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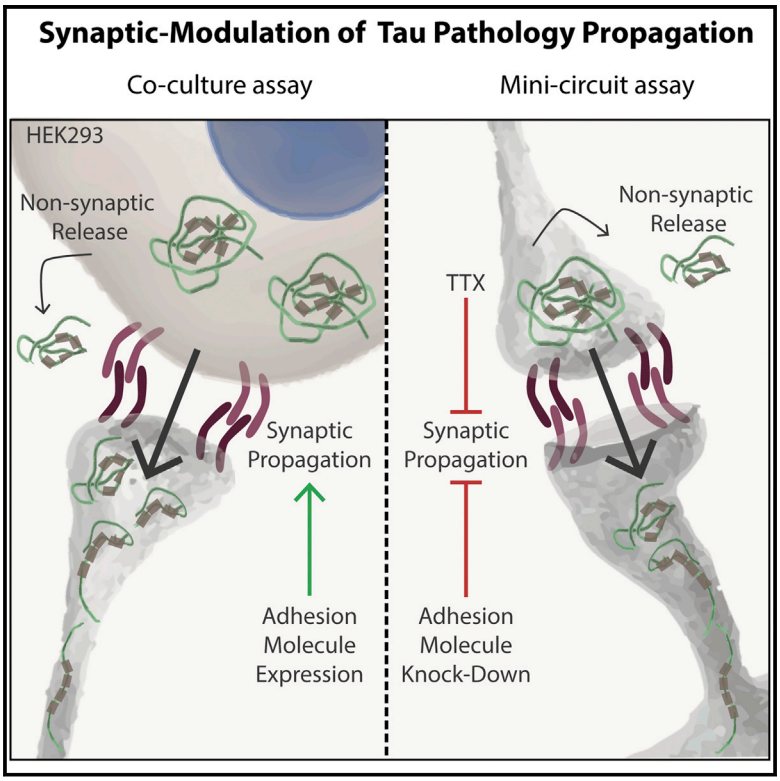
Synaptic Contacts Enhance Cell-to-Cell Tau Pathology Propagation

Graphical Abstract



Highlights

- Synaptic and non-synaptic mechanisms each contribute to tau pathology spreading
- Synaptic contacts, but not mere close proximity, enhance tau pathology spreading
- Quantitative in vitro cellular synaptic systems allow modeling of tau spreading
- Synaptic cell adhesion proteins and neuronal activity promote tau spreading

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In Brief

Tau dysfunction causes neurodegeneration, and tau pathology spreading appears to occur along synaptically connected neurons. However, it is not known whether the actual presence of synapses enhances spreading. Using artificial neuronal circuits in vitro, Calafate et al. show that synaptic contacts promote spreading and that synaptic and non-synaptic mechanisms act in parallel.

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Synaptic Contacts Enhance Cell-to-Cell Tau Pathology Propagation

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SUMMARY

Accumulation of insoluble Tau protein aggregates and stereotypical propagation of Tau pathology through the brain are common hallmarks of tauopathies, including Alzheimer's disease (AD). Propagation of Tau pathology appears to occur along connected neurons, but whether synaptic contacts between neurons are facilitating propagation has not been demonstrated. Using quantitative in vitro models, we demonstrate that, in parallel to non-synaptic mechanisms, synapses, but not merely the close distance between the cells, enhance the propagation of Tau pathology between acceptor hippocampal neurons and Tau donor cells. Similarly, in an artificial neuronal network using microfluidic devices, synapses and synaptic activity are promoting neuronal Tau pathology propagation in parallel to the non-synaptic mechanisms. Our work indicates that the physical presence of synaptic contacts between neurons facilitate Tau pathology propagation. These findings can have implications for synaptic repair therapies, which may turn out to have adverse effects by promoting propagation of Tau pathology.

INTRODUCTION

Tauopathies are a diverse group of neurodegenerative diseases defined by the accumulation of misfolded cytosolic aggregates of the microtubule-associated protein Tau. Alzheimer's disease (AD) is the most common tauopathy (Holtzman et al., 2011). Tau aggregates first appear in the locus coeruleus and gradually propagate via the entorhinal cortex to hippocampus and the neocortex. This typical temporo-spatial progression of Tau pathology provides the basis for the classical Braak disease staging in AD (Braak and Del Tredici, 2011, 2012; Jucker and Walker, 2013). Recent evidence suggests that the progression of Tau pathology is determined by intrinsic characteristics and vulnerability of anatomically connected neurons (Hardy and Revesz, 2012), supporting the idea that intracellular Tau aggregates are released from the originating neuron and taken up by a neighboring connected neuron. These observations indicate that neuronal synaptic contacts may be involved in Tau pathology propagation (Brettschneider et al., 2015), but this idea has not been experimentally addressed.

In vitro studies show that neuronal activity can modulate Tau protein secretion into the extracellular space (Pooler et al., 2013; Yamada et al., 2014). Extracellular Tau aggregates can directly penetrate adjacent cell membranes or can be internalized via fluid-phase endocytosis or receptor-mediated endocytosis (Clavaguera et al., 2009; Frost et al., 2009; Guo and Lee, 2011, 2013; Holmes et al., 2013). In addition, recombinant preformed synthetic Tau fibrils move anterogradely and retrogradely within a neuron (Scholz and Mandelkow, 2014; Wu et al., 2013). A recent in vivo study provides further support for the concept that Tau pathology progression involves circuit-based transfer of Tau aggregates (de Calignon et al., 2012; Liu et al., 2012). However, it remains to be determined whether Tau pathology propagation is a consequence of cell damage or is mediated by synaptic regulated mechanisms.

Here, we use in vitro models where Tau pathology propagates from a donor clonal cell line that persistently harbors Tau aggregates to acceptor hippocampal neurons. We find that this transfer is significantly potentiated when we induce the formation of presynaptic contacts from neurons onto the clonal cells. Furthermore, by generating artificial neuronal networks in microfluidic devices that allow separation of the somatodendritic compartments from the axonal compartments, we find that synaptic contacts and neuronal activity potentiate neuron-to-neuron propagation of Tau pathology.

RESULTS

HEK293 Cells Persistently Form Tau Aggregates

To study the propagation of Tau pathology in a cellular system, we developed a clonal cell-to-neuron transmission model. We co-cultured HEK293 cells persistently harboring intracellular Tau aggregates with rat hippocampal neurons. These HEK293 cells with Tau aggregates were derived from a polyclonal HEK293 cell line with inducible expression of a GFP-tagged human mutant P301L Tau (TauP301L-GFP; "clone 1"; Figure S1A). Aggregation of TauP301L-GFP was induced by adding

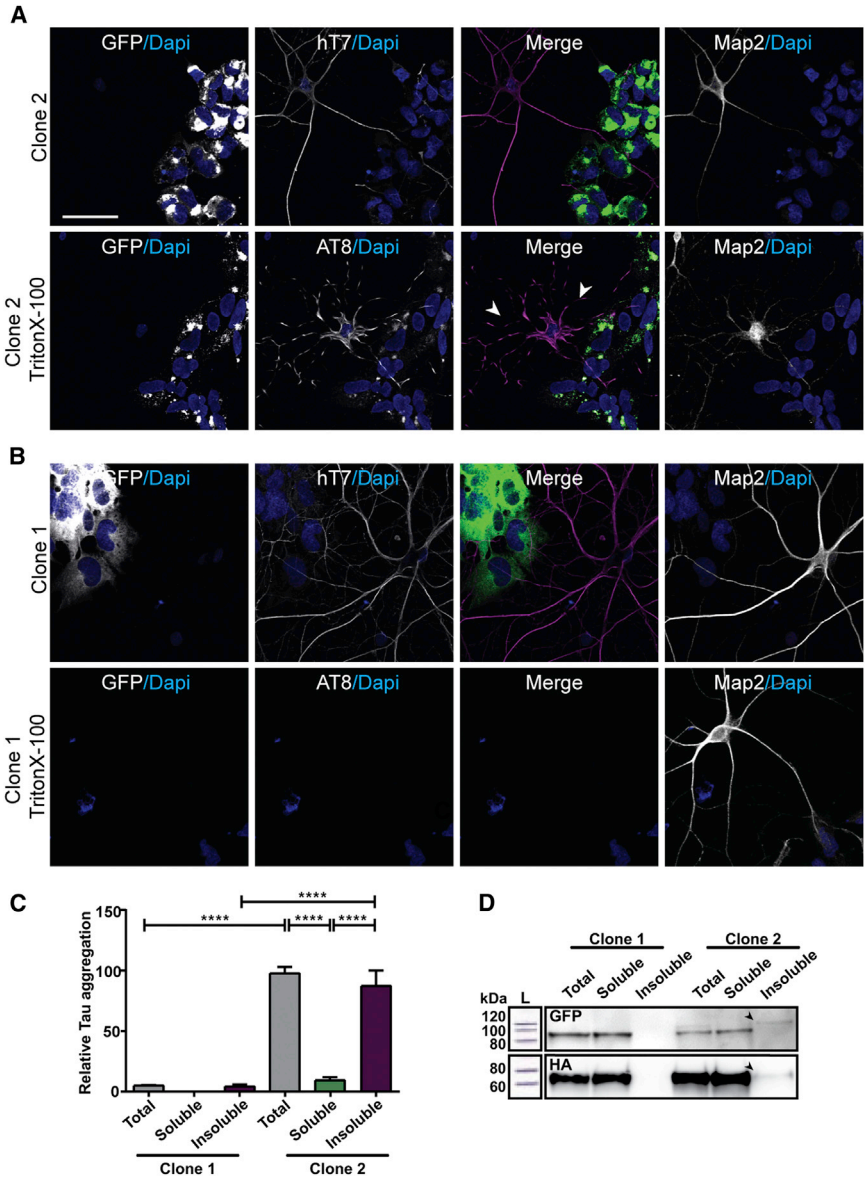

Figure 1. Tau Pathology Propagates from HEK293 with Aggregates to Hippocampal Neurons

(A and B) Confocal analysis of clone 1 or clone 2 cells co-cultured at DIV7 with hippocampal neurons expressing TauP301L and fixed at DIV12–14 in 4% PFA or 4% PFA 1% Triton X-100 immunolabeled for total Tau using hT7 or for phospho-Tau using AT8 antibody (magenta) and MAP2 (white) antibodies. DAPI, nuclei (blue). The scale bar represents 50 μm. (A) Hippocampal neurons co-cultured with clone 2 show Triton-X-100-resistant TauP301L aggregates in hippocampal neurons (magenta; arrow). (B) Hippocampal neurons co-cultured with clone 1 cells do not show AT8 immunoreactivity.

(C) AlphaLISA for aggregated HA-tagged neuronal Tau of fractions of hippocampal neurons expressing HA-TauP301L co-cultured with clone 2 at DIV7 and lysed in 1% Triton X-100 (error bars: SEM; n = 4; ****$p < 0.0001$).

(D) Fractions as in (C) analyzed by western blot. Total HA-TauP301L (expressed in neurons) was detected using anti-HA, and total TauP301L-GFP (expressed in HEK293 cells) was detected using anti-GFP. Arrows, insoluble HA-TauP301L and TauP301L-GFP. n = 4.

Related to Figure S1.

recombinant preformed synthetic HA-K18P301L fibrils as described before (Guo and Lee, 2011). These cells were diluted, and sub-clones were isolated. To assess whether clones that stably replicate Tau aggregates can be identified (now in the absence of exogenously added HA-K18P301L fibrils), cells were fixed and soluble protein extracted and remaining insoluble inclusions were labeled with AT8, a monoclonal anti-Tau antibody that recognizes hyperphosphorylated Tau protein (Vandermeeren et al., 1993). One of the clones (“clone 2”) constitutively showed AT8-positive juxtanuclear aggregates and cytosolic speckles (Figure S1B), indicating that Tau aggregates are self-replicative over cellular generations. Biochemical characterization of clone 1 and 2 using semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) showed that, along with monomeric Tau, clone 2 also contains higher-molecular-weight Tau species (Figure S1C). The aggregated nature of these higher-molecular-weight Tau species was further demonstrated by sedimentation analysis and confirmed by western blot (Figure S1D). Our results indicate that, once Tau forms aggregates in cells, they are replicated over generations (Clavaguera et al., 2013; Sanders et al., 2014).

Clonal Cell-to-Neuron Tau Pathology Propagation

To examine whether Tau pathology can propagate from clonal HEK293 donor cells to a neuronal acceptor population, we co-cultured clone 2 cells with hippocampal neurons (DIV7) expressing human mutant P301L Tau (TauP301L; not GFP tagged). About 10% of the neurons developed Tau aggregates as evident from the AT8-positive and Triton X-100 extraction-resistant nature of the inclusions after 5–7 days of co-culture (Figure 1A). These aggregates were GFP negative and co-localized with MAP2, indicating that these represented aggregates derived from human TauP301L expressed in these neurons. In contrast, neurons co-cultured with clone 1 did not develop Tau aggregates (Figure 1B).

Next, we further explored the biochemical properties of the Tau aggregates found in hippocampal neurons. Clone 1 and clone 2 cells were co-cultured with hippocampal neurons (DIV7) expressing HA-tagged TauP301L (HA-TauP301L), and total lysates were fractionated into soluble and insoluble fractions at 5–7 days of co-culture. The fractions were then analyzed by an AlphaLISA assay (see Experimental Procedures) that selectively

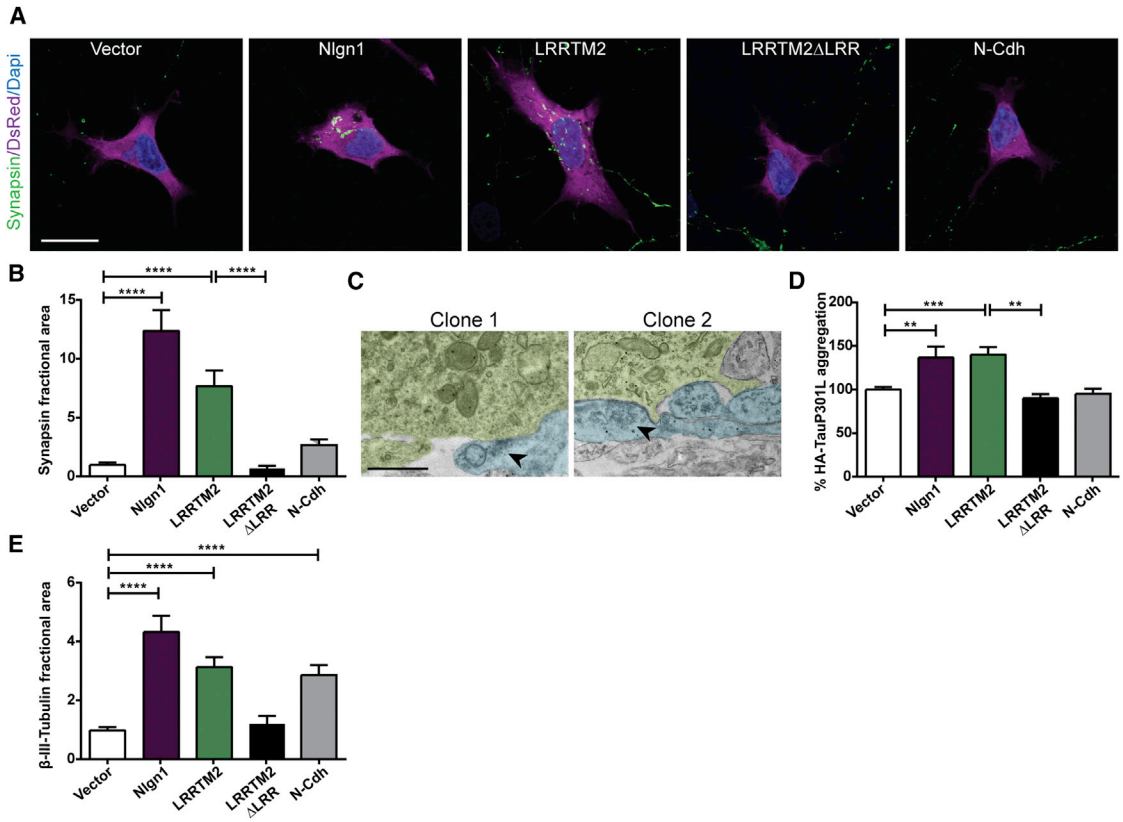

Figure 2. Nlgn1 and LRRTM2 Induce Presynaptic Contacts and Enhance Tau Pathology Propagation

(A) Synapsin labeling (green) of primary hippocampal neurons transduced with TauP301L at DIV2 and co-cultured with HEK293 clone 2 cells transfected to express adhesion molecules and dsRed (magenta) at DIV7. The scale bar represents 20 μm.

(B) Quantification of the area occupied by synapsin on the HEK293 cell surface. Error bars: SEM; vector (n = 74); N-Cdh (n = 80); Nlgn1 (n = 40); LRRTM2 (n = 74); DLRR (n = 40). ANOVA/Tukey's: ****p < 0.0001.

(C) TEM of co-cultures as in (A), pseudocolored to reveal presynaptic contacts (blue; synaptic vesicles, arrow) onto clone 1 or 2 cells expressing LRRTM2 (green). The scale bar represents 100 nm.

(D) AlphaLISA of aggregated HA-Tau of co-cultures of primary hippocampal neurons transduced with HA-TauP301L at DIV2 and HEK293 clone 2 cells transfected to express adhesion molecules. Error bars: SEM. Vector (n = 8); N-Cdh (n = 6); Nlgn1 (n = 6); LRRTM2 (n = 6); LRRTM2ΔLRR (n = 4). ANOVA/Tukey's: **p < 0.01; ***p < 0.001.

(E) Quantification of the area occupied by β-III-tubulin as in (B). Error bars: SEM; vector (n = 74); N-Cdh (n = 80); Nlgn1 (n = 40); LRRTM2 (n = 74); LRRTM2ΔLRR (n = 40). ANOVA/Tukey's: ****p < 0.0001.

Related to Figure S2.

detects aggregated HA-TauP301L from the neurons, but not clone-2-derived Tau aggregates (TauP301L-GFP). High levels of HA-TauP301L aggregates were only detected using the AlphaLISA assay in the total and insoluble fractions from the co-culture of hippocampal neurons with clone 2, but not with clone 1 (Figure 1C). These results were further confirmed by western blot (Figure 1D). These results indicate that Tau pathology can propagate from a clonal cell to a primary neuronal cell.

Synaptogenic Molecules Potentiate Clonal Cell-to-Neuron Tau Pathology Propagation

To test whether Tau propagation is enhanced by the formation of synaptic contacts, we expressed, in the clonal cells, the synaptic cell adhesion molecules neuroligin 1 (Nlgn1) and leucine-rich repeat trans-membrane protein 2 (LRRTM2) that bind neurexin and are expected to induce presynaptic differentiation in contacting axons of co-cultured neurons (Linhoff et al., 2009; Scheiffele et al., 2000; Südhof, 2008; de Wit et al., 2009). Indeed, immunolabeling of the co-cultures indicated the accumulation of presynaptic markers (synapsin) when HEK293 cells harboring constant Tau aggregation were expressing Nlgn1 or LRRTM2, but not when the cells were transfected with an empty vector (Figures 2A and 2B). In addition, transmission electron microscopy revealed neuron-to-clone 2 cells synaptic contacts with presynaptic densities and clusters of synaptic vesicles in proximity to the presynaptic membranes, indicating a presence of active zones (Figure 2C).

We then used our AlphaLISA assay to assess whether HA-TauP301L aggregated in the hippocampal neurons. As expected, co-culturing the Nlgn1- or LRRTM2-expressing clone 1 cells with HA-TauP301L-expressing hippocampal neurons did not result in Tau aggregates (Figure S2A). By contrast, when

we co-cultured Nlgn1- or LRRTM2-expressing clone 2 cells with HA-TauP301L-expressing hippocampal neurons, we observed HA-TauP301L aggregation (Figure 2D). The amount of Tau aggregation in neurons after generating synaptic contacts using Nlgn1 or LRRTM2 was almost 50% higher than when the neurons were co-cultured with clone 2 cells transfected with a control vector (Figure 2D). This increase in HA-TauP301L aggregation was not simply due to differences in TauP301L-GFP secreted into culture medium or expression levels in donor clone 2 cells transfected with the adhesion molecules (Figures S2B–S2D) but due to the increased number of synaptic contacts. Indeed, when we co-cultured the hippocampal neurons with clone 2 cells that express LRRMT2ΔLRR, an LRRTM2 mutant that fails to induce synaptogenesis (Figures 2A and 2B) (de Wit et al., 2009), we did not observe the increase in HA-TauP301L aggregation (Figure 2D). Finally, the increased HA-TauP301L aggregation is also not merely the result of the increased cellular contact surface. Here, we used expression of cell adhesion molecule N-Cadherin (N-Cdh) that induced an increase in cell-neuron contacts, but not an increased number of synapses (Scheiffele et al., 2000) (Figures 2B and 2E), and this condition did not result in an increase in HA-TauP301L aggregation (Figure 2D). Hence, these results suggest that synaptic contacts induced by expression of synaptogenic adhesion proteins enables an additional and distinct mechanism of Tau propagation above and beyond the baseline propagation observed in this cell-to-neuron model in the absence of synaptic contacts.

Neuron-to-Neuron Tau Pathology Propagation Is Facilitated by Synaptic Connectivity

We next sought to assess whether synaptic contacts between neurons also promote Tau pathology propagation. Hippocampal neurons expressing human mutant P301L Tau (TauP301L) were exposed to synthetic HA-K18P301L fibrils at DIV5 (Guo and Lee, 2013; Sanders et al., 2014). As expected, these neurons developed Tau aggregates derived from the expressed human TauP301L, as was evident from robust AT8-positive and Triton X-100 extraction-resistant TauP301L inclusions (the AT8 epitope is absent in HA-K18P301L fibrils; Figure S3A). To examine the propagation of Tau aggregation in neuronal cultures, we resorted to microfluidic culture devices that allowed us to physically separate the somatodendritic and axonal areas (Taylor et al., 2005). The devices consist of three fluidic isolated chambers (C1–3) connected by microgrooves. Axons, but not dendrites, can project through microgrooves, and axons are expected to form synaptic contacts with the somatodendritic compartment of the neuron in the chambers to where they extend, thereby forming an artificial neuronal circuit between the chambers. Axons of neurons plated in C3 reach C2 at DIV15, but at this stage, we never detected neurons that reached over to C1 (Figure S3B). We plated hippocampal neurons in all three chambers (C1, 2, and 3) and transduced the cells with TauP301L at DIV2. Next, Tau pathology was induced in C1 by seeding with HA-K18P301L fibrils at DIV5. At DIV12, Tau aggregates were detected in neurites as well as neuronal cell bodies in both C1 and C2 whereas they were absent in C3 cell bodies (Figure 3A). At DIV15, lower quantities of Tau aggregates were also detectable in neurites as well as neuronal cell bodies in C3 (Figure 3B). This effect is not because the HA-K18P301L fibrils added in C1 are diffusing across chambers because (1) pressure is higher in C3 than in C1 blocking diffusion to C3 and (2) even after prolonged time periods (DIV15), we were unable to detect these exogenously added HA-K18P301L fibrils in C2 or C3 (Figure S3C).

To test the importance of synaptic contacts for the propagation of Tau pathology, we sought to interrupt the neuronal circuit formed between these chambers. We only plated neurons in C1 and C3 because, in this situation, the absence of somatodendritic compartments in C2 prevents the neurons that project from C1 and C3 from forming synapses in C2 (Figure 3C). HA-K18P301L fibrils were added to C1, and robust induction of Tau aggregation was observed in somatodendritic areas of neurons in C1 and within the axons that projected from C1 to C2. However, at DIV15, we did not detect substantial Tau aggregation in C3 neurons (Figures 3C–3E). Hence, Tau pathology propagation to neurons in C3 is enhanced when synapses are allowed to form in C2.

Efficient Neuron-to-Neuron Tau Pathology Propagation Is Promoted by Synaptic Connections and Neuronal Activity

To further address the involvement of synaptic connectivity in promoting neuron-to-neuron Tau propagation, we blocked the formation of synapses with neurons in C2. We used shRNA to knock down the synaptogenic cell adhesion molecules Nlgn1, 2, and 3 (sh-Nlgn1, sh-Nlgn2, and sh-Nlgn3; Figure S4A). Reduced expression of Nlgn1, 2, or 3 resulted in synaptic density decrease (Figures S4B and S4B') as observed previously (Chih et al., 2005) without inducing cellular toxicity (Figure S4D). Hippocampal neurons were plated in all three chambers (C1-2-3) and transduced with TauP301L. Neurons in C2 and C3 were also additionally transduced with shRNA for Nlgn1, 2, or 3 or a control shRNA at DIV2. Neurons in C1 were seeded with HA-K18P301L fibrils at DIV5, and Tau aggregation in the three chambers was assessed by alphaLISA. Reduction of synaptic connectivity upon expression of shNlgn1, 2, and 3 resulted in a significant decrease of total aggregated Tau in C3 as compared to expression of a control shRNA (Figures S4A and 4A).

To examine the potential role of synaptic activity within the neurons of this mini-circuit, we incubated the cells in C2 and C3 with the sodium channel blocker tetrodotoxin (TTX), a manipulation that blocks neuronal activity (Murthy et al., 2001) but does not affect synapse density (Figure S4C) or cause cellular toxicity (Figure S4E). We then induced Tau aggregation by incubating the cells in C1 with HA-K18P301L fibrils and observed a significant decrease in the amount of aggregated Tau developed in the neurons in C3 at DIV15 (Figure 4B). We believe Tau aggregation levels in C2 are not affected by Nlgn knockdown or TTX because these neurons can project into C1, taking up HA-K18P301L. Thus, neuronal propagation of Tau pathology is promoted by synaptic connectivity as well as neuronal activity.

DISCUSSION

The observations reported here show that Tau propagation between cells is significantly facilitated by synaptic connections.

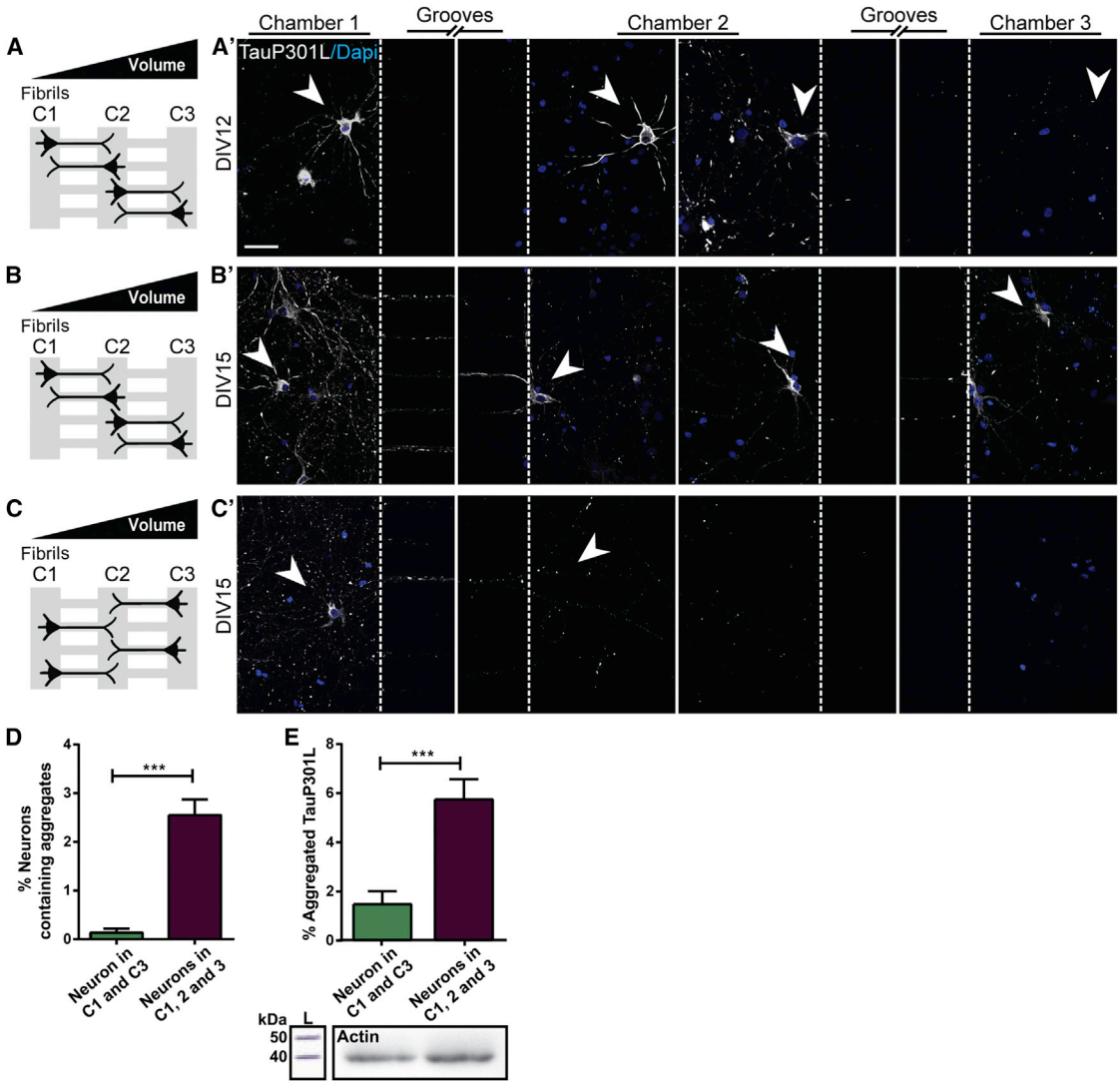

Figure 3. Neuron-to-Neuron Tau Pathology Propagation Is Facilitated by Synapses

(A) Hippocampal neurons incubated with HA-K18P301L fibrils (in C1 at DIV5) and transduced with TauP301L (C1–3; DIV2), fixed and extracted with 4% PFA and 1% Triton X-100 (DIV12), and labeled with phospho-Tau AT8 antibody (white) and DAPI (nuclei). The scale bar represents 50 μm.

(B) Experiment as in (A), but cells were fixed at DIV15.

(C) Experiment as in (A) without neurons in C2.

(D) Quantification of hippocampal neurons containing TauP301L aggregates in C3 from conditions (B) and (C). Error bars: SEM; C1, 3 (n = 4); C1, 2, 3 (n = 3). Student's t test; ***$p < 0.001$.

(E) Quantification of the TauP301L pathology propagation to neurons in C3 using AlphaLISA from conditions (B) and (C) normalized to actin protein loading control and expressed as percentage of aggregated TauP301L. Error bars: SEM; C1, 3 (n = 8); C1, 2, 3 (n = 10). Student's t test; **$p < 0.01$.

Related to Figure S3.

Using a unique in vitro model for Tau pathology propagation, we find that the expression of synaptogenic proteins in heterologous cells containing Tau aggregates potentiates Tau propagation from these cells to co-cultured hippocampal neurons. We extend these findings to demonstrate Tau pathology propagation between synaptically connected neurons using the microfluidic chamber setup. Additionally, we show decreased synaptic density or suppressed synaptic activity weakens neuron-to-neuron Tau pathology propagation.

In one of our co-culture models, Tau pathology propagates from the clonal HEK293 donor cell to the hippocampal neuron acceptor cells that consequently develop Tau aggregation. In this baseline condition, propagation is mediated via an unknown mechanism that might involve the release of a "seed" from the donor cell to the extracellular medium followed by entry into the recipient neuron (Kfoury et al., 2012). The stereotypical propagation of Tau pathology within the brain between connected neurons has led to the postulation that the propagation can be

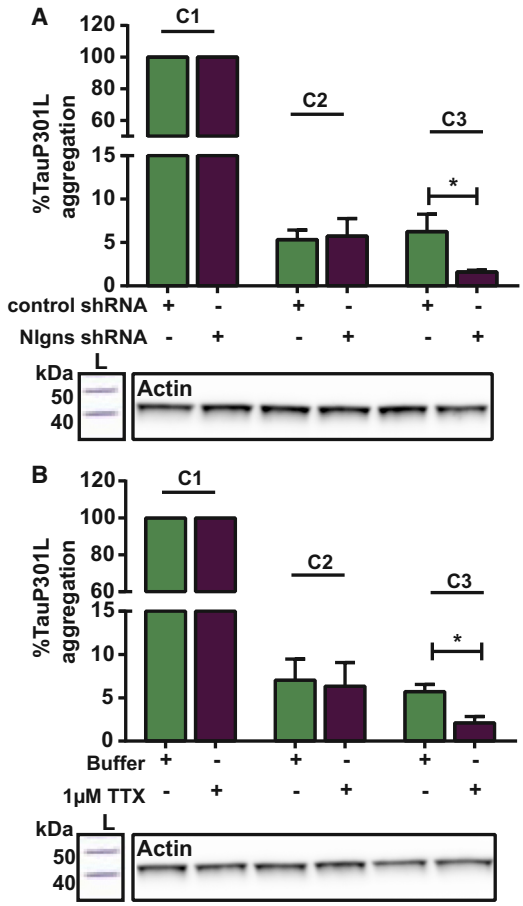

Figure 4. Decrease in Synapse Formation and Synapse Activity Reduces Neuron-to-Neuron Tau Pathology Propagation

Hippocampal neurons in C1, 2, and 3 transduced with TauP301L on DIV2 and HA-K18P301L fibrils added on DIV5 in C1.

(A) Hippocampal neurons in C2 and C3 were also transduced with control shRNA or shRNA for Nlgn1, 2, and 3. Cells from each channel were lysed, and aggregated TauP301L was quantified by AlphaLISA. Error bars: SEM; n = 6. Student's t test; *p < 0.05.

(B) Hippocampal neurons in C2 and C3 were chronically exposed to TTX from DIV5 to 15, and aggregated TauP301L was quantified by AlphaLISA.

Aggregated TauP301L in C3 in (A) and (B) is normalized to actin protein loading control and expressed as percentage of aggregated TauP301L; error bars: SEM; control (n = 10); TTX (n = 5). Student's t test: *p < 0.01. Related to Figure S4.

synapse mediated (Braak and Del Tredici, 2011; de Calignon et al., 2012; Liu et al., 2012). Here, we now provide several lines of evidence in favor of this idea because we are able to follow Tau propagation while specifically modulating the synaptic contacts between cells. First, the generation of synaptic contacts between donor cells and the acceptor neuron causes the Tau pathology propagation to increase by almost 50%. Furthermore, we demonstrate that synaptogenic mechanisms also promoted Tau pathology dissemination within an artificial neuronal network. The presence of abnormal Tau within the pre- and post-synaptic compartments of AD brains supports the idea that this species can be a substrate for *trans*-synaptic transmission (Tai et al., 2014). We therefore hypothesize that a form of Tau-seeding species that is present at the synapse can be *trans*-synaptically transmitted to connected neurons, thereby disseminating Tau pathology by inducing aggregation of soluble Tau (Guo and Lee, 2014; Tai et al., 2014). In future work, the mechanism by which such seeding species are transmitted across the synapse can now be discovered.

The co-culture systems presented here are a considerable improvement over the standard Tau-fibril-seeding models that require constant addition of exogenously added seeds. The cell-neuron model could potentially be employed in unbiased screenings to unveil potential pre- and post-synaptic players that drive Tau propagation, such as adhesion molecules, membrane receptors, as well as other proteins of the synaptic machinery that sustain synaptic activity. In addition, the neuronal model allows for the generation of a neuronal network that enables the study of seeding and propagation of Tau pathology in a more physiologically relevant system.

Evidence converges on the idea that synaptic degeneration is a major pathophysiological feature of neurodegenerative diseases (Lu et al., 2013; Serrano-Pozo et al., 2011). In AD, synapse loss strongly correlates with cognitive decline and both Aβ and Tau are identified key pathogenic players (Selkoe, 2002; Spires-Jones and Hyman, 2014). Therefore, disease-modifying therapies that focus on rescuing synaptic dysfunction and synapse rejuvenation are emerging (Lu et al., 2013; Peretti et al., 2015). However, our data suggest that synapses can also be an avenue for enhanced Tau propagation. Therefore, therapeutic strategies that are designed to target pathophysiology should be accompanied by strategies that target pathogenesis, for example, by specifically blocking synapse-mediated Tau propagation or by boosting clearance of Tau aggregates that are internalized at the synapse. Our observations are consistent with the "cell-to-cell transmission hypothesis" of Tau pathology and reveals that synapses are actively involved in the transmission of Tau pathology.

EXPERIMENTAL PROCEDURES

Primary Antibodies

Primary antibodies are mouse anti-hT7 (Sigma-Aldrich; S5062); mouse anti-human Tau 10 (hTau10; epitope 29–36); mouse anti-AT8 (pSer202/Thr205); rabbit anti-synapsin (Millipore; AB1543P); rabbit anti-PSD95 (Millipore; MA1-046); chicken anti-Map2 (Abcam; ab5392); chicken anti-β-III-tubulin (Abcam; ab41489); mouse anti-III β-tubulin (Covance; mms-435p); rabbit anti-HA (Invitrogen; 71-5500); rabbit anti-FLAG (Sigma-Aldrich; F3165); mouse anti-GFP (Sigma-Aldrich; G1546); mouse anti-LRRTM2 (NeuroMab; 73-364); mouse anti-actin (Millipore; MAB1501); and rabbit anti-Nlgn1, 2, and 3 (Synaptic Systems; 129013, 129203, and 129103). Immunolabeling, western blots, and semi-denaturing PAGE were performed as described in the Supplemental Information.

Primary Cultures

Hippocampal neurons were cultured from E17 to E18 Wistar rat embryos (Charles River Laboratories). Transductions with the plasmids of interest (see Supplemental Experimental Procedures) were performed at DIV2. For biochemistry, neurons were plated in poly-d-lysine-coated plates (BD BioCoat). For immunofluorescence, neurons were plated on coverslips coated with poly-D-lysine (Sigma-Aldrich) and laminin (Sigma-Aldrich). Neurons were maintained in Neurobasal-A medium (Invitrogen) supplemented with B27and glutamine. All cultures were maintained at 37°C and 5% CO_2. Cytotoxicity assays were performed as described in the Supplemental Information.

Microfluidic Chambers

Triple-compartment microfluidic chambers (Xona Microfluidics) were placed on coated coverslips, and 60,000 neurons were plated per channel. Transductions with plasmids of interest (see Supplemental Experimental Procedures) were performed at DIV2. Cultures were maintained at 37°C and 5% CO_2 up to 15 days.

Fibril Preparation and Transduction

Truncated human Tau fragments containing the four microtubule-binding repeat domains (K18; residues Q244–E372 of the longest human Tau isoform) with a P301L mutation and N-terminal HA tag (HA-K18P301L) were produced in *Escherichia coli* (Tebu-bio). For in vitro fibril production, a solution of 40 μM HA-K18P301L, 40 μM low-molecular-weight heparin (Sigma-Aldrich), and 2 mM DTT in 100 mM sodium acetate buffer (pH 7.0) was incubated at 37°C for 3 days. The mixture was centrifuged at 100,000 × *g* for 30 min at 4°C, and the pellet, which contained the HA-K18P301L fibrils, was re-suspended in the same volume of sodium acetate buffer. HA-K18P301L fibrils were sonicated with 25 pulses of 2 s and added to culture medium in a final concentration of 50 nM.

Mixed Culture

Mixed-culture assays were performed as described in Biederer and Scheiffele (2007). In brief, clones 1 and 2 (see Supplemental Experimental Procedures) were transfected with the plasmid of interest (Supplemental Experimental Procedures) using Fugene6 (Promega). 24 hr after, they were trypsinized for 5 min at 37°C and re-suspended in DMEM medium. Transfected cells were co-cultured with DIV7 hippocampal neurons for 7 to 8 days. For synaptic contacts analysis, transfected cells were co-cultured with DIV7 hippocampal neurons for 48 hr.

Sedimentation

Cells were lysed in PBS containing 1% Triton and cOmplete mini protease inhibitor tablet (Roche) and clarified by centrifugation (1,000 × *g*) for 10 min. Part of the lysate was kept for total fraction, and the other part was centrifuged at 100,000 × *g* at 4°C for 1 hr. The supernatant was used as soluble fraction. The pellet was re-suspended in 1% Triton, and a second centrifugation at 100,000 × *g* at 4°C for 1 hr was done. Supernatant was discarded and the pellet re-suspended in 1% LDS buffer and used as insoluble fraction.

AlphaLISA Immunoassay

Culture medium or pre-cleared lysates (see Sedimentation) sample was incubated for 2 hr at 37°C with the AlphaLISA capturing mixture containing antibody conjugated biotin and acceptor beads. Next, AlphaLISA detection mixture containing streptavidin-conjugated donor beads was added and incubated for 1 hr at 37°C. AlphaLISA signal was detected with Envision plate reader (PerkinElmer). To specifically detect aggregated Tau, components of capturing mixture (biotin/acceptor beads) were conjugated to the same antibody: for untagged TauP301L, mouse anti-hT10 was used, and for aggregated HA-TauP301L, mouse anti HA (Roche Life Science; 12CA5) was used.

TTX Treatment of Hippocampal Neuronal Cultures

Hippocampal neurons in microfluidic devices were chronically treated with 1 μM TTX (Sigma) in normal medium from DIV5 to DIV15.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.04.043.

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