Acta Neuropathol (2016) 131:27–48
DOI 10.1007/s00401-015-1507-z

REVIEW

Propagation of tau pathology: hypotheses, discoveries, and yet unresolved questions from experimental and human brain studies

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Received: 8 July 2015 / Revised: 4 November 2015 / Accepted: 5 November 2015 / Published online: 17 November 2015

Abstract Tau is a microtubule-associated protein and a key regulator of microtubule stabilization as well as the main component of neurofibrillary tangles—a principle neuropathological hallmark of Alzheimer's disease (AD)—as well as pleomorphic neuronal and glial inclusions in neurodegenerative tauopathies. Cross-sectional studies of neurofibrillary pathology in AD reveal a stereotypic spatiotemporal pattern of neuronal vulnerability that correlates with disease severity; however, the relationship of this pattern to disease progression is less certain and exceptions to the typical pattern have been described in a subset of AD patients. The basis for the selective vulnerability of specific populations of neurons to tau pathology and cell death is largely unknown, although there have been a number of hypotheses based upon shared properties of vulnerable neurons (e.g., degree of axonal myelination or synaptic plasticity). A recent hypothesis for selective vulnerability takes into account the emerging science of functional connectivity based upon resting state functional magnetic resonance imaging, where subsets of neurons that fire synchronously define patterns of degeneration similar to specific neurodegenerative disorders, including various tauopathies. In the past 6 years, the concept of tau propagation has emerged from numerous studies in cell and animal models suggesting that tau moves from cell-to-cell and that this may trigger aggregation and region-to-region spread of tau pathology within the brain. How the spread of tau pathology relates to functional connectivity is an area of active investigation. Observations of templated folding and propagation of tau have prompted comparisons of tau to prions, the pathogenic proteins in transmissible spongiform encephalopathies. In this review, we discuss the most compelling studies in the field, discuss their shortcomings and consider their implications with respect to human tauopathies as well as the controversy that tauopathies may be prion-like disorders.

Keywords Conformational templating · Macropinocytosis · Neurofibrillary tangles · Propagation · Prion · Seeding · Selective vulnerability · Tau

Introduction

The microtubule-associated protein tau is the major structural protein of neurofibrillary tangles (NFT) in Alzheimer disease (AD) [45]. Tau protein that accumulates in NFT has a number of physicochemical properties that differ from normal tau. While normal tau is a heat-stable, unfolded protein that is protease sensitive, tau in NFT is highly ordered and protease resistant with beta-sheet structure similar to amyloid. Normal tau is a phospho-protein that promotes microtubule polymerization and stabilization, but tau in NFT has increased and abnormal phosphorylation, is dissociated from microtubules and is inefficient in promoting microtubule polymerization [54]. Once considered to be relatively restricted to neurons [10], it is now known that tau also accumulates in glia in a wide range of neurodegenerative disorders and in the aging brain [33]. Disorders in which tau pathology is the major neuropathologic characteristic are referred to as "primary tauopathies." On the

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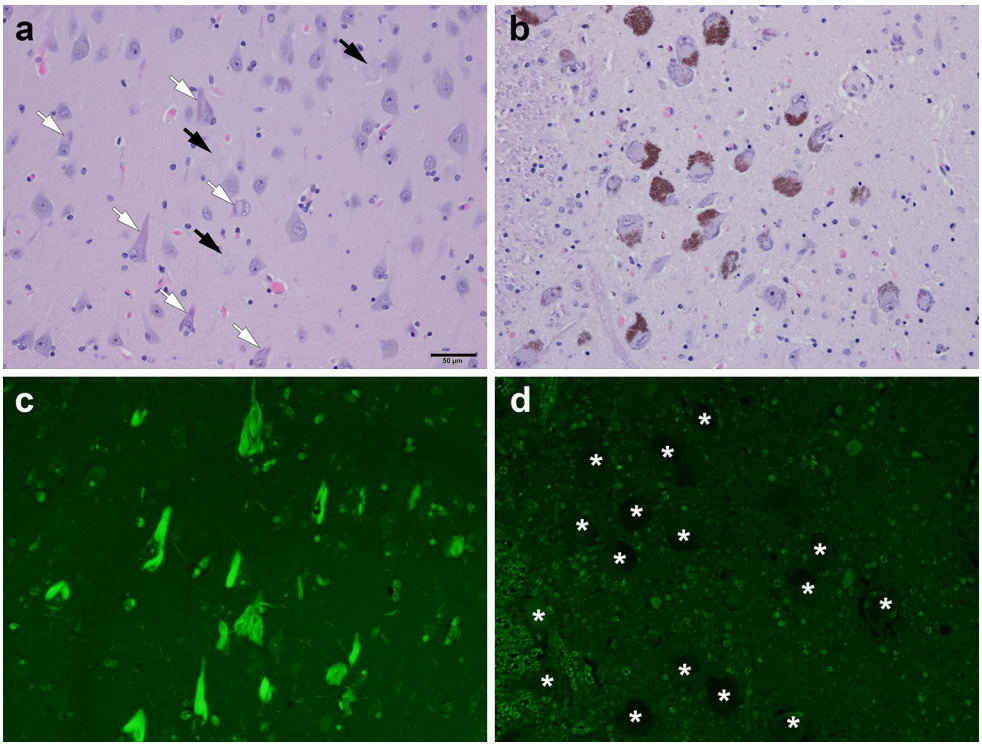

Fig. 1 An 83-year-old woman with PART has NFT and neuronal loss in CA1 sector of hippocampus (**a**, **c**), but none in the locus ceruleus (**b**, **d**) with hematoxylin and eosin (**a**, **b**) and thioflavin S fluorescent microscopy (**c**, **d**). *White arrows* in **a** indicate intracellular NFT and *black arrows*, extracellular ("ghost") NFT. *Asterisks* in **d** indicate location of neurons that have no evidence of thioflavin S-positive NFT, which contrasts with numerous NFT in **c**. *Brown* pigment in **b** is neuromelanin pigment, which accumulates in noradrenergic neurons with aging. *Bar* in **a** = 50 μm

other hand, tau that accumulates in disorders considered to have another driving force—often another amyloid protein (Aβ, PrP, ABri), including familial and sporadic AD, familial Gerstmann–Sträussler–Scheinker disease [41] and familial British dementia [84]—are referred to as "secondary tauopathies." Tau protein in the brain is heterogeneous due to alternative splicing of exons 2, 3 and 10. Alternative splicing of exon 10 generates tau species with either three or four conserved ~32 amino acid repeats in the microtubule-binding domain of tau protein [5], referred to as 3R and 4R tau. There is preferential accumulation of 3R or 4R tau in various tauopathies, providing a subclassification of the tauopathies [63]. In secondary tauopathies, including AD, tau is composed of an equimolar ratio of 3R and 4R tau (3R + 4R tau) [42], while primary tauopathies may preferentially accumulate 3R, 4R, or 3R + 4R tau, depending upon the disorder [21].

NFT that occur in AD (and in some of the other secondary tauopathies) have a predilection for medial temporal lobe structures, including the hippocampus, entorhinal cortex and amygdala, as well as subcortical nuclei with long projections to the neocortex, including cholinergic neurons of the basal forebrain, serotonergic neurons of the raphe nuclei, and noradrenergic neurons of the locus ceruleus. The topography of neurons vulnerable to NFT was described by Hirano and Zimmerman over 50 years ago [49], and it was placed into a hierarchical scheme by Braak and Braak nearly 25 years ago [15]. Specifically, they proposed a sequential progression of neurofibrillary pathology, with initial deposits in the anteromedial temporal lobe, progressing to hippocampus proper and subsequently to multimodal association areas and finally secondary and primary modality cortices. More recently, Braak et al. have revised the staging scheme after examining the brains of younger cohorts and discovering NFT in the locus ceruleus of a subset of individuals [18], leading to the hypothesis that subcortical nuclei may actually be the site of the initial seed for tau propagation. Clearly, this hypothesis needs testing in other cohorts. It may not apply to NFT that occur in the aging brain in the absence of amyloid deposition, in other words as a primary tauopathy. The proposed term for the latter process is "primary age-related tauopathy" (PART) [29]. If the Braak hypothesis is correct, then neurons should be affected in the locus ceruleus before they are found in the medial temporal lobe. As illustrated in Fig. 1, this is not always the case. Illustrated is one such example of an 83-year-old woman with PART with NFT in entorhinal cortex and hippocampus, but

none in the locus ceruleus. Moreover, there is neuronal loss accompanying NFT in the hippocampus, but no neuronal loss in the locus ceruleus. It remains to be determined if PART is unique in this regard. An extreme form of PART is NFT-dominant dementia (NFTD), where NFT and neuronal loss are severe in medial temporal lobe, with minimal "propagation" to higher order cortical neurons [57], which clearly does not fit with the typical Braak NFT staging scheme. It is also increasingly clear that AD is clinically and pathologically heterogeneous and that some individuals do not show a marked predilection for medial temporal lobe structures, but instead neocortical neurons seem to be the primary target for NFT [73]. These individuals do not have typical amnestic syndromes, but may instead have syndromes of behavioral variant frontotemporal dementia, progressive aphasia or posterior cortical atrophy [73]. Yet another AD variant is associated with severe limbic system neurofibrillary degeneration with much less tau pathology in multimodal association cortices, suggesting that this variant may be associated with the relative arrest of tau propagation to anteromedial temporal lobe and associated areas. Models of tau propagation in experimental systems often do not consider or even acknowledge heterogeneity of the human condition that they purport to model.

Nevertheless, it should be clearly acknowledged that the majority of AD patients show the stereotypical pattern of regional densities of NFT. The explanation for the selective vulnerability of a subset of neurons to NFT has been the subject of speculation that has been fueled in recent years by the concept that tau may propagate from one neuron to the next, which is the focus of this review. Other explanations for selective involvement include the hypothesis that neurons selectively vulnerable to NFT have high levels of plasticity that inversely recapitulates ontogenetic and phylogenetic brain development [6] or that they are long unmyelinated or thinly myelinated axons with vulnerability following the inverse sequence of cortical myelination [17]. More recently, with the advent of resting state functional magnetic resonance brain imaging, it is possible to define specific networks of brain regions, whose activity levels are synchronous, differing from one another by the location of the "seed" region of interest, which has led to the hypothesis that "what wires together, fires together and degenerates together" [89, 108]. Whether this functional connectivity model fits with tau propagation remains hypothetical.

The spatiotemporal sequence of NFT pathology in AD [15] and updated more recently [18] leads to the hypothesis that progression is related to cell-to-cell propagation [16]. The concept has been developed mostly from cell and animal models and the following is a critical review of these models and the veracity of the tau propagation hypothesis. According to this hypothesis, tau pathology radiates through the brain along synaptically connected pathways as the disease progresses.

The tau propagation hypothesis

In 2009, the concept of "tau propagation" was introduced as a potential mechanism through which tau pathology may systematically develop and progress through different regions of the brain [37]. In its most rudimentary form, tau propagation has been used to refer to a tau "seed" being introduced or formed, which thereby is transferred into other cells, subsequently promoting additional intracellular aggregation of tau. The newly affected cells then proceed to propagate tau misfolding within other naïve cells.

Tau propagation has become such a groundbreaking and controversial topic that approximately half of the publications on the subject have been reviews or commentaries. The concept of disease spread by templated propagation had its origin in experimental studies of human and animal models of transmissible spongiform encephalopathies, including Creutzfeldt–Jakob disease in humans and scrapie in sheep [54]. These disorders have been shown to be transmissible through the introduction of tissue or extract from an affected individual into a naïve animal, with the ability to be repeatedly passaged to other animals. The term "prion" was coined by Prusiner to describe the transmissible agent, which had properties of a *pro*tein and yet was *in*fectious [83]. The field did not originally embrace the concept that prions could be infectious and propagate based solely on a protein. Similarly, the proposal that tau propagation plays a major role in human tauopathies has elicited doubt among tau biologists and many investigators find substantial questions that must be carefully answered before the term "prion" [47] can be comfortably ascribed to tau. For example, what form of tau functions as a "seed" and how are these seeds transferred into other cells? What factors influence the ability of tau seeds to spread to other cells? Can tau propagate within new hosts? Have we suitably demonstrated that tau can propagate? If so, in which systems can tau propagate? How does tau propagate and is the manner of propagation the same across all systems? If tau can propagate, does it propagate in humans as part of the disease process? If so, how does tau propagation change our concept of at-risk cells, at-risk individuals, and therapeutic development? The current review will address each of these topics, highlight potential discrepancies or confounds in published studies, and then suggest potential roads forward.

Is it all semantics?

As the concept that tau pathology may somehow spread from cell to cell, region to region, and potentially from human to human has emerged, we have quickly adapted language from the prion field [28, 36, 86]. However, our use of language has been imprecise or poorly defined in some cases, with the term "propagation" often being used as a catch-all, sometimes interchangeable, term for many of the individual events that have been proposed to occur. To fully address the literature, we must first discuss terminology. Some forms of tau have been suggested to function as "seeds." Specifically, this refers to the concept of conformational templating, a property characteristic of amyloids in general [38]. In this particular case, tau molecules that function like a seed adopt a specific conformation and subsequently have the ability to directly interact and draw naïve tau protein into that conformation. Based on the prion field, one would anticipate that protein with this same conformation could be subsequently extracted from infected animals and be able to be re-introduced into naïve animals to produce protein of the same conformation. Importantly, the term "seed" does not imply specific localization—tau seeds could be present within intracellular or extracellular compartments. Tau "spreading" can refer to the macroenvironment of tau pathology spreading from region to region, such as along interconnected axonal projects, as suggested by Braak et al. [16]. It may also refer to the microenvironment, whereby tau has been proposed to spread from cell to cell. To refine this language, we will use the term "transcellular" to refer to tau abnormalities that spread from one cell to another. There are a number of ways that tau has been proposed to move from one cell to another. We will utilize the term "trans-synaptic" when discussing the possibility that tau is directly transmitted across a synapse. We will reserve "propagation" for the entirety of the process—from conformational templating, to transfer from one affected cell into a naïve cell, and for subsequent affected cells to have the same capacity. Finally, we reserve the term prion-like to refer to tau with a specific conformational profile that permits propagation across various cells within a host animal that can then be repeatedly and stably re-derived in affected animals to subsequently infect and elicit the same tau conformation within naïve animals, with the most rigorous definition requiring that the naïve animals not bear a mutant form the protein.

What was the initial evidence for tau seeding and transcellular spreading in cell culture?

Tau is considered to be an intracellular protein whose major function is to promote microtubule assembly and stability within the cell; however, some of the first cell culture experiments to examine the influence of extracellular tau on intracellular tau are almost 40 years old. De Boni and Crapper [30] had previously exposed human fetal cortical cultures to extract from AD brain, and utilized electron microscopy to demonstrate the presence of filaments that were ultrastructurally similar to paired helical filaments (PHFs). Importantly, the authors noted in their proof that the occasional appearance of the same type of filaments was observed in control experiments, potentially suggesting that the phenomenon was not specifically due to the presence of PHFs in the extract. In 2009, Frost et al. hypothesized that extracellular tau may be able to enter naïve cells and promote tau aggregation within those cells [37]. To test this hypothesis, the authors expressed tagged full length and truncated 4R tau in cell culture and demonstrated that truncated tau readily aggregated. Cellular uptake of tau showed significant bias for uptake of aggregated versus monomeric tau. Based on the co-localization of these aggregates with dextran, the authors suggested that aggregates entered the cell via fluid phase endocytosis. Once the tau aggregates entered the cell, they appeared to elicit aggregation of host cytoplasmic tau. Neither monomeric truncated tau nor aggregates of an expanded huntingtin protein produced similar results, indicating relative specificity of tau seeding. Finally, Frost et al. co-cultured cells in which the origin of the tau could be traced with fluorescent tags, allowing the authors to determine the ability of tau to leave one cell and be taken up by a second cell, creating tau aggregates of mixed origins. Extracellular tau was able to enter the cell and conformationally template intracellular tau in less than 2 % of the cells.

The study from Frost et al. stimulated debate and excitement about the possibility that tau had prion-like properties; however, there were many issues with regard to these findings. It was not clear how closely the aggregates resembled tau in human NFT. This issue was partially addressed by a subsequent work by Guo and Lee [46], who found that introduction of minimal levels of sonicated pre-formed fibrils of recombinant tau could shift endogenous tau into the detergent-insoluble fraction and into an abnormal conformation recognized by a monoclonal antibody (MC1) specific to abnormal conformation in human NFT [99]. The authors also asserted that their cellular system "robustly develop[ed] authentic NFT-like tau aggregates," a claim that they supported by demonstrating that the aggregated tau stained with thioflavin S (ThioS), indicative of the beta-pleated sheet conformation that is found in some but not all NFT pathology in human tauopathies [94, 95]. They used recombinant 4R-tau, which in human tauopathies has weak amyloid-like properties [95], in contrast to Alzheimer type tau that has a mixture of 3R and 4R tau. Despite this claim, the ultrastructural studies presented by Guo and Lee were not thorough, relying on immunoperoxidase staining with one phospho-tau antibody. A more comprehensive

analysis using immunogold labeling with multiple antibodies would have been useful to demonstrate that fibrils were aggregated tau rather than tau bound to microtubules and that the phosphorylation, the periodicity, and length of filamentous tau were similar to those found in human tauopathies. Surprisingly, in contrast to the inefficiency of tau in conformationally templating and transcellular spread in the study by Frost et al., Guo and Lee identified aggregates in up to 35 % of the cells exposed to sonicated preformed tau fibrils.

What influences tau conformational templating?

A large number of studies have attempted to determine the factors that could influence the ability of tau to seed, potentially providing an explanation for differences in efficiency between published studies. These attempts mirror the lack of consensus between investigators in the long-standing search for the toxic tau species. Sonication of the samples is often an integral step in generating toxic species, but standardization of this technique is often not addressed. Other factors such as the basic form of the seed, tau mutation, isoform of both the seed and the seeded tau, and the source of the tau seed as discussed below have been reported to play a role in the ability of tau to conformationally template.

What is the basic form of a seed?

Frost et al. suggested that an efficient tau seed was a fibril rather than a soluble monomer; however, the prerequisite qualities of tau fibrils were ill defined [36]. Mirbaha et al. recently suggested that tau trimers were the minimal particle size that could be taken up by a cell to serve as a conformational template for intracellular tau [69]. In contrast, Michel et al. utilized super-resolution imaging to provide evidence that monomeric tau could function as an efficient seed [68]. In 2013, Wu et al. [102] demonstrated that low molecular weight (LMW) tau (~10–40 nm), which was about the size of tau dimers and trimers, and short tau filaments (~40–250 nm) were taken up by the cell from the extracellular space for transport into the axonal terminals; however, uptake of monomeric tau was not observed, even when the concentration was shifted to dramatically favor the uptake of monomeric tau. Lack of consensus in studies performed in the controlled environment of cell culture makes it difficult to weigh the importance of each study.

Does efficient tau seeding rely on mutation?

Guo and Lee demonstrated that the frequency of tau seeding appeared to depend, in part, on the mutational status of the transfected tau and the transduced fibrils [46]. Subsequent studies have also suggested that mutant tau can influence the ability of tau to serve as a seed or be seeded—forms of tau that are more aggregation competent are also more efficient in these roles while tau that is an aggregation incompetent neither serves as a conformational template nor can be conformationally templated, even in primary neuronal cultures [35, 60, 67, 86, 92]. It is tempting to consider the human implications of these studies. Firstly, the majority of human tauopathies occur in the absence of mutant tau; therefore, the relevance of tau seeding to the majority of tauopathies could be questioned if mutant tau is required for an efficient process. If, however, tau seeding contributes in a tangible manner to human tauopathies in general, sequence variants in tau that reduce the ability of tau to seed or be seeded could be protective against the development or progression of tauopathy. Such variants remain to be discovered, but they might explain the phenotypic diversity of human tauopathies [51, 86].

Is efficient tau seeding dependent on the tau isoform?

Many of the published studies on tau propagation use truncated rather than full-length tau. The microtubule binding domains within the truncated recombinant tau utilized for some of these studies are also a primary driving force for binding of tau to microtubules [61, 62]. Furthermore, a role in tau pathology has been repeatedly suggested for truncated tau, though the exact role remains the source of some debate [56, 59, 76, 79, 85, 98, 107]. A recent study by Sokolow et al. [90] demonstrated that C-terminally truncated tau is the primary tau species in the synapse and C-terminally cleaved tau is elevated in the synaptosomal compartment in AD compared to controls. Additionally, they demonstrated that potassium chloride depolarization of the synaptosomal fractions from control and AD led to release of tau, with higher rates from AD than control. If truncated tau is preferentially released and thus available for uptake into naïve cells in humans, then the use of truncated tau in seeding studies may be completely justified. Such studies, however, fail to consider that fact that tau is present in multiple isoforms in the human brain and those isoforms are reflective of alternative splicing that affects both the N- and C-terminus. To address the influence of isoforms on tau seeding, Dinkel et al. [34] found that in vitro filament growth was dependent on tau isoforms. They were unable to seed aggregation of 3R tau when using 4R tau as the conformational template. Nonaka et al. [75] were unable to seed tau aggregation with 1N4R tau (4R tau with 1 N-terminal insert) in cells expressing 1N3R tau or with 1N3R tau in cells expressing 1N4R. Interestingly,

Gerson et al. [40] purified oligomers from brains of individuals with PSP, a 4R tauopathy, and were able to effectively utilize these oligomers for in vitro seeding of both 3R and 4R tau. In human tauopathies, tau aggregates can be composed of only 3R, only 4R or of a mixture of both 3R and 4R tau, depending on the disease. Further, 3R, 4R, and mixed 3R + 4R tauopathies all occur in the presence of expression of both 3R and 4R tau. It is imperative that the field explores how tau seeding could specifically play in the development of each type of tauopathy rather than relying on what is convenient in the laboratory setting.

Is efficient tau seeding dependent on source of seeds?

Differing technical details may explain some of the discrepancies across studies. One obvious factor to consider is the source of tau. Logic would dictate that seeding capacity of tau filaments derived from human tauopathies would likely to be more relevant to human disease than recombinant tau. Both the aforementioned studies by Dinkel et al. [34] and Nonaka et al. [75] utilized recombinant tau, whereas Gerson et al. [40] used tau from human tissue. Likewise, Santa-Maria et al. [87] isolated tau from AD brains and successfully demonstrated the ability to seed full-length tau aggregation in cultured cells within structures that resembled aggresomes. Morozova et al. [72] reported conformational differences between PHFs from AD and recombinant tau fibrils and showed that human PHFs could be used to conformationally template recombinant tau into filaments that were similar to the "native" human PHFs. Similarly, Falcon et al. [35] demonstrated that seeds derived from the brains of a mouse model of tauopathy (P301S), which they termed a "native" source, were able to adopt a different conformation than those derived from recombinant tau. Furthermore, seeds derived from the P301S model could template recombinant tau into a conformation similar to the "native" species. These results suggest that studies utilizing native tau may be more relevant for human tauopathies, but other scientists may argue about the physiological relevance of native tau fibrils derived from mouse models as opposed to tau from human tissues.

Are tau seeds strain-specific?

One critical feature of genuine prion proteins is their ability to stably propagate as distinct strain(s) that can then be recovered from the cell or host organism and subsequently utilized to produce the same pathology when reintroduced into other naïve cells or organisms. Sanders et al. [86] sought to determine if tau possessed this prion-like ability. HEK293 cells that were stably expressing various forms of truncated tau were exposed to fibrillar truncated tau. Focusing on the cell line expressing an aggregation-prone, truncated form of tau that contained both the P301L and V337M mutations, the authors then established 20 monoclonal lines. The 20 different cells lines had tau aggregates of different morphologies and biochemical profiles. Clone 9, which initially contained frequent aggregates, had the highest seeding capacity and the greatest toxicity, whereas, clone 10, which initially contained less frequent larger aggregates, showed less toxicity and reduced ability to seed compared to clone 9. The inclusions in clone 10 had features of aggresomes, similar to those reported by Santa Maria et al. [87]. The authors were able to continuously passage the cells and their associated unique morphologies over 6 months. They then exposed naïve cells to the stable pro-aggregation truncated tau and demonstrated that the lysates were able to transmit the conformations that were uniquely associated with the original clones. This approximated the cellular equivalent of prion-like transmissibility for tau. Importantly, lysates from the original clones were able to seed inclusions in primary neurons expressing mutant full-length tau.

Two particularly interesting pieces of information were derived from this study. First, the authors were able to repress stable expression of the mutant tau and reverse aggregation within HEK293 cell line. These results were similar to those published by Polydoro et al. [81], who were able to reverse seeded tau pathology in inducible transgenic mice, indicating that seeded tau pathology, if it exists in humans, could be reversible. Second, the authors were able to seed the aggregation of full-length P301S tau, but not wild-type tau, in primary neurons with lysates from either clones 9 or 10, which presumably contain seeds composed of truncated tau containing the P301L and V337M mutations. Tau containing both the P301L and V337M mutations has not been reported in humans; therefore, this system is artificial and its relevance to human tauopathies could be questioned. On the other hand, humans carrying the P301L mutation develop a 4R tauopathy [32], with neuronal inclusions that selectively deposit mutant tau [70], suggesting that P301L tau is similarly unable to conformationally template wild-type human tau. Interestingly, the "seeding barrier," as they termed this phenomenon, appears to be unidirectional since truncated wild-type tau was able to seed both truncated wild-type and P301 mutant tau.

How does tau move from one cell to the next?

The ability of tau to seed in a prion-like manner ultimately depends on its ability to move from an affected cell into a naïve cell. Several cell culture studies have attempted

to identify the cellular doors through which tau exits and enters, as well as the factors that influence the choice of doors utilized. Much of the current data, as discussed below, implicate macropinocytosis and synaptic transmission as likely doors that are used for tau seeds to enter into new neurons.

To understand how tau may exit cells, Kfoury et al. [60] demonstrated that the transcellular movement of tau likely occurs through the secretion of the tau into the media, since changes in culture media altered this capability. Introduction of an anti-tau antibody reduced the uptake of tau from the media and allowed purification of tau fibrils that likely represented the tau seeds. The ability of secreted tau to serve as a seed could help explain why in vivo studies have shown that active and passive immunization against tau slows the progression of tauopathy in mouse models [8, 9, 13, 14, 24].

In contrast, Frost et al. [37] originally suggested that tau fibrils might enter naïve cells through fluid phase endocytosis (macropinocytosis; bulk endocytosis). Santa-Maria et al. [87] and Wu et al. [102] also found that much of the induced tau aggregates co-localized with dextran, a marker for fluid phase endocytosis. Internalized tau could be transported either anterogradely to the axonal terminal or retrogradely to the soma as was later suggested by Ahmed et al. [1] with their in vivo observations. The entry into the somatodendritic compartment was not dependent upon the presence of pre- and post-synaptic connections, suggesting that tau aggregates could transfer trans-cellularly, rather than exclusively trans-synaptically. If this is true, it would suggest that tau aggregation could spread to adjacent cells within the brain, rather than only between synaptically connected regions, as hypothesized by Braak et al. [16]. In addition to this finding, the authors observed uptake of LMW tau by axons and retrograde transport of LMW species.

In 2013, Holmes et al. [50] suggested that extracellular tau attached to heparin sulfate proteoglycans (HSPGs) to gain entry into cells, a mechanism that was previously observed with prions. Using electron microscopy, they were able to image this process at multiple stages—from the association of fibrillar actin with truncated tau to association with the cellular membrane to the formation of aggregates within vacuoles within the cell. Macropinosome inhibitors reduced the uptake of extracellular truncated tau, further validating their observations. The authors found that cellular uptake of full-length tau was more sensitive to the concentration of HSPGs than truncated tau. It was not clear if this sensitivity may be due to a reduced ability of full-length tau to stimulate macropinocytosis. One of the most surprising results presented by Holmes et al. was that extracellular tau was able to stimulate dextran uptake and that this uptake was proportional to the concentration of tau fibrils. This experiment strongly suggested that extracellular tau could stimulate its own cellular uptake via its activation of macropinocytosis. The potential implications of tau being able to influence its own uptake in human tauopathies depend on the source and availability of the tau seeds. For example, if the tau seeds are derived from dying cells, then traumatic or disease events in humans that result in considerable cell death over a short period of time could then trigger a rapid domino effect in terms of tau seeding and spreading.

Does synaptic activity influence transcellular propagation of tau?

To navigate away from the use of exogenously applied tau seeds and to explain if the synaptic transmission could underlie tau passage between cells, Calafate et al. [22] utilized an in vitro system based on donor cells that consistently developed tau aggregates in the absence of truncated tau partnered with acceptor neurons to explore tau propagation. They identified P301L-GFP HEK293 line (termed line 2) that consistently developed tau aggregates, and used them as a consistent source of tau aggregates for co-cultured rat hippocampal neurons that expressed P301L tau. About 10 % of the rat neurons co-cultured in this manner developed phospho-tau (AT8)-positive aggregates. In this relatively "pure" culture, since only about a 10 % of the acceptor cells developed tau aggregates, tau propagation by this method also appeared relatively inefficient. Importantly, control neurons that were co-cultured with a clonal HEK293 cell line that did not express mutant tau failed to develop tau aggregates. To determine if synaptic connectivity increased the induction of tau aggregation in acceptor cells, the authors induced presynaptic differentiation of the P301L-GFP HEK293 lines, finding about a 50 % increase in the number of acceptor cells with tau compared to those neurons cultured with the P301L-GFP HEK293 cells that were expressing vector only.

These experiments suggest that synaptic connectivity increased tau aggregation. To help determine the role that synaptic activity plays in tau propagation, the authors then combined microfluidic multi-chambers to isolate neurons from each other with transduction of P301L tau and introduction of P301L tau fibrils. The authors demonstrated that blocking either synaptic connectivity or synaptic activity similarly blocked tau aggregation in the downstream chamber. This microfluidic chamber data strongly support the existence of a trans-synaptic pathway for movement of tau between cells; however, it does not exclude the possibility that other pathways exist as well.

Does tau propagate in vivo?

Given the compelling in vitro and cell culture data, it was a logical transition to exploit the growing wealth of in vivo models that are available to study tau propagation. Indeed, there is now a large body of data indicating that it is possible to promote tau propagation in vivo, at least in transgenic models.

In 2009, Clavaguera et al. [26] provided the first evidence that tauopathy could be induced in vivo, presumably through extracellular tau seeds. It should be noted that transgenic mice expressing a single isoform of wild-type human tau have yet to be documented to develop robust tau pathology or neurodegeneration. Clavaguera et al. utilized the ALZ17 mouse line that expresses the wild-type human 2N4R tau isoform under the control of the Thy-1 promoter [29] as a base model for their studies. They also relied on a second mouse model that overexpressed human 0N4R tau with P301S mutation with Thy1 promoter [3] as their source of extracellular tau. Homozygous P301S transgenic mice have robust tau pathology at 6 months of age, primarily in the spinal cord and brainstem. The authors utilized extracts from brainstems of 6-month-old P301S mice and prepared a sample in which they immunodepleted tau using a mixture of antibodies (HT7 for human tau, AT8 for tau phosphorylated at S202/T205, and Tau-5 for both murine and human tau) to theoretically clear the tau from the sample. Whole and immunodepleted extracts were then injected into hippocampi and cortices of 3-month-old ALZ17 mice. Mice were analyzed for biochemical and pathological changes at 6, 12, and 15 months post-injection. The mice were compared with uninjected ALZ17 mice as well as ALZ17 mice that had received extracts from non-transgenic mice. The ALZ17 mice that had received the P301S extract developed inclusions within the hippocampus, with a significant difference at 12 months of post-injection for neuropil threads and coiled bodies, compared to 6 months post-injection and 15 months post-injection for neurofibrillary tangles compared to 12 months post-injection. ALZ17 mice that received the P301S immunodepleted extract failed to develop Gallyas-positive tau pathology in the hippocampal dentate gyrus, subiculum and fimbria up to 6 months post-injection, the oldest time point studied.

The authors did not directly compare the Gallyas results from ALZ17 mice that were injected with P301S extract and the ones that received the immunodepleted P301S extract; however, there was no qualitative difference between the experimental and control cohorts. These studies would have been more informative if the control group had been the same age as the 15 month post-injection time point in the experimental group. Additionally, it should be noted that the results of immunostaining in the dentate gyrus were not presented for the experimental group, making comparisons difficult. The authors were able to show that the ALZ17 mice injected with the P301S extract developed Gallyas-positive inclusions that included the 2N4R tau isoform, indicating that at least some of the tau within these inclusions was derived from the wild-type tau expressed in the ALZ17 mouse and not from the injected P301S mouse. No details were provided regarding the frequency of overlap between these two stains; therefore, it is not clear if there was complete overlap between the two.

Interestingly, the bulk of the pathology appeared to be attributable to insoluble rather than soluble tau, consistent with cell culture data in which insoluble tau was more efficient at seeding. Aside from differences in tau inclusions, neither gliosis nor neuronal loss was triggered by the changes in tau pathology, a critical difference in comparison to both human tauopathies and mouse models of tauopathy. The authors found that injection of the P301S extract into nontransgenic mice was able to induce some pathological changes in the form of tau threads and coiled bodies.

Another important observation from this paper that has been confirmed in subsequent in vivo studies is that human tau overexpression helps to facilitate seeding and spreading. If tau overexpression is a critical determinant for the ability of tau to seed, this would suggest that the process would be remarkably inefficient in humans who do not overexpress tau.

Does tau propagate within the medial temporal lobe?

By February 2012, two groups reported the creation of nearly identical models of tauopathy relying on a bigenic tetracycline transactivator (tTA) system to drive conditional expression of the 0N4R human P301L tau protein. Using this system, the tau responder mouse line originally utilized in the rTg4510 mouse model, a conditional mouse model of tauopathy expressing P301L human tau [88], was alternatively put under the control of a tTA effector transgene driven by the neuropsin promoter to yield bigenic mice, termed rTgTauEC by de Calignon et al. [31] and NT mice (for neuropsin and tau) by Liu et al. [65]. Mice in both studies expressed the tau transgene seemingly restricted to the entorhinal cortex, providing a good platform to determine if tau pathology could spread from the entorhinal cortex along synaptically connected regions to the dentate fascia through the perforant pathway. In the rTgTauEC model, abnormally folded tau (detected by the abnormal conformation-specific monoclonal antibody, Alz50 [101]) within axon terminal in the molecular layer of the dentate gyrus was observed at 3 months of age, progressing to somatic staining in the medial entorhinal cortex at 6 months of age. Inclusions in the entorhinal cortex that was immunopositive

for PHF1 (a monoclonal antibody that detects phospho-tau at Ser396/404) were apparent at 12 months of age, followed by argyrophilic staining at 18 months. In addition to pathology observed in the entorhinal cortex, PHF-1 and Alz50-immunopositive inclusions were found in the dentate gyrus at 18 months of age and in CA1 and CA2/3 hippocampal sectors after 21 months of age. Liu et al. found similar timing and regional spread of tau abnormalities in their cohorts of NT mice with a panel of tau antibodies (MC1, CP27 and AT8) as well as with thioflavin S fluorescent microscopy and silver stains. Neuronal loss was detected in layer two of the entorhinal cortex and in the subiculum by de Calignon et al. [31] at 24 months, but similar loss was not found in downstream regions, suggesting that the downstream transmission of tau was not sufficient to drive neuronal loss or that the degree of neuronal loss was relatively small. Liu et al. [65] did not assess neuronal loss.

Both de Calignon et al. and Liu et al. interpreted their findings to indicate that tau pathology in these mice initiated in the entorhinal cortex, the region with focal tau transgene expression, and then spread to synaptically connected regions. As a confounding variable, neither group sufficiently addressed alternative explanations for how downstream neurons might contain human tau. The Tg4510 (tau responder) line that was utilized in the creation of the rTgTauEC and NT models has been previously reported to "leak" human tau expression at levels of approximately twofold above endogenous levels [88]. Utilizing a fluorescent in situ hybridization (FISH) assay, de Calignon et al. [31] failed to identify tau leakiness in regions outside of those in which neuropsin-tTA is expressed. This could have been related to the sensitivity of their detection method. In contrast, Liu et al. [65] performed laser capture micro-dissection followed by qPCR and identified leaky tau expression; however, since they pooled a large number of neurons, it was not clear if the leak was uniform across all neurons in the region or if only a few neurons expressed tau, heavily influencing the signal. Importantly, a third group [48], who generated essentially the same model used by de Calignon et al. [31] and Liu et al. [65], which they termed EC-hTau, was able to detect leaky tau expression in 4–5-month bigenic tau mice. This leaky signal could indeed predispose a neuron to the formation of tau pathology under pressure of an additional insult. On the other hand, Harris et al. [48] aged Tg4510 tau responders without observing tau pathology, indicating that this low level of P301L tau expression is not sufficient to drive tauopathy on its own. These results from aged Tg4510 responder mice mirror unpublished results from our own laboratory (J.L.).

The studies detailed in these papers are also complicated by a recent publication in which Yetman et al. [105] demonstrated that tTA expression in the neuropsin-tTA line can drive the expression of a LacZ reporter outside of the entorhinal cortex and subiculum. For example, "promiscuous" expression of the reporter transgene can be seen in hippocampal pyramidal layer and the dentate gyrus—regions that the aforementioned studies cited as areas into which tau had propagated. If this holds true in the rTg-TauEC and NT models, any promiscuous tau expression outside of entorhinal cortex and subiculum would be additive to the tau "leakiness." Given this, the additive nature of both the tau leakiness and promiscuous tTA-driven expression of tau could result in a subset of neurons within the perforant pathway expressing tau at levels high enough to drive tau pathology. While this does not exclude the possibility of trans-neuronal spread of tau from the entorhinal cortex, it does raise issues to consider in rigorously interpreting these results.

Finally, neither group carefully considered other possibilities for how neurons might lack human tau mRNA yet still contain human tau. For example, as tau aggregates, neurons could become dysfunctional in a manner that reduces transcription [23]. Since tau has been repeatedly shown to have a long half-life, these cells could conceivably contain tau protein, but no longer have detectable levels of human tau mRNA. Alternatively, perhaps both groups miscalculated the true extent of tau mRNA levels because they focused on the cell body rather than the axon—tau mRNA has been shown in the axons and local protein synthesis may occur from that mRNA [7]. Finally, a tau feedback loop could exist that might regulate stability of its own message; though, the latter possibility seems unlikely given that the tau transgene in these mice is driven by an exogenous promoter and from a cDNA. In aggregate, there are other possibilities that should be considered to explain the appearance of trans-neuronal spread of tau reported in these studies.

Does tau propagate outside of the medial temporal lobe?

Modeling extracellular seeding studies performed in cell culture, Iba et al. [53] and Ahmed et al. [1] introduced extracellular fibril tau derived from recombinant full-length (P301S) and truncated (P301L) tau [73] or brain extract derived from an end-stage P301S mouse brain [1] into the brains of two different lines of P301S mice [3, 106]. Both groups found that tau pathology spread into regions that shared the strongest synaptic connection with the site of injection. Ahmed et al. suggested that tau could be spread in both anterograde and retrograde directions mirroring an observation made by Wu et al. [102]. This builds on previous studies that also suggested that synaptic connections were key mediators of the transcellular spread of tau [31, 48, 65]. Neither group observed neurodegeneration akin

to that observed in humans, though the failure to observe robust neurodegeneration may simply reflect the inability to age the mice to a point that neuronal loss could develop.

Peeraer et al. [80] obtained similar results to Iba et al. [53] through the injection of fibrils of recombinant truncated P301L tau into the brains of transgenic mice expressing P301L human tau [93], with an important difference—Peeraer et al. observed neuronal loss and gliosis. The authors also injected the same recombinant tau into transgenic mice expressing wild-type tau; however, they did not observe the same tau or neuronal changes as they observed in the P301L mice—a finding seemingly counter to the findings of Clavaguera et al. [26, 27].

Stancu et al. [92] recently reported that they were able to both seed tau aggregation in primary cell and hippocampal slice cultures from a P301S transgenic model and affect neuronal function. In addition, they were able to seed an aggregation of tau in P301S mice, developed originally by Yoshiyama et al. [106]. In the seeded P301S mice, tau aggregation progressively spread from the original site of injection (the entorhinal cortex) to the contralateral entorhinal cortex as well as to functionally connected regions including the subiculum, hippocampal formation, amygdala, thalamus, and frontal cortex. The authors were not able to detect neurodegeneration through the use of small animal FDG-PET; however, they were able to detect post-synaptic dysfunction in the seeded animals as well as deficits in object recognition memory following bilateral, but not unilateral, injections into the entorhinal cortex. Moreover, injection of tau seeds in the basal ganglia of P301S mice resulted in tau aggregation within the striatum, thalamus, brain stem, and cortex showing that within the same model, the authors could manipulate the distribution of pathology based on the site of tau seeding. The authors also showed that motor performance was altered by the introduction of seeds into the basal ganglia; however, given the age at which this was analyzed and the fact that this model develops a motor phenotype on its own, these data were not particularly compelling. The authors inferred that the neuronal network disturbances that they observed in seeded primary and hippocampal cultures were likely due to early tau aggregates rather than mature aggregates, given the relative paucity of mature NFT-like inclusions in these systems following seeding. These studies were correlative and did not provide definitive mechanistic evidence needed to establish a clear connection between tau species and neuronal dysfunction. Importantly, the authors could not seed tau aggregation in non-transgenic cultures or in non-transgenic mice using paradigms that were successfully used in P301S mice. These data bring into question the studies reported by Clavaguera et al. [26, 27], which suggested that tau aggregation could be seeded in non-transgenic mice.

Can tauopathy be repeatedly passaged in vivo?

A key feature of prion disorders is the ability of the prions to be derived from affected animals, injected into naïve animals to elicit the same conformational change, which can be re-derived and re-injected [20]. While there is some literature indicating that tauopathy may be repeatedly passaged in vivo, this feature of tau propagation remains to be conclusively demonstrated. As previously detailed, Sanders et al. [86] demonstrated passaging with tau in cell culture and subsequently sought to demonstrate this phenomenon in vivo. Previously, it had been shown that fibrils composed of recombinant, truncated tau could be used to induce tau aggregation into the P301S transgenic mouse model of tauopathy [53] and this strategy was utilized as the positive control in the study by Sanders et al. [86]. Using lysate derived from clonal cell lines that consistently developed tau aggregates of different morphologies and biochemical properties, the authors injected the brains of the P301S transgenic model of tauopathy. Further, the unique pathology could be replicated across multiple passages through mouse brain and could be isolated and reintroduced into cells to elicit the same unique morphology of tau aggregation. They also saw evidence that tau aggregation spread along anatomically connected regions in vivo.

What factors influence in vivo tau propagation?

A number of factors, as discussed below, may influence in vivo tau propagation including source of seeds, the passage of time, tau isoforms and tau species. The main factor that appears to influence the ability of tau to propagate in vivo is tau mutation.

In 2013, Clavaguera et al. [27] injected total brain homogenate from six different tauopathies [AD, tangle-only dementia (TOD), Pick's disease (PiD), argyrophilic grain disease (AGD), progressive supranuclear palsy (PSP), and corticobasal degeneration(CBD)] into the ALZ17 mouse line using the same paradigm as in their original 2009 paper [26]. The authors reported that tau inclusions were observed as early as 6 months post-injection, progressing and spreading with time. No evidence of neuroinflammation was observed. Control injections of homogenate from a mouse model of amyloidosis failed to elicit tau or amyloid beta accumulation within the ALZ17 mice. Interestingly, animals that received homogenate from PiD developed minimal pathology, with neuronal lesions failing to resemble Pick bodies. Because PiD is a 3R tauopathy and the homogenate was injected into a mouse that overexpresses a 4R human tau isoform, this result could suggest that 3R tau is not capable of seeding 4R tau pathology. In contrast, results by Dinkel et al. suggest that 3R tau

is capable of seeding 4R tau aggregation, at least in vitro [34]. Alternatively, it could suggest that the tau pathology in PiD is composed of tau filaments that have reached a terminal state through a post-transcriptional or aggregation-related process. In contrast to many of the studies that utilized mutant tau transgenic mouse models of tauopathy, Clavaguera et al. [27] were unable to confirm the presence of filamentous tau in the sarkosyl-insoluble fractions from the injected mice, even after 15 months. The authors suggested that the filaments were likely at subthreshold levels for the assay. An alternative explanation could be that the tau inclusions were composed of tau that was still partially soluble. This would correlate to the observations by Polydoro et al. [81] in which tau suppression was able to reverse tau pathology in rTgTauEC model when they suppressed transgenic tau expression.

In addition to their injections into the ALZ17 model, Clavaguera et al. injected homogenate from human brain affected by AD, NFTD, AGD, and PSP into the hippocampus of 3-month-old C57BL6 mice and performed analysis at 6 and 15 months post-injection [27]. Inclusions composed of material that was silver-positive and immunopositive for antibodies for phospho-tau epitopes were seen as early as 6 months post-injection. Interestingly, the authors were subsequently able to induce tau abnormalities in ALZ17 mice when injected with homogenate from either ALZ17 or B6 mice that had previously been injected with either homogenate from P301S mice or human tauopathy, respectively. The authors suggested that this was evidence of their ability to propagate tauopathy from one animal to the next, even in the absence of tau overexpression. Importantly, the authors did not observe neurodegeneration in any of the mice up to 18 months post-injection. Although the authors note that the relatively short lifespan could contribute to the lack of neuronal loss, this seems unlikely since neuronal loss can be observed in other tau transgenic mouse models. Alternatively, it may be that the conformations of tau that are induced through seeding are simply not neurotoxic at the levels at which they are present.

Boluda et al. [12] performed similar experiments using a P301S model that eventually develops tau pathology on its own [106], in the absence of exogenous seeding. The authors were able to demonstrate that injection of tau preparations from CBD (4R tau) and AD (3R + 4R tau) into brains of P301S mice produced unique pathologic findings. Mice injected with CBD extract developed mostly oligodendroglial pathology, showed no neuronal loss, and had tau changes in a regionally distinct pattern when compared to those injected with AD, which showed primarily neuronal pathology and CA3 neuronal loss. These data would suggest that tau seeds from human tauopathies might exist as "strains" as suggested by Clavaguera et al. [27] and Sanders et al. [86]. Identification of those strains could allow more accurate diagnosis and/or treatment for tauopathies that often show overlapping symptomology. Interpretation of the relevance of these results for human tauopathy, however, could be complicated by the fact that the P301S model expresses 4R human tau containing a mutation in one of the microtubule-binding domains and 4R murine tau—in stark contrast to the six main tau isoforms expressed in the human brain. The distribution of expression of the human 4R mutant transgene may create an artificial system in which seeds composed of 4R tau, derived from CBD, interact differently with the 4R transgenic tau versus the tau seeds from the 3R + 4R tau, derived from AD.

As mentioned previously, Wu et al. [102] demonstrated that aggregated LMW and small fibrillar species of full-length tau could be taken up by cells in primary cultures using microfluidic chambers. As a follow-up to their studies to demonstrate that specific forms of tau could function as seeds, Wu et al. also injected the aggregated LMW species of full-length tau unilaterally into the brains of 4-week-old rTg4510 mice while the contralateral side was injected with vehicle. At 11 weeks post-injection (final age 15 weeks), the authors reported an increase of ~50 % in MC1-labeled tau inclusions compared to the vehicle-injected side. As this was an initial proof of concept study, there were little details provided on this study including the sex of the mice, the number of animals in each group, the statistical analyses, and characterization of tau hyperphosphorylation and reactive gliosis. While these were potentially exciting findings, there are a number of confounds that must be considered when evaluating these results. Based on our experience with the rTg4510 model [88], they used mice that had entered a growth phase in the development of tau pathology and a larger sample size would have been needed to evaluate the significance of any observed changes during this period. A better design would be to perform pathological analyses at ~5.5 months of age to determine the effect that treatment might have on the development of tauopathy in this model.

Does amyloid beta influence tau propagation?

Amyloid beta may influence tau propagation in vivo; however, there are some concerns with the relatively small body of literature that have explored this specific question. In 2015, Pooler et al. [82] bred the rTgTauEC model with the APP/PS1 mouse model of AD to determine if amyloid beta could impact tau propagation in vivo. Consistent with previous reports on APP/PS1 mice alone, 16-month-old rTgTauEC × APP/PS1 mice developed robust cortical amyloid deposits. Importantly, the 16-month-old rTgTauEC × APP/PS1 also showed a 20-fold increase in the number of

neurons within the dentate gyrus containing human tau aggregates as well as a significant increase in the number of cells within the DG that were immunopositive for Alz50. In these same mice, an elevated number of CA1 neurons was also positive for human tau in comparison to the rTgTauEC mice alone. Additionally, the rTgTauEC × APP/PS1 mice contained neurons within the somatosensory cortex and the accessory olfactory areas that were immunopositive for human tau—a finding that was missing from the rTgTauEC mice alone. These data strongly suggest that amyloid potentiates the degree and distribution of tau spread within the brain. Interestingly, the enhancement of propagation was variable in the rTgTauEC × APP/PS1.

Given the mixed, inconsistent genetic background of the experimental animals, the authors attempted to utilize genetic profiling to uncover a genetic factor that could differentiate the rTgTauEC × APP/PS1 animals that showed robust propagation compared to the rTgTauEC × APP/PS1 mice that did not. Unfortunately, no Mendelian cause was identified. In addition to the marked increase in trans-synaptic spread, there was over 90 % loss in cells that were positive for tau mRNA within the entorhinal cortex in 16-month-old rTgTauEC mice compared to those tau mice on the APP/PS1 background. While the authors did not cite the actual percentage decrease in neurons in the entorhinal cortex, there appeared to only be ~12 % overall loss in neurons in layer II of the entorhinal cortex. An additional finding in this rTgTauEC model on the APP/PS1 background was that tau expression and/or pathology generally increased amyloid beta deposition. A cursory evaluation by the authors suggested that an increase in APP levels was not responsible for this finding; however, the authors did not assess the levels of APP over time, and they did not assess the levels of amyloid beta; therefore, these factors could have confounded the results.

Years before this publication, our group demonstrated that APP and/or amyloid beta levels enhanced tau pathology [64] and a back-to-back publication from Gotz et al. [43] demonstrated that a similar enhancement was achieved through amyloid beta. Therefore, the observation that tau pathology is increased in the context of amyloid pathology in the rTgTauEC × APP/PS1 mice is not particularly novel. In contrast, Pooler et al. suggested that this enhanced pathology arose in cells that were not expressing the tau transgene, but instead contained tau propagated through trans-synaptic spread. Pooler et al. utilized FISH to determine the presence or absence of transgenic tau expression, a caveat in studies by de Calignon et al. [31] that we previously discussed. Since the authors were unable to detect the "leak" level that is found in mice containing the tau responder alone, there remains a concern that the authors' assay did not have a low enough threshold of detection in this study.

What are the commonalities or discrepancies between experimental seeding and human studies?

The literature now overwhelmingly suggests that tau aggregation can be seeded in vitro, in cells and in mice and there are some common themes that run across multiple studies, which are conveyed in Fig. 2. We now need to determine the extent to which the knowledge gained in the experimental studies can be directly translated to our understanding of human tauopathy. In the experimental studies, the form of tau to seed and to be seeded appears to critically influence the efficacy of the process. Typically, the forms of tau that are able to aggregate more readily (i.e., pro-aggregation mutants or truncated species) appear to be efficient seeds, whereas tau that is aggregation impaired or incompetent due to mutation prohibits conformational templating. It is possible that age of onset of tauopathy in humans reflects efficient tau seeding—individuals with tau mutations present with tauopathy between the 4th and 6th decades of life; however, individuals who develop AD and lack tau mutations rarely show onset of clinically significant tauopathy before the age of 65. A caveat to that interpretation is that preclinical tau pathology may begin at a much earlier age [18].

Some experimental studies suggest a species barrier, as has been reported for prion disorders, though this finding has not been without controversy [28]. Experimental studies by Dinkel et al. [34], Nonaka et al. [75], and Sanders et al. [86] demonstrated that seeding was influenced by the degree of match between seed and template. On the other hand, Clavaguera et al. [26, 27] were able to seed non-transgenic murine tau with tau seeds derived from three different sources—a mouse model of 4R tauopathy, 4R human tauopathy as well as 3R + 4R human tauopathy. Evidence that a seed barrier does exist can, however, also be derived from those same studies by Clavaguera et al. [27]. Extracts from PiD (a 3R tauopathy) failed to efficiently seed Pick-like pathology in mice, perhaps owing to the presence of only 4R tau in mouse brain. It is possible that a seed barrier could explain the diversity of tauopathy in humans—3R and 4R tau aggregate in AD and some forms of frontotemporal dementia with tau pathology (FTD-tau); 4R tau aggregates in PSP, CBD, and some cases of FTD-tau; and 3R tau aggregates in PiD (Table 1; [21]). We could easily envision how a species barrier could exist in humans in certain cases, whereby a 3R seed ultimately causes PiD and a 4R seed ultimately gives rise to a 4R tauopathy such as PSP. Importantly, all human tauopathies develop in the context of both 3R and 4R tau expression, a critical difference between the experimental and human systems that must be highlighted. If a seed barrier exists in humans, then we would not expect to see 3R/4R tauopathies unless the seed for the mixed tauopathy were fundamentally different than

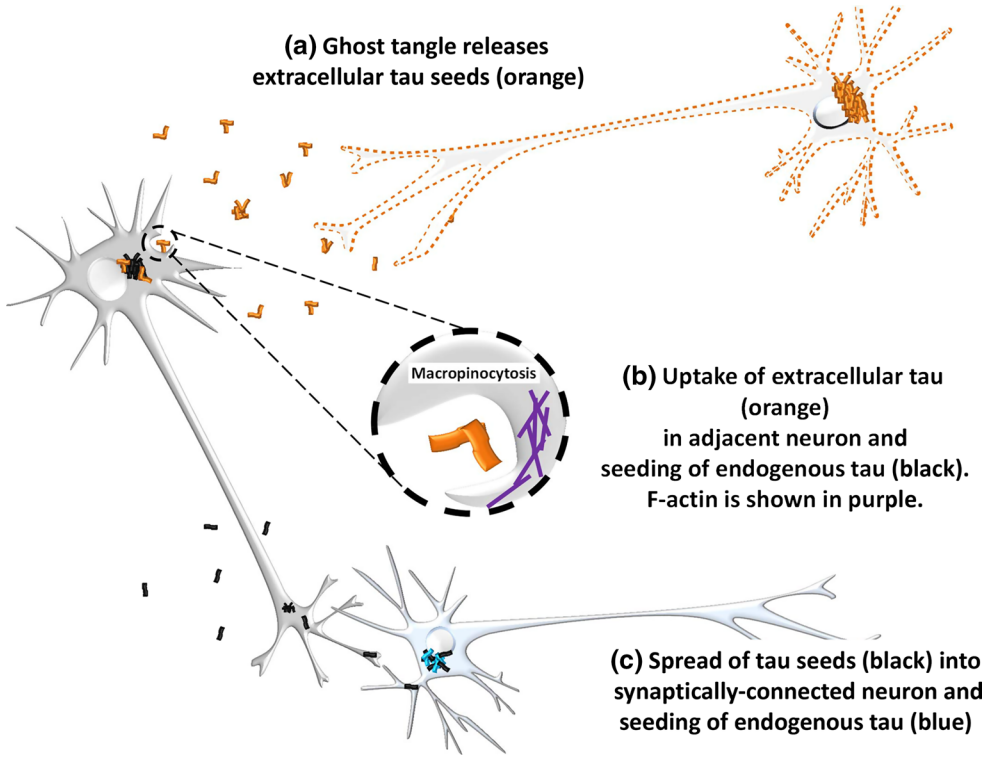

Fig. 2 Commonalities across tau propagation studies in cell and mouse models. Tau aggregates form neurofibrillary tangles and smaller fibrils within neurons, which eventually die. The aggregated tau (*orange*) is released from extra-neuronal tangles (ghost tangles), remnants of dying neurons, and may be one source for extracellular tau seeds. Tau is also present in cerebrospinal fluid and in interstitial fluid (not shown), released by physiological and pathological processes (e.g., synaptic activity) independent of neurodegeneration. Each of these could provide sources of extracellular tau. Many studies suggest that tau can be taken up by macropinocytosis (f-actin—*purple*). Additionally, tau could spread from cell-to-cell trans-synaptically (*black*) or by local diffusion. Tau seeds subsequently serve as templates for conformational changes and aggregation of normal intracellular tau (*black* and *blue* seeds) within naïve cells

those that promoted 3R or 4R tauopathy. As a step forward, we suggest that more work should be performed using the Htau model which lacks murine tau [4], but in which both 3R and 4R human tau are spliced and translated, albeit in a different ratio than observed in either humans or mice. This will allow the field to determine if the natural background of 4R tau in murine brain might give artificial or incomplete results when introducing various forms of tau seeds. The previous characterization of this model already provides a compelling argument for a tau species barrier—Htau mice do not develop robust tauopathy until the murine tau is removed, suggesting that human tau either cannot form a seed or extend fibrils in the presence of murine tau.

What are the commonalities and discrepancies between experimental propagation and human studies?

In many experimental studies, synaptic connections appear to facilitate transcellular spread of tau; though, other methods of cellular uptake cannot be ruled out. Fluid phase endocytosis appears to be a major door through which tau can enter the cell, and one study suggests that tau can trigger its own uptake through macropinocytosis [50]. In human AD, the earliest stage of tau cell-to-cell spread is postulated to be the locus ceruleus, but the locus ceruleus is not affected in all cases (see Fig. 1). This would suggest at the very least that there are alternative initiating sites. The transentorhinal cortex is postulated to be the initial cortical site for tau pathology in AD, and this was the basis of three different propagation studies which used the neuropsin promoter to conditionally drive mutant tau expression in mice [31, 48, 65]. The neurons in the entorhinal cortex project to the dentate gyrus in the hippocampus proper via the perforant pathway. In the neuropsin tau mice, the second neuron in the chain of cell-to-cell spread is dentate granular neurons. In contrast, dentate granular neurons are not affected until end-stage disease (Braak stage VI) in humans [15]. If the cell-to-cell spread modeled in animals applied to humans, there should be involvement of dentate granular neurons at stage III, not VI. This is not the case. It suggests, at the least, that propagation may skip a node and affect it only in later stages of the disease process.

Table 1 Clinicopathologic features of major primary and secondary tauopathies

	Major clinical syndrome	Class	Tau	Distribution of tau pathology	Neuronal tau	Glial tau
Primary tauopathies						
PART	Normal or mild cognitive impairment	Tau	3R + 4R	Anteromedial temporal lobe; hippocampus and amygdala	NFT	
NFTD	Amnestic dementia	Tau	3R + 4R	Anteromedial temporal lobe; hippocampus and amygdala	NFT	
PSP	Atypical Parkinsonism with gaze palsy (other: pure akinesia with freezing of gait, progressive nonfluent aphasia)	Tau	4R	Frontal multimodal association cortices; pallido-nigral-Luysian, neostriatal, and olivopontocerebellar systems	NFT, pretangles and threads	Tufted astrocytes and coiled bodies
CBD	Asymmetrical rigidity and apraxia (other: progressive nonfluent aphasia, frontotemporal dementia)	Tau	4R	Frontoparietal association cortices, neostriatum, pallidonigral system	Pretangles, threads and ballooned neurons	Astrocytic plaques
AGD	Mild cognitive impairment	Tau	4R	Anteromedial temporal lobe; hippocampus and amygdala	Pretangles and grains	Ramified astrocytes and coiled bodies
PiD	Frontotemporal dementia (other: progressive aphasia, progressive apraxia)	Tau	3R	Frontotemporal multimodal association cortices, anteromedial temporal lobe, limbic structures, neostriatum, basal forebrain, reticular formation	Pick bodies and ballooned neurons	Ramified astrocytes and Pick body-like oligodendroglia
Secondary tauopathies						
AD, typical	Progressive amnestic dementia with apraxia and agnosia	Aβ	3R + 4R	Temporoparietal multimodal association cortices, anteromedial temporal lobe, limbic structures, basal forebrain, isodendritic core and reticular formation	NFT, threads, neuritic plaques	
AD, HpSp	Non-amnestic focal cortical syndromes (bvFTD, PPA, PCA, CBS)	Aβ	3R + 4R	Multimodal association cortices, basal forebrain, isodendritic core and reticular formation	NFT, threads, neuritic plaques	
AD, LP	Amnestic dementia	Aβ	3R + 4R	Inferior and anteromedial temporal lobe; hippocampus and amygdala, isodendritic core and reticular formation	NFT, threads, neuritic plaques	
Familial British dementia	Progressive amnestic dementia, frontobehavioral, progressive spasticity	ABri	3R + 4R	Anteromedial temporal lobe	NFT, threads, neuritic plaques	
Familial GSS	Progressive amnestic dementia with ataxia and parkinsonism	PrP	3R + 4R	Anteromedial temporal lobe, isodendritic core and reticular formation	NFT, threads, neuritic plaques	

PART primary age-related tauopathy, *NFTD* NFT predominant dementia, *PSP* progressive supranuclear palsy, *CBD* corticobasal degeneration, *AGD* argyrophilic grain disease, *PiD* Pick's disease, *AD* Alzheimer's disease, *HpSp* hippocampal-sparing, *LP* limbic predominant, *GSS* Gerstmann-Sträussler-Scheinker disease

AD is pathologically and clinically heterogeneous [73], yet in all forms; early involvement of entorhinal cortex is the rule. On the other hand, a subset of AD has relative sparing of the hippocampus, even at end-stage disease when the cerebral cortical areas have very severe tau pathology. This argues against the inevitability of spread to directly interconnected neuronal populations. In another subset of atypical AD, the tau pathology is relatively limited to the medial temporal lobe, the so-called limbic predominant AD [73]. In this variant, there is progressive neurodegeneration in the hippocampus leading to severe neuronal loss and many extracellular neurofibrillary tangles, yet the propagation to other interconnected neurons is minimal.

The concept of "systems degeneration" is a better fit for many neurodegenerative tauopathies than is direct cell-to-cell transmission from a single point of origin. A question that is often neglected by proponents of the cell-to-cell propagation is why there should be a point of origin of the disease process and more importantly, what defines the vulnerability of this point of origin. The propagation hypothesis, as usually presented, does not typically allow for a multifocal origin of the disease process. A current model of tau propagation in AD has its site of origin in the locus ceruleus and subsequent spread to medial temporal lobe cortices [18]. The model does not account for the fact that not only the noradrenergic neurons of the locus ceruleus [44], but also the basal forebrain cholinergic neurons are also affected early [66], and that they have no direct connection to the locus ceruleus.

The basis for selective vulnerably of specific systems to neurodegeneration is unknown, but there are hypotheses other than cell-to-cell spread. The concept of functional connectivity based upon functionally related brain regions based upon the concordance of firing during resting state fMRI is a modern hypothesis as mentioned previously [39, 89, 108] and warrants further study. Given the evidence that tau is not only an axonal protein, but also one that is present in the synaptic terminals, closely associated with synaptic membranes, and released from synaptic termini upon synaptic activity [90]. Thus, the functional connectively hypothesis argues that neurons that synaptically fire together define a set of neurons that are vulnerable to degeneration, even if they are not directly connected in a cell-to-cell manner. As an example of a common non-AD tauopathy, PSP has selective vulnerability of several functionally connected systems—the dentatorubrothalamic pathway, striatonigral pathway, pallidonigral pathway—that are not easily defined as nuclei that have direct cell-to-cell contact. Interestingly, when the "seed" (defined as the brain region that is the point of reference for other brain regions that have concordant activity) in resting state fMRI is placed in the midbrain tegmentum [39], a region markedly vulnerable to PSP pathology and probably linked the impairment of vertical eye movement that is one of the hallmark clinical features of PSP, the regions that are functionally connected including motor cortex, globus pallidus, subthalamic nucleus, substantia nigra and cerebellar dentate nucleus are highlighted. The fact that brain regions that are functionally connected, but not necessarily directly synaptically connected, degenerate as part of a disease process, suggest that an experimental paradigm that models propagation from a single point source may be simplistic experimental paradigm for systems degenerations typical of the various human neurodegenerative tauopathies.

It remains to be seen if functional connectivity can explain focal cortical degenerations associated with tau pathology, as in PiD and CBD. Another issue that remains to be explained by the functional connectivity hypothesis and the cell-to-cell propagation hypothesis is the marked asymmetry that is the hallmark of some of the tauopathies (PiD and CBD, in particular). While some degree of asymmetry of tau pathology is common in AD, it is far less than in frontotemporal degenerations.

Do experimental propagation studies address heterogeneity of tauopathies?

The cell-to-cell propagation hypothesis does not adequately account for clinicopathologic heterogeneity of tauopathies (Table 1), including not only AD, but also PSP, CBD and PiD. In contrast to AD, the latter tauopathies are uncommon and only evaluation of case series of sufficient size permits the recognition of distinct clinicopathologic subtypes. For PSP, the subtypes include those with predominant cortical pathology presenting as corticobasal syndrome [58] and those with severe involvement of the globus pallidus, substantia nigra and subthalamic nucleus (pallido-nigro-Luysian form) presenting as pure akinesia with freezing of gait [2, 100]. In all cases, there is complete overlap in regions affected by tau pathology, but the severity of associated neuronal loss and gliosis is different. Clearly, factors other than cell-to-cell propagation must be considered in understanding vulnerability of specific sets of neuronal populations to neurodegeneration in primary tauopathies, such as PSP. Furthermore, the primary tauopathies are characterized by not merely neuronal tau pathology, but also variable glial pathology. How involvement of separate cell types fits with the cell-to-cell propagation hypothesis is not readily explained. For example, in PSP, some brain regions are especially vulnerable to astrocytic pathology (e.g., caudate nucleus), and they typically do not show significant neuronal loss or gliosis. This would be consistent with in vivo model studies of tau propagation that, for the most part, lack robust neuronal loss or gliosis. On the other hand, some nuclei (e.g., subthalamic nucleus

Table 2 Example of selective vulnerability in a primary tauopathy, progressive supranuclear palsy (PSP)

Mismatch between neuronal and astroglial tau pathology and neuronal loss			
Region	Tufted astrocytes	Pretangles/NFT	Neuronal loss
Superior frontal gyrus	++	+	+
Motor cortex	+++	++	+
Caudate nucleus	+++	++	+
Basal nucleus of Meynert	–	+++	+
Locus ceruleus	–	+++	+
Subthalamic nucleus	+	+++	+++
Substantia nigra	+	+++	+++
Mismatch between oligodendroglial tau pathology and myelinated fiber loss			
Region	Coiled bodies	Atrophy/axonal loss	
Internal capsule	+++	+	
Cerebral peduncle	++	–	
Superior cerebellar peduncle	–	+++	

Neuronal tau pathology differs from glial tau pathology in terms of regional distribution, which in turn differs from distribution of neuronal loss. In general, neuronal loss more closely parallels neuronal tau pathology. White matter degeneration also does not match with tau pathology. Semiquantitative severity score: – = none, + = mild, ++ = moderate, +++ = severe

and substantial nigra) are vulnerable to neuronal tau pathology and show severe neuronal loss and gliosis, while other nuclei and their fiber tracts (e.g., cerebellar dentate nucleus and the superior cerebellar peduncle) have degeneration but minimal tau pathology (Table 2; Fig. 3). These observations suggest that selective vulnerability in tauopathies, with respect to not only the cytopathology, but also the consequences in terms of neuronal loss, is complicated and not readily explained by a model of simple cell-to-cell transmission. While this construct may, to some extent, account for the anatomical distribution of pathology, it does not explain why some affected areas are more susceptible to neuronal loss.

Is there a physiologically relevant source of tau seeds in humans?

If tau does not enter the cell through a trans-synaptic mechanism, what is the source of the extracellular tau? There is one well-defined example of extracellular tau termed "ghost tangles" which are extracellular, aggregated tau found in the brains of individuals with tauopathy. Ghost tangles provide a potential, physiologically relevant source for tau seeds in humans (Fig. 2). An interesting point is that ghost tangles have been shown to lose most of their hyperphosphorylation as well as their N-terminus [19]; therefore, in many ways, these extracellular remnants of aggregated tau in the extracellular space are reminiscent of the truncated tau that has been used as a tau seed in many of the studies investigating tau propagation. While extracellular tangles can be abundant in AD, they are not common in primary tauopathies, such as PSP and CBD, perhaps owing to their less amyloid-like properties [95].

It is also conceivable that tau could be taken up from the cerebrospinal fluid (CSF)—tau has repeatedly been identified in the CSF, and this knowledge led to the development of CSF tau as a potential biomarker for AD [71, 74, 96, 97]. A third potential source for extracellular tau was reported by Yamada et al. [103] to be tau within the interstitial fluid (ISF) of both non-transgenic mice and a transgenic (P301S) model of tauopathy [106]. This finding suggests that the presence of tau in the ISF is neither dependent on nor inherently indicative of neurodegeneration. In fact, levels of ISF tau appeared to depend on two factors: degree of tau aggregation and level of tau expression. Interestingly, Holmes et al. [50] suggested that tau could trigger its expression on macropinocytosis uptake, and this uptake was proportional to the concentration of tau fibrils. If levels of tau (over)expression, such as in a transgenic mouse model, increase the amount of tau in the ISF, one could also envision that these same animals may show a higher level of uptake and subsequent seeding and may not accurately reflect the human condition. Indeed, the P301S mice initially showed higher levels of ISF tau than non-transgenic counterparts; however, levels of ISF tau in the P301S mice decreased with the development of intracellular tau aggregation. If ISF is an important source of extracellular tau as a driver of tau seeding and propagation, then it should be noted that Yamada et al. [103] primarily detected full-length, monomeric tau in the ISF fraction, rather than the truncated tau utilized in the bulk of the tau seeding studies.

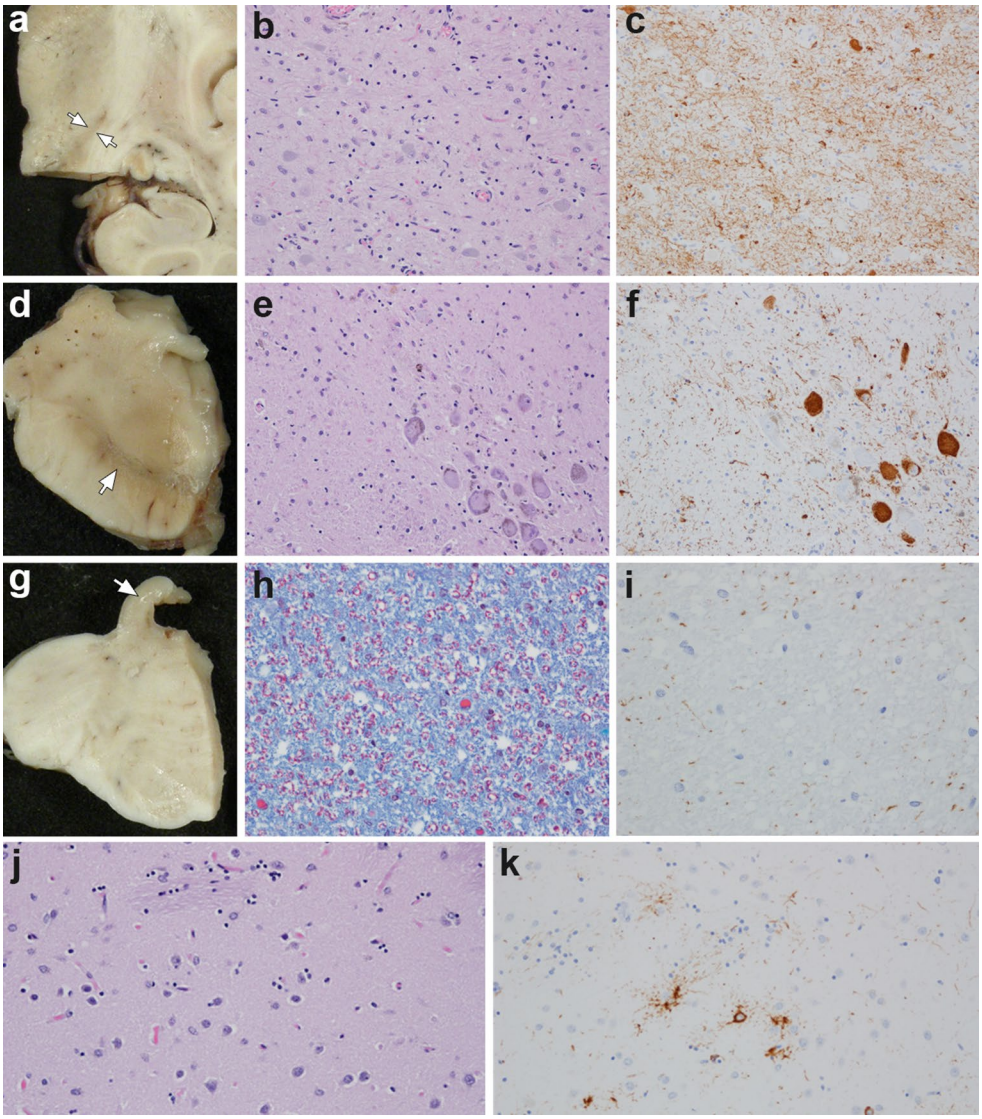

Fig. 3 In primary tauopathies such as PSP, tau pathology is heterogeneous in terms of morphology and distribution. In some areas, such as the subthalamic nucleus (**a**), there is severe atrophy (*arrows*) as well as neuronal loss and gliosis (**b**) and significant tau pathology in neurons, glia and cell processes (**c**). In other areas, such as the substantia nigra (**d**), there is loss of neuromelanin pigment (*arrow*) due to neuronal loss (**e**) and prominent neurofibrillary pathology, but minimal glial pathology (**f**). In some fiber tracts, such as the superior cerebellar peduncle (**g**), there is marked atrophy (*arrow*) and severe myelinated fiber loss (**h**), but minimal tau pathology (**i**). Finally, brain regions most vulnerable to astrocytic tau pathology, such as the caudate nucleus (**j**), are histologically unremarkable, but show prominent astrocytic tau pathology (**k**). These observations suggest that selective vulnerability in PSP is not easily explained by simple cell-to-cell propagation of abnormal tau conformers spreading from a single point of origin and must take into account the specific properties of each affected and unaffected brain region during disease pathogenesis. **b**, **e**, **j** Hematoxylin and eosin; **c**, **f**, **i**, **k** phospho-tau (CP13); **h** trichrome. *Bar* in **k** = 50 μm for **b**–**e**, **f**, **j**, **k**; *bar* in **i** = 20 μm for **h** and **i**

Is neurodegeneration observed in context of tau propagation?

A major discrepancy exists between much of experimental literature and human tauopathies. Tauopathies are neurodegenerative in nature, presenting with both neuronal loss with varying degrees of gliosis, depending on the brain regions and the specific tauopathy. Signs of neurodegeneration are generally lacking from the published tau experimental propagation studies with few exceptions. Failure of published studies to consistently replicate neuronal loss observed in human tauopathies casts doubt on the concept that the prion-like properties ascribed to tau are physiologically relevant. It seems unlikely that the lack of neuronal loss and gliosis is due to the short lifespan of mice when there are already multiple examples of transgenic

mouse models of tauopathy that develop neuronal loss, even in short timeframes such as seen for the rTg4510 model [88]. Rather, the lack of robust neurodegeneration in the majority of the in vivo propagation studies suggests that the tau pathology that is induced in these studies may not represent the toxic tau forms that drive human tauopathies.

How do tau propagation studies influence tau therapies?

Multiple studies have reported some degree of efficacy of tau immunotherapy in mouse models of tauopathy [3, 8, 9, 14, 24]; however, it is unclear how these approaches impact a disease caused by an intracellular protein. As previously discussed, tau appears to have some capability to transfer from cell to cell. Kfoury et al. [60] demonstrated that administration of a tau antibody into the media of tau expressing cells blocked uptake of a tau fibrillar species. In 2013, this same group [104] published an elegant study in which they utilized a cell-based biosensor assay to identify antibodies that could clear the seeding capability of extracts from P301S mouse brain. This ability subsequently corresponded with the degree to which the antibodies could clear tau, block microglial activation, and alleviate downstream cognitive aspects of the seeding in the P301S mouse model of tauopathy. The concept of tau propagation partnered with our greater awareness that tau may be secreted into the extracellular space in both abnormal and normal conditions provides a potential explanation for how immunotherapy may prove effective.

Are we at risk for tau infection?

All successful tau propagation studies modeled events that happen after fibrils are formed, not the initial formation of the tau seed. If we can extend these lessons to humans, tau propagation is likely to only be relevant in human disease after the onset of a tauopathy and is unlikely to explain the processes that set the disease in motion. Simply put—no seed, no propagation. Once the seed has been introduced into the brain through an environmental or genetic risk factor, though, it is conceivable that tau could conformationally template and that trans-cellular movement of tau might influence disease progression, even in humans. There are two important caveats. (1) Most of the tau propagation studies involve the overexpression of mutant tau or large quantities of toxic tau seeds, frequently generated by sonicating preparations. With the exception of rare splicing mutations in tau that lead to increased 4R tau production [52, 91], this permissive environment is absent in human brain. (2) The amount of tau seeds utilized in published studies may exceed levels of any physiologically relevant source, though we cannot say this for certain until the specific source is identified in human tauopathies.

It appears unlikely that exogenous sources of tau seeds could prove dangerous to humans as no evidence of human-to-human transmission of tauopathy through routine routes of potential infection has been discovered [11, 55, 77]. Furthermore, Chakrabarty et al. [25] recently reported that they were unable to seed tau propagation by either intramuscular or intracisternal injections of tau fibrils in a permissive mouse model of tauopathy. This demonstrates that there may be physiological barriers against transmission of tauopathy. Given this, it seems unlikely that exogenous introduction of tau through exposure to patients or mice with tauopathy or even to tau tissue or fibrils would prove risky.

Since we cannot discount that cannibalism which contributed to Kuru could also potentially allow transmissibility of tauopathy in humans, we will provide sage advice given by editorial board of JAMA in 1968 [78]—just in case, do not eat your neighbor.

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