse model. *Am. J. Pathol.* 177, 1977–1988.
6053 <https://doi.org/10.2353/ajpath.2010.100346>
- 6054 Iannuzzi, F., Sirabella, R., Canu, N., Maier, T.J., Annunziato, L., Matrone, C., 2020. Fyn Tyrosine
6055 Kinase Elicits Amyloid Precursor Protein Tyr682 Phosphorylation in Neurons from
6056 Alzheimer's Disease Patients. *Cells* 9, 1–21. <https://doi.org/10.3390/cells9081807>
- 6057 Iqbal, K., Del C. Alonso, A., Chen, S., Chohan, M.O., El-Akkad, E., Gong, C.X., Khatoon, S., Li,
6058 B., Liu, F., Rahman, A., Tanimukai, H., Grundke-Iqbal, I., 2005. Tau pathology in Alzheimer
6059 disease and other tauopathies. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1739, 198–210.
6060 <https://doi.org/10.1016/j.bbadis.2004.09.008>
- 6061 Iqbal, K., Liu, F., Gong, C.-X., Grundke-Iqbal, I., 2010. Tau in Alzheimer Disease and Related
6062 Tauopathies. *Curr. Alzheimer Res.* 7, 656–664.
6063 <https://doi.org/10.2174/156720510793611592>
- 6064 Iqbal, K., Liu, F., Gong, C.X., 2016. Tau and neurodegenerative disease: The story so far. *Nat.*
6065 *Rev. Neurol.* 12, 15–27. <https://doi.org/10.1038/nrneurol.2015.225>
- 6066 Iqbal, K., Zaidi, T., Wen, G., Grundke-Iqbal, I., Merz, P., Shaikh, S., Wisniewski, H., Alafuzoff,
6067 I., Winblad, B., 1986. DEFECTIVE BRAIN MICROTUBULE ASSEMBLY IN
6068 ALZHEIMER'S DISEASE. *Lancet* 328, 421–426. [https://doi.org/10.1016/S0140-6736\(86\)92134-3](https://doi.org/10.1016/S0140-6736(86)92134-3)
- 6069
- 6070 Ittner, A., Chua, S.W., Bertz, J., Volkerling, A., van der Hoven, J., Gladbach, A., Przybyla, M.,
6071 Bi, M., van Hummel, A., Stevens, C.H., Ippati, S., Suh, L.S., Macmillan, A., Sutherland, G.,
6072 Kril, J.J., Silva, A.P.G., Mackay, J.P., Poljak, A., Delerue, F., Ke, Y.D., Ittner, L.M., 2016.
6073 Site-specific phosphorylation of tau inhibits amyloid- β toxicity in Alzheimer's mice. *Science*
6074 (80-.). 354, 904–908. <https://doi.org/10.1126/science.aah6205>
- 6075 Ittner, L.M., Götz, J., 2011. Amyloid- β and tau - A toxic pas de deux in Alzheimer's disease. *Nat.*
6076 *Rev. Neurosci.* 12, 67–72. <https://doi.org/10.1038/nrn2967>
- 6077 Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., Wölfling, H., Chieng, B.C.,
6078 Christie, M.J., Napier, I.A., Eckert, A., Staufenbiel, M., Hardeman, E., Götz, J., 2010.
6079 Dendritic function of tau mediates amyloid- β toxicity in Alzheimer's disease mouse models.
6080 *Cell* 142, 387–397. <https://doi.org/10.1016/j.cell.2010.06.036>
- 6081 Iulita, M.F., Allard, S., Richter, L., Munter, L.-M., Ducatenzeiler, A., Weise, C., Do Carmo, S.,

- 6082 Klein, W.L., Multhaup, G., Cuellar, A.C., 2014. Intracellular A β pathology and early cognitive
6083 impairments in a transgenic rat overexpressing human amyloid precursor protein: a
6084 multidimensional study. *Acta Neuropathol. Commun.* 2, 61. [https://doi.org/10.1186/2051-](https://doi.org/10.1186/2051-5960-2-61)
6085 5960-2-61
- 6086 J. Whitehouse, P., L. Price, D., W. Clark, A., T. Coyle, J., R. DeLong, M., 2004. Alzheimer
6087 disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann.*
6088 *Neurol.* 10, 122–126. <https://doi.org/doi:10.1002/ana.410100203>
- 6089 Jablonski, A.M., Warren, L., Usenovic, M., Zhou, H., Sugam, J., Parmentier-Batteur, S., Voleti,
6090 B., 2021. Astrocytic expression of the Alzheimer's disease risk allele, ApoE ϵ 4, potentiates
6091 neuronal tau pathology in multiple preclinical models. *Sci. Rep.* 11, 3438.
6092 <https://doi.org/10.1038/s41598-021-82901-1>
- 6093 Jack, C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman,
6094 D.M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J.L., Montine, T., Phelps, C.,
6095 Rankin, K.P., Rowe, C.C., Scheltens, P., Siemers, E., Snyder, H.M., Sperling, R., Elliott, C.,
6096 Masliah, E., Ryan, L., Silverberg, N., 2018. NIA-AA Research Framework: Toward a
6097 biological definition of Alzheimer's disease. *Alzheimer's Dement.*
6098 <https://doi.org/10.1016/j.jalz.2018.02.018>
- 6099 Jack, C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Feldman, H.H., Frisoni, G.B., Hampel, H.,
6100 Jagust, W.J., Johnson, K.A., Knopman, D.S., Petersen, R.C., Scheltens, P., Sperling, R.A.,
6101 Dubois, B., 2016. A/T/N: An unbiased descriptive classification scheme for Alzheimer
6102 disease biomarkers. *Neurology* 87, 539–547.
6103 <https://doi.org/10.1212/WNL.0000000000002923>
- 6104 Jack, C.R., Holtzman, D.M., 2013. Biomarker modeling of alzheimer's disease. *Neuron* 80, 1347–
6105 1358. <https://doi.org/10.1016/j.neuron.2013.12.003>
- 6106 Jack, C.R., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M.,
6107 Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C.,
6108 Trojanowski, J.Q., 2013. Tracking pathophysiological processes in Alzheimer's disease: An
6109 updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12, 207–216.
6110 [https://doi.org/10.1016/S1474-4422\(12\)70291-0](https://doi.org/10.1016/S1474-4422(12)70291-0)
- 6111 Jack, C.R., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C.,
6112 Trojanowski, J.Q., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's
6113 pathological cascade. *Lancet Neurol.* 9, 119–128. [https://doi.org/10.1016/S1474-](https://doi.org/10.1016/S1474-4422(09)70299-6)
6114 4422(09)70299-6
- 6115 Jack, C.R., Vemuri, P., Wiste, H.J., Weigand, S.D., Aisen, P.S., Trojanowsky, J.Q., Shaw, L.M.,
6116 Bernstein, M.A., Petersen, R.C., Weiner, M.W., Knopman, D.S., Alzheimers Disease
6117 Neuroimaging Initiative, 2011. Evidence for Ordering of Alzheimer Disease Biomarkers.
6118 *Arch. Neurol.* 68, 1526. <https://doi.org/10.1001/archneurol.2011.183>
- 6119 Jack, C.R., Wiste, H.J., Weigand, S.D., Therneau, T.M., Lowe, V.J., Knopman, D.S., Botha, H.,
6120 Graff-Radford, J., Jones, D.T., Ferman, T.J., Boeve, B.F., Kantarci, K., Vemuri, P., Mielke,
6121 M.M., Whitwell, J., Josephs, K., Schwarz, C.G., Senjem, M.L., Gunter, J.L., Petersen, R.C.,
6122 2020. Predicting future rates of tau accumulation on PET. *Brain* 143, 3136–3150.
6123 <https://doi.org/10.1093/brain/awaa248>
- 6124 Jacob, H.J., 1999. Functional genomics and rat models. *Genome Res.* 9, 1013–1016.
6125 <https://doi.org/10.1101/gr.9.11.1013>
- 6126 Jahn, H., 2013. Memory loss in alzheimer's disease. *Dialogues Clin. Neurosci.* 15, 445–454.
6127 <https://doi.org/10.31887/dcns.2013.15.4/hjahn>

- 6128 Jankowsky, J.L., Fadale, D.J., Anderson, J., Xu, G.M., Gonzales, V., Jenkins, N.A., Copeland,
6129 N.G., Lee, M.K., Younkin, L.H., Wagner, S.L., Younkin, S.G., Borchelt, D.R., 2004. Mutant
6130 presenilins specifically elevate the levels of the 42 residue β -amyloid peptide in vivo:
6131 Evidence for augmentation of a 42-specific γ secretase. *Hum. Mol. Genet.* 13, 159–170.
6132 <https://doi.org/10.1093/hmg/ddh019>
- 6133 Jansen, W.J., Ossenkoppele, R., Knol, D.L., Tijms, B.M., Scheltens, P., Verhey, F.R.J., Visser,
6134 P.J., Aalten, P., Aarsland, D., Alcolea, D., Alexander, M., Almdahl, I.S., Arnold, S.E.,
6135 Baldeiras, I., Barthel, H., van Berckel, B.N.M., Bibeau, K., Blennow, K., Brooks, D.J., van
6136 Buchem, M.A., Camus, V., Cavedo, E., Chen, K., Chetelat, G., Cohen, A.D., Drzezga, A.,
6137 Engelborghs, S., Fagan, A.M., Fladby, T., Fleisher, A.S., van der Flier, W.M., Ford, L.,
6138 Förster, S., Fortea, J., Fosskett, N., Frederiksen, K.S., Freund-Levi, Y., Frisoni, G.B., Froelich,
6139 L., Gabryelewicz, T., Gill, K.D., Gkatzima, O., Gómez-Tortosa, E., Gordon, M.F., Grimmer,
6140 T., Hampel, H., Hausner, L., Hellwig, S., Herukka, S.-K., Hildebrandt, H., Ishihara, L.,
6141 Ivanoiu, A., Jagust, W.J., Johannsen, P., Kandimalla, R., Kapaki, E., Klimkowicz-Mrowiec,
6142 A., Klunk, W.E., Köhler, S., Koglin, N., Kornhuber, J., Kramberger, M.G., Van Laere, K.,
6143 Landau, S.M., Lee, D.Y., de Leon, M., Lisetti, V., Lleó, A., Madsen, K., Maier, W.,
6144 Marcusson, J., Mattsson, N., de Mendonça, A., Meulenbroek, O., Meyer, P.T., Mintun, M.A.,
6145 Mok, V., Molinuevo, J.L., Møllergård, H.M., Morris, J.C., Mroczko, B., Van der Mussele,
6146 S., Na, D.L., Newberg, A., Nordberg, A., Nordlund, A., Novak, G.P., Paraskevas, G.P.,
6147 Parnetti, L., Perera, G., Peters, O., Popp, J., Prabhakar, S., Rabinovici, G.D., Ramakers,
6148 I.H.G.B., Rami, L., Resende de Oliveira, C., Rinne, J.O., Rodrigue, K.M., Rodríguez-
6149 Rodríguez, E., Roe, C.M., Rot, U., Rowe, C.C., Rütger, E., Sabri, O., Sanchez-Juan, P.,
6150 Santana, I., Sarazin, M., Schröder, J., Schütte, C., Seo, S.W., Soetewey, F., Soininen, H.,
6151 Spira, L., Struyfs, H., Teunissen, C.E., Tsolaki, M., Vandenberghe, R., Verbeek, M.M.,
6152 Villemagne, V.L., Vos, S.J.B., van Waalwijk van Doorn, L.J.C., Waldemar, G., Wallin, A.,
6153 Wallin, Å.K., Wiltfang, J., Wolk, D.A., Zboch, M., Zetterberg, H., 2015. Prevalence of
6154 Cerebral Amyloid Pathology in Persons Without Dementia. *JAMA* 313, 1924.
6155 <https://doi.org/10.1001/jama.2015.4668>
- 6156 Javonillo, D.I., Tran, K.M., Phan, J., Hingco, E., Kramár, E.A., da Cunha, C., Forner, S.,
6157 Kawauchi, S., Milinkeviciute, G., Gomez-Arboledas, A., Neumann, J., Banh, C.E., Huynh,
6158 M., Matheos, D.P., Rezaie, N., Alcantara, J.A., Mortazavi, A., Wood, M.A., Tenner, A.J.,
6159 MacGregor, G.R., Green, K.N., LaFerla, F.M., 2022. Systematic Phenotyping and
6160 Characterization of the 3xTg-AD Mouse Model of Alzheimer's Disease. *Front. Neurosci.* 15.
6161 <https://doi.org/10.3389/fnins.2021.785276>
- 6162 Jawhar, S., Trawicka, A., Jenneckens, C., Bayer, T.A., Wirths, O., 2012. Motor deficits, neuron
6163 loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal A β
6164 aggregation in the 5XFAD mouse model of Alzheimer's disease. *Neurobiol. Aging* 33,
6165 196.e29-196.e40. <https://doi.org/10.1016/j.neurobiolaging.2010.05.027>
- 6166 Jaya Prasanthi, R.P., Schommer, E., Thomasson, S., Thompson, A., Feist, G., Ghribi, O., 2008.
6167 Regulation of β -amyloid levels in the brain of cholesterol-fed rabbit, a model system for
6168 sporadic Alzheimer's disease. *Mech. Ageing Dev.* 129, 649–655.
6169 <https://doi.org/10.1016/j.mad.2008.09.002>
- 6170 Jayaraj, R.L., Azimullah, S., Beiram, R., 2020. Diabetes as a risk factor for Alzheimer's disease
6171 in the Middle East and its shared pathological mediators. *Saudi J. Biol. Sci.* 27, 736–750.
6172 <https://doi.org/10.1016/j.sjbs.2019.12.028>
- 6173 Jeganathan, S., Hascher, A., Chinnathambi, S., Biernat, J., Mandelkow, E.M., Mandelkow, E.,

2008. Proline-directed pseudo-phosphorylation at AT8 and PHF1 epitopes induces a compaction of the paperclip folding of tau and generates a pathological (MC-1) conformation. *J. Biol. Chem.* 283, 32066–32076. <https://doi.org/10.1074/jbc.M805300200>
- Jicha, G.A., Bowser, R., Kazam, I.G., Davies, P., 1997. Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J. Neurosci. Res.* 48, 128–132. [https://doi.org/10.1002/\(SICI\)1097-4547\(19970415\)48:2<128::AID-JNR5>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-4547(19970415)48:2<128::AID-JNR5>3.0.CO;2-E)
- Jin, R., Chan, A.K.Y., Wu, J., Lee, T.M.C., 2022. Relationships between Inflammation and Age-Related Neurocognitive Changes. *Int. J. Mol. Sci.* 23. <https://doi.org/10.3390/ijms232012573>
- Johnson, E.C.B., Carter, E.K., Dammer, E.B., Duong, D.M., Gerasimov, E.S., Liu, Y., Liu, J., Betarbet, R., Ping, L., Yin, L., Serrano, G.E., Beach, T.G., Peng, J., De Jager, P.L., Haroutunian, V., Zhang, B., Gaiteri, C., Bennett, D.A., Gearing, M., Wingo, T.S., Wingo, A.P., Lah, J.J., Levey, A.I., Seyfried, N.T., 2022. Large-scale deep multi-layer analysis of Alzheimer’s disease brain reveals strong proteomic disease-related changes not observed at the RNA level. *Nat. Neurosci.* 25, 213–225. <https://doi.org/10.1038/s41593-021-00999-y>
- Joie, R. La, Visani, A. V., Baker, S.L., Brown, J.A., Bourakova, V., Cha, J., Chaudhary, K., Edwards, L., Iaccarino, L., Janabi, M., Lesman-Segev, O.H., Miller, Z.A., Perry, D.C., O’Neil, J.P., Pham, J., Rojas, J.C., Rosen, H.J., Seeley, W.W., Tsai, R.M., Miller, B.L., Jagust, W.J., Rabinovici, G.D., 2020. Prospective longitudinal atrophy in Alzheimer’s disease correlates with the intensity and topography of baseline tau-PET. *Sci. Transl. Med.* 12, 1–13. <https://doi.org/10.1126/scitranslmed.aau5732>
- Jones, M.J., Goodman, S.J., Kobor, M.S., 2015. DNA methylation and healthy human aging. *Aging Cell* 14, 924–932. <https://doi.org/10.1111/accel.12349>
- Jonsson, T., Atwal, J.K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., Stefansson, H., Sulem, P., Gudbjartsson, D., Maloney, J., Hoyte, K., Gustafson, A., Liu, Y., Lu, Y., Bhangale, T., Graham, R.R., Huttenlocher, J., Bjornsdottir, G., Andreassen, O.A., Jonsson, E.G., Palotie, A., Behrens, T.W., Magnusson, O.T., Kong, A., Thorsteinsdottir, U., Watts, R.J., Stefansson, K., 2012. A mutation in APP protects against Alzheimer’s disease and age-related cognitive decline. *Nature* 488, 96. <https://doi.org/10.1038/nature11283>
- Jørgensen, M.B., Finsen, B.R., Jensen, M.B., Castellano, B., Diemer, N.H., Zimmer, J., 1993. Microglial and Astroglial Reactions to Ischemic and Kainic Acid-Induced Lesions of the Adult Rat Hippocampus. *Exp. Neurol.* <https://doi.org/10.1006/exnr.1993.1041>
- Jouanne, M., Rault, S., Voisin-Chiret, A.-S.S., 2017. Tau protein aggregation in Alzheimer’s disease: An attractive target for the development of novel therapeutic agents. *Eur. J. Med. Chem.* 139, 153–167. <https://doi.org/10.1016/j.ejmech.2017.07.070>
- Juárez-Muñoz, Y., Rivera-Olvera, A., Ramos-Languren, L.E., Escobar, M.L., 2017. CaMKII requirement for the persistence of in vivo hippocampal mossy fiber synaptic plasticity and structural reorganization. *Neurobiol. Learn. Mem.* 139, 56–62. <https://doi.org/10.1016/j.nlm.2016.12.015>
- Kadavath, H., Hofele, R. V., Biernat, J., Kumar, S., Tepper, K., Urlaub, H., Mandelkow, E., Zweckstetter, M., 2015. Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. *Proc. Natl. Acad. Sci.* 112, 7501–7506. <https://doi.org/10.1073/pnas.1504081112>
- Kalaria, R.N., Deramecourt, V., 2012. Staging and natural history of cerebrovascular pathology in dementia. *Neurology* 79, 107.
- Kametani, F., Hasegawa, M., 2018. Reconsideration of amyloid hypothesis and tau hypothesis in

- Alzheimer's disease. *Front. Neurosci.* 12. <https://doi.org/10.3389/fnins.2018.00025>
- Kanekiyo, T., Bu, G., 2014. The low-density lipoprotein receptor-related protein 1 and amyloid- β clearance in Alzheimer's disease. *Front. Aging Neurosci.* 6, 1–12. <https://doi.org/10.3389/fnagi.2014.00093>
- Karch, C.M., Goate, A.M., 2015. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol. Psychiatry* 77, 43–51. <https://doi.org/10.1016/j.biopsych.2014.05.006>
- Karlawish, J., Jack, C.R., Rocca, W.A., Snyder, H.M., Carrillo, M.C., 2017. Alzheimer's disease: The next frontier—Special Report 2017. *Alzheimer's Dement.* 13, 374–380. <https://doi.org/10.1016/j.jalz.2017.02.006>
- Kaufman, S.K., Del Tredici, K., Thomas, T.L., Braak, H., Diamond, M.I., 2018. Tau seeding activity begins in the transentorhinal/entorhinal regions and anticipates phospho-tau pathology in Alzheimer's disease and PART. *Acta Neuropathol.* 1–11. <https://doi.org/10.1007/s00401-018-1855-6>
- Kayed, R., Lasagna-Reeves, C.A., 2013. Amyloid Hypothesis: Molecular and Cellular Aspects of Toxicity, in: *Alzheimer's Disease: Insights into Low Molecular Weight and Cytotoxic Aggregates from In Vitro and Computer Experiments*. pp. 3–28.
- Kelmer Sacramento, E., Kirkpatrick, J.M., Mazzetto, M., Baumgart, M., Bartolome, A., Di Sanzo, S., Caterino, C., Sanguanini, M., Papaevgeniou, N., Lefaki, M., Childs, D., Bagnoli, S., Terzibasi Tozzini, E., Di Fraia, D., Romanov, N., Sudmant, P.H., Huber, W., Chondrogianni, N., Vendruscolo, M., Cellerino, A., Ori, A., 2020. Reduced proteasome activity in the aging brain results in ribosome stoichiometry loss and aggregation. *Mol. Syst. Biol.* 16, 1–22. <https://doi.org/10.15252/msb.20209596>
- Kempermann, G., 2015. Activity Dependency and Aging in the Regulation of Adult Neurogenesis. *Cold Spring Harb. Perspect. Biol.* 7, a018929. <https://doi.org/10.1101/cshperspect.a018929>
- Khan, T.K., 2016a. Clinical Diagnosis of Alzheimer's Disease, in: *Biomarkers in Alzheimer's Disease*. pp. 27–48. <https://doi.org/10.1016/B978-0-12-804832-0.00002-X>
- Khan, T.K., 2016b. Neuroimaging Biomarkers in Alzheimer's Disease, in: *Biomarkers in Alzheimer's Disease*. pp. 51–100. <https://doi.org/10.1016/B978-0-12-804832-0/00003-1>
- Kim, J., Choi, I.Y., Michaelis, M.L., Lee, P., 2011. Quantitative in vivo measurement of early axonal transport deficits in a triple transgenic mouse model of Alzheimer's disease using manganese-enhanced MRI. *Neuroimage* 56, 1286–1292. <https://doi.org/10.1016/j.neuroimage.2011.02.039>
- Kimura, T., Whitcomb, D.J., Jo, J., Regan, P., Piers, T., Heo, S., Brown, C., Hashikawa, T., Murayama, M., Seok, H., Sotiropoulos, I., Kim, E., Collingridge, G.L., Takashima, A., Cho, K., 2013. Microtubule-associated protein tau is essential for long-term depression in the hippocampus. *Philos. Trans. R. Soc. B Biol. Sci.* 369, 20130144–20130144. <https://doi.org/10.1098/rstb.2013.0144>
- Kinirons, M.T., O'Mahony, M.S., 2004. Drug metabolism and ageing. *Br. J. Clin. Pharmacol.* 57, 540–544. <https://doi.org/10.1111/j.1365-2125.2004.02096.x>
- Kinney, J.W., Bemiller, S.M., Murtishaw, A.S., Leisgang, A.M., Salazar, A.M., Lamb, B.T., 2018. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's Dement. Transl. Res. Clin. Interv.* 4, 575–590. <https://doi.org/10.1016/j.trci.2018.06.014>
- Klunk, W.E., Savitcheva, I., Bergström, M., Mathis, C.A., Lopresti, B.J., Koivisto, P., Antoni, G., Holt, D.P., Barletta, J., Price, J.C., Huang, G.-F., Wang, Y., Wall, A., Ausén, B., Debnath, M.L., Nordberg, A., Långström, B., Blomqvist, G., Engler, H., Sandell, J., Estrada, S., 2004. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann. Neurol.*

- 55, 306–319. <https://doi.org/10.1002/ana.20009>
- 6267 Klyubin, I., Walsh, D.M., Cullen, W.K., Fadeeva, J. V., Anwyl, R., Selkoe, D.J., Rowan, M.J.,
6268 2004. Soluble Arctic amyloid β protein inhibits hippocampal long-term potentiation in vivo.
6269 *Eur. J. Neurosci.* 19, 2839–2846. <https://doi.org/10.1111/j.1460-9568.2004.03389.x>
- 6270 Kneynsberg, A., Combs, B., Christensen, K., Morfini, G., Kanaan, N.M., 2017. Axonal
6271 degeneration in tauopathies: Disease relevance and underlying mechanisms. *Front. Neurosci.*
6272 11, 1–14. <https://doi.org/10.3389/fnins.2017.00572>
- 6273 Knopman, D.S., Lundt, E.S., Therneau, T.M., Vemuri, P., Lowe, V.J., Kantarci, K., Gunter, J.L.,
6274 Senjem, M.L., Mielke, M.M., Machulda, M.M., Boeve, B.F., Jones, D.T., Graff-Radford, J.,
6275 Albertson, S.M., Schwarz, C.G., Petersen, R.C., Jack, C.R., 2019. Entorhinal cortex tau,
6276 amyloid- β , cortical thickness and memory performance in non-demented subjects. *Brain* 1–
6277 13. <https://doi.org/10.1093/brain/awz025>
- 6278 Koffie, R.M., Meyer-Luehmann, M., Hashimoto, T., Adams, K.W., Mielke, M.L., Garcia-Alloza,
6279 M., Micheva, K.D., Smith, S.J., Kim, M.L., Lee, V.M., Hyman, B.T., Spires-Jones, T.L.,
6280 2009. Oligomeric amyloid beta associates with postsynaptic densities and correlates with
6281 excitatory synapse loss near senile plaques. *Proc. Natl. Acad. Sci. U. S. A.* 106, 4012–7.
6282 <https://doi.org/10.1073/pnas.0811698106>
- 6283 Kojima, N., Wang, J., Mansu, I.M., Grant, S.G.N., Mayford, M., Kandel, E.R., 1997. Rescuing
6284 impairment of long-term potentiation in fyn-deficient mice by introducing Fyn transgene.
6285 *Proc. Natl. Acad. Sci. U. S. A.* 94, 4761–4765. <https://doi.org/10.1073/pnas.94.9.4761>
- 6286 Kopeikina, K.J., Polydoro, M., Tai, H.C., Yaeger, E., Carlson, G.A., Pitstick, R., Hyman, B.T.,
6287 Spires-Jones, T.L., 2013. Synaptic alterations in the rTg4510 mouse model of tauopathy. *J.*
6288 *Comp. Neurol.* 521, 1334–1353. <https://doi.org/10.1002/cne.23234>
- 6289 Korhonen, P., van Groen, T., Thornell, A., Kyrylenko, S., Soininen, M.L., Ojala, J., Peltomaa, E.,
6290 Tanila, H., Salminen, A., Mandelkow, E.M., Soininen, H., 2011. Characterization of a novel
6291 transgenic rat carrying human tau with mutation P301L. *Neurobiol. Aging* 32, 2314–2315.
6292 <https://doi.org/10.1016/j.neurobiolaging.2009.12.022>
- 6293 Kosik, K., Orecchio, L., Binder, L., Trojanowski, J.Q., Lee, V.M.-Y., Lee, G., 1988. Epitopes that
6294 span the tau molecule are shared with paired helical filaments. *Neuron* 1, 817–825.
6295 [https://doi.org/10.1016/0896-6273\(88\)90129-8](https://doi.org/10.1016/0896-6273(88)90129-8)
- 6296 Kosik, K.S., Joachim, C.L., Selkoe, D.J., 1986. Microtubule-associated protein tau (τ) is a major
6297 antigenic component of paired helical filaments in Alzheimer disease. *Proc. Natl. Acad. Sci.*
6298 *U. S. A.* 83, 4044–4048. <https://doi.org/10.1073/pnas.83.11.4044>
- 6299 Kovacs, G.G., 2015. Invited review: Neuropathology of tauopathies: principles and practice.
6300 *Neuropathol. Appl. Neurobiol.* 41, 3–23. <https://doi.org/10.1111/NAN.12208>
- 6301 Kovacs, G.G., Lukic, M.J., Irwin, D.J., Arzberger, T., Respondek, G., Lee, E.B., Coughlin, D.,
6302 Giese, A., Grossman, M., Kurz, C., McMillan, C.T., Gelpi, E., Compta, Y., van Swieten, J.C.,
6303 Laat, L.D., Troakes, C., Al-Sarraj, S., Robinson, J.L., Roeber, S., Xie, S.X., Lee, V.M.Y.,
6304 Trojanowski, J.Q., Höglinger, G.U., 2020. Distribution patterns of tau pathology in
6305 progressive supranuclear palsy. *Acta Neuropathol.* 140, 99–119.
6306 <https://doi.org/10.1007/s00401-020-02158-2>
- 6307 Kowalski, G.M., Bruce, C.R., 2014. The regulation of glucose metabolism: Implications and
6308 considerations for the assessment of glucose homeostasis in rodents. *Am. J. Physiol. -*
6309 *Endocrinol. Metab.* 307, E859–E871. <https://doi.org/10.1152/ajpendo.00165.2014>
- 6310 Kremen, W.S., Elman, J.A., Panizzon, M.S., Eglit, G.M.L., Sanderson-Cimino, M., Williams,
6311 M.E., Lyons, M.J., Franz, C.E., 2022. Cognitive Reserve and Related Constructs: A Unified

- 6312 Framework Across Cognitive and Brain Dimensions of Aging. *Front. Aging Neurosci.* 14, 1–
 6313 10. <https://doi.org/10.3389/fnagi.2022.834765>
- 6314 Krüger, L., Mandelkow, E.M., 2016. Tau neurotoxicity and rescue in animal models of human
 6315 Tauopathies. *Curr. Opin. Neurobiol.* 36, 52–58. <https://doi.org/10.1016/j.conb.2015.09.004>
- 6316 Kummer, K.K., Hofhansel, L., Barwitz, C.M., Schardl, A., Prast, J.M., Salti, A., El Rawas, R.,
 6317 Zernig, G., 2014. Differences in social interaction- vs. cocaine reward in mouse vs. rat. *Front.*
 6318 *Behav. Neurosci.* 8, 1–7. <https://doi.org/10.3389/fnbeh.2014.00363>
- 6319 LaDu, M.J., Lukens, J.R., Reardon, C.A., Getz, G.S., 1997. Association of human, rat, and rabbit
 6320 apolipoprotein E with β -amyloid. *J. Neurosci. Res.* 49, 9–18.
 6321 [https://doi.org/10.1002/\(SICI\)1097-4547\(19970701\)49:1<9::AID-JNR2>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-4547(19970701)49:1<9::AID-JNR2>3.0.CO;2-H)
- 6322 LaFerla, F.M., Green, K.N., Oddo, S., 2007. Intracellular amyloid- β in Alzheimer's disease. *Nat.*
 6323 *Rev. Neurosci.* 8, 499–509. <https://doi.org/10.1038/nrn2168>
- 6324 Lambert, M.P., Barlow, A.K., Chromy, B.A., Edwards, C., Freed, R., Liosatos, M., Morgan, T.E.,
 6325 Rozovsky, I., Trommer, B., Viola, K.L., Wals, P., Zhang, C., Finch, C.E., Krafft, G.A., Klein,
 6326 W.L., 1998. Diffusible, nonfibrillar ligands derived from A 1-42 are potent central nervous
 6327 system neurotoxins. *Proc. Natl. Acad. Sci.* 95, 6448–6453.
 6328 <https://doi.org/10.1073/pnas.95.11.6448>
- 6329 Lan, Y.L., Zhao, J., Ma, T., Li, S., 2016. The Potential Roles of Aquaporin 4 in Alzheimer's
 6330 Disease. *Mol. Neurobiol.* 53, 5300–5309. <https://doi.org/10.1007/s12035-015-9446-1>
- 6331 Lasagna-Reeves, C.A., Castillo-Carranza, D.L., Sengupta, U., Sarmiento, J., Troncoso, J., Jackson,
 6332 G.R., Kaye, R., Lasagna-Reeves, C.A., Castillo-Carranza, D.L., Sengupta, U., Sarmiento,
 6333 J., Troncoso, J., Jackson, G.R., Kaye, R., 2012. Identification of oligomers at early stages of
 6334 tau aggregation in Alzheimer's disease. *FASEB J.* 26, 1946–1959.
 6335 <https://doi.org/10.1096/fj.11-199851>
- 6336 Lasagna-Reeves, C.A., de Haro, M., Hao, S., Park, J., Rousseaux, M.W.C., Al-Ramahi, I., Jafar-
 6337 Nejad, P., Vilanova-Velez, L., See, L., De Maio, A., Nitschke, L., Wu, Z., Troncoso, J.C.,
 6338 Westbrook, T.F., Tang, J., Botas, J., Zoghbi, H.Y., 2016. Reduction of Nuak1 Decreases Tau
 6339 and Reverses Phenotypes in a Tauopathy Mouse Model. *Neuron* 92, 407–418.
 6340 <https://doi.org/10.1016/j.neuron.2016.09.022>
- 6341 Latimer, C.S., Shively, C.A., Keene, C.D., Jorgensen, M.J., Andrews, R.N., Register, T.C.,
 6342 Montine, T.J., Wilson, A.M., Neth, B.J., Mintz, A., Maldjian, J.A., Whitlow, C.T., Kaplan,
 6343 J.R., Craft, S., 2019. A nonhuman primate model of early Alzheimer's disease pathologic
 6344 change: Implications for disease pathogenesis. *Alzheimer's Dement.* 15, 93–105.
 6345 <https://doi.org/10.1016/j.jalz.2018.06.3057>
- 6346 Laurent, C., Buée, L., Blum, D., 2018. Tau and neuroinflammation: What impact for Alzheimer's
 6347 Disease and Tauopathies? *Biomed. J.* 41, 21–33. <https://doi.org/10.1016/j.bj.2018.01.003>
- 6348 Lazarov, O., Marr, R.A., 2013. Of mice and men: Neurogenesis, cognition, and Alzheimer's
 6349 disease. *Front. Aging Neurosci.* 5, 1–8. <https://doi.org/10.3389/fnagi.2013.00043>
- 6350 Lee, V.M.-Y., Goedert, M., Trojanowski, J.Q., 2001. Neurodegenerative Tauopathies. *Annu. Rev.*
 6351 *Neurosci.* 24, 1121–1159. <https://doi.org/10.1146/annurev.neuro.24.1.1121>
- 6352 Lee, V.M.Y., Kenyon, T.K., Trojanowski, J.Q., 2005. Transgenic animal models of tauopathies.
 6353 *Biochim. Biophys. Acta - Mol. Basis Dis.* 1739, 251–259.
 6354 <https://doi.org/10.1016/j.bbadis.2004.06.014>
- 6355 Leon, W.C., Canneva, F., Partridge, V., Allard, S., Ferretti, M.T., Dewilde, A., Vercauteren, F.,
 6356 Atifeh, R., Ducatenzeiler, A., Klein, W., Szyf, M., Alhonen, L., Cuellar, A.C., 2010. A novel
 6357 transgenic rat model with a full Alzheimer's-Like amyloid pathology displays pre-plaque

- intracellular amyloid-B-Associated cognitive impairment. *J. Alzheimer's Dis.* 20, 113–126. <https://doi.org/10.3233/JAD-2010-1349>
- Leroy, K., Yilmaz, Z., Brion, J.P., 2007. Increased level of active GSK-3 β in Alzheimer's disease and accumulation in argyrophilic grains and in neurones at different stages of neurofibrillary degeneration. *Neuropathol. Appl. Neurobiol.* 33, 43–55. <https://doi.org/10.1111/j.1365-2990.2006.00795.x>
- Leung, C., Cao, F., Nguyen, R., Joshi, K., Agrabawi, A.J., Xia, S., Cortez, M.A., Snead, O.C., Kim, J.C., Jia, Z., 2018a. Activation of Entorhinal Cortical Projections to the Dentate Gyrus Underlies Social Memory Retrieval. *Cell Rep.* 23, 2379–2391. <https://doi.org/10.1016/j.celrep.2018.04.073>
- Leung, C., Kim, J., Jia, Z., 2018b. Three-chamber Social Approach Task with Optogenetic Stimulation (Mice). *Bio-Protocol* 8, 1–12. <https://doi.org/10.21769/bioprotoc.3120>
- Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., Van Duinen, S.G., Bots, G.T.A.M., Luyendijk, W., Frangione, B., 1990. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* (80-.). 248, 1124–1126. <https://doi.org/10.1126/science.2111584>
- Lewis, J., Dickson, D.W., Lin, W., Chisholm, L., Corral, A., Jones, G., Yen, S., Sahara, N., Skipper, L., Yager, D., Eckman, C., Hutton, M., McGowan, E., Hardy, J., 2001. Neurofibrillary Degeneration in Transgenic Mice Expressing Mutant tau and APP. *Science* (80-.). 293, 1487–1491.
- Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Van Slegtenhorst, M., Gwinn-Hardy, K., Murphy, M.P., Baker, M., Yu, X., Duff, K., Hardy, J., Corral, A., Lin, W.L., Yen, S.H., Dickson, D.W., Davies, P., Hutton, M., 2000. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L)tau protein. *Nat. Genet.* 25, 402–405. <https://doi.org/10.1038/78078>
- Li, H., Jia, X., Qi, Z., Fan, X., Ma, T., Pang, R., Ni, H., Li, C. shan R., Lu, J., Li, K., 2018. Disrupted functional connectivity of cornu ammonis subregions in amnesic mild cognitive impairment: A longitudinal resting-state fMRI study. *Front. Hum. Neurosci.* 12, 1–9. <https://doi.org/10.3389/fnhum.2018.00413>
- Li, S., Jin, M., Koeglsperger, T., Shepardson, N.E., Shankar, G.M., Selkoe, D.J., 2011. Soluble A Oligomers Inhibit Long-Term Potentiation through a Mechanism Involving Excessive Activation of Extrasynaptic NR2B-Containing NMDA Receptors. *J. Neurosci.* 31, 6627–6638. <https://doi.org/10.1523/JNEUROSCI.0203-11.2011>
- Lin, Y.T., Seo, J., Gao, F., Feldman, H.M., Wen, H.L., Penney, J., Cam, H.P., Gjoneska, E., Raja, W.K., Cheng, J., Rueda, R., Kritskiy, O., Abdurrob, F., Peng, Z., Milo, B., Yu, C.J., Elmsaouri, S., Dey, D., Ko, T., Yankner, B.A., Tsai, L.H., 2018. APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types. *Neuron* 98, 1141–1154.e7. <https://doi.org/10.1016/j.neuron.2018.05.008>
- Littau, J.L., Velilla, L., Hase, Y., Villalba-Moreno, N.D., Hagel, C., Drexler, D., Osorio Restrepo, S., Villegas, A., Lopera, F., Vargas, S., Glatzel, M., Krasemann, S., Quiroz, Y.T., Arboleda-Velasquez, J.F., Kalara, R., Sepulveda-Falla, D., Villalba-Moreno, N.D., Hagel, C., Drexler, D., Osorio Restrepo, S., Villegas, A., Lopera, F., Vargas, S., Glatzel, M., Krasemann, S., Quiroz, Y.T., Arboleda-Velasquez, J.F., Kalara, R., Sepulveda-Falla, D., 2022. Evidence of beta amyloid independent small vessel disease in familial Alzheimer's disease. *Brain Pathol.* 1–16. <https://doi.org/10.1111/bpa.13097>

- 6404 Liu, F., Grundke-Iqbal, I., Iqbal, K., Gong, C.X., 2005. Contributions of protein phosphatases PP1,
6405 PP2A, PP2B and PP5 to the regulation of tau phosphorylation. *Eur. J. Neurosci.* 22, 1942–
6406 1950. <https://doi.org/10.1111/j.1460-9568.2005.04391.x>
- 6407 Liu, G., Fiock, K.L., Levites, Y., Golde, T.E., Hefti, M.M., Lee, G., 2020. Fyn depletion
6408 ameliorates tauP301L-induced neuropathology. *Acta Neuropathol. Commun.* 8, 1–15.
6409 <https://doi.org/10.1186/s40478-020-00979-6>
- 6410 Liu, R., 2022. Aging, Cellular Senescence, and Alzheimer's Disease. *Int. J. Mol. Sci.* 23, 1989.
6411 <https://doi.org/10.3390/ijms23041989>
- 6412 Liu, Y., Braidy, N., Poljak, A., Chan, D.K.Y., Sachdev, P., 2018. Cerebral small vessel disease
6413 and the risk of Alzheimer's disease: A systematic review. *Ageing Res. Rev.* 47, 41–48.
6414 <https://doi.org/10.1016/j.arr.2018.06.002>
- 6415 Lopez, E.M., Bell, K.F.S., Ribeiro-da-Silva, a, Cuello, a C., 2004. Early changes in neurons of
6416 the hippocampus and neocortex in transgenic rats expressing intracellular human a-beta. *J.*
6417 *Alzheimers. Dis.* 6, 421–431; discussion 443–449.
- 6418 Lozupone, M., Imbimbo, B. Pietro, Balducci, C., Lo Vecchio, F., Bisceglia, P., Latino, R.R.,
6419 Leone, M., Dibello, V., Solfrizzi, V., Greco, A., Daniele, A., Watling, M., Seripa, D., Panza,
6420 F., 2023. Does the imbalance in the apolipoprotein E isoforms underlie the pathophysiological
6421 process of sporadic Alzheimer's disease? *Alzheimer's Dement.* 19, 353–368.
6422 <https://doi.org/https://doi.org/10.1002/alz.12728>
- 6423 Lue, L.-F., Kuo, Y.-M., Roher, A.E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J.H., Rydel,
6424 R.E., Rogers, J., 1999. Soluble Amyloid β Peptide Concentration as a Predictor of Synaptic
6425 Change in Alzheimer's Disease. *Am. J. Pathol.* 155, 853–862. [https://doi.org/10.1016/S0002-](https://doi.org/10.1016/S0002-9440(10)65184-X)
6426 [9440\(10\)65184-X](https://doi.org/10.1016/S0002-9440(10)65184-X)
- 6427 Luna-Muñoz, J., Chávez-Macías, L., García-Sierra, F., Mena, R., 2007. Earliest stages of tau
6428 conformational changes are related to the appearance of a sequence of specific phospho-
6429 dependent tau epitopes in Alzheimer's disease. *J. Alzheimer's Dis.* 12, 365–375.
6430 <https://doi.org/10.3233/JAD-2007-12410>
- 6431 Luna-Muñoz, J., García-Sierra, F., Falcón, V., Menéndez, I., Chávez-Macías, L., Mena, R., 2005.
6432 Regional conformational change involving phosphorylation of tau protein at the Thr231,
6433 precedes the structural change detected by Alz-50 antibody in Alzheimer's disease. *J.*
6434 *Alzheimer's Dis.* 8, 29–41. <https://doi.org/10.3233/JAD-2005-8104>
- 6435 Lyketsos, C.G., Breitner, J.C.S., Green, R.C., Martin, B.K., Meinert, C., Piantadosi, S., Sabbagh,
6436 M., 2007. Naproxen and celecoxib do not prevent AD in early results from a randomized
6437 controlled trial. *Neurology* 68, 1800–1808.
6438 <https://doi.org/10.1212/01.wnl.0000260269.93245.d2>
- 6439 Mahmoud, S., Gharagozloo, M., Simard, C., Gris, D., 2019. Astrocytes maintain glutamate
6440 homeostasis in the cns by controlling the balance between glutamate uptake and release. *Cells*
6441 8, 1–27. <https://doi.org/10.3390/cells8020184>
- 6442 Mair, W., Muntel, J., Tepper, K., Tang, S., Biernat, J., Seeley, W.W., Kosik, K.S., Mandelkow,
6443 E., Steen, H., Steen, J.A., 2016. FLEXITau: Quantifying Post-translational Modifications of
6444 Tau Protein in Vitro and in Human Disease. *Anal. Chem.* 88, 3704–3714.
6445 <https://doi.org/10.1021/acs.analchem.5b04509>
- 6446 Majid, T., Ali, Y.O., Venkitaramani, D. V., Jang, M.K., Lu, H.C., Pautler, R.G., 2014. In vivo
6447 axonal transport deficits in a mouse model of fronto-temporal dementia. *NeuroImage Clin.* 4,
6448 711–717. <https://doi.org/10.1016/j.nicl.2014.02.005>
- 6449 Malcolm, J.C., Breuillaud, L., Do Carmo, S., Hall, H., Welikovitch, L.A., Macdonald, J.A.,

- Goedert, M., Cuello, A.C., 2019. Neuropathological changes and cognitive deficits in rats transgenic for human mutant tau recapitulate human tauopathy. *Neurobiol. Dis.* 127, 323–338. <https://doi.org/10.1016/j.nbd.2019.03.018>
- Malin, D.H., Crothers, M.K., Lake, J.R., Goyarzu, P., Plotner, R.E., Garcia, S.A., Spell, S.H., Tomsic, B.J., Giordano, T., Kowall, N.W., 2001. Hippocampal injections of amyloid β -peptide 1-40 impair subsequent one-trial/day reward learning. *Neurobiol. Learn. Mem.* 76, 125–137. <https://doi.org/10.1006/nlme.2000.3991>
- Maloney, B., Ge, Y.W., Alley, G.M., Lahiri, D.K., 2007. Important differences between human and mouse APOE gene promoters: Limitation of mouse APOE model in studying Alzheimer's disease. *J. Neurochem.* 103, 1237–1257. <https://doi.org/10.1111/j.1471-4159.2007.04831.x>
- Manczak, M., Reddy, P.H., 2013. Abnormal interaction of oligomeric amyloid-B with phosphorylated tau: Implications to synaptic dysfunction and neuronal damage. *J. Alzheimer's Dis.* 36, 285–295. <https://doi.org/10.3233/JAD-130275>
- Mandelkow, E.-M., Mandelkow, E., 2012. Biochemistry and Cell Biology of Tau Protein in Neurofibrillary Degeneration. *Cold Spring Harb. Perspect. Med.* 2, a006247–a006247. <https://doi.org/10.1101/cshperspect.a006247>
- Mandelkow, E.E.M., Von Bergen, M., Biernat, J., Mandelkow, E.E.M., 2007. Structural principles of tau and the paired helical filaments of Alzheimer's disease. *Brain Pathol.* 17, 83–90. <https://doi.org/10.1111/j.1750-3639.2007.00053.x>
- Mandelkow, E.M., Mandelkow, E., 1998. Tau in Alzheimer's disease. *Trends Cell Biol.* 8, 425–427. [https://doi.org/10.1016/S0962-8924\(98\)01368-3](https://doi.org/10.1016/S0962-8924(98)01368-3)
- Maness, L.M., Banks, W.A., Podlisny, M.B., Selkoe, D.J., Kastin, A.J., 1994. Passage of human amyloid β -protein 1-40 across the murine blood-brain barrier. *Life Sci.* 55, 1643–1650. [https://doi.org/10.1016/0024-3205\(94\)00331-9](https://doi.org/10.1016/0024-3205(94)00331-9)
- Mangoni, A.A., Jackson, S.H.D., 2004. Age-related changes in pharmacokinetics and pharmacodynamics: Basic principles and practical applications. *Br. J. Clin. Pharmacol.* 57, 6–14. <https://doi.org/10.1046/j.1365-2125.2003.02007.x>
- Maragakis, N.J., Dykes-Hoberg, M., Rothstein, J.D., 2004. Altered Expression of the Glutamate Transporter EAAT2b in Neurological Disease. *Ann. Neurol.* 55, 469–477. <https://doi.org/10.1002/ana.20003>
- Marcus, J.N., Schachter, J., 2011. Targeting Post-translational Modifications on Tau as a Therapeutic Strategy for Alzheimer's Disease. *J. Neurogenet.* 25, 127–133. <https://doi.org/10.3109/01677063.2011.626471>
- Marr, R.A., Guan, H., Rockenstein, E., Kindy, M., Gage, F.H., Verma, I., Masliah, E., Hersh, L.B., 2004. Neprilysin regulates amyloid β peptide levels. *J. Mol. Neurosci.* <https://doi.org/10.1385/JMN:22:1-2:5>
- Martin, L., Latypova, X., Terro, F., 2011. Post-translational modifications of tau protein: Implications for Alzheimer's disease. *Neurochem. Int.* 58, 458–471. <https://doi.org/10.1016/j.neuint.2010.12.023>
- Martínez-Cué, C., Rueda, N., 2020. Cellular Senescence in Neurodegenerative Diseases. *Front. Cell. Neurosci.* 14. <https://doi.org/10.3389/fncel.2020.00016>
- Masliah, E., Mallory, M., Hansen, L., Richard, D.T., Alford, M., Terry, R., 1994. Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci. Lett.* 174, 67–72. [https://doi.org/10.1016/0304-3940\(94\)90121-X](https://doi.org/10.1016/0304-3940(94)90121-X)
- Masliah, E., Terry, R.D., DeTeresa, R.M., Hansen, L.A., 1989. Immunohistochemical

- quantification of the synapse-related protein synaptophysin in Alzheimer disease. *Neurosci. Lett.* 103, 234–239. [https://doi.org/10.1016/0304-3940\(89\)90582-X](https://doi.org/10.1016/0304-3940(89)90582-X)
- Masters, C.L., Multhaup, G., Simms, G., Pottgiesser, J., Martins, R.N., Beyreuther, K., 1985a. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J.* 4, 2757–63.
- Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L., Beyreuther, K., 1985b. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci.* 82, 4245–4249. <https://doi.org/10.1073/pnas.82.12.4245>
- Matrone, C., Petrillo, F., Nasso, R., Ferretti, G., 2020. Fyn tyrosine kinase as harmonizing factor in neuronal functions and dysfunctions. *Int. J. Mol. Sci.* <https://doi.org/10.3390/ijms21124444>
- Maurer, K., Volk, S., Gerbaldo, H., 1997. Auguste D and Alzheimer's disease. *Lancet* 349, 1546–1549. [https://doi.org/10.1016/S0140-6736\(96\)10203-8](https://doi.org/10.1016/S0140-6736(96)10203-8)
- McDade, E., Wang, G., Gordon, B.A., Hassenstab, J., Benzinger, T.L.S., Buckles, V., Fagan, A.M., Holtzman, D.M., Cairns, N.J., Goate, A.M., Marcus, D.S., Morris, J.C., Paumier, K., Xiong, C., Allegri, R., Berman, S.B., Klunk, W., Noble, J., Ringman, J., Ghetti, B., Farlow, M., Sperling, R.A., Chhatwal, J., Salloway, S., Graff-Radford, N.R., Schofield, P.R., Masters, C., Rossor, M.N., Fox, N.C., Levin, J., Jucker, M., Bateman, R.J., 2018. Longitudinal cognitive and biomarker changes in dominantly inherited Alzheimer disease. *Neurology* 91, E1295–E1306. <https://doi.org/10.1212/WNL.00000000000006277>
- McGeer, P.L., Akiyama, H., Itagaki, S., McGeer, E.G., 1989. Immune system response in Alzheimer's disease. *Can. J. Neurol. Sci. Le J. Can. des Sci. Neurol.* 16, 516–527. <https://doi.org/10.1017/s0317167100029863>
- McGeer, P.L., McGeer, E.G., 1995. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res. Rev.* 21, 195–218. [https://doi.org/10.1016/0165-0173\(95\)00011-9](https://doi.org/10.1016/0165-0173(95)00011-9)
- McHugh, P.R., 1975. Mini-mental state: A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.
- McKean, N.E., Handley, R.R., Snell, R.G., 2021. A review of the current mammalian models of alzheimer's disease and challenges that need to be overcome. *Int. J. Mol. Sci.* 22. <https://doi.org/10.3390/ijms222313168>
- McLean, C.A., Cherny, R.A., Fraser, F.W., Fuller, S.J., Smith, M.J., Konrad Vbeyreuther, Bush, A.I., Masters, C.L., Cherny, R.A., Konrad Vbeyreuther, Fuller, S.J., Fraser, F.W., McLean, C.A., Cherny, R.A., Fraser, F.W., Fuller, S.J., Smith, M.J., Konrad Vbeyreuther, Bush, A.I., Masters, C.L., 1999. Soluble pool of AB amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* 46, 860–866. [https://doi.org/10.1002/1531-8249\(199912\)46:6<860::AID-ANA8>3.0.CO;2-M](https://doi.org/10.1002/1531-8249(199912)46:6<860::AID-ANA8>3.0.CO;2-M)
- Meng, Q., Lin, M.S., Tzeng, I.S., 2020. Relationship Between Exercise and Alzheimer's Disease: A Narrative Literature Review. *Front. Neurosci.* 14, 1–6. <https://doi.org/10.3389/fnins.2020.00131>
- Merino-Serrais, P., Benavides-Piccione, R., Blazquez-Llorca, L., Kastanauskaite, A., Rábano, A., Avila, J., Defelipe, J., 2013. The influence of phospho-tau on dendritic spines of cortical pyramidal neurons in patients with Alzheimer's disease. *Brain* 136, 1913–1928. <https://doi.org/10.1093/brain/awt088>
- Merlini, M., Wanner, D., Nitsch, R.M., 2016. Tau pathology-dependent remodelling of cerebral arteries precedes Alzheimer's disease-related microvascular cerebral amyloid angiopathy.

- Acta Neuropathol. 131, 737–752. <https://doi.org/10.1007/s00401-016-1560-2>
- Mestas, J., Hughes, C.C.W., 2004. Of Mice and Not Men: Differences between Mouse and Human Immunology. *J. Immunol.* 172, 2731–2738. <https://doi.org/10.4049/jimmunol.172.5.2731>
- Michalíková, A., Majerová, P., Kováč, A., 2020. Tau Protein and Its Role in Blood–Brain Barrier Dysfunction. *Front. Mol. Neurosci.* 13, 1–13. <https://doi.org/10.3389/fnmol.2020.570045>
- Mielke, M.M., Aakre, J.A., Algeciras-Schimnich, A., Proctor, N.K., Machulda, M.M., Eichenlaub, U., Knopman, D.S., Vemuri, P., Graff-Radford, J., Jack, C.R., Petersen, R.C., Dage, J.L., 2022. Comparison of CSF phosphorylated tau 181 and 217 for cognitive decline. *Alzheimer's Dement.* 18, 602–611. <https://doi.org/10.1002/alz.12415>
- Milà-Alomà, M., Ashton, N.J., Shekari, M., Salvadó, G., Ortiz-Romero, P., Montoliu-Gaya, L., Benedet, A.L., Karikari, T.K., Lantero-Rodriguez, J., Vanmechelen, E., Day, T.A., González-Escalante, A., Sánchez-Benavides, G., Minguillon, C., Fauria, K., Molinuevo, J.L., Dage, J.L., Zetterberg, H., Gispert, J.D., Suárez-Calvet, M., Blennow, K., 2022. Plasma p-tau231 and p-tau217 as state markers of amyloid- β pathology in preclinical Alzheimer's disease. *Nat. Med.* 28, 1797–1801. <https://doi.org/10.1038/s41591-022-01925-w>
- Miller, Y., Ma, B., Nussinov, R., 2011. Synergistic interactions between repeats in tau protein and A β amyloids may be responsible for accelerated aggregation via polymorphic States. *Biochemistry* 50, 5172–5181. <https://doi.org/10.1021/bi200400u>
- Min, S.W., Cho, S.H., Zhou, Y., Schroeder, S., Haroutunian, V., Seeley, W.W., Huang, E.J., Shen, Y., Masliah, E., Mukherjee, C., Meyers, D., Cole, P.A., Ott, M., Gan, L., 2010. Acetylation of tau inhibits its degradation and contributes to tauopathy. *Neuron* 67, 953–966. <https://doi.org/10.1016/j.neuron.2010.08.044>
- Mintun, M.A., Lo, A.C., Duggan Evans, C., Wessels, A.M., Ardayfio, P.A., Andersen, S.W., Shcherbinin, S., Sparks, J., Sims, J.R., Brys, M., Apostolova, L.G., Salloway, S.P., Skovronsky, D.M., 2021. Donanemab in Early Alzheimer's Disease. *N. Engl. J. Med.* 384, 1691–1704. <https://doi.org/10.1056/NEJMoa2100708>
- Mioshi, E., Dawson, K., Mitchell, J., Arnold, R., Hodges, J.R., 2006. The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. *Int. J. Geriatr. Psychiatry* 21, 1078–1085. <https://doi.org/10.1002/gps.1610>
- Moftakhar, P., Lynch, M.D., Pomakian, J.L., Vinters, H. V., 2010. Aquaporin expression in the brains of patients with or without cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol.* 69, 1201–1209. <https://doi.org/10.1097/NEN.0b013e3181fd252c>
- Molinuevo, J.L., Ayton, S., Batrla, R., Bednar, M.M., Bittner, T., Cummings, J., Fagan, A.M., Hampel, H., Mielke, M.M., Mikulskis, A., O'Bryant, S., Scheltens, P., Sevigny, J., Shaw, L.M., Soares, H.D., Tong, G., Trojanowski, J.Q., Zetterberg, H., Blennow, K., 2018. Current state of Alzheimer's fluid biomarkers. *Acta Neuropathol.* 136, 821–853. <https://doi.org/10.1007/s00401-018-1932-x>
- Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L., Harrington, M.G., Chui, H.C., Law, M., Zlokovic, B. V., 2015. Blood-Brain barrier breakdown in the aging human hippocampus. *Neuron* 85, 296–302. <https://doi.org/10.1016/j.neuron.2014.12.032>
- Montagne, A., Nation, D.A., Sagare, A.P., Barisano, G., Sweeney, M.D., Chakhoyan, A., Pachicano, M., Joe, E., Nelson, A.R., D'Orazio, L.M., Buennagel, D.P., Harrington, M.G., Benzinger, T.L.S., Fagan, A.M., Ringman, J.M., Schneider, L.S., Morris, J.C., Reiman, E.M., Caselli, R.J., Chui, H.C., Tcw, J., Chen, Y., Pa, J., Conti, P.S., Law, M., Toga, A.W., Zlokovic, B. V., 2020. APOE4 leads to blood–brain barrier dysfunction predicting cognitive

- 6588 decline. *Nature* 581, 71–76. <https://doi.org/10.1038/s41586-020-2247-3>
- 6589 Moreno, H., Yu, E., Pigino, G., Hernandez, A.I., Kim, N., Moreira, J.E., Sugimori, M., Llinas,
6590 R.R., 2009. Synaptic transmission block by presynaptic injection of oligomeric amyloid beta.
6591 *Proc. Natl. Acad. Sci.* 106, 5901–5906. <https://doi.org/10.1073/pnas.0900944106>
- 6592 Morfini, G.A., Burns, M., Binder, L.I., Kanaan, N.M., Lapointe, N., Bosco, D.A., Brown, R.H.,
6593 Brown, H., Tiwari, A., Hayward, L., Edgar, J., Nave, K.A., Garberrn, J., Atagi, Y., Song, Y.,
6594 Pigino, G., Brady, S.T., 2009. Axonal transport defects in neurodegenerative diseases. *J.*
6595 *Neurosci.* 29, 12776–12786. <https://doi.org/10.1523/JNEUROSCI.3463-09.2009>
- 6596 Morris, M.C., 2009. The role of nutrition in Alzheimer’s disease: Epidemiological evidence. *Eur.*
6597 *J. Neurol.* 16, 1–7. <https://doi.org/10.1111/j.1468-1331.2009.02735.x>
- 6598 Morris, M.S., 2003. Homocysteine and Alzheimer’s disease. *Lancet Neurol.* 2, 425–428.
6599 [https://doi.org/10.1016/S1474-4422\(03\)00438-1](https://doi.org/10.1016/S1474-4422(03)00438-1)
- 6600 Morsch, R., Simon, W., Coleman, P.D., 1999. Neurons may live for decades with neurofibrillary
6601 tangles. *J. Neuropathol. Exp. Neurol.* 58, 188–197. [https://doi.org/10.1097/00005072-](https://doi.org/10.1097/00005072-199902000-00008)
6602 199902000-00008
- 6603 Mosconi, L., 2013. Glucose metabolism in normal aging and Alzheimer’s disease: Methodological
6604 and physiological considerations for PET studies. *Clin. Transl. Imaging* 1, 217–233.
6605 <https://doi.org/10.1007/s40336-013-0026-y>
- 6606 Mosconi, L., Mistur, R., Switalski, R., Tsui, W.H., Glodzik, L., Li, Y., Pirraglia, E., De Santi, S.,
6607 Reisberg, B., Wisniewski, T., De Leon, M.J., 2009. FDG-PET changes in brain glucose
6608 metabolism from normal cognition to pathologically verified Alzheimer’s disease. *Eur. J.*
6609 *Nucl. Med. Mol. Imaging* 36, 811–822. <https://doi.org/10.1007/s00259-008-1039-z>
- 6610 Mucke, L., Selkoe, D.J., 2012a. Neurotoxicity of Amyloid -Protein: Synaptic and Network
6611 Dysfunction. *Cold Spring Harb. Perspect. Med.* 2, a006338–a006338.
6612 <https://doi.org/10.1101/cshperspect.a006338>
- 6613 Mucke, L., Selkoe, D.J., 2012b. Neurotoxicity of amyloid β -protein: Synaptic and network
6614 dysfunction. *Cold Spring Harb. Perspect. Med.* 2, 1–17.
6615 <https://doi.org/10.1101/cshperspect.a006338>
- 6616 Mudher, A., Colin, M., Dujardin, S., Medina, M., Dewachter, I., Naini, S.M.A., Mandelkow, E.-
6617 M., Mandelkow, E., Buée, L., Goedert, M., Brion, J.-P., 2017. What is the evidence that tau
6618 pathology spreads through prion-like propagation? *Acta Neuropathol. Commun.* 5, 99.
6619 <https://doi.org/10.1186/s40478-017-0488-7>
- 6620 Mufson, E.J., Ginsberg, S.D., Ikonomic, M.D., DeKosky, S.T., 2003. Human cholinergic basal
6621 forebrain: Chemoanatomy and neurologic dysfunction. *J. Chem. Neuroanat.* 26, 233–242.
6622 [https://doi.org/10.1016/S0891-0618\(03\)00068-1](https://doi.org/10.1016/S0891-0618(03)00068-1)
- 6623 Mufson, E.J., Ma, S.Y., Cochran, E.J., Bennett, D.A., Beckett, L.A., Jaffar, S., Saragovi, H.U.,
6624 Kordower, J.H., 2000. Loss of nucleus basalis neurons containing trkA immunoreactivity in
6625 individuals with mild cognitive impairment and early Alzheimer’s disease. *J. Comp. Neurol.*
6626 427, 19–30.
- 6627 Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B., Lannfelt, L., 1992.
6628 A pathogenic mutation for probable Alzheimer’s disease in the APP gene at the N-terminus
6629 of β -amyloid. *Nat. Genet.* 1, 345–347. <https://doi.org/10.1038/ng0892-345>
- 6630 Multhaup, G., Schlicksupp, A., Hesse, L., Beher, D., Ruppert, T., Masters, C.L., Beyreuther, K.,
6631 1996. The amyloid precursor protein of Alzheimer’s disease in the reduction of copper (II
6632) to copper (I). *Science* (80-.). 271, 1406–1409.
- 6633 Murrell, J., Farlow, M., Ghetti, B., Benson, M.D., 1991. A mutation in the Amyloid Precursor

- Protein Associated with Hereditary Alzheimer's Disease. *Science* (80-.). 254, 97–99. <https://doi.org/10.1126/science.1925564>
- Necula, M., Kuret, J., 2004. Pseudophosphorylation and Glycation of Tau Protein Enhance but Do Not Trigger Fibrillization in Vitro *. *J. Biol. Chem.* 279, 49694–49703. <https://doi.org/10.1074/jbc.M405527200>
- Nicoll, R.A., Schmitz, D., 2005. Synaptic plasticity at hippocampal mossy fibre synapses. *Nat. Rev. Neurosci.* 6, 863–876. <https://doi.org/10.1038/nrn1786>
- Niewiadomska, G., Niewiadomski, W., Steczkowska, M., Gasiorowska, A., 2021. Tau Oligomers Neurotoxicity. *Life* 11, 28. <https://doi.org/10.3390/life11010028>
- Nilson, A.N., English, K.C., Gerson, J.E., Barton Whittle, T., Nicolas Crain, C., Xue, J., Sengupta, U., Castillo-Carranza, D.L., Zhang, W., Gupta, P., Kaye, R., 2017. Tau oligomers associate with inflammation in the brain and retina of tauopathy mice and in neurodegenerative diseases. *J. Alzheimer's Dis.* 55, 1083–1099. <https://doi.org/10.3233/JAD-160912>
- Nilsson, C., Waldö, M.L., Nilsson, K., Santillo, A., Vestberg, S., 2014. Age-related incidence and family history in Frontotemporal dementia: Data from the Swedish Dementia Registry. *PLoS One* 9, 4–9. <https://doi.org/10.1371/journal.pone.0094901>
- Nisbet, R.M., Polanco, J.C., Ittner, L.M., Götz, J., 2015. Tau aggregation and its interplay with amyloid- β . *Acta Neuropathol.* 129, 207–220. <https://doi.org/10.1007/s00401-014-1371-2>
- Novak, M., Wischik, C.M., Edwards, P., Pannell, R., Milstein, C., 1989. Characterisation of the first monoclonal antibody against the pronase resistant core of the Alzheimer PHF. *Prog. Clin. Biol. Res.* 317, 755–761.
- Novak, P., Kovacech, B., Katina, S., Schmidt, R., Scheltens, P., Kontseikova, E., Ropele, S., Fialova, L., Kramberger, M., Paulenka-Ivanovova, N., Smisek, M., Hanes, J., Stevens, E., Kovac, A., Sutovsky, S., Parrak, V., Koson, P., Prcina, M., Galba, J., Cente, M., Hromadka, T., Filipcik, P., Piestansky, J., Samcova, M., Prenn-Gologranc, C., Sivak, R., Froelich, L., Fresser, M., Rakusa, M., Harrison, J., Hort, J., Otto, M., Tosun, D., Ondrus, M., Winblad, B., Novak, M., Zilka, N., 2021. ADAMANT: a placebo-controlled randomized phase 2 study of AADvac1, an active immunotherapy against pathological tau in Alzheimer's disease. *Nat. Aging* 1, 521–534. <https://doi.org/10.1038/s43587-021-00070-2>
- Novo, M., Freire, S., Al-Soufi, W., 2018. Critical aggregation concentration for the formation of early Amyloid-beta (1-42) oligomers. *Sci. Rep.* 8, 1783. <https://doi.org/10.1038/s41598-018-19961-3>
- Nygaard, H.B., Dyck, C.H. Van, Strittmatter, S.M., 2014. Fyn Kinase Inhibition as a novel therapy for AD. *Alzheimers. Res. Ther.* 6, 1–8.
- Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., Guillozet-Bongaarts, A., Ohno, M., Disterhoft, J., Van Eldik, L., Berry, R., Vassar, R., 2006. Intraneuronal β -amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. *J. Neurosci.* 26, 10129–10140. <https://doi.org/10.1523/JNEUROSCI.1202-06.2006>
- Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kaye, R., Metherate, R., Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003. Triple-transgenic model of Alzheimer's Disease with plaques and tangles: Intracellular A β and synaptic dysfunction. *Neuron* 39, 409–421. [https://doi.org/10.1016/S0896-6273\(03\)00434-3](https://doi.org/10.1016/S0896-6273(03)00434-3)
- Okamura, N., Harada, R., Ishiki, A., Kikuchi, A., Nakamura, T., Kudo, Y., 2018. The development and validation of tau PET tracers: current status and future directions. *Clin. Transl. Imaging* 6, 305–316. <https://doi.org/10.1007/s40336-018-0290-y>

- 6680 Olsson, A., Vanderstichele, H., Andreasen, N., De Meyer, G., Wallin, A., Holmberg, B.,
 6681 Rosengren, L., Vanmechelen, E., Blennow, K., 2005. Simultaneous measurement of β -
 6682 amyloid(1-42), total Tau, and phosphorylated Tau (Thr181) in cerebrospinal fluid by the
 6683 xMAP technology. *Clin. Chem.* 51, 336–345. <https://doi.org/10.1373/clinchem.2004.039347>
- 6684 Ossenkoppele, R., Schonhaut, D.R., Schöll, M., Lockhart, S.N., Ayakta, N., Baker, S.L., O’Neil,
 6685 J.P., Janabi, M., Lazaris, A., Cantwell, A., Vogel, J., Santos, M., Miller, Z.A., Bettcher, B.M.,
 6686 Vossel, K.A., Kramer, J.H., Gorno-Tempini, M.L., Miller, B.L., Jagust, W.J., Rabinovici,
 6687 G.D., 2016. Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer’s
 6688 disease. *Brain* 139, 1551–1567. <https://doi.org/10.1093/brain/aww027>
- 6689 Ossenkoppele, R., van der Kant, R., Hansson, O., 2022. Tau biomarkers in Alzheimer’s disease:
 6690 towards implementation in clinical practice and trials. *Lancet Neurol.* 21, 726–734.
 6691 [https://doi.org/10.1016/S1474-4422\(22\)00168-5](https://doi.org/10.1016/S1474-4422(22)00168-5)
- 6692 Padmanabhan, P., Martínez-Mármol, R., Xia, D., Götz, J., Meunier, F.A., 2019. Frontotemporal
 6693 dementia mutant Tau promotes aberrant Fyn nanoclustering in hippocampal dendritic spines.
 6694 *Elife* 8, 1–25. <https://doi.org/10.7554/eLife.45040.001>
- 6695 Padurariu, M., Ciobica, A., Mavroudis, I., Fotiou, D., Baloyannis, S., 2012. Hippocampal neuronal
 6696 loss in the CA1 and CA3 areas of Alzheimer’s disease patients. *Psychiatr Danub* 24, 152–
 6697 158.
- 6698 Pampuscenko, K., Morkuniene, R., Krasauskas, L., Smirnovas, V., Tomita, T., Borutaite, V., 2021.
 6699 Distinct Neurotoxic Effects of Extracellular Tau Species in Primary Neuronal-Glial Cultures.
 6700 *Mol. Neurobiol.* 58, 658–667. <https://doi.org/10.1007/s12035-020-02150-7>
- 6701 Pang, K., Jiang, R., Zhang, W., Yang, Z., Li, L.L., Shimozaawa, M., Tambaro, S., Mayer, J., Zhang,
 6702 B., Li, M., Wang, J., Liu, H., Yang, A., Chen, X., Liu, J., Winblad, B., Han, H., Jiang, T.,
 6703 Wang, W., Nilsson, P., Guo, W., Lu, B., 2022. An App knock-in rat model for Alzheimer’s
 6704 disease exhibiting A β and tau pathologies, neuronal death and cognitive impairments. *Cell*
 6705 *Res.* 32, 157–175. <https://doi.org/10.1038/s41422-021-00582-x>
- 6706 Pannérec, A., Migliavacca, E., De Castro, A., Michaud, J., Karaz, S., Goulet, L., Rezzi, S., Ng,
 6707 T.P., Bosco, N., Larbi, A., Feige, J.N., 2018. Vitamin B12 deficiency and impaired expression
 6708 of amnionless during aging. *J. Cachexia. Sarcopenia Muscle* 9, 41–52.
 6709 <https://doi.org/10.1002/jcsm.12260>
- 6710 Papassotiropoulos, A., Fountoulakis, M., Dunckley, T., Stephan, D.A., Reiman, E.M., 2006.
 6711 Genetics, transcriptomics, and proteomics of Alzheimer’s disease, *Journal of Clinical*
 6712 *Psychiatry.* <https://doi.org/10.4088/JCP.v67n0418>
- 6713 Parent, M.J., Zimmer, E.R., Shin, M., Kang, M.S., Fonov, V.S., Mathieu, A., Aliaga, A., Kostikov,
 6714 A., Do Carmo, S., Dea, D., Poirier, J., Soucy, J.-P., Gauthier, S., Cuellar, A.C., Rosa-Neto, P.,
 6715 2017. Multimodal Imaging in Rat Model Recapitulates Alzheimer’s Disease Biomarkers
 6716 Abnormalities. *J. Neurosci.* 37, 12263–12271. [https://doi.org/10.1523/JNEUROSCI.1346-](https://doi.org/10.1523/JNEUROSCI.1346-17.2017)
 6717 [17.2017](https://doi.org/10.1523/JNEUROSCI.1346-17.2017)
- 6718 Pascoal, T.A., Benedet, A.L., Tudorascu, D.L., Therriault, J., Mathotaarachchi, S., Savard, M.,
 6719 Lussier, F.Z., Tissot, C., Chamoun, M., Kang, M.S., Stevenson, J., Massarweh, G., Guiot,
 6720 M.-C., Soucy, J.-P., Gauthier, S., Rosa-Neto, P., 2021. Longitudinal 18F-MK-6240 tau
 6721 tangles accumulation follows Braak stages. *Brain* 144, 3517–3528.
 6722 <https://doi.org/10.1093/brain/awab248>
- 6723 Pascoal, T.A., Leuzy, A., Therriault, J., Chamoun, M., Lussier, F., Tissot, C., Strandberg, O.,
 6724 Palmqvist, S., Stomrud, E., Ferreira, P.C.L., Ferrari-Souza, J.P., Smith, R., Benedet, A.L.,
 6725 Gauthier, S., Hansson, O., Rosa-Neto, P., 2023. Discriminative accuracy of the A/T/N scheme

- 6726 to identify cognitive impairment due to Alzheimer's disease. *Alzheimer's Dement.*
 6727 *Diagnosis, Assess. Dis. Monit.* 15, 1–9. <https://doi.org/10.1002/dad2.12390>
- 6728 Pascoal, T.A., Mathotaarachchi, S., Mohades, S., Benedet, A.L., Chung, C.-O., Shin, M., Wang,
 6729 S., Beaudry, T., Kang, M.S., Soucy, J.-P., Labbe, A., Gauthier, S., Rosa-Neto, P., 2017a.
 6730 Amyloid- β and hyperphosphorylated tau synergy drives metabolic decline in preclinical
 6731 Alzheimer's disease. *Mol. Psychiatry* 22, 306–311. <https://doi.org/10.1038/mp.2016.37>
- 6732 Pascoal, T.A., Mathotaarachchi, S., Shin, M., Benedet, A.L., Mohades, S., Wang, S., Beaudry, T.,
 6733 Kang, M.S., Soucy, J., Labbe, A., Gauthier, S., Rosa-Neto, P., 2017b. Synergistic interaction
 6734 between amyloid and tau predicts the progression to dementia. *Alzheimer's Dement.* 13, 644–
 6735 653. <https://doi.org/10.1016/j.jalz.2016.11.005>
- 6736 Pascoal, T.A., Therriault, J., Benedet, A.L., Savard, M., Lussier, F.Z., Chamoun, M., Tissot, C.,
 6737 Qureshi, M.N.I., Kang, M.S., Mathotaarachchi, S., Stevenson, J., Hopewell, R., Massarweh,
 6738 G., Soucy, J.-P., Gauthier, S., Rosa-Neto, P., 2020. 18F-MK-6240 PET for early and late
 6739 detection of neurofibrillary tangles. *Brain* 1–13. <https://doi.org/10.1093/brain/awaa180>
- 6740 Patterson, C., Feightner, J.W., Garcia, A., Hsiung, G.-Y.R., MacKnight, C., Sadovnick, A.D.,
 6741 2008. Diagnosis and treatment of dementia: 1. Risk assessment and primary prevention of
 6742 Alzheimer disease. *C. Can. Med. Assoc. J.* 178, 548–56.
 6743 <https://doi.org/10.1503/cmaj.070796>
- 6744 Pearson, R.C.A., Sofroniew, M. V., Cuello, A.C., Powell, T.P.S., Eckenstein, F., Esiri, M.M.,
 6745 Wilcock, G.K., 1983. Persistence of cholinergic neurons in the basal nucleus in a brain with
 6746 senile dementia of the Alzheimer's type demonstrated by immunohistochemical staining for
 6747 choline acetyltransferase. *Brain Res.* 289, 375–379. [https://doi.org/10.1016/0006-8993\(83\)90046-X](https://doi.org/10.1016/0006-8993(83)90046-X)
- 6749 Perlman, R.L., 2016. Mouse Models of Human Disease: An Evolutionary Perspective. *Evol. Med.*
 6750 *Public Heal.* eow014. <https://doi.org/10.1093/emph/eow014>
- 6751 Perry, R.H., Bergmann, K., Tomlinson, B.E., Blessed, G., Gibson, P.H., 1978. Correlation of
 6752 cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br.*
 6753 *Med. J.* 2, 1457–1459. <https://doi.org/10.1136/bmj.2.6150.1457>
- 6754 Peters, D.G., Pollack, A.N., Cheng, K.C., Sun, D., Saido, T., Haaf, M.P., Yang, Q.X., Connor,
 6755 J.R., Meadowcroft, M.D., 2018. Dietary lipophilic iron alters amyloidogenesis and microglial
 6756 morphology in Alzheimer's disease knock-in APP mice. *Metallomics* 10, 426–443.
 6757 <https://doi.org/10.1039/c8mt00004b>
- 6758 Pickett, E.K., Herrmann, A.G., McQueen, J., Abt, K., Dando, O., Tulloch, J., Jain, P., Dunnett, S.,
 6759 Sohrabi, S., Fjeldstad, M.P., Calkin, W., Murison, L., Jackson, R.J., Tzioras, M., Stevenson,
 6760 A., d'Orange, M., Hooley, M., Davies, C., Colom-Cadena, M., Anton-Fernandez, A., King,
 6761 D., Oren, I., Rose, J., McKenzie, C.A., Allison, E., Smith, C., Hardt, O., Henstridge, C.M.,
 6762 Hardingham, G.E., Spires-Jones, T.L., 2019. Amyloid Beta and Tau Cooperate to Cause
 6763 Reversible Behavioral and Transcriptional Deficits in a Model of Alzheimer's Disease. *Cell*
 6764 *Rep.* 29, 3592–3604.e5. <https://doi.org/10.1016/j.celrep.2019.11.044>
- 6765 Ponce-Lopez, T., Hong, E., Abascal-Díaz, M., Meneses, A., 2017. Role of GSK3B and PP2A on
 6766 Regulation of Tau Phosphorylation in Hippocampus and Memory Impairment in ICV-STZ
 6767 Animal Model of Alzheimer's Disease. *Adv. Alzheimer's Dis.* 06, 13–31.
 6768 <https://doi.org/10.4236/aad.2017.61002>
- 6769 Pontecorvo, M.J., Devous, M.D., Kennedy, I., Navitsky, M., Lu, M., Galante, N., Salloway, S.,
 6770 Murali Doraiswamy, P., Southekal, S., Arora, A.K., McGeehan, A., Lim, N.C., Xiong, H.,
 6771 Truocchio, S.P., Joshi, A.D., Shcherbinin, S., Teske, B., Fleisher, A.S., Mintun, M.A., 2019.

- 6772 A multicentre longitudinal study of flortaucipir (18F) in normal ageing, mild cognitive
 6773 impairment and Alzheimer's disease dementia. *Brain* 142, 1723–1735.
 6774 <https://doi.org/10.1093/brain/awz090>
- 6775 Pooler, A.M., Polydoro, M., Maury, E.A., Nicholls, S.B., Reddy, S.M., Wegmann, S., William,
 6776 C., Saqran, L., Cagsal-Getkin, O., Pitstick, R., Beier, D.R., Carlson, G.A., Spires-Jones, T.L.,
 6777 Hyman, B.T., 2015. Amyloid accelerates tau propagation and toxicity in a model of early
 6778 Alzheimer's disease. *Acta Neuropathol. Commun.* 3, 14. [https://doi.org/10.1186/s40478-](https://doi.org/10.1186/s40478-015-0199-x)
 6779 015-0199-x
- 6780 Preische, O., Schultz, S.A., Apel, A., Kuhle, J., Kaeser, S.A., Barro, C., Gräber, S., Kuder-Bulletta,
 6781 E., LaFougere, C., Laske, C., Vöglein, J., Levin, J., Masters, C.L., Martins, R., Schofield,
 6782 P.R., Rossor, M.N., Graff-Radford, N.R., Salloway, S., Ghetti, B., Ringman, J.M., Noble,
 6783 J.M., Chhatwal, J., Goate, A.M., Benzinger, T.L.S., Morris, J.C., Bateman, R.J., Wang, G.,
 6784 Fagan, A.M., McDade, E.M., Gordon, B.A., Jucker, M., 2019. Serum neurofilament
 6785 dynamics predicts neurodegeneration and clinical progression in presymptomatic
 6786 Alzheimer's disease. *Nat. Med.* 2019 1. <https://doi.org/10.1038/s41591-018-0304-3>
- 6787 Price, J.L., McKeel, D.W., Buckles, V.D., Roe, C.M., Xiong, C., Grundman, M., Hansen, L.A.,
 6788 Petersen, R.C., Parisi, J.E., Dickson, D.W., Smith, C.D., Davis, D.G., Schmitt, F.A.,
 6789 Markesbery, W.R., Kaye, J., Kurlan, R., Hulette, C., Kurland, B.F., Higdon, R., Kukull, W.,
 6790 Morris, J.C., 2009. Neuropathology of nondemented aging: Presumptive evidence for
 6791 preclinical Alzheimer disease. *Neurobiol. Aging* 30, 1026–1036.
 6792 <https://doi.org/10.1016/j.neurobiolaging.2009.04.002>
- 6793 Probst, A., Langui, D., Ulrich, J., 1991. Alzheimer's Disease: A Description of the Structural
 6794 Lesions. *Brain Pathol.* 1, 229–239. <https://doi.org/10.1111/j.1750-3639.1991.tb00666.x>
- 6795 Puzzo, D., Piacentini, R., Fá, M., Gulisano, W., Li Puma, D.D., Staniszewski, A., Zhang, H.,
 6796 Tropea, M.R., Cocco, S., Palmeri, A., Fraser, P., D'Adamio, L., Grassi, C., Arancio, O., 2017.
 6797 LTP and memory impairment caused by extracellular A β and tau oligomers is APP-
 6798 dependent. *Elife* 6, 1–21. <https://doi.org/10.7554/eLife.26991.001>
- 6799 Qi, G., Mi, Y., Shi, X., Gu, H., Brinton, R.D., Yin, F., 2021. ApoE4 Impairs Neuron-Astrocyte
 6800 Coupling of Fatty Acid Metabolism. *Cell Rep.* 34, 108572.
 6801 <https://doi.org/10.1016/j.celrep.2020.108572>
- 6802 Qi, Y., Klyubin, I., Harney, S.C., Hu, N., Cullen, W.K., Grant, M.K., Steffen, J., Wilson, E.N., Do
 6803 Carmo, S., Remy, S., Fuhrmann, M., Ashe, K.H., Cuellar, A.C., Rowan, M.J., 2014a.
 6804 Longitudinal testing of hippocampal plasticity reveals the onset and maintenance of
 6805 endogenous human A beta-induced synaptic dysfunction in individual freely behaving pre-
 6806 plaque transgenic rats: rapid reversal by anti-A beta agents. *ACTA Neuropathol. Commun.*
 6807 2. <https://doi.org/10.1186/s40478-014-0175-x>
- 6808 Qi, Y., Klyubin, I., Harney, S.C., Hu, N., Cullen, W.K., Grant, M.K., Steffen, J., Wilson, E.N., Do
 6809 Carmo, S., Remy, S., Fuhrmann, M., Ashe, K.H., Cuellar, A.C., Rowan, M.J., 2014b.
 6810 Longitudinal testing of hippocampal plasticity reveals the onset and maintenance of
 6811 endogenous human A β -induced synaptic dysfunction in individual freely behaving pre-
 6812 plaque transgenic rats: rapid reversal by anti-A β agents. *Acta Neuropathol. Commun.* 2, 175.
 6813 <https://doi.org/10.1186/s40478-014-0175-x>
- 6814 Qi, Y., Klyubin, I., Hu, N.W., Ondrejcek, T., Rowan, M.J., 2019. Pre-plaque A β -Mediated
 6815 Impairment of Synaptic Depotentiation in a Transgenic Rat Model of Alzheimer's Disease
 6816 Amyloidosis. *Front. Neurosci.* 13, 1–13. <https://doi.org/10.3389/fnins.2019.00861>
- 6817 Quiroz, Y.T., Sperling, R.A., Norton, D.J., Baena, A., Arboleda-Velasquez, J.F., Cosio, D.,

- Schultz, A., Lapoint, M., Guzman-Velez, E., Miller, J.B., Kim, L.A., Chen, K., Tariot, P.N., Lopera, F., Reiman, E.M., Johnson, K.A., 2018. Association Between Amyloid and Tau Accumulation in Young Adults With Autosomal Dominant Alzheimer Disease. *JAMA Neurol.* 02129, 1–9. <https://doi.org/10.1001/jamaneurol.2017.4907>
- Radde, R., Bolmont, T., Kaeser, S.A., Coomaraswamy, J., Lindau, D., Stoltze, L., Calhoun, M.E., Jäggi, F., Wolburg, H., Gengler, S., Haass, C., Ghetti, B., Czech, C., Hölscher, C., Mathews, P.M., Jucker, M., 2006. A β 42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep.* 7, 940–946. <https://doi.org/10.1038/sj.embor.7400784>
- Ramalho, R.M., Viana, R.J.S., Castro, R.E., Steer, C.J., Low, W.C., Rodrigues, C.M.P., 2008. Apoptosis in transgenic mice expressing the P301L mutated form of human tau. *Mol. Med.* 14, 309–317. <https://doi.org/10.2119/2007-00133.Ramalho>
- Ramos-Cejudo, J., Wisniewski, T., Marmar, C., Zetterberg, H., Blennow, K., de Leon, M.J., Fossati, S., 2018. Traumatic Brain Injury and Alzheimer’s Disease: The Cerebrovascular Link. *EBioMedicine* 28, 21–30. <https://doi.org/10.1016/j.ebiom.2018.01.021>
- Ramsden, M., Kotilinek, L., Forster, C., Paulson, J., McGowan, E., SantaCruz, K., Guimaraes, A., Yue, M., Lewis, J., Carlson, G., Hutton, M., Ashe, K.H., 2005. Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L). *J. Neurosci.* 25, 10637–10647. <https://doi.org/10.1523/JNEUROSCI.3279-05.2005>
- Rao, S.S., Adlard, P.A., 2018. Untangling Tau and Iron: Exploring the Interaction Between Iron and Tau in Neurodegeneration. *Front. Mol. Neurosci.* 11, 1–13. <https://doi.org/10.3389/fnmol.2018.00276>
- Rapoport, M., Dawson, H.N., Binder, L.I., Vitek, M.P., Ferreira, A., 2002. Tau is essential to B-amyloid-induced neurotoxicity. *Proc. Natl. Acad. Sci.* 99, 6364–6369. <https://doi.org/10.1073/pnas.092136199>
- Rauch, J.N., Luna, G., Guzman, E., Audouard, M., Challis, C., Sibih, Y.E., Leshuk, C., Hernandez, I., Wegmann, S., Hyman, B.T., Gradinaru, V., Kampmann, M., Kosik, K.S., 2020. LRP1 is a master regulator of tau uptake and spread. *Nature* 580, 381–385. <https://doi.org/10.1038/s41586-020-2156-5>
- Raz, Y., Miller, Y., 2013. Interactions between A β and Mutated Tau Lead to Polymorphism and Induce Aggregation of A β -Mutated Tau Oligomeric Complexes. *PLoS One* 8, 1–14. <https://doi.org/10.1371/journal.pone.0073303>
- Reese, T.S., Karnovsky, M.J., 1967. Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J. Cell Biol.* 34, 207–217. <https://doi.org/10.1083/jcb.34.1.207>
- Regan, P., Piers, T., Yi, J.-H., Kim, D.-H., Huh, S., Park, S.J., Ryu, J.H., Whitcomb, D.J., Cho, K., 2015. Tau Phosphorylation at Serine 396 Residue Is Required for Hippocampal LTD. *J. Neurosci.* 35, 4804–4812. <https://doi.org/10.1523/JNEUROSCI.2842-14.2015>
- Rhein, V., Song, X., Wiesner, A., Ittner, L.M., Baysang, G., Meier, F., Ozmen, L., Bluethmann, H., Drose, S., Brandt, U., Savaskan, E., Czech, C., Gotz, J., Eckert, A., 2009. Amyloid- and tau synergistically impair the oxidative phosphorylation system in triple transgenic Alzheimer’s disease mice. *Proc. Natl. Acad. Sci.* 106, 20057–20062. <https://doi.org/10.1073/pnas.0905529106>
- Ribé, E.M., Pérez, M., Puig, B., Gich, I., Lim, F., Cuadrado, M., Sesma, T., Catena, S., Sánchez, B., Nieto, M., Gómez-Ramos, P., Morán, M.A., Cabodevilla, F., Samaranch, L., Ortiz, L., Pérez, A., Ferrer, I., Avila, J., Gómez-Isla, T., 2005. Accelerated amyloid deposition, neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice. *Neurobiol. Dis.* 20, 814–822. <https://doi.org/10.1016/j.nbd.2005.05.027>

- Ribeiro-da-Silva, A., Priestley, J. V., Cuellar, A.C., 1993. Pre-embedding ultrastructural immunocytochemistry, in: Cuellar, A.C. (Ed.), *Immunocytochemistry II*. John Wiley & Sons, Chichester, pp. 181–227.
- Riederer, F., 2021. Donanemab in early Alzheimer's Disease. *J. fur Neurol. Neurochir. und Psychiatr.* 22, 142–143. <https://doi.org/10.1056/nejmoa2212948>
- Roberson, E.D., Scarce-Levie, K., Palop, J.J., Yan, F., Cheng, I.H., Wu, T., Gerstein, H., Yu, G.-Q., Mucke, L., 2007. Reducing Endogenous Tau Ameliorates Amyloid-Induced Deficits in an Alzheimer's Disease Mouse Model. *Science* (80-.). 316, 750–754. <https://doi.org/10.1126/science.1141736>
- Roberts, K.F., Elbert, D.L., Kasten, T.P., Patterson, B.W., Sigurdson, W.C., Connors, R.E., Ovod, V., Munsell, L.Y., Mawuenyega, K.G., Miller-Thomas, M.M., Moran, C.J., Cross, D.T., Derdeyn, C.P., Bateman, R.J., 2014. Amyloid- β efflux from the central nervous system into the plasma. *Ann. Neurol.* 76, 837–844. <https://doi.org/10.1002/ana.24270>
- Rodríguez-Arellano, J.J., Parpura, V., Zorec, R., Verkhratsky, A., 2016. Astrocytes in physiological aging and Alzheimer's disease. *Neuroscience* 323, 170–182. <https://doi.org/10.1016/j.neuroscience.2015.01.007>
- Rodriguez, R.D., Grinberg, L.T., 2015. Argyrophilic grain disease: An underestimated tauopathy. *Dement. Neuropsychol.* 9, 2–8. <https://doi.org/10.1590/S1980-57642015DN91000002>
- Rohn, T.T., Head, E., Su, J.H., Anderson, A.J., Bahr, B.A., Cotman, C.W., Cribbs, D.H., 2001. Correlation between caspase activation and neurofibrillary tangle formation in Alzheimer's disease. *Am. J. Pathol.* 158, 189–198. [https://doi.org/10.1016/S0002-9440\(10\)63957-0](https://doi.org/10.1016/S0002-9440(10)63957-0)
- Ruiz-Opazo, N., Kosik, K.S., Lopez, L. V., Bagamasbad, P., Ponce, L.R., Herrera, V.L., 2004. Attenuated hippocampus-dependent learning and memory decline in transgenic TgAPPsw Fischer-344 rats. *Mol. Med.* 10, 36–44.
- Saido, T., Leissring, M.A., 2012. Proteolytic Degradation of Amyloid -Protein. *Cold Spring Harb. Perspect. Med.* 2, a006379–a006379. <https://doi.org/10.1101/cshperspect.a006379>
- Saito, T., Matsuba, Y., Mihira, N., Takano, J., Nilsson, P., Itohara, S., Iwata, N., Saido, T.C., 2014. Single App knock-in mouse models of Alzheimer's disease. *Nat. Neurosci.* 17, 661–663. <https://doi.org/10.1038/nn.3697>
- Saito, T., Matsuba, Y., Yamazaki, N., Hashimoto, S., Saido, T.C., 2016. Calpain activation in Alzheimer's model mice is an artifact of APP and presenilin overexpression. *J. Neurosci.* 36, 9933–9936. <https://doi.org/10.1523/JNEUROSCI.1907-16.2016>
- Saito, T., Mihira, N., Matsuba, Y., Sasaguri, H., Hashimoto, S., Narasimhan, S., Zhang, B., Murayama, S., Higuchi, M., Lee, V.M.Y., Trojanowski, J.Q., Saido, T.C., 2019. Humanization of the entire murine Mapt gene provides a murine model of pathological human tau propagation. *J. Biol. Chem.* 294, 12754–12765. <https://doi.org/10.1074/jbc.RA119.009487>
- Saito, Y., Ruberu, N.N., Sawabe, M., Arai, T., Tanaka, N., Kakuta, Y., Yamanouchi, H., Murayama, S., 2004. Staging of argyrophilic grains: An age-associated tauopathy. *J. Neuropathol. Exp. Neurol.* 63, 911–918. <https://doi.org/10.1093/jnen/63.9.911>
- Salloway, S., Sperling, R., Fox, N.C., Blennow, K., Klunk, W., Raskind, M., Sabbagh, M., Honig, L.S., Porsteinsson, A.P., Ferris, S., Reichert, M., Ketter, N., Nejadnik, B., Guenzler, V., Miloslavsky, M., Wang, D., Lu, Y., Lull, J., Tudor, I.C., Liu, E., Grundman, M., Yuen, E., Black, R., Brashear, H.R., Bapineuzumab 301 and 302 Clinical Trial Investigators, 2014. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N. Engl. J. Med.* 370, 322–33. <https://doi.org/10.1056/NEJMoa1304839>

- 6910 Santos, J.R.F., Bauer, C., Schuchhardt, J., Wedekind, D., Waniek, K., Lachmann, I., Wiltfang, J.,
 6911 Vogelgsang, J., 2019. Validation of a prototype tau Thr231 phosphorylation CSF ELISA as
 6912 a potential biomarker for Alzheimer's disease. *J. Neural Transm.* 126, 339–348.
 6913 <https://doi.org/10.1007/s00702-019-01982-5>
- 6914 Saroja, S.R., Gorbachev, K., Julia, T., Goate, A.M., Pereira, A.C., 2022. Astrocyte-secreted
 6915 glypican-4 drives APOE4-dependent tau hyperphosphorylation. *Proc. Natl. Acad. Sci.* 119,
 6916 1–12. <https://doi.org/10.1073/pnas.2108870119>
- 6917 Schedin-Weiss, S., Winblad, B., Tjernberg, L.O., 2014. The role of protein glycosylation in
 6918 Alzheimer disease. *FEBS J.* 281, 46–62. <https://doi.org/10.1111/febs.12590>
- 6919 Scheff, S.W., Price, D.A., Schmitt, F.A., Dekosky, S.T., Mufson, E.J., 2007. Synaptic alterations
 6920 in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* 68, 1501–1508.
 6921 <https://doi.org/10.1212/01.wnl.0000260698.46517.8f>
- 6922 Scheff, S.W., Price, D.A., Schmitt, F.A., Mufson, E.J., 2006. Hippocampal synaptic loss in early
 6923 Alzheimer's disease and mild cognitive impairment. *Neurobiol. Aging* 27, 1372–1384.
 6924 <https://doi.org/10.1016/j.neurobiolaging.2005.09.012>
- 6925 Schindowski, K., Bretteville, A., Leroy, K., Bégard, S., Brion, J.P., Hamdane, M., Buée, L., 2006.
 6926 Alzheimer's disease-like tau neuropathology leads to memory deficits and loss of functional
 6927 synapses in a novel mutated tau transgenic mouse without any motor deficits. *Am. J. Pathol.*
 6928 169, 599–616. <https://doi.org/10.2353/ajpath.2006.060002>
- 6929 Schöll, M., Lockhart, S.N., Schonhaut, D.R., O'Neil, J.P., Janabi, M., Ossenkoppele, R., Baker,
 6930 S.L., Vogel, J.W., Faria, J., Schwimmer, H.D., Rabinovici, G.D., Jagust, W.J., 2016. PET
 6931 Imaging of Tau Deposition in the Aging Human Brain. *Neuron* 89, 971–982.
 6932 <https://doi.org/10.1016/j.neuron.2016.01.028>
- 6933 Sebastián-Serrano, Á., de Diego-García, L., Díaz-Hernández, M., 2018. The Neurotoxic Role of
 6934 Extracellular Tau Protein. *Int. J. Mol. Sci.* 19, 998. <https://doi.org/10.3390/ijms19040998>
- 6935 Selkoe, D.J., 2001. Alzheimer's Disease: Genes, Proteins, and Therapy. *Physiol. Rev.* 81, 741–
 6936 766. <https://doi.org/10.1152/physrev.2001.81.2.741>
- 6937 Selkoe, D.J., Hardy, J., 2016. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO*
 6938 *Mol. Med.* 8, 595–608. <https://doi.org/10.15252/emmm.201606210>
- 6939 Sengillo, J.D., Winkler, E.A., Walker, C.T., Sullivan, J.S., Johnson, M., Zlokovic, B. V., 2013.
 6940 Deficiency in mural vascular cells coincides with blood-brain barrier disruption in
 6941 alzheimer's disease. *Brain Pathol.* 23, 303–310. <https://doi.org/10.1111/bpa.12004>
- 6942 Sengupta, U., Portelius, E., Hansson, O., Farmer, K., Castillo-Carranza, D., Woltjer, R.,
 6943 Zetterberg, H., Galasko, D., Blennow, K., Kayed, R., 2017. Tau oligomers in cerebrospinal
 6944 fluid in Alzheimer's disease. *Ann. Clin. Transl. Neurol.* 4, 226–235.
 6945 <https://doi.org/10.1002/acn3.382>
- 6946 Seok, J., Warren, H.S., Cuenca, A.G., Mindrinos, M.N., Baker, H. V., Xu, W., Richards, D.R.,
 6947 McDonald-Smith, G.P., Gao, H., Hennessy, L., Finnerty, C.C., López, C.M., Honari, S.,
 6948 Moore, E.E., Minei, J.P., Cuschieri, J., Bankey, P.E., Johnson, J.L., Sperry, J., Nathens, A.B.,
 6949 Billiar, T.R., West, M.A., Jeschke, M.G., Klein, M.B., Gamelli, R.L., Gibran, N.S.,
 6950 Brownstein, B.H., Miller-Graziano, C., Calvano, S.E., Mason, P.H., Cobb, J.P., Rahme, L.G.,
 6951 Lowry, S.F., Maier, R. V., Moldawer, L.L., Herndon, D.N., Davis, R.W., Xiao, W.,
 6952 Tompkins, R.G., Abouhamze, A., Balis, U.G.J., Camp, D.G., De, A.K., Harbrecht, B.G.,
 6953 Hayden, D.L., Kaushal, A., O'Keefe, G.E., Kotz, K.T., Qian, W., Schoenfeld, D.A., Shapiro,
 6954 M.B., Silver, G.M., Smith, R.D., Storey, J.D., Tibshirani, R., Toner, M., Wilhelmy, J.,
 6955 Wispelwey, B., Wong, W.H., 2013. Genomic responses in mouse models poorly mimic

- human inflammatory diseases. *Proc. Natl. Acad. Sci.* 110, 3507–3512.
<https://doi.org/10.1073/pnas.1222878110>
- Serra-Batiste, M., Ninot-Pedrosa, M., Bayoumi, M., Gairí, M., Maglia, G., Carulla, N., 2016. A β 42 assembles into specific β -barrel pore-forming oligomers in membrane-mimicking environments. *Proc. Natl. Acad. Sci.* 113, 10866–10871.
<https://doi.org/10.1073/pnas.1605104113>
- Serrano-Pozo, A., Mielke, M.L., Gómez-Isla, T., Betensky, R.A., Growdon, J.H., Frosch, M.P., Hyman, B.T., 2011. Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. *Am. J. Pathol.* 179, 1373–1384.
<https://doi.org/10.1016/j.ajpath.2011.05.047>
- Serrano-Pozo, A., Qian, J., Muzikansky, A., Monsell, S.E., Montine, T.J., Frosch, M.P., Betensky, R.A., Hyman, B.T., 2016. Thal amyloid stages do not significantly impact the correlation between neuropathological change and cognition in the Alzheimer disease continuum. *J. Neuropathol. Exp. Neurol.* 75, 516–526. <https://doi.org/10.1093/jnen/nlw026>
- Sevigny, J., Chiao, P., Bussière, T., Weinreb, P.H., Williams, L., Maier, M., Dunstan, R., Salloway, S., Chen, T., Ling, Y., O'Gorman, J., Qian, F., Arastu, M., Li, M., Chollate, S., Brennan, M.S., Quintero-Monzon, O., Scannevin, R.H., Arnold, H.M., Engber, T., Rhodes, K., Ferrero, J., Hang, Y., Mikulskis, A., Grimm, J., Hock, C., Nitsch, R.M., Sandrock, A., 2016. The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature* 537, 50–6. <https://doi.org/10.1038/nature19323>
- Seward, M.E., Swanson, E., Norambuena, A., Reimann, A., Cochran, J.N., Li, R., Roberson, E.D., Bloom, G.S., 2013. Amyloid- signals through tau to drive ectopic neuronal cell cycle re-entry in Alzheimer's disease. *J. Cell Sci.* 126, 1278–1286.
<https://doi.org/10.1242/jcs.1125880>
- Shafiei, S.S., Guerrero-Muñoz, M.J., Castillo-Carranza, D.L., 2017. Tau oligomers: Cytotoxicity, propagation, and mitochondrial damage. *Front. Aging Neurosci.* 9, 1–9.
<https://doi.org/10.3389/fnagi.2017.00083>
- Shammas, S.L., Garcia, G.A., Kumar, S., Kjaergaard, M., Horrocks, M.H., Shivji, N., Mandelkow, E.E.E., Knowles, T.P.J., Mandelkow, E.E.E., Klennerman, D., 2015. A mechanistic model of tau amyloid aggregation based on direct observation of oligomers. *Nat. Commun.* 6, 1–10.
<https://doi.org/10.1038/ncomms8025>
- Sharman, M.J., Moussavi Nik, S.H., Chen, M.M., Ong, D., Wijaya, L., Laws, S.M., Taddei, K., Newman, M., Lardelli, M., Martins, R.N., Verdile, G., 2013. The Guinea Pig as a Model for Sporadic Alzheimer's Disease (AD): The Impact of Cholesterol Intake on Expression of AD-Related Genes. *PLoS One* 8. <https://doi.org/10.1371/journal.pone.0066235>
- Sharp, E.S., Gatz, M., 2011. Relationship between education and dementia: An updated systematic review. *Alzheimer Dis. Assoc. Disord.* 25, 289–304.
<https://doi.org/10.1097/WAD.0b013e318211c83c>
- Shea, Y.F., Chu, L.W., Chan, A.O.K., Ha, J., Li, Y., Song, Y.Q., 2016. A systematic review of familial Alzheimer's disease: Differences in presentation of clinical features among three mutated genes and potential ethnic differences. *J. Formos. Med. Assoc.* 115, 67–75.
<https://doi.org/10.1016/j.jfma.2015.08.004>
- Shi, Y., Yamada, K., Liddelow, S.A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S., Grinberg, L.T., Rojas, J.C., Gallardo, G., Wang, K., Roh, J., Robinson, G., Finn, M.B., Jiang, H., Sullivan, P.M., Baufeld, C., Wood, M.W., Sutphen, C., McCue, L., Xiong, C., Del-Aguila, J.L., Morris, J.C., Cruchaga, C., Fagan, A.M., Miller, B.L., Boxer, A.L., Seeley, W.W.,

- Butovsky, O., Barres, B.A., Paul, S.M., Holtzman, D.M., 2017. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* 549, 523–527. <https://doi.org/10.1038/nature24016>
- Shibata, M., Yamada, S., Kumar, S.R., Calero, M., Bading, J., Frangione, B., Holtzman, D.M., Miller, C.A., Strickland, D.K., Ghiso, J., Zlokovic, B. V., 2000. Clearance of Alzheimer's amyloid- β 1-40 peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J. Clin. Invest.* 106, 1489–1499. <https://doi.org/10.1172/JCI10498>
- Shipton, O.A., Leitz, J.R., Dworzak, J., Acton, C.E.J., Tunbridge, E.M., Denk, F., Dawson, H.N., Vitek, M.P., Wade-Martins, R., Paulsen, O., Vargas-Caballero, M., 2011. Tau Protein Is Required for Amyloid β -Induced Impairment of Hippocampal Long-Term Potentiation. *J. Neurosci.* 31, 1688–1692. <https://doi.org/10.1523/JNEUROSCI.2610-10.2011>
- Shirazi, S.K., Wood, J.G., 1993. The protein tyrosine kinase, fyn, in Alzheimer's disease pathology. *Neuroreport* 4, 435–437. <https://doi.org/10.1097/00001756-199304000-00024>
- Shirotani, K., Tsubuki, S., Iwata, N., Takaki, Y., Harigaya, W., Maruyama, K., Kiryu-Seo, S., Kiyama, H., Iwata, H., Tomita, T., Iwatsubo, T., Saido, T.C., 2001. Neprilysin Degrades Both Amyloid β Peptides 1-40 and 1-42 Most Rapidly and Efficiently among Thiorphan- and Phosphoramidon-sensitive Endopeptidases. *J. Biol. Chem.* 276, 21895–21901. <https://doi.org/10.1074/jbc.M008511200>
- Sidoryk-Wegrzynowicz, M., Gerber, Y.N., Ries, M., Sastre, M., Tolkovsky, A.M., Spillantini, M.G., 2017. Astrocytes in mouse models of tauopathies acquire early deficits and lose neurosupportive functions. *Acta Neuropathol. Commun.* 5, 89. <https://doi.org/10.1186/s40478-017-0478-9>
- Sikora, E., Bielak-Zmijewska, A., Dudkowska, M., Krzystyniak, A., Mosieniak, G., Wesierska, M., Wlodarczyk, J., 2021. Cellular Senescence in Brain Aging. *Front. Aging Neurosci.* 13, 1–23. <https://doi.org/10.3389/fnagi.2021.646924>
- Silva, I., Silva, J., Ferreira, R., Trigo, D., 2021. Glymphatic system, AQP4, and their implications in Alzheimer's disease. *Neurol. Res. Pract.* 3. <https://doi.org/10.1186/s42466-021-00102-7>
- Skjolding, A.D., Rowland, I.J., S gaard, L. V., Praetorius, J., Penkowa, M., Juhler, M., 2010. Hydrocephalus induces dynamic spatiotemporal regulation of aquaporin-4 expression in the rat brain. *Cerebrospinal Fluid Res.* 7, 1–13. <https://doi.org/10.1186/1743-8454-7-20>
- Smith, M.A., Harris, P.L.R., Sayre, L.M., Perry, G., 1997. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9866–9868. <https://doi.org/10.1073/pnas.94.18.9866>
- Smolek, T., Jadhav, S., Brezovakova, V., Cubinkova, V., Valachova, B., Novak, P., Zilka, N., 2019. First-in-Rat Study of Human Alzheimer's Disease Tau Propagation. *Mol. Neurobiol.* 56, 621–631. <https://doi.org/10.1007/s12035-018-1102-0>
- Sokoloff, L., Reivich, M., Kennedy, C., Rosiers, M.H.D., Patlak, C.S., Pettigrew, K.D., Sakurada, O., Shinohara, M., 1977. the [14C]Deoxyglucose Method for the Measurement of Local Cerebral Glucose Utilization: Theory, Procedure, and Normal Values in the Conscious and Anesthetized Albino Rat. *J. Neurochem.* 28, 897–916. <https://doi.org/10.1111/j.1471-4159.1977.tb10649.x>
- Sowell, E.R., Peterson, B.S., Thompson, P.M., Welcome, S.E., Henkenius, A.L., Toga, A.W., 2003. Mapping cortical change across the human life span. *Nat. Neurosci.* 6, 309–315. <https://doi.org/10.1038/nn1008>
- Sperling, R.A., Aisen, P.S., Beckett, L.A., Bennett, D.A., Craft, S., Fagan, A.M., Iwatsubo, T., Jack, C.R., Kaye, J., Montine, T.J., Park, D.C., Reiman, E.M., Rowe, C.C., Siemers, E., Stern,

- 7048 Y., Yaffe, K., Carrillo, M.C., Thies, B., Morrison-Bogorad, M., Wagster, M. V., Phelps, C.H.,
 7049 2011. Toward defining the preclinical stages of Alzheimer's disease: Recommendations from
 7050 the National Institute on Aging-Alzheimer's Association workgroups on diagnostic
 7051 guidelines for Alzheimer's disease. *Alzheimer's Dement.* 7, 280–292.
 7052 <https://doi.org/10.1016/j.jalz.2011.03.003>
- 7053 Spiegel, J., Pirraglia, E., Osorio, R.S., Glodzik, L., Li, Y., Louis, L.A. Saint, Randall, C., Butler,
 7054 T., Xu, J., Raymond, P., Zetterberg, H., Fortea, J., Fossati, S., Wisniewski, T., Blennow, K.,
 7055 Leon, M.J. De, Radiology, D., 2015. Greater Specificity for Cerebrospinal Fluid P-tau231
 7056 over P- tau181 in the Differentiation of Healthy Controls from Alzheimer's Disease. *J.*
 7057 *Alzheimer's Dis.* 49, 93–100. <https://doi.org/10.3233/JAD-150167>.Greater
- 7058 Spillantini, M.G., Goedert, M., 1998. Tau protein pathology in neurodegenerative diseases. *Trends*
 7059 *Neurosci.* 21, 428–433. [https://doi.org/10.1016/S0166-2236\(98\)01337-X](https://doi.org/10.1016/S0166-2236(98)01337-X)
- 7060 Spires, T.L., Orne, J.D., SantaCruz, K., Pitstick, R., Carlson, G.A., Ashe, K.H., Hyman, B.T.,
 7061 2006. Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse
 7062 model of tauopathy. *Am. J. Pathol.* 168, 1598–1607.
 7063 <https://doi.org/10.2353/ajpath.2006.050840>
- 7064 Spotorno, N., Acosta-Cabronero, J., Stomrud, E., Lampinen, B., Strandberg, O.T., van Westen,
 7065 D., Hansson, O., 2020. Relationship between cortical iron and tau aggregation in Alzheimer's
 7066 disease. *Brain* 143, 1341–1349. <https://doi.org/10.1093/brain/awaa089>
- 7067 Stancu, I.C., Vasconcelos, B., Terwel, D., Dewachter, I., 2014. Models of B-amyloid induced Tau-
 7068 pathology: the long and “folded” road to understand the mechanism. *Mol. Neurodegener.* 9,
 7069 51. <https://doi.org/10.1186/1750-1326-9-51>
- 7070 Steffener, J., Stern, Y., 2012. Exploring the neural basis of cognitive reserve in aging. *Biochim.*
 7071 *Biophys. Acta - Mol. Basis Dis.* 1822, 467–473. <https://doi.org/10.1016/j.bbadis.2011.09.012>
- 7072 Stern, Y., 2013. Cognitive reserve in ageing. *Lancet Neurol.* 11, 1006–1012.
 7073 [https://doi.org/10.1016/S1474-4422\(12\)70191-6](https://doi.org/10.1016/S1474-4422(12)70191-6).Cognitive
- 7074 Sterniczuk, R., Antle, M.C., Laferla, F.M., Dyck, R.H., 2010. Characterization of the 3xTg-AD
 7075 mouse model of Alzheimer's disease: Part 2. Behavioral and cognitive changes. *Brain Res.*
 7076 1348, 149–155. <https://doi.org/10.1016/j.brainres.2010.06.011>
- 7077 Strang, K.H., Croft, C.L., Sorrentino, Z.A., Chakrabarty, P., Golde, T.E., Giasson, B.I., 2018.
 7078 Distinct differences in prion-like seeding and aggregation between Tau protein variants
 7079 provide mechanistic insights into tauopathies. *J. Biol. Chem.* 293, 2408–2421.
 7080 <https://doi.org/10.1074/jbc.M117.815357>
- 7081 Streit, W.J., Xue, Q.S., Tischer, J., Bechmann, I., 2014. Microglial pathology. *Acta Neuropathol.*
 7082 *Commun.* 2, 1–17. <https://doi.org/10.1186/s40478-014-0142-6>
- 7083 Strozyk, D., Blennow, K., White, L.R., Launer, L.J., 2003. CSF Aβ 42 levels correlate with
 7084 amyloid-neuropathology in a population-based autopsy study. *Neurology* 60, 652–656.
 7085 <https://doi.org/10.1212/01.WNL.0000046581.81650.D0>
- 7086 Sturchler-Pierrat, C., Abramowski, D., Duke, M., Wiederhold, K.H., Mistl, C., Rothacher, S.,
 7087 Ledermann, B., Bürki, K., Frey, P., Paganetti, P.A., Waridel, C., Calhoun, M.E., Jucker, M.,
 7088 Probst, A., Staufenbiel, M., Sommer, B., 1997. Two amyloid precursor protein transgenic
 7089 mouse models with Alzheimer disease-like pathology. *Proc. Natl. Acad. Sci. U. S. A.* 94,
 7090 13287–92.
- 7091 Su, J.H., Zhao, M., Anderson, A.J., Srinivasan, A., Cotman, C.W., 2001. Activated caspase-3
 7092 expression in Alzheimer's and aged control brain: Correlation with Alzheimer pathology.
 7093 *Brain Res.* 898, 350–357. [https://doi.org/10.1016/S0006-8993\(01\)02018-2](https://doi.org/10.1016/S0006-8993(01)02018-2)

- 7094 Suárez-Calvet, M., Karikari, T.K., Ashton, N.J., Lantero Rodríguez, J., Milà-Alomà, M., Gispert,
7095 J.D., Salvadó, G., Minguillon, C., Fauria, K., Shekari, M., Grau-Rivera, O., Arenaza-Urquijo,
7096 E.M., Sala-Vila, A., Sánchez-Benavides, G., González-de-Echávarri, J.M., Kollmorgen, G.,
7097 Stoops, E., Vanmechelen, E., Zetterberg, H., Blennow, K., Molinuevo, J.L., Beteta, A.,
7098 Cacciaglia, R., Cañas, A., Deulofeu, C., Cumplido, I., Dominguez, R., Emilio, M., Falcon,
7099 C., Fuentes, S., Hernandez, L., Huesa, G., Huguet, J., Marne, P., Menchón, T., Operto, G.,
7100 Polo, A., Pradas, S., Soteras, A., Vilanova, M., Vilor-Tejedor, N., 2020. Novel tau biomarkers
7101 phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's
7102 continuum when only subtle changes in A β pathology are detected. *EMBO Mol. Med.* 12, 1–
7103 19. <https://doi.org/10.15252/emmm.202012921>
- 7104 Tai, H.C., Serrano-Pozo, A., Hashimoto, T., Frosch, M.P., Spires-Jones, T.L., Hyman, B.T., 2012.
7105 The synaptic accumulation of hyperphosphorylated tau oligomers in alzheimer disease is
7106 associated with dysfunction of the ubiquitin-proteasome system. *Am. J. Pathol.* 181, 1426–
7107 1435. <https://doi.org/10.1016/j.ajpath.2012.06.033>
- 7108 Takahashi, M., Miyata, H., Kametani, F., Nonaka, T., Akiyama, H., Hisanaga, S. ichi, Hasegawa,
7109 M., 2015. Extracellular association of APP and tau fibrils induces intracellular aggregate
7110 formation of tau. *Acta Neuropathol.* 129, 895–907. [https://doi.org/10.1007/s00401-015-](https://doi.org/10.1007/s00401-015-1415-2)
7111 1415-2
- 7112 Takahashi, M., Tsujioka, Y., Yamada, T., Tsuboi, Y., Okada, H., Yamamoto, T., Liposits, Z.,
7113 1999. Glycosylation of microtubule-associated protein tau in Alzheimer's disease brain. *Acta*
7114 *Neuropathol.* 97, 635-641 ST-Glycosylation of microtubule-associa.
7115 <https://doi.org/10.1007/s004010051040>
- 7116 Takashima, A., 2016. Mechanism of neurodegeneration through tau and therapy for Alzheimer's
7117 disease. *J. Sport Heal. Sci.* 5, 391–392. <https://doi.org/10.1016/j.jshs.2016.08.009>
- 7118 Tam, W.Y., Ma, C.H.E., 2014. Bipolar/rod-shaped microglia are proliferating microglia with
7119 distinct M1/M2 phenotypes. *Sci. Rep.* 4, 1–7. <https://doi.org/10.1038/srep07279>
- 7120 Tang, H., Mao, X.O., Xie, L., Greenberg, D.A., Jin, K., 2013. Expression level of vascular
7121 endothelial growth factor in hippocampus is associated with cognitive impairment in patients
7122 with Alzheimer's disease. *Neurobiol. Aging* 34, 1412–1415.
7123 <https://doi.org/10.1016/j.neurobiolaging.2012.10.029>
- 7124 Taylor, S.E., Morganti-Kossmann, C., Lifshitz, J., Ziebell, J.M., 2014. Rod microglia: A
7125 morphological definition. *PLoS One* 9. <https://doi.org/10.1371/journal.pone.0097096>
- 7126 Terry, R.D., Peck, A., DeTeresa, R., Schechter, R., Horoupian, D.S., 1981. Some morphometric
7127 aspects of the brain in senile dementia of the alzheimer type. *Ann. Neurol.* 10, 184–192.
7128 <https://doi.org/10.1002/ana.410100209>
- 7129 Terwel, D., Muyllaert, D., Dewachter, I., Borghgraef, P., Croes, S., Devijver, H., Van Leuven, F.,
7130 2008. Amyloid activates GSK-3 β to aggravate neuronal tauopathy in bigenic mice. *Am. J.*
7131 *Pathol.* 172, 786–798. <https://doi.org/10.2353/ajpath.2008.070904>
- 7132 Thal, D.R., Rüb, U., Orantes, M., Braak, H., 2002. Phases of A β -deposition in the human brain
7133 and its relevance for the development of AD. *Neurology* 58, 1791–1800.
7134 <https://doi.org/10.1212/WNL.58.12.1791>
- 7135 Therriault, J., Benedet, A.L., Pascoal, T.A., Mathotaarachchi, S., Chamoun, M., Savard, M.,
7136 Thomas, E., Kang, M.S., Lussier, F., Tissot, C., Parsons, M., Qureshi, M.N.I., Vitali, P.,
7137 Massarweh, G., Soucy, J.P., Rej, S., Saha-Chaudhuri, P., Gauthier, S., Rosa-Neto, P., 2020.
7138 Association of Apolipoprotein e ϵ 4 with Medial Temporal Tau Independent of Amyloid- β .
7139 *JAMA Neurol.* 77, 470–479. <https://doi.org/10.1001/jamaneurol.2019.4421>

- Thibault, O., Landfield, P.W., 1996. Increase in single L-type calcium channels in hippocampal neurons during aging. *Science* (80-.). 272, 1017–1020. <https://doi.org/10.1126/science.272.5264.1017>
- Thijssen, E.H., La Joie, R., Wolf, A., Strom, A., Wang, P., Iaccarino, L., Bourakova, V., Cobigo, Y., Heuer, H., Spina, S., VandeVrede, L., Chai, X., Proctor, N.K., Airey, D.C., Shcherbinin, S., Duggan Evans, C., Sims, J.R., Zetterberg, H., Blennow, K., Karydas, A.M., Teunissen, C.E., Kramer, J.H., Grinberg, L.T., Seeley, W.W., Rosen, H., Boeve, B.F., Miller, B.L., Rabinovici, G.D., Dage, J.L., Rojas, J.C., Boxer, A.L., Forsberg, L., Knopman, D.S., Graff-Radford, N., Grossman, M., Huey, E.H., Onyike, C., Kaufer, D., Roberson, E., Ghoshal, N., Weintraub, S., Appleby, B., Litvan, I., Kerwin, D., Mendez, M., Bordelon, Y., Coppola, G., Ramos, E.M., Tartaglia, M.C., Hsiung, G.Y., MacKenzie, I., Domoto-Reilly, K., Foroud, T., Dickerson, B.C., 2020. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat. Med.* 26, 387–397. <https://doi.org/10.1038/s41591-020-0762-2>
- Thomas, T., Miners, S., Love, S., 2015. Post-mortem assessment of hypoperfusion of cerebral cortex in Alzheimer's disease and vascular dementia. *Brain* 138, 1059–1069. <https://doi.org/10.1093/brain/awv025>
- Town, T., Zolton, J., Shaffner, R., Schnell, B., Crescentini, R., Wu, Y., Zeng, J., DelleDonne, A., Obregon, D., Tan, J., Mullan, M., 2002. p35/Cdk5 pathway mediates soluble amyloid- β peptide-induced tau phosphorylation in vitro. *J. Neurosci. Res.* 69, 362–372. <https://doi.org/10.1002/jnr.10299>
- Tran, D., DiGiacomo, P., Born, D.E., Georgiadis, M., Zeineh, M., 2022. Iron and Alzheimer's Disease: From Pathology to Imaging. *Front. Hum. Neurosci.* 16, 1–19. <https://doi.org/10.3389/fnhum.2022.838692>
- Tran, T.N., Kim, S.H., Gallo, C., Amaya, M., Kyees, J., Narayanaswami, V., 2013. Biochemical and biophysical characterization of recombinant rat apolipoprotein E: Similarities to human apolipoprotein E3. *Arch. Biochem. Biophys.* 529, 18–25. <https://doi.org/10.1016/j.abb.2012.10.007>
- Tseng, J.H., Xie, L., Song, S., Xie, Y., Allen, L., Ajit, D., Hong, J.S., Chen, X., Meeker, R.B., Cohen, T.J., 2017. The Deacetylase HDAC6 Mediates Endogenous Neuritic Tau Pathology. *Cell Rep.* 20, 2169–2183. <https://doi.org/10.1016/j.celrep.2017.07.082>
- Tsoi, K.K.F., Chan, J.Y.C., Hirai, H.W., Wong, S.Y.S., Kwok, T.C.Y., 2015. Cognitive Tests to Detect Dementia A Systematic Review and Meta-analysis. *JAMA Intern. Med.* 175, 1450–1458. <https://doi.org/10.1001/jamainternmed.2015.2152>
- Usenovic, M., Niroomand, S., Drolet, R.E., Yao, L., Gaspar, R.C., Hatcher, N.G., Schachter, J., Renger, J.J., Parmentier-Batteur, S., 2015. Internalized tau oligomers cause neurodegeneration by inducing accumulation of pathogenic tau in human neurons derived from induced pluripotent stem cells. *J. Neurosci.* 35, 14234–14250. <https://doi.org/10.1523/JNEUROSCI.1523-15.2015>
- Valenza, M., Facchinetti, R., Steardo, L., Scuderi, C., 2020. Altered waste disposal system in aging and Alzheimer's disease: Focus on astrocytic aquaporin-4. *Front. Pharmacol.* 10, 1–7. <https://doi.org/10.3389/fphar.2019.01656>
- Van Broeckhoven, C., Haan, J., Bakker, E., Hardy, J.A., Van Hul, W., Wehnert, A., Vegter-Van Der Vlis, M., Roos, R.A.C., 1990. Amyloid β protein precursor gene and hereditary cerebral hemorrhage with amyloidosis (Dutch). *Science* (80-.). 248, 1120–1122. <https://doi.org/10.1126/science.1971458>

- van de Haar, H.J., Jansen, J.F.A., van Osch, M.J.P., van Buchem, M.A., Muller, M., Wong, S.M., Hofman, P.A.M., Burgmans, S., Verhey, F.R.J., Backes, W.H., 2016. Neurovascular unit impairment in early Alzheimer's disease measured with magnetic resonance imaging. *Neurobiol. Aging* 45, 190–196. <https://doi.org/10.1016/j.neurobiolaging.2016.06.006>
- van der Kant, R., Goldstein, L.S.B., 2015. Cellular Functions of the Amyloid Precursor Protein from Development to Dementia. *Dev. Cell* 32, 502–515. <https://doi.org/10.1016/j.devcel.2015.01.022>
- Vandamme, T., 2014. Use of rodents as models of human diseases. *J. Pharm. Bioallied Sci.* 6, 2–9. <https://doi.org/10.4103/0975-7406.124301>
- Vanden Dries, V., Stygelbout, V., Pierrot, N., Yilmaz, Z., Suain, V., De Decker, R., Buée, L., Octave, J.N., Brion, J.P., Leroy, K., 2017. Amyloid precursor protein reduction enhances the formation of neurofibrillary tangles in a mutant tau transgenic mouse model. *Neurobiol. Aging* 55, 202–212. <https://doi.org/10.1016/j.neurobiolaging.2017.03.031>
- Vergara, C., Houben, S., Suain, V., Yilmaz, Z., De Decker, R., Vanden Dries, V., Boom, A., Mansour, S., Leroy, K., Ando, K., Brion, J.-P., 2019. Amyloid- β pathology enhances pathological fibrillary tau seeding induced by Alzheimer PHF in vivo. *Acta Neuropathol.* 137, 397–412. <https://doi.org/10.1007/s00401-018-1953-5>
- Vilchez, D., Saez, I., Dillin, A., 2014. The role of protein clearance mechanisms in organismal ageing and age-related diseases. *Nat. Commun.* 5. <https://doi.org/10.1038/ncomms6659>
- Villemagne, V.L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K.A., Salvado, O., Szoëke, C., Macaulay, S.L., Martins, R., Maruff, P., Ames, D., Rowe, C.C., Masters, C.L., Australian Imaging Biomarkers and Lifestyle (AIBL) Research Group, 2013. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet. Neurol.* 12, 357–67. [https://doi.org/10.1016/S1474-4422\(13\)70044-9](https://doi.org/10.1016/S1474-4422(13)70044-9)
- Vilor-Tejedor, N., Ciampa, I., Operto, G., Falcón, C., Suárez-Calvet, M., Crous-Bou, M., Shekari, M., Arenaza-Urquijo, E.M., Milà-Alomà, M., Grau-Rivera, O., Minguillon, C., Kollmorgen, G., Zetterberg, H., Blennow, K., Guigo, R., Molinuevo, J.L., Gispert, J.D., Beteta, A., Bruguat, A., Cacciaglia, R., Cañas, A., Deulofeu, C., Cumplido, I., Dominguez, R., Emilio, M., Fauria, K., Fuentes, S., Hernandez, L., Huesa, G., Huguet, J., Marne, P., Menchón, T., Polo, A., Pradas, S., Rodriguez-Fernandez, B., Sala-Vila, A., Sánchez-Benavides, G., Salvadó, G., Soteras, A., Vilanova, M., 2021. Perivascular spaces are associated with tau pathophysiology and synaptic dysfunction in early Alzheimer's continuum. *Alzheimers. Res. Ther.* 13, 135. <https://doi.org/10.1186/s13195-021-00878-5>
- Violet, M., Delattre, L., Tardivel, M., Sultan, A., Chauderlier, A., Caillierez, R., Talahari, S., Nesslany, F., Lefebvre, B., Bonnefoy, E., Buée, L., Galas, M.C., 2014. A major role for Tau in neuronal DNA and RNA protection in vivo under physiological and hyperthermic conditions. *Front. Cell. Neurosci.* 8, 1–11. <https://doi.org/10.3389/fncel.2014.00084>
- Vitek, M.P., Araujo, J.A., Fosse, M., Greenberg, B.D., Howell, G.R., Rizzo, S.J.S., Seyfried, N.T., Tenner, A.J., Territo, P.R., Windisch, M., Bain, L.J., Ross, A., Carrillo, M.C., Lamb, B.T., Edelmayer, R.M., 2020. Translational animal models for Alzheimer's disease: An Alzheimer's Association Business Consortium Think Tank. *Alzheimer's Dement. Transl. Res. Clin. Interv.* 6, 1–12. <https://doi.org/10.1002/trc2.12114>
- Vogelsberg-Ragaglia, V., Schuck, T., Trojanowski, J.Q., Lee, V.M.-Y., 2001. PP2A mRNA Expression Is Quantitatively Decreased in Alzheimer's Disease Hippocampus. *Exp. Neurol.* 168, 402–412. <https://doi.org/10.1006/exnr.2001.7630>
- Volianskis, A., Køstner, R., Mølgaard, M., Hass, S., Jensen, M.S., 2010. Episodic memory deficits

- are not related to altered glutamatergic synaptic transmission and plasticity in the CA1 hippocampus of the APP^{swe}/PS1 Δ E9-deleted transgenic mice model of β -amyloidosis. *Neurobiol. Aging* 31, 1173–1187. <https://doi.org/10.1016/j.neurobiolaging.2008.08.005>
- Vossel, K.A., Zhang, K., Brodbeck, J., Daub, A.C., Sharma, P., Finkbeiner, S., Cui, B., Mucke, L., 2010. Tau reduction prevents A β -induced defects in axonal transport. *Science* (80-.). 330, 8–10. <https://doi.org/10.1126/science.1194653>
- Walsh, D.M., Klyubin, I., Fadeeva, J. V., Cullen, W.K., Anwyl, R., Wolfe, M.S., Rowan, M.J., Selkoe, D.J., 2002. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416, 535–539. <https://doi.org/10.1038/416535a>
- Walsh, D.M., Tseng, B.P., Rydel, R.E., Podlisny, M.B., Selkoe, D.J., 2000. The oligomerization of amyloid β -protein begins intracellularly in cells derived from human brain. *Biochemistry* 39, 10831–10839. <https://doi.org/10.1021/bi001048s>
- Walters, A., Phillips, E., Zheng, R., Biju, M., Kuruvilla, T., 2016. Evidence for neuroinflammation in Alzheimer's disease. *Prog. Neurol. Psychiatry* 20, 25–31. <https://doi.org/10.1002/pnp.444>
- Wang, H.-Y., Li, W., Benedetti, N.J., Lee, D.H.S., 2003. α 7 Nicotinic Acetylcholine Receptors Mediate β -Amyloid Peptide-induced Tau Protein Phosphorylation. *J. Biol. Chem.* 278, 31547–31553. <https://doi.org/10.1074/jbc.M212532200>
- Wang, H., Ardiles, A.O., Yang, S., Tran, T., Posada-Duque, R., Valdivia, G., Baek, M., Chuang, Y.A., Palacios, A.G., Gallagher, M., Worley, P., Kirkwood, A., 2016. Metabotropic glutamate receptors induce a form of LTP controlled by translation and arc signaling in the hippocampus. *J. Neurosci.* 36, 1723–1729. <https://doi.org/10.1523/JNEUROSCI.0878-15.2016>
- Wang, H.W., Pasternak, J.F., Kuo, H., Ristic, H., Lambert, M.P., Chromy, B., Viola, K.L., Klein, W.L., Stine, W.B., Krafft, G.A., Trommer, B.L., 2002. Soluble oligomers of β amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Res.* 924, 133–140. [https://doi.org/10.1016/S0006-8993\(01\)03058-X](https://doi.org/10.1016/S0006-8993(01)03058-X)
- Wang, J., Grundke-Iqbal, I., Iqbal, K., 1996. Glycosylation of microtubule-associated protein tau: An abnormal posttranslational modification in Alzheimer's disease. *Nat. Med.* 2, 871–875. <https://doi.org/10.1038/nm0896-871>
- Wang, J., Jin, W.S., Bu, X. Le, Zeng, F., Huang, Z.L., Li, W.W., Shen, L.L., Zhuang, Z.Q., Fang, Y., Sun, B.L., Zhu, J., Yao, X.Q., Zeng, G.H., Dong, Z.F., Yu, J.T., Hu, Z., Song, W., Zhou, H.D., Jiang, J.X., Liu, Y.H., Wang, Y.J., 2018. Physiological clearance of tau in the periphery and its therapeutic potential for tauopathies. *Acta Neuropathol.* 136, 525–536. <https://doi.org/10.1007/s00401-018-1891-2>
- Wang, J.Z., Wang, Z.H., Tian, Q., 2014. Tau hyperphosphorylation induces apoptotic escape and triggers neurodegeneration in Alzheimer's disease. *Neurosci. Bull.* 30, 359–366. <https://doi.org/10.1007/s12264-013-1415-y>
- Wang, L., Benzinger, T.L., Su, Y., Christensen, J., Friedrichsen, K., Aldea, P., McConathy, J., Cairns, N.J., Fagan, A.M., Morris, J.C., Ances, B.M., Louis, S., Wang, M., Charles, T.F., Knight Alzheimer, J., 2016. Evaluation of Tau Imaging in Staging Alzheimer Disease and Revealing Interactions Between β -Amyloid and Tauopathy. *JAMA Neurol* 73, 1070–1077. <https://doi.org/10.1001/jamaneurol.2016.2078>
- Wang, X.-J., Cao, Q., Zhang, Y., Su, X.-D., 2015. Activation and Regulation of Caspase-6 and Its Role in Neurodegenerative Diseases. *Annu. Rev. Pharmacol. Toxicol.* 55, 553–572. <https://doi.org/10.1146/annurev-pharmtox-010814-124414>

- Wang, X., Zhang, C., Szabo, G., Sun, Q.-Q., 2013. Distribution of CaMKII α expression in the brain in vivo, studied by CaMKII α -GFP mice. *Brain Res.* 1518, 9–25. <https://doi.org/10.3174/ajnr.A1256>. Functional
- Wang, X.L., Li, L., 2021. Cell type-specific potential pathogenic genes and functional pathways in Alzheimer's Disease. *BMC Neurol.* 21, 1–18. <https://doi.org/10.1186/s12883-021-02407-1>
- Wang, Y., Mandelkow, E., 2015. Tau in physiology and pathology. *Nat. Rev. Neurosci.* 17, 22–35. <https://doi.org/10.1038/nrn.2015.1>
- Ward, S.M., Himmelstein, D.S., Lancia, J.K., Binder, L.I., 2012. Tau oligomers and tau toxicity in neurodegenerative disease. *Biochem. Soc. Trans.* 40, 667–671. <https://doi.org/10.1042/BST20120134>
- Wardlaw, J.M., Makin, S.J., Valdés Hernández, M.C., Armitage, P.A., Heye, A.K., Chappell, F.M., Muñoz-Maniega, S., Sakka, E., Shuler, K., Dennis, M.S., Thrippleton, M.J., 2017. Blood-brain barrier failure as a core mechanism in cerebral small vessel disease and dementia: evidence from a cohort study. *Alzheimer's Dement.* 13, 634–643. <https://doi.org/10.1016/j.jalz.2016.09.006>
- Wardlaw, J.M., Smith, E.E., Biessels, G.J., Cordonnier, C., Fazekas, F., Frayne, R., Lindley, R.I., O'Brien, J.T., Barkhof, F., Benavente, O.R., Black, S.E., Brayne, C., Breteler, M., Chabriat, H., DeCarli, C., de Leeuw, F.E., Doubal, F., Duering, M., Fox, N.C., Greenberg, S., Hachinski, V., Kilimann, I., Mok, V., Oostenbrugge, R. van, Pantoni, L., Speck, O., Stephan, B.C.M., Teipel, S., Viswanathan, A., Werring, D., Chen, C., Smith, C., van Buchem, M., Norrving, B., Gorelick, P.B., Dichgans, M., 2013. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol.* 12, 822–838. [https://doi.org/10.1016/S1474-4422\(13\)70124-8](https://doi.org/10.1016/S1474-4422(13)70124-8)
- Warren, J.T.J., Roses, A.D., Peacock, M.L., Warren, J.T.J., Roses, A.D., Fink, J.K., 1993. Novel polymorphism in the A4 region of the amyloid precursor protein gene in a patient without Alzheimer's disease. *LK* - <https://mcgill.on.worldcat.org/oclc/117074751>. *Neurol. TA* - TT - 43, 1254–1256.
- Weaver, C.L., Espinoza, M., Kress, Y., Davies, P., 2000. Conformational change as one of the earliest alterations of tau in Alzheimer's disease. *Neurobiol. Aging* 21, 719–727. [https://doi.org/10.1016/S0197-4580\(00\)00157-3](https://doi.org/10.1016/S0197-4580(00)00157-3)
- Weingarten, M.D., Lockwood, A.H., Hwo, S., Kirschner, M.W., 1975. A Protein factor essential for microtubule assembly. *Proc Nat Acad Sci USA* 72, 1858–1862.
- Welikovitch, L.A., Do Carmo, S., Maglóczy, Z., Szocsics, P., Lőke, J., Freund, T., Cuello, A.C., 2018. Evidence of intraneuronal A β accumulation preceding tau pathology in the entorhinal cortex. *Acta Neuropathol.* 136, 901–917. <https://doi.org/10.1007/s00401-018-1922-z>
- West, M.J., Kawas, C.H., Stewart, W.F., Rudow, G.L., Troncoso, J.C., 2004. Hippocampal neurons in pre-clinical Alzheimer's disease. *Neurobiol. Aging* 25, 1205–1212. <https://doi.org/10.1016/j.neurobiolaging.2003.12.005>
- Whishaw, I.Q., Tomie, J., 1996. Of Mice and Mazes: Similarities Between Mice and Rats on Dry Land But Not Water Mazes. *Physiol. Behav.* 60, 1191–1197. [https://doi.org/10.1016/S0031-9384\(96\)00176-X](https://doi.org/10.1016/S0031-9384(96)00176-X)
- Wienhard, K., Pawlik, G., Herholz, K., Wagner, R., Heiss, W.D., 1985. Estimation of local cerebral glucose utilization by positron emission tomography of [18F]2-fluoro-2-deoxy-D-glucose: A critical appraisal of optimization procedures. *J. Cereb. Blood Flow Metab.* 5, 115–125. <https://doi.org/10.1038/jcbfm.1985.15>

- Wilcock, D.M., Vitek, M.P., Colton, C.A., 2009. Vascular amyloid alters astrocytic water and potassium channels in mouse models and humans with Alzheimer's disease. *Neuroscience* 159, 1055–1069. <https://doi.org/10.1016/j.neuroscience.2009.01.023>
- Wilcock, G.K., Gauthier, S., Frisoni, G.B., Jia, J., Hardlund, J.H., Moebius, H.J., Bentham, P., Kook, K.A., Schelter, B.O., Wischik, D.J., Davis, C.S., Staff, R.T., Vuksanovic, V., Ahearn, T., Bracoud, L., Shamsi, K., Marek, K., Seibyl, J., Riedel, G., Storey, J.M.D., Harrington, C.R., Wischik, C.M., 2018. Potential of Low Dose Leuco-Methylthioninium Bis(Hydromethanesulphonate) (LMTM) Monotherapy for Treatment of Mild Alzheimer's Disease: Cohort Analysis as Modified Primary Outcome in a Phase III Clinical Trial. *J. Alzheimer's Dis.* 61, 435–457. <https://doi.org/10.3233/JAD-170560>
- Wildner, G., 2019. Are rats more human than mice? *Immunobiology* 224, 172–176. <https://doi.org/10.1016/j.imbio.2018.09.002>
- Wilson, E.N., Abela, A.R., Do Carmo, S., Allard, S., Marks, A.R., Welikovitch, L.A., Ducatenzeiler, A., Chudasama, Y., Cuellar, A.C., 2016. Intraneuronal Amyloid Beta Accumulation Disrupts Hippocampal CRTC1-Dependent Gene Expression and Cognitive Function in a Rat Model of Alzheimer Disease. *Cereb Cortex* 27, 1–11. <https://doi.org/10.1093/cercor/bhv332>
- Wilson, R.S., Krueger, K.R., Arnold, S.E., Schneider, J.A., Kelly, J.F., Barnes, L.L.L., Tang, Y., Bennett, D.A., 2007. Loneliness and Risk of Alzheimer Disease. *Arch. Gen. Psychiatry* 64, 234. <https://doi.org/10.1001/archpsyc.64.2.234>
- Windisch, M., 2014. We can treat alzheimer's disease successfully in mice but not in men: Failure in translation? A perspective. *Neurodegener. Dis.* 13, 147–150. <https://doi.org/10.1159/000357568>
- Wirths, O., Weis, J., Szczygielski, J., Multhaup, G., Bayer, T.A., 2006. Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. *Acta Neuropathol.* 111, 312–319. <https://doi.org/10.1007/s00401-006-0041-4>
- Wisniewski, H.M., Kozlowski, P.B., 1982. Evidence for Blood-Brain Barrier Changes in Senile Dementia of the Alzheimer Type (SDAT). *Ann. N. Y. Acad. Sci.* 396, 119–129. <https://doi.org/10.1111/j.1749-6632.1982.tb26848.x>
- Wisniewski, H.M., Vorbrodt, A.W., Wegiel, J., 1997. Amyloid angiopathy and blood-brain barrier changes in Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 826, 161–172. <https://doi.org/10.1111/j.1749-6632.1997.tb48468.x>
- Xia, X., Jiang, Q., McDermott, J., Han, J.D.J., 2018. Aging and Alzheimer's disease: Comparison and associations from molecular to system level. *Aging Cell* 17, 1–14. <https://doi.org/10.1111/accel.12802>
- Xia, Y., Prokop, S., Bell, B.M., Gorion, K.M.M., Croft, C.L., Nasif, L., Xu, G., Riffe, C.J., Manaois, A.N., Strang, K.H., Quintin, S.S., Paterno, G., Tansey, M.G., Borchelt, D.R., Golde, T.E., Giasson, B.I., 2022. Pathogenic tau recruits wild-type tau into brain inclusions and induces gut degeneration in transgenic SPAM mice. *Commun. Biol.* 5, 1–18. <https://doi.org/10.1038/s42003-022-03373-1>
- Xiang, Y., Bu, X., Le, Liu, Y.H., Zhu, C., Shen, L.L., Jiao, S.S., Zhu, X.Y., Giunta, B., Tan, J., Song, W.H., Zhou, H.D., Zhou, X.F., Wang, Y.J., 2015. Physiological amyloid-beta clearance in the periphery and its therapeutic potential for Alzheimer's disease. *Acta Neuropathol.* 130, 487–499. <https://doi.org/10.1007/s00401-015-1477-1>
- Yamamoto, A., Shin, R.W., Hasegawa, K., Naiki, H., Sato, H., Yoshimasu, F., Kitamoto, T., 2002. Iron (III) induces aggregation of hyperphosphorylated τ and its reduction to iron (II) reverses

- the aggregation: Implications in the formation of neurofibrillary tangles of Alzheimer's disease. *J. Neurochem.* 82, 1137–1147. <https://doi.org/10.1046/j.1471-4159.2002.01061.x>
- Yang, M., Silverman, J.L., Crawley, J.N., 2011. Automated Three-Chambered Social Approach Task for Mice, in: *Current Protocols in Neuroscience*. John Wiley & Sons, Inc., Hoboken, NJ, USA, pp. 1–16. <https://doi.org/10.1002/0471142301.ns0826s56>
- Yankner, B.A., Duffy, L.K., Kirschner, D.A., 1990. Neurotrophic and Neurotoxic Effects of Amyloid β Protein: Reversal by Tachykinin Neuropeptides. *Science* (80-.). 250, 279–282. <https://doi.org/10.1126/science.2218531>
- Yap, S.Y., Frias, B., Wren, M.C., Schöll, M., Fox, N.C., Årstad, E., Lashley, T., Sander, K., 2021. Discriminatory ability of next-generation tau PET tracers for Alzheimer's disease. *Brain* 144, 2284–2290. <https://doi.org/10.1093/brain/awab120>
- Yoshida, M., 2014. Astrocytic inclusions in progressive supranuclear palsy and corticobasal degeneration. *Neuropathology* 34, 555–570. <https://doi.org/10.1111/neup.12143>
- Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S.M., Iwata, N., Saido, T.C., Maeda, J., Suhara, T., Trojanowski, J.Q., Lee, V.M.Y., 2007. Synapse Loss and Microglial Activation Precede Tangles in a P301S Tauopathy Mouse Model. *Neuron* 53, 337–351. <https://doi.org/10.1016/j.neuron.2007.01.010>
- Yousef, A., Robinson, J.L., Irwin, D.J., Byrne, M.D., Kwong, L.K., Lee, E.B., Xu, Y., Xie, S.X., Rennert, L., Suh, E.R., Van Deerlin, V.M., Grossman, M., Lee, V.M.Y., Trojanowski, J.Q., 2017. Neuron loss and degeneration in the progression of TDP-43 in frontotemporal lobar degeneration. *Acta Neuropathol. Commun.* 5, 68. <https://doi.org/10.1186/s40478-017-0471-3>
- Zempel, H., Luedtke, J., Kumar, Y., Biernat, J., Dawson, H., Mandelkow, E., Mandelkow, E.M., 2013. Amyloid- β oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin. *EMBO J.* 32, 2920–2937. <https://doi.org/10.1038/emboj.2013.207>
- Zempel, H., Mandelkow, E., 2019. Mechanisms of Axonal Sorting of Tau and Influence of the Axon Initial Segment on Tau Cell Polarity, in: Takashima, A., Wolozin, B., Buee, L. (Eds.), . Springer Singapore, Singapore, pp. 69–77. https://doi.org/10.1007/978-981-32-9358-8_6
- Zempel, H., Thies, E., Mandelkow, E.-M., Mandelkow, E.-M., 2010. AB Oligomers Cause Localized Ca^{2+} Elevation, Missorting of Endogenous Tau into Dendrites, Tau Phosphorylation, and Destruction of Microtubules and Spines. *J. Neurosci.* 30, 11938–11950. <https://doi.org/10.1523/JNEUROSCI.2357-10.2010>
- Zeppenfeld, D.M., Simon, M., Haswell, J.D., D'Abreo, D., Murchison, C., Quinn, J.F., Grafe, M.R., Woltjer, R.L., Kaye, J., Iliff, J.J., 2017. Association of perivascular localization of aquaporin-4 with cognition and Alzheimer disease in aging brains. *JAMA Neurol.* 74, 91–99. <https://doi.org/10.1001/jamaneurol.2016.4370>
- Zhang, X., Song, W., 2013. The role of APP and BACE1 trafficking in APP processing and amyloid- β generation. *Alzheimer's Res. Ther.* 5, 1–8. <https://doi.org/10.1186/alzrt211>
- Zhao, Q., Liu, M., Ha, L., Zhou, Y., 2019. Quantitative 18F-AV1451 brain tau PET imaging in cognitively normal older adults, mild cognitive impairment, and Alzheimer's disease patients. *Front. Neurol.* 10, 1–12. <https://doi.org/10.3389/fneur.2019.00486>
- Zheng-Fischhöfer, Q., Biernat, J., Mandelkow, E.M., Illenberger, S., Godemann, R., Mandelkow, E., 1998. Sequential phosphorylation of Tau by glycogen synthase kinase-3 β and protein kinase A at Thr212 and Ser214 generates the Alzheimer-specific epitope of antibody AT100 and requires a paired-helical-filament-like conformation. *Eur. J. Biochem.* 252, 542–552. <https://doi.org/10.1046/j.1432-1327.1998.2520542.x>

- 7416 Zheng, J., Li, H.L., Tian, N., Liu, F., Wang, L., Yin, Y., Yue, L., Ma, L., Wan, Y., Wang, J.Z.,
 7417 2020. Interneuron Accumulation of Phosphorylated tau Impairs Adult Hippocampal
 7418 Neurogenesis by Suppressing GABAergic Transmission. *Cell Stem Cell* 26, 331-345.e6.
 7419 <https://doi.org/10.1016/j.stem.2019.12.015>
- 7420 Zhou, L., McInnes, J., Wierda, K., Holt, M., Herrmann, A.G., Jackson, R.J., Wang, Y.-C., Swerts,
 7421 J., Beyens, J., Miskiewicz, K., Vilain, S., Dewachter, I., Moechars, D., De Strooper, B.,
 7422 Spires-Jones, T.L., De Wit, J., Verstreken, P., 2017. Tau association with synaptic vesicles
 7423 causes presynaptic dysfunction. *Nat. Commun.* 8, 15295.
 7424 <https://doi.org/10.1038/ncomms15295>
- 7425 Zhou, Y., Lin, W., Rao, T., Zheng, J., Zhang, T., Zhang, M., Lin, Z., 2022. Ferroptosis and Its
 7426 Potential Role in the Nervous System Diseases. *J. Inflamm. Res.* 15, 1555–1574.
 7427 <https://doi.org/10.2147/JIR.S351799>
- 7428 Zhou, Y., Song, W.M., Andhey, P.S., Swain, A., Levy, T., Miller, K.R., Poliani, P.L., Cominelli,
 7429 M., Grover, S., Gilfillan, S., Cella, M., Ulland, T.K., Zaitsev, K., Miyashita, A., Ikeuchi, T.,
 7430 Sainouchi, M., Kakita, A., Bennett, D.A., Schneider, J.A., Nichols, M.R., Beausoleil, S.A.,
 7431 Ulrich, J.D., Holtzman, D.M., Artyomov, M.N., Colonna, M., 2020. Human and mouse
 7432 single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular
 7433 responses in Alzheimer's disease. *Nat. Med.* 26, 131–142. [https://doi.org/10.1038/s41591-](https://doi.org/10.1038/s41591-019-0695-9)
 7434 [019-0695-9](https://doi.org/10.1038/s41591-019-0695-9)
- 7435 Zilka, N., Filipcik, P., Koson, P., Fialova, L., Skrabana, R., Zilkova, M., Rolkova, G., Kontseikova,
 7436 E., Novak, M., 2006. Truncated tau from sporadic Alzheimer's disease suffices to drive
 7437 neurofibrillary degeneration in vivo. *FEBS Lett.* 580, 3582–3588.
 7438 <https://doi.org/10.1016/j.febslet.2006.05.029>
- 7439 Zimmer, E.R., Parent, M.J., Cuellar, A.C., Gauthier, S., Rosa-Neto, P., 2014. MicroPET imaging
 7440 and transgenic models: a blueprint for Alzheimer's disease clinical research. *Trends Neurosci.*
 7441 37, 629–641. <https://doi.org/10.1016/j.tins.2014.07.002>
 7442