Role of Amyloid-beta and Tau proteins in Alzheimer's disease: Confuting the Amyloid Cascade

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Running title (45 characters or less, including spaces)

Abstract

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by dementia, defined as a deficit of mnestic function associated with at least one cognitive disorder (language, praxic function, gnosis function, executive function, judgment and abstract thought) without alteration of the state of consciousness. In the last decades, AD has gained rising attention for its growing incidence in the elderly, with a prevalence of 46.8 million cases of people affected by the pathology worldwide, a number expected to increase up to 74.7 million in 2030 and 131.5 million in 2050. Besides representing a serious health and social problem, the disease causes exorbitant costs for the healthcare system estimated in 604 billion dollars in 2010 that already reached a 35.4% increase in only 5 years (Martin Prince et al. 2015; Wimo et al. 2017). Despite the numerous efforts to counteract the disease, no therapies are so far available to prevent AD onset and progression.

To date, data from thousands of basic, pre-clinical and clinical studies have identified Amyloid-β peptide (Aβ) and tau protein as the main actors in the pathophysiology of AD, mainly because of their deposition in the characteristic histopathological brain lesions, i.e. senile plaques for Aβ and neurofibrillary tangles (NFTs) for tau, and the increase of their soluble forms in the brain of AD patients. However, all the therapeutic approaches aimed to decrease Aβ levels that have been completed so far, have failed. Similarly, tau-based clinical trials have not yet produced positive findings. The overall goal of this review is to provide a critical assessment of literature on mechanisms underlying the disease occurrence and progression. Specifically, we will revisit the studies on Aβ and tau, as well as on their interaction, challenging the amyloid hypothesis which establishes Aβ as the *primum movens* in a cascade of pathological events that include tau at the downstream level of Aβ, and proposing to rearrange the intricate puzzle of AD pathogenesis by placing soluble forms of Aβ and tau in parallel and upstream of amyloid-precursor protein (APP).

Such a view will necessarily change the approach to therapies against AD, paving the way for new therapeutic strategies.

Amyloid β-peptide and Alzheimer's disease: more than one century of research

Aβ derives from a complex cleavage of APP, a type I single-pass transmembrane protein constituted by 639-770 amino acids in humans, highly expressed in the central nervous system where it exerts a variety of physiological functions (Müller & Zheng 2012). APP is initially cleaved by α-secretase or β-secretase, generating soluble (s) and carboxyterminal fragments (CTF). α-secretase activity leads to the formation of sAPPα and CTF83, whereas β-secretase generates sAPPβ and CTF99. Thus, γ-secretase intervenes further cleaving CTF83 and CTF99, generating the intracellular peptide AICD/AID (amyloid intracellular domain) and a small p3 peptide from CTF83, and AICD/AID and Aβ from CTF99. Based on this biochemical processing, the cascade initiated by α-secretase has been considered neuroprotective when compared with the β-secretase cleavage, leading to the amyloidogenic cascade and the formation of Aβ (Zhang et al. 2011). Based on the γ-secretase site of cutting, different isoforms of Aβ can be generated, composed by 38-43 amino acids. $A\beta_{40}$ is the most represented, whereas $A\beta_{42}$ is present at lower concentrations but has received more attention in the AD field due to its propensity to form aggregates. However, in the brain of AD patients, $A\beta_{38}$ and truncated forms at N-terminal region, i.e. $A\beta_{15}$, $A\beta_{16}$ and $A\beta_{17}$, have been also detected (Mawuenyega et al. 2013).

Aβ is undoubtedly the most studied protein in AD and its putative role in the pathogenesis of the disease has oriented drug development and clinical trials for several decades. But, how and why did the AD amyloidogenic theory emerge?

From an historical perspective, it was at the beginning of the last century when Alois Alzheimer and other European neuropsychiatrists, as Gaetano Perusini, attributed a nosographic identity to a form of "mental" disorder characterized by memory loss, hallucinations and disorientation. At that time, the most influent personalities in psychiatry, Sigmund Freud and Emilin Kraeplin, fervently disputed on the origin of psychiatric illness, respectively emphasizing the role of the psyche or of organic and genetic factors. The mind/brain diatribe led several scientists to seek for the "material" causes of mental diseases. In this context, Alzheimer and Perusini, strongly supported by Kraeplin, observed that the psychiatric symptoms of dementia could be correlated to peculiar histological lesions in post-mortem brains. In the autopsy of the first Alzheimer's patient, Auguste Deter, cortical atrophy, neurons filled with neurofibrils, and extracellular miliar foci of an unknown substance were observed. After Alzheimer's death, research studies on the disease slowed down until the 1980s, when epidemiological studies revealed an increase of patients affected by primary dementia. It was during these years that key discoveries were made, fated to influence research in the field until today. Based on Alzheimer's histological descriptions, Aβ and tau were recognized as the main components of extracellular foci (senile plaques) and intracellular neurofibrils (NFTs), respectively (Glenner & Wong 1984a; Glenner & Wong 1984b; Grundke-Iqbal et al. 1986). In the same period, the first genetic mutation linked to dementia was identified on chromosome 21 coding for the Amyloid Precursor Protein (APP) (Levy et al. 1990) . This autosomal dominant disease was responsible of an early onset AD (EOAD) characterized by high levels of Aβ. Other genetic mutations were identified in Familiar Alzheimer's disease (FAD), involving genes responsible for Aβ production such as presenilin 1 (PS1) on chromosome 14, which mutation is the most diffuse cause of EOAD, and presenilin 2 (PS2) on chromosome 1. Consisting with these findings, the presence of an AD-like pathology in patients affected by Down's Syndrome, due to a trisomy of

chromosome 21, reinforced the idea that the increase of Aβ played a major role in AD pathogenesis. Based on these data, in 1995 the first mouse model of AD carrying an APP mutation was created (Games et al. 1995) and, over time, different models for pre-clinical studies have been generated based on the most common mutations observed in FAD (Puzzo et al. 2015).

These findings contributed to the excitement around the "Amyloid Cascade Hypothesis" (Hardy & Allsop 1991; Hardy & Higgins 1992; Hardy et al. 1998), recognized as the pathogenetic mechanism underlying AD. Because insoluble fibrils of Aβ were present in AD plaques, and could be formed *in vitro* from synthetic Aβ, they have dominated the scene until a fundamental breakthrough signed by several *in vitro* and *in vivo* studies indicating that Aβ might be also present in the brain in soluble forms (Wisniewski et al. 1994; Lambert et al. 1998). Aβ soluble aggregates range from monomers to oligomers and pre-clinical studies confirmed that dimers, trimers, tetramers, dodecamers and high molecular weight oligomers were all able to induce neurotoxic effects as well as to induce an immediate impairment of synaptic plasticity, and in particular of hippocampal long-term potentiation (LTP), thought to be the molecular correlate of memory (for a review on the role of Aβ oligomers see Walsh and Selkoe, 2007). Moreover, oligomers better correlated with cognitive impairment than plaques and their presence in human CSF could be already recognized decades before AD onset (Fukumoto et al. 2010). These data led to the formulation of another theory, the "Oligomer Hypothesis" (Hardy & Selkoe 2002; Ferreira & Klein 2011), according to which Aβ oligomers but not monomers or fibrils were responsible of AD pathogenesis. This further changed the aim of AD drug discovery so that therapies clearing Aβ plaques were abandoned in favor of new drugs aimed at specifically targeting Aβ oligomers.

Unfortunately, while the “Oligomer Hypothesis” is still a matter of investigation, and data are being gathered to test the grounds of its premises, the clinical failure of most of the anti-Aβ drugs has strongly destabilized this concept. Careful analyses of clinical trials indicate that, despite successful results obtained in animal models of AD, anti-Aβ drugs do not modify cognition in humans although they might be able to reduce plaque or amyloid burden. Till now (based on Medline database search and ClinicalTrials.gov): i) active immunization (i.e. ANI 1792 and CAD 106) has failed or has been interrupted for severe side effects and, apart from safety studies, no clear information were given on the effective improvement of cognition; ii) passive immunization with monoclonal antibodies bapinezumab, solanezumab, and gantenerumab has failed; a recent clinical trial with aducanumab has shown a dose-dependent reduction of Aβ plaques, however the study was not sufficiently powered to detect clinical changes and therefore still needs a confirmation of an effect against the cognitive decline, furthermore patients displayed a dose-dependent occurrence of ARIA, a medically important serious adverse effect (Sevigny et al. 2016); iii) drugs aimed at preventing Aβ formation by inhibiting β- or γ- secretases have also failed or were interrupted; among these, clinical trials for the γ -secretase inhibitors semagacestat and avagacestat were terminated for severe side effects, whereas the EPOCH trial with the newest β-secretase inhibitor verubecestat stopped for the lack of any positive effect. Notwithstanding these discouraging results, several scientists pointed out that the failure of Aβ tailored drugs might be justified by the fact that treatment was started in a late phase of the disease when Aβ-induced damage cannot be reversible.

Due to the numerous failures of clinical trials, several evidences that have been underestimated for a long time are now gaining ground, questioning the actual role of Aβ in AD pathogenesis. First,

late onset AD (LOAD), representing the 95% of AD cases, is not linked to genetic anomalies leading to a direct overproduction of Aβ, as in FAD, although the phenotype might be comparable. Importantly, pre-clinical studies on AD mouse models have been almost entirely performed on mice presenting FAD-like mutations leading to an increase of Aβ. Second, the poor correlation between Aβ deposits and the clinical degree of dementia among affected individuals is heavily reported since the 90's (Terry et al. 1991; Arriagada et al. 1992; Dickson et al. 1995; Sloane et al. 1997), as the presence of brain plaques in individuals that show no sign of dementia (Katzman et al. 1988; Delaère et al. 1990; Dickson et al. 1995). Furthermore, recent studies pointed out that plaques formation is a reactive process (Reitz 2012) that might even have a protective role by decreasing oligomers level (Cheng et al. 2007). Finally, a vast literature claims that Aβ exerts a physiological role in the CNS interfering with neuronal growth, neurotransmitter release, synaptic function and memory formation (for a review see (Puzzo & Arancio 2013; D Puzzo et al. 2015). Indeed, our group and others have previously demonstrated that administration of low concentration of oligomeric Aβ positively modulate synaptic function (Puzzo et al. 2008; Morley et al. 2010; Lawrence et al. 2014) and, conversely, blocking endogenous Aβ in the healthy brain resulted in an impairment of synaptic plasticity and memory (Puzzo et al. 2011; Morley et al. 2010).

In conclusion, taking into account almost one century of researches, it emerges that the Aβ model of AD is insufficient (Herrup 2015) and needs to be deeply reconsidered (D Puzzo et al. 2015).

A revaluated player in Alzheimer's disease pathogenesis: Tau protein

As described above, the intricate story of Aβ and tau began with the brain of Auguste Deter, but

most of the research efforts have been directed towards Aβ. Recently, the discontent generated by too many therapeutic failures has induced several groups to re-focus on tau.

Tau is a microtubule-associated protein originally described as a heat stable protein essential for microtubule assembly and stabilization (Weingarten et al. 1975). It is present in the human brain in six main isoforms, deriving from the alternative splicing of exons 2,3 and 10 of microtubule-associated protein tau (MAPT) gene. This process appears to be of particular interest for exon 10 splicing which determines the presence of tau isoforms containing 3 (3R) or 4 repeats (4R) of ~32 amino acids sequence in the microtubule binding domain (MBD) (Dickson et al. 2011). Moreover, the splicing process of exons 2 and 3 determines the number of 29-residue near-amino-terminal inserts which results in isoforms containing 0, 1 or 2 inserts (0N, 1N, 2N) (Liu & Gotz 2013). Both -R and -N repeats are capable of microtubule-binding and assembly-promoting activity, whereas the flanking regions are more likely involved in binding processes (Trinczek et al. 1995; Kar et al. 2003). In the last decades, many studies have demonstrated tau physiological involvement at different subcellular localizations: i) at axonal level, by regulating axonal elongation, maturation and transport (Dawson 2001; Vershinin et al. 2007; Yuan et al. 2008; Ittner et al. 2009); ii) in dendrites, participating in synaptic plasticity (Frandemiche 2014; Qu et al. 2017); iii) in nucleus, maintaining the integrity of genomic DNA, cytoplasmic and nuclear RNA (Sultan 2011; Violet et al. 2014).

From a functional point of view, tau can be divided in four different regions consisting of a N-terminal domain, a proline-rich domain, a MBD and a C-terminal domain (for reviews see Morris et al. 2011; Wang & Mandelkow 2015; Arendt et al. 2016). The N-terminal domain is rich of

negative charges devoted to separation of different microtubules by electrostatic repulsion when tau is bound to a certain microtubule (Mukhopadhyay & Hoh 2001; Amos 2004; Kar et al. 2003). Interestingly, the C-terminal domain, besides its key role in regulation of microtubule polymerization induction and interaction with plasmatic membranes (Brandt & Lee 1993; Maas et al. 2000; Eidenmüller et al. 2001; Reynolds et al. 2008), creates an overall asymmetry in the molecule contributing to this microtubule spacing function thanks to its neutral charge. The proline-rich domain and the MBD with their multiple aminoacidic acceptor residues are more involved in interactions with different signaling proteins, which can be scaffolded by tau or can modify tau conformational status and activity itself (Morris et al. 2011).

The presence of multiple binding sites confers to tau many interaction possibilities in regards of cell signaling, of great interest in terms of post-translational modifications. The flanking region of MBD contains the majority of phosphate acceptor residues, and the phosphorylation of these sites is relevant for regulating microtubule polymerization (Lee et al. 1989; Butner & Kirschner 1991; Goode et al. 1997; Mukrasch et al. 2005), regulation of axonal transport (Xia et al. 2015) and neurotransmitter receptors (Cardona-Gomez et al. 2006; Miyamoto et al. 2017), interference with vesicles trafficking (Ebneth et al. 1998) and apolipoprotein E (Strittmatter et al. 1994), interaction with Src-family kinases (Brandt et al. 1995; Bhaskar et al. 2005; Lee 2005; Qi et al. 2015; Reynolds et al. 2008) and many others (for reviews see Morris et al. 2011; Wang & Mandelkow 2015; Arendt et al. 2016).

The multiple roles of tau in neuronal physiology have been widely studied and, undoubtedly, a fine regulation is needed to maintain tau structure and function. Accordingly, a wide range of

neurodegenerative disorders known as tauopathies have been recognized and classified with respect to the predominant species of tau that accumulates: i) 3R tauopathies (i.e. Pick's disease); ii) 4R tauopathies (i.e. corticobasal degeneration and progressive supranuclear palsy); iii) 3R + 4R tauopathies (i.e. AD) (Dickson et al. 2011).

Biochemical studies have demonstrated that deposition of insoluble tau aggregates in NFTs depends upon a dysregulated phosphorylation process of the flanking regions of tau. In fact, while two phosphates per molecule of tau are normally present (Kanemaru et al. 1992), analysis of tau from AD brains has revealed the presence of about eight phosphates per molecule of tau (Kopke 1993). For this reason, tau phosphorylation abnormalities have been linked to misfolding and deposition of the protein in the diseased brain (Smith 2002). Despite tau has been defined as a "natively unfolded" protein with a low tendency to aggregation (Mukrasch 2009), phosphorylation of certain residues or detachment from microtubules (Schneider et al. 1999; von Bergen 2000; Mukrasch 2009) might change its conformational status and consequently its aggregation propensity. However, the undefined structure of tau in solution has precluded crystallographic analyses leaving a lack of knowledge about the protein structure (Jeganathan et al. 2006). Moreover, notwithstanding electron microscopic visualization of tau bound to microtubules demonstrated a linear alignment lengthwise to the protofilament ridges, the protein structure keeps holding a disordered state (Al-Bassam et al. 2002; Goux et al. 2004). Interestingly, when in a solution, tau spontaneously tends to modify its conformation in favor of a paperclip-like structure that might prevent its aggregation (Jeganathan et al. 2006; Wang & Mandelkow 2015), unlike Aβ that has a high tendency to aggregate in a solution due to its biochemical properties. Thus, alterations of tau (i.e. hyperphosphorylation, truncated forms) could inhibit the constitution of the

paperclip-like structure leading to paired helical filaments (PHFs) and NFT formation (Wegmann et al. 2013). In this context, tau hyperphosphorylation has been widely studied and the sequence hyperphosphorylation→PHFs→NFTs linked to AD, even if it is unlikely to represent by itself the main pathogenetic event for several reasons. First, tau phosphorylation has been demonstrated to be responsible for aggregation only when occurring at certain residues (Vega 2005), whereas in other sites it has the opposite effect thus preventing aggregation (Schneider et al. 1999). Moreover, tau hyperphosphorylation is not a prerogative of AD, since it occurs in several other conditions such as hypothermia (Planel 2004), starvation (Yanagisawa et al. 1999), chronic stress (Sotiropoulos et al. 2011) and anesthesia (Le Freche et al. 2012; Whittington et al. 2013).

Interestingly, as previously shown for Aβ fibrils and senile plaques, the amount of PHFs and NFTs is only slightly related to the severity of neuronal damage and cognitive impairment. Experiments on regulatable mouse models of tauopathy demonstrated that a variant of human tau with the pro-aggregant mutation ΔK280 developed synaptic and memory impairment as well as tau hyperphosphorylation and pre-tangle formation. However, when the pro-aggregant tau was turned off, synaptic deficit was rescued even if insoluble tau was still present (Van der Jeugd 2012). Other studies on transgenic mice expressing mutant tau (P301L mutation) that could be suppressed with doxycycline, demonstrated that behavioral impairment and neuronal loss were recovered when suppressing transgenic tau, whereas NFTs accumulation continued (Santacruz 2005). Moreover, in the P301S model of tauopathy, synaptic damage and cognitive impairment occurred before the emergence of NFTs (Yoshiyama et al. 2007). Some authors also reported that, *in vitro*, abnormally phosphorylated tau can sequester normal tau into tangles of filaments, leading the hypothesis that tau accumulation into PHFs might initially be neuroprotective until it starts compromising

neuronal function as a space-occupying lesion (Alonso et al. 2001).

The observations that synaptic and memory impairment is not mediated by NFTs, and that insoluble deposition of tau might be a compensatory protective mechanism suggested that synaptic failure might be sought in soluble oligomeric species of tau, resembling the "Oligomeric Hypothesis" already formulated for Aβ. Indeed, we have recently demonstrated that an acute exposure to tau oligomers (but not monomers) both *in vitro* and *in vivo* is detrimental for LTP and memory (M. Fá et al. 2016). Noteworthy, this toxic effect was exerted by different preparation of oligomeric tau, i.e. recombinant tau 4R/2N, tau derived from AD patients, Tau derived from h-tau mice (M. Fá et al. 2016). These results were in agreement with other observations reporting that tau oligomers impair synaptic function and memory in wild type mice (Lasagna-Reeves et al., 2011), correlate with cognitive impairment in rTg4510 mice (Berger et al. 2007), and accelerate pathology in hTau mice (Gerson et al., 2016).

Pre-clinical findings were confirmed by studies on humans showing the increase of oligomeric forms of tau in the brain of AD patients compared to controls, occurring before NFT formation and clinical symptoms (Maeda et al. 2006). Interestingly, tau oligomers have been also found in other tauopathies such as progressive supranuclear palsy, dementia with Lewy bodies and Huntington's diseases (Gerson et al. 2016; Sengupta et al. 2017; Vuono et al. 2015). In AD brain homogenates tau dimers are also markedly elevated, suggesting that tau aggregation might be a hierarchical process that passes through distinct phases, i.e. monomers, dimers, oligomers, pre-tangles, tangles (Patterson et al. 2011). Notably, the time-course leading from monomers to insoluble deposits is comparable to that already described for Aβ, with soluble forms of the peptide

increasing in an initial phase of the disease.

Based on the findings described above and considering the urgent need to find more valuable biomarkers for an early diagnosis, the possibility of detecting tau oligomers in CSF of living patients is appealing. Hence, we have conducted a pilot study to verify that soluble aggregated forms of tau are detectable outside neurons in the CSF of living people and therefore they are not necessarily the byproduct of pathological alterations occurring in post-mortem evaluations. We characterized tau immunoreactivity by western blot in CSF samples (Meredith et al. 2013) from a cohort of 11 patients with probable AD and 11 healthy controls (HC) individuals at the time of harvesting CSF (Table 1). High molecular immunoreactive species for total tau were observed in all the samples (Fig. 1A, B). However, a significant change in intensity of different bands was found, with an increase in the high molecular weight bands, presumably corresponding to oligomers, coincident with a decrease at 31-38 kDa in AD patient CSF compared to HC (Fig. 1A, B).

Interestingly, when we dissociated tau by treating the CSF samples with the reducing agent beta-mercaptoethanol (βME) to disrupt the thiol bonds between tau molecules, the signal intensity of high molecular weight tau immunoreactivity became undetectable, whereas a clear signal was present for monomeric tau, suggesting that the presence of oligomers was linked to disulfide bridges involving tau molecules (Fig. 1C). This study leads to important considerations. First, the possibility to evaluate the presence of extracellular oligomeric tau in clinical lumbar CSF specimens could be useful as a possible early biomarker of the disease, in agreement with other findings (Saman et al. 2012; Sengupta et al. 2017). Second, the observations that oligomers of tau are also present in HC and that monomers/oligomers are differently distributed in AD and control

CSF suggest that the biological significance of tau species should be further investigated. These aspects should be taken into account when designing new drugs aimed at targeting tau, to avoid the same issues already experienced with anti-Aβ treatment.

PATIENTS #	DIAGNOSIS	AGE	GENDER	MMSE
13	HC	65	W	30
14	HC	64	W	27
15	HC	69	W	27
16	HC	57	W	27
17	HC	66	M	29
18	HC	55	M	28
19	HC	73	M	26
20	HC	58	W	30
21	HC	83	M	28
22	HC	73	W	28
24	HC	79	W	29
2	AD	76	M	18
3	AD	72	M	28
4	AD	58	M	23
5	AD	68	M	17
6	AD	66	M	25
7	AD	54	M	25
8	AD	81	M	26
9	AD	71	M	27
10	AD	69	M	24
11	AD	64	W	25
12	AD	68	M	26

Table 1 - Patients Characteristics: The diagnosis of probable AD or control for healthy individual was done according to the NINCDS-ADRDA Alzheimer's Criteria. Diagnosis was determined after full neurological history and examination including testing of mental status. All diagnoses were made by an experienced neurologist, psychiatrist, or a consensus conference including

neurologists and neuropsychologists. Cerebrospinal fluid samples were collected in the morning after overnight fast at Columbia University, according to the IRB-Human Subjects Review Committee protocol. All patients gave an informed consent to the study. Only samples tested negative for human immunodeficiency virus and hepatitis B and C, Creutzfeldt–Jakob disease were included in the study. HC: range 55-83 yrs, average: 67.45 ± 2.72; probable AD: range: 54-81 yrs, average: 67.91 ± 2.29 yrs. Mini Mental State Examination: MMSE.

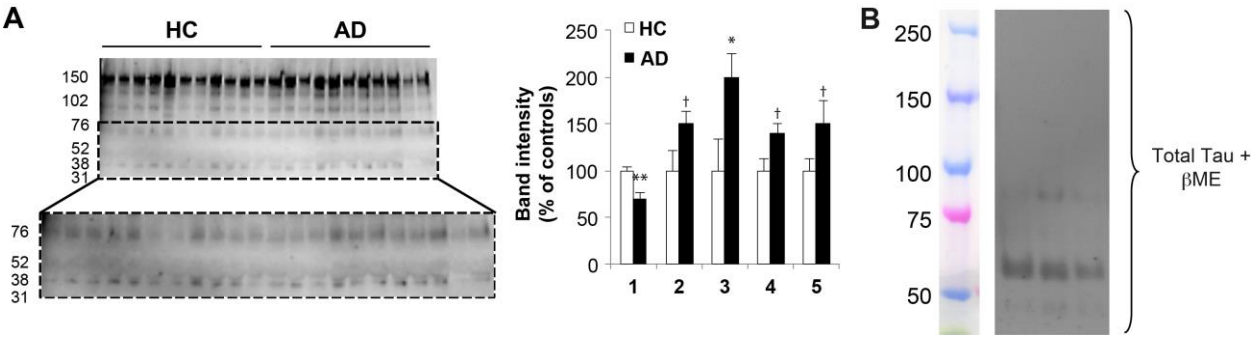

Figure 1 - Oligomeric tau is present in the CSF of AD patients and healthy individuals. A) Western blot showing total tau levels in CSF samples of healthy individuals (HC) and probable AD patients (higher magnification view of the lower molecular weight bands on the lower part of the panel. Different band intensity is quantified on the right graph (31-38 kDa: $p = 0.009$, 50-54 kDa: $p = 0.003$, 74-78 kDa: $p = 0.04$, 100-104 kDa: $p = 0.002$ and 120-150 kDa: $p = 0.003$). Fresh CSF specimens from subjects listed in Table 1 were thoroughly mixed, de-identified, and underwent one freeze–thaw cycle before standard aliquoting in 1.5 ml portions in polypropylene screw-cap tubes and storage at -80ºC. To verify the oligomerization status of tau, we ran samples

on a western blotting. Immunoreactivity towards total tau was measured in each of the CSF aliquots. Equal amounts of protein (8 μg) were fractionated by Tris-Acetate gradient gels (3-8%) and transferred to nitrocellulose membranes (Millipore). Tau immunoreactivity was detected using anti-total tau polyclonal antibody (1:2000; Epitomics). Immunoblot data were quantified by measuring the band intensity using imaging software (NIH ImageJ). Statistical analyses was performed by ANOVA plus post-hoc multiple comparisons test using Prism (GraphPad) software. **B)** Immunoreactivity for total tau in samples from probable AD patients reduced with β-mercaptoethanol (βME). βME zeroed the high molecular weight signal revealed by tau antibodies while intensifying the signals in the monomeric range.

Notwithstanding the increase of Tau oligomers in the AD brain and CSF, drugs aimed at inhibiting tau aggregation or dissolving existing aggregates, i.e. methylthioninium chloride and its second-generation derivatives such as TRx0237, did not produced the expected results in clinical trials. A Phase 2 study with TRx0237 was terminated after few months for "administrative" reasons, whereas Phase 3 studies have reported negative results on cognitive improvement so far (see clinicaltrials.gov for details). This make us wonder whether the increase of tau oligomers in AD patients should be better considered as a pathogenetic marker of the disease rather then a target of therapeutical strategies.

Aβ and tau oligomers: a game at the synapse

How do Aβ and tau induce memory loss? According to most of the studies, the answer should be sought at the synapse. Despite cortical atrophy and synaptic loss have been reported in AD brains, mainly due to a structural damage imputable to plaques and tangles in a later stage of the disease,

a subtle effect exerted by soluble forms of Aβ and tau at the synapse seems to be the earlier event underlying memory loss (Selkoe 2002; Selkoe 2008; M. Fá et al. 2016). Several studies have demonstrated that administration of different preparations of oligomeric Aβ and tau (synthetic, from transgenic mice, from AD brains) impaired synaptic plasticity and memory. The role of soluble oligomers also emerged in studies performed on AD mouse models, since synaptic and memory dysfunction was present before the appearance of plaques or tangles (for a review on the role of oligomers see Walsh & Selkoe 2007; Guerrero-Muñoz et al. 2015).

In vitro and *in vivo* studies have shown that Aβ and tau derange molecular signaling pathways crucial for synaptic plasticity at both pre- and post- synaptic sites. Both Aβ and tau interfere with the transcription factor cAMP response element-binding protein (CREB), whose phosphorylation at Ser133 is thought to be one of the fundamental events in memory formation (Silva et al. 1998; Lonze & Ginty 2002; Carlezon et al. 2005). In particular, Aβ inhibits the physiological increase of CREB phosphorylation during LTP (Vitolo et al. 2002; Puzzo et al. 2005; for a review see Teich et al. 2015), causing a downregulation of both the NO/cGMP/PKG and the cAMP/PKA pathways, two cascades converging on CREB. Tau overexpression and hyperphosphorylation was also found to be accompanied by a reduction of CREB phosphorylation at Ser133, mediated by a decrease of phosphorylation of NR2B (Tyr1472) (Xie et al. 2017). Moreover, synaptic plasticity and memory impairment caused by h-tau overexpression was reported to be related to nuclear dephosphorylation/inactivation of CREB (Yin et al. 2016).

Aβ and tau also target other molecules upstream of CREB, among which the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), another key molecule needed for LTP and memory

formation (Fink & Meyer 2002). CaMKII is dysregulated in the hippocampus of AD mouse models and patients (for a review see Ghosh & Giese 2015) and it has been demonstrated that Aβ oligomers interfere with its phosphorylation leading to AMPA receptor dysfunction (Zhao et al, 2004; Gu et al. 2009; Whitcomb et al., 2015). On the other hand, evidences for the interaction tau-CaMKII have been reported since the late '80s in works analyzing the ability of CaMKII to induce an AD-like tau phosphorylation (Baudier & Cole 1987; Baudier & Cole 1988). CaMKII phosphorylates tau at different sites and this might prevent tau binding to microtubule (Singh et al. 1996) and modify tau structure leading to PHFs formation (Steiner et al. 1990). Indeed, CaMKII colocalizes with tau mRNA, PHFs, NFTs in AD brains (for a review see Ghosh & Giese 2015). Recently, in a drosophila model of tauopathy, suppression of tau phosphorylation at Ser262/356 inhibited tau toxicity through a mechanism involving calcium homeostasis dysregulation driven by CaMKII (Oka et al. 2017).

The deleterious effects of Aβ and tau also involved BDNF, a critical factor linked to neuronal survival and function that is needed for synaptic plasticity and memory. A decrease of BDNF levels in serum and brains of AD patients highly correlate with cognitive impairment and BDNF polymorphisms have been involved in AD pathogenesis (for review see Song et al. 2014). Moreover, several *in vitro* and *in vivo* studies have confirmed that Aβ-induced LTP and cognitive dysfunction are associated with a reduction of BDNF levels (Song et al. 2014). Recently, a loss of BDNF has been also reported in THY-Tau22 and P301L mouse models of tau pathology (Burnouf et al. 2013; Jiao et al. 2016).

Taken all together, these findings suggest that restoring synaptic-related molecules and second

messenger systems regulating memory mechanisms might be a viable therapeutic strategy to counteract AD (Teich et al. 2015).

Aβ and tau activity-dependent secretion, neuronal uptake and spreading of the disease

Because Aβ and tau interfere with the synaptic machinery, another relevant subject of investigation has been to determine whether they act via extracellular or intracellular mechanisms. Based on the localization of insoluble deposits, for several years Aβ has been considered an extracellular protein and tau an intracellular one. However, it is now clear that this rigid vision is no more applicable, since both Aβ and tau can be found inside and outside neurons. Notwithstanding most of the studies have been performed on models of disease, the extra- and intracellular presence of Aβ and tau is the result of a physiological dynamic process in which the two proteins are secreted at the synapse and internalized by neurons. A relevant body of data has supported the hypothesis that neurons are able to secrete Aβ in an activity dependent fashion. *In vitro* studies performed by applying drugs that decrease (i.e. tetrodotoxin or high magnesium) or increase (i.e. picrotoxin) neuronal activity have shown a concomitant decrease or increase of Aβ secretion in organotypic slices overexpressing human APP Swedish mutation (Kamenetz et al. 2003). An *in vivo* approach by using microdialysis also revealed an increase of Aβ levels in the brain interstitial fluid concomitant to the increase of synaptic activity (Cirrito et al. 2005) or paralleling the neurological status (Brody et al. 2008). An increase of Aβ secretion has also been found during learning in healthy wild-type mice (Puzzo et al. 2011). Based on the fact that synaptic activity stimulates Aβ secretion, and that extracellular Aβ is known to reduce synaptic plasticity, it has been proposed a theory according to which an increase of synaptic (and cognitive) activity is linked to AD pathogenesis. However, although an increase of brain activity in AD could be supported by data indicating

hyperexcitability in transgenic mice and human AD patients, this activity-dependent role of Aβ should be better viewed as a physiological mechanism occurring within the healthy brain, especially because levels of Aβ secreted during activity are in the picomolar range and are not neurotoxic (Palmeri et al. 2017; Puzzo et al. 2008; Puzzo et al. 2011). Thus, the high increase of extracellular Aβ during AD might be due to a derangement of this physiological loop or it could be a consequence of degeneration of neurons that have previously accumulated Aβ at intracellular level (for a review see Tampellini 2015). Whether the impairment of synaptic function is directly mediated by these high extracellular Aβ levels or by Aβ accumulated inside neurons, is still a matter of debate. Surely, these two pools are strictly interconnected, since extracellular Aβ induced the accumulation of intracellular Aβ by stimulating APP processing (Tampellini et al. 2009) or by a direct APP-mediated internalization (Puzzo et al. 2017); in turn, intracellular Aβ disrupts synaptic transmission and plasticity (Ripoli et al. 2014).

Interestingly, also tau undergoes the same dynamic flux characterized by activity-dependent secretion and neuronal internalization. Indeed, application of KCl or glutamate to cultured neurons resulted in an increase of tau secretion (Pooler et al. 2013; M Fá et al. 2016) mediated by AMPA receptor activation. *In vivo* studies reported an increase of tau in brain interstitial fluid when stimulating neurons with high K^+ perfusion or after administration of NMDARs agonist, whereas a decrease of its secretion followed picrotoxin administration (Yamada et al. 2014). An increase of tau secretion also paralleled the increase of glutamate release induced by an antagonist of metabotropic glutamate receptors 2/3 (Yamada et al. 2014). The phenomenon was further confirmed in different cultured neural cell lines where extracellular tau levels were modified proportionally to synaptic activity (Wu et al. 2016). On the other hand, several pre-clinical studies

have demonstrated that exogenously applied tau can be internalized by neurons (Frost et al. 2009; Guo & Lee 2011; Guo & Lee 2013; Wu et al. 2013; M Fá et al. 2016) and glial cells (Luo et al. 2015; Bolós et al. 2017; Piacentini et al. 2017) with different mechanisms involving bulk endocytosis (Wu et al. 2013), binding to heparan sulfate proteoglycans (Holmes et al. 2013) or to APP (Puzzo et al. 2017).

Activity dependent secretion and neuronal uptake of Aβ and tau have been related to the diffusion of the disease throughout the brain, a process known as spreading which refers to the capability of neurotoxic proteins to diffuse from a neuron to another, expanding the disease from a restricted area to the entire brain. This type of dissemination, defined as "trans-synaptic spreading", is thought to occur among different brain areas functionally connected (Braak & Del Tredici 2011; Liu et al. 2012) and is supported by observations on post-mortem AD brains as well as by clinical studies exploiting computerized x-ray tomography (CT) and magnetic resonance imaging (MRI) techniques, that allow tracing different neuropathological markers such as atrophy of certain brain areas, brain ventricles enlargement, and deposition of amyloid plaques and NFTs (for a review see Smith 2002).

Evidences for AD spreading and progression throughout the cortex were reported more than 30 years ago, based on tangle distribution in the proximity of the same pyramidal neurons that give connectivity to other brain areas (Pearson et al. 1985). At the present day, neither the cause that initiates spreading nor its underlying mechanisms have been identified, but useful information has come from pre-clinical studies. Notwithstanding tau has been under the spotlight for many years, one of the first evidences of spreading in AD dates back to the 90's and involves Aβ (for reviews

see Jarrett & Lansbury 1993; Lv et al. 2017). When trying to unravel the causes of Aβ diffusion, studies have often focused on the first area affected in AD, the medial temporal lobe, and in particular in the entorhinal cortex (EC). EC superficial layer is susceptible to Aβ-dependent neurodegeneration, and this can negatively affect its primary afferent regions resulting in a disruption of the whole circuitry in both mouse models and AD patients (Palop et al. 2003; Scarmeas et al. 2009). Consistently, an increase of mutant APP in layer II/III neurons of EC has been shown to elicit a molecular and functional disruption in the CA1 area of the hippocampus with presence of soluble Aβ in the dentate gyrus, Aβ deposits in the performant pathway, and epileptiform activity in the parietal cortex (Harris et al. 2010). Further studies in mutant human mhAPP mice have reported an age-dependent progressive deterioration of synaptic plasticity and memory spreading from the EC to the hippocampus (Criscuolo et al. 2017), a phenomenon mediated by microglial RAGE activation and subsequent increase in p38MAPK phosphorylation (Criscuolo et al. 2017). Consistently, other studies reported the capability of reactive microglia in secreting Aβ through microvesicles, which in turn would promote Aβ toxicity to neurons through their axons (Joshi et al. 2014; Prada et al. 2016; Söllvander et al. 2016). Accordingly, other supporting evidences indicate that after administration of fluorescent oligomeric Aβ to neurons, an higher percentage of the protein was found surrounding neurons, and this process needed the presence of differentiated neuritis to occur (Nath et al. 2012). Cell-to-cell transfer mechanism has been reported for different Aβ species (i.e. oAβ1–42 TMR, oAβ3(pE)–40TMR, oAβ1–40TMR, and oAβ11–42TMR), and this prion-like spreading was attributed to an insufficient activity of cellular clearance degradation systems (Domert et al. 2014). Another mechanisms proposed for Aβ spreading relies on the presence of tunneling nanotubes (TNTs) consisting in cellular membrane extensions creating a direct connection between cells (Rustom et al. 2004) TNTs have

been demonstrated to mediate high-speed transfer of Aβ among neurons, through a p53/EGFR/Akt/PI3K/mTOR pathway that, in turn, would trigger F-actin polymerization promoting TNTs formation (Wang et al. 2011). However, Aβ has been shown to be secreted by neurons through exosomes (Rajendran et al. 2006) that could be internalized and stored from the acceptor neuron as lysosomal vesicles through a macroautophagy mediated mechanism (Zheng et al. 2011; Nath et al. 2012). In any case, despite these numerous evidences, there is not a uniform consensus about the causes or mechanisms underlying Aβ spreading.

On the other hand, a growing body of evidence refers to tau spreading as a prion-like propagation, which fascinatingly occurs in different directions among the many forms of tauopathies (Sanders et al. 2014). Also tau pathology is likely to begin in EC then moving to the hippocampus, and ultimately invading the cortex, following an overlapping path existing among functionally connected areas (Braak & Braak 1991; Braak & Del Tredici 2011; Liu et al. 2012; Wang & Mandelkow 2015). These evidences are consistent with data coming from studies on non-human primates in which bilateral lesions of EC induce a functional impairment of declarative memory accompanied by long-lasting hypometabolism in temporal and parietal lobes, demonstrating a functional connection starting from EC (Meunier et al. 1993). Accordingly, in a transgenic mouse model differentially expressing pathological human tau in EC (EC-tau), the localization of tauopathy was investigated at different time points, demonstrating a progression of the pathology through anatomically and functionally connected brain areas (Liu et al. 2012). Interestingly, *in vivo* chemogenetic stimulation of EC in EC-tau mice induced additional pathology in synaptically connected areas (e.g. dentate gyrus) (Wu et al. 2016). Consistent with this finding, tau has been found in exosomes that might lead to its diffusion to adjacent cells (Asai et al. 2015; Saman et al.

2012). Further works demonstrated that cell-to-cell contact was not necessarily needed for tau spreading *in vitro* given that the administration of neuronal-derived tau media to neuronal cultures was sufficient for tau transfer and internalization, even though it is not known whether tau in the media was vesicle bound or free (Wu et al. 2016). Other studies suggested that pathologic tau requires TNTs to be transferred from a neuron to another one (Gousset et al. 2009). However, whether the mechanism underlying tau propagation is mediated by TNTs, non-vesicular direct translocation or through secretory lysosomes into extracellular space (Simón et al. 2012; Dujardin et al. 2014; Lv et al. 2017; Saman et al. 2012) is still under investigation. Another interesting feature of tau transmission is the possibility that it can move both anterogradely and retrogradely, meaning that it can be internalized both at the somatodendritic compartment and axon terminals, and can be transported in either directions to disseminate tauopathy (Lv et al. 2017; Wu et al. 2013). In conclusion, it is widely accepted that Aβ and tau spreading are crucial for the progression of the disease, but mechanisms underlying this phenomenon are still debated.

Alzheimer's disease: rearranging the puzzle

As described so far, Aβ and tau share several features (Table 2), even if different structural and biochemical properties regulate their propensity to aggregate and interaction with molecular pathways. By now, a variety of studies have demonstrated that soluble oligomeric forms more than plaques increased in the diseased brain, are detectable in the CSF, and are highly correlated with cognitive impairment. The deleterious effect of Aβ and tau is exerted at the synapse, where they interfere with molecular pathways needed for synaptic plasticity and memory. The capability of neuronal and glial cells to release and internalize Aβ and tau contribute to spread the disease from specific areas, such as EC and the hippocampus, to the entire brain. Despite these studies have

certainly clarified several aspects of AD onset and progression, the crosstalk between Aβ and tau in the diseased brain is still a matter of debate.

	AMYLOID-β PEPTIDE	TAU PROTEIN
Physiological functions	• Neuronal growth • Neurotransmitter release • Synaptic transmission and plasticity • Memory formation • Immune response • Anti-oxidant properties	• Microtubule assembly and stabilization • Axon elongation • Synaptic plasticity • Nuclear function
Isoforms	Aβ40, Aβ42, other fragments	3R-4R, 0N-1N-2N
Aggregation sequence	Monomers → Oligomers → Fibrils → Senile plaques	Tau hyperphosphorylation → PHFs → NFTs
Insoluble and soluble forms	• No correlation between senile plaques and cognitive impairment • Oligomers induce synaptic dysfunction and memory loss • Oligomers increase in brains and CSF of AD patients vs. controls	• Poor correlation between NFTs and cognitive impairment • Oligomers induce synaptic dysfunction and memory los • Oligomers increase in brains and CSF of AD patients vs. controls
Genetic mutations	APP, PS1 and PS2 linked to FAD	MAPT linked to FTDP-17, PSP, CBD
Synaptic target	CREB, CamKII, BDNF among others	CREB, CamKII, BDNF among others
Extra- and intracellular dynamic	• Activity dependent secretion • Neuronal and glia uptake • Extracellular toxicity • Intracellular toxicity	• Activity dependent secretion • Neuronal and glia uptake • Extracellular toxicity • Intracellular toxicity
Spreading	EC → Hippocampus → Cortex	EC → Hippocampus → Cortex
APP-dependent mechanism	• APP binding • Neuronal and glial uptake • Synaptic plasticity impairment • Memory impairment	• APP binding • Neuronal and glial uptake • Synaptic plasticity impairment • Memory impairment

Table 2 – **Similarities and differences between Aβ and Tau.** PHFs = paired helical filaments; NFTs = neurofibrillary tangles; CSF = cerebrospinal fluid; APP = Amyloid Precursor Protein; PS = Presenilin; FAD = Familiar Alzheimer's disease; MAPT = microtubule-associated protein tau; FTPD-17 = Frontotemporal dementia with parkinsonism-17; PSP = Progressive Supranuclear Palsy; CBD = corticobasal degeneration; CREB = cAMP response element binding protein; CaMKII = Ca2+ /calmodulin-dependent protein kinase II; BDNF = Brain-derived neurotrophic factor; EC = enthorinal cortex.

The most common idea in the AD field is based on the "Amyloid Cascade Hypothesis" and suggests that the initial increase of Aβ induces amyloidosis and tau pathology over time (Figure 2). This temporal sequence derives from studies in patients with FAD, where the genetic-driven increase of Aβ is followed by NFTs accumulation (Bateman et al. 2012), whereas the increase of tau, as in tauopathies, is not associated with amyloidosis. Preclinical studies have confirmed that oligomers of Aβ can trigger tau pathology (Shankar et al. 2008) and, conversely, when knocking down tau, Aβ toxic effects are prevented (Jin et al. 2011; Roberson et al. 2007). Interestingly, a recent work has demonstrated that Aβ acutely induces tubulin posttranslational modifications and stabilizes dynamic microtubules promoting tau-dependent loss of dendritic spines and tau hyperphosphorylation (Qu et al. 2017). Thus, Aβ has been thought to act upstream of tau in the pathogenesis of the disease. However, our recent works have challenged this scenario. We have demonstrated that oligomers of both Aβ and tau produced an immediate reduction of synaptic plasticity and memory when extracellularly applied and that these detrimental effects occurred not only with high concentrations of Aβ or tau alone, but also when sub-toxic doses of oligomeric Aβ were combined with sub-toxic doses of oligomeric tau (M Fá et al. 2016). These observations suggested that: i) Aβ and tau might act at the same level or on different targets that later converge

on a common molecular mechanism; ii) the two proteins are able to impair synaptic plasticity and memory *per se*, i.e. regardless of the presence of high concentrations of one another; iii) elevated levels of Aβ are not needed to initiate the tau-mediated toxic events leading to synaptic dysfunction. Inspired by these data, we have recently focused on the possible common mechanism of action for extracellular Aβ and tau oligomers to impair LTP and memory. We have found that both oligomers of Aβ and tau require APP to exert its deleterious effect at the synapse (Puzzo et al. 2017), in agreement with previous observations indicating that APP mediates extracellular Aβ neurotoxicity (Lorenzo et al. 2000; Shaked et al. 2006; Tampellini et al. 2009) and is involved in AD hippocampal hyperactivity (Bakker et al. 2012; Busche et al. 2012; Palop et al. 2007; Verret et al. 2012; Vossel et al. 2013). Previous papers have already shown that oligomeric Aβ was able to bind APP (Fogel et al. 2014), whereas APP and tau interaction was studied several years ago in the context of NFTs (Smith et al. 1995; Giaccone et al. 1996; Islam & Levy 1997), but we have now provided evidences that also soluble oligomeric tau is able to bind APP (Puzzo et al. 2017). This binding might be related to the APP-mediated uptake of Aβ and tau, since APP influences accumulation of tau fibrils in cultured cells (Takahashi et al. 2015) and is needed for the entrance of oligomeric Aβ and tau into neurons (Puzzo et al. 2017) and astrocytes (Piacentini et al. 2017). Based on these findings, we hypothesize that APP-mediated oligomers uptake plays a role in AD pathogenesis. Indeed, because Aβ and tau do not impair synaptic plasticity and memory in APP KO mice, APP binding and/or APP-mediated internalization of the two proteins should lead to LTP and memory reduction, even if one cannot exclude the possibility that Aβ and tau act on other targets, or that their intraneuronal accumulation does not directly inhibit the synaptic machinery. However, previous observation indicating that blocking intracellular Aβ rescues the LTP impairment induced by administration of extracellular Aβ (Ripoli et al. 2014) supports the

hypothesis that Aβ intraneuronal uptake is critical for the impairment of synaptic plasticity. On the other hand, recent studies have evidenced that the APP-dependent accumulation of extracellular tau oligomers in astrocytes induces a disruption of calcium signaling which in turn disrupts synaptic function in neighboring neurons (Piacentini et al. 2017). Interestingly, while it has been previously demonstrated that extracellular Aβ requires APP cleavage to permit intraneuronal Aβ accumulation (Tampellini et al. 2009), our evidences have excluded that the toxicity of extracellular Aβ and tau oligomers on LTP depends upon amyloidogenic processing of APP since BACE KO mice still present the impairment of LTP induced by the two oligomeric proteins (Puzzo et al. 2017).

Whether APP acts as a channel (Fraser et al. 1996; Fraser et al. 1997), or induces the formation of pores across the membrane to let oligomers enter the cell (Kayed et al. 2004), or promotes endocytosis (Xu et al. 2016) is still under investigation. Another possibility is that when Aβ and tau oligomers bind APP, they lead to the activation of its intracellular portion, AID/AICD, triggering either a structural change, for example inducing a different APP conformation, or a functional effect, for example activating or inhibiting molecular cascades involved in synaptic plasticity and memory. Interestingly, it is known that AID/AICD might stimulate transcription by forming a multimeric complex with the nuclear adaptor protein Fe65 and the histone acetyltransferase Tip60 (Cao 2001). Another possible mechanisms might involve APP phosphorylation at specific intracellular sites. For example, it has been demonstrated that APP phosphorylation of Thr668, which regulates docking sites for intracellular proteins that interact with APP, is increased in AD cases (Shin et al. 2007) and knock-in mouse bearing a Thr(668)Ala mutation preventing phosphorylation at this site protect against abnormal synaptic plasticity and

memory when crossed with a mouse model of dementia (Lombino et al. 2013).

Our model placing Aβ and tau in parallel and upstream of APP does not exclude the possibility that the two proteins use other molecules in addition to APP to produce their detrimental effects, nor the possibility that some deleterious effects need the other protein for the effect itself to be present (i.e. Aβ might require tau for some of the pathologies to occur). Consistent with this scenario, AD is a complex condition involving multiple aspects in addition to memory, a phenomenon that is likely dependent upon synaptic activity and that has greatly influenced our critical analysis of the current literature because it represents the clinical hallmark of AD. Furthermore, as shown in table 2, some of the physiological functions of the two proteins are different with Aβ playing a major role in neuronal growth and synaptic plasticity and tau in axonal elongation and microtubule assembly and stabilization. Then, in light of the different affinities that Aβ has towards its multiple targets, it is likely that as the concentration of the peptide increases with worsening of the pathology new pathways are interested by the disease.

In any case, demonstrating that APP serves as a trojan horse to mediate synaptic plasticity and memory impairment by oligomers of both Aβ and tau, challenges the prevailing hypothesis in the AD field stating that Aβ triggers tau pathology. According to our findings, Aβ and tau do not act in series but in parallel, both through APP (Figure 2). Now, it would be desirable to understand whether and how the involvement of APP is limited to Aβ and tau entrance into cells or also underlies the derangement of molecular mechanisms involved in synaptic plasticity and memory. In any case, this new player might be taken into consideration when studying the pathogenesis of AD. For example, further studies should be performed to understand the exact mechanisms of

APP-mediated entrance of oligomers inside neurons and glial cells and whether this might initially represent a compensatory mechanism aimed at clearing toxic oligomers from the synaptic cleft.

The consequences of the model underlying AD pathology proposed in the current review are notable from a drug discovery point of view. The first thought is that therapies aimed at tau might not work similar to anti-Aβ therapies, as Aβ might still exercise its detrimental effects independent of tau. Most important, given the convergence of Aβ and tau onto APP, a fascinating possibility is that therapies acting onto APP might be more efficacious than those acting solely against Aβ or tau. Furthermore, an approach directed against APP would have the advantage of overcoming obstacles offered by the physiological functions of Aβ and tau that might occur independently of their action onto APP, and might still be present if one decides to simultaneously target Aβ and tau, an approach that is also suggested by our model. A strategy directed against APP will likely have its own drawbacks including physiological functions of full length APP (Müller & Zheng 2012). Nevertheless, APP offers the flexibility of having multiple sites that could be exploited as a tool to selectively affect a putative toxic role of the full length protein. To this end, the APP phosphorylation at Thr668 is very interesting because it has been suggested that averting its noxious role in synaptic plasticity and memory might serve as a therapeutic strategy for human dementias (Lombino et al. 2013) . Certainly, our hypothesis paves the way to an increased interest towards APP, a molecule that has been taken into account mostly for its role as an Aβ generator, being, on our opinion, unfortunately overshadowed by its own child, Aβ.

In conclusion, after more than one century of research in the AD field, several questions remain to be answered especially on the role of the two main actors, Aβ and tau, in the pathogenesis of the

disease. It is certain that their interactions at the synapse need to be further elucidated and new players such as APP should enter the stage to get a clearer picture of this intricate disease.

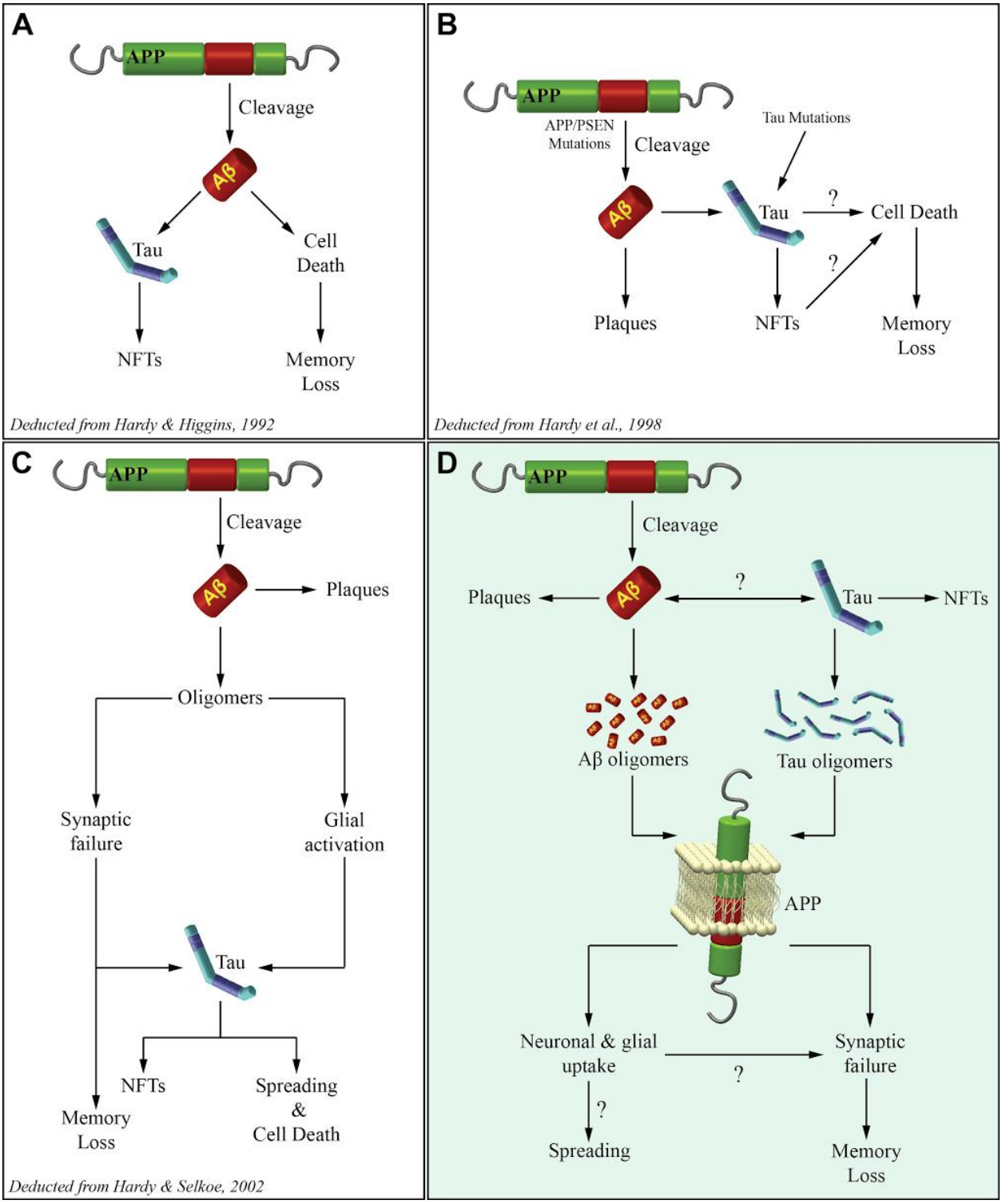

Figure 2 – **Different visions of Aβ and tau interaction in AD pathogenesis.** The Amyloid cascade hypothesis has dominated the AD field for several years. This picture describes how it has

been updated over time from the beginning **(A)**, to the discovery of genetic mutations involving both Aβ and tau production **(B)**, to a more complex vision recognizing oligomers as the toxic Aβ species **(C)**. Notably, in A-C Aβ acts upstream tau. **D)** According to our novel vision, both oligomers of Aβ and tau exert a neurotoxic effect mediated by APP leading to synaptic and memory dysfunction. APP also mediates oligomers entrance into neurons and glial cells, a mechanism probably contributing to the spreading of the disease throughout the brain.

References

Al-Bassam, J. et al., 2002. MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments. *The Journal of Cell Biology*, 157(7), pp.1187–1196. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12082079.

Alonso, A. et al., 2001. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc. Natl Acad. Sci. USA*, 98, pp.6923–6928.

Amos, L.A., 2004. Microtubule structure and its stabilisation. *Organic & Biomolecular Chemistry*, 2(15), p.2153.

Arendt, T., Stieler, J.T. & Holzer, M., 2016. Tau and tauopathies. *Brain Research Bulletin*, 126, pp.238–292.

Arriagada, P. V et al., 1992. Neurofibrillary Tangles But Not Senile Plaques Parallel Duration and Severity of Alzheimers-Disease. *Neurology*, 42(3), pp.631–639.

Asai, H. et al., 2015. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. *Nature Neuroscience*, 18(11), pp.1584–1593. Available at: http://www.nature.com/doifinder/10.1038/nn.4132.

Bakker, A. et al., 2012. Reduction of Hippocampal Hyperactivity Improves Cognition in Amnestic Mild Cognitive Impairment. *Neuron*, 74(3), pp.467–474.

Bateman, R.J. et al., 2012. Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *New England Journal of Medicine*, 367(9), pp.795–804. Available at: http://www.nejm.org/doi/abs/10.1056/NEJMoa1202753.

Baudier, J. & Cole, R.D., 1988. Interactions between the microtubule-associated tau proteins and S100b regulate tau phosphorylation by the Ca2+/calmodulin-dependent protein kinase II. *The Journal of biological chemistry*, 263(12), pp.5876–83.

Baudier, J. & Cole, R.D., 1987. Phosphorylation of tau proteins to a state like that in Alzheimer's brain is catalyzed by a calcium/calmodulin-dependent kinase and modulated by phospholipids. *The Journal of biological chemistry*, 262(36), pp.17577–83.

von Bergen, M., 2000. Assembly of [tau] protein into Alzheimer paired helical filaments depends on a local sequence motif (306VQIVYK311) forming [beta] structure. *Proc. Natl Acad. Sci. USA*, 97, pp.5129–5134.

Berger, Z. et al., 2007. Accumulation of pathological tau species and memory loss in a

conditional model of tauopathy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(14), pp.3650–62.

Bhaskar, K., Yen, S.-H. & Lee, G., 2005. Disease-related modifications in tau affect the interaction between Fyn and Tau. *The Journal of biological chemistry*, 280(42), pp.35119–25.

Bolós, M., Perea, J.R. & Avila, J., 2017. Alzheimer's disease as an inflammatory disease. *Biomolecular concepts*, 8(1), pp.37–43.

Braak, H. & Braak, E., 1991. Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica*, 82(4), pp.239–259. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1759558.

Braak, H. & Del Tredici, K., 2011. Alzheimer's pathogenesis: is there neuron-to-neuron propagation? *Acta Neuropathologica*, 121(5), pp.589–595.

Brandt, R. & Lee, G., 1993. Functional organization of microtubule-associated protein tau. Identification of regions which affect microtubule growth, nucleation, and bundle formation in vitro. *The Journal of biological chemistry*, 268(5), pp.3414–9.

Brandt, R., Léger, J. & Lee, G., 1995. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. *The Journal of cell biology*, 131(5), pp.1327–40.

Brody, D.L. et al., 2008. Amyloid-beta dynamics correlate with neurological status in the injured human brain. *Science (New York, N.Y.)*, 321(5893), pp.1221–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18755980.

Burnouf, S. et al., 2013. NMDA receptor dysfunction contributes to impaired brain-derived neurotrophic factor-induced facilitation of hippocampal synaptic transmission in a Tau transgenic model. *Aging Cell*, 12(1), pp.11–23.

Busche, M.A. et al., 2012. Critical role of soluble amyloid-β for early hippocampal hyperactivity in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, 109(22), pp.8740–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22592800%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3365221.

Butner, K.A. & Kirschner, M.W., 1991. Tau protein binds to microtubules through a flexible array of distributed weak sites. *The Journal of cell biology*, 115(3), pp.717–30.

Cao, X., 2001. A Transcriptively Active Complex of APP with Fe65 and Histone Acetyltransferase Tip60. *Science*, 293(5527), pp.115–120. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1058783.

Cardona-Gomez, G.P. et al., 2006. Estrogen dissociates Tau and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor subunit in postischemic hippocampus. *NeuroReport*, 17(12), pp.1337–1341.

Carlezon, W.A., Duman, R.S. & Nestler, E.J., 2005. The many faces of CREB. *Trends in neurosciences*, 28(8), pp.436–45.

Cheng, I.H. et al., 2007. Accelerating amyloid-beta fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. *J Biol Chem*, 282(33), pp.23818–23828. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17548355%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/17548355.

Cirrito, J.R. et al., 2008. Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. *Neuron*, 58(1), pp.42–51.

Cirrito, J.R. et al., 2005. Synaptic Activity Regulates Interstitial Fluid Amyloid-β Levels In Vivo. *Neuron*, 48(6), pp.913–922.

Criscuolo, C. et al., 2017. Entorhinal Cortex dysfunction can be rescued by inhibition of microglial RAGE in an Alzheimer's disease mouse model. *Scientific Reports*, 7, p.42370. Available at: http://www.nature.com/articles/srep42370.

Dawson, H.N., 2001. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. *J. Cell Sci.*, 114, pp.1179–1187.

Delaère, P. et al., 1990. Large amounts of neocortical ??A4 deposits without neuritic plaques nor tangles in a psychometrically assessed, non-demented person. *Neuroscience Letters*, 116(1–2), pp.87–93.

Dickson, D.W. et al., 1995. Correlations of synaptic and pathological markers with cognition of the elderly. *Neurobiology of Aging*, 16(3), pp.285–298.

Dickson, D.W. et al., 2011. Neuropathology of frontotemporal lobar degeneration-tau (FTLD-tau). *Journal of molecular neuroscience : MN*, 45(3), pp.384–9.

Domert, J. et al., 2014. Spreading of amyloid-$β$ peptides via neuritic cell-to-cell transfer is dependent on insufficient cellular clearance. *Neurobiology of Disease*, 65, pp.82–92.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/24412310.

Dujardin, S. et al., 2014. Ectosomes: A New Mechanism for Non-Exosomal Secretion of Tau Protein J. Avila, ed. *PLoS ONE*, 9(6), p.e100760. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24971751.

Ebneth, A. et al., 1998. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *The Journal of cell biology*, 143(3), pp.777–94.

Eidenmüller, J. et al., 2001. Phosphorylation-mimicking glutamate clusters in the proline-rich region are sufficient to simulate the functional deficiencies of hyperphosphorylated tau protein. *The Biochemical journal*, 357(Pt 3), pp.759–67.

Fá, M. et al., 2016. Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory. *Scientific Reports*, 6(1), p.19393. Available at: http://www.nature.com/articles/srep19393.

Fá, M. et al., 2016. Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory. *Scientific Reports*, 6(1), p.19393. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26786552.

Ferreira, S.T. & Klein, W.L., 2011. The A?? oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. *Neurobiology of Learning and Memory*, 96(4), pp.529–543.

Fink, C.C. & Meyer, T., 2002. Molecular mechanisms of CaMKII activation in neuronal plasticity. *Current opinion in neurobiology*, 12(3), pp.293–9.

Fogel, H. et al., 2014. APP homodimers transduce an amyloid-??-mediated increase in release probability at excitatory synapses. *Cell Reports*, 7(5), pp.1560–1576.

Frandemiche, M.L., 2014. Activity-dependent tau protein translocation to excitatory synapse is disrupted by exposure to amyloid-[beta] oligomers. *J. Neurosci.*, 34, pp.6084–6097. Available at: http://dx.doi.org/10.1523/JNEUROSCI.4261-13.2014.

Fraser, S.P. et al., 1996. Membrane Currents Induced In Xenopus Oocytes By the C-Terminal Fragment Of the Beta-Amyloid Precursor Protein. *Journal of Neurochemistry*, 66(5), pp.2034–2040.

Fraser, S.P., Suh, Y.H. & Djamgoz, M.B.A., 1997. Ionic effects of the Alzheimer's disease ??-amyloid precursor protein and its metabolic fragments. *Trends in Neurosciences*, 20(2),

pp.67–72.

Le Freche, H. et al., 2012. Tau Phosphorylation and Sevoflurane Anesthesia. *Anesthesiology*, 116(4), pp.779–787.

Frost, B., Jacks, R.L. & Diamond, M.I., 2009. Propagation of Tau Misfolding from the Outside to the Inside of a Cell. *Journal of Biological Chemistry*, 284(19), pp.12845–12852.

Fukumoto, H. et al., 2010. High-molecular-weight beta-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 24(8), pp.2716–2726.

Games, D. et al., 1995. Alzheimer-type neuropathology in transgenic mice overexpressing V717F b-amyloid precursor protein. *Letters to Nature*, 373(9), pp.523–527.

Gerson, J. et al., 2016. Tau Oligomers Derived from Traumatic Brain Injury Cause Cognitive Impairment and Accelerate Onset of Pathology in Htau Mice. *Journal of Neurotrauma*, 33(22), pp.2034–2043. Available at: http://online.liebertpub.com/doi/10.1089/neu.2015.4262.

Ghosh, A. & Giese, K.P., 2015. Calcium/calmodulin-dependent kinase II and Alzheimer's disease. *Molecular Brain*, 8(1), p.78. Available at: http://molecularbrain.biomedcentral.com/articles/10.1186/s13041-015-0166-2.

Giaccone, G. et al., 1996. beta PP and Tau interaction. A possible link between amyloid and neurofibrillary tangles in Alzheimer's disease. *The American journal of pathology*, 148(1), pp.79–87. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1861592&tool=pmcentrez&rendertype=abstract.

Glenner, G.G. & Wong, C.W., 1984a. Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications*, 120(3), pp.885–890.

Glenner, G.G. & Wong, C.W., 1984b. Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochemical and Biophysical Research Communications*, 122(3), pp.1131–1135.

Goode, B.L. et al., 1997. Functional interactions between the proline-rich and repeat regions of tau enhance microtubule binding and assembly. *Molecular biology of the cell*, 8(2), pp.353–65.

Gousset, K. et al., 2009. Prions hijack tunnelling nanotubes for intercellular spread. *Nature Cell Biology*, 11(3), pp.328–336. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19198598.

Goux, W.J. et al., 2004. The formation of straight and twisted filaments from short tau peptides. *The Journal of biological chemistry*, 279(26), pp.26868–26875. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15100221.

Grundke-Iqbal, I. et al., 1986. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J Biol Chem*, 261(13), pp.6084–6089. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3084478.

Gu, Z., Liu, W. & Yan, Z., 2009. {beta}-Amyloid impairs AMPA receptor trafficking and function by reducing Ca2+/calmodulin-dependent protein kinase II synaptic distribution. *The Journal of biological chemistry*, 284(16), pp.10639–49.

Guerrero-Muñoz, M.J., Gerson, J. & Castillo-Carranza, D.L., 2015. Tau Oligomers: The Toxic Player at Synapses in Alzheimer's Disease. *Frontiers in Cellular Neuroscience*, 9. Available at: http://journal.frontiersin.org/Article/10.3389/fncel.2015.00464/abstract.

Guo, J.L. & Lee, V.M., 2011. Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. *J. Biol. Chem.*, 286, pp.15317–15331. Available at: http://dx.doi.org/10.1074/jbc.M110.209296.

Guo, J.L. & Lee, V.M.Y., 2013. Neurofibrillary tangle-like tau pathology induced by synthetic tau fibrils in primary neurons over-expressing mutant tau. *FEBS Letters*, 587(6), pp.717–723.

Hardy, J. et al., 1998. Genetic dissection of Alzheimer's disease and related dementias: amyloid and its relationship to tau. *Nature neuroscience*, pp.355–8.

Hardy, J. & Allsop, D., 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends in Pharmacological Sciences*, 12(C), pp.383–388.

Hardy, J. & Higgins, G., 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science*, 256(5054), pp.184–185. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1566067.

Hardy, J. & Selkoe, D.J., 2002. The Amyloid Hypothesis of Alzheimer ' s Disease : Progress and Problems on the Road to Therapeutics. *Science*, 297(July), pp.353–357.

Harris, J.A. et al., 2010. Transsynaptic progression of amyloid-$β$-induced neuronal dysfunction within the entorhinal-hippocampal network. *Neuron*, 68(3), pp.428–441. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/21040845.

Herrup, K., 2015. The case for rejecting the amyloid cascade hypothesis. *Nature Neuroscience*, 18(6), pp.794–799. Available at: http://www.nature.com/doifinder/10.1038/nn.4017.

Holmes, B.B. et al., 2013. Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proceedings of the National Academy of Sciences*, 110(33), pp.E3138--E3147. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23898162.

Islam, K. & Levy, E., 1997. Carboxyl-terminal fragments of beta-amyloid precursor protein bind to microtubules and the associated protein tau. *Am J Pathol*, 151(1), pp.265–271. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9212751.

Ittner, L.M., Ke, Y.D. & Gotz, J., 2009. Phosphorylated Tau interacts with c-Jun N-terminal kinase-interacting protein 1 (JIP1) in Alzheimer disease. *J. Biol. Chem.*, 284, pp.20909–20916. Available at: http://dx.doi.org/10.1074/jbc.M109.014472.

Jarrett, J.T. & Lansbury, P.T., 1993. Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell*, 73(6), pp.1055–1058. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8513491.

Jeganathan, S. et al., 2006. Global hairpin folding of tau in solution. *Biochemistry*, 45, pp.2283–2293. Available at: http://dx.doi.org/10.1021/bi0521543.

Jiao, S.-S. et al., 2016. Brain-derived neurotrophic factor protects against tau-related neurodegeneration of Alzheimer's disease. *Translational Psychiatry*, 6(10), p.e907.

Jin, M. et al., 2011. Soluble amyloid -protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. *Proceedings of the National Academy of Sciences*, 108(14), pp.5819–5824. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.1017033108.

Joshi, P. et al., 2014. Microglia convert aggregated amyloid-$β$ into neurotoxic forms through the shedding of microvesicles. *Cell Death and Differentiation*, 21(4), pp.582–593. Available at: http://www.nature.com/doifinder/10.1038/cdd.2013.180.

Kamenetz, F. et al., 2003. APP processing and synaptic function. *Neuron*, 37(6), pp.925–37.

Kanemaru, K. et al., 1992. Fetal-type phosphorylation of the tau in paired helical filaments. *J. Neurochem.*, 58, pp.1667–1675.

Kar, S. et al., 2003. Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. *The EMBO journal*, 22(1), pp.70–7.

Katzman, R. et al., 1988. Clinical, pathological, and neurochemical changes in dementia: A subgroup with preserved mental status and numerous neocortical plaques. *Annals of Neurology*, 23(2), pp.138–144.

Kayed, R. et al., 2004. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. *Journal of Biological Chemistry*, 279(45), pp.46363–46366.

Kopke, E., 1993. Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J. Biol. Chem.*, 268, pp.24374–24384.

Lambert, M.P. et al., 1998. Diffusible, nonfibrillar ligands derived from A 1-42 are potent central nervous system neurotoxins. *Proceedings of the National Academy of Sciences*, 95(11), pp.6448–6453. Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.95.11.6448.

Lawrence, J.L.M. et al., 2014. Regulation of Presynaptic Ca2+, Synaptic Plasticity and Contextual Fear Conditioning by a N-terminal -Amyloid Fragment. *Journal of Neuroscience*, 34(43), pp.14210–14218. Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.0326-14.2014.

Lee, G., 2005. Tau and src family tyrosine kinases. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1739(2–3), pp.323–330.

Lee, G., Neve, R.L. & Kosik, K.S., 1989. The microtubule binding domain of tau protein. *Neuron*, 2(6), pp.1615–24.

Levy, E. et al., 1990. Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science (New York, N.Y.)*, 248(4959), pp.1124–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2111584.

Liu, C. & Gotz, J., 2013. Profiling murine tau with 0N, 1N and 2N isoform-specific antibodies in brain and peripheral organs reveals distinct subcellular localization, with the 1N isoform being enriched in the nucleus. *PLoS ONE*, 8, p.e84849. Available at: http://dx.doi.org/10.1371/journal.pone.0084849.

Liu, L. et al., 2012. Trans-Synaptic Spread of Tau Pathology In Vivo T. Ikezu, ed. *PLoS ONE*, 7(2), p.e31302.

Lombino, F. et al., 2013. An Intracellular Threonine of Amyloid-β Precursor Protein Mediates

Synaptic Plasticity Deficits and Memory Loss. *PLoS ONE*, 8(2).

Lonze, B.E. & Ginty, D.D., 2002. Function and regulation of CREB family transcription factors in the nervous system. *Neuron*, 35(4), pp.605–23.

Lorenzo, A. et al., 2000. Amyloid beta interacts with the amyloid precursor protein: a potential toxic mechanism in Alzheimer's disease. *Nature neuroscience*, 3(5), pp.460–464.

Luo, W. et al., 2015. Microglial internalization and degradation of pathological tau is enhanced by an anti-tau monoclonal antibody. *Scientific reports*, 5(1), p.11161.

Lv, Z.-Y. et al., 2017. Spreading of Pathology in Alzheimer's Disease. *Neurotoxicity Research*, 32(4), pp.707–722. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28623460.

Maas, T., Eidenmüller, J. & Brandt, R., 2000. Interaction of Tau with the Neural Membrane Cortex Is Regulated by Phosphorylation at Sites That Are Modified in Paired Helical Filaments. *Journal of Biological Chemistry*, 275(21), pp.15733–15740.

Maeda, S. et al., 2006. Increased levels of granular tau oligomers: An early sign of brain aging and Alzheimer's disease. *Neuroscience Research*, 54(3), pp.197–201.

Martin Prince, A. et al., 2015. World Alzheimer Report 2015 The Global Impact of Dementia An AnAlysIs of prevAlence, IncIDence, cosT AnD TrenDs.

Mawuenyega, K.G. et al., 2013. Amyloid-beta isoform metabolism quantitation by stable isotope-labeled kinetics. *Analytical Biochemistry*, 440(1), pp.56–62.

Meredith, J.E. et al., 2013. Characterization of Novel CSF Tau and ptau Biomarkers for Alzheimer's Disease. *PLoS ONE*, 8(10).

Meunier, M. et al., 1993. Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 13(12), pp.5418–5432. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8254384.

Miyamoto, T. et al., 2017. Phosphorylation of tau at Y18, but not tau-fyn binding, is required for tau to modulate NMDA receptor-dependent excitotoxicity in primary neuronal culture. *Molecular Neurodegeneration*, 12(1), p.41.

Morley, J.E. et al., 2010. A physiological role for amyloid-β protein: Enhancement of learning and memory. *Journal of Alzheimer's Disease*, 19(2), pp.441–449.

Morris, M. et al., 2011. The Many Faces of Tau. *Neuron*, 70(3), pp.410–426.

Mukhopadhyay, R. & Hoh, J.H., 2001. AFM force measurements on microtubule-associated proteins: the projection domain exerts a long-range repulsive force. *FEBS Letters*, 505(3), pp.374–378.

Mukrasch, M.D. et al., 2005. Sites of tau important for aggregation populate {beta}-structure and bind to microtubules and polyanions. *The Journal of biological chemistry*, 280(26), pp.24978–86.

Mukrasch, M.D., 2009. Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biol.*, 7, p.e34.

Müller, U.C. & Zheng, H., 2012. Physiological functions of APP family proteins. *Cold Spring Harbor Perspectives in Medicine*, 2(2).

Nath, S. et al., 2012. Spreading of Neurodegenerative Pathology via Neuron-to-Neuron Transmission of -Amyloid. *Journal of Neuroscience*, 32(26), pp.8767–8777. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22745479.

Oka, M. et al., 2017. Ca2+/calmodulin-dependent protein kinase II promotes neurodegeneration caused by tau phosphorylated at Ser262/356 in a transgenic Drosophila model of tauopathy. *The Journal of Biochemistry*, 162(5), pp.335–342.

Palmeri, A. et al., 2017. Amyloid-β Peptide Is Needed for cGMP-Induced Long-Term Potentiation and Memory. *The Journal of Neuroscience*, 37(29), pp.6926–6937. Available at: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.3607-16.2017.

Palop, J.J. et al., 2007. Aberrant Excitatory Neuronal Activity and Compensatory Remodeling of Inhibitory Hippocampal Circuits in Mouse Models of Alzheimer's Disease. *Neuron*, 55(5), pp.697–711.

Palop, J.J. et al., 2003. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. *Proceedings of the National Academy of Sciences of the United States of America*, 100(16), pp.9572–9577. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12881482.

Patterson, K.R. et al., 2011. Characterization of Prefibrillar Tau Oligomers *in Vitro* and in Alzheimer Disease. *Journal of Biological Chemistry*, 286(26), pp.23063–23076.

Pearson, R.C. et al., 1985. Anatomical correlates of the distribution of the pathological changes in the neocortex in Alzheimer disease. *Proceedings of the National Academy of Sciences of*

the United States of America, 82(13), pp.4531–4534. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3859874.

Piacentini, R. et al., 2017. Reduced gliotransmitter release from astrocytes mediates tau-induced synaptic dysfunction in cultured hippocampal neurons. *GLIA*, 65(8), pp.1302–1316.

Planel, E., 2004. Alterations in glucose metabolism induce hypothermia leading to tau hyperphosphorylation through differential inhibition of kinase and phosphatase activities: implications for Alzheimer's disease. *J. Neurosci.*, 24, pp.2401–2411.

Pooler, A.M. et al., 2013. Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.*, 14, pp.389–394.

Prada, I. et al., 2016. A new approach to follow a single extracellular vesicle–cell interaction using optical tweezers. *BioTechniques*, 60(1), pp.35–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26757810.

Puzzo, D. et al., 2005. Amyloid- Peptide Inhibits Activation of the Nitric Oxide/cGMP/cAMP-Responsive Element-Binding Protein Pathway during Hippocampal Synaptic Plasticity. *Journal of Neuroscience*, 25(29), pp.6887–6897. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16033898.

Puzzo, D. et al., 2011. Endogenous amyloid-β is necessary for hippocampal synaptic plasticity and memory. *Annals of Neurology*, 69(5), pp.819–830.

Puzzo, D. et al., 2017. LTP and memory impairment caused by extracellular Aβ and Tau oligomers is APP-dependent. *eLife*, 6, p.e26991. Available at: http://elifesciences.org/lookup/doi/10.7554/eLife.26991 [Accessed September 18, 2017].

Puzzo, D. et al., 2008. Picomolar Amyloid- Positively Modulates Synaptic Plasticity and Memory in Hippocampus. *Journal of Neuroscience*, 28(53), pp.14537–14545. Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.2692-08.2008.

Puzzo, D. et al., 2015. Rodent models for Alzheimer's disease drug discovery. *Expert Opinion on Drug Discovery*, 10(7), pp.703–711. Available at: http://www.tandfonline.com/doi/full/10.1517/17460441.2015.1041913.

Puzzo, D. et al., 2015. The keystone of Alzheimer pathogenesis might be sought in A$β$ physiology. *Neuroscience*, 307, pp.26–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26314631.

Puzzo, D. & Arancio, O., 2013. Amyloid-β peptide: Dr. Jekyll or Mr. Hyde? *Journal of*

Alzheimer's Disease, 33(SUPPL. 1).

Qi, H. et al., 2015. Nuclear Magnetic Resonance Spectroscopy Characterization of Interaction of Tau with DNA and Its Regulation by Phosphorylation. *Biochemistry*, 54(7), pp.1525–1533.

Qu, X. et al., 2017. Stabilization of dynamic microtubules by mDia1 drives Tau-dependent Aβ1-42synaptotoxicity. *Journal of Cell Biology*, 216(10), pp.3161–3178.

Rajendran, L. et al., 2006. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proceedings of the National Academy of Sciences of the United States of America*, 103(30), pp.11172–11177. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16837572.

Reitz, C., 2012. Alzheimer's disease and the amyloid cascade hypothesis: A critical review. *International Journal of Alzheimer's Disease*, 2012.

Reynolds, C.H. et al., 2008. Phosphorylation regulates tau interactions with Src homology 3 domains of phosphatidylinositol 3-kinase, phospholipase Cgamma1, Grb2, and Src family kinases. *The Journal of biological chemistry*, 283(26), pp.18177–86.

Ripoli, C. et al., 2014. Intracellular Accumulation of Amyloid- (A) Protein Plays a Major Role in A -Induced Alterations of Glutamatergic Synaptic Transmission and Plasticity. *Journal of Neuroscience*, 34(38), pp.12893–12903. Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1201-14.2014.

Roberson, E.D. et al., 2007. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science (New York, N.Y.)*, 316(5825), pp.750–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17478722.

Rustom, A. et al., 2004. Nanotubular Highways for Intercellular Organelle Transport. *Science*, 303(5660), pp.1007–1010. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14963329.

Saman, S. et al., 2012. Exosome-associated Tau Is Secreted in Tauopathy Models and Is Selectively Phosphorylated in Cerebrospinal Fluid in Early Alzheimer Disease. *Journal of Biological Chemistry*, 287(6), pp.3842–3849. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22057275.

Sanders, D.W. et al., 2014. Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron*, 82(6), pp.1271–1288. Available at: http://dx.doi.org/10.1016/j.neuron.2014.04.047.

Scarmeas, N. et al., 2009. Seizures in Alzheimer Disease. *Archives of Neurology*, 66(8), pp.992–

997. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19667221.

Schneider, A. et al., 1999. Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into Alzheimer paired helical filaments. *Biochemistry*, 38, pp.3549–3558.

Selkoe, D.J., 2002. Alzheimer's Disease Is a Synaptic Failure. *Science*, 298(5594), pp.789–791. Available at: http://www.sciencemag.org/cgi/doi/10.1126/science.1074069.

Selkoe, D.J., 2008. Soluble oligomers of the amyloid β-protein impair synaptic plasticity and behavior. *Behavioural Brain Research*, 192(1), pp.106–113.

Sengupta, U. et al., 2017. Tau oligomers in cerebrospinal fluid in Alzheimer's disease. *Annals of Clinical and Translational Neurology*, 4(4), pp.226–235. Available at: http://doi.wiley.com/10.1002/acn3.382.

Sevigny, J. et al., 2016. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature*, 537(7618), pp.50–56. Available at: http://www.nature.com/doifinder/10.1038/nature19323.

Shaked, G.M. et al., 2006. Abeta induces cell death by direct interaction with its cognate extracellular domain on APP (APP 597-624). *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 20(8), pp.1254–1256.

Shankar, G.M. et al., 2008. Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature Medicine*, 14(8), pp.837–842. Available at: http://www.nature.com/doifinder/10.1038/nm1782.

Shin, R.-W. et al., 2007. Amyloid precursor protein cytoplasmic domain with phospho-Thr668 accumulates in Alzheimer's disease and its transgenic models: a role to mediate interaction of Abeta and tau. *Acta neuropathologica*, 113(6), pp.627–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17431643.

Silva, A.J. et al., 1998. CREB AND MEMORY. *Annual Review of Neuroscience*, 21(1), pp.127–148.

Simón, D. et al., 2012. Tau Overexpression Results in Its Secretion via Membrane Vesicles. *Neurodegenerative Diseases*, 10(1–4), pp.73–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22269430.

Singh, T.J. et al., 1996. Calcium/calmodulin-dependent protein kinase II phosphorylates tau at Ser-262 but only partially inhibits its binding to microtubules. *FEBS Letters*, 387(2–3),

pp.145–148.

Sloane, J.A. et al., 1997. Lack of correlation between plaque burden and cognition in the aged monkey. *Acta Neuropathologica*, 94(5), pp.471–478.

Smith, A.D., 2002. Imaging the progression of Alzheimer pathology through the brain. *Proceedings of the National Academy of Sciences of the United States of America*, 99(7), pp.4135–7.

Smith, M.A. et al., 1995. Tau Protein Directly Interacts With the Amyloid Beta-Protein Precursor - Implications For Alzheimers Disease. *Nature Medicine*, 1(4), pp.365–369.

Söllvander, S. et al., 2016. Accumulation of amyloid-$β$ by astrocytes result in enlarged endosomes and microvesicle-induced apoptosis of neurons. *Molecular Neurodegeneration*, 11(1), p.38. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27176225.

Song, J.-H., Yu, J.-T. & Tan, L., 2014. Brain-Derived Neurotrophic Factor in Alzheimer's Disease: Risk, Mechanisms, and Therapy. *Molecular neurobiology*, (2015), pp.1477–1493. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25354497.

Sotiropoulos, I. et al., 2011. Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(21), pp.7840–7.

Steiner, B. et al., 1990. Phosphorylation of microtubule-associated protein tau: identification of the site for Ca2(+)-calmodulin dependent kinase and relationship with tau phosphorylation in Alzheimer tangles. *The EMBO journal*, 9(11), pp.3539–44. Available at: /pmc/articles/PMC552103/?report=abstract.

Strittmatter, W.J. et al., 1994. Isoform-specific interactions of apolipoprotein E with microtubule-associated protein tau: implications for Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, 91(23), pp.11183–6.

Sultan, A., 2011. Nuclear tau, a key player in neuronal DNA protection. *J. Biol. Chem.*, 286, pp.4566–4575. Available at: http://dx.doi.org/10.1074/jbc.M110.199976.

Takahashi, M. et al., 2015. Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. *Acta Neuropathologica*, 129(6), pp.895–907.

Tampellini, D., 2015. Synaptic activity and Alzheimer's disease: A critical update. *Frontiers in Neuroscience*, 9(NOV), pp.1–7.

Tampellini, D. et al., 2009. Synaptic Activity Reduces Intraneuronal A , Promotes APP Transport to Synapses, and Protects against A -Related Synaptic Alterations. *Journal of Neuroscience*, 29(31), pp.9704–9713. Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.2292-09.2009.

Teich, A.F. et al., 2015. Synaptic Therapy in Alzheimer's Disease: A CREB-centric Approach. *Neurotherapeutics*, 12(1), pp.29–41.

Terry, R.D. et al., 1991. Physical basis of cognitive alterations in alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Annals of Neurology*, 30(4), pp.572–580.

Trinczek, B. et al., 1995. Domains of tau protein, differential phosphorylation, and dynamic instability of microtubules. *Molecular biology of the cell*, 6(12), pp.1887–902.

Vega, I.E., 2005. Increase in tau tyrosine phosphorylation correlates with the formation of tau aggregates. *Brain Res. Mol. Brain Res.*, 138, pp.135–144. Available at: http://dx.doi.org/10.1016/j.molbrainres.2005.04.015.

Verret, L. et al., 2012. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in alzheimer model. *Cell*, 149(3), pp.708–721.

Vershinin, M. et al., 2007. Multiple-motor based transport and its regulation by Tau. *Proc. Natl Acad. Sci. USA*, 104, pp.87–92. Available at: http://dx.doi.org/10.1073/pnas.0607919104.

Violet, M. et al., 2014. A major role for Tau in neuronal DNA and RNA protection in vivo under physiological and hyperthermic conditions. *Frontiers in cellular neuroscience*, 8, p.84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24672431.

Vitolo, O. V et al., 2002. Amyloid beta -peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 99(20), pp.13217–13221. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12244210.

Vossel, K.A. et al., 2013. Seizures and Epileptiform Activity in the Early Stages of Alzheimer Disease. *JAMA Neurology*, 70(9), p.1158. Available at: http://archneur.jamanetwork.com/article.aspx?doi=10.1001/jamaneurol.2013.136.

Vuono, R. et al., 2015. The role of tau in the pathological process and clinical expression of Huntington's disease. *Brain*, 138(7), pp.1907–1918.

Walsh, D.M. & Selkoe, D.J., 2007. Aβ oligomers - A decade of discovery. *Journal of*

Neurochemistry, 101(5), pp.1172–1184.

Wang, Y. et al., 2011. Tunneling-nanotube development in astrocytes depends on p53 activation. *Cell death and differentiation*, 18(4), pp.732–742. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21113142.

Wang, Y. & Mandelkow, E., 2015. Tau in physiology and pathology. *Nature Reviews Neuroscience*, 17(1), pp.22–35.

Wegmann, S. et al., 2013. The fuzzy coat of pathological human Tau fibrils is a two-layered polyelectrolyte brush. *Proc. Natl Acad. Sci. USA*, 110, pp.E313–E321.

Weingarten, M.D. et al., 1975. A protein factor essential for microtubule assembly. *Proc. Natl Acad. Sci. USA*, 72, pp.1858–1862. Available at: http://dx.doi.org/10.1073/pnas.72.5.1858.

Whitcomb, D.J. et al., 2015. Intracellular oligomeric amyloid-beta rapidly regulates GluA1 subunit of AMPA receptor in the hippocampus. *Scientific reports*, 5(February), p.10934. Available at: http://www.nature.com/doifinder/10.1038/srep10934%5Cnpapers3://publication/doi/10.1038/srep10934.

Whittington, R.A. et al., 2013. Anesthesia and tau pathology. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 47, pp.147–155.

Wimo, A. et al., 2017. The worldwide costs of dementia 2015 and comparisons with 2010. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, 13(1), pp.1–7.

Wisniewski, T., Ghiso, J. & Frangione, B., 1994. Alzheimer's disease and soluble A beta. *Neurobiology of aging*, 15(2), pp.143–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7838284.

Wu, J.W. et al., 2016. Neuronal activity enhances tau propagation and tau pathology in vivo. *Nature Neuroscience*, 19(8), pp.1085–1092.

Wu, J.W. et al., 2013. Small Misfolded Tau Species Are Internalized via Bulk Endocytosis and Anterogradely and Retrogradely Transported in Neurons. *Journal of Biological Chemistry*, 288(3), pp.1856–1870. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23188818.

Xia, D., Li, C. & Götz, J., 2015. Pseudophosphorylation of Tau at distinct epitopes or the presence of the P301L mutation targets the microtubule-associated protein Tau to dendritic spines. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1852(5), pp.913–924.

Xie, M. et al., 2017. The Involvement of NR2B and tau Protein in MG132-Induced CREB Dephosphorylation. *Journal of Molecular Neuroscience*, 62(2), pp.154–162.

Xu, W. et al., 2016. Amyloid precursor protein-mediated endocytic pathway disruption induces axonal dysfunction and neurodegeneration. *Journal of Clinical Investigation*, 126(5), pp.1815–1833.

Yamada, K. et al., 2014. Neuronal activity regulates extracellular tau in vivo. *The Journal of Experimental Medicine*, 211(3), pp.387–393.

Yanagisawa, M. et al., 1999. Starvation induces tau hyperphosphorylation in mouse brain: implications for Alzheimer's disease. *FEBS Letters*, 461(3), pp.329–333.

Yin, Y. et al., 2016. Tau accumulation induces synaptic impairment and memory deficit by calcineurin-mediated inactivation of nuclear CaMKIV/CREB signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 113(26), pp.E3773-81.

Yoshiyama, Y. et al., 2007. Synapse Loss and Microglial Activation Precede Tangles in a P301S Tauopathy Mouse Model. *Neuron*, 53(3), pp.337–351.

Yuan, A. et al., 2008. Axonal transport rates in vivo are unaffected by tau deletion or overexpression in mice. *J. Neurosci.*, 28, pp.1682–1687. Available at: http://dx.doi.org/10.1523/JNEUROSCI.5242-07.2008.

Zhang, Y. et al., 2011. APP processing in Alzheimer's disease. *Molecular Brain*, 4(1), p.3. Available at: http://molecularbrain.biomedcentral.com/articles/10.1186/1756-6606-4-3.

Zhao, D., Watson, J.B. & Xie, C.-W., 2004. Amyloid beta prevents activation of calcium/calmodulin-dependent protein kinase II and AMPA receptor phosphorylation during hippocampal long-term potentiation. *Journal of neurophysiology*, 92(5), pp.2853–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15212428.

Zheng, L. et al., 2011. Macroautophagy-generated increase of lysosomal amyloid $β$-protein mediates oxidant-induced apoptosis of cultured neuroblastoma cells. *Autophagy*, 7(12), pp.1528–1545. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22108004.