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# Transmission of Tau Pathology from Human to Rodent Brain: How to Humanise Animal Models for Alzheimer's Disease Research

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## Abstract

Tauopathies represent a group of neurodegenerative disorders characterised by the accumulation of conformationally altered tau protein. Alzheimer's disease (AD) is the most prevalent primary tauopathy. In AD, tau pathology progressively spreads across a stereotypical sequence of anatomically connected brain regions. In early stages, the disease manifests in the locus coeruleus and entorhinal cortex; at later stages it spreads through the hippocampus to cortical brain areas. Recent studies suggest that spreading of pathological tau occurs predominantly through neuron-to-neuron transmission; however, glial cells can also be involved in this process. Propagation depends on the conformational state and post-translational modifications of tau protein of various tau strains. Abnormal tau can subsequently act as a seed, misfolding and aggregating normal tau proteins inside the cells. Several research groups have successfully recapitulated tau transmission in animal models. Currently, we are able to induce and drive tau neurodegeneration by using tau species isolated from diseased human brains. Such state-of-the-art "humanised" animal models represent a powerful tool for development of new drug leads and diagnostics for human tauopathies.

**Keywords:** Tau spreading; Tau aggregates; Tauons; Tau propagation; Tauopathies

## Brief History on Transmission of Infectious Proteins in Neurodegenerative Diseases

Scrapie, a slowly progressive neurologic disease of sheep and goats, was found to be transmissible by injecting healthy animals with extracts from diseased sheep [1]. Ensuing studies on transmissibility of scrapie into mice [2] or of Kuru, a human neurodegenerative disease, into chimpanzees [3-5] strengthened the evidence for the presence of an infectious entity in these disorders. Later, it was shown that infectivity in spongiform encephalopathy, a neurologic disease of cattle, was mainly caused by a proteinaceous agent [5,6]. However, the scientific community initially rejected the possibility of the existence of an infectious agent that is devoid of any genetic information and it was not until 1982 that Prusiner et al. purified and isolated this proteinaceous infectious agent and coined the term "prion" [7].

Prion protein in physiological cellular form (PrP$^C$) is a cell-surface glycoprotein which is predominantly arranged in alpha-helical sheets, with unknown function. However, in its diseased state, the misfolding of PrP$^C$ results in a conformationally altered PrP scrapie form (PrP$^{SC}$) with high β-sheet content, which bestows both replicative and infectious properties. Although the exact mechanism of prion infectivity is unknown, PrP$^{SC}$ demonstrates polymorphic and self-aggregation propensity [8]. PrP$^{SC}$ aggregates extracellularly by forming protease-resistant filaments called 'amyloid', which propagate to different regions in the brain via repeated cycles of filament elongation and breakage [9]. A few mechanisms for this transmission have been proposed, such as synaptic or vesicular transport [10]. Furthermore, prion proteins demonstrate a unique inter-individual transmissibility, being capable of dissemination though saliva, milk, urine, or other biological excretes [9,11].

Based on the prion hypothesis, numerous authors proposed that other misfolded proteins involved in neurodegeneration, such as tau, amyloid-β, or α-synuclein, might be transmissible from cell to cell [12-15]. This theory is being fervently investigated in Alzheimer's disease (AD) and in multiple system atrophy (MSA), a progressive neurodegenerative synucleinopathy [16], due to similarities in their clinical symptoms with Creutzfeldt-Jakob disease (CJD) [17]. Moreover, similar to prion diseases, these disorders are also tied to pathological intra and extracellular aggregates: Misfolded tau and β-amyloid in AD [18-22] and neuronal and oligodendroglial inclusions of α-synuclein in MSA [23,24], hinting at closer similarities between these diseases. Recent experimental evidence demonstrates that misfolded tau protein exhibits prion-like properties, inducing and propagating neurofibrillary pathology in healthy rodent brains [25-30]. Particularly, increasing attention is given to factors which may play key roles in the seeding and propagation of tau pathology in human neurodegenerative diseases.

## Spreading of Pathology in Tauopathies - The Inside Story

Misfolded tau protein, a hallmark of human tauopathies, displays prion-like features [31,32]. Tau pathology in AD and some other tauopathies such as argyrophilic grain disease and Pick's disease initially manifests in the entorhinal cortex (EC) and spreads along the hippocampus (HP) to other cortical areas [33-37]. Braak and Braak [33] first showed that in AD there is a characteristic progressive hierarchical distribution of misfolded tau protein in neuroanatomically connected brain areas. More specifically, the initial "pre-tangle" cytoskeletal alterations (i.e., accumulation of hyperphosphorylated tau protein) may spread from the trans-entorhinal region (anteromedial temporal

isocortex), the magnocellular nuclei of the basal forebrain and the locus coeruleus to other brain regions [38]. This characteristic spreading is also observed *in vivo* using Tau-PET scans of AD patients at various Braak stages [39].

In transgenic mice, the tau spreading was successfully recapitulated. The pattern of tau spreading follows a synaptically connected circuit, which is either monosynaptic (as in the hippocampus) or trans-synaptic (as in the cortex) [40]. A transgenic mice model expressing human tau in the EC also recapitulates the trans-synaptic spread of tau pathology [41].

Besides humans, the intraneuronal accumulation of hyper phosphorylated tau has been reported in brains of dogs, Rhesus monkeys and gorillas, bisons, rabbits, reindeer, wolverines, bears, goats, sheep and cats. Most affected neurons have been identified as pyramidal neurons of the hippocampus and entorhinal cortex [42-52]. These findings suggest a selective vulnerability of this brain region and anatomical conservation of the disease progression in various animal species.

The factors that influence this characteristic spread of tau in the brain are less known. Recently, a brain-wide transcriptomic analysis from healthy individuals revealed an expression signature in regions vulnerable to AD pathology (Braak regions), when compared to non-Braak regions [53]. These regions displayed elevated expression of proteins that co-aggregate with tangles and plaques in AD, such as 14-3-3ζ, 14-3-3ε, Cathepsin D, etc. Moreover, the levels of proteins that prevent aggregation of amyloid and tau, for example, chaperones such as HSPA1A, HSPA1B, HSPA1L and GRP78 were lower in AD-vulnerable regions when compared to non-AD regions. On the other hand, the expression levels of proteins which promote aggregation of tau and amyloid, such as Cdc37, GSK3α, GSK3β and FKBP5 were good predictors of tissue vulnerability to AD and closely mimicked the staging of AD pathogenesis [53]. This study is the first in line to investigate the susceptibility of certain brain regions in correlation with the Braak staging in the brain, necessary raising the question whether transcriptomic levels of certain proteins associated with AD pathogenesis are deregulated, how different the proteomic picture is, especially considering the array of post-translational modifications that are found in AD protein lesions. The post-mortem delay in humans prevents the examination of more delicate pathological features. As a rodent's brain can be preserved within minutes of sacrifice, evaluating transcriptomic and proteomic pattern in animal models recapitulating the spread of tau pathology may provide otherwise unobtainable evidence about region-specific changes in the diseased brain.

## Tauons and their Infamous Cousins – The Molecular Abyss

Intrinsically disordered proteins (IDPs) such as tau, β-amyloid, α-synuclein etc. are considered to be key players in the neurodegenerative cascade in human neurodegenerative disorders [54]. These proteins naturally lack characteristic secondary and tertiary structures unless bound to a partner. Another unique property of these proteins is their ability to adopt β-structures which contribute to formation of amyloid-like fibrils [55], alleged to be involved in seeding and aggregation of these proteins [56,57].

Recently it was shown that a major factor that contributes to formation of amyloid-like fibrils is the presence of segments with high fibrillisation propensity (HFP) that form "steric zippers", or self-complementary beta sheets, that serve as the backbone for the nascent aggregates [58]. These regions are particularly enriched with valine (V), isoleucine (I), alanine (A) and serine (S). Aggregation of tau protein into PHF is driven by its transition to β sheet structure [59]. Based on "PASTA 2.0", an advanced algorithm for prediction of amyloid-like structural aggregation depending on aggregation-prone regions (http://protein.bio.unipd.it/pasta2/) [60], we observed that tau protein (2N4R; longest tau isoform, 441aa) has 17.69% of sequences with potential for beta-strand formation (Table 1). Moreover, several HFP sequences are distributed along the length of tau protein, mainly in the microtubule binding repeat domain (MTBR), indicating a propensity for the formation of prion-like motifs in tau protein. For example, $^{aa306}$VQIVYK$^{311}$ (based on single letter amino acid code) in the third MTBR of tau is identical to a segment globally classified as having high beta-sheet aggregation propensity [58]. This sequence is known to be involved in assembly of PHF [61]. Another HFP sequence, $^{aa274}$KVQIIN$^{279}$, is also present in the microtubule binding region of tau. Post-translational modifications lead to the structural changes of HFP segments that may allow oligomerisation of tau molecules. Recently, independent studies on PHFs and straight filaments (SF) from AD conclusively demonstrated that the 3rd repeat and 4th repeat binding domains of tau occupy the common core of these filaments, with mixture of 1st and 2nd repeats [62,63]. These are arranged as eight beta-sheet structures with N-terminal ends formed by $^{aa306}$VQIVYK$^{311}$ sequence which complement with $^{aa}$373-378 residues. In addition, the purified fraction of sarcosyl-insoluble tau was successful in seeding aggregation *in vitro* [63]. Put together, this evidence strongly implicates HFP sequences in seeding of aggregation and highlights their potential for propagation of tau pathology.

It is proposed that these disease-modified tau proteins termed "tauons" can undergo self-assembly and aggregate healthy tau protein

| Protein | Length (aa) | % disorder | % α-helix | % β-strand | % coil |
|---|---|---|---|---|---|
| Prion* | 246 | 35.77 | 26.42 | 14.63 | 58.94 |
| Tau | 441 | 62.35 | 6.58 | 17.69 | 75.74 |
| α-synuclein | 140 | 34.28 | 26.43 | 22.14 | 51.43 |
| Amyloid β40 | 40 | 30.0 | 0.0 | 52.5 | 47.5 |
| Amyloid β42 | 42 | 30.95 | 0.0 | 40.48 | 59.52 |
| TDP43 | 414 | 34.05 | 16.18 | 21.5 | 62.32 |

Using web-based algorithm, PASTA 2.0 (http://protein.bio.unipd.it/pasta2/) for prediction of amyloid structural aggregation, high fibrillation propensity motifs (HFP) were identified. Threshold score of -5.0 pasta energy units was set (1.0 Pasta Energy Unit=1.192 Kcal/mol). HFP sequences which are have propensity to form β-sheet structures are observed in all intrinsically disordered proteins. The percentage of disorder and percentage of sequences with potency to form α-helix, β-strands and coils are given. *partial sequence. aa-Amino acids; Amyloid β40- Amyloid beta 40; Amyloid β42-Amyloid beta 42; HFP- High fibrillation propensity motifs; TDP43- Transactive response DNA binding protein 43 kDa; α-helix- Alpha helix, β-strand- Beta strand

**Uniprot id:** Prion-D4P3Q7; Tau-P10636-8; Amyloid β40 and 42 were obtained from APP-P05067 residues aa688-711 and aa688-713 respectively; alpha-synuclein-P37840; TDP 43-Q13148

**Table 1:** High fibrillation propensity motifs on intrinsically disordered protein.

in a non-saturable fashion and spread through the brain, resulting in infectivity and disease progression [12]. Tau is plagued by a wide range of pathological post-translational modifications, such as truncation, hyper phosphorylation and ubiquitination, which promote structural misfolding of the protein [12,19,20,64-67]. Along with the presence of HFP sequences which allow tau's aggregation into beta-sheets this means that pathologically altered protein tau is an elegant agent for initiation and propagation of disease pathogenesis in tauopathies.

## Experimental Seeding of Tau Protein Lead to Progression of Pathology to Defined Brain Areas

An early study on transmissibility of AD was performed on hamsters which were inoculated with a buffy coat preparation from AD patients [68]. These animals developed CJD-like histopathological alterations. However, later studies strived to mimic the spreading of tau pathology by direct injection of diseased brain extracts from animal models, AD or other tauopathies [69-72], mainly into the hippocampus, a region highly vulnerable to tau pathology. The initial study after injection of insoluble tau (hyper phosphorylated tau) from AD brain into wild type rats showed accumulation of amyloid beta, ubiquitin and α1-antichymotrypsin along with accumulation of tau filaments in these animals [69]. However, it was uncertain whether insoluble tau alone contributed to the formation of filaments, or whether the accumulation of these proteins was due to the presence of other proteins. The research groups of Boluda et al. were the first who clearly demonstrated the ability of misfolded tau to induce tau neurodegeneration [71]. The study showed that the injection of brain extract from tau P301S mice line into ALZ17 mice which express a single isoform of human wild type tau induced full-blown tau pathology. Prion-like properties of human misfolded tau were observed also after inoculation of purified tau oligomers from human AD into C57BL/6 mice. However, due to the lack of comprehensive immunohistochemical staining this study does not represent the final proof of the infectivity of tau oligomers [27].

Similar to prions, "tauon" strains are specific for different tauopathies [70,72,73]. Misfolded tau isolated from different tauopathies, such as argyrophilic grain disease (AGD), tangle-only dementia (TD), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) also induced and drove tau pathology in injected animals [70,72]. The morphology of tau inclusions was diverse and identical to their respective human counterparts. For example, injection of AD or TD material induced the typical neurofibrillary pathology to these diseases [70,72]. On the other hand, CBD tau induced glial tau pathology in the white matter tracts and hippocampus [72]. Inoculation of AGD into ALZ17 mice induced small spherical or comma-shaped argyrophilic grains identical to human AGD [70]. Moreover, seeding of tau from different tauopathies into the PS19 mouse line also affected the specific cell types seen in these human tauopathies i.e., neuronal pathology was initiated by AD extracts and predominant oligodendroglial pathology by material from CBD [72]. Furthermore, injection of material from other tauopathies (AGD and PSP) into ALZ17 mice also resulted in lesions similar to these human pathological diseases. However, formation or propagation of classical Pick bodies was not observed after injection of homogenates from Pick's disease [70,72]. One of the reasons for this selective infectivity could be the absence of a proper "sparring partner" in the inoculated organism, analogous to the species barrier to prion transmission. We showed that injection of brain extract from the transgenic rat SHR24 line, which expresses 3R form of human truncated tau, into the SHR72 transgenic rat line, which expresses 4R human truncated tau, developed tau pathology at the site of injection, but no propagation of pathology to other regions was observed [28].

However, administration of misfolded tau from SHR72 transgenic rats not only induced pathology in cortical neurons in these animals, but also induced neurofibrillary tangles in adjacent brain areas. Since the Alz17 line used by Clavaguera et al. [70] and Boluda et al. [71] mainly expresses human four-repeat tau protein, the three-repeat tau inclusions from PiD lacked the substrate necessary for the propagation of pathology. These results clearly show that pathologically altered tau protein requires a proper substrate in the target organism in order to manifest its infectious and spreading properties i.e., 3R tau or 4R tau for the respective tauopathies. The tau isoforms from diseased human or rodent brains differ in their specificity and infectivity, which accounts for the pathological differences between 3R and 4R tauopathies.

Most studies on the tau spreading phenomenon have been performed on transgenic rodent models; however, few studies have investigated the spreading of misfolded tau in wild type mice. Wild type animals injected with tau extracts from human P301S mice resulted in Gallyas silver and AT100 positive threads and coiled bodies at the site of injection [71], but no NFTs or progression of disease were observed at 6 and 12 months post-injection. In a later study, injection of up to 8 μg of tau protein induced tau pathology, as well as spreading in non-transgenic mice [74]. Furthermore, neuropil threads and oligodendroglial coiled bodies were observed in wild type mice following injection of AD, TD or PSP brain extracts; only astroglial tau inclusions positive for AT8 or AT100 were observed post injection of CBD brain extracts [70]. A recent study clearly demonstrated that by injecting pathological tau extracted from AD, PSP and CBD patients into different brain regions of wild-type mice, endogenous mouse tau aggregates were inducted and propagated [75]. Interestingly, PSP and CBD tau strains induced astroglial and oligodendroglial tau inclusions as was shown by Clavaguera et al. [70]. These findings suggest that higher levels of pathological tau injected into the brain of wild type mice may break the protective barrier of the endogenous mouse tau proteome.

## Mechanisms of Tau Release and Spreading

A growing body of evidence strongly supports the notion that extracellular tau plays a key role in spreading of tau pathology [76,77]. Tau is constitutively released from neurons under physiological conditions [71,76-88]. The secretion is multifaceted, involving diverse intracellular components such as autophagosomes, Golgi, endosomes, ectosomes, lysosomes, microsomes and endoplasmic reticulum [89]. In the diseased brain, extracellular tau additionally originates from dead or dying neurons as well as a result of active secretion [76,90] and yet unknown mechanisms. Therefore, the modes of tau release in pathological conditions are constantly being investigated.

Recently, it was shown that synaptic terminals release tau upon depolarisation, however, the levels of released tau were higher in synaptic terminals from AD brains [91,92]. Calafate et al. [92] demonstrated that hippocampal neurons expressing human truncated tau K18 (aaQ244-E372) with the P301L mutation develop intracellular tau aggregates on exposure to K18P301L fibrils aggregated with synthetic heparin. When cultured in a microfluidic chamber, these cells triggered tau pathology in non-treated neurons cultured in a distant compartment via synaptically connected neurons in the intermediate chamber. This process of tau release was also observed *in vivo*, where enhanced neuronal firing increased the release of tau in the rTg4510 line. Chemo- or optogenetical stimulations of left hippocampi of rTg4510 demonstrated enhanced tau pathology mainly in the CA1 and CA3 hippocampal neurons in this hemisphere when compared to the

unstimulated right hemisphere [92,93]. The depolarisation of synaptic terminals from AD brains is enhanced in comparison to control human synapses. Moreover, AD synaptic terminals contain tau oligomers [94], C-terminally truncated tau along with 20 kDa tau fragment [91], which may be released after activation. In addition, tau is reported as a key component of tunnelling nanotubes (TNT) which are tubular structures that facilitate cell-cell communication under stress [95]. In addition, extracellular tau increases the formation of these tunnelling nanotubes, therefore aggravating transfer of tau between neurons [95].

The presence of extracellular tau in the CSF of AD patients and transgenic models strongly advocates that at least a portion of extracellular tau (N terminal fragments) may exit the brain [90,96]. It was recently shown that mTor activity is involved in tau secretion into the extracellular space [97]. On the other hand, tau can be uptake by neuronal and non-neuronal cells. HEK293 cells expressing aggregation prone tau species produced fibrillary tau aggregated which were released into extracellular medium and was seeded in neighbouring cells [77]. Moreover, HEK293 cells expressing human mutant P301L tau develop intracellular tau aggregates and co-culture of these cells with hippocampal neurons induced tau pathology in these neurons [93], indicating a possibility transmission of tau pathology from non-neuronal donor to neuronal cell. Interestingly, the infectivity of prions and α-synuclein between distal non-neuronal cells and the brain has also been reported [98-100]. It would be interesting to study whether similar infectivity of tau is observed *in vivo* in transgenic models or AD.

In an elegant study by Holmes et al. [101], it was shown that the uptake and propagation of tau is mediated by heparin sulphate proteoglycans via micropinocytosis. Increased tau propagation was observed following down regulation of amphiphysin 2 (BIN1), a regulator of clathrin-mediated endocytosis [102]. The sizes of tau fibrils or aggregates also play a crucial role in the process of active endocytosis. Aggregated full-length tau or truncated tau forms are readily endocytosed by neuronal and non-neuronal cells; tau monomers, long fibrils or filaments are not [78,81,87]. Put together, the ability to seed, induce aggregation and propagate tau pathology in specific cells and cell compartments depends on tau conformation, its post-translational modification/s and size and involves a complex of known and unknown secretory and uptake mechanisms.

## The Role of Misfolded Tau in the Seeding and Propagation

Various forms of tau protein like fibrils [87,103,104], filaments [28,72,105,106] and oligomers [27] or monomers [107,108] display different seeding properties despite the difference in the origin of the seeding tau protein. Studies also showed differences in propagation (brain areas affected, distance of spreading) after injection of recombinant tau [87,103,104] or native forms [105,107]. Interestingly, tau from human tauopathies brain extracts induced tau pathology mimicking the parent neurodegenerative diseases (Table 2). Moreover, in most of these cases tau pathology in the form of NFTs, NTs and argyrophilic grains were observed. On the contrary, few studies only demonstrated presence of hyper phosphorylated tau inclusions after seeding of tau from human AD without formation of NFTs [27,72,74,106]. In these studies, spreading of tau pathology from site of injection was observed. The only exception was extract from Pick's disease, which induced tau pathology only at the site of injection [70].

Similarly, various forms of pathological tau protein extracted from rodent brain induced tau pathology when injected into the rodent brains (Table 2). Majority of the studies showed the presence of mature NFTs

and NTs in these models [28,71,107]. In a first rat study, we also showed the formation of NFTs in cortical areas and striatum after injection of insoluble tau isolated from two transgenic rat lines expressing human truncated tau [28]. All these studies suggest that the misfolded tau from transgenic rodent models display similar seeding potency as human misfolded tau.

In contrast to tau from rodent and human brain extracts, various forms of synthetic tau oligomers or filaments did not induce NFTs or NTs; however, in all the cases the presence of hyper phosphorylated tau was reported [74,103,104,108-110]. Only one study showed NFT like tau pathology and presence of conformationally modified tau protein in the tau inclusions [103]. Thus, synthetic tau does not fully recapitulate the seeding and spreading potency of human or rodent pathological tau [111-114].

Finally, Sanders et al. [72] and Kaufman et al. [115] elegantly demonstrated the presence of different tau strains in the brain of human tauopathies. Isolated tau strains from human tauopathies induced different forms of pathological aggregates in HEK293 cells. These aggregates were able to induce AT8 positive tangle-like pathology if they were injected into the mouse brain. Contralateral spreading of tau pathology was also reported in both cases implicating the seeding and propagation potency of various strains of misfolded tau. To sum up, the 'tauons' retain their pathological potential even in cell cultures and successfully seed and transmit pathology when reintroduced in brains.

Although the propagation of tau pathology in the brain is observed, less is known about the intrinsic property of tau protein responsible for its seeding and propagation. Recently it was shown that the conformation of tau protein determines the templating potency and assembly into filaments. Interestingly, *in vitro* heparin induced synthetic tau aggregates displayed higher stability to guanidium hydrochloride solubility than native sarcosyl-insoluble tau aggregates from TgP301S mice. This was attributed to the difference in conformations between the two preparations [112]. However, the insoluble tau aggregates from TgP301S mice displayed higher potency for seeding when compared to *in vitro* heparin generated tau aggregates. Several other groups also report a weak seeding potency of soluble tau [71,109], suggesting that insoluble tau predominates as the infectious component of the tau proteome in spreading of tau pathology. Apparently, the misfolded conformation is encoded more stably in insoluble and/or aggregated tau protein and aggregates find it easier to convert and aggregate healthy tau protein than soluble pathological tau molecules do.

## Concluding Remarks

Based on evidence from *in vitro* and *in vivo* studies, the spread of tau pathology displays the following characteristic features:

1) Pathological misfolded tau from a diseased brain can induce tau pathology in the brain of transgenic and wild type animals

2) Spreading is a dynamic process which is time and dose-dependent and propagates through synaptic connectivity

3) Specific tauopathy brain extracts induced cell tau pathology in injected brains and the induced pathology is analogous in appearance to the parent tauopathy (AD – neuronal lesions, CBD, PSP – astroglial and oligodendroglial lesions etc.)

4) A high amount of tau, of either exogenous or endogenous origin, seems to be essential for induction and spread of tau pathology in mouse models

| Origin of tau | Ref | Age (injection time/line) | Sampling time | Injection region (coordinates from bregma) | Concentration/ Total Volume | Speed of application | Positive brain areas for tau pathology after seeding/notes |
|---|---|---|---|---|---|---|---|
| **Brain extracts from human tauopathies** | [27] | 3 months C57BL/6 | 11 months PI | **Hippocampus** A/P-2.06 mm, L ± 1.75 mm, D/V - 2.5 mm | **AD tau oligomers or PHF** 0.3 μg/ μl Total 2 μl | 0.2 μl/min | **Injection of tau oligomers: Few tau inclusions** in the hippocampus, corpus callosum, hypothalamus, cortex **Injection of PHF: Tau inclusions** in the hippocampus (only on the place of injection) |
| | [70] | 3 months ALZ17 C57BL/6 | 6, 12, months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V-1.8 mm **Overlying cortex** A/P-2.5mm, L+2 mm, D/V-0.8 mm | **AD, TD, AGD CBD, PSP, PiD tau** Total 2.5 μl | 1.25 μl/min | **Injection of AD, TD, AGD, PSP, CBD, NFts, NTs, argyrophilic grains and nerve cell body inclusions** in fimbria, optic tract, medial lemniscus, dorsal thalamus, cerebral peduncle amygdala, thalamus, internal capsule, entorhinal cortex , Fornix **Injection of PiD, Short, thick NTs and filamentous tau** only in injection site |
| | [72] | 2-5 months PS19 | 1, 3 and 6 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V-2 mm **Cortex** A/P-2.5mm, L+2 mm, D/V-0.8 mm | **CBD tau**- 50ng, **AD tau**-10.5μg, **DSAD tau**-12.5μg Total 5.0 μl | NA | **Injection of CBD AT8 positive tau inclusions** in fimbria (also **tau inclusions positive for MC1, TG3, ThS negative**), alveus, subcortical white mater, neurons in hippocampus CA1, CA3, dentate gyrus, subiculum (predominantly glial tau pathology) **Injection of AD/DSAD AT8 positive tau inclusions** in the lateral septal nuclei, subiculum, entorhinal cortex, locus coeruleus, raphe nuclei,ipsilateral CA1, dentate gyrus and also contralateral site CA3, entorhinal cortex (neuronal tau pathology) **Injection of DSAD ThS positive tau inclusions** in CA3 |
| | [74] | 2-3 months 15-19 months WT | 2,7 d 1, 3, 6, 9, months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V-2.4 mm **Cortex** A/P-2.5 mm, L+2 mm, D/V-1.4 mm | **AD-tau**-0.4 μg/μl total 1 μg, and 1.6 μg/μl Total 4 μg/site | NA | **AT8 positive tau pathology** in ipsilateral (**positive tau inclusions for MC1, TG3, ThS**) and contralateral hippocampus, entorhinal cortex, locus coeruleus, corpus callosum, raphe nucleus, mammillary area |
| | [75] | 2-3 months WT (C57Bl/6) | 1, 3, 6, or 9 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V-2.4 mm **Cortex** A/P-2.5 mm, L+2 mm, D/V-1.4 mm **Thalamus** A/P-2.5 mm, L+2 mm, D/V-3.4 mm | **AD, CBD, PSP tau** 2.5 μl (Hippocampus and Cortex) 4 μl (Thalamus) | NA | **Injection of AD, CBD, PSP, Tau aggregates** in the hippocampus (ventral, dorsal), entorhinal cortex, fimbria, corpus callosum mammillary area, thalamus, olfactory bulb |
| | [106] | 3 months THY-Tau22, WT | 3 months PI | **Hippocampus** A/P-2.1 mm, L+1.5 mm, D/V-2.0 mm | **AD tau** 0.5 μg/μl Total 2 μl | 0.2 μl/min | **No NFTs, but Gallyas phospho and conformational - dependent tau antibodies positive tau inclusions (grains, granules,NT,CB)** in ipsilateral hippocampal hilus, stratum oriens, stratum radiatum, alveus, fimbria, corpus callosum |
| | [116] | 3 months hTau tg mice | 6, 9, 11 months PI | **Hippocampus** A/P-2.5 mm; L ± 2.0 mm; D/V-1.8 mm | **AD tau** 0.12 μg /2.5 μL | 1.25 μl/min | **NFTs and NT positive for phospho - antibodies and ThS** in the hippocampus (CA1, CA2, CA3) dentate gyrus), entorhinal cortex, amygdala, corpus callosum, neocortex, septal nuclei (**AD-P tau**-NFTs and NTs Dephosphorylated AD-P tau - argyrophilic grain-like tau pathology) |
| | [116] | 2-3 months T40PL-GFP tg mice WT | 3 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm; D/V-2.4 mm | **AD tau** 0.8 μg/μl Total 2.5 μl | NA | **AT8 positive tau pathology** in CA3, subiculum, retrosplenial granular cortex, dentate gyrus, more cortical tau pathology, especially entorhinal cortex, contralateral and ipsilateral site (Better seeding tau pathology in WT than in Tg mice) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Brain extracts from rodent models** | [28] | 2 months rat lines SHR72 and SHR24 | 100-175 d PI | **Motor cortex 1** A/P+3 mm, L+2 mm, D/V -0.75 mm | **Rat brain extract (SHR72- 4R tau)** 20 ng/µl **(SHR 24- 3R tau)** 50 ng/µl Total 2 µl | 0.5 µl/min | **NFTs** in cortical areas and striatum |
| | [71] | 3 months ALZ17 C57BL/6 | 6,12 and 15 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V -1.8 mm **Cortex** A/P-2.5mm, L+2 mm, D/V- 0.8 mm | **Mice brain extract (P301S)** Total 2.5 µl | 1.25 µl/min | **CB, NT and NFTs** in the hippocampus, fimbria, optic tract, thalamus, internal capsule, medial lemniscus, zona incerta, cerebral peduncle, hypothalamus, caudate putamen, somatosensory cortex, amygdala, superior colliculus, substantia nigra, entorhinal cortex, visual cortex, mesencephalic nuclei, pontine nuclei some brain region in contralateral site |
| | [87] | 1 month rTg4510 | 4 and 11 weeks PI | **Cortex** A/P-2.5mm, L+/–2 mm, D/V- 1 mm | **hTau SFs** 2µg /µl Total 2.5 µl | 0.5 µl/min | **MC1 positive tangles** on injection site |
| | [107] | 2 months P301S | 1d, 2 weeks, 1 months, 2 months, 2,5 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V -1.8 mm **Cortex/white matter** A/P-2.5mm, L+2 mm, D/V- 0.8 mm | **Mice brain extract (P301S)** Total 2.5 µl | 1.25 µl/min | **NFTs, NTs** in the hippocampal formation (CA1, CA3, dentate gyrus, subiculum) para-hippocampal region,cortex (retrosplenial, ventrolateral), mammillary nucleus, supramammillary nucleus, thalamus (anterio-dorsal, anterio-ventral), lateral septal nucleus, nucleus accumbens |
| | [109] | 2-3 months rTg4510 mice | 3 weeks PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V -1.8 mm | **Mice brain extract (rTg4510) HMW or LMW tau** Total 2.5 µl | 0.2 µl/min | **Injection of HMW tau** **AT8 positive neurons** in ipsilateral dentate gyrus **Injection of LMW tau** **no AT8 positive neurons** |
| | [114] | 10 weeks months TgP301S | 10 weeks months PI | **Hippocampus** A/P-2.5 mm; L+2.0 mm, D/V- 1.8 mm **Cortex** A/P-2.5 mm, L+2 mm, D/V-0.8 mm | **Mice brain extract (P301S)** Total 2.5 µl | 1.25 µl/min | **PG5 positive tangle like tau pathology** in the hippocampus (Ipsilateral, contralateral) retrosplenial cortex, mamillary nucleus/ |
| **Synthetic tau fibrils** | [74] | 2-3 months 15-19 months WT | 3, 6, 9, 18, 24 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V- 2.4 mm **Cortex** A/P-2.5 mm, L+2 mm, D/V- 1.4 mm | **Synthetic tau fibrils** 1,8 µg/ µl total 4.5 µg/site | NA | **AT8 positive tau inclusions** in ipsilateral and contralateral hippocampus |
| | [103] | 2-3 months PS19 | 1, 2, 4 weeks 1, 3, 6, 9 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V- 1.8 mm **Cortex** A/P+0.2 mm, L+2 mm, D/V- 0.8 mm **Striatum** A/P+ 0.2 mm, L+2 mm, D/V- 2.6 mm | **Synthetic tau PFFs** 0.02-0.2 and 2 µg/µl Total 2.5 µl | NA | **NFTs like tau pathology positive for phospho, conformational dependent antibodies and ThS** in ipsilateral and contralateral hippocampus, locus coeruleus, entorhinal cortex, substantia nigra, thalamus, white mater tracts in corpus callosum |
| | [104] | 3 months P301L | 2 d, 1, 2 weeks 1, 2 and 3 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V-2.4 mm **Frontal cortex** A/P+2 mm, L+2 mm, D/V-2.7 mm | **Synthetic tau PFFs** varied 2-5 µl | 1 µl/min | **Injection into hippocampus** **AT8 and ThS positive tau pathology** in the hippocampus, cortex above hippocampus and piriform cortex **Injection into frontal cortex** **AT8 and ThS positive tau pathology** in frontal cortex, hippocampus, amygdala, thalamus, midbrain, brainstem and also to contralateral hippocampus, midbrain, brainstem |
| | [110] | 2-3 months T40PL-GFP tg mice WT | 3 months PI | **Hippocampus** A/P-2.5 mm, L+2 mm; D/V- 2.4 mm | **Synthetic tau fibrils** 0.8 µg/µl Total 2.5 µl | NA | **AT8 positive tangle like tau pathology** in CA3, subiculum, retrosplenial granular cortex, dentate gyrus, contralateral and ipsilateral site (less tau pathology in WT than in Tg mice) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Cell lysates** | [73] | 3 months P301S WT | 3 and 5 weeks PI | **Hippocampus** A/P-2.5 mm, L ± 2 mm, D/V -1.8 mm | **Clone 1, 9,10** 5 µg/µl Total 2 µl | 0.2 µl/min | **AT8 positive tangle like tau pathology** in the hippocampus (ipsilateral, contralateral), retrosplenial cortex, entorhinal cortex (ipsilateral, contralateral), ipsilateral subiculum and dentate gyrus |
| | [111] | Young PS19 Age NA | 4, 8, 12 weeks PI 5 weeks PI | **Hippocampus** A/P-2.5 mm, L+2 mm, D/V -1.8 mm **SC, CP, VC, Hip, Thal, IC** Exact coordinates NA | **DS1-DS19 strains** 5 µg/µl Total 2 µl | NA | **AT8 positive tangle like tau pathology** in the hippocampus (Ipsilateral, contralateral) (DS6, and DS9 the most potent tau strains in seeding **AT8 positive tau pathology** in all injected regions) |

**Table 2:** Overview of *in vivo* studies on propagation of tau pathology in rodent models.

5) The spreading of tau pathology in the brain of rodent models is a combined process involving injected human/rodent tau and misfolding and aggregation of endogenous tau

6) Most importantly, the induction and propagation of misfolded tau requires the presence of proper substrate, or "sparring partner" for template-mediated conversion, namely 3R or 4R tau for respective tauopathies respectively.

In conclusion, the variable infectious potential of tau strains depends on the origin of the seed protein, its post-translational, conformational and morphological state. Moreover, the difference in ability to induce a pathological process confirms the existence of various tau strains or "tauons" in diseased brains [12,113]. Major questions that yet remain to be thoroughly understood are the mode of propagation of pathology and how tau aggregates from one neuron drive aggregation and fibrillation in another neuron. Although some mechanisms of secretion have been identified (synaptic, exosomal, or vesicular) and a few other mechanisms of secretion and spreading have been speculated, a clear picture of the role of various cell compartments and cell types in propagating tau pathology is missing.

Although prion-like transmission of tau protein is widely being investigated in mouse models, some argue that tau protein cannot behave like a genuine prion [114]. This is mainly due to the fact that prion diseases are transmitted from animals to humans or humans to humans. Interestingly, cadaver-derived human growth hormone containing neurodegenerative disease-associated proteins such as tau, Aβ and α-synuclein when injected into humans did not show any risk for Alzheimer's disease or Parkinson's disease in these recipients indicating the lack of transmissivity properties in these extracts [115,116]. This may also be due to the lack of potential seeding strains of tau or other proteins in these extracts or due to the lack of required incubation period for the disorders to manifest and mature. Therefore, elaborate studies are required to thoroughly address the discrepancies in human to human transmission of tauopathies using similar approaches. However, documented *in vitro* and *in vivo* evidence on cell lines and rodent models universally supports the seeding and propagation of misfolded tau. Future studies will define the nature of the tau seed in different tauopathies and delineate the various models of tau transmission in the diseased brain.

## Using Future Applications of Humanise Models

The usefulness of the above findings becomes clear when one considers the root cause of many development failures in AD drug research: inappropriate models. To illustrate, some drug research in AD is conducted on toxin-based models; in these models, toxic lesions to the hippocampus produce a phenotype dominated by learning disability, overtly AD-like. Yet, the brains of these models contain neither tau nor amyloid pathology and thus are utterly unsuitable for the development of disease-modifying therapies that would halt the

progression of these pathologies. In contrast, models based on the spreading of injected material are very faithful to the human disorder: the pathology is induced and driven by genuine pathological tau from human tauopathies, progresses as in humans and the conformation-based nature of tau protein seeds means that the pathology induced in the animals retains important molecular characteristics. Models with such human-like pathology constitute an excellent tool for the study of therapeutic and diagnostic candidates, with increased predictive value for subsequent human studies and therapeutic development. Similarly, generating genuine tau pathology in animals makes them excellent screening platforms for novel tau PET ligands and may be helpful in selection of tau ligands dedicated for various human tauopathies.

A potential shortcoming of spreading-based models is the increased variability, which arises from the interaction of inter-individual variability of the model animals and inter-individual variability of donors of tau seeding material; this has to be overcome through rigorous characterisation of the injected tau seeds and through an increased understanding of what makes a particular tau strain a strong seed.

To sum up, beside the scientific value, the rodent models of human-like tau spreading open up previously unavailable vistas for the effective preclinical development of novel drugs and diagnostic tools.

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