Initiation and spreading of Tau pathology: is β-Amyloid the only key?

Inauguraldissertation

Zur Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

Florence Clavaguera

Aus Frankreich

Basel, 2009

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag von

Prof. Dr. Markus Rüegg
Prof. Dr. med. Markus Tolnay
Dr. Matthias Staufenbiel

Basel, den 2. Juli 2008

Prof. Dr. Hans-Peter Hauri
Dekan der Philosophisch-Naturwissenschaftlichen Fakultät

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

First and foremost I wish to acknowledge my supervisor Professor Markus Tolnay for providing me the opportunity to carry out my PhD in his laboratory and for all his guidance and support during my doctoral work as well as for his humanity and understanding. I would also like to thank Professor Markus Rüegg and Dr. Matthias Staufenbiel for their participation in my dissertation and for their academic support. Furthermore, I would like to express my gratitude to Professor Michael Mihatsch for hosting my work at the Institute of Pathology. My sincere thanks also go to Professor Mathias Jucker for advising me as PhD student and for hosting me in his team during his time in Basel. I also want to convey my deepest thanks to Dr. Anne Stalder for her support from the very beginning, her advice and her generosity.

From the Neuropathology laboratory, Sabine Ipsen, Myriam Vonlanthen, Bea Maurer, Edith Hui Bon Hoa, Sabina Weingärtner and Markus Bänziger are especially acknowledged for their immense help with cutting and staining thousands of tissue sections. I am grateful to Emeritus Professor Alphonse Probst for his enthusiasm towards my research project, his intellectual input and his psychological support. Many thanks are due to PD Dr. Stephan Frank for his kindness and advice as well as for proofreading the manuscript. In addition, I would like to acknowledge people from the animal facility, especially Katharina Meyer-von-Bergen for her competence and availability.

I would like to express my thanks to Dorothee Abramowski from the research group of Matthias Staufenbiel, for her friendship and constant professional collaboration. Likewise, I am indebted to Dr. Kumlesh Dev, Maribel Osinde, Rachel May-Nass, and Professor Michel Goedert for their generous collaborative support. I would like to thank my colleagues from the Jucker lab with whom I enjoyed the first year here in Basel: Dr. Melanie Meyer-Luehmann, Dr. Martin Hertzig, Dr. Luca Bondolfi, Stephan Käser, Esther Kohler, Dr. Sonia Boncristiano, and Dr. Florian Ermini. Dr. Tristan Bolmont also deserves special thanks for having me taught a lot for my project and having accompanied my first steps in Alsace.

All my thoughts are for Janaky Coomaraswamy with whom I shared the first moments in Basel and whom I miss a lot. My warmest thanks are due to Caroline Bourgau, Michelle

Larson, Virginie Rhein and my friends in Alsace, in particular Nadine, Alex, Claude, Steph, Georges and Béa. I wish to thank all my friends from the South of France despite the long distance between us, especially Béné, Aude, Greg and Sophie.

My sincere gratitude goes to my parents, my sister Fanny, Cristel, my grandmother and my whole family for their support and encouragement.

Last but not least, because they were integral part of all the studies presented herein, thanks and respect are due to the mice.

Finally, I am forever indebted to Fred for following me in this adventure, without him none of this would have been possible.

I wish to dedicate this work to the memory of my grandparents, Lucette and François Clavaguera.

SUMMARY

Neurodegenerative diseases associated with dementia affect 5-10 % of individuals over the age of 65 in the Western world and represent one of the main health-related socio-economic burdens. The most common form of dementia, Alzheimer's disease (AD), affects 98000 people in Switzerland, with 23000 newly diagnosed cases each year additionally underscoring the importance of understanding its pathogenesis with the ultimate goal of developing efficient therapeutic strategies.

Neuropathologically, AD is characterized by the extracellular deposition of Amyloid-β peptide (Aβ) and the intracellular aggregation of hyperphosphorylated Tau protein. Early-onset AD, which accounts for only 6%-7% of all AD cases, is generally defined as occurring before the age of 60 and is due to hereditary mutations in genes that promote the deposition of Aβ. Based on these familial AD cases (FAD), the 'Amyloid cascade' hypothesis was formulated, postulating that deposition of Aβ, which triggers subsequent Tau pathology, is at the heart of AD pathogenesis. However, somewhat contradictory to this hypothesis, abundant filamentous Tau deposits and neuronal damage can also occur in the absence of Aβ pathology and are in fact key features of a heterogeneous group of neurodegenerative disorders termed 'tauopathies'. In the late nineties, the discovery of multiple mutations in the *Tau* gene underlying a particular condition called *frontotemporal dementia with parkinsonism linked to chromosome 17* (FTDP-17) provided direct evidence that mutant Tau dysfunction *per se* is sufficient to cause neurodegeneration. Of note, in AD and many other tauopathies, Tau pathology spreads intracerebrally following a stereotypical temporo-spatial pattern that correlates well with the cognitive impairment of affected patients. Intriguingly, however, the mechanisms by which Tau pathology initiates and spreads within the brain remain largely unknown so far.

The present research project therefore focused on the experimental induction and the spreading of Tau pathology. Taking advantage of several transgenic mouse models that exhibit neuropathological characteristics of AD and tauopathies, we aimed to elucidate the relative contributions of Aβ and Tau, respectively, to disease pathogenesis.

In particular, we employed the APP23 mouse model where a mutated human amyloid precursor protein (APP), the so-called Swedish mutation, is transgenically expressed. These mice develop Aβ plaques at 6 months of age in association with the typical AD-associated pathology including cerebral amyloid angiopathy (CAA), neuron loss, glial activation, and cognitive impairment.

Moreover, we also studied various Tau transgenic mouse models including the JNPL3 strain, which harbours the human FTDP-17-associated P301L Tau mutation and which develops NFTs by the age of 6.5 months. We backcrossed this line to the C57Bl/6J background (B6/P301L). B6/P301L heterozygous females developed a delayed filamentous Tau pathology, occurring at around 17 months of age, which might represent a particularly suitable animal model to experimentally modulate the onset of tauopathy. Surprisingly, heterozygous B6/P301L male mice developed only little Tau pathology by the age of 26 months, restricted to the spinal cord and the brain stem.

In addition, we took advantage of the P301S mouse line transgenically expressing the FTDP-17-linked P301S mutation. These mice develop abundant filaments made of hyperphosphorylated Tau protein and become severely paralyzed by 5-6 months of age due to a high load of fibrillar Tau pathology in the brain stem and spinal cord. Finally, we used the ALZ17 mouse model which transgenically expresses the human wild-type Tau protein. These mice develop abnormal Tau hyperphosphorylation in the absence of Tau filaments or neurodegeneration, and therefore lend themselves as so-called 'pre-tangle' mice. This model is of particular interest for studying the pathological steps leading to Tau fibrils formation.

The first part of the experimental studies was centred on the *in vivo* relationship between Aβ and Tau pathologies. In our initial study, we used lentivirus technology for its ability to infect nondividing cells and to stably maintain long-term transgene expression in specific brain regions. Lentivirus-mediated expression of the mutated human Tau protein (LV-hTauP301S) was assessed in the brains of both wild-type and APP23 mice. Injection of LV-hTauP301S into the hippocampus of wild-type as well as APP23 mice led to strong and stable transgene expression for more than one year. In addition, hyperphosphorylation of Tau was induced in both mouse lines as early as 3 months post-

injection, while no Tau aggregation was observed in wild-type animals up to 13 months of mutated Tau transgene expression. In contrast, APP23 mice injected with LV-hTauP301S developed Gallyas-positive neurons indicative of the formation of Tau filaments. We concluded from these findings that Tau aggregation can only be induced in an environment rich in Aβ deposits, also supporting the notion that APP and/or Aβ promote Tau pathology.

In another set of experiments, we tested alternative approaches to study the induction of Tau pathology. In particular, we performed a series of cortical as well as intrahippocampal injections of brain extracts bearing Aβ pathology and/or Tau pathology into young B6/P301L animals. Aβ extracts were prepared from old APP23 mice whereas Tau extracts were derived from aged B6/P301L animals. Brain extracts from human AD patients (containing both Aβ and Tau pathology) were also injected into P301L/B6 mice. Six months after infusion with Aβ-containing extracts, we found an induction of Tau pathology not only in the injected hippocampus but also in the entorhinal cortex and amygdala. In contrast, intracerebral injection of Tau-containing extracts produced only limited Tau deposition. In parallel to these brain extract injection experiments, a breeding approach was used to study the relationship between Aβ and Tau pathology by mating B6/P301L with APP23 transgenic mice. Significantly, double-transgenic mice developed increased fibrillar Tau pathology when compared to single B6/P301L-transgenic mice, especially in areas with Aβ deposition. Collectively, these results demonstrated that both injection of Aβ-containing extract and deposition of Aβ fibrils can induce intracellular aggregation of Tau.

Finally, we investigated the Aβ-independent induction of tauopathy by injecting murine P301S brainstem extract rich in Tau filaments into the cortex and hippocampus of young ALZ17 mice expressing wild-type human Tau. We observed filamentous Tau pathology as early as six months after injection with fibrillar Tau induction occurring not only in neurons but also in oligodendrocytes. Strikingly, filamentous Tau was not restricted to injection sites but also stereotypically developed in discrete brains regions over time, a finding which – for the first time – clearly demonstrated the transmission and spreading of filamentous Tau pathology *in vivo*.

Altogether, the studies comprised by the present thesis aim to contribute to a better understanding of Tau pathology induction and its pathogenic relation to Aβ. We have not only demonstrated that Aβ deposits are able to potentiate Tau pathology but also that pathological Tau *per se* can induce Tau aggregation. The latter observation may provide the basis for future studies of the transmissibility of Tau pathology with special regard to potential similarities and differences in comparison to classical prion diseases.

1. INTRODUCTION

1.1. Tau protein

In 1975, Weingarten et al. isolated a factor that induces microtubule assembly and called it Tau for its ability to promote tubule formation [1]. Tau was described as an extremely heat stable protein that controls the polymerization of microtubules as major component of nerve axons implicated in transport and neurotransmission. This discovery was the first example of a protein other than tubulin that was later proven to be essential for the assembly of microtubules. However, Tau remained largely neglected by the field until it was found to be associated with the neurofibrillary lesions encountered in the brains of patients who suffer from Alzheimer's disease [2-6], a discovery that led many research groups to study the metabolism and function of Tau protein.

1.1.1. The *Tau* gene gives rise to different Tau isoforms

A unique gene encodes for Tau protein in the human [7], rat [8] and bovine [9] genomes. In human, where it contains 16 exons, it has been localized to chromosome 17q21.1 [7] (Figure 1). In analogy to more than 60% of human genes [10], *Tau* is alternatively spliced, giving rise to three transcripts of 2, 6, and 9 kb of distinct exon composition encoding multiple different protein isoforms [11-16].

Nuclear Tau

A tau transcript of 2 kb has been detected in cultured human cells and human frontal cortex [11]. The 2 kb message encodes for ubiquitous nuclear Tau and arises from the original tau transcript [11, 17-19]. This nuclear isoform is highly insoluble and present in mitotic cells within nuclear organizer regions of the acrocentric chromosomes (numbers 13, 14, 15, 21 and 22), where rRNA genes are located [20]. Nuclear Tau specifically binds to AT-rich satellite DNA sequences, which form the centromere of mammalian chromosomes [21], and may function in nuclear remodelling and/or ribosomal synthesis.

Tau in the peripheral nervous system (PNS)

A 9 kb mRNA restricted to the retina and the PNS encodes for a much larger-sized variant of Tau that has an apparent molecular mass of 110 kDa [12, 15, 18]. In this mRNA exons 2 and 3 are constitutively expressed as well as exon 4A, which by itself codes for 254 amino acids [12, 15, 22]. This Tau variant has been identified in various cell types, including mature dorsal root ganglion cells, sympathetic neurons, as well as the rat pheochromocytoma cell line PC12 [23-26], where it is thought to serve an important role in stabilizing the neuronal cytoskeleton [22, 25].

Tau in the central nervous system (CNS)

The human 6 kb mRNA is restricted to the CNS and codes for axonal Tau. Among the 16 exons of the primary transcript, exons 1, 4, 5, 7, 9, 11, 12, and 13 are constitutive in CNS tau, whereas exons 2, 3, and 10 are alternatively spliced and are adult-specific [13, 14, 27]. The mRNA undergoes complex splicing leading to the production of six Tau isoforms (Figure 1) [13, 14, 28]. On the protein level, these variants range from 352 to 441 amino acids with a molecular weight from 45 to 65 kDa, with further complexity arising from the presence (or absence) of a 29- or 58-amino acid insert in the amino terminal (N-terminal) half of the protein, encoded by exon 2 and/or 3 [13, 29, 30]. Whereas exon 2 can be expressed without exon 3, expression of exon 3 is always coupled to that of exon 2. All six CNS Tau isoforms contain at least three repeat domains (R1, R3 and R4) in the carboxy terminal (C-terminal) part and the inclusion of exon 10 leads to three isoforms each containing a fourth repeat domain (R2) of 31 amino acids [13, 29, 30]. Tau expression is developmentally regulated and only the shortest Tau isoform, characterized by the absence of N-terminal inserts and the presence of three C-terminal repeats is expressed in developing human brain [14, 30]. The six isoforms are not equally expressed in neurons: for instance, tau mRNA containing exon 10 is not found in granule cells of the dentate gyrus [14].

1.1.2. Structure and functions of the different domains of Tau

Tau is a tripartite protein composed of a C-terminal region harbouring varying numbers of repeat domains, a constant middle domain rich in proline, and variable N-terminal domains (Figure 1) [29].

The C-terminal domain

As previously mentioned, Tau proteins are microtubule-binding proteins that promote tubulin assembly [1, 31]. This property is attributable to repetitive sequences of 31 or 32 residues located within in the C-terminal half of the protein [29, 32, 33]. These repeats can be divided in two parts, one composed of an 18 amino acid sequence containing the region with tubulin binding activity and a second composed of 13 or 14 amino acid inter-repeat regions [9, 13, 14, 29, 30, 34]. The repeat sequences are encoded by exons 9-12 among which exon 10 is alternatively spliced [14, 35] and leads to the insertion of a fourth repeat, R2, which increases the affinity of Tau for microtubules [14, 30, 33, 34]. Each repeat binds to an adjacent tubulin molecule, thereby tethering microtubule together and limiting their freedom of movement relative to one another [36]. Expression of exon 10 is developmentally regulated and may be important during the transition from the more fluid foetal cytoskeleton, which requires plasticity, to the more stable adult one.

The N-terminal domain

The two 29 amino acids inserts encoded by exons 2 and 3 result in different lengths of the N-terminal domain of Tau and render it more acidic [37]. This part of Tau is called the projection domain as it forms short arm-like projections important for Tau anchoring to microtubules [38]. This structural property determines microtubule spacing and influences axon diameter [37], which is of particular importance in the PNS, where large diameter axons are required. It depends on the expression of exon 4A in PNS Tau, which gives rise to projection domains twice as long as those of CNS Tau [28, 39]. Through these molecular modifications Tau functions as an important regulator of axonal size and stabilizer of the axonal cytoskeleton of different axon types. In addition, the N-terminal domain of Tau interacts with the plasma membrane and cytoskeletal elements such as spectrin, actin, and

neurofilaments [40-46]. Via these interactions, Tau mediates associations between microtubules and other components of the cytoskeleton. Interestingly, Tau also binds to the plasma membrane at the periphery of growth cone-like structures in differentiating PC12 cells suggesting a role of Tau in neuritic development [40].

The proline-rich middle domain

The middle domain of Tau (proline-rich) interacts with so-called SH3 domains present in several proteins. These SH3 domains, first identified in the non-receptor tyrosine kinase src family, mediate protein-protein interactions and recognize proline-rich regions [47, 48]. For instance, Tau interacts with fyn, a member of the src family, by a PXXP (residues 233-236) motif that binds to the SH3 domain of fyn [49, 50]. This interaction takes place just beneath the plasma membrane in association with actin filaments [49]. Thus, Tau is assumed to be a key component of a fyn-associated signalling pathway that modulates cell shape by impinging on the submembranous actin cytoskeleton [49]. Proline-rich sequences of Tau also interact with the SH3 domain of phospholipase C-γ (PLC), localized in the plasma membrane [51]. Tau activates PLC-γ towards phosphatidylinositol 4,5 bisphosphate (PIP2), which is potentiated by the presence of arachidonic acid [51, 52]. Interestingly, the middle domain of Tau also plays a role in microtubule binding. The proline-rich domain causes a strong increase in the efficiency of microtubule assembly when joined to the inter-repeat sequences between R1 and R2 (Figure 1) [53]. These flanking regions are important for Tau intramolecular interactions [54, 55]. Interestingly, the proline-rich region *per se* binds very weakly to microtubules but its deletion from Tau dramatically decreases microtubule-affinity *in vitro* and *in vivo* [32, 56-59].

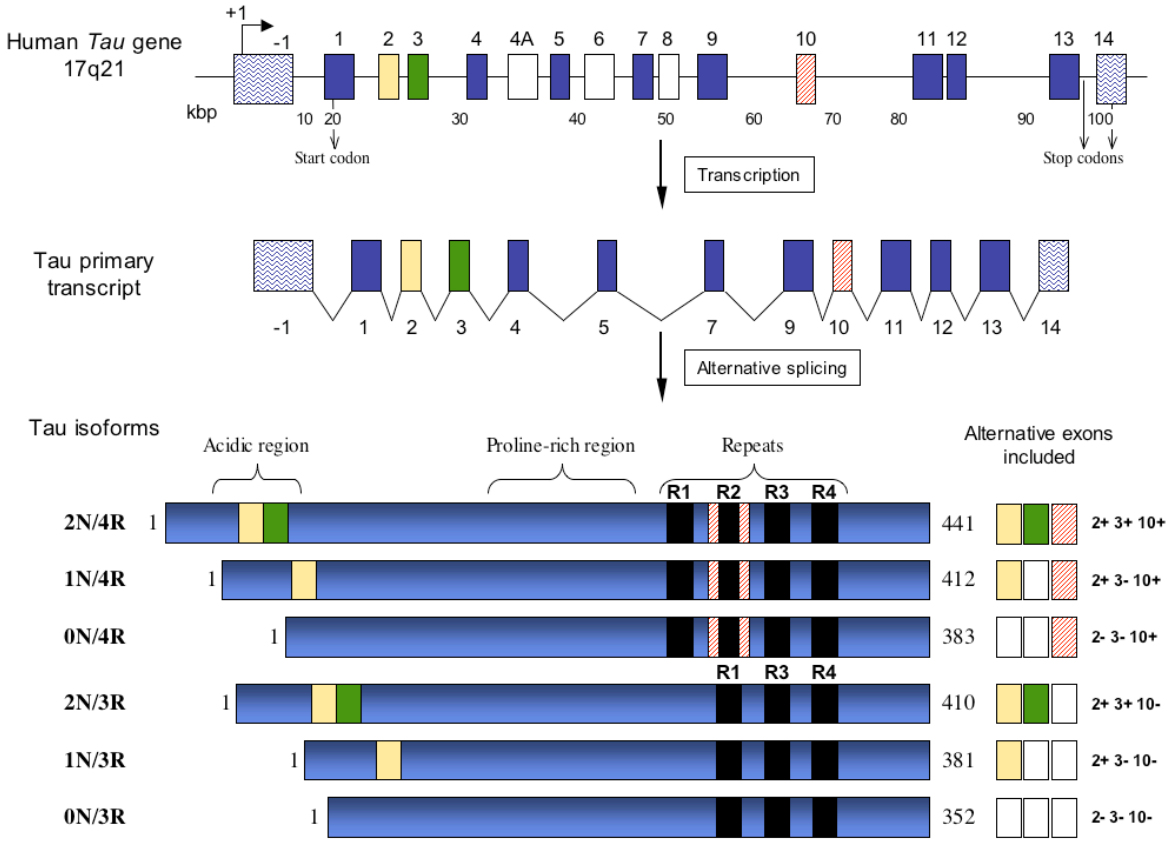


Figure 1. *Tau* gene, primary transcript and isoforms of human brain Tau
The human *Tau* gene spans over 100kbp on chromosome 17 at the position q21. It contains 16 exons, exon -1 being part of the promoter. The start codon is located in exon 1 and the initiation of the transcription is indicated by +1. Exons 4A, 6 and 8 are generally not spliced into human brain tau mRNA and most transcripts retain the intron between exons 13 and 14. **(upper panel)**. In the CNS, the human primary transcript contains 13 exons. **(middle panel)**. The alternative splicing of exons 2 (yellow box), 3 (green box) and 10 (slashed box) gives rise to 6 different CNS adult Tau isoforms **(lower panel)**. They range from 352 to 441 amino acids and differ by the presence or absence of 1 or 2 N-terminal inserts (N) in combination with either 3 or 4 repeats (R, black boxes) in the C-terminal region of the protein. They are composed of three domains, a N-terminal acidic domain, a proline-rich region and a C-terminal repeat domain. The splicing in exon 10 leads to the insertion of the second repeat (R2). Its presence increases the binding to microtubules. The shortest 352 amino acids isoform (0N/3R) is the unique Tau protein present in the foetal brain. (Adapted from Buée *et al*, 2000 [60]).

1.1.3. Tau phosphorylation

The phosphorylation of Tau constitutes its main post-translational modification [44, 61]. Phosphorylation sites on the Tau molecule include serine (Ser) or threonine (Thr) residues followed by proline (Pro) (the longest form of human brain Tau has eighty Ser or Thr residues) [13]. A large number of potential phosphorylation sites are easily accessible due to the open structure of Tau. This feature suggests implication of a subgroup of kinases specific for Ser-Pro or Thr-Pro motifs, so-called proline-directed protein kinases (PDPK). On the other hand, Tau phosphorylation can also occur on Ser or Thr residues that are not followed by Pro, by non-proline-directed protein kinases (non-PDPK). Although more than a dozen protein kinases have been shown to phosphorylate Tau *in vitro*, only few appear to be suitable candidates for *in vivo* phosphorylation.

Glycogen synthase kinase 3β (GSK3β)

This kinase, which phosphorylates both non-Ser/Thr-Pro and Ser/Thr-Pro sites, is expressed at high levels in neurons where it associates with microtubules, and thus is present in the appropriate compartment to access Tau [62-64]. Currently, it appears to be the most obvious candidate for *in vivo* phosphorylation of Tau. Overexpression of GSK3β in cells or in the brains of transgenic mice leads to an increase of Tau phosphorylation [65-69]. In addition, treatment of cells with lithium, which inhibits GSK3β, significantly reduces Tau phosphorylation [70-72]. However, as GSK3β acts on different other proteins, additional studies need to define the direct or/and indirect pathways of GSK3β-mediated Tau phosphorylation [73-76].

Cyclin-dependent kinase 5 (Cdk5)

As a member of the cyclin-dependent kinase family, Cdk5 is apparently not involved in cell-cycle regulation. Its activity is high in neurons due to the presence of its regulator p35 [77, 78]. Truncation of p35 produces the more stable p25, which forms a complex with Cdk5, resulting in Tau phosphorylation at specific sites [79-81]. However, conflicting data emerged concerning the activity of Cdk5 on Tau and it remains to be determined if this kinase directly contributes to Tau phosphorylation [82].

The non-receptor tyrosine kinase fyn

In addition to phosphorylation at Ser and Thr, Tau is also phosphorylated at tyrosine (Tyr) residues [83]. As fyn binds to Tau, it has been hypothesized to be the kinase that phosphorylates Tyr^{18} [49, 83]. In addition, fyn phosphorylates GSK3β, which in turn becomes activated and increases Tau phosphorylation [84]. Therefore, fyn not only mediates phosphorylates of Tau at Tyr^{18} but also indirectly increases Tau phosphorylation via GSK3β.

Additional kinases implicated in Tau phosphorylation

In addition to the kinases discussed above, it has been shown *in vivo* that Tau can also be phosphorylated at the KXGS motifs present in each microtubule-binding repeat of the protein [85]. Microtubule-affinity-regulating kinase (MARK) phosphorylates KXGS motifs more efficiently than other protein kinases suggesting that MARK could be a Tau kinase [85-88]. Other data exist to indicate that Tau is phosphorylated by cAMP-dependent kinase (PKA) or calcium/calmodulin-dependent kinase II (CamKII) *in vivo* [89-92]. In addition, members of the mitogen-activated protein kinase family (MAPK) have also been linked with Tau phosphorylation. They include the stress-activated C-Jun N-terminal kinase (SAPK/JNK), p38 mitogen-activated protein kinase (p38 MAPK), and the extracellular signal-regulated kinase 2 (ERK2) [93-95].

However, the role of potential Tau kinases in modulating Tau phosphorylation under various physiological and pathophysiological conditions still needs to be established *in vivo* using appropriate experimental models.

Phosphatases

All major protein phosphatases (PPs) have been implicated in Tau dephosphorylation [96-99]. PP1 indirectly dephosphorylates several Tau residues via regulation of GSK3β and Cdk5, whereas PP2A appears to dephosphorylate the same sites directly [100]. In particular, PP2A has been shown to act by attaching to Tau microtubule-binding domains thereby rendering the binding of Tau to microtubules impossible [101]. In addition, PP2A has also been shown to dephosphorylate Tau indirectly through CamKII inhibition [102]. Phosphatase activity on Tau has also been demonstrated for PP2B, affecting other sites

than those targeted by PP1 and PP2A [89]. Finally, PP5, a phosphatase highly expressed in brain neurons, was shown to counteract Tau phosphorylation by PKA and GSK3β [103]. Additional candidates, which efficiently dephosphorylate Tau in vivo, still wait identification.

1.1.4. Physiological roles of Tau phosphorylation

Phosphorylation of Tau is developmentally regulated. For example, the foetal short Tau isoform is highly phosphorylated, especially at sites important for microtubule binding [85, 104-106]. Tau, when phosphorylated within the C-terminal repeat domains, has a reduced ability to bind to and polymerize microtubules when compared to non-phosphorylated Tau [65, 107-111]. This is of particular importance during the cell cycle where Tau phosphorylation is synchronized with cell division phases. During interphase, the majority of Tau proteins are poorly phosphorylated and associate with microtubules to maintain the cytoskeleton, whereas during mitosis Tau phosphorylation increases [112] and the protein subsequently detaches from microtubules which enables their reorganization [113]. Interestingly, during axon formation, there is a gradient in Tau phosphorylation from proximal where Tau is highly phosphorylated, to distal where only 20% of Tau is phosphorylated [114, 115]. The modulation of this proximo-distal gradient in Tau phosphorylation could contribute to (or even underlie) neuronal polarity. Furthermore, neurite outgrowth is locally regulated by phosphorylation of Tau within its microtubule-binding domains [88, 116, 117].

These observations reflect the highly dynamic structure of microtubules during development and underline the important role of Tau phosphorylation in neuronal differentiation and plasticity.

1.1.5. Pathological phosphorylation and aggregation of Tau

Whereas phosphorylation is fundamental for Tau function in physiological conditions, a large number of neurodegenerative diseases features neurofibrillary lesions composed of Tau in an abnormal phosphorylated state (see chapter 1.2.). This pathological phosphorylation of Tau, assumed to result from deregulation of Tau kinases or/and phosphatases, can occur either at sites that are normally phosphorylated, or at residues that do not undergo phosphorylation under physiological conditions [80, 102, 106, 118-124]. Abnormal phosphorylation of Tau dissociates it from microtubules, causing its redistribution from axonal to somatodendritic compartments, which is characteristic of the 'pretangle stage'. Consequently, the increased pool of hyperphosphorylated Tau is thought to promote the 'tangle stage'-specific Tau aggregation [125]. Whereas both pretangle and tangle stages can be visualized by phosphorylation-dependent antibodies like AT8 [126], only the tangle stage can be specifically detected by silver staining techniques such as the Bielschowsky and Gallyas methods [127, 128] or by conformation-dependent antibodies such as AT100 [129] (Figure 2).

Ultrastructurally, Tau aggregates into filaments that mainly appear as two strands twisted around each other thereby forming the so-called paired-helical filaments (PHF), whereas a minority of filaments are straight, as if two strands ran parallel to each other, or twisted ribbons filaments [130-134]. Electron microscopy reveals that PHFs are composed of a 'core' region composed of the repeat domains that consists of cross-β structure, and a largely disordered 'fuzzy' coat made of the rest of the protein that retains its natively unfolded character [135-137]. This aggregation is triggered by its dimerization, which occurs via nascent β-structures present within the repeat domains and is accompanied by a conformational change that brings the N-terminal domain into close proximity to the microtubule binding repeats [138-147]. Assembly studies revealed that after formation of Tau dimers a kinetic barrier prevents the rapid formation of filaments, thus suggesting a nucleation step [148, 149]. After the formation of the nucleus, filaments grow in an exponential growth phase with predominant filament extension, followed by a plateau phase, during which the rates of promoter association to and dissociation from filament ends balance each other [150, 151].

A feature of many amyloid deposits is that protein fibrils are enriched in β-sheet content [152]. Thus, abnormal Tau filaments formed in neurodegenerative diseases can be considered as 'amyloids' and understanding the mechanisms leading to Tau aggregation will undoubtedly contribute to the development of strategies aimed at preventing neuronal degeneration.

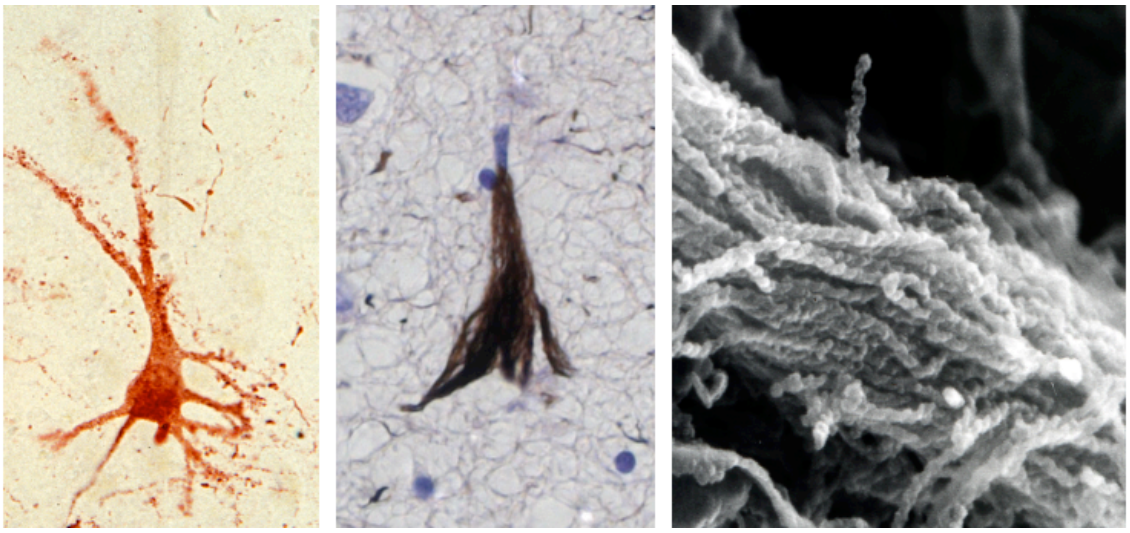


Figure 2. Hyperphosphorylation of Tau precedes its aggregation
Pretangle neuron containing Tau in a hyperphosphorylated but non-aggregated state visualized by phosphorylation-dependent antibody AT8; this neuron is Gallyas negative **(left panel)**. Neurofibrillary tangle, composed of aggregated Tau, stained by Gallyas silver technique **(middle panel)**. The image on the **right** is a scanning electron microscopy picture of paired-helical filaments (picture from Itoh et al. 1997 [153]).

1.2. Neurodegenerative disorders with Tau pathology

Pathological Tau aggregates are encountered in a large number of neurodegenerative diseases grouped under the convenient term 'tauopathies' (Table 1) [154]. Only the most frequent of them will be shortly described here.

Table 1 Diseases with Tau-based neurofibrillary pathology

Alzheimer's disease
Amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam
Argyrophillic grain disease
Corticobasal degeneration
Creutzfeld-Jakob disease
Dementia pugilistica
Diffuse neurofibrillary tangles with calcification
Down's syndrome
Familial British dementia
Frontal variant of AD
Frontotemporal dementia with parkinsonism linked to chromosome 17
Gerstmann-Sträussler-Scheinker disease
Hippocampal sclerosis
Hallervorden-Spatz disease
Myotonic dystrophy
Neurofibrillary tangle dementia
Niemann-Pick disease, type C
Non-Guamanian motor neuron disease with neurofibrillary tangles
Parkinson with dementia of Guadeloupe
Pick's disease
Postencephalic parkinsonism
Prion protein cerebral amyloid angiopathy
Progressive subcortical gliosis
Progressive supranuclear palsy
Sporadic multiple system atrophy
Subacute sclerosing panencephalitis

(Adapted from Lee et al. 2001 [154])

1.2.1. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia affecting millions of people worldwide. The history of AD started in 1901 when the German psychiatrist Alois Alzheimer (1864-1915) (Figure 3) began to be involved in the observation of a 51-year-old female patient, Auguste D (Figure 3) [155]. Her first symptoms included changes in personality with strong feelings of jealousy toward her husband. Over one year, this patient developed a serious impairment in new memory formation that was followed by hallucinations, paranoid ideas, speech difficulties and behavioural problems [155]. After her death in 1906, Alzheimer published a clinicopathological description of her illness [156-158]. By the use of a silver staining method described by Max Bielschowsky (1869-1940), he found abundant senile plaques and neurofibrillary tangles (NFT) in the cerebral cortex [159]. Blocq and Marinesco, however, had described these plaques already previously in an epileptic patient but Alzheimer was the first to discover the tangle pathology [160] (Figure 3).

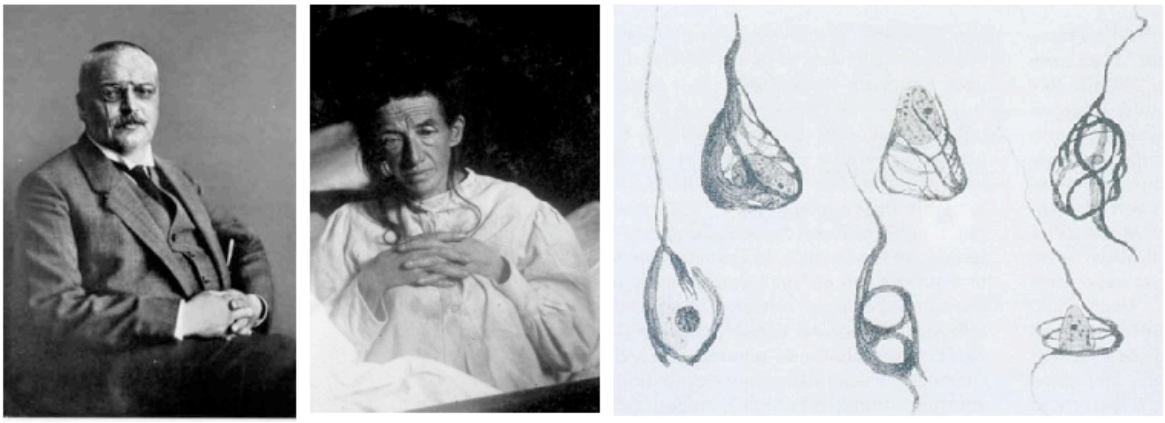


Figure 3. Alois Alzheimer and Auguste D.
Alois Alzheimer (1864-1912) **(left image)** and his first patient, Auguste D., in whom he described the typical lesions of the disease now bearing his name. On the **right** the original drawing by Alois Alzheimer showing the neurofibrillary lesions present in this patient's brain is depicted.

1.2.1.1. Neuropathological hallmarks

The plaques and tangles that were observed in the brain of Alzheimer's original patient are still the two undisputed histopathological hallmarks of AD. Neuropathologically, they are accompanied by variable neuronal losses in the entorhinal cortex, hippocampus, and cerebral cortex [161]; synaptic damage [162-164]; loss of cholinergic innervation [165]; and signs of inflammation [166].

Aβ deposits and APP processing

The senile plaques are composed of extracellular deposits of aggregated amyloid-β protein (Aβ), surrounded by dystrophic neurites, activated microglial cells and reactive astrocytes [167, 168]. Aβ deposits are also encountered in diffuse plaques that present a more amorphous profile and lack Aβ fibrils [169, 170] and in the walls of cerebral and leptomeningeal blood vessels, also called cerebral amyloid angiopathy (CAA) [171, 172] (Figure 4).

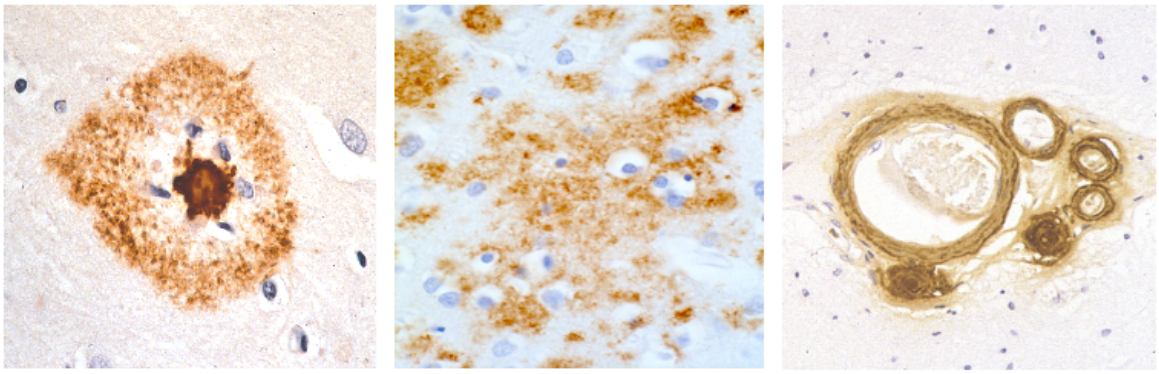


Figure 4. Extracellular deposition of Aβ
Immunohistochemistry for Aβ showing the main types of extracellular deposits encountered in AD. Senile plaque with amyloid core **(left)**, diffuse plaque **(middle)** and Aβ deposition in vessel walls (cerebral amyloid angiopathy; **right**).

The major component of β-amyloid plaques is the Aβ peptide, which results from the proteolysis of the amyloid precursor protein (APP) [173-176]. APP is a transmembrane glycoprotein that can be proteolytically processed by α-, β-, and γ-secretases. The α-secretase constitutively cleaves APP within the Aβ region to produce soluble α-APP and the 83-residue C-terminal fragment, C83 [177, 178]. In contrast to α-secretase cleavage, cleavage by β-secretase is amyloidogenic, generating the soluble β-APP and the C99 fragment [179]. Both C83 and C99 are membrane-anchored and may become degraded by γ-secretase to produce either p3 or Aβ, respectively (Figure 5). γ-secretase forms a large heterotypic complex required for its activity, which is composed of presenilin (PS), nicastrin, PEN-2, and APH-1 [180-184]. Presenilins act as catalytic subunits of γ-secretase [185]. γ-secretase cleavage is not sequence-specific, and produces various Aβ peptides with Aβ40 being the most common, followed by Aβ42. The latter is the predominant form in senile plaque cores, more prone to aggregate into toxic fibrils than Aβ40 [186-188]. The formation of Aβ fibrils follows a nucleation or seeding mechanism that implies the formation of oligomers [189]. These oligomers are thought to act as seeds and to recruit further monomers that self-organize into protofibrils and fibrils made of β-sheet structures [190]. In AD, oligomeric Aβ is more toxic than its monomeric form and fibrils made of both Aβ peptides (Aβ40, Aβ42) contain β-sheet structures classifying them as amyloids [191-194].

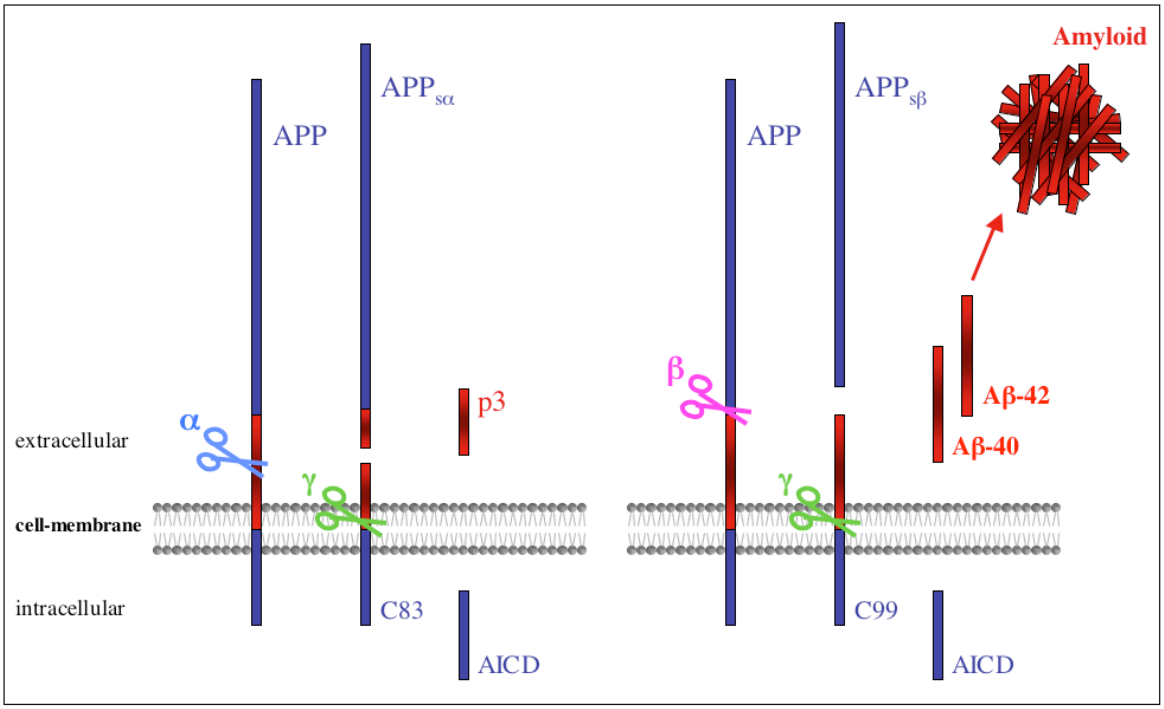


Figure 5. APP processing
The amyloid peptide includes 28 residues outside the membrane and its first 12-14 residues of the transmembrane domain. Cleavage by α-secretase **(left panel)** secretes the large and soluble APP intracellular domain (AICD) into the cytoplasm and retention of the 83-residue C-terminal fragment (C83) in the membrane. C83 can undergo cleavage by γ-secretase to release the p3 peptide. Cleavage by β-secretase **(right panel)** results in the secretion of AICD and the retention of a 99-residue C-terminal fragment in the membrane. C99 can also undergo cleavage by γ-secretase to release the Aβ peptides that can aggregate into amyloid deposits. Amyloidogenic peptides in red. (Adapted from Kahle *et al*, 2003 [195]).

Neurofibrillary lesions

The second hallmark of AD is the presence of intracellular deposits of hyperphosphorylated Tau proteins in neurofibrillary tangles (NFT), neuropil threads (NT) and dystrophic neurites that are associated with senile plaques. In NFTs, Tau lesions are located in the somatodendritic compartment, whereas in NTs, they are found in distal dendrites and axons (Figure 6).

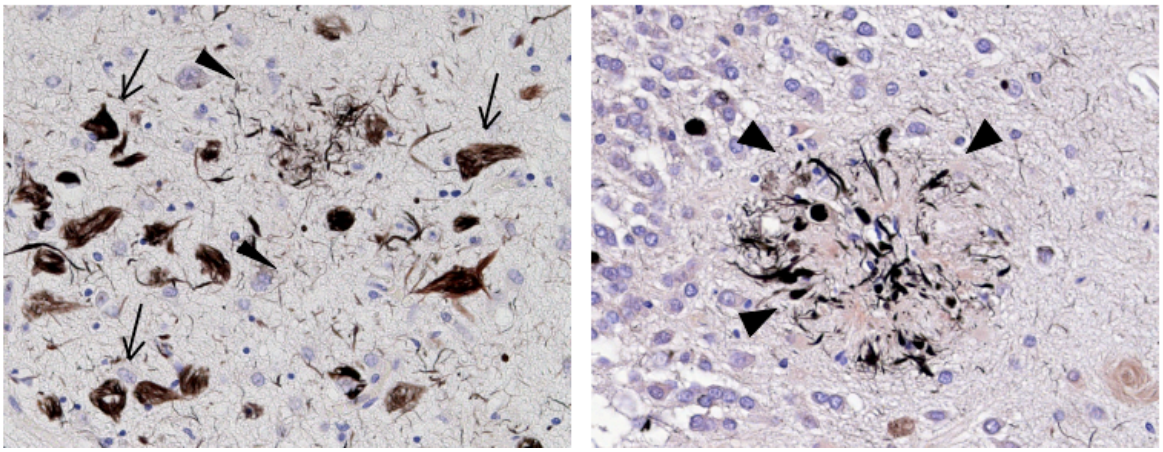


Figure 6. Intracellular lesions made of Tau protein
Gallyas silver staining in the neocortex of an AD brain. **Left panel:** neurofibrillary tangles (arrows) and neuropil threads (arrowheads). **Right panel:** dystrophic neurites surrounding senile plaques (arrowheads).

1.2.1.2. *Aβ cascade hypothesis*

Aβ has been in the main focus of AD research since it is generally considered as the upstream causative factor for tau pathology and other neurodegenerative changes [196]. This 'amyloid cascade hypothesis' derives from molecular genetic studies that had identified three genes associated with early onset familial AD (FAD) cases: APP, PS1, and PS2 [197, 198]. Mutations in one of these genes cause FAD in an autosomal-dominant manner by affecting the processing of APP and Aβ production. Mutations in the APP gene, located on chromosome 21, lead to an increase in total Aβ levels [199]. In addition, in Down's syndrome (trisomy 21), the extra copy of chromosome 21 results in increased APP expression and/or Aβ deposition [200]. Mutations in the PS1 or PS2 genes located on chromosomes 14 and 1, respectively, enhance the production of Aβ42 [201].

1.2.1.3. Spreading of Tau pathology

It is important to specify that, in AD, Tau pathology starts and spreads through the brain along a stereotypical pattern that correlates with cognitive decline. This pattern is the basis of a neuropathological AD staging that defines six neuropathological stages, the so-called 'Braak stages' [202, 203]. Braak stages I and II (or 'entorhinal stages') correspond to the appearance of NFTs in the transentorhinal (medial part of the perirhinal cortex) and entorhinal cortex and are not associated with clinical dementia. The more pronounced involvement of both the transentorhinal and entorhinal regions and the formation of NFTs in CA1 (cornu ammonis 1) of the hippocampus are characteristics of stages III-IV (or 'limbic stages'). The degree of neuronal damage at stages III-IV may lead to the appearance of first clinical symptoms. Stages V and VI (or 'isocortical stages') correspond to an abundant spreading of NFTs to isocortical association areas. Patients with Braak stages V and VI are severely demented, and meet the neuropathological criteria for the diagnosis of AD (Figure 7).

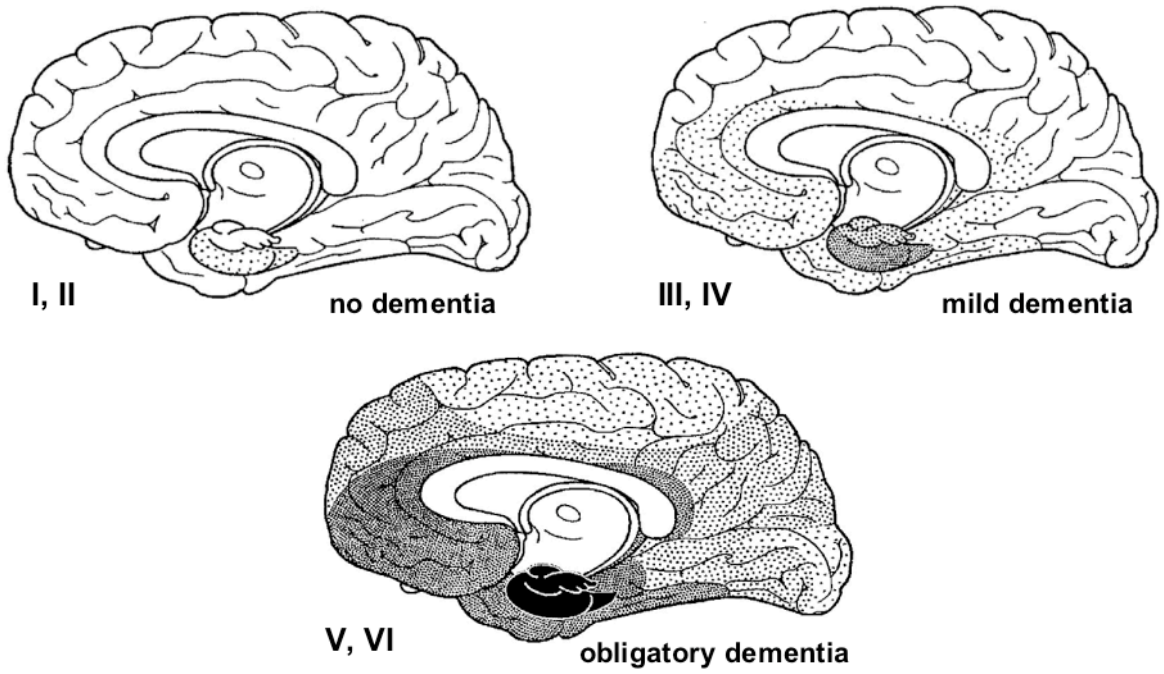


Figure 7. Braak stages
Braak stages I and II correspond to the appearance of NFT in the transentorhinal and entorhinal cortex and are not associated with clinical dementia. Stages III-IV represent the additional appearance of NFT in the limbic system and may lead to the appearance of first clinical symptoms. Stages V and VI correspond to an abundant spreading of NFT to isocortical association areas and patients are severely demented.

1.2.2. Neurodegenerative tauopathies other than AD

Abundant pathological Tau inclusions in the absence of Aβ deposits characterize a number of neurodegenerative diseases known as tauopathies. Although they may share common lesions such as gross brain atrophy, neuronal loss, gliosis, superficial spongiosis, ballooned neurons, and abnormal glial cells, they show disease-specific Tau pathology.

Progressive supranuclear palsy

Progressive supranuclear palsy (PSP), or Steele-Richardson-Olszewski disease, is a multisystem disorder clinically characterized by a parkinsonism component [204]. Cognitive problems, mainly in the form of slowing of thought, may be present as well as motor disturbances including axial rigidity and vertical gaze palsy [205]. At autopsy, marked midbrain atrophy with discoloration of the substantia nigra, atrophy of the superior cerebellar peduncles, subthalamic nucleus and pallidum can be found. Characteristic Tau inclusions are present in different brain regions as globose-type NFTs and NTs [206] but also in astrocytes (tuft-shaped astrocytes) and oligodendrocytes (coiled bodies) (Figure 8).

Corticobasal degeneration

Corticobasal degeneration (CBD) also presents with Parkinsonian features resistant to dopaminergic drugs, associated with cognitive and behavioural features [205, 207, 208]. Macroscopically, the brain shows asymmetric frontoparietal atrophy and depigmentation of the substantia nigra. Intracytoplasmic pathological Tau is observed in NTs, pretangle neurons or small NFTs as well as in astrocytic plaques and oligodendroglial coiled bodies [209-211] (Figure 8).

Pick's disease

Pick's disease (PiD) is a presenile syndrome with a variably progressive clinical course characterized by aphasic symptoms, language impairment, and behaviour changes [212]. The most distinctive pathological feature is a marked cortical atrophy of the frontal and

anterior temporal lobes, also known as ‘knife-edge’ atrophy due to severe neuronal loss and tissue shrinkage in respective brain areas. Argyrophilic (but Gallyas negative) Pick bodies, first described by Alois Alzheimer [158], are present in neocortical, hippocampal and subcortical neurons. Abnormally phosphorylated Tau was demonstrated in Pick bodies and axons [213] (Figure 8).

Argyrophilic grain disease

Argyrophilic grain disease (AgD) is a sporadic late-onset dementia that accounts for approximately 5% of all cases of dementia. The clinical features of AgD are still poorly understood, and there might be difficulties to distinguish AgD from AD. A subset of AgD patients may present clinical features of frontotemporal dementia [214]. Hallmark lesions of AgD are abundant argyrophilic grains (ArGs) in neuronal processes and coiled bodies in oligodendrocytes. ArGs are densely distributed through various parts of the limbic area including the CA1 sector of the hippocampus, layers II and III of the entorhinal and transentorhinal cortices, the amygdala and the hypothalamic lateral tuberal nuclei (Figure 8). In addition to ArGs, there are abundant pretangle neurons in limbic areas as well as glial tau pathology, both in astrocytes and oligodendrocytes [215].

Hereditary frontotemporal dementia and parkinsonism due to tau gene mutations

In 1994, a familial form of frontotemporal dementia and parkinsonism was linked to chromosome 17q21-22 [216]. During the following years other autosomal dominantly inherited familial forms of frontotemporal dementia were identified that were linked to the same region. Based on the similarities of the clinical, neuropathological, and genetic findings, distinct familial cases have been grouped under the umbrella of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [217]. In 1998, tau gene mutations (FTDP-17*T*) were reported in the majority of these families [218-220]. Clinically, FTDP-17*T* is characterized by personality changes, motor symptoms and cognitive decline. The early presenting symptoms are usually disinhibition, loss of initiative, obsessive-compulsive behavior, and/or psychosis. These changes are followed by cognitive decline leading to profound dementia. There may be, however, substantial intra- and interfamilial variation in clinical phenotype [221, 222]. Neuropathologically,

FTDP-17*T* presents with severe neuronal loss, astrocytic gliosis, and spongiosis in cortical and subcortical structures. Filamentous tau inclusions affect both neuronal and glial cells. Depending on the mutation site, tau aggregates may be composed predominantly of 3R, 4R or an admixture of 3R and 4R tau isoforms. At present, more than 40 mutations have been described in 117 families. The mutations in the tau gene, and the mechanisms by which they impair tau functions and generate filamentous tau inclusions in FTDP-17*T*, have recently been reviewed extensively [222-225].

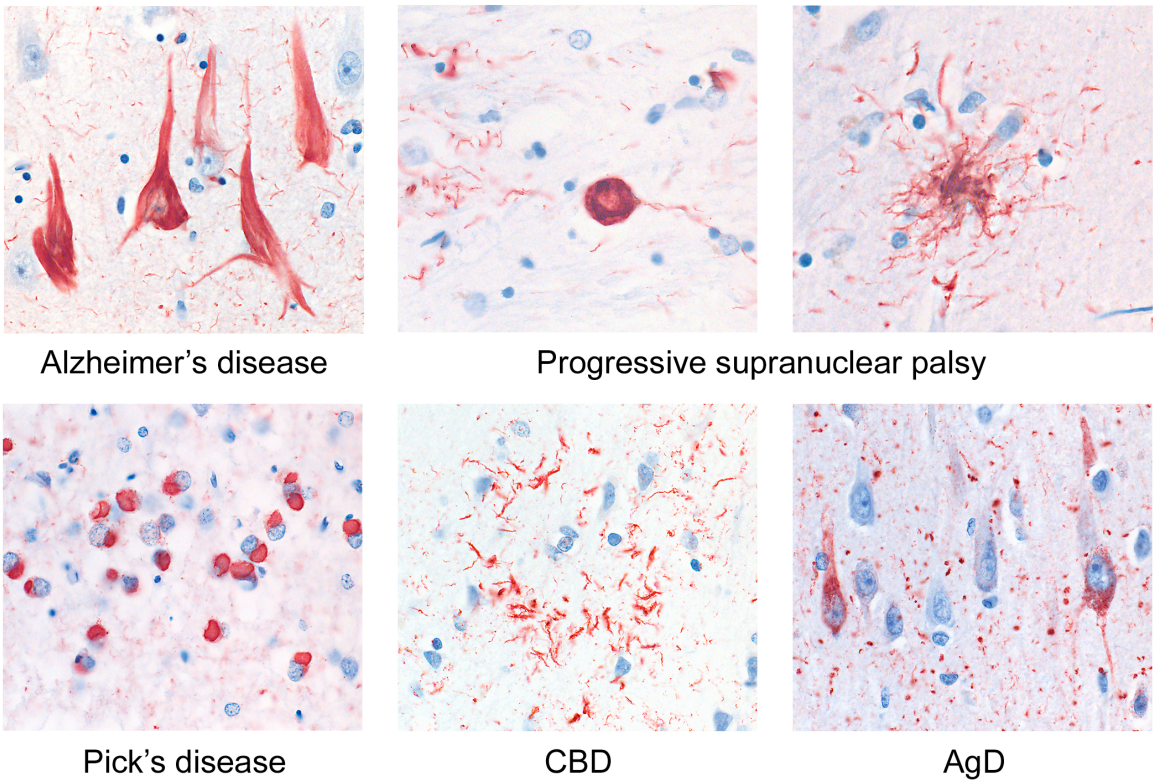


Figure 8. Different types of Tau pathology in tauopathies
Immunohistochemistry with phosphorylation-dependent anti-tau antibody. **From left to right, upper panel:** classical neurofibrillary tangles encountered in AD, globose-type neurofibrillary tangles and tuft-shaped astrocytes in PSP. **Lower panel:** Pick bodies in PiD, astrocytic plaques in CBD, and argyrophilic grains and pretangle neurons in AgD.

1.2.3. Biochemical heterogeneity among tauopathies

The biochemistry of Tau aggregates specific for the various tauopathies reflects different Tau profiles differing in isoform phosphorylation and content, thereby allowing a molecular classification. On SDS gels, phosphorylated Tau migrates more slowly than non-phosphorylated Tau and, after dephosphorylation, it is possible to characterize the Tau isoforms associated with specific diseases [54, 226-228]. Four Tau classes have been established [229] (Figure 9).

The first class (class I) is characterized by a pathological Tau quartet at 60, 64 and 69 kDa with a minor pathological Tau form at 72/74 kDa. This corresponds to the aggregation of the six different brain Tau isoforms [230, 231], a profile that is typical for AD and some FTDP-17*T* forms (Figure 9). Class II corresponds to a triplet of pathological Tau at 64, 69, and a minor 74 kDa forms, indicative of the aggregation of 4R Tau isoforms as it occurs in CBD, AgD, PSP, and in some FTDP-17*T* variants [232, 233] (Figure 9). For the third class (class III), the typical profile corresponds to a triplet of aggregated Tau at 60, 64, and 69 kDa. This class of tauopathies includes PiD and some FTDP-17*T* variants, and comprises tauopathies mainly composed of 3R Tau isoforms (Figure 9). Class IV tauopathies are represented by a single neurological disorder, myotonic dystrophy (DM). DM is the most common form of adult-onset muscular dystrophy but is also a tauopathy since the central nervous system is affected and presents neurofibrillary lesions such as NFTs [234-236]. The pathological Tau profile of DM is characterized by a strong pathological Tau band at 60 kDa and, to a lesser extent, pathological Tau bands at 64 and 69 kDa. These DM-associated aggregates are composed of Tau isoforms lacking N terminal inserts [237] (Figure 9).

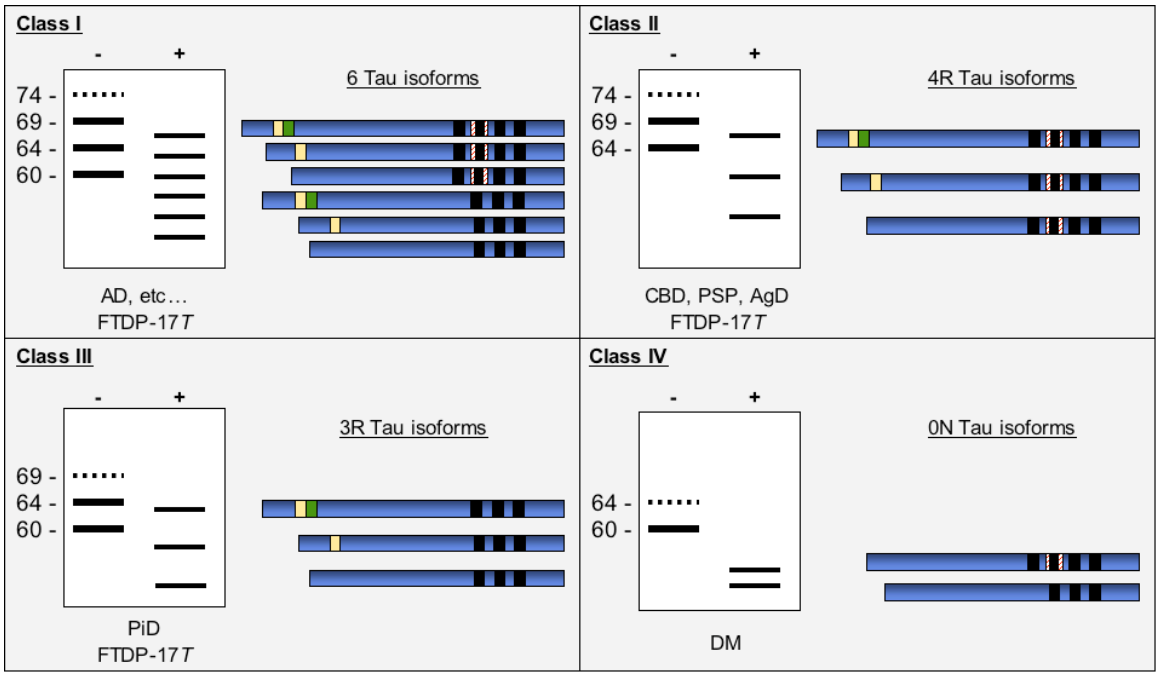


Figure 9. Biochemical classification of tauopathies
Schematic representation of Western blots banding pattern from different tauopathies before (-) and after dephosphorylation (+). **Class I** presents nondephosphorylated aggregated Tau proteins as a major triplet of 69, 64, and 60 kDa and a minor band at 74 kDa that, upon dephosphorylation, correspond to the six Tau isoforms. This profile is encountered in the brain of patients with AD but also in the case of normal aging (>75 years), dementia pugilistica, Down's syndrome and some FTDP-17*T*. **Class II** groups the diseases predominantly composed of 4R aggregated Tau isoforms such as corticobasal degeneration, progressive supranuclear palsy, argyrophilic grain disease and some FTDP-17*T*. Pick's disease and some FTDP-17*T* are part of the **Class III**, which consists of tauopathies with 3R Tau aggregates. Finally, **Class IV** corresponds to the myotonic dystrophy that is essentially composed of aggregated Tau isoforms without N-terminal inserts (0N). (Adapted from Sergeant *et al*, 2008 [229]).

1.2.4. Transgenic mouse models of Aβ and Tau pathologies

Establishment of appropriate animal models is not only essential for understanding the pathogenesis of human diseases but also for the development of potential therapeutic compounds. This is of particular importance for neurodegenerative disorders, like AD and tauopathies, as CNS tissue biopsies are not normally performed for diagnostic reasons precluding analyses of the first pathogenesis steps in human patients. The generation of transgenic mice has been instrumental for experimentally recapitulating key pathological features of AD and tauopathies. Many different transgenic models have been developed so far, selected examples of which will be reported here.

Aβ transgenic mice

The first transgenic mice expressing high levels of the human APP V717F mutation encountered in FAD were generated in 1995 by Games and colleagues [238]. These mice develop robust amyloid deposits and neuritic dystrophy. In 1996, transgenic mice overexpressing the Swedish APP double mutation (KM670/671NL) that leads to a rise in overall Aβ generation [239, 240], known as the 'Tg2576' mouse model, develop Congo red-positive plaques and age-dependent correlative memory deficits [241]. The well-characterized APP23 model expresses human APP with the Swedish double mutation at sevenfold higher levels compared to endogenous mouse APP [242]. Aβ deposition in these mice starts at around 6 months of age in the cerebral cortex, and dramatically increases with age to occupy a substantial area of the cerebral cortex and hippocampus in 24-month-old mice. In these mice, the first amyloid accumulation in leptomeningeal vessels can be detected 3 months after the first plaques and results in extensive damage to the vascular smooth muscle layer of CAA-affected vessels [243]. The Aβ deposits in APP23 mice are extracellular and mostly of the compact plaque type. Almost all plaques as well as the vascular amyloid deposits display Thioflavin S fluorescence and are Congo Red birefringent in polarized light [242]. Massive astrogliosis can be demonstrated in plaque-containing brain areas such as neocortex and hippocampus [242, 244]. In close vicinity to essentially all compact amyloid deposits an accumulation of microglial cells is found [245, 246]. In the hippocampal pyramidal cell layer, a reduction in neuron number adjacent to

the Aβ deposits is apparent and lower CA1 neuron numbers have been reported for 14-18-month-old APP23 transgenic mice [247]. Moreover, in these mice, AT8 immunohistochemistry reveals hyperphosphorylated Tau to be exclusively associated with Congo Red-positive plaques. In addition, APP23 mice exhibit an age-dependent cognitive decline in visual and spatial learning capacities [248-250].

Tau transgenic mice

The first Tau-transgenic mouse models were generated before the discovery of human *Tau* mutations (Table 2). Several Tau-transgenic mice expressing the longest human Tau isoform were analyzed and led to similar observations: transgenic Tau was found to be expressed in neurons and, interestingly, phosphorylated at the same sites that are hyperphosphorylated in human AD brains. However, these mice never developed Tau filaments, thus defining a 'pretangle' phenotype. For instance, ALZ17 mice show prominent somatodendritic staining for phosphorylated tau in nerve cells from brain and spinal cord [251]. However, these animals fail to develop Tau aggregates as evidenced by the absence of tau filaments upon immunoelectron microscopic examination of brain extracts from 17- 20 month-old mice, and the absence of staining by the Gallyas silver impregnation technique [127]. In addition, these mice develop axonopathy when they age and present motor deficits [251].

Mice expressing the shortest Tau isoform were also created and mainly presented the same pretangle phenotype than the 4R-Tau transgenic mice [252, 253]. However, one of these models showed, for the first time, a time-dependent assembly of Gallyas-positive Tau filaments appearing at 12-24 months of age [254]. Transgenic models that are more relevant to the human condition in terms of Tau pathogenesis were established by expressing the six Tau isoforms but no Tau filaments were observed [255]. This was overcome by the generation of a sophisticated mouse model that expressed all six human Tau isoforms in absence of endogenous murine Tau [256]. By the age of 9 months, these mice develop Tau filaments affecting neocortical neurons and pyramidal hippocampal cells, reminiscent of AD [256]. However, neither *bona fide* NFTs nor significant neuronal loss occurred in these mice, which highlighted the limitations of wild-type Tau-transgenic mice in fully reproducing human tauopathy features.

The discovery of human *Tau* mutations, pathogenetically linked to FTDP-17*T* [222] triggered the establishment of various transgenic mice in which NFT formation was recapitulated (Table 3). For instance, missense mutations leading to amino acid substitutions, such as P301L or P301S, were expressed in mice and resulted in severely impaired motor phenotypes as well as in neurofibrillary tangle formation. The JNPL3 line expressing the P301L Tau mutation under the control of the mouse prion promoter develops motor and behavioural disturbances histologically accompanied by morphologically heterogeneous tau-positive inclusions resembling those encountered in human tauopathies [257]. In addition, the fibrillar Tau pathology in these mice can also be demonstrated at the ultrastructural level. Moreover, the spinal cord pathology observed in the JNPL3 mice correlates with motor dysfunction. Interestingly, for reasons unknown, these mice show a gender difference, with higher levels of filamentous Tau pathology observed in females. Another very similar model is the P301S mouse line that expresses the transgene under the control of the murine Thy 1.2 promoter [258]. These mice develop severe paraparesis, mainly attributable to the presence of abundant filamentous inclusions in the brainstem and spinal cord that consist of phosphorylated Tau. Filaments extracted from the CNS of these mice resemble the half-twisted ribbons described in cases of FTDP-17*T* with a minority being similar to the PHF of AD. Neurodegeneration in the spinal cord results in an almost 50% loss of motor neurons.

These transgenic animals expressing mutated Tau demonstrate, for instance, the impact of the P301L and P301S mutations on Tau pathology and provide models in which potential therapeutic agents can be assessed.

Alternative approaches, e.g. transgenic expression of Tau kinases in mice, are also aimed at reproducing Tau pathology *in vivo*. For instance, mice overexpressing P25 or inducible expression of GSK3-β in animals double transgenic for CamKII and GSK3-β (see chapter 1.1.3) develop Tau hyperphosphorylation but lack formation of neurofibrillary pathology [68, 81]. Taken together, these mice, in which potential Tau kinases are expressed, have reproduced some of the initial features of the Tau pathology observed in human diseases.

Table 2: Transgenic mouse strains modeling tauopathy through human wt tau expression

Transgene	Promoter	Histopathology	Phenotype	Remarks
4R/2N	Human Thy-1	Phosphorylated Tau; somatodendritic Tau distribution		Relatively low transgene expression, pretangle Tau pathology, no NFTs
4R/2N	mThy-1 (mThy-1.2 in [69])	Somatodendritic staining for hyperphosporylated Tau, early axonopathy with degeneration in brain and spinal cord; astrogliosis	Progressive motor deficits; unable to spread hindlimbs when lifted by the tail; neurogenic muscle atrophy, normal life span	Pretangle Tau pathology, no NFT formation
4R/2N x GSK3ß	mThy-1	Axonopathy rescued		
4R/2N	MoPrP	Weak somatodendritic Tau staining		No NFT formation
3R/0N	mHMG-CoA reductase	Tg Tau expressed in neurons (cell bodies and dendrites) and few astrocytes		Pretangle Tau pathology, no NFT formation
All 3R isoforms of human Tau	mtubulin Tα1	Accumulation of Tau in astrocytes and oligodendrocytes with subsequent cell death; disruption of myelin sheaths; no neuronal Tau lesions	Motor deficits (weakness in hind- and forelimbs) associated with age-dependent accumulation of insoluble hyperphosphorylated human Tau	Glial Tau lesions similar to astrocytic plaques and oligodendroglial coiled bodies in old mice
3R/0N	MoPrP	Rare NFT in aged mice (24 months); axonal degeneration	Progressive motor deficits	
All six isoforms of human Tau, endogenous murine Tau background	Human Tau	Tau-immunoreactive axonal swellings in spinal cord	Hind-limb abnormality	High 3R:4R ratio, no NFT
Same as above, but on murine Tau-null background	Human Tau	Hyperphosphorylated Tau accumulating as PHF	Mice fertile and viable	Filamentous Tau pathology in spatiotemporally relevant distribution
4R/2N on murine Tau-null background	mThy-1	No obvious pathology; significant increases in hippocampal volume and neuronal number	Minor motor phenotype late in life, normal mean life span; improved cognitive function	4R Tau important for hippocampal development by controlling neuronal precursor proliferation and differentiation

Frank, Clavaguera and Tolnay, 2008 [259]

Table 3: Transgenic mouse strains modeling tauopathy through human mutant tau expression

Transgene	Promoter	Histopathology	Phenotype	Remarks
P301L (4R/0N)	MoPrP	Age- and dose-dependent NFT development, starting at 4.5 months; neuronal loss, neurogenic muscle atrophy	Severe motor and behavioral deficits, premature death	NFT pathology linked to neuronal loss
P301L (4R/2N)	mThy-1.2	NFT-like, sarkosyl-insoluble, short filament structures with hyperphosphorylated tau at 8 months; gliosis, TUNEL-positive neurons		
P301L (4R/2N)	mThy-1	Widespread NFTs at 6 months; no axonopathy	Late minor motor phenotype, muscle and tissue wasting, death prior to age of 13 months	Tau hyperphosphorylation, progressive aggregation
P301L (4R/0N) (tet-regulatable)	CaMK-II	High transgene expression levels in forebrain with progressive, age-related NFTs and neuronal loss; pretangles starting at 2.5 months	Early cognitive impairment	Upon transgene suppression, significant improvement of memory function, despite ongoing NFT accumulation
P301L (4R/1N)	2', 3'-CNP	Impaired axonal transport before axonal degeneration; disruption of myelin and axons preceding filamentous oligodendroglial tau inclusions	Weight loss and progressive motor weakness, presumably as a consequence of neurogenic muscle atrophy	Oligodendroglial inclusions Gallyas-positive
P301S (4R/0N)	mThy-1.2	Abundant filamentous tau in brain stem and spinal cord; significant reduction of spinal cord motor neurons with neurogenic muscle atrophy	Severe paraparesis at 5-6 months	Soluble Tau demonstrated to be phosphorylated prior to filament assembly; microglia adjacent to Tau-positive neurons
P301S (4R/1N)	MoPrP	Early synaptic pathology with dysfunction at 6 months of age, before neuronal loss and NFT formation; hippocampal and cortical atrophy by 9-12 months of age	Limb weakness, progressing to paralysis ; median survival 9 months	Early prominent microglia activation, prior to tangle formation; immuno-suppression attenuates Tau pathology
G272V (4R/2N) (tet-regulatable)	MoPrP	Transgene expression in neurons and oligodendrocytes	No obvious neurological deficits	Tau filament formation
G272V/P301S double mutant (4R/1N)	mThy-1.2	NFT; pathology starting at 6 months of age in hippocampus (CA1) and neocortex; ghost tangles at 12 months	Decreased synaptic trans-mission; behavioral abnormalities (anxiety, cognition, memory); no motor deficits	
G272V/P301L/R406W triple mutant (4R/2N)	mThy-1	High concentration of hyperphosphorylated somatodendritic Tau in cortex and hippocampus; sarcosyl-insoluble filament formation		Lysosomal abnormalities observed in forebrain
V337M (4R/2N)	PDGF-ß	Degenerating hippocampal neurons, immunoreactive for PHF-associated Tau; neuronal cell death	Decreased hippocampal neural activity, cognitive deficits (elevated plus maze)	
R406W (4R/2N)	MoPrP	Tau lesions with advancing age in neuronal perikarya	Hindleg weakness in older mice	
R406W (4R/2N)	CaMK-II	Congophilic hyperphosphorylated Tau in forebrain at 18 months of age; insoluble Tau filaments in aged mice only	Impaired associative memory (fear conditioning), no obvious sensorimotor deficit	
R406W (4R/2N)	Syrian hamster PrP	Phosphorylated, sarkosyl-insoluble, Gallyas-positive Tau starting to accumulate in hippocampus and amygdala at 6 months of age	In <20% of tg mice, motor symptoms and progressive acquired memory loss between 10-12 months	In more than 80% of tg mice, no significant behavioral or neuropatho-logical phenotype; reason for variable expression/ penetrance unclear
APPsw x P301L (4R/0N)	MoPrP	NFT pathology in limbic system and olfactory cortex at 6-9 months of age (enhanced as compared to P301L mice); starts at 3 months of age in spinal cord and pons	Motor symptoms similar to P301L mice [51], with progressive hindlimb weakness, hunched posture, eye irritations, etc.	APP (and/or Aß) promote NFT formation
APPsw x PS1M146V x P301L	mThy-1.2	Progressive development of plaque and tangle pathology	Synaptic dysfunction prior to onset of Aß and Tau pathology	
hAPP x Tau +/- hAPP x Tau -/-	-	No changes in hAPP or Aß (soluble or aggregated) levels observed	Cognitive deficits of APP mice prevented by decreased levels of endogenous Tau expression	Study demonstrates link between neuronal overexcitation and cognitive impairment

Frank, Clavaguera and Tolnay, 2008 [259]

1.2.5. Therapeutic approaches to AD and tauopathies

The development of therapeutic approaches for the treatment of AD is the ultimate goal of researchers. However, only few compounds with limited efficacy are currently available.

Symptomatic approach

Currently available therapeutic compounds for AD have shown efficacy in reducing cognitive symptoms as well as behavioural abnormalities for cases of mild to moderate AD. They are aimed at reducing the cholinergic deficits [260, 261] or glutamate-mediated neurotoxicity [262, 263] observed in AD. The four cholinesterase inhibitors approved by the US Food and Drug Administration (FDA) for the treatment of AD are Donepezil, rivastigmine, galantamine, and tacrine. Among them, only rivastigmine has been approved by the European Agency for the Evaluation of Medication (EMEA). Another drug approved by both FDA and EMEA is memantine, a non-competitive NMDA receptor, which appears to be protective to neurons [264]. The efficacy of these drugs is regularly reported in clinical studies but further investigation is needed for the development of practice guidelines [265-267].

Therapeutic strategies directed against Aβ

According to the Amyloid cascade hypothesis strategies aimed at reducing Aβ production and/or deposition are promising approaches for the treatment of AD.

In this respect, several studies of active or passive vaccination against Aβ peptide have been performed pre-clinically. In transgenic mice these efforts have led to a reduction in Aβ deposition and the prevention of Aβ aggregation, accompanied by functional improvements [268-273]. Following these promising results, Elan Corporation started a clinical trial of AD vaccination which had to be stopped in 2002 in phase IIa because approximately 6% of the treated patients developed meningoencephalitis [274, 275]. However, evidence for efficacy of Aβ immunotherapy has been found in autopsies of vaccinated patients. Whereas a reduction in the number of Amyloid plaques as well as

their associated dystrophic neurites and astrocytes has been reported, CAA and NFTs were not reduced [276-278].

Other therapeutic strategies are aimed at modulating Aβ production by targeting γ-secretase in order to prevent the formation of amyloidogenic Aβ peptides. A γ-secretase compound that showed a reduction of Aβ plasma levels has been tested [279]. However, this strategy can have significant side effects because γ-secretase also cleaves Notch1, which has important functions in gene regulation [280]. This limitation can be overcome by the use of certain non-steroidal anti-inflammatory drugs (NSAIDs) that preferentially reduce the formation of Aβ42 species without affecting Notch cleavage [281]. Aggregation of Aβ can also be prevented by small molecules that bind to its oligomeric forms. For instance, tamiprosate, an Aβ antagonist which preferentially binds to soluble Aβ, interferes with β-sheet formation and has shown successful results [282].

Therapeutic strategies against pathological Tau

Nowadays, targeting abnormal Tau phosphorylation and aggregation is another very promising strategy. Since hyperphosphorylation of Tau is thought to be a first event in the formation of fibrillar Tau pathology, interfering with this process might be possible via targeting potential Tau kinases and phosphatases. In an experimental approach, the administration of an inhibitor of ERK2 to JNPL3 mice was effective not only at significantly reducing hyperphosphorylated Tau but also in preventing the typical motor deficits in these animals [283]. In addition, administration of lithium-chloride, an inhibitor of GSK-3 activity, resulted in decreased Tau pathology in JNPL3 mice which strongly correlated with markedly reduced axonal degeneration [284].

Other alternative approaches could be aimed at interfering in the Tau-aggregation process. Methods for screening Tau aggregation inhibitors have been developed and some candidate small molecule compounds have been identified [150, 285].

Stabilizing microtubules by agents such as taxols might represent another potential therapeutic avenue [286]. As such agents act on dividing cells, adverse side effects can hardly be avoided. Nevertheless, treatment of transgenic mice expressing the shortest isoform of human wild-type Tau with paclitaxel not only results in the restoration of fast axonal transport but also leads to the improvement of motor deficits [287].

Finally, immunization strategies can also be of benefit since a recent study has shown that active Tau-immunization, in JNPL3 mice, reduces cerebral Tau aggregation and slows the progression of the behavioural phenotype of these animals [288].

1.2.6. Experimental approaches to model Aβ and Tau pathologies

Protein misfolding and formation of amyloids are common features of the majority of neurodegenerative disorders, including AD, tauopathies, Parkinson's disease and prionoses. It becomes increasingly evident that these cerebral proteopathies share pathogenic similarities. Understanding the pathological mechanisms might be effective to establish therapeutic strategies interfering with the proteopathic cascade in AD.

To determine whether Aβ deposition can be induced *in vivo* brain extracts prepared from the neocortex of AD patients were unilaterally injected into the hippocampus and neocortex of 3-month-old APP-transgenic mice (Tg2576) that normally develop Aβ deposits at the age of 9 months [289]. Analysis of animals 1 month after injection did not reveal any Aβ deposition. In contrast, 5 months after injection, the injected extract induced Aβ-immunoreactive senile plaques and vascular deposits in the injected hippocampus and to a lesser extent in the neocortex. Aβ deposits were only detected in 8-month-old Tg2576 mice that were injected with AD extracts, but not in age-matched Tg2576 mice or in Tg2576 mice injected with brain extract prepared from young human control. There was no evidence of Aβ immunoreactivity in mice sacrificed 5 days, 2 weeks, or 4 weeks after injection with AD extracts, which confirms that the Aβ-immunoreactivity detected 4 months post-injection did not result from the injectate itself. A subsequent long-term analysis of this study showed that Aβ deposits were more numerous in the hemisphere of Tg2576 mice injected with AD extract when compared to the contralateral (non-injected) side [290]. Hyperphosphorylated Tau was present in the brain of AD extract-injected Tg2576 mice, especially at sites of intense Aβ immunoreactivity, whereas no NFTs were detected. Based on these results, the authors speculated that an abundant nucleating seed was present in the AD brain for the ordered aggregation of Aβ [290].

More detailed seeding experiments were performed by intracerebral injection of various Aβ-containing brain extracts in different Aβ-depositing mouse models [291]. Injection of AD extract or APP23 brain extract into the brain of male APP23 mice resulted in robust deposition of Aβ essentially in the hippocampus at 4 months after injection. No Aβ-deposits were observed in APP23 mice injected with PBS or with extracts prepared from brains of aged wild-type mice. In addition, the injection of APP23-extract into the brain of wild-type mice did not produce any Aβ deposition confirming that the Aβ-deposits observed in APP23-injected animals did not simply represent the injected material. APP23 brain extracts were also injected into the brain of APP-presenilin-1 (APPxPS1) double-transgenic mice and vice versa. APPxPS1 mice start to develop Aβ deposits at 3 to 4 months of age, whereas amyloid deposition in APP23 males starts at around 9 months of age [242, 292]. The injection of APPxPS1 extract into the brain of APP23 mice resulted in a coarse pattern of compact, punctate Aβ deposits whereas the Aβ deposits induced-by APP23 extracts were more of the diffuse type. When APPxPS1 extract was injected into APPxPS1 mice, the observed Aβ deposits were even more coarse and punctate than those observed in APP23 hosts whereas the injection of APP23 extract into APPxPS1 mice resulted in a mixture of both diffuse and compact Aβ deposits. In addition, the first Aβ deposits in APPxPS1 animals were observed as soon as 1 month post-injection with Aβ-containing extracts whereas no deposits were detected before 2 months after injection in APP23 mice. Injection of Aβ extracts that were either Aβ-immunodepleted or treated with formic acid did not induce Aβ deposition suggesting that the seeding agent consisted of an aggregated Aβ species. This study demonstrated that both, agent and host are critical for *in vivo* Aβ seeding.

This experimental approach was also useful to assess the relationship between Aβ and Tau pathologies in AD. This was first addressed by using non-transgenic species such as monkeys and rats [293]. Intracerebral injection of fibrillar but not of soluble Aβ induced neuronal loss, Tau phosphorylation, and microglial activation in the cerebral cortex of rhesus monkeys. This observation was age- and species-specific, since the same Aβ preparations were not toxic in young monkeys or in rats of different ages [293]. Similar approaches were used in transgenic mice to directly manipulate the interaction between

Aβ and Tau. The injection of synthetic preparations of fibrillar Aβ42 in Tau-transgenic mice (P301L) resulted in a five-fold increase of NFTs in the amygdala of transgenic mice from where neurons project to the injection sites [294]. In this study, the induction of NFTs was observed as early as 18 days after Aβ42 injection. NFTs were identified by Gallyas staining as well as by the conformation-specific antibody AT100 and were composed of twisted filaments. Another breeding approach validated these observations by mating APP (Tg2576) and Tau (P301L) transgenic mice [295]. These double-transgenic mice developed a more than sevenfold increase in NFT numbers in various regions of the brain, whereas plaque numbers were unchanged [295]. Altogether, these experiments demonstrate pathogenic interactions between Aβ and Tau and seem to confirm the Aβ cascade hypothesis. However, they still fail to reproduce sporadic AD since wild-type human Tau was not influenced by Aβ pathology. In addition, they do not address the pathogenesis of 'pure' tauopathies.

1.3. References

1. Weingarten, M.D., et al., *A protein factor essential for microtubule assembly.* Proc Natl Acad Sci U S A, 1975. 72(5): p. 1858-62.
2. Delacourte, A. and A. Defossez, *Alzheimer's disease: Tau proteins, the promoting factors of microtubule assembly, are major components of paired helical filaments.* J Neurol Sci, 1986. 76(2-3): p. 173-86.
3. Grundke-Iqbal, I., et al., *Microtubule-associated protein tau. A component of Alzheimer paired helical filaments.* J Biol Chem, 1986. 261(13): p. 6084-9.
4. Kosik, K.S., C.L. Joachim, and D.J. Selkoe, *Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease.* Proc Natl Acad Sci U S A, 1986. 83(11): p. 4044-8.
5. Nukina, N. and Y. Ihara, *One of the antigenic determinants of paired helical filaments is related to tau protein.* J Biochem, 1986. 99(5): p. 1541-4.
6. Wood, J.G., et al., *Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau).* Proc Natl Acad Sci U S A, 1986. 83(11): p. 4040-3.
7. Neve, R.L., et al., *Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2.* Brain Res, 1986. 387(3): p. 271-80.
8. Drubin, D. and M. Kirschner, *Purification of tau protein from brain.* Methods Enzymol, 1986. 134: p. 156-60.
9. Himmler, A., *Structure of the bovine tau gene: alternatively spliced transcripts generate a protein family.* Mol Cell Biol, 1989. 9(4): p. 1389-96.
10. Herbert, A., *The four Rs of RNA-directed evolution.* Nat Genet, 2004. 36(1): p. 19-25.
11. Wang, Y., et al., *A novel tau transcript in cultured human neuroblastoma cells expressing nuclear tau.* J Cell Biol, 1993. 121(2): p. 257-67.
12. Couchie, D., et al., *Primary structure of high molecular weight tau present in the peripheral nervous system.* Proc Natl Acad Sci U S A, 1992. 89(10): p. 4378-81.
13. Goedert, M., et al., *Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease.* Neuron, 1989. 3(4): p. 519-26.
14. Goedert, M., et al., *Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain.* Embo J, 1989. 8(2): p. 393-9.
15. Goedert, M., M.G. Spillantini, and R.A. Crowther, *Cloning of a big tau microtubule-associated protein characteristic of the peripheral nervous system.* Proc Natl Acad Sci U S A, 1992. 89(5): p. 1983-7.
16. Nunez, J. and I. Fischer, *Microtubule-associated proteins (MAPs) in the peripheral nervous system during development and regeneration.* J Mol Neurosci, 1997. 8(3): p. 207-22.
17. Brady, R.M., R.P. Zinkowski, and L.I. Binder, *Presence of tau in isolated nuclei from human brain.* Neurobiol Aging, 1995. 16(3): p. 479-86.
18. Andreadis, A., et al., *A tau promoter region without neuronal specificity.* J Neurochem, 1996. 66(6): p. 2257-63.

19. Thurston, V.C., et al., *Nucleolar localization of the microtubule-associated protein tau in neuroblastomas using sense and anti-sense transfection strategies.* Cell Motil Cytoskeleton, 1997. 38(1): p. 100-10.
20. Loomis, P.A., et al., *Identification of nuclear tau isoforms in human neuroblastoma cells.* Proc Natl Acad Sci U S A, 1990. 87(21): p. 8422-6.
21. Sjoberg, M.K., et al., *Tau protein binds to pericentromeric DNA: a putative role for nuclear tau in nucleolar organization.* J Cell Sci, 2006. 119(Pt 10): p. 2025-34.
22. Oblinger, M.M., et al., *Tau gene expression in rat sensory neurons during development and regeneration.* J Neurosci, 1991. 11(8): p. 2453-9.
23. Drubin, D.G., D. Caput, and M.W. Kirschner, *Studies on the expression of the microtubule-associated protein, tau, during mouse brain development, with newly isolated complementary DNA probes.* J Cell Biol, 1984. 98(3): p. 1090-7.
24. Drubin, D., S. Kobayashi, and M. Kirschner, *Association of tau protein with microtubules in living cells.* Ann N Y Acad Sci, 1986. 466: p. 257-68.
25. Drubin, D., et al., *Regulation of microtubule protein levels during cellular morphogenesis in nerve growth factor-treated PC12 cells.* J Cell Biol, 1988. 106(5): p. 1583-91.
26. Peng, I., L.I. Binder, and M.M. Black, *Biochemical and immunological analyses of cytoskeletal domains of neurons.* J Cell Biol, 1986. 102(1): p. 252-62.
27. Andreadis, A., J.A. Broderick, and K.S. Kosik, *Relative exon affinities and suboptimal splice site signals lead to non-equivalence of two cassette exons.* Nucleic Acids Res, 1995. 23(17): p. 3585-93.
28. Andreadis, A., W.M. Brown, and K.S. Kosik, *Structure and novel exons of the human tau gene.* Biochemistry, 1992. 31(43): p. 10626-33.
29. Himmler, A., et al., *Tau consists of a set of proteins with repeated C-terminal microtubule-binding domains and variable N-terminal domains.* Mol Cell Biol, 1989. 9(4): p. 1381-8.
30. Kosik, K.S., et al., *Developmentally regulated expression of specific tau sequences.* Neuron, 1989. 2(4): p. 1389-97.
31. Drubin, D.G. and M.W. Kirschner, *Tau protein function in living cells.* J Cell Biol, 1986. 103(6 Pt 2): p. 2739-46.
32. Lewis, S.A., et al., *Organization of microtubules in dendrites and axons is determined by a short hydrophobic zipper in microtubule-associated proteins MAP2 and tau.* Nature, 1989. 342(6249): p. 498-505.
33. Lee, G., R.L. Neve, and K.S. Kosik, *The microtubule binding domain of tau protein.* Neuron, 1989. 2(6): p. 1615-24.
34. Lee, G., N. Cowan, and M. Kirschner, *The primary structure and heterogeneity of tau protein from mouse brain.* Science, 1988. 239(4837): p. 285-8.
35. Gao, Q.S., et al., *Complex regulation of tau exon 10, whose missplicing causes frontotemporal dementia.* J Neurochem, 2000. 74(2): p. 490-500.
36. Matus, A., *Stiff microtubules and neuronal morphology.* Trends Neurosci, 1994. 17(1): p. 19-22.
37. Chen, J., et al., *Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons.* Nature, 1992. 360(6405): p. 674-7.

38. Hirokawa, N., Y. Shiomura, and S. Okabe, *Tau proteins: the molecular structure and mode of binding on microtubules.* J Cell Biol, 1988. 107(4): p. 1449-59.
39. Georgieff, I.S., et al., *Expression of high molecular weight tau in the central and peripheral nervous systems.* J Cell Sci, 1993. 105 (Pt 3): p. 729-37.
40. Brandt, R., J. Leger, and G. Lee, *Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain.* J Cell Biol, 1995. 131(5): p. 1327-40.
41. Carlier, M.F., et al., *Interaction between microtubule-associated protein tau and spectrin.* Biochimie, 1984. 66(4): p. 305-11.
42. Correas, I., R. Padilla, and J. Avila, *The tubulin-binding sequence of brain microtubule-associated proteins, tau and MAP-2, is also involved in actin binding.* Biochem J, 1990. 269(1): p. 61-4.
43. Henriquez, J.P., et al., *Subpopulations of tau interact with microtubules and actin filaments in various cell types.* Cell Biochem Funct, 1995. 13(4): p. 239-50.
44. Selden, S.C. and T.D. Pollard, *Phosphorylation of microtubule-associated proteins regulates their interaction with actin filaments.* J Biol Chem, 1983. 258(11): p. 7064-71.
45. Miyata, Y., et al., *Binding of microtubule-associated protein 2 and tau to the intermediate filament reassembled from neurofilament 70-kDa subunit protein. Its regulation by calmodulin.* J Biol Chem, 1986. 261(28): p. 13026-30.
46. Aamodt, E.J. and R.C. Williams, Jr., *Microtubule-associated proteins connect microtubules and neurofilaments in vitro.* Biochemistry, 1984. 23(25): p. 6023-31.
47. Mayer, B.J., *SH3 domains: complexity in moderation.* J Cell Sci, 2001. 114(Pt 7): p. 1253-63.
48. Kay, B.K., M.P. Williamson, and M. Sudol, *The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains.* Faseb J, 2000. 14(2): p. 231-41.
49. Lee, G., et al., *Tau interacts with src-family non-receptor tyrosine kinases.* J Cell Sci, 1998. 111 (Pt 21): p. 3167-77.
50. Williamson, R., et al., *Rapid tyrosine phosphorylation of neuronal proteins including tau and focal adhesion kinase in response to amyloid-beta peptide exposure: involvement of Src family protein kinases.* J Neurosci, 2002. 22(1): p. 10-20.
51. Hwang, S.C., et al., *Activation of phospholipase C-gamma by the concerted action of tau proteins and arachidonic acid.* J Biol Chem, 1996. 271(31): p. 18342-9.
52. Jenkins, S.M. and G.V. Johnson, *Tau complexes with phospholipase C-gamma in situ.* Neuroreport, 1998. 9(1): p. 67-71.
53. Goode, B.L., et al., *Functional interactions between the proline-rich and repeat regions of tau enhance microtubule binding and assembly.* Mol Biol Cell, 1997. 8(2): p. 353-65.
54. Gustke, N., et al., *The Alzheimer-like phosphorylation of tau protein reduces microtubule binding and involves Ser-Pro and Thr-Pro motifs.* FEBS Lett, 1992. 307(2): p. 199-205.

55. Sengupta, A., et al., *Phosphorylation of tau at both Thr 231 and Ser 262 is required for maximal inhibition of its binding to microtubules.* Arch Biochem Biophys, 1998. 357(2): p. 299-309.
56. Gustke, N., et al., *Domains of tau protein and interactions with microtubules.* Biochemistry, 1994. 33(32): p. 9511-22.
57. Lee, G. and S.L. Rook, *Expression of tau protein in non-neuronal cells: microtubule binding and stabilization.* J Cell Sci, 1992. 102 (Pt 2): p. 227-37.
58. Butner, K.A. and M.W. Kirschner, *Tau protein binds to microtubules through a flexible array of distributed weak sites.* J Cell Biol, 1991. 115(3): p. 717-30.
59. Goode, B.L. and S.C. Feinstein, *Identification of a novel microtubule binding and assembly domain in the developmentally regulated inter-repeat region of tau.* J Cell Biol, 1994. 124(5): p. 769-82.
60. Buee, L., et al., *Tau protein isoforms, phosphorylation and role in neurodegenerative disorders.* Brain Res Brain Res Rev, 2000. 33(1): p. 95-130.
61. Cleveland, D.W., S.Y. Hwo, and M.W. Kirschner, *Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly.* J Mol Biol, 1977. 116(2): p. 227-47.
62. Woodgett, J.R., *Molecular cloning and expression of glycogen synthase kinase-3/factor A.* Embo J, 1990. 9(8): p. 2431-8.
63. Ishiguro, K., et al., *Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments.* FEBS Lett, 1993. 325(3): p. 167-72.
64. Leroy, K. and J.P. Brion, *Developmental expression and localization of glycogen synthase kinase-3beta in rat brain.* J Chem Neuroanat, 1999. 16(4): p. 279-93.
65. Cho, J.H. and G.V. Johnson, *Glycogen synthase kinase 3beta phosphorylates tau at both primed and unprimed sites. Differential impact on microtubule binding.* J Biol Chem, 2003. 278(1): p. 187-93.
66. Lovestone, S., et al., *Phosphorylation of tau by glycogen synthase kinase-3 beta in intact mammalian cells: the effects on the organization and stability of microtubules.* Neuroscience, 1996. 73(4): p. 1145-57.
67. Wagner, U., et al., *Cellular phosphorylation of tau by GSK-3 beta influences tau binding to microtubules and microtubule organisation.* J Cell Sci, 1996. 109 (Pt 6): p. 1537-43.
68. Lucas, J.J., et al., *Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice.* Embo J, 2001. 20(1-2): p. 27-39.
69. Spittaels, K., et al., *Glycogen synthase kinase-3beta phosphorylates protein tau and rescues the axonopathy in the central nervous system of human four-repeat tau transgenic mice.* J Biol Chem, 2000. 275(52): p. 41340-9.
70. Hong, M., et al., *Lithium reduces tau phosphorylation by inhibition of glycogen synthase kinase-3.* J Biol Chem, 1997. 272(40): p. 25326-32.
71. Lovestone, S., et al., *Lithium reduces tau phosphorylation: effects in living cells and in neurons at therapeutic concentrations.* Biol Psychiatry, 1999. 45(8): p. 995-1003.

72. Stambolic, V., L. Ruel, and J.R. Woodgett, *Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells.* Curr Biol, 1996. 6(12): p. 1664-8.
73. Mudher, A., et al., *Dishevelled regulates the metabolism of amyloid precursor protein via protein kinase C/mitogen-activated protein kinase and c-Jun terminal kinase.* J Neurosci, 2001. 21(14): p. 4987-95.
74. Li, L., et al., *Axin and Frat1 interact with dvl and GSK, bridging Dvl to GSK in Wnt-mediated regulation of LEF-1.* Embo J, 1999. 18(15): p. 4233-40.
75. Culbert, A.A., et al., *GSK-3 inhibition by adenoviral FRAT1 overexpression is neuroprotective and induces Tau dephosphorylation and beta-catenin stabilisation without elevation of glycogen synthase activity.* FEBS Lett, 2001. 507(3): p. 288-94.
76. Jope, R.S. and G.V. Johnson, *The glamour and gloom of glycogen synthase kinase-3.* Trends Biochem Sci, 2004. 29(2): p. 95-102.
77. Dhavan, R. and L.H. Tsai, *A decade of CDK5.* Nat Rev Mol Cell Biol, 2001. 2(10): p. 749-59.
78. Shelton, S.B., P. Krishnamurthy, and G.V. Johnson, *Effects of cyclin-dependent kinase-5 activity on apoptosis and tau phosphorylation in immortalized mouse brain cortical cells.* J Neurosci Res, 2004. 76(1): p. 110-20.
79. Hamdane, M., et al., *Mitotic-like tau phosphorylation by p25-Cdk5 kinase complex.* J Biol Chem, 2003. 278(36): p. 34026-34.
80. Patrick, G.N., et al., *Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration.* Nature, 1999. 402(6762): p. 615-22.
81. Ahlijanian, M.K., et al., *Hyperphosphorylated tau and neurofilament and cytoskeletal disruptions in mice overexpressing human p25, an activator of cdk5.* Proc Natl Acad Sci U S A, 2000. 97(6): p. 2910-5.
82. Hallows, J.L., et al., *Decreased cyclin-dependent kinase 5 (cdk5) activity is accompanied by redistribution of cdk5 and cytoskeletal proteins and increased cytoskeletal protein phosphorylation in p35 null mice.* J Neurosci, 2003. 23(33): p. 10633-44.
83. Lee, G., et al., *Phosphorylation of tau by fyn: implications for Alzheimer's disease.* J Neurosci, 2004. 24(9): p. 2304-12.
84. Lesort, M., R.S. Jope, and G.V. Johnson, *Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase.* J Neurochem, 1999. 72(2): p. 576-84.
85. Seubert, P., et al., *Detection of phosphorylated Ser262 in fetal tau, adult tau, and paired helical filament tau.* J Biol Chem, 1995. 270(32): p. 18917-22.
86. Drewes, G., et al., *MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption.* Cell, 1997. 89(2): p. 297-308.
87. Drewes, G., et al., *Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262.* J Biol Chem, 1995. 270(13): p. 7679-88.
88. Biernat, J., et al., *Protein kinase MARK/PAR-1 is required for neurite outgrowth and establishment of neuronal polarity.* Mol Biol Cell, 2002. 13(11): p. 4013-28.

89. Fleming, L.M. and G.V. Johnson, *Modulation of the phosphorylation state of tau in situ: the roles of calcium and cyclic AMP.* Biochem J, 1995. 309 (Pt 1): p. 41-7.
90. Litersky, J.M., et al., *Tau protein is phosphorylated by cyclic AMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase II within its microtubule-binding domains at Ser-262 and Ser-356.* Biochem J, 1996. 316 (Pt 2): p. 655-60.
91. Baudier, J. and R.D. Cole, *Interactions between the microtubule-associated tau proteins and S100b regulate tau phosphorylation by the Ca2+/calmodulin-dependent protein kinase II.* J Biol Chem, 1988. 263(12): p. 5876-83.
92. Steiner, B., et al., *Phosphorylation of microtubule-associated protein tau: identification of the site for Ca2(+)-calmodulin dependent kinase and relationship with tau phosphorylation in Alzheimer tangles.* Embo J, 1990. 9(11): p. 3539-44.
93. Yoshida, H., et al., *Phosphorylation of microtubule-associated protein tau by isoforms of c-Jun N-terminal kinase (JNK).* J Neurochem, 2004. 90(2): p. 352-8.
94. Feijoo, C., et al., *Evidence that phosphorylation of the microtubule-associated protein Tau by SAPK4/p38delta at Thr50 promotes microtubule assembly.* J Cell Sci, 2005. 118(Pt 2): p. 397-408.
95. Drewes, G., et al., *Mitogen activated protein (MAP) kinase transforms tau protein into an Alzheimer-like state.* Embo J, 1992. 11(6): p. 2131-8.
96. Iqbal, K. and I. Grundke-Iqbal, *Elevated levels of tau and ubiquitin in brain and cerebrospinal fluid in Alzheimer's disease.* Int Psychogeriatr, 1997. 9 Suppl 1: p. 289-96; discussion 317-21.
97. Sergeant, N., A. Delacourte, and L. Buee, *Tau protein as a differential biomarker of tauopathies.* Biochim Biophys Acta, 2005. 1739(2-3): p. 179-97.
98. Lau, L.F., et al., *Tau protein phosphorylation as a therapeutic target in Alzheimer's disease.* Curr Top Med Chem, 2002. 2(4): p. 395-415.
99. Avila, J., et al., *Tau in neurodegenerative diseases: tau phosphorylation and assembly.* Neurotox Res, 2004. 6(6): p. 477-82.
100. Bennecib, M., et al., *Role of protein phosphatase-2A and -1 in the regulation of GSK-3, cdk5 and cdc2 and the phosphorylation of tau in rat forebrain.* FEBS Lett, 2000. 485(1): p. 87-93.
101. Sontag, E., et al., *Molecular interactions among protein phosphatase 2A, tau, and microtubules. Implications for the regulation of tau phosphorylation and the development of tauopathies.* J Biol Chem, 1999. 274(36): p. 25490-8.
102. Bennecib, M., et al., *Inhibition of PP-2A upregulates CaMKII in rat forebrain and induces hyperphosphorylation of tau at Ser 262/356.* FEBS Lett, 2001. 490(1-2): p. 15-22.
103. Gong, C.X., et al., *Dephosphorylation of microtubule-associated protein tau by protein phosphatase 5.* J Neurochem, 2004. 88(2): p. 298-310.
104. Brion, J.P., et al., *Developmental changes in tau phosphorylation: fetal tau is transiently phosphorylated in a manner similar to paired helical filament-tau characteristic of Alzheimer's disease.* J Neurochem, 1993. 61(6): p. 2071-80.
105. Kenessey, A. and S.H. Yen, *The extent of phosphorylation of fetal tau is comparable to that of PHF-tau from Alzheimer paired helical filaments.* Brain Res, 1993. 629(1): p. 40-6.

106. Liu, W.K. and S.H. Yen, *The state of phosphorylation of normal adult brain tau, fetal tau, and tau from Alzheimer paired helical filaments at amino acid residue Ser262.* J Neurochem, 1996. 66(3): p. 1131-9.
107. Brandt, R., et al., *Differential effect of phosphorylation and substrate modulation on tau's ability to promote microtubule growth and nucleation.* J Biol Chem, 1994. 269(16): p. 11776-82.
108. Utton, M.A., et al., *Phosphorylation of tau by glycogen synthase kinase 3beta affects the ability of tau to promote microtubule self-assembly.* Biochem J, 1997. 323 (Pt 3): p. 741-7.
109. Xie, H., et al., *The interrelationship between selective tau phosphorylation and microtubule association.* Brain Res, 1998. 798(1-2): p. 173-83.
110. Biernat, J., et al., *Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubule binding.* Neuron, 1993. 11(1): p. 153-63.
111. Cho, J.H. and G.V. Johnson, *Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3beta (GSK3beta) plays a critical role in regulating tau's ability to bind and stabilize microtubules.* J Neurochem, 2004. 88(2): p. 349-58.
112. Pope, W.B., et al., *Microtubule-associated protein tau is hyperphosphorylated during mitosis in the human neuroblastoma cell line SH-SY5Y.* Exp Neurol, 1994. 126(2): p. 185-94.
113. Preuss, U., et al., *Cell cycle-dependent phosphorylation and microtubule binding of tau protein stably transfected into Chinese hamster ovary cells.* Mol Biol Cell, 1995. 6(10): p. 1397-410.
114. Mandell, J.W. and G.A. Banker, *A spatial gradient of tau protein phosphorylation in nascent axons.* J Neurosci, 1996. 16(18): p. 5727-40.
115. Black, M.M., et al., *Tau is enriched on dynamic microtubules in the distal region of growing axons.* J Neurosci, 1996. 16(11): p. 3601-19.
116. Biernat, J. and E.M. Mandelkow, *The development of cell processes induced by tau protein requires phosphorylation of serine 262 and 356 in the repeat domain and is inhibited by phosphorylation in the proline-rich domains.* Mol Biol Cell, 1999. 10(3): p. 727-40.
117. Sayas, C.L., J. Avila, and F. Wandosell, *Glycogen synthase kinase-3 is activated in neuronal cells by Galpha12 and Galpha13 by Rho-independent and Rho-dependent mechanisms.* J Neurosci, 2002. 22(16): p. 6863-75.
118. Buee Scherrer, V., et al., *Hyperphosphorylated tau proteins differentiate corticobasal degeneration and Pick's disease.* Acta Neuropathol, 1996. 91(4): p. 351-9.
119. Hanger, D.P., et al., *New phosphorylation sites identified in hyperphosphorylated tau (paired helical filament-tau) from Alzheimer's disease brain using nanoelectrospray mass spectrometry.* J Neurochem, 1998. 71(6): p. 2465-76.
120. Tomizawa, K., et al., *Tau-tubulin kinase phosphorylates tau at Ser-208 and Ser-210, sites found in paired helical filament-tau.* FEBS Lett, 2001. 492(3): p. 221-7.
121. Gong, C.X., et al., *Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain.* J Neurochem, 1995. 65(2): p. 732-8.

122. Gong, C.X., et al., *Phosphoprotein phosphatase activities in Alzheimer disease brain.* J Neurochem, 1993. 61(3): p. 921-7.
123. Pei, J.J., et al., *Okadaic-acid-induced inhibition of protein phosphatase 2A produces activation of mitogen-activated protein kinases ERK1/2, MEK1/2, and p70 S6, similar to that in Alzheimer's disease.* Am J Pathol, 2003. 163(3): p. 845-58.
124. Kins, S., et al., *Activation of the ERK and JNK signaling pathways caused by neuron-specific inhibition of PP2A in transgenic mice.* Am J Pathol, 2003. 163(3): p. 833-43.
125. Chen, F., et al., *Posttranslational modifications of tau--role in human tauopathies and modeling in transgenic animals.* Curr Drug Targets, 2004. 5(6): p. 503-15.
126. Goedert, M., R. Jakes, and E. Vanmechelen, *Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205.* Neurosci Lett, 1995. 189(3): p. 167-9.
127. Gallyas, F., *Silver staining of Alzheimer's neurofibrillary changes by means of physical development.* Acta Morphol Acad Sci Hung, 1971. 19(1): p. 1-8.
128. Braak, H., et al., *Silver impregnation of Alzheimer's neurofibrillary changes counterstained for basophilic material and lipofuscin pigment.* Stain Technol, 1988. 63(4): p. 197-200.
129. Yoshida, H. and M. Goedert, *Sequential phosphorylation of tau protein by cAMP-dependent protein kinase and SAPK4/p38delta or JNK2 in the presence of heparin generates the AT100 epitope.* J Neurochem, 2006. 99(1): p. 154-64.
130. Kidd, M., *Paired helical filaments in electron microscopy of Alzheimer's disease.* Nature, 1963. 197: p. 192-3.
131. Kidd, M., *Alzheimer's Disease--an Electron Microscopical Study.* Brain, 1964. 87: p. 307-20.
132. Crowther, R.A. and C.M. Wischik, *Image reconstruction of the Alzheimer paired helical filament.* Embo J, 1985. 4(13B): p. 3661-5.
133. Crowther, R.A., *Straight and paired helical filaments in Alzheimer disease have a common structural unit.* Proc Natl Acad Sci U S A, 1991. 88(6): p. 2288-92.
134. Ruben, G.C., et al., *The organization of the microtubule associated protein tau in Alzheimer paired helical filaments.* Brain Res, 1993. 602(1): p. 1-13.
135. Crowther, T., M. Goedert, and C.M. Wischik, *The repeat region of microtubule-associated protein tau forms part of the core of the paired helical filament of Alzheimer's disease.* Ann Med, 1989. 21(2): p. 127-32.
136. Wischik, C.M., et al., *Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease.* Proc Natl Acad Sci U S A, 1988. 85(12): p. 4506-10.
137. Barghorn, S., P. Davies, and E. Mandelkow, *Tau paired helical filaments from Alzheimer's disease brain and assembled in vitro are based on beta-structure in the core domain.* Biochemistry, 2004. 43(6): p. 1694-703.
138. Wille, H., et al., *Alzheimer-like paired helical filaments and antiparallel dimers formed from microtubule-associated protein tau in vitro.* J Cell Biol, 1992. 118(3): p. 573-84.

139. Schweers, O., et al., *Oxidation of cysteine-322 in the repeat domain of microtubule-associated protein tau controls the in vitro assembly of paired helical filaments.* Proc Natl Acad Sci U S A, 1995. 92(18): p. 8463-7.
140. Schweers, O., et al., *Structural studies of tau protein and Alzheimer paired helical filaments show no evidence for beta-structure.* J Biol Chem, 1994. 269(39): p. 24290-7.
141. Eliezer, D., et al., *Residual structure in the repeat domain of tau: echoes of microtubule binding and paired helical filament formation.* Biochemistry, 2005. 44(3): p. 1026-36.
142. Mukrasch, M.D., et al., *Sites of tau important for aggregation populate {beta}-structure and bind to microtubules and polyanions.* J Biol Chem, 2005. 280(26): p. 24978-86.
143. von Bergen, M., et al., *Tau aggregation is driven by a transition from random coil to beta sheet structure.* Biochim Biophys Acta, 2005. 1739(2-3): p. 158-66.
144. von Bergen, M., et al., *Assembly of tau protein into Alzheimer paired helical filaments depends on a local sequence motif ((306)VQIVYK(311)) forming beta structure.* Proc Natl Acad Sci U S A, 2000. 97(10): p. 5129-34.
145. Goux, W.J., et al., *The formation of straight and twisted filaments from short tau peptides.* J Biol Chem, 2004. 279(26): p. 26868-75.
146. Inouye, H., et al., *Structure of core domain of fibril-forming PHF/Tau fragments.* Biophys J, 2006. 90(5): p. 1774-89.
147. Carmel, G., et al., *The structural basis of monoclonal antibody Alz50's selectivity for Alzheimer's disease pathology.* J Biol Chem, 1996. 271(51): p. 32789-95.
148. Goedert, M., et al., *Assembly of microtubule-associated protein tau into Alzheimer-like filaments induced by sulphated glycosaminoglycans.* Nature, 1996. 383(6600): p. 550-3.
149. Friedhoff, P., et al., *A nucleated assembly mechanism of Alzheimer paired helical filaments.* Proc Natl Acad Sci U S A, 1998. 95(26): p. 15712-7.
150. Chirita, C., M. Necula, and J. Kuret, *Ligand-dependent inhibition and reversal of tau filament formation.* Biochemistry, 2004. 43(10): p. 2879-87.
151. Chirita, C.N., et al., *Triggers of full-length tau aggregation: a role for partially folded intermediates.* Biochemistry, 2005. 44(15): p. 5862-72.
152. Lansbury, P.T., Jr., *Evolution of amyloid: what normal protein folding may tell us about fibrillogenesis and disease.* Proc Natl Acad Sci U S A, 1999. 96(7): p. 3342-4.
153. Itoh, Y., et al., *Scanning electron microscopical study of the neurofibrillary tangles of Alzheimer's disease.* Acta Neuropathol, 1997. 94(1): p. 78-86.
154. Lee, V.M., M. Goedert, and J.Q. Trojanowski, *Neurodegenerative tauopathies.* Annu Rev Neurosci, 2001. 24: p. 1121-59.
155. Maurer, K., S. Volk, and H. Gerbaldo, *Auguste D and Alzheimer's disease.* Lancet, 1997. 349(9064): p. 1546-9.
156. Alzheimer, A., et al., *An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde".* Clin Anat, 1995. 8(6): p. 429-31.
157. Alzheimer, A., *Über eine eigenartige Erkrankung der Hirnrinde.* Allg Z Psychiat, 1907. 64: p. 146-148.

158. Alzheimer, A., *Über eigenartige Krankheitsfälle des späteren Alters.* Z ges Neurol Psychiat, 1911. 4: p. 356-385.
159. Bielschowsky, M., *Die Silberimprägnation der Achsenzylinder.* Neurol Centralbl, 1902. 21: p. 579-584.
160. Blocq, P. and G. Marinesco, *Sur les lésions et la pathogénie de l'épilepsie dite essentielle.* Sem Méd, 1892. 12: p. 445-446.
161. Mouton, P.R., et al., *Cognitive decline strongly correlates with cortical atrophy in Alzheimer's dementia.* Neurobiol Aging, 1998. 19(5): p. 371-7.
162. Terry, R.D., et al., *Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment.* Ann Neurol, 1991. 30(4): p. 572-80.
163. West, M.J., et al., *Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease.* Lancet, 1994. 344(8925): p. 769-72.
164. Gomez-Isla, T., et al., *Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease.* J Neurosci, 1996. 16(14): p. 4491-500.
165. Coyle, J.T., D.L. Price, and M.R. DeLong, *Alzheimer's disease: a disorder of cortical cholinergic innervation.* Science, 1983. 219(4589): p. 1184-90.
166. Eikelenboom, P., et al., *Complement activation in amyloid plaques in Alzheimer's dementia.* Virchows Arch B Cell Pathol Incl Mol Pathol, 1989. 56(4): p. 259-62.
167. Itagaki, S., et al., *Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease.* J Neuroimmunol, 1989. 24(3): p. 173-82.
168. Wisniewski, H.M., et al., *Spectrum of morphological appearance of amyloid deposits in Alzheimer's disease.* Acta Neuropathol, 1989. 78(4): p. 337-47.
169. Tagliavini, F., et al., *Preamyloid deposits in the cerebral cortex of patients with Alzheimer's disease and nondemented individuals.* Neurosci Lett, 1988. 93(2-3): p. 191-6.
170. Yamaguchi, H., et al., *Electron micrograph of diffuse plaques. Initial stage of senile plaque formation in the Alzheimer brain.* Am J Pathol, 1989. 135(4): p. 593-7.
171. Mandybur, T.I., *The incidence of cerebral amyloid angiopathy in Alzheimer's disease.* Neurology, 1975. 25(2): p. 120-6.
172. Vinters, H.V., et al., *Immunohistochemical study of cerebral amyloid angiopathy. II. Enhancement of immunostaining using formic acid pretreatment of tissue sections.* Am J Pathol, 1988. 133(1): p. 150-62.
173. Glenner, G.G. and C.W. Wong, *Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein.* Biochem Biophys Res Commun, 1984. 120(3): p. 885-90.
174. Masters, C.L., et al., *Amyloid plaque core protein in Alzheimer disease and Down syndrome.* Proc Natl Acad Sci U S A, 1985. 82(12): p. 4245-9.
175. Wong, C.W., V. Quaranta, and G.G. Glenner, *Neuritic plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related.* Proc Natl Acad Sci U S A, 1985. 82(24): p. 8729-32.
176. Kang, J., et al., *The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor.* Nature, 1987. 325(6106): p. 733-6.
177. Esch, F.S., et al., *Cleavage of amyloid beta peptide during constitutive processing of its precursor.* Science, 1990. 248(4959): p. 1122-4.

178. Sisodia, S.S., *Beta-amyloid precursor protein cleavage by a membrane-bound protease.* Proc Natl Acad Sci U S A, 1992. 89(13): p. 6075-9.
179. Seubert, P., et al., *Secretion of beta-amyloid precursor protein cleaved at the amino terminus of the beta-amyloid peptide.* Nature, 1993. 361(6409): p. 260-3.
180. Steiner, H. and C. Haass, *Intramembrane proteolysis by presenilins.* Nat Rev Mol Cell Biol, 2000. 1(3): p. 217-24.
181. Steiner, H., et al., *PEN-2 is an integral component of the gamma-secretase complex required for coordinated expression of presenilin and nicastrin.* J Biol Chem, 2002. 277(42): p. 39062-5.
182. Yu, G., et al., *Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing.* Nature, 2000. 407(6800): p. 48-54.
183. Francis, R., et al., *aph-1 and pen-2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation.* Dev Cell, 2002. 3(1): p. 85-97.
184. Lee, S.F., et al., *Mammalian APH-1 interacts with presenilin and nicastrin and is required for intramembrane proteolysis of amyloid-beta precursor protein and Notch.* J Biol Chem, 2002. 277(47): p. 45013-9.
185. Marjaux, E., D. Hartmann, and B. De Strooper, *Presenilins in memory, Alzheimer's disease, and therapy.* Neuron, 2004. 42(2): p. 189-92.
186. Jarrett, J.T., E.P. Berger, and P.T. Lansbury, Jr., *The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease.* Biochemistry, 1993. 32(18): p. 4693-7.
187. Burdick, D., et al., *Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs.* J Biol Chem, 1992. 267(1): p. 546-54.
188. Fukumoto, H., et al., *Amyloid beta protein deposition in normal aging has the same characteristics as that in Alzheimer's disease. Predominance of A beta 42(43) and association of A beta 40 with cored plaques.* Am J Pathol, 1996. 148(1): p. 259-65.
189. Jarrett, J.T. and P.T. Lansbury, Jr., *Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie?* Cell, 1993. 73(6): p. 1055-8.
190. Walsh, D.M., et al., *Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates.* J Biol Chem, 1999. 274(36): p. 25945-52.
191. Pike, C.J., et al., *Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state.* J Neurosci, 1993. 13(4): p. 1676-87.
192. Dahlgren, K.N., et al., *Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability.* J Biol Chem, 2002. 277(35): p. 32046-53.
193. Petkova, A.T., W.M. Yau, and R. Tycko, *Experimental constraints on quaternary structure in Alzheimer's beta-amyloid fibrils.* Biochemistry, 2006. 45(2): p. 498-512.
194. Luhrs, T., et al., *3D structure of Alzheimer's amyloid-beta(1-42) fibrils.* Proc Natl Acad Sci U S A, 2005. 102(48): p. 17342-7.
195. Kahle, P.J. and B. De Strooper, *Attack on amyloid.* EMBO Rep, 2003. 4(8): p. 747-51.

196. Hardy, J. and D.J. Selkoe, *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics.* Science, 2002. 297(5580): p. 353-6.
197. Hardy, J., *Amyloid, the presenilins and Alzheimer's disease.* Trends Neurosci, 1997. 20(4): p. 154-9.
198. Tilley, L., K. Morgan, and N. Kalsheker, *Genetic risk factors in Alzheimer's disease.* Mol Pathol, 1998. 51(6): p. 293-304.
199. Jankowsky, J.L., et al., *Mutant presenilins specifically elevate the levels of the 42 residue beta-amyloid peptide in vivo: evidence for augmentation of a 42-specific gamma secretase.* Hum Mol Genet, 2004. 13(2): p. 159-70.
200. Mori, C., et al., *Intraneuronal Abeta42 accumulation in Down syndrome brain.* Amyloid, 2002. 9(2): p. 88-102.
201. Tomita, T., et al., *The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid beta protein ending at the 42nd (or 43rd) residue.* Proc Natl Acad Sci U S A, 1997. 94(5): p. 2025-30.
202. Braak, H. and E. Braak, *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol, 1991. 82(4): p. 239-59.
203. Gertz, H.J., et al., *Examination of the validity of the hierarchical model of neuropathological staging in normal aging and Alzheimer's disease.* Acta Neuropathol, 1998. 95(2): p. 154-8.
204. Steele, J.C., J.C. Richardson, and J. Olszewski, *Progressive Supranuclear Palsy. A Heterogeneous Degeneration Involving the Brain Stem, Basal Ganglia and Cerebellum with Vertical Gaze and Pseudobulbar Palsy, Nuchal Dystonia and Dementia.* Arch Neurol, 1964. 10: p. 333-59.
205. Sha, S., et al., *Are frontotemporal lobar degeneration, progressive supranuclear palsy and corticobasal degeneration distinct diseases?* Nat Clin Pract Neurol, 2006. 2(12): p. 658-65.
206. Probst, A., et al., *Progressive supranuclear palsy: extensive neuropil threads in addition to neurofibrillary tangles. Very similar antigenicity of subcortical neuronal pathology in progressive supranuclear palsy and Alzheimer's disease.* Acta Neuropathol, 1988. 77(1): p. 61-8.
207. Rebeiz, J.J., E.H. Kolodny, and E.P. Richardson, Jr., *Corticodentatonigral degeneration with neuronal achromasia.* Arch Neurol, 1968. 18(1): p. 20-33.
208. Grimes, D.A., A.E. Lang, and C.B. Bergeron, *Dementia as the most common presentation of cortical-basal ganglionic degeneration.* Neurology, 1999. 53(9): p. 1969-74.
209. Tolnay, M. and A. Probst, *Frontotemporal lobar degeneration--tau as a pied piper?* Neurogenetics, 2002. 4(2): p. 63-75.
210. Uchihara, T., et al., *Discrepancy between tau immunoreactivity and argyrophilia by the Bodian method in neocortical neurons of corticobasal degeneration.* Acta Neuropathol, 1998. 96(6): p. 553-7.
211. Ikeda, K., et al., *Pick-body-like inclusions in corticobasal degeneration differ from Pick bodies in Pick's disease.* Acta Neuropathol, 2002. 103(2): p. 115-8.
212. Pick, A., *Über die Beziehungen der senilen Atrophie zur Aphasie.* Prager Med Wochenschr, 1892. 17: p. 165-167.

213. Probst, A., et al., *Pick's disease: hyperphosphorylated tau protein segregates to the somatoaxonal compartment.* Acta Neuropathol, 1996. 92(6): p. 588-96.
214. Tolnay, M. and S. Frank, *Pathology and genetics of frontotemporal lobar degeneration: an update.* Clin Neuropathol, 2007. 26(4): p. 143-56.
215. Tolnay, M. and F. Clavaguera, *Argyrophilic grain disease: a late-onset dementia with distinctive features among tauopathies.* Neuropathology, 2004. 24(4): p. 269-83.
216. Wilhelmsen, K.C., et al., *Localization of disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22.* Am J Hum Genet, 1994. 55(6): p. 1159-65.
217. Foster, N.L., et al., *Frontotemporal dementia and parkinsonism linked to chromosome 17: a consensus conference. Conference Participants.* Ann Neurol, 1997. 41(6): p. 706-15.
218. Hutton, M., et al., *Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17.* Nature, 1998. 393(6686): p. 702-5.
219. Poorkaj, P., et al., *Tau is a candidate gene for chromosome 17 frontotemporal dementia.* Ann Neurol, 1998. 43(6): p. 815-25.
220. Spillantini, M.G., T.D. Bird, and B. Ghetti, *Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies.* Brain Pathol, 1998. 8(2): p. 387-402.
221. Spillantini, M.G., J.C. Van Swieten, and M. Goedert, *Tau gene mutations in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17).* Neurogenetics, 2000. 2(4): p. 193-205.
222. van Swieten, J. and M.G. Spillantini, *Hereditary frontotemporal dementia caused by Tau gene mutations.* Brain Pathol, 2007. 17(1): p. 63-73.
223. Rademakers, R., M. Cruts, and C. van Broeckhoven, *The role of tau (MAPT) in frontotemporal dementia and related tauopathies.* Hum Mutat, 2004. 24(4): p. 277-95.
224. Goedert, M., *Tau gene mutations and their effects.* Mov Disord, 2005. 20 Suppl 12: p. S45-52.
225. Goedert, M. and M.G. Spillantini, *A century of Alzheimer's disease.* Science, 2006. 314(5800): p. 777-81.
226. Lindwall, G. and R.D. Cole, *The purification of tau protein and the occurrence of two phosphorylation states of tau in brain.* J Biol Chem, 1984. 259(19): p. 12241-5.
227. Lichtenberg-Kraag, B., et al., *Phosphorylation-dependent epitopes of neurofilament antibodies on tau protein and relationship with Alzheimer tau.* Proc Natl Acad Sci U S A, 1992. 89(12): p. 5384-8.
228. Mulot, S.F., et al., *PHF-tau from Alzheimer's brain comprises four species on SDS-PAGE which can be mimicked by in vitro phosphorylation of human brain tau by glycogen synthase kinase-3 beta.* FEBS Lett, 1994. 349(3): p. 359-64.
229. Sergeant, N., et al., *Biochemistry of Tau in Alzheimer's disease and related neurological disorders.* Expert Rev Proteomics, 2008. 5(2): p. 207-24.
230. Sergeant, N., et al., *Two-dimensional characterization of paired helical filament-tau from Alzheimer's disease: demonstration of an additional 74-kDa component and age-related biochemical modifications.* J Neurochem, 1997. 69(2): p. 834-44.

231. Goedert, M., et al., *Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms.* Neuron, 1992. 8(1): p. 159-68.
232. Sergeant, N., A. Wattez, and A. Delacourte, *Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively "exon 10" isoforms.* J Neurochem, 1999. 72(3): p. 1243-9.
233. Tolnay, M., et al., *Argyrophilic grain disease and Alzheimer's disease are distinguished by their different distribution of tau protein isoforms.* Acta Neuropathol, 2002. 104(4): p. 425-34.
234. Vermersch, P., et al., *Specific tau variants in the brains of patients with myotonic dystrophy.* Neurology, 1996. 47(3): p. 711-7.
235. Yoshimura, N., *Alzheimer's neurofibrillary changes in the olfactory bulb in myotonic dystrophy.* Clin Neuropathol, 1990. 9(5): p. 240-3.
236. Yoshimura, N., et al., *Topography of Alzheimer's neurofibrillary change distribution in myotonic dystrophy.* Clin Neuropathol, 1990. 9(5): p. 234-9.
237. Maurage, C.A., et al., *Similar brain tau pathology in DM2/PROMM and DM1/Steinert disease.* Neurology, 2005. 65(10): p. 1636-8.
238. Games, D., et al., *Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein.* Nature, 1995. 373(6514): p. 523-7.
239. Mullan, M., et al., *A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid.* Nat Genet, 1992. 1(5): p. 345-7.
240. Citron, M., et al., *Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production.* Nature, 1992. 360(6405): p. 672-4.
241. Hsiao, K., et al., *Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice.* Science, 1996. 274(5284): p. 99-102.
242. Sturchler-Pierrat, C., et al., *Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology.* Proc Natl Acad Sci U S A, 1997. 94(24): p. 13287-92.
243. Winkler, D.T., et al., *Spontaneous hemorrhagic stroke in a mouse model of cerebral amyloid angiopathy.* J Neurosci, 2001. 21(5): p. 1619-27.
244. Sommer, B. and M. Staufenbiel, *A beta peptide deposition in the brains of transgenic mice: evidence for a key event in Alzheimer's disease pathogenesis.* Mol Psychiatry, 1998. 3(4): p. 284-6, 282-3.
245. Stalder, M., et al., *Association of microglia with amyloid plaques in brains of APP23 transgenic mice.* Am J Pathol, 1999. 154(6): p. 1673-84.
246. Bornemann, K.D., et al., *Abeta-induced inflammatory processes in microglia cells of APP23 transgenic mice.* Am J Pathol, 2001. 158(1): p. 63-73.
247. Calhoun, M.E., et al., *Neuron loss in APP transgenic mice.* Nature, 1998. 395(6704): p. 755-6.
248. Dumont, M., et al., *Spatial learning and exploration of environmental stimuli in 24-month-old female APP23 transgenic mice with the Swedish mutation.* Brain Res, 2004. 1024(1-2): p. 113-21.
249. Kelly, P.H., et al., *Progressive age-related impairment of cognitive behavior in APP23 transgenic mice.* Neurobiol Aging, 2003. 24(2): p. 365-78.

250. Van Dam, D., et al., *Age-dependent cognitive decline in the APP23 model precedes amyloid deposition.* Eur J Neurosci, 2003. 17(2): p. 388-96.
251. Probst, A., et al., *Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein.* Acta Neuropathol, 2000. 99(5): p. 469-81.
252. Brion, J.P., G. Tremp, and J.N. Octave, *Transgenic expression of the shortest human tau affects its compartmentalization and its phosphorylation as in the pretangle stage of Alzheimer's disease.* Am J Pathol, 1999. 154(1): p. 255-70.
253. Ishihara, T., et al., *Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform.* Neuron, 1999. 24(3): p. 751-62.
254. Ishihara, T., et al., *Age-dependent induction of congophilic neurofibrillary tau inclusions in tau transgenic mice.* Am J Pathol, 2001. 158(2): p. 555-62.
255. Duff, K., et al., *Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes.* Neurobiol Dis, 2000. 7(2): p. 87-98.
256. Andorfer, C., et al., *Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms.* J Neurochem, 2003. 86(3): p. 582-90.
257. Lewis, J., et al., *Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein.* Nat Genet, 2000. 25(4): p. 402-5.
258. Allen, B., et al., *Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein.* J Neurosci, 2002. 22(21): p. 9340-51.
259. Frank, S., F. Clavaguera, and M. Tolnay, *Tauopathy models and human neuropathology: similarities and differences.* Acta Neuropathol, 2008. 115(1): p. 39-53.
260. Bartus, R.T., et al., *The cholinergic hypothesis of geriatric memory dysfunction.* Science, 1982. 217(4558): p. 408-14.
261. Cummings, J.L. and C. Back, *The cholinergic hypothesis of neuropsychiatric symptoms in Alzheimer's disease.* Am J Geriatr Psychiatry, 1998. 6(2 Suppl 1): p. S64-78.
262. Bleich, S., et al., *Glutamate and the glutamate receptor system: a target for drug action.* Int J Geriatr Psychiatry, 2003. 18(Suppl 1): p. S33-40.
263. Hynd, M.R., H.L. Scott, and P.R. Dodd, *Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease.* Neurochem Int, 2004. 45(5): p. 583-95.
264. Kornhuber, J., et al., *Memantine displaces [3H]MK-801 at therapeutic concentrations in postmortem human frontal cortex.* Eur J Pharmacol, 1989. 166(3): p. 589-90.
265. Areosa, S.A., F. Sherriff, and R. McShane, *Memantine for dementia.* Cochrane Database Syst Rev, 2005(3): p. CD003154.
266. Areosa, S.A., F. Sherriff, and R. McShane, *Memantine for dementia.* Cochrane Database Syst Rev, 2005(2): p. CD003154.
267. Bentue-Ferrer, D., et al., *Clinically significant drug interactions with cholinesterase inhibitors: a guide for neurologists.* CNS Drugs, 2003. 17(13): p. 947-63.

268. Schenk, D., et al., *Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse.* Nature, 1999. 400(6740): p. 173-7.
269. Weiner, H.L., et al., *Nasal administration of amyloid-beta peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease.* Ann Neurol, 2000. 48(4): p. 567-79.
270. Bard, F., et al., *Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease.* Nat Med, 2000. 6(8): p. 916-9.
271. Janus, C., et al., *A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease.* Nature, 2000. 408(6815): p. 979-82.
272. Morgan, D., et al., *A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease.* Nature, 2000. 408(6815): p. 982-5.
273. DeMattos, R.B., et al., *Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease.* Proc Natl Acad Sci U S A, 2001. 98(15): p. 8850-5.
274. Orgogozo, J.M., et al., *Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization.* Neurology, 2003. 61(1): p. 46-54.
275. Schenk, D., *Amyloid-beta immunotherapy for Alzheimer's disease: the end of the beginning.* Nat Rev Neurosci, 2002. 3(10): p. 824-8.
276. Nicoll, J.A., et al., *Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report.* Nat Med, 2003. 9(4): p. 448-52.
277. Ferrer, I., et al., *Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease.* Brain Pathol, 2004. 14(1): p. 11-20.
278. Masliah, E., et al., *Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease.* Neurology, 2005. 64(1): p. 129-31.
279. Siemers, E., et al., *Safety, tolerability, and changes in amyloid beta concentrations after administration of a gamma-secretase inhibitor in volunteers.* Clin Neuropharmacol, 2005. 28(3): p. 126-32.
280. De Strooper, B. and J. Woodgett, *Alzheimer's disease: Mental plaque removal.* Nature, 2003. 423(6938): p. 392-3.
281. Weggen, S., et al., *A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity.* Nature, 2001. 414(6860): p. 212-6.
282. Citron, M., *Strategies for disease modification in Alzheimer's disease.* Nat Rev Neurosci, 2004. 5(9): p. 677-85.
283. Le Corre, S., et al., *An inhibitor of tau hyperphosphorylation prevents severe motor impairments in tau transgenic mice.* Proc Natl Acad Sci U S A, 2006. 103(25): p. 9673-8.
284. Noble, W., et al., *Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo.* Proc Natl Acad Sci U S A, 2005. 102(19): p. 6990-5.
285. Pickhardt, M., et al., *Screening for inhibitors of tau polymerization.* Curr Alzheimer Res, 2005. 2(2): p. 219-26.

286. Tolnay, M. and A. Probst, *The neuropathological spectrum of neurodegenerative tauopathies.* IUBMB Life, 2003. 55(6): p. 299-305.
287. Zhang, B., et al., *Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model.* Proc Natl Acad Sci U S A, 2005. 102(1): p. 227-31.
288. Asuni, A.A., et al., *Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements.* J Neurosci, 2007. 27(34): p. 9115-29.
289. Kane, M.D., et al., *Evidence for seeding of beta -amyloid by intracerebral infusion of Alzheimer brain extracts in beta -amyloid precursor protein-transgenic mice.* J Neurosci, 2000. 20(10): p. 3606-11.
290. Walker, L.C., et al., *Exogenous induction of cerebral beta-amyloidosis in betaAPP-transgenic mice.* Peptides, 2002. 23(7): p. 1241-7.
291. Meyer-Luehmann, M., et al., *Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host.* Science, 2006. 313(5794): p. 1781-4.
292. Radde, R., et al., *Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology.* EMBO Rep, 2006. 7(9): p. 940-6.
293. Geula, C., et al., *Aging renders the brain vulnerable to amyloid beta-protein neurotoxicity.* Nat Med, 1998. 4(7): p. 827-31.
294. Gotz, J., et al., *Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils.* Science, 2001. 293(5534): p. 1491-5.
295. Lewis, J., et al., *Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP.* Science, 2001. 293(5534): p. 1487-91.

2. LENTIVIRUS TAU (P301S) EXPRESSION IN ADULT AMYLOID PRECURSOR PROTEIN (APP)-TRANSGENIC MICE LEADS TO TANGLE FORMATION

Maribel Osinde[1*], Florence Clavaguera[1†], Rachel May-Nass[*], Markus Tolnay[†], and Kumlesh K Dev[*, ¥, §¶]

[*] Department of Neuroscience, Novartis Institutes for BioMedical Research, Novartis Pharma, Basel, Switzerland, [†] Pathology Institute, Neuropathology, 4031 Basel, Switzerland, [¥] Department of Anatomy, Molecular Neuropharmacology, University College Cork, Windle Building, Cork, Ireland, [§] Biosciences Institute, University College Cork, Cork, Ireland

[1] These authors contributed equally

Published in Neuropathology and Applied Neurobiology, 2008

[Epub ahead of print 14. Feb.]

ABSTRACT

AIMS: In this study we aimed to investigate the interaction between amyloid- and Tau-associated pathologies to gain further insights into the development of Alzheimer's disease. We examined the formation of neurofibillary tangles (NFT) in adult mouse brain without the prior overexpression of Tau at embryonic or early post-natal stages to dissociate any developmental mechanisms. METHODS: Lentivirus technology was used to examine the effects of overexpressing mutant Tau-P301S in the adult mouse brains of both wild-type and amyloid precursor-protein (APP)-transgenic mice. RESULTS: We find that injection of lentivirus Tau-P301S into the hippocampus of adult wild-type mice increases levels of hyperphosphorylated Tau, as early as 3 months post injection. However, no NFT are found even after 13 months of Tau expression. In contrast, the overexpression of Tau-P301S in adult APP-transgenic animals results in the formation of Gallyas stained NFT. CONCLUSIONS: Our current findings are the first to show that overexpression of Tau-P301S in adult mice overexpressing APP, but not wild-type mice, leads to enhanced Tau-related pathology.

INTRODUCTION

Tau is a microtubule-associated protein that polymerizes tubulin and is involved in intracellular trafficking [1-2]. This protein is located in axons under normal conditions but is hyperphosphorylated and found in cell bodies and dendrites in various Tauopathies including Alzheimer's disease (AD). Neurofibillary tangles (NFT) are composed of filaments of hyperphosphorylated Tau and the temporospatial spreading of NFT in AD correlates well with the cognitive decline [3]. In addition, mutations in the *Tau* gene are associated with a subset of frontotemporal dementias with parkinsonism linked to chromosome 17 (FTDP-17*T*) [4-6].

A number of Tau transgenic mouse models show that overexpression of wild-type Tau results in higher levels of its hyperphosphorylated form (so called the pretangle stage) as seen in human Tauopathies [7], but no or limited formation of NFT [8-12]. In contrast, robust NFT pathology develops in transgenic mice expressing a FTDP-17*T* linked P301L Tau mutant [13-14]. Additionally, abundant filaments made of hyperphosphorylated Tau can be observed in transgenic mice expressing the FTDP-17*T* linked P301S Tau mutant [15]. NFT are also found in transgenic mice overexpressing V337M Tau [16] and R406W Tau [17]. Not surprisingly, transgenic mice with double mutations in Tau (G272V, P301S) [18] or triple mutations (G272V, P301L, R406W) [19] also display NFT formation.

In AD pathogenesis, the 'Amyloid cascade hypothesis' holds that accumulation of Amyloid-β-peptide in the brain is an early and critical event that triggers a cascade of events leading to hyperphosphorylation and somatodendritic segregation of Tau, and formation of NFT [20]**.** To examine the interaction between Amyloid- and Tau-associated pathologies, two approaches have been used: (i) Amyloid precursor protein (APP)-transgenic animals have been mated with Tau-transgenic mice [21-23] and (ii) seeding studies with injected Aβ-containing preparations into Tau transgenic animals [21, 24]. Collectively these studies indicate that Tau-related pathology is augmented in the presence of abnormal levels of Aβ. Although conditional Tau transgenic animals exist, they have not been crossed with APP transgenics or used in seeding studies. These mice

express Tau P301L at an early age starting at P7 and develop NFT [25-26]. Discontinuing Tau expression in these mice in adult stages results in stabilisation of neuronal numbers and recovery of memory function but, surprisingly, NFT continue to accumulate [25].

To our knowledge, there has been no study that examines the effects of Aβ and/or APP on the formation of NFT without the overexpression of Tau at the embryonic or early postnatal stage. To dissociate any effects of Tau overexpression during development, we examined the effects of overexpressing Tau-P301S (a FTDP-17*T* linked mutant) starting at a much later age in adult brains of wild-type and APP-transgenic animals by starting expression at 6 months of age using lentivirus technology.

RESULTS

Distribution of lentivirus-mediated transgene expression – To determine the distribution of protein expression mediated by lentivirus in mouse brain, the expression of an EGFP transgene was evaluated under the control of the PGK promoter (LV-EGFP). When the LV-EGFP was delivered into the hippocampus, EGFP expression spread over a region of approximately 5 mm cubic area (Figure 1A). At the cellular level, EGFP expression occurred in cell bodies of pyramidal layers and the dentate gyrus as well as axons and dendrites (Figure 1A). On comparison, the injection of LV-EGFP into the corpus callosum gave a more extensive distribution which is likely explained by a spread of EGFP throughout the fibre tracts of the corpus callosum (Figure 1B). When injected into the corpus callosum, expression also reached adjacent cortical cells and the pyramidal cells of hippocampal subfield CA1 (Figure 1B). The EGFP expression was additionally found in the ventricular linings, most probably the ependymal cells, when injected into the corpus callosum (Figure 1B).

The LV-hTauP301S drives stable expression of Tau in neuronal cultures and in the hippocampus – Lentivirus-mediated expression of the human TauP301S (LV-hTauP301S) was driven by the PGK promoter, similar to LV-EGFP. The LV-hTauP301S was first tested for its ability to drive expression *in vitro*. CHO cells transduced with LV-hTauP301S stained positively with an anti-Tau T5 antibody 72h post-transduction (Figure 2A). In addition, rat cortical cultures (E18) transduced at DIV 7 with LV-hTauP301S showed immunoreactivity with a human specific anti-Tau T14 antibody at 48h (*data not shown*) and 96 hours post-transduction (Figure 2B). Furthermore, Western blot analysis of rat cortical cultures transduced with LV-hTauP301S showed expression of hTauP301S, where the expression level correlated with the amount of lentivirus used for transduction (Figure 2C).

The LV-hTauP301S was injected unilaterally into the hippocampus of wild-type mice and expression level of total hTauP301S was detected in lysates prepared from each isolated hippocampus by Western blotting using N-Tau5 (total Tau) and HT7 (human specific) antibodies (Figure 3). Hippocampi, injected and non-injected side, from the

same animals were used as positive and negative controls, respectively. There was an increase in total Tau expression as detected by the N-Tau5 antibody in the injected hippocampi as compared with the non-injected hippocampi. Accordingly, there was a dramatic increase in HT7 immunoreactivity in lysates prepared from injected hippocampi compared with non-injected, indicating the overexpression of hTauP301S. The expression of hyperphosphorylated Tau was also determined using the AT8 antibody (Figure 3). Levels of hyperphosphorylated Tau were markedly augmented in LV-hTauP301S injected side compared to non-injected side controls. The expression of hTauP301S remained strong as detected by all three antibodies until thirteen months post-injection compared to non-injected side controls (Figure 3).

Notably, the T14 and HT7 (human specific) antibodies showed weak immunoreactivity in non-infected neuronal cultures (Figure 2) and non-injected hippocampi (Figure 3), respectively. In the neuronal cultures, the non-transfected and transfected cells were prepared in different wells; the weak staining found in the non-transfected samples is likely due to cross-reactivity of the T14 antibody with rodent Tau (Figure 2). There is a possibility that the weak immunoreactivity found in non-injected hippocampal samples using the HT7 antibody was due to the partial spread of the virus in the mouse brain to the non-injected hippocampus (Figure 3). In light of the finding that all three N-Tau5, HT7 and AT8 antibodies showed increased immunoreactive bands in the LV-hTauP301S injected side, the increased immunoreactivity is likely a consequence of Tau overexpression and a subsequent increase in phosphorylated Tau levels, compared with the non-injected side.

Overexpression of hTauP301S in adult APP-transgenic mice promotes tangle formation – Histological analysis using the T14 antibody was performed to examine distribution of total hTauP301S expression after injection of LV-hTauP301S in wild-type animals (Figure 4A). Total hTauP301S expression started to appear at 2 months post injection and increased, remaining strong at 7 months (*not shown*) and 13 months post injection (Figure 4A). Injection of the LV-hTauP301S towards the CA3 region of the hippocampus resulted in the expression of hTauP301S predominantly in the CA3 subfield of the hippocampus and on occasion in the dentate gyrus (Figure 4A). The hTauP301S

expression was found in cell bodies and axonal tracts of the mossy fibres in the dentate gyrus. The AT8 antibody was used to determine the distribution of hyperphosphorylated Tau (Figure 4B). Few neurons expressing hyperphosphorylated Tau were found at 2 months post injection, which was abundant at 7 months (*not shown*) and also 13 months (Figure 4B). Hyperphosphorylated Tau was found predominantly in the areas of high total hTauP301S expression, mainly in the CA3 region. These data were in agreement with Western blotting results (Figure 3) showing that the number of neurons expressing hyperphosphorylated Tau was proportional to those expressing total hTauP301S levels.

To determine synergy between Aβ deposition and hyperphosphorylation or aggregation of Tau, the LV-TauP301S was injected into APP23-transgenic mice, which overexpresses human APP751 with the Swedish double mutation (K670N/M671L) under the control of a neuron-specific Thy-1 promoter [31]. Animals were injected with LV-hTauP301S (and with LV-EGFP for control groups) before onset of plaques at 6 months of age and examined 13 months post-injection, where plaque pathology was evident. The extent of Aβ deposition was examined using an antibody specific for Aβ40 (NT12 antibody). Plaques were detected mainly in the cortical area of APP23 mice whereas no staining was observed in the brain of wild-type animals (*not shown*). The expression and distribution of total human TauP301S (T14 antibody) was similar in wild-type (Figure 4A) and APP23 (Figure 4D) mice injected with LV-TauP301S, but more intense cytoplasmic AT8 staining was observed in APP23 mice **(**Figure 4E) when compared with wild-type animals (Figure 4B). No or very few NFT were detected using the Gallyas staining in wild-type animals injected with LV-TauP301S, suggesting that Tau pathology in these mice remained in a 'pretangle stage' (Figure 4C). In contrast, APP23 transgenic mice injected with LV-TauP301S revealed a high number of Gallyas positive cells indicating that formation of NFT is augmented in animals overexpressing APP (Figure 4F). No difference was found in the number of plaques in the hippocampus of APP23 transgenic mice injected with LV-TauP301S compared with those injected with LV-EGFP, indicating that expression of mutated Tau does not influence Aβ deposition (*not shown*).

DISCUSSION

To our knowledge, this is the first report showing that the overexpression of Tau starting in adult mouse brain leads to the formation of NFT in APP-transgenic mice but not wild-type mice. The development of NFT was seen at 13 months after expression of LV-TauP301S, occurring at a later age than other mouse models expressing mutated forms of Tau [13-19], possibly due to differences in expression levels and/or the fact that we avoid expression of Tau at embryonic or early post-natal stages, which may increase the rate development of NFT. The findings also show that directed overexpression of TauP301S can result in the development of tangles in the CA3 region of the hippocampus. These results are in agreement with previous reports, confirming that while APP and/or Aβ augments Tau pathology, the overexpression of Tau has little impact on plaque pathology.

The first Tau transgenic models that overexpressed wild-type Tau showed limited or no NFT pathology despite an elevated level of hyperphosphorylation of Tau, reminiscent of a 'pretangle' phenotype [8-12]. In contrast, a number of transgenic animals that overexpress pathogenic Tau mutants have been shown to develop NFT pathology. Two sets of studies, namely, the direct injection of Aβ fibrils and the overexpression of APP, support the notion that Aβ and/or APP can increase NFT formation. In particular, the injection of fibrillar Aβ42 into the cortex and the CA1 region of the hippocampus increases phosphorylation of Tau and the formation of NFT in P301L mice but not mice expressing wild-type Tau; the NFT are found in cell bodies of the amygdala, where the injected neurons are known to project [24]. To examine the interaction of amyloid- and Tau-associated pathologies, APP transgenic animals (Tg2576 mice) have been mated with Tau P301L-transgenic mice (JNPL3 mice) [22]. These double-transgenic animals (TAPP mice) develop both plaques and tangles and show that Tau overexpression has no effect on amyloid pathology while APP overexpression enhances NFT formation [22], in agreement with our findings. In TAPP mice, NFT develop by around 6 months of age at an increased number compared with P301L single-transgenic mice [22]. In a triple-transgenic model, the 3xTg-AD mice harbour mutations of APP (Swedish), presenilin PS1 (M146V), and Tau (P301L) and develop plaques in the neocortex at 3 months of age

and in the hippocampus by 6 months. NFT appear in the hippocampus at 12 months, then spread to the cortex [23]. In this mouse model, Aβ immunotherapy reduces extracellular Aβ plaques and intracellular Tau pathology. Tau clearance is thought to be mediated by the proteasome and to be dependent on the phosphorylation state of Tau; hyperphosphorylated Tau aggregates are unaffected by Aβ antibody treatment [35]. The regulation of Tau pathology by amyloid is in agreement with our current findings.

In summary therefore, a number of studies have shown that Tau expression (mutant forms) leads to NFT formation and that amyloid pathology increases the levels of NFT. These studies have used mainly transgenic animals where APP and/or Tau are expressed at ontogeny. In the current study we have shown the overexpression of mutant Tau (P301S) in the brains of adult transgenic mice overexpressing APP (but not wild-type mice) leads to NFT formation. Our studies support the notion that APP and/or Aβ can promote Tau-related pathology even in the adult brain, indicating that amyloid-induced NFT formation can be independent of embryonic and/or post-natal developmental mechanisms.

MATERIALS AND METHODS

Lentiviral vector and virus preparation – The vector was constructed based on the backbone of pLL3.7 [27] using standard molecular-cloning techniques. The cytomegalovirus (CMV) promoter was replaced by phospho-glycerate-kinase (PGK) promoter (obtained by polymerase chain reaction amplification of the PGK promoter from pSIREN-Retro Q vector) (BD biosciences, NJ, USA) generating the enhanced green fluorescent protein (EGFP) pLL4.0-PKG lentiviral (LV) transfer vector. The cDNA encoding the isoform (4R0N) [28] of the human Tau mutant P301S was then cloned into the pLL4.0-PGK vector (LV-hTauP301S) by replacing the EGFP with hTauP301S. The viral particles were produced as described previously for both LV-hTauP301S and LV-EGFP [29]. The viral suspension was concentrated by ultracentrifugation and re-suspended in phosphate buffer saline (PBS) with 2 % bovine serum albumin.

Analysis of Tau expression in cell cultures – Chinese hamster ovary (CHO) cells were cultured in HAM F12 media supplemented with 10% foetal calf serum and 1% penicillin and streptomycin (Invitrogen, Carlsbad, CA, USA). Cells were plated on glass coverslips in 12-well plates at $1.5x10^5$ cells per ml. Two days after, transduction was performed with LV-hTauP301S using one transfecting particle per cell. Three days post transduction, the cells were fixed in 4% paraformaldehyde in PBS (PFA), permeabilized in 0.2% Triton X-100 (TX-100) followed by incubation in blocking buffer (2% BSA and 5% goat serum in PBS) for 2h at room temperature before addition of primary antibody. Anti-Tau (Tau-5) antibody (Abcam, Cambridge, UK) was used (1:2000) for human Tau detection. Alexa fluor 488 anti-mouse (1:1000) (Molecular Probes, Invitrogen, CA, USA) was used as secondary antibody. Coverslips were mounted with Vectashield mounting media and pictures taken with an ORCA-AG CCD camera (Hamamatsu, Massy, France). Neuronal cortical cultures were prepared from embryonic day 18 Sprague Dawley rats as previously described [30]. The cortices were dissected in cold Hank's buffered salt solution (Invitrogen), and dissociated using trypsin for 10 min at 37°C. Cultures were plated onto poly-D lysine culture dishes (BD biosciences) or chamber slides (BD biosciences) and maintained at 37°C, 5% CO_2 in Neurobasal media (Invitrogen) with B27 supplement (Invitrogen). The neuronal cultures were transfected after 7 days *in vitro*

(DIV) and immunocytochemistry was performed 2-3 days after addition of lentivirus as described for CHO cells.

Stereotaxic Injection – Lentiviruses (either LV-EGFP or LV-hTauP301S) were stereotaxically injected into the right hippocampus of adult male C57Bl/6 mice (Charles River Laboratories, l'Arbresle, France), APP23-transgenic animals and wild-type littermates of 6 months of age [31]. Viral suspensions (1 µl volume) were injected with a 10 µl Hamilton syringe at a speed of 0.4 µl / min with an automatic injector (Stoelting, Wood Dale, IL, USA), and the needle was left in place for an additional 5 min before being withdrawn. Stereotaxic injections were delivered within the hippocampus with the following coordinates in millimeters: posterior, 1.75; lateral, 1.5; and ventral, 2.3. The posterior and lateral coordinates were calculated from the Bregma and the ventral coordinates were calculated from the skull surface. Animal studies were conducted in accordance with Swiss animal welfare authorities.

Histology – At the time points indicated post injection of lentivirus, animals were anaesthetized with sodium pentobarbital and transcardially perfused with saline and 4% PFA. Brains were removed, kept overnight at 4°C in 4% PFA and paraffin-embedded (Medite TPC 15 Duo, Nunningen, Switzerland). Sections of five microns were made with a microtome (Microm HM 340, Volketswil, Switzerland) and placed onto Superfrost glass slides (Menzel Gläser, Braunschweig, Germany). Sections were rehydrated and additionally heated at 90°C for 10-30 min in citrate buffer (Zymed, Invitrogen, Carlsbad, CA, USA) for epitope retrieval for T14 and AT8 antibodies. Permeabilization was performed in 0.2% TX-100 followed by incubation in blocking buffer (2% BSA and 5% horse serum in PBS) for two hours at room temperature before addition of primary antibodies. Primary antibodies to detect Tau expression were anti-human Tau T14 clone (1:1000) (Zymed) and anti-phosphorylated Tau AT8 (1:1000) (Pierce, Rockford, IL, USA) and to detect EGFP expression was anti-EGFP (1:1000) (Molecular Probes). Secondary antibody anti-mouse IgG conjugated to biotin (1:200) (Vector) was applied 1 hour at room temperature. Sections were stained with ABC kit (Vector) and AEC (Sigma, St Louis, MO, USA) following manufacturer instructions. Additional haematoxylin staining was performed to visualize nuclei and general morphology. After mounting

(Vectamount, Vector), sections were visualized with Zeiss microscope. Gallyas silver technique, which detects most NFT in human Tauopathies [32-34] was used to detect NFT in injected mouse brains.

Analysis of Tau expression in hippocampal samples – Thirteen months post-injection of lentivirus, mice were anaesthetized with sodium pentobarbital and decapitated. Brains were removed, left and right hippocampi were separately isolated and homogenized on ice. Lysis buffer containing 50 mM Tris, 150mM NaCl, 1% TX-100, 10% Glycerin, protease (Roche, Mannheim, Germany) and phosphatase inhibitor cocktails (Calbiochem, Merk, Nottingam, UK) was added at a ratio of 100 mg tissue/ml buffer and the homogenates were sonicated repeatedly at maximum 50 Watts. After 1h rotation on a wheel at 4°C, the total homogenates were aliquoted and stored at -80°C until used for Western blotting.

Western blotting – Chinease hamster ovary, neuronal cultures and hippocampal samples were run on NuPAGE 7% or 4-20% Tris-Acetate pre-cast gels (Invitrogen), transferred onto PVDF membrane (Invitrogen) and blocked with 5% non-fat milk powder in Tris-Buffered Saline supplemented with 0.05% Tween-20 (TBST). Membranes were then incubated overnight at 4°C with primary antibodies in 2.5% non-fat milk powder in TBST. Following washing in TBST, membranes were incubated for 1 hour in 2.5% non-fat milk powder in TBST with horseradish peroxidase (HRP)-conjugated secondary antibodies. When required, blots were stripped using Restore Western Blot Buffer (Pierce). The primary antibodies were: rabbit polyclonal anti-total Tau, N-Tau5 (1:2000; in-house antibody raised against a Tau peptide, amino acids 1-16) [35], mouse monoclonal anti-human Tau clone HT7 (1:1000; Innogenetics, Gent, Belgium), mouse monoclonal anti-phospho Tau clone AT8 (1:1000; Pierce); anti-human Tau T14 clone (1:1000; Zymed) and mouse monoclonal anti-βTubulin isotype III clone SDL.3D10 (1:2000; Sigma). The secondary antibodies were: goat anti-rabbit IgG (Sigma) and goat anti-mouse IgG (Sigma). Supersignal West Femto (Pierce) was used for HRP detection on Hyperfilm ECL (Amersham, Bucking-hamshire, UK).

ACKNOWLEDGMENTS

We wish to thank André Schade, Simone Danner and Dorothee Abramowski for technical assistance and Karl-Heinz Wiederhold for advice on histological analysis. We are also grateful to Dr. Matthias Staufenbiel for the kind gift of the APP23 transgenic animals and appreciate Dr. Peter Frey for insightful discussions. FC and MT are supported by the Swiss National Science Foundation (3100-068328).

REFERENCES

1. Weingarten M.D., Lockwood A.H., Hwo S.Y., Kirschner M.W., *A protein factor essential for microtubule assembly.* Proc Natl Acad Sci USA 1975; 72: 1858-1862
2. Friedhoff P., von Bergen M., Mandelkow E.M., Mandelkow E., *Structure of Tau protein and assembly into paired helical filaments.* Biochim Biophys Acta 2000; 1502: 122-132
3. Braak H., Braak E., *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol 1991; 82: 239-259
4. Hutton M., et al., *Association of missense and 5'-splice-site mutations in Tau with the inherited dementia FTDP-17.* Nature 1998; 393: 702-705
5. Bugiani O., *FTDP-17: phenotypical heterogeneity within P301S.* Ann Neurol. 2000; 48: 126
6. Van Swieten J., Spillantini M.G., *Hereditary frontotemporal dementia caused by Tau gene mutations.* Brain Pathol 2007; 17: 63-73
7. Tolnay M., Probst A., *The neuropathological spectrum of neurodegenerative Tauopathies.* IUBM Life 2003; 55: 299-305
8. Götz J., et al., *Somatodendritic localization and hyperphosphorylation of Tau protein in transgenic mice expressing the longest human brain Tau isoform.* EMBO J. 1995; 14: 1304-13
9. Probst A., et al., *Axonopathy and amyotrophy in mice transgenic for human four-repeat Tau protein.* Acta Neuropathol (Berl). 2000; 99: 469-81
10. Brion J.P., Tremp G., Octave J.N., *Transgenic expression of the shortest human Tau affects its compartmentalization and its phosphorylation as in the pretangle stage of Alzheimer's disease.* Am J Pathol 1999; 154: 255-270
11. Ishihara T., et al., *Age-dependent emergence and progression of a Tauopathy in transgenic mice overexpressing the shortest human Tau isoform.* Neuron 1999; 24: 751-762
12. Spittaels K., et al., *Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human Tau protein.* Am J Pathol 1999; 155: 2153-2165
13. Götz J., Chen F., Barmettler R., Nitsch R.M., *Tau filament formation in transgenic mice expressing P301L Tau.* J Biol Chem 2001; 276: 529-534
14. Lewis J., et al., *Neurofibillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) Tau protein.* Nat Genet 2000; 25: 402-405
15. Allen .B, et al., *Abundant Tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S Tau protein.* J Neurosci 2002; 22: 9340-9351
16. Tanemura K., et al., *Neurodegeneration with Tau accumulation in a transgenic mouse expressing V337M human Tau.* J Neurosci 2002; 22: 133-141
17. Tatebayashi Y., et al., *Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human Tau.* Proc Natl Acad Sci USA 2002; 99: 13896-13901

18. Schindowski K., et al., *Alzheimer's disease-like Tau neuropathology leads to memory deficits and loss of functional synapses in a novel mutated Tau transgenic mouse without any motor deficits.* Am J Pathol 2006; 169: 599-616
19. Lim F., et al., *FTDP-17 mutations in Tau transgenic mice provoke lysosomal abnormalities and Tau filaments in forebrain.* Mol Cell Neurosci 2001; 18: 702-714
20. Hardy J., Selkoe D.J., *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics.* Science 2002, 297: 353-356
21. Bolmont T., et al., *Induction of tau pathology by intracerebral infusion of Aβ-containing brain extract and by amyloid in APP x tau transgenic mice.* Am J Pathol. 2007; 171(6): 2012-20
22. Lewis J., et al., *Enhanced neurofibillary degeneration in transgenic mice expressing mutant Tau and APP.* Science 2001; 293: 1487-1491
23. Oddo S., et al., *Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction.* Neuron 2003; 39: 409-421
24. Götz J., Chen F., van Dorpe J., Nitsch R.M., *Formation of neurofibillary tangles in P301L Tau transgenic mice induced by Abeta 42 fibrils.* Science 2001; 293: 1491-1495
25. Santacruz K., et al., *Tau suppression in a neurodegenerative mouse model improves memory function.* Science 2005; 309: 476-481
26. Ramsden M., et al., *Age-dependent neurofibillary tangle formation, neuron loss, and memory impairment in a mouse model of human Tauopathy (P301L).* J Neurosci 2005; 25: 10637-10647
27. Rubinson D.A., et al., *A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference.* Nat Genet 2003; 33: 401-406
28. Spillantini M.G., Goedert M., *Tau protein pathology in neurodegenerative diseases.* Trends Neurosci. 1998; 21: 428–433
29. Deglon N., et al., *Self-inactivating lentiviral vectors with enhanced transgene expression as potential gene transfer system in Parkinson's disease.* Hum Gene Ther 2000; 11: 179-190
30. Osinde M., Mullershausen F., Dev K.K., *Phosphorylated FTY720 stimulates ERK phosphorylation in astrocytes via S1P receptors.* Neuropharmacology 2007; 52: 1210-1218
31. Sturchler-Pierrat C., et al., *Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology.* Proc Natl Acad Sci USA 1997; 94: 13287-13292
32. Uchihara T., *Silver diagnosis in neuropathology: principles, practice and revised interpretation.* Acta Neuropathol 2007; 113: 483-499
33. Tolnay M., Monsch A.U., Staehelin H.B., Probst A., *Argyrophilic grain disease: a disorder distinct from Alzheimer's disease.* Pathologe 1999; 20: 159-168
34. Gallyas F., *Silver staining of Alzheimer's neurofibrillary changes by means of physical development.* Acta Morphol Acad Sci Hung 1971; 19: 1-8
35. Oddo S., Billings L., Kesslak J.P., Cribbs D.H., LaFerla F.M., Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated Tau aggregates via the proteasome. Neuron 2004; 43: 321-332

FIGURE LEGENDS

Figure 1. PGK-EGFP lentivirus expression in mouse brain. Wild-type B6 mice were injected with 2µl lentivirus expressing EGFP under PGK promoter into the hippocampus. After two weeks, the animals were perfused with 4% PFA . 50 micron sections were analysed for EGFP expression by immunostaining using the EGFP antibody. (**A**) Above, strong EGFP signal was observed in the injected side of the hippocampus. Below, higher magnification of the hippocampus (just to precise) (**B**) Lentivirus was injected in the Corpus callosum showing a large spreading along the fibres tracts and some pyramidal neurons of CA1 as well as ependymal cells of the ventricles. Cortex (c), corpus callosum (cc), CA1/3 field of hippocampus (ca1/3), hippocampus (h), dentate gyrus (dg), ventricle (v), 2.5x and 5x magnification.

Figure 2. Lentivirus-hTauP301S mediates stable expression of Tau in cell cultures. (**A**) CHO cells were infected with lentivirus TauP301S for 48 hours, fixed and incubated with anti-Tau (T5) antibody. No endogenous Tau was detected in non-infected CHO cells while detectable expression was found in the infected cells. (**B**) Same transduction procedure was applied on primary cortical cultures incubated with anti-human Tau (T14) antibody. (**C**) Primary cortical culture lysates were analysed on Western blot with anti-human Tau (T14) antibody. Expression levels were proportional to the amount of transducing particles used. The amount of LV-hTauP301S used for transfection is indicated, none (non-transfected), 2 µl and 4 µl.

Figure 3. Lentivirus-hTauP301S mediates stable expression of Tau in the hippocampus. Hippocampi of wt mice were dissected 13 months post-injection and lysates of injected and non-injected side analysed by Western blot. Tau protein expression was measured with N-Tau5 that detects total Tau, HT7 for human Tau detection and AT8 for human hyperphosphorylated Tau. H1 and H2 are hippocampus from two different mice. Beta-tubulin showed equal protein amounts loaded.

Figure 4. Expression of Tau P301S in APP23 mice drives tangles formation. Images are from wild-type or APP23 mice sacrificed 13 months after injection with lentivirus

hTauP301S. T14 immunostaining (1:1000; x 100 magnification) showed the expression of human Tau P310S in the hippocampus of wild-type (**a**) and APP23 (**d**) mice. Hyperphosphorylated Tau was detected using the AT8 antibody (1:1000; x 100 magnification) in both wild-type (**b**) and APP23 (**e**) animals. Inserts in **b** and **e** (x630) show high magnification of AT8 positive neurons in the CA3 region of the hippocampus. Note that more intense cytoplasmic AT8 staining in the APP23 animal. Numerous Gallyas staining (x400 magnification, CA3 region of the hippocampus) neurofibrillary tangles were found in the APP23 (**c**) but not in the wild-type (**f**) mice. The figures shown are representatives of 5-6 animals for each condition.

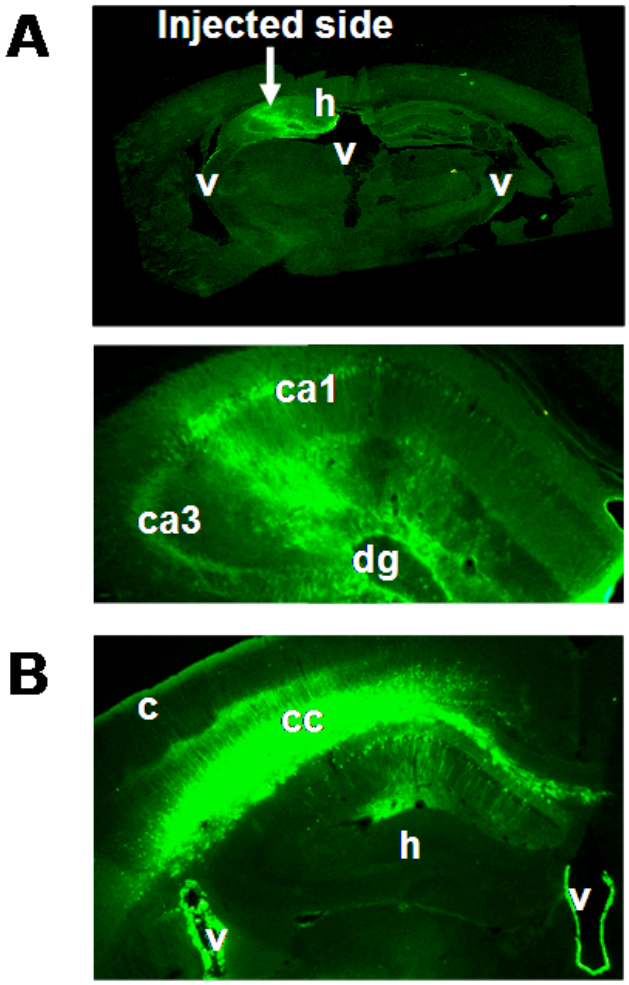


Figure 1.

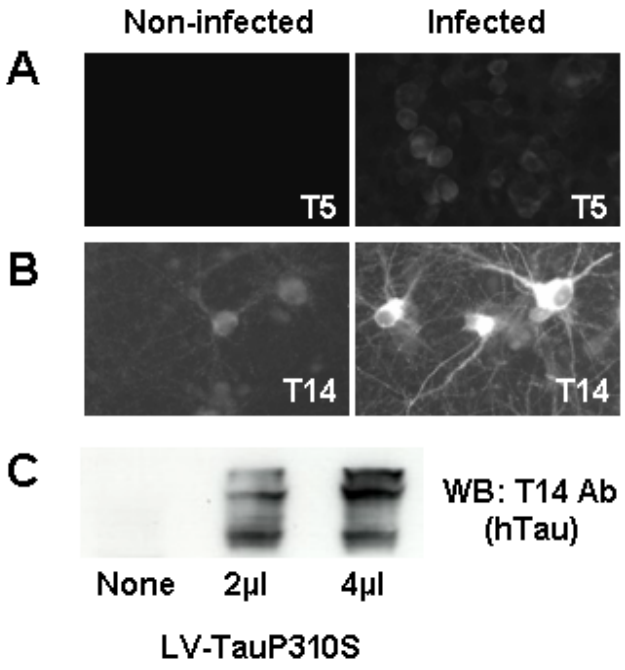

Non-infected
Infected
A
T5
T5
B
T14
T14
C
WB: T14 Ab
(hTau)
None
2µl
4µl
LV-TauP310S

Figure 2.

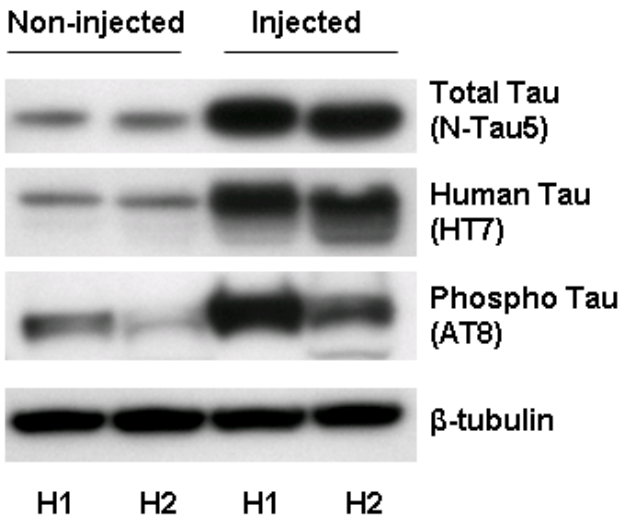

Non-injected
Injected
Total Tau
(N-Tau5)
Human Tau
(HT7)
Phospho Tau
(AT8)
β-tubulin
H1
H2
H1
H2

Figure 3.

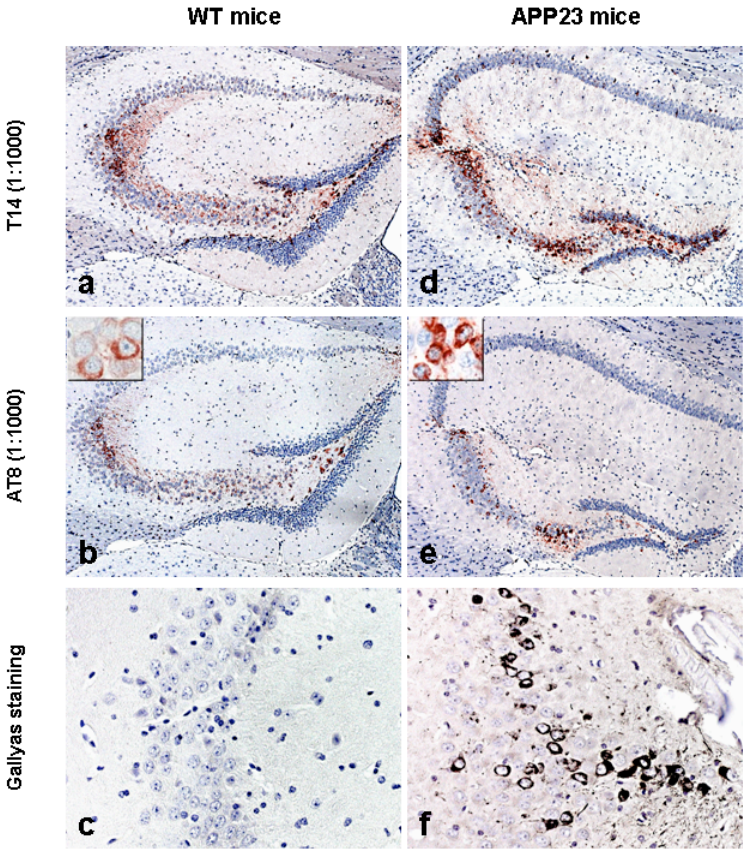

WT mice
APP23 mice
T14 (1:1000)
AT8 (1:1000)
Gallyas staining
a
b
c
d
e
f

Figure 4.

3. INDUCTION OF TAU PATHOLOGY BY INTRACEREBRAL INFUSION OF Aβ-CONTAINING BRAIN EXTRACT AND BY AMYLOID IN APP x TAU TRANSGENIC MICE

Tristan Bolmont[1*], Florence Clavaguera[2*], Melanie Meyer-Luehmann[1], Martin C. Herzig[1], Rebecca Radde[1], Matthias Staufenbiel[3], Jada Lewis[4], Mike Hutton[4], Markus Tolnay[2], Mathias Jucker[1]

[1]Department of Cellular Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Otfried-Müller Str. 27, D-72076 Tübingen, Germany; [2]Department of Neuropathology, Institute of Pathology, University of Basel, CH-4003 Basel, Switzerland; [3]Novartis Institutes for Biomedical Research Basel, CH-4002 Basel, Switzerland; [4]Department of Neuroscience, Mayo Clinic Jacksonville, Jacksonville, Florida, USA,

* These authors contributed equally

Published in American Journal of Pathology, 2007. 171(6): p. 2012-2020

ABSTRACT

Alzheimer's disease presents morphologically with senile plaques, primarily made of extracellular amyloid-β (Aβ) deposits, and neurofibrillary lesions, which consist of intracellular aggregates of hyperphosphorylated tau protein. To study the *in vivo* induction of tau pathology, dilute brain extract from aged Aβ-depositing APP23 transgenic mice were intracerebrally infused in young B6/P301L tau transgenic mice. Six months after the infusion, tau pathology was induced in the injected hippocampus but also in brain regions well beyond the injection sites such as the entorhinal cortex and amygdala, areas with neuronal projection to the injection site. No or only modest tau induction was observed when brain extracts from aged non-transgenic control mice and aged tau-depositing B6/P301L-transgenic mice were infused. To further study Aβ-induced tau lesions B6/P301L tau transgenic mice were crossed with APP23 mice. Although Aβ deposition in double-transgenic mice did not differ from single APP23-transgenic mice, double-transgenic mice revealed increased tau pathology compared to single B6/P301L tau-transgenic mice predominately in areas with high Aβ plaque load. The present results suggest that both extract-derived Aβ species and deposited fibrillary Aβ can induce the formation of neurofibrillary pathology. The observation that infused Aβ can trigger the tau pathology in the absence of Aβ deposits provides an explanation for the discrepancy between the neuroanatomical location of Aβ deposits and the development and spreading of tau lesions in Alzheimer's disease brain.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that is pathologically characterized by senile plaques, primarily composed of extracellular deposits of amyloid-β (Aβ) peptide, and neurofibrillary tangles, composed of intracellular aggregates of hyperphosphorylated tau protein [1]. The amyloid cascade hypothesis of AD pathogenesis holds that accumulation of polymerised forms of Aβ in the brain is an early and critical event that triggers a cascade of events leading to hyperphosphorylation and somatodendritic segregation of tau and formation of neurofibrillary tangles [2]. Consistent with this view, previous *in vivo* studies have revealed an interaction between Aβ and tau pathology in vulnerable brain regions [3-5].

Previously we have shown that intracerebral injection of dilute Aβ-containing human and murine brain extracts induces cerebral amyloidosis in young, predepositing amyloid precursor protein (APP23) transgenic mice [6]. In the present study we examined whether the same Aβ-containing brain extracts would also induce tau pathology in young B6/P301L mutated tau-transgenic mice and thus confirm and expand previous studies in which synthetic Aβ was intracerebrally infused in tau transgenic mice [4]. However, in contrast to this latter study in which a high concentration of fibrillary synthetic Aβ was injected and gave rise to amyloid deposits in the host at the injection site, the Aβ in the brain extract was at low concentration and did not form amyloid deposits in the injected host [6]. To complement this exogenous induction of tau pathology, we also crossbred B6/P301L tau transgenic mice with APP23 transgenic mice to study the induction of tau pathology by (genetic) expression of APP/Aβ. A similar cross breeding strategy has previously been reported to induce tau pathology in selected brain regions [3]. Our results suggest that both the infusion of an Aβ-containing extract and the deposition of fibrillary Aβ can induce the formation of neurofibrillary pathology.

RESULTS

Tau pathology in P301L tau transgenic mice on C57BL/6J background – On a mixed C57BL/DBA2/SW background, heterozygous P301L transgenic female mice develop tau pathology between 7 to 11 months of age, whereas disease onset in males is between 11 to 15 months [3,7]. To minimize immunological consequences and to minimize genetic variability in brain extract infusion studies and cross-breeding (see below), P301L transgenic mice were backcrossed to a C57BL/6J genetic background for at least seven times (thereafter termed B6/P301L mice; see Materials and Methods). Surprisingly, B6/P301L mice revealed a substantial delay in the development of tau pathology compared to the original P301L mice.

In female B6/P301L mice intracellular tau deposition (assessed with phosphorylation-dependent AT8 antibody) was not apparent in a group of 11- to 12-month-old mice (mean age 11.5 ± 0.3 month; n=5; indicated is the SEM) (Figure 1, A-E). At ~ 18 months of age, female B6/P301L mice became symptomatic and were unable to spread their hindlimbs when lifted by the tail. Within 1 to 3 months of disease onset, mice showed weight loss, and there were signs of dystonic posture. At that stage another group of mice (mean age, 19.8 ± 0.6 months; n = 6) was sacrificed and analyzed. Neuropathologically, similar to the original report of P301L mice on the mixed background [7], a substantial amount of hyperphosphorylated tau protein accumulated in affected neurons of the spinal cord, brain stem nuclei, and also of the hippocampus, entorhinal cortex, and amygdala (Figure 1, F-J). In the spinal cord, amygdala and brain stem (Figure 1, I, J), the majority (~ 80 to 90%) of these tau lesions were Gallyas-positive similar to neurofibrillary tangles found in AD brain. These changes were followed by axonal degeneration in descending fibre tracts of the spinal cord and by neurogenic atrophy in skeletal muscles (not shown), all overall similar as described previously in P301L mice on a mixed genetic background [7].

Male heterozygous B6/P301L mice did not show any clinical symptoms up to 30 months of age. Consistently, a group of 27 month-old B6/P301L mice (26.5 ± 1.1 months; n = 6) revealed only little tau pathology in the spinal cord and brain stem with no signs of tau lesions in other areas of the brain.

Characterization of murine brain extracts – Brain homogenates were prepared from the neocortex of aged, Aβ-depositing APP23 mice and an aged-matched non-transgenic control littermate (Figure 2A and B). Western blotting of the APP23 brain extract revealed Aβ levels of ~ 1 to 10 ng/μl with more Aβ1-40 compared to Aβ1-42 (Figure 2D) consistent with previous findings [6,9]. As an additional control, an extract from the brain stem and diencephalon of an aged B6/P301L transgenic mouse was used. The presence of tau lesions and of the shortest 4-repeat human tau isoform was demonstrated by immunohistochemistry and Western blotting (Figure 2, C and D).

Intracerebral injection of Aβ-containing brain extract induces tau pathology in B6/P301L tau transgenic mice – Extracts were unilaterally injected into the right hippocampus and overlying somatosensory cortex of young (5- to 6-month-old) heterozygous B6/P301L female mice. All mice were analyzed 6 months later, ie, when host mice were 11 to 12 months of age. At that age, uninjected B6/P301L female mice have not yet developed significant tau pathology (see Figure 1, A-E).

Intracerebral injection of the Aβ-rich APP23 extract induced robust deposition of tau throughout the injected hippocampus (assessed by phosphorylation-dependent AT8 antibody; Figure 3, A and B). In contrast injection of non-transgenic brain extract or PBS did not induce any tau lesions (Figure 3, C and E). Modest tau pathology was also induced with the B6/P301L extract, but this was not statistically significant (Figure 3 D and Figure 4A). APP23 extracts injected into non-transgenic mice did not induce any tau deposits (Figure 3F), indicating that the expression of human tau was necessary for the induction of tau lesions by the Aβ-containing extracts. All these observations were supported by quantitative analysis (Figure 4A).

No tau lesions were observed around the injection site of the neocortex. Tau pathology, however, was induced in brain regions farther away from the injection sites, such as the entorhinal cortex and the amygdala, but not brain stem (Figure 3A; Figure 4, B-E). In most regions tau induction appeared confined to the injected hemisphere. However, in the amygdala the induction, albeit to a lesser degree, also occurred contralateral to the injection site (Figure 4C).

Immunostaining for Aβ at any time after the infusion of the Aβ-containing extracts did not reveal any Aβ deposition (data not shown), consistent with previous studies [6]. Gliosis was limited to the needle track and was similar among all mice independent whether of they were injected with PBS or brain extracts.

Increased tau pathology in double APP23 x B6/P301L tau transgenic mice – B6/P301L tau transgenic mice were crossed with APP23 transgenic mice. Before Aβ deposition, double-transgenic B6/P301L x APP23 mice did not exhibit increased tau pathology compared to the single transgenic B6/P301L littermate control mice (results not shown). However, at the time of significant amyloid deposition, a robust increase of tau pathology (assessed by phosphorylation-dependent AT8 antibody) in the entorhinal cortex, hippocampus, and to a lower extent in the amygdala but not in brain stem regions was noted in the double transgenic B6/P301L x APP23 mice compared to the single transgenic B6/P301L control mice. This was true for the female double-transgenic mice (21.6 ± 0.9 months old; data not shown) and particularly appreciable in male double-transgenic mice (27.6 ± 0.7 months old; Figure 5A) since single-transgenic male B6/P301L tau mice do not develop tau pathology up to this age (Figure 5B). In the entorhinal cortex and hippocampus, a subset (~ 50 %) of these induced tau lesions was Gallyas-positive (Figure 5I). Stereological analysis of the amyloid load did not reveal any differences between double B6/P301L x APP23 and single-transgenic APP23 male mice. Notably, however, there was a great region-specific variability of amyloid deposition with neocortex and hippocampus revealing the highest amyloid load, followed by the amygdala and the brain stem, with the latter showing virtually no amyloid deposition (Figure 5D). Tau lesions were typically induced in vicinity of congophilic plaques in the entorhinal cortex and hippocampus (Figure 5, E and F); however, in the amygdala there was a clear separation with induced tau lesions in the basolateral part and amyloid deposition in the dorso- and ventrolateral parts (Fig. 5, H). In areas not prone to develop tau pathology in B6/P301L mice, such as the piriform and somatosensory cortex, there was no or only modest tau pathology induction in the double-transgenic mice despite a high plaque load (Figure 5G). In all mice congophilic amyloid deposits were surrounded

by a robust activation of microglia and astroglia (not shown) similar to previous results in APP23 mice [8,14].

DISCUSSION

The present study was undertaken to confirm and expand previous *in vivo* studies demonstrating a link between Aβ and the induction of neurofibrillary pathology [3,4]. We have shown that tau pathology can be induced in the brain of young B6/P301L tau-transgenic mice by a single injection of a dilute Aβ-containing extract, of a transgenic APP23 mouse brain, but not by PBS, non-transgenic control extract or extract from a transgenic B6/P301L mouse brain with tau lesions. These observations suggest that Aβ is key to an effective induction of the tau pathology.

A similar induction of tau pathology, albeit only in the amyloid, has been described after the infusion of synthetic Aβ1-42 into the somatosensory cortex and hippocampus of a tau transgenic mouse model [4]. However, several important differences between the two studies emerge. i) Gotz and colleagues [4] injected fibrillary synthetic Aβ at a high concentration (1 μg/μl) and the injected Aβ was readily detectable as amyloid and persisted at the injection sites. In contrast, a highly dilute extract, with a 100- to 1000-fold lower Aβ concentration (1 to 10 ng/μl) was used in the present study. ii) Gotz and colleagues [4] used as host a mouse model (albeit with the same P301L mutation) that develops tau pathology earlier (5 to 6 months of age) than the B6/P301L mouse model used in the present study. This may explain the induction of tau lesions already 3 weeks after the injection whereas in the present study analysis was done 6 months post-infusion. iii) Gotz and colleagues, whereas reported that the induced tau lesions were Gallyas-positive whereas the majority of the induced tau-lesion in the present study were Gallyas-negative and of 'pretangle' stage. Again, this difference may be explained by the difference in susceptibility of developing neurofibrillary lesions of the two transgenic hosts or the concentration of the injected Aβ.

We have previously shown that extracts identical to the APP23 extracts have potent Aβ-amyloid-inducing activity whereas synthetic Aβ did not reveal similar activity [6]. From these results we suggested that the amyloid-inducing activity in brain represents a brain-specific conformation or modification of Aβ that is different from synthetic Aβ. Thus

from this latter study and the work of Gotz and colleagues [4], a dissociation of the Aβ-amyloid-inducing activity and tau lesion-inducing activity of Aβ species could be speculated, ie, synthetic Aβ is sufficient for the induction of the tau pathology but not for the induction of Aβ-amyloid [6]. However, because of the above mentioned differences between the present study and that of Gotz and colleagues [4], the conclusion that synthetic Aβ and Aβ-containing brain extract are equally potent inducers of tau pathology appears premature. Thus, to rigorously test the hypothesis of a dissociation of the amyloid-inducing activity of Aβ from the neurofibrillary-inducing activity of Aβ, future studies will be necessary in which brain extract versus synthetic Aβ will be tested in the same transgenic model.

The observation that tau lesions were induced not only at the hippocampal injection site, but also in remote areas such as entorhinal cortex and amygdala, and at the contralateral sites might be explained by the axonal projections of these brain regions to the injection site [4,15]. Various Aβ species are known to interact with synapses and to affect adversely their structure and function [16], which in turn may lead to retrograde signaling, damage and induction of tau lesions in the remote cell bodies. It is also possible that Aβ is taken up and transported retrogradely to the soma where Aβ interacts with tau and induces tau phosphorylation and aggregation [17-19]. Independent of the exact mechanism, the idea of synaptic damage with retrogradely induced tau lesions might at least partly explain why induction of tau lesions was found in the entorhinal cortex and amygdala but not in the brain stem, which exhibits only diffuse projection to the injection sites. The lack of induction of tau pathology in the somatosensory cortex may simply be a result of low susceptibility of this region to form tau lesions in such tau transgenic mice [4,7,20]. In addition, or alternatively to such a retrograde model of tau pathology propagation, the infused Aβ may have been transported via blood and/or via interstitial fluid pathways to the contralateral site and remote areas [21,22].

Tau pathology was also induced in B6/P301L transgenic mice when crossed with APP23 mice, confirming a previous study in which the same tau transgenic mouse model, albeit on a mixed genetic background, was crossed with Tg2576 transgenic mice [3]. Although in the infusion experiment exogenously applied Aβ species have triggered the tau

pathology, in the double B6/P301L X APP23 transgenic mice, it may be argued that the tau lesions were initiated by an intraneuronal (indirect) interaction of tau and Aβ, because both the Thy1 promoter (in case of the APP23 mice) and PrP promoter (in case of the B6/P301L mice) are expressed to a great extent in similar neuronal populations [23,24]. However, because the induction of the tau lesions appeared concomittant with the appearance of significant cerebral amyloidosis, it is more likely that the extracellular deposition of amyloid is the trigger of the neurofibrillary pathology.

The idea of a local induction of the tau pathology by the amyloid deposits is supported by the current region-specific analysis of tau pathology induction versus amyloid load. The most robust induction of tau pathology was in the entorhinal cortex, followed by hippocampus, amygdala, and brain stem. Consistently, we found the highest plaque load in the entorhinal cortex, followed by the hippocampus, the amygdala, and brain stem. However, consistent with previous findings [3], neurofibrillary tangle bearing neurons were not necessarily in close vicinity of the amyloid deposits exemplified in the basolateral amygdala, with a robust induction of tau pathology but virtually no amyloid deposition. These observations suggest that in the double transgenic mice tau induction in areas without amyloid aggregates may be triggered from remote areas with amyloid deposits similar to the suggested mechanism with the Aβ-containing brain extracts.

Although the infusion experiment strongly suggests Aβ species as being the trigger of the induced tau pathology in remote areas, the mechanism of 'local' tau induction in the crossed mice is more open for speculation. Congophilic amyloid deposition is always accompanied by a strong neuroinflammatory response [14,25,26] and microglia activation has recently been linked to the progression of tau pathology in transgenic mice [27]. It has also been shown that congophilic plaques induce local axonal dystrophy and synaptic abnormalities [15,28], which in turn may promote tau aggregation.

In conclusion, our results suggest that Aβ can induce neurofibrillary pathology by different mechanisms. In particular the observation that infused Aβ can trigger the tau pathology in the absence of amyloid deposits provides an explanation for the discrepancy between the neuroanatomical location of amyloid deposits, or absence thereof, and the development and spreading of tau lesions in AD brain.

MATERIALS AND METHODS

Transgenic mice – Hemyzygous B6/P301L transgenic mice and non-transgenic wild-type littermate controls were used. B6/P301L mice were obtained by backcrossing the original P301L transgenic mice (JNPL3 mice) [7] with C57BL/6J mice for at least seven generations. JNPL3 mice express the shortest four-repeat (4R0N) tau with the P301L mutation under control of the mouse prion promoter. B6/P301L transgenic mice were also crossbred with APP23 transgenic mice expressing human KM670/671NL mutant APP [8].

Stereotaxic surgery – Host mice were anaesthetized with a mixture of ketamine (10 mg/kg body weight) and xylazine (20 mg/kg body weight) in saline. Unilateral stereotaxic injections of 2.5 µl brain extracts were placed with a 5-µl Hamilton syringe into the right neocortex (A/P, –2.5 mm from bregma; L, –2.0 mm; D/V, –0.8 mm) and hippocampus (A/P, –2.5 mm from bregma; L, –2.0 mm; D/V, –1.8 mm). Injection speed was 1.25 µl/minute, and the needle was kept in place for an additional 3 minutes before it was slowly withdrawn. The surgical area was cleaned with sterile saline, and the incision was sutured. Operated animals were monitored weekly in their cages for signs of behavioural changes. No seizure activity was apparent during or after the infusion. All experiments were in compliance with protocols approved by the local animal care and use committee.

Preparation of brain extract – Brain extracts were prepared by first dissecting out the neocortex of a 24 month-old APP23 transgenic female mouse (APP23 extract) and an aged matched female non-transgenic littermate control. Brain extract was also prepared from the brainstem and diencephalon of a 21,5 month-old B6/P301L transgenic female mouse (B6/P301L extract). Tissue samples were frozen and stored at –80°C until use. The tissue pieces were then homogenized at 10% (w/v) in sterile phosphate-buffered saline (PBS), vortexed, sonicated for 5 seconds, and centrifuged at 3000 X *g* for 5 minutes as previously described [6]. The supernatant was recovered and immediately frozen (-80°C).

Western blot analysis of brain homogenates – To determine Aβ levels in the brain homogenates [9], samples were diluted 1 to 4 in sample buffer [0,48 mol/l Bis-Tris, 0,21 mol/l Bicine, 1,32 % (w/v) sodium dodecyl sulfate, 20 % (w/v) sucrose, 3,33 % (v/v) 2-mercaptoethanol, 0,0053 % (w/v) bromophenol blue]. Samples were then subjected to 10 % Bicine-Tris 8 mol/l urea sodium dodecyl sulfate-polyacrilamide gel electrophoresis [10]. Synthetic Aβ1-40 and 1-42 (Bachem, Bubendorf, Switzerland) were used as controls. Proteins were transferred onto a polyvinylidene difluoride membrane and probed with monoclonal antibody 6E10 specific to human Aβ (diluted 1:500; Covance, Emeryville, CA). The secondary antibody was horseradish peroxidase-conjugated goat anti-mouse IgG (Chemicon, Temecula, CA). Bands were visualized using SuperSignal (Pierce, Rockford, IL) and developed onto Kodak X-OMAT AR film (Eastman-Kodak, Rochester, NY).

To determine tau levels in the brain homogenates, samples were dissolved in sample buffer and run on 10 % sodium dodecyl sulfate-polyacrilamide gel electrophoresis Tris-tricine gels. Proteins were electrophoretically transferred onto a polyvinylidene difluoride membrane and probed with monoclonal antibody HT7 (diluted 1:1000, recognizes amino acids 159 to 163 of tau phosphorylated and non phosphorylated epitopes; Pierce). The secondary antibody was horseradish peroxidase-conjugated sheep anti-rabbit IgG (NA931; Amersham-Biosciences, Uppsala, Sweden). Bands were visualized using SuperSignal and developed onto Kodak X-OMAT AR film.

Histology and immunohistochemistry – Host mice were deeply anesthetized and they were perfused transcardially with 4% paraformaldehyde in sodium phosphate buffer (pH 7.4) and processed for paraffin embedding. Immunohistological stainings were done on 5-µm coronal paraffin sections throughout areas of interest (see quantification), according to standard procedures [11]. The following antibodies were used: monoclonal AT8 antibody (1:1000; Innogenetics, Gent, Belgium) specific to tau phosphorylated at ser-202 and thr-205; polyclonal anti-Aβ antibody NT12 (1:1000; courtesy of P. Paganetti, Basel, Switzerland) or DW6 (1:1000; courtesy of D. Walsh, Dublin, Ireland); polyclonal antibody to GFAP (1:20000; Sigma Aldrich); and polyclonal antibody to Iba-1 (1:1000; Wako, Neuss, Germany). Secondary antibodies were obtained from Vector Laboratories,

Burlingame, CA (Vectastain ABC kits), for peroxidase staining. In addition sections were stained histologically with silver-impregnation by the Gallyas protocol [12].

Stereological assessment of tau deposition – For selection of sampling sites for the estimation of tau-immunopositive area fraction in the brain regions of interest stereological sampling rules were applied [13]. Every 72th section through the hippocampus (from positions AP -1.0 mm to AP – 4.0 mm from bregma), entorhinal cortex (AP -2.5 mm to -4.0 mm), sensorimotor cortex (AP -1.0 mm to AP -3.5 mm), amygdala (AP -1.0 mm to AP -3.5 mm), and brainstem (AP -4.0 mm to AP -5.0 mm) was immunostained with AT8. Therefore, eight to nine sections through the hippocampus, four to five through the entorhinal cortex, six to seven through the sensorimotor cortex and amygdala, and two to three through the brain stem were analyzed per animal. The brain regions were delineated at low magnification according to cytoarchitectural criteria (Paxinos and Franklin, 1997). Within a single section, a systematic random sampling scheme was performed to capture video images for measurement of AT8-positive staining (percent area). All images were captured with an X40 objective using a Zeiss Axioskop microscope (Zeiss, Göttingen, Germany) with a motorized x-y-z stage coupled to a Sony CCD-IRIS color camera. To analyze the individual images for AT8-positive burden, 24-bit color images (20 to 50 images per brain region) were converted to 8-bit gray scale images by using conversion algorithms associated with Adobe Photoshop CS (Adobe, San Jose, CA). Calculation of the percent area occupied by tau immunopositive area fraction was performed using the public domain NIH ImageJ. Statistical analysis included analysis of variance and post-hoc testing with the help of Statistica 5.0 (StatSoft, Tulsa, OK).

Stereological assessment of amyloid deposition – To assess the burden of Aβ deposition in brains, we have used the same stereological sampling as described above for the assessment of AT8-positive deposits. Briefly, every 144th section through the hippocampus (from positions AP -1.0 mm to AP -4.0 mm from bregma), neocortex (AP -1.0 mm to AP -4.0 mm) amygdala (AP -1.0 mm to AP -3.5 mm) and brain stem (AP -4.0 mm to AP -5.5 mm) was immunostained with DW6. Therefore, four to five sections

through the hippocampus and neocortex, three to four through the amygdala and one to two through the brain stem were analyzed per animal. Using a systematic random sampling scheme, images were captured with an X20 objective and converted to 8-bit gray scale for calculating the percent area occupied by Aβ immunoreactive pixels. Statistical analysis included analysis of variance and post-hoc testing with the help of Statistica 5.0.

ACKNOWLEDGEMENTS

We thank Michael Calhoun (Tübingen, Germany) for helpful discussions and P. Paganetti (Basel, Switzerland) and D. Walsh (Dublin, Ireland) for the generous donation of antibodies.

REFERENCES

1. Goedert M., Spillantini M.G., *A century of Alzheimer's disease*. Science 2006, 314:777-781
2. Hardy J., Selkoe D.J., *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics*. Science 2002, 297:353-356
3. Lewis J., Dickson D.W., et al., *Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP*. Science 2001, 293:1487-1491
4. Gotz J., Chen F., van Dorpe J., Nitsch R.M., *Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils*. Science 2001, 293:1491-1495
5. Oddo S., Billings L., Kesslak J.P., Cribbs D.H., LaFerla F.M., *Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome*. Neuron 2004, 43:321-332
6. Meyer-Luehmann M., et al., *Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host*. Science 2006, 313:1781-1784
7. Lewis J., et al., *Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein*. Nat Genet 2000, 25:402-405
8. Sturchler-Pierrat C., et al., *Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology*. Proc Natl Acad Sci U S A 1997, 94:13287-13292
9. Herzig M.C., et al., *Abeta is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis*. Nat Neurosci 2004, 7:954-960
10. Wiltfang J., et al., *Improved electrophoretic separation and immunoblotting of beta-amyloid (A beta) peptides 1-40, 1-42, and 1-43*. Electrophoresis 1997, 18:527-532
11. Bondolfi L., et al., *Amyloid-associated neuron loss and gliogenesis in the neocortex of amyloid precursor protein transgenic mice*. J Neurosci 2002, 22:515-522
12. Gallyas F., *Silver staining of Alzheimer's neurofibrillary changes by means of physical development*. Acta Morphol Acad Sci Hung 1971, 19:1-8
13. West M.J., Slomianka L., Gundersen H.J., *Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator*. Anat Rec 1991, 231:482-497
14. Stalder M., et al., *Association of microglia with amyloid plaques in brains of APP23 transgenic mice*. Am J Pathol 1999, 154:1673-1684
15. Phinney A.L., et al., *Cerebral amyloid induces aberrant axonal sprouting and ectopic terminal formation in amyloid precursor protein transgenic mice*. J Neurosci 1999, 19:8552-8559
16. Haass C., Selkoe D.J., *Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide*. Nat Rev Mol Cell Biol 2007, 8:101-112
17. Busciglio J., Lorenzo A., Yeh J., Yankner B.A., *beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding*. Neuron 1995, 14:879-888

18. Walker L.C., et al., *Exogenous induction of cerebral beta-amyloidosis in betaAPP-transgenic mice*. Peptides 2002, 23:1241-1247
19. Guo J.P., Arai T., Miklossy J., McGeer P.L., *Abeta and tau form soluble complexes that may promote self aggregation of both into the insoluble forms observed in Alzheimer's disease*. Proc Natl Acad Sci U S A 2006, 103:1953-1958
20. Allen B., et al., *Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein*. J Neurosci 2002, 22:9340-9351
21. Cirrito J.R., et al., *In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life*. J Neurosci 2003, 23:8844-8853
22. Zlokovic B.V., *Neurovascular mechanisms of Alzheimer's neurodegeneration*. Trends Neurosci 2005, 28:202-208
23. Borchelt D.R., et al., *A vector for expressing foreign genes in the brains and hearts of transgenic mice*. Genet Anal 1996, 13:159-163
24. Calhoun M.E., et al., *Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid*. Proc Natl Acad Sci U S A 1999, 96:14088-14093
25. Akiyama H., et al., *Inflammation and Alzheimer's disease*. Neurobiol Aging 2000, 21:383-421
26. Stalder A.K., et al., *Invasion of hematopoietic cells into the brain of amyloid precursor protein transgenic mice*. J Neurosci 2005, 25:11125-11132
27. Yoshiyama Y., et al., *Synapse Loss and Microglial Activation Precede Tangles in a P301S Tauopathy Mouse Model*. Neuron 2007, 53:337-351
28. Tsai J., Grutzendler J., Duff K., Gan W.B., *Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches*. Nat Neurosci 2004, 7:1181-1183

FIGURES LEGENDS

Figure 1. Tau pathology in heterozygous P301L tau transgenic mice on the genetic C57BL6/J background (B6/P301L). (**A, F**) Overview picture taken from an AT8-stained section shows the distribution of tau pathology in female B6/P301L mice at 12 months (**A**) and 21 months (**F**) of age. (**B-E**) and (**G-J**) are high magnification pictures taken from (**A**) and (**F**) and reveal in the 21 month-old but not 12 month-old mice a substantial amount of tau lesions in the hippocampus (**B**, **G**; box top middle in **A** and **F**), lateral entorhinal cortex (**C**, **H**; box bottom right in **A** and **F**), amygdala (**D**, **I**) and deep mesencephalic nucleus (**E**, **J**; box top left in **A** and **F**). Inserts in **I** and **J** show Gallyas-positive tangles, indicating that a large subset of the accumulating tau proteins form insoluble aggregates. Bar in (**G**) is 100 μm. All panels have same magnification.

Figure 2. Histological and biochemical analysis of the injected brain extracts. (**A**) Aβ-immunostaining of neocortex of a 24 month-old APP23 transgenic mouse, similar to the one from which the extract was prepared, reveals extensive cerebral amyloid deposition. (**B**) No amyloid was found in a non-transgenic age-matched littermate control brain. (**C**) AT8-immunostaining of the brainstem of a female 21 month-old B6/P301L transgenic mouse, similar to the one from which the tau extract was prepared, reveals numerous tau-positive deposits. Bar is 50 μm. All panels have the same magnification. (**D**) Left: Immunoblotting with antibody 6E10 specific to human Aβ: *lane 1*, synthetic Aβ1-40 and 1-42 (1ng/μl each); *lane 2*, APP23 brain extract, *lane 3*, non-transgenic APP23 littermate control. Right: Western blotting analysis with HT7 antibody specific to human tau: *lane 1*, AD brain extract reveals the various human tau isoforms (arrowheads); *lane 2*, B6/P301L brain extract shows the transgenic 4R0N human isoform.

Figure 3. Intracerebral injection of Aβ-containing mouse brain extract induces tau pathology in the hippocampus of young B6/P301L tau transgenic mice. Young 5-6 month-old B6/P301L transgenic mice and control littermates were injected unilaterally in the hippocampus and overlying neocortex with various brain extracts and analyzed 6 months later. (**A**) Overview picture taken from an AT8-stained section shows the pattern

of induced tau deposits throughout the injected hippocampus but also entorhinal cortex (asterisk) of a B6/P301L transgenic mouse injected with the APP23 brain extract (the injected site is the right one in the panel). (**B**) The injected hippocampal area, box in A, is shown in higher magnification. (**C**) Injection with the non-transgenic control extract did not induce tau pathology in the hippocampus. (**D**) Modest induction of tau pathology was seen with the B6/P301L extract, while (**E**) no induced tau deposits were observed following PBS injection. (**F**) When Aβ-containing APP23 extract was injected into a non-transgenic B6/P301L mouse no tau lesions were induced. Bar is 100 μm.

Figure 4. Tau pathology induction in various regions in B6/P301L tau tg mice. (**A**) Stereological analysis of % area occupied by AT8-positive staining (two-way ANOVA, extract x hemisphere) in the hippocampus (HPS) confirms the significant induction of tau deposition by the APP23 extract compared to PBS ($^{+}$ n=5/group; $p<0.0001$; Newmann-Keuls postdoc tests; indicated is SEM) and compared to the contralateral site (* $p<0.0001$). Tau induction also occurred in some brain regions away from the injection site, such as (**B**) the entorhinal cortex (ENT) and (**C**) the amygdala (AMG). Analysis revealed again significant induction of the APP23 extract compared to PBS ($^{+}$ n=5/group, $p<0.0001$) and to the contralateral site (* $p<0.05$) which, for the amygdala also showed some induction compared to PBS ($p<0.001$). No induced tau pathology was found in the sensorimotor cortex (CTX) and brain stem (BS) (n=5/group; $p>0.05$). APP23=extract from an aged APP23 mouse brain; WT=wild-type non-transgenic extract; P301L=extract from an aged B6/P301L mouse brain.

Figure 5. Induced tau lesions in B6/P301LxAPP23 mice. (**A, B**) AT8-stained coronal sections show tau pathology in the entorhinal cortex and adjacent CA1 field of an aged male 28 mo-old double transgenic B6/P301L x APP23 mouse (**A**) while no pathology was observed in a male aged-matched B6/P301L mouse (**B**). (**C**) Stereological analysis of tau immunoreactivity (AT8 antibody) in the entorhinal cortex (ENT), hippocampus (HPS), and amygdala (AMG) revealed tau induction in B6/P301L x APP23 transgenic animals (n=7) compared to both single transgenic B6/P301L mice (n=7) and to APP23 littermates (n=4) (one-way ANOVA for genotype: * ENT, $p<0.01$; HPS, $p<0.05$; AMG,

$p<0.001$; indicated is SEM) but not in the brain stem. (**D**) Stereological analysis of amyloid load in the neocortex (CTX), hippocampus (HPS), amygdala (AMG) and brain stem (BS) revealed no difference between the double transgenic mice and the single APP23 transgenic littermates in any of the brain region analyzed ($p>0.05$). (**E-H**) High magnification of an adjacent section to (**A**) double immunostained for AT8 and Aβ shows the distribution of tau pathology in close vicinity of amyloid deposits in the entorhinal cortex (**E**), and hippocampus (**F**) (see also boxed areas in **A**). In the pirform cortex (**G**), and dorso- and ventrolateral amygdala (asteriks in **H**) no significant induction of tau pathology was observed despite robust amyloid load. Notably in the basolateral part of the amygdala substantial induction of tau lesions were observed (arrowheads in **H**) although no significant amyloid deposition was apparent in this area. Bars are 75 μm. Panels (**E-G**) have same magnification. (**I**) Gallyas silver staining shows that many of the induced tau deposits in the entorhinal cortex were of fibrillary nature.

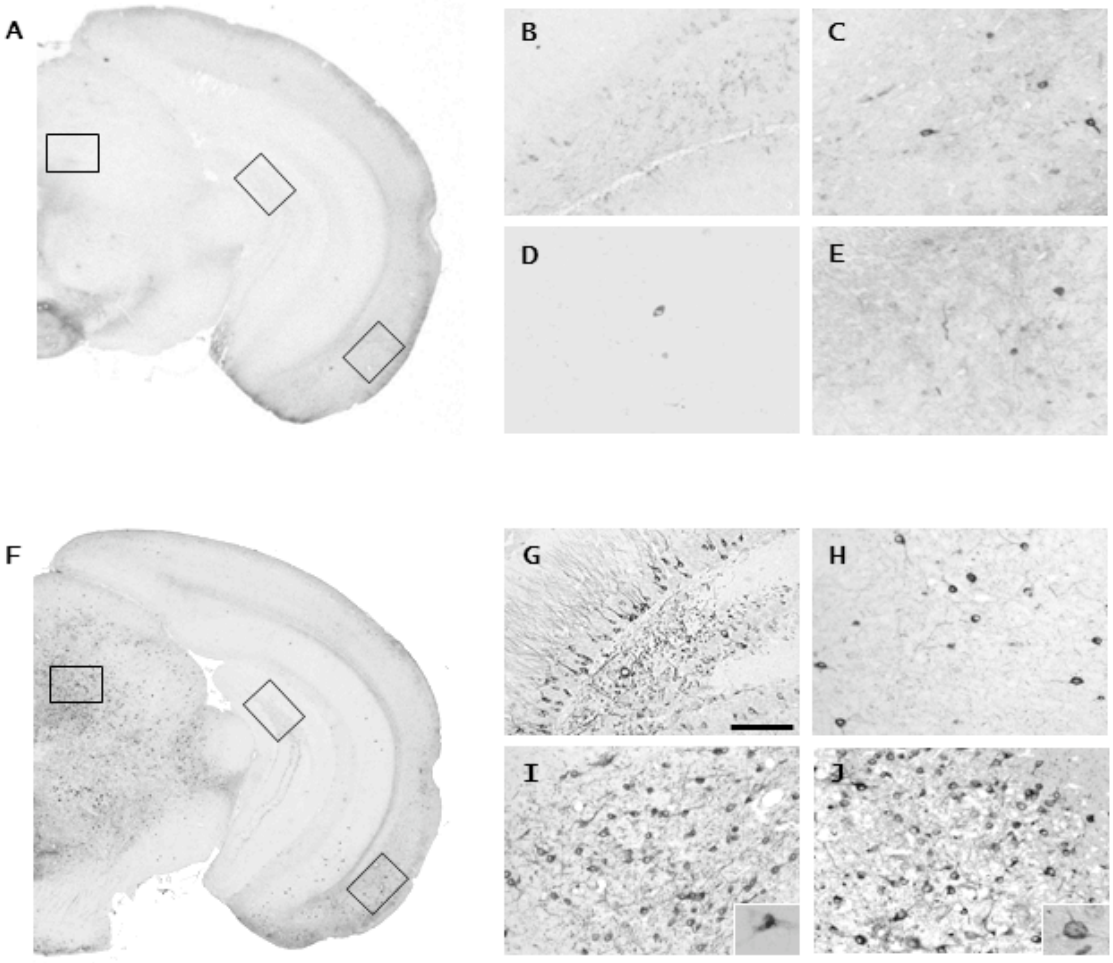


Figure 1.

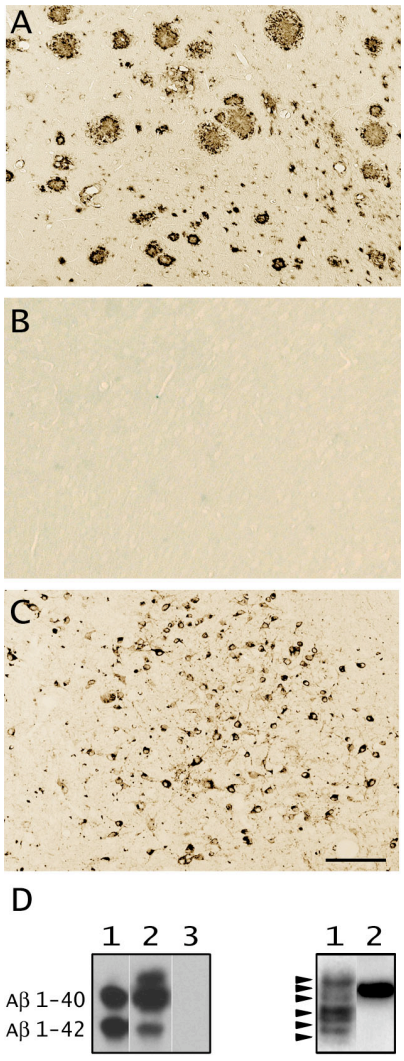


Figure 2.

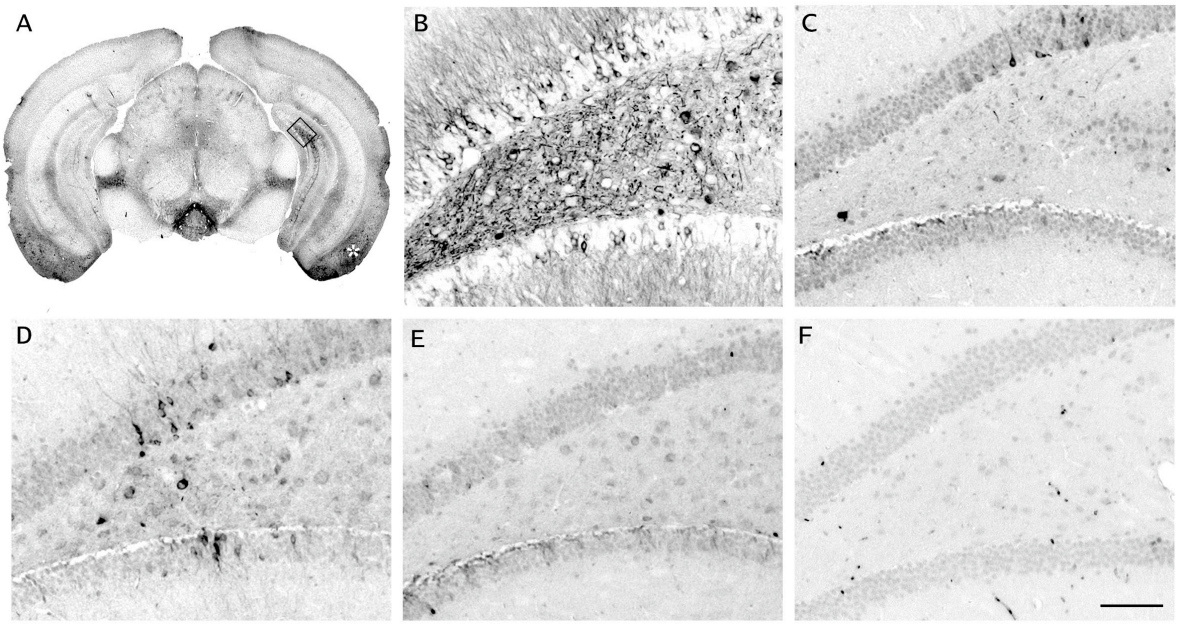


Figure 3.

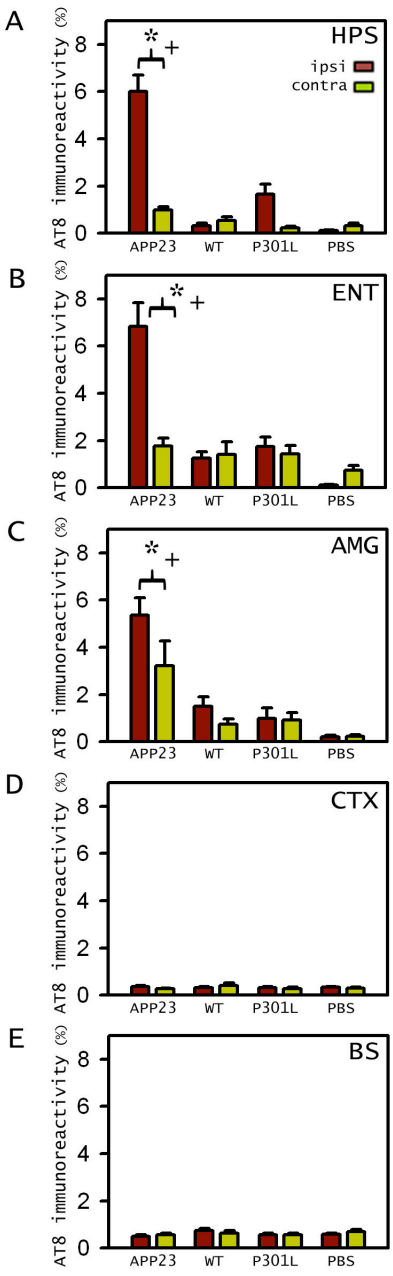

A
HPS
ipsi
contra
B
ENT
C
AMG
D
CTX
E
BS
AT8 immunoreactivity (%)
0
2
4
6
8
APP23
WT
P301L
PBS

Figure 4.

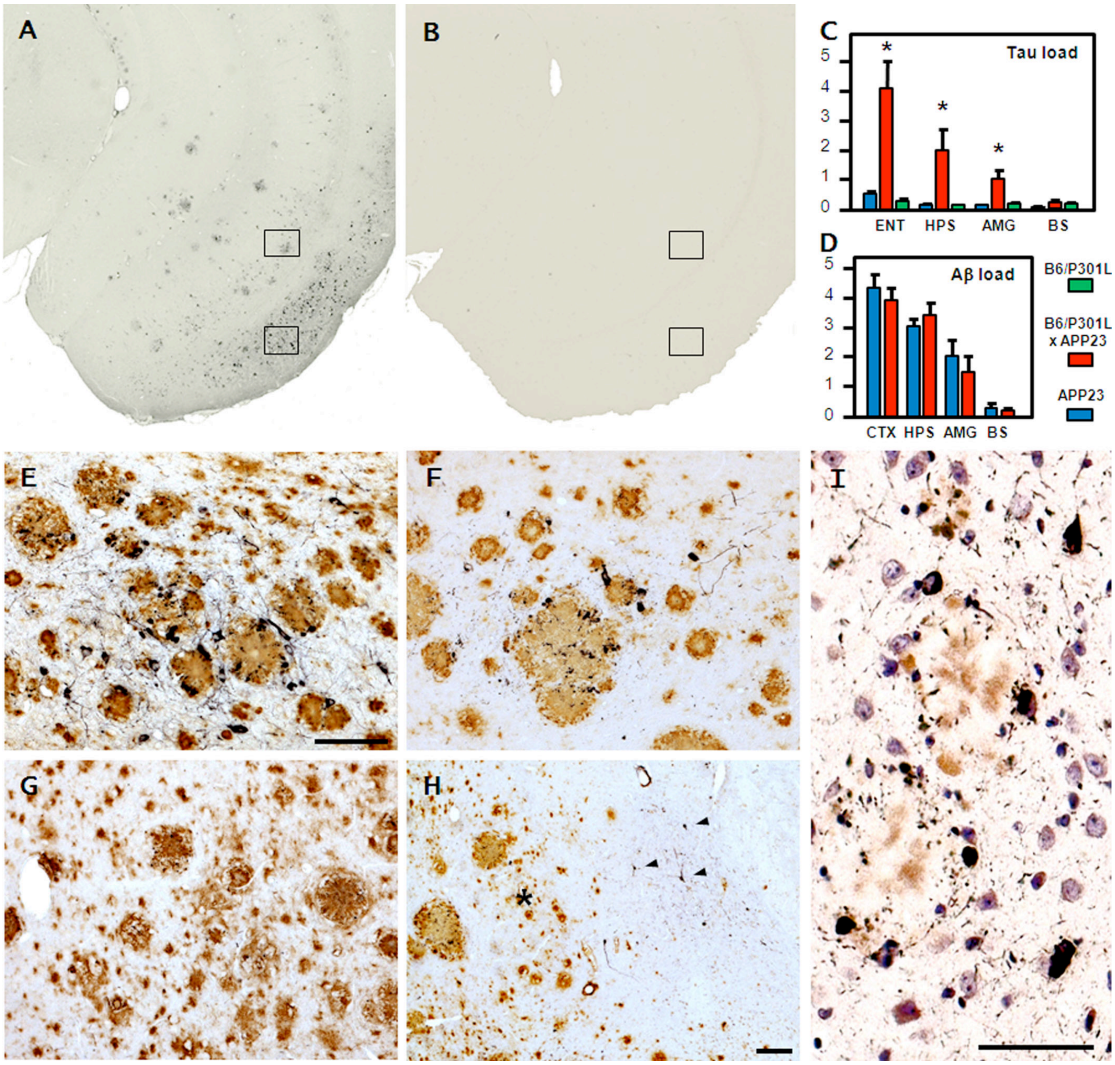

A
B
C
Tau load
ENT HPS AMG BS
D
Aβ load
CTX HPS AMG BS
B6/P301L
B6/P301L x APP23
APP23
E
F
G
H
I

Figure 5.

SUPPLEMENTARY INFORMATION

INDUCTION OF TAU PATHOLOGY BY INTRACEREBRAL INFUSION OF Aβ-CONTAINING BRAIN EXTRACT AND BY AMYLOID IN APP x TAU TRANSGENIC MICE

Tristan Bolmont [1*], Florence Clavaguera [2*], Melanie Meyer-Luehmann [1], Martin C. Herzig [1], Rebecca Radde [1], Matthias Staufenbiel [3], Jada Lewis [4], Mike Hutton [4], Markus Tolnay [2], Mathias Jucker [1]

[1]Department of Cellular Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Otfried-Müller Str. 27, D-72076 Tübingen, Germany; [2]Department of Neuropathology, Institute of Pathology, University of Basel, CH-4003 Basel, Switzerland; [3]Novartis Institutes for Biomedical Research Basel, CH-4002 Basel, Switzerland; [4]Department of Neuroscience, Mayo Clinic Jacksonville, Jacksonville, Florida, USA,

* These authors contributed equally

Unpublished

Supplementary study

To determine whether neurofibrillary pathology can be induced *in vivo* by material derived from human disease the previous study was extended by injection of brain extracts derived from human patients with Alzheimer's disease (AD). 5 to 6 month-old B6/P301L mice were unilaterally injected with AD brain homogenates unilaterally into the hippocampus and neocortex. To assess the importance of soluble Aβ species for exogenous induction of tau pathogenesis, we injected AD extract with either low (brain extract prepared from the amygdala) or high Aβ levels (brain extract prepared from the frontal cortex) in parallel to the injection of brain extracts derived from old APP23- and B6/P301L-transgenic mice.

Brain homogenates were prepared from the superior frontal cortex of an AD patient (ADF extract) with both extensive β-amyloid deposition and NFT (Supplementary figure 1A), and from the amygdala (ADA extract) of an AD patient with extensive NFT but almost complete absence of β-amyloid deposition (Supplementary figure 1B). Brain homogenate was also prepared from an aged, β-amyloid-laden APP23 mouse (Supplementary figure 1C) and from an aged, tau-depositing B6/P301L transgenic mouse (Supplementary figure 1D). Western blotting confirmed significant Aβ levels (approx. 1-10 ng/μl) in the ADF and APP23 extracts. The ADF extract contained significantly more Aβ1-42 than Aβ1-40, while the APP23 mouse extract had more Aβ1-40 than Aβ1-42 (Supplementary figure 1E). Immunoblot analysis with human tau-specific antibody detected all six human tau isoforms in the ADF and ADA human brain homogenates (Supplementary figure 1F). The 4R0N human tau isoform was detected in extracts prepared from an aged B6/P301L transgenic mouse consistent with the expression of this shortest 4 repeat tau isoform in P301L transgenic mice (Supplementary figure 1F).

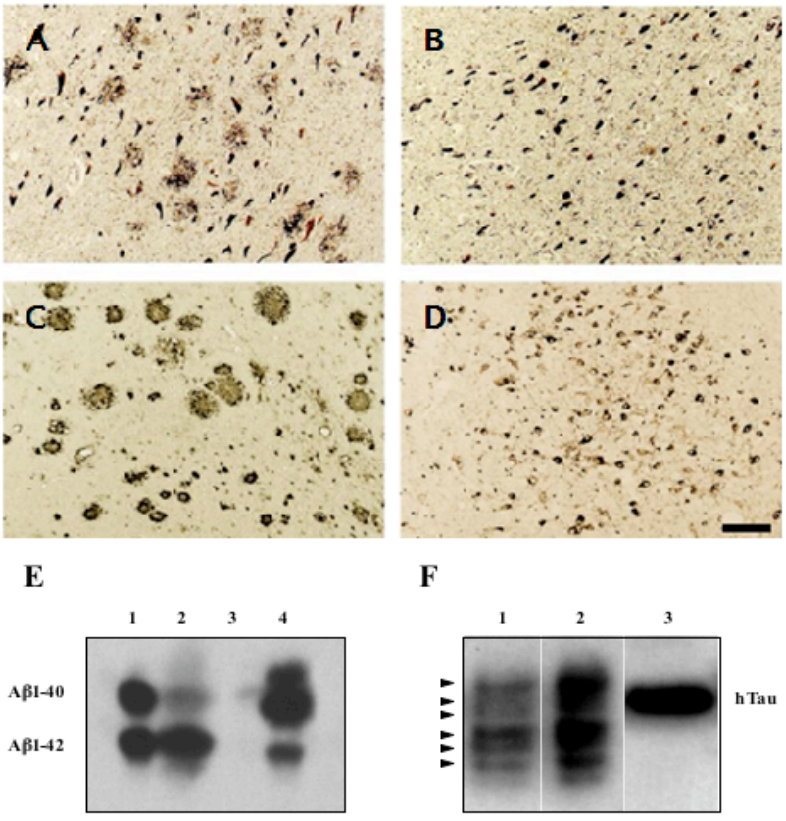
Intracerebral injection of Aβ-rich human ADF extract induced robust deposition of tau in the hippocampus (assessed by phosphorylation-dependent AT8 antibody; Supplementary figure 2A). Tau deposition was induced primarily throughout the injected hippocampus

and, to a lesser degree, in the contralateral hippocampus (data not shown). In addition, Tau pathology was also induced in brain regions farther away from the injection sites, such as the amygdala (Supplementary figure 2B) and the entorhinal cortex (Supplementary figure 2C). No tau deposits were observed around the injection site in the neocortex (data not shown). ADF extracts injected into non-transgenic mice did not induce any tau deposits, indicating that the expression of human tau was necessary for the induction of tau lesions by the Aβ-containing extracts (data not shown).
Intracerebral injections of ADA extract induced only modest and non-significant tau deposition (Supplementary figure 2D-F), consistent with the low β-amyloid load in the tissue from which the extract was derived.

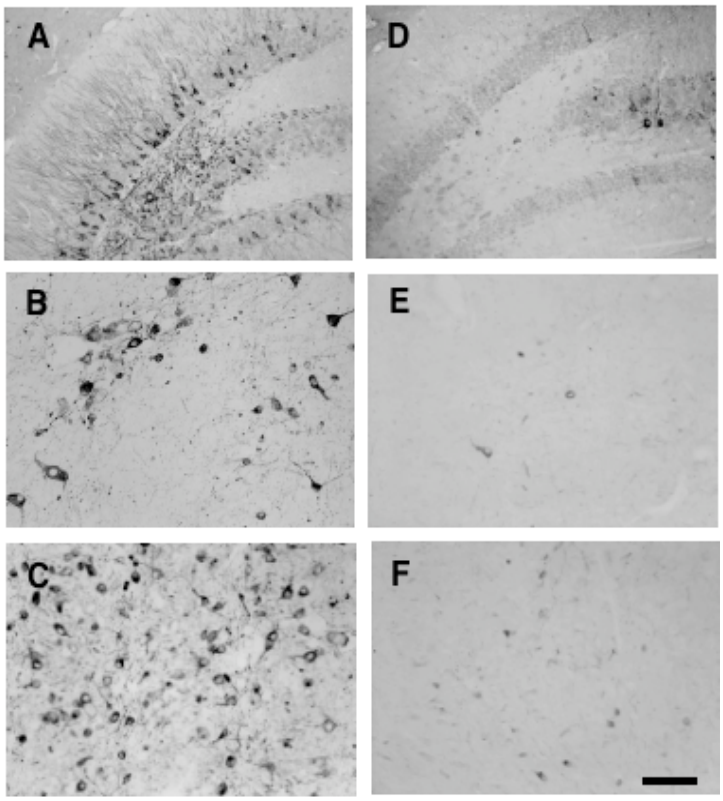
The finding that extracts with low Aβ levels have only modest tau-inducing activity while extracts with high Aβ load reveals robust activity suggests that Aβ is key to an effective induction of the tau pathology. Because the ADF extract (in which Aβ42 predominates) and the APP23 extract (in which Aβ40 predominates) were both potent inducers of the tau pathology with qualitative and quantitative overall similar appearance, it is likely that various Aβ species of different length are potent in inducing neurofibrillary pathology *in vivo*.

Supplementary figure 1. Histological and biochemical characterization of the injected extracts. (**A**) Gallyas staining of the superior frontal cortex of a 85 year-old AD patient reveals amyloid plaques and neurofibrillary tangles. (**B**) Gallyas staining of basolateral amygdala of a 96 year-old AD patient reveals significant neurofibrillary lesions but almost no amyloid deposits. These sections were taken from an area directly adjacent to the tissue from which the extracts (ADF = AD frontal cortex extract; ADA = AD amygdala extract) were derived. (**C**) Aβ-immunostaining of neocortex of a 24 month-old APP23 transgenic mouse. (**D**) Immunostaning with AT8 antibody reveals numerous tau-positive deposits in the brainstem of a 21 month-old B6/P301L transgenic mouse. Bar in (**D**) is 50 µm. All panels have same magnification. (**E**) Immunoblotting with antibody 6E10 specific to human Aβ: *lane 1*, synthetic Aβ1-40 and 1-42 (1ng/µl each); *lane 2*, ADF brain extract; *lane 3,* ADA brain extract; *lane 4:* APP23 brain extract. (**F**) Western blotting analysis with HT7 antibody specific to human tau: *lane 1*, ADF brain extract; *lane 2,* ADA brain extract; *lane 3*, B6/P301L brain extract. Indicated are the various human tau isoforms (arrowheads).

Supplementary figure 2. Intracerebral injection of Aβ-containing human brain extract induces tau pathology in B6/P301L tau tg mice. Adult 5-6 month-old B6/P301L transgenic mice and control littermates were injected unilaterally in the hippocampus and overlying neocortex and analyzed 6 months later. The ADF-injected B6/P301L mice show an enhanced limbic neurofibrillary pathology in the hippocampus (**A**), Amygdala (**B**), and entorhinal cortex (**C**). In contrast, injection with the ADA extract did not significantly induce tau pathology in the brain of B6/P301L mice. Bar is 100 μm. All panels have same magnification.



Supplementary figure 1.



Supplementary figure 2.

SUPPORTING MATERIAL

Preparation of brain extract – Tissues for human brain extracts were derived at autopsy from the superior frontal gyrus (ADF extract, patient 1) and the basolateral amygdala (ADA extract, patient 2) of two female patients (85 and 96 years old; post mortem delays 7 and 11 hours; respectively) who have died of confirmed Alzheimer's disease. The tissue samples were fresh frozen and stored at -80°C until use. The tissue pieces were then homogenized at 10 % (w/v) in sterile PBS, vortexed, sonicated for 5 seconds and centrifuged at 3000x g for 5 minutes. The supernatant was aliquoted and immediately frozen (-80°C).

4. TRANSMISSION AND SPREADING OF TAUOPATHY IN TRANSGENIC MOUSE BRAIN

Florence Clavaguera [1], Tristan Bolmont [2], R. Anthony Crowther [3], Dorothee Abramowski [4], Stephan Frank [1], Alphonse Probst [1], Graham Fraser [3], Anna K. Stalder [5], Martin Beibel [4], Matthias Staufenbiel [4], Mathias Jucker [2], Michel Goedert [3*] & Markus Tolnay [1*]

[1] Department of Neuropathology, Institute of Pathology, University Hospital, Basel, Switzerland. [2] Department of Cellular Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Germany. [3] MRC Laboratory of Molecular Biology, Cambridge, UK. [4] Novartis Institutes for Biomedical Research, Basel, Switzerland. [5] Neurology and Neurobiology, University Hospital, Basel, Switzerland.

* These authors contributed equally

Under revision (submitted to Nature)

ABSTRACT

Tau protein in a hyperphosphorylated state makes up the filamentous intracellular inclusions of several neurodegenerative diseases, including Alzheimer's disease [1]. In the disease process neuronal tau inclusions first appear in transentorhinal cortex, from where they spread to hippocampal formation and neocortex [2]. Cognitive impairment becomes manifest when inclusions reach the hippocampal formation, with abundant neocortical tau inclusions and extracellular β-amyloid deposits being the defining pathological hallmarks of Alzheimer's disease. Abundant tau inclusions, in the absence of β-amyloid deposits, define Pick's disease, progressive supranuclear palsy, corticobasal degeneration and other diseases [1]. Mutations in *Tau* cause familial forms of frontotemporal dementia, establishing that dysfunction of tau protein is sufficient to cause neurodegeneration and dementia [3-5]. Thus, transgenic mice that express mutant (e.g. P301S) human tau in nerve cells exhibit the essential features of tauopathies, including abundant filaments made of hyperphosphorylated tau protein and neurodegeneration [6,7]. In contrast, mouse lines expressing single isoforms of wild-type human tau do not produce tau filaments or display neurodegeneration [7,8]. Here we have used tau-expressing lines to investigate whether experimental tauopathy can be transmitted. We show that the injection of brain extract from mutant P301S tau-expressing mice into the brain of transgenic wild-type tau-expressing animals induces the assembly of wild-type human tau protein into filaments and the spreading of pathology from the site of injection to neighbouring brain regions.

Transgenic mouse lines ALZ17 and P301S tau were used [6,8]. Mice from line ALZ17, which express the longest human brain tau isoform (441 amino acids) do not exhibit filamentous tau aggregates (Supplementary Fig. 1). By contrast, mice from line P301S tau, which express the 383 amino acid human tau isoform with the P301S mutation that causes inherited frontotemporal dementia, develop abundant filamentous tau inclusions (Supplementary Fig. 1). Both the 383 and 441 amino acid tau isoforms contain 4 microtubule-binding repeats, but they differ by the presence of 2 alternatively spliced N-terminal inserts of 29 amino acids each [9].

To investigate whether aggregation of tau can be transmitted, we injected diluted extracts of brainstem homogenates from 5 month-old human P301S tau mice into the hippocampus and the overlying cerebral cortex of 3 month-old ALZ17 mice. Prior to injection, the homogenates were analyzed by immunoblotting and immunoelectron microscopy. Human tau protein bands of 55-64 kDa were detected by Western blotting (Supplementary Fig. 2a). The slowest migrating tau species were immunoreactive with antibody AT100 (Supplementary Fig. 2a) and other phosphorylation-dependent anti-tau antibodies (not shown). By immunoelectron microscopy, tau filaments were present in the tissue extracts (Supplementary Fig. 2b). Injection of brain extract from human P301S tau mice induced filamentous tau pathology in ALZ17 mice, as indicated by the appearance of Gallyas-Braak silver staining [10,11] (Fig. 1a,b) and the presence of tau filaments by immunoelectron microscopy (Fig. 1c). Gallyas-Braak staining was present intracellularly 6, 12 and 15 months after the injection of brain extract. In contrast, no silver-positive lesions were observed in age-matched 18 month-old non-injected ALZ17 mice or in ALZ17 mice 15 months after the injection of brain extract from non-transgenic control mice. In addition to silver-positive nerve cell bodies and processes, the injection of brain extract from P301S tau mice resulted in the appearance of immunoreactivity with antibody AT100, indicative of tau filaments [6]. In contrast to the absence of AT100 staining, AT8-immunoreactivity (reflecting tau hyperphosphorylation) was present in the hippocampus of 18 month-old ALZ17 mice, as previously reported [8]. Following the injection of brain extract from human P301S tau mice, AT8-immunoreactivity became more widespread, indicating the promotion of tau hyperphosphorylation (Fig. 1a).

Filamentous tau pathology in P301S tau-injected ALZ17 mice was induced in different cell types. Silver-positive structures morphologically indistinguishable from those found in human tauopathies were observed in the brains of injected ALZ17 mice (Fig. 1b). They included neurofibrillary tangles (arrows in panels 1 and 2), neuropil threads (arrowheads in panels 1 and 2) and oligodendroglial coiled bodies (arrows in panels 3 and 4). The silver-positive structures were also immunoreactive for phosphorylated tau (Fig. 1b, panels 2 and 3). Filaments extracted from injected brains were decorated by phosphorylation-dependent anti-tau antibodies and by antibodies specific for tau isoforms with N-terminal inserts (Fig. 1c). It follows that the filaments had formed from the wild-type 441 amino acid human tau isoform expressed in line ALZ17 and were not derived from the injected material. This is also consistent with the observation that no silver staining was observed in ALZ17 mice 1 day after the injection of P301S tau brain extract (not shown).

The induction of filamentous tau in ALZ17 mice was time- and brain region-dependent. Quantitative assessment in the hippocampus revealed a significant increase in the number of silver-positive lesions between 6, 12 and 15 months after injection (Fig. 2). Neuropil threads were most abundant, followed by coiled bodies and neurofibrillary tangles (Fig. 3). The same was true for the cerebral cortex, although fewer silver-positive structures developed there over time (Fig. 2).

Positive silver staining did not remain confined to the injected areas, but spread also to neighbouring brain regions (Fig. 4, Table 1). At the injection level, abundant silver staining was present in subiculum, fimbria, optic tract and thalamus, with fewer abnormal structures in more distant regions, such as medial lemniscus, zona incerta and cerebral peduncle. The region most distant (4 mm ventral from the injection sites), where filamentous tau pathology developed, was the hypothalamus. Almost 2 mm anterior to the injection level, filamentous tau pathology was restricted to the fimbria and the thalamus 6 months after injection, where it increased over time (Supplementary Fig. 3). After 12 months, aggregated tau was found in the internal capsule, the caudate-putamen and the somatosensory cortex. At 15 months post-injection, the hypothalamus and the amygdala were also affected. More than 1 mm posterior to the injection level, the cerebral peduncle, the hippocampus, the superior colliculus and the substantia nigra

exhibited filamentous lesions starting 6 months after the injection of brain extract from human P301S tau mice (Supplementary Fig. 4). At 12 months, silver-positive structures appeared in the entorhinal cortex and in the deep mesencephalic nucleus, before they were seen in the pontine nuclei at 15 months. A small amount of filamentous tau pathology was observed in some brain regions of the contralateral, non-injected hemisphere (Supplementary Fig. 5).

The present findings show that the intracerebral injection of brain extract from mice with a filamentous tau pathology induces the formation of silver-positive aggregates made of hyperphosphorylated tau in mice transgenic for wild-type tau, demonstrating the experimental transmission of tauopathy. From the increase in silver-positive structures over time, it appears likely that nerve cell processes and oligodendrocytes are major sites of filament induction. These findings are reminiscent of previous work using mouse models of prion diseases, β-amyloid deposition and some peripheral amyloidoses [12-19]. However, unlike the extracellular location of filamentous deposits in those other diseases, tau inclusions are intracellular. Mechanisms must therefore exist by which tau aggregates can either gain access to the inside of cells or activate filament-inducing cascades from the outside. Inside cells, tau aggregates could function as seeds for the ordered assembly of transgenic wild-type human tau into silver- and AT100-positive filamentous deposits. Intracellular filamentous tau pathology could also be induced by extracellular tau aggregates. Previous work has indeed shown potentiation of tau pathology by extracellular β-amyloid aggregates in mice transgenic for mutant human tau [15,16]. However, no such effect was observed in mice transgenic for wild-type human tau [20].

A combined neuronal and glial tau pathology is the defining feature of a number of human neurodegenerative diseases characterized by the assembly of wild-type four-repeat tau isoforms into filaments. They include progressive supranuclear palsy, corticobasal degeneration and argyrophilic grain disease [21,22]. By contrast, in Pick's disease, three-repeat tau isoforms are found in the mostly neuronal inclusions and in Alzheimer's disease, both three- and four-repeat tau isoforms make up neurofibrillary tangles [23,24]. In addition, the morphologies of tau filaments vary widely in different tauopathies [25]. Together with the findings reported here showing transmission and spreading of filamentous tau pathology, this is reminiscent of mammalian and yeast

prions, for which different strains have been described, based on the existence of separate conformers of assembled protein [26]. It is tempting to speculate that distinct tau strains may underlie the pathogenesis of different sporadic tauopathies. This hypothesis can now be tested experimentally by injecting ALZ17 mice with brain extracts from patients with sporadic tauopathies.

During the process leading to Alzheimer's disease, neuronal tau pathology forms in a stereotypical fashion in transentorhinal cortex, from where it spreads to hippocampal formation and neocortex [2], consistent with a uniform biological process. We also show the appearance over time of silver-positive structures at sites that are at a distance from the injection sites in hippocampus and cerebral cortex. Brain regions that develop pathology are connected anatomically to the injection sites or to each other. This, together with the stereotypical appearance of silver-positive structures in defined brain regions over time (Table 1), is clearly indicative of the active induction and spreading of pathology rather than passive diffusion of tau aggregates from the injection sites to more distant regions.

It has been suggested that misfolded superoxide dismutase, which causes a subset of familial forms of amyotrophic lateral sclerosis, is secreted from motor neurons [27]. By analogy, spreading of tau pathology may also result from the release of aggregates from affected nerve cells and glial cells. It will be important to investigate whether such mechanisms exist for misfolded tau. In addition to active release mechanisms, the death of nerve cells could also contribute to the accumulation of misfolded proteins in the extracellular space and their uptake by neighbouring cells. The formation of tau aggregates in a single brain cell and their subsequent spreading may therefore be at the origin of sporadic tauopathies. Consequently, the presence of tau aggregates in the extracellular space would be an obligatory step in the events leading to disease. Immunisation strategies may prevent this process [28].

The present findings demonstrate the transmission of tauopathy between transgenic mouse lines and describe an experimental system in which to investigate the spreading of pathology and the existence of tau strains. It will be important to identify the tau species capable of inducing transmission, aggregation and spreading. Similar mechanisms may also underlie Parkinson's disease and dementia with Lewy bodies,

where α-synuclein pathology spreads from brainstem areas to midbrain and neocortex [29]. Unlike prion diseases, human tauopathies are not believed to be infectious [30]. Experimental model systems of the type described here now make it possible to dissect the similarities and differences between tauopathies and prion diseases.

REFERENCES

1. Goedert, M. & Spillantini, M.G. *A century of Alzheimer's disease.* Science 314, 777-781 (2006).
2. Braak, H. & Braak, E. *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol. 82, 239-259 (1991).
3. Poorkaj, P. et al. *Tau is a candidate gene for chromosome 17 frontotemporal dementia.* Ann. Neurol. 43, 815-825 (1998).
4. Hutton, M. et al. *Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17.* Nature 393, 702-705 (1998).
5. Spillantini, M.G. et al. *Mutation in the tau gene in familial multiple system tauopathy with presenile dementia.* Proc. Natl. Acad. Sci. USA 95, 7737-7741 (1998).
6. Allen, B. et al. *Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein.* J. Neurosci. 22, 9340-9351 (2002).
7. Frank, S., Clavaguera F., Tolnay, M. *Tauopathy models and human neuropathology: similarities and differences.* Acta Neuropathol. 115, 39-53 (2008).
8. Probst, A. et al. *Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein.* Acta Neuropathol. 99, 469-481 (2000).
9. Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. & Crowther, R.A. *Multiple isoforms of human microtubule-associated protein tau: Sequences and localization in neurofibrillary tangles of Alzheimer's disease.* Neuron 3, 519-526 (1989).
10. Gallyas, F. *Silver staining of Alzheimer's neurofibrillary changes by means of physical development.* Acta Morphol. Acad. Sci. Hung. 19, 1-8 (1971).
11. Braak, H., Braak, E., Ohm, T. & Bohl, J. *Silver impregnation of Alzheimer's neurofibrillary changes counterstained for basophilic material and lipofuscin pigment.* Stain Technol. 63, 197-200 (1988).
12. Legname, G. et al. *Synthetic mammalian prions.* Science 305, 673-676 (2004).
13. Kane, M.D. et al. *Evidence for seeding of β-amyloid by intracerebral infusion of Alzheimer brain extracts in β-amyloid precursor protein-transgenic mice.* J. Neurosci. 15, 3606-3611 (2000).
14. Meyer-Luehmann, M. et al. *Exogenous induction of cerebral beta-amyloidosis is governed by agent and host.* Science 313, 1781-1784 (2006).
15. Götz, J., Chen, F., van Dorpe, J. & Nitsch, R.M. *Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Abeta 42 fibrils.* Science 293, 1491-1495 (2001).
16. Bolmont, T. et al. *Induction of tau pathology by intracerebral infusion of amyloid-β-containing brain extract and by amyloid-β deposition in APP x tau transgenic mice.* Am. J. Pathol. 171, 2012-2020 (2007).
17. Xing, Y. et al. *Transmission of mouse senile amyloidosis.* Lab. Invest. 81, 493-499 (2001).
18. Lundmark, K. et al. *Transmissibility of systemic amyloidosis by a prion-like mechanism.* Proc. Natl. Acad. Sci. USA 99, 6979-6984 (2002).
19. Walker, L.C., LeVine III, H., Mattson, M.P. & Jucker, M. *Inducible proteopathies.* Trends Neurosci. 29, 438-443 (2006).

20. Boutajangout, et al. *Characterisation of cytoskeletal abnormalities in mice transgenic for wild-type human tau and familial Alzheimer's disease mutants of APP and presenilin-1.* Neurobiol. Dis. 15, 47-60 (2004).
21. Sergeant, N., Wattez, A. & Delacourte, A. *Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: Tau pathologies with exclusively "exon 10" isoforms.* J. Neurochem. 72, 1243-1249 (1999).
22. Togo, T. et al. *Argyrophilic grain disease is a sporadic 4-repeat tauopathy.* J. Neuropathol. Exp. Neurol. 61, 547-556 (2002).
23. Delacourte, A. et al. *Specific pathological tau protein variants characterize Pick's disease.* J. Neuropathol. Exp. Neurol. 55, 159-168 (1996).
24. Goedert, M., Spillantini, M.G., Cairns, N.J. & Crowther, R.A. *Tau proteins of Alzheimer paired helical filaments: Abnormal phosphorylation of all six brain isoforms.* Neuron 8, 159-168 (1992).
25. Crowther, R.A. & Goedert, M. *Abnormal tau-containing filaments in neurodegenerative diseases.* J. Struct. Biol. 130, 271-279 (2000).
26. Prusiner, S.B. *Prions.* Proc. Natl. Acad. Sci. USA 95, 13363-13383 (1998).
27. Urushitani, M. et al. *Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis.* Nature Neurosci. 9, 108-118 (2006).
28. Asuni, A.A., Boutajangout, A., Quartermain, D. & Sigurdson, E.M. *Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements.* J. Neurosci. 27, 9115-9129 (2007).
29. Braak, H. et al. *Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol. Aging 24, 197-211 (2003).*
30. Brown, P. et al. *Human spongiform encephalopathy: The National Institutes of Health series of 300 cases of experimentally transmitted disease.* Ann. Neurol. 35, 513-529 (1994).

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Science Foundation (3100-06838.02) (M.T.); the Alzheimer Association (ZEN-06-27341), the German National Genome Network (NGFN2) and the German Competence Network in Degenerative Dementias (01GI0705) (M.J.); the U.K. Medical Research Council and the U.K. Alzheimer's Research Trust (M.G.). We thank K.H. Wiederhold (Novartis Institutes for Biomedical Research, Basel) and N. Schaeren-Wiemers (University Hospital Basel) for antibodies and helpful discussions.

FIGURE LEGENDS

Figure 1. Induction of filamentous tau pathology in ALZ17 mice injected with brain extract from mice transgenic for human P301S tau. **(a)** Staining of the hippocampal CA3 region from 18 month-old ALZ17 mice with anti-tau antibody AT8, Gallyas-Braak silver or anti-tau antibody AT100. Non-injected (left), 15 months after injection with brain extract from non-transgenic control mice (middle) and 15 months after injection with brain extract from 5 month-old mice transgenic for human P301S tau protein (right). The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels). **(b)** Different types of filamentous tau pathology are found in ALZ17 brains injected with P301S tau brain extract: (1) neurofibrillary tangle (arrow) and neuropil threads (arrowheads) visualized by silver staining; (2,3) double staining with silver and AT8 (red) shows neurofibrillary tangles (2, arrows), neuropil threads (2, arrowhead) and coiled bodies (3, arrow). (4) Double staining of coiled bodies (arrow) with silver and antibody Olig2 (red). The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels). **(c)** Immunoelectron microscopy of filaments extracted from the brain of an ALZ17 mouse 15 months after the injection of brain extract from mice transgenic for human P301S tau. Labelling with anti-tau sera 134, 189 and 304, and antibody AT100. Scale bar, 100 nm.

Figure 2. Temporal increase in the number of Gallyas-Braak-positive structures at the injection sites (- 2.5 mm from bregma) in ALZ17 mice. A statistically significant increase in silver-positive structures was observed in hippocampus between 6 and 12 months and between 12 and 15 months. In cerebral cortex, a significant increase was present between 12 and 15 months. The results are expressed as means ± S.E.M. (n=5). *$p<0.05$.

Figure 3. Temporal increase in the number of neuropil threads, coiled bodies and neurofibrillary tangles at the injection sites (- 2.5 mm from bregma) in ALZ17 mice. Significant increases in neuropil threads and coiled bodies were observed between 6 and 12 months, and between 12 and 15 months. For neurofibrillary tangles, a significant

increase was observed between 12 and 15 months. The results are expressed as means ± S.E.M. (n=5). *p<0.05; **p<0.001; ***p<0.0001.

Figure 4. Spreading of filamentous tau pathology in ALZ17 mice injected with brain extract from mice transgenic for human P301S tau. Gallyas-Braak silver staining of brain regions at a distance from the injection sites 15 months post-injection. The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels).

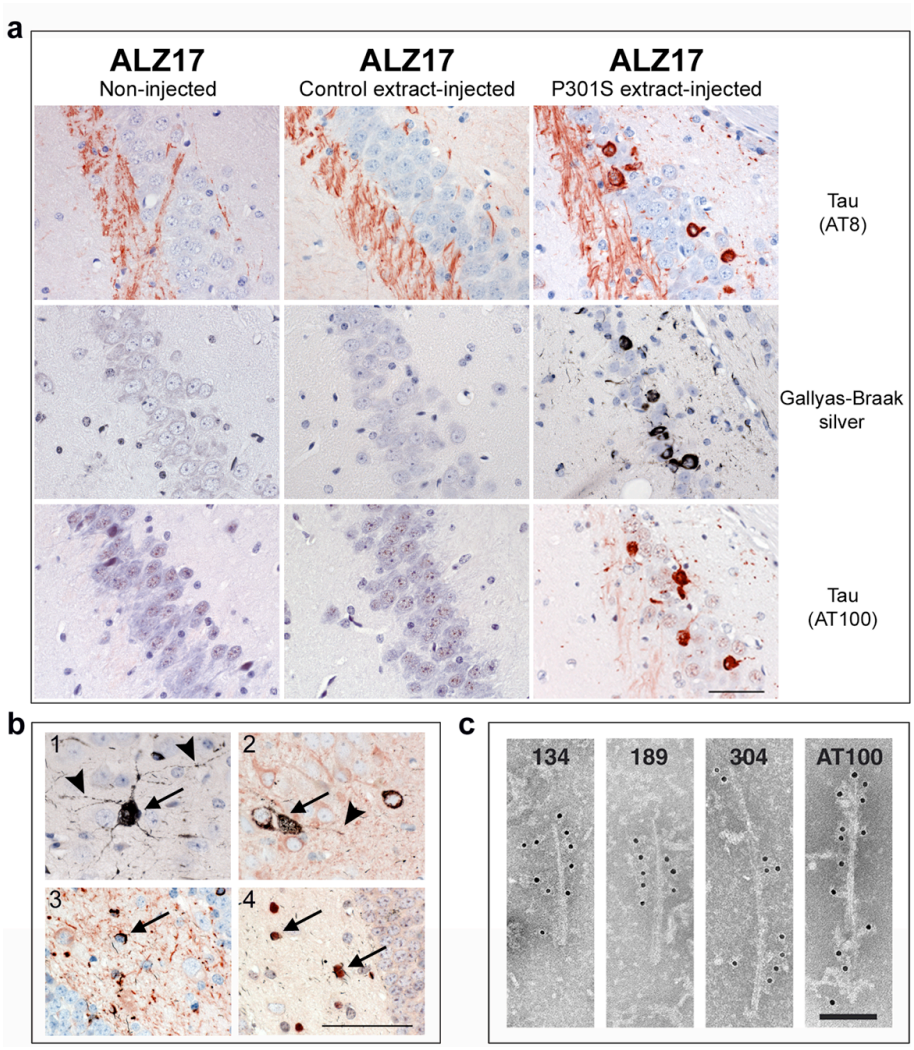

a
ALZ17
Non-injected
ALZ17
Control extract-injected
ALZ17
P301S extract-injected
Tau
(AT8)
Gallyas-Braak
silver
Tau
(AT100)
b
1
2
3
4
c
134
189
304
AT100

Figure 1.

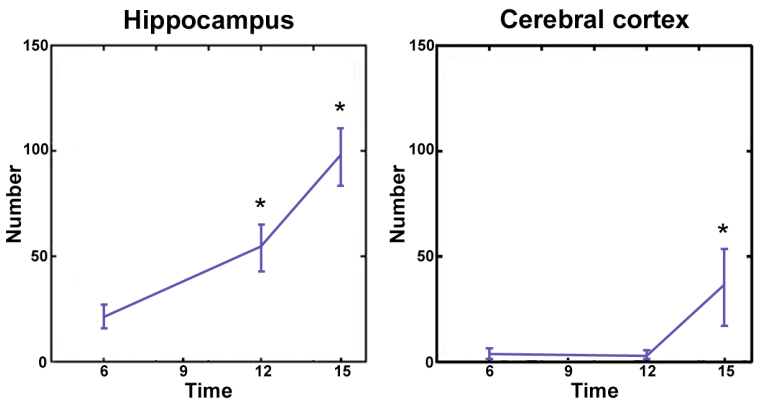

Hippocampus
Cerebral cortex
Number
Time
150
100
50
0
6
9
12
15
*

Figure 2.

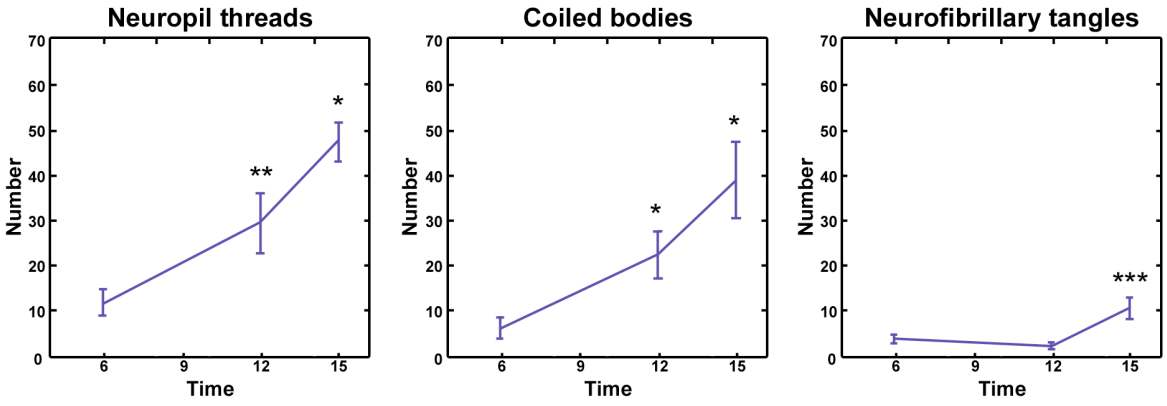

Neuropil threads
Coiled bodies
Neurofibrillary tangles
Number
Time
0
10
20
30
40
50
60
70
6
9
12
15
**
*
*
*
***

Figure 3.

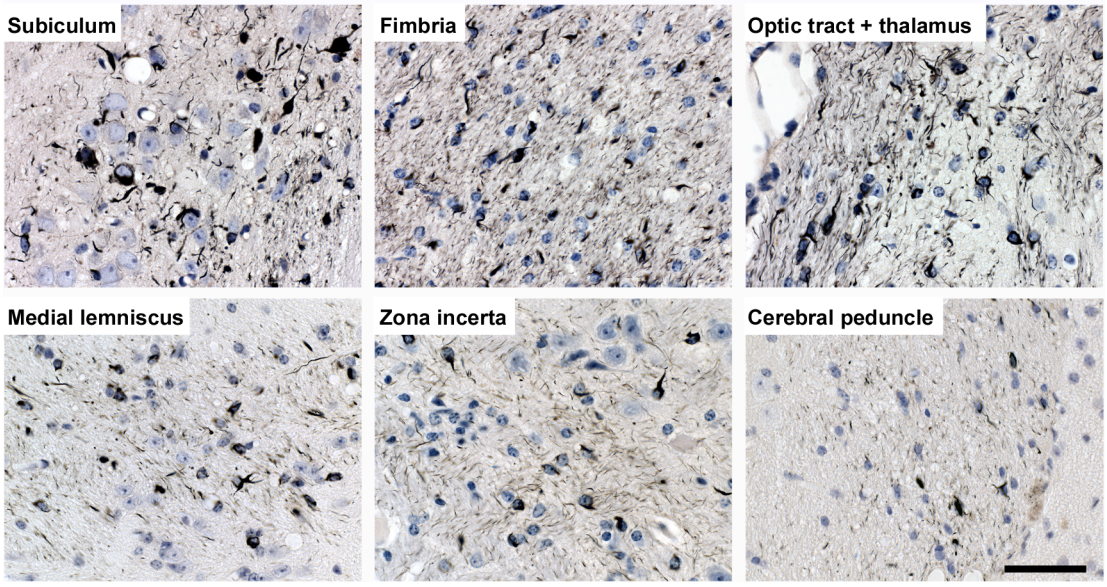

Subiculum
Fimbria
Optic tract + thalamus
Medial lemniscus
Zona incerta
Cerebral peduncle

Figure 4.

SUPPORTING MATERIAL

MATERIALS AND METHODS

Mice – Homozygous human wild-type tau ALZ17 transgenic mice [8], homozygous human P301S tau transgenic mice [6] and non-transgenic control mice, all females on a C57BL/6J background, were used. Experiments were performed in compliance with protocols approved by the local Basel Committee for Animal Care and Animal Use.

Preparation of brain extracts – Five month-old mice transgenic for human P301S tau and age-matched non-transgenic control mice were deeply anaesthetized with pentobarbital (100 mg/kg) and killed by decapitation. Brainstems were dissected, snap-frozen in liquid nitrogen and stored at -80° C. Brainstems from 3 mice were combined and homogenized at 10% (w/v) in sterile phosphate-buffered saline (PBS), sonicated (Branson 450, output 2, 90% cycle = 5 times 0.9 s) and centrifuged at 3,000xg at 4° C for 5 min. The supernatants were aliquoted, snap-frozen and kept at -80° C until use.

Western blotting – Brain extracts were run on 8% tris-glycine SDS-PAGE transferred onto a PVDF membrane (Immobilon P, Millipore, Zug, Switzerland) and probed with anti-tau antibodies HT7 (1:1,000, Pierce, Rockford, IL), AT8 (1:1,000, Pierce) and AT100 (1:200, Pierce). HT7 recognizes human, but not mouse tau [31], AT8 labels tau phosphorylated at S202 and T205 [32], and AT100 detects tau phosphorylated at T212, S214 and T217 [33]. The secondary antibody was goat anti-mouse IgG (Chemicon, Temecula, CA). For signal detection, ECL plus (Amersham, Piscataway, NJ) was used in conjunction with Amersham Hyperfilm ECL.

Stereotaxic surgery – Three month-old ALZ17 mice were anaesthetized with a mixture of ketamine (10 mg/kg) and xylazine (20 mg/kg). When deeply anaesthetized, the mice were placed on a heating pad to maintain body temperature during surgery. Using a Hamilton syringe, the hippocampus (A/P, -2.5 mm from bregma; L, +/- 2.0 mm; D/V, -1.8 mm) and the overlying cerebral cortex (A/P, -2.5 mm from bregma; L, +/- 2.0 mm; D/V, -0.8 mm) each received a unilateral (right hemisphere) stereotaxic injection of 2.5 μl brain extract, at a speed of 1.25 μl/min. Following injection, the needle was kept in

place for an additional 3 min before gentle withdrawal. The surgical area was cleaned with sterile saline and the incision sutured. Mice were monitored until recovery from anaesthesia and checked weekly following surgery.

Histology and immunohistochemistry – ALZ17 mice were deeply anaesthetized with pentobarbital (100 mg/kg) and killed by transcardial perfusion with 20 ml cold PBS, followed by 20 ml 4% paraformaldehyde in PBS. The brain was dissected and post-fixed overnight. Following paraffin embedding, 5 μm coronal sections were prepared. Sections were silver-impregnated following the method of Gallyas-Braak to visualize filamentous tau pathology [9,10]. For immunohistochemistry, the following anti-tau antibodies were used: T14 (1:1,000, Zymed, San Francisco, CA), AT8 (1:1,000) and AT100 (1:1,000). T14 is specific for human tau [34]. Antibody Olig2 (1:500, Chemicon, Temecula, CA) was used to stain oligodendrocytes. Secondary antibodies were from Vector Laboratories, Burlingame, CA (Vectastain ABC kit).

Immunoelectron microscopy – Immunoelectron microscopy of injected P301S brain extract using anti-tau antibody BR134 was performed as described [35]. BR134 was raised against a synthetic peptide corresponding to amino acids 428-441 of tau (numbering corresponding to the longest human brain tau isoform) [11]. In some experiments, sarkosyl-insoluble tau was prepared from injected ALZ17 brains, as described [24]. Anti-tau antibodies BR134, BR189, BR304 and AT100 were used to identify tau filaments. BR189 and BR304 were raised against amino acids 76-87 and 45-73 of tau, respectively [24]. They are specific for tau isoforms with one (BR304) or two (BR304 and BR189) N-terminal inserts.

Quantitative analysis of tau pathology – Five ALZ17 mice injected with P301S brain extract were analyzed at 6, 12 and 15 months post-injection. Gallyas-stained coronal sections were selected at identical brain coordinates [36] (anterior to the injection level, - 0.82 mm from bregma; injection level, - 2.5 mm from bregma; posterior to the injection level, - 3.8 mm from bregma). Silver-positive structures (neurofibrillary tangles, neuropil threads and coiled bodies) were counted at these 3 levels with a x20 objective (Zeiss,

Feldbach, Switzerland). At the injection level (- 2.5 mm from bregma), total counts of silver-positive structures were assessed in the injection sites hippocampus (CA1, CA2, CA3, dentate gyrus and subiculum) and visual cortex. In the visual cortex, silver-positive lesions developed just around and at a very short distance from the needle tract. Statistical analysis was performed using the Poisson regression algorithm with the help of the SAS 8.02 software. Link function was the logarithm and scale was estimated by deviance (to account for overdispersion). For semi-quantitative assessment, Gallyas-Braak staining was graded as follows: (-), no silver-positive structures; (+), < 20 silver-positive structures; (++), 20-40 silver-positive structures; (+++), > 40 silver-positive structures.

REFERENCES OF SUPPORTING MATERIAL

31. Mercken M. et al., *Affinity purification of human tau proteins and the construction of a sensitive sandwich enzyme-linked immunosorbent assay for human tau detection.* J. Neurochem. 58, 548-553 (1992).
32. Goedert M., Jakes R., Vanmechelen E., *Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205.* Neurosci. Lett. 189, 167-170 (1995).
33. Yoshida H., Goedert M., *Sequential phosphorylation of tau protein by cAMP-dependent protein kinase and SAPK4/p38δ or JNK2 in the presence of heparin generates the AT100 epitope.* J. Neurochem. 99, 154-164 (2006).
34. Kosik K.S., et al., *Epitopes that span the tau molecule are shared with paired helical filaments.* Neuron 1, 817-825 (1988).
35. Crowther R.A., *Straight and paired helical filaments in Alzheimer disease have a common structural unit.* Proc. Natl. Acad. Sci. USA 88, 2288-2292 (1991).
36. Franklin K.B.J., Paxinos G., *The mouse brain in stereotaxic coordinates*. Academic Press, New York (2001).

LEGENDS OF SUPPLEMENTARY FIGURES

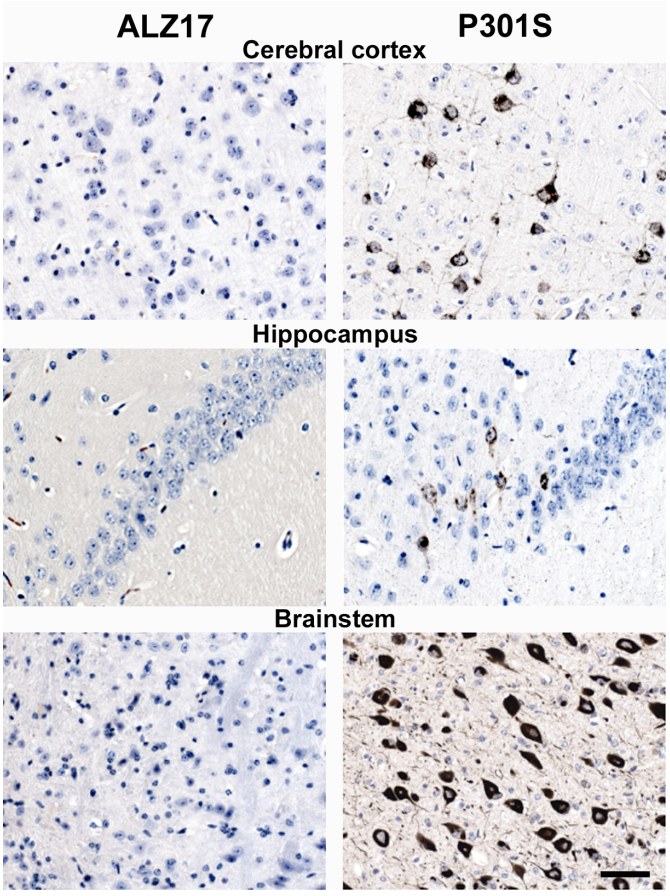
Figure 1. Lines ALZ17 and P301S tau. Staining of cerebral cortex, hippocampus and brainstem of an 18 month-old ALZ17 mouse (left) and a 5 month-old mouse transgenic for human P301S tau (right). Note the absence of silver staining in the ALZ17 mice and the strong staining in the P301S mouse. The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels).

Figure 2. Characterization of mouse brain extracts. **(a)** Immunoblotting of mouse brain extracts with anti-tau antibodies HT7 (specific for human tau, phosphorylation-independent) and AT100 (phosphorylation-dependent). Lanes: 1, brainstem extract (125 μg tissue) from non-transgenic control mice; 2, brainstem extract used for injection (125 μg tissue) from mice transgenic for human P301S tau; 3, forebrain extract (125 μg tissue) from 18 month-old ALZ17 mice; 4, recombinant human tau isoform mixture (10 ng). **(b)** Immunoelectron microscopy using anti-tau serum BR134 to decorate filaments in the brain extract used for injection. Scale bar, 100 nm.

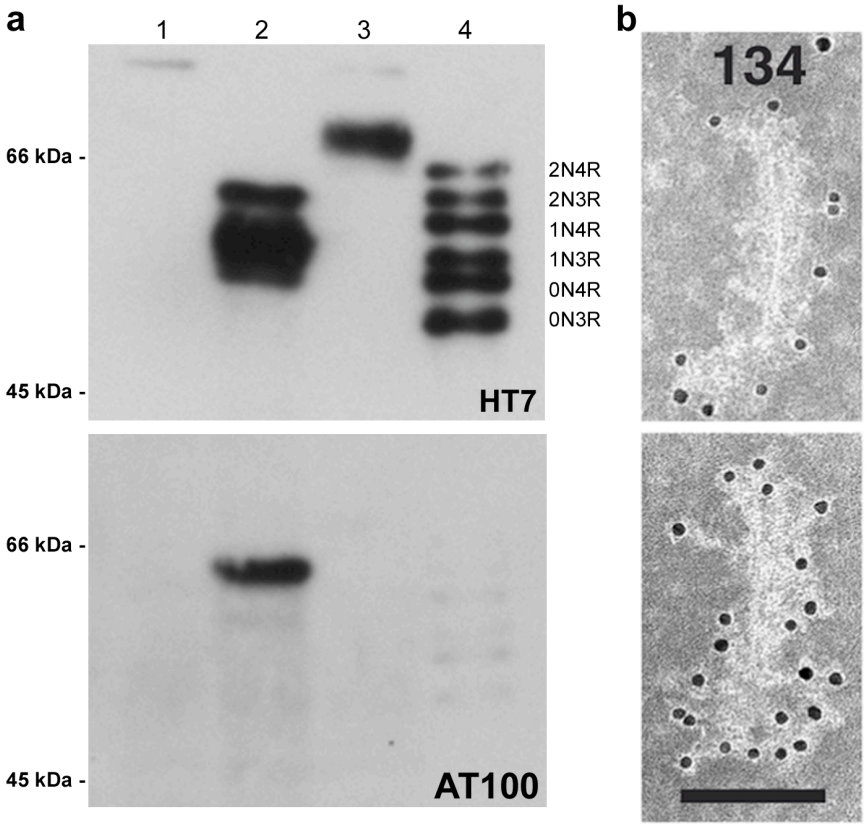
Figure 3. Spreading of filamentous tau pathology anterior to the injection level. **(a,b)** Gallyas-Braak staining of the fimbria from ALZ17 mice injected with P301S brain extract (- 0.82 mm from bregma) 6 months **(a)** and 15 months **(b)** after the injection of brain extract from mice transgenic for human P301S tau. The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels).

Figure 4. Spreading of filamentous tau pathology posterior to the injection level. **(a,b)** Gallyas-Braak staining of the polymorphic layer of the hippocampus from ALZ17 mice injected with P301S brain extract posterior (- 3.8 mm from bregma) to the injection site 6 months **(a)** and 15 months **(b)** after the injection of brain extract from mice transgenic for human P301S tau. The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels).

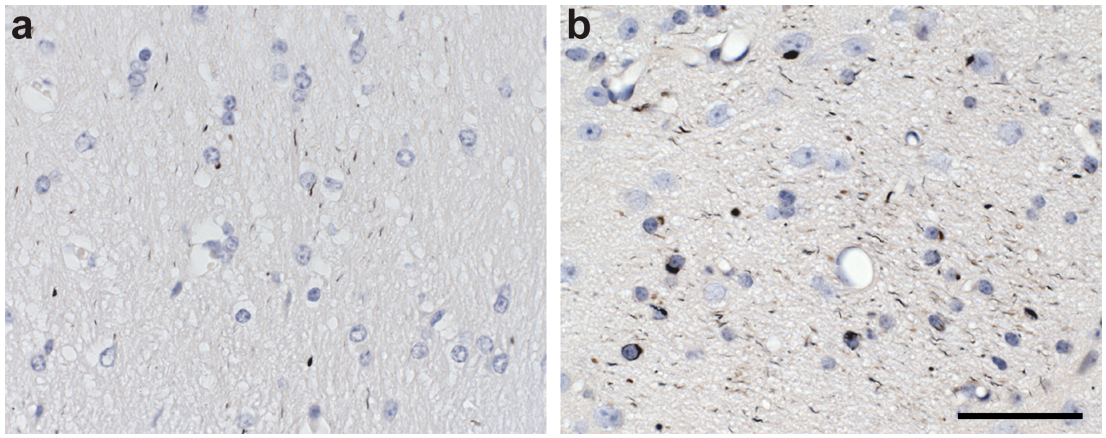
Figure 5. Modest contralateral spreading of filamentous tau pathology. **(a,b)** Gallyas-Braak staining of the ipsilateral **(a)** and contralateral **(b)** fimbria of an ALZ17 mouse 15 months after the unilateral injection of brain extract from mice transgenic for human P301S tau (injection level). Note the few scattered silver-positive lesions in the contralateral side (arrow). The sections were counterstained with haematoxylin. Scale bar, 50 μm (same magnification in all panels).



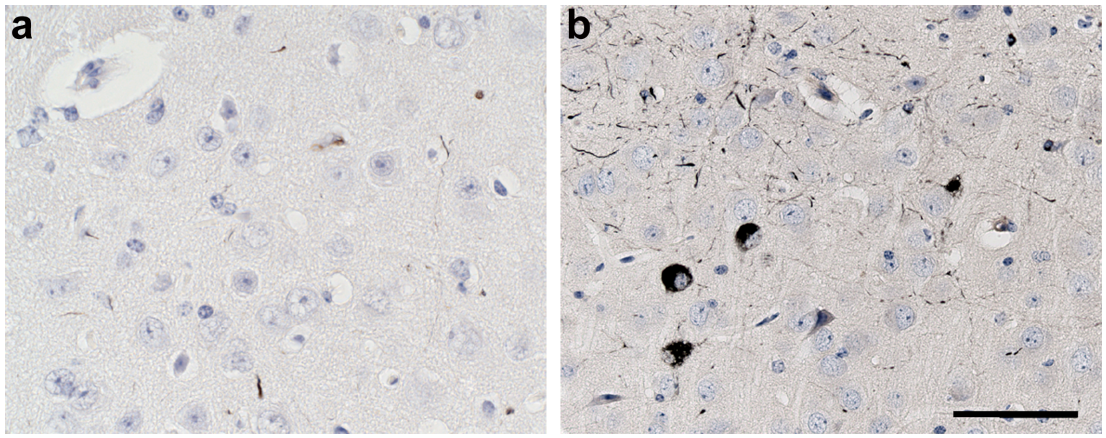
Supplementary figure 1.



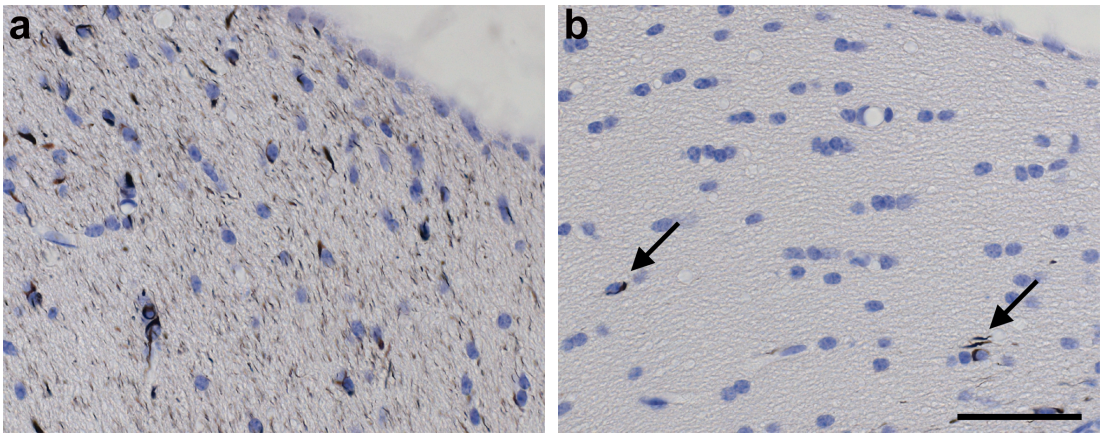
Supplementary figure 2.



Supplementary figure 3.



Supplementary figure 4.



Supplementary figure 5.

SEMI-QUANTITATIVE GRADING OF FILAMENTOUS TAU PATHOLOGY IN ALZ17 MICE INJECTED WITH P301S BRAIN EXTRACT

Anterior (1.6 mm from injection level)			
Region \ Time	6 months	12 months	15 months
fimbria	++	+++	+++
thalamus	+	++	++
internal capsule	-	+	++
caudate putamen	-	+	+
somatosensory cortex	-	+	+
hypothalamus	-	-	+
amygdala	-	-	+
Injection level			
Region \ Time	6 months	12 months	15 months
hippocampus *[1]	++	+++	+++
optic tract	++	++	+++
fimbria	+	++	+++
medial lemniscus	+	++	+++
zona incerta	+	++	+++
thalamus	+	++	++
cerebral peduncle	+	++	++
visual cortex [2]	+	+	++
hypothalamus	+	+	+
amygdala	-	+	+
Posterior (1.3 mm from injection level)			
Region \ Time	6 months	12 months	15 months
cerebral peduncle	++	+++	+++
hippocampus *	+	++	+++
superior colliculus	+	++	++
substantia nigra	+	+	+
entorhinal cortex	-	+	++
deep mesencephalic nucleus	-	+	++
pontine nuclei	-	-	+

- : no Gallyas-positive structures; + : 0-20; ++ : 20-40; +++ : >40

*: CA1, CA2, CA3, dentate gyrus and subiculum

1,2 : injection sites

Table 1.

5. CONCLUSION

Tau, which is abundantly expressed in both central and peripheral nervous system, binds to and stabilizes microtubules as fundamental structures for neuronal integrity. For currently unknown reasons, Tau becomes abnormally phosphorylated and aggregates in many 'tauopathies', clinically characterized by dementia and/or movement disorders. Pathological Tau detaches from microtubules, which in turn disassemble, leading to cell death.

Alzheimer's disease (AD), the most common form of dementia in the elderly, is histologically characterized not only by intracellular aggregation of Tau but also by the extracellular deposition of Amyloid-β (Aβ) peptide [1, 2]. In cases of familial AD, the 'Amyloid cascade' hypothesis holds that production of Aβ deposits precedes and triggers Tau pathology [2, 3, 4]. In this thesis, we have first validated this hypothesis by using different experimental approaches taking the advantage of transgenic mouse models. Through lentivirus-mediated expression of P301S mutant Tau in both wild-type and APP23 mice we have obtained stable Tau expression for up to thirteen months. Neurofibrillary tangles were that way induced in APP23 mice; in contrast, Tau remained in a hyperphosphorylated state in wild-type animals, validating the pathogenic influence of Aβ on the induction of neurofibrillar Tau pathology [5].

Using a 'seeding' approach it has been shown previously that injection of Aβ42 fibrils into the somatosensory cortex of Tau P301L mice results in the formation of NFTs in the amygdala, a region projecting to the injection site [6]. In the present thesis, we confirmed this finding by injection of dilute brain extract from aged, Aβ-laden APP23 transgenic mice into the brains of young B6/P301L Tau transgenic mice. Six months after injection, Tau pathology was induced not only in the injected hippocampus but also in the entorhinal cortex and amygdala, areas with neuronal projections to the injection site [7]. These data additionally support the hypothesis that Aβ-induced damage of presynaptic terminals or axons that project to the injection site may cause subsequent NFT formation in respective somatic compartments.

Transgenic mouse models of AD, where mutant APP is expressed, develop senile plaques but fail to reproduce neurofibrillary Tau pathology probably due to the lack of human

Tau expression. To overcome this problem, transgenic mice expressing mutant APP have been bred with the transgenic JNPL3 mice carrying the P301L Tau mutation [8]. Interestingly, Aβ pathogenesis was not modified in double transgenic animals compared to single APP-transgenic mice, but the density of NFTs was significantly higher than in single JNPL3 Tau-transgenic female animals [8]. We confirmed these findings by crossbreeding our B6/P301L Tau-transgenic mice with APP23-transgenic animals. In our double transgenic mice, NFTs were induced in males whereas B6/P301L single-transgenic males did not develop Tau pathology up to 30 months of age. In addition, double-transgenic female mice showed an acceleration of Tau pathology that appeared more pronounced when compared to the original B6/P301L single-transgenic mouse model [7]. Collectively, our findings demonstrate that filamentous Tau pathology can be exogenously induced by Aβ deposition and provide further evidence for the putative effect of Aβ as a causative pathogenic factor of AD.

In AD, Tau pathology spreads within the brain following a stereotypical pattern, which is clinically paralleled by a progressive cognitive decline but occurs independently of development and spreading of Aβ deposits [9, 10, 11]. In addition, somewhat contradictory to the 'Amyloid cascade' hypothesis, most tauopathies -devoid of any Aβ deposition- feature pathological Tau lesions and even exhibit an intriguing spatiotemporal spreading of Tau pathology [12, 13, 14, 15]. Furthermore, mutations in the *Tau* gene identified in FTDP-17 patients demonstrate that Tau dysfunction *per se* is sufficient to cause neurodegeneration [16, 17, 18]. However, the conditions by which Tau dysfunction leads to neurodegeneration and dementia are poorly understood, especially in the absence of detectable Aβ deposition. To investigate a potential role of Tau in the initiation and spreading of Tau pathology, we have performed seeding studies. We injected brain homogenates from aged Tau-depositing P301S mice into the somatosensory cortex and the hippocampus of young ALZ17 mice which transgenically express wild-type human Tau but do not develop filamentous Tau deposits. Significantly, injected ALZ17 animals developed a robust filamentous Tau pathology that increased with time and appeared in regions with neuronal projections to the injection sites. These results demonstrate for the first time the experimental transmission and spreading of tauopathy in transgenic mouse lines and open up new perspectives for future studies aimed at dissecting the fibrillogenic

agent present in the P301S extract. Moreover, injection of brain extracts from human sporadic tauopathies would allow to elucidate their potentially infectious nature and thus also enable to compare similarities and differences between tauopathies and prion diseases. Last but not least, therapeutic strategies such as immunotherapy-based approaches could be tested in such experimental seeding models to prevent the process of Tau fibril formation [19].

References

1. Selkoe D.J., *Translating cell biology into therapeutic advances in Alzheimer's disease.* Nature, 1999. 399(6738 Suppl): p. A23-31.
2. Haass C., Selkoe D.J., *Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide.* Nat Rev Mol Cell Biol, 2007. 8(2): p. 101-12.
3. Hardy J., Selkoe D.J., *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics.* Science, 2002. 297(5580): p. 353-6.
4. Hardy J., *Has the amyloid cascade hypothesis for Alzheimer's disease been proved?* Curr Alzheimer Res, 2006. 3(1): p. 71-3.
5. Osinde M., et al., *Lentivirus Tau (P301S) expression in adult amyloid precursor protein (APP)-transgenic mice leads to tangle formation.* Neuropathol Appl Neurobiol, 2008.
6. Gotz J., et al., *Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils.* Science, 2001. 293(5534): p. 1491-5.
7. Bolmont T., et al., *Induction of tau pathology by intracerebral infusion of amyloid-beta -containing brain extract and by amyloid-beta deposition in APP x Tau transgenic mice.* Am J Pathol, 2007. 171(6): p. 2012-20.
8. Lewis J., et al., *Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP.* Science, 2001. 293(5534): p. 1487-91.
9. Braak H., Braak E., *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol, 1991. 82(4): p. 239-59.
10. Gertz H.J., et al., *Examination of the validity of the hierarchical model of neuropathological staging in normal aging and Alzheimer's disease.* Acta Neuropathol, 1998. 95(2): p. 154-8.
11. Thal D.R., et al., *The development of amyloid beta protein deposits in the aged brain.* Sci Aging Knowledge Environ, 2006. 2006(6): p. re1.
12. Verny M., Duyckaerts C., Hauw J.J., *[Cortical lesions in progressive supranuclear palsy (Steele-Richardson-Olszewski disease)].* Rev Neurol (Paris), 1999. 155(1): p. 15-26.
13. Delacourte A., *The molecular parameters of tau pathology. Tau as a killer and a witness.* Adv Exp Med Biol, 2001. 487: p. 5-19.
14. Jellinger K.A., Attems J., *Neurofibrillary tangle-predominant dementia: comparison with classical Alzheimer disease.* Acta Neuropathol, 2007. 113(2): p. 107-17.
15. Tolnay M., Clavaguera F., *Argyrophilic grain disease: a late-onset dementia with distinctive features among tauopathies.* Neuropathology, 2004. 24(4): p. 269-83.
16. Hutton M., et al., *Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17.* Nature, 1998. 393(6686): p. 702-5.
17. Poorkaj P., et al., *Tau is a candidate gene for chromosome 17 frontotemporal dementia.* Ann Neurol, 1998. 43(6): p. 815-25.
18. Spillantini M.G., Bird T.D., Ghetti B., *Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies.* Brain Pathol, 1998. 8(2): p. 387-402.

19. Asuni, A.A., et al., *Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements.* J Neurosci, 2007. 27(34): p. 9115-29.

ABBREVIATIONS

Aβ	Amyloid-beta peptide
AD	Alzheimer's disease
AgD	Argyrophilic grain disease
APP	Amyloid precursor protein
CA	Cornu ammonis
CAA	Cerebral amyloid angiopathy
CamKII	Calcium/calmodulin-dependent kinase II
CBD	Corticobasal degeneration
Cdk5	Cyclin-dependent kinase 5
CNS	Central nervous system
C-terminal	Carboxy-terminal
DM	Myotonic dystrophy
EMEA	European agency for the evaluation of medication
ERK2	Extracellular signal-regulated kinase 2
FAD	Familial Alzheimer's disease
FDA	Food and drug administration
FTDP-17	Frontotemporal dementia with parkinsonism linked to chromosome 17
GSK3β	Glycogen synthase kinase 3β
MAPK	Mitogen-activated protein kinase
MARK	Microtubule-affinity-regulating kinase
NFT(s)	Neurofibrillary tangles
N-terminal	Amino-terminal
Non-PDPK	Non-proline-directed protein kinase
NSAIDs	Non-steroidal anti-inflammatory drugs
NT(s)	Neuropil threads
PDPK	Proline-directed protein kinase
PHF(s)	Paired-helical filament
PiD	Pick's disease

PIP2	Phosphatidylinositol 4,5 bisphosphate
PKA	cAMP-dependent kinase
PLC	Phospholipase C-γ
PNS	Peripheral nervous system
PP(s)	Protein phosphatase
PS	Presenilin
PSP	Progressive supranuclear palsy
SAPK/JNK	Stress-activated C-Jun N-terminal kinase

CURRICULUM VITAE

Florence Clavaguera

Education

- **2008** **PhD in Neurobiology**
  PhD Student at the Institute of Pathology, University of Basel
  Laboratory of Prof. Dr. M. Tolnay. Department of Neuropathology, University Hospital Basel, Switzerland

- **2002** **Diplôme d'Etudes Approfondies de neurobiologie des processus de communication et d'intégration (Masters degree)**
  Diploma thesis in the laboratory of Prof. Dr. A. Privat, INSERM U 436 (Title: Sodium salicylate as a neuroprotective agent in a model of ischemic spinal cord injury).
  Faculté des Sciences et Techniques du Languedoc, Montpellier, France

- **2001** **Bachelor of Cellular and Physiological Sciences**
  Practical training in electrophysiology in the laboratory of Prof. Dr. G. Vassort, INSERM U 390
  Faculté des Sciences et Techniques du Languedoc, Montpellier, France

- **1999** **DEUG of Biological Sciences**
  Faculté des Sciences et Techniques du Languedoc, Montpellier, France

- **1995** **High School diploma: Graduation in Sciences**
  Minor in Biology and Geology
  Lycée Ferdinand Fabre, Bédarieux (Hérault, 34), France

<u>Publications:</u> 2003 - Mai 2008

Peer-reviewed Journals

1. **Clavaguera F.**, Bolmont T., Crowther R.A., Abramowski D., Frank S., Probst A., Fraser G., Stalder A.K., Beibel M., Staufenbiel M., Jucker M., Goedert M., Tolnay M. :*Transmission and spreading of tauopathy in transgenic mouse brain.* Submitted

2. Osinde M.*, **Clavaguera F.***, May-Nass R., Tolnay M., Dev K.K. : *Lentivirus Tau (P301S) expression in adult amyloid precursor protein (APP)-transgenic mice leads to tangle formation.* Neuropathol Appl Neurobiol, 2008. [Epub ahead of print 14.Feb.2008]
   * Contributed equally

3. Bolmont T.*, **Clavaguera F**.*, Meyer-Luhemann M., Herzig M., Radde R., Staufenbiel M., Lewis J., Hutton M., Tolnay M., Jucker M. : *Induction of Tau pathology by intracerebral infusion of Amyloid-β-containing brain extract and by Amyloid-β deposition in APPxTau transgenic mice.* Am J Pathol, 2007. 171(6): p. 2012-20.
   * Contributed equally

4. Miserez AR, **Clavaguera F**, Monsch AU, Probst A, Tolnay M.: *Argyrophilic grain disease: molecular genetic difference to other four-repeat tauopathies.* Acta Neuropathol (Berl). 2003 Oct; 106(4): 363-6.

Reviews

1. Frank S., **Clavaguera F.**, and Tolnay, M.: *Tauopathy models and human neuropathology: similarities and differences.* Acta Neuropathol. 115, 39-53 (2008).

2. Tolnay M, **Clavaguera F.**: *Argyrophilic grain disease: a late-onset dementia with distinctive features among tauopathies.* Neuropathology. 2004 Dec; 24(4): 269-83.

Abstracts

1. **Clavaguera F.**, Bolmont T., Crowther R.A., Abramowski D., Frank S., Probst A., Fraser G., Stalder A.K., Beibel M., Staufenbiel M., Jucker M., Goedert M., and Tolnay M.: *Transmission and spreading of tauopathy in transgenic mouse brain.* Swiss Society for Neuropathology, St-Moritz, 2008.

2. Osinde M., **Clavaguera F.**, Schade A., Stoehr N., Pun S., Tolnay M.,Wiederhold K.H., Staufenbiel M., Dev K.K.: *Pathology study in wild-type and APP23-transgenic animals overexpressing Human Tau P301S.* 10th Conference on AD disease and related disorders, Madrid, 2006.

3. Bolmont T., **Clavaguera F.**, Meyer-Luhemann M., Herzig M., Lewis J., Hutton M., Staufenbiel M., Jucker M., Tolnay M.: *Neurofibrillary pathology in mutant tau transgenic mice is triggered by intracerebral infusion of human Alzheimer's disease brain extract.* Swiss Society for Neuropathology, St-Moritz, 2005.

Presentations

1. **Clavaguera F.**: Induction of tauopathy through the seeded aggregation of Tau protein in transgenic mouse brain. *Neurobiology Program of the University of Basel*, December 2007.
2. **Clavaguera F.**: Induction and spreading of Tau pathology. *Neurobiology Program of the University of Basel*, June 2007.

3. **Clavaguera F.**: Neurofibrillary pathology is triggered in mutant tau transgenic mice by intracerebral infusion of material from diseased brains. *Neurobiology Program of the University of Basel*, July 2006.