Universitat Autònoma de Barcelona

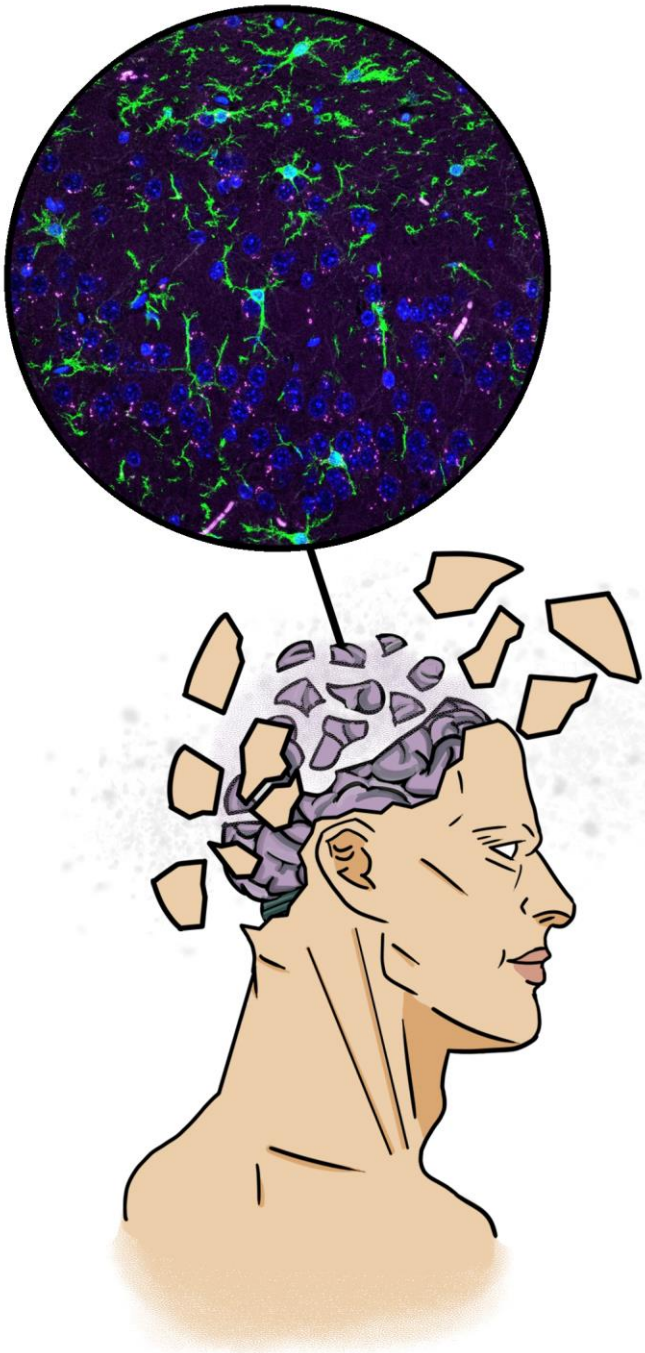
Molecular mechanisms underlying presenilin-dependent tau pathology in neurodegeneration

Carlos Manuel Soto Faguás

PhD dissertation

Molecular mechanisms underlying presenilin-dependent tau pathology in neurodegeneration

Carlos Manuel Soto Faguás



UAB
Universitat Autònoma de Barcelona

Bellaterra, 2021

INc Institut de Neurociències

Institut de Neurociències

Departament de Bioquímica i Biologia Molecular

Unitat de Bioquímica i Biologia Molecular

Facultat de Medicina

Universitat Autònoma de Barcelona

Molecular mechanisms underlying presenilin-dependent tau pathology in neurodegeneration

Mecanismos moleculares implicados en la patología de tau regulados por las presenilinas en neurodegeneración

Carlos Manuel Soto Faguás

Doctoral thesis

Bellaterra, Julio 2021

Institut de Neurociències

Departament de Bioquímica i Biologia Molecular

Universitat Autònoma de Barcelona

Molecular mechanisms underlying presenilin-dependent tau pathology in neurodegeneration

Mecanismos moleculares implicados en la patología de tau regulados por las presenilinas en neurodegeneración

Memoria de tesis doctoral presentada por *Carlos Manuel Soto Faguás* para optar al grado de Doctor en Neurociencias por la Universitat Autònoma de Barcelona.

Trabajo realizado en la Unitat de Bioquímica i Biologia Molecular de la Facultat de Medicina del Departament de Bioquímica i Biologia Molecular y el Institut de Neurociències de la Universitat Autònoma de Barcelona, bajo la dirección del Doctor Carlos Alberto Saura Antolín.

El trabajo realizado en esta tesis doctoral ha estado financiado por los proyectos de investigación del Ministerio de Ciencia e Innovación con financiación del Fondo Europeo del Desarrollo Regional (FEDER): SAF2016-80027-R y PID2019-106615RB-100, Instituto de Salud Carlos III: CIBERNED CB06/05/0042 y por la Generalitat de Catalunya: 2017 SGR749 y beca de formación de doctores FI-AGAUR: 2017 FI_B00326.

Bellaterra, Julio de 2021

Doctorando — Director de tesis

Carlos Manuel Soto Faguás — Dr. Carlos Alberto Saura Antolín

A mis padres,
a mis hermanos
y a Paula

“Cuando recordar no pueda,
¿dónde mi recuerdo irá?
Una cosa es el recuerdo
y otra cosa recordar”

Antonio Machado

I. Index

II. List of abbreviations

ΔC	ΔCre-recombinase
μFTIR	Fourier transform infrared microspectroscopy
a.u.	Arbitrary units
Aβ	Amyloid β
aa	Amino acid
ACQ	Acquisition
AD	Alzheimer's disease
AMPK	AMP-activated protein kinase
Aph-1	Anterior pharynx-defective-1
APOE	Apolipoprotein-e
APP	Amyloid precursor protein
Asp	Aspartate
BV-FTD	Behavioral variant frontotemporal dementia
C	Cre-recombinase
CBD	Corticobasal degeneration
CBP	CREB binding protein
CC	Corpus callosum
Cdk5	Cyclin-dependent kinase-5
CFC	Contextual fear conditioning
CHAP	Chicago Health and Aging Project
CQ	Chloroquine
CREB	cAMP response element-binding
CRTC1	CREB regulated transcription coactivator 1
CS	Conditioned stimulus
CSF	Cerebrospinal fluid
CTF	Carboxy-terminal fragment
CuedFC	Cued fear conditioning
DCX	Doublecortin
DG	Dentate gyrus
DIV	Days in vitro
EC	Entorhinal cortex
EDTA	Ethylenediamine tetraacetic

Erk	Extracellular signal-regulated kinase
FAD	Familial Alzheimer's disease
FBS	Fetal bovine serum
FFT	Fast Fourier transform
FLD	Frontotemporal lobar dementia
FTD	Frontotemporal dementia
FUS	Fused-in-sarcoma
GRN	Progranulin
GSK3β	Glycogen synthase kinase 3β
ICD	Intracellular domain
IR	Infrared
LC3B	Microtubule-associated proteins 1A/1B light chain 3B
LQ	Left quadrant
LV-PPA	Logopenic variant of primary progressive aphasia
MAPK	Mitogen-activated protein kinases
MAPT/tau	Microtubule-associated protein tau
MCI	Mild cognitive impairments
MCT	Mercury cadmium telluride
MoPrP	Mouse prion promoter
MTBR	Microtubule binding region
MWM	Morris water maze
Nct	Nicastrin
NF	Neurofilament
NFκB	Nuclear factor kappa B
NF-H	Neurofilament high chain
NF-L	Neurofilament light chain
NF-M	Neurofilament medium chain
NFT	Neurofibrillary tangles
NFV-PPA	Non-fluent variant primary progressive aphasia
NOR	Novel object recognition
NTF	Amino-terminal fragment
OQ	Opposite quadrant

PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
Pen-2	Presenilin enhancer-2
PET	Positron emission tomography
PFA	Paraformaldehyde
PHF	Paired helical filaments
PICALM/CALM	Phosphatidylinositol-binding clathrin assembly protein
PKA	Protein kinase A
PP2A	Protein phosphatase 2A
PRAS40	Proline-rich Akt substrate
PS/PSEN	Presenilin
PS cDKO	Presenilin conditional double knockout
PS1 cKO	Presenilin 1 conditional knockout
PSD95	Postsynaptic density protein 95
PSP	Progressive supranuclear palsy
RQ	Right quadrant
RSC	Retrosplenial cortex
RT	Room temperature
SR	Synchrotron radiation
SV-PPA	Semantic-variant primary progressive aphasia
TB	Tris-HCl buffer
TBS	Tris-HCl buffer saline
TDP	TAR DNA-binding protein
TQ	Target quadrant
TTBK	Tau-tubulin kinase
UPS	Ubiquitin-proteasome system
US	Unconditioned stimulus
VGLUT1	Vesicular glutamate transporter 1
WT	Wild type

III. Abstract

Alzheimer's disease (AD), the most common cause of dementia worldwide, is characterized by gradual loss of cognition, especially memory, and behavioral and neuropsychiatric impairments. Neuropathological features of AD include amyloid-β (Aβ)-containing plaques, neurofibrillary tangles (NFT) composed of hyperphosphorylated tau protein, neuronal loss, neuroinflammation and autophagic dysfunction, among others. In contrast to late-onset AD, most familial AD (FAD) cases are caused by dominantly inherited mutations in the Presenilin (*PS*/*PSEN: PS1* and *PS2*) genes, the catalytic subunit of γ-secretase complex that is responsible for Aβ generation. Notably, mutations in PS1 are also linked to frontotemporal dementia (FTD) characterized by cerebral accumulation of aggregated phosphorylated tau but not amyloid plaques. Although the mechanisms by which PS mutations affect Aβ are well defined, the precise role of PS/γ-secretase on tau pathology independently of Aβ during neurodegeneration is largely unclear. The **hypothesis** of this doctoral thesis is that loss of PS function caused by dementia-linked mutations contributes to tau hyperphosphorylation, aggregation and/or spreading by affecting specific signaling and autophagy pathways. **The objective** of this study was to investigate the role of PS on tau pathology during synaptic dysfunction and neurodegeneration, and the mechanisms involved. To address this objective, I performed biochemical and structural protein analyses, including synchrotron infrared microspectroscopy, histological and behavioral approaches in novel neuronal-specific PS1 and double PS (PS1/PS2) conditional knockout (cKO) mice expressing either endogenous tau or overexpressing the FTD-linked (P301S) mutant human tau (Tau) gene. Our **results** show that loss of PS function results in age-dependent tau phosphorylation in the cortex and the hippocampus of PS cDKO mice associated with increased activation of p25/Cdk5, PKA/AMPK and Akt. As a result of neuronal PS inactivation, pathological phosphorylated tau accumulates in excitatory neurons, astrocytes, microglia, and oligodendrocytes. Interestingly, genetic inactivation of PS in human tau transgenic mice results in accelerated and enhanced tau phosphorylation and aggregation in memory and emotional neuronal circuits linked to AD pathology, including the entorhinal and restrosplenial cortices, hippocampus and amygdala, a phenotype associated to exacerbated memory and learning impairments. Biochemical analyses of purified synaptosomes from human AD and mouse transgenic brains also revealed abnormal synaptic accumulation of tau and decreased synaptic proteins and synaptonuclear factors (e.g., CRTC1) suggesting pre- and post-synaptic mechanisms impaired in brain of AD and AD mouse models. Remarkably, loss of PS function causes abnormally aggregated neurofilament protein together with increased aggregated and oligomeric β-sheet protein structures in PS1 cKO;Tau and PS cDKO;Tau mice. At the molecular level, I found that PS is required for correct mTOR activity and autophagic flux, maintaining autophagosome-lysosome fusion and tau degradation. Conversely, genetic inactivation of *PS1* or both *PS* genes in human tau transgenic mice increases autophagic markers (LC3-II/I, p62…) and impairs and enhances phosphorylated and aggregated tau in autophagosomes. Taken together these results provide further evidence that partial loss of PS function leads to increased hyperphosphorylated and aggregated tau, inflammatory responses, and neurodegeneration, and that synapse dysfunction and memory deficits caused by PS inactivation suggest mediated by synaptic tau. Our findings could indicate that tau phosphorylation and aggregation are key pathological processes that may underlie neurodegeneration caused by familial AD-linked PS mutations.

IV. Introduction

1. Dementia disorders

1.1. Alzheimer's disease

1.1.1. Epidemiology

Alzheimer's disease (AD) is the main cause of dementia worldwide, accounting for 60-80% of all cases. The World Alzheimer's Report 2009 estimated that 36 million people had dementia in 2010 worldwide, a number that will double every 20 years, reaching 66 million people by 2030 and 115 million by 2050. In the most recent study, The World Alzheimer's Report 2015, the estimation was that 46.8 million people worldwide are living with dementia reaching 74.7 million and 131.5 by 2030 and 2050, respectively, being 12-13% higher than the estimated 2009 report. The overall incidence of dementia is almost 10 million per year, diagnosing a new case every 3.2 seconds. The estimation of the global economic cost of dementia reached 818 billion US dollars, increasing the importance of the research for its prevention and treatment (World Alzheimer's Report, 2009, 2015).

In the USA, AD affects 5.8 million people and by 2050 the expectation is to reach 13.8 million. AD cases increase intensely with age. A study using the latest data from the 2019 US Census and the Chicago Health and Aging Project (CHAP) showed that in 2011 the incidence in people of 65-74 years was 0.4%, in people with 75-84 was 3.2% and in 85 and older was 7.6% (Rajan et al., 2019). Importantly, AD affects differentially women and men. Almost 2/3 of cases of AD are women in the US, probably due to women have a longer life than men and age is the greatest risk factor for AD. However, there are some differences in the risk of dementia also depending on genetics, geographic region and/or even educational level (due to lower educational attainment in women in the 20th century) (Hebert et al., 2013).

AD is driven by a complex interplay between genetic and environmental factors. The main risk factor of AD is age, and indeed, most people with AD are 65 years or older, excluding familial AD cases (FAD). There are also genetic risk factors being apolipoprotein-e (APOE-ε2/ε3/ε4) isoform the most well studied. The presence of an allele of the ε3 isoform increases the risk of developing AD one's compared to ε2, and ε4 allele increases the risk of the ε3 allele. People with the two ε4 alleles show between 8 and 12 times more risk of having AD than those with two copies of ε3 (Holtzman et al., 2012; Loy et al., 2014). Furthermore, up to 60 environmental AD risk factors have been identified (Killin et al., 2016). These non-genetic factors include healthy food habits, exposition to toxics or infectious agents, physical exercise, traumatic brain

injury, mitochondrial dysfunction, immune system, vascular disease, smoking or education (Armstrong, 2019).

1.1.2. Clinical characteristics

AD is a progressive neurodegenerative disorder comprising three different phases: preclinical, mild cognitive impairment (MCI) and dementia. The duration of these phases varies between patients and is also affected by many factors such as age, genetics, gender, etc. (Vermunt et al., 2019). Symptoms of each stage are summarized in figure 1.

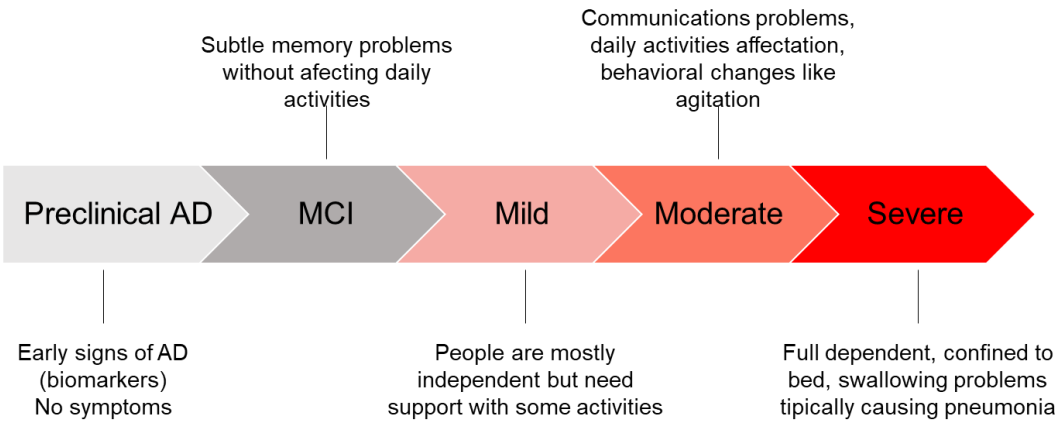


Figure 1. Classification of AD clinical progression and symptomatology: preclinical AD, MCI and mild, moderate and severe dementia (Modified from Alzheimer's Dementia, 2020**).**

The main signs of AD are:

- Lack of memory that interferes daily routine: forget names or places.
- Difficulties doing familiar tasks.
- Time and/or place confusion.
- Visual and spatial relationships problems.
- Communication complications.
- Misplacing and losing things.
- Decrease in the capacity of making decisions.
- Behavioral changes like confusion, depression, anxiety, irritability and/or upset.

1.1.3. Familial AD

AD is classified as either late-onset, being sporadic and in people over the age of 65 years, and early-onset, known as familial AD (FAD) caused by genetic inheritance, usually in people affected under 65 years of age. FAD cases represent less than 1-5% of all AD cases and are caused by dominantly inherited mutations in the amyloid-β (Aβ) precursor protein (*APP*), presenilin 1 (*PSEN1/PS1*) and presenilin 2 (*PSEN2/PS2*) genes (Hutton and Hardy, 1997).

APP mutations were originally found in large families with early-onset AD (45-65 years) (Goate et al., 1991). These mutations are located close or flanking the sequence of Aβ peptide affecting APP processing to generate more Aβ or more pathogenic Aβ42 (43). The vast majority of the mutations of FAD cases are in *PSEN1* gene causing the earliest and most aggressive pathology (28-60 years) (Cruts et al., 1996). Mutations in *PSEN2* cause FAD in older (35-82 years) and more variable onset-age than *PSEN1* mutations (Levy-Lahad et al., 1995). In FAD-linked *PSEN* cases, there is an alteration of γ-secretase activity affecting APP processing and generation of Aβ leading to accelerated and/or increased Aβ accumulation and phosphorylation and aggregation of the microtubule-associated protein tau (MAPT/tau). Interestingly, this later pathological marker is also present in *PSEN1* mutations in families with clinical symptoms of frontotemporal dementia (FTD), atypical dementia with FTD phenotype and dementia with Lewy Bodies (Hutton, 2004) causing severe neurodegeneration and dementia in the absence of Aβ pathology. Therefore, mutations on *PSEN* genes accelerate the AD onset-age and cause an earlier and more severe neurodegeneration (Saura, 2010). In support of PS involvement in tau pathology and neurodegeneration independently of Aβ, genetic inactivation of both PS (*PS1/PS2*) in the postnatal mouse brain of PS conditional double knockout (PS cDKO) mice leads to an age-dependent increase in phosphorylated tau protein, neurodegeneration and memory impairments (Saura, 2004).

1.1.4. Neuropathology: Aβ and tau

The brain of AD patients is characterized by progressive atrophy of cortical and subcortical structures, as well as two major pathological hallmarks: extracellular accumulation of Aβ in plaques and intracellular neurofibrillary tangles (NFT) composed of aberrantly hyperphosphorylated tau protein (Gorevic et al., 1986; Grundke-Iqbal et al., 1986a; Grundke-Iqbal et al., 1986b, **Fig. 2**). AD neuropathology also includes synapse loss, astrogliosis, microgliosis, vascular disease, neuronal death, oxidative stress, and loss of white matter between others.

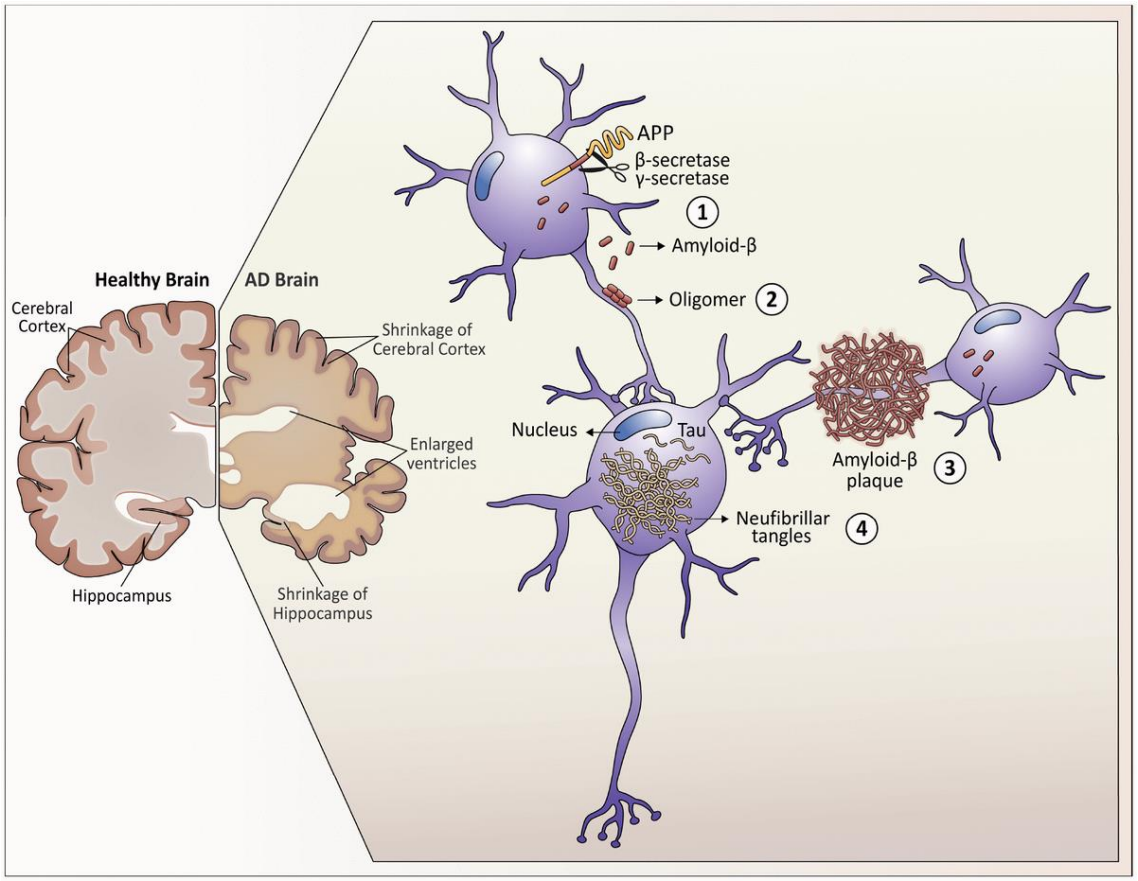


Figure 2. Anatomical structure and neuronal pathology in AD brain. AD brain is characterized by cortical and hippocampal shrinkage and ventricles enlargement. Pathologically, there is an accumulation of extracellular Aβ plaques (red) and intracellular accumulation of neurofibrillary tangles of tau protein (brown, Gomez et al., 2020).

Amyloid plaques are mainly composed of abnormally folded Aβ peptides. Aβ42 is the most abundant form because of its insolubility and fibrillization tendency. According to the amyloid hypothesis, cerebral Aβ accumulation is the primary cause of AD and the rest of the pathology results from an imbalance between production and clearance of Aβ (Hardy and Selkoe, 2002). However, amyloid accumulation does not always correlate with the severity or the progression of the disease. In fact, it can be found even in healthy people without dementia symptoms (Hardy and Selkoe, 2002; Price and Morris, 1999; Rodrigue et al., 2012). Furthermore, there is a high number of clinical trials using monoclonal antibodies against Aβ that have shown decrease of amyloid accumulation but have failed suggesting that Aβ is useful for AD diagnosis, but it is not sufficient for AD dementia.

On the other hand, NFT consist of paired helical filaments (PHF) formed by hyperphosphorylated tau protein. Usually, tau pathology affects memory-related neural circuits, starting in the entorhinal cortex, spreading first to the hippocampus and then to associative and limbic cortical regions (**Fig. 3**). Tau pathology correlates with the clinical symptoms and the severity of the disease but also with neuronal and synapse

loss (Serrano-Pozo et al., 2011; de Calignon et al., 2012). Tau pathology has allowed the classification of AD in six different pathological stages: Braak I to VI (Braak and Braak, 1991; Braak et al., 2006). Interestingly, tau mutations alone do not cause AD, but they lead to tau accumulation causing a diversity of dementias within the frontotemporal dementia spectrum (Lashley et al., 2015).

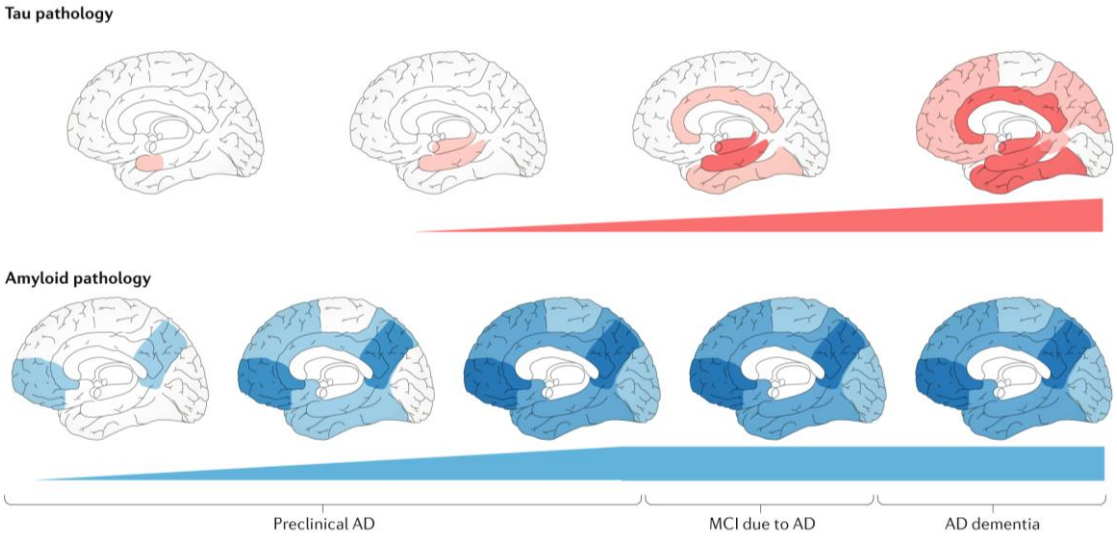


Figure 3. Progression of tau and Aβ pathologies in AD. Spatiotemporal patterns of tau (top, red) and Aβ (bottom, blue) pathologies in AD using positron emission tomography (PET). Tau pathology starts in the entorhinal cortex and spreads to, first, the hippocampus and limbic areas, then to neocortical areas. In contrast, Aβ pathology emerges in neocortex and propagates to allocortex (van der Kant et al., 2020).

Recent studies show that tau and Aβ induce protein conformational changes as occurs in prion diseases and they may be trans-synaptically transferred between neurons (Jucker and Walker, 2013). Thus, numerous studies have focused on Aβ and tau propagation in the last decade. They show that exogenous Aβ/tau can induce endogenous Aβ/tau pathology and propagation *in vitro* and *in vivo* (Eisele et al., 2009; Narasimhan et al., 2017; Reilly et al., 2017). Furthermore, there are different tau species and different secretion mechanisms involved (Pérez et al., 2018) (see below).

Recent reports suggest that the immune system could link Aβ and tau pathologies. Neuroinflammation precedes and increases during AD clinical decline, microglial markers colocalize with AD aggregates and recent identified AD-risks genes are involved in the immune system (Hollingworth et al., 2011; Calsolaro and Edison, 2016). AD brain shows increased inflammatory factors such as IL-1β, IL-6, TNF-α or complement receptors (Ng et al., 2018) and the inhibition of complement receptors reduces microglial overactivation leading to neuronal protection and rescuing tau pathology and dysfunctional phagocytosis (Rogers et al., 2006; Crehan et al., 2013; Litvinchuk et al., 2018). Furthermore, Aβ and tau aggregates are eliminated by

autophagy and phagocyte by microglia (Fu et al., 2014). All this data hints the importance of the connection between neuroimmune system and Aβ/tau pathologies.

1.1.5. Therapeutic treatments

At present, no effective therapeutic treatments block or reverse the pathology and clinical progression of AD. The use of acetylcholinesterase inhibitors is the main symptomatic treatment with proved short beneficial effects in mild and moderate stages (Birks, 2006). Memantine, an antagonist of N-methyl-d-aspartate receptor and dopamine agonist, decreases glutamate excitatory neurotoxicity having a small but beneficial effect on cognition and behavioral decline. Combined treatment did not show strong cognition enhancement but there was an improvement in the behavioral symptoms (Schmidt et al., 2015).

Despite the depression and anxious symptoms of AD, drugs targeting these signs can result detrimental causing more confusion. They are only used in specific situations with severe psychosis (The Lancet Neurology, 2009). Non-pharmacological treatments such as physical exercise or music therapy have proved some beneficial effects (Livingston et al., 2014).

Novel treatment strategies are focused on two different time-points: disease-prevention or disease-modifying. For disease-prevention approaches is important to develop methods that allow us to identify individuals with AD risk before symptoms onset. The development of non-invasive and well predictive biomarkers is essential. For disease-modifying treatment, different treatment targets, including Aβ, tau or neuroinflammation, have been developed. Thus, there are some approaches in preclinical phase based on amyloid and/or tau clearance using monoclonal antibodies or preventing the generation of the pathological forms (Lane et al., 2018; Weller and Budson, 2018).

1.2. Frontotemporal dementia

1.2.1. Epidemiology

Frontotemporal dementia (FTD), also named Pick's disease and Frontotemporal lobar dementia (FLD), is a term that includes a set of neurodegenerative disorders comprising the second cause of early-onset dementia (under 65 years) and the third one for all groups of age, after AD and dementia with Lewy bodies (Vieira et al., 2013). FTD represents 3-26% of dementia cases in people under 65 years old. The 10% of the people with FTD is under 45 years, 60% between 45 and 64 years and 30% above 64 years (Knopman and Roberts, 2011; Lambert et al., 2014). FTD has an estimated

incidence between 1.61 and 4.1 cases per 100000 people every year (Coyle-Gilchrist et al., 2016; Knopman and Roberts, 2011). Unlike AD, FTD seems to affect equally men and women and is frequently underdiagnosed because of the deficient recognition and the similarity to a variety of psychiatric disorders (Lanata & Miller, 2016).

1.2.2. Clinical characteristics

FTD is a heterogeneous disease classified into three clinical variants: 1) Behavioral-variant FTD (BV-FTD) characterized by early behavioral deficits, 2) non-fluent variant primary progressive aphasia (NFV-PPA) associated with progressive deficits in talking and grammar communication skills, and 3) semantic-variant primary progressive aphasia (SV-PPA) consisting of progressive shortage of semantic knowledge and naming. There is also a fourth category, called logopenic variant of primary progressive aphasia (LV-PPA), characterized by fluent simple sequences but frequent pauses to find words (Gorno-Tempini et al., 2011). At the beginning, the degeneration is focal and different for each type, but with time, it becomes more diffuse and can spread affecting large brain regions. With progression, symptoms can converge, and patients develop global cognitive and motor deficits. In advanced stages, FTD patients have problems moving, eating, and swallowing. Death is usually caused by pneumonia or other secondary infection 8 years after the symptom onset. The criteria for clinical diagnosis of FTD is summarize in table 1. Of note, there are also related FTD diseases like FTD with motor neuron disease, FTD with Parkinsonism, progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD).

BV-FTD	NFV-PPA	SV-PPA
•Behavioural disinhibition •Apathy or inertia •Stereotypical or compulsive. •Dietary changes •Executive deficits combined with affected visuospatial and memory skills	•Prominent language impairment with aphasia •Agrammatism or apraxia •Impaired comprehension of word, sentences or object knowledge	•Prominent language impairment with aphasia •Impaired confrontation naming •Impaired single word comprehension •Spare repetition and speech or object knowledge •Dyslexia or dysgraphia

Table 1. Criteria for the diagnosis of the three main FTD variants (Adapted from Bang et al., 2015**).**

1.2.3. Pathology

FTD is a progressive syndrome characterized by neuronal degeneration, inflammation, and microvascular changes particularly in frontal and anterior temporal lobe and anterior cingulate and insular cortex. Von Economo neurons and fork cells are the responsible of the initial generation. FTD is neuropathologically subdivided depending on the abnormal accumulation of different proteins: MAPT/tau, TAR DNA-binding protein (TDP-43), fused-in-sarcoma (FUS) and in a lesser extent ubiquitin-only or p62 only (Mackenzie et al., 2010). Frontotemporal lobar degeneration-tau (FTD-tau) represent between 36% and 50% of all cases of FTD (Baborie et al., 2011). There are three different FTD-tau syndromes: (1) Pick's disease: characterized by the presence of Pick bodies and neurodegeneration in frontal, temporal and cingulate gyri. (2) CBD: characterized by the accumulation of pre-tangles, neuritic threads, ballooned neurons, astrocytic plaques and oligodendroglial coiled bodies mainly localized in dorsal prefrontal cortex, supplemental motor area, peri-Rolandic cortex and subcortical nuclei. (3) PSP: characterized by neuronal granular inclusions, tufted astrocytes, and globose tangles and atrophy of the frontal convexity globus pallidus, subthalamic nucleus and brainstem nuclei (Dickson et al., 2011).

1.2.4. Genetics

Around 40% of FTD patients have dementia family history. Only 10% of cases are due to autosomal dominant heritance in *C9orf72*, *MAPT* and progranulin (*GRN*) genes, representing the 60% of all familial cases (Rohrer et al., 2009). The prevalence of the mutations in each gene depends on world-region. *C9orf72* mutations are more frequent in northern Europe and Scandinavia, and they are involved in the dysregulation of the endosomal trafficking, impaired transcription processing and protein toxicity. Mutations in *MAPT* gene are more common in Northwest Europe and USA and they cause alteration of microtubule stabilization and increasing tau aggregation. *GRN* mutations are mainly present in southern Europe and they affect neurotrophic function and the degradation mediated by lysosome and causing inflammation (Bang et al., 2015).

1.2.5. Therapeutic treatments

Current pharmacological treatments do not slow or halt FTD progression. Selective serotonin reuptake inhibitors are used for the treatment of impulsivity, eating changes, agitation, and aggressiveness (Lebert et al., 2004). Behavioral anomalies are treated with low doses of antipsychotics. Furthermore, physical, behavioral, and environmental practices contribute to improve cognitive function (Merrilees, 2007; Cheng et al., 2014).

In contrast to AD, cholinesterase inhibitors and memantine have not shown beneficial effects on FTD patients (Mendez et al., 2007; Boxer et al., 2013a).

Progress in the knowledge of the neuropathology of FTD has allowed to identify new potential targets for disease-modifying treatments. In fact, there are some ongoing clinical trials based on blockage of tau aggregation, stabilization and spreading or tau elimination. For example, methylene blue and tau vaccines have been used to inhibit tau aggregation (Wischik et al., 2014). Moreover, antisense oligonucleotides targeting the toxic gain function of progranulin are used for *GRN* and *C9orf72* FTD cases (Boxer et al., 2013b; Boxer et al., 2013c; Lagier-Tourenne et al., 2013).

2. Presenilin/γ-secretase

Presenilins (PS: PSEN1 and 2) are the catalytic subunits of the γ-secretase complex, an aspartyl protease implicated in the intramembranous cleavage of transmembrane type I proteins (De Strooper, 2010, De Strooper and Annaert, 2010). The γ-secretase complex, formed by nicastrin (Nct), anterior pharynx-defective-1 (Aph-1) and presenilin enhancer-2 (Pen-2), is involved in the processing of more than 90 substrates and reaching up to 149 substrates to date, including APP, Notch and cadherins (LLeó and Saura, 2011; Güner and Lichtenthaler, 2020). Notably, PS generate the Aβ peptides after APP processing by β-secretase (amyloidogenic pathway). By contrast, α-secretase cleaves APP in the middle of the Aβ sequence, preventing Aβ generation (non-amyloidogenic pathway; Hutton and Hardy, 1997). Briefly, APP processing by the amyloidogenic pathway consists of a first cleavage mediated by β-secretase that generates a soluble APP ectodomain (APPsβ) and a carboxy-terminal fragment (CTF) anchored to the membrane (β-stub). Then, the APP CTF is proteolyzed by PS/γ-secretase releasing an intracellular domain fragment (ICD) and the Aβ peptide (**Fig. 4**). Interestingly, substrate processing mediated by PS/γ-secretase is affected by different molecular mechanisms such as calcium influx or ligand binding, whereas sex differences in γ-secretase activity were reported in aged mouse brains (Mumm et al., 2000; Litterst et al., 2007; Placanica et al., 2009).

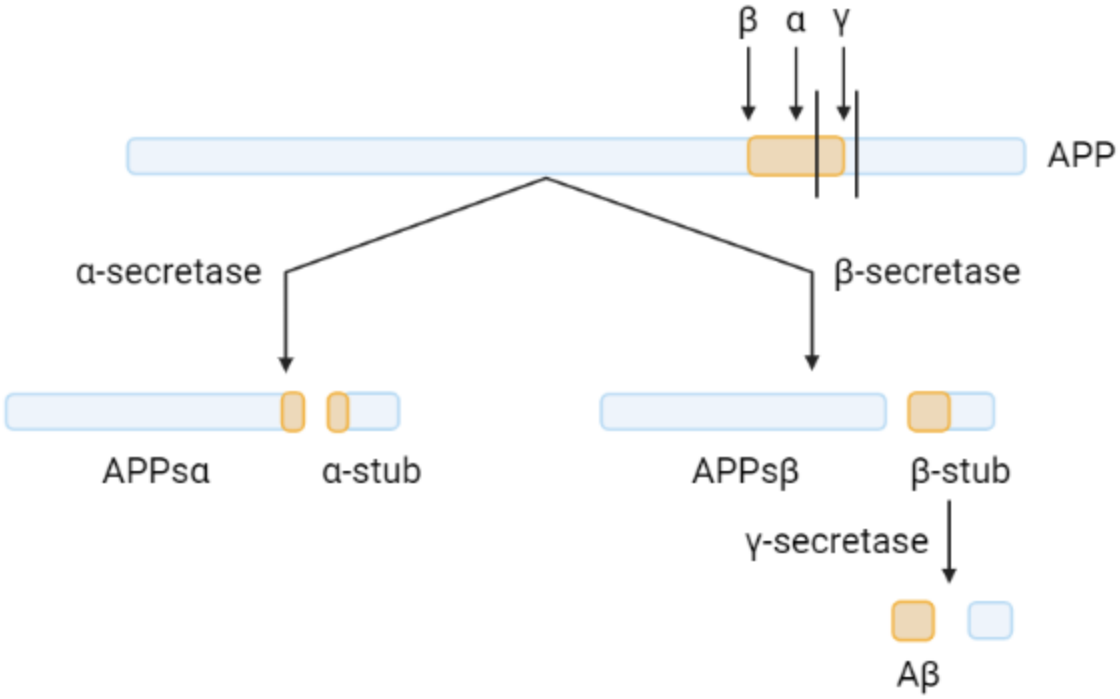


Figure 4. APP processing by β- and γ-secretases leads to Aβ generation.

2.1. PS/γ-secretase biology

γ-secretase is a hetero-tetrameric protein complex formed by PS, the proteolytically active subunit, and three non-proteolytic subunits Nct, Aph-1 and Pen-2. Aph-1 is encoded by two homologous genes in humans (*Aph-1a* and *Aph-1b*) and an additional homologous gene in rodents (*Aph-1c*). Aph-1 seems to be implicated in the assembly of the complex and in the substrate interaction. Pen-2 is essential for PS endoproteolytic activation and complex stability (Ahn et al., 2010). All the subunits have multiple transmembrane domains, except for Nct, that is connected to a large extracellular domain giving an essential structure to the complex that helps the substrate specificity (**Fig. 5**). The combination of different subunits makes possible the formation of homologous complexes differing in its expression levels and subcellular localization (Güner and Lichtenthaler, 2020). The absence of one subunit causes the rapid degradation or the retention of the other substrates in the endoplasmic reticulum (Escamilla-Ayala et al., 2020).

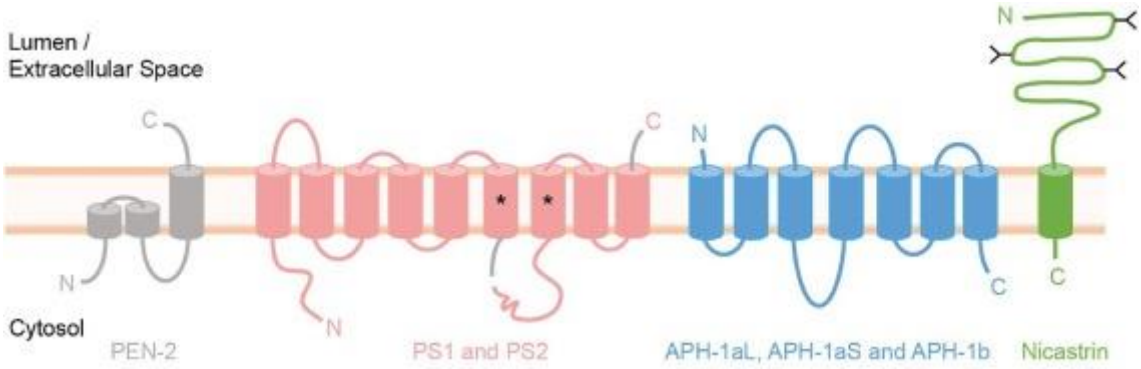


Figure 5. γ-secretase complex. γ-secretase complex is formed by 4 subunits: the proteolytic subunit PS1 and PS2, and the non-proteolytic subunits Pen-2, Aph-1 and Nct. Asterisks correspond to the catalytic aspartic acid residues (Güner and Lichtenthaler, 2020).

PS1 and PS2 proteins are encoded by *PSEN1* and *PSEN2* localized in chromosomes 14 and 1, respectively (De Strooper et al., 2012). In the adult brain, PS are highly expressed in hippocampal and entorhinal cortex neurons but also in interneurons and motoneurons. Although PS mRNAs are present at low levels in glial cells in basal conditions, the expression is upregulated in some pathological conditions, such as AD (Saura, 2010).

PS are hypothesized to contain 9 transmembrane domains with the N-terminus localized in the cytosol and the C-terminus in the lumen. The catalytic site of PS is thought to rely on the hydrophobic domains six and seven that contain two key aspartate residues (Asp) at positions 257 and 385 (**Fig. 6**) (Wolfe et al., 1999; Laudon et al., 2005). PS are first generated as a precursor of 50 kDa that are

endoproteolytically cleaved to become active (Thinakaran et al. 1996). This processing occurs in the cytoplasmatic loop, generating an N-terminal fragment (NTF) of 27-28 kDa and a CTF of 17-18 kDa (Saura et al., 1999). Finally, PS proteins are modified post-translationally through phosphorylation, ubiquitination, or caspase cleavage, modulating their function (Duggan and McCarthy, 2016).

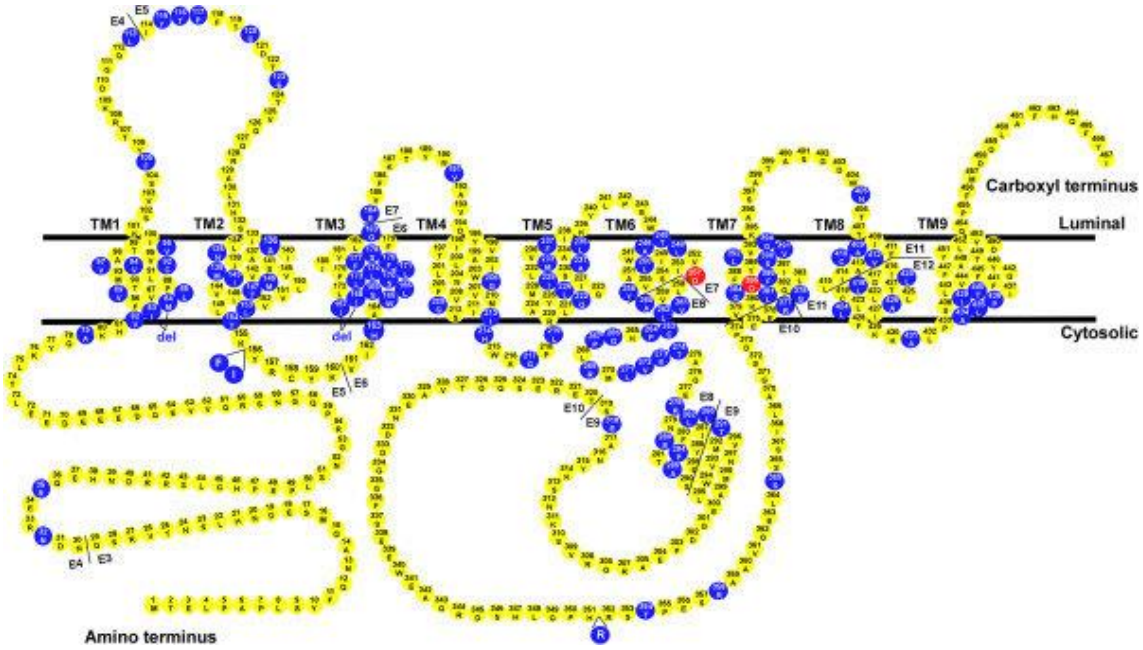


Figure 6. PS1 topological structure. Human PS1 structure showing catalytic active Asp (red) and FAD-associated mutations (blue) (Zhang et al., 2013).

2.2. Physiological and pathological functions of PS/γ-secretase: APP processing

PS are ubiquitous proteins with important biological functions during development and adulthood. PS1 plays an essential role during brain development as PS1 deficiency during embryogenesis results in axial skeleton defects, brain hemorrhages, ventricle atrophy, impaired neurogenesis and neuronal loss, triggering premature death (Shen et al., 1997). In the adult brain, PS function is necessary for synaptic plasticity, learning and memory and neuronal survival (Saura et al., 2004). PS/γ-secretase participates in important biological functions including regulation of cell differentiation, neurite outgrowth, cell adhesion, protein trafficking, calcium homeostasis or β-catenin signaling (Czech et al., 2000). Some of these functions could involve the processing of one or more of the 140 known PS/γ-secretase substrates and the function of ICDs, generated after PS/γ-secretase cleavage, which translocate to the nucleus regulating gene expression (Lleó and Saura, 2011; Güner and Lichtenthaler, 2020). Interestingly, APP was the first PS/γ-secretase substrate identified. PS/γ-secretase APP processing leads

to the generation of Aβ of different length. Mostly of the Aβ generated has 40 amino acids, but there is a more pathogenic form with 42 residues (Aβ42) characterized by higher aggregation tendency (Scheuner et al. 1996). FAD-PS mutations and low/moderate doses of pharmacological γ-secretase inhibitors increase the production of the pathogenic Aβ42 and the levels of phosphorylated tau enhancing the important role of PS/γ-secretase on Aβ and tau pathologies (Shen and Kelleher, 2007; Beglopoulos et al., 2004; Saura et al., 2004; Zhang et al., 2010). However, despite the well described role of PS in APP processing and Aβ generation, the molecular mechanisms linking PS and tau pathology remains largely unclear.

2.3. Loss of PS function in neurodegeneration

Mutations in *PS1* or *PS2* cause the majority of FAD and some of FTD cases. Mutations in *PS* affect PS/γ-secretase activity leading to altered APP processing and Aβ42/Aβ40 levels (De Strooper, 2007). Furthermore, PS mutations anticipate the age of AD-onset and cause earlier and more severe neurodegeneration (Saura, 2010). In addition, FTD-linked PS1 mutations cause severe dementia and neurodegeneration characterized by accumulation of tau in the absence of Aβ pathology. Taken together, it is possible that PS mutations may cause tau pathology and neurodegeneration independently of Aβ pathology.

To overcome the lethality of PS1 null mice, Saura et al. used the Cre-loxP system under de CamKIIα promoter to generate brain-specific PS1/2 conditional double knockout (cDKO) mice (Saura et al., 2004). In PS cDKO mice, genetic inactivation of PS1 in the adult brain is restricted temporally and spatially, starting at postnatal day 18 in excitatory glutamatergic neurons of the forebrain (Yu et al., 2001). PS deficiency in PS cDKO mice leads to age-dependent synaptic dysfunction, learning and memory deficits and neurodegeneration (Saura et al., 2004). At 6 months of age PS cDKO mice develop cortical atrophy, microgliosis and astrogliosis and tau hyperphosphorylation (Beglopoulos et al., 2004; Saura et al., 2004). At the age of 9 months, the pathology is more severe and neurodegeneration is more apparent. Neuronal PS deficiency alters the cAMP response element-binding protein (CREB)-regulated transcription coactivator 1/ (CRTC1/CREB) signaling pathway contributing to memory loss in PS cDKO mice (Parra-Damas et al., 2017). Of note, Aβ pathology has also shown to dysregulated CRTC1/CREB transcriptional pathway during aging (Parra-Damas et al., 2014). All this data demonstrates that loss of PS function causes tau pathology, but the molecular mechanisms linking PS and tau pathology are largely unknown.

3. Microtubule associated tau protein (MAPT/tau)

3.1. *MAPT*/tau gene and protein

MAPT gene, located in chromosome 17, encodes the microtubule-associated protein tau. *Tau* gene contains 16 exons that suffers complex and regulated alternative splicing resulting in several mRNA species. Exons 1, 4, 5, 7, 11, 12 and 13 are constitutive and exons 0, 4a, 6, 8 and 14 are not transcribed in the human brain. There are six different tau isoforms in the adult human brain depending on the presence of exons 2, 3 and/or 10 (Alavi and Soussi-Yanicostas, 2015) (**Fig. 7**). They differ in the N-terminal sequence because of the addition of 29 amino acids (1N) or repetitions (2N = 58 amino acids) encoded by exons 2 and 3. The sequence encoded by exon 3 is only present with exon 2 sequence. 2N isoform has a low expression in human brain. Tau sequence also contains a microtubule binding region (MTBR) repeated (R) three times (3R: R1, R3, R4) or four times (4R: R1-R4). Exon 10 is the responsible of the insertion of the MTBR R2 forming the 4R isoform. Although both isoforms are expressed in a similar proportion, they have a specific cellular localization in the human brain. Thus, granular neurons of the dentate gyrus only express 3R isoform mRNA (Jadhav et al., 2019). Furthermore, isoform expression is not conserved as adult mouse brain only express 4R isoform switched from 3R between postnatal day 8 and 18 (Tuerde et al., 2018). Interestingly, tauopathies can be distinguished by 3R/4R tau ratio. For example, AD is characterized by a mix of 3R and 4R isoforms with NFT, while Pick's disease or CBD are characterized by specific 3R and 4R tau accumulation, respectively.

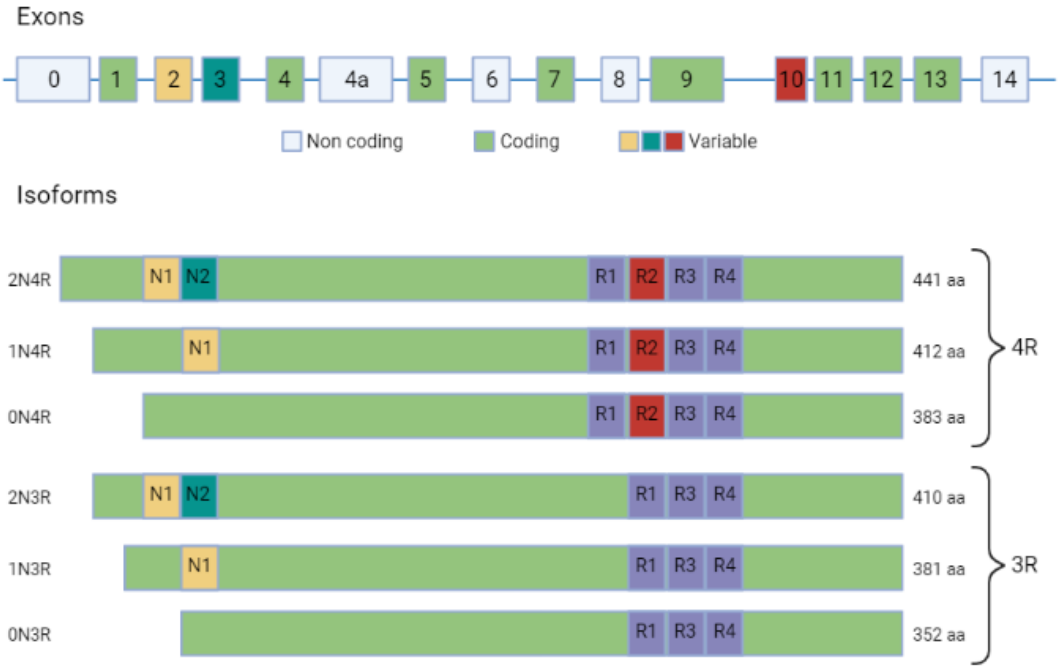


Figure 7. Scheme of tau isoforms expressed in the human brain. Non-coding sequences are represented in light blue and constitutive exons in green. The variable exons that determine the N- terminal sequence are in yellow (N1) and blue (N2) and the variable exon that determine the 3R/4R isoform is in red (R2). The length of each isoform depends on the number of amino acids (aa).

MAPT gene is characterized by two haplotypes due to the inversion (H1) or non-inversion (H2) of a 900 kb sequence. H1 haplotype is a risk factor for Parkinson's disease, PSP, CBD, and amyotrophic lateral sclerosis but not for Pick's disease (Stenfansson et al., 2005).

Tau protein was discovered in 1975 as a monomer with intrinsically disordered structure. After a correct assembly structure, filaments are formed. Tauopathies are characterized by neuronal progression of tau pathology from the axon to the soma and dendrites (Bancher et al., 1987; Hoover et al., 2010). Accumulation of tau in glial cells occurs in some neurodegenerative diseases, but the types and the percentage of glial cells with tau pathology are variable. However, the pathological role of this glial tau pathology is still unexplored (Kahlson and Colodner, 2016).

Tau protein can suffer from several post-translational modifications such as phosphorylation, acetylation, glycation, methylation, nitration, sumoylation, ubiquitination, isomerization, O-Glc-NAcylation and truncation (Goedert et al., 2017). Phosphorylation is implicated in the regulation of tau binding to microtubules. Abnormal or hyper-phosphorylated tau causes its self-aggregation constituting a pathological hallmark of numerous neurodegenerative diseases (Iqbal et al., 2016). Tau acetylation in lysine residues affects its assembly and phosphorylation. Depending on the

acetylated residue, the effects may vary and can increase or decrease both tau phosphorylation and aggregation (Cohem et al., 2011; Cook et al., 2014). Finally, tau protein can be found in many intracellular compartments but also in the interstitial and the cerebrospinal fluid, what makes tau an actual biomarker of tauopathies as AD.

3.2. Mutations of *MAPT* in tauopathies

MAPT mutations are associated with several neurodegenerative diseases, particularly within the FTD spectrum disorders, including FTD with parkisionism-17, CBD and PSP (Ballatore et al., 2007). There are 27 different tauopathies described to date (Ling, 2018). In familial FTD with parkisionism-17 cases have been found 60 different mutations in *MAPT*, usually within exons 9-12, that affect either protein levels or the alternative splicing of mRNA tau (Hasegawa et al., 1998; Hutton et al., 1998). FTD-linked MAPT mutations increase tau phosphorylation, aggregation and pathogenic effects leading to neurodegeneration. Particularly, P301S mutation on exon 10 results in overexpression of 4R tau and increased tau phosphorylation and aggregation causing early-onset FTD with parkinsonism (Yasuda et al., 2005). Heterozygous overexpression of the human tau isoform (1N4R) harboring the P301S FTD-linked mutation causes progressive accumulation of phosphorylated tau in the hippocampus, amygdala, entorhinal cortex, and spinal cord of P301S (PS19) transgenic mice at 6 months (Yoshiyama et al., 2007). The expression of the human protein is under the mouse prion promoter (MoPrP) and is 5-fold times higher than the expression of the endogenous tau (Borchelt et al., 1996). P301S tau mice is characterized by an age-dependent neurodegeneration with ventricle enlargement and cortical and hippocampal atrophy starting at 9 months. Interestingly, synaptic impairment, microgliosis and astrogliosis are found in these mice at 3 months before tau pathology (Yoshiyama et al., 2007). Behavioral abnormalities such as clasping and limb retraction in hang tail test can be found followed by loss of strength in the limbs and ending with paralysis and hunched-back posture by 9 months. Life expectancy is around 12 months, although the median was around 9 months (Yoshiyama et al., 2007). Remarkably, sexual dimorphism in behavior, neuropathology and inflammatory molecules are evident in tau P301S transgenic mice (Sun et al., 2020). In addition, microglia transcriptional responses to tau or amyloid pathologies differ between male and females, and microglial miRNAs affects differentially to tau pathology in male and females (Sala Frigerio et al., 2019; Kodama et al., 2020).

3.3. Biological function of tau

Tau is a highly dynamic protein presenting multiple structural conformations that are involved in a diverse range of cellular functions. Tau is expressed in the central and peripheral nervous system, especially in neurons and glial cells (Weingarten et al., 1975). In the human brain, tau is expressed mainly in axons but also, in a much lesser extent, in somatodendritic compartments such as plasmatic membrane, nucleus, dendrites and mitochondria (Li et al., 2016). The classical function of tau is the assembly and the stabilization of microtubules in axons by binding to α- and β-tubulin heterodimers. However, tau also participates in the regulation of axonal transport influencing the function of dynein and kinesin (Stamer et al., 2002). The physiological presence and function of tau in dendrites is still under debate. It has been proposed that tau is implicated in synapse regulation and reduction of tau leads to loss of synapses (Frandemiche et al., 2014). Tau also participate in cellular signaling, so it can be phosphorylated in 85 sites acting as a signaling buffer (Chen et al., 2012). Furthermore, tau plays a protective role on DNA and RNA damage caused by reactive oxygen species and denaturation induced by heat stress (Violet et al., 2014). Tau is also a scaffolding protein for phosphotransferases involved on neuron activity, synaptic plasticity, and actin bundling (Wang and Mandelkow, 2016). Finally, tau is required for iron export, hippocampal long-term depression and neurogenesis in the adult hippocampus (Hong et al., 2010; Lei et al., 2012; Kimura et al., 2013).

3.4. Tau kinases and phosphatases

Tau is phosphorylated in multiple Ser/Thr and Tyr residues in physiological and pathological conditions. Tau kinases are divided in three groups: 1) Proline-directed serine/threonine-protein kinases such as cyclin-dependent kinase-5 (Cdk5), glycogen synthase kinase 3β (GSK3β) and mitogen-activated protein kinases (MAPKs); 2) Non-proline-directed serine/threonine-protein kinases like tau-tubulin kinase 1/2 (TTBK1/2) and protein kinase B/Akt, and 3) Protein kinases specific for tyrosine residues as Fyn, Syk and Src (Martin et al., 2011). Here, I will focus on tau phosphorylation by GSK3β, Cdk5, and Akt kinases. GSK3β can phosphorylate tau in more than 40 residues. In AD, GSK3β activity contributes to tau hyperphosphorylation and aggregation and it colocalizes and correlates with NFT (Hanger et al., 1992; Leroy et al., 2002). GSK3β overexpression causes increased phosphorylated tau, neurodegeneration and spatial learning deficits (Hernández et al., 2002). In contrast, GSK3β inhibition reduces tau pathology and neurodegeneration (Caccamo et al., 2007; Serenó et al., 2009) and regulates tau splicing increasing tau mRNA with exon 10 (Hernández et al., 2004).

Notably, clinical trials targeting GSK3β in AD patients have shown no positive effects (Noble et al., 2013). On the other hand, Cdk5 activity is associated with tau hyperphosphorylation. Aberrant hyperactivation of Cdk5 through overexpression of its coactivator p25, causes progressive tau pathology and neurodegeneration (Cruz et al., 2003; Noble et al., 2013). Furthermore, some reports have confirmed the direct phosphorylation of tau by Akt in two different residues: Thr212 and Ser214. Due to the role of Akt in the anti-apoptotic signaling PI3K/Akt, this may have important effects on neurodegeneration (Ksiezak-Reding et al., 2003; Kyoung Pyo et al., 2004).

Tau is mainly dephosphorylated by protein phosphatase 2A (PP2A). In AD brain, PP2A activity is decreased by 50% (Liu et al., 2005), although PP2A upregulation can also cause the abnormal accumulation of tau in the brain (Planel et al., 2001). This result shows that both, hyperphosphorylation or excessive dephosphorylation of tau are detrimental. PP2A has been related to GSK3β, Akt and mTOR suggesting that dysregulation of this pathway can contribute to tau pathology (Cross et al., 1995; Meske et al., 2008; Yao et al., 2011).

3.5. Cellular elimination of tau: proteasome and autophagy

A cellular mechanism that can lead to tau accumulation is the failure of its elimination. Tau is degraded by different intracellular mechanisms, including proteasome or autophagy and extracellularly by proteases, blood brain barrier or glymphatic system. Intracellular tau clearance is mainly mediated by the ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (Boland et al., 2018). The UPS, which controls the quality of the proteins removing abnormal or damage proteins, is implicated in the selective elimination of unfolded and soluble tau in an ubiquitin-independent manner (David et al., 2002). Different ubiquitination sites have been identified on soluble and insoluble tau suggesting clearance of this tau forms by UPS (Lee et al., 2013). Furthermore, tauopathy models and AD patients have shown decreased proteasome activity (Keller et al., 2000; Myeku et al., 2016). Aggregated tau binds to and affects the proteasome, but it is not proved if UPS alteration precedes or is induced by aggregated tau (Keck et al., 2003).

Pathological tau is mainly cleared by autophagy. There are three different types of autophagy: microautophagy, chaperone-mediated autophagy and macroautophagy. Here, I will focus on macroautophagy that I will refer as autophagy. Autophagy is a self-degradative process of long-lived proteins and organelles delivered from vacuoles, called autophagosomes, to lysosomes. Briefly, autophagy begins with the formation and elongation of a structure with double membrane called phagophore that ends

closing around cargos and forming the autophagosome. After its maturation, autophagosomes fuse with lysosomes forming autolysosomes. Otherwise, autophagosome can fuse previously with endosomes constituting an amphisome that also ends fusing with lysosome. The purpose of this degradation is to provide recycled components or energy to the cell. Although is elevated in glial cells, autophagy is constitutively active in neurons and its components are found in the soma, axons, dendrites, pre- and post-synaptic terminals (Kulkarni and Maday, 2018). Remarkably, the expression of autophagy induction proteins, such as Beclin-1, ATG5 and ATG7, are reduced during aging and in neurodegenerative diseases, including AD, suggesting a close relation between altered autophagy and neurodegeneration (Lipinski et al., 2010).

In vitro studies have shown the involvement of autophagy in tau clearance. Pharmacological inhibition of the autophagosome-lysosome fusion in hippocampal slices with chloroquine results in elevated total and phosphorylated tau levels (Bendiske and Bahr, 2003). Autophagy stimulation by mTOR inhibition through nutrient deprivation or rapamycin treatment causes the reduction of tau inclusions in SH-SY5Y cells and fibroblasts expressing mutant tau (Wong et al., 2008). N2A cells overexpressing tau with a FTD mutation treated with trehalose, an mTOR-independent activator of autophagy, results in reduction of aggregated, soluble, and insoluble tau. Primary rat neurons treated with proteasomal inhibitors unexpectedly result in reduced tau levels due to an upregulation of autophagy (Krüger et al., 2012). *In vivo* activation of autophagy with methylene blue or trehalose treatments causes the reduction of phosphorylated and insoluble tau in P301L and P301S transgenic mice, respectively (Congdon et al., 2012; Schaeffer and Goedert, 2012). Importantly, pathological tau colocalizes with autophagic markers in the frontal cortex of AD patients harboring FAD-linked APP mutants or suffering PSP and CBD (Piras et al., 2016).

The mechanisms of autophagy-mediated tau elimination are largely unclear. However, recent studies have revealed that autophagy receptors are involved in the selective autophagy elimination of tau. Remarkably, p62 seems to bind to tau aggregates colocalizing with NDP52 autophagy receptor (Falcon et al., 2018). Furthermore, BAG3 is a contributor of phosphorylated tau clearance by autophagy, especially in dendrites and post-synaptic terminal of inhibitory neurons. This aspect may be implicated in the cell-specific accumulation of tau pathology in excitatory neurons in AD or FTD (Fu et al., 2019). Finally, phosphatidylinositol-binding clathrin assembly protein (PICALM/CALM), a recent reported genetic risk factor for AD, regulates endocytosis, different steps of autophagy and tau specific clearance supporting an important role of autophagy in tau specific elimination in AD (Moreau et al., 2014).

In summary, autophagy mediates elimination of pathological tau, and deregulated autophagy, characterized by accumulation of autophagosomes and lysosomes, seems to play a pivotal role in the initiation and/or progression of neurodegeneration in AD and FTD (Nixon and Cataldo, 2006; Ghavami et al., 2014).

3.6. Tau secretion and propagation

Tauopathies are characterized by progressive spreading of tau pathology through affected brain regions. AD neuropathology is classified in six Braak stages (I-VI) depending on tau accumulation in affected brain regions. At early stages, AD patients show tau accumulation in the entorhinal cortex (EC) and then the lesion reaches the hippocampus. Finally, tau pathology extends to other cortical regions such as motor, associative and sensory regions and affecting frontal, temporal and parietal lobes (Braak and Braak, 1991; Braak et al., 2006). The mechanisms of tau spreading in the brain are still under investigation and it is not clear whether tau propagates from one to other brain regions or tau pathology is developed independently in each region.

There is compelling evidence of tau propagation through secretion and synaptic mechanisms. *In vitro* and *in vivo* studies have revealed that tau propagation appears to be most likely from the secretion (free molecules, exosomes…) by a presynaptic or donor cell and the uptake by a postsynaptic or recipient cell, resulting in generation of new abnormal tau (Frost et al., 2009; Yamada et al., 2011). The lack of secretory signal sequence in tau suggests that tau is released by unconventional secretion pathways (Nickel and Rabouille, 2009). Some studies suggest that extracellular tau is mostly free and that phosphorylated tau can cross the plasma membrane through type I unconventional protein secretion mechanism or through chaperone-mediated release (Fontaine et al., 2016; Katsinelos et al., 2018). The presence of pathological tau in pre- and post-synaptic terminals supports the trans-synaptic spreading of tau. Aggregated tau can travel along axons and dendrites in an antero- and retrograde manner, affecting monomeric tau in a similar manner than prions (Calafe et al., 2015). Furthermore, injected- aggregated tau propagate to synaptically connected areas affecting neurons and oligodendrocytes in a time-dependent manner (Clavaguera et al., 2009; Lasagna-Reeves et al., 2012; Clavaguera et al., 2014). The fact that mice expressing human tau specifically in the EC show both human and endogenous tau aggregates in the hippocampus supports tau trans-synaptic propagation (de Calignon et al., 2012).

Extracellular vesicles containing tau were recently suggested as a compensatory mechanism for tau clearance. Microvesicles, also called ectosomes, and exosomes are

the two types of extracellular vesicles containing tau. These vesicles share most of the markers making difficult its differentiation, but they have a slightly different origin. Both types of vesicles are present in all cells of the central nervous system and they are increased in tauopathies, supporting the role of microvesicles in tau propagation (Rajendran et al., 2006). Microvesicles secretion is stimulated by intracellular levels of calcium, inflammatory molecules, and oxidative stress in neurodegenerative diseases (Piccin et al., 2007). I*n vitro* and *in vivo* studies of a rat model of tauopathy showed the presence of tau in microvesicles in extracellular space (Dujardin et al., 2014). On the other hand, exosomes are smaller vesicles usually with higher levels of lipid raft elements (Record et al., 2014). Phosphorylated and unbound tau are associated to vesicles, especially in intracellular compartments with disordered microtubules, and colocalize with lipid-raft associated tyrosine kinase, fyn, identified in exosomes (Lee et al., 2012). Additionally, tau is found in isolated exosomes in cerebrospinal fluid (CSF) and blood of AD patients and AD mice models (Barten et al., 2011; Saman et al., 2012; Fiandaca et al., 2015). Interestingly, the injection of exosomes isolated form tau transgenic mice or the injection of microglia-derived exosomes results in tau phosphorylation, aggregation and propagation (Asai et al., 2015; Baker et al., 2016). These results indicate the involvement of extracellular vesicles on pathological tau propagation, not only in neurons, but also in microglia supporting the role of the immune system in tau pathology.

Intracellular tubular connections are bridges connecting remote cells formed by actin-based transient cytoplasmic extensions (Rustom et al., 2004). Recent studies have shown that tau are specific markers of these channels and that tau species can stimulate their formation enabling tau transference in pathological situations contributing to tau propagation (Tardivel et al., 2016). Tau secretion is also regulated by several cellular processes such as starvation, lysosomal dysfunction or neuronal stimulation (Pooler et al., 2013; Mohamed et al., 2014). MAPT mutations can affect the form and the rate of tau release (Nacharaju et al., 1999). Interestingly, injection of aggregated tau in transgenic mice containing β-amyloid plaques causes tau seeding, which suggests that Aβ can increases tau pathology in AD (He et al., 2018).

Tau species involved in tau spreading are under debate. Soluble monomers, large fibrils, full-length or truncated tau forms are detected in the extracellular fluid (Chai et al., 2012; Kfoury et al., 2012; Meredith et al., 2013; Takeda et al., 2015). However, CSF of control and AD patients show N-terminal tau fragments but not C-terminal fragment or full-length tau protein (Meredith et al., 2013).

Recent evidence indicates cellular internalization of tau after addition of tau to the cell media or the injection of tau into the brain causing tau pathology and neuronal death (Diaz-Hernandez et al., 2010; Lasagna-Reeves et al., 2012). Surprisingly, intraperitoneally injections of tau aggregates cause cerebral tau pathology in tau transgenic mice (Clavaguera et al., 2014b). Furthermore, several reports show endocytosis of tau by either macropinocytosis or clathrin-mediated mechanisms (Michel et al., 2014), and tau aggregates are uptaked into cells through heparin sulfate (Holmes et al., 2013). Interestingly, not only neurons but microglia have also shown to internalize tau (Bolós et al., 2016).

In summary, different tau species and secretion/internalization mechanisms are implicated in the spreading of tau in physiological and pathological conditions. Clarifying these mechanisms are important for targeting tau propagation (tau secretion/uptake) as novel therapeutic strategy for tauopathies, including AD (**Fig. 8**).

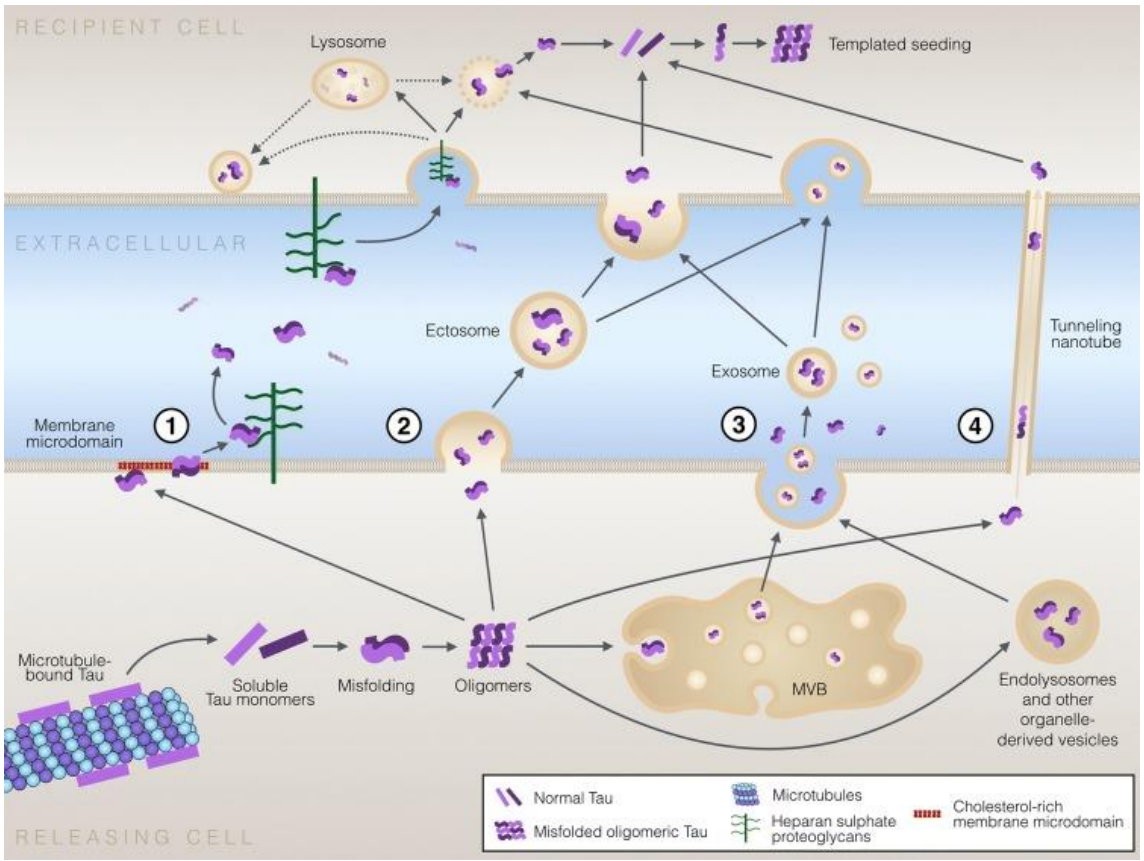


Figure 8. Mechanisms of cell-to-cell tau protein transfer. (1) Unconventional secretion of tau protein directly through plasmatic membrane. Tau secretion by ectosomes (2) and exosomes (3). After release, both share uptake mechanisms. (4) Tau transfer through intracellular tubular connections. MVB: multivesicular bodies (Brunello et al., 2020).

3.7. Therapeutic strategies for tauopathies

Current strategies therapies for tauopathies are based on modifying or reducing tau expression, alternative splicing, post-translational modifications (phosphorylation, acetylation, aggregation…) and metabolism.

The reduction of tau expression via tau silencing with antisense oligonucleotides, siRNA or microRNAs results in reduced tau pathology, improvement of behavior and electrophysiological properties in AD mouse models (Roberson et al., 2007; DeVos et al., 2017). For FTD cases caused by mutations on exon 10 of *MAPT* gene, experimental therapies are focused on the modification of the alternative splicing through the inclusion or exclusion of the exon 10. The correction of mis-splicing or splicing reduction represents a novel therapy strategy for specific tauopathies characterized by abnormal tau RNA processing (Kalbfuss et al., 2001; Avale et al., 2013).

Acetylation and phosphorylation are the main post-translational tau modifications associated to NFT in AD patients. Acetylation of phosphorylated tau reduces tau degradation resulting in increased tau aggregation and accumulation (Min et al., 2010). Reduction of tau phosphorylation with GSK3β inhibitors such as lithium chloride has shown promising results in mice (Forlenza et al., 2011; Ahmed et al., 2015), but it showed no benefits on AD patients (Hampel et al., 2009; Hampel et al., 2015). Our own results revealed that sodium selenate treatment increases the activity of PP2A reducing tau pathology and memory impairments in mice (Corcoran et al., 2010; Van der Jeugd et al., 2018). Furthermore, fyn inhibitor and a selective inhibitor of O-GlcNAcase that prevents tau phosphorylation are currently on clinical trials with promising results in mice (Selnick et al., 2019; Wang et al., 2020).

Some drugs have been used to promote tau proteolysis or degradation. Trehalose or pharmacological farnesyltransferase inhibition reduce tau pathology in mice through the activation of lysosomal tau degradation (Schaeffer et al., 2012; Hernandez et al., 2019). Methylthioninium and derivates reduce tau aggregation and pathology in vitro and in transgenic mice. Clinical trial on mild to moderate AD patients showed some benefits but there was no improvement in patients co-treated with cholinesterase inhibitor and/or memantine and further studies are necessary (Wischik et al., 2015; Gauthier et al., 2016; Wilcock et al. 2018). Active and passive immunization focused on reducing tau propagation have shown to reduce tau uptake and seeding *in vitro* and tau pathology in transgenic mice (Yanamadra et al., 2013; Castillo-Carranza et al., 2014; Nobuhara et al., 2017). Currently, active and passive tau immunization targeting

different residues and forms of tau are ongoing on clinical trial for mild to moderate AD (Theunis et al., 2013; Kontsekova et al., 2014; Courade et al., 2018; Albert et al., 2019).

To sum up, tau has been proposed as a therapeutic target for the treatment of tauopathies and several approaches targeting tau pathology are currently ongoing in clinical trials. A better understanding of the mechanisms regulating tau phosphorylation, aggregation, degradation and secretion will help to investigate its role in physiological and pathological conditions.

V. Hypothesis

The hypothesis of this doctoral thesis is that **loss of PS function in neurons contributes to tau hyperphosphorylation, aggregation and/or spreading.**

VI. Objectives

The objectives of this doctoral thesis are the following:

1. To investigate the role of PS on tau phosphorylation, aggregation and transmission
2. To study the molecular and cellular mechanisms regulated by PS in tau pathology in tauopathy mouse models
3. To examine the effects of tau in synapse pathology

VII. Materials and Methods

1. Mouse models

PS cDKO mice (C57BL/6/129 background) lacking expression of both PS (PS1 and PS2) genes in forebrain glutamatergic neurons were previously described (Saura et al., 2004). Briefly, Littermate control (PS1 f/f; $PS2^{+/+}$ or PS1 f/f; $PS2^{+/-}$; f:floxed), PS1 cKO (PS1 f/f; CamKIIα-Cre) and PS cDKO (PS1 f/f; $PS2^{-/-}$; CaMKIIα-Cre) mice were obtained by crossing floxed PS1/$PS2^{-/-}$ (PS1 f/f; $PS2^{-/-}$) or $PS2^{+/-}$ (PS1 f/f; $PS2^{+/-}$) males to heterozygous PS1 cKO; $PS2^{+/-}$ females (PS1 f/f; $PS2^{+/-}$; CaMKIIα-Cre, Steiner et al., 1999; Yu et al., 2001). To investigate the role of PS in human tau pathology we generated novel non-transgenic control (PS1 f/f; Tau-), tau (PS1 f/f; Tau+), PS1 cKO;Tau (PS1 f/f; CamKIIα-Cre; Tau+) and PS cKO;Tau (PS1 f/f; $PS2^{-/-}$; CamKIIα-Cre; Tau+) mice by crossing PS1 cKO or PS cDKO with Tau P301S Tg (PS19) mice (JAX #008169; C57BL/6). Tau P301S mice expressing the FTDP-17-linked P301S *MAPT* gene under the neuron-specific prion protein promoter (MoPrP) show behavior alterations and phosphorylated tau and NFT pathologies at 5-8 months (Yoshiyama et al., 2007). Mice were maintained at the Animal Core facility of the Universitat Autònoma de Barcelona, on a 12h-light/dark cycle with ad libitum food and water. All experimental procedures were conducted according to the Animal and Human Ethical Committee of the Universitat Autònoma de Barcelona (CEEAH 2896) and Generalitat de Catalunya (DMAH 8787) following the experimental European Union guidelines and regulations (2010/63/EU).

2. Mouse genotyping

DNA extraction was performed digesting approximately 2 mm of the mouse tail in 50mM NaOH during 30 min at 95ºC. Purified DNA was amplified by Polymerase Chain Reaction (PCR) using 2 µl of DNA sample, 2.5 µl of 10X PCR buffer (Biotools), 0.5 µl dNTP (10mM; Biotools), 0.5 µl $MgCl_2$ (50 mM; Biotools), 0.2 µl Taq DNA polymerase (5 U/ml; Biotools), forward and reverse primers (0.5 µM; Life Technologies). We checked the *PS1*, *Cre*, *PS2* and *Mapt* genes. All the primer sequences used for PCR are in table 2.

Gene	Primer	Sequence (5'-3')	PCR conditions
PS1	P139	GGTTTCCCTCCATCTTGGTTG	94°C, 4 min
	P140	TCAACTCCTCCAGAGTCAGG	94°C, 1 min
	P158	TGCCCCCTCTCCATTTTCTC	60°C, 1 min – 40 cycles
Cre	P156	GCCTGCATTACCGGTCGATGCAACGA	72°C, 1 min
	P157	GTGGCAGATGGCGCGGCAACACCATT	72°C, 7 min
PS2	P162	CATCTACACGCCCTTCACGG	94°C, 4 min
	P163	CACACAGAGAGGCTCAAGATC	94°C, 1 min 65°C, 1 min – 35 cycles 72°C, 1 min
	P164	AAGGGCCAGCTCATTCCTCC	72°C, 7 min
Mapt	P16134	GGCATCTCAGCAATGTCTCC	94°C, 4 min
	P12473	GGTATTAGCCTATGGGGGACAC	94°C, 1 min
	OIMR 8744	CAAATGTTGCTTGTCTGGTG	65°C, 1 min – 35 cycles 72°C, 1 min
	OIMR 8745	GTCAGTCGAGTGCACAGTTT	72°C, 7 min

Table 2. Primer sequences and conditions used for mouse genotyping.

3. Behavioral tests

3.1. Tail suspension test

To evaluate significant motor deficits, we measured clasping scores suspending the mouse from the tail for 5 min. Measures of movement of hind/forelimbs and clasping behavior are measured according to the severity of the hindlimb clasping on a scale from 0 to 4 (0 = no clasping; 1-4: clasping of one-four toes).

3.2. Contextual fear conditioning (CFC)

Mice handled for three days (3 min/day) were placed in a conditioning chamber (15.9 x 14 x 12.7 cm; Med Associates, St. Albans, Vermont) for 3 min, foot-shocked (1s/1mA) and retained in the chamber for 2 min (immediate freezing, España et al., 2010). Fear memory was tested as freezing behavior, defined as a complete cessation of all

movement except for respiration, in the same conditioning chamber for 4 min 24 h after training using Video Freeze Software (Med Associates).

3.3. Cued fear conditioning (CuedFC)

Animals were positioned in the same chamber as CFC with white light during 3 min to measure the neophobia. After that, the animals were exposed to a sound stimulus (conditioned stimulus: CS) of 2800 Hz and 80 dB for 30 seconds. The last 2 seconds of the CS were accompanied by an electric shock of 0.8 mA (unconditioned stimulus: US). The immobilization response was measured during the consecutive 2 min after de shock as the immediate freezing. 24h after, the animals were introduced in a modified chamber with dark light to avoid the visual recognition during 2 min. Then, the CS were presented during 3 min without electric shock. The immobilization response (freezing) before the CS (pre-CS) and during the CS were recorded using the same software as the CFC.

3.4. Novel Object Recognition (NOR)

Mice were placed in a square apparatus for 1 min for context habituation. Then, during the training phase, mice were exposed to two identical objects during 5 min. To study short-term memory, 2 hours after, on the test phase, one object were replaced for a different object and mice were exposed for 5 min. Finally, to analyze long-term memory, 24 h after training phase, mice were exposed to the first object and a new one during 5 min. We analyzed the preference for both objects and the number and latency of rearing in each phase.

3.5. Morris water maze (MWM)

Mice were put in a circular pool with 90 cm of diameter with water (22ºC). White paint was used to make the water opaque. Four visible cued were place in the cardinal points for be used for the mice to locate the hidden platform (6,5 cm). The pool was virtually divided in 4 zones: target quadrant where the platform was located (TQ), opposite quadrant (OQ) and adjacent quadrants (LQ and RQ). The experiment was divided in three phases: easy task, learning and memory probe. In the easy task, a flag was anchored in the platform to facilitate its localization and this phase consists in 6 trials for each animal using different entrance in each trial. The learning phase consist in 3 trials of 2 consecutive test during the next 5 days. The protocol was the same as easy task phase but without the flag and varying the order of the entrances. If the mouse were able to reach the platform, it was picked after 10 seconds. If the mice were not able to find the platform after 60 seconds, the mice were guided to the platform and

placed there for 10 seconds. To analyze the learning, the latency (s) was measured in each probe of each day. The memory probe consists in a single trial of 60 seconds with the removal of the platform. The hippocampal dependent memory was measured according to the time spent in, number of crossings and latency to the target quadrant and the number of crossings of the area where the platform was during the learning phase. All the sessions were recorded using the any-maze behavioral tracking software.

4. Tissue processing

4.1. Biochemical analysis

For biochemistry experiments by western blotting, animals were euthanized by cervical dislocation and brains were dissected in order to obtain the cortex and the hippocampus. Then, a total tau extraction protocol was used (Planel et al., 2001). Cortex and hippocampus were dissected and individually homogenized in 100 µl of O+ buffer for 1 mg of weight and boiled immediately in boiling water during 5 min, to isolate proteins resistant to heat, including tau (Petry et al., 2014). Samples were sonicated for 15 seconds and centrifuged for 15 min at 14,000 rpm and 4ºC. Then, we took the supernatant and we used a quantitative protein assay kit (Invitrogen) to measure the protein concentration in each sample.

4.2. Histological analysis

For histological analysis by immunohistochemistry, mice were killed using CO2 and perfused intracardially with 4% of paraformaldehyde (PFA) in Tris-HCl buffer saline (TBS) pH 7.4 at room temperature (RT). Brains were removed and immerse in 10% PFA for 2 h at RT and then rinsed in TBS before dehydration in a graded series of ethanol followed by xylene. Then the tissue was embedded in paraffin, sectioned at 10 µm and mounted on glass slides. Sections from all the mice groups of each age were mounted on the same slide in order to assure maximum homogeneity during immunostaining procedure.

4.3. Synchrotron analysis

For synchrotron based-µFTIR analysis, animals were euthanized and perfused with TB and 4% PFA. Brains were extracted and postfixed during 2 h at RT in 4% PFA and cryoprotected with 30% sucrose solution in 0,1 M TB for 48 h at 4ºC and, finally, frozen in ice-cold methylbutane (320404, Sigma-Aldrich) and stored at -80ºC. Series of coronal parallel sections (8-µm-thick) between bregma 0.86 mm and bregma -1.22 mm coordinates were cut on a cryostat (CM3050S Leica) and mounted onto polished

barium fluoride (BaF2) optical windows (BAFP16-1, Crystran, U.K). BaF2 windows containing brain sections were stored at room temperature (RT) in vacuum chamber and protected from light.

5. Gallyas staining

To detect the neurofibrillary tangles (NFT) composed of aggregated and phosphorylated tau, we used the simplification of the modified Gallyas staining, a silver-staining method useful for histological detection of pathological accumulation in the central nervous system (Kuninaka et al., 2015). More concretely, the Gallyas method is an impregnation technique of silver with high sensitivity for argyrophilic structure like NFT. Sections were deparaffinized in xylene followed by a rehydration in a graded series of ethanol (2x3 min in xylene, 2x3 min in 100%, 3 min in 95%, 3 min in 70%, 3 min in 50% ethanol) and 3 min in H2Od. Then, the slides were submerged in potassium permanganate 0.3% (Sigma 223468) during 10 min followed by 2 rinses in H2Od of 3 min. After that, samples were treated during 1 min in oxalic acid 1% (Sigma 75688) and washed for 5 min in H2Od. After 1 min of alkaline silver iodide treatment, sections were washed 3 times with acetic acid 0.5% during 1 min each. Then, slides were submerged in developer solution, prepared immediately before its use, during 6-7 min depends on temperature and reaction time. This solution is composed by solution A (1000 ml: 2 g silver nitrate, 2 g ammonium nitrate, 10 g tungstosilicic acid and 5.1 ml of paraformaldehyde 35% in H2Od) and solution B (50 g of anhydrous sodium carbonate (Sigma 223484) in 1000 ml H2Od). Again, samples were washed 3 times in acetic acid 1% during 1 min. Then, gold chloride 0.5% (Sigma 254169) was used during 5 min and washed with H2Od during 1 min. We used an incubation of 3 min in nuclear fast red 0.1% as a counterstain to stain the nucleus without interfering the NFT. Finally, slides were dehydrate using the same graded series of ethanol in the opposite way and in xylene and mounted with DPX Mountant for histology (SIGMA 06522).

6. Purification and injection of tau extracts

Parietal cortex (7 g) of two pathologically confirmed sporadic AD cases (two females, 80 and 89 years old) were homogenized in 10 volumes of cold H buffer (10mM Tris, 1mM EGTA, 0.8 M NaCl and 10% sucrose at pH 7.4) and then purified by ultracentrifugation as described (Greenberg and Davies, 1990). After collecting the extracts from the 35% sucrose layer and 35-50% of interface, the purified material was centrifuged at 55,000 rpm for 1h in a Sorvall S55A rotor. The pellet containing PHFs was resuspended in 100-200 µl of PBS and stored at -80°C. PHFs extracts (10 µl) were resolved in SDS-PAGE and analyzed by Western blotting with monoclonal PHF-1

antibody (Ser396/Ser404). Tau extract samples were also characterized by transmission electron microscopy by using the air-dried negative staining method. Briefly, 8 µl of the mixture were placed on carbon-coated copper grids and incubated at room temperature for 1 min. Samples were stained negatively with uranyl acetate (2%) for 1 min. Images were obtained with a JEOL JEM-1400 80KV microscope. Purified tau extracts (3µl; 10µg/µl; 0.5µl/min) or vehicle were injected unilaterally stereotaxically into the hippocampus of mice anesthetized with isoflurane. The injection coordinates were (in mm) according to Paxinos and Franklin's brain atlas: anterior 0.2 caudal to bregma; 0.18 lateral to bregma; depth 0.18 ventral to dural surface. Mice were analyzed 3 months post-injection (n = 4-8 mice/genotype).

7. In vitro experiments

7.1. HEK293T

Human embryonic kidney cell 293, expressing the SV40 T-antigen to promote the replication of the transfected plasmids, were used for the lentiviral particle generation. HEK293T cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS; Invitrogen-Gibco 10106-169) and penicillin/streptomycin (5000 U/ml, Life Technologies 15070-063) at 37ºC in a humidified 5% CO_2 incubator. Cells were washed with pre-warmed phosphate buffe saline (PBS), treated with 0.25% trypsin-ethylenediamine tetraacetic (EDTA) and incubated for 3 min at 37ºC. Then. Cells were centrifuged at 1000 rpm during 5 min and seeded at the required cell density.

7.2. Lentiviral generation

HEK293T cells were transfected using the Calphos Mammalian Transfection Kit (Clontech). Briefly, 30 µg of plasmid containing the sequence of interest (ΔCre and Cre), 15 µg of plasmid containing the viral genes of packaging (psPAX2) and 10 µg of a plasmid with viral genes of envelope sequence (pM2D.G) were mixed with 250 mM $CaCl_2$. Then, DNA solution was added to 1.5 ml of HBS buffer, softly mixed and incubated for 20 min at room temperature. Medium of cells were replaced by OptiMEM (Invitrogen, 31985-062) supplemented with 25 µM chloroquine. After the 20 min, 1 ml of the transfection mix was added to the cells and incubated for 7-8 h at 37ºC in 5% CO_2. 24, 36 and 48h after, the culture medium was recollected and centrifuged at 100000 x *g* at 4ºC during 2h. Pellet, containing the lentiviral particles, was resuspended in 10 µl of PBS in agitation at 4ºC overnight. Lentiviral particles were aliquot and stored at -80ºC.

Taking advantage of the GFP-expressing cassette present in the ΔCre and Cre lentiviral plasmid, lentiviral particles generated were tittered by flow cytometry. HEK293T cells were cultured in 24-well plates at 1500 cells/cm^2 density and 24h later, transduced with a range dilution of lentiviral particles (2 µl, 1 µL, 0.5 µl, 0.25 µl, 0.125 µl, 0.0625 µl and 0.03125 µl). Cells were maintained for 48h at 37ºC in 5% CO_2. Cells were washed twice with PBS, trypsinized, recollected and centrifuged at 10000 rpm during 3 min. The pellet containing the cells were washed twice with PBS and the GFP fluorescence of 10000 cells was measured in a flow cytometer (Cytomics FC 500, Beckman Coulter). The final titer (infective particles/ml) was calculated according to the formula:

$$\frac{Number\ of\ GFP\ positive\ cells\ x\ 1000\ x\ number\ of\ cells\ at\ the\ transduction\ moment}{Viral\ dilution\ x\ 100}$$

7.3. Primary neuronal cultures

6-wells plates were treated with 50 µg/ml poly-D-lysine incubated at 37ºC for 3-24h and washed twice with PBS.

Brains from mouse embryos (E15.5), obtained from PS1 f/f; PS2$^{+/-}$; Tau+ and PS1 f/f; PS2$^{-/-}$ crossing, were dissected in PBS supplemented with 0.6% glucose and cortices of each embryo were transferred separately to a 2 ml of solution 1 (Krebs buffer [120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ 14.3 mM glucose] with 0.3% bovine serum albumin and 0.03% Mg_2SO_4) and centrifuged at 300 x *g* during 1 min. The pellet was incubated at 37ºC during 10 min in 2 ml solution 2 (solution 1 supplemented with 0.0025% trypsin) and solution 3 (solution 1 supplemented with 0.0056% trypsin inhibitor, 0.008% DNase and 0.03% Mg_2SO_4) was added to stop the trypsin reaction by centrifugation at 300 x *g* for 1 min. Then, pellet was resuspended in solution 4 (84% solution 1 and 16% solution 3), disrupted using a Pasteur pipette and filtered with a nylon mesh of 40 µm pore size. Finally, solution 5 (solution 1 supplemented with 0.03% Mg_2SO_4 and 0.0014% $CaCl_2$) was added to cell suspension and centrifuged for 5 min at 250 x *g*. Cell pellet was resuspended in supplemented DMEM medium and cells were stained with trypan blue and counted in a Neubauer chamber. Neurons were cultured at 75000 cells/cm^2 density in supplemented DMEM containing 5% FBS and penicillin/streptomycin in poly-D-lysine coated dishes for 2-3h and then in Neurobasal medium containing B27 supplement and glutamine (Life Technologies). The genotype of each embryo was determined by PCR (Section 2 of *Materials and methods)* For PS1 inactivation, half of the conditioned medium was kept and neurons (4 days in vitro, DIV) were transduced with lentiviral Cre-deficient or Cre-recombinase vectors overnight as described (1-2 transducing units/cell) (Watanabe et

al., 2009). Next day, the medium was replaced by the kept conditioned medium plus fresh neurobasal medium. Culture medium was changed at 8 DIV and 11 DIV neurons were lysed with cold RIPA-DOC (50 mM Tris-hydrochloride, pH 7.4, 150 mM sodium chloride, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 2.5 mM EDTA, 1 mM Na3VO4, 25 mM sodium fluoride). Then, samples were sonicated and centrifuged for 20 min at 20.000 x *g* at 4ºC.

8. In vitro treatments

For neuronal stimulation, proteasome inhibition and modulation of autophagy and protein exocytosis, after 2 hours in fresh media, 10-11 DIV cortical neurons were treated with chloroquine (30 µM) during 24h, nutrient deprivation in DMEM free glucose for 30 min to 24h and/or MG132 (1 µM) for 24h. After the treatment, neurons were lysed as describe above.

9. Biochemical analysis

For biochemical analysis, half cortex and hippocampi were lysed in cold-lysis buffer (62.5 mM Tris-hydrochloride, pH 6.8, 10% glycerol, 5% β-mercaptoethanol, 2.3% sodium dodecyl sulfate [SDS], 5 mM NaF, 100 µM Na_3VO_4, 1 mM EDTA, 1mM ethylene glycol tetraacetic acid) containing protease and phosphatase inhibitors (Roche España, Barcelona, Spain) and boiled at 100°C (Planel et al., 2001). Protein content was quantified with the BCA protein assay kit (Thermo Fisher Scientific), resolved on SDS-polyacrylamide gel electrophoresis and detected by Western blotting with the antibodies listed on table 3. Bands detected with secondary antibodies coupled to peroxidase (Bio-Rad) and enhanced chemiluminescent reagent were captured in ChemiDoc MP System (Bio-Rad) and quantified in a linear range using the ImageLab 5.2.1 software (Bio-Rad).

Antibody	Specificity	Source	Cat number	Host	Dilution
β-actin	β-actin	Sigma-Aldrich	A5441	Mouse	1:60000
Akt	Protein kinase B	Santa Cruz	Sc-1618	Goat	1:1000
p-Akt	Phosphorylated Thr 308 Akt	CellSignaling	#4056	Rabbit	1:1000
p-Akt	Phosphorylated Ser473 Akt	CellSignaling	#9271	Rabbit	1:1000
AMPK	Total AMP-activated protein kinase	CellSignaling	#2532	Rabbit	1:1000

p-AMPK	Phosphorylated Thr172 AMPK	CellSignaling	#2531	Rabbit	1:1000
AT8	Phosphorylated Ser202/Thr 205 tau	LifeTechnologies	BR-00390206	Mouse	1:200
Cdk5	Total Cdk5	Santa Cruz	Sc-6247	Mouse	1:300
CP13	Phosphorylated Ser202 tau	P. Davies	-	Mouse	1:250
CRTC1	CREB-regulated transcription factor 1	Millipore	ABE1952	Rabbit	1:2000
D1M9X	Total tau	CellSignaling	#46687	Rabbit	1:1000
Doublecortin	Doublecortin	ABCAM	#AB18723	Rabbit	1:1000
Erk	Total p44/42 MAPK	CellSignaling	#9102	Rabbit	1:2000
p-Erk	Phosphorylated Thr202/Tyr204 p44/p42 MAPK	CellSignaling	#9101S	Rabbit	1:1000
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	LifeTechnologies	AM4300	Mouse	1:100000
GFAP	Glial fibrillary acid protein	Dako	Z0334	Rabbit	1:5000
GSK3β	Total GSK3β	BD Biosciences	#610202	Mouse	1:2500
p-GSK3β	Phosphorylated Ser9 GSK3β	CellSignaling	#9336	Rabbit	1:1000
Iba1	Ionized calcium binding adaptor molecule 1	Wako	019-19741	Rabbit	1:1000
Lamin-β1	Lamin-β1	Zymed	33-2000	Mouse	1:1000
LC3B	Microtubule-associated proteins 1A/1B light chain 3	Abcam	#AB48394	Rabbit	1:1000
MC1	Conformational tau (aa 312-322)	P. Davies	-	Mouse	1:250
mTOR	Mammalian target of rapamycin	CellSignaling	#2972	Rabbit	1:1000
p-mTOR	Phosphorylated Ser2448 mTOR	CellSignaling	#2971	Rabbit	1:1000

NFκB	Nuclear factor Kappa B	Santa Cruz	Sc-372	Rabbit	1:500
NF-L	Neurofilament light chain	Millipore	#AB9586	Rabbit	1:1000
p35	p35/p25	Santa Cruz	Sc-820	Rabbit	1:500
p70S6K	Ribosomal protein S6 kinase	CellSignaling	#9202	Rabbit	1:1000
p-p70S6K	Phosphorylated Thr389 p70S6K	CellSignaling	#9205	Rabbit	1:1000
PG5	Phosphorylated Ser409 tau	P. Davies	-	Mouse	1:250
PHF-1	Phosphorylated Ser396/404 tau	P. Davies	-	Mouse	1:250
p-PRAS40	Phosphorylated Thr246 PRAS40	CellSignaling	#2997	Rabbit	1:1000
PS1-CTF (loop)	C-terminal fragment-Presenilin 1	Chemicon	MAB5232	Rabbit	1:500
PS1-NTF	N-terminal fragment-Presenilin 1	Calbiochem	529591	Rabbit	1:10000
PSD95	Postsynaptic density protein 95	BD Biosciences	610496	Mouse	1:2500
SMI312	Neurofilament heavy and medium chain	Biolegend	#837904	Mouse	1:500
Synaptophysin	Synaptophysin	Sigma-Aldrich	S5768	Mouse	1:100000
Syntaxin 1	Syntaxin 1	Santa Cruz	Sc-12736	Mouse	1:1000
Tau17025	Total tau	V. Lee	-	Rabbit	1:5000
TG5	Total tau	P. Davies	-	Mouse	1:500
β-tubulin	β-tubulin	Sigma-Aldrich	T7816	Mouse	1:20000
VGLUT1	Vesicular glutamate transporter 1	Synaptic Systems	135302	Rabbit	1:1000

Table 3. List of antibodies used for Western blotting.

10. *Synchrotron based-μFTIR analysis*

10.1. Acquisition of the data

Fourier transform infrared microspectroscopy (μFTIR) coupled to synchrotron radiation (SR) was performed at the beamline MIRAS at ALBA's synchrotron (Catalonia, Spain). A Hyperion 3000 microscope equipped with a 36x magnification objective and couple to a Vertex 70 spectrometer (Bruker) was used. The measuring range was 600-4000 cm-1 wavenumber, and the spectra collection was carried out in transmission mode at 4 cm-1 resolution, 10 μm x 10 μm aperture dimensions and 128 scans. Zero filling was performed with fast Fourier transform (FFT), so that, in the final spectra, there was one point every 2 cm^{-1}. Background spectra were collected from a clean area of each BaF2 window every 7,5 min. A mercury cadmium telluride (MCT) detector was used, and both the microscope and spectrometer were continuously purged with liquid nitrogen. For each animal, a minimum of 100 spectra with a step size of 30 μm x 30 μm were acquired in the granular layer and the hilus of the hippocampus, medial corpus callosum and in the retrosplenial cortex area. A total of 4 animals by group was used for this experimental procedure. All the spectra were obtained by means of Opus 7.5 software (Bruker).

10.2. Synchrotron based-μFTIR spectra analysis

The spectra obtained from the tissue samples were firstly visualized in Opus 7.5 software (Bruker) and those exhibiting a low signal to noise ratio were eliminated. For data processing, The Unscrambler X software (CAMO Software, Oslo, Norway) was used. Second derivative of the spectra was calculated using a Savitsky-Golay algorithm with an eleven-point smoothing filter and a polynomial order of 5 to eliminate the baseline contribution and enhance narrow bands. The spectroscopic infrared (IR) peaks at different wavenumbers are well stablished to correspond with biomolecules and functional organic groups (**Fig. 9**). In this study, we focused the analysis on the determination of the amount of lipid respect to the total amount of protein in the tissue using the ratios for Amide I/CH_2 ($A_{1635+1656}/A_{2921}$). Second, the lipid oxidation was determined by calculating the increase of the carbonyl group (C=O) using the ratio A_{1741}/A_{2921} and the increase of the unsaturated olefinic group (C=CH) calculating the ratio A_{3014}/A_{2921}. Third, the α- and β- secondary structures of proteins determined by $A_{1656}/A_{1635+1656}$ and $A_{1635}/A_{1635+1656}$, respectively. Forth, the unordered structures determined by $A_{1644}/A_{1635+1656}$, proteins with intermolecular β-sheet and antiparallel β-sheet structures determined by $A_{1625}/A_{1635+1656}$ and $A_{1695}/A_{1635+1656}$, respectively (Rice-Evans, 1994; Benseny-Cases et al., 2018; Sanchez-Molina et al., 2020).

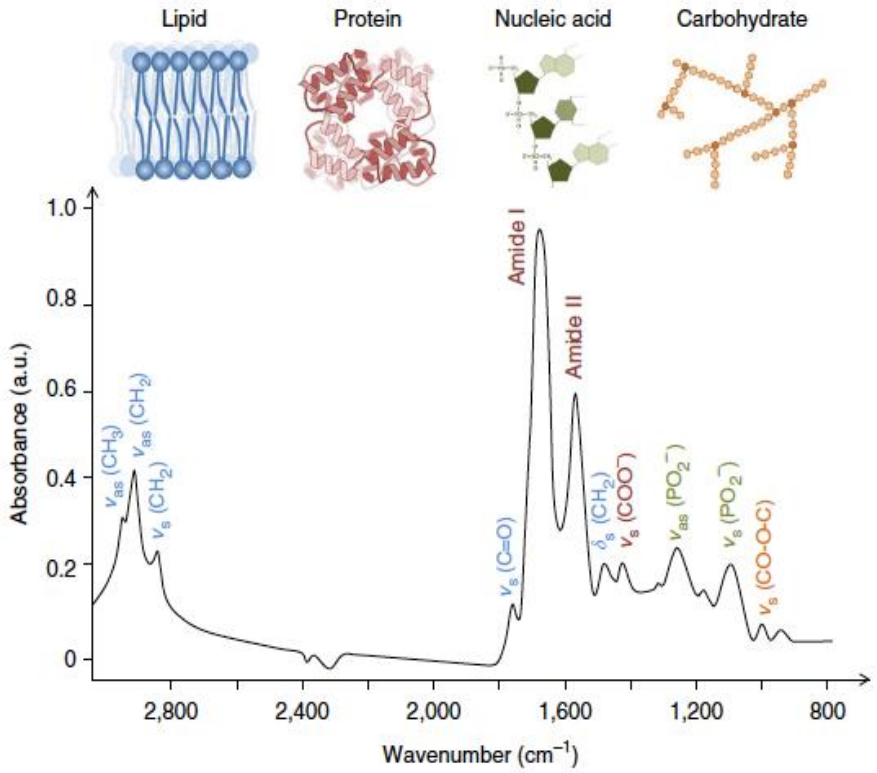


Figure 9. Representative infrared biochemical profile of biological tissue. Peaks represent chemical functional groups of lipids, proteins, nucleic acids and carbohydrates (Baker et al., 2014).

11. Immunohistochemistry (Immunofluorescence and DAB staining)

Mice were perfused transcardially with TBS and 4% phosphate-buffered paraformaldehyde. After 2h immersion in 4% PFA and 2 washes with TBS, brains were embedded in paraffin. Sagittal brain sections (5-10 µm) cut in the microtome were deparaffinized in xylene, rehydrated and microwave heated for 10 min in citrate buffer (10 mM, pH=6.0). Sections were incubated overnight at 4ºC with primary antibody listed on table 4. Immunofluorescence slides were incubated with AlexaFluor-488/594/658-conjugated goat IgGs (1:400) during 90 min followed by 30 min Hoechst (1:10,000; Thermo Fisher Scientific) incubation. For DAB staining, sections were incubated with a biotin conjugated anti-mouse and anti-rabbit secondary antibody (1:200, Vector laboratories) during 30 min at RT. Slides were incubated with ABC kit (Vector laboratories) during 30 min at RT. Then, slides were incubated with DAB peroxidase substrate kit (Vector laboratories) during a specific time according to the primary antibody. Slides were immersed in Harris Hematoxylin (Sigma) to stain cell nuclei and dehydrated in a graded series of ethanol and xylene. Finally, sections were coverslipped using Fluoroprep mounting media for immunofluorescence (BioMérieux) or DPX Mountant for histology (Sigma). Confocal images (40x; zoom 1 or 2) were obtained with a Zeiss Axio Examiner D1 LSM700 laser scanning microscope (Carl

Zeiss Microcopy, Jena, Germany) and immunoperoxidase staining images were captured using a Nikon Eclipse 80i microscope. All images were analyzed with ImageJ software.

Antibody	Specificity	Source	Cat number	Host	Dilution
CP13	Phosphorylated Ser202 tau	P. Davies	-	Mouse	1:50
GFAP	Glial fibrillary acid protein	Dako	Z0334	Rabbit	1:500
Iba1	Ionized calcium binding adaptor molecule 1	Wako	019-19741	Rabbit	1:500
MC1	Conformational tau (aa 312-322)	P. Davies	-	Mouse	1:50
NeuN	Neuronal marker	Chemicon	#ABN78	Rabbit	1:1000
NF-L	Neurofilament light chain	Millipore	#9586	Rabbit	1:800
Olig2	Oligodendrocyte transcription factor 2	Millipore	#AB9610	Rabbit	1:500
PG5	Phosphorylated Ser409 tau	P. Davies	-	Mouse	1:100
PHF-1	Phosphorylated Ser396/404 tau	P. Davies	-	Mouse	1:200
SMI312	Neurofilament heavy and medium chain	Biolegend	#837904	Mouse	1:500

Table 4. Primary antibodies used for immunohistochemistry.

12. Nuclear and synaptosome purification

Two hippocampi were lysate in 4 ml of ice-cold solution A (5mM HEPES pH 7.4, 0.32M sucrose, 1mM NAHCO5, 1mM MgCl2, 0.5mM CaCl2) on ice. 50 µl were kept as the lysate fraction. Then, lysate sample were centrifuged in 15 ml falcon tubes at 1400g for 10 min (3600rpm, Eppendorf 5810R centrifuge). Supernatant was kept and pellet was resuspended and homogenized with 2 ml of solution A. After that, samples were centrifuged at 710g for 10 min (1900rpm, Eppendorf 5417R). Supernatant was kept and combined with the previous one for synaptosomal purification while, pellet was resuspended with 400 µl of NHB (250mM sucrose, 10mM TEA, 10nM acetic acid, 1mM MgCl2, 0.5mM ZnCl2) and homogenized. 1.45 ml of 2.2M sucrose were added to the

sample and homogenized. Sucrose gradient was prepared at the moment. From bottom to top: 2ml 2.2M sucrose, 2ml 2M sucrose, 2 ml 1.8M sucrose, 2 ml sample and 2 ml 1.4 sucrose. Samples were centrifuged at 75000g (30000rpm) in SW41 rotor for 35 min at 4ºC. Nuclear pellet was resuspended in 75 µl of lysis buffer (150mM NaCl, 25mM Tris HCl, 1% NP40, 10% glycerol). Combined supernatant (6 ml) was centrifuged at 13800 g for 10 min (14000rpm, Beckman Coulter) in 70Ti rotor. Pellet was resuspended and homogenized in 3 ml of solution B (6mM Tris HCl pH 8.0, 0.32M sucrose, 1mM NAHCO3). Samples were added to a sucrose gradient prepared at the moment (from bottom to top: 1.2M sucrose, 1M sucrose, 0.85M sucrose, 2,5 ml sample) and centrifuged at 82500 g for 2h at 4ºC (26000rpm, Beckman Coulter) SW41Ti rotor. The synaptosome fraction (1 ml) was taken form interface between 1M and 1.2M sucrose gradients. 200 µl of synaptosomal fraction was kept. 0.5 ml of solution C was added to the rest of the sample (12mM Tris HCl pH 8, 1% triton X-100) and samples were incubated on ice for 15 min. Then, samples were centrifuged at 32800g for 60 min at 4ºC (21000rpm, Sorvall M120SE mini-ultracentrifuge). The supernatant was kept as the presynaptic fraction and the pellet was resuspended in 75 µl of solution D (40mM Tris HCl pH 8, 1% NP40) and kept as the postsynaptic fraction. All the buffers contained protease and phosphatase inhibitors (Roche), Na3VO4 and NaF. Sucrose gradients were prepared in gradient buffer (10mM TEA, 10mM acetic acid, 1mM MgCl2, pH 7.4).

13. Statistical analysis

Statistical analysis of behavioral, biochemical and pathological studies was performed using one or two-way ANOVA followed by Sidak's for same age comparison or Tukey's post hoc test for multiple comparisons and unpaired student's t-test was used when only a specific age was analyzed when the standard deviation was equal between groups. When standard deviation was significantly different between groups, the statistical analysis was performed using Brown-Forsythe and Welch's ANOVA test followed by Dunnett T3 post hoc tests. Values represent mean ± s.e.m. All the statistical analysis was carried out using Prism software (GraphPad, La Jolla, CA). Differences with $P < 0.05$ were considered significant.

VIII. Results

1. Loss of PS function causes age-dependent neurodegeneration and behavioral abnormalities

Previous studies have revealed that loss of neuronal PS function in mice caused progressive brain atrophy as assayed by Nissl staining (Saura et al., 2004). To confirm the effects of PS deficiency on brain degeneration we examined the brain, cortex and hippocampus of wild-type (WT) and PS cDKO mice at 2, 6, 9 and 12 months of age. We found an age-dependent decreased in total weight of brain (genotype effect: $P < 0.0001$, age effect: $P < 0.01$, genotype x age interaction effect: $P < 0.0001$), cortex (genotype effect: $P < 0.0001$, age effect: $P = 0.055$) and hippocampus (genotype effect: $P < 0.001$) of PS cDKO mice starting at 6 months of age **(Figs. 10A,B**). Brain degeneration is not present from the birth as there are no significant differences at 2 months. We performed a tail suspension test to analyze the clasping finding significant motor deficits in PS cDKO mice at 9 months of age. Furthermore, PS cDKO mice develop abnormal body posture at late stages (**Fig. 10C**). These results remark the importance of PS function in neuronal survival in the adult mouse brain.

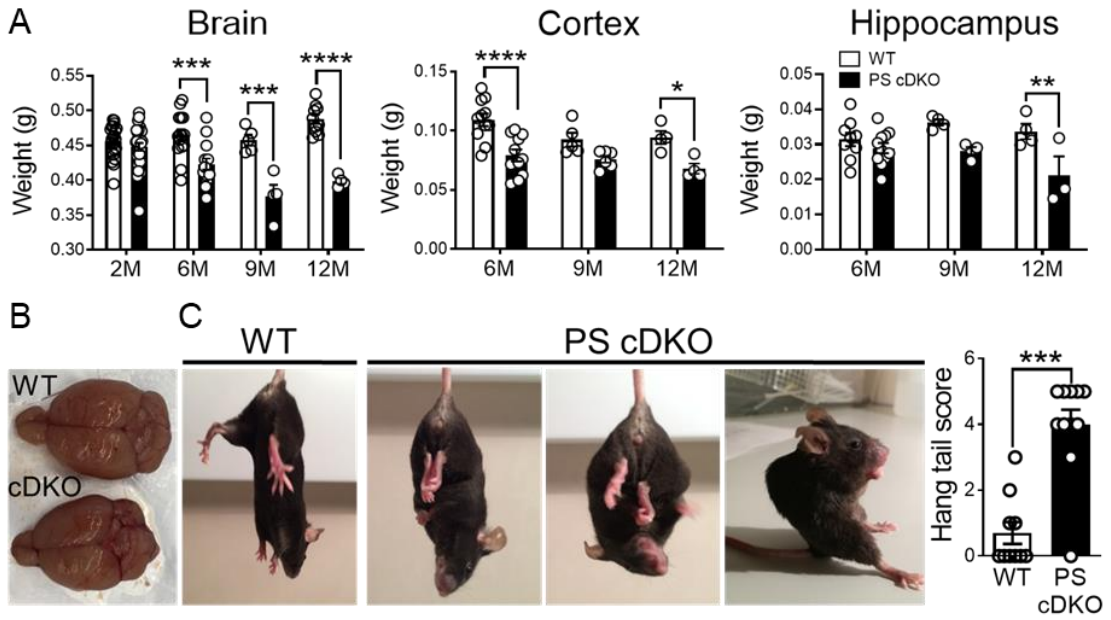


Figure 10. Analysis of brain degeneration and behavior in PS cDKO mice. (A) Age-dependent decrease in brain (left), cortex (middle) and hippocampus (right) weight in PS cDKO mice. **(B)** Brain atrophy in PS cDKO mice. (**C**) Motor and postural alterations in PS cDKO mice (left). Tail suspension test (right) shows severe clasping behavior in PS cDKO mice starting at 9 months of age. Values represent mean ± s.e.m. (n = 4-30 mice/group). Statistical analysis was determined by two-way ANOVA followed by Sidak's post hoc tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

2. Age-dependent tau phosphorylation in the cortex and hippocampus of PS cDKO mice

To investigate whether neurodegeneration was accompanied by tau pathology in PS cDKO mice, we analyzed levels of phosphorylated tau in the cortex and hippocampus of WT and PS cDKO mice at 6, 9 and 12 months of age. We performed Western blot analyses using specific anti-total and phosphorylated (p) tau antibodies and found a significant age-dependent increase in pSer202 (CP13, genotype effect: $P < 0.01$), pSer202/pThr205 (AT8, genotype effect: $P < 0.01$, age effect: $P < 0.05$) and pSer396/404 (PHF-1, genotype effect: $P < 0.05$) but no changes in total tau levels (Tau17025) in the cortex of PS cDKO mice. However, low migrating hyperphosphorylated band was significantly increased in PS cDKO mice at 12 months suggesting increased hyperphosphorylation (**Fig. 11**). In the hippocampus, pSer202 (CP13) and pSer202/pThr205 (AT8) tau levels tend to increase at 12 months and pSer396/404 (PHF-1) were significantly increased in PS cDKO mice (genotype effect: $P < 0.05$) without changes in total tau levels (Tau17025).

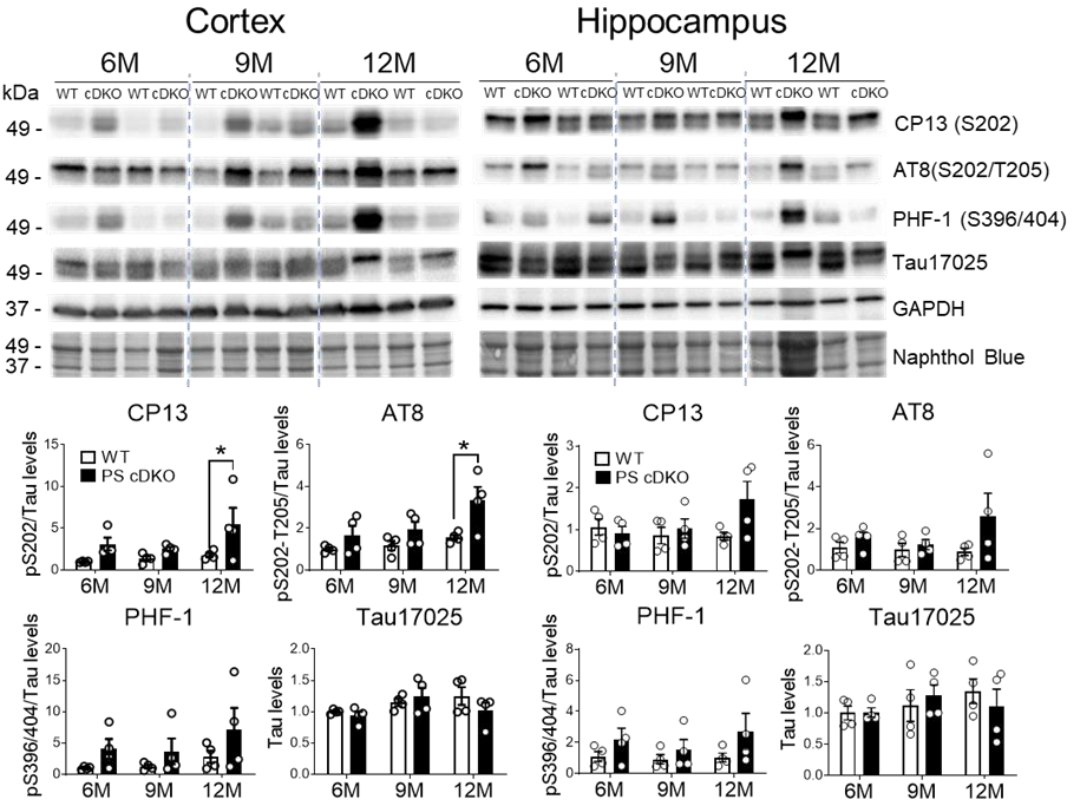
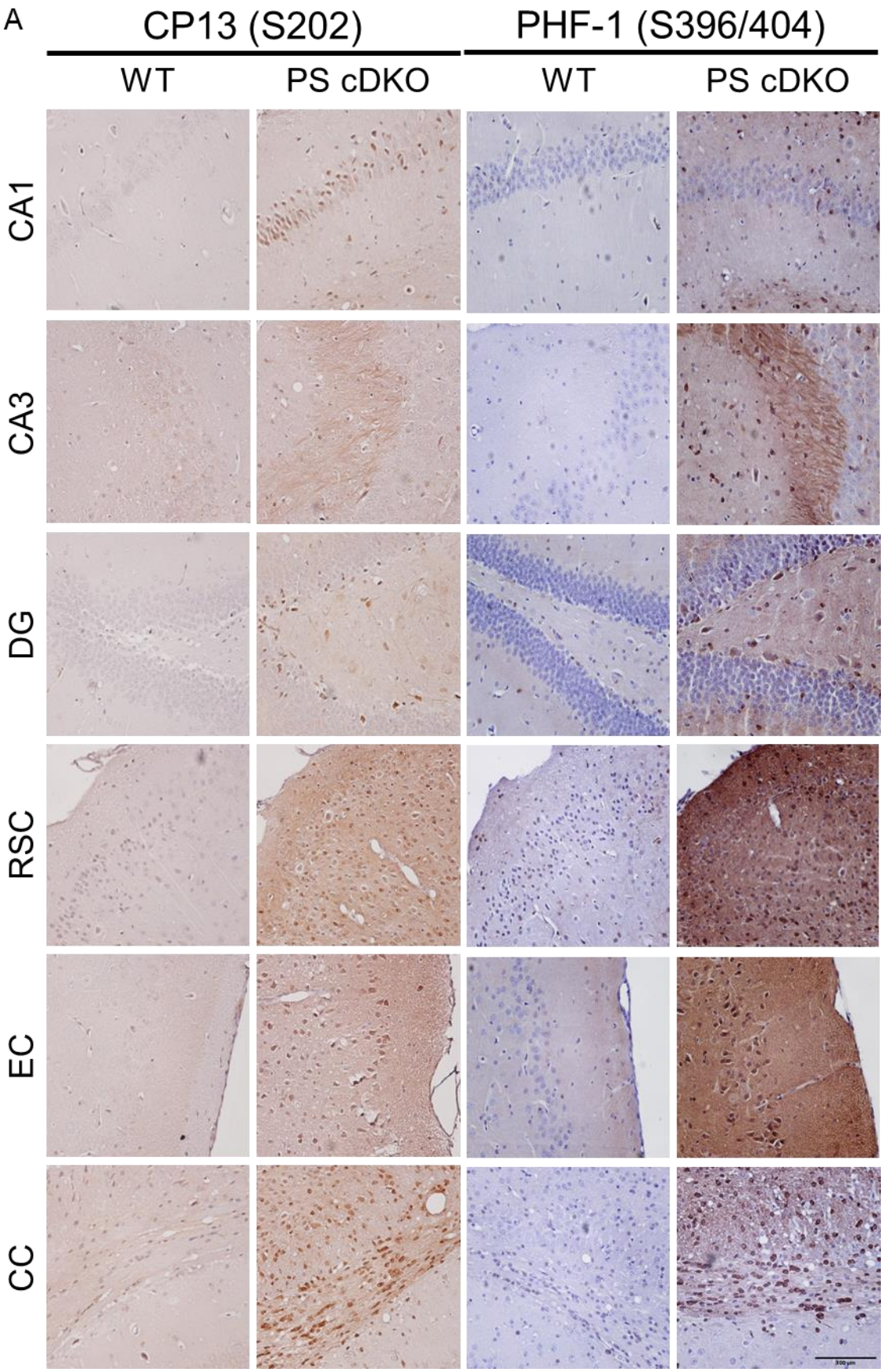


Figure 11. Biochemical analysis of total and phosphorylated tau in the cortex and hippocampus of PS cDKO mice. Western blot images (top) and quantitative analysis (bottom) of phosphorylated tau (CP13, AT8 and PHF-1) and total tau (tau 17025) in cortex (left) and hippocampus (right) of WT and PS cDKO mice. Values represent mean fold ± s.e.m. (n = 4 mice/group). Phosphorylated tau levels were normalized to total tau levels and total tau to GAPDH. Statistical analysis was determined by two-way ANOVA followed by Sidak's post hoc tests. *$P < 0.05$.

To confirm this result, we performed histological analysis of 12 month-old WT and PS cDKO mice using CP13 and PHF1 antibodies. Increased number of tau-positive cells and total phosphorylated tau staining was found in the retrosplenial (RSC) and entorhinal cortex (EC), corpus callosum (CC) and CA1, CA3 and dentate gyrus (DG) regions of PS cDKO hippocampus ($P < 0.05$; **Fig. 12**). These results suggest that loss of PS function in neurons results in tau pathology and brain degeneration.

3. Partial loss of PS does not affect tau phosphorylation

To examine whether tau pathology was dependent on presenilin gene dose, we performed biochemical analysis of cortical and hippocampal lysates of 9 month-old WT, $PS2^{-/-}$, PS1 cKO and PS cDKO mice. We found increased phosphorylated tau levels in the cortex of PS cDKO but not in $PS2^{-/-}$ or PS1 cKO mice. In the hippocampus, we found increased phosphorylated tau at Ser202 (CP13), Ser202/Thr205 (AT8) and Ser396/404 (PHF-1, genotype effect: $P < 0.05$) and no changes in total tau in PS cDKO mice but not $PS2^{-/-}$ or PS1 cKO mice (**Fig. 13**). This result suggests that lack of a single PS gene does not significantly affect total or phosphorylated mouse tau in the cortex and hippocampus of adult mice (**Fig. 13**).


A
CP13 (S202)
PHF-1 (S396/404)
WT
PS cDKO
WT
PS cDKO
CA1
CA3
DG
RSC
EC
CC

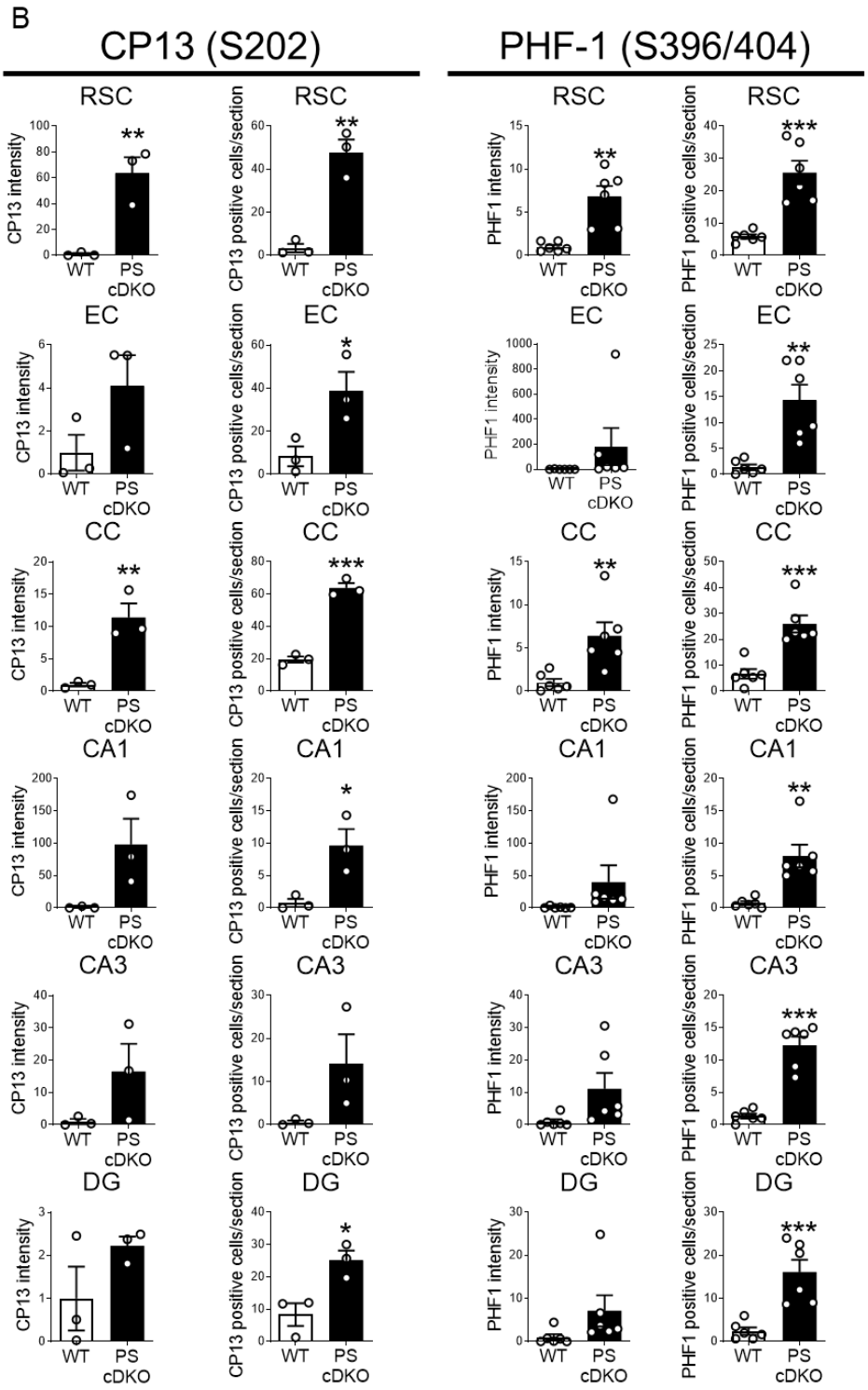


Figure 12. Histological analysis of phosphorylated tau in 12 month-old PS cDKO mice. (A) Representative images of immunohistological analysis of phosphorylated Ser202 (CP13, left) and Ser396/404 (PHF-1, right) tau in 12 months-old control (WT) and PS cDKO mice. Scale bar: 100 µm. **(B)** Quantitative analysis of total intensity and number of positive cells of CP13 (left) and PHF-1 (right) immunohistochemistry. Values represent mean ± s.e.m. (n = 3 slices/mice, 3-6 mice/group). Statistical analysis was determined by unpaired student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

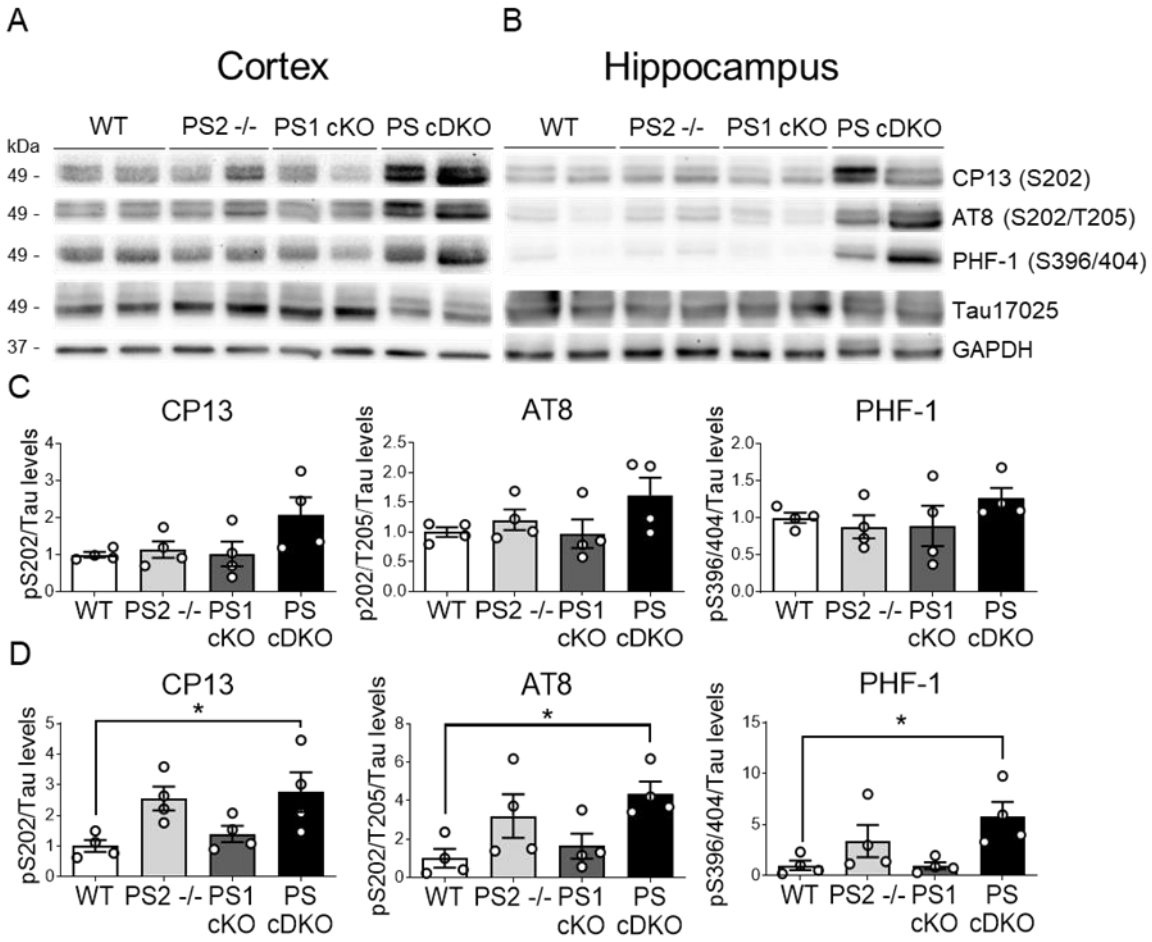


Figure 13. Biochemical analysis of phosphorylated tau in PS2$^{-/-}$, PS1 cKO and PS cDKO mice. Lysates of cortex (**A**) and hippocampus (**B**) of WT, PS2$^{-/-}$, PS1 cKO and PS cDKO mice at 9 months were analyzed by Western blotting using anti-phosphorylated tau (CP13, AT8, PHF-1) and total tau (Tau17025) antibodies. Quantitative analysis of phosphorylated tau in cortex (**C**) and hippocampus (**D**). Phosphorylated tau levels were normalized to total tau. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05.

4. Neuronal PS deficiency causes accumulation of phosphorylated tau in neurons and glial cells

To study the specific cell types affected by tau pathology, we performed double immunostaining with CP13 antibody and cell specific markers. We used NeuN as marker of neurons, Iba-1 as a microglial marker, Olig2 as an oligodendrocyte marker and GFAP as an astrocyte marker. Confocal imaging analyses of immunofluorescence images revealed colocalization of phosphorylated tau (CP13) with neuronal (NeuN), microglial (Iba1), oligodendroglial (Olig2) and astroglial markers (GFAP). Phosphorylated tau was accumulated in ≈60-70% of neurons, ≈40% of oligodendrocytes, ≈15% of microglia and ≈6-8% of astrocytes in PS cDKO mice (**Fig. 14**). These results demonstrate that loss of neuronal PS leads to phosphorylated tau accumulation in neurons and glial cells.

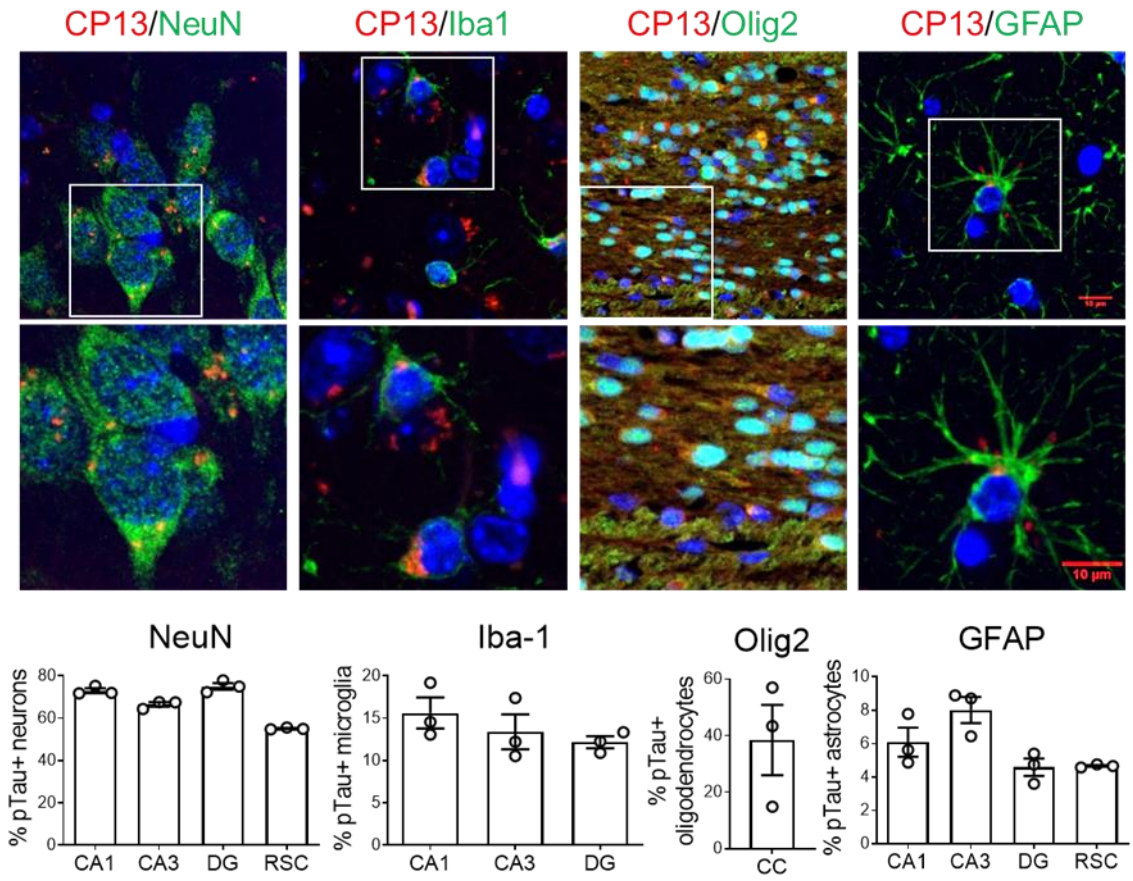


Figure 14. Accumulation of phosphorylated tau in neurons and glial cells in PS cDKO mice. Top and middle: Representative immunohistofluorescence images showing the presence of phosphorylated tau (CP13: pSer202, red) in hippocampal neurons (NeuN, green), microglia (Iba1, green), oligodendrocytes (Olig2, green; corpus callosum) and astrocytes (GFAP) in PS cDKO mice at 12 months. Scale bar: 10 µm. Bottom: Quantification of percentage of cells per section containing phosphorylated tau (n = 3 mice, n = 5 sections/mouse).

5. P301S tau transgenic mice show increased total and phosphorylated tau in the cortex and hippocampus

To investigate how PS/γ-secretase contributes to human tau pathology we next characterized tau P301S transgenic (PS19; Tau Tg) mice that overexpress the human tau protein under the prion protein promoter (MoPrP, Yoshiyama et al., 2007). We performed biochemical analysis in the cortex and the hippocampus of WT and Tau transgenic mice at 2 months of age using specific phosphorylated and total tau antibodies. Levels of total tau (TG5; 2.5-fold, $P < 0.001$) and phosphorylated tau at Ser202 (CP13, $P < 0.01$), Ser396/404 (PHF-1, $P < 0.001$) and Ser202/Thr205 (AT8) (2-fold) were significantly increased in the cortex of Tau transgenic mice. In the hippocampus, total tau (2.5-fold, $P < 0.001$) and phosphorylated tau by CP13 (5-fold, $P < 0.001$), PHF1 (1.5-fold, $P < 0.05$) and AT8 (7-fold, $P < 0.01$) were significantly increased (**Fig. 15**).

6. Loss of PS function exacerbates tau phosphorylation and aggregation in hTau transgenic mice

We next examined the effect of PS on human tau pathology by crossing PS1 cKO and PS cDKO mice with tau P301S transgenic mice to obtain WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau and PS cDKO;Tau littermates. Interestingly, we found an increase of phosphorylated tau in 6 month-old PS1 cKO;Tau and PS cDKO;Tau mice compared to tau transgenic group in both cortex (CP13: tau effect: $P < 0.0001$, PS effect: $P < 0.05$; AT8: tau effect: $P < 0.001$, PS effect: $P < 0.05$; PHF-1: tau effect: $P < 0.01$, PS effect: $P < 0.01$) and hippocampus (CP13: tau effect: $P < 0.0001$, PS effect $P < 0.01$, tau x PS interaction effect: $P < 0.01$; AT8: tau effect: $P < 0.0001$, PS effect: $P < 0.01$, tau x PS interaction effect: $P < 0.01$; PHF-1: tau effect: $P < 0.0001$). By contrast, total tau levels (TG5) were similarly increased (≈10-fold) in all tau transgenic groups independently of PS expression (**Fig. 16**). This result was confirmed by immunohistochemistry. There was no positive phosphorylated tau antibody (CP13) in the control mice. Tau and PS1 cKO;Tau mice show positive staining in neuronal projections of the hilus. PS1 cKO;Tau and PS cDKO;Tau mice present positive somatic staining in the hippocampus (CA3, DG) and in the cortex (RSC) suggesting anticipated tau pathology (Zempel and Mandelkow, 2014). Furthermore, amygdala images also showed somatic staining in Tau and, especially, PS1 cKO;Tau and PS cDKO;Tau mice. CP13 staining revealed an increase of pSer202 tau in the hippocampus (CA3, genotype effect: $P < 0.05$; DG, genotype effect: $P < 0.01$; CA1, genotype effect: $P < 0.05$ data not shown), cortex (RSC, genotype effect: $P < 0.01$; EC, genotype effect: $P < 0.05$ data not shown) and amygdala (genotype effect: $P < 0.01$) of PS1 cKO;Tau and PS cDKO;Tau mice (**Fig. 17**).

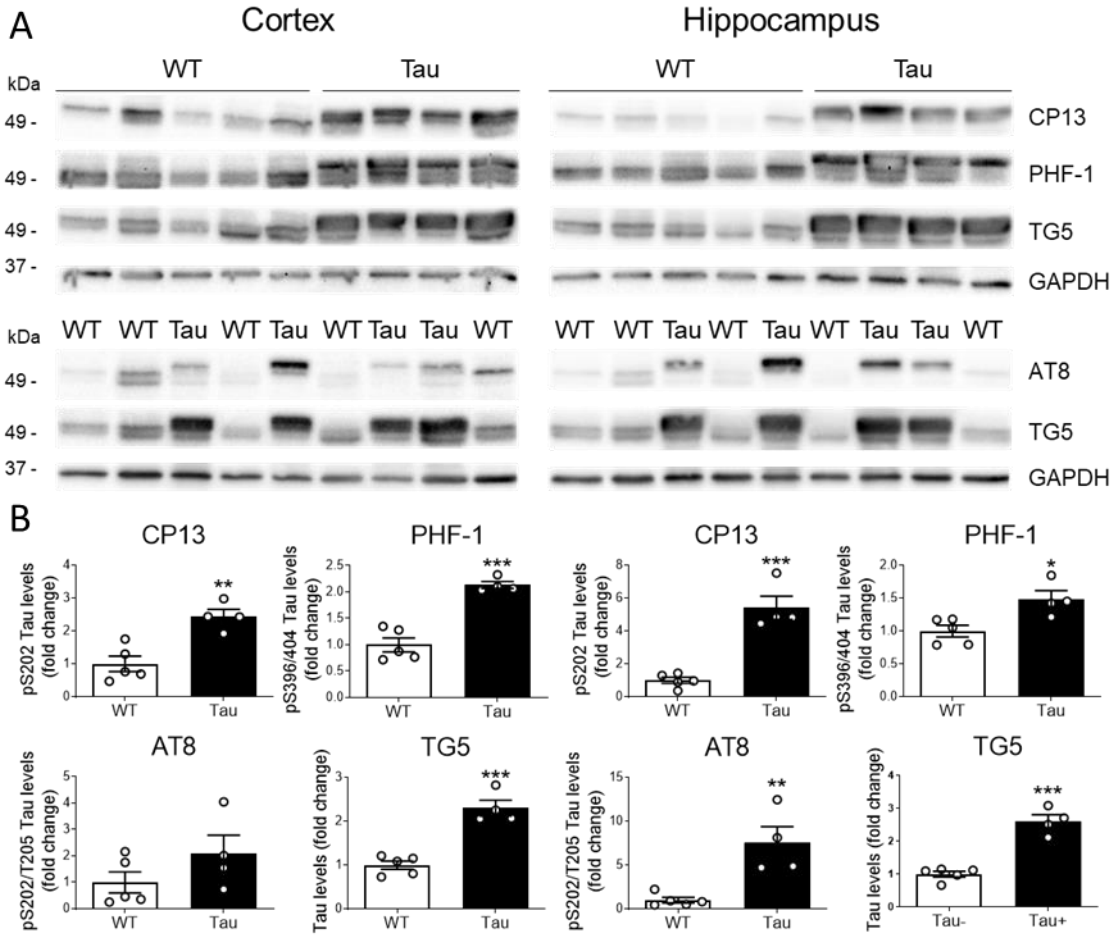


Figure 15. Increased phosphorylated and total tau in P301S tau transgenic mice. Cortical (left) and hippocampal (right) lysates of control (WT) and P301S tau transgenic mice (tau) of 2 months were analyzed by Western blotting. (Top) Images of phosphorylated (CP13, PHF-1, AT8) and total tau (TG5) in the cortex (left) and hippocampus (right) are shown. (Bottom) Quantitative analysis of phosphorylated and total tau levels in the cortex (left) and hippocampus (right) of 2 month-old WT and Tau mice. Values represent mean fold ± s.e.m. (n = 4-5 mice/group). Phosphorylated and total protein levels were normalized to GAPDH levels. Statistical analysis was determined by unpaired student's t-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.0001.

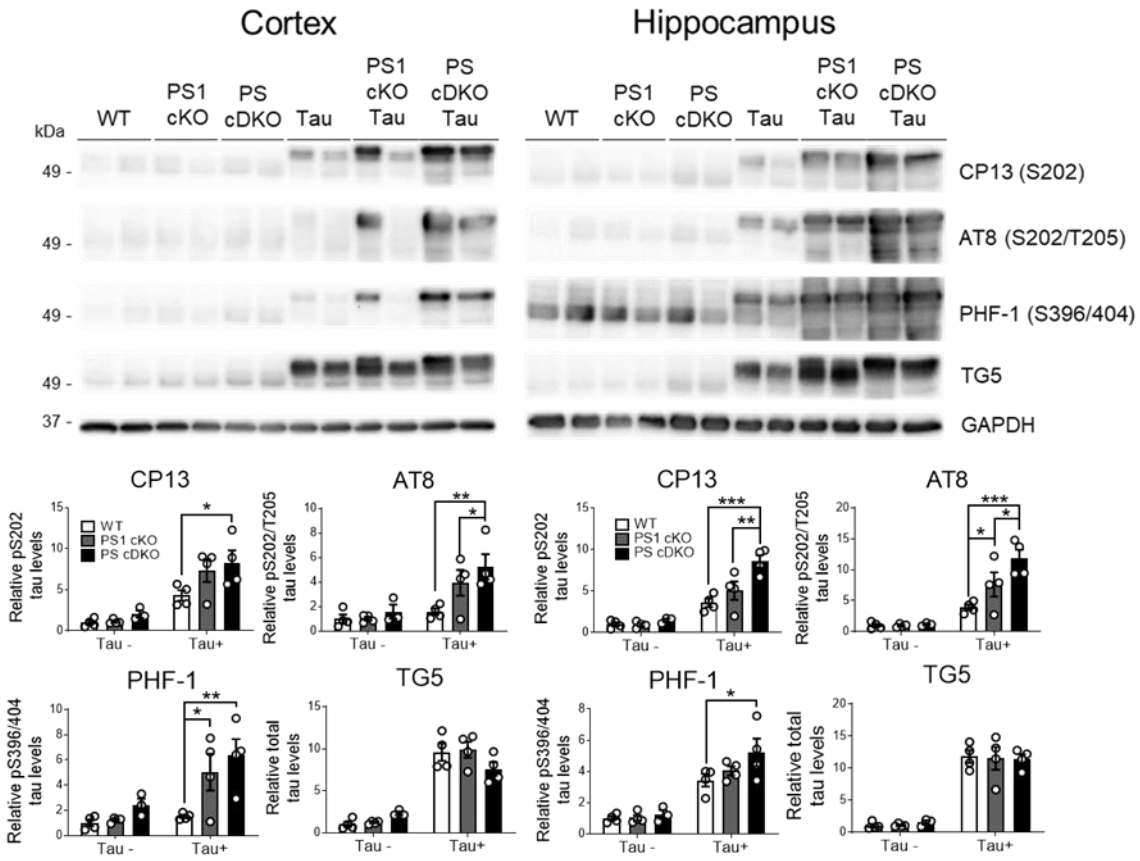


Figure 16. Increased phosphorylated tau in human tau Tg mice lacking PS. (Top) Western blot images of phosphorylated (CP13, PHF1, AT8) and total tau (TG5) in the cortex (left) and hippocampus (right) of non-transgenic mice (Tau-): control (WT), PS1 cKO and PS cDKO, tau; and tau transgenic mice (Tau+): control (WT), PS1 cKO;Tau and PS cDKO;Tau of 6 months. (Bottom) Quantitative analysis of phosphorylated and total tau levels in the cortex (left) and hippocampus (right). Phosphorylated and total protein levels were normalized to GAPDH levels. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

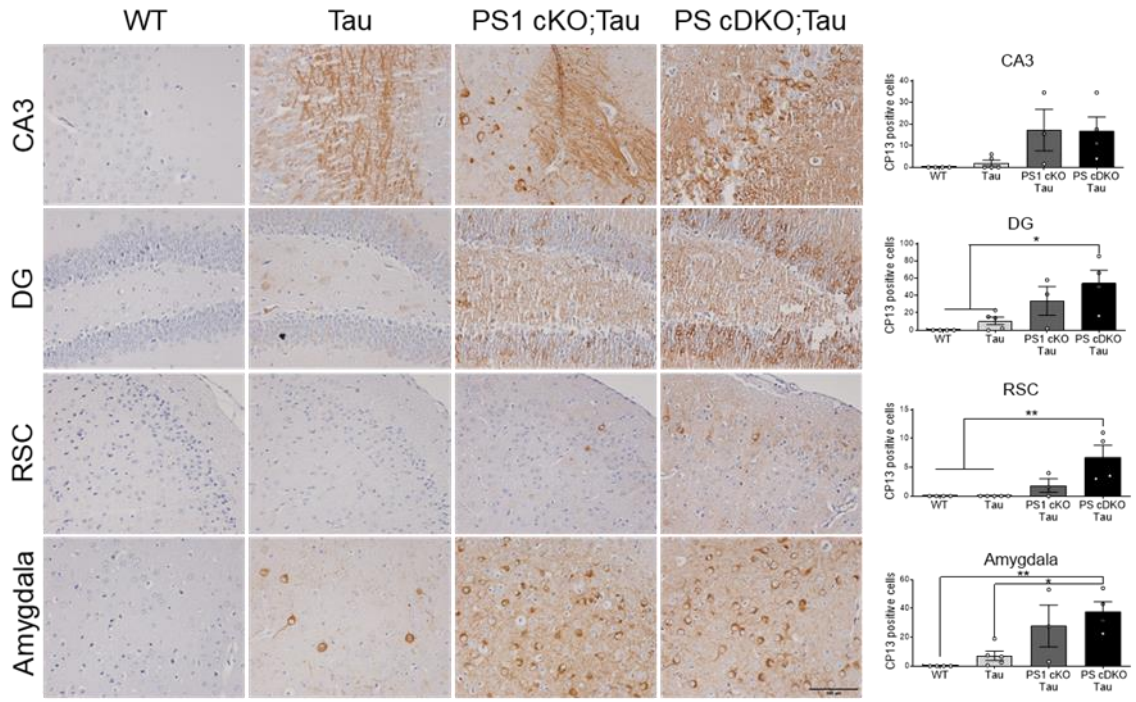


Figure 17. Increased pSer202 tau in PS1 cKO;Tau and PS cDKO;Tau mice. (Left) Representative immunohistochemical images of phosphorylated tau (pSer202, CP13) in 6 month-old non- and tau transgenic mice. Scale bar: 100 µm. (Right) Quantitative analysis of number of CP13 positive cells. Values represent mean ± s.e.m. (n = 3 slices per mice, 4-5 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01.

Interestingly, PG5 antibody, which recognizes protein kinase A (PKA)-dependent tau phosphorylation at Ser409 present in PHFs (Vingtdeux et al 2011), detected a low-migrating prominent band significantly increased in the hippocampus of PS cDKO;Tau mice (tau effect: $P < 0.001$, PS effect: $P < 0.0001$, tau x PS interaction effect: $P < 0.01$) (**Fig. 18**). Indeed, immunohistochemical analyses showed significant increase of PG5-positive cells in the CA1 (genotype effect: $P < 0.05$), CA3 (genotype effect: $P < 0.01$) and DG (genotype effect: $P < 0.01$) of the hippocampus, in the RSC (genotype effect: $P < 0.05$) and EC (genotype effect: $P < 0.0001$) and in the amygdala (genotype effect: $P < 0.0001$) in PS1 cKO;Tau and PS cDKO;Tau mice (**Fig. 19**).

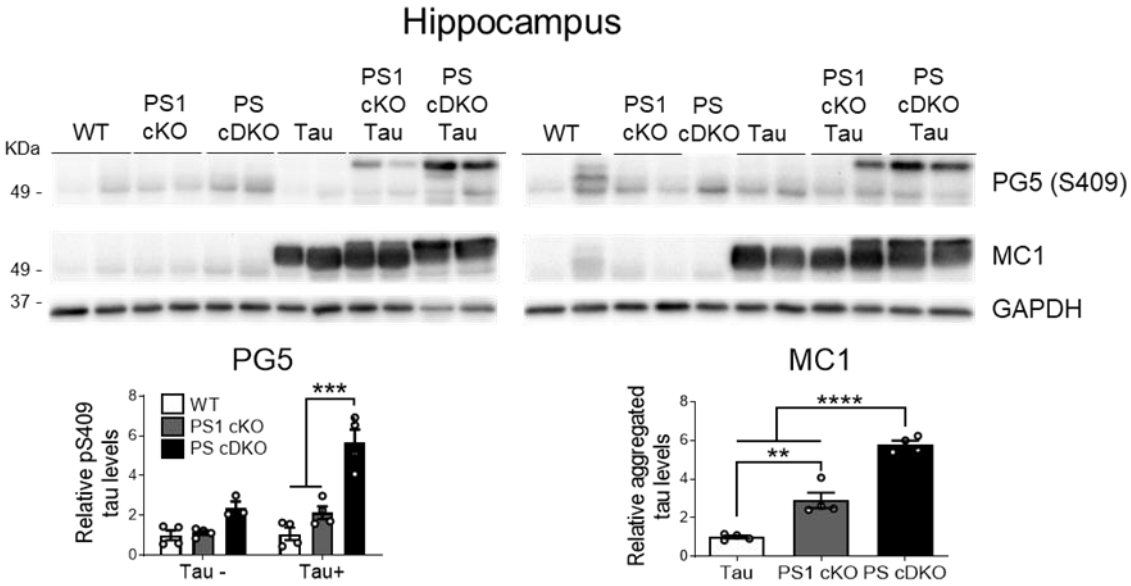


Figure 18. PS deficiency increases pSer409 and aggregated (MC1) tau in tau transgenic mice. Hippocampal lysates of non-transgenic mice (Tau-): control (WT), PS1 cKO and PS cDKO, tau; and tau transgenic mice (Tau+): Tau, PS1 cKO;Tau and PS cDKO;Tau of 6 months. Western blot images of pSer409 (PG5) and aggregated tau (MC1) in the hippocampus (top). Quantitative analysis of phosphorylated and aggregated tau levels in hippocampal lysates (bottom). Values represent mean fold ± s.e.m. (n = 4 mice/group). Protein levels were normalized to GAPDH levels. Statistical analysis was determined by one- or two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

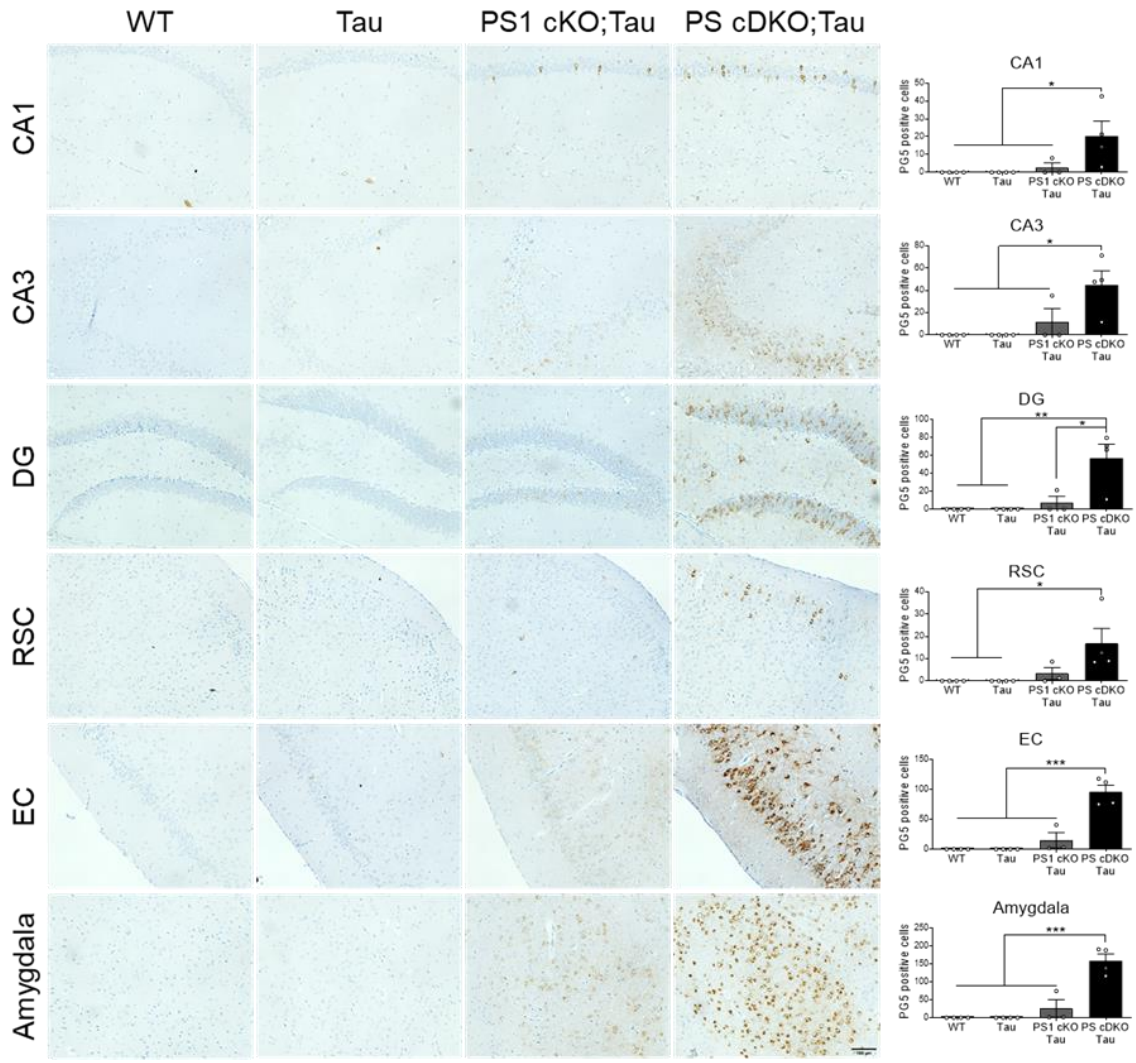


Figure 19. Loss of PS function increases pathological pSer404 tau in human tau Tg mice. Representative immunohistochemical images of pSer409 (PG5) tau in 6 months-old non- and tau transgenic mice. Scale bar: 100 µm (left). Quantitative analysis of number of PG5 positive cells in RSC, EC and amygdala of control (WT), Tau, PS1 cKO;Tau and PS cDKO;Tau mice (right). Values represent mean PG5+ cells/section ± s.e.m. (n = 3 slices per mice, 4 mice/group). Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ****P* < 0.001.

To investigate the role of PS on tau aggregation, we next used MC1, a specific antibody against conformational pathological tau (aa 312-322). Biochemical analysis of cortex (data not shown) and hippocampus showed a significant increase of aggregated tau in PS1 cKO;Tau and PS cDKO;Tau mice compared to tau mice (genotype effect: *P* < 0.0001; **Fig. 18**). Immunohistochemical analysis showed that PS deficiency significantly enhanced aggregated tau in neurons of different brain regions including CA3 (genotype effect: *P* < 0.001), DG (genotype effect: *P* < 0.05, data not shown), RSC (genotype effect: *P* < 0.01, data not shown), EC (genotype effect: *P* < 0.0001) and amygdala (genotype effect: *P* < 0.0001, **Fig. 20**). Indeed, both biochemical and immunohistochemical analyses revealed that MC1 staining was dependent on PS

dosage as revealed by statistical differences on MC1 staining between PS1 cKO;Tau and PS cDKO;Tau mice in CA3 (P <0.05), RSC (P < 0.05), EC (P < 0.001) and amygdala (P < 0.01).

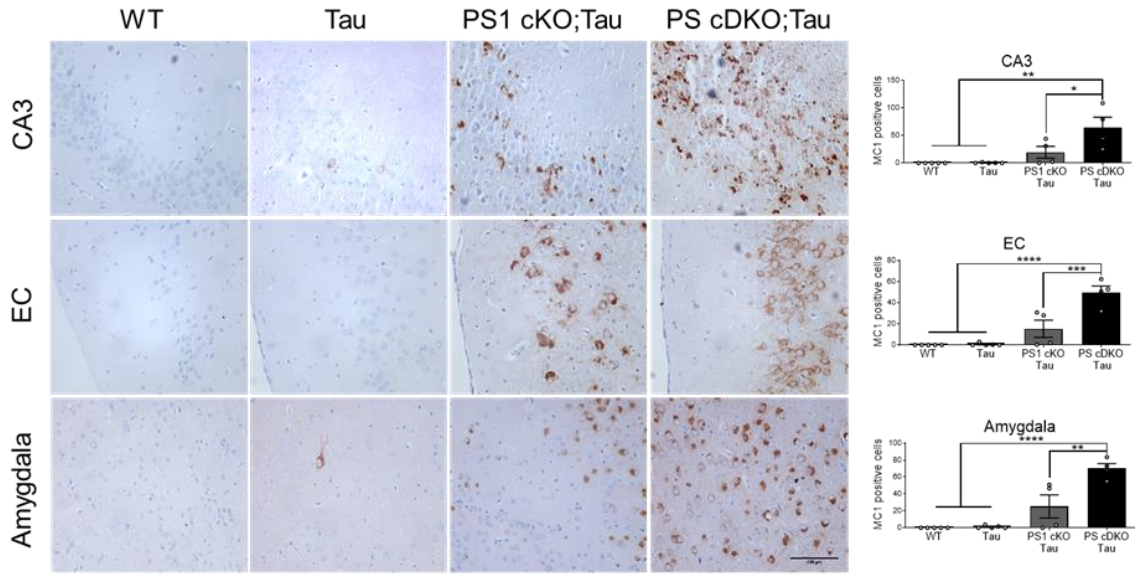


Figure 20. PS deficiency increases tau aggregation in tau transgenic mice. Representative immunohistochemical images of aggregated tau (MC1) in 6 month-old non- and tau transgenic mice. Scale bar: 100 µm (left). Quantitative analysis of number of MC1 positive cells per section in CA3, EC and amygdala of control (WT), Tau, PS1 cKO;Tau and PS cDKO;Tau mice (right). Values represent mean MC1 positive cells/section ± s.e.m. (n = 3 slices per mice, 4 mice/group). Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

To confirm the above results, we next performed Gallyas staining that detects neurofibrillary tangles in AD brain (Kuninaka et al., 2015). Histological analysis showed absence of staining in non-transgenic and Tau mice. By contrast, Gallyas staining revealed increased conformational aggregated tau in CA3 (genotype effect: P < 0.05), EC (genotype effect: P < 0.01) and amygdala (genotype effect: P < 0.05) of both PS1 cKO;Tau and PS cDKO;Tau mice at 6 months (**Fig. 21**). These results indicate that partial and total loss of PS leads to pathological tau aggregation.

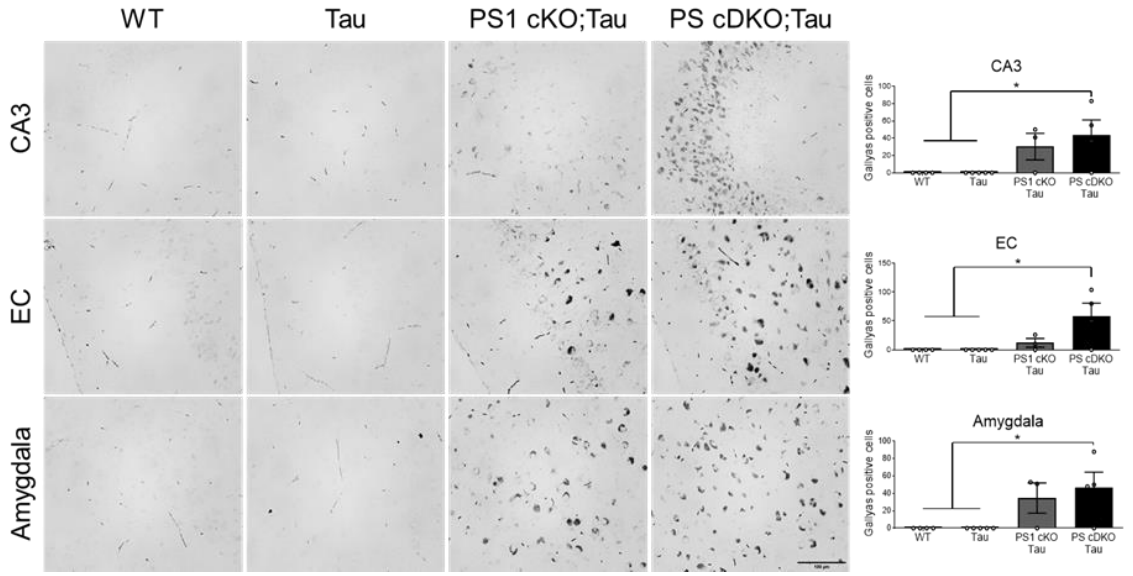


Figure 21. Gallyas staining reveals aggregated tau in tau mice lacking PS1 and both PS. Representative images (left) and quantitative analysis (right) of Gallyas stained sections in 6 month-old control (WT), tau, PS1 cKO;Tau and PS cDKO;Tau transgenic mice. Scale bar: 100 µm. Values represent mean of Gallyas-positive cells/section ± s.e.m. (n = 3 slices per mice, 4 mice/group). Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05.

7. Synchrotron based-µFTIR analysis shows increased protein aggregation pattern in PS cDKO mice

To analyze the aggregated protein pattern of tau in depth and the biochemical changes caused by loss of PS function, we performed synchrotron-based Fourier transform infrared microspectroscopy (µFTIR) analysis in histological slices of 6 month-old WT, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. First, we focused on the granular layer of CA3 and the hilus of the hippocampus. Unexpectedly, similar biochemical profiles in the lipid oxidation (C=O and C=CH), protein/lipid ratio and physiological protein structures (α-helix and β-sheet) were found among groups in each area, except for a tendency to decrease in lipid oxidation levels ($C=O/CH_2$ and $C=CH/CH_2$) and to increase in antiparallel β-sheet structures in the granular layer of PS cDKO;Tau mice (**Fig. 22**).

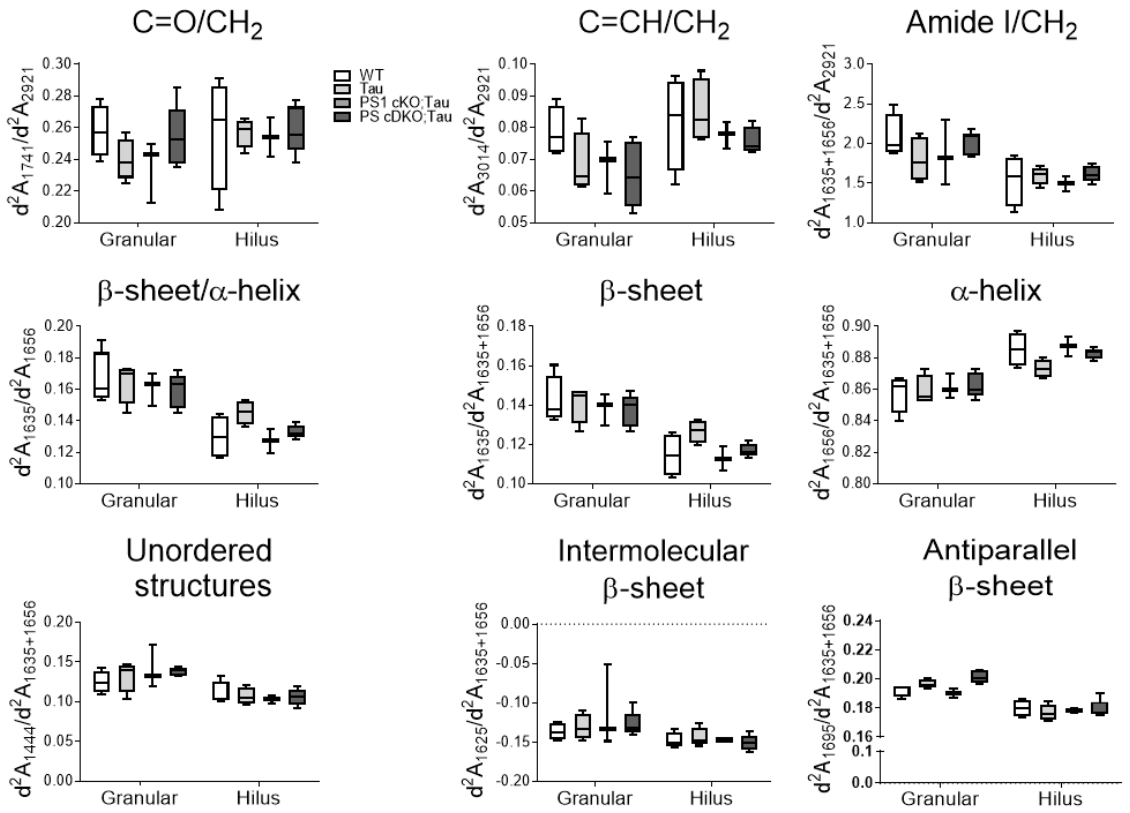


Figure 22. Brain regional differences in protein profiles measured by synchrotron-µFTIR. Synchrotron-based µFTIR analysis of the hippocampal CA3 granular layer and hilus of WT, Tau, PS1 cKO;Tau and PS cDKO;Tau mice at 6 months of age. Lipid oxidation was measured by $C=O/CH_2$ (A_{1741}/A_{2921}) and $C=CH/CH_2$ (A_{3014}/A_{2921}) ratios. Protein levels (Amide I, $A_{1656+1635}$), lipids levels (CH_2, A_{2921}), α-helix proteins (A_{1656}), β-sheet proteins (A_{1635}), unordered structures (A_{1444}), intermolecular β-sheet (A_{1625}) and antiparallel β-sheet (A_{1695}) were also analyzed. Values represent the minimum, the maximum and the median of the mean of 100-200 spectrums/mice and 4-5 mice/group. Statistical analysis was determined by one-way ANOVA followed by Sidak's post hoc tests.

To examine differential effects of PS on secondary protein structures in gray and white matters, we next performed *in situ* synchrotron infrared radiation analysis in brain sections of WT and PS cDKO;Tau mice RSC and the corpus callosum, respectively. Representative infrared spectra in the RSC and the CC of WT and PS cDKO;Tau mice is shown in **Figure 23A**. We found that, despite the different biochemical profile of the two studied brain regions such as increased lipid oxidation, protein/lipid ratio and β-sheet/α-helix structure proteins in the RSC compared to the CC (area effect: $P < 0.0001$), there were no major changes among genotypes ($P > 0.05$). However, we found increased intermolecular (genotype effect: $P < 0.05$, area effect: $P < 0.0001$) and antiparallel (genotype effect: $P < 0.0001$, area effect: $P < 0.0001$, genotype x area interaction effect: $P < 0.0001$) β-sheet structures in PS cDKO;Tau mice in the CC but not in RSC (**Fig. 23B**).

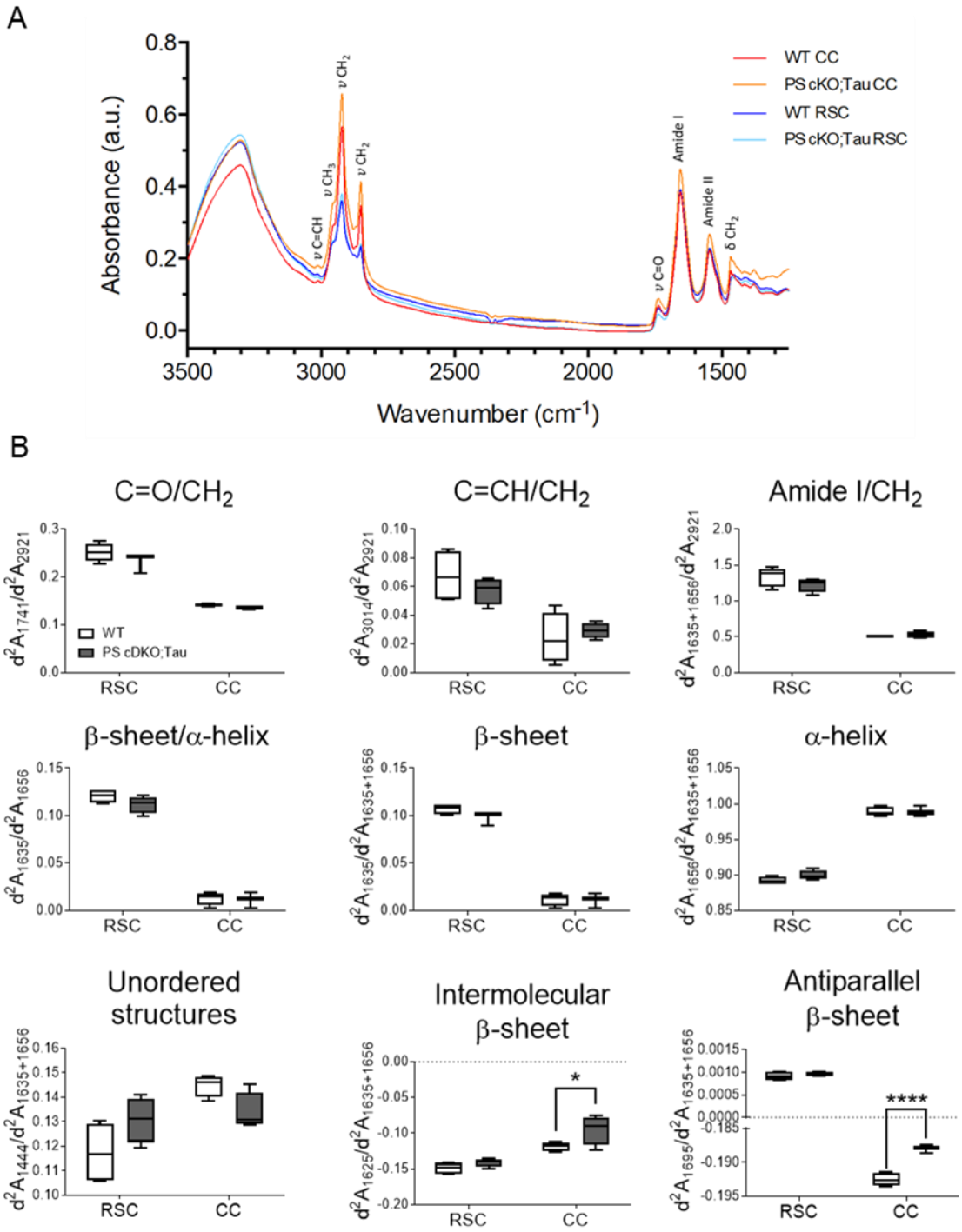


Figure 23. Increased pathological protein structures in corpus callosum of PS cDKO;Tau mice. Synchrotron-based µFTIR analysis of the retrosplenial cortex (RSC) and corpus callosum (CC) of WT and PS cDKO;Tau mice at 6 months. (**A**) Representative infrared spectra average of WT and PS cKO;Tau mice in the RSC and the CC indicating the main wavelength absorptions peaks with their corresponding chemical functional groups. a.u.: arbitrary units. (**B**) Lipid oxidation was measured by C=O/CH2 (A_{1741}/A_{2921}) and C=CH/CH_2 (A_{3014}/A_{2921}) ratios. Protein levels (Amide I, $A_{1656+1635}$), lipids levels (CH_2, A_{2921}), α-helix proteins (A_{1656}), β-sheet proteins (A_{1635}), unordered structures (A_{1444}), intermolecular β-sheet (A_{1625}) and antiparallel β-sheet (A_{1695}) proteins were also analyzed. Values represent the minimum, the maximum and the median of the mean of 100-200 spectrums/mice and 4 mice/group. Statistical analysis was determined by one-way ANOVA followed by Sidak's post hoc tests. **P* < 0.05, *****P* < 0.0001.

In agreement to these pathological protein structures, MC1 immunohistological analysis of consecutive brain sections used for the synchrotron study revealed increased aggregated tau in PS1 cKO;Tau and PS cDKO;Tau mice in the hippocampus (**Fig. 24A**), cortex (**Fig. 24B**) and corpus callosum (**Fig. 24C**).

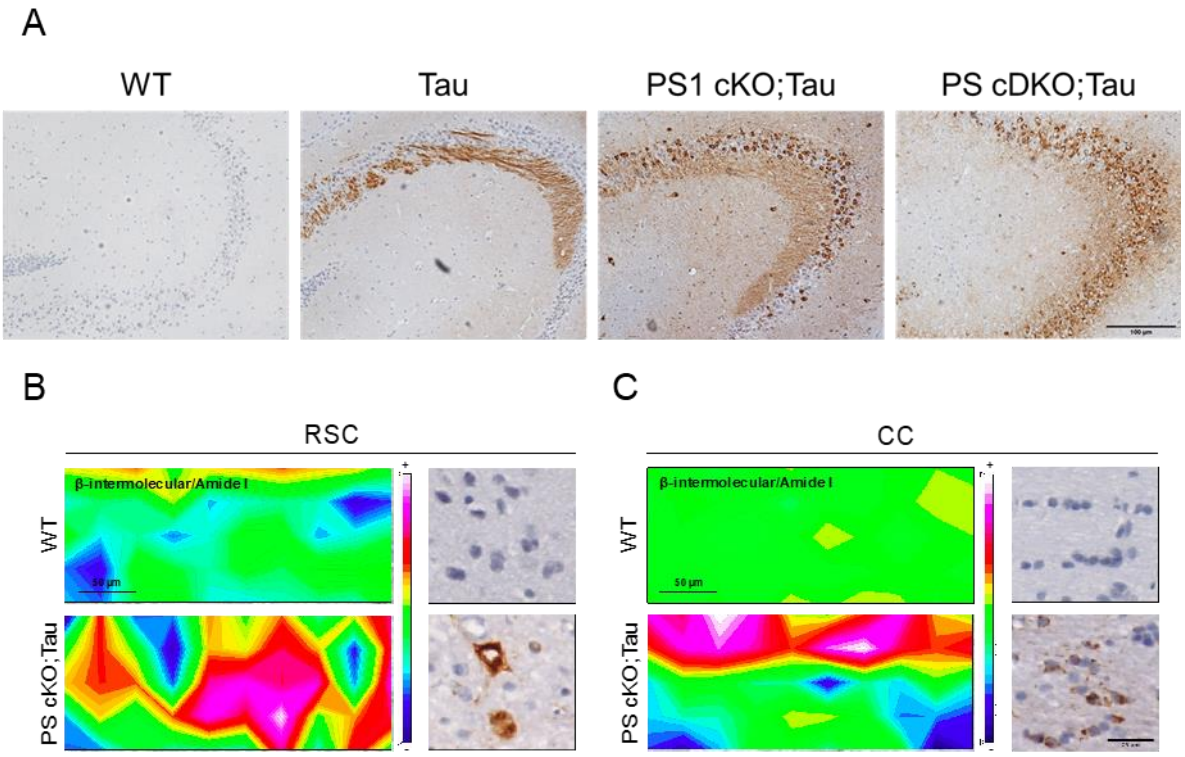


Figure 24. Increased aggregated tau in CA3, RSC and CC of PS-deficient tau mice. (A) Representative immunohistochemical images of aggregated tau (MC1) in 6 month-old WT, tau, PS1 cKO;Tau and PS cDKO;Tau mice. Scale bar: 100 µm. **(B-C)** Representative heat maps of the β-intermolecular/Amide I structure (left) and MC1 immunostaining images (right) in the retrosplenial cortex (RSC, **B**) and corpus callosum (CC, **C**). Scale bar: 25 µm.

8. Loss of PS function triggers increased astrocytic and microglial markers in cortex and hippocampus

To study the effects of loss of PS function on brain inflammation, I next performed biochemical analysis of GFAP (astrocyte) and Iba1 (microglia) in cortical and hippocampal lysates of WT and PS cDKO mice at 6, 9 and 12 months (**Fig. 25**). Quantification and statistical analyses revealed a genotype effect in GFP and Iba1 levels in the hippocampus ($P < 0.05$) and cortex ($P < 0.01$) in PS cDKO mice compared to WT mice (**Fig. 25**).

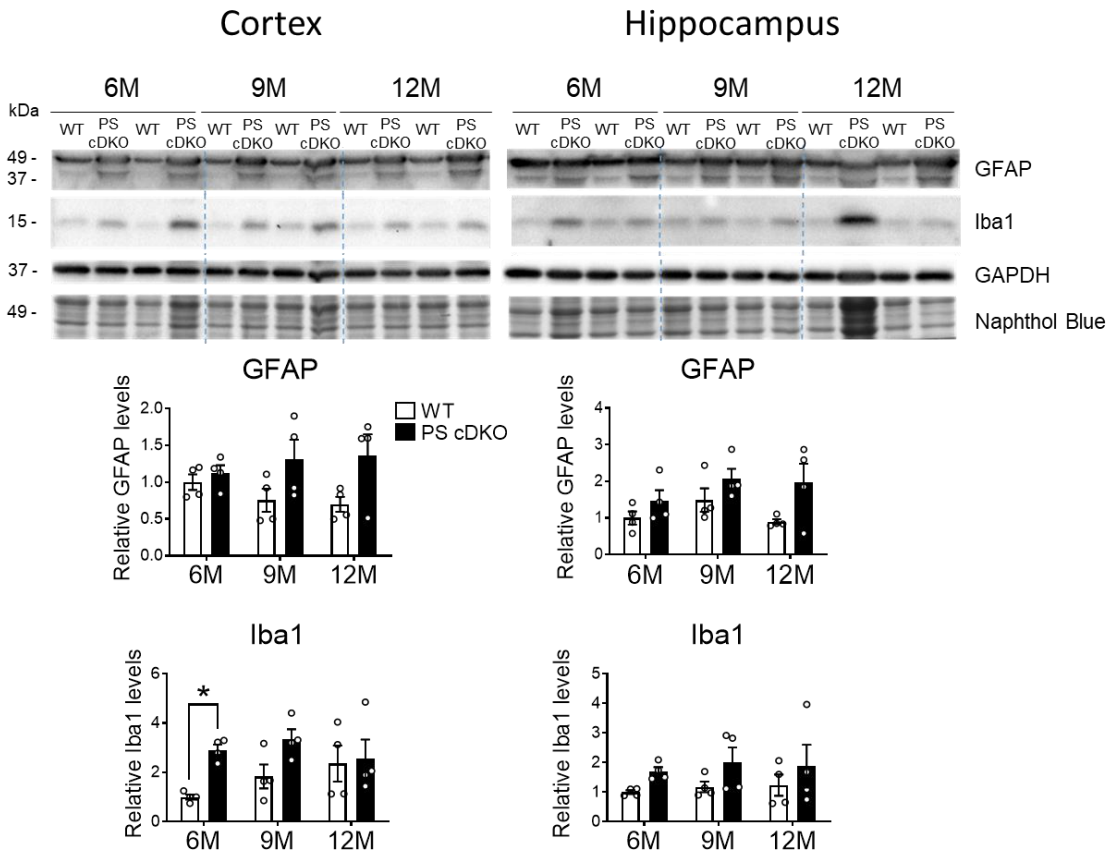


Figure 25. Increased neuroinflammatory levels in PS cDKO mice. Western blot images (top) and quantitative analysis (bottom) of astrocytic (GFAP) and microglia (Iba1) markers in cortical (left) and hippocampal (right) lysates of WT and PS cDKO mice at 6, 9 and 12 months of age. Protein levels were normalized to GAPDH. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Sidak's post hoc tests. *$P < 0.05$.

To examine whether inflammatory responses are dependent on PS dosage, I next analyzed GFP and Iba1 levels in the hippocampus of 9 month-old WT, PS2$^{-/-}$, PS1 cKO and PS cDKO mice (**Fig. 26**). Quantitative and statistical analyses revealed a genotype effect in GFAP ($P < 0.05$) and Iba1 ($P < 0.01$) levels with significant changes only in PS cDKO mice (**Fig. 26**).

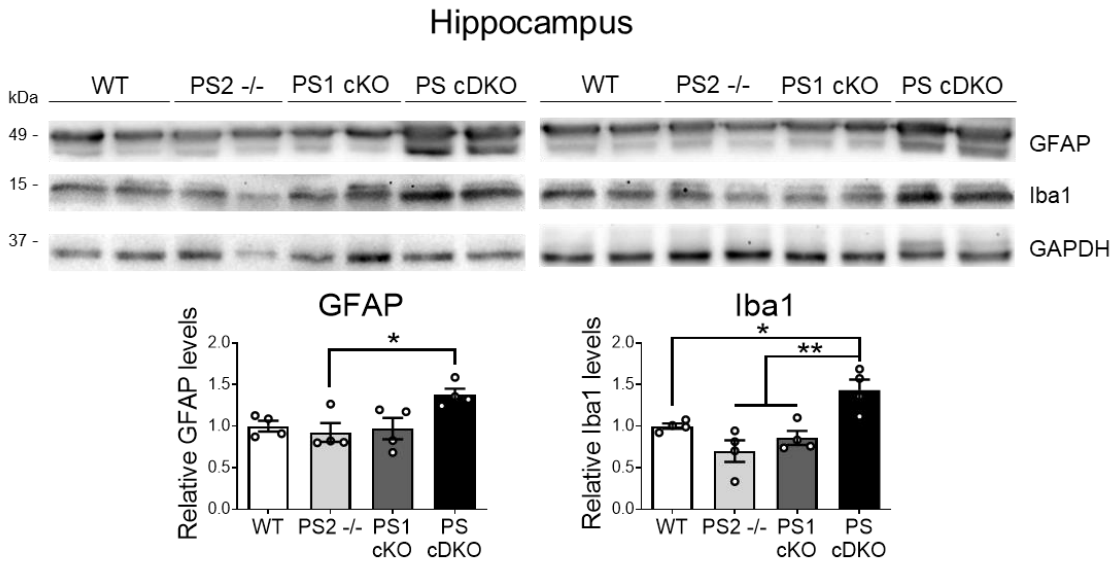


Figure 26. Partial loss of PS function does not affect brain inflammation. Western blot images (top) and quantitative analysis (bottom) of astrocytic (GFAP) and microglia (Iba1) markers in the hippocampus of WT, PS2$^{-/-}$, PS1 cKO and PS cDKO mice at 9 months of age. Protein levels were normalized to GAPDH. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01.

Similarly, quantification of GFAP levels in tau transgenic mice revealed a main PS effect (*P* < 0.0001) in the cortex and main effect of tau and PS (*P* < 0.0001) and tau x PS interaction effect (*P* < 0.01) in the hippocampus. Post-hoc analyses revealed significant increase of GFAP levels in Tau Tg mice lacking PS1 and both PS in the hippocampus (**Fig. 27**).

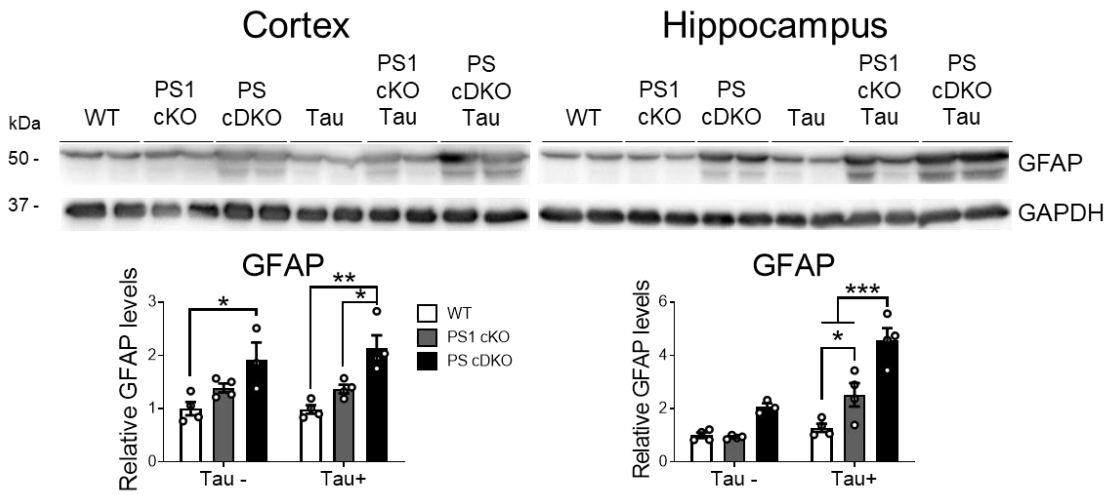
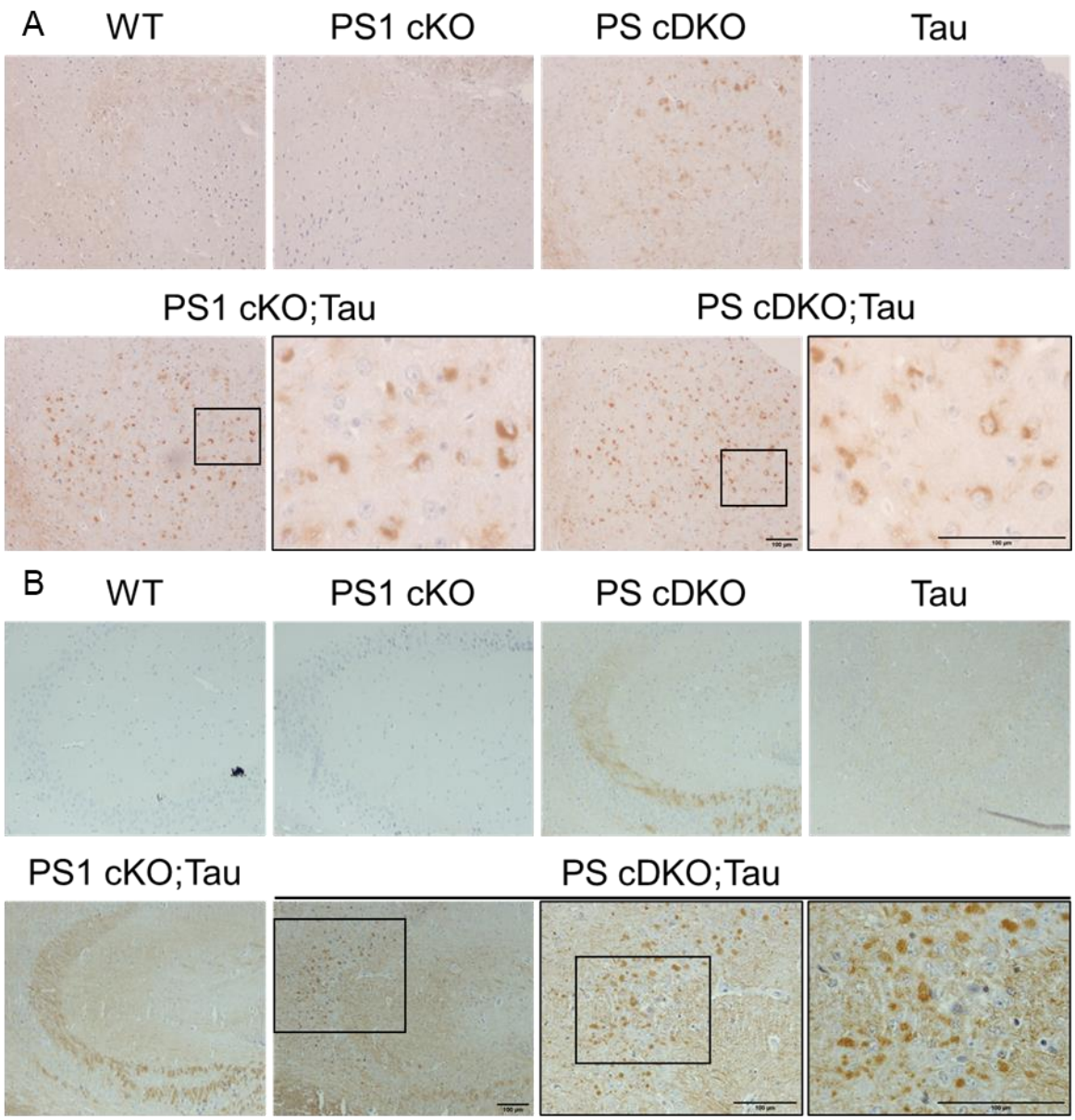


Figure 27. PS deficiency increases GFAP levels in tau Tg mice. Western blot images (top) and quantification (bottom) of astrocytic marker (GFAP) in the cortex (left) and the hippocampus (right) of non-transgenic mice (Tau-): control (WT), PS1 cKO and PS cDKO and tau transgenic mice (Tau+): Tau, PS1 cKO;Tau and PS cDKO;Tau at 6 months of age. GFAP levels were normalized to GAPDH levels. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

9. Absence of PS increases the aggregation pattern of neurofilament

PS1 regulates the assembly of neurofilaments (NF) and *PSEN1* mutations cause cytoskeleton alterations characterized by tau-positive NFTs labeled with the SMI312, an antibody specific for phosphorylated axonal NF, including NF medium (NF-M) and high (NF-H) chains forms and phosphorylated tau proteins (Dowjat et al., 2001; Woodhouse et al., 2009). Interestingly, neurofilament light chain (NF-L) could be a promising neurodegeneration biomarker for pre-symptomatic AD patients that predicts clinical progression (Mattsson et al., 2017). To examine whether neuronal PS could regulate NF dynamics, I next performed immunohistochemical analysis using SMI312 and NF-L antibodies in 6 month-old mice. Notably, imaging analysis of SMI312 staining showed enhanced NF staining in CA3 hippocampal projections in PS cDKO, tau and PS1 cKO;Tau mice and clear somatic staining in PS cDKO;Tau mice, a pattern not observed in WT and tau transgenic mice (**Fig. 28A**). NF-L staining revealed abnormal-NF accumulation in the neuronal soma in EC of PS cDKO, PS1 cKO;Tau and PS cDKO;Tau mice (**Fig. 28B**). Western blot analysis revealed that PS deficiency cause a decrease in the levels of NF-L (PS effect: $P < 0.05$) and a tendency to decrease in NF-H levels in the hippocampus of PS1 cKO;Tau and PS cDKO;Tau mice. Interestingly, in these two mice groups we found a non-reported band (≈70kDA) that was highly increased (tau effect: $P < 0.0001$, PS effect: $P < 0.001$, tau x PS interaction effect: $P < 0.001$) and may be related to the histological aggregated pattern (**Fig. 28C**).


A
WT
PS1 cKO
PS cDKO
Tau
PS1 cKO;Tau
PS cDKO;Tau
B
WT
PS1 cKO
PS cDKO
Tau
PS1 cKO;Tau
PS cDKO;Tau

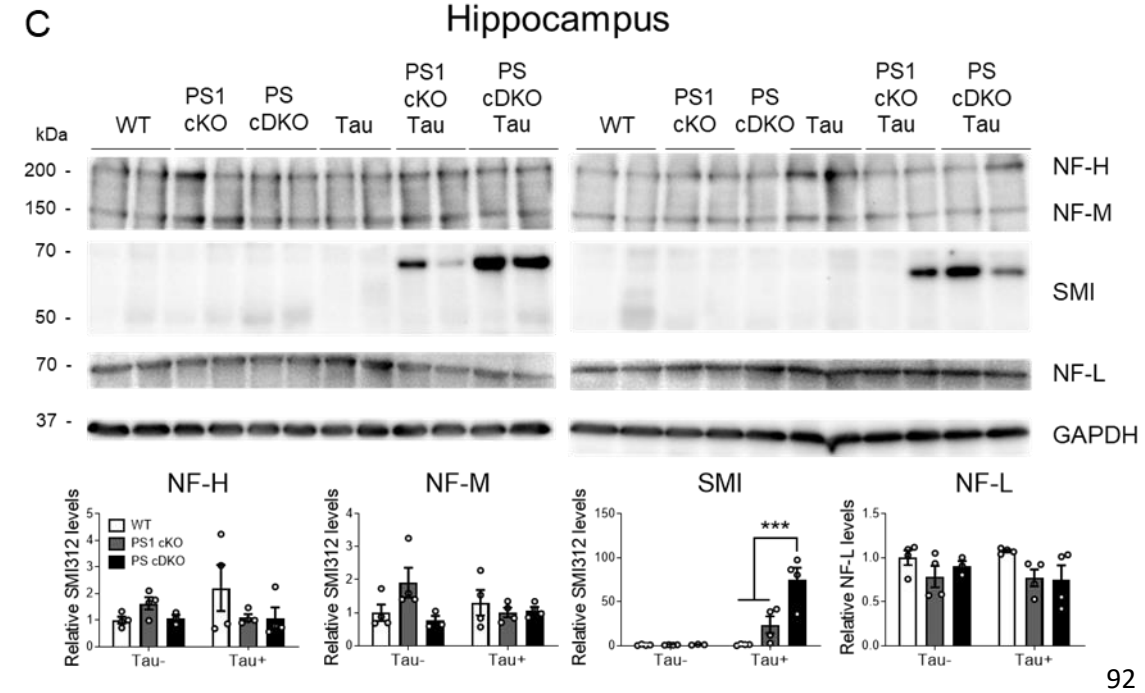

C
Hippocampus
kDa
WT
PS1 cKO
PS cDKO
Tau
PS1 cKO Tau
PS cDKO Tau
200 -
150 -
70 -
50 -
70 -
37 -
NF-H
NF-M
SMI
NF-L
GAPDH
NF-H
NF-M
SMI
NF-L
Relative SMI312 levels
Relative NF-L levels
WT
PS1 cKO
PS cDKO
Tau-
Tau+
***

Figure 28. PS deficiency causes abnormal NF staining and levels. (A) Representative SMI312 immunohistological images of the basolateral amygdala of 6 month-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. Higher magnified images showing SMI312 somatic staining of PS1 cKO;Tau and PS cDKO;Tau mice are shown (insets). Scale bars: 100 µm. **(B)** Representative immunohistological images of NF-L of hippocampus of WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau, PS cDKO;Tau mice of 6 months of age. Scale bars: 100 µm **(C)** Biochemical analysis of hippocampal lysates of 6 month-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau, PS cDKO;Tau mice. Western blot images (top) and quantification (bottom) of NF-H (200 kDa), NF-M (150 kDa) and SMI-labeled (60-70 kDa) bands in the hippocampus of experimental groups. Protein levels were normalized to GAPDH levels. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. ****P* < 0.001.

10. PS deficiency increases doublecortin levels in tau transgenic mice

We next evaluated hippocampal neurogenesis changes by examining doublecortin (DCX) levels in hippocampal lysates of WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau and PS cDKO;Tau mice at 6 months of age. Unexpectedly, we found statistical differences in DCX levels (tau x PS interaction effect: $P < 0.0001$), particularly increased levels in tau mice (tau effect: $P < 0.0001$) and those lacking PS (PS effect: $P < 0.0001$), which suggests increased neurogenesis in PS cDKO;Tau mice (**Fig. 29**).

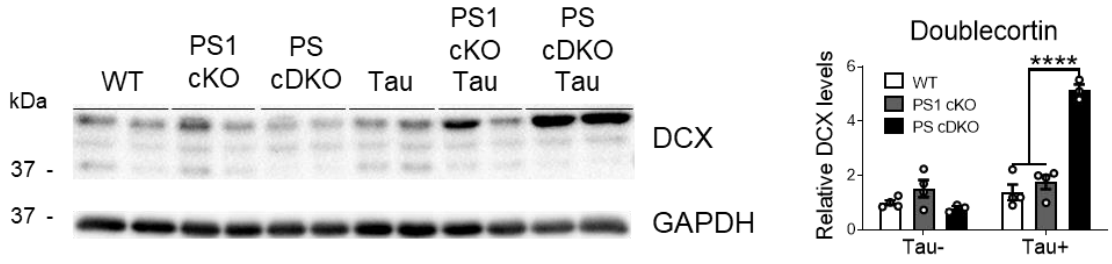


Figure 29. Increased doublecortin levels in PS cDKO;Tau mice. Biochemical analysis of hippocampal lysates of 6 month-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau, PS cDKO;Tau mice. Western blot images (left) and quantitative analysis (right) of doublecortin (DCX) levels in the hippocampus. Doublecortin levels were normalized to GAPDH levels. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. *****P* < 0.0001.

11. Loss of PS function results in exacerbated memory impairments in tau transgenic mice

The presence of tau pathology in memory-related regions in PS1 cKO;Tau and PS cDKO;Tau mice raises the possibility for hippocampal memory and learning deficits in these mice. To assess this issue, we next assayed our experimental mouse groups to distinct memory-related behavioral tests, including Morris water maze, novel object recognition and contextual fear conditioning (CFC). Morris water maze test consisted in a first day of 6 individual trials with a visible platform and five consecutives acquisition days (AQC) of training with 6 individual trials per day in which a hidden platform was present in the target quadrant. 24h after last training trial, we performed a memory-probe test (1 min) without the platform. During the visible platform test, swimming

speed was similar among all groups ($P > 0.05$) excluding the possibility of motor and visual deficits. Despite no differences were found in the first trial, significant latency changes were detected between genotype and trial ($P < 0.0001$), especially in PS cDKO;Tau mice suggesting deficits in easy task (**Fig. 30A**). The results during the learning phase showed increased latencies and slower learning capacity in PS cDKO and PS cDKO;Tau mice compared to WT mice (genotype effect $P < 0.0001$, AQC effect $P < 0.001$) (**Fig. 30B**). Furthermore, in the memory probe test, PS cDKO;Tau mice spent significant more time to find the target quadrant ($P < 0.05$) and crossed to ($P < 0.05$) and spent less time in the target quadrant than the rest of the groups ($P < 0.05$) (**Fig. 30C,D**).

The novel object recognition test consisted of four different phases. First, the mice were habituated to the context during 1 min, after that, two equal objects were added to the context for the training phase during 5 min. 2h after the training phase, one object was replaced for a new object to evaluate the short-term memory according to the preference for the novel object for 5 min. Furthermore, 24h after the training phase the new object was replaced for a second new object to analyze the long-term memory depending on the preference for this novel object during 5 min. In 2 h and 24 h phases, we measured the latency and the time spent in the new and the old object. Discrimination index (time spent in new object vs old object) and latency to the new object did not show significant differences between groups ($P > 0.05$). However, the latency to the old object present significant differences among groups (genotype effect: $P < 0.05$; phase effect: $P < 0.001$), especially in the 24h-phase, being PS cDKO;Tau mice the lowest latency in exploring the old object (**Fig. 31**).

Finally, we perform a CFC in which the mice were placed in a novel chamber and exposed to a specific context during the training phase of 5 min. The freezing time during the first 3 min were considered a measure of neophobia. After this, mice were shocked (1 sec, 0.8 mA) and immediate freezing was recorded (2 min). Freezing time at 24 h (4 min) in the same chamber without shock was used as a measure of long-term associative memory. Freezing time during immediate freezing was similar with no significant changes in all experimental groups ($P > 0.05$). By contrast, freezing was significantly decreased in PS cDKO and PS cDKO;Tau mice compared to WT confirming hippocampal-dependent memory deficits caused by PS deficiency (genotype effect: $P < 0.0001$, time effect: $P < 0.0001$, genotype x time interaction effect: $P < 0.001$). Surprisingly, PS1 cKO and PS1 cKO;Tau mice showed elevated freezing behavior suggesting increased anxious-related behavior (**Fig. 32**).

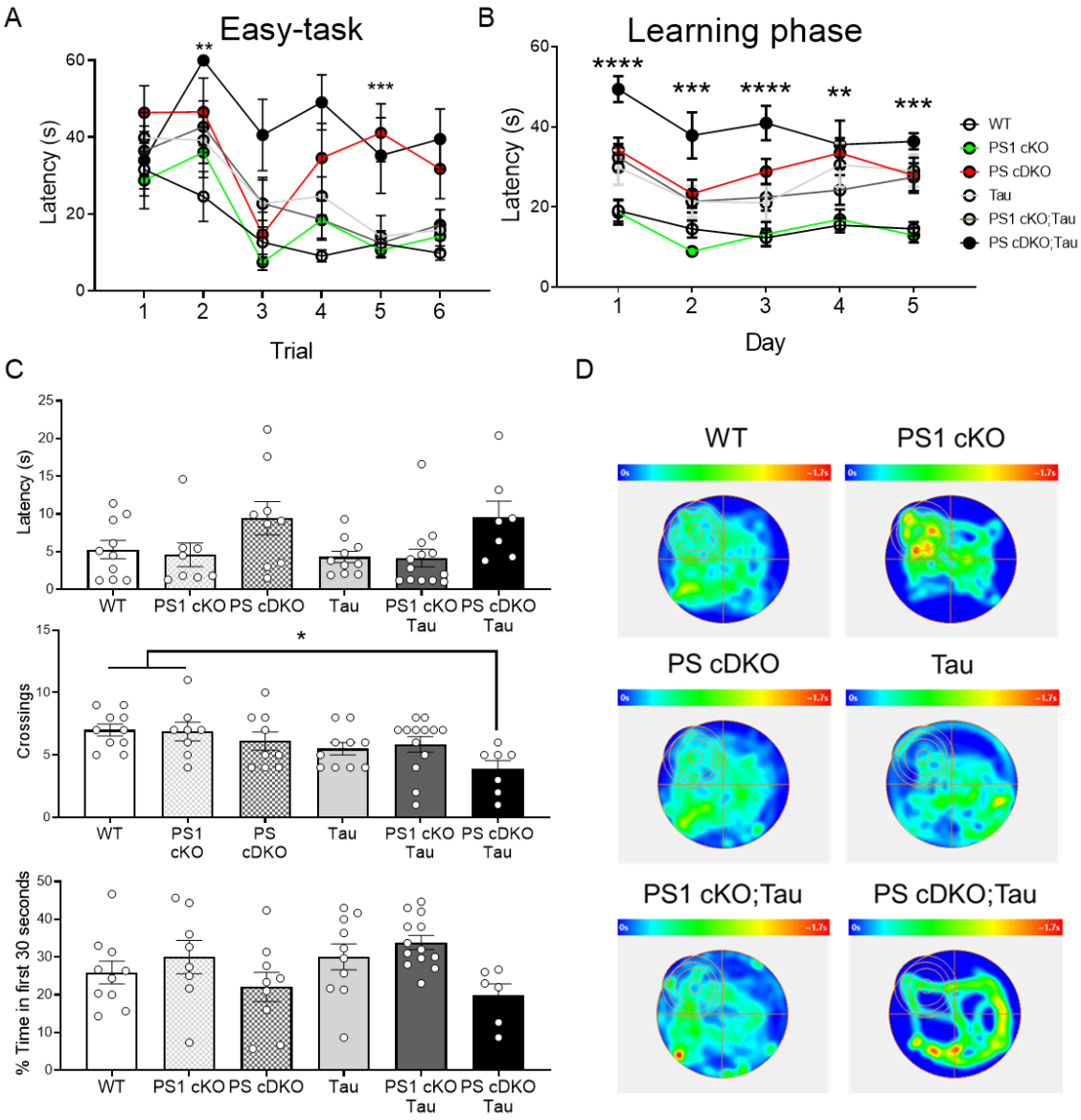


Figure 30. Loss of PS function causes impaired hippocampal-dependent memory and learning. Morris water maze was performed with 6 months-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. **(A)** During visible platform phase, which consisted in six trials, latency to the platform in seconds was measured. **(B)** During learning phase, which consisted in six trials during five consecutive days, latency to the platform in seconds was measured. **(C)** During the probe test, latency to (top), number of crossings to (middle) and percentage of time during the first 30 seconds in (bottom) the target quadrant were measured. **(D)** Representative heat map of all experimental groups showing the time spent in each quadrant during the probe trial. Values represent mean ± s.e.m. (n = 7-10 mice/group). Statistical analysis was determined by one or two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

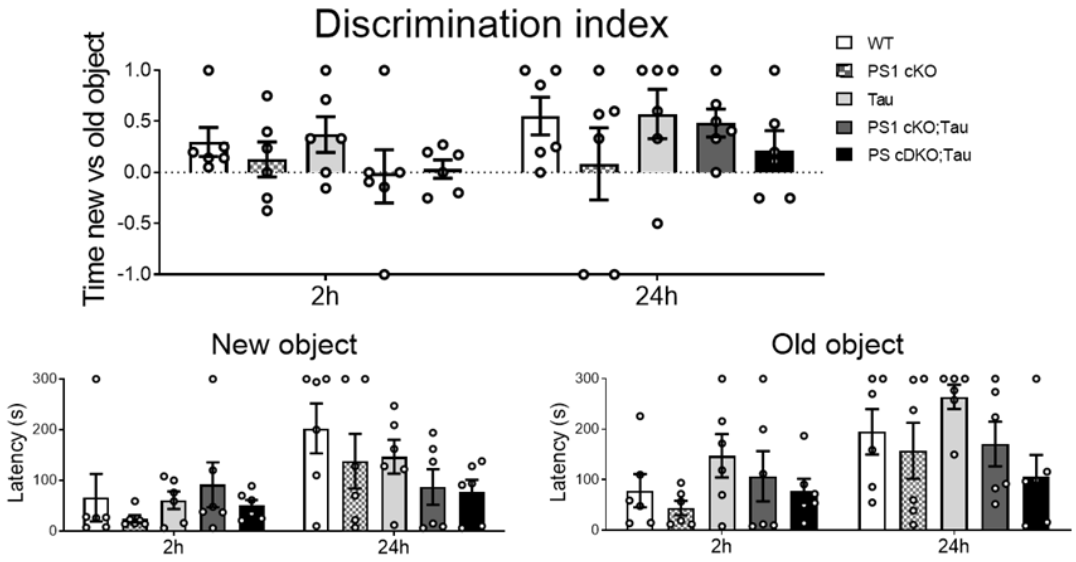


Figure 31. Unchanged visual object recognition in PS mutant mice. Visual object recognition was performed in 6 month-old WT, PS1 cKO, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. Discrimination index (time spent in the new object vs old object) and latency to new and old object was measured 2 and 24h after the habituation phase. Values represent mean ± s.e.m. (n = 6 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests.

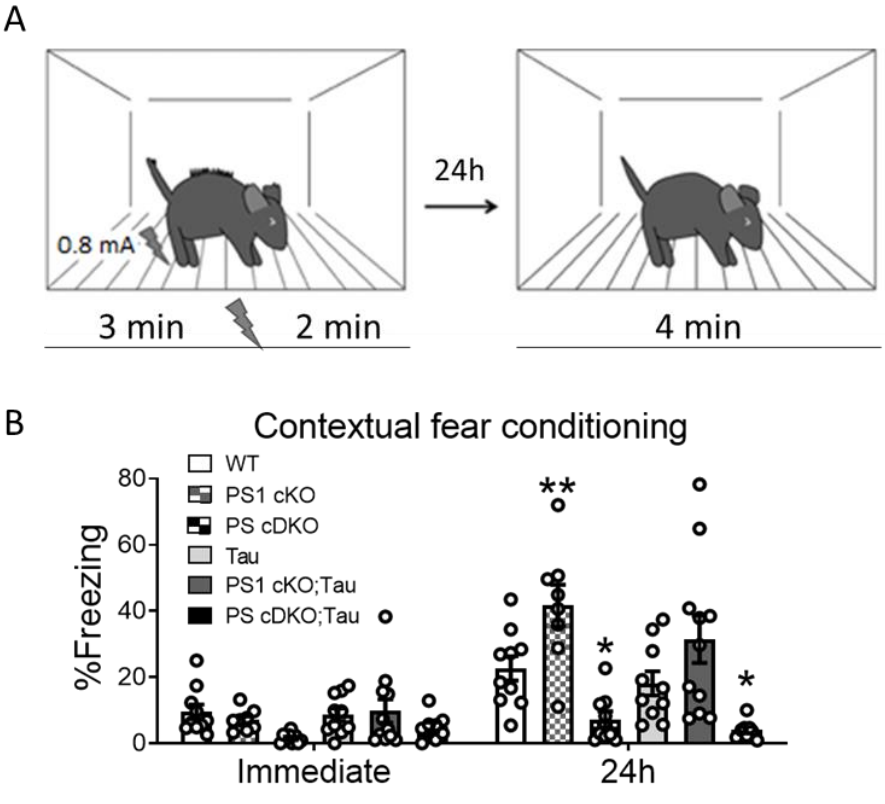


Figure 32. PS deficiency impairs hippocampal-dependent associative memory. Contextual fear conditioning was carried out in 6 month-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. **(A)** Representative image of the methodology used for the CFC. **(B)** Analysis of the percentage of freezing (number of episodes and duration) during the immediate and 24h-after phases. Values represent mean ± s.e.m. (n = 10 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01.

12. Loss of PS1 increases fear-anxiety behavior in mice

To analyze in depth the increased fear-related memory in PS1 cKO and PS1 cKO;Tau mice, we performed a cued-fear conditioning in which a non-aversive stimulus (CS), a sound, was paired to an aversive stimulus (US), a footshock, to measure the anxiety phenotype of these mice. 24h after, mice were exposed to a new context and the same sound was presented without the footshock to measure freezing response. PS1 cKO mice showed a tendency (not significant) of freezing increase. Surprisingly, PS1 cKO showed significant higher freezing response three months later than the other groups when the sound was presented (genotype effect: $P < 0.01$, phase effect: $P < 0.05$). This result suggests that PS1 deficiency may be affecting anxiety-related behavior (**Fig. 33**).

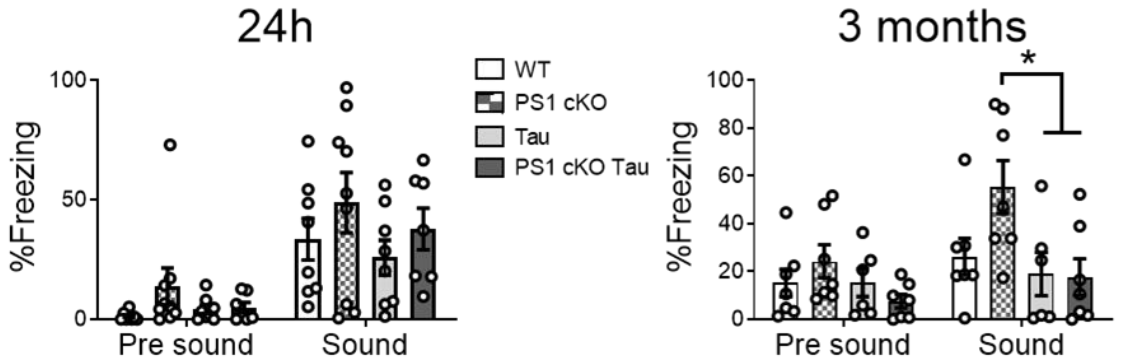


Figure 33. PS1 deficiency increases anxiety-related behavior in mice. Cued fear conditioning was performed in 6 month-old WT, PS1 cKO, Tau and PS1 cKO;Tau mice. Percentage of freezing was measured 24h (left) and 3 months (right) after the paired of conditioned (sound) and unconditioned stimulus (footshock). Values represent mean ± s.e.m. (n = 7 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. *$P < 0.05$.

13. PS deficiency dysregulates tau kinases

To investigate the molecular mechanisms underlying tau pathology, I analyzed next protein levels of the main tau kinases, including Cdk5, GSK3β and Akt, in cortical lysates of WT and PS cDKO mice at 6, 9 and 12 months. Biochemical analyses revealed no changes in Cdk5, although levels of an unknown Cdk5-derived lower migrating band were increased in PS cDKO mice (genotype effect: $P < 0.05$, age effect: $P < 0.05$), accompanied by an increase of p25/p35 ratio (genotype effect: $P < 0.001$). Furthermore, the levels of calpain, the protease implicated in the processing of p35 to p25, were increased in PS cDKO mice (genotype effect: $P < 0.05$). PS deficiency caused an increase of GSK3β phosphorylation at Ser9 (genotype effect: $P < 0.01$) and Akt at Thr308 (genotype effect: $P < 0.05$, age effect: $P < 0.05$) and Ser473

(genotype effect: $P < 0.0001$). These results indicate that loss neuronal PS results in increased p25/p35 and elevated levels of active Akt and inactive GSK3β (**Fig. 34**).

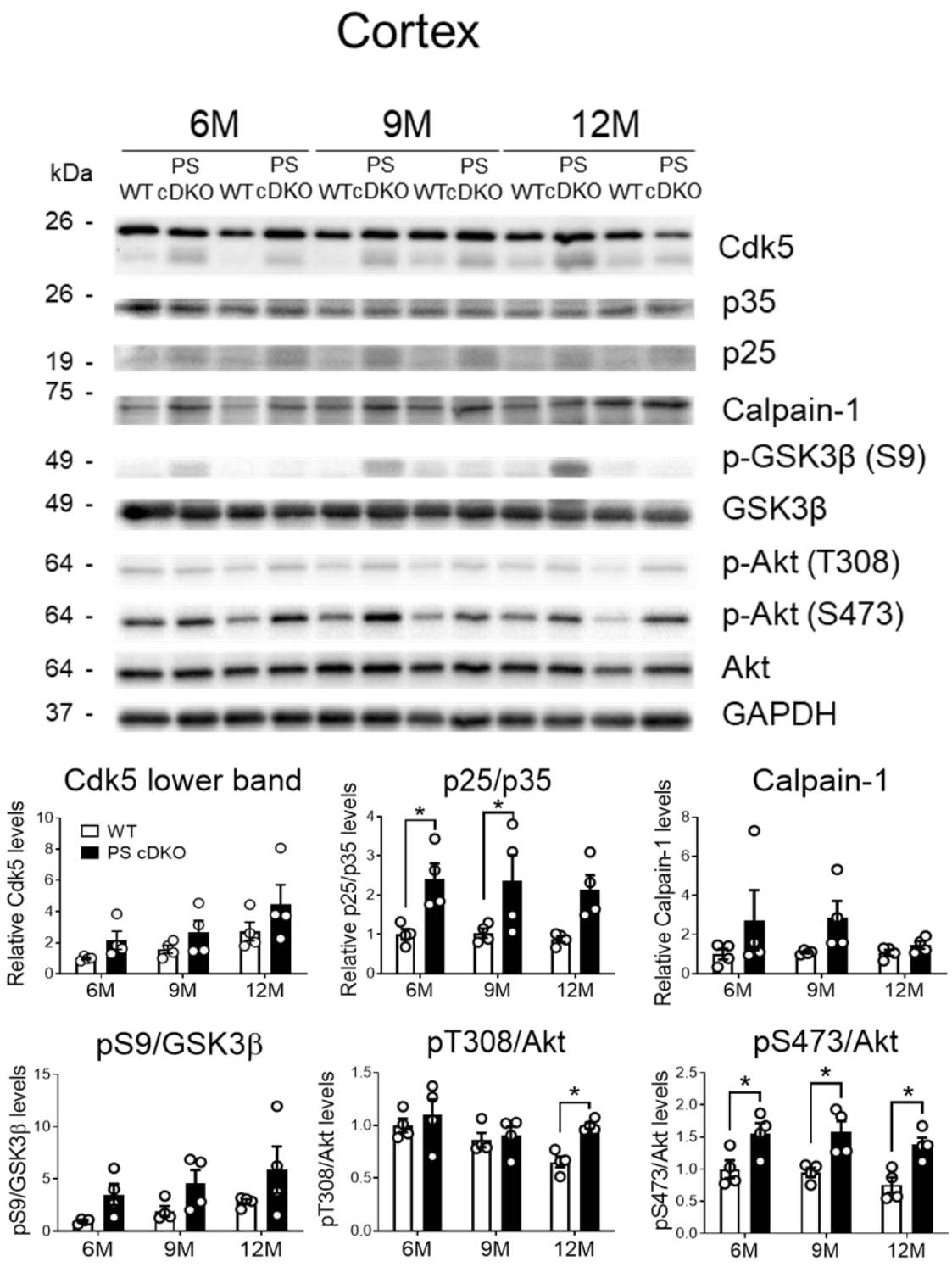


Figure 34. PS deficiency alters levels of tau kinases and effectors. Western blot images (top) and quantitative analysis (bottom) of tau kinases and effectors: Cdk5, p25/p35, calpain-1, phosphorylated (Ser9) and total GSK3β and phosphorylated (Thr308 and Ser473) and total Akt in cortex of WT and PS cDKO mice of 6, 9 and 12 months of age. Phosphorylated protein levels were normalized to total protein levels, and total protein was normalized to GAPDH. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Sidak's post hoc tests. *$P < 0.05$.

14. Loss of PS function impairs autophagy

Another mechanism that may be involved in PS-dependent tau phosphorylation and aggregation is a failure of tau elimination caused by loss of PS function. Since pathological tau is eliminated via autophagy (Wang et al., 2009; Krüger et al., 2012; Vaz-Silva et al., 2018; Jiang and Bhaskar, 2020), we next examined whether loss of PS function causes changes in autophagy by analyzing the following classical autophagy markers: LC3 and p62. Biochemical analyses revealed increased LC3-II/LC3-I (genotype effect: $P < 0.0001$) and p62 (genotype effect: $P < 0.05$) in cortical lysates of PS cDKO mice during aging (**Fig. 35**).

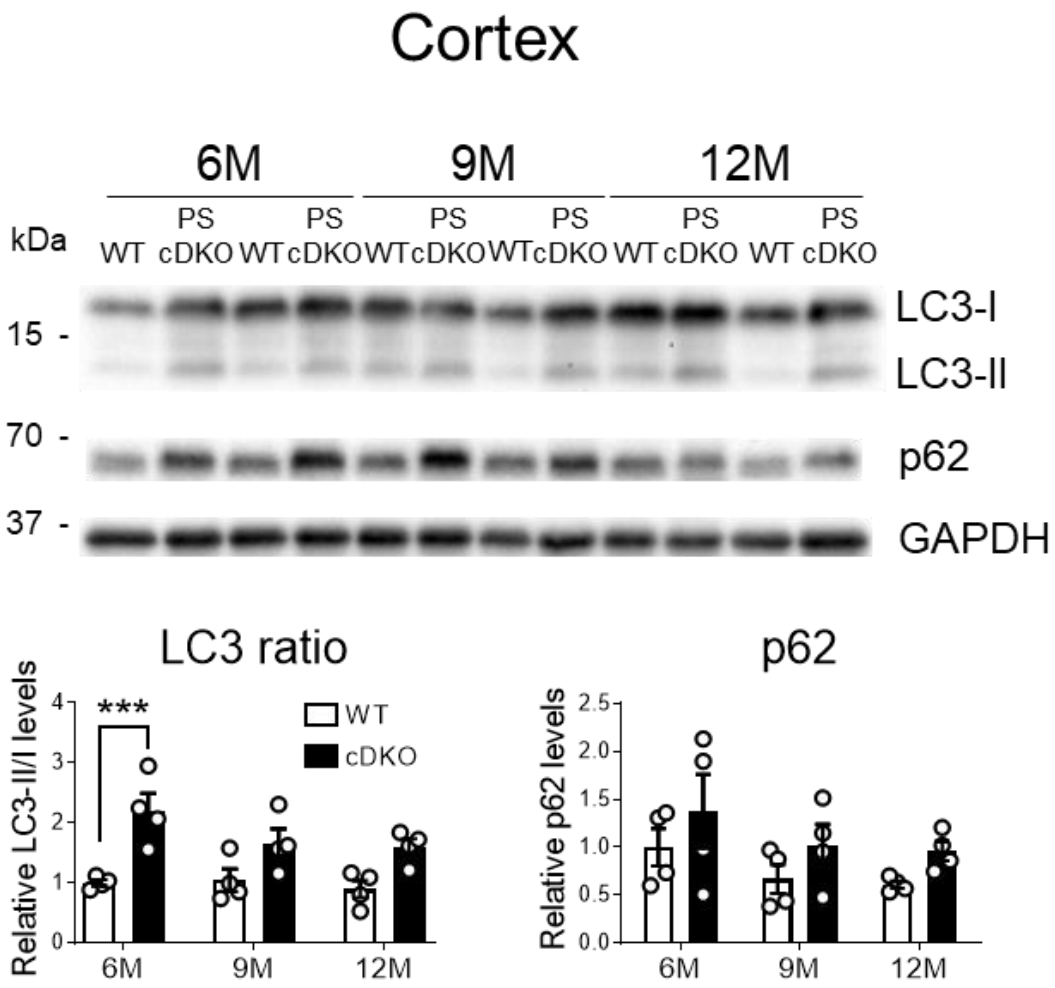


Figure 35. Loss of PS function increases autophagosome markers. Western blot images (top) and quantitative analysis (bottom) of autophagosome markers (LC3 and p62) in cortical lysates of WT and PS cDKO mice of 6, 9 and 12 months of age. p62 levels are normalized to GAPDH. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Sidak's post hoc tests. ***$P < 0.001$.

Partial PS deficiency showed an increase in autophagosomes and lysosomes number in PS cDKO fibroblast and SHSY5Y cells *in vitro* (Neely et al., 2011). To investigate whether the effect of PS in autophagy could be mediated by PS1 and/or PS2, I performed biochemical analysis of cortical and hippocampal lysates of WT, PS1 cKO, PS2$^{-/-}$ and PS cDKO mice at 9 months of age. Whereas LC3-II/I ratio levels were not significantly affected in the cortex of PS mutant mice, they were significantly increased specifically in the hippocampus of PS cDKO mice ($P < 0.05$; **Fig. 36**).

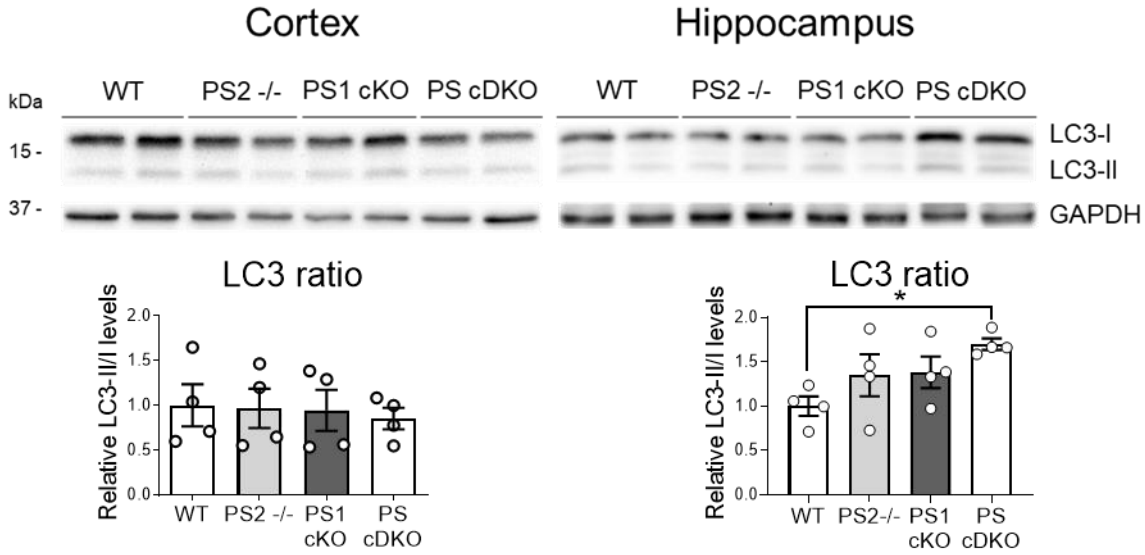


Figure 36. Effect of partial PS deficiency in autophagy. Western blot images (top) of autophagosome marker LC3 in the cortex (left) and the hippocampus (right) of WT, PS2$^{-/-}$, PS1 cKO and PS cDKO mice at 9 months of age. Quantitative analysis (bottom) of LC3-II/I ratio in WT, PS2$^{-/-}$, PS1 cKO and PS cDKO mice. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. *$P < 0.05$.

To analyze in depth the autophagy pathway, we next examined the levels of several key autophagy regulators. Although total and phosphorylated mTOR were unchanged ($P > 0.05$), there was a significant decrease in phosphorylated p70S6K (Thr389; (genotype effect: $P < 0.0001$), the main direct substrate of mTOR, in the cortex of PS cDKO mice. Moreover, PS cDKO showed increased phosphorylated proline-rich Akt substrate (PRAS40, Thr246; genotype effect: $P < 0.05$) and total (genotype effect: $P < 0.001$, age effect: $P < 0.01$) and phosphorylated (Thr172; genotype effect: $P < 0.05$) AMP-activated protein kinase (AMPK), which suggests altered autophagy in PS cDKO mice at different levels (**Fig. 37**).

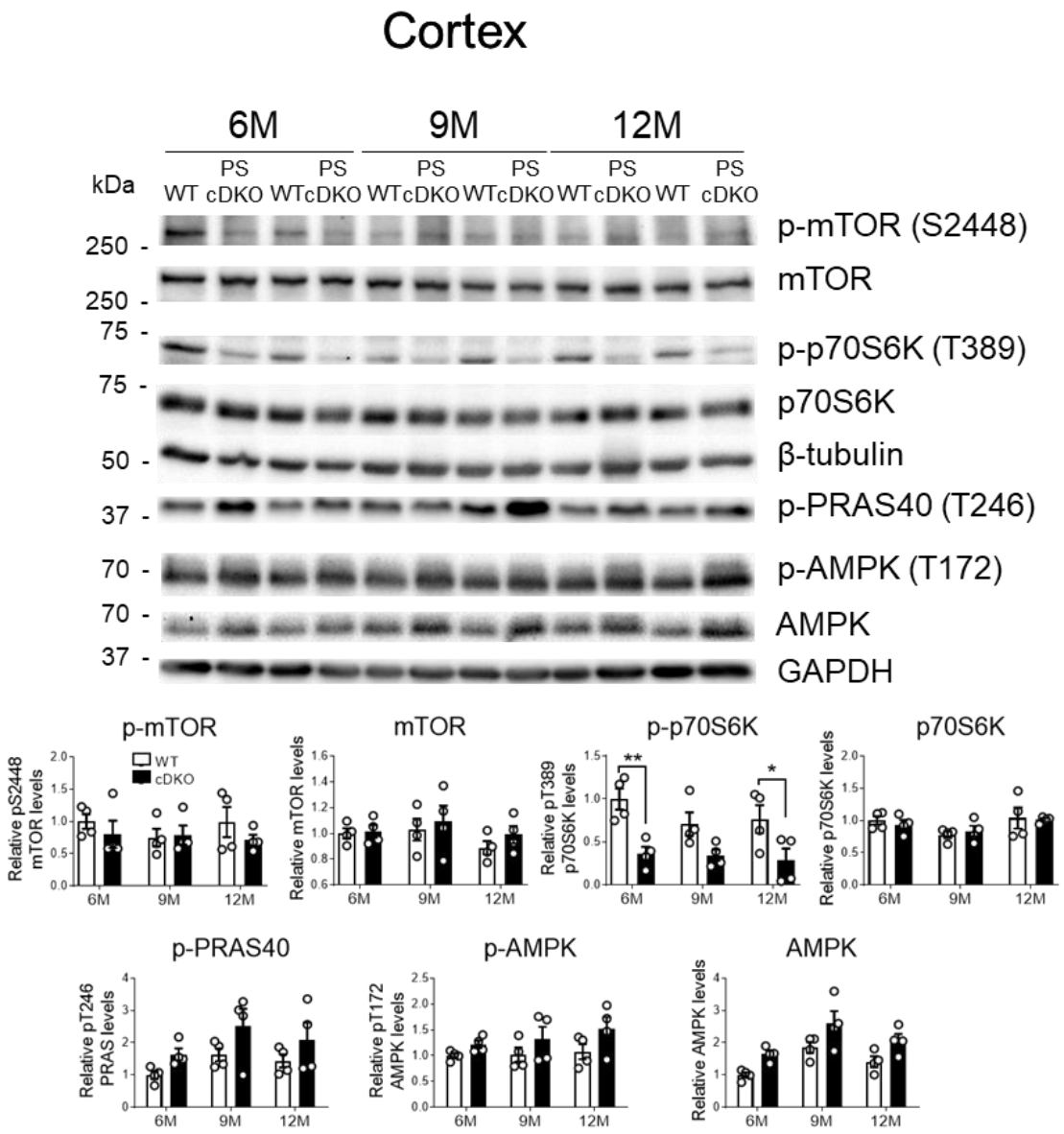


Figure 37. PS deficiency disrupts autophagy pathway. Western blot images (top) and quantitative analysis (bottom) of phosphorylated and/or total mTOR, p70S6K, PRAS40 and AMPK in cortical lysates of WT and PS cDKO mice at 6, 9 and 12 months of age. Phosphorylated proteins are normalized to total levels (except for pPRAS40 and p-AMPK that were normalized to GAPDH) and total levels are normalized to GAPDH or β-tubulin. Values represent mean fold ± s.e.m. (n = 4 mice/group). Statistical analysis was determined by two-way ANOVA followed by Sidak's post hoc tests. **P* < 0.05, ***P* < 0.01.

We next performed similar biochemical analysis of hippocampal lysates of 6 month-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. Interestingly, we found increased Akt-mediated phosphorylated GSK3β (Ser9; PS effect: $P < 0.05$), p62 (genotype effect: $P < 0.05$), LC3-II/I ratio (tau effect: $P < 0.01$, PS effect: $P < 0.01$), phosphorylated PRAS40 (Thr246; tau effect: $P < 0.05$, PS effect: $P < 0.05$), and total and phosphorylated AMPK (Thr172; PS effect: $P < 0.05$, PS x tau interaction effect: $P < 0.05$), suggesting dysregulation of autophagy flux in the hippocampus of PS cDKO;Tau mice (**Fig. 38**).

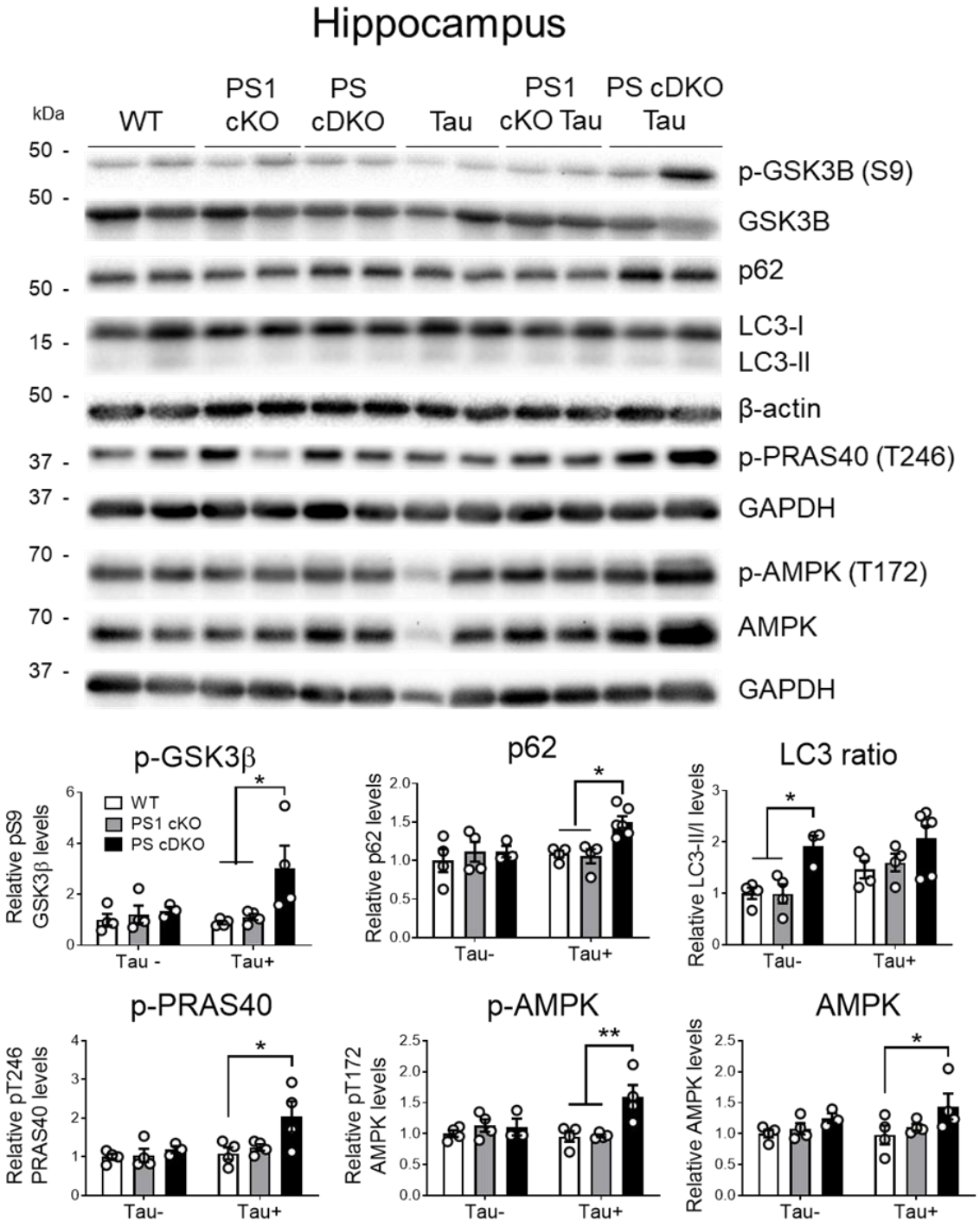


Figure 38. Loss of PS dysregulates autophagy markers in the hippocampus of tau transgenic mice. Western blot images (top) and quantitative analysis (bottom) of phosphorylated and/or total GSK3β, p62, LC3B, PRAS40 and AMPK in the hippocampus of 6 month-old WT, PS1 cKO, PS cDKO, Tau, PS1 cKO;Tau, PS cDKO;Tau mice. Phosphorylated proteins were normalized to total levels (except for pPRAS40 and p-AMPK that were normalized to GAPDH) and total levels were normalized to GAPDH or β-actin. Values represent mean fold ± s.e.m. (n = 4-5 mice/group). Statistical analysis was determined by two-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01.

15. PS inactivation results in increased phosphorylated tau and LC3-II/I in primary neurons

I next established primary cortical neuronal cultures from non-transgenic (Tau-) and tau transgenic (Tau+) embryos harboring PS1 f/f; $PS2^{-/-}$ alleles, which allows to silence conditionally PS1 after lentiviral Cre-recombinase transduction. Consistent with *in vivo* results, lentiviral-Cre recombinase-mediated PS inactivation in human Tau neurons (see decreased PS1 NTF/CTFs) resulted in increased phosphorylated tau (CP13) and LC3-II/I ratio (Brown-Forsythe ANOVA: $P < 0.05$, Welch's ANOVA: $P < 0.001$) (**Fig. 39**).

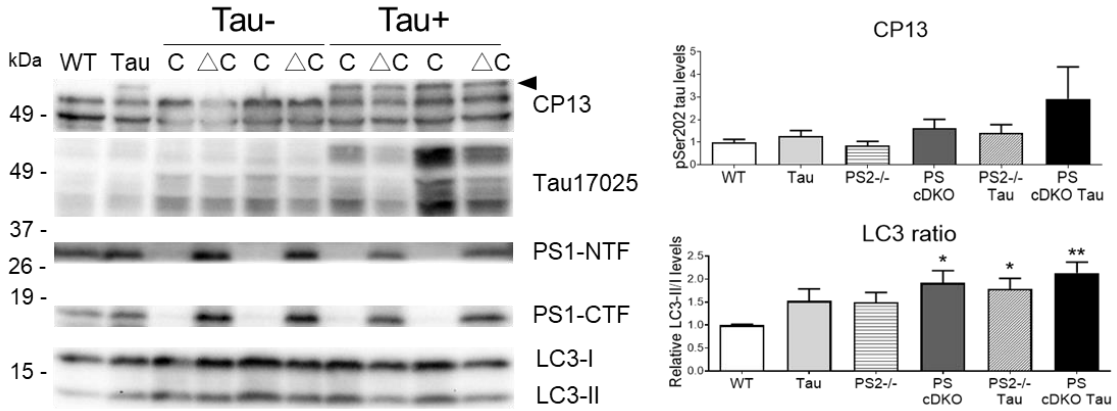


Figure 39. Increased phosphorylated human tau and LC3-II/I levels in PS-deficient Tau neurons. Western blot images (left) and quantitative analysis (right) of phosphorylated (CP13) and total (Tau17025) tau, PS1-NTF and CTF and LC3-II/I in cultured cortical neurons from PS1 f/f; $PS2^{+/-}$ (control, WT) expressing or not human Tau (black arrowhead), and PS1 f/f; $PS2^{-/-}$ expressing or not human Tau, transduced with lentiviral vectors containing Cre-recombinase (C) or ΔCre-recombinase (ΔC). Values represent mean fold ± s.e.m. (n = 7-11 embryos/group of 6 independent cultures). SD was significantly different between groups. Statistical analysis was determined by Brown-Forsythe and Welch's ANOVA test followed by Dunnett T3 post hoc tests. **P* < 0.05, ***P* < 0.01.

16. Nutrient deprivation decreases intracellular phosphorylated tau levels in primary neurons

To analyze the response capacity of cultured control and PS-deficient tau expressing neurons to different stimulus, we activated autophagy with nutrient deprivation for 0.5-24h. Surprisingly, despite no increase in autophagy markers, there was a time-dependent decrease in phosphorylated and total tau levels in neurons after nutrient deprivation treatment independently of PS1/2 expression (**Fig. 40**), indicating that nutrient deprivation reduces tau levels without affecting autophagy in primary cultured neurons.

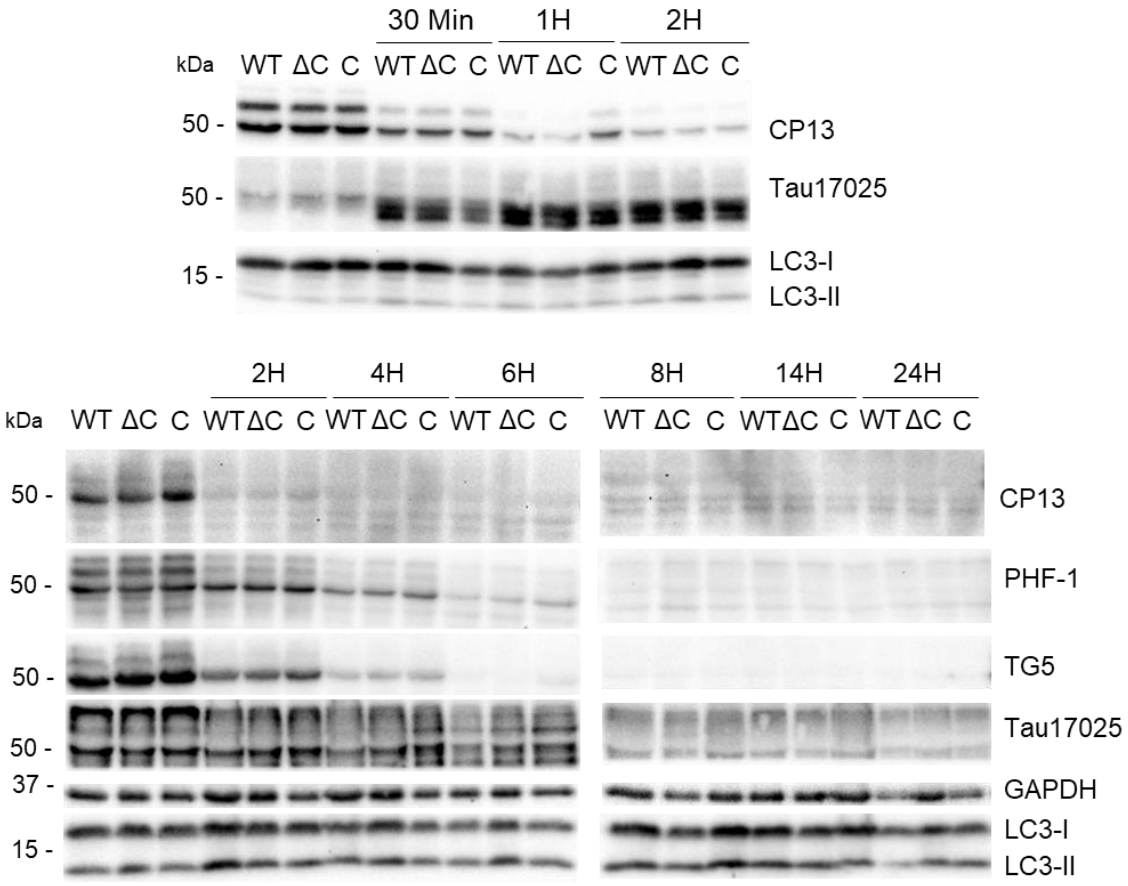


Figure 40. Nutrient deprivation decreases phosphorylated and total tau levels. Western blot images of phosphorylated (CP13 and PHF-1) and total (TG5 and Tau17025) tau and LC3 levels in htau expressing cortical neurons (11 DIV) from PS1 f/f; PS2$^{-/-}$ mouse embryos transduced with Cre-recombinase (C) or ΔCre-recombinase (ΔC) lentivirus in basal and 0.5-24h of nutrient deprivation conditions.

17. Lysosomal and/or proteasomal inhibition decreases intracellular phosphorylated tau levels in primary neurons

We next studied tau levels in response to autophagy and/or proteasomal inhibition. We used chloroquine (CQ) and MG132 to block the autophagosome/lysosome fusion and proteasome, respectively. Unexpectedly, autophagy or proteasome inhibition and, especially blocking both pathways, caused a reduction in phosphorylated and total tau levels independently of PS expression (**Fig. 41**).

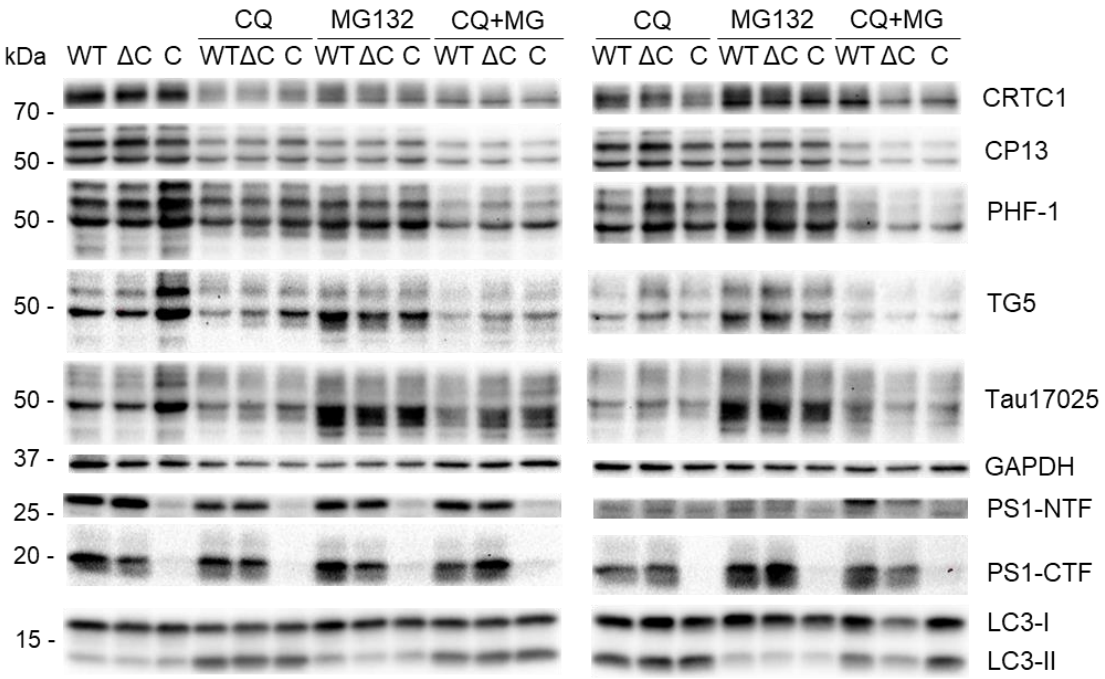


Figure 41. Autophagy and/or proteasomal inhibition decreases intracellular phosphorylated and total tau levels. Western blot images of CRTC1, phosphorylated (CP13 and PHF-1) and total (TG5 and Tau17025) tau, PS1-NTF and CTF and autophagosome marker (LC3) in htau expressing cortical neurons (11 DIV) from PS1 f/f; $PS2^{+/-}$ (control, WT) and PS1 f/f; $PS2^{-/-}$ mouse embryos transduced with ΔCre-recombinase (ΔC) or Cre-recombinase (C) lentivirus in basal conditions or treated with chloroquine (CQ) and/or MG132.

18. Injection of human purified-PHF into the hippocampus of PS cDKO mice increases hippocampal-dependent memory deficits

To investigate tau propagation and its potential induction by PS deficiency, we purified PHFs from parietal cortex of confirmed AD brain (see Methods). Electron microscopy showed the presence of PHFs in our purified samples (**Fig. 42A**). Then, we performed hippocampal injections of purified-PHFs or vehicle in 3 month-old WT and PS cDKO mice, and memory was tested 3 months after injection. Although PHF did not caused significant changes in associative memory retention in WT mice, PS cDKO injected with PHF showed decreased freezing compared to PS cDKO injected with vehicle (genotype effect: $P < 0.01$, phase effect: $P < 0.001$, genotype x phase interaction effect: $P < 0.05$) (**Fig. 42B**). This result indicates that PHFs potentiated associative memory loss caused by loss of neuronal PS function.

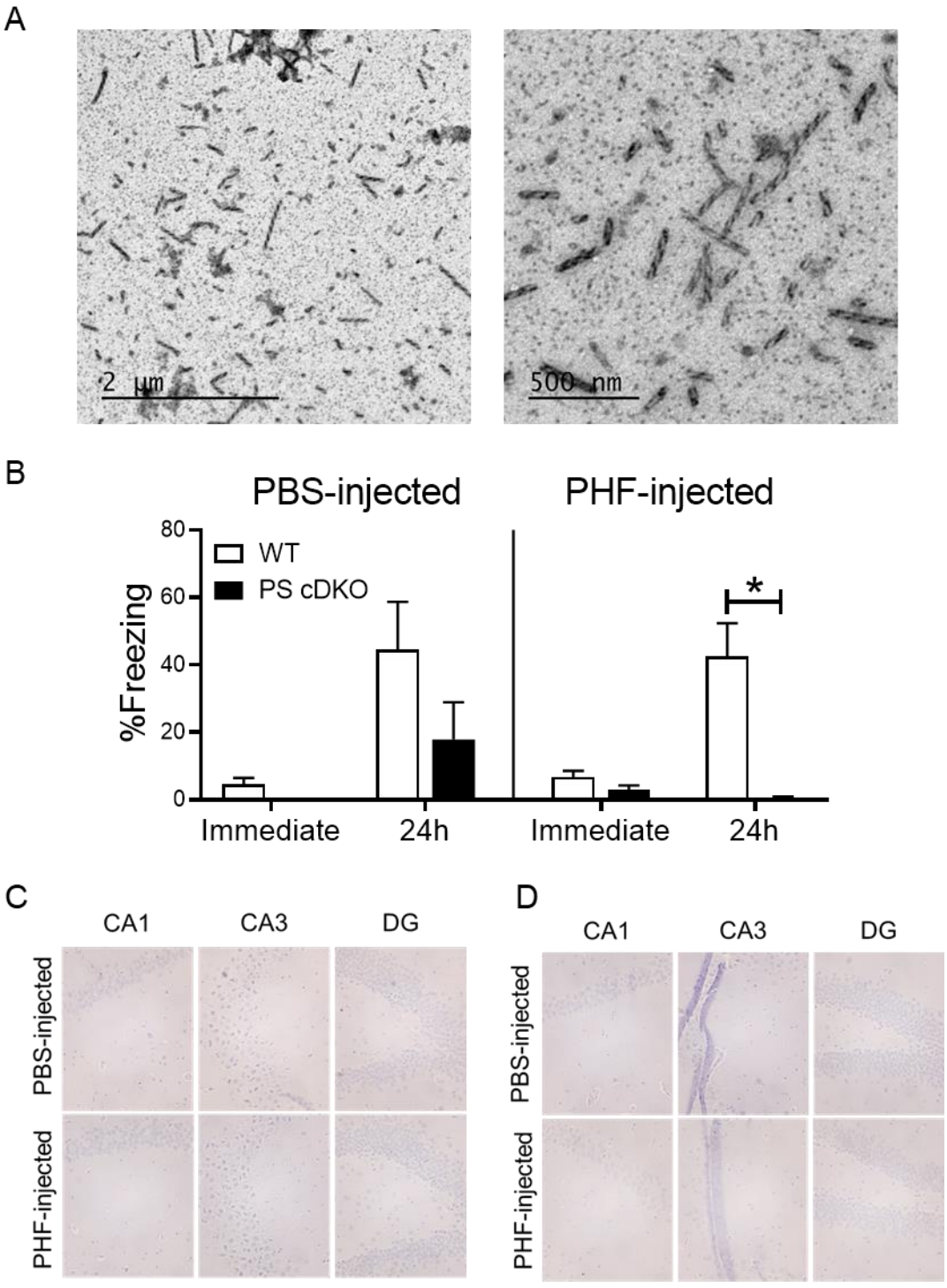


Figure 42. Human PHF-injection caused exacerbated memory deficits in PS cDKO mice. **(A)** Electron microscopy image of paired helical filaments (PHFs) purified from parietal cortex of human AD brain. Scale bars 2 µm (left image) and 500 nm (right image). **(B)** Contextual fear conditioning was carried out in 6 month-old WT and PS cDKO mice 3 months after PHF injection. Graph shows the analysis of the percentage of freezing (number of episodes and duration) during the immediate and 24h-after phases. Values represent mean ± s.e.m. (n = 4-8 mice/group). Statistical analysis was determined by three-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01. Representative images of immunohistological analysis of **(C)** phosphorylated tau (CP13) and **(D)** aggregated tau (MC1) in 6 month-old PS cDKO mice, 3 months after PHF/PBS injection.

19. No changes in tau levels in injected mice

To analyze the effects of PHF injection on tau pathology and the influence of PS deficiency on tau propagation, we performed histological analysis of WT and PS cDKO injected with vehicle or PHF. Despite the significant differences found in fear-related memory, no tau pathology was observed in WT or PS cDKO mice after PHF injection (**Figs. 42C,D**).

20. Synaptic tau accumulation and decreased synaptic proteins in tau transgenic mice and synapse pathology in human AD brain

To investigate the effects of tau pathology on synapse, we performed tissue fractionation of hippocampal tissue of 6-9 month-old WT and Tau mice to obtain nuclear, synaptosome, presynaptic and postsynaptic fractions. Biochemical analysis showed decreased of postsynaptic density protein 95 (PSD95) (synaptosome: $P < 0.05$, postsynaptic: $P < 0.05$) and synaptophysin (presynaptic: $P < 0.05$, synaptosome: $P < 0.05$), postsynaptic CRTC1 ($P < 0.05$) and the presence of phosphorylated tau (PHF1, synaptosome: $P < 0.05$, presynaptic: $P < 0.05$, postsynaptic $P < 0.05$) in synaptic compartments in tau Tg mice compared to WT mice (**Fig. 43**).

Next, we repeated this experiment using an independent group of WT and tau transgenic mice at 6 months of age. We found decreased levels of PSD95, vesicular glutamate transporter 1(VGLUT1) and synaptophysin and increased phosphorylated (CP13) and total tau (D1M9X) in total lysates of tau Tg mice (**Fig. 44)**.

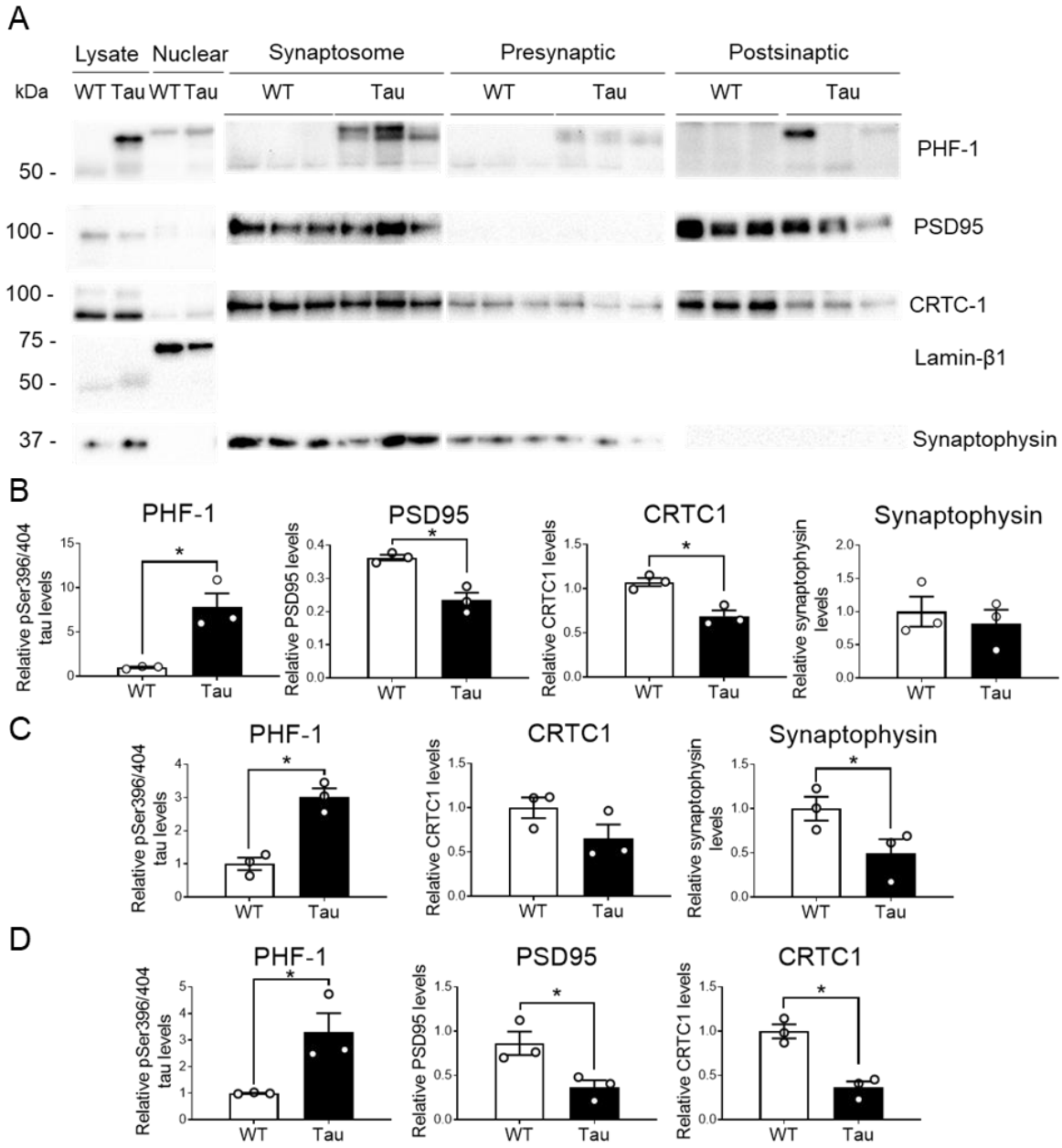


Figure 43. Synaptic phosphorylated tau in Tau Tg mice. (A) Western blot images of total lysate and nuclear, synaptosome, presynaptic and postsynaptic fractions after synaptosomal purification in the hippocampus of 6-9 months-old WT and Tau mice. **(B-D)** Quantitative analysis of phosphorylated tau (PHF-1), CRTC1 PSD95, and synaptophysin in synaptosomal **(B)**, presynaptic **(C)** and postsynaptic **(D)** fractions. Values represent mean fold ± s.e.m. (n = 3 mice/group). Statistical analysis was determined by unpaired student's *t*-test. **P* < 0.05.

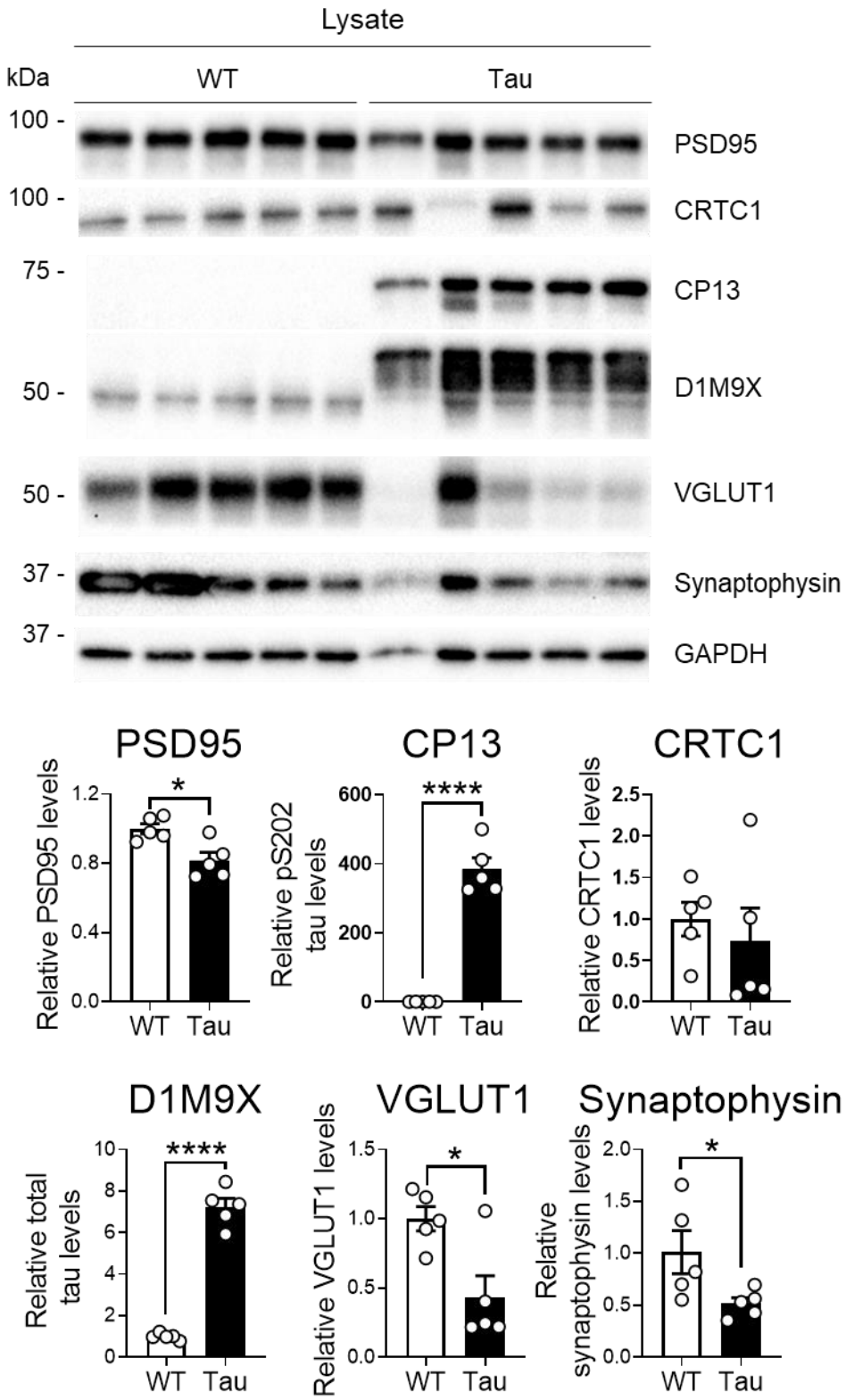


Figure 44. P301S mice showed increased tau and synaptic pathology. Western blot images (top) of total hippocampal lysates of 9 month-old WT and Tau mice. Quantitative analysis (bottom) of phosphorylated (CP13) and total (D1M9X) tau, CRTC1 and synaptic markers (PSD95, VGLUT1 and synaptophysin). Values represent mean fold ± s.e.m. (n = 5 mice/group). Protein levels were normalized to GAPDH. Statistical analysis was determined by unpaired student's *t*-test. **P* < 0.05, *****P* < 0.0001.

Interestingly, abnormal tau accumulation was associated with decreased VGLUT1 and synaptophysin but no changes in PSD95 in purified synaptosomes (**Fig. 45**). Thus, presynaptic fractions showed tau accumulation ($P < 0.0001$), decreased VGLUT1 ($P < 0.05$) and a tendency to decrease in synaptophysin levels, but no changes in CRTC1 ($P > 0.05$). Postsynaptic fraction contained tau ($P < 0.001$), decreased VGLUT1 ($P < 0.05$) and CRTC1 ($P < 0.01$) and increased PSD95 ($P < 0.05$). These results suggest that human tau overexpression in mice causes abnormal tau accumulation at synaptic compartment causing changes in pre- and post-synaptic proteins (**Fig. 46**).

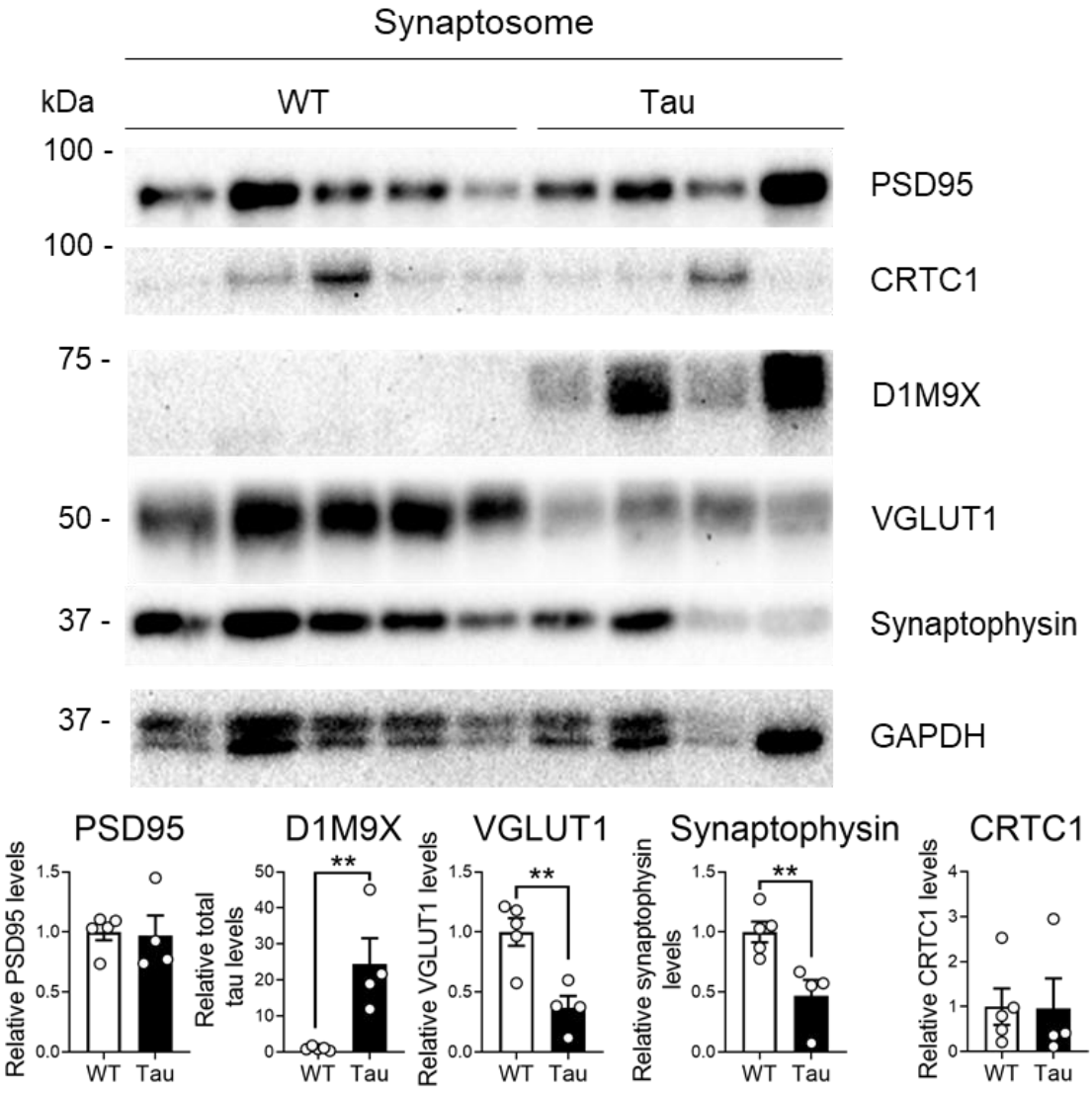


Fig. 45. Tau accumulation and decreased synaptic proteins in hippocampal synaptosomes of Tau mice. Western blot images (top) and quantification of total tau (D1M9X), CRTC1 and synaptic markers (PSD95, VGLUT1 and synaptophysin, bottom) of hippocampal synaptosomes from 9 month-old WT and Tau mice. Values represent mean fold ± s.e.m. (n = 4-5 mice/group). Protein levels were normalized to GAPDH. Statistical analysis was determined by unpaired student's *t*-test. ***P* < 0.01.

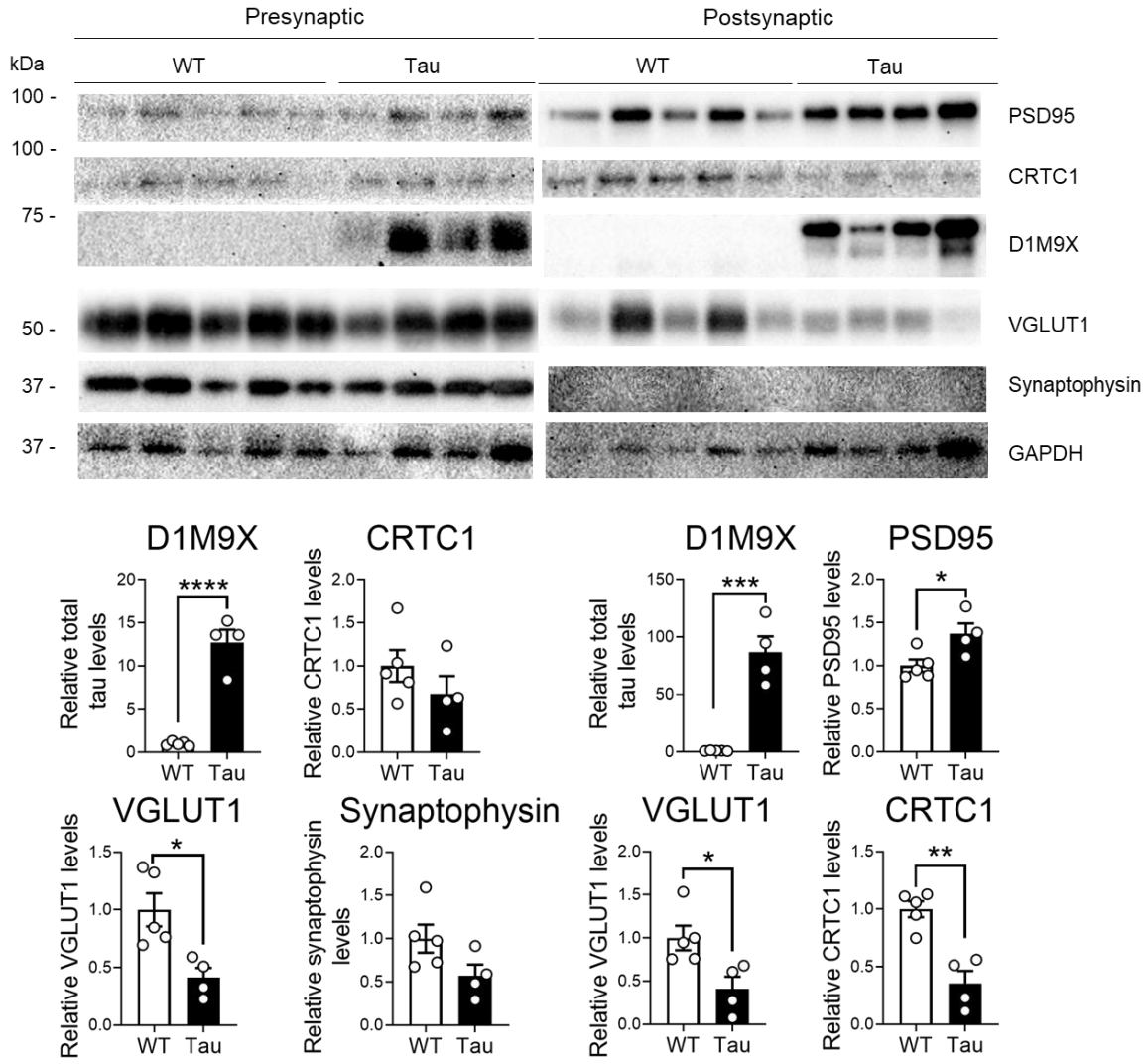


Figure 46. Synaptic tau accumulation and synaptic pathology. Western blot images (top) of purified presynaptic (left) and postsynaptic (right) fractions obtained from hippocampal tissue of 9 month-old WT and Tau mice. Quantitative analysis (bottom) of total tau (D1M9X), CRTC1 and synaptic markers (PSD95, VGLUT1 and synaptophysin). Values represent mean fold ± s.e.m. (n = 4-5 mice/group). Protein levels were normalized to GAPDH. Statistical analysis was determined by unpaired student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Next, we analyzed synaptic proteins in lysates and purified synaptosomal fractions from human parietal cortex of control (no pathology or Braak I/II) and Braak III/IV and V/VI stages. Biochemical analysis revealed a significant decrease in CRTC1, extracellular signal-regulated kinase ½ (Erk1/2), PSD95 and synaptophysin (pathology effect, $P < 0.05$) in total lysates at late AD stages. Furthermore, there is a tendency to increase in CRTC1, phosphorylated (Tyr202/204) and total Erk1/2 levels in the cytosolic fraction (**Fig. 47**). Nuclear fraction revealed decreased CRTC1 and nuclear factor kappa B (NFκB) levels and increased lamin-β1 levels, whereas synaptosome fraction contains decreased phosphorylated (Tyr202/204) Erk and β-tubulin levels (**Fig. 48**). Finally, synaptophysin levels were reduced in presynaptic fractions (pathology effect, $P < 0.01$), and CRTC1, phosphorylated (Tyr202/204) Erk1/2, NFκB and PSD95

were significantly decreased (pathology effect, $P < 0.05$) in postsynaptic fractions (**Fig. 49**). These results demonstrate synaptic pathology and alteration of synaptonuclear factors (CRTC1, Erk1/2, NFκB) in human brain during AD pathology.

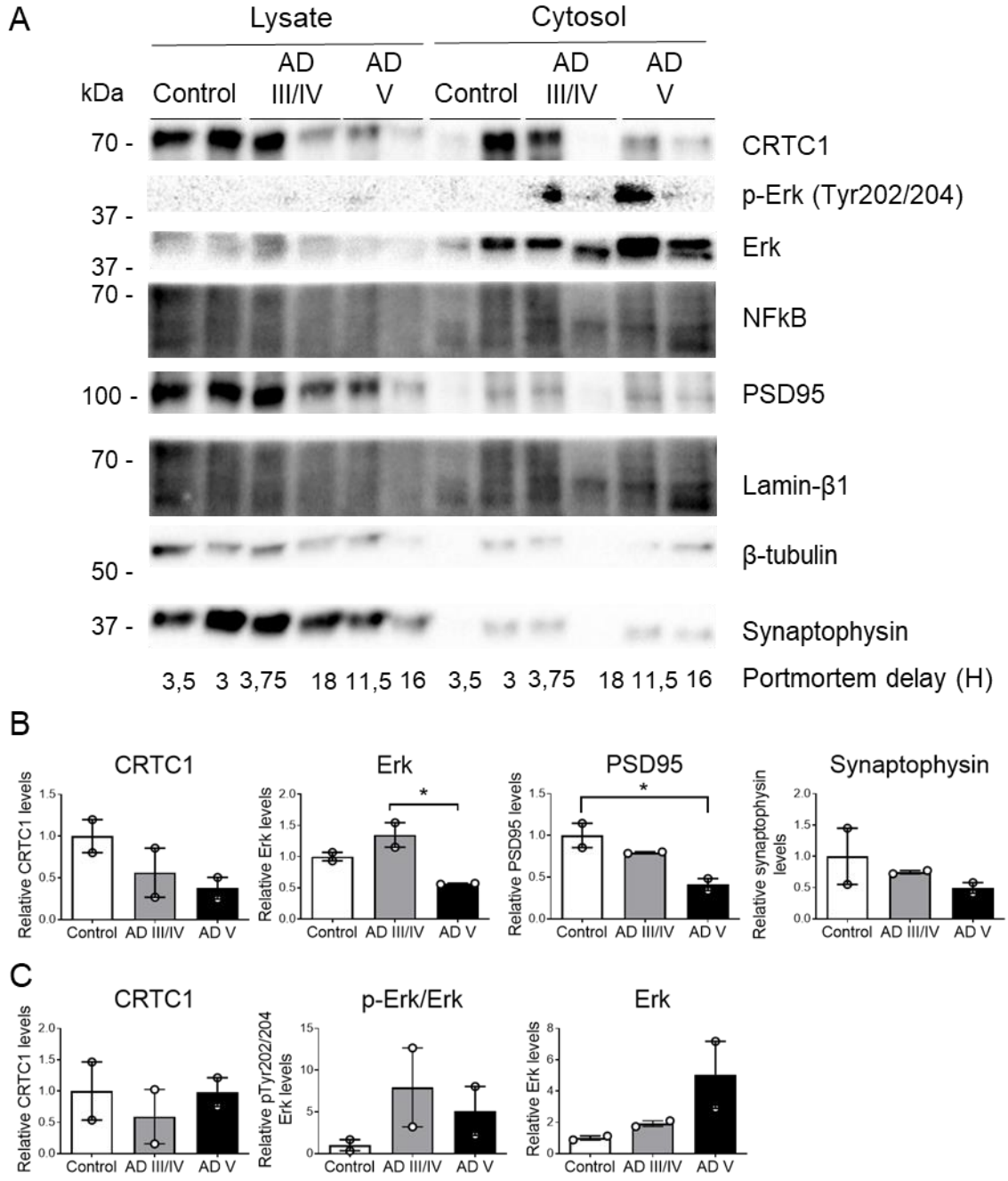


Figure 47. Human brain shows changes in synaptic proteins during AD pathology. (A) Western blot images of total lysate (left) and cytosolic (right) fractions obtained from human parietal cortex of control and AD patients. **(B)** Quantitative analysis of CRTC1, phosphorylated (Tyr202/204) and total Erk and synaptic markers (PSD95 and synaptophysin) in total parietal cortex. **(C)** Quantitative analysis of CRTC1 and phosphorylated (Tyr202/204) and total Erk1/2 levels in cytosolic fraction. Values represent mean fold ± s.e.m. (n = 2 /group). Protein levels were normalized to GAPDH. Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. *$P < 0.05$.

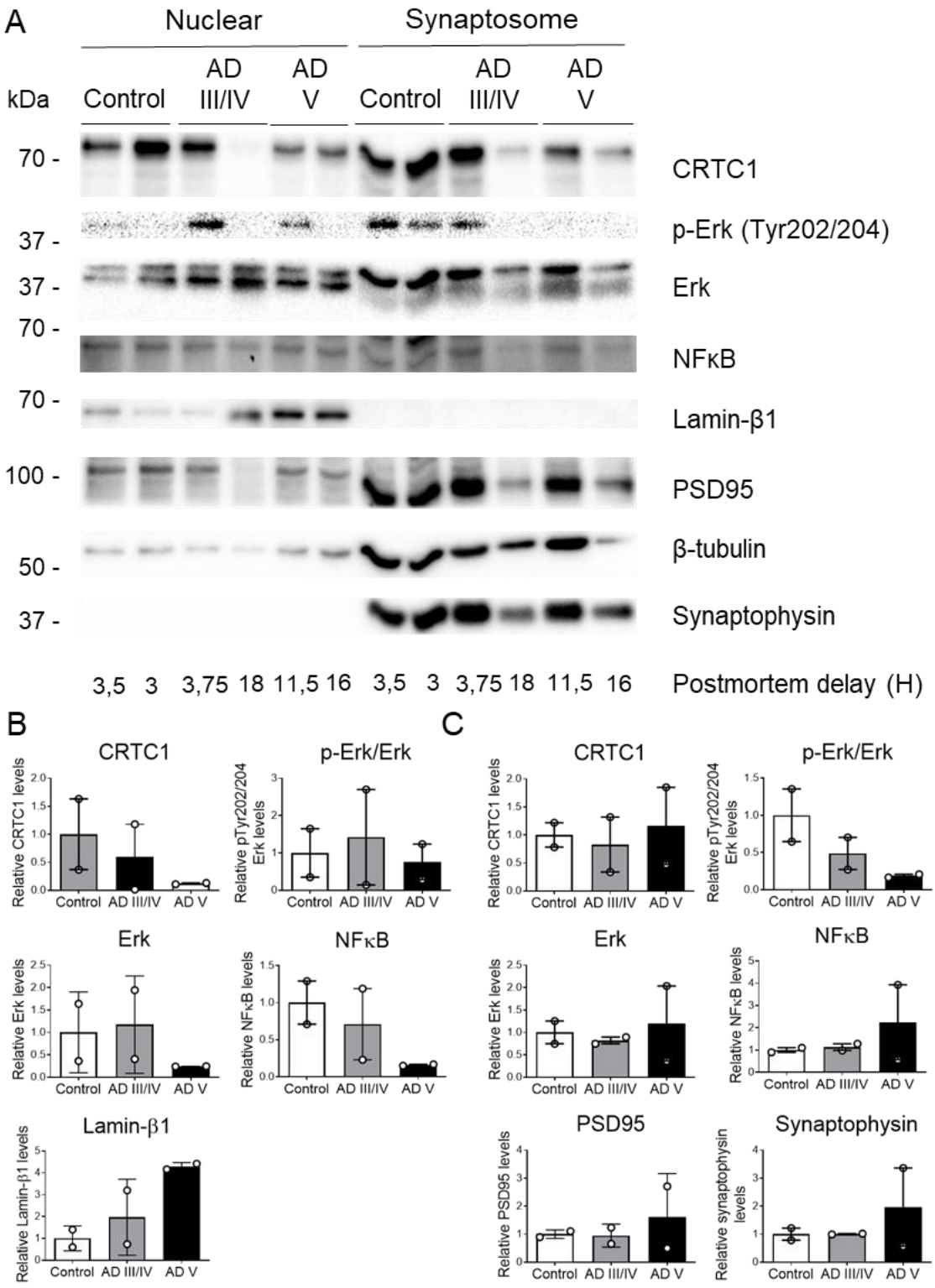


Figure 48. Altered synaptic proteins in nuclear and synaptosome fractions in human brain. (A) Western blot images of nuclear (left) and synaptosomal (right) fractions obtained from human parietal cortex of control, ADIII/IV and V. **(B)** Quantitative analysis of CRTC1, phosphorylated (Tyr202/204) and total Erk, NFκB and lamin-β1 levels in the nucleus. **(C)** Quantitative analysis of CRTC1, phosphorylated (Tyr202/204) and total Erk, NFκB and synaptic markers (PSD95 and synaptophysin) levels in synaptosome fraction. Values represent mean fold ± s.e.m. (n = 2 /group). Nuclear protein levels were normalized to lamin-β1 levels and synaptosomal protein levels to β-tubulin levels. Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests.

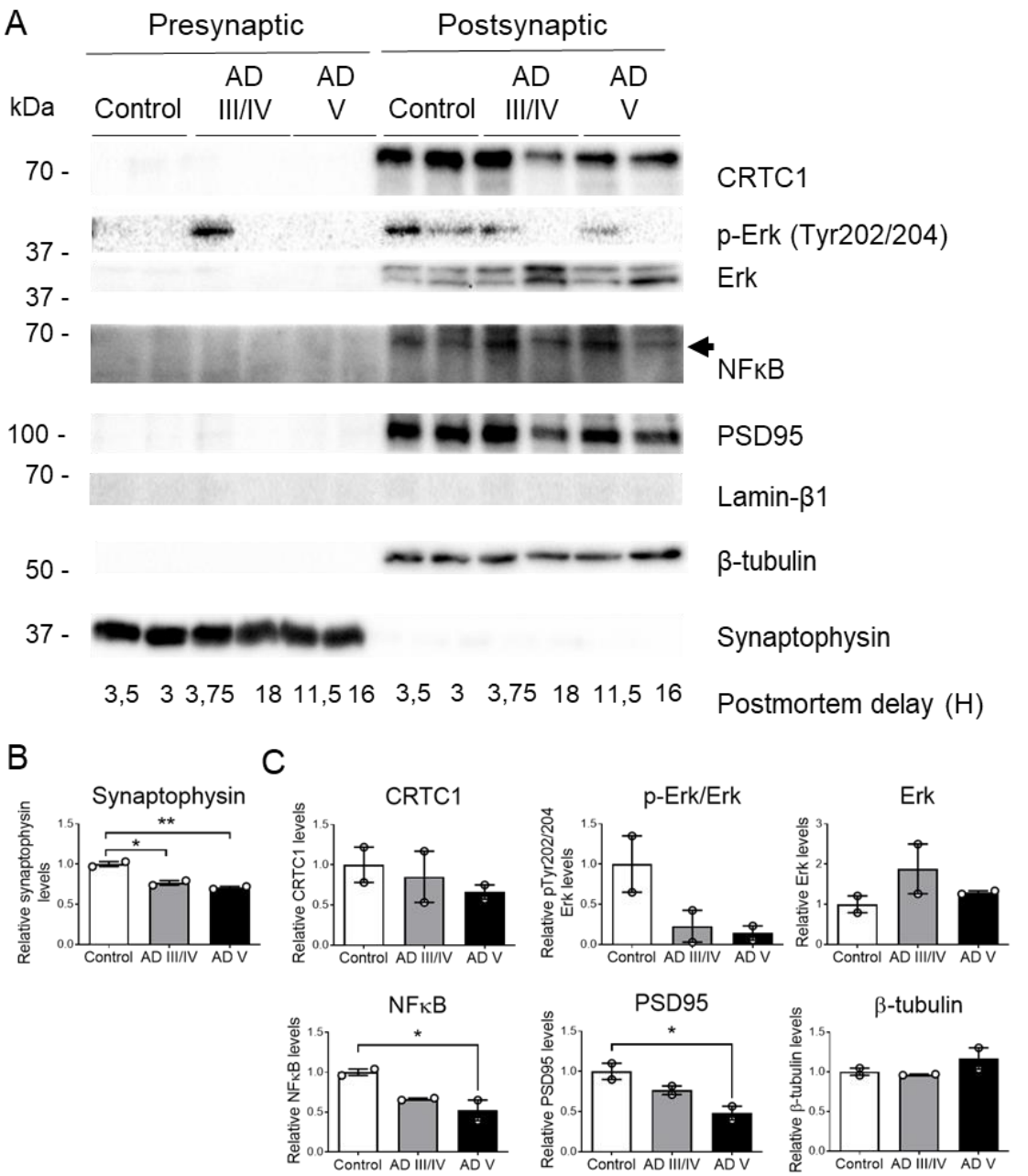


Figure 49. Synaptic alteration in pre and postsynaptic terminals of AD patients. (A) Western blot images of pre (left) and postsynaptic (right) fractions obtained from human parietal cortex of control and AD patients. **(B)** Quantitative analysis of synaptophysin levels in the presynaptic fraction. **(C)** Quantitative analysis of CRTC1, phosphorylated (Tyr202/204) and total Erk, NFκB, PSD95 and β-tubulin levels in postsynaptic terminal. Values represent mean fold ± s.e.m. (n = 2 /group). Postsynaptic protein levels were normalized to β-tubulin levels. Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01.

Finally, we evaluated the effects of PS on synaptic tau accumulation and synaptic pathology. We analyzed the levels of phosphorylated and total tau levels and pre- and postsynaptic markers in purified synaptosomes obtained from hippocampal lysates of 6 month-old WT, Tau, PS1 cKO;Tau and PS cDKO;Tau mice. We found a significant increase in phosphorylated and total tau levels (genotype effect, $P < 0.05$) and a decrease in PSD95 and syntaxin 1 (genotype effect, $P < 0.01$) in all tau transgenic groups compared to WT mice. Levels of the presynaptic marker synaptophysin were also reduced, especially in the PS cDKO;Tau mice (genotype effect, $P < 0.05$). Levels of the synaptonuclear factor CRTC1 tend to decrease in transgenic mice although was not significant ($P > 0.05$, **Fig. 50A**). Analysis of purified postsynaptic fractions revealed abnormal tau accumulation in Tau transgenic mice lacking PS1 or both PS (genotype effect, $P < 0.05$) and a tendency to decrease in synaptic markers, including PSD95 and β-actin. Notably, a young 3 month-old PS cDKO;Tau mice (indicated with #) did not show synaptic tau or changes in synaptic proteins (**Fig. 50B**). These results suggest that synaptic tau accumulation is associated with synaptic pathology, which is exacerbated by neuronal PS1 and PS deficiency.

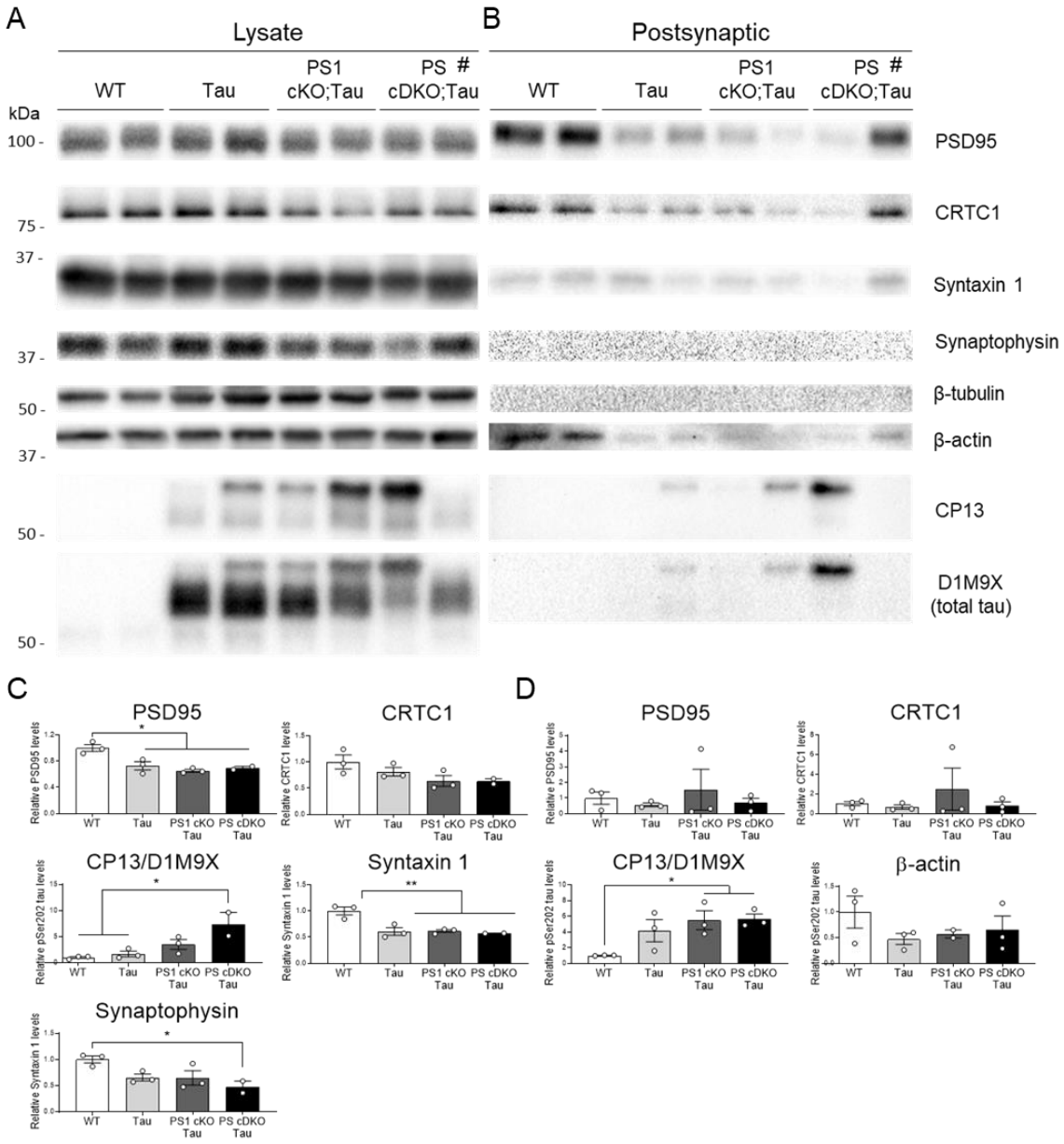


Figure 50. Loss of neuronal PS function in Tau mice increases synaptic tau accumulation and synaptic pathology. Western blot images of hippocampal lysates (**A**) and postsynaptic fraction (**B**) obtained from purified synaptosomes of 6 months-old WT, Tau, PS1 cKO;Tau and PS cDKO;Tau. Quantitative analysis (bottom) of total hippocampal lysates (**C**) and postsynaptic fraction (**D**). Levels of PSD95, CRTC1, phosphorylated (Ser202, CP13) and total tau (D1M9X), syntaxin 1, synaptophysin, β-actin and β-tubulin levels were analyzed. Values represent mean fold ± s.e.m. (n = 3 people/group). Protein levels of total lysates were normalized to β-tubulin levels. Statistical analysis was determined by one-way ANOVA followed by Tukey's post hoc tests. **P* < 0.05, ***P* < 0.01.

IX. Discussion

Loss of PS function leads to tau pathology and neurodegeneration

Tau pathology is the main pathological hallmark of distinct neurodegenerative tauopathies, including sporadic and familial AD. *PSEN1* mutations cause neurodegeneration and tauopathy in familial AD and FTD (Bernardi et al., 2009). The molecular and cellular mechanisms linking PS to tau pathology in neurodegeneration were largely unclear at the beginning of this doctoral thesis. This issue is important in dementia since unraveling the mechanistic link(s) between PS mutations and tau phosphorylation and aggregation are essential for developing new targets for tauopathies such as AD or FTD. In this doctoral thesis, I demonstrate the involvement of PS genes in tau homeostasis, including regulation of tau phosphorylation, aggregation and elimination.

Previous studies from our lab revealed that genetic PS inactivation in mice causes tau hyperphosphorylation, inflammation, synaptic loss, neurodegeneration and learning and memory deficits (Beglopoulos et al., 2004; Saura et al., 2004). PS deficiency triggers presynaptic impairment and postsynaptic dysfunction, oxidative damage and age-related neuropsychiatric symptoms (Zhang et al., 2009; Zhang et al., 2010; Yan et al., 2013). In this doctoral thesis, we replicated some of these results, and found that loss of neuronal PS function in adult mice leads to postural and behavioral alterations associated with brain atrophy indicative of severe neurodegeneration. Importantly, neuronal PS deficiency causes age-dependent neurodegeneration in the cortex and hippocampus, which is associated with progressive tau phosphorylation and aggregation. Biochemical and imaging analyses revealed increased phosphorylated tau at different residues in different brain regions in PS cDKO mice. Tau pathology was restricted to specific brain areas related to memory formation and consolidation and affected in AD patients, including the hippocampus and EC and RSC (Braak and Braak, 1991; Braak et al., 2006; Serrano-Pozo et al., 2011). On the contrary, PS1 cKO and PS2 deficient mice do not apparently show tau pathology or neuroinflammation in the cortex or the hippocampus of 9 months. These results suggest that PS1 and PS2 can share some important functions or compensate each other so that the presence of one PS can overcome the effects of the absence of the other at early stages (Lai et al., 2003; Lessard et al., 2019; Pimenova and Goate, 2020).

In contrast to endogenous murine tau, neuronal PS1 or PS1/PS2 inactivation in human Tau expressing mice results in increased tau phosphorylation and aggregation in memory-associated circuits affected early in AD. However, it is possible that the presence of a more pathogenic tau breaks the homeostatic mechanisms anticipating

the pathological features in PS1 cKO;Tau mice. In agreement, and in contrast to PS1 cKO mice, PS1 cKO mice lacking one PS2 allele develop marked neurodegeneration at 16 months of age (Watanabe et al., 2014). PS1 mutations accelerate and potentiate tau pathology compared to sporadic AD cases (Gómez-Isla et al., 1999; Woodhouse et al., 2009), so it is possible that FAD- or FTD-linked PSEN1 mutations can lead to tau phosphorylation and accumulation through a partial PS loss of function mechanism. It is intriguing that familial PS1 mutations cause cerebral tau accumulation in the presence or absence of Aβ deposits in familial AD and FTD, respectively (Hutton, 2004; Larner and Doran, 2006). How pathological *PSEN* mutations differentially affect Aβ generation/accumulation whereas having a similar effect on tau phosphorylation and aggregation is unclear. It is possible that both types of mutations affect similar cellular or molecular mechanisms leading to tau phosphorylation/aggregation, whereas only FAD-linked PS mutations affect APP processing increasing the Aβ42/40 ratio (Kumar-Singh et al., 2006; Sun et al., 2017). Particularly, the *PSEN1* L435F mutation decreases total Aβ while enhancing Aβ42/Aβ40, amyloid deposition and tau phosphorylation causing synaptic deficits and neurodegeneration in mice (Xia et al., 2015). Whether mutant PS1 affects tau pathology through Aβ or other cellular effectors is still unclear. Nonetheless, our results showing increased phosphorylated and aggregated tau in PS1 and PS-deficient Tau mice indicate that PS affects tau pathology independently of Aβ.

Previous studies from our lab demonstrated that transcriptome dysregulation and memory impairment in PS cDKO mice occur via CRTC1 deregulation (Parra-Damas et al., 2017). Here, we show that loss of PS function in mice results in hippocampal memory and learning deficits and in anticipated and exacerbated memory impairments in human tau transgenic mice. Interestingly, PS1 cKO mice do not present memory impairment but showed increased higher levels of freezing compared to control mice in CFC, suggesting an anxious-related behavior. Interestingly, AD patients show early neuropsychiatric symptoms, including anxiety, related to tau pathology rather than amyloid load or neurodegeneration in parietal, temporal and frontal lobes, and these symptoms are related to tau-dependent affectation of AD-vulnerable areas, including the amygdala among others (Tissot et al., 2021). Here, we have reported that although PS1 cKO mice do not show tau pathology, PS1 cKO;Tau mice showed increased tau phosphorylation and aggregation, especially in the amygdala. As anxiety is an early AD clinical symptom, this result suggests the involvement of PS1 in emotion, whereas PS deficiency may result in anxiety linked to tau pathology in the amygdala.

Interestingly, mice harboring FAD-linked PS1 mutants or lacking PS develop neuropsychiatric symptoms, including irritability, apathy, depression, aggressiveness or anxiety (España et al., 2010). This phenotype is not present in other AD mice models such as 5xFAD, P301L tau or APP/PS1 mice increasing the implication of PS-dependent tau pathology in AD-related neuropsychiatric symptoms (Yan et al., 2013; Ortega-Martinez et al., 2019).

Mechanisms regulating PS-dependent tau phosphorylation

Which are the mechanisms of PS-dependent tau phosphorylation? Our results show that neuronal PS deficiency elevates p25 levels and Akt phosphorylation, two signaling mechanisms involved in tau phosphorylation. Indeed, loss of PS increases phosphorylation of tau at Ser/Thr epitopes phosphorylated by Cdk5 and GSK3β, which are recognized by AT8, CP13 and PHF-1 antibodies. Increased levels of p25, a physiological activator of Cdk5, increases tau phosphorylation in AD (Patrick et al., 1999; Seo et al., 2017), suggesting that increased Cdk5 activity could mediate tau phosphorylation in PS-deficient mice. Alternatively, mutant PS1 increases GSK3β-mediated tau phosphorylation (Takashima et al., 1998; Dewachter et al., 2008). It is plausible that PS1 deficiency could potentially affect GSK3β-mediated tau phosphorylation. However, activation of Akt usually decreases GSK3β activity, as revealed by increased phosphorylated inactive GSK3β (Ser 9), although this residue is also a physiological substrate of PKA (Jope and Johnson, 2004). In physiological conditions, PS seems to tightly control tau phosphorylation by maintaining a correct balance between GSK3β activation and Cdk5 inactivation. In pathological conditions such as those occurring in the absence of neuronal PS, it is possible that dysregulation of Cdk5 and GSK3β could lead tau phosphorylation and neurodegeneration.

Remarkably, PS1 and PS-deficient tau transgenic mice show elevated levels of PKA/AMPK-mediated tau phosphorylation at Ser409. This phosphorylation in tau is present in axons but not cell bodies or dendrites of neurons at early developmental stages (Postnatal day 1-11) but not in mouse and human adult brains (Andorfer and Davies, 2000). By contrast, phosphorylated tau at Ser409 is found in AD patients preceding or coinciding with initial tau aggregation, which suggested PKA-mediated tau phosphorylation at the early stages of tau pathology (Jicha et al., 1999). Indeed, tau phosphorylation at Ser409 was present mainly in human Tau transgenic lines lacking PS1 or both PS1/PS2 genes but they are absent in WT, PS1 cKO and PS1 cKO mice, and only slightly increased in PS cDKO mice. This result demonstrates that PS1 and total PS deficiency cause an increase in PKA-mediated tau phosphorylation. In support

of this idea, FAD-linked PS1 mutations and PS1 genetic inactivation promote neurite outgrowth by enhancing cAMP/PKA signaling (Deyts et al., 2016). An alternative mechanism of PKA-dependent tau phosphorylation could involve calcium since a recent study reported that calcium dysregulation mediates PKA-induced tau hyperphosphorylation (Carlyle et al., 2014; Datta et al., 2021). Due to the reported calcium homeostasis dysregulation caused by FAD-linked PS1 and PS2 mutations (Deaton and Johnson, 2020; Pizzo et al., 2020), loss of PS function could induce PKA-mediated tau phosphorylation by exacerbating age-related calcium disturbance. In agreement, PS regulates mitochondrial Ca^{2+} homeostasis and modulates intracellular Ca^{2+} release in presynaptic terminals (Zhang et al., 2009b; Lee et al., 2017). Notably, FAD-linked PS mutations, such as *PSEN1* M146L and *PSEN2* N141I have shown to increase Ca^{2+} signaling and endoplasmic reticulum release through the interaction with the inositol 1,4,5-triphosphate receptor (Cheung et al., 2008). It would be interesting to measure the intracellular Ca^{2+} levels in neurons of PS-deficient mice and its correlation with PKA-mediated Ser409 tau phosphorylation levels. Alternatively, AMPK also phosphorylates tau at Ser409 in a lesser extent in AD and other tauopathies (Vingtdeux et al., 2011). Interestingly, PS deficient mice show increased phosphorylated Thr172 AMPK suggesting increased AMPK activity that could result as well in tau phosphorylation at Ser409. By contrast, AMPK deficiency in P301S tau transgenic mice results in reduced tau pathology suggesting that targeting abnormal AMPK activation could represent a good strategy to reduce tau pathology (Domise et al., 2016).

Phosphorylated tau accumulates in neurons and glial cells

Tau pathology in PS cDKO mice is accompanied by brain inflammation as revealed by increased inflammatory astrocytic and microglia markers. It is interesting that, despite restricting PS1 inactivation to glutamatergic neurons, tau pathology is also accumulated in microglia, astrocytes and oligodendrocytes of PS cDKO mice. Microglial and astrocytic tau accumulation occurs in the aging brain and in neurodegenerative diseases such as AD (Kovacs et al., 2017; Perea et al., 2018). Possibly, this process is due to neuronal tau release or tau-mediated neuronal death leading to tau uptake by glial cells. Recently, internalization of extracellular tau by microglia and astrocytes has been reported (Bolós et al., 2017; Martini-Stoica et al., 2018). Moreover, crosstalk between astrocytic complement C3 and microglial receptor C3aR mediates neuronal tau pathology in AD and P301S tau transgenic mice (Litvinchuk et al., 2018). The consequences of tau pathology in glia are still unclear, but it seems that they can contribute to glial degeneration, tau spreading and synapse dysfunction (Higuchi et al., 2002; Piacentini et al., 2017; Martini-Stoica et al., 2018). AD

patients show tau accumulation in astrocytes of the dentate gyrus, and overexpression of tau in astrocytes of the dentate gyrus causes neuron and synapse loss and memory impairments (Richetin et al., 2020). It will be important to determine the mechanisms of tau accumulation in glia and its effects on AD and FTD pathology. On the other hand, increased microglia activation in PS cDKO mice occurs at the beginning of tau pathology (6 months), suggesting that microglia can have a role triggering tau pathology and then becomes inefficient for tau elimination and degenerates or does not proliferate more at latter stages, while control mice show an age-dependent increase in Iba1 levels. On the other hand, the increase in astrocytic activation correlates with age-dependent tau pathology and degeneration suggesting that astrocyte can either be implicated in the pathology initiation and/or progression or have a neuroprotective role against tau pathology. Further studies are needed to unravel the beneficial and/or detrimental role of glial cells in tau pathology.

PS regulates tau and neurofilament cytoskeleton structures

Our synchrotron-based infrared microspectroscopy assays revealed intermolecular and antiparallel β-sheet structures in the corpus callosum of PS-deficient tau mice. These abnormal protein structures seem to be related to tau aggregates as histological analysis of the consecutive slice confirmed tau aggregation. Besides these protein biochemical changes, control and AD mice model show an unexpected similar biochemical profile in physiological parameters. On the other hand, the biochemical profile of the white matter (corpus callosum) and the gray matter (cortex) was completely different as recently reported (Sanchez-Molina et al., 2020). Synchrotron-based infrared microspectroscopy was previously used for amyloid plaques detection and tau aggregates (Leskovjan et al., 2011; Miller et al., 2013; Benseny-Cases et al., 2018). However, tau fibrils aggregation by infrared microspectroscopy was detected *in vitro* using purified PHFs but not in human or animal models tissue samples (Mandelkow et al., 1995; von Bergen et al., 2005; Ramachandran, 2016). Importantly, we detected intermolecular and antiparallel β-sheet structures, especially in the corpus callosum. Although, synchrotron infrared microspectroscopy is not probably the best method to detect PHF *in vivo*, we were able to detect a protein aggregation pattern possibly related to tau protein.

The cytoskeleton neurofilament (NF) light chain was recently proposed as a promising biomarker for several neurodegenerative diseases (Zetterberg, 2016). Increased cerebrospinal fluid (CSF)/serum neurofilament light levels correlates with cerebral phosphorylated tau, neurofibrillary tangles and neurodegeneration, but not with Aβ42

levels, in AD and FTD (Rohrer et al., 2016; Sánchez-Valle et al., 2018; Ashton et al., 2019). Furthermore, pre-symptomatic PS1-FAD or FTD patients show significant increased CSF/serum neurofilament light levels correlating with clinical progression and, more importantly, predicting the onset of clinical symptoms (Sanchez-Valle et al., 2018; Preische et al., 2019). Despite the close association of pathological tau and neurofilament light chain in biological fluids of PS mutant carriers, the relationship between PS, tau and neurofilament changes is currently not clear. Notably, PS1 regulates neurofilament assembly, whereas FAD-linked PS1 mutations cause aberrant neurofilament structures associated with tau phosphorylation and release from microtubules (Dowjat et al., 2001; Pigino et al., 2001; Woodhouse et al., 2009). Similarly, we demonstrate that loss of PS function in mice causes reduced hippocampal NF levels and abnormal pattern of staining or aggregation of neurofilament light, medium and heavy chains, especially in tau pathology-affected areas such as the amygdala or the hippocampus. These results indicate that, similar to FAD-PS1 mutations, PS deficiency can cause aberrant cerebral NF accumulation and reduced hippocampal NF levels. Thus, increased NF secretion to CSF and serum could represent a good biomarker for tauopathies (Sanchez-Valle et al., 2018; Preische et al., 2019). It would be interesting in the future to analyze NF levels in the CSF and serum of PS deficient mice and its correlation with age-dependent tau pathology and neurodegeneration.

PS regulates autophagy-mediated tau elimination

Neurodegenerative diseases are characterized by abnormal accumulation of aggregated proteins in specific cells and brain regions (Dugger and Dickson, 2017). Intracellular tau degradation includes proteasomal and autophagic processes (Boland et al., 2018). Growing evidence points out the relationship between altered autophagy and neurodegeneration. Autophagy dysregulation is related to the initiation and the progression of tauopathies such as AD and FTD (Nixon et al., 2005; Ghavami et al., 2014). Of note, autophagy induction proteins are reduced during aging and neurodegenerative diseases (Lipinski et al., 2010), different tau species are eliminated by selective autophagy (Falcon et al., 2018; Fu et al., 2019) and recent genetic AD risk factors, such as PICALM/CALM, have been identified (Moreau et al., 2014).

In AD and FTD, tau pathology mainly occurs in excitatory neurons of memory-related circuits, but the mechanisms of this vulnerability are not fully understood (Spina et al., 2008). Some studies have suggested differential factors such as cell size and location, calcium signaling, energy homeostasis, metabolism of disease-specific proteins,

mechanisms of stress resistance and protein homeostasis dysfunction (Kaushik and Cuervo, 2015; Freer et al., 2016). Interestingly, inhibitory neurons seem to have more effective mechanisms of tau clearance (Fu et al., 2019). Thus, the understanding of the mechanisms that leads to excitatory neurons vulnerability and inhibitory neurons resistance, such as autophagy disparity, is necessary for the development of novel therapeutic strategies based on cell-specific stimulation of intrinsic defense mechanism for neurodegenerative diseases.

Previous reports have suggested that PS play an important role on autophagy, especially, regulating autophagy/lysosomal genes expression and maintaining lysosomal acidification and fusion with autophagosome (Lee et al., 2010; Neely et al., 2011; Hung and Livesey, 2018). In the present thesis, we demonstrate that PS maintains autophagy induction by inhibiting mTORC1 through increased activation of AMPK and Akt. This result supports the reduction of mTORC1 and its complex proteins and its excessive attachment to lysosomes in PS-deficient fibroblast (Reddy et al., 2016). In agreement with previous reports (Lee et al., 2010; Neely et al., 2011), we found increased autophagosome number due to increased LC3 ratio and p62 levels in PS cDKO mice and PS deficient neurons, suggesting altered autophagic flux caused by loss of PS function. Interestingly, PS1 mutations impair autophagosome-lysosome fusion, lysosomal acidification and reduce autophagy-related genes expression (Dobrowolski et al., 2012; Chong et al., 2018). In agreement with PS1 involvement on pathological protein elimination by autophagy, PS1 promotes autophagic βCTF elimination (Bustos et al., 2017) and FAD-linked PS1 mutations lead to Aβ accumulation in iPSCs-derived neurons (Peric and Annaert, 2015; Hung and Livesey, 2018). Interestingly, we could not find a significant alteration in the cortical autophagosome number neither in PS1 cKO nor $PS2^{-/-}$ mice at 9 months of age. However, the LC3 ratio tends to increase in the hippocampal lysates of PS1 cKO and $PS2^{-/-}$ compared to WT mice suggesting a PS dose-dependent effect on autophagy.

Importantly, PS-dependent autophagy disruption causes abnormal tau accumulation and increased tau secretion (Mohamed et al., 2014). We have shown that either stimulation or inhibition of autophagy, even with simultaneous proteasomal inhibition, strongly reduce hyperphosphorylated and total tau levels in PS-deficient neurons through likely increased tau secretion and boosting tau propagation. Notably, this may have important biological and therapeutic consequences.

In agreement to the important role of autophagy in tau pathology, activators of the autophagy pathway reduce pathological tau progression and toxicity in cellular and tau

transgenic mouse models (Kruger et al., 2012; Schaeffer et al., 2012; Ozcelik et al., 2013; Silva et al., 2020). Moreover, autophagy disruption caused by p300/CREB binding protein (CBP) inhibition promotes tau release and propagation in neurons (Chen et al., 2020). Considering that PS tightly controls CBP levels and activity (Marambaud et al., 2003; Saura et al., 2004), it is plausible that PS could regulate autophagy-dependent tau degradation via CBP.

In summary, these findings strength the role of PS on autophagy by strongly suggest that loss of PS function (e.g., familial AD-linked mutations) alters autophagy function triggering tau accumulation aggregation and/or secretion**.**

PS deficiency regulates tau secretion and propagation

There is much debate about whether cell tau transmission is a physiological mechanism or is only present in pathological conditions. The identification of the transmission of non-hyperphosphorylated or non-misfolding tau suggests that tau trans-synaptic transference can be a physiological mechanism (Dujardin et al., 2018). In addition, whether physiological trans-synaptic tau transference share the same or different mechanisms than pathological tau. Thus, tau secretion by exosomes appears to occur only with aggregated tau (Dujardin et al., 2014). Furthermore, tau uptake is mostly restricted to aggregated forms (Mirbaha et al., 2015). However, some studies have shown that secreted tau is mostly monomeric, soluble, dephosphorylated and truncated suggesting physiological secretion of tau. In agreement to physiological tau secretion, tau can be detected in the CSF of healthy human (Hall et al., 2012). Clarifying the physiological and pathological tau secretion and uptake mechanisms, which have an important role in tau spreading, will facilitate the development of promising novel treatment therapies for tauopathies.

Brain injections of purified tau oligomers from AD human or mice model have been shown to induce tau accumulation and propagation in transgenic and control mice (Clavaguera et al., 2009; Lasagna-Reeves et al., 2012). Surprisingly, intraperitoneally injection of aggregated tau causes brain tau pathology (Clavaguera et al., 2014b). However, some reports show that tau oligomers are more pathogenic forms than tau aggregates (Sanders et al., 2014; Gerson et al., 2016). Thus, the pathogenic potential of each tau form remains controversial. Here, we have shown increased memory deficits in PS cDKO mice after hippocampal injection of human purified PHF. However, there were no differences between PHF- and vehicle-injected WT mice. Furthermore, we could not see any significant increase in tau pathology or propagation after PHF

injection, probably due to the elimination of the injected PHF, a problem with the sample procedure and/or the experimental design.

Nutrient deprivation has shown to promote autophagy in several tissues, including the brain (Kuma et al., 2004; Mizushima et al., 2004). Therefore, we perform nutrient deprivation in cultured primary neurons to enhance autophagy. Interestingly, neurons show a strong reduction in phosphorylated and total levels despite no LC3-II/I major changes. Contrast to what we expected, autophagy inhibition with chloroquine also shows reduced phosphorylated and total tau levels either in presence or absence of the proteasomal inhibitor MG132. These results suggest the involvement of other mechanism in tau decrease such as tau secretion as it is reported to be increased by lysosomal inhibition and starvation in primary cortical neurons (Mohamed et al., 2014). We hypothesize that PS deficiency alters autophagy reducing tau elimination and, very likely, promoting tau secretion and/or propagation. Thus, it will be necessary to analyze the levels of tau in the medium of PS-deficient neurons in basal and treatment conditions.

Synaptic tau accumulation in AD brain and Tau and PS-deficient Tau mice

Pathological tau causes synaptic dysfunction through different mechanisms, including altered dendritic spines, LTP impairment or decreased levels of PSD-95 and glutamate receptors (Yoshiyama et al., 2007; Hoover et al., 2010; Crimins et al., 2012; Warmus et al., 2014). Thus, tau pathology triggers loss of synapsis causing memory impairments in tauopathies mouse models. Other studies have focused on synaptonuclear factors that have local functions at the synapsis and translocate to the nucleus promoting gene expression (Parra-Damas and Saura, 2019). However, the role of tau pathology on synaptonuclear factors are still unexplored. Here, we have shown synaptic tau accumulation in purified hippocampal synaptosomes of Tau transgenic mice. The consequences of tau accumulation may be synapse dysfunction or loss as reported (Ittner et al., 2010; Pickett et al., 2019). In correlation with previous data, we have seen that tau pathology mediates the reduction of pre- and postsynaptic markers such as synaptophysin and PSD95, respectively (Yoshiyama et al., 2007; Jackson et al., 2017; Dejanovic et al., 2018). Interestingly, tau pathology reduces synaptic CRTC1 levels in tau transgenic mice whereas CRTC1, Erk and NFκB are also reduce in synaptosomes obtained from AD patient's brain. Finally, PS1/PS deficiency on tau transgenic mice exacerbates the synaptic tau pathology and synapse pathology increasing the evidence of the correlation of tau, synaptic loss and memory impairments. Hence, more investigation of the pathological effects on synapsis and specifically, on synaptonuclear

factors is needed to decipher the synaptic alteration and memory impairments caused by tau pathology. Furthermore, it would be interesting to investigate the effects of PS FAD-linked mutations on synaptic alteration and its contribution to memory loss using FAD brain samples.

In summary, the data presented in this thesis strengthen the effects of PS deficiency on (1) dysregulation of GSK3β, PKA and Cdk5 kinases, resulting in increased phosphorylated tau levels, (2) alteration of autophagy though mTOR inhibition, increased autophagosome accumulation due to inhibition of lysosomal/autophagosome fusion, triggering tau aggregation and secretion (**Fig. 51A**), (3) synaptic tau accumulation mediating synaptic loss, neuroinflammation, neurodegeneration and memory impairments (**Fig. 51B**).

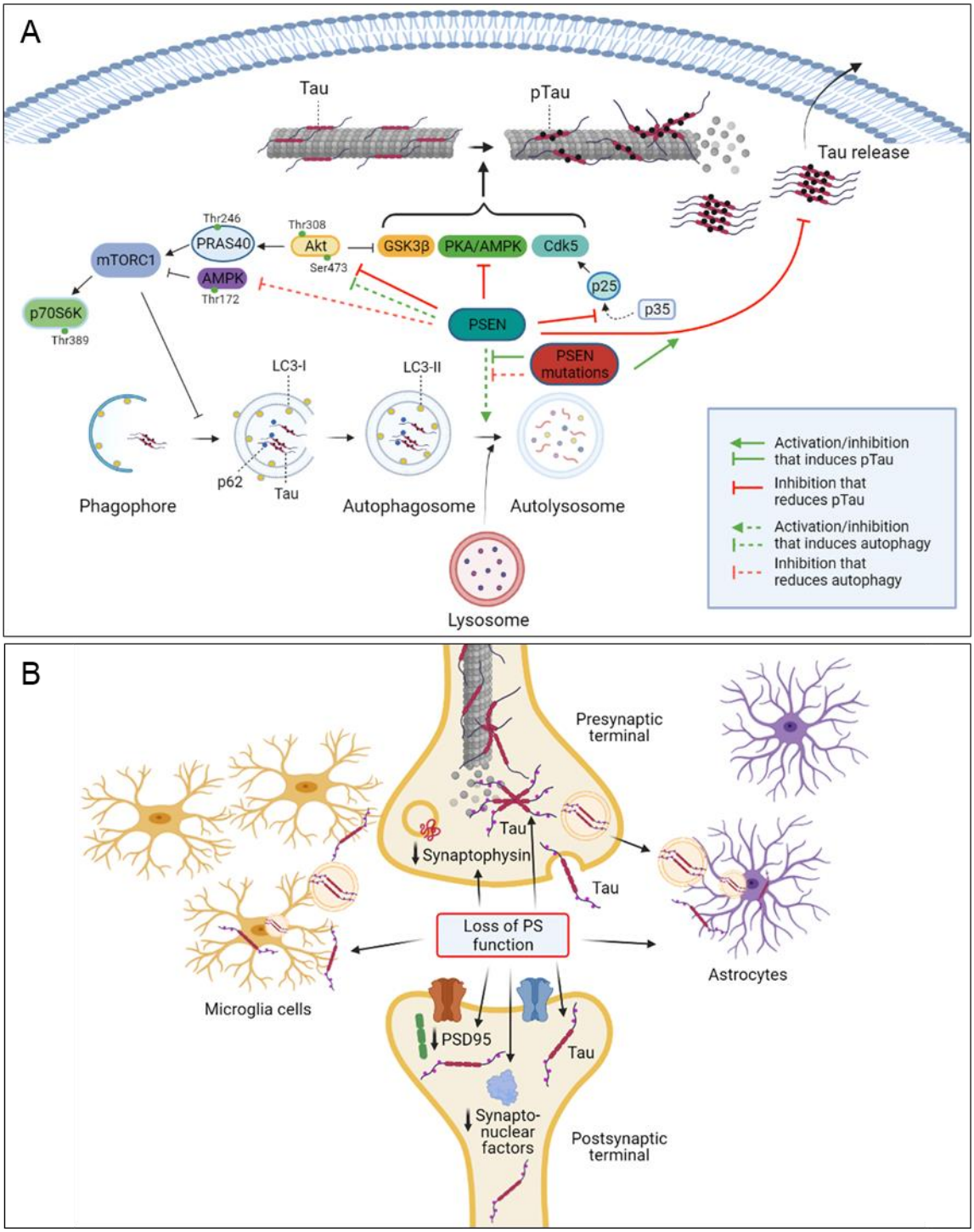


Figure 51. (**A**) **Proposed model of PS/γ-secretase-dependent regulation of autophagy and tau pathology.** PS regulates tightly tau kinases (Cdk5, PKA and GSK3β) maintaining basal tau levels. PS inhibit phosphorylation of Akt and reduces AMPK activation regulating mTORC1 activity. PS is required for a proper autophagy flux and tau elimination and secretion. By contrast, PS deficiency dysregulates tau kinases activity, increasing phosphorylated tau levels, and autophagy pathway and disrupts autophagosome-lysosome fusion causing increased phosphorylated and aggregated tau and, probably, increasing tau secretion. (**B**) **Hypothetical model of PS-dependent regulation of tau-mediated inflammation, synaptic function and neurodegeneration.** Lack of PS causes abnormal tau accumulation at synaptic terminals decreasing pre- and postsynaptic proteins (synaptophysin, PSD95…) and synaptonuclear factors (CRTC1) which is associated with cerebral inflammation, synaptic loss and exacerbated memory impairments.

X. Conclusions

- Loss of presenilin function in neurons results in age-dependent neurodegeneration, behavioral abnormalities and tau pathology in memory-related brain regions.

- Genetic inactivation of both presenilin genes in glutamatergic neurons triggers neuroinflammation and accumulation of phosphorylated tau in neurons, microglia, oligodendrocytes and astrocytes.

- Presenilins repress cerebral tau pathology, whereas partial and total loss of presenilin function accelerates human tau phosphorylation and aggregation in soma and dendrites of neurons in memory-related brain regions coinciding with exacerbated memory deficits.

- Presenilins regulate tau kinases, so its disruption increases Cdk5- and PKA/AMPK-mediated tau phosphorylation.

- Synchrotron infrared microspectroscopy demonstrates intermolecular and antiparallel β-sheet protein structures in amyloid plaque-free transgenic mice associated with tau and neurofilament aggregates

- Stimulation and inhibition of autophagy decrease intracellular tau levels *in vitro* suggesting that autophagy regulates tau secretion and/or propagation.

- Presenilins contributes to tau elimination and secretion through autophagy flux regulation by blocking mTORC1 and activating autophagosome-lysosome fusion.

- Synaptic pathology is associated with accumulation of synaptic tau in the hippocampus of Tau transgenic mouse brains.

- Neuronal PS deficiency in Tau transgenic mice exacerbates synaptic tau accumulation and synaptic pathology linking PS dependent-tau pathology, synaptic loss and hippocampal memory impairments.

XI. Bibliography

2020 Alzheimer's disease facts and figures. Alzheimers Dement. 2020;10.1002/alz.12068. doi:10.1002/alz.12068.

A Armstrong R. Risk factors for Alzheimer's disease. Folia Neuropathol. 2019;57(2):87-105. doi:10.5114/fn.2019.85929.

Ahmed T, Blum D, Burnouf S, Demeyer D, Buée-Scherrer V, D'Hooge R, Buée L, Balschun D. Rescue of impaired late-phase long-term depression in a tau transgenic mouse model. Neurobiol Aging. 2015;36(2):730-739. doi:10.1016/j.neurobiolaging.2014.09.015.

Ahn K, Shelton CC, Tian Y, Zhang X, Gilchrist ML, Sisodia SS, Li YM. Activation and intrinsic gamma-secretase activity of presenilin 1. Proc Natl Acad Sci U S A. 2010;107(50):21435-21440. doi:10.1073/pnas.1013246107.

Alavi Naini SM, Soussi-Yanicostas N. Tau Hyperphosphorylation and Oxidative Stress, a Critical Vicious Circle in Neurodegenerative Tauopathies?. Oxid Med Cell Longev. 2015;2015:151979. doi:10.1155/2015/151979.

Albert M, Mairet-Coello G, Danis C, Lieger S, Caillierez R, Carrier S, Skrobala E, Landrieu I, Michel A, Schmitt M, Citron M, Downey P, Courade JP, Buée L, Colin M. Prevention of tau seeding and propagation by immunotherapy with a central tau epitope antibody. Brain. 2019;142(6):1736-1750. doi:10.1093/brain/awz100.

Andorfer CA, Davies P. PKA phosphorylations on tau: developmental studies in the mouse. Dev Neurosci. 2000;22(4):303-309. doi:10.1159/000017454.

Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, Wolozin B, Butovsky O, Kügler S, Ikezu T. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci. 2015;18(11):1584-1593. doi:10.1038/nn.4132.

Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobágyi T, Höglund K, Aarsland D, Lovestone S, Schöll M, Blennow K, Zetterberg H, Hye A. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. Acta Neuropathol Commun. 2019;7(1):5. Published 2019 Jan 9. doi:10.1186/s40478-018-0649-3.

Avale ME, Rodríguez-Martín T, Gallo JM. Trans-splicing correction of tau isoform imbalance in a mouse model of tau mis-splicing. Hum Mol Genet. 2013;22(13):2603-2611. doi:10.1093/hmg/ddt108.

Baborie A, Griffiths TD, Jaros E, McKeith IG, Burn DJ, Richardson A, Ferrari R, Moreno J, Momeni P, Duplessis D, Pal P, Rollinson S, Pickering-Brown S, Thompson JC, Neary D, Snowden JS, Perry R, Mann DM. Pathological correlates of frontotemporal lobar degeneration in the elderly. Acta Neuropathol. 2011;121(3):365-371. doi:10.1007/s00401-010-0765-z.

Baker MJ, Trevisan J, Bassan P, hargava R, Butler HJ, Dorling KM, Fielden PR, Fogarty SW, Fullwood NJ, Heys KA, Hughes C, Lasch P, Martin-Hirsch PL, Obinaju B, Sockalingum GD, Sulé-Suso J, Strong RJ, Walsh MJ, Wood BR, Gardner P, Martin FL. Using Fourier transform IR spectroscopy to analyze biological materials. Nat Protoc. 2014;9(8):1771-1791. doi:10.1038/nprot.2014.110.

Baker S, Polanco JC, Götz J. Extracellular Vesicles Containing P301L Mutant Tau Accelerate Pathological Tau Phosphorylation and Oligomer Formation but Do Not Seed Mature Neurofibrillary Tangles in ALZ17 Mice. J Alzheimers Dis. 2016;54(3):1207-1217. doi:10.3233/JAD-160371.

Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci. 2007;8(9):663-672. doi:10.1038/nrn2194.

Bancher C, Lassmann H, Budka H, Grundke-Iqbal I, Iqbal K, Wiche G, Seitelberger F, Wisniewski HM. Neurofibrillary tangles in Alzheimer's disease and progressive supranuclear palsy: antigenic similarities and differences. Microtubule-associated protein tau antigenicity is prominent in all types of tangles. Acta Neuropathol. 1987;74(1):39-46. doi:10.1007/BF00688336.

Bang J, Spina S, Miller BL. Frontotemporal dementia. Lancet. 2015;386(10004):1672-1682. doi:10.1016/S0140-6736(15)00461-4.

Barten DM, Cadelina GW, Hoque N, DeCarr LB, Guss VL, Yang L, Sankaranarayanan S, Wes PD, Flynn ME, Meredith JE, Ahlijanian MK, Albright CF. Tau transgenic mice as models for cerebrospinal fluid tau biomarkers. J Alzheimers Dis. 2011;24 Suppl 2:127-141. doi:10.3233/JAD-2011-110161.

Beglopoulos V, Sun X, Saura CA, Lemere CA, Kim RD, Shen J. Reduced beta-amyloid production and increased inflammatory responses in presenilin conditional knock-out mice. J Biol Chem. 2004;279(45):46907-46914. doi:10.1074/jbc.M409544200.

Bendiske J, Bahr BA. Lysosomal activation is a compensatory response against protein accumulation and associated synaptopathogenesis--an approach for slowing Alzheimer disease?. J Neuropathol Exp Neurol. 2003;62(5):451-463. doi:10.1093/jnen/62.5.451.

Benseny-Cases N, Álvarez-Marimon E, Castillo-Michel H, Cotte M, Falcon C, Cladera J. Synchrotron-Based Fourier Transform Infrared Microspectroscopy (μFTIR) Study on the Effect of Alzheimer's Aβ Amorphous and Fibrillar Aggregates on PC12 Cells. Anal Chem. 2018;90(4):2772-2779. doi:10.1021/acs.analchem.7b04818.

Bernardi L, Tomaino C, Anfossi M, Gallo M, Geracitano S, Costanzo A, Colao R, Puccio G, Frangipane F, Curcio SA, Mirabelli M, Smirne N, Iapaolo D, Maletta RG, Bruni AC. Novel PSEN1 and PGRN mutations in early-onset familial frontotemporal dementia. Neurobiol Aging. 2009;30(11):1825-1833. doi:10.1016/j.neurobiolaging.2008.01.005.

Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev. 2006;(1):CD005593. Published 2006 Jan 25. doi:10.1002/14651858.CD005593.

Boland B, Yu WH, Corti O, Mollereau B, Henriques A, Bezard E, Pastores GM, Rubinsztein DC, Nixon RA, Duchen MR, Mallucci GR, Kroemer G, Levine B, Eskelinen EL, Mochel F, Spedding M, Louis C, Martin OR, Millan MJ. Promoting the clearance of neurotoxic proteins in neurodegenerative disorders of ageing. Nat Rev Drug Discov. 2018;17(9):660-688. doi:10.1038/nrd.2018.109.

Bolós M, Llorens-Martín M, Jurado-Arjona J, Hernández F, Rábano A, Avila J. Direct Evidence of Internalization of Tau by Microglia In Vitro and In Vivo. J Alzheimers Dis. 2016;50(1):77-87. doi:10.3233/JAD-150704.

Bolós M, Llorens-Martín M, Perea JR, Jurado-Arjona J, Rábano A, Hernández F, Avila J. Absence of CX3CR1 impairs the internalization of Tau by microglia. Mol Neurodegener. 2017;12(1):59. Published 2017 Aug 15. doi:10.1186/s13024-017-0200-1.

Borchelt DR, Davis J, Fischer M, Lee MK, Slunt HH, Ratovitsky T, Regard J, Copeland NG, Jenkins NA, Sisodia SS, Price DL. A vector for expressing foreign genes in the brains and hearts of transgenic mice. Genet Anal. 1996;13(6):159-163. doi:10.1016/s1050-3862(96)00167-2.

Boxer AL, Knopman DS, Kaufer DI, Grossman M, Onyike C, Graf-Radford N, Mendez M, Kerwin D, Lerner A, Wu CK, Koestler M, Shapira J, Sullivan K, Klepac K, Lipowski K, Ullah J, Fields S, Kramer JH, Merrilees J, Neuhaus J, Mesulam MM, Miller BL. Memantine in patients with frontotemporal lobar degeneration: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2013a;12(2):149-156. doi:10.1016/S1474-4422(12)70320-4.

Boxer AL, Gold M, Huey E, Gao FB, Burton EA, Chow T, Kao A, Leavitt BR, Lamb B, Grether M, Knopman D, Cairns NJ, Mackenzie IR, Mitic L, Roberson ED, Van Kammen D, Cantillon M, Zahs K, Salloway S, Morris J, Tong G, Feldman H, Fillit H, Dickinson S, Khachaturian Z, Sutherland M, Farese R, Miller BL, Cummings J. Frontotemporal degeneration, the next therapeutic frontier: molecules and animal models for frontotemporal degeneration drug development. Alzheimers Dement. 2013b;9(2):176-188. doi:10.1016/j.jalz.2012.03.002.

Boxer AL, Gold M, Huey E, Hu WT, Rosen H, Kramer J, Gao FB, Burton EA, Chow T, Kao A, Leavitt BR, Lamb B, Grether M, Knopman D, Cairns NJ, Mackenzie IR, Mitic L, Roberson ED, Van Kammen D, Cantillon M, Zahs K, Jackson G, Salloway S, Morris J, Tong G, Feldman H, Fillit H, Dickinson S, Khachaturian ZS, Sutherland M, Abushakra S, Lewcock J, Farese R, Kenet RO, Laferla F, Perrin S, Whitaker S, Honig L, Mesulam MM, Boeve B, Grossman M, Miller BL, Cummings JL. The advantages of frontotemporal degeneration drug development (part 2 of frontotemporal degeneration: the next therapeutic frontier). Alzheimers Dement. 2013c;9(2):189-198. doi:10.1016/j.jalz.2012.03.003.

Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239-259. doi:10.1007/BF00308809.

Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006;112(4):389-404. doi:10.1007/s00401-006-0127-z.

Brunello CA, Merezhko M, Uronen RL, Huttunen HJ. Mechanisms of secretion and spreading of pathological tau protein. Cell Mol Life Sci. 2020;77(9):1721-1744. doi:10.1007/s00018-019-03349-1.

Bustos V, Pulina MV, Kelahmetoglu Y, Sinha SC, Gorelick FS, Flajolet M, Greengard P. Bidirectional regulation of Aβ levels by Presenilin 1. Proc Natl Acad Sci U S A. 2017;114(27):7142-7147. doi:10.1073/pnas.1705235114.

Caccamo A, Oddo S, Tran LX, LaFerla FM. Lithium reduces tau phosphorylation but not A beta or working memory deficits in a transgenic model with both plaques and tangles. Am J Pathol. 2007;170(5):1669-1675. doi:10.2353/ajpath.2007.061178.

Calafate S, Buist A, Miskiewicz K, Vijayan V, Daneels G, de Strooper B, de Wit J, Verstreken P, Moechars D. Synaptic Contacts Enhance Cell-to-Cell Tau Pathology Propagation. Cell Rep. 2015;11(8):1176-1183. doi:10.1016/j.celrep.2015.04.043.

Calsolaro V, Edison P. Neuroinflammation in Alzheimer's disease: Current evidence and future directions. Alzheimers Dement. 2016;12(6):719-732. doi:10.1016/j.jalz.2016.02.010.

Carlyle BC, Nairn AC, Wang M, Yang Y, Jin LE, Simen AA, Ramos BP, Bordner KA, Craft GE, Davies P, Pletikos M, Šestan N, Arnsten AF, Paspalas CD. cAMP-PKA phosphorylation of tau confers risk for degeneration in aging association cortex. Proc Natl Acad Sci U S A. 2014;111(13):5036-5041. doi:10.1073/pnas.1322360111.

Castillo-Carranza DL, Gerson JE, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Kayed R. Specific targeting of tau oligomers in Htau mice prevents cognitive impairment and tau toxicity following injection with brain-derived tau oligomeric seeds. J Alzheimers Dis. 2014;40 Suppl 1:S97-S111. doi:10.3233/JAD-132477.

Chai X, Dage JL, Citron M. Constitutive secretion of tau protein by an unconventional mechanism. Neurobiol Dis. 2012;48(3):356-366. doi:10.1016/j.nbd.2012.05.021.

Chen Q, Zhou Z, Zhang L, Wang Y, Zhang YW, Zhong M, Xu SC, Chen CH, Li L, Yu ZP. Tau protein is involved in morphological plasticity in hippocampal neurons in response to BDNF. Neurochem Int. 2012;60(3):233-242. doi:10.1016/j.neuint.2011.12.013.

Chen X, Li Y, Wang C, Tang Y, Mok SA, Tsai RM, Rojas JC, Karydas A, Miller BL, Boxer AL, Gestwicki JE, Arkin M, Cuervo AM, Gan L. Promoting tau secretion and propagation by hyperactive p300/CBP via autophagy-lysosomal pathway in tauopathy. Mol Neurodegener. 2020;15(1):2. Published 2020 Jan 6. doi:10.1186/s13024-019-0354-0.

Cheng ST, Chow PK, Song YQ, Yu EC, Chan AC, Lee TM, Lam JH. Mental and physical activities delay cognitive decline in older persons with dementia. Am J Geriatr Psychiatry. 2014;22(1):63-74. doi:10.1016/j.jagp.2013.01.060.

Cheung KH, Shineman D, Müller M, Cárdenas C, Mei L, Yang J, Tomita T, Iwatsubo T, Lee VM, Foskett JK. Mechanism of Ca2+ disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. Neuron. 2008;58(6):871-883. doi:10.1016/j.neuron.2008.04.015.

Chong CM, Ke M, Tan Y, Huang Z, Zhang K, Ai N, Ge W, Qin D, Lu JH, Su H. Presenilin 1 deficiency suppresses autophagy in human neural stem cells through reducing γ-secretase-independent ERK/CREB signaling. Cell Death Dis. 2018;9(9):879. Published 2018 Aug 29. doi:10.1038/s41419-018-0945-7.

Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, Stalder AK, Beibel M, Staufenbiel M, Jucker M, Goedert M, Tolnay M. Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol. 2009;11(7):909-913. doi:10.1038/ncb1901.

Clavaguera F, Grueninger F, Tolnay M. Intercellular transfer of tau aggregates and spreading of tau pathology: Implications for therapeutic strategies. Neuropharmacology. 2014;76 Pt A:9-15. doi:10.1016/j.neuropharm.2013.08.037.

Clavaguera F, Hench J, Lavenir I, Schweighauser G, Frank S, Goedert M, Tolnay M. Peripheral administration of tau aggregates triggers intracerebral tauopathy in transgenic mice. Acta Neuropathol. 2014b;127(2):299-301. doi:10.1007/s00401-013-1231-5.

Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, Lee VM. The acetylation of tau inhibits its function and promotes pathological tau aggregation. Nat Commun. 2011;2:252. doi:10.1038/ncomms1255.

Congdon EE, Wu JW, Myeku N, Figueroa YH, Herman M, Marinec PS, Gestwicki JE, Dickey CA, Yu WH, Duff KE. Methylthioninium chloride (methylene blue) induces autophagy and attenuates tauopathy in vitro and in vivo. Autophagy. 2012;8(4):609-622. doi:10.4161/auto.19048.

Cook C, Carlomagno Y, Gendron TF, Dunmore J, Scheffel K, Stetler C, Davis M, Dickson D, Jarpe M, DeTure M, Petrucelli L. Acetylation of the KXGS motifs in tau is a critical determinant in modulation of tau aggregation and clearance. Hum Mol Genet. 2014;23(1):104-116. doi:10.1093/hmg/ddt402.

Corcoran NM, Martin D, Hutter-Paier B, Windisch M, Nguyen T, Nheu L, Sundstrom LE, Costello AJ, Hovens CM. Sodium selenate specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits in an Alzheimer's disease model. J Clin Neurosci. 2010;17(8):1025-1033. doi:10.1016/j.jocn.2010.04.020.

Courade JP, Angers R, Mairet-Coello G, Pacico N, Tyson K, Lightwood D, Munro R, McMillan D, Griffin R, Baker T, Starkie D, Nan R, Westwood M, Mushikiwabo ML, Jung S, Odede G, Sweeney B, Popplewell A, Burgess G, Downey P, Citron M. Epitope determines efficacy of therapeutic anti-Tau antibodies in a functional assay with human Alzheimer Tau. Acta Neuropathol. 2018;136(5):729-745. doi:10.1007/s00401-018-1911-2.

Coyle-Gilchrist IT, Dick KM, Patterson K, Vázquez Rodríquez P, Wehmann E, Wilcox A, Lansdall CJ, Dawson KE, Wiggins J, Mead S, Brayne C, Rowe JB. Prevalence, characteristics, and survival of frontotemporal lobar degeneration syndromes. Neurology. 2016;86(18):1736-1743. doi:10.1212/WNL.0000000000002638.

Crehan H, Hardy J, Pocock J. Blockage of CR1 prevents activation of rodent microglia. Neurobiol Dis. 2013;54:139-149. doi:10.1016/j.nbd.2013.02.003.

Crimins JL, Rocher AB, Luebke JI. Electrophysiological changes precede morphological changes to frontal cortical pyramidal neurons in the rTg4510 mouse model of progressive tauopathy. Acta Neuropathol. 2012;124(6):777-795. doi:10.1007/s00401-012-1038-9.

Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature. 1995;378(6559):785-789. doi:10.1038/378785a0.

Cruts M, Hendriks L, Van Broeckhoven C. The presenilin genes: a new gene family involved in Alzheimer disease pathology. Hum Mol Genet. 1996;5 Spec No:1449-1455. doi:10.1093/hmg/5.supplement_1.1449.

Cruz JC, Tseng HC, Goldman JA, Shih H, Tsai LH. Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. Neuron. 2003;40(3):471-483. doi:10.1016/s0896-6273(03)00627-5.

Czech C, Tremp G, Pradier L. Presenilins and Alzheimer's disease: biological functions and pathogenic mechanisms. Prog Neurobiol. 2000;60(4):363-384. doi:10.1016/s0301-0082(99)00033-7.

Datta D, Leslie SN, Wang M, Morozov YM, Yang S, Mentone S, Zeiss C, Duque A, Rakic P, Horvath TL, van Dyck CH, Nairn AC, Arnsten AFT. Age-related calcium dysregulation linked with tau pathology and impaired cognition in non-human primates. Alzheimers Dement. 2021;17(6):920-932. doi:10.1002/alz.12325.

David DC, Layfield R, Serpell L, Narain Y, Goedert M, Spillantini MG. Proteasomal degradation of tau protein. J Neurochem. 2002;83(1):176-185. doi:10.1046/j.1471-4159.2002.01137.x.

de Calignon A, Polydoro M, Suárez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, Pitstick R, Sahara N, Ashe KH, Carlson GA, Spires-Jones TL, Hyman BT. Propagation of tau pathology in a model of early Alzheimer's disease [published correction appears in Neuron. 2012 Oct 18;76(2):461]. Neuron. 2012;73(4):685-697. doi:10.1016/j.neuron.2011.11.033.

De Strooper B. Loss-of-function presenilin mutations in Alzheimer disease. Talking Point on the role of presenilin mutations in Alzheimer disease. EMBO Rep. 2007;8(2):141-146. doi:10.1038/sj.embor.7400897.

De Strooper B. Proteases and proteolysis in Alzheimer disease: a multifactorial view on the disease process. Physiol Rev. 2010;90(2):465-494. doi:10.1152/physrev.00023.2009.

De Strooper B, Annaert W. Novel research horizons for presenilins and γ-secretases in cell biology and disease. Annu Rev Cell Dev Biol. 2010;26:235-260. doi:10.1146/annurev-cellbio-100109-104117.

De Strooper B, Iwatsubo T, Wolfe MS. Presenilins and γ-secretase: structure, function, and role in Alzheimer Disease. Cold Spring Harb Perspect Med. 2012;2(1):a006304. doi:10.1101/cshperspect.a006304.

Deaton CA, Johnson GVW. Presenilin 1 Regulates Membrane Homeostatic Pathways that are Dysregulated in Alzheimer's Disease. J Alzheimers Dis. 2020;77(3):961-977. doi:10.3233/JAD-200598.

Dejanovic B, Huntley MA, De Mazière A, Meilandt WJ, Wu T, Srinivasan K, Jiang Z, Gandham V, Friedman BA, Ngu H, Foreman O, Carano RAD, Chih B, Klumperman J, Bakalarski C, Hanson JE, Sheng M. Changes in the Synaptic Proteome in Tauopathy and Rescue of Tau-Induced Synapse Loss by C1q Antibodies. Neuron. 2018;100(6):1322-1336.e7. doi:10.1016/j.neuron.2018.10.014.

DeVos SL, Miller RL, Schoch KM, Holmes BB, Kebodeaux CS, Wegener AJ, Chen G, Shen T, Tran H, Nichols B, Zanardi TA, Kordasiewicz HB, Swayze EE, Bennett CF, Diamond MI, Miller TM. Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy. Sci Transl Med. 2017;9(374):eaag0481. doi:10.1126/scitranslmed.aag0481.

Dewachter I, Ris L, Croes S, Borghgraef P, Devijver H, Voets T, Nilius B, Godaux E, Van Leuven F. Modulation of synaptic plasticity and Tau phosphorylation by wild-type and mutant presenilin1. Neurobiol Aging. 2008;29(5):639-652. doi:10.1016/j.neurobiolaging.2006.11.019.

Deyts C, Clutter M, Herrera S, Jovanovic N, Goddi A, Parent AT. Loss of presenilin function is associated with a selective gain of APP function. Elife. 2016;5:e15645. Published 2016 May 19. doi:10.7554/eLife.15645.

Díaz-Hernández M, Gómez-Ramos A, Rubio A, Gómez-Villafuertes R, Naranjo JR, Miras-Portugal MT, Avila J. Tissue-nonspecific alkaline phosphatase promotes the neurotoxicity effect of extracellular tau. J Biol Chem. 2010;285(42):32539-32548. doi:10.1074/jbc.M110.145003.

Dickson, D.W., Hauw, J.-J., Agid, Y. and Litvan, I. (2011). Progressive Supranuclear Palsy and Corticobasal Degeneration. In Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders (eds D.W. Dickson and R.O. Weller).

Dobrowolski R, Vick P, Ploper D, Gumper I, Snitkin H, Sabatini DD, De Robertis EM. Presenilin deficiency or lysosomal inhibition enhances Wnt signaling through relocalization of GSK3 to the late-endosomal compartment. Cell Rep. 2012;2(5):1316-1328. doi:10.1016/j.celrep.2012.09.026.

Domise M, Didier S, Marinangeli C, Zhao H, Chandakkar P, Buée L, Viollet B, Davies P, Marambaud P, Vingtdeux V. AMP-activated protein kinase modulates tau phosphorylation and tau pathology in vivo. Sci Rep. 2016;6:26758. Published 2016 May 27. doi:10.1038/srep26758.

Dowjat WK, Wisniewski H, Wisniewski T. Alzheimer's disease presenilin-1 expression modulates the assembly of neurofilaments. Neuroscience. 2001;103(1):1-8. doi:10.1016/s0306-4522(00)00550-9.

Duggan SP, McCarthy JV. Beyond γ-secretase activity: The multifunctional nature of presenilins in cell signalling pathways. Cell Signal. 2016;28(1):1-11. doi:10.1016/j.cellsig.2015.10.006.

Dugger BN, Dickson DW. Pathology of Neurodegenerative Diseases. Cold Spring Harb Perspect Biol. 2017;9(7):a028035. Published 2017 Jul 5. doi:10.1101/cshperspect.a028035.

Dujardin S, Bégard S, Caillierez R, Lachaud C, Delattre L, Carrier S, Loyens A, Galas MC, Bousset L, Melki R, Aurégan G, Hantraye P, Brouillet E, Buée L, Colin M. Ectosomes: a new mechanism for non-exosomal secretion of tau protein. PLoS One. 2014;9(6):e100760. Published 2014 Jun 27. doi:10.1371/journal.pone.0100760.

Dujardin S, Bégard S, Caillierez R, Lachaud C, Carrier S, Lieger S, Gonzalez JA, Deramecourt V, Déglon N, Maurage CA, Frosch MP, Hyman BT, Colin M, Buée L. Different tau species lead to heterogeneous tau pathology propagation and misfolding. Acta Neuropathol Commun. 2018;6(1):132. Published 2018 Nov 29. doi:10.1186/s40478-018-0637-7.

Eisele YS, Bolmont T, Heikenwalder M, Langer F, Jacobson LH, Yan ZX, Roth K, Aguzzi A, Staufenbiel M, Walker LC, Jucker M. Induction of cerebral beta-amyloidosis: intracerebral versus systemic Abeta inoculation. Proc Natl Acad Sci U S A. 2009;106(31):12926-12931. doi:10.1073/pnas.0903200106.

Escamilla-Ayala A, Wouters R, Sannerud R, Annaert W. Contribution of the Presenilins in the cell biology, structure and function of γ-secretase. Semin Cell Dev Biol. 2020;105:12-26. doi:10.1016/j.semcdb.2020.02.005.

España J, Giménez-Llort L, Valero J, Miñano A, Rábano A, Rodriguez-Alvarez J, LaFerla FM, Saura CA. Intraneuronal beta-amyloid accumulation in the amygdala enhances fear and anxiety in Alzheimer's disease transgenic mice. Biol Psychiatry. 2010;67(6):513-521. doi:10.1016/j.biopsych.2009.06.015.

Falcon B, Noad J, McMahon H, Randow F, Goedert M. Galectin-8-mediated selective autophagy protects against seeded tau aggregation. J Biol Chem. 2018;293(7):2438-2451. doi:10.1074/jbc.M117.809293.

Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB, Abner EL, Petersen RC, Federoff HJ, Miller BL, Goetzl EJ. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. Alzheimers Dement. 2015;11(6):600-7.e1. doi:10.1016/j.jalz.2014.06.008.

Fontaine SN, Zheng D, Sabbagh JJ, Martin MD, Chaput D, Darling A, Trotter JH, Stothert AR, Nordhues BA, Lussier A, Baker J, Shelton L, Kahn M, Blair LJ, Stevens SM Jr, Dickey CA. DnaJ/Hsc70 chaperone complexes control the extracellular release of neurodegenerative-associated proteins. EMBO J. 2016;35(14):1537-1549. doi:10.15252/embj.201593489.

Forlenza OV, Diniz BS, Radanovic M, Santos FS, Talib LL, Gattaz WF. Disease-modifying properties of long-term lithium treatment for amnestic mild cognitive impairment: randomised controlled trial. Br J Psychiatry. 2011;198(5):351-356. doi:10.1192/bjp.bp.110.080044.

Frandemiche ML, De Seranno S, Rush T, Borel E, Elie A, Arnal I, Lanté F, Buisson A. Activity-dependent tau protein translocation to excitatory synapse is disrupted by exposure to amyloid-beta oligomers. J Neurosci. 2014;34(17):6084-6097. doi:10.1523/JNEUROSCI.4261-13.2014.

Freer R, Sormanni P, Vecchi G, Ciryam P, Dobson CM, Vendruscolo M. A protein homeostasis signature in healthy brains recapitulates tissue vulnerability to Alzheimer's disease. Sci Adv. 2016;2(8):e1600947. Published 2016 Aug 10. doi:10.1126/sciadv.1600947.

Frost B, Jacks RL, Diamond MI. Propagation of tau misfolding from the outside to the inside of a cell. J Biol Chem. 2009;284(19):12845-12852. doi:10.1074/jbc.M808759200.

Fu R, Shen Q, Xu P, Luo JJ, Tang Y. Phagocytosis of microglia in the central nervous system diseases. Mol Neurobiol. 2014;49(3):1422-1434. doi:10.1007/s12035-013-8620-6.

Fu H, Possenti A, Freer R, Nakano Y, Hernandez Villegas NC, Tang M, Cauhy PVM, Lassus BA, Chen S, Fowler SL, Figueroa HY, Huey ED, Johnson GVW, Vendruscolo M, Duff KE. A tau homeostasis signature is linked with the cellular and regional vulnerability of excitatory neurons to tau pathology. Nat Neurosci. 2019;22(1):47-56. doi:10.1038/s41593-018-0298-7.

Gauthier S, Feldman HH, Schneider LS, Wilcock GK, Frisoni GB, Hardlund JH, Moebius HJ, Bentham P, Kook KA, Wischik DJ, Schelter BO, Davis CS, Staff RT, Bracoud L, Shamsi K, Storey JM, Harrington CR, Wischik CM. Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomised, controlled, double-blind, parallel-arm, phase 3 trial. Lancet. 2016;388(10062):2873-2884. doi:10.1016/S0140-6736(16)31275-2.

Gerson JE, Mudher A, Kayed R. Potential mechanisms and implications for the formation of tau oligomeric strains. Crit Rev Biochem Mol Biol. 2016;51(6):482-496. doi:10.1080/10409238.2016.1226251.

Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, Christoffersson J, Chaabane W, Moghadam AR, Kashani HH, Hashemi M, Owji AA, Łos MJ. Autophagy and apoptosis dysfunction in neurodegenerative disorders. Prog Neurobiol. 2014;112:24-49. doi:10.1016/j.pneurobio.2013.10.004.

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature. 1991;349(6311):704-706. doi:10.1038/349704a0.

Goedert M, Eisenberg DS, Crowther RA. Propagation of Tau Aggregates and Neurodegeneration. Annu Rev Neurosci. 2017;40:189-210. doi:10.1146/annurev-neuro-072116-031153.

Gomez W, Morales R, Maracaja-Coutinho V, Parra V, Nassif M. Down syndrome and Alzheimer's disease: common molecular traits beyond the amyloid precursor protein. Aging (Albany NY). 2020;12(1):1011-1033. doi:10.18632/aging.102677.

Gómez-Isla T, Growdon WB, McNamara MJ, Nochlin D, Bird TD, Arango JC, Lopera F, Kosik KS, Lantos PL, Cairns NJ, Hyman BT. The impact of different presenilin 1 andpresenilin 2 mutations on amyloid deposition, neurofibrillary changes and neuronal loss in the familial Alzheimer's disease brain: evidence for other phenotype-modifying factors. Brain. 1999;122 (Pt 9):1709-1719. doi:10.1093/brain/122.9.1709.

Gorevic PD, Goñi F, Pons-Estel B, Alvarez F, Peress NS, Frangione B. Isolation and partial characterization of neurofibrillary tangles and amyloid plaque core in Alzheimer's disease: immunohistological studies. J Neuropathol Exp Neurol. 1986;45(6):647-664. doi:10.1097/00005072-198611000-00004.

Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, Ogar JM, Rohrer JD, Black S, Boeve BF, Manes F, Dronkers NF, Vandenberghe R, Rascovsky K, Patterson K, Miller BL, Knopman DS, Hodges JR, Mesulam MM, Grossman M. Classification of primary progressive aphasia and its variants. Neurology. 2011;76(11):1006-1014. doi:10.1212/WNL.0b013e31821103e6.

Greenberg SG, Davies P. A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. Proc Natl Acad Sci U S A. 1990;87(15):5827-5831. doi:10.1073/pnas.87.15.5827.

Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem. 1986a;261(13):6084-6089.

Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A. 1986b;83(13):4913-4917. doi:10.1073/pnas.83.13.4913.

Güner G, Lichtenthaler SF. The substrate repertoire of γ-secretase/presenilin. Semin Cell Dev Biol. 2020;105:27-42. doi:10.1016/j.semcdb.2020.05.019.

Hall S, Öhrfelt A, Constantinescu R, Andreasson U, Surova Y, Bostrom F, Nilsson C, Håkan W, Decraemer H, Någga K, Minthon L, Londos E, Vanmechelen E, Holmberg B, Zetterberg H, Blennow K, Hansson O. Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. Arch Neurol. 2012;69(11):1445-1452. doi:10.1001/archneurol.2012.1654.

Hampel H, Ewers M, Bürger K, Annas P, Mörtberg A, Bogstedt A, Frölich L, Schröder J, Schönknecht P, Riepe MW, Kraft I, Gasser T, Leyhe T, Möller HJ, Kurz A, Basun H. Lithium trial in Alzheimer's disease: a randomized, single-blind, placebo-controlled, multicenter 10-week study. J Clin Psychiatry. 2009;70(6):922-931.

Hampel H, Schneider LS, Giacobini E, Kivipelto M, Sindi S, Dubois B, Broich K, Nisticò R, Aisen PS, Lista S. Advances in the therapy of Alzheimer's disease: targeting amyloid beta and tau and perspectives for the future. Expert Rev Neurother. 2015;15(1):83-105. doi:10.1586/14737175.2015.995637.

Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH. Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. Neurosci Lett. 1992;147(1):58-62. doi:10.1016/0304-3940(92)90774-2.

Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics [published correction appears in Science 2002 Sep 27;297(5590):2209]. Science. 2002;297(5580):353-356. doi:10.1126/science.1072994.

Hasegawa M, Smith MJ, Goedert M. Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. FEBS Lett. 1998;437(3):207-210. doi:10.1016/s0014-5793(98)01217-4.

He Z, Guo JL, McBride JD, Narasimhan S, Kim H, Changolkar L, Zhang B, Gathagan RJ, Yue C, Dengler C, Stieber A, Nitla M, Coulter DA, Abel T, Brunden KR, Trojanowski JQ, Lee VM. Amyloid-β plaques enhance Alzheimer's brain tau-seeded pathologies by facilitating neuritic plaque tau aggregation. Nat Med. 2018;24(1):29-38. doi:10.1038/nm.4443

Hebert LE, Weuve J, Scherr PA, Evans DA. Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology. 2013;80(19):1778-1783. doi:10.1212/WNL.0b013e31828726f5.

Hernández F, Borrell J, Guaza C, Avila J, Lucas JJ. Spatial learning deficit in transgenic mice that conditionally over-express GSK-3beta in the brain but do not form tau filaments. J Neurochem. 2002;83(6):1529-1533. doi:10.1046/j.1471-4159.2002.01269.x.

Hernández F, Pérez M, Lucas JJ, Mata AM, Bhat R, Avila J. Glycogen synthase kinase-3 plays a crucial role in tau exon 10 splicing and intranuclear distribution of SC35. Implications for Alzheimer's disease. J Biol Chem. 2004;279(5):3801-3806. doi:10.1074/jbc.M311512200.

Hernandez I, Luna G, Rauch JN, Reis SA, Giroux M, Karch CM, Boctor D, Sibih YE, Storm NJ, Diaz A, Kaushik S, Zekanowski C, Kang AA, Hinman CR, Cerovac V, Guzman E, Zhou H, Haggarty SJ, Goate AM, Fisher SK, Cuervo AM, Kosik KS. A farnesyltransferase inhibitor activates lysosomes and reduces tau pathology in mice with tauopathy. Sci Transl Med. 2019;11(485):eaat3005. doi:10.1126/scitranslmed.aat3005.

Higuchi M, Ishihara T, Zhang B, Hong M, Andreadis A, Trojanowski J, Lee VM. Transgenic mouse model of tauopathies with glial pathology and nervous system degeneration. Neuron. 2002;35(3):433-446. doi:10.1016/s0896-6273(02)00789-4.

Hollingworth P, Harold D, Sims R, errish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Rüther E, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Gallacher J, Hüll M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC; Alzheimer's Disease Neuroimaging Initiative, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S; CHARGE consortium, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alpérovitch A, Lathrop M; EADI1 consortium, Feulner TM, Friedrich P, Riehle C,

Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Björnsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossù P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet. 2011;43(5):429-435. doi:10.1038/ng.803.

Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP, Kotzbauer PT, Miller TM, Papy-Garcia D, Diamond MI. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. Proc Natl Acad Sci U S A. 2013;110(33):E3138-E3147. doi:10.1073/pnas.1301440110.

Holtzman DM, Herz J, Bu G. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harb Perspect Med. 2012;2(3):a006312. doi:10.1101/cshperspect.a006312.

Hong XP, Peng CX, Wei W, Tian Q, Liu YH, Yao XQ, Zhang Y, Cao FY, Wang Q, Wang JZ. Essential role of tau phosphorylation in adult hippocampal neurogenesis. Hippocampus. 2010;20(12):1339-1349. doi:10.1002/hipo.20712.

Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, Pitstick R, Carlson GA, Lanier LM, Yuan LL, Ashe KH, Liao D. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. Neuron. 2010;68(6):1067-1081. doi:10.1016/j.neuron.2010.11.030.

Hung COY, Livesey FJ. Altered γ-Secretase Processing of APP Disrupts Lysosome and Autophagosome Function in Monogenic Alzheimer's Disease. Cell Rep. 2018;25(13):3647-3660.e2. doi:10.1016/j.celrep.2018.11.095.

Hutton M, Hardy J. The presenilins and Alzheimer's disease. Hum Mol Genet. 1997;6(10):1639-1646. doi:10.1093/hmg/6.10.1639.

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature. 1998;393(6686):702-705. doi:10.1038/31508.

Hutton M. Presenilin mutations associated with fronto-temporal dementia. Ann Neurol. 2004;55(5):604-606. doi:10.1002/ana.20103.

Iqbal K, Liu F, Gong CX. Tau and neurodegenerative disease: the story so far. Nat Rev Neurol. 2016;12(1):15-27. doi:10.1038/nrneurol.2015.225.

Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Wölfing H, Chieng BC, Christie MJ, Napier IA, Eckert A, Staufenbiel M, Hardeman E, Götz J. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell. 2010;142(3):387-397. doi:10.1016/j.cell.2010.06.036.

Jackson JS, Witton J, Johnson JD, Ahmed Z, Ward M, Randall AD, Hutton ML, Isaac JT, O'Neill MJ, Ashby MC. Altered Synapse Stability in the Early Stages of Tauopathy. Cell Rep. 2017;18(13):3063-3068. doi:10.1016/j.celrep.2017.03.013.

Jadhav S, Avila J, Schöll M, Kovacs GG, Kövari E, Skrabana R, Evans LD, Kontsekova E, Malawska B, de Silva R, Buee L, Zilka N. A walk through tau therapeutic strategies. Acta Neuropathol Commun. 2019;7(1):22. Published 2019 Feb 15. doi:10.1186/s40478-019-0664-z.

Jiang S, Bhaskar K. Degradation and Transmission of Tau by Autophagic-Endolysosomal Networks and Potential Therapeutic Targets for Tauopathy. Front Mol Neurosci. 2020;13:586731. Published 2020 Oct 16. doi:10.3389/fnmol.2020.586731.

Jicha GA, Weaver C, Lane E, Vianna C, Kress Y, Rockwood J, Davies P. cAMP-dependent protein kinase phosphorylations on tau in Alzheimer's disease. J Neurosci. 1999;19(17):7486-7494. doi:10.1523/JNEUROSCI.19-17-07486.1999.

Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci. 2004;29(2):95-102. doi:10.1016/j.tibs.2003.12.004.

Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature. 2013;501(7465):45-51. doi:10.1038/nature12481.

Kahlson MA, Colodner KJ. Glial Tau Pathology in Tauopathies: Functional Consequences. J Exp Neurosci. 2016;9(Suppl 2):43-50. Published 2016 Feb 10. doi:10.4137/JEN.S25515.

Kalbfuss B, Mabon SA, Misteli T. Correction of alternative splicing of tau in frontotemporal dementia and parkinsonism linked to chromosome 17. J Biol Chem. 2001;276(46):42986-42993. doi:10.1074/jbc.M105113200.

Katsinelos T, Zeitler M, Dimou E, Karakatsani A, Müller HM, Nachman E, Steringer JP, Ruiz de Almodovar C, Nickel W, Jahn TR. Unconventional Secretion Mediates the Trans-cellular Spreading of Tau. Cell Rep. 2018;23(7):2039-2055. doi:10.1016/j.celrep.2018.04.056.

Kaushik S, Cuervo AM. Proteostasis and aging. Nat Med. 2015;21(12):1406-1415. doi:10.1038/nm.4001.

Keck S, Nitsch R, Grune T, Ullrich O. Proteasome inhibition by paired helical filament-tau in brains of patients with Alzheimer's disease. J Neurochem. 2003;85(1):115-122. doi:10.1046/j.1471-4159.2003.01642.x.

Keller JN, Hanni KB, Markesbery WR. Impaired proteasome function in Alzheimer's disease. J Neurochem. 2000;75(1):436-439. doi:10.1046/j.1471-4159.2000.0750436.x.

Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Trans-cellular propagation of Tau aggregation by fibrillar species. J Biol Chem. 2012;287(23):19440-19451. doi:10.1074/jbc.M112.346072.

Killin LO, Starr JM, Shiue IJ, Russ TC. Environmental risk factors for dementia: a systematic review. BMC Geriatr. 2016 Oct 12;16(1):175. doi: 10.1186/s12877-016-0342-y. PMID: 27729011; PMCID: PMC5059894.

Kimura T, Whitcomb DJ, Jo J, Regan P, Piers T, Heo S, Brown C, Hashikawa T, Murayama M, Seok H, Sotiropoulos I, Kim E, Collingridge GL, Takashima A, Cho K. Microtubule-associated protein tau is essential for long-term depression in the hippocampus. Philos Trans R Soc Lond B Biol Sci. 2013;369(1633):20130144. Published 2013 Dec 2. doi:10.1098/rstb.2013.0144.

Knopman DS, Roberts RO. Estimating the number of persons with frontotemporal lobar degeneration in the US population. J Mol Neurosci. 2011;45(3):330-335. doi:10.1007/s12031-011-9538-y.

Kodama L, Guzman E, Etchegaray JI, Li Y, Sayed FA, Zhou L, Zhou Y, Zhan L, Le D, Udeochu JC, Clelland CD, Cheng Z, Yu G, Li Q, Kosik KS, Gan L. Microglial microRNAs mediate sex-specific responses to tau pathology. Nat Neurosci. 2020;23(2):167-171. doi:10.1038/s41593-019-0560-7.

Kontsekova E, Zilka N, Kovacech B, Novak P, Novak M. First-in-man tau vaccine targeting structural determinants essential for pathological tau-tau interaction reduces tau oligomerisation and neurofibrillary degeneration in an Alzheimer's disease model. Alzheimers Res Ther. 2014;6(4):44. Published 2014 Aug 1. doi:10.1186/alzrt278.

Kovacs GG, Lee VM, Trojanowski JQ. Protein astrogliopathies in human neurodegenerative diseases and aging. Brain Pathol. 2017;27(5):675-690. doi:10.1111/bpa.12536

Krüger U, Wang Y, Kumar S, Mandelkow EM. Autophagic degradation of tau in primary neurons and its enhancement by trehalose. Neurobiol Aging. 2012;33(10):2291-2305. doi:10.1016/j.neurobiolaging.2011.11.009

Ksiezak-Reding H, Pyo HK, Feinstein B, Pasinetti GM. Akt/PKB kinase phosphorylates separately Thr212 and Ser214 of tau protein in vitro. Biochim Biophys Acta. 2003;1639(3):159-168. doi:10.1016/j.bbadis.2003.09.001.

Kulkarni VV, Maday S. Compartment-specific dynamics and functions of autophagy in neurons. Dev Neurobiol. 2018;78(3):298-310. doi:10.1002/dneu.22562.

Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N. The role of autophagy during the early neonatal starvation period. Nature. 2004;432(7020):1032-1036. doi:10.1038/nature03029.

Kumar-Singh S, Theuns J, Van Broeck B, Pirici D, Vennekens K, Corsmit E, Cruts M, Dermaut B, Wang R, Van Broeckhoven C. Mean age-of-onset of familial alzheimer disease caused by presenilin mutations correlates with both increased Abeta42 and decreased Abeta40. Hum Mutat. 2006;27(7):686-695. doi:10.1002/humu.20336.

Kuninaka N, Kawaguchi M, Ogawa M, Sato A, Arima K, Murayama S, Saito Y. Simplification of the modified Gallyas method. Neuropathology. 2015;35(1):10-15. doi:10.1111/neup.12144.

Kyoung Pyo H, Lovati E, Pasinetti GM, Ksiezak-Reding H. Phosphorylation of tau at THR212 and SER214 in human neuronal and glial cultures: the role of AKT. Neuroscience. 2004;127(3):649-658. doi:10.1016/j.neuroscience.2004.05.036.

Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li HR, Jiang J, Watt AT, Chun S, Katz M, Qiu J, Sun Y, Ling SC, Zhu Q, Polymenidou M, Drenner K, Artates JW, McAlonis-Downes M, Markmiller S, Hutt KR, Pizzo DP, Cady J, Harms MB, Baloh RH, Vandenberg SR, Yeo GW, Fu XD, Bennett CF, Cleveland DW, Ravits J. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci U S A. 2013;110(47):E4530-E4539. doi:10.1073/pnas.1318835110.

Lai MT, Chen E, Crouthamel MC, DiMuzio-Mower J, Xu M, Huang Q, Price E, Register RB, Shi XP, Donoviel DB, Bernstein A, Hazuda D, Gardell SJ, Li YM. Presenilin-1 and presenilin-2 exhibit distinct yet overlapping gamma-secretase activities. J Biol Chem. 2003;278(25):22475-22481. doi:10.1074/jbc.M300974200.

Lambert MA, Bickel H, Prince M, Fratiglioni L, Von Strauss E, Frydecka D, Kiejna A, Georges J, Reynish EL. Estimating the burden of early onset dementia; systematic review of disease prevalence. Eur J Neurol. 2014;21(4):563-569. doi:10.1111/ene.12325.

Lanata SC, Miller BL. The behavioural variant frontotemporal dementia (bvFTD) syndrome in psychiatry. J Neurol Neurosurg Psychiatry. 2016;87(5):501-511. doi:10.1136/jnnp-2015-310697.

Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018;25(1):59-70. doi:10.1111/ene.13439.

Larner AJ, Doran M. Clinical phenotypic heterogeneity of Alzheimer's disease associated with mutations of the presenilin-1 gene. J Neurol. 2006;253(2):139-158. doi:10.1007/s00415-005-0019-5.

Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, Jackson GR, Kayed R. Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. Sci Rep. 2012;2:700. doi:10.1038/srep00700.

Lashley T, Rohrer JD, Mead S, Revesz T. Review: an update on clinical, genetic and pathological aspects of frontotemporal lobar degenerations. Neuropathol Appl Neurobiol. 2015;41(7):858-881. doi:10.1111/nan.12250.

Laudon H, Hansson EM, Melén K, Bergman A, Farmery MR, Winblad B, Lendahl U, von Heijne G, Näslund J. A nine-transmembrane domain topology for presenilin 1. J Biol Chem. 2005;280(42):35352-35360. doi:10.1074/jbc.M507217200.

Lebert F, Stekke W, Hasenbroekx C, Pasquier F. Frontotemporal dementia: a randomised, controlled trial with trazodone. Dement Geriatr Cogn Disord. 2004;17(4):355-359. doi:10.1159/000077171.

Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, Wolfe DM, Martinez-Vicente M, Massey AC, Sovak G, Uchiyama Y, Westaway D, Cuervo AM, Nixon RA. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell. 2010;141(7):1146-1158. doi:10.1016/j.cell.2010.05.008.

Lee S, Kim W, Li Z, Hall GF. Accumulation of vesicle-associated human tau in distal dendrites drives degeneration and tau secretion in an in situ cellular tauopathy model. Int J Alzheimers Dis. 2012;2012:172837. doi:10.1155/2012/172837.

Lee MJ, Lee JH, Rubinsztein DC. Tau degradation: the ubiquitin-proteasome system versus the autophagy-lysosome system. Prog Neurobiol. 2013;105:49-59. doi:10.1016/j.pneurobio.2013.03.001.

Lee SH, Lutz D, Mossalam M, Bolshakov VY, Frotscher M, Shen J. Presenilins regulate synaptic plasticity and mitochondrial calcium homeostasis in the hippocampal mossy fiber pathway. Mol Neurodegener. 2017;12(1):48. Published 2017 Jun 15. doi:10.1186/s13024-017-0189-5.

Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, Wong BX, Adlard PA, Cherny RA, Lam LQ, Roberts BR, Volitakis I, Egan GF, McLean CA, Cappai R, Duce JA, Bush AI. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. Nat Med. 2012;18(2):291-295. Published 2012 Jan 29. doi:10.1038/nm.2613.

Leroy K, Boutajangout A, Authelet M, Woodgett JR, Anderton BH, Brion JP. The active form of glycogen synthase kinase-3beta is associated with granulovacuolar degeneration in neurons in Alzheimer's disease. Acta Neuropathol. 2002;103(2):91-99. doi:10.1007/s004010100435.

Lessard CB, Rodriguez E, Ladd TB, Minter LM, Osborne BA, Miele L, Golde TE, Ran Y. Individual and combined presenilin 1 and 2 knockouts reveal that both have highly overlapping functions in HEK293T cells. J Biol Chem. 2019;294(29):11276-11285. doi:10.1074/jbc.RA119.008041.

Leskovjan AC, Kretlow A, Lanzirotti A, Barrea R, Vogt S, Miller LM. Increased brain iron coincides with early plaque formation in a mouse model of Alzheimer's disease. Neuroimage. 2011;55(1):32-38. doi:10.1016/j.neuroimage.2010.11.073.

Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science. 1995;269(5226):973-977. doi:10.1126/science.7638622.

Li XC, Hu Y, Wang ZH, Luo Y, Zhang Y, Liu XP, Feng Q, Wang Q, Ye K, Liu GP, Wang JZ. Human wild-type full-length tau accumulation disrupts mitochondrial dynamics and the functions via increasing mitofusins. Sci Rep. 2016;6:24756. Published 2016 Apr 21. doi:10.1038/srep24756.

Ling H. Untangling the tauopathies: Current concepts of tau pathology and neurodegeneration. Parkinsonism Relat Disord. 2018;46 Suppl 1:S34-S38. doi:10.1016/j.parkreldis.2017.07.031.

Lipinski MM, Zheng B, Lu T, Yan Z, Py BF, Ng A, Xavier RJ, Li C, Yankner BA, Scherzer CR, Yuan J. Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease. Proc Natl Acad Sci U S A. 2010;107(32):14164-14169. doi:10.1073/pnas.1009485107.

Litterst C, Georgakopoulos A, Shioi J, Ghersi E, Wisniewski T, Wang R, Ludwig A, Robakis NK. Ligand binding and calcium influx induce distinct ectodomain/gamma-secretase-processing pathways of EphB2 receptor. J Biol Chem. 2007;282(22):16155-16163. doi:10.1074/jbc.M611449200.

Litvinchuk A, Wan YW, Swartzlander DB, Chen F, Cole A, Propson NE, Wang Q, Zhang B, Liu Z, Zheng H. Complement C3aR Inactivation Attenuates Tau Pathology and Reverses an Immune Network Deregulated in Tauopathy Models and Alzheimer's Disease. Neuron. 2018;100(6):1337-1353.e5. doi:10.1016/j.neuron.2018.10.031.

Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. Eur J Neurosci. 2005;22(8):1942-1950. doi:10.1111/j.1460-9568.2005.04391.x.

Livingston G, Kelly L, Lewis-Holmes E, Baio G, Morris S, Patel N, Omar RZ, Katona C, Cooper C. Non-pharmacological interventions for agitation in dementia: systematic review of randomised controlled trials. Br J Psychiatry. 2014;205(6):436-442. doi:10.1192/bjp.bp.113.141119.

Lleó A, Saura CA. γ-secretase substrates and their implications for drug development in Alzheimer's disease. Curr Top Med Chem. 2011;11(12):1513-1527. doi:10.2174/156802611795861004.

Loy CT, Schofield PR, Turner AM, Kwok JB. Genetics of dementia. Lancet. 2014 Mar 1;383(9919):828-40. doi:10.1016/S0140-6736(13)60630-3. Epub 2013 Aug 6. PMID: 23927914.

Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, Kovacs GG, Ghetti B, Halliday G, Holm IE, Ince PG, Kamphorst W, Revesz T, Rozemuller AJ, Kumar-Singh S, Akiyama H, Baborie A, Spina S, Dickson DW, Trojanowski JQ, Mann DM. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol. 2010;119(1):1-4. doi:10.1007/s00401-009-0612-2.

Mandelkow E, Song YH, Schweers O, Marx A, Mandelkow EM. On the structure of microtubules, tau, and paired helical filaments. Neurobiol Aging. 1995;16(3):347-354. doi:10.1016/0197-4580(95)00026-b.

Marambaud P, Wen PH, Dutt A, Shioi J, Takashima A, Siman R, Robakis NK. A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. Cell. 2003;114(5):635-645. doi:10.1016/j.cell.2003.08.008.

Martin L, Latypova X, Terro F. Post-translational modifications of tau protein: implications for Alzheimer's disease. Neurochem Int. 2011;58(4):458-471. doi:10.1016/j.neuint.2010.12.023.

Martini-Stoica H, Cole AL, Swartzlander DB, Chen F, Wan YW, Bajaj L, Bader DA, Lee VMY, Trojanowski JQ, Liu Z, Sardiello M, Zheng H. TFEB enhances astroglial uptake of extracellular tau species and reduces tau spreading. J Exp Med. 2018;215(9):2355-2377. doi:10.1084/jem.20172158.

Mattsson N, Andreasson U, Zetterberg H, Blennow K; Alzheimer's Disease Neuroimaging Initiative. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. JAMA Neurol. 2017;74(5):557-566. doi:10.1001/jamaneurol.2016.6117.

Mendez MF, Shapira JS, McMurtray A, Licht E. Preliminary findings: behavioral worsening on donepezil in patients with frontotemporal dementia. Am J Geriatr Psychiatry. 2007;15(1):84-87. doi:10.1097/01.JGP.0000231744.69631.33.

Meredith JE Jr, Sankaranarayanan S, Guss V, Lanzetti AJ, Berisha F, Neely RJ, Slemmon JR, Portelius E, Zetterberg H, Blennow K, Soares H, Ahlijanian M, Albright CF. Characterization of novel CSF Tau and ptau biomarkers for Alzheimer's disease. PLoS One. 2013;8(10):e76523. Published 2013 Oct 7. doi:10.1371/journal.pone.0076523.

Merrilees J. A model for management of behavioral symptoms in frontotemporal lobar degeneration. Alzheimer Dis Assoc Disord. 2007;21(4):S64-S69. doi:10.1097/WAD.0b013e31815bf774.

Meske V, Albert F, Ohm TG. Coupling of mammalian target of rapamycin with phosphoinositide 3-kinase signaling pathway regulates protein phosphatase 2A- and glycogen synthase kinase-3 -dependent phosphorylation of Tau. J Biol Chem. 2008;283(1):100-109. doi:10.1074/jbc.M704292200.

Michel CH, Kumar S, Pinotsi D, Tunnacliffe A, St George-Hyslop P, Mandelkow E, Mandelkow EM, Kaminski CF, Kaminski Schierle GS. Extracellular monomeric tau protein is sufficient to initiate the spread of tau protein pathology. J Biol Chem. 2014;289(2):956-967. doi:10.1074/jbc.M113.515445.

Miller LM, Bourassa MW, Smith RJ. FTIR spectroscopic imaging of protein aggregation in living cells. Biochim Biophys Acta. 2013;1828(10):2339-2346. doi:10.1016/j.bbamem.2013.01.014.

Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, Huang EJ, Shen Y, Masliah E, Mukherjee C, Meyers D, Cole PA, Ott M, Gan L. Acetylation of tau inhibits its degradation and contributes to tauopathy [published correction appears in Neuron. 2010 Nov 18;68(4):801]. Neuron. 2010;67(6):953-966. doi:10.1016/j.neuron.2010.08.044.

Mirbaha H, Holmes BB, Sanders DW, Bieschke J, Diamond MI. Tau Trimers Are the Minimal Propagation Unit Spontaneously Internalized to Seed Intracellular Aggregation. J Biol Chem. 2015;290(24):14893-14903. doi:10.1074/jbc.M115.652693.

Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell. 2004;15(3):1101-1111. doi:10.1091/mbc.e03-09-0704.

Mohamed NV, Plouffe V, Rémillard-Labrosse G, Planel E, Leclerc N. Starvation and inhibition of lysosomal function increased tau secretion by primary cortical neurons. Sci Rep. 2014;4:5715. Published 2014 Jul 17. doi:10.1038/srep05715.

Moreau K, Fleming A, Imarisio S, Lopez Ramirez A, Mercer JL, Jimenez-Sanchez M, Bento CF, Puri C, Zavodszky E, Siddiqi F, Lavau CP, Betton M, O'Kane CJ, Wechsler DS, Rubinsztein DC. PICALM modulates autophagy activity and tau accumulation. Nat Commun. 2014;5:4998. Published 2014 Sep 22. doi:10.1038/ncomms5998.

Mumm JS, Schroeter EH, Saxena MT, Griesemer A, Tian X, Pan DJ, Ray WJ, Kopan R. A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. Mol Cell. 2000;5(2):197-206. doi:10.1016/s1097-2765(00)80416-5.

Myeku N, Clelland CL, Emrani S, Kukushkin NV, Yu WH, Goldberg AL, Duff KE. Tau-driven 26S proteasome impairment and cognitive dysfunction can be prevented early in disease by activating cAMP-PKA signaling. Nat Med. 2016;22(1):46-53. doi:10.1038/nm.4011.

Nacharaju P, Lewis J, Easson C, Yen S, Hackett J, Hutton M, Yen SH. Accelerated filament formation from tau protein with specific FTDP-17 missense mutations. FEBS Lett. 1999;447(2-3):195-199. doi:10.1016/s0014-5793(99)00294-x.

Narasimhan S, Guo JL, Changolkar L, Stieber A, McBride JD, Silva LV, He Z, Zhang B, Gathagan RJ, Trojanowski JQ, Lee VMY. Pathological Tau Strains from Human Brains Recapitulate the Diversity of Tauopathies in Nontransgenic Mouse Brain. J Neurosci. 2017;37(47):11406-11423. doi:10.1523/JNEUROSCI.1230-17.2017.

Neely KM, Green KN, LaFerla FM. Presenilin is necessary for efficient proteolysis through the autophagy-lysosome system in a γ-secretase-independent manner. J Neurosci. 2011;31(8):2781-2791. doi:10.1523/JNEUROSCI.5156-10.2010.

Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, Ho RC. IL-1β, IL-6, TNF- α and CRP in Elderly Patients with Depression or Alzheimer's disease: Systematic Review and Meta-Analysis. Sci Rep. 2018;8(1):12050. Published 2018 Aug 13. doi:10.1038/s41598-018-30487-6.

Nickel W, Rabouille C. Mechanisms of regulated unconventional protein secretion [published correction appears in Nat Rev Mol Cell Biol. 2009 Mar;10(3):234]. Nat Rev Mol Cell Biol. 2009;10(2):148-155. doi:10.1038/nrm2617.

Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol. 2005;64(2):113-122. doi:10.1093/jnen/64.2.113.

Nixon RA, Cataldo AM. Lysosomal system pathways: genes to neurodegeneration in Alzheimer's disease. J Alzheimers Dis. 2006;9(3 Suppl):277-289. doi:10.3233/jad-2006-9s331.

Noble W, Hanger DP, Miller CC, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. Front Neurol. 2013;4:83. Published 2013 Jul 1. doi:10.3389/fneur.2013.00083.

Nobuhara CK, DeVos SL, Commins C, Wegmann S, Moore BD, Roe AD, Costantino I, Frosch MP, Pitstick R, Carlson GA, Hock C, Nitsch RM, Montrasio F, Grimm J, Cheung AE, Dunah AW, Wittmann M, Bussiere T, Weinreb PH, Hyman BT, Takeda S. Tau Antibody Targeting Pathological Species Blocks Neuronal Uptake and Interneuron Propagation of Tau in Vitro. Am J Pathol. 2017;187(6):1399-1412. doi:10.1016/j.ajpath.2017.01.022.

Ortega-Martinez S, Palla N, Zhang X, Lipman E, Sisodia SS. Deficits in Enrichment-Dependent Neurogenesis and Enhanced Anxiety Behaviors Mediated by Expression of Alzheimer's Disease-Linked Ps1 Variants Are Rescued by Microglial Depletion. J Neurosci. 2019;39(34):6766-6780. doi:10.1523/JNEUROSCI.0884-19.2019.

Ozcelik S, Fraser G, Castets P, Schaeffer V, Skachokova Z, Breu K, Clavaguera F, Sinnreich M, Kappos L, Goedert M, Tolnay M, Winkler DT. Rapamycin attenuates the progression of tau pathology in P301S tau transgenic mice. PLoS One. 2013;8(5):e62459. Published 2013 May 7. doi:10.1371/journal.pone.0062459.

Parra-Damas A, Valero J, Chen M, España J, Martín E, Ferrer I, Rodríguez-Alvarez J, Saura CA. Crtc1 activates a transcriptional program deregulated at early Alzheimer's disease-related stages. J Neurosci. 2014;34(17):5776-5787. doi:10.1523/JNEUROSCI.5288-13.2014.

Parra-Damas A, Chen M, Enriquez-Barreto L, Ortega L, Acosta S, Perna JC, Fullana MN, Aguilera J, Rodríguez-Alvarez J, Saura CA. CRTC1 Function During Memory Encoding Is Disrupted in Neurodegeneration. Biol Psychiatry. 2017;81(2):111-123. doi:10.1016/j.biopsych.2016.06.025.

Parra-Damas A, Saura CA. Synapse-to-Nucleus Signaling in Neurodegenerative and Neuropsychiatric Disorders. Biol Psychiatry. 2019;86(2):87-96. doi:10.1016/j.biopsych.2019.01.006.

Patrick GN, Zukerberg L, Nikolic M, de la Monte S, Dikkes P, Tsai LH. Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. Nature. 1999;402(6762):615-622. doi:10.1038/45159.

Perea JR, Llorens-Martín M, Ávila J, Bolós M. The Role of Microglia in the Spread of Tau: Relevance for Tauopathies. Front Cell Neurosci. 2018;12:172. Published 2018 Jul 10. doi:10.3389/fncel.2018.00172.

Pérez M, Medina M, Hernández F, Avila J. Secretion of full-length Tau or Tau fragments in cell culture models. Propagation of Tau in vivo and in vitro. Biomol Concepts. 2018;9(1):1-11. Published 2018 Mar 5. doi:10.1515/bmc-2018-0001.

Peric A, Annaert W. Early etiology of Alzheimer's disease: tipping the balance toward autophagy or endosomal dysfunction?. Acta Neuropathol. 2015;129(3):363-381. doi:10.1007/s00401-014-1379-7.

Petry FR, Pelletier J, Bretteville A, Morin F, Calon F, Hébert SS, Whittington RA, Planel E. Specificity of anti-tau antibodies when analyzing mice models of Alzheimer's disease: problems and solutions. PLoS One. 2014;9(5):e94251. Published 2014 May 2. doi:10.1371/journal.pone.0094251.

Piacentini R, Li Puma DD, Mainardi M, Lazzarino G, Tavazzi B, Arancio O, Grassi C. Reduced gliotransmitter release from astrocytes mediates tau-induced synaptic dysfunction in cultured hippocampal neurons. Glia. 2017;65(8):1302-1316. doi:10.1002/glia.23163.

Piccin A, Murphy WG, Smith OP. Circulating microparticles: pathophysiology and clinical implications. Blood Rev. 2007;21(3):157-171. doi:10.1016/j.blre.2006.09.001.

Pickett EK, Herrmann AG, McQueen J, Abt K, Dando O, Tulloch J, Jain P, Dunnett S, Sohrabi S, Fjeldstad MP, Calkin W, Murison L, Jackson RJ, Tzioras M, Stevenson A, d'Orange M, Hooley M, Davies C, Colom-Cadena M, Anton-Fernandez A, King D, Oren I, Rose J, McKenzie CA, Allison E, Smith C, Hardt O, Henstridge CM, Hardingham GE, Spires-Jones TL. Amyloid Beta and Tau Cooperate to Cause Reversible Behavioral and Transcriptional Deficits in a Model of Alzheimer's Disease. Cell Rep. 2019;29(11):3592-3604.e5. doi:10.1016/j.celrep.2019.11.044.

Pigino G, Pelsman A, Mori H, Busciglio J. Presenilin-1 mutations reduce cytoskeletal association, deregulate neurite growth, and potentiate neuronal dystrophy and tau phosphorylation. J Neurosci. 2001;21(3):834-842. doi:10.1523/JNEUROSCI.21-03-00834.2001.

Pimenova AA, Goate AM. Novel presenilin 1 and 2 double knock-out cell line for in vitro validation of PSEN1 and PSEN2 mutations. Neurobiol Dis. 2020;138:104785. doi:10.1016/j.nbd.2020.104785.

Piras A, Collin L, Grüninger F, Graff C, Rönnbäck A. Autophagic and lysosomal defects in human tauopathies: analysis of post-mortem brain from patients with familial Alzheimer disease, corticobasal degeneration and progressive supranuclear palsy. Acta Neuropathol Commun. 2016;4:22. Published 2016 Mar 2. doi:10.1186/s40478-016-0292-9.

Pizzo P, Basso E, Filadi R, Greotti E, Leparulo A, Pendin D, Redolfi N, Rossini M, Vajente N, Pozzan T, Fasolato C. Presenilin-2 and Calcium Handling: Molecules, Organelles, Cells and Brain Networks. Cells. 2020;9(10):2166. Published 2020 Sep 25. doi:10.3390/cells9102166.

Placanica L, Zhu L, Li YM. Gender- and age-dependent gamma-secretase activity in mouse brain and its implication in sporadic Alzheimer disease. PLoS One. 2009;4(4):e5088. doi:10.1371/journal.pone.0005088.

Planel E, Yasutake K, Fujita SC, Ishiguro K. Inhibition of protein phosphatase 2A overrides tau protein kinase I/glycogen synthase kinase 3 beta and cyclin-dependent kinase 5 inhibition and results in tau hyperphosphorylation in the hippocampus of starved mouse. J Biol Chem. 2001;276(36):34298-34306. doi:10.1074/jbc.M102780200.

Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP. Physiological release of endogenous tau is stimulated by neuronal activity. EMBO Rep. 2013;14(4):389-394. doi:10.1038/embor.2013.15.

Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, Gräber S, Kuder-Buletta E, LaFougere C, Laske C, Vöglein J, Levin J, Masters CL, Martins R, Schofield PR, Rossor MN, Graff-Radford NR, Salloway S, Ghetti B, Ringman JM, Noble JM, Chhatwal J, Goate AM, Benzinger TLS, Morris JC, Bateman RJ, Wang G, Fagan AM, McDade EM, Gordon BA, Jucker M. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med. 2019;25(2):277-283. doi:10.1038/s41591-018-0304-3.

Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol. 1999;45(3):358-368. doi:10.1002/1531-8249(199903)45:3<358::aid-ana12>3.0.co;2-x.

Prince MJ and Jackson J. World Alzheimer Report 2009: The Global Prevalence of Dementia. Alzheimer's Disease International. 2009.

Prince MJ, Anders W, Guerchet MM, Ali GC, Wu YT, Prina M. World Alzheimer Report 2015: The Global Impact of Dementia. An Analysis of Prevalence, Incidence, Cost and Trends. Alzheimer's Disease International. 2015.

Rajan KB, Weuve J, Barnes LL, Wilson RS, Evans DA. Prevalence and incidence of clinically diagnosed Alzheimer's disease dementia from 1994 to 2012 in a population study. Alzheimers Dement. 2019;15(1):1-7. doi:10.1016/j.jalz.2018.07.216.

Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P, Simons K. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proc Natl Acad Sci U S A. 2006;103(30):11172-11177. doi:10.1073/pnas.0603838103.

Ramachandran G. Fourier Transform Infrared (FTIR) Spectroscopy, Ultraviolet Resonance Raman (UVRR) Spectroscopy, and Atomic Force Microscopy (AFM) for Study of the Kinetics of Formation and Structural Characterization of Tau Fibrils. Methods Mol Biol. 2017;1523:113-128. doi:10.1007/978-1-4939-6598-4_7.

Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochim Biophys Acta. 2014;1841(1):108-120. doi:10.1016/j.bbalip.2013.10.004.

Reddy K, Cusack CL, Nnah IC, Khayati K, Saqcena C, Huynh TB, Noggle SA, Ballabio A, Dobrowolski R. Dysregulation of Nutrient Sensing and CLEARance in Presenilin Deficiency. Cell Rep. 2016;14(9):2166-2179. doi:10.1016/j.celrep.2016.02.006.

Reilly P, Winston CN, Baron KR, Trejo M, Rockenstein EM, Akers JC, Kfoury N, Diamond M, Masliah E, Rissman RA, Yuan SH. Novel human neuronal tau model exhibiting neurofibrillary tangles and transcellular propagation. Neurobiol Dis. 2017;106:222-234. doi:10.1016/j.nbd.2017.06.005.

Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Methods Enzymol. 1994;234:279-293. doi:10.1016/0076-6879(94)34095-1.

Richetin K, Steullet P, Pachoud M, Perbet R, Parietti E, Maheswaran M, Eddarkaoui S, Bégard S, Pythoud C, Rey M, Caillierez R, Q Do K, Halliez S, Bezzi P, Buée L, Leuba G, Colin M, Toni N, Déglon N. Tau accumulation in astrocytes of the dentate gyrus induces neuronal dysfunction and memory deficits in Alzheimer's disease. Nat Neurosci. 2020;23(12):1567-1579. doi:10.1038/s41593-020-00728-x.

Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science. 2007;316(5825):750-754. doi:10.1126/science.1141736.

Rodrigue KM, Kennedy KM, Devous MD Sr, Rieck JR, Hebrank AC, Diaz-Arrastia R, Mathews D, Park DC. β-Amyloid burden in healthy aging: regional distribution and cognitive consequences. Neurology. 2012;78(6):387-395. doi:10.1212/WNL.0b013e318245d295.

Rogers LM, Mott SL, Smith BJ, Link BK, Sahin D, Weiner GJ. Complement-Regulatory Proteins CFHR1 and CFHR3 and Patient Response to Anti-CD20 Monoclonal Antibody Therapy. Clin Cancer Res. 2017;23(4):954-961. doi:10.1158/1078-0432.CCR-16-1275.

Rohrer JD, Guerreiro R, Vandrovcova J, Uphill J, Reiman D, Beck J, Isaacs AM, Authier A, Ferrari R, Fox NC, Mackenzie IR, Warren JD, de Silva R, Holton J, Revesz T, Hardy J, Mead S, Rossor MN. The heritability and genetics of frontotemporal lobar degeneration. Neurology. 2009;73(18):1451-1456. doi:10.1212/WNL.0b013e3181bf997a.

Rohrer JD, Woollacott IO, Dick KM, Brotherhood E, Gordon E, Fellows A, Toombs J, Druyeh R, Cardoso MJ, Ourselin S, Nicholas JM, Norgren N, Mead S, Andreasson U, Blennow K, Schott JM, Fox NC, Warren JD, Zetterberg H. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology. 2016;87(13):1329-1336. doi:10.1212/WNL.0000000000003154.

Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. Science. 2004;303(5660):1007-1010. doi:10.1126/science.1093133

Sala Frigerio C, Wolfs L, Fattorelli N, Thrupp N, Voytyuk I, Schmidt I, Mancuso R, Chen WT, Woodbury ME, Srivastava G, Möller T, Hudry E, Das S, Saido T, Karran E, Hyman B, Perry VH, Fiers M, De Strooper B. The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Aβ Plaques. Cell Rep. 2019;27(4):1293-1306.e6. doi:10.1016/j.celrep.2019.03.099.

Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF. Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem. 2012;287(6):3842-3849. doi:10.1074/jbc.M111.277061.

Sanchez-Molina P, Kreuzer M, Benseny-Cases N, Valente T, Almolda B, González B, Castellano B, Perálvarez-Marín A. From Mouse to Human: Comparative Analysis between Grey and White Matter by Synchrotron-Fourier Transformed Infrared Microspectroscopy. Biomolecules. 2020;10(8):1099. Published 2020 Jul 24. doi:10.3390/biom10081099.

Sánchez-Valle R, Heslegrave A, Foiani MS, Bosch B, Antonell A, Balasa M, Lladó A, Zetterberg H, Fox NC. Serum neurofilament light levels correlate with severity measures and neurodegeneration markers in autosomal dominant Alzheimer's disease. Alzheimers Res Ther. 2018;10(1):113. Published 2018 Nov 3. doi:10.1186/s13195-018-0439-y.

Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, Barker SJ, Foley AC, Thorpe JR, Serpell LC, Miller TM, Grinberg LT, Seeley WW, Diamond MI. Distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron. 2014;82(6):1271-1288. doi:10.1016/j.neuron.2014.04.047.

Saura CA, Tomita T, Davenport F, Harris CL, Iwatsubo T, Thinakaran G. Evidence that intramolecular associations between presenilin domains are obligatory for endoproteolytic processing. J Biol Chem. 1999;274(20):13818-13823. doi:10.1074/jbc.274.20.13818.

Saura CA, Choi SY, Beglopoulos V, Malkani S, Zhang D, Shankaranarayana Rao BS, Chattarji S, Kelleher RJ 3rd, Kandel ER, Duff K, Kirkwood A, Shen J. Loss of presenilin function causes impairments of memory and synaptic plasticity followed by age-dependent neurodegeneration. Neuron. 2004;42(1):23-36. doi:10.1016/s0896-6273(04)00182-5.

Saura CA. Presenilin/gamma-Secretase and Inflammation. Front Aging Neurosci. 2010;2:16. Published 2010 May 18. doi:10.3389/fnagi.2010.00016.

Schaeffer V, Goedert M. Stimulation of autophagy is neuroprotective in a mouse model of human tauopathy. Autophagy. 2012;8(11):1686-1687. doi:10.4161/auto.21488.

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med. 1996;2(8):864-870. doi:10.1038/nm0896-864.

Schmidt R, Hofer E, Bouwman FH, Buerger K, Cordonnier C, Fladby T, Galimberti D, Georges J, Heneka MT, Hort J, Laczó J, Molinuevo JL, O'Brien JT, Religa D, Scheltens P, Schott JM, Sorbi S. EFNS-ENS/EAN Guideline on concomitant use of cholinesterase inhibitors and memantine in moderate to severe Alzheimer's disease. Eur J Neurol. 2015;22(6):889-898. doi:10.1111/ene.12707.

Selnick HG, Hess JF, Tang C, Liu K, Schachter JB, Ballard JE, Marcus J, Klein DJ, Wang X, Pearson M, Savage MJ, Kaul R, Li TS, Vocadlo DJ, Zhou Y, Zhu Y, Mu C, Wang Y, Wei Z, Bai C, Duffy JL, McEachern EJ. Discovery of MK-8719, a Potent O-GlcNAcase Inhibitor as a Potential Treatment for Tauopathies. J Med Chem. 2019;62(22):10062-10097. doi:10.1021/acs.jmedchem.9b01090.

Seo J, Kritskiy O, Watson LA, Barker SJ, Dey D, Raja WK, Lin YT, Ko T, Cho S, Penney J, Silva MC, Sheridan SD, Lucente D, Gusella JF, Dickerson BC, Haggarty SJ, Tsai LH. Inhibition of p25/Cdk5 Attenuates Tauopathy in Mouse and iPSC Models of Frontotemporal Dementia. J Neurosci. 2017;37(41):9917-9924. doi:10.1523/JNEUROSCI.0621-17.2017.

Serenó L, Coma M, Rodríguez M, Sánchez-Ferrer P, Sánchez MB, Gich I, Agulló JM, Pérez M, Avila J, Guardia-Laguarta C, Clarimón J, Lleó A, Gómez-Isla T. A novel GSK-3beta inhibitor reduces Alzheimer's pathology and rescues neuronal loss in vivo. Neurobiol Dis. 2009;35(3):359-367. doi:10.1016/j.nbd.2009.05.025.

Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011;1(1):a006189. doi:10.1101/cshperspect.a006189.

Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. Skeletal and CNS defects in Presenilin-1-deficient mice. Cell. 1997;89(4):629-639. doi:10.1016/s0092-8674(00)80244-5.

Shen J, Kelleher RJ 3rd. The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. Proc Natl Acad Sci U S A. 2007;104(2):403-409. doi:10.1073/pnas.0608332104.

Silva MC, Nandi GA, Tentarelli S, Gurrell IK, Jamier T, Lucente D, Dickerson BC, Brown DG, Brandon NJ, Haggarty SJ. Prolonged tau clearance and stress vulnerability rescue by pharmacological activation of autophagy in tauopathy neurons. Nat Commun. 2020;11(1):3258. Published 2020 Jun 26. doi:10.1038/s41467-020-16984-1.

Spina S, Farlow MR, Unverzagt FW, Kareken DA, Murrell JR, Fraser G, Epperson F, Crowther RA, Spillantini MG, Goedert M, Ghetti B. The tauopathy associated with mutation +3 in intron 10 of Tau: characterization of the MSTD family. Brain. 2008;131(Pt 1):72-89. doi:10.1093/brain/awm280.

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J Cell Biol. 2002;156(6):1051-1063. doi:10.1083/jcb.200108057.

Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, Baker A, Jonasdottir A, Ingason A, Gudnadottir VG, Desnica N, Hicks A, Gylfason A, Gudbjartsson DF, Jonsdottir GM, Sainz J, Agnarsson K, Birgisdottir B, Ghosh S, Olafsdottir A, Cazier JB, Kristjansson K, Frigge ML, Thorgeirsson TE, Gulcher JR, Kong A, Stefansson K. A common inversion under selection in Europeans. Nat Genet. 2005;37(2):129-137. doi:10.1038/ng1508.

Steiner H, Duff K, Capell A, Romig H, Grim MG, Lincoln S, Hardy J, Yu X, Picciano M, Fechteler K, Citron M, Kopan R, Pesold B, Keck S, Baader M, Tomita T, Iwatsubo T, Baumeister R, Haass C. A loss of function mutation of presenilin-2 interferes with amyloid beta-peptide production and notch signaling. J Biol Chem. 1999;274(40):28669-28673. doi:10.1074/jbc.274.40.28669.

Sun L, Zhou R, Yang G, Shi Y. Analysis of 138 pathogenic mutations in presenilin-1 on the in vitro production of Aβ42 and Aβ40 peptides by γ-secretase. Proc Natl Acad Sci U S A. 2017;114(4):E476-E485. doi:10.1073/pnas.1618657114.

Sun Y, Guo Y, Feng X, Jia M, Ai N, Dong Y, Zheng Y, Fu L, Yu B, Zhang H, Wu J, Yu X, Wu H, Kong W. The behavioural and neuropathologic sexual dimorphism and absence of MIP-3α in tau P301S mouse model of Alzheimer's disease. J Neuroinflammation. 2020;17(1):72. Published 2020 Feb 24. doi:10.1186/s12974-020-01749-w.

Takashima A, Murayama M, Murayama O, Kohno T, Honda T, Yasutake K, Nihonmatsu N, Mercken M, Yamaguchi H, Sugihara S, Wolozin B. Presenilin 1 associates with glycogen synthase kinase-3beta and its substrate tau. Proc Natl Acad Sci U S A. 1998;95(16):9637-9641. doi:10.1073/pnas.95.16.9637.

Takeda S, Wegmann S, Cho H, DeVos SL, Commins C, Roe AD, Nicholls SB, Carlson GA, Pitstick R, Nobuhara CK, Costantino I, Frosch MP, Müller DJ, Irimia D, Hyman BT. Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain. Nat Commun. 2015;6:8490. Published 2015 Oct 13. doi:10.1038/ncomms9490.

Tardivel M, Bégard S, Bousset L, Dujardin S, Coens A, Melki R, Buée L, Colin M. Tunneling nanotube (TNT)-mediated neuron-to neuron transfer of pathological Tau protein assemblies. Acta Neuropathol Commun. 2016;4(1):117. Published 2016 Nov 4. doi:10.1186/s40478-016-0386-4.

The Lancet Neurology. Antipsychotic drugs for dementia: a balancing act. Lancet Neurol 2009; 8: 125.

Theunis C, Crespo-Biel N, Gafner V, Pihlgren M, López-Deber MP, Reis P, Hickman DT, Adolfsson O, Chuard N, Ndao DM, Borghgraef P, Devijver H, Van Leuven F, Pfeifer A, Muhs A. Efficacy and safety of a liposome-based vaccine against protein Tau, assessed in tau.P301L mice that model tauopathy. PLoS One. 2013;8(8):e72301. Published 2013 Aug 19. doi:10.1371/journal.pone.0072301.

Thinakaran G, Borchelt DR, Lee MK, Slunt HH, Spitzer L, Kim G, Ratovitsky T, Davenport F, Nordstedt C, Seeger M, Hardy J, Levey AI, Gandy SE, Jenkins NA, Copeland NG, Price DL, Sisodia SS. Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. Neuron. 1996;17(1):181-190. doi:10.1016/s0896-6273(00)80291-3.

Tissot C, Therriault J, Pascoal TA, Chamoun M, Lussier FZ, Savard M, Mathotaarachchi SS, L Benedet A, Thomas EM, Parsons M, Nasreddine Z, Rosa-Neto P, Gauthier S. Association between regional tau pathology and neuropsychiatric symptoms in aging and dementia due to Alzheimer's disease. Alzheimers Dement (N Y). 2021;7(1):e12154. Published 2021 Mar 31. doi:10.1002/trc2.12154.

Tuerde D, Kimura T, Miyasaka T, Furusawa K, Shimozawa A, Hasegawa M, Ando K, Hisanaga SI. Isoform-independent and -dependent phosphorylation of microtubule-associated protein tau in mouse brain during postnatal development. J Biol Chem. 2018;293(5):1781-1793. doi:10.1074/jbc.M117.798918.

van der Jeugd A, Parra-Damas A, Baeta-Corral R, Soto-Faguás CM, Ahmed T, LaFerla FM, Giménez-Llort L, D'Hooge R, Saura CA. Reversal of memory and neuropsychiatric symptoms and reduced tau pathology by selenium in 3xTg-AD mice. Sci Rep. 2018;8(1):6431. Published 2018 Apr 24. doi:10.1038/s41598-018-24741-0.

van der Kant R, Goldstein LSB, Ossenkoppele R. Amyloid-β-independent regulators of tau pathology in Alzheimer disease. Nat Rev Neurosci. 2020;21(1):21-35. doi:10.1038/s41583-019-0240-3.

Vaz-Silva J, Gomes P, Jin Q, Zhu M, Zhuravleva V, Quintremil S, Meira T, Silva J, Dioli C, Soares-Cunha C, Daskalakis NP, Sousa N, Sotiropoulos I, Waites CL. Endolysosomal degradation of Tau and its role in glucocorticoid-driven hippocampal malfunction. EMBO J. 2018;37(20):e99084. doi:10.15252/embj.201899084.

Vermunt L, Sikkes SAM, van den Hout A, Handels R, Bos I, van der Flier WM, Kern S, Ousset PJ, Maruff P, Skoog I, Verhey FRJ, Freund-Levi Y, Tsolaki M, Wallin ÅK, Olde Rikkert M, Soininen H, Spiru L, Zetterberg H, Blennow K, Scheltens P, Muniz-Terrera G, Visser PJ. Duration of preclinical, prodromal, and dementia stages of Alzheimer's disease in relation to age, sex, and APOE genotype. Alzheimers Dement. 2019;15(7):888-898. doi:10.1016/j.jalz.2019.04.001.

Vieira RT, Caixeta L, Machado S, Silva AC, Nardi AE, Arias-Carrión O, Carta MG. Epidemiology of early-onset dementia: a review of the literature. Clin Pract Epidemiol Ment Health. 2013;9:88-95. Published 2013 Jun 14. doi:10.2174/1745017901309010088.

Vingtdeux V, Davies P, Dickson DW, Marambaud P. AMPK is abnormally activated in tangle- and pre-tangle-bearing neurons in Alzheimer's disease and other tauopathies. Acta Neuropathol. 2011;121(3):337-349. doi:10.1007/s00401-010-0759-x.

Violet M, Delattre L, Tardivel M, Sultan A, Chauderlier A, Caillierez R, Talahari S, Nesslany F, Lefebvre B, Bonnefoy E, Buée L, Galas MC. A major role for Tau in neuronal DNA and RNA protection in vivo under physiological and hyperthermic conditions. Front Cell Neurosci. 2014;8:84. Published 2014 Mar 18. doi:10.3389/fncel.2014.00084.

von Bergen M, Barghorn S, Biernat J, Mandelkow EM, Mandelkow E. Tau aggregation is driven by a transition from random coil to beta sheet structure. Biochim Biophys Acta. 2005;1739(2-3):158-166. doi:10.1016/j.bbadis.2004.09.010.

Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM, Mandelkow E. Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. Hum Mol Genet. 2009;18(21):4153-4170. doi:10.1093/hmg/ddp367.

Wang Y, Mandelkow E. Tau in physiology and pathology. Nat Rev Neurosci. 2016;17(1):5-21. doi:10.1038/nrn.2015.1.

Wang X, Li W, Marcus J, Pearson M, Song L, Smith K, Terracina G, Lee J, Hong KK, Lu SX, Hyde L, Chen SC, Kinsley D, Melchor JP, Rubins DJ, Meng X, Hostetler E, Sur C, Zhang L, Schachter JB, Hess JF, Selnick HG, Vocadlo DJ, McEachern EJ, Uslaner JM, Duffy JL, Smith SM. MK-8719, a Novel and Selective O-GlcNAcase Inhibitor That Reduces the Formation of Pathological Tau and Ameliorates Neurodegeneration in a

Mouse Model of Tauopathy. J Pharmacol Exp Ther. 2020;374(2):252-263. doi:10.1124/jpet.120.266122.

Warmus BA, Sekar DR, McCutchen E, Schellenberg GD, Roberts RC, McMahon LL, Roberson ED. Tau-mediated NMDA receptor impairment underlies dysfunction of a selectively vulnerable network in a mouse model of frontotemporal dementia. J Neurosci. 2014;34(49):16482-16495. doi:10.1523/JNEUROSCI.3418-14.2014.

Watanabe H, Smith MJ, Heilig E, Beglopoulos V, Kelleher RJ 3rd, Shen J. Indirect regulation of presenilins in CREB-mediated transcription. J Biol Chem. 2009;284(20):13705-13713. doi:10.1074/jbc.M809168200.

Watanabe H, Iqbal M, Zheng J, Wines-Samuelson M, Shen J. Partial loss of presenilin impairs age-dependent neuronal survival in the cerebral cortex. J Neurosci. 2014;34(48):15912-15922. doi:10.1523/JNEUROSCI.3261-14.2014.

Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. Proc Natl Acad Sci U S A. 1975;72(5):1858-1862. doi:10.1073/pnas.72.5.1858.

Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. F1000Res. 2018;7:F1000 Faculty Rev-1161. Published 2018 Jul 31. doi:10.12688/f1000research.14506.1.

Wilcock GK, Gauthier S, Frisoni GB, Jia J, Hardlund JH, Moebius HJ, Bentham P, Kook KA, Schelter BO, Wischik DJ, Davis CS, Staff RT, Vuksanovic V, Ahearn T, Bracoud L, Shamsi K, Marek K, Seibyl J, Riedel G, Storey JMD, Harrington CR, Wischik CM. 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 Alzheimers Dis. 2018;61(1):435-457. doi:10.3233/JAD-170560.

Wischik CM, Harrington CR, Storey JM. Tau-aggregation inhibitor therapy for Alzheimer's disease. Biochem Pharmacol. 2014;88(4):529-539. doi:10.1016/j.bcp.2013.12.008.

Wischik CM, Staff RT, Wischik DJ, Bentham P, Murray AD, Storey JM, Kook KA, Harrington CR. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. J Alzheimers Dis. 2015;44(2):705-720. doi:10.3233/JAD-142874.

Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature. 1999;398(6727):513-517. doi:10.1038/19077.

Wong ES, Tan JM, Soong WE, Hussein K, Nukina N, Dawson VL, Dawson TM, Cuervo AM, Lim KL. Autophagy-mediated clearance of aggresomes is not a universal phenomenon. Hum Mol Genet. 2008;17(16):2570-2582. doi:10.1093/hmg/ddn157.

Woodhouse A, Shepherd CE, Sokolova A, Carroll VL, King AE, Halliday GM, Dickson TC, Vickers JC. Cytoskeletal alterations differentiate presenilin-1 and sporadic Alzheimer's disease. Acta Neuropathol. 2009;117(1):19-29. doi:10.1007/s00401-008-0458-z.

Xia D, Watanabe H, Wu B, Lee SH, Li Y, Tsvetkov E, Bolshakov VY, Shen J, Kelleher RJ 3rd. Presenilin-1 knockin mice reveal loss-of-function mechanism for familial Alzheimer's disease. Neuron. 2015;85(5):967-981. doi:10.1016/j.neuron.2015.02.010.

Yamada K, Cirrito JR, Stewart FR, Jiang H, Finn MB, Holmes BB, Binder LI, Mandelkow EM, Diamond MI, Lee VM, Holtzman DM. In vivo microdialysis reveals age-

dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. J Neurosci. 2011;31(37):13110-13117. doi:10.1523/JNEUROSCI.2569-11.2011.

Yan L, Li L, Han W, Pan B, Xue X, Mei B. Age-related neuropsychiatric symptoms in presenilins conditional double knockout mice. Brain Res Bull. 2013;97:104-111. doi:10.1016/j.brainresbull.2013.06.002.

Yanamandra K, Kfoury N, Jiang H, Mahan TE, Ma S, Maloney SE, Wozniak DF, Diamond MI, Holtzman DM. Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo. Neuron. 2013;80(2):402-414. doi:10.1016/j.neuron.2013.07.046.

Yao XQ, Zhang XX, Yin YY, Liu B, Luo DJ, Liu D, Chen NN, Ni ZF, Wang X, Wang Q, Wang JZ, Liu GP. Glycogen synthase kinase-3β regulates Tyr307 phosphorylation of protein phosphatase-2A via protein tyrosine phosphatase 1B but not Src. Biochem J. 2011;437(2):335-344. doi:10.1042/BJ20110347.

Yasuda M, Nakamura Y, Kawamata T, Kaneyuki H, Maeda K, Komure O. Phenotypic heterogeneity within a new family with the MAPT p301s mutation. Ann Neurol. 2005;58(6):920-928. doi:10.1002/ana.20668.

Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T, Trojanowski JQ, Lee VM. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model [published correction appears in Neuron. 2007 Apr 19;54(2):343-4]. Neuron. 2007;53(3):337-351. doi:10.1016/j.neuron.2007.01.010.

Yu H, Saura CA, Choi SY, Sun LD, Yang X, Handler M, Kawarabayashi T, Younkin L, Fedeles B, Wilson MA, Younkin S, Kandel ER, Kirkwood A, Shen J. APP processing and synaptic plasticity in presenilin-1 conditional knockout mice. Neuron. 2001;31(5):713-726. doi:10.1016/s0896-6273(01)00417-2.

Zempel H, Mandelkow E. Lost after translation: missorting of Tau protein and consequences for Alzheimer disease. Trends Neurosci. 2014;37(12):721-732. doi:10.1016/j.tins.2014.08.004.

Zetterberg H. Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration. Neuron. 2016;91(1):1-3. doi:10.1016/j.neuron.2016.06.030.

Zhang DL, Chen YQ, Jiang X, Ji TT, Mei B. Oxidative damage increased in presenilin1/presenilin2 conditional double knockout mice. Neurosci Bull. 2009a;25(3):131-137. doi:10.1007/s12264-009-0114-1.

Zhang C, Wu B, Beglopoulos V, Wines-Samuelson M, Zhang D, Dragatsis I, Südhof TC, Shen J. Presenilins are essential for regulating neurotransmitter release. Nature. 2009b;460(7255):632-636. doi:10.1038/nature08177.

Zhang D, Zhang C, Ho A, Kirkwood A, Südhof TC, Shen J. Inactivation of presenilins causes pre-synaptic impairment prior to post-synaptic dysfunction. J Neurochem. 2010;115(5):1215-1221. doi:10.1111/j.1471-4159.2010.07011.x.

Zhang Y, Xing S, Zhang J, Li J, Li C, Pei Z, Zeng J. Reduction of β-amyloid deposits by γ-secretase inhibitor is associated with the attenuation of secondary damage in the ipsilateral thalamus and sensory functional improvement after focal cortical infarction in hypertensive rats. J Cereb Blood Flow Metab. 2011;31(2):572-579. doi:10.1038/jcbfm.2010.127.

Zhang S, Zhang M, Cai F, Song W. Biological function of Presenilin and its role in AD pathogenesis. Transl Neurodegener. 2013;2(1):15. Published 2013 Jul 17. doi:10.1186/2047-9158-2-15.

XII. **Agradecimientos**

Los que mejor me conocéis sabéis que nunca se me han dado bien estas cosas. No me suele ser fácil expresar mis sentimientos y, mucho menos, escribirlos. Dada la ocasión, habrá que hacer un esfuerzo.

En primer lugar, me gustaría acordarme de cuatro personas que, por desgracia, no están hoy aquí. Mis abuelos. Pepita, Remedios, Carlos y Manolo. Me hubiera gustado haber podido pasar muchísimo más tiempo con vosotros, pero, aun así, os debo mucho por todo lo que me enseñasteis. En especial, quiero recordar a mi abuela Pepita. Aunque siempre he estado interesado en la ciencia y en la medicina, y, en especial, en el cerebro, los últimos años que viví contigo hicieron que me decidiera por investigar sobre las enfermedades neurodegenerativas y, en concreto, por el Alzheimer. Los años te quitaron el recuerdo, pero siempre me quedaré con que, aunque no supieses quiénes éramos, siempre nos sonreías cuando nos veías. “El Alzheimer borra la memoria, no los sentimientos” *Pasqual Maragall*.

Después, quiero agradecer a los principales responsables de que haya llegado hasta aquí y de que sea lo que soy hoy en día. Mis padres, Concha y Pepe. No hay palabras de agradecimiento que puedan expresar todo lo que os debo. Nunca me ha faltado de nada y siempre me habéis apoyado al máximo en cada paso que he querido dar, ya fuera personal, académico, económico o deportivo. Me habéis inculcado todos los valores para llegar a ser el mejor tipo de persona que se puede ser. No hay tiempo ni forma para devolver todo el cariño recibido y todo lo que me habéis enseñado durante toda mi vida. Sentíos orgullosos de vosotros mismos, porque esto también es, en gran medida, vuestro.

También quiero acordarme de mis tres hermanos: Inma, José y Pablo. Quizá los otros responsables de que sea como soy hoy. Independientemente de alguna que otra peleilla típica de hermanos, siempre habéis estado ahí apoyándome y enseñándome cosas desde muy pequeño. He tenido la suerte de tener dos hermanos mayores y uno pequeño que me han hecho no sentirme nunca sólo y que cada uno, a su manera, me ha enseñado y me ha hecho convertirme en lo que soy hoy. Aprovecho estas líneas para dar agradecerle por la portada y darle un toque al más pequeño. Espero que no tardes en darte cuenta y aprendas a valorar todo lo te han dado, enseñado y luchado por ti papá y mamá. Es hora de que despiertes y empieces a formar tu propia vida. Al final, cuando te des cuenta y con lo que has estudiado (gracias al esfuerzo de nuestros padres) serás el que mejor vivas. Solo es cuestión de que te des cuenta y empieces.

Tampoco quiero olvidarme de mis cuñados Isi y Pedro, de mis sobrinos Gonzalo, Pedro, Fernando y, de mi ahijado, Bruno. Hay pocas cosas por las que me cuesta y me duele estar fuera de casa, pero sin duda, vosotros sois una de ellas. Me gustaría haber pasado y poder pasar mucho más tiempo con vosotros, sobre todo en vuestros primeros años de vida. Solamente espero que en el futuro pueda recuperar el tiempo “perdido”.

Dejando la familia a un lado, tengo que agradecerte la oportunidad de estar hoy aquí, después de casi 6 años. Carles, me acogiste para formar parte de tu laboratorio y hacer las prácticas del máster cuando comenzaba en esto de la investigación y no sabía nada, y luego, me diste la oportunidad de realizar la tesis. Me has hecho formarme como investigador, pero también como persona. Siempre te agradeceré todo lo que me has enseñado y que siempre hayamos podido discutir sobre ciencia y valoro mucho que siempre tuvieras en cuenta mis opiniones. Siento mucha admiración por cómo eres capaz de llevar tantas cosas (clases, proyectos, investigación…) y supervisar a tantas personas a la vez. Es una pena que los científicos no tengamos el reconocimiento y la consideración que nos merecemos y por eso, no sé si quiero ni si llegaré a ser algún día IP, pero sí tengo claro y puedo decir que, si ese momento llega, me gustaría parecerme a ti.

Ahora me gustaría acordarme de toda la gente con la que he coincidido en el lab. Espero no dejarme a nadie. Cuando llegué al laboratorio empecé trabajando con Muriel. Al final compartimos menos tiempo del esperado, pero siempre recordaré que fue contigo con quien hice mis primeras perfusiones y westerns. También quiero acordarme de Lilian. Aunque mi

proyecto no tuviera nada que ver, siempre estabas ahí para ayudarnos en lo científico y en lo personal. También estaba Arnaldo, aunque casi no coincidimos porque prácticamente te quedaba leer la tesis. Tras tu paso por Alemania, sí que pude conocer a esa persona de la que tan bien me habían hablado. No sólo me quedo con tus conocimientos y consejos científicos, también con las pizzas para cenar en el lab, las vueltas a casa en nitbus y, por supuesto, el SAF, las cervezas y quedadas. Por aquel entonces sólo había dos estudiantes de doctorado: Laura y Miriam. Laura, aunque tardaste un poco en abrirte, al final sí que pude llegar a conocer tu lado más gracioso y siempre recordaré las risas además de todo lo que me ayudaste y me enseñaste. A ti, Miriam, siempre te agradeceré cómo me acogiste en el laboratorio de los Saura's y todo lo que me ensañaste y ayudaste con los experimentos. Además, siempre recordaré todos los momentos que pasamos fuera del lab, como en la Feria o en las Santas.

Detrás de mí, llegó Anna (no sé si era del Ser o del Saber). Llegaste siendo esa chica tímida y seca de la España profunda, pero después de casi 5 años casi no queda nada de eso. Además de toda la ayuda que me has dado, especialmente con nuestro mejor amigo el cloning y los lentivirus, quiero agradecerte el apoyo que has sido cuando las cosas no salían o cuando necesitábamos desconectar. Te mereces lo mejor y pase lo que pase, no te desanimes y sigue trabajando como lo has hecho hasta ahora que seguro que al final, acabarán saliendo las cosas. También llegó Óscar como técnico de prácticas. Además de por tu ayuda con el genotipaje y con el Gallyas, quiero agradecerte el buen rollo, las bromas y las risas que has aportado durante el tiempo que estábamos en el laboratorio y fuera de él, enseñándonos antros que sólo tú conocías. Luego llegó Lola. Tú, sí que caíste de pie en el laboratorio y desde el primer día también aportaste alegría y buen rollo al lab, algo imprescindible para poder superar los altibajos de la tesis. Aunque la vida te atropelle, sigue con la dedicación que has puesto hasta ahora que seguro que todo te acabará saliendo bien. También me quedaré con los ratitos dentro y fuera del lab, sobre todo el viaje a Burdeos donde "sólo pasaste la mayor vergüenza de tu vida". Después llegó Ángel. A pesar de tu timidez e introversión siempre has estado ahí para echar una mano y siempre con una sonrisa. Te deseo lo mejor para lo que todavía te queda de doctorado. En este último curso, llegó Mar. Ninguno nos esperábamos tu llegada y ojalá hubieras llegado antes. Desde el primer día aportaste positividad, alegría, energía y motivación al laboratorio y, quizás, en uno de los momentos que más falta hacía. Me hubiera encantado haber podido compartir mucho más tiempo juntos, por tus consejos y sabiduría científica, pero sobre todo por tu calidad como persona. La ciencia actual necesita mucha más gente como tú para que lo positivo supere siempre lo negativo.

No quiero olvidarme de todos los técnicos y estudiantes de máster con los que he coincido y que también han contribuido a que esto sea posible: Alejandra, Evelyn, Navera, Gemma, Irene, Sara, Marc, Edgar, Rafa, Laura, Noelia, Luis, Paula, Oihane…

Quiero agradecer también a todos aquellos a los que he conocido en el departamento y que me han ayudado de una forma u otra: Irene, Judith, Cristina, Elena, Paola, Meri, Laura, Nora, Pau, Eli, Laura, Raquel, Anna, Mireia, Montse… Especialmente, quiero agradecer los buenos ratos que hemos pasado a Guille y Sergio y por acogerme en su equipo de fútbol. También quiero acordarme de los antiguos Galea's: Carmen, Arantxa y Raquel, y de Dolo. Además de que me acogisteis y me ayudasteis cuando llegué, me llevo las cervezas, calçotadas, salidas a enfants, etc. Especialmente, quiero acordarme de Abel, por acogerme desde el principio y por todas las cosas que hemos compartido desde entonces, ya sean charlas de fútbol, catán, Burdeos, Cádiz o que te pusieras por primera vez traje y corbata para la Feria de Sevilla.

Me gustaría agradecerle a Núria su ayuda tanto en el proyecto como en el propio sincrotrón. También al grupo de Andero, en especial a Raúl y Antonio, el haber contado y confiado en nosotros para poder llevar a cabo esa magnífica colaboración. También quiero dar las gracias a todo el personal de la unidad de bioquímica y del instituto de neurociencias por su ayuda, en especial a Cris, Neus, Susana, Núria, Mar y Roser.

Quiero agradecer a la gente que ha hecho que no sienta tan lejos mi casa y que me ha hecho mucho más fácil la estancia en Barcelona: Marina, Manolo, Carmen, Dani, Mayk, Ángel, Neus, Fran, Asun, Guille... En especial quiero acordarme de Loren y las dos personas del máster que me llevo como amigos: Sergio y Efren. Loren, el único amigo de Sevilla con el que he coincidido viviendo en Barcelona. También tengo que agradecerte el que me hayas hecho sentir como en casa y las pechás de reír con tus anécdotas, especialmente con el catalán. A Sergio, fue una pena y me hubiera encantado que te hubieras podido quedar en Barcelona

para hacer la tesis, pero a pesar de eso, hemos mantenido contacto y nos hemos seguido viendo siempre que hemos podido y espero que lo sigamos haciendo. Me guardo nuestras quedadas por Barcelona para hacer deporte o cervecear, mi visita al "centro" geográfico de la península y con tu primera Feria de Sevilla. Te deseo lo mejor para el futuro en la ciencia (aunque sé que no lo necesitas por tu vocación y constancia) y en la vida. A Efren, aunque nos conociéramos más tarde y nuestra amistad se formara después del máster, acabamos congeniando mucho y te agradezco el haber podido compartir mis últimos años de baloncesto contigo, además de muchas salidas, cenas y risas. También recordaré tu cara de sorpresa y admiración al llegar a Sevilla y a la Feria y lo agradecido que siempre has estado por haberte invitado. Suerte con tu tesis y con lo que decidas para el futuro.

Quiero acordarme de los que han estado apoyando desde la distancia: Borja, Rubén, Andrés, Vallado, Salva, Raúl, todos los Bambis, todo el Coti…

También me gustaría agradecer todo el esfuerzo y valores que me ha enseñado el Col·lectiu de Doctorands en Lluita. Gracias a sus reivindicaciones he podido disfrutar del cuarto año de tesis y he aprendido que las injusticias no se superan solas, sino luchando. Al igual que a la lucha de Sci-hub por eliminar todas las barreras que existen para que la ciencia sea pública, accesible y universal.

Por último, quiero y tengo que darle las gracias a Paula. Seguramente la parte más importante para que estos casi 6 años hayan sido posibles. Lejos quedan ya las dudas que teníamos cuando vinimos en 2015 a hacer el máster. Nos independizábamos por primera vez, salíamos de nuestra ciudad, nos separábamos de nuestros amigos y nos íbamos a vivir juntos, aunque no llevásemos mucho tiempo. Demasiados cambios y muchas incertidumbres. Al final, dijimos que mejor probar y darnos cuenta pronto que tarde. Al final, resultó ser lo mejor que podíamos haber hecho y siempre nos quedará nuestro "nidito". Difícilmente podría haber aguantado tanto tiempo lejos de familia y amigos sin que estuvieses tú a mi lado, sobre todo en los momentos más duros por los que todo el mundo pasa mientras hace el doctorado. Porque claro que se hace duro hacer una tesis y la verdad es que no nos lo han puesto nada fácil. Nos ha tocado vivir una pandemia, confinamientos, 4 meses separados por estancia… Aun así, echo la vista atrás y no tengo malos recuerdos, solo buenos y eso es, en gran medida, culpa tuya. Me entra mucha nostalgia al pensar que esto se acaba y eso solo puede reflejar lo a gusto que hemos estado. Tenemos la suerte (aunque no siempre sea bueno) de compartir casi absolutamente todo (menos mal que soy yo el del SFC) y de ir al mismo tiempo en nuestra carrera científica, con todo lo bueno y lo malo que eso conlleva. Espero que podamos seguir teniendo esa "suerte" y que las siguientes etapas sean lo más parecidas a lo que ha sido esta.

Espero que no sea un adiós, sino un hasta luego, Barcelona.