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Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo

Highlights

- Tau forms multiple, unique prion strains with distinct biochemical properties
- Tau strains induce diverse pathological phenotypes in vitro and in vivo
- Tau strains target different brain regions and propagate pathology at unique rates
- We present a framework to understand the diversity of human tauopathies

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In Brief

Kaufman et al. observe that tau forms multiple self-propagating amyloid strains. The introduction of each into transgenic mouse brain creates a distinct pathology that targets different brain regions and induces unique rates of spread. Thus, strain variation can account for the diversity of human tauopathies.



Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo

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SUMMARY

Tauopathies are neurodegenerative disorders that affect distinct brain regions, progress at different rates, and exhibit specific patterns of tau accumulation. The source of this diversity is unknown. We previously characterized two tau strains that stably maintain unique conformations in vitro and in vivo, but did not determine the relationship of each strain to parameters that discriminate between tauopathies such as regional vulnerability or rate of spread. We have now isolated and characterized 18 tau strains in cells based on detailed biochemical and biological criteria. Inoculation of PS19 transgenic tau (P301S) mice with these strains causes strain-specific intracellular pathology in distinct cell types and brain regions, and induces different rates of network propagation. In this system, strains alone are sufficient to account for diverse neuropathological presentations, similar to those that define human tauopathies. Further study of these strains can thus establish a structural logic that governs these biological effects.

INTRODUCTION

Tauopathies are a diverse group of neurodegenerative diseases characterized by clinical heterogeneity, progressive deposition of tau protein aggregates in characteristic brain regions, and distinct cellular pathologies (Lee et al., 2001). The etiology of this clinical and pathological diversity is unknown, but may hold the key to accurate diagnosis, prognosis, and therapy. Tauopathies include Alzheimer's disease (AD), frontotemporal dementias (FTDs), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and others (Lee et al., 2001). Most tauopathies are sporadic, but dominantly inherited mutations in the *MAPT* gene, which encodes the tau protein, lead to specific

FTD syndromes (Hutton et al., 1998). Disease-associated mutations enhance the ability of tau to form amyloids, which are ordered protein assemblies rich in cross beta sheet structure (Knowles et al., 2014), and support a causal role for tau aggregation in pathogenesis (Barghorn et al., 2000).

In AD, the most common tauopathy (Lee et al., 2001), tau amyloid deposition occurs in an orderly fashion, beginning in the transentorhinal cortex, spreading to synaptically connected regions such as the hippocampus, and eventually moving to more distant regions of the neocortex (Braak and Braak, 1991). Multiple studies have now documented tau aggregate uptake, "seeding" (i.e., aggregate serving as a template for the conversion of monomer to a fibrillar form) and transfer of aggregates among cultured cells (Frost et al., 2009a; Guo and Lee, 2011; Holmes et al., 2013; Nonaka et al., 2010). Experimental evidence suggests that "propagation", or the movement of tau aggregates between connected neurons with seeding of tau monomer in recipient cells, mediates this progression in vivo (Sanders et al., 2016; Walker and Jucker, 2015). Importantly, injection of tau aggregates into mice that express human tau protein induces tau pathology that spreads outward along known brain networks (Clavaguera et al., 2009; Iba et al., 2013). Transgenic mice that limit the expression of tau to the entorhinal cortex also show spread of tau pathology to distant, connected brain regions (de Calignon et al., 2012; Liu et al., 2012). Together, these studies suggest that propagation of an aggregated state underlies the progression of tau pathology. These observations match the established mechanisms of propagation of pathological prion protein (PrP) (Prusiner, 1998).

The pathology of tauopathies occurs in distinct brain regions (Arnold et al., 2013), involves disparate brain networks (Raj et al., 2012; Zhou et al., 2012), and features unique tau inclusions in various cell types (Kovacs, 2015). Individuals may develop rapid or slow neurodegeneration even within the same syndrome (Armstrong et al., 2014; Thalhauser and Komarova, 2012). The basis of these diverse disease patterns is unknown.

We initially observed that tau adopts multiple, stably propagating conformers in vitro and speculated that structural variation in amyloids could underlie different tauopathies (Frost

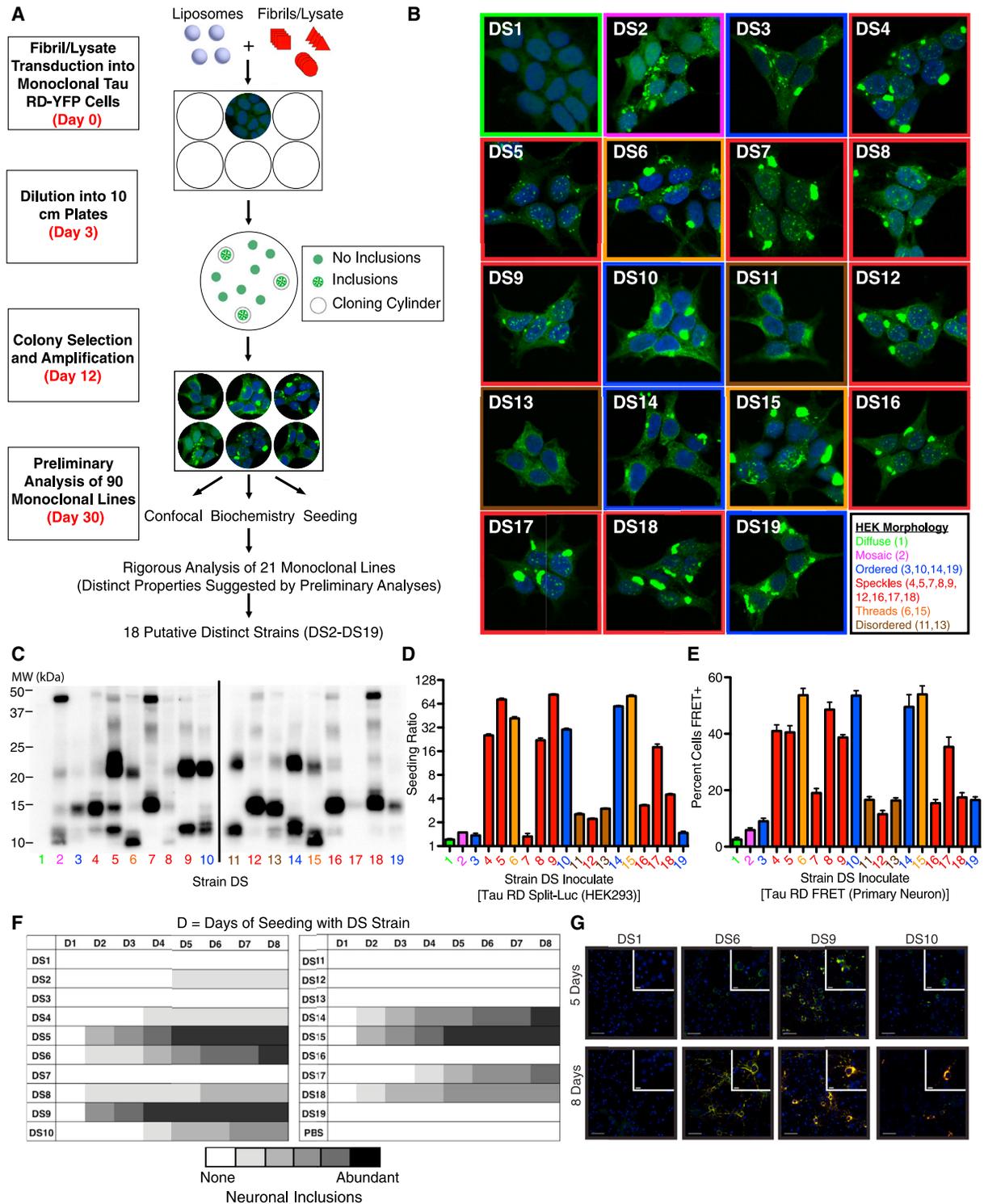


Figure 1. Generation and Characterization of a Tau Prion Strain Library

(A) A monoclonal HEK293 line stably expressing tau RD(P301L/V337M) (“LM”)-YFP (DS1) was treated with diverse sources of fibrillar tau seeds. 90 monoclonal lines that stably propagated tau inclusions were derived and characterized by the indicated metrics. 18 strains were differentiated based on their unique properties in the indicated assays. See [Figure S1A](#) for origin of inoculates used to derive each strain.

(B) Several tau inclusion phenotypes were identified in the monoclonal strains: mosaic (magenta), ordered (blue), speckles (red), threads (orange), and disordered (brown). With the exception of the mosaic phenotype, these inclusion phenotypes stably propagate to daughter cells over months of division. A negative control cell line (DS1) features diffuse tau (green). See [Figures S2F–S2H](#) for data regarding stability of specific strains upon passage into DS1.

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et al., 2009b). We subsequently determined that tau forms discrete prion “strains” that propagate with remarkable fidelity through living systems (Sanders et al., 2014). The concept of prion strains derived from a realization that PrP prions can induce distinct transmissible spongiform encephalopathies with reproducible incubation times and patterns of neuropathology (Collinge and Clarke, 2007). It is now clear that PrP prion strains derive from different PrP amyloid conformations and produce predictable incubation times and neurodegenerative phenotypes upon serial passage in vivo. Moreover, distinct PrP strains probably account for the myriad features of individual PrP prion diseases (Collinge et al., 1996). The concept of a strain as a stably propagating structure that induces a specific and reproducible phenotype is critical, as it anticipates and enables mechanistic, diagnostic, and therapeutic insights based on knowledge of a defined molecular assembly (Sanders et al., 2016).

In addition to the distinct morphologies of tau fibrils and isoform composition of amyloid deposits in tauopathies (Lee et al., 2001), prior studies have suggested that unique tau amyloid structures might account for some aspects of clinical variation. Injection of homogenate from different tauopathy brains into a mouse model that expresses full-length human tau induced pathology that closely resembled that of the human source cases (Clavaguera et al., 2013). In a similar study, tau aggregates purified either from AD or CBD brains induced distinct patterns of tau pathology that affected different cell populations in transgenic mice that express 1N4R tau with a P301S mutation (PS19) (Boluda et al., 2015). However, both works relied upon a limited number of patient samples that likely contain a heterogeneous mixture of tau aggregate conformations (Sanders et al., 2014). Thus, the structure and biochemical properties of injected tau aggregates could not be well defined, making it impossible to directly link an amyloid structure to pathology.

Like PrP, tau forms bona fide prion strains that propagate in cells and animals (Sanders et al., 2014). We have now isolated 18 putative tau prion strains derived from recombinant, mouse, or human sources. We have studied them extensively in vivo and find that they can account for diverse and predictable patterns of neuropathology. This work thus develops a framework to understand the relationship of tau prion structure to distinct tauopathy syndromes.

RESULTS

Generation of a Library of Tau Strains

We previously created a monoclonal HEK293 cell line (Clone 1/DS1) that stably expresses the repeat domain (amino acids 244–372) of 2N4R tau with two disease-associated mutations (P301L and V337M), which allows us to indefinitely propagate tau prion strains derived from a variety of sources (Sanders et al., 2014). We treated DS1 cells with tau aggregates from diverse recombinant, mouse, and human brain samples (Figure 1A). We isolated and amplified 90 monoclonal cell lines that stably propagated tau aggregates and froze them for later study. Following preliminary analyses by several assays (inclusion morphology, limited proteolysis, and seeding by tau split-luciferase complementation, as described previously; Sanders et al., 2014), we isolated 18 putatively distinct strains (DS2–DS19; see Figure S1A for origin of each strain). These strains featured several striking differences in their subcellular distribution of aggregated tau (Figure 1B): a single, large juxtanuclear inclusion (ordered: DS3, DS10, DS14, and DS19), prominent nuclear inclusions (speckles: DS4, DS5, DS7, DS8, DS9, DS12, DS16, DS17, and DS18), aggregated tau that failed to organize into ordered inclusions (disordered: DS11 and DS13), fibril-like ribbons of aggregated tau throughout the cytoplasm (threads: DS6 and DS15), and one strain that sectorized with time, reverting from the aggregated state to the soluble diffuse state (mosaic: DS2). Importantly, with the exception of the mosaic strain DS2, every daughter cell featured the same inclusion morphology even after months of passage, suggesting that each monoclonal cell line stably propagated a single strain.

To examine whether the tau inclusions were composed of structurally distinct tau amyloids, we used limited proteolysis, an assay previously shown to differentiate prion strains derived from PrP (Bessen and Marsh, 1994) and tau (Sanders et al., 2014). We thus determined a “fingerprint” for each putative strain based on the regions of tau protected from digestion by pronase (Figure 1C). These digestion patterns were stable even upon dilution with HEK lysate (Figure S1B), confirming their independence from the amount of aggregated tau present in each sample. This assay suggested that while cell lines with different inclusion morphologies always propagated different

(C) Limited proteolysis using pronase differentiates the protected fibrillar cores in individual tau strains. Unique fingerprints along with other metrics indicated structurally distinct tau prion strains. See Figure S1B for pronase digestion of strains diluted with HEK lysate.

(D) Seeding activity of strains in a split-luciferase assay. A tau RD(P301S) split-luciferase assay based on enzymatic complementation following aggregation demonstrates differences in strain seeding activities following introduction into the cytoplasm using lipofectamine. The seeding ratio indicates luminescence relative to sham treatment. The biological quadruplicates with saturating quantities of lysate were averaged. The error bars represent SEM for biological quadruplicates.

(E) Strain seeding activities replicate in primary neurons expressing tau RD. Primary hippocampal neurons expressing tau RD(P301S)-CFP and tau RD(P301S)-YFP were treated with lysates derived from various strains. After 96 hr, neurons were fixed and the percentage of cells featuring seeded aggregates was determined by FRET flow cytometry. The error bars represent SEM for biological quintuplicates.

(F) Strain seeding activities replicate in primary neurons expressing full-length tau. Primary hippocampal neurons expressing 1N4R tau(P301S)-YFP were exposed to lysates from each strain and the extent of seeding was semiquantitatively determined at various time points (D = number of days) based on the extent of visible YFP puncta (0–5: 0 = none and 5 = abundant inclusions). The strains show variable lag times and extent of seeding, which correlates with the split-luciferase complementation assay.

(G) Strains differentially induce the formation of insoluble tau aggregates in primary neurons. Triton X-100 was used to remove soluble tau, and the primary neurons were stained for conformationally altered tau (MC1) 5 or 8 days following seeding. The strains show significant differences in seeding of aggregation in neurons. This parallels differences in the split-luciferase complementation assay. The scale bars represent 50 μ m for the wide view and 10 μ m for the inset images. See Figure S1C for representative images for all strains.

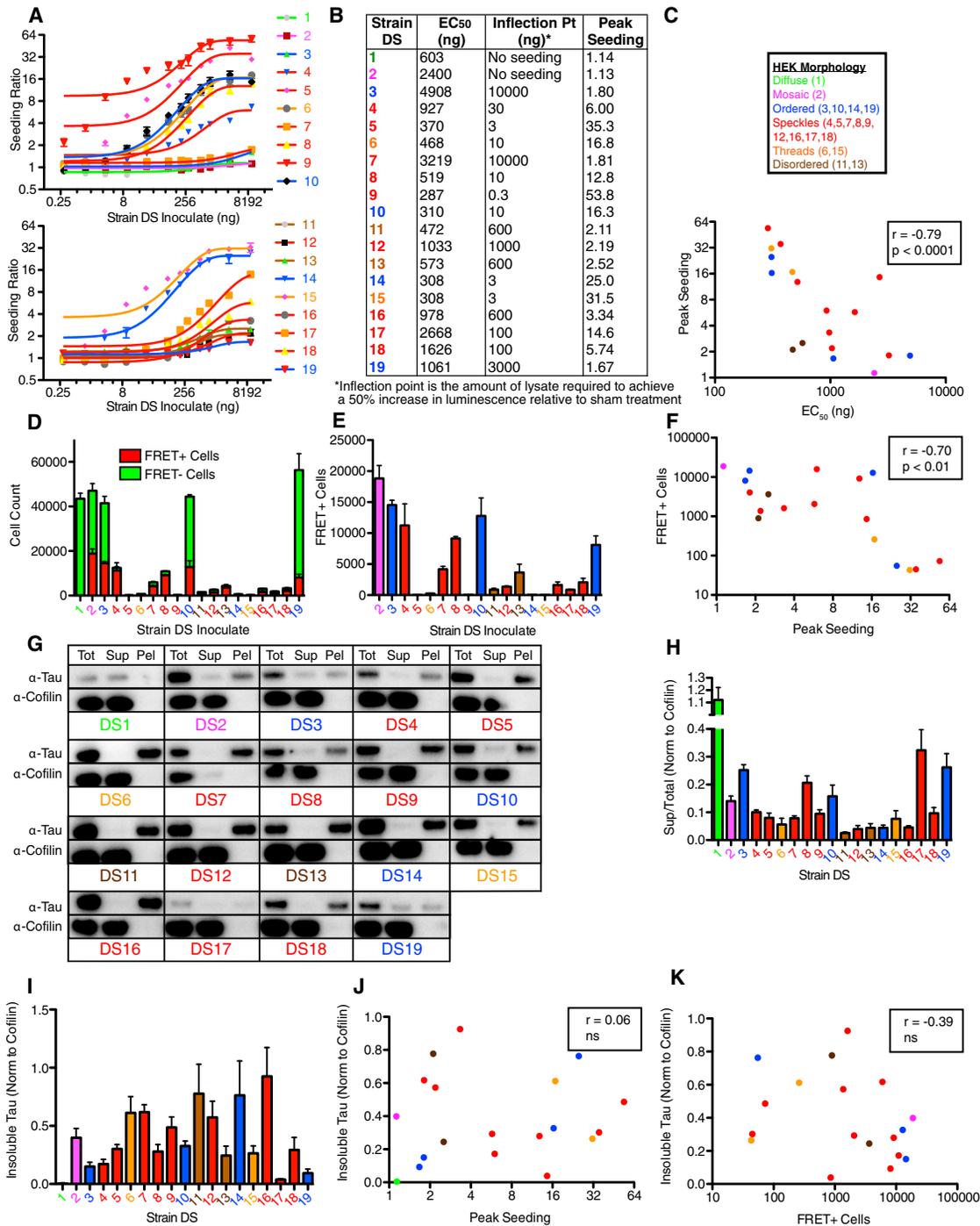


Figure 2. Seeding Activity, but Not Insoluble Tau, Correlates with Strain Toxicity In Vitro

(A) Strains have large differences in seeding of monomeric tau as determined by a tau split-luciferase complementation assay. The strain lysates were transduced into tau RD(P301S) split-luciferase cells, the seeding ratios relative to sham treatment were determined, and the titration curves were plotted using non-linear regression with a one-phase decay fit. The curves are plotted on two separate graphs for clarity. The error bars represent SEM for biological quadruplicates. (B) Based on titration curves in the tau split-luciferase complementation assay, the EC₅₀, infection point, and peak seeding ratio were determined for each strain. The infection point represents the amount of lysate required to achieve a 50% increase in luminescence relative to sham treatment. (C) Peak seeding significantly correlates with EC₅₀s for the strain library in the tau split-luciferase complementation assay. (D) Strains display significant differences in toxicity. The strains were transduced in biological triplicates into cells overexpressing both tau RD(LM)-CFP and tau RD(LM)-YFP. After 72 hr, equivalent numbers of aggregate-containing (FRET+) cells were sorted for each condition by FRET flow cytometry. For the negative control (DS1), aggregate-negative (FRET-) cells were sorted. The sorted cells were allowed to proliferate in technical sextuplicates for 1 week.

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conformations, inclusion morphology alone could not discriminate all strains.

Tau Strains Show Unique Seeding Profiles in Dividing Cells and Neurons

Next, we examined the ability of each putative strain to seed tau monomer using a cell line that expresses tau RD(P301S) fused to the N or C terminus of click beetle green luciferase enzyme (Mirbaha et al., 2015; Sanders et al., 2014). When saturating concentrations of lysate were transduced directly into the cytoplasm of these cells, induction of luminescence ranged from 0.3- to 80-fold increase in seeding at saturation versus background, termed the “seeding ratio” (Figure 1D). We observed no association between inclusion morphology and seeding. For example, the four ordered strains showed different seeding activity (DS3, DS10, DS14, and DS19). The relative seeding abilities of individual tau strains were largely recapitulated when lysates were applied to primary hippocampal neurons expressing tau RD(P301S) fluorescence resonance energy transfer (FRET) biosensor proteins (Holmes et al., 2014) (Figure 1E). We also observed these relative differences upon exposure of strains to primary neurons expressing full-length 1N4R P301S-YFP, suggesting common effects on either truncated or full-length tau. Strains also showed different lag phases to induce inclusions in neurons that express full-length tau (Figures 1F, 1G, and S1C).

Seeding Activity Correlates with Toxicity in Dividing Cells

To investigate the relationship between seeding activity and toxicity, we first performed a detailed titration of cell lysates (30 pg to 10 μ g) from the 18 putatively distinct strains. We determined the EC_{50} and peak seeding ratio for each strain using the tau split-luciferase complementation assay. Strains differentially seeded tau monomer in cell culture as reflected by their peak seeding ratios (Figures 2A and 2B). Different strains displayed $>10\times$ range for EC_{50} (DS9: 287 ng and DS3: 4908 ng) (Figure 2B). Peak seeding and EC_{50} strongly anti-correlated (Figure 1C), suggesting that peak seeding accurately reflects a strain’s potency in triggering tau aggregation.

We then compared the toxicity of each putative strain to their seeding potential. We generated a cell line (LM10) that expresses

a mutant tau RD FRET pair (CFP/YFP) at high levels and assessed the growth potential of cells propagating various strains after first isolating aggregate-positive cells by FRET fluorescence-activated cell sorting (FACS) (Holmes et al., 2014). Several ordered and mosaic strains (DS2, DS3, DS10, and DS19) lost the aggregated state (“sectored”) with repeated cell division (Figure 2D). All others stably propagated the aggregated state, but exhibited growth defects relative to LM10 cells that lacked tau aggregates (Figure 2E). Strains that sectored, a possible correlate of low seeding, were the least toxic. All three seeding metrics (peak seeding, EC_{50} and inflection point) correlated with inhibition of growth (Figures 2F, S2A, and S2B). In other words, strains that seeded more efficiently were significantly more toxic to cells that express high levels of monomeric tau.

We next performed sedimentation analyses to determine the level of soluble, insoluble, and total tau for all 18 putative strains (Figure 2G). While each strain (DS2–DS19) contained the majority of tau in the insoluble fraction (Figures 2G and 2H), different strains featured variable levels of insoluble (Figure 2I) and total (Figure S2C) tau. Neither total nor insoluble tau levels correlated with seeding (Figures 2J and S2D) or toxicity (Figures 2K and S2E). Thus, structural differences among strains, rather than soluble/insoluble tau levels per se, account for seeding activity and toxicity in dividing cells.

Diversity of Pathology Induced by Tau Strains

We hypothesized that to account for variation in tauopathies, individual tau strains should produce a wide array of pathological phenotypes in vivo. To test whether these putative strains can produce such diversity, we inoculated cell lysate from each line (DS1–DS19) into the PS19 mouse model that expresses 1N4R tau with the FTDP-17-associated P301S mutation from the prion promoter (Yoshiyama et al., 2007) (Figure 3A) and examined tau pathology induced 8 weeks after inoculation (Figures 3B–3J).

Pathology varied greatly between putative strains and was often, but not always, consistent with seeding activity observed in culture (Figures 1D–1G). Strains with low seeding activity (DS2, DS3, DS11, and DS19) produced a “rare seeding” phenotype in vivo, with limited AT8 pathology localized in CA1 of the ipsilateral hippocampus (Figures 3G and S3A). These strains appear

Aggregate-positive (FRET+) and aggregate-negative (FRET-) cells were then quantified by flow cytometry. The presence of FRET- cells in certain conditions reflects the fact that some strains lose the aggregated state with cell division. Technical sextuplicates were averaged for each biological replicate. The error bars represent SEM of biological triplicates.

(E) Aggregate-positive (FRET+) cells were quantified and plotted after 1 week of growth. This highlights the variable growth defects in aggregate-containing cells. The error bars represent SEM of biological triplicates.

(F) Toxicity correlates with seeding activity. The number of aggregate-positive (FRET+) cells for a strain was plotted against its peak seeding ratio in the tau split-luciferase complementation assay. The strains that seed more efficiently are associated with reduced growth of aggregate-positive cells. See Figures S2A and S2B for data indicating the correlation between a strain’s toxicity, EC_{50} , and inflection point in the seeding assay.

(G) Sedimentation analysis of strains. Lysates were ultracentrifuged and tau as well as a loading control protein (cofilin) were probed in the total, supernatant, and pellet fractions (total = Tot, supernatant = Sup, and pellet = Pel). The blots are representative of biological quadruplicates.

(H) Strains feature the majority of tau in the insoluble fraction. Densitometric analysis of tau in the total, supernatant, and pellet fractions was used to calculate supernatant to total ratios (a higher ratio indicates a smaller proportion of tau in the insoluble pellet). The error bars represent SEM of biological quadruplicates.

(I) Densitometric analyses highlight variation in insoluble tau in the various strains. The error bars represent SEM of biological quadruplicates. See Figure S2C for quantification of tau in the total fractions.

(J) Lack of correlation between insoluble tau and seeding activity as measured by peak seeding ratio. See Figure S2D for data indicating lack of correlation between total tau and seeding.

(K) Lack of correlation between total tau and seeding activity. See Figure S2E for data indicating lack of correlation between total tau and toxicity.

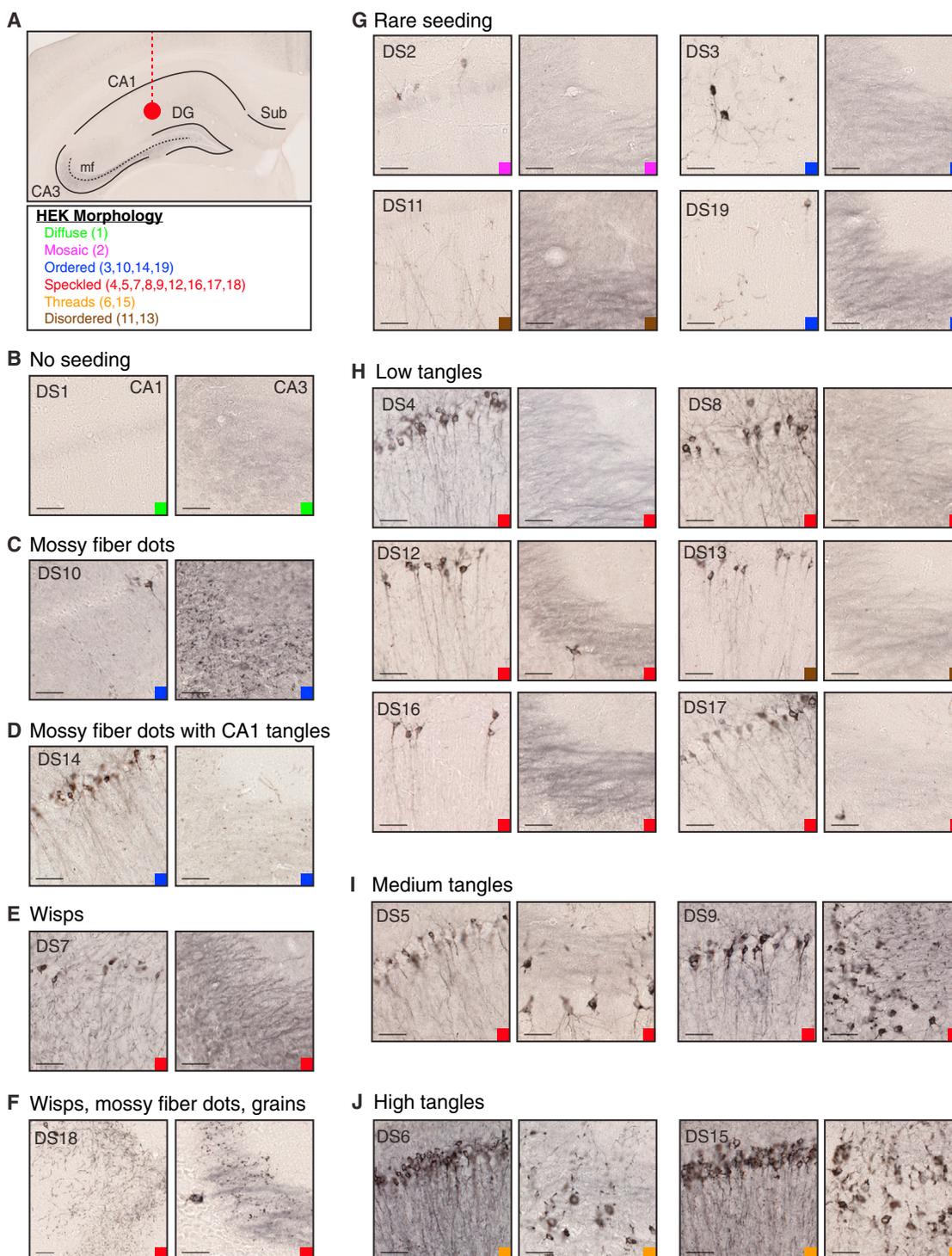


Figure 3. Tau Prion Strains Induce Diverse Patterns of Hippocampal Tau Pathology

(A) Tau strains (10 μ g) were injected into the left hippocampus of young PS19 mice ($n = 3$ per condition, see [Table S1](#)). Mouse brains were collected 8 weeks after injection. The relevant regions are indicated on a representative mouse hippocampus (dentate gyrus, DG; mossy fibers, mf; and subiculum, Sub). The HEK morphology table indicates the inclusion patterns in various strains, grouped by text color. The color-coded squares indicate these HEK cell-associated patterns in all images (B–J).

(B) DS1 injection produces no AT8 tau pathology. The representative images of CA1 and CA3 are displayed. The scale bars represent 50 μ m. See [Figure S3A](#) for whole hippocampal images for DS1–DS19.

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different in terms of their AT8 subcellular localization (soma versus axonal pathology in DS2 and DS11), but this may reflect different levels of maturation of tau aggregates (e.g., pretangles and tangles). Several strains induced low level, yet consistent, tangle-like pathology in CA1 of the hippocampus (DS4, DS8, DS12, DS13, DS16, and DS17) (Figures 3H and S3A). Strains with the highest seeding activity in culture (DS5, DS6, DS9, and DS15) caused widespread tangle-like tau pathology throughout several hippocampal regions (Figures 3I and 3J). Pathology from these robust strains spread to distant regions such as the entorhinal cortex (EC) and contralateral hippocampus (Figure S3B).

Several putative strains induced unique pathology in the hippocampus. DS10 typically produced AT8-positive “dots” in the mossy fiber tracts of the ipsilateral and contralateral hippocampus, while mostly sparing CA1 pyramidal neurons (Figures 3C and S3C) as was observed in previous work (Sanders et al., 2014). DS14, which shared the same ordered cellular phenotype as DS10, also produced mossy fiber dots (Figure 3D). However, this strain showed higher seeding activity in culture and additionally induced CA1 tangle-like pathology in the ipsilateral and contralateral hippocampus (Figure S3C).

DS7 produced “wisps” that resemble neuropil threads (Figure 3E), while inducing weaker AT8 pathology in the main axon branches. This contrasted with several other speckled phenotype strains, which primarily induced AT8 pathology in the main axon (Figures 3H and 3I). DS18 produced wisps, mossy fiber dots, and “grains”, which are AT8 positive puncta found throughout the hippocampus (Figure 3F). DS18-inoculated mice also developed grain pathology in the contralateral hippocampus and wisps in the EC, indicating these specific phenotypic features can spread to distant regions (Figure S3D).

Critically, strains with matching limited proteolysis fingerprints produced similar histopathology in vivo (DS3 and 19; DS6 and DS15; and DS12 and DS16) (Figures 3F, 3G, and 3I). These pairs of similar strains (which may propagate identical tau aggregate conformations) displayed similar seeding activity and toxicity levels and induced similar phenotypes in primary neuron culture. Importantly, DS6 and DS15 derive from distinct aggregate sources (aged PS19 mice and recombinant fibrils, respectively), indicating that these strain-based phenotypes are conformation-specific rather than source-specific.

Stability of Distinct Tau Prion Strains

We previously demonstrated that DS9 and DS10 propagate unique conformations and produce identical phenotypes upon reintroduction into DS1 cells. To test whether other strains meet these same criteria for stable prion strains (Sanders et al., 2014), we transduced cell lysate from strains with distinct cellular morphology, seeding activity, and/or in vivo phenotypes into naive DS1 cells (DS1, DS4, DS6, DS7, DS9, DS10, or DS11). We first performed a blinded analysis of cell morphology from a polyclonal population at 5 and 8 days after transduction. The original DS1 and secondary polyclonal DS1 cell lines contained no aggregate-positive cells (Figures S2F–S2H). Blinded counts of DS4, DS7, and DS9 demonstrated the polyclonal population maintained the nuclear speckled phenotype, while DS10 and DS11 secondary lines were readily scored as ordered and disordered. DS6 threads that project from a large juxtannuclear aggregate are only readily apparent when assessing morphology on a population level rather than within individual cells. However, transduction of this cell line reliably induced overt “threads” in the vast majority of secondary cells at 5 days after transduction. By 8 days, tau aggregates in DS6 secondary cells appeared to mature, and the cellular morphology and blinded scoring results resembled that of the original DS6 cell line (Figures S2F–S2H).

To further examine the stability of each strain phenotype, monoclonal secondary cell lines that stably propagate aggregates were isolated by unbiased single-cell sorting at 4 days after transduction of DS4, DS6, DS7, DS9, DS10, and DS11 into the LM1 cell line. Secondary strains displayed the same cellular morphology as the original cell lines (Figure S7A). We tested the seeding activity of each secondary cell line compared to the original strains by transducing cell lysate into a biosensor cell line that expresses tau RD(P301S)-CFP/YFP and quantifying FRET by flow cytometry (Holmes et al., 2014). Secondary cell lines produced seeding activity similar to their respective source strain (Figure S7C). Thus, these are stable strains that induce unique cellular phenotypes even upon serial passage in culture.

Specific Strains Reliably Induce Astrocytic Pathology

Several tauopathies, including CBD and PSP, feature unique glial cell pathology (Kovacs, 2015). While the mechanisms that underlie these patterns are not known, previous work suggested that inoculation of CBD patient lysate into PS19 mice is sufficient to

(C) DS10 produces AT8 positive mossy fiber dot pathology, with limited CA1 pathology as observed previously (Sanders et al., 2014). See Figure S3C for contralateral mf pathology.

(D) DS14 seeds mossy fiber dots similar to DS10, as well as tangle-like pathology, indicating that it is a distinct strain despite its other similar features to DS10. See Figure S3C for contralateral mossy fiber and CA1 pathology.

(E) DS7 induces wisps that resemble neuropil threads, but may fall within axon terminals and the dendritic tree of pyramidal neurons.

(F) DS18 pathology includes wisps and mossy fiber dots similar to DS7 and DS10, respectively, as well as grains that are found throughout much of the hippocampus. See Figure S3D for data indicating that these phenotypes spread to distant synaptically connected locations including the entorhinal cortex.

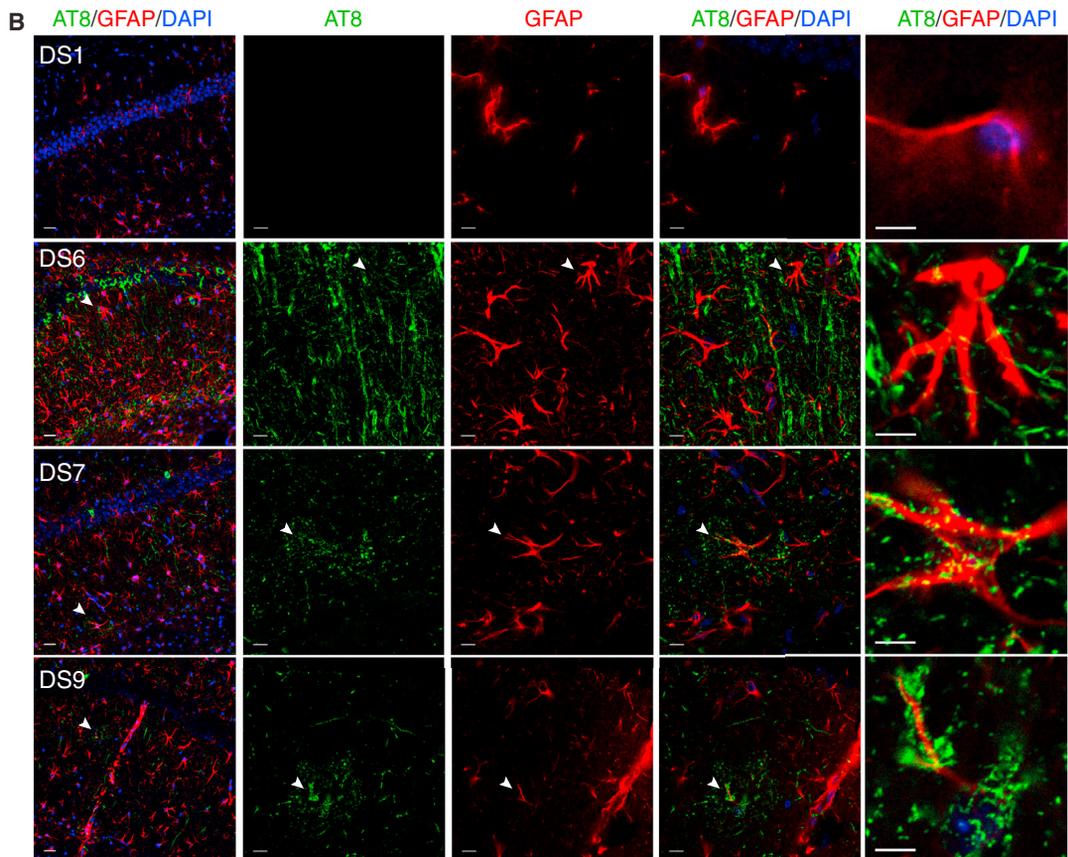
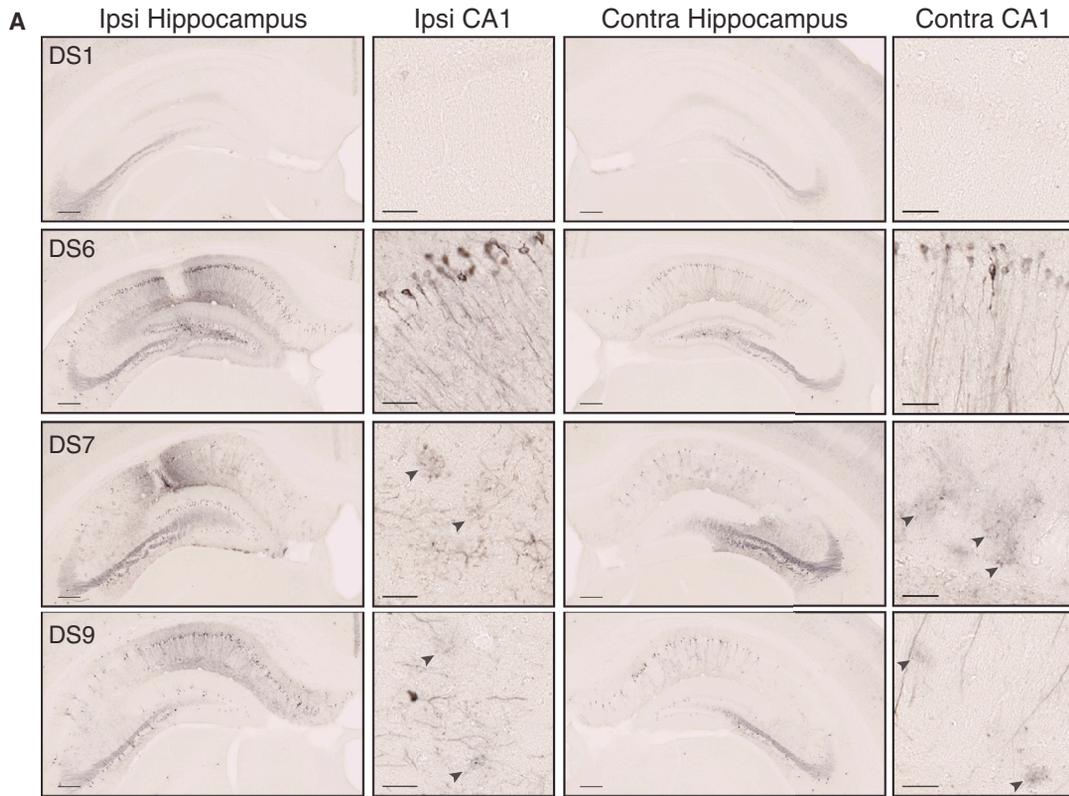
(G–J) Several strains produce different levels of tangle-like AT8 pathology in CA1 and CA3 of the hippocampus.

(G) DS2, DS3, DS11, and DS19 induce rare AT8 pathology in pyramidal CA1 neurons. The localization of AT8 staining varies in certain cases (cell body versus axonal pathology in DS2 and DS11, respectively).

(H) DS4, DS8, DS12, DS13, DS16, and DS17 induce slightly stronger tangle-like pathology in CA1 of the hippocampus (“low tangles”). CA3 shows limited or no tangle pathology at this time point.

(I) DS5 and DS9 produces AT8 tangle-like tau pathology that reaches CA3 of the hippocampus as well as CA1 pyramidal cells (“medium tangles”). Tangles appear relatively consolidated within the soma of neurons. See Figure S3B for spread of tau pathology to the contralateral hippocampus and ipsilateral EC.

(J) DS6 and DS15 display the highest level of tangle-like AT8 pathology (“high tangles”). Highly consolidated pathology was observed throughout cell bodies and axons of CA1 and CA3 neurons. See Figure S3B for spread of tau pathology to the contralateral hippocampus and ipsilateral EC.



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induce astrocytic pathology (Boluda et al., 2015). Interpretation of this result was limited by the potential for individual patient brains to contain multiple strains (Sanders et al., 2014). Further, two different paradigms were used for purification of AD versus CBD derived tau, which might also have affected the observed phenotypes (Boluda et al., 2015). We thus assessed astrocytic pathology after inoculation with isolated, individual tau strains.

At 8 weeks after inoculation with DS7 and DS9, we observed tau pathology reminiscent of astrocytic plaques in multiple animals, as noted by small AT8-positive inclusions arranged in ring-like structures (Figure 4A) (Yoshida, 2014). Strains that produced higher levels of tau pathology such as DS6 did not show similar plaque pathology at this time point (Figure 4A). Co-staining with GFAP and AT8 indicated that these accumulations consist of phospho-tau within or directly adjacent to astrocytes as is typical of astrocytic plaques (Figure 4B) (Yoshida, 2014). DS12, DS15, DS16, and DS18 induced a small degree of astrocytic plaque-like pathology at 8 weeks (Figures S4A and S4B). Given these findings, we retrospectively quantified the number of animals with astrocytic plaque-like pathology after completion of the time course injection experiment described below. DS7 or DS9 inoculations induced astrocytic plaque-like pathology in the majority of inoculated mice by 8 weeks (Figure S4A). In contrast, DS4 and DS6 induced limited plaque pathology by 12 weeks, which was far less robust than the level observed in DS7 and DS9 inoculated animals (Figures S4A and S4C). Thus, this phenotype is likely independent of seeding activity and suggests specific tau conformations preferentially and predictably induce astrocytic tau pathology.

While two CBD-derived strains showed a small degree of astrocytic plaque-like pathology (DS12 and DS16), they were sparse at this time point (Figures S4A and S4B). We examined mice inoculated with DS12 and DS16 at 6 months after injection and observed increased levels of astrocytic pathology (Figure S4D). This suggests these two CBD-derived strains produce robust astrocytic plaque pathology after an extended incubation period compared to DS7 or DS9. DS11 and DS13, which were also isolated from CBD patients, did not induce astrocytic plaque pathology 8 weeks after injection. Individual CBD patients likely have multiple tau strains present in their brains (Sanders et al., 2014), which may give rise to the overall pattern of histopathology observed in patients. Alternatively, DS11 and DS13 may simply require more time to produce robust astrocytic plaque pathology in this mouse model, as was observed for DS12 and DS16 (Figures S4B and S4D).

Regional Vulnerability to Specific Strains

Tauopathies feature accumulation of tau pathology in distinct brain regions (Arnold et al., 2013), yet the mechanisms that un-

derlie these patterns are not well understood. To test whether strains differentially induce pathology in specific brain regions, we inoculated DS1, DS4, DS6, DS7, DS9, DS10, or DS11 into six locations per mouse: sensory cortex (SC), caudate/putamen (CP), visual cortex (VC), hippocampus (Hip), thalamus (Thal), and inferior colliculus (IC) (Figure 5A). We chose these strains based on their unique limited proteolysis patterns, different tau pathology induced in the hippocampus, and their low (DS7 and DS11), medium (DS4 and DS10), or high (DS6 and DS9) seeding activity in culture. Further, DS4 and DS11 derive from AD and CBD brain homogenates, respectively. Patients with these diseases have different patterns of tau deposition (Arnold et al., 2013).

After 5 weeks, we quantified the level of AT8 pathology these strains induced at each injection site in a blinded fashion (Figure 5B). All produced hippocampal pathology consistent with the previous injection paradigm, illustrating the reproducibility of these phenotypes (Figure 5C). Strains with the strongest seeding activity in culture (DS6 and DS9) produced pathology in every injected region. DS4, a medium-seeding strain, induced moderate pathology in each region except the IC. DS11 pathology was entirely limited to the hippocampus (Figures 5B and 5C).

DS10 again induced pathology specific to the mossy fiber tracts of the hippocampus (Figure S5A), with limited pathology in the caudate/putamen and thalamus. Of note, it did not produce any pathology in the injected cortical regions. In contrast, DS7 produced limited AT8 pathology in each targeted brain region (Figures 5B and 5C). The specificity of DS10, despite its strong seeding activity, and promiscuity of DS7, despite its weak seeding activity (Figures 1D–1G), were remarkable. These studies indicate tropism of certain strains for specific brain regions (mossy fiber tracts, cortical structures, and IC) that is independent of simple metrics such as seeding activity.

Strains Induce Different Rates of Spread of Tau Pathology along Neuronal Networks

Even within a single clinical syndrome, tauopathy patients experience rapid or slow rates of progression (Armstrong et al., 2014; Thalhauser and Komarova, 2012). PrP strains show different lag phases and rates of neurodegeneration in animal models of prion diseases (Collinge and Clarke, 2007), suggesting this phenomenon may be linked to specific aggregate conformations. While several factors may contribute to the rate of degeneration observed in tauopathy patients, rapid spread of tau pathology likely accelerates this process. Thus, we tested the relationship between strain characteristics and rates of spread of tau pathology.

To control for differences in insoluble material, we first quantified the insoluble tau present in lysate from DS1, DS4, DS6, DS7, DS9, and DS10 prepared for this time course experiment. As

Figure 4. Specific Strains Induce Astrocytic Tau Pathology

(A) AT8 tau pathology 8 weeks after injection with DS1, DS6, DS7, or DS9. DS1 does not induce tau pathology. DS6, DS7, and DS9 develop strong AT8 staining in ipsilateral and contralateral hippocampi. DS7 and DS9 develop diffuse, circular-shaped accumulations of AT8 staining that do not appear to localize to a neuronal cell body (black arrow heads). The scale bars represent 250 μ m for the whole hippocampus and 50 μ m for CA1.

(B) Co-staining of AT8 (green) for phospho-tau, GFAP (red) for astrocytes, and DAPI (blue) for cell nuclei. DS1 shows limited GFAP staining and no AT8 pathology. DS6 shows strong AT8 staining with limited overlap of AT8 staining. DS7 and DS9 injected mice display astrocytic plaque-like pathology that either deposits within or around GFAP-positive processes of astrocytes. The scale bars represent 25 μ m for left column and 10 μ m for all remaining images. For further quantification and representative images of other strains that display limited astrocytic plaque pathology, see Figure S4.

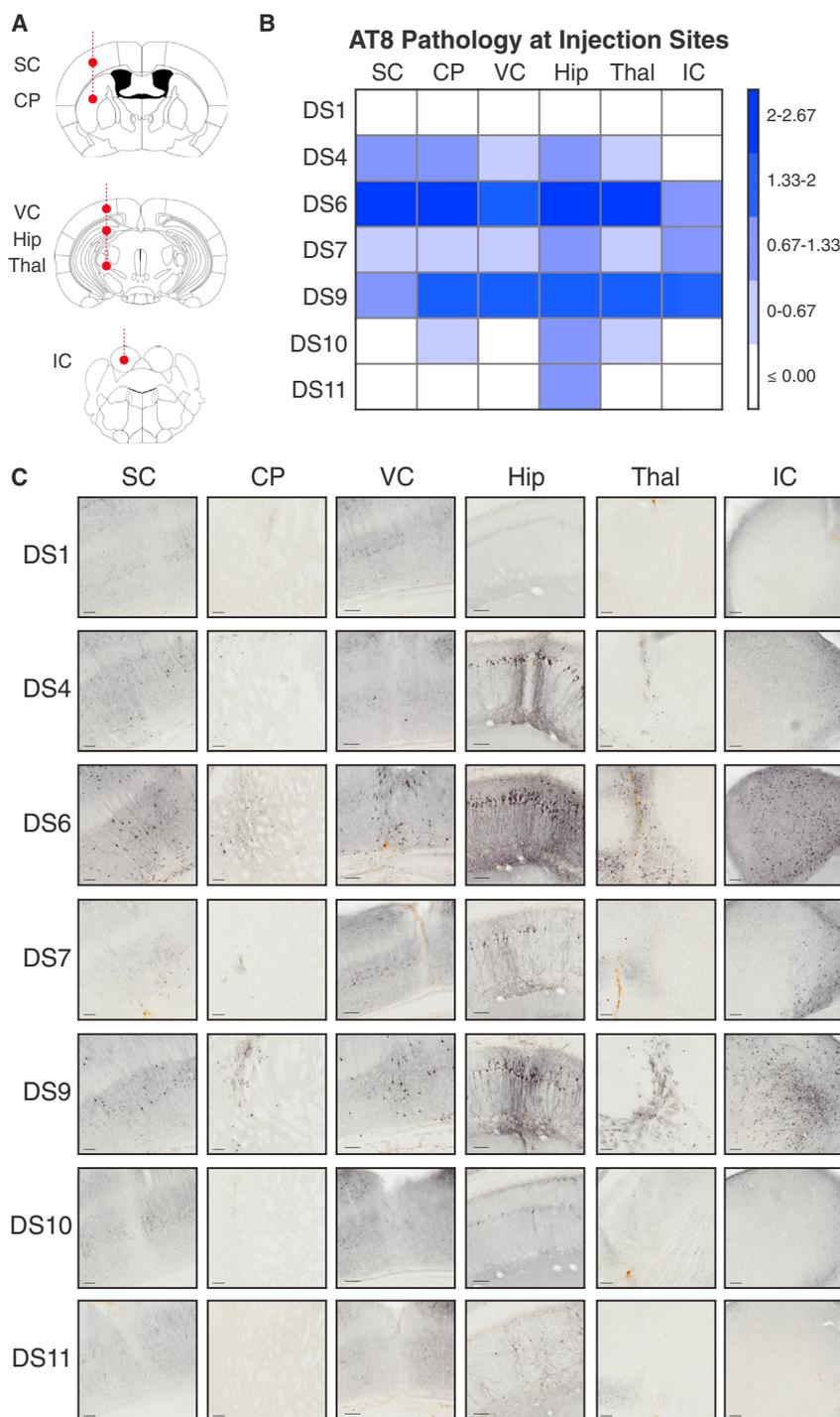


Figure 5. Tau Strains Preferentially Seed Pathology in Specific Brain Regions

(A) Six tau strains were injected simultaneously into six brain regions: SC; CP; VC; Hip; Thal; and IC ($5 \mu\text{g}$ per region). The mice that received DS1 (negative control), DS4, DS6, DS7, DS9, DS10, or DS11 strain injections were kept for 5 weeks post-inoculation before assessment of AT8 tau pathology ($n = 3$ per condition).

(B) Strains preferentially induced tau pathology in specific brain regions. Slices that contained the injection sites were stained for AT8 phospho-tau. Each injection site was assessed in a blinded fashion for tau pathology on a 0–3 scale (none, low, medium, and high). The level of background AT8 pathology at each injection site was accounted for by subtracting the level of pathology present in DS1 mice within each brain region. A binned heatmap represents the level of pathology observed at the injection site for each strain. Note differences in regional vulnerability.

(C) Representative images are displayed for each brain region injected with the different tau strains. The scale bars represent $100 \mu\text{m}$. DS10 mossy fiber pathology is shown in [Figure S5A](#).

levels of insoluble tau. To test this hypothesis, we included DS6 and DS9 lysate diluted 1:10 as part of this time course experiment.

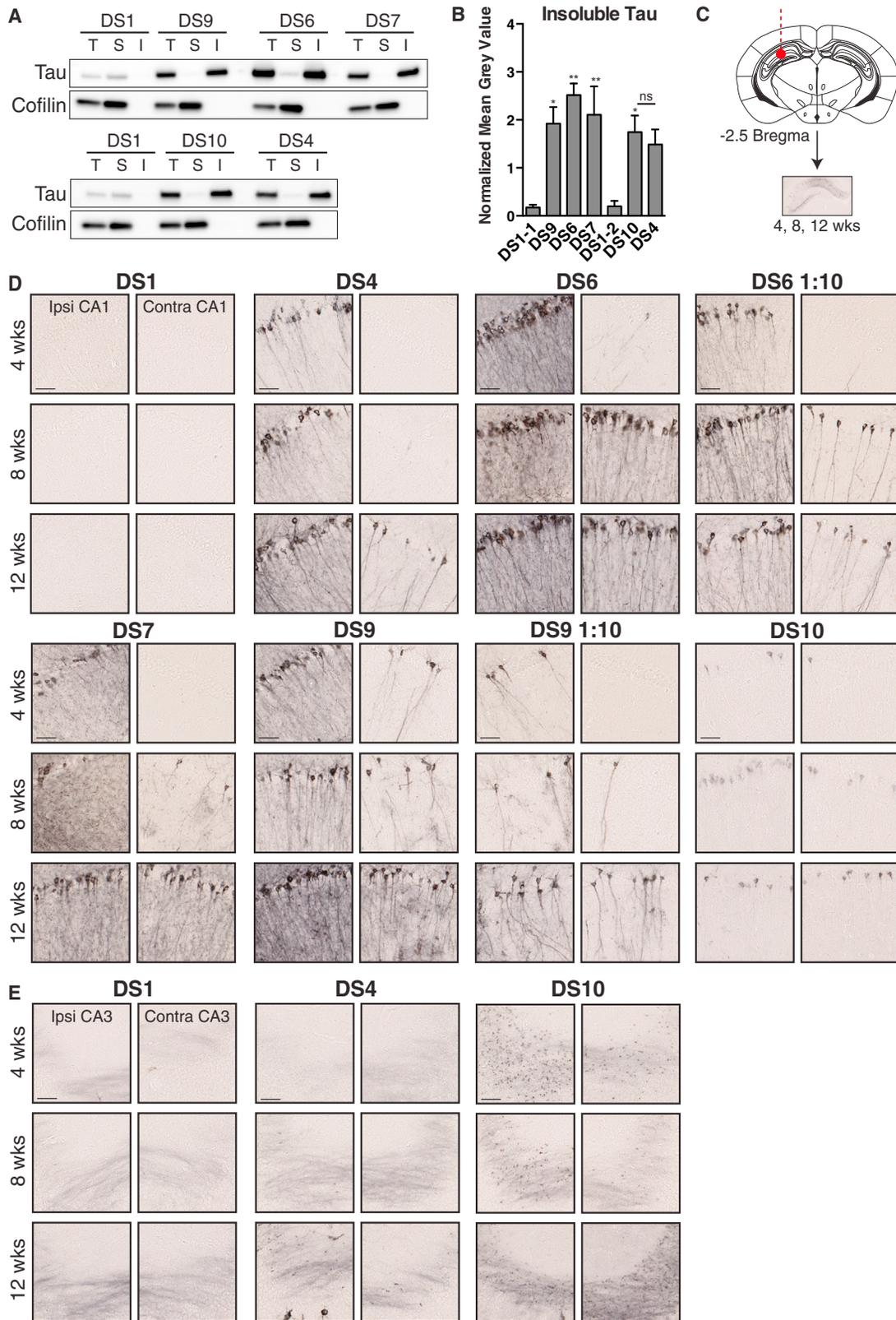
We injected cell lysate from each condition into the hippocampus of young PS19 mice and collected brains at 4, 8, and 12 weeks post-injection ([Figure 6C](#)). While each strain induced tau pathology in the contralateral hippocampus, this occurred at different time points ([Figures 6D and 6E](#)). DS6 and DS9 pathology progressed rapidly to the contralateral hippocampus, beginning as early as 4 weeks ([Figure 6D](#)). DS10 induced mossy fiber dots and limited CA1 pathology in the ipsilateral and contralateral hippocampus by 8 weeks ([Figures 6D and 6E](#)). DS7 wisp pathology also spread to the contralateral hippocampus by 8 weeks ([Figure 6D](#)).

While dilution of DS6 and DS9 decreased the initial level of pathology induced upon inoculation, we observed tau pathology in the contralateral hippocampus at 8 weeks. In contrast, DS4 did not show tau pathology in the contralateral

hippocampus until 12 weeks ([Figures 6D and 6E](#)). Thus, stronger strains induced more rapid spread of pathology even with a reduced amount of insoluble tau inoculum, presumably due to more efficient seeding and spread of endogenous tau aggregates.

While these inoculations induced robust neuronal tau pathology and strain-specific patterns of astrocytic tau pathology, we

expected, each strain contained a large amount of insoluble tau ([Figures 6A and 6B](#)). We hypothesized that a strain's ability to seed aggregation of endogenously expressed monomeric tau would primarily determine the rate of spread of pathology. We predicted that strains such as DS6 and DS9 with high seeding activity in culture and in vivo ([Figures 1D, 5B, and 5C](#)) would produce rapid spread of pathology even after inoculation of reduced



(legend on next page)

did not observe overt neuronal loss at these time points (data not shown). However, to begin to assess the functional effects of distinct strains, we examined the patterns of microglial pathology induced at 12 weeks after inoculation. Compared to DS1 inoculated mice, several strains displayed Iba1-positive rod microglial phenotype, with elongated projections that align along axons of CA1 pyramidal neurons (Figures S6A–S6C). We performed blinded quantification of ramified and rod microglia ipsilateral and contralateral to the site of inoculation. DS6 induced the largest degree of rod microgliosis, while DS7 produced very limited rod microglial pathology. DS4, DS9, and DS10 also produced rod microglia, but we observed this phenotype in the contralateral hippocampus primarily in DS6 and DS10 inoculated mice.

To test the stability of tau strains *in vivo*, we transduced brain homogenate from the hippocampus of mice at 8 weeks after inoculation into the naive DS1 cell line. DS4, DS6, DS9, and DS10 produced morphologies consistent with the original inoculum (Figures S2F–S2H and S7D). However, brain-derived DS7 produced a mixture of cellular morphologies. This is in contrast to DS7 cell lysate, which stably induces its cellular phenotype in culture, suggesting DS7 may imperfectly template its conformation onto full-length P301S tau in this mouse model.

We isolated monoclonal lines derived from inoculated mouse tissue by transducing brain homogenate into the DS1 line and sorting single aggregate-containing cells. Tau derived from mice inoculated with DS6 strain readily produced thread-containing inclusions in the population of converted cells (Figures S2F and S7D). However, isolation of monoclonal lines was not possible due to toxicity (0/36 individual colonies survived). In contrast, transduction of mouse-derived tissue inoculated with DS7 produced few inclusions in the population overall, with only one resultant monoclonal line. This is consistent with the low seeding activity of the original DS7 line. Multiple secondary lines were derived from brains originally inoculated with DS4, DS9, and DS10 (Figure S7B). FRET-based seeding activity of the secondary cell lines resembled that of the original lines (Figure S7C). Thus, several of these strains stably propagate their phenotype even upon passage through mice.

To assess AT8 pathology induced by each strain, we performed a blinded analysis of AT8 staining in slices at the level

of the locus coeruleus, hippocampal injection site, and caudate/putamen. We averaged AT8 pathology rankings for each region and displayed them as a heatmap to visualize the spread of tau pathology (Figure 7A). We subsequently created a limited heatmap for each strain that focuses on specific brain regions, several of which developed pathology over time (Figure 7B). DS10 once again displayed marked neuronal specificity, with strong pathology only in the mossy fiber tracts (Figures 7A and 7B). Blinded analysis also confirmed that DS4 exhibits slower kinetics than DS6 or DS9 even when the latter strains are diluted 10-fold (Figure 7B). DS4 did not develop strong pathology outside the ipsilateral hippocampus until 12 weeks after injection, while the stronger strains showed AT8 staining in distant brain regions by 8 weeks. DS7 induced robust pathology at the injection site as observed previously (Figures 3 and 5). However, the spread of DS7 pathology was relatively slow and appeared limited to the hippocampus (Figures 7A and 7B).

DS6 and 9 spread pathology rapidly to specific brain regions after 4 weeks. DS6 strongly targeted the ipsilateral retrosplenial cortex and LC, while DS9 pathology spread most strongly to the ipsilateral entorhinal cortex and thalamus. Despite these initial differences, by 8 weeks the patterns of pathology induced by these strains largely resembled one another (Figure 7B). While diluted lysate of DS6 and DS9 induced lower levels of pathology at early time points, they induced pathology that spread faster and farther than DS4, DS7, or DS10 and followed similar patterns to that of undiluted DS6 and DS9 lysate (Figure 7B).

We next performed a seeding assay on tissue from the ipsilateral and contralateral hippocampus, thalamus, and sensory cortex at 8 weeks after injection as a second metric of tau pathology. We transduced homogenized brain regions into the FRET biosensor cell line (Holmes et al., 2014) and quantified seeding after 2 days in culture. We have observed that all seeding activity measured after inoculation of DS9 into the hippocampus of tau knockout mice completely dissipates by approximately 6 weeks (data not shown), which suggests any signal identified at this time point likely derives from induced aggregation of endogenous tau expressed in this mouse line.

Several strains displayed robust seeding in the ipsilateral hippocampus (DS4, DS6, DS9, DS10, and 1:10 diluted DS6 and

Figure 6. Strains Induce Different Rates of Tau Pathology Spread

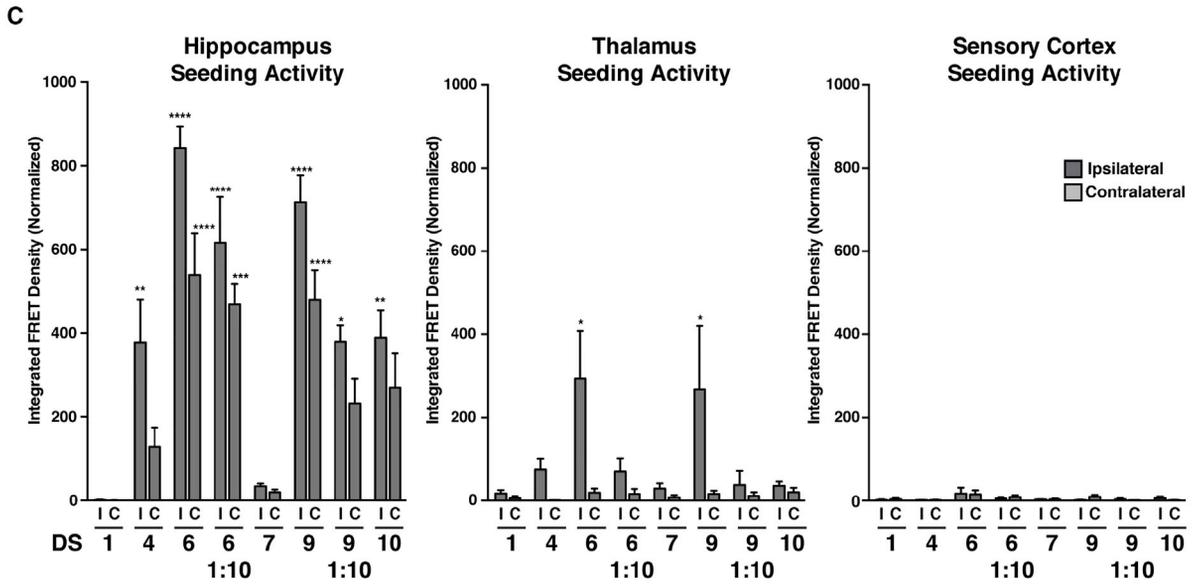
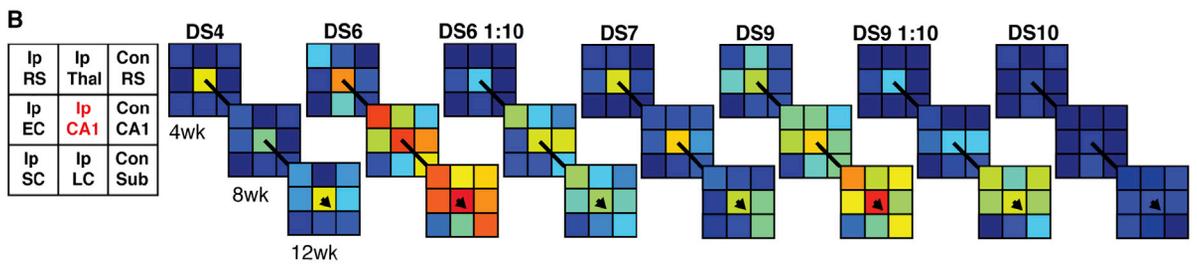
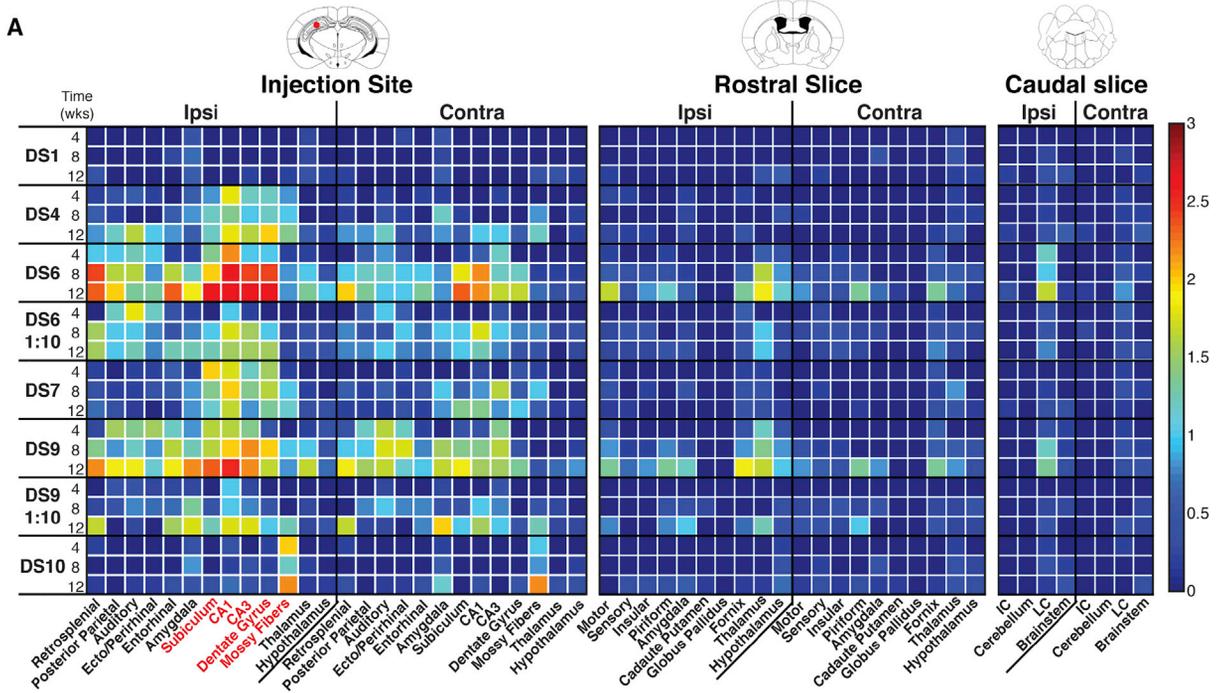
(A) Sedimentation analysis was performed on cell lysate used for the time course inoculation experiment. Each strain contains a large amount of insoluble material (total, T; soluble, S; and insoluble, I). Western blot analysis of insoluble tau was performed on three biological replicates. For each experiment, the soluble fraction was loaded at 2× the concentration of the total and insoluble fractions. A cofilin loading control was performed on the blots to verify the same amount of cell lysate was added for each strain.

(B) The level of insoluble tau present in each strain was quantified by measuring the mean gray value of the insoluble tau western blot band. The samples were normalized to the mean gray value of cofilin in the total cell lysate fraction. DS1-1 and DS1-2 represent biological replicates of DS1. ANOVA shows strains have significantly more insoluble tau than DS1. A two-way t test demonstrates DS10 and DS4 do not contain significantly different levels of insoluble tau (ns for $p > 0.5$; * for $p \leq 0.05$; and ** for $p \leq 0.01$). The error bars represent SEM of biological triplicates.

(C) Strains were inoculated into the hippocampus of young PS19 mice ($n = 5$ – 6 per condition per time point, see Table S1). DS6 and DS9 lysate diluted 1:10 in HEK293 cell lysate were also injected ($n = 4$ – 5 per condition per time point). The mice were collected at 4, 8, or 12 weeks.

(D) Representative images of ipsilateral and contralateral CA1 are displayed for each strain at 4, 8, and 12 weeks post-injection. AT8-positive tau pathology spreads to the contralateral hippocampus at different time points. The diluted DS6 and DS9 lysate show faster spread than concentrated DS4 and more robust spread than DS7 and DS10 at 8 weeks post-injection. The scale bars represent 50 μm . See Figure S6 for data regarding strain-specific rod microglial phenotype present at 12 weeks after inoculation.

(E) Spread of mossy fiber dot pathology occurs by 8 weeks in DS10 mice. The dot pathology appears eventually to develop in DS4 mice, but spread appears delayed compared to DS10.



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DS9). While each of these strains spread to the contralateral hippocampus, DS4 showed distinctly less contralateral seeding activity as a percentage of the ipsilateral hippocampus (Figure 7C). Further, DS6 and DS9 showed variable yet significant seeding activity in the ipsilateral thalamus. The level of seeding observed in DS1 inoculated mice is consistent with the level observed in this mouse model at 4–5 months of age, suggesting this signal is due to spontaneous aggregation of endogenous tau that normally occurs within this model (Holmes et al., 2014). DS7 showed remarkably low seeding activity, while DS10 induced relatively robust seeding from the hippocampus despite only inducing mossy fiber AT8 tau pathology (Figures 6E and 7C). This is consistent with the seeding activity for the original inoculum as observed by the split-luciferase assay (Figures 1D and 2G–2I). These results are also consistent with the different rates of spread observed by AT8 immunohistochemistry for DS4 versus DS6 and DS9, while providing a separate metric to quantify the spread of pathology for strains such as DS10.

DISCUSSION

Overview of Findings

We have tested whether specific tau prion strains can account for critical neuropathological features that are used to discriminate tauopathies. We characterized strains by various biochemical and biological metrics, including inclusion morphology, seeding activity in dividing cells and primary neurons, detergent solubility, cellular toxicity, limited proteolysis, and reintroduction into reporter cells. We inoculated all 18 strains individually into the hippocampi of a transgenic tauopathy mouse model, causing distinct patterns of tau pathology in cell bodies, axons, and dendrites. To test for regional vulnerability, we injected six strains into six different brain regions and waited 5 weeks to evaluate pathology. Many induced pathology in all regions (DS6, DS7, and DS9), while others exhibited restricted patterns in which very little or no pathology occurred despite direct exposure of neurons to a particular strain (DS4, DS10, and DS11). We next tested whether strains exhibited unique rates of propagation through the brain. In this case, we observed correlations with *in vitro* parameters: strains with high seeding activity tended to spread more rapidly through the brain, with one important exception—strain DS10—which only spread to the contralateral mossy fiber tracts of the hippocampus. These observations suggest that distinct tau prion strains could

account for many of the features observed in human tauopathies.

Limitations of this Experimental Approach

It is impossible at this time to directly control tau strain production *in vivo* using transgenic mice or to propagate strains faithfully and indefinitely *in vitro* without using cultured cells. This work relies on a cell model that expresses a truncated form of tau with a fluorescent protein tag, an imperfect system that has nonetheless allowed us to propagate distinct tau prion strains indefinitely. Likewise, in mice, we utilized a model that expresses full-length human tau (1N4R) that contains a disease-associated mutation (P301S) (Yoshiyama et al., 2007). While this obviously deviates from sporadic tauopathy that occurs in most individuals, it has enabled us to rapidly and reliably induce unique tau pathology based on local inoculation. We cannot exclude the possibility that inoculated tau prions themselves are moving throughout the brain and inducing pathology based on local uptake rather than true trans-neuronal propagation (Rey et al., 2013). Nonetheless, our observations suggest a fundamental disease mechanism whereby strain-specific differences govern seeding, propagation, and specific regional vulnerability.

The Utility of Studying Isolated Strains

Our prior work has indicated that human tauopathy brains, even those carefully defined by histopathology, exhibit enormously diverse strain content within and between individuals (Sanders et al., 2014). Thus syndromes that appear to be clinically and neuropathologically identical are potentially quite distinct in terms of their strain composition. This presents obvious challenges when attempting to define strains present in human disease based principally on inoculation of purified aggregates into mice. Fibril preparations created *in vitro* also exhibit tremendous conformational heterogeneity, as clearly illustrated by studies of α -synuclein in which one dominant fibril structure shifts to another upon serial seeding reactions (Guo et al., 2013). In this work, we have stably propagated strains with specific biochemical properties in a simple culture system based on expression of tau RD-YFP. While technical limitations have restricted our ability to define the specific structures of tau aggregates present in these strains, this work suggests a single dominant structure is faithfully propagated in each line. This has enabled us to make predictions about phenotypes *in vivo* based on properties observed *in vitro*.

Figure 7. Strain Dictates the Rate and Pattern of Spread of Tau Pathology

(A) Slices from mice injected with each strain at each time point were stained for AT8 pathology. Tau pathology was quantified in a blinded fashion on a 0–3 scale and averaged for each location within a given condition ($n = 5–6$ per condition). A continuous heatmap was generated. Note differential rates of spread and regional vulnerability. The regions are listed on the x axis and conditions/time points are on the y axis.

(B) Limited heatmaps were generated from the above data set (Figure 7A). Ipsilateral (Ip) and contralateral (Con) regions were included to assess patterns and rates of spread of pathology (retrosplenial cortex, RS; EC; SC; Thal; CA1 of hippocampus, CA1; locus coeruleus, LC; and subiculum, Sub). Time points are arranged in order from earliest (4 weeks) to latest (12 weeks). Diluted DS6 and DS9 lysates are also displayed (DS6 1:10 and DS9 1:10).

(C) Homogenized tissue from the hippocampus, thalamus, or sensory cortex of mice 8 weeks after inoculation with strains was applied to tau biosensor cell lines. After 48 hr, cells were collected and flow cytometry was performed to quantify the level of seeding activity in each region by integrated FRET density (percent FRET-positive cells*median fluorescent intensity of FRET positive cells = IFD) (Holmes et al., 2014). DS4 induces lower spread of seeding activity to the contralateral hippocampus at 8 weeks. DS10 induces high seeding activity despite limited AT8 pathology, while DS7 induces low seeding activity despite high AT8 pathology. DS6 and DS9 also induce seeding activity in the ipsilateral thalamus. A one-way ANOVA with Bonferroni correction for multiple comparisons was performed between ipsilateral DS1 and every other sample within a given region (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; and **** $p \leq 0.0001$). The error bars represent SEM, $n = 4–5$. See Figure S7 for data regarding secondary cell line isolation of strains derived from inoculated mice or strain cell lysate.

Prion Strains Characterized In Vitro

PrP prion strains have distinct, yet reproducible patterns of incubation time, neuropathology, and behavioral phenotypes (Collinge and Clarke, 2007). Strains are presumably distinct amyloid structures that faithfully replicate in a living system and produce well-defined pathology. Thus, if a strain is identified, it is possible to predict incubation time and resultant pathology (Collinge and Clarke, 2007). In this work, we characterized multiple tau strains from recombinant, mouse, and patient sources in vitro and in vivo. Their detailed characterization in vitro using several metrics (inclusion morphology, solubility, seeding efficiency, and limited proteolysis) allowed us to make predictions regarding their effects in vivo. For instance, DS6 and DS9 induced robust spread of tau pathology even upon dilution, which was predicted by in vitro seeding activity. Importantly, while seeding activity correlated best with induction of local and distant pathology, this is an imperfect metric. For example, DS10 inoculation produces very limited pathology in vivo despite its relatively strong seeding activity in vitro. This indicates a major influence of other as yet unidentified strain-specific parameters. With further, more detailed study, we hope to link specific structural characteristics to various steps in pathogenesis, i.e., to define the “logic” that predicts biological effects. For instance, cell-type specificity (or at least preference) might be based on differential strain binding affinities to heparan sulfate proteoglycans (Holmes et al., 2013). Likewise, post-translational modifications of monomeric tau within a target cell might render it more or less vulnerable to conversion by a specific strain.

Distinct Cellular Pathologies In Vivo

Tauopathies are defined histopathologically by several criteria, especially the pattern of intracellular tau accumulation: neurofibrillary tangles, Pick bodies, threads, grains, axonal puncta, etc. (Kovacs, 2015). Although we have readily observed patterns of pathology reminiscent of those described in patients, we have not attempted to link human patterns of pathology to those in P301S mice, which express only a single isoform of mutant tau. Instead, we wish to emphasize how conformational differences in tau prion strains are sufficient to create an enormous pathological diversity: neurofibrillary tangles, soma versus axonal accumulation, grain-like structures, dendritic and axonal terminal deposits that resemble threads, and astrocytic plaques.

The presence or absence of specific glial pathology also contributes to the definition of tauopathies (Yoshida, 2014). We observed that certain strains produce AT8-positive pathology in patterns reminiscent of astrocytic plaques described in tauopathies, with localization of phospho-tau inclusions along the processes of GFAP-positive astrocytes (Yoshida, 2014). We cannot attribute these effects to tau seeding activity or toxicity, as we observed these phenomena in DS7 (low seeding and low toxicity) and DS9 (high seeding and high toxicity). Distinct strains also induced different levels of rod microglial pathology.

Given that inoculates were identical with the exception of tau structure, and the transgenic mouse model expresses only a single tau isoform, we conclude that tau prion strains them-

selves dictate the resultant glial pathology. Further, three strain pairs (DS3/DS19; DS12/DS16; and DS6/DS15) that displayed similar biochemical features and limited proteolysis patterns produced similar patterns of neuropathology in vivo. These strains served to internally validate the methods we developed and highlight the close relationship of strain type to induced pathology.

Rates of Propagation In Vivo

Neurodegenerative diseases progress at different rates for unknown reasons. Similar to previous observations regarding PrP prion strains (Legname et al., 2006), our data indicate that the characteristics of an individual tau prion strain are sufficient to dictate the rate at which pathology spreads throughout the nervous system. Seeding activity correlates with this phenomenon, but cannot completely explain it. Instead, the rate of spread appears to reflect a unique interaction of specific strains with vulnerable cells. For example, strain DS10 seeds very strongly in vitro, but fails to propagate pathology outside of the hippocampal mossy fibers. Likewise, DS6 seeds very strongly in vitro, but exhibits a longer lag phase as it spreads to specific brain regions such as the entorhinal cortex versus the retrosplenial cortex. In contrast, DS9 showed more rapid spread to the entorhinal than the retrosplenial cortex. Taken together, our results suggest that the rate of propagation must be strongly influenced by raw seeding potential, i.e., the ability to convert monomeric tau upon direct introduction to the cytoplasm via lipofectamine, but also the ability of a given strain to spontaneously enter and replicate within a vulnerable cell.

Distinct Regional Vulnerabilities

Regional or “selective” neuronal vulnerability in neurodegenerative diseases has long mystified investigators. PrP prion strains appear to account for differential regional involvement of the brain (Collinge and Clarke, 2007). Within the limits of our experimental system, our identification and characterization of distinct tau strains has allowed us to test whether aggregate structure itself defines regional vulnerability. We have found striking strain-specific regional differences, both in the pattern of spread from a single hippocampal inoculum and also in vulnerability to a direct injection. These effects correlated with individual tau strains, independent of an inoculum dose that could have accounted for vulnerability. For example, 10-fold dilution of a potent strain, DS9, produced patterns of spreading pathology very similar to a full dose. Further, low-dose DS9 spread at a faster rate than a full dose of DS4, even though the initial “seed burden” of DS9 was less. By contrast, strain DS10 has relatively high seeding activity, yet it selectively targets the mossy fibers of the hippocampus and does not convert tau in several other brain regions even after direct inoculation.

Others previously observed that different tau fibril preparations produce unique patterns of pathology based on inoculation of tau purified from tauopathy brains or crude homogenates (Boluda et al., 2015; Clavaguera et al., 2013), and two groups have inoculated unique α -synuclein preparations (Bousset et al., 2013; Guo et al., 2013; Peelaerts et al., 2015). However, no prior studies can attribute these effects to a specific, well-characterized strain or structure and make predictions about

the behavior of a strain in vivo from the biochemical properties of that inoculum. Given that we can now link specific pathology patterns to single tau prion strains, the experiments described here should enable new approaches to define how the structural characteristics of prions dictate neuronal vulnerability.

Implications for Diagnosis and Therapy

We propose that tau prion strains will explain the diversity of human neuropathology and will be required for mechanistic understanding of disease. Precise diagnosis of tauopathy now depends on histopathology, yet we have observed different strain composition patterns in putatively identical pathological syndromes (Sanders et al., 2014). Further, diversity and evolution may confound efforts to target a specific strain, as for PrP (Giles et al., 2010; Weissmann et al., 2011). Tau prion strains as defined by their specific conformations should have enormous power to help elucidate the structural determinants that underlie and predict the pathological patterns of diverse human tauopathies and to devise appropriate therapies.

EXPERIMENTAL PROCEDURES

Generation of Monoclonal Strain Library

Cells stably expressing tau RD(P301L/V337M) fused to YFP (DS1) were treated with recombinant tau fibrils or cell/brain homogenate. Resultant monoclonal lines were isolated and analyzed for inclusion morphology, seeding by split luciferase assay, and protease digestion as previously described (Sanders et al., 2014). These analyses identified 18 putatively distinct strains (DS2–DS19).

Limited Proteolysis

Cell lysates (60 μ g) in PBS/Triton X-100 were digested with pronase (30 μ g/mL) for 1 hr at 37°C, then resolved by SDS-PAGE and western blot probed with primary anti-tau antibody 2B11.

Sedimentation Analysis of Strain Library

Cell lysates were spun at 186,000 $\times g$ for 60 min. Pellets were washed with 1 mL lysis buffer and spun for an additional 30 min. Samples were resolved by SDS-PAGE and western blot using rabbit polyclonal anti-tau antibody ab64193 (Abcam).

Toxicity and Seeding Assay

LM10 cells, a monoclonal cell line expressing high levels of tau RD(P301L/V337M)-CFP and tau RD(P301L/V337M)-YFP, were transduced with 20 μ g of clarified cell lysate. After 72 hr, FRET-positive cells were sorted by FACS (Holmes et al., 2014) and replated. After 7 days, the number of FRET-positive and FRET-negative cells was quantified by flow cytometry.

Animal Maintenance and Inoculation Experiments

PS19 mice expressing 4R1N P301S human tau under the murine prion promoter (Yoshiyama et al., 2007) were used. Strains were inoculated intracerebrally via 10 μ L gas-tight Hamilton syringes. All experiments involving animals were approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee.

Histology and Quantification of AT8 Pathology

50 μ m frozen sections were used. For DAB stain, biotinylated AT8 primary antibody (Thermo Scientific) was used. Images of AT8-stained slices were collected via Olympus Nanoscope 2.0-HT (Hamamatsu). The level of tau pathology present in each region was determined by blinded analysis with a semiquantitative 0–3 scale (no pathology, mild, moderate, and severe). Pathology was averaged among biological replicates and plotted as a heatmap using MATLAB as described.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures, and three tables and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2016.09.055>.

AUTHOR CONTRIBUTIONS

S.K.K. designed and performed all in vivo and primary neuron experiments, inoculum sedimentation analysis, secondary cell line isolation and characterization, and brain immunohistochemistry. D.W.S. designed and performed all in vitro cell culture and biochemistry experiments to characterize DS1–DS19. Both authors assisted in data analysis, writing, and figure preparation for this manuscript. T.L.T. provided technical assistance with stereotaxic injections, tissue collection, sectioning, and immunohistochemistry. A.R. conducted blinded scoring of AT8 pathology and microglial pathology. A.M.S. performed limited proteolysis experiments. J.V.-A. assisted with development of primary neuron culture experiments and MATLAB analysis of pathology. T.M.M. provided guidance and reagents for in vivo experiments. M.I.D. provided guidance for in vitro and in vivo experiments and assisted in the writing and preparation of figures for this manuscript.

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