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Amyloid-β, tau, and the cholinergic system in Alzheimer's disease: seeking direction in a tangle of clues

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Abstract: The link between histopathological hallmarks of Alzheimer's disease (AD), i.e. amyloid plaques, and neurofibrillary tangles, and AD-associated cognitive impairment, has long been established. However, the introduction of interactions between amyloid-beta (Aβ) as well as hyperphosphorylated tau, and the cholinergic system to the territory of descriptive neuropathology has drastically changed this field by adding the theory of synaptic neurotransmission to the toxic pas de deux in AD. Accumulating data show that a multitarget approach involving all amyloid, tau, and cholinergic hypotheses could better explain the evolution of events happening in AD. Various species of both Aβ and tau could be traced in cholinergic neurons of the basal forebrain system early in the course of the disease. These molecules induce degeneration in the neurons of this system. Reciprocally, aberrant cholinergic system modulation promotes changes in amyloid precursor protein (APP) metabolism and tau phosphorylation, resulting in neurotoxicity, neuroinflammation, and neuronal death. Altogether, these changes may better correlate with the clinical findings and cognitive impairment detected in AD patients. Failure of several of Aβ- and tau-related therapies further highlights the need for special attention to molecules that target all of these mentioned pathologic changes. Another noteworthy fact here is that none of the popular hypotheses of AD such as amyloidopathy or tauopathy seem to be responsible for the changes observed in AD alone. Thus, the main culprit should be sought higher in the stream somewhere in APP metabolism or Wnt signaling in the cholinergic system of the basal forebrain. Future studies should target these pathological events.

Keywords: Alzheimer's disease; amyloid-beta; cholinergic system; hypothesis; tau.

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Introduction

The relationship between the histopathological hallmarks of Alzheimer's disease (AD), i.e. amyloid plaques (APs), and neurofibrillary tangles (NFTs), and AD-associated cognitive impairment, has long been established (Bloom, 2014). However, the introduction of interactions between amyloid-beta (Aβ) as well as hyperphosphorylated tau, and the cholinergic system to the territory of descriptive neuropathology has drastically changed this field by adding the theory of synaptic neurotransmission to the toxic pas de deux in AD (Hampel et al., 2018). Accordingly, these changes have each become the basis for a theory that tries to explain which changes are the trigger and which ones are the bullets.

It has been widely accepted that Aβ is a crucial factor for the initiation and progression of AD (Sadigh-Eteghad et al., 2015a,b). Aβ hypothesis has until now been the most commonly accepted hypothesis which tries to justify AD pathophysiology. Soluble $A\beta_{(1-42)}$ oligomers, which are thought to play a crucial role in this hypothesis, are the result of the action of beta and gamma secretases on amyloid precursor protein (APP) (Anand et al., 2019). These toxic molecules are internalized by Aβ-related receptors and initiate a series of interactions that lead to tau hyperphosphorylation (Sadigh-Eteghad et al., 2015a,b). Evidence suggests that Aβ deteriorates cognitive function by both hijacking normal cholinergic mechanisms [i.e. Aβ internalization by α7 nicotinic acetylcholine receptor (α7nAChR)] and degrading brain cholinergic system (Wang et al., 2000; Nagele et al., 2002). However, this hypothesis suffers from several issues.

First, Aβ accumulation also occurs in cognitively healthy subjects. Second, there is a weak link between APs and the severity of cognitive impairment in AD patients. Third, the onset of cognitive decline in patients with Down syndrome is temporally variable. Nevertheless, all of these subjects possess APs almost in the fifth decade of their lives (Morris et al., 2014). These inconsistencies cast doubt over the fact that Aβ is the trigger in this cause-and-effect affair.

Tau is a microtubule-linked protein that modulates the stability of tubulin assemblies. In pathological conditions such as AD, however, it aggregates in inclusions (NFTs and threads) in a hyperphosphorylated state (Kametani and Hasegawa, 2018). Evidence shows that spatial pattern of tau accumulation in the brain has a close relationship with neural death and clinical presentation in AD (Bejanin et al., 2017). Tau lesions are also found earlier than Aβ accumulations (Johnson et al., 2016), leading to cellular signaling, transport and cytoskeletal systems, and mitochondrial function defects (Kametani and Hasegawa, 2018). However, tau pathology is seen in other neurodegenerative disorders (Hasegawa, 2016). Also, the development of tau and amyloid pathologies in AD brains is temporally and spatially separated. This explains that another pathologic factor may influence both pathologic processes (Mudher and Lovestone, 2002).

The cholinergic hypothesis is based on the progressive and continuous loss of limbic and neocortical cholinergic innervation as the primary culprit in AD phenomenology (Majdi et al., 2017; Hampel et al., 2018). This hypothesis is supported by three facts: first, presynaptic cholinergic markers drastically diminish in the cerebral cortex of AD patients; second, the nucleus basalis of Meynert (NbM) in the basal forebrain, as the source of cholinergic innervation, greatly suffers from degeneration in AD; and third, cholinesterase inhibitors significantly alleviate symptoms in AD patients (Hampel et al., 2018). Imaging studies suggest that nAChR impairment is an early finding in AD, indicating the significance of nAChRs as a potential culprit in AD pathophysiology (Nordberg, 2001). Nevertheless, the cholinergic hypothesis has received considerably less attention than its other counterparts.

This review aims to gather the most recent and old evidence over the various hypotheses of AD and their relevance, interconnections, and interactions with each other, which ultimately lead to clinical AD. Giving direction to the evidence in a tangle of clues, rather than finding the main culprit, is the main purpose of this study.

The amyloid hypothesis

General comments

The amyloid hypothesis, also recognized as the amyloid cascade hypothesis, has been up until now the mainstream by which the pathogenesis of AD is justified (LaFerla et al., 2007; Sadigh-Eteghad et al., 2015a,b; Anand et al., 2019). This idea is well supported by the fact that $A\beta_{42}$ oligomers take part in all identified forms of familial AD (Takahashi et al., 2017). Although there is a continuing dispute over this hypothesis, new experimental and clinical data further indicate that an imbalance between $A\beta_{42}$ production and clearance is responsible for the initiation and progression of AD (Selkoe and Hardy, 2016).

The origins of Aβ and amyloidopathy

Aβ was first isolated from the vasculature of the AD brain (Glenner and Wong, 1984). Accordingly, its upstream molecule, i.e. the APP, was discovered (Kang et al., 1987). APP is one of the APP family members, which is composed of APP and APP-like protein 1 and 2 (APLP1 and APLP2) (van der Kant and Goldstein, 2015). APP is a type I transmembrane protein that is cleaved by α, β, and γ secretase enzymes. The Aβ section is located within both the ectodomain and transmembrane parts of the APP molecule and is composed of approximately 40–43 amino acids (Takahashi et al., 2017). Intriguingly, the disease-causing form of Aβ, i.e. $A\beta_{42}$, constitutes only around 5–10% of all Aβ forms produced in the human brain (Gouras et al., 2000).

After synthesis within the endoplasmic reticulum and shipping to the cell membrane during a secretory process, APP is cleaved by the α secretase enzyme [one of a disintegrin and metalloproteinase domain (ADAM) 9, ADAM10, or ADAM17]. This process leads to the production of soluble APPα (sAPPα) and αC-terminal membrane-bound fragment (αCTF)/C83. Accordingly, γ secretase cleaves the remaining molecule to P3 and APP intracellular domain (AICD). This pathway is called non-amyloidogenic because α secretase acts on the Aβ domain of APP precluding Aβ production (van der Kant and Goldstein, 2015).

Sequentially, APP also undergoes cleavage by the β and γ secretases enzymes [the latter is composed of presenilin (PS)1 or PS2, nicastrin, anterior pharynx-defective (APH)1, and presenilin enhancer 2] at the N and C terminals of the Aβ domain, respectively, leading to Aβ production in a process called the amyloidogenic pathway. Briefly, β-site APP cleaving enzyme (BACE1) acts on APP

and produces sAPPβ and βCTF/C99. Accordingly, C99 is cleaved by γ secretase and releases Aβ to the intracellular space and generation of AICD. Not all cuts by the γ secretase complex are at the same place. Thus, Aβ peptides with a variable number of amino acids ranging from 34 to 50 are produced. It has been found that approximately 90% of all Aβ molecules are $A\beta_{40}$. These molecules are the predominant form of Aβ species under normal conditions. However, fragments of $A\beta_{42}$ and $A\beta_{43}$ peptides, which are more toxic and prone to oligomerization and aggregation could be found in APs. Aβ, then, takes part in a variety of physiological and pathological processes, and the AICD regulates gene expression and Ca^{2+} signaling (Bates et al., 2009; Kummer and Heneka, 2014; Takahashi et al., 2017).

Aβ clearance from the brain

Under physiological conditions, Aβ is cleared from the brain through the blood-brain barrier. The mechanisms by which Aβ is washed out from the brain are either non-enzymatic or enzymatic.

In the non-enzymatic mechanism, the interstitial fluid is drained into the cerebrospinal fluid (CSF) or it passes through the perivascular basement membranes (or Virchow-Robin arterial spaces) into the blood vessels (Yoon and Jo, 2012). Also, several vascular Aβ receptors expressed by endothelial cells such as the low-density lipoprotein receptor-related protein-1 (LRP-1), apolipoprotein J, P-glycoprotein, and very-low-density lipoprotein receptor are responsible for the clearance of Aβ from the brain (transcytosis). Aβ receptors also facilitate Aβ clearance through glial cell-mediated phagocytosis by astrocytes (Nagele et al., 2003) and bone marrow-derived microglia. The process is primarily age dependent and is mediated by several receptors such as scavenger receptor class B member 1, the macrophage receptor with collagenous structure, and LRP-1 (Paresce et al., 1996).

Enzymatic mechanisms include multiple proteases of Aβ-degrading enzymes (ADEs) such as (1) thiol-dependent metalloendopeptidase [insulin-degrading enzyme (IDE)], (2) cysteine proteases (cathepsin B, D, and S), (3) serine proteases [plasmin, myelin basic protein, acylpeptide hydrolase], (4) matrix metalloproteinase (MMP-9 and MMP-2), (5) zinc metalloendopeptidase [NEP-1 and NEP-2, endothelin-converting enzyme-1 and -2, angiotensin-converting enzyme (ACE)], and (6) enzymes from miscellaneous groups (glutamate carboxypeptidase II, aminopeptidase A, and mitochondrial peptidasome) (Nalivaeva et al., 2012; Yoon and Jo, 2012).

However, in pathological conditions such as AD, failure of ADEs (e.g. reduction in NEP and IDE mRNA levels) (Miners et al., 2009) and transport processes (e.g. LRP-1 receptor reduction and impaired interaction between LRP-1 and Aβ) (Bates et al., 2009) leads to accumulation of Aβ peptides in the brain. These changes signify the importance of Aβ turnover in the pathophysiology of the disease.

From Aβ to AD

'Our hypothesis is that deposition of amyloid-β protein (AβP), the main component of the plaques, is the causative agent of Alzheimer's pathology and that the neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition.' These sentences are considered as the establishment of the amyloid hypothesis by Hardy and Higgins in 1992 (Hardy and Higgins, 1992).

As mentioned earlier, APP cleavage in a stepwise manner leads to the production of Aβ peptides containing around 39–42 amino acids. These peptides are vulnerable to aggregation, forming dimers, oligomers, and fibrils (Haass and Selkoe, 2007). There is a debate over the fact which molecules are more toxic to the neurons and synaptic compartments. However, a consensus has been reached that soluble Aβ forms and not insoluble fibrillar aggregates are the critical role players in the damages observed in AD (Ricciarelli and Fedele, 2017).

According to the amyloid hypothesis, failure of mechanisms responsible for Aβ clearance leads to Aβ accumulation and oligomerization primarily in limbic areas and associative regions with increasing age. Afterward, these oligomers affect synaptic complexes and decrease their efficacy. As time passes, the faulty mechanisms mentioned above result in diffuse plaque formation and microglial and astrocytic cell activation. This aggravates inflammatory responses and oxidative stress and disrupts neuronal ionic homeostasis. Along with that, altered kinase/phosphatase activities intensify tangle formation and result in severe neurodegeneration and synaptic loss (Selkoe 2002; Selkoe and Hardy, 2016).

Synaptic toxicity

It has been accepted that all Aβ species induce damage primarily in the synaptic complex and reduce its efficacy (Selkoe 2002; Ferreira and Klein, 2011). Because of the crucial role of synapses in AD, it has been labeled

as a disease of synaptic failure or a disease in which synapses go cold (Selkoe 2002; Koffie et al., 2011). The defects in synapses are presented in several forms such as changes in neurotransmitter uptake/release, alterations in the localization of receptors in cells and prevention of synaptic plasticity, disruption of long-term potentiation, propagation of long-term depression (LTD), and, last but not least, cytoskeletal defects at synaptic levels (Ferreira and Klein, 2011; Koffie et al., 2011; Ferreira et al., 2015). This could be due to the shift in Wnt signaling from the canonical pathway, which promotes synaptic plasticity, to the non-canonical pathway, which is in favor of synaptic instability and retraction. The process could be mediated by the canonical Wnt inhibitor Dickkopf-1, which is itself activated by Aβ. On the other hand, the association is thought to be bidirectional where induction of non-canonical Wnt signaling favors Aβ generation, whereas activation of canonical signaling prevents Aβ production (Elliott et al., 2018).

Other changes also occur in the postsynaptic compartment, including Aβ-induced changes in α_7nAChRs and metabotropic glutamate receptors (mGluRs), specifically N-methyl-D-aspartic acid receptors (NMDARs) (Chang et al., 2016; Farhat and Ahmed, 2017). Toxic levels of Aβ oligomers partially prevent synaptic NMDARs through either receptor desensitization or internalization, activate extrasynaptic mGluRs, NMDARs, and downstream aberrant pathways involving calcineurin/STEP/cofilin, p38 mitogen-activated protein kinase (MAPK), and glycogen synthase kinase 3β (GSK-3β) molecules, resulting in LTD and dendritic spine and synaptic loss (Shankar et al., 2007; Li et al., 2009; Tackenberg and Brandt, 2009).

These synaptotoxic changes correlate well with cortical Aβ levels, but not with Aβ plaque burden or APP levels (Mucke et al., 2000; Mucke and Selkoe, 2012). In line with that, the alterations are more severe where soluble Aβ levels (soluble pool of cortical Aβ including oligomers) escalate, whence no plaque formation has ever happened (Naslund et al., 2000; DaRocha-Souto et al., 2011). The synaptic loss is linked to these soluble forms, even in the very early or preclinical stages of AD (Lue et al., 1999). Apart from that, several lines of evidence indicate that Aβ concentration in the synapses is an essential determiner in Aβ-mediated synaptic dysfunction. For example, low/picomolar doses of $A\beta_{42}$ significantly enhance synaptic activity, whereas its high/nanomolar levels induce the mentioned synaptic dysfunction and loss. This is, thus, considered as a 'bell-shaped association' or 'double-edged-sword relationship' between extracellular Aβ and synaptic activity (Laird et al., 2005; Puzzo et al., 2008; Abramov et al., 2009; Lasala et al., 2019).

Excitotoxicity

Excitotoxicity was once thought to be a late finding in AD. However, recent studies have shown that it is part of the early alterations occurring in AD. A clear link exists between glutamate-induced excitotoxicity and memory impairment in AD (Esposito et al., 2013; Majdi et al., 2016; Pallo et al., 2016). Aβ-induced overactivation of NMDARs is one of the central mechanisms by which Aβ exerts its toxic effects on neurons and causes neuronal damage in AD. However, the evidence for a direct interaction between these receptors and Aβ lacks (Roberson et al., 2007). Accumulating data show that soluble Aβ oligomers are implicated in the induction of excitotoxicity in AD (Šišková et al., 2014). Evidence suggests that soluble Aβ oligomers disrupt NMDA signaling and induce the internalization of postsynaptic NMDA/NR2A subunits. Also, Aβ interacts with excitatory amino acid transporter (EAAT) on astrocyte, which leads to an increase in the concentration of glutamate in the synaptic cleft. Escalated glutamate levels induce the activity of extrasynaptic NMDA/NR2B receptors and increase intracellular calcium levels, which ultimately lead to synaptic loss and neuronal cell death. Of interest, Aβ-induced excitotoxicity results in p38 MAPK, GSK-3β, and Janus kinase (JNK) activation, leading to cytoskeletal tau hyperphosphorylation and neurodegeneration (Esposito et al., 2013). The moderate efficacy of memantine (an NMDA receptor antagonist) in the symptomatic treatment of AD proposes the rationale for the Aβ-induced glutamate increase/excitotoxicity hypothesis (Danysz and Parsons, 2012).

Oxidative stress

Oxidative stress is a significant role player in the AD-induced neurodegeneration (Huang et al., 2016; Majdi et al., 2016). Oxidative stress has also been implicated in the pathogenesis of the earlier stages of AD, i.e. amnestic mild cognitive impairment (aMCI). Aβ-induced oxidative stress leads to a 'quadrilateral of neuronal death.' This includes changes in the proteostasis network, protein phosphorylation, mammalian target of rapamycin (mTOR) activation, and glucose metabolism. Oxidative stress has also a close relationship with synaptic loss and excitotoxicity, where each component is simultaneously the cause and effect of a vicious cycle in AD.

Evidence shows that oxidative stress and its resulting lipid peroxidation commonly happen in $A\beta_{1-42}$ oligomer-dense areas of the brain, but not in $A\beta_{1-42}$-poor regions (Butterfield and Boyd-Kimball, 2018). In line with that, $A\beta_{25-35}$, $A\beta_{1-40}$, and $A\beta_{1-42}$ oligomers are shown to increase protein oxidation, as indicated by the elevated levels of protein carbonyls (PCs) in cortical synaptosomes (Ansari et al., 2006). It has been verified that levels of PCs are higher in Aβ-rich regions as opposed to Aβ-poor areas of the AD brains (Hensley et al., 1995). Further, Aβ-mediated oxidative stress disrupts mitochondrial function, reduces ATP production, and decreases glucose metabolism (hypometabolism) in AD and aMCI brains (Butterfield, 2014). Besides, Aβ-mediated oxidative stress triggers mTOR activation, causes aggregation of cellular detritus and damaged organelles inside neurons, and leads to neuronal cell death (Tramutola et al., 2015).

Aβ coordination by active metal ions, particularly copper (Cu) either in the +I or +II oxidative states and iron [Fe (II)], also directly participates in reactive oxygen species production and oxidative stress, thus launching a connection between AD and oxidative stress (Cheignon et al., 2018).

Neuroinflammation

Microglial dysfunction and the resulting neuroinflammation are significant contributors to the pathogenesis of AD. Accordingly, the relationship between diffusible Aβ oligomers and microglial cells is extensively investigated (Salminen et al., 2009; Xu et al., 2016). Activation of microglia by Aβ increases the release of neurotoxic pro-inflammatory mediators (M1 activation), which causes neurodegeneration (Liu et al., 2012a,b). Aβ also induces neuronal death by activation of astrocytes and release of nitric oxide (NO), cytokines, and chemokines, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and growth-related oncogene. The mentioned molecules then activate caspases and induce neuronal apoptosis (Dorey et al., 2014). They also activate pro-oligomeric pathways by regulating APP processing. This then creates a vicious cycle where neuroinflammation triggers Aβ oligomers production and vice versa (Blasko et al., 2004; Alasmari et al., 2018). Also, Aβ oligomers and fibrils are considered foreign molecules by the innate immunity system; thus, they influence the activity of the pattern recognition receptors such as Toll-like receptors, NOD-like receptors, formyl peptide receptors, receptor for advanced glycation end products, scavenger receptors, complement receptors, and pentraxin 3. Activation of these receptors induces the activity of several signaling pathways and causes neuroinflammation. Aβ oligomers can also produce calcium-permeable ion channels (amyloid channels) on neuronal membranes (Aβ calcium channel hypothesis, which is discussed under the Apoptosis section). These pores escalate potassium efflux, induce IL-1β release, and cause neuroinflammation (Arispe et al., 2007; Salminen et al., 2009; Liu et al., 2012a,b). Cross-talks between neuroinflammation and AD pathology, i.e. the formation of neuritic plaques and NFTs, and neuronal cell death have been demonstrated (Salminen et al., 2009). However, AD treatment using anti-inflammatory agents such as nonsteroidal anti-inflammatory drugs has not shown promising results yet (Meyer et al., 2019).

Apoptosis

Apoptosis is an essential aspect of AD neurodegeneration. Aβ activates pro-apoptotic pathways in AD; however, the exact mechanisms are under investigation (Loo et al., 1993; Simon et al., 2011). One of the key features of Aβ-induced apoptosis is the fast accumulation of GD3, a disialoganglioside, in lipid raft microdomains including caveolae before mitochondrial repositioning. Following GD3 overproduction, cell cycle activation and apoptosis are inevitable (Copani et al., 2002; Kim et al., 2010). Evidence also suggests that $A\beta_{1-42}$ oligomers induce mitochondrial fission by protein Drp1 upregulation and prevent mitochondrial fusion by proteins Mfn1/2 and OPA1 downregulation. Accordingly, because of mitochondrial dynamics disruption, neuronal apoptosis occurs. Further, $A\beta_{1-42}$ oligomers alter mitochondrial membrane potentials, making mitochondria vulnerable to damage and mitophagy (Han et al., 2017). $A\beta_{1-42}$ also accumulates inside the mitochondria through clathrin-mediated endocytosis and results in mitochondrial function disruption and neuronal apoptosis. This is mediated by activation of the CD95/Fas apoptotic pathway, escalated Bax/Bcl-2 ratio, and cytochrome C discharge from the mitochondria (Morishima et al., 2001; Cha et al., 2012). Additionally, accumulation of large amounts of protofibrillar $A\beta_{1-42}$ by astrocytes causes significant astrocytic endosome/lysosome damages and microvesicle-mediated neuronal apoptosis in AD (Söllvander et al., 2016). In addition, $A\beta_{1-42}$ promotes apoptosis by increasing the expression of Meg3 long noncoding RNA and activates p53-dependent and -independent pathways (Huang and Liu, 2015). $A\beta_{1-42}$ (but not $A\beta_{1-40}$)-induced apoptosis could be also mediated by Aβ channels. These pores cause abnormal elevations of the intracellular calcium levels inside both neuronal

and mitochondrial membranes. Increased intracellular calcium, in turn, increases Ca^{2+} flux via voltage-sensitive Ca^{2+} channels in the plasma membrane and triggers several apoptotic pathways (Kagan et al., 2002). Because Aβ peptides tend to form channels in low PH conditions, they also reside on lysosomes, release toxic lysosomal contents into the cytosol, and cause apoptosis (Knauer et al., 1992).

Cholinergic dysfunction

Aβ-induced cholinergic system dysfunction, especially in the basal forebrain cholinergic neurons, is an early hallmark of AD (Nunes-Tavares et al., 2012). Aβ peptides reduce acetylcholine (ACh) production and discharge, prevent its axonal transportation via the inhibition of vesicular ACh transporter, and disrupt its degradation. Along with that, a decrease in the choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities, and the net amounts of nAChRs and muscarinic receptors have been reported in AD (Nyakas et al., 2011). Of interest, although the total level of AChE decreases in the AD, its activity increases around plaques due to the effects of $A\beta_{1-42}$ on oxidative stress and excitotoxicity through α7nAChRs and voltage-dependent calcium channels, which are permeable to Ca^{2+} (Fodero et al., 2004). Cholinergic dysfunction also results from the direct effects of Aβ on aberrant neuritic sprouting. The latter induces cholinergic fiber swelling and formation of grape-like structures (Nyakas et al., 2011).

AChE, on the other hand, induces Aβ aggregation via forming binding sites (at the ACE peripheral anionic site, which is different from the catalytic site of the enzyme) with Aβ molecules by reducing the lag phase of the peptide aggregation. This indicates that AChE acts as a chaperone which facilitates Aβ oligomer formation with high structural stability (Carvajal and Inestrosa, 2011). It also increases the neurotoxicity of Aβ aggregates. For example, neurons injected with Aβ-AChE complexes reveal a much-distorted neurite network as opposed to neurons administered with Aβ alone. This is, at least in part, mediated by the effects of Aβ-AChE complexes on mitochondrial membrane potential. Intriguingly, the impact is shown to be greater compared to Aβ alone (Dinamarca et al., 2010). Also, M1 and M3 muscarinic receptor subtypes escalate APP production via the induction of the phospholipase C/protein kinase C pathway and increase BACE expression in the AD brain (Nitsch et al., 1992; Zuchner et al., 2004).

Amyloid hypothesis: pros and cons

Pros

Several lines of evidence presented above strongly indicate the crucial role of soluble Aβ oligomers in all aspects of AD pathology. Here, the conformation and also endogenous Aβ species concentration are of paramount significance. Thus, neglecting these factors may lead to the current controversy over the ingenuity of the amyloid hypothesis. Besides, other factors also support the amyloid hypothesis of AD: (1) carriers of autosomal-dominant mutations in the APP or the γ secretase complex proteins presenilin 1/2 (PSEN1/2) clearly show AD pathology, (2) a large number of patients with Down syndrome show AD-like clinical findings early in life due to the overexpression of the APP gene located on chromosome 21, (3) several rodent and non-rodent models based on APP or APP/PS1 mutations mimic the critical structural and behavioral features of AD, (4) several genetical, immunological, and pharmacological strategies trying to decrease the cerebral Aβ burden in AD model of mice have alleviated AD pathology and manifestations (Gotz et al., 2004; Li et al., 2013; Puzzo et al., 2014; Ricciarelli and Fedele, 2017). Other evidence also supports the amyloid hypothesis of AD. It has been demonstrated that mutations in APP and PSEN1/2 genes primarily result in early-onset Aβ deposition ensued by NFT formation. This evidence clearly states that AP formation chronologically precedes NFT deposition in AD. However, tau mutations leading to some types of frontotemporal dementia do not lead to Aβ accumulation in the brain (Selkoe and Hardy, 2016).

Further, the crossing of hAPP tg mice with hTau tg mice [double mutant (tau/APP) progeny] meaningfully escalates NFT formation without exerting any effects on AP deposition (Lewis et al., 2001). On the other hand, the crossing of APP tg mice with tau knockout animals decreases behavioral impairment expressed by animals compared with the animal in which tau is expressed (Roberson et al., 2007). Compelling evidence also shows that injection of soluble Aβ oligomers results in tau hyperphosphorylation and NFT formation. However, this does not happen when tau is primarily knocked down in the animals (Jin et al., 2011). These data are further supported by a locus recognized in chromosome 10, which is associated with increased Aβ production and is linked to late-onset AD (LOAD) (Mudher and Lovestone, 2002). It seems that tau is an essential factor in AD pathogenesis. However, it is a downstream agent in amyloid cascade.

This role thus appears to be 'permissive' than 'causative' (Maruyama et al., 2013).

Cons

The opponents of amyloid hypothesis argue that although carriers of autosomal-dominant mutations in the APP or the γ secretase complex proteins presenilin 1/2 (PSEN1/2) clearly show AD pathology, this does not mean that familial cases of AD (FAD) or early-onset AD share the same features with LOAD or sporadic AD patients. Besides, no correlation exists between cerebral amyloid burden or CSF levels of $A\beta_{1-42}$ and cognitive decline pattern, where a considerable number of subjects expressing massive cerebral amyloid deposition do not exhibit AD-like cognitive impairment (Aizenstein et al., 2008; Fagan et al., 2009; Klunk et al., 2009; Villemagne et al., 2011; Harrison and Owen, 2016). On the other hand, the cerebral distribution pattern of tau shows a better correlation with AD symptomology than Aβ. Additionally, no temporal and spatial correlations exist between these two pathologic factors. Indeed, some evidence suggests that tangle formation may occur before plaque deposition in the brain (Braak and Braak, 1991; Price et al., 1991; Schonheit et al., 2004; Herrup 2015).

Further evidence proposes that transgenic mice models of FAD, which show severe amyloid pathology, do not exhibit considerable neuronal damage, nor tangle formation, as expected by the amyloid hypothesis (Herrup 2015; Makin 2018). The same thing could be shown in humans, where the massive amyloid burden is found in the cerebellum, but no subsequent NFT formation nor neuronal loss could be demonstrated (Joachim et al., 1989; Jacobs et al., 2017).

Amyloid hypothesis: where do we stand?

Failure of clinical trials

No convincing conclusion could be drawn from the arguments made by opponents and proponents of the amyloid hypothesis. A definitive response might be elicited from the Aβ-directed immunotherapies for AD. Several studies are on the go to assess the impacts of active or passive Aβ vaccination in AD patients (Relkin et al., 2009; Bohrmann et al., 2012; Farlow et al., 2015). Monoclonal antibodies such as bapineuzumab, gantenerumab, solanezumab, aducanumab, crenezumab, and N3pG-Aβ are being or have been tried in AD patients. However, several of these trials have failed, and new ones are being designed (Table 1) (Liu et al., 2016; Mullard 2016; Mullane and Williams, 2018). To further address the problems observed in these studies, the mentioned drugs were tried in the earlier and asymptomatic phases of AD (NCT02008357 and NCT02760602 trials) or in the at-risk population (NCT01998841 trial) where no significant neuronal damage could be found in the brain (Ricciarelli and Fedele, 2017). If the elimination of amyloid pathology halts NFT formation, then the cascade-based hypothesis of Aβ is confirmed. The second possibility could be that vaccination eliminates amyloid pathology and reverses dementia but exerts no effects of NFT formation. In this case, Aβ is the sole role player in AD, and the cascade hypothesis should be revised. Conversely, if the elimination of Aβ affects neither clinical manifestations of AD nor NFT formation, then the hypothesis should be thoroughly revised (Mudher and Lovestone, 2002). Unfortunately, the results of clinical trials prove the latter case in which the Aβ hypothesis requires a radical revision, which will be discussed to some extent in what follows.

Reconsideration of the role of APP metabolism products

The wealth of evidence presented earlier supports the critical role of Aβ in AD. However, the results of anti-Aβ trials (which have been directed against various forms of Aβ) indicate that Aβ may not be the pathogenetic factor we are looking for, and it may just have a participating role in the progression of AD. This might be very bold a claim, however. Some other downstream molecules resulting from APP processing, i.e. accumulation of APP C-terminal fragments such as C83, C99, and AICD, may be involved in AD pathology (Pera et al., 2013; Svedruzic et al., 2015; Kametani and Hasegawa, 2018). Recently, a clinical trial that used a γ secretase inhibitor (semagacestat) in AD patients showed a decrease in Aβ production, along with an increase in APP C-terminal fragments in the brain. This was in tandem with the worsened symptoms in the selected AD patients (Doody et al., 2013). Several studies have investigated the link between APP C-terminal fragments and accumulation of phosphorylated tau, impaired axonal vesicle trafficking, synaptic failure, and memory impairment (Ghosal et al., 2009; Szpankowski et al., 2012; Tamayev et al., 2012), suggesting the role of APP C-terminal fragments in the pathogenesis of sporadic and familial AD. Here, we may conclude that the weight should be shifted from dysregulated Aβ production to impaired APP metabolism and its resulting by-products.

Table 1: Summary of selected anti-Aβ immunotherapies in patients suffering from various stages of AD.

Drug	Target	Site of action	Clinical phase	Result(s)		
				Patients	Efficacy	Adverse effects
Solanezumab	Mainly monomers	Mid-domain	Phase III	Mild AD	Decrease in Aβ burden (S) and improved cognitive function (S)	None
Gammagard IVIG	Oligomers and Aβ fibrils	Aβ aggregates	Phase III	Mild to moderate AD	Mainly negative results; Improved cognitive function in moderate AD and APOE e4 carriers	?
Gantenerumab	Monomers, oligomers, and fibrils	Central region and N terminus	Phase III	Prodromal to mild AD	Decrease in Aβ burden (NS)	None
Bapineuzumab	Mainly monomers, fibrils, and deposited APs	N terminus	Phase III	Mild to moderate AD	Decrease in Aβ burden (S) and improved cognitive function (NS)	Vasogenic edema, amyloid-related imaging abnormalities
Ponezumab	Mainly $A\beta_{1-40}$ species	C terminus	Phase II	Mild to moderate AD	Decrease in Aβ burden (NS) and improved cognitive function (NS)	None
BAN2401	Protofibrils	Protofibrils	Phase I	Mild AD	_	None
Crenezumab	Oligomers, fibrils, and plaques	Pyroglutamate-$A\beta_{3-15}$	Phase II	Mild AD	Decrease in Aβ burden (S) and improved cognitive function (S)	None
Aducanumab	Oligomers and fibrils	N terminus	Phase Ib	Prodromal to mild AD	Decrease in Aβ burden (S) and improved cognitive function (S)	Amyloid-related imaging abnormalities
Donanemab[a]	APs	$A\beta_{3-42}$	Phase I	Mild AD	Decrease in Aβ burden	Strong immunogenicity
UB311	?	N terminus of $A\beta_{1-14}$	Phase I	Mild to moderate AD	Improved cognitive function (S)	None
Gamunex	?	?	Phase II/III	Mild to moderate AD	?	?
GSK-933776	APP, monomers, oligomers, protofibrils, fibrils, and APs	N terminus	Phase I	Mild AD	Decrease in Aβ burden (NS)	?
Affitope AD02	?	N terminus	Phase I	Mild to moderate AD	None	None

[a]S, Significant; NS, non-significant.
Commonly known as N3pG-Aβ monoclonal antibody or LY3002813. Data are obtained from https://www.alzforum.org/therapeutics.
This table does not include data for other Aβ-directed therapies such as BACE1 inhibitors.

The role of $A\beta_{1-42}$ monomers

$A\beta_{1-42}$ monomers are exceedingly prone to aggregation, thus forming species ranging from soluble oligomers to large prefibrillar assemblies (Ha et al., 2007). In normal conditions, $A\beta_{1-42}$ monomers possess neuroprotective characteristics, where they are involved in glucose uptake by neurons (Giuffrida et al., 2015). However, these molecules are also dominantly found in the lag phase of AD (Arosio et al., 2015). Although it has been demonstrated that neuronal toxicity of oligomeric forms of Aβ is higher than monomeric forms. Monomers can modulate APP metabolism and BACE1 activity, leading to increased Aβ production and tau hyperphosphorylation (Tamagno

et al., 2018). These findings may further shed light on the fact that any Aβ-based immunotherapy not only should be directed against oligomeric forms but also should target monomeric species. This may fundamentally change the position where we stand now.

The tangle and tau hypothesis

General comments

'Whenever a theory appears to you as the only possible one, take this as a sign that you have neither understood the theory nor the problem which it was intended to solve.' This remark by Karl Popper in his famous book 'Objective Knowledge: An Evolutionary Approach' in 1972 might be generalized to the amyloid hypothesis, which has been the mainstream concept of AD pathology over the last 20 years. The results of Aβ-directed immunotherapy trials have yielded nothing but failure. Thus, is it fair to propose that tau is the main culprit or at least a permissive factor in AD initiation and progression (Kametani and Hasegawa, 2018)?

The origins of tau and tauopathy in AD

Tau is a microtubule-associated protein that takes part in the stabilization of tubulin assemblies. In the adult brain, two different isoforms of tau, namely, 3-repeat (3R) and 4-repeat (4R), are expressed. These isoforms are found in the neuronal axons in normal conditions (Goedert et al., 1989). However, in AD, some unique alterations in tau structure, i.e. hyperphosphorylation due to discrepancy between activities of kinases (such as GSK3β and CDK5) and phosphatases (including PP1, PP2A, PP2B, and PP5), truncation of tau, glycosylation, glycation, nitration, ubiquitination, lysine methylation, and sumoylation, disrupt microtubule assembly and provoke tau aggregation in a pair helical filament form called NFTs (Patterson et al., 2011). In a perion-like manner, abnormal tau then converts normal tau to the abnormal molecule (Kametani and Hasegawa, 2018). Evidence suggests that hyperphosphorylation increases tau affinity to the microtubule, dissociating the cargos from the kinesin and thus preventing regular axonal transport (Brady and Sperry, 1995). Sequentially, tau is first hyperphosphorylated. Then, it produces tau dimers and oligomers. These oligomers tend to aggregate and form the paired helical filaments (PHF) or related straight filaments. The final assembly of these structures ultimately leads to the formation of pathological inclusions, which are called either, NFT if formed in the neuronal soma, or threads, if formed in dendrites or axons (Zempel and Mandelkow, 2014; Chong et al., 2018). Tau oligomers are more toxic than the larger assemblies and spread trans-synaptically from one neuron to the other, and also from one area to another in the AD brain. Tau oligomers are also present in the extracellular space, taking part in LTD and memory impairment (Liu et al., 2012a,b; Fa et al., 2016). However, the order of these steps is not universally accepted, and some argue that tau phosphorylation happens after aggregation, and conformational changes in tau protein are responsible for tau aggregation (Mena et al., 1996).

From tau to AD

Pathological tau assemblies are first formed in a limited number of neurons in specific areas of the brain and accordingly spread to other regions. This stereotypical spatiotemporal spreading happens in a well-defined manner via three distinct mechanisms, namely, endocytosis, macropinocytosis, and exosomes (Simic et al., 2016). According to Braak and Braak, tau pathology is first detectable in the transentorhinal area (stages I and II). Subsequently, it spreads to the limbic area (stages III and IV) and neocortical regions (stages V and IV) (Braak and Braak, 1991). This widespread involvement of various brain areas correlates well with clinical features of AD, but not amyloid burden (Bejanin et al., 2017). This is accompanied by extensive loss of synapses and their function in areas most severely affected by tau pathology (Dejanovic et al., 2018). Several mechanisms have been proposed by which pathological tau impairs synaptic function and exacerbates neurodegeneration. This will be discussed in detail in what follows.

Synaptic loss

Synaptic loss as a central finding in AD has a better association with tau pathology than with the load of APs (Weiner et al., 2013). Several lines of evidence link tau to synaptic dysfunction in AD. First, tau detachment from microtubules interferes with kinesin motors and impairs the transportation of mitochondria in the neuronal axon. It also disrupts cargo deliveries to the dendritic spines. Second, tau accumulation inside neurons has destructive effects on synaptic structures such as postsynaptic density (PSD) of excitatory synapses. This is induced by disrupted

regulation of F-actin (which is an essential element in the growth and stability of PSD) and Fyn kinase (which is essential for NMDAR stimulation) in neurons. Third, NFT aggregation inside neurons in the AD brain decreases synaptophysin, a synaptic protein, mRNA by 35–37% (Dixit et al., 2008; Hoover et al., 2010; Chai et al., 2011). Fourth, PSDs of neurons damaged by tau aggregations become devoid of specific intracellular signaling proteins, i.e. small GTPase regulators. The latter plays an essential role in the postsynaptic compartment by regulating the dynamics of F-actin in spines. On the other hand, PSDs may be loci for complement C1q accumulation, which has a role in innate immunity. All these changes may not be detected using conventional methods but play a role in tau-mediated synaptic dysfunction (Spence and Soderling, 2015; Dejanovic et al., 2018).

Oxidative stress

Tau prevents the kinesin-dependent carriage of mitochondria, neurofilaments, Golgi vesicles, and peroxisomes into neurites. This makes neurons highly susceptible to oxidative damage. As a result of deficient transportation of peroxisomes to neurites, they cannot defend against oxidative stress, and thus, these neurons have shorter and fewer neurites (Stamer et al., 2002). Also, it has been revealed that some tau fragments induce copper reduction, promoting H_2O_2 production and oxidative stress (Su et al., 2007). On the other hand, Aβ-induced oxidative stress exacerbates tau hyperphosphorylation and aggregation at paired helical filament (PHF-1) epitope (serine 396/404) by blocking glutathione synthesis activity and upregulating GSK-3β activity. This creates a vicious cycle that leads to neurodegeneration in AD (Feng et al., 2013; Alavi Naini and Soussi-Yanicostas, 2015).

Mitochondrial dysfunction and apoptosis

Mitochondrial dysfunction is a universal finding in AD (Moreira et al., 2010). It has been found that pathologic tau molecules disrupt normal mitochondrial function in several ways. Both *in vivo* and *in vitro* studies show that tau reduces mitochondrial complexes (particularly complex I and V) and antioxidant enzyme activities, and thus prevents ATP production. Mitochondrial membrane potential impairment also happens as a result of N-terminal-truncated tau aggregation in mitochondria (Eckert et al., 2011; Wang et al., 2015; Li et al., 2016). Phosphorylated tau also interacts with voltage-dependent anion channel 1 protein (VDAC1) in the AD brain and impairs the opening and closure of mitochondrial pores, leading to mitochondrial dysfunction and apoptosis (Kobayashi et al., 2003; Manczak and Reddy, 2012). Mitochondrial dysfunction then aggravates the aforementioned oxidative stress. It should be noted that mitochondrial dysfunction increases pathologic tau phosphorylation, exacerbating the events described above (Cheng and Bai, 2018).

Neuroinflammation

Intraneuronal tau pathology provokes neuronal damage and reactivates resting microglia and their transformation into phagocytic microglia. Consequently, these cells aggregate and form clusters. Also, expression of specific markers such as lymphocytic antigen CD4 and integrins CD45, CD68, CD18, CD11a, b, and c by activated microglia is escalated. Accordingly, the infiltration of leukocytes from blood into the brain tissue exacerbates the mentioned inflammatory changes (Zilka et al., 2012). Intraneuronal tau could also be released into the interstitial fluid without overt neurodegeneration. The released molecules activate the innate immune response through the MAPK pathway, increase the release of NO and inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and escalate tissue inhibitor of metalloproteinase-1 production by astroglia-microglia cells (Kovac et al., 2011). On the other hand, neuroinflammation induces conformational changes in tau protein and increases misfolded hyperphosphorylated tau protein levels (Zilka et al., 2012).

Tau hypothesis: pros and cons

Pros

Evidence provided above shows that tau pathology plays an active and crucial role in the progression of AD. Besides, several other facts support the tau hypothesis of AD. The number and burden of tangles are in close association with cognitive impairment detected in AD patients (Nagy et al., 1995). Besides, increase in tau burden most commonly occurs in areas that are thought to have a pivotal role in memory formation (Naslund et al., 2000).

Along with that, hyperphosphorylated tau levels in CSF are in a close association with the memory-associated areas of the brain, such as the hippocampus, in patients with MCI (de Leon et al., 2006; Fagan and Holtzman, 2010). Also, AD-inducing variants of triggering receptor expressed on myeloid cells 2 (TREM2) and apolipoprotein E4 (ApoE4) promote cerebral atrophy mainly in the hippocampus and piriform/entorhinal cortex. These

changes are independent from Aβ pathology and depend mainly on tau aggregations (Krasemann et al., 2017; Shi et al., 2017; Ulland et al., 2017).

Cons

Until now, 15 drugs (active and passive immunizations) have been developed to target hyperphosphorylated tau. However, the results are either unavailable or discouraging (Table 2). Only two vaccines [namely, methylthioninium (MT), a tau aggregation inhibitor (TAI) registered under NCT00515333 code and tideglusib, a GSK-3 inhibitor registered under NCT01350362 code] have shown promising preliminary results, which should be validated across larger-scale studies (del Ser et al., 2013; Wischik et al., 2015).

Tau picture is further complicated when studies show that tau mutations could give rise to tau inclusions and NFTs, but they cannot produce APs. However, a mutation in APP could create both plaques and tangles. Thus, it could be inferred that amyloid pathology, wherever it is, is upstream to tau pathology. Double-mutant mice that possess both amyloid and tau mutations have shown more brain tangles than those with only tau mutations (Götz et al., 2001; Lewis et al., 2001). Other evidence indicates that APP, and not Aβ, serves as a receptor that facilitates intracellular aggregation of tau (Takahashi et al., 2015).

Aβ or tau: finding the bullet and trigger!

There are three potential ways of interaction between Aβ and tau. First, Aβ induces an aberrant process which leads to tau hyperphosphorylation and synaptic toxicity. Second, tau is critically involved in the mediation of Aβ toxicity, and its presence is necessary for Aβ-induced neuronal damage. Third, Aβ and tau act in parallel and synergistically, each one targeting specific cellular processes

Table 2: Summary of selected anti-tau immunotherapies in patients suffering from various stages of AD.

Medication	Target	Other names	Clinical phase	Therapy category	Efficacy
RO7105705	Tau	MTAU9937A and RG6100	Phase II	Passive immunotherapy	Unavailable
AADvac-1	Tau	Axon peptide 108 conjugated to KLH	Phase II	Active immunotherapy	Unavailable
ACI-35	Tau	–	Phase I	Active immunotherapy	Unavailable
BIIB076	Tau	NI-105 and 6C5 huIgG1/l	Phase I	Passive immunotherapy	Unavailable
BIIB080	Tau	IONIS-MAPTRx and ISIS 814907	Phase I	RNA based	Unavailable
BIIB092	Tau	BMS-986168 and IPN007	Phase II	Passive immunotherapy	Unavailable
C2N 8E12	Tau	ABBV-8E12	Phase II	Passive immunotherapy	Unavailable
Epothilone D	Tau	BMS-241027	Discontinued	Small molecule	Unavailable
JNJ-63733657	Tau	–	Phase I	Passive immunotherapy	Unavailable
LMTM	Tau	TRx0237, LMT-X, methylene blue, and TAI	Phase III	Small molecule	Negative results in all phases
LY3303560	Tau	–	Phase II	Passive immunotherapy	–
RG7345	Tau	RO6926496	Discontinued	Passive immunotherapy	–
Rember TM	Tau	Methylene blue, MT, TRx-0014, and TAI	Discontinued	Small molecule	Benefit was observed on the ADAS-cog scale in both mild and moderate AD subjects
TPI 287	Tau	–	Phase I	Small molecule	
Tideglusib	Tau	NP031112, Nypta®, Zentylor™, GSK-3 inhibitor, and NP12	Discontinued	Small molecule	Positive trends in MMSE, ADAS-cog, GDS, and GCA without statistical significance

MMSE, Mini-mental state examination; ADAS-cog, Alzheimer's disease assessment scale–cognitive subscale; AD, Alzheimer's disease; GDS, global deterioration scale; GCA, global cortical atrophy; KLH, keyhole limpet hemocyanin. Data are obtained from https://www.alzforum.org/therapeutics.

mentioned above, thus fortifying each other's destructive impacts (Ittner and Gotz, 2011). But what is the bullet and what is the trigger? There remain other explanations.

One possibility is that APP protein and its metabolism products such as Aβ are responsible for the worsening of tau aggregation inside neurons. Here, we, along with Kametani et al. (Kametani and Hasegawa, 2018), assume that neither tau nor Aβ is sufficient to produce AD-like pathology, but the problem should be sought in the APP and its metabolism level. This could be supported by the fact that APP metabolism impairment increases APP C-terminal fragments level, which leads to axonal and synaptic defects and misplacement of tau proteins. This, in turn, exacerbates synaptic defects mentioned earlier (Goldsbury et al., 2006; Rodrigues et al., 2012). Another direct consequence of APP metabolism failure is Aβ amyloidosis, which increases tau aggregation, induces oxidative stress, neuroinflammation, and apoptosis, and worsens AD pathology (Leyns et al., 2017). At this stage, it does not seem such bold a claim to state that AD might be a dysfunction of APP and its metabolism, and not merely Aβ or tau pathology. The latter factors are necessary for AD initiation and progression but do not appear to be sufficient. They are indeed permissive and not causative.

Another possibility is the Wnt signaling pathway whose dysfunction is upstream to both Aβ and tau, and its dysregulation could mediate both amyloid and tau pathologies (Anderton et al., 2000; De Ferrari et al., 2014). Apart from these effects, aberrant Wnt signaling disrupts apolipoprotein E function, and glucose metabolism in the brain (De Ferrari et al., 2014; Cisternas and Inestrosa, 2017). It has been shown that Wnt signaling is essential for sAPPα production and tau phosphorylation modulation in normal conditions. These effects are mediated through the interaction between Wnt and the destruction complex. This complex is composed of the Fz receptor, LRP5/6, as well as Dvl. The complex inhibits GSK-3β, prevents β-catenin degradation, and decreases BACE1 expression. However, dysregulation of the Wnt signaling pathway decreases the non-amyloidogenic metabolism of APP (thus shifting the balance toward amyloidogenic pathway) and increases tau phosphorylation through GSK-3β activation (leading to tau hyperphosphorylation) and β-catenin degradation (leading to increased BACE1 expression) (Ittner and Gotz, 2011; Tapia-Rojas and Inestrosa, 2018) (Figure 1).

These are just possibilities whose authenticity should be validated across experimental studies and clinical trials.

The cholinergic hypothesis

General comments

The cholinergic system and ACh play a crucial role in the formation of memory. They also have an essential role in learning, attention, and other higher brain tasks

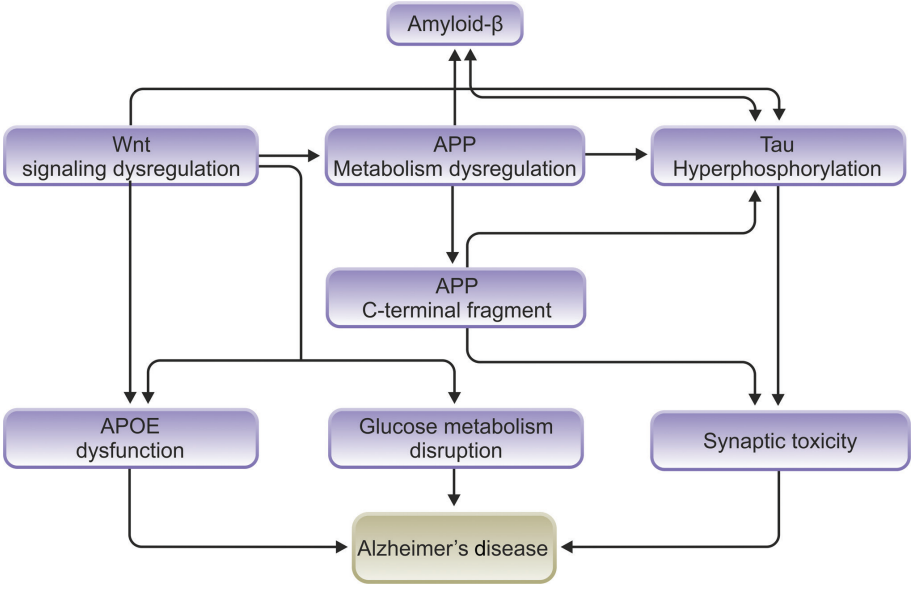

Figure 1: The interaction of Aβ, tau, APP, and Wnt signaling pathway in the pathophysiology of AD. Ab, amyloid-b; APP, amyloid precursor protein; AD, Alzheimer's disease.

(Hampel et al., 2018). Progressive presynaptic rather than postsynaptic loss of limbic and neocortical cholinergic innervation is an early pathogenic incident in the AD brain, which is interconnected with the severity of the cognitive decline. The wealth of evidence led to the proposal of the cholinergic hypothesis of AD and the invention of cholinesterase inhibitor drugs (Hampel et al., 2019). A distinctive feature, which distinguishes the cholinergic hypothesis of AD from others, is its emphasis on the recent theory of synaptic neurotransmission rather than traditional descriptive neuropathology. Three main pillars of this theory are as follows: first, a selective decrease in the presynaptic cholinergic activity in the brain of AD subjects (Davies and Maloney, 1976); second, prominent atrophy of the NbM, as the core of cortical cholinergic neurons, in the basal forebrain in AD patients. This results in severe memory and learning impairment (Whitehouse et al., 1982; Mesulam 2012). Third, the established efficacy of cholinergic agonists, specifically cholinesterase inhibitors, in the improvement of AD symptoms and the deteriorating impacts of cholinergic antagonists in the same settings (Drachman and Leavitt, 1974; Summers et al., 1986).

Compelling evidence suggests that cholinergic lesion occurs at asymptomatic or prodromal stages of the disease, indicating its primary role in the pathogenesis of AD (Hampel et al., 2018).

Cholinergic lesion and AD

Involvement of the cholinergic system in AD

Cholinergic NbM neurons, known as Ch4, project fibers to all cerebral cortex in primates. These cells are extremely vulnerable to the neurodegeneration observed in AD. Accumulating data propose that a massive load of NFTs could be traced in Ch4 neurons, especially in the anterolateral (Ch4al) and posterior (Ch4p) sections, in both early (MCI) and advanced stages of AD. Strikingly, NFT density is in good correlation with the severity of AD symptoms and cognitive impairment (Mesulam et al., 2004). Very low amounts of NFTs could also be found in healthy individuals who are at risk for AD. This suggests that the involvement of Ch4 neurons of NbM is an early incident in AD (Mesulam, 2004). NFTs are not the only pathological findings in Ch4 neurons. It has been shown that loss of cholinergic projections in the inferior temporal gyrus and entorhinal cortex is linked to the burden of Aβ detected in these regions. Further evidence shows that amyloid burden is correlated with the degeneration level of NbM (Beach et al., 1997; Kerbler et al., 2014; Grothe et al., 2016). Additionally, synaptic complexes of the cholinergic system are highly susceptible to Aβ oligomers, being damaged early in the course of the disease (Bell et al., 2006; Ferreira-Vieira et al., 2016). Altogether, these facts provide compelling evidence that the cholinergic system is a key role player in AD pathophysiology.

AChE

AChE tailed splice variant (AChET) or G4 is the principal form in the central nervous system, which has a C-terminal α-helix peptide of 40 amino acids named T peptide. Through disulfur bonding between these peptides, homodimers and homotetramers of AChET are generated, which constitute the functional units at the cholinergic synapse (Henderson et al., 2010; Hicks et al., 2011). It has been shown that AChE activity and levels are affected by AD (García-Ayllón et al., 2011). A general loss in the AChE protein levels and enzyme activity has been detected in all areas of the AD brain. Also, a selective and predominant loss of tetramers (G4) of AChE has been reported in Brodmann areas 9, 10, 11, 21, 22, and 40, and the amygdala of AD patients. The mentioned change had a good correlation with the degeneration of presynaptic elements. This occurs along with preservation or even escalation of lighter species of this molecule (Fishman et al., 1986; Saez-Valero et al., 1999). The same changes could be traced in CSF and blood samples of patients with AD (Saez-Valero et al., 2000; Garcia-Ayllon et al., 2010). These alterations might resemble the expression pattern of AChE in the embryonic stage, indicating the AChE-mediated stimulation of a neuronal restoration system in the AD (Layer, 1995).

Evidence suggests that both hyperphosphorylated tau and Aβ interact with AChE. It has been revealed that Aβ imposes increasing effects on AChE level, explaining why the latter is higher around Aβ plaques (Sberna et al., 1997). Also, it has been found that P-tau can activate AChE expression and increase its level (Silveyra et al., 2012). However, these changes are accompanied by an extreme loss of AChE activity in AD. AChE directly interacts with soluble Aβ and facilitates its precipitation around plaques (Rees et al., 2003). AChE overexpression also changes APP metabolism, increases PS1 levels, and promotes the amyloidogenic APP pathway (Silveyra et al., 2012).

On the other hand, AChE increases GSK-3β activity and tau hyperphosphorylation (Toiber et al., 2008). In a vicious cycle, escalated levels of AChE promote both the amyloidogenic pathway and tau hyperphosphorylation in

AD (García-Ayllón et al., 2011). Other studies have found that re-treatment of SH-SY5Y cells with siRNA AChE could halt Aβ42-mediated PS1 escalation. The contradictory findings may indicate the fact that several forms, tetrameric versus monomeric, or T-variant versus R-variant or N-extended variant, of AChE, are present in AD brain, each one having specific roles and functions (Silveyra et al., 2012).

ChAT

Aβ also interacts with ChAT in AD. It has been found that single intracerebroventricular (i.c.v.) injection of $A\beta_{25-35}$ to the mice brain reduces ChAT activity in the cortex, hippocampus, and medial septum, but not in the basal forebrain (Yamaguchi and Kawashima, 2001; Ikonomovic et al., 2005; Fgaier et al., 2015). However, other studies failed to recapitulate these results and showed no effect of i.c.v. injections of $A\beta_{25-35}$ on ChAT activity (Pavia et al., 2000). This might be due to the differences in route, duration, dose, and species of Aβ being administered (Kar et al., 1998). In agreement with the former assumption, reduced ChAT activity is accompanied by decreased ACh production (Nunes-Tavares et al., 2012) and choline uptake in AD (Parikh et al., 2014). In line with that, a decrease in ChAT activity has been linked to the burden of NFTs in AD (Wilcock et al., 1982).

Muscarinic AChRs

AChRs have an important role in AD. For instance, M_1 and M_2 ACh receptors or M_1/G-protein coupling dramatically decrease in the hippocampus and cortex. Also, the number of M_4 receptors decreases in the cortex of AD subjects, contributing to the symptomology of the disease (Tsang et al., 2006; Lebois et al., 2018). The severity of muscarinic receptors' loss is in association with the severity of AD dementia (Tsang et al., 2006). It has been found that the hypofunction of M_1/M_3 receptors results in the activation of the amyloidogenic pathway of APP metabolism and alteration of proteolysis of APP, ultimately increasing Aβ generation (Caccamo et al., 2006). Among these receptors, the M_1 subtype is of paramount importance, as it is copious in the areas associated with memory, i.e. the hippocampus, and is linked to short-term memory (Konar et al., 2019). Also, activation of M_1 receptors stimulates α secretase activity and shifts the balance of APP processing toward the non-amyloidogenic pathway (Welt et al., 2015). Indeed, muscarinic agonists reduce Aβ aggregation and tau phosphorylation, and improve cognitive outcomes (Nitsch et al., 2000; Beach et al., 2001; Jones et al., 2008; Zhao et al., 2019).

nAChRs

nAChRs are another major component of the cholinergic system, playing a significant role in its pathophysiology. Research shows that $\alpha_4\beta_2$ and α_7 receptors are the most common subtypes in the human brain (Sadigh-Eteghad et al., 2015a,b). Among these, the α_7 subtype is of special importance due to its abundance in the basal forebrain neuronal projections to the hippocampus and its role in memory and learning (Sadigh-Eteghad et al., 2016). Conflicting results have been presented about the change in the mass of nAChRs in AD, some of which showing their increase and others indicating their decrease throughout the disease (Banerjee et al., 2000; Counts et al., 2007; Pandya and Yakel, 2011). However, the contradicting results may originate from the fact that some studies assessed the mRNA levels of the receptors, while others evaluated the protein levels. A post-translational change in this regard would justify the observed differences between studies. Thus, a reduction in the expression of $\alpha_4\beta_2$ and α_7 receptors can potentially happen in AD, which leads to cognitive impairment (Wevers et al., 1999; Burghaus et al., 2000).

It has been found that in the early stages of AD, Aβ aggregation interacts with the α_7 receptors in the basal forebrain with high affinity compared to other subtypes and alters its expression pattern (Pettit et al., 2001; Parri et al., 2011). Evidence shows that high micromolar concentrations of Aβ seen in AD inactivate α_7 receptors, impair cell signaling pathways, and cause nAChR-mediated Aβ internalization. The mentioned cascade finally impairs synaptic plasticity and cognition (Pettit et al., 2001; Gu and Yakel, 2011). Additionally, internalization of the Aβ-α_7 nAChR complex by endocytosis leads to Aβ accumulation inside neurons and triggers upregulation of the α_7 nAChRs. The latter has been shown to have toxic effects on neurons (Liu et al., 2015). α_7 nAChR inactivation by Aβ, on the other hand, changes APP metabolism post-translationally and increases plaque burden. Further, it has been found that nAChR modulation by Aβ causes tau hyperphosphorylation and PHFs, as well as NFT formation (Ahmed et al., 2017). Conversely, low pico/nanomolar doses of Aβ bind to α_7 nAChRs and activate these receptors and downstream cell survival signaling pathways, inducing neuroprotection (Sadigh-Eteghad et al., 2014).

Astrocytic and microglial nAChRs participate in Aβ phagocytosis and degradation. These receptors are also involved in the Aβ-associated oxidative stress and neuronal death and protect neurons against these insults via a trophic factor-mediated pathway. Further, glial nAChRs indirectly interact with NMDA and AMPA receptors and modulate gamma-aminobutyric acid and intracellular calcium levels (Sadigh-Eteghad et al., 2016).

Linking the cholinergic system to Aβ and tau

Early in the course of AD, cholinergic neurons of the basal forebrain system undergo severe degeneration, leading to cholinergic atrophy in the mentioned area. Along with that, cortical loss of glutaminergic neurotransmission occurs. Accordingly, because of the alterations in muscarinic and nicotinic AChRs, tau hyperphosphorylation, NFT formation, altered APP metabolism, and increased Aβ production occur, possibly leading to AD (Babic 1999; Sivaprakasam, 2006). However, the trigger and bullet mystery remain largely an imbroglio unresolved.

Targeting the cholinergic system in AD

Because of its popularity, the cholinergic system has been the target of treatment in AD for a long time. Searching ALZFORUM (https://www.alzforum.org/therapeutics), we came across 30 different cholinergic-based therapeutics that have been designed to partially or entirely target the cholinergic system in AD. Eighteen of these molecules have been discontinued for several reasons. However, the remaining drugs such as donepezil, galantamine, rivastigmine, and tacrine are almost the only treatments providing partial symptomatic relief for patients with varying degrees of AD. Others such as GLN-1062 (Memogain), HTL0018318, Ladostigil, and SUVN-G3031 are under phase 1/2 clinical trials. Among these, Ladostigil has shown promising preliminary results in MCI patients in imaging findings and selected cognitive tests (http://www.avphar.com/ladostigil/clinical-data/). To our surprise, nicotine, another cholinergic drug, and its metabolite cotinine do not exist in this list. Several clinical trials using nicotine patches have been conducted or are being performed in AD and MCI patients. Unlike other trials, the results of these trials have been promising (Wilson et al., 1995; White and Levin, 1999; Gold et al., 2012). Based on convincing evidence, we suggest that nicotine and cotinine could both be potentially promising leaders in the treatment of AD, and more attention should be directed toward these agents (Majdi et al., 2017, 2018).

Concluding remarks

Accumulating data show that a multitarget approach involving all amyloid, tau, and cholinergic hypotheses could better explain the evolution of events happening in AD. Early in the course of the disease, various species of Aβ and tau could be traced in cholinergic neurons of basal forebrain system. These molecules induce degeneration of cholinergic neurons. Reciprocally, aberrant cholinergic system modulation promotes changes in APP metabolism and tau phosphorylation, resulting in neurotoxicity, neuroinflammation, and neuronal death. Altogether, these changes may better correlate with the clinical findings and cognitive impairment detected in AD patients. Failure of several of Aβ- and tau-related therapies further highlights the need for special attention to molecules that target all of these mentioned pathologic changes. None of the popular hypotheses of AD, i.e. amyloid nor tau, seem to be responsible for the changes observed in AD alone. Thus, the main culprit should be sought higher in the stream somewhere in APP metabolism or Wnt signaling in the cholinergic system of the basal forebrain. Future studies should aim at targeting these pathological events.

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References

Abramov, E., Dolev, I., Fogel, H., Ciccotosto, G.D., Ruff, E., and Slutsky, I. (2009). Amyloid-β as a positive endogenous regulator of release probability at hippocampal synapses. Nat. Neurosci. *12*, 1567–1576.

Ahmed, T., Zahid, S., Mahboob, A., and Farhat, S.M. (2017). Cholinergic system and post-translational modifications: an insight on the role in Alzheimer's disease. Curr. Neuropharmacol. *15*, 480–494.

Aizenstein, H.J., Nebes, R.D., Saxton, J.A., Price, J.C., Mathis, C.A., Tsopelas, N.D., Ziolko, S.K., James, J.A., Snitz, B.E., Houck, P.R., et al. (2008). Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch. Neurol. *65*, 1509–1517.

Alasmari, F., Alshammari, M.A., Alasmari, A.F., Alanazi, W.A., and Alhazzani, K. (2018). Neuroinflammatory cytokines induce amyloid beta neurotoxicity through modulating amyloid precursor protein levels/metabolism. biomed. Res. Int. *2018*, 3087475.

Alavi Naini, S.M. and Soussi-Yanicostas, N. (2015). Tau hyperphosphorylation and oxidative stress, a critical vicious circle in neurodegenerative tauopathies? Oxid. Med. Cell Longevit. *2015*, 151979.

Anand, A., Sharma, N., Gulati, M., and Khurana, N. (2019). Amyloid beta: the foremost protagonist in Alzheimer's disease. In: Handbook of Research on Critical Examinations of Neurodegenerative Disorders. M. Uddin and M. Amran, eds. (Hershey, PA: IGI Global). pp. 235–251.

Anderton, B.H., Dayanandan, R., Killick, R., and Lovestone, S. (2000). Does dysregulation of the Notch and wingless/Wnt pathways underlie the pathogenesis of Alzheimer's disease? Mol. Med. Today *6*, 54–59.

Ansari, M.A., Joshi, G., Huang, Q., Opii, W.O., Abdul, H.M., Sultana, R., and Butterfield, D.A. (2006). *In vivo* administration of D609 leads to protection of subsequently isolated gerbil brain mitochondria subjected to *in vitro* oxidative stress induced by amyloid beta-peptide and other oxidative stressors: relevance to Alzheimer's disease and other oxidative stress-related neurodegenerative disorders. Free Radic. Biol. Med. *41*, 1694–1703.

Arispe, N., Diaz, J.C., and Simakova, O. (2007). Aβ ion channels. Prospects for treating Alzheimer's disease with Aβ channel blockers. Biochim. Biophys. Acta Biomembr. *1768*, 1952–1965.

Arosio, P., Knowles, T.P., and Linse, S. (2015). On the lag phase in amyloid fibril formation. Phys. Chem. Chem. Phys. *17*, 7606–7618.

Babic, T. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. J. Neurol. Neurosurg. Psychiatry *67*, 558.

Banerjee, C., Nyengaard, J.R., Wevers, A., de Vos, R.A., Jansen Steur, E.N., Lindstrom, J., Pilz, K., Nowacki, S., Bloch, W., and Schroder, H. (2000). Cellular expression of alpha7 nicotinic acetylcholine receptor protein in the temporal cortex in Alzheimer's and Parkinson's disease – a stereological approach. Neurobiol. Dis. *7*, 666–672.

Bates, K.A., Verdile, G., Li, Q.X., Ames, D., Hudson, P., Masters, C.L., and Martins, R.N. (2009). Clearance mechanisms of Alzheimer's amyloid-beta peptide: implications for therapeutic design and diagnostic tests. Mol. Psychiatry. *14*, 469–486.

Beach, T.G., Honer, W.G., and Hughes, L.H. (1997). Cholinergic fibre loss associated with diffuse plaques in the non-demented elderly: the preclinical stage of Alzheimer's disease? Acta Neuropathol. *93*, 146–153.

Beach, T.G., Walker, D.G., Potter, P.E., Sue, L.I., and Fisher, A. (2001). Reduction of cerebrospinal fluid amyloid beta after systemic administration of M1 muscarinic agonists. Brain Res. *905*, 220–223.

Bejanin, A., Schonhaut, D.R., La Joie, R., Kramer, J.H., Baker, S.L., Sosa, N., Ayakta, N., Cantwell, A., Janabi, M., Lauriola, M., et al. (2017). Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. Brain *140*, 3286–3300.

Bell, K.F., Ducatenzeiler, A., Ribeiro-da-Silva, A., Duff, K.,Bennett, D.A., and Cuello, A.C. (2006). The amyloid pathology progresses in a neurotransmitter-specific manner. Neurobiol. Aging *27*, 1644–1657.

Blasko, I., Stampfer-Kountchev, M., Robatscher, P., Veerhuis, R., Eikelenboom, P., and Grubeck-Loebenstein, B. (2004). How chronic inflammation can affect the brain and support the development of Alzheimer's disease in old age: the role of microglia and astrocytes. Aging Cell *3*, 169–176.

Bloom, G.S. (2014). Amyloid-beta and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol. *71*, 505–508.

Bohrmann, B., Baumann, K., Benz, J., Gerber, F., Huber, W., Knoflach, F., Messer, J., Oroszlan, K., Rauchenberger, R., Richter, W.F., et al. (2012). Gantenerumab: a novel human anti-Abeta antibody demonstrates sustained cerebral amyloid-beta binding and elicits cell-mediated removal of human amyloid-beta. J. Alzheimers. Dis. *28*, 49–69.

Braak, H. and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. *82*, 239–259.

Brady, S.T. and Sperry, A.O. (1995). Biochemical and functional diversity of microtubule motors in the nervous system. Curr. Opin. Neurobiol. *5*, 551–558.

Burghaus, L., Schutz, U., Krempel, U., de Vos, R.A., Jansen Steur, E.N., Wevers, A., Lindstrom, J., and Schroder, H. (2000). Quantitative assessment of nicotinic acetylcholine receptor proteins in the cerebral cortex of Alzheimer patients. Brain Res. Mol. Brain Res. *76*, 385–388.

Butterfield, D.A. (2014). The 2013 SFRBM discovery award: selected discoveries from the butterfield laboratory of oxidative stress and its sequela in brain in cognitive disorders exemplified by Alzheimer disease and chemotherapy induced cognitive impairment. Free Radic. Biol. Med. *74*, 157–174.

Butterfield, D.A. and Boyd-Kimball, D. (2018). Oxidative stress, amyloid-beta peptide, and altered key molecular pathways in the pathogenesis and progression of Alzheimer's disease. J. Alzheimers Dis. *62*, 1345–1367.

Caccamo, A., Oddo, S., Billings, L.M., Green, K.N., Martinez-Coria, H., Fisher, A., and LaFerla, F.M. (2006). M1 receptors play a central role in modulating AD-like pathology in transgenic mice. Neuron *49*, 671–682.

Carvajal, F.J. and Inestrosa, N.C. (2011). Interactions of AChE with Aβ aggregates in Alzheimer's brain: therapeutic relevance of IDN 5706. Front. Mol. Neurosci. *4*, 19.

Cha, M.Y., Han, S.H., Son, S.M., Hong, H.S., Choi, Y.J., Byun, J., and Mook-Jung, I. (2012). Mitochondria-specific accumulation of amyloid beta induces mitochondrial dysfunction leading to apoptotic cell death. PLoS One *7*, e34929.

Chai, X., Wu, S., Murray, T.K., Kinley, R., Cella, C.V., Sims, H., Buckner, N., Hanmer, J., Davies, P., and O'Neill, M.J. (2011). Passive immunization with anti-tau antibodies in two transgenic models reduction of tau pathology and delay of disease progression. J. Biol. Chem. *286*, 34457–34467.

Chang, L., Zhang, Y., Liu, J., Song, Y., Lv, A., Li, Y., Zhou, W., Yan, Z., Almeida, O.F., and Wu, Y. (2016). Differential regulation of N-methyl-D-aspartate receptor subunits is an early event in the actions of soluble amyloid-β(1-40) oligomers on hippocampal neurons. J. Alzheimers Dis. *51*, 197–212.

Cheignon, C., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C., and Collin, F. (2018). Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biol. *14*, 450–464.

Cheng, Y. and Bai, F. (2018). The association of tau with mitochondrial dysfunction in Alzheimer's disease. Front Neurosci. *12*, 163.

Chong, F.P., Ng, K.Y., Koh, R.Y., and Chye, S.M. (2018). Tau proteins and tauopathies in Alzheimer's disease. Cell. Mol. Neurobiol. *38*, 965–980.

Cisternas, P. and Inestrosa, N.C. (2017). Brain glucose metabolism: role of Wnt signaling in the metabolic impairment in Alzheimer's disease. Neurosci. Biobehav. Rev. *80*, 316–328.

Copani, A., Melchiorri, D., Caricasole, A., Martini, F., Sale, P., Carnevale, R., Gradini, R., Sortino, M.A., Lenti, L., De Maria, R., et al. (2002). Beta-amyloid-induced synthesis of the ganglioside GD3 is a requisite for cell cycle reactivation and apoptosis in neurons. J. Neurosci. *22*, 3963–3968.

Counts, S.E., He, B., Che, S., Ikonomovic, M.D., DeKosky, S.T., Ginsberg, S.D., and Mufson, E.J. (2007). Alpha7 nicotinic

receptor up-regulation in cholinergic basal forebrain neurons in Alzheimer disease. Arch. Neurol. *64*, 1771–1776.

Danysz, W. and Parsons, C.G. (2012). Alzheimer's disease, beta-amyloid, glutamate, NMDA receptors and memantine – searching for the connections. Br. J. Pharmacol. *167*, 324–352.

DaRocha-Souto, B., Scotton, T.C., Coma, M., Serrano-Pozo, A., Hashimoto, T., Serenó, L., Rodríguez, M., Sánchez, B., Hyman, B.T., and Gómez-Isla, T. (2011). Brain oligomeric β-amyloid but not total amyloid plaque burden correlates with neuronal loss and astrocyte inflammatory response in amyloid precursor protein/tau transgenic mice. J. Neuropathol. Exp. Neurol. *70*, 360–376.

Davies, P. and Maloney, A.J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet *2*, 1403.

De Ferrari, G.V., Avila, M.E., Medina, M.A., Perez-Palma, E., Bustos, B.I., and Alarcon, M.A. (2014). Wnt/beta-catenin signaling in Alzheimer's disease. CNS Neurol. Disord. Drug Targets *13*, 745–754.

de Leon, M.J., DeSanti, S., Zinkowski, R., Mehta, P.D., Pratico, D., Segal, S., Rusinek, H., Li, J., Tsui, W., Saint Louis, L.A., et al. (2006). Longitudinal CSF and MRI biomarkers improve the diagnosis of mild cognitive impairment. Neurobiol. Aging *27*, 394–401.

Dejanovic, B., Huntley, M.A., De Mazière, A., Meilandt, W.J., Wu, T., Srinivasan, K., Jiang, Z., Gandham, V., Friedman, B.A., Ngu, H., et al. (2018). Changes in the synaptic proteome in tauopathy and rescue of tau-induced synapse loss by C1q antibodies. Neuron *100*, 1322–1336.e1327.

del Ser, T., Steinwachs, K.C., Gertz, H.J., Andres, M.V., Gomez-Carrillo, B., Medina, M., Vericat, J.A., Redondo, P., Fleet, D., and Leon, T. (2013). Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. J. Alzheimers Dis. *33*, 205–215.

Dinamarca, M.C., Sagal, J.P., Quintanilla, R.A., Godoy, J.A., Arrázola, M.S., and Inestrosa, N.C. (2010). Amyloid-β-acetylcholinesterase complexes potentiate neurodegenerative changes induced by the Aβ peptide. Implications for the pathogenesis of Alzheimer's disease. Mol. Neurodegener. *5*, 4.

Dixit, R., Ross, J.L., Goldman, Y.E., and Holzbaur, E.L.F. (2008). Differential regulation of dynein and kinesin motor proteins by tau. Science *319*, 1086–1089.

Doody, R.S., Raman, R., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, S., Kieburtz, K., He, F., Sun, X., Thomas, R.G., et al. (2013). A phase 3 trial of semagacestat for treatment of Alzheimer's disease. N. Engl. J. Med. *369*, 341–350.

Dorey, E., Chang, N., Liu, Q.Y., Yang, Z., and Zhang, W. (2014). Apolipoprotein E, amyloid-β, and neuroinflammation in Alzheimer's disease. Neurosci. Bull. *30*, 317–330.

Drachman, D.A. and Leavitt, J. (1974). Human memory and the cholinergic system. A relationship to aging? Arch. Neurol. *30*, 113–121.

Eckert, A., Schmitt, K., and Gotz, J. (2011). Mitochondrial dysfunction – the beginning of the end in Alzheimer's disease? Separate and synergistic modes of tau and amyloid-beta toxicity. Alzheimers Res. Ther. *3*, 15.

Elliott, C., Rojo, A.I., Ribe, E., Broadstock, M., Xia, W., Morin, P., Semenov, M., Baillie, G., Cuadrado, A., Al-Shawi, R., et al. (2018). A role for APP in Wnt signalling links synapse loss with β-amyloid production. Transl. Psychiatry *8*, 179.

Esposito, Z., Belli, L., Toniolo, S., Sancesario, G., Bianconi, C., and Martorana, A. (2013). Amyloid beta, glutamate, excitotoxicity in Alzheimer's disease: are we on the right track? CNS Neurosci. Ther. *19*, 549–555.

Fa, M., Puzzo, D., Piacentini, R., Staniszewski, A., Zhang, H., Baltrons, M.A., Li Puma, D.D., Chatterjee, I., Li, J., Saeed, F., et al. (2016). Extracellular tau oligomers produce an immediate impairment of LTP and memory. Sci. Rep. *6*, 19393.

Fagan, A.M. and Holtzman, D.M. (2010). Cerebrospinal fluid biomarkers of Alzheimer's disease. Biomark Med. *4*, 51–63.

Fagan, A.M., Mintun, M.A., Shah, A.R., Aldea, P., Roe, C.M., Mach, R.H., Marcus, D., Morris, J.C., and Holtzman, D.M. (2009). Cerebrospinal fluid tau and ptau(181) increase with cortical amyloid deposition in cognitively normal individuals: implications for future clinical trials of Alzheimer's disease. EMBO Mol. Med. *1*, 371–380.

Farhat, S.M. and Ahmed, T. (2017). Neuroprotective and neurotoxic implications of alpha7 nicotinic acetylcholine receptor and Aβ interaction: therapeutic options in Alzheimer's disease. Curr. Drug Targets *18*, 1537–1544.

Farlow, M.R., Andreasen, N., Riviere, M.E., Vostiar, I., Vitaliti, A., Sovago, J., Caputo, A., Winblad, B., and Graf, A. (2015). Long-term treatment with active Abeta immunotherapy with CAD106 in mild Alzheimer's disease. Alzheimers Res Ther. *7*, 23.

Feng, Y., Xia, Y., Yu, G., Shu, X., Ge, H., Zeng, K., Wang, J., and Wang, X. (2013). Cleavage of GSK-3beta by calpain counteracts the inhibitory effect of Ser9 phosphorylation on GSK-3beta activity induced by H_2O_2. J. Neurochem. *126*, 234–242.

Ferreira, S.T. and Klein, W.L. (2011). The Aβ oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. Neurobiol. Learn Mem. *96*, 529–543.

Ferreira, S.T., Lourenco, M.V., Oliveira, M.M., and De Felice, F.G. (2015). Soluble amyloid-beta oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. Front. Cell. Neurosci. *9*, 191.

Ferreira-Vieira, T.H., Guimaraes, I.M., Silva, F.R., and Ribeiro, F.M. (2016). Alzheimer's disease: targeting the cholinergic system. Curr. Neuropharmacol. *14*, 101–115.

Fgaier, H., Mustafa, I.H.I., Awad, A.A.R., and Elkamel, A. (2015). Modeling the interaction between β-amyloid aggregates and choline acetyltransferase activity and its relation with cholinergic dysfunction through two-enzyme/two-compartment model. Comput. Math. Method Med. *2015*, 923762.

Fishman, E.B., Siek, G.C., MacCallum, R.D., Bird, E.D., Volicer, L., and Marquis, J.K. (1986). Distribution of the molecular forms of acetylcholinesterase in human brain: alterations in dementia of the Alzheimer type. Ann Neurol. *19*, 246–252.

Fodero, L.R., Mok, S.S., Losic, D., Martin, L.L., Aguilar, M.I., Barrow, C.J., Livett, B.G., and Small, D.H. (2004). Alpha7-nicotinic acetylcholine receptors mediate an Abeta(1-42)-induced increase in the level of acetylcholinesterase in primary cortical neurones. J. Neurochem. *88*, 1186–1193.

Garcia-Ayllon, M.S., Riba-Llena, I., Serra-Basante, C., Alom, J., Boopathy, R., and Saez-Valero, J. (2010). Altered levels of acetylcholinesterase in Alzheimer plasma. PLoS One *5*, e8701.

García-Ayllón, M.-S., Small, D.H., Avila, J., and Sáez-Valero, J. (2011). Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with P-tau and β-amyloid. Front. Mol. Neurosci. *4*, 22.

Ghosal, K., Vogt, D.L., Liang, M., Shen, Y., Lamb, B.T., and Pimplikar, S.W. (2009). Alzheimer's disease-like pathological features in transgenic mice expressing the APP intracellular domain. Proc. Natl. Acad. Sci. U.S.A. *106*, 18367–18372.

Giuffrida, M.L., Tomasello, M.F., Pandini, G., Caraci, F., Battaglia, G., Busceti, C., Di Pietro, P., Pappalardo, G., Attanasio, F., Chiechio, S., et al. (2015). Monomeric ss-amyloid interacts with type-1 insulin-like growth factor receptors to provide energy supply to neurons. Front. Cell. Neurosci. *9*, 297.

Glenner, G.G. and Wong, C.W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. *120*, 885–890.

Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D., and Crowther, R.A. (1989). Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron *3*, 519–526.

Gold, M., Newhouse, P.A., Howard, D., and Kryscio, R.J. (2012). Nicotine treatment of mild cognitive impairment: a 6-month double-blind pilot clinical trial. Neurology *78*, 1895; author reply 1895.

Goldsbury, C., Mocanu, M.M., Thies, E., Kaether, C., Haass, C., Keller, P., Biernat, J., Mandelkow, E., and Mandelkow, E.M. (2006). Inhibition of APP trafficking by tau protein does not increase the generation of amyloid-β peptides. Traffic *7*, 873–888.

Götz, J., Chen, F.V., Van Dorpe, J., and Nitsch, R. (2001). Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ42 fibrils. Science *293*, 1491–1495.

Gotz, J., Streffer, J.R., David, D., Schild, A., Hoerndli, F., Pennanen, L., Kurosinski, P., and Chen, F. (2004). Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. Mol Psychiatry *9*, 664–683.

Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J.P., Haroutunian, V., Buxbaum, J.D., Xu, H., et al. (2000). Intraneuronal Aβ42 accumulation in human brain. Am. J. Pathol. *156*, 15–20.

Grothe, M.J., Heinsen, H., Amaro, E., Jr., Grinberg, L.T., and Teipel, S.J. (2016). Cognitive correlates of basal forebrain atrophy and associated cortical hypometabolism in mild cognitive impairment. Cereb. Cortex *26*, 2411–2426.

Gu, Z. and Yakel, J.L. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. Neuron *71*, 155–165.

Ha, C., Ryu, J., and Park, C.B. (2007). Metal ions differentially influence the aggregation and deposition of Alzheimer's β-amyloid on a solid template. Biochemistry *46*, 6118–6125.

Haass, C. and Selkoe, D.J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat. Rev. Mol. Cell. Biol. *8*, 101–112.

Hampel, H., Mesulam, M.-M., Cuello, A.C., Farlow, M.R., Giacobini, E., Grossberg, G.T., Khachaturian, A.S., Vergallo, A., Cavedo, E., and Snyder, P.J. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain *141*, 1917–1933.

Hampel, H., Mesulam, M.M., Cuello, A.C., Khachaturian, A.S., Vergallo, A., Farlow, M.R., Snyder, P.J., Giacobini, E., and Khachaturian, Z.S. (2019). Revisiting the cholinergic hypothesis in Alzheimer's disease: emerging evidence from translational and clinical research. J. Prev. Alzheimers Dis. *6*, 2–15.

Han, X.-J., Hu, Y.-Y., Yang, Z.-J., Jiang, L.-P., Shi, S.-L., Li, Y.-R., Guo, M.-Y., Wu, H.-L., and Wan, Y.-Y. (2017). Amyloid β-42 induces neuronal apoptosis by targeting mitochondria. Mol. Med. Rep. *16*, 4521–4528.

Hardy, J.A. and Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. Science *256*, 184–185.

Harrison, J.R. and Owen, M.J. (2016). Alzheimer's disease: the amyloid hypothesis on trial. Br. J. Psychiatry *208*, 1–3.

Hasegawa, M. (2016). Molecular mechanisms in the pathogenesis of Alzheimer's disease and tauopathies-prion-like seeded aggregation and phosphorylation. Biomolecules *6*, 24.

Henderson, Z., Matto, N., John, D., Nalivaeva, N.N., and Turner, A.J. (2010). Co-localization of PRiMA with acetylcholinesterase in cholinergic neurons of rat brain: an immunocytochemical study. Brain Res. *1344*, 34–42.

Hensley, K., Hall, N., Subramaniam, R., Cole, P., Harris, M., Aksenov, M., Aksenova, M., Gabbita, S.P., Wu, J.F., Carney, J.M., et al. (1995). Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J. Neurochem. *65*, 2146–2156.

Herrup, K. (2015). The case for rejecting the amyloid cascade hypothesis. Nat. Neurosci. *18*, 794–799.

Hicks, D., John, D., Makova, N.Z., Henderson, Z., Nalivaeva, N.N., and Turner, A.J. (2011). Membrane targeting, shedding and protein interactions of brain acetylcholinesterase. J. Neurochem. *116*, 742–746.

Hoover, B.R., Reed, M.N., Su, J., Penrod, R.D., Kotilinek, L.A., Grant, M.K., Pitstick, R., Carlson, G.A., Lanier, L.M., Yuan, L.-L., et al. (2010). Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. Neuron *68*, 1067–1081.

Huang, Y. and Liu, T. (2015). Amyloid beta peptide 1-42 induces SH-SY5Y cell apoptosis via the promotion of Meg3 long noncoding RNA expression. Integ. Med. Int. *2*, 73–79.

Huang, W.-J., Zhang, X., and Chen, W.-W. (2016). Role of oxidative stress in Alzheimer's disease. Biomed. Rep. *4*, 519–522.

Ikonomovic, M.D., Mufson, E.J., Wuu, J., Bennett, D.A., and DeKosky, S.T. (2005). Reduction of choline acetyltransferase activity in primary visual cortex in mild to moderate Alzheimer's disease. Arch. Neurol. *62*, 425–430.

Ittner, L.M. and Gotz, J. (2011). Amyloid-β and tau – a toxic pas de deux in Alzheimer's disease. Nat. Rev. Neurosci. *12*, 65–72.

Jacobs, H.I.L., Hopkins, D.A., Mayrhofer, H.C., Bruner, E., van Leeuwen, F.W., Raaijmakers, W., and Schmahmann, J.D. (2017). The cerebellum in Alzheimer's disease: evaluating its role in cognitive decline. Brain *141*, 37–47.

Jin, M., Shepardson, N., Yang, T., Chen, G., Walsh, D., and Selkoe, D.J. (2011). Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. Proc. Natl. Acad. Sci. U.S.A. *108*, 5819–5824.

Joachim, C.L., Morris, J.H., and Selkoe, D.J. (1989). Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. Am. J. Pathol. *135*, 309–319.

Johnson, K.A., Schultz, A., Betensky, R.A., Becker, J.A., Sepulcre, J., Rentz, D., Mormino, E., Chhatwal, J., Amariglio, R., Papp, K., et al. (2016). Tau positron emission tomographic imaging in aging and early Alzheimer disease. Ann. Neurol. *79*, 110–119.

Jones, C.K., Brady, A.E., Davis, A.A., Xiang, Z., Bubser, M., Tantawy, M.N., Kane, A.S., Bridges, T.M., Kennedy, J.P., Bradley, S.R., et al. (2008). Novel selective allosteric activator of the M1 muscarinic acetylcholine receptor regulates amyloid processing and produces antipsychotic-like activity in rats. J. Neurosci. *28*, 10422–10433.

Kagan, B.L., Hirakura, Y., Azimov, R., Azimova, R., and Lin, M.-C. (2002). The channel hypothesis of Alzheimer's disease: current status. Peptides *23*, 1311–1315.

Kametani, F. and Hasegawa, M. (2018). Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. Front. Neurosci. *12*, 25.

Kang, J., Lemaire, H.G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature *325*, 733–736.

Kar, S., Issa, A.M., Seto, D., Auld, D.S., Collier, B., and Quirion, R. (1998). Amyloid beta-peptide inhibits high-affinity choline uptake and acetylcholine release in rat hippocampal slices. J. Neurochem. *70*, 2179–2187.

Kerbler, G.M., Fripp, J., Rowe, C.C., Villemagne, V.L., Salvado, O., Rose, S., Coulson, E.J., and Alzheimer's Disease Neuroimaging Initiative. (2014). Basal forebrain atrophy correlates with amyloid β burden in Alzheimer's disease. Neuroimage Clin. *7*, 105–113.

Kim, J.-K., Kim, S.-H., Cho, H.-Y., Shin, H.-S., Sung, H.-R., Jung, J.-R., Quan, M.-L., Jiang, D.-H., and Bae, H.-R. (2010). GD3 accumulation in cell surface lipid rafts prior to mitochondrial targeting contributes to amyloid-β-induced apoptosis. J. Korean Med. Sci. *25*, 1492–1498.

Klunk, W.E., Mathis, C.A., Price, J.C., DeKosky, S.T., Lopresti, B.J., Tsopelas, N.D., Saxton, J.A., and Nebes, R.D. (2009). Amyloid imaging with PET in Alzheimer's disease, mild cognitive impairment, and clinically unimpaired subjects. In: PET in the Evaluation of Alzheimer's Disease and Related Disorders. D. Silverman, ed. (New York, NY: Springer). pp. 119–147.

Knauer, M.F., Soreghan, B., Burdick, D., Kosmoski, J., and Glabe, C.G. (1992). Intracellular accumulation and resistance to degradation of the Alzheimer amyloid A4/beta protein. Proc. Natl. Acad. Sci. U.S.A. *89*, 7437–7441.

Kobayashi, K., Nakano, H., Hayashi, M., Shimazaki, M., Fukutani, Y., Sasaki, K., Sugimori, K., and Koshino, Y. (2003). Association of phosphorylation site of tau protein with neuronal apoptosis in Alzheimer's disease. J. Neurol. Sci. *208*, 17–24.

Koffie, R.M., Hyman, B.T., and Spires-Jones, T.L. (2011). Alzheimer's disease: synapses gone cold. Mol. Neurodegener. *6*, 63.

Konar, A., Gupta, R., Shukla, R.K., Maloney, B., Khanna, V.K., Wadhwa, R., Lahiri, D.K., and Thakur, M.K. (2019). M1 muscarinic receptor is a key target of neuroprotection, neuroregeneration and memory recovery by i-Extract from Withania somnifera. Sci. Rep. *9*, 13990.

Kovac, A., Zilka, N., Kazmerova, Z., Cente, M., Zilkova, M., and Novak, M. (2011). Misfolded truncated protein tau induces innate immune response via MAPK pathway. J. Immunol. *187*, 2732–2739.

Krasemann, S., Madore, C., Cialic, R., Baufeld, C., Calcagno, N., El Fatimy, R., Beckers, L., O'Loughlin, E., Xu, Y., and Fanek, Z. (2017). The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity *47*, 566–581.e569.

Kummer, M.P. and Heneka, M.T. (2014). Truncated and modified amyloid-beta species. Alzheimers Res. Ther. *6*, 28.

LaFerla, F.M., Green, K.N., and Oddo, S. (2007). Intracellular amyloid-beta in Alzheimer's disease. Nat. Rev. Neurosci. *8*, 499–509.

Laird, F.M., Cai, H., Savonenko, A.V., Farah, M.H., He, K., Melnikova, T., Wen, H., Chiang, H.C., Xu, G., Koliatsos, V.E., et al. (2005). BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions. J. Neurosci. *25*, 11693–11709.

Lasala, M., Fabiani, C., Corradi, J., Antollini, S., and Bouzat, C. (2019). Molecular modulation of human alpha7 nicotinic receptor by amyloid-beta peptides. Front. Cell. Neurosci. *13*, 37.

Layer, P.G. (1995). Nonclassical roles of cholinesterases in the embryonic brain and possible links to Alzheimer disease. Alzheimer Dis. Assoc. Disord. *9*(Suppl 2), 29–36.

Lebois, E.P., Thorn, C., Edgerton, J.R., Popiolek, M., and Xi, S. (2018). Muscarinic receptor subtype distribution in the central nervous system and relevance to aging and Alzheimer's disease. Neuropharmacology *136*, 362–373.

Lewis, J., Dickson, D.W., Lin, W.-L., Chisholm, L., Corral, A., Jones, G., Yen, S.-H., Sahara, N., Skipper, L., and Yager, D. (2001). Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science *293*, 1487–1491.

Leyns, C.E., Ulrich, J.D., Finn, M.B., Stewart, F.R., Koscal, L.J., Serrano, J.R., Robinson, G.O., Anderson, E., Colonna, M., and Holtzman, D.M. (2017). TREM2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. Proc. Natl. Acad. Sci. U.S.A. *114*, 11524–11529.

Li, S., Hong, S., Shepardson, N.E., Walsh, D.M., Shankar, G.M., and Selkoe, D. (2009). Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron *62*, 788–801.

Li, C., Ebrahimi, A., and Schluesener, H. (2013). Drug pipeline in neurodegeneration based on transgenic mice models of Alzheimer's disease. Ageing Res. Rev. *12*, 116–140.

Li, X.C., Hu, Y., Wang, Z.H., Luo, Y., Zhang, Y., Liu, X.P., Feng, Q., Wang, Q., Ye, K., Liu, G.P., et al. (2016). Human wild-type full-length tau accumulation disrupts mitochondrial dynamics and the functions via increasing mitofusins. Sci Rep. *6*, 24756.

Liu, L., Drouet, V., Wu, J.W., Witter, M.P., Small, S.A., Clelland, C., and Duff, K. (2012a). Trans-synaptic spread of tau pathology *in vivo*. PLoS One *7*, e31302.

Liu, S., Liu, Y., Hao, W., Wolf, L., Kiliaan, A.J., Penke, B., Rube, C.E., Walter, J., Heneka, M.T., Hartmann, T., et al. (2012b). TLR2 is a primary receptor for Alzheimer's amyloid beta peptide to trigger neuroinflammatory activation. J. Immunol. *188*, 1098–1107.

Liu, Q., Xie, X., Emadi, S., Sierks, M.R., and Wu, J. (2015). A novel nicotinic mechanism underlies β-amyloid-induced neurotoxicity. Neuropharmacology *97*, 457–463.

Liu, J., Yang, B., Ke, J., Li, W., and Suen, W.C. (2016). Antibody-based drugs and approaches against amyloid-β species for Alzheimer's disease immunotherapy. Drugs Aging *33*, 685–697.

Loo, D.T., Copani, A., Pike, C.J., Whittemore, E.R., Walencewicz, A.J., and Cotman, C.W. (1993). Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. Proc. Nat. Acad. Sci. U.S.A. *90*, 7951–7955.

Lue, L.F., Kuo, Y.M., Roher, A.E., Brachova, L., Shen, Y., Sue, L., Beach, T., Kurth, J.H., Rydel, R.E., and Rogers, J. (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. *155*, 853–862.

Majdi, A., Mahmoudi, J., Sadigh-Eteghad, S., Golzari, S.E., Sabermarouf, B., and Reyhani-Rad, S. (2016). Permissive role of cytosolic pH acidification in neurodegeneration: a closer look at its causes and consequences. J. Neurosci. Res. *94*, 879–887.

Majdi, A., Kamari, F., Vafaee, M.S., and Sadigh-Eteghad, S. (2017). Revisiting nicotine's role in the ageing brain and cognitive impairment. Rev. Neurosci. *28*, 767–781.

Majdi, A., Kamari, F., Sadigh-Eteghad, S., and Gjedde, A. (2018). Molecular insights into memory-enhancing metabolites of nicotine in brain: a systematic review. Front. Neurosci. *12*, 1002.

Makin, S. (2018). The amyloid hypothesis on trial. Nature. *559*, S4.

Manczak, M. and Reddy, P.H. (2012). Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease. Hum. Mol. Genet. *21*, 5131–5146.

Maruyama, M., Shimada, H., Suhara, T., Shinotoh, H., Ji, B., Maeda, J., Zhang, M.R., Trojanowski, J.Q., Lee, V.M., Ono, M., et al. (2013). Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. Neuron *79*, 1094–1108.

Mena, R., Edwards, P.C., Harrington, C.R., Mukaetova-Ladinska, E.B., and Wischik, C.M. (1996). Staging the pathological assembly of truncated tau protein into paired helical filaments in Alzheimer's disease. Acta Neuropathol. *91*, 633–641.

Mesulam, M. (2012). Cholinergic aspects of aging and Alzheimer's disease. Biol. Psychiatry *71*, 760–761.

Mesulam, M. (2004). The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? Learn Mem. *11*, 43–49.

Mesulam, M., Shaw, P., Mash, D., and Weintraub, S. (2004). Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. Ann. Neurol. *55*, 815–828.

Meyer, P.F., Tremblay-Mercier, J., Leoutsakos, J., Madjar, C., Lafaille-Maignan, M.E., Savard, M., Rosa-Neto, P., Poirier, J., Etienne, P., and Breitner, J. (2019). INTREPAD: a randomized trial of naproxen to slow progress of presymptomatic Alzheimer disease. Neurology *92*, e2070–e2080.

Miners, J.S., Baig, S., Tayler, H., Kehoe, P.G., and Love, S. (2009). Neprilysin and insulin-degrading enzyme levels are increased in Alzheimer disease in relation to disease severity. J. Neuropathol. Exp. Neurol. *68*, 902–914.

Moreira, P.I., Carvalho, C., Zhu, X., Smith, M.A., and Perry, G. (2010). Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. Biochim. Biophys. Acta *1802*, 2–10.

Morishima, Y., Gotoh, Y., Zieg, J., Barrett, T., Takano, H., Flavell, R., Davis, R.J., Shirasaki, Y., and Greenberg, M.E. (2001). β-Amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. J. Neurosci. *21*, 7551–7560.

Morris, G.P., Clark, I.A., and Vissel, B. (2014). Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer's disease. Acta Neuropathol. Commun. *2*, 135.

Mucke, L. and Selkoe, D.J. (2012). Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb. Perspect. Med. *2*, a006338.

Mucke, L., Masliah, E., Yu, G.Q., Mallory, M., Rockenstein, E.M., Tatsuno, G., Hu, K., Kholodenko, D., Johnson-Wood, K., and McConlogue, L. (2000). High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J. Neurosci. *20*, 4050–4058.

Mudher, A. and Lovestone, S. (2002). Alzheimer's disease – do tauists and baptists finally shake hands? Trends Neurosci. *25*, 22–26.

Mullane, K. and Williams, M. (2018). Alzheimer's disease (AD) therapeutics – 1: repeated clinical failures continue to question the amyloid hypothesis of AD and the current understanding of AD causality. Biochem. Pharmacol. *158*, 359–375.

Mullard, A. (2016). Alzheimer amyloid hypothesis lives on. Nat. Rev. Drug Discov. *16*, 3–5.

Nagele, R.G., D'Andrea, M.R., Anderson, W.J., and Wang, H.Y. (2002). Intracellular accumulation of β-amyloid1–42 in neurons is facilitated by the α7 nicotinic acetylcholine receptor in Alzheimer's disease. Neuroscience *110*, 199–211.

Nagele, R.G., D'Andrea, M.R., Lee, H., Venkataraman, V., and Wang, H.Y. (2003). Astrocytes accumulate A beta 42 and give rise to astrocytic amyloid plaques in Alzheimer disease brains. Brain Res. *971*, 197–209.

Nagy, Z., Esiri, M., Jobst, K., Morris, J., King, E.-F., McDonald, B., Litchfield, S., Smith, A., Barnetson, L., and Smith, A. (1995). Relative roles of plaques and tangles in the dementia of Alzheimer's disease: correlations using three sets of neuropathological criteria. Dement. Geriatr. Cogn. Disord. *6*, 21–31.

Nalivaeva, N.N., Beckett, C., Belyaev, N.D., and Turner, A.J. (2012). Are amyloid-degrading enzymes viable therapeutic targets in Alzheimer's disease? J. Neurochem. *120*(Suppl 1), 167–185.

Naslund, J., Haroutunian, V., Mohs, R., Davis, K.L., Davies, P., Greengard, P., and Buxbaum, J.D. (2000). Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. JAMA Neurol. *283*, 1571–1577.

Nitsch, R.M., Slack, B.E., Wurtman, R.J., and Growdon, J.H. (1992). Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. Science *258*, 304–307.

Nitsch, R.M., Deng, M., Tennis, M., Schoenfeld, D., and Growdon, J.H. (2000). The selective muscarinic M1 agonist AF102B decreases levels of total Abeta in cerebrospinal fluid of patients with Alzheimer's disease. Ann. Neurol. *48*, 913–918.

Nordberg, A. (2001). Nicotinic receptor abnormalities of Alzheimer's disease: therapeutic implications. Biol. Psychiatry *49*, 200–210.

Nunes-Tavares, N., Santos, L.E., Stutz, B., Brito-Moreira, J., Klein, W.L., Ferreira, S.T., and de Mello, F.G. (2012). Inhibition of choline acetyltransferase as a mechanism for cholinergic dysfunction induced by amyloid-β peptide oligomers. J. Biol. Chem. *287*, 19377–19385.

Nyakas, C., Granic, I., Halmy, L.G., Banerjee, P., and Luiten, P.G.M. (2011). The basal forebrain cholinergic system in aging and dementia. Rescuing cholinergic neurons from neurotoxic amyloid-β42 with memantine. Behav. Brain Res. *221*, 594–603.

Pallo, S.P., DiMaio, J., Cook, A., Nilsson, B., and Johnson, G.V.W. (2016). Mechanisms of tau and Aβ-induced excitotoxicity. Brain Res. *1634*, 119–131.

Pandya, A. and Yakel, J.L. (2011). Allosteric modulator Desformylflustrabromine relieves the inhibition of α2β2 and α4β2 nicotinic acetylcholine receptors by β-amyloid(1-42) peptide. J. Mol. Neurosci. *45*, 42–47.

Paresce, D.M., Ghosh, R.N., and Maxfield, F.R. (1996). Microglial cells internalize aggregates of the Alzheimer's disease amyloid β-protein via a scavenger receptor. Neuron. *17*, 553–565.

Parikh, V., Bernard, C.S., Naughton, S.X., and Yegla, B. (2014). Interactions between Aβ oligomers and presynaptic cholinergic signaling: age-dependent effects on attentional capacities. Behav. Brain Res. *274*, 30–42.

Parri, H.R., Hernandez, C.M., and Dineley, K.T. (2011). Research update: α7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. Biochem. Pharmacol. *82*, 931–942.

Patterson, K.R., Remmers, C., Fu, Y., Brooker, S., Kanaan, N.M., Vana, L., Ward, S., Reyes, J.F., Philibert, K., Glucksman, M.J., et al. (2011). Characterization of prefibrillar tau oligomers *in vitro* and in Alzheimer disease. J. Biol. Chem. *286*, 23063–23076.

Pavia, J., Alberch, J., Alvarez, I., Toledano, A., and de Ceballos, M.L. (2000). Repeated intracerebroventricular administration of β-amyloid(25-35) to rats decreases muscarinic receptors in cerebral cortex. Neurosci. Lett. *278*, 69–72.

Pera, M., Alcolea, D., Sanchez-Valle, R., Guardia-Laguarta, C., Colom-Cadena, M., Badiola, N., Suarez-Calvet, M., Llado, A., Barrera-Ocampo, A.A., Sepulveda-Falla, D., et al. (2013). Distinct patterns of APP processing in the CNS in autosomal-dominant and sporadic Alzheimer disease. Acta Neuropathol. *125*, 201–213.

Pettit, D.L., Shao, Z., and Yakel, J.L. (2001). β-Amyloid(1-42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. J. Neurosci. *21*, Rc120.

Price, J.L., Davis, P.B., Morris, J.C., and White, D.L. (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. Neurobiol. Aging *12*, 295–312.

Puzzo, D., Privitera, L., Leznik, E., Fa, M., Staniszewski, A., Palmeri, A., and Arancio, O. (2008). Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. J. Neurosci. *28*, 14537–14545.

Puzzo, D., Lee, L., Palmeri, A., Calabrese, G., and Arancio, O. (2014). Behavioral assays with mouse models of Alzheimer's disease: practical considerations and guidelines. Biochem. Pharmacol. *88*, 450–467.

Rees, T., Hammond, P.I., Soreq, H., Younkin, S., and Brimijoin, S. (2003). Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex. Neurobiol. Aging *24*, 777–787.

Relkin, N.R., Szabo, P., Adamiak, B., Burgut, T., Monthe, C., Lent, R.W., Younkin, S., Younkin, L., Schiff, R., and Weksler, M.E. (2009). 18-Month study of intravenous immunoglobulin for treatment of mild Alzheimer disease. Neurobiol. Aging *30*, 1728–1736.

Ricciarelli, R. and Fedele, E. (2017). The amyloid cascade hypothesis in Alzheimer's disease: it's time to change our mind. Curr. Neuropharmacol. *15*, 926–935.

Roberson, E.D., Scearce-Levie, K., Palop, J.J., Yan, F., Cheng, I.H., Wu, T., Gerstein, H., Yu, G.Q., and Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. Science *316*, 750–754.

Rodrigues, E.M., Weissmiller, A.M., and Goldstein, L.S. (2012). Enhanced β-secretase processing alters APP axonal transport and leads to axonal defects. Hum. Mol. Genet. *21*, 4587–4601.

Sadigh-Eteghad, S., Talebi, M., Farhoudi, M., Golzari, S.E.J., Sabermarouf, B., and Mahmoudi, J. (2014). Beta-amyloid exhibits antagonistic effects on alpha 7 nicotinic acetylcholine receptors in orchestrated manner. J. Med. Hypotheses Ideas *8*, 49–52.

Sadigh-Eteghad, S., Majdi, A., Talebi, M., Mahmoudi, J., and Babri, S. (2015a). Regulation of nicotinic acetylcholine receptors in Alzheimers disease: a possible role of chaperones. Eur. J. Pharmacol. *755*, 34–41.

Sadigh-Eteghad, S., Sabermarouf, B., Majdi, A., Talebi, M., Farhoudi, M., and Mahmoudi, J. (2015b). Amyloid-β: a crucial factor in Alzheimer's disease. Med. Princ. Pract. *24*, 1–10.

Sadigh-Eteghad, S., Majdi, A., Mahmoudi, J., Golzari, S.E.J., and Talebi, M. (2016). Astrocytic and microglial nicotinic acetylcholine receptors: an overlooked issue in Alzheimer's disease. J. Neural. Transm. (Vienna) *123*, 1359–1367.

Saez-Valero, J., Sberna, G., McLean, C.A., and Small, D.H. (1999). Molecular isoform distribution and glycosylation of acetylcholinesterase are altered in brain and cerebrospinal fluid of patients with Alzheimer's disease. J. Neurochem. *72*, 1600–1608.

Saez-Valero, J., Barquero, M.S., Marcos, A., McLean, C.A., and Small, D.H. (2000). Altered glycosylation of acetylcholinesterase in lumbar cerebrospinal fluid of patients with Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry *69*, 664–667.

Salminen, A., Ojala, J., Kauppinen, A., Kaarniranta, K., and Suuronen, T. (2009). Inflammation in Alzheimer's disease: amyloid-β oligomers trigger innate immunity defence via pattern recognition receptors. Prog. Neurobiol. *87*, 181–194.

Sberna, G., Saez-Valero, J., Beyreuther, K., Masters, C.L., and Small, D.H. (1997). The amyloid beta-protein of Alzheimer's disease increases acetylcholinesterase expression by increasing intracellular calcium in embryonal carcinoma P19 cells. J. Neurochem. *69*, 1177–1184.

Schonheit, B., Zarski, R., and Ohm, T.G. (2004). Spatial and temporal relationships between plaques and tangles in Alzheimer-pathology. Neurobiol. Aging *25*, 697–711.

Selkoe, D.J. (2002). Alzheimer's disease is a synaptic failure. Science *298*, 789–791.

Selkoe, D.J. and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol. Med. *8*, 595–608.

Shankar, G.M., Bloodgood, B.L., Townsend, M., Walsh, D.M., Selkoe, D.J., and Sabatini, B.L. (2007). Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. J. Neurosci. *27*, 2866–2875.

Shi, Y., Yamada, K., Liddelow, S.A., Smith, S.T., Zhao, L., Luo, W., Tsai, R.M., Spina, S., Grinberg, L.T., and Rojas, J.C. (2017). ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature *549*, 523.

Silveyra, M.X., Garcia-Ayllon, M.S., de Barreda, E.G., Small, D.H., Martinez, S., Avila, J., and Saez-Valero, J. (2012). Altered expression of brain acetylcholinesterase in FTDP-17 human tau transgenic mice. Neurobiol. Aging *33*, 624.e623–634.

Simic, G., Babic Leko, M., Wray, S., Harrington, C., Delalle, I., Jovanov-Milosevic, N., Bazadona, D., Buee, L., de Silva, R., Di Giovanni, G., et al. (2016). Tau protein hyperphosphorylation and aggregation in Alzheimer's disease and other tauopathies, and possible neuroprotective strategies. Biomolecules *6*, 6.

Simon, D., Medina, M., Avila, J., and Wandosell, F. (2011). Overcoming cell death and tau phosphorylation mediated by PI3K-inhibition: a cell assay to measure neuroprotection. CNS Neurol. Disord. Drug Targets *10*, 208–214.

Šišková, Z., Justus, D., Kaneko, H., Friedrichs, D., Henneberg, N., Beutel, T., Pitsch, J., Schoch, S., Becker, A., von der Kammer, H., et al. (2014). Dendritic structural degeneration is functionally linked to cellular hyperexcitability in a mouse model of Alzheimer's disease. Neuron *84*, 1023–1033.

Sivaprakasam, K. (2006). Towards a unifying hypothesis of Alzheimer's disease: cholinergic system linked to plaques, tangles and neuroinflammation. Curr. Med. Chem. *13*, 2179–2188.

Söllvander, S., Nikitidou, E., Brolin, R., Söderberg, L., Sehlin, D., Lannfelt, L., and Erlandsson, A. (2016). Accumulation of amyloid-β by astrocytes result in enlarged endosomes and microvesicle-induced apoptosis of neurons. Mol. Neurodegener. *11*, 38.

Spence, E.F. and Soderling, S.H. (2015). Actin out: regulation of the synaptic cytoskeleton. J. Biol. Chem. *290*, 28613–28622.

Stamer, K., Vogel, R., Thies, E., Mandelkow, E., and Mandelkow, E.M. (2002). Tau blocks traffic of organelles, neurofilaments,

and APP vesicles in neurons and enhances oxidative stress. J. Cell. Biol. *156*, 1051–1063.

Su, X.Y., Wu, W.H., Huang, Z.P., Hu, J., Lei, P., Yu, C.H., Zhao, Y.F., and Li, Y.M. (2007). Hydrogen peroxide can be generated by tau in the presence of Cu(II). Biochem. Biophys. Res. Commun. *358*, 661–665.

Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K., and Kling, A. (1986). Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. N. Engl. J. Med. *315*, 1241–1245.

Svedruzic, Z.M., Popovic, K., and Sendula-Jengic, V. (2015). Decrease in catalytic capacity of gamma-secretase can facilitate pathogenesis in sporadic and familial Alzheimer's disease. Mol. Cell. Neurosci. *67*, 55–65.

Szpankowski, L., Encalada, S.E., and Goldstein, L.S. (2012). Subpixel colocalization reveals amyloid precursor protein-dependent kinesin-1 and dynein association with axonal vesicles. Proc. Natl. Acad. Sci. U.S.A. *109*, 8582–8587.

Tackenberg, C. and Brandt, R. (2009). Divergent pathways mediate spine alterations and cell death induced by amyloid-beta, wild-type tau, and R406W tau. J. Neurosci. *29*, 14439–14450.

Takahashi, M., Miyata, H., Kametani, F., Nonaka, T., Akiyama, H., Hisanaga, S.-I., and Hasegawa, M. (2015). Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. Acta Neuropathol. *129*, 895–907.

Takahashi, R.H., Nagao, T., and Gouras, G.K. (2017). Plaque formation and the intraneuronal accumulation of beta-amyloid in Alzheimer's disease. Pathol. Int. *67*, 185–193.

Tamagno, E., Guglielmotto, M., Monteleone, D., Manassero, G., Vasciaveo, V., and Tabaton, M. (2018). The unexpected role of Aβ1-42 monomers in the pathogenesis of Alzheimer's disease. J. Alzheimers Dis. *62*, 1241–1245.

Tamayev, R., Matsuda, S., Arancio, O., and D'Adamio, L. (2012). β- but not γ-secretase proteolysis of APP causes synaptic and memory deficits in a mouse model of dementia. EMBO Mol. Med. *4*, 171–179.

Tapia-Rojas, C. and Inestrosa, N.C. (2018). Loss of canonical Wnt signaling is involved in the pathogenesis of Alzheimer's disease. Neural. Regen. Res. *13*, 1705–1710.

Toiber, D., Berson, A., Greenberg, D., Melamed-Book, N., Diamant, S., and Soreq, H. (2008). N-acetylcholinesterase-induced apoptosis in Alzheimer's disease. PLoS One *3*, e3108.

Tramutola, A., Triplett, J.C., Di Domenico, F., Niedowicz, D.M., Murphy, M.P., Coccia, R., Perluigi, M., and Butterfield, D.A. (2015). Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD. J. Neurochem. *133*, 739–749.

Tsang, S.W., Lai, M.K., Kirvell, S., Francis, P.T., Esiri, M.M., Hope, T., Chen, C.P., and Wong, P.T. (2006). Impaired coupling of muscarinic M1 receptors to G-proteins in the neocortex is associated with severity of dementia in Alzheimer's disease. Neurobiol. Aging *27*, 1216–1223.

Ulland, T.K., Song, W.M., Huang, S.C.-C., Ulrich, J.D., Sergushichev, A., Beatty, W.L., Loboda, A.A., Zhou, Y., Cairns, N.J., and Kambal, A. (2017). TREM2 maintains microglial metabolic fitness in Alzheimer's disease. Cell *170*, 649–663.e613.

van der Kant, R. and Goldstein, L.S.B. (2015). Cellular functions of the amyloid precursor protein from development to dementia. Dev. Cell. *32*, 502–515.

Villemagne, V.L., Pike, K.E., Chetelat, G., Ellis, K.A., Mulligan, R.S., Bourgeat, P., Ackermann, U., Jones, G., Szoeke, C., Salvado, O., et al. (2011). Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. Ann. Neurol. *69*, 181–192.

Wang, H.Y., Lee, D.H., D'Andrea, M.R., Peterson, P.A., Shank, R.P., and Reitz, A.B. (2000). β-Amyloid(1-42) binds to α7 nicotinic acetylcholine receptor with high affinity. Implications for Alzheimer's disease pathology. J. Biol. Chem. *275*, 5626–5632.

Wang, Z.X., Tan, L., and Yu, J.T. (2015). Axonal transport defects in Alzheimer's disease. Mol. Neurobiol. *51*, 1309–1321.

Weiner, M.W., Veitch, D.P., Aisen, P.S., Beckett, L.A., Cairns, N.J., Green, R.C., Harvey, D., Jack, C.R., Jagust, W., Liu, E., et al. (2013). The Alzheimer's Disease Neuroimaging Initiative: a review of papers published since its inception. Alzheimers Dement. *9*, e111–e194.

Welt, T., Kulic, L., Hoey, S.E., McAfoose, J., Spani, C., Chadha, A.S., Fisher, A., and Nitsch, R.M. (2015). Acute effects of muscarinic M1 receptor modulation on AβPP metabolism and amyloid-β levels *in vivo:* a microdialysis study. J. Alzheimers Dis. *46*, 971–982.

Wevers, A., Monteggia, L., Nowacki, S., Bloch, W., Schutz, U., Lindstrom, J., Pereira, E.F., Eisenberg, H., Giacobini, E., de Vos, R.A., et al. (1999). Expression of nicotinic acetylcholine receptor subunits in the cerebral cortex in Alzheimer's disease: histotopographical correlation with amyloid plaques and hyperphosphorylated-tau protein. Eur. J. Neurosci. *11*, 2551–2565.

White, H.K. and Levin, E.D. (1999). Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. Psychopharmacology (Berl.) *143*, 158–165.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., and Delon, M.R. (1982). Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. Science *215*, 1237–1239.

Wilcock, G.K., Esiri, M.M., Bowen, D.M., and Smith, C.C. (1982). Alzheimer's disease. Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. J. Neurol. Sci. *57*, 407–417.

Wilson, A.L., Langley, L.K., Monley, J., Bauer, T., Rottunda, S., McFalls, E., Kovera, C., and McCarten, J.R. (1995). Nicotine patches in Alzheimer's disease: pilot study on learning, memory, and safety. Pharmacol. Biochem. Behav. *51*, 509–514.

Wischik, C.M., Staff, R.T., Wischik, D.J., Bentham, P., Murray, A.D., Storey, J.M., Kook, K.A., and Harrington, C.R. (2015). Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. J. Alzheimers. Dis. *44*, 705–720.

Xu, H., Gelyana, E., Rajsombath, M., Yang, T., Li, S., and Selkoe, D. (2016). Environmental enrichment potently prevents microglia-mediated neuroinflammation by human amyloid β-protein oligomers. J. Neurosci. *36*, 9041–9056.

Yamaguchi, Y. and Kawashima, S. (2001). Effects of amyloid-β-(25–35) on passive avoidance, radial-arm maze learning and choline acetyltransferase activity in the rat. Eur. J. Pharmacol. *412*, 265–272.

Yoon, S.-S. and Jo, S.A. (2012). Mechanisms of amyloid-β peptide clearance: potential therapeutic targets for Alzheimer's disease. Biomol. Ther. *20*, 245–255.

Zempel, H. and Mandelkow, E. (2014). Lost after translation: missorting of tau protein and consequences for Alzheimer disease. Trends Neurosci. *37*, 721–732.

Zhao, L.-X., Chen, M.-W., Qian, Y., Yang, Q.-H., Ge, Y.-H., Chen, H.-Z., and Qiu, Y. (2019). M1 muscarinic receptor activation rescues β-amyloid-induced cognitive impairment through AMPA receptor GluA1 subunit. Neuroscience *408*, 239–247.

Zilka, N., Kazmerova, Z., Jadhav, S., Neradil, P., Madari, A., Obetkova, D., Bugos, O., and Novak, M. (2012). Who fans the flames of Alzheimer's disease brains? Misfolded tau on the crossroad of neurodegenerative and inflammatory pathways. J. Neuroinflamm. *9*, 47.

Zuchner, T., Perez-Polo, J.R., and Schliebs, R. (2004). Beta-secretase BACE1 is differentially controlled through muscarinic acetylcholine receptor signaling. J. Neurosci. Res. *77*, 250–257.