CHAPTER

15 The use of mouse models to study cell-to-cell transmission of pathological tau

Sneha Narasimhan, Virginia M.Y. Lee[1]

Institute on Aging and Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine, Philadelphia, PA, United States

[1]*Corresponding author: e-mail address: vmylee@upenn.edu*

CHAPTER OUTLINE

Abstract

Tau protein aggregates are found in a variety of neurodegenerative diseases known as tauopathies. Emerging evidence shows tau can propagate from cell-to-cell by seeding endogenous tau to aggregate. Studies in tau transgenic mice showed intracerebrally injecting misfolded tau seeds initiates and transmits tau pathology across the mouse brain. However, transgenic mice that overexpress human tau with disease-associated mutations do not fully recapitulate sporadic tauopathies. Here, we present our method for developing a sporadic tauopathy model using pathological tau extracted from human Alzheimer's disease (AD) brains. We describe a novel

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method for sequentially extracting tau pathologies from human AD brain in high yield and purity. We then describe how to intracerebrally inject this extract into a nontransgenic mouse brain and analyze the transmission of tau pathology. This novel sporadic tauopathy model can be used to study the transmission of tau aggregates and test new tau-directed therapies.

1 INTRODUCTION

Tau is a microtubule-associated protein primarily expressed in neuronal axons where it promotes the assembly and stability of microtubules (Cleveland, Hwo, & Kirschner, 1977; Drechsel, Hyman, Cobb, & Kirschner, 1992). Tau is normally a highly soluble protein without well-defined secondary or tertiary structures; however, in a pathological state, it can misfold and form fibrillar aggregates. Tau aggregates are found in the brains of a variety of neurodegenerative diseases collectively known as tauopathies, the most common of which is Alzheimer's disease (AD). Interestingly, tauopathies exhibit great heterogeneity in clinical symptoms and neuropathological changes, despite the fact that the tau protein is aggregating in all these neurodegenerative diseases (Lee, Goedert, & Trojanowski, 2001). The exact mechanism of tau pathogenesis in these diseases is still unknown.

However, the emerging hypothesis in the field is that tau protein transmits in a prion-like manner through the cell-to-cell propagation of misfolded tau seeds that underlies the progression of tauopathies (referred to as the "transmission" hypothesis; see Guo & Lee, 2014 for review). Several published studies, including those from our lab, have now shown that misfolded tau can "seed" endogenous tau to form pathological tau aggregates in cell culture models (Ahmed et al., 2014; Frost, Jacks, & Diamond, 2009; Guo, Buist, et al., 2016; Guo & Lee, 2011, 2013; Holmes et al., 2013; Kfoury, Holmes, Jiang, Holtzman, & Diamond, 2012; Nonaka, Watanabe, Iwatsubo, & Hasegawa, 2010; Sanders et al., 2014; Takahashi et al., 2015; Usenovic et al., 2015). However, the most convincing evidence in support of the transmission hypothesis comes from studies using mouse models of tauopathies. Here, we will briefly summarize the literature on mouse models of transmission of pathological tau, while also highlighting some of the challenges in using these models (see Table 1). We will then describe a new method we have developed to address some of these challenges to better understand the mechanism of cell-to-cell propagation of tau in vivo.

Almost all of the studies describing tau transmission in vivo have used transgenic mouse models that overexpress different forms of human tau, often with a *MAPT* (tau) mutation found in familial forms of tauopathies (Table 1). The most common transgenic mice used include the PS19 mouse model developed in our lab (1N4R human tau with P301S mutation), and the Alz17 mouse model (2N4R human wild-type (WT) tau). While some of these transgenic mouse models develop tau aggregates on their own over long periods of time, intracerebral injection of pathological tau seeds hasten the formation and spreading of tau aggregates in the mouse brain (Ahmed et al., 2014; Boluda et al., 2015; Clavaguera et al., 2013, 2009; Falcon et al., 2015; Holmes et al., 2014; Hu et al., 2016; Iba et al., 2013, 2015; Jackson et al., 2016;

Table 1 Tau Transmission Studies Using Transgenic Mouse Models

Transgenic Mouse Line	Tau Expression	References	Mode of Transmission	Type of Seeds
Alz17	2N4R human WT tau	Clavaguera et al. (2009)	Intracerebral injection	Brain extract from P301S transgenic mouse
		Clavaguera et al. (2013)	Intracerebral injection	Human tauopathy brain lysate
PS19	1N4R human tau with P301S mutation	Iba et al. (2013, 2015) and Stancu et al. (2015)	Intracerebral injection	PFFs from recombinant tau
		Holmes et al. (2014), Kaufman et al. (2016), and Sanders et al. (2014)	Intracerebral injection	Lysates from HEK293 cells with tau aggregates
		Boluda et al. (2015)	Intracerebral injection	Human tauopathy brain lysate
		Ahmed et al. (2014), Falcon et al. (2015), and Jackson et al. (2016)	Intracerebral injection	Mouse brain lysate
P301L	2N4R human tau with P301L mutation	Peeraer et al. (2015)	Intracerebral injection	PFFs from recombinant tau
rTg4510	Inducible 0N4R human tau with P301L mutation	Polanco, Scicluna, Hill, and Gotz (2016)	Intracerebral injection	Exosomes from same transgenic mouse brains
Htau	All six human tau isoforms with mouse tau KO	Hu et al. (2016)	Intracerebral injection	Human AD brain lysate
rTgTauEC	Entorhinal cortex-specific expression of human tau with P301L mutation	de Calignon et al. (2012), Pooler et al. (2015), and Wegmann et al. (2015)	Restricted expression of tau	Endogenously expressed human tau
AAV overexpression of human tau	AAV with human 2N4R tau with P301L mutations	Asai et al. (2015)	Restricted expression of tau	AAV expressed human tau

Kaufman et al., 2016; Peeraer et al., 2015; Polanco et al., 2016; Sanders et al., 2014; Stancu et al., 2015). Studies have used different sources of tau seeds, including synthetic tau preformed fibrils (PFFs), extracts from transgenic mouse brains, or extracts from human tauopathy brains, all with reasonable success in seeding tau pathology in transgenic mice.

Another model of tau transmission uses restricted overexpression of human tau in one specific brain region and describes the spread of tau aggregates to other regions over time. The most prominent model for this is the rTgTauEC mouse, which restricts expression of human tau with the P301L mutation expression to the entorhinal cortex, but shows tau aggregates forming in anatomically connected regions as the mouse ages (de Calignon et al., 2012; Pooler et al., 2015; Wegmann et al., 2015). Injection of AAV virus overexpressing human 2N4R tau with the P301L mutation into specific brain regions has also been used to study tau propagation over time (Asai et al., 2015). While transgenic mouse models have been used widely to study pathological tau transmission, they have inherent limitations: they use heterologous promoters to drive tau overexpression, making it difficult to understand how tau pathology spreads in the human brain; furthermore, they use the overexpression of human tau, and with familial tau mutations, which does not fully recapitulate the more common sporadic tauopathies. Despite the widespread use of these transgenic models, there has yet to be a disease-modifying therapy for tauopathies in clinical practice.

It has been challenging to develop a nontransgenic (nonTg) mouse model of tau propagation that fully recapitulates the phenotypes of tauopathies. A few studies have tried to intracerebrally inject different tau seeds into a nonTg mouse brain, but with limited success in developing a few endogenous tau aggregates, and only after long periods of incubation (Clavaguera et al., 2013; Lasagna-Reeves et al., 2012). While one of those studies used human tauopathy brain lysates, they used a crude homogenate with no quantification of the amount of pathological tau in their lysate; thus, low amounts of tau seeds likely account for their limited ability to induce tau pathology in nonTg mice (Clavaguera et al., 2013).

Recent studies have shown that different structural conformations of tau exist, known as tau strains, and these tau strains may underlie the phenotypic diversity of tauopathies (Boluda et al., 2015; Guo, Narasimhan, et al., 2016; Kaufman et al., 2016; Sanders et al., 2014). Most interestingly, we have found different structural conformations between synthetic preformed tau fibrils and insoluble tau extracted from human AD brains; only tau extracted from human AD brains can seed endogenous mouse tau aggregates in a nonTg primary neurons and nonTg mouse brain (Guo, Narasimhan, et al., 2016). We hypothesize the different strains of tau seeds account for why it has been challenging to develop a nonTg mouse model of tau transmission. Here, we will describe our novel method of sequentially extracting pathological tau from the human AD brain in higher yield and purity than previous studies (Clavaguera et al., 2013). We will then describe how to use this pathological tau-enriched lysate to develop a sporadic tauopathy mouse model.

2 METHODS

2.1 EXTRACTION OF PATHOLOGICAL TAU FROM HUMAN AD BRAINS

Methods adapted from Guo, Narasimhan, et al. (2016).

Materials

High-salt extraction buffer with 0.1% sarkosyl: 10 mM Tris pH 7.4, 10% sucrose, 0.8 M NaCl, 1 mM EDTA, 0.1% sarkosyl
Protease inhibitor cocktail (Sigma; 1:1000): 25 mg Pepstatin, 25 mg Leupeptin, 25 mg TPCK, 25 mg TLCK, 25 mg Trypsin inhibitor, 0.5 mL 0.5 M EDTA made in 25 mL dH_2O
Phosphatase inhibitor cocktail (1:100): 200 mM Imidazole, 100 mM NaF, 100 mM NaOrthovanadate, 500 mM PMSF (1:500), 200 mM DTT (1:200; final concentration 2 mM)
Sterile Dulbecco's Phosphate-Buffered Saline (dPBS; Corning)
25% sarkosyl in dH_2O
Autoclaved materials (sterile):
- Glass homogenizers and pestles
- Pipette tips
- Forceps
- Beakers
- 1.7 mL Eppendorf tubes
- 100 cm Petri dish

Sterile scalpel blades
Ultracentrifuge with Ti45 and Ti60 rotors and polycarbonate centrifuge tubes
Personal protective equipment: lab coat, gloves, sleeves, face shield

Methods

1. Refer to Fig. 1A for schematic of extraction (each step is labeled with an abbreviation, which is also indicated in the methods later).
2. Select AD cases with abundant (3+ on a scale of 0–3) tau pathology in frontal cortex (Braak stage VI on I–VI scale).
3. On ice, place brain tissue (gray and white matter) on weighed sterile Petri dish to let thaw.
4. After the brain is mostly thawed (soft enough to touch, but not completely thawed), use forceps to dissect out meninges and blood vessels and separate gray from white matter by scraping the gray matter with a sterile scalpel (discard white matter).
5. Weigh the remaining gray matter to determine how much high-salt buffer with 0.1% sarkosyl is needed to homogenize in 9 volumes (v/w).
6. Homogenize the tissue in glass homogenizers on ice in 9 volumes of high-salt buffer with 0.1% sarkosyl. Take an aliquot for biochemical analysis (**T**).

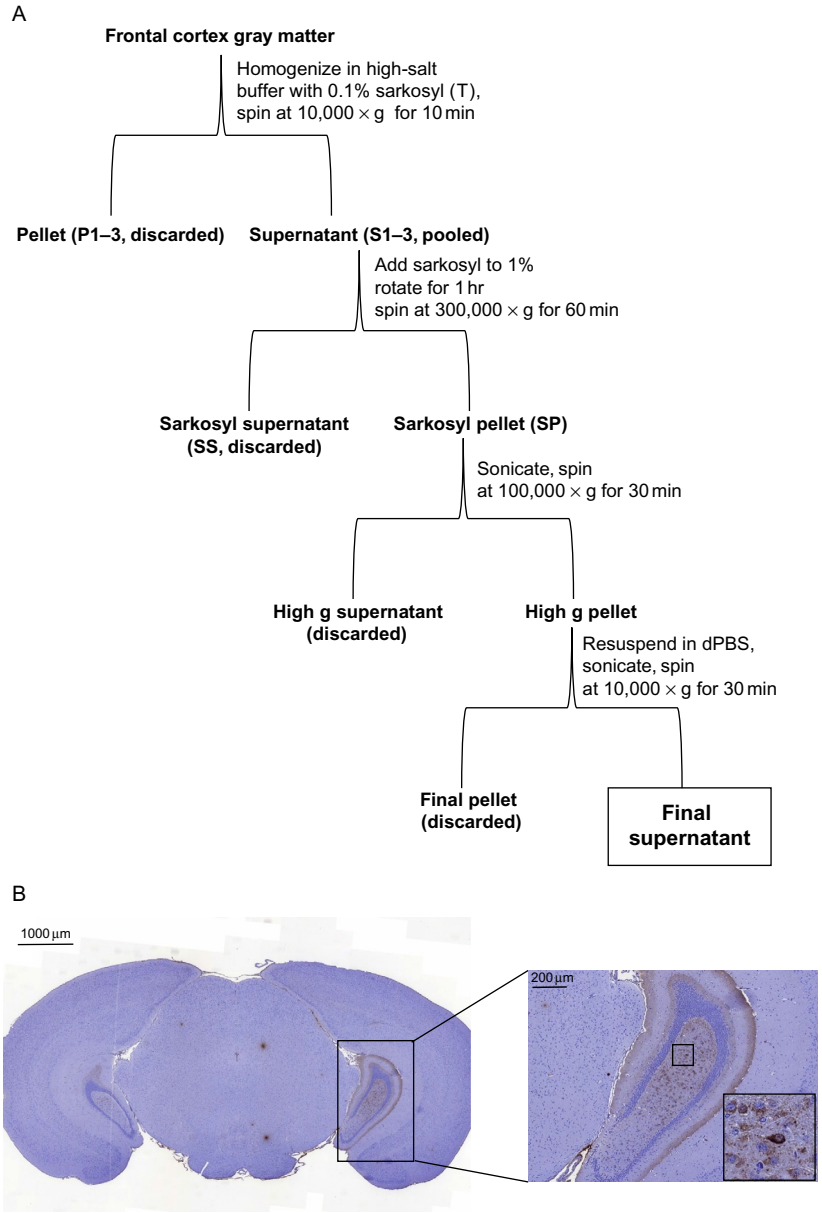

FIG. 1

(A) Diagram of sequential extraction protocol of pathological tau from human AD brain tissue. Following abbreviations at each of the main steps are also included and bolded in the details of the methods for reference. (B) Representative images from scanned slides of IHC for AT8 antibody on AD-tau injected nonTg mice 3 months postinjection. Image is from the ventral hilus of the hippocampus.

Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice.
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7. Centrifuge the homogenate at 10,000 × *g* for 10 min at 4°C. Carefully remove the supernatant (pellet is loose) and filter through a Kimwipe into conical tubes on ice. Take a sample of the supernatant for analysis (**S1**).
8. Repeat steps 6–7 one or two more times (see *Notes*) and collect all supernatants in a beaker (9 volumes (v/w) calculated from original gray matter weight). Take an aliquot of subsequent homogenization steps (**P1–3**), and take an aliquot of each supernatant after ultracentrifugation (**S2–3**).
9. Add 25% sarkosyl to all three combined supernatant fractions to increase final sarkosyl concentration to 1% (from 0.1% sarkosyl). Stir the mixture in 1% sarkosyl in a beaker for 1–1.5 h at room temperature.
10. Centrifuge the mixture at 300,000 × *g* for 60 min at 4°C. Remove the supernatant and float myelin carefully so as to not disturb the pellet (supernatant can be discarded, but retain an aliquot for biochemical analysis—**SS**).
11. Rinse the pellet with 6 mL dPBS (see *Materials*) with gentle swirling. Aspirate the PBS (if pellet becomes loose, carefully aspirate around it).
12. Repeat step 11 with 2 mL of dPBS. Then add 1 mL dPBS and dislodge the pellet with a pipette tip.
13. Carefully transfer the pellet using a transfer pipette to a Ti60 centrifuge tube. Add a small amount of dPBS and break up the pellet using a pipette tip or transfer pipette. Fill up the centrifuge tube with dPBS.
14. Centrifuge the samples at 250,000 × *g* for 30 min at 4°C. Remove/discard the rest of the supernatant.
15. Add 0.1 mL dPBS per gram of original gray matter and break up the pellet into small pieces using a P1000 pipette tip. The pellet is very sticky so be careful to recover all the material once broken up. Transfer the small pellet pieces in dPBS to a sterile Beckman centrifuge tube.
16. Rock the tube at room temperature overnight.
17. The next day, centrifuge the tube at 1000 × *g* for 1 min to consolidate the pellet at the bottom of the tube.
18. Pass the suspension through a 27-gage needle to homogenize the pellet in dPBS (already in tube).
19. Sonicate pellet on ice using a hand-held sonicator (QSonica) for 20 short pulses on 1–2 power. Remove sample for analysis (**SP**).
20. Centrifuge pellet at 100,000 × *g* for 30 min at 4°C. Remove supernatant and save in another tube for analysis (**high g sup**).
21. Resuspend pellet in fivefold reduced volume of fresh dPBS to concentrate final pellet. Sonicate for 60 pulses using hand-held probe at 1–2 power settings on ice. Save a small aliquot of the homogenate for analysis (**high g pell**).
22. Centrifuge the high g pellet at 10,000 × *g* for 30 min at 4°C. Transfer the supernatant to a sterile Eppendorf tube (**final supernatant**)—this contains the pathological insoluble tau of interest.
23. Add an equal volume of dPBS as in step 21 to the final pellet and sonicate 20–30 pulses using a hand-held probe on ice. Store all samples at −80°C until further use (can flash freeze using dry ice/100% ethanol; see below for analysis and intracerebral injection into mouse brain).

Notes

- It is important to select cases with abundant tau pathology (at least 3+ tau score) to have high yield and purity in the final supernatant.
- Normal age-matched brains with no tau pathology can be used as controls using the same extraction protocol.
- High-salt extraction buffer can be made ahead of time, but add 0.1% sarkosyl, DTT, PMSF, protease inhibitors, and phosphatase inhibitors the day of extraction.
- Prepare autoclaved materials before the day of extraction. Let brain tissue equilibrate to −20 or −30°C the night before extraction.
- Two rounds of homogenization in high-salt buffer with 0.1% sarkosyl is sufficient to extract >90% of insoluble tau from AD frontal cortex. If final yield is low, add another round of homogenization.
- If the needle suspension on day 2 is difficult, try initially passing sample through a 19-gage needle.

2.2 ANALYSIS OF SEQUENTIAL EXTRACTION OF PATHOLOGICAL TAU FROM HUMAN BRAIN

Western blot analysis

1. For SDS-PAGE gel 1: Dilute samples from total homogenate to sarkosyl pellet (**T, S1–3, P1–3, SS, SP**) 35 µL sample in 15 µL 5 × sample buffer. Load 7 µL of each samples onto gel. Add 10–30 ng of recombinant tau protein T40 (2N4R) and T39 (2N3R) as loading controls.
2. For gel 2, load 3 µL pf **SP and high g sup** onto gel (no dilution). Dilute samples **high g pellet and final sup** fivefold and load 3 µL of each onto gel. Add 10–30 ng of recombinant tau protein T40 (2N4R) and T39 (2N3R) as loading controls.
3. Run samples on 7.5% SDS gels. Transfer the protein onto 0.2 µm nitrocellulose membranes (blots) for 1 h and 15 min at 100 V at 4°C (transfer buffer: 10% 10 × running buffer (400 g Tris, 1430 glycine, 1 L dH_2O), 10% methanol, up to 1 L dH_2O.
4. Incubate the blots in Ponceau red stain for 1–2 min to estimate protein loading. Scan the blot for future reference.
5. Block the blots in 5% milk in TBS for 1 h at room temperature. Incubate in primary antibodies 17,025 (Covance; total human tau; 1:1000) and PHF-1 (gift from Peter Davies; pS396/T404; 1:1000) overnight at 4°C.
6. The next day, wash the blot in TBS-T (1 L 10 × TBS, 10 mL Tween-20, 10 L dH_2O) for 10 min on a rocker at room temperature. Then wash 3 × in TBS for 10 min each at room temperature.
7. Incubate the blots in fluorescent LiCor secondary antibodies diluted 1:20,000 in blocking buffer (Rockland, Blocking buffer for fluorescent Western blotting) for 1–2 h at room temperature (goat antimouse IRDye 800CW for PHF-1 and goat antirabbit IRDye 680RD for 17,025).

8. Repeat the washes in step 5. Image the blots on the LiCor imaging system and save images for future reference (refer to Guo, Narasimhan, et al., 2016) for sample images of Western blots).

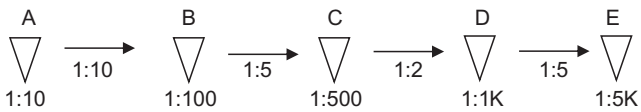
2.2.1 Tau ELISA and BCA analysis

Materials:

Coating buffer:
- 0.1 M $NaHCO_3$
- 0.1 M Na_2CO_3
- pH 9.6
- Block Ace: 0.1% Blockace powder (Serotec)
- 0.05% NaN_3
- 1 × PBS (heat to 56°C for 40 min)

C buffer:
- 0.02 M sodium phosphate buffer
- 2 mM EDTA
- 0.4 M NaCl
- 1% bovine serum albumin (BSA)
- 0.005% Thimerisol

1. The day before ELISA, prepare Tau5 (Covance) coating buffer in a 50 mL tube at a 5 µg/mL concentration using coating buffer.
2. Coat a 384-well plate with 30 µL/well of Tau5 coating buffer. Seal the plate and centrifuge at 1000 × *g* for 2 min at 4°C. Store plate at 4°C overnight.
3. The next day, prepare Block Ace at 90 µL/well. Wash plates on plate washer (PBS with 0.1% Tween), then centrifuge at 1000 × *g* for 2 min at 4°C.
4. Add Block Ace as in step 3 to each well, and then seal plate. Refrigerate at 4°C for at least 1 week before use (reduces background).
5. For day 1 of ELISA, prepare T40 recombinant tau standard diluted in 0.2% BSA in PBS at following concentrations: 0 (just buffer), 37.5, 75, 150, 300, 600, 1200, and 2400 pg/mL.
6. Dilute samples with serial dilution in 0.2% BSA in PBS as shown in diagram below (starting at A—need 30 µL of each sample in four replicates):



7. Remove block buffer from plate and add 5 µL of 0.2% BSA in PBS to each well to prevent drying. Keep plate and samples on ice.
8. Load 30 µL of diluted samples in quadruplicate to wells. Seal the plate, centrifuge at 1000 × *g* for 2 min at 4°C and store at 4°C overnight.

9. The next day, prepare BT2 (1:3200) and HT7 (1:6000) reporters in C buffer (Biotin labeled HT7 MN1000B; BT2 MN1010B Thermo Scientific).
10. Wash plate, and add 30 μL of BT2/HT7 to each well. Seal and centrifuge at $1000 \times g$ for 2 min at 4°C and store at 4°C overnight.
11. The next day, prepare HRP-labeled Streptavidin (Thermo, Pierce High Sensitivity Streptavidin HRP #21130) 1:8000 in C buffer.
12. Wash plate and add 30 μL of Streptavidin to each well. Incubate at 25°C for 1 h.
13. Make developing solution as follows: Peroxidase substrate solution B 7 mL & TMB Peroxidase substrate 7 mL.
14. Wash the plate and add 30 μL of developing solution to each well. 10–20 min later, add 30 μL of 10% phosphoric acid (color changes to yellow).
15. Measure OD on plate reader using 450 nm absorbance. Graph the standards on a logarithmic scale (should be sigmoidal curve) and determine concentrations of samples based on standards.
16. For BCA analysis (total protein concentration), prepare standards using BSA at the following concentrations: 0, 0.2, 0.4, 0.8, 1, 1.2, and 2 μg/μL.
    a. Prepare final supernatant samples at following dilutions: 1:20 and 1:10. Run BCA according to BCA kit with samples and standards in triplicate (Pierce-Emsco).
    b. Use plate reader with absorbance of 562 nm to analyze samples. Calculate standard curve from standards and use to estimate total protein concentrations in samples.

Notes

- Western blot for 17,025 can be used to determine relative amounts of human tau in each step of sequential extraction—can compare to a loading control of recombinant T40 tau (2N4R) for estimated concentration of tau in final supernatant.
- Western blot for PHF-1 can be used to determine relative amounts of phosphorylated human tau in each step of extraction.
- Additional tau antibodies can be used for Western blot analysis: RD3 (Millipore) and 4R–tau (Cosmo Bio) to detect 3R and 4R isoforms of tau, respectively.
- Tau ELISA is used to determine tau concentration. Similar procedures can be used to estimate levels of Aβ and α-syn in final supernatant (see Table 2 for examples; Guo, Narasimhan, et al., 2016).
- Final tau purity for AD extractions ranges from 15% to 20%. Final tau yield can range from 100 to 200 μg of tau per 5–10 g of AD gray matter (Table 2).
- This method yields both high purity and high amounts of insoluble tau in the final supernatant, which is essential for mouse intracerebral injections described below.

Table 2 Characterization of Final Supernatant Fraction From Human Brain Extractions

Preparation No.	Concentration of Tau by ELISA (μg/mL)	Total Protein Concentration by BCA Assay (mg/mL)	Purity (tau/total protein, %)	Concentration of Aβ 1–40 by ELISA (μg/mL)	Concentration of Aβ 1–42 by ELISA (μg/mL)	Concentration of α-syn by ELISA (μg/mL)
AD1	1800	6.5	27.7	0.030	0.468	1.22
AD2	1600	9.7	16.5	0.019	0.349	1.08
AD3	1500	10	15.0	0.056	0.324	1.01
AD4	1270	8.3	15.3	Not detected	0.262	0.88
AD5	1360	12.5	10.9	0.054	0.261	0.95
AD6	1650	10.6	15.6	0.114	0.416	0.92
AD7	2300	12.6	18.3	0.071	0.069	4.95
AD8	1500	7.2	20.8	Not detected	0.066	0.86
Control 1	5	10.3	0.049%	Not detected	0.004	5.80
Control 2	2	12.2	0.016%	0.002	0.010	7.60

This table shows protein composition of representative final supernatant fractions following our purification protocol.
Reproduced from Guo, J. L., Narasimhan, S., Changolkar, L., He, Z., Stieber, A., Zhang, B., … Lee, V. M. (2016). Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice. Journal of Experimental Medicine, 213*(12), 2635–2654. doi:10.1084/jem.20160833.*

2.2.2 Transduction of nonTg primary hippocampal neurons

Materials

CD1 pregnant females (Charles River)
Papain (Worthington Biochemical Corporation)
Falcon cell strainer (BD)
poly-D-lysine 2 μg/μL (Sigma Aldrich)
Borate buffer (0.05 M boric acid, pH 8.5)
DE/DI Lonza water for tissue culture (Fisher)
Coverslips (Deckglaser, 12 mm No. 1)
Neuron media: Neurobasal medium (Invitrogen), 1% penicillin–streptomycin (Invitrogen), 1% L-glutamine (Glutamax, Invitrogen), 2% B27 (Thermo Fisher)
Hand-held probe for sonication (QSonica)
Methanol
Bovine serum albumin (BSA; Fisher)
Fetal bovine serum (FBS; Atlanta Biologicals)
Sterile Dulbecco's Phosphate-Buffered Saline (dPBS; Corning)
Fluoromount-G containing DAPI (SouthernBiotech)
Alexa-594 or 488 (Invitrogen)

1. Two days before plating of neurons, prepare coverslips as follows:
   a. Sterilize coverslips in methanol for 15–20 min.
   b. Let coverslips dry in 24-well plate for 15–20 min.
   c. Add 0.5 mL of poly-D-lysine (diluted to 100 μg/μL in borate buffer) to each coverslip.
   d. Wrap plate in parafilm and store for 24 h at room temperature to coat coverslips.
   e. Next day, add 1 mL Lonza water to each coverslip on top of poly-D-lysine. Aspirate all liquid from each well, and add another 1 mL Lonza water to each well. Repeat washes three more times (total of five washes).
   f. For last two washes, make sure to aspirate all the liquid from each well.
   g. Let coverslips dry at room temperature for at least 30 min.
   h. Add 0.5 mL of neuron media (see materials) and store in 37°C incubator until plating of neurons.
2. Prepare primary hippocampal neuron cultures by digesting hippocampal tissues from E16 to E18 embryos of CD1 mice in papain.
3. Triturate neurons and strain into single neurons using a Falcon cell strainer. Plate neurons on previously prepared coverslips (see step 1) at 100,000 cells per 24-well coverslip in neuron media (see *Materials*).
4. After 6 or 7 days in vitro, treat neurons with tau fibrils in final supernatant after sonication at 60 pulses using hand-held probe.
5. Perform immunocytochemistry 15 days after transduction as follows:
   a. Fix and extract neurons in ice-cold methanol for 15 min.
   b. Block neurons with 3% BSA and 3% FBS in sterile dPBS for 1 h at room temperature.

c. Incubate neurons with primary antibodies in blocking buffer overnight at 4°C: T49 1:1000 (mouse tau antibody; generated in-house) and MAP2 1:5000 (generated in-house).
d. The next day, incubate neurons with secondary antibody conjugated to Alexa-594 or 488 for 2 h at room temperature.
e. Mount coverslips onto glass slides using Fluoromount-G containing DAPI. Slides can be imaged on fluorescent microscope.

Notes

- Pathological tau around 0.5 μg/coverslip yields robust tau pathology, but can use as low as 0.15 μg/coverslip to detect tau aggregates on 24-well coverslips.
- T49 antibody distinguishes endogenous mouse tau aggregates from added human tau lysate, which often sticks to the coverslip and can still be visualized after extraction. Other tau antibodies that recognize both human and mouse tau can give a high background signal because of this.

2.3 TRANSMISSION OF HUMAN PATHOLOGICAL TAU IN NONTG MOUSE BRAIN

2.3.1 Intracerebral injection of human pathological tau into nonTg mouse brain

Materials

Hamilton syringe (5 μL, model 75 SN SYR cemented NDL)
C57Bl/6 Mice (Charles River)
Ketamine (100 mg/mL, controlled substance with DEA license needed)
Xylazine (20 mg/mL)
Stereotaxic frame (David Kopf instruments)
Betadyne

1. Prepare a Hamilton syringe with the human tau final supernatant (sonicated 30–60 pulses before injection) in 2.5 μL volume per site (5 μL total; see *Notes* for concentrations of insoluble tau to use).
2. Obtain 2–3 month-old C57Bl/6 mice and deeply anesthetize them with ketamine/xylazine mixture (100 and 10 mg/kg).
3. Immobilize the mice on a stereotaxic frame with preset coordinates for hippocampus and overlaying cortex (bregma: −2.5 mm; lateral: +2 mm; depth: −2.4 mm; and −1.4 mm from the skull).
4. Disinfect skin with betadyne solution and open head skin to expose skull. Drill holes according to coordinates for injection (see above; inject one side only).
5. Set the syringe to the coordinates above using the stereotaxic frame. Infuse the human lysate into the hippocampus first, then overlaying cortex over 2 min. Keep syringe in place for additional 5 min to prevent leakage of lysate.
6. Stitch the skin closed after surgery and keep mice warm until fully awake from anesthesia.

Notes

- Make sure final supernatant (human tau lysate) is fully homogenized with sonication (at least 30 pulses, add additional pulses if not homogeneous).
- For AD-derived insoluble tau, 0.4 μg/μL (1 μg/site) is the minimum concentration needed to induce significant mouse tau aggregates at 3 months postinjection. Higher concentrations yield more mature tau aggregates over time (up to 1.6 μg/μL or 4 μg/site has been tested).

2.3.2 Analysis of propagated tau aggregates using immunohistochemistry

Materials

Ketamine (100 mg/mL, controlled substance with DEA license needed)
Xylazine (20 mg/mL)
Acepromazine (10 mg/mL)
0.01 M PBS (made from 0.4 M PBS stock: Add monobasic solution from 110.4 g NaH_2PO_4 in 2 L of dH_2O to dibasic solution of 459.8 g Na_2HPO_4 in 7 L dH_2O to pH 7.3)
10% neutral-buffered formalin (NBF)
Precision Brain slicer (Braintree Scientific)
100% ethanol (to make subsequent ethanol dilutions)
Xylene (Fisher Scientific)
Paraffin wax (Leica Biosystems)
Methanol (Fisher Scientific)
Hydrogen peroxide (Fisher Scientific)
0.1 M Tris pH 7.6
0.1 M Tris pH 7.6 with 2% fetal bovine serum (FBS, Atlanta Biologicals)
Polymer detection kit-mega volume (Biogenex)
ImmPACT DAB (3,3′-diaminobenzidine) substrate kit (Vector Laboratories)—Note: DAB is a suspected carcinogen and appropriate care should be exercised when using (gloves, eye protection, lab coats, and good laboratory procedures)
Hematoxylin (Biogenex)

1. After designated periods of incubation, anesthetize mice using ketamine/xylazine/acepromazine mixture (4:2:1).
2. Intracardially perfuse mice with 30 mL of PBS at a rate of 120 mL/h. Remove the brain and spinal cord and fix in 10% NBF overnight at 4°C.
3. Slice mouse brain coronally into 2 mm pieces using a brain slicer and prepare for further processing in tissue processor (Thermo Electron) overnight using following protocol:
   a. 70% ethanol for 2 h at room temperature
   b. 80% ethanol for 1 h at room temperature
   c. 95% ethanol for 1 h at room temperature
   d. 95% ethanol for 2 h at room temperature
   e. 100% ethanol for 2 h at room temperature
   f. 100% ethanol for 2 h at room temperature

   g. 100% ethanol for 2 h at room temperature
   h. Xylene for 30 min at room temperature
   i. Xylene for 1 h at room temperature
   j. Xylene for 1.5 h at room temperature
   k. Wax for 1 h at 62°C
   l. Wax for 1 h at 62°C
   m. Wax for 1 h at 62°C
4. Embed the coronal pieces in paraffin wax in a mold for microtome sectioning. Section the whole brain at 6 μm coronal sections and place sections on glass slides to dry overnight. Number slides sequentially to keep track of every section. Slides can be stored at room temperature indefinitely.
5. Hydrate every 20th slide with tissue 2× in xylene for 5 min each and in 100%, 100%, 95%, 80%, and 70% ethanol for 1 min each. Incubate in dH_2O for 1 min.
6. Incubate slides in 150 mL methanol/30 mL hydrogen peroxide to quench the endogenous peroxidases for 30 min at room temperature.
7. Wash slides under running water for 10 min, then wash in 0.1 M Tris buffer (pH 7.6) for 5 min.
8. Block tissue in 0.1 M Tris/2% FBS for at least 5 min. Add primary antibodies of interest directly to slide and incubate in humidifying chamber overnight at 4°C.
   a. AT8 (p202/205): 1:10,000 (Thermo Scientific)
   b. AT180 (pT231): 1:1000 (Thermo Scientific)
   c. MC1 (conformation-specific antibody): 1:1000 (gift from Peter Davies)
   d. TG3 (conformation-specific antibody): 1:250 (gift from Peter Davies)
9. The next day, wash slides in 0.1 M Tris for 5 min, then block in 0.1 M Tris/2% FBS for 5 min.
10. Use polymer horseradish peroxidase detection system (Biogenex) as follows: add enhancer directly to tissue on slides and incubate for exactly 20 min.
11. Wash slides in 0.1 M Tris for 5 min, then block in 0.1 M Tris/2% FBS for at least 5 min.
12. Add poly-HRP from Biogenex system directly onto slides and incubate for exactly 30 min.
13. Wash slides in 0.1 M Tris for 5 min. Then add DAB directly onto slides and incubate for exactly 8 min. A brown color should develop where antibody has bound detecting tau pathology.
14. Wash slides in 0.1 M Tris for 5 min. Then add hematoxylin directly onto slides and incubate for 1 min per slide.
15. Wash slides in running tap water for 10 min to remove excess hematoxylin. Then dehydrate slides as follows: 70%, 80%, 95%, 95%, 100%, and 100% ethanol for 1 min each, then 2× in xylene for 5 min each.
16. Coverslip slides with Cytoseal and let dry overnight. Slides can be imaged using Brightfield microscopy or scanned using Lamina™ Multilabel Slide Scanner (PerkinElmer). Refer to Fig. 1B for representative images from scanned slides of IHC for AT8.

Notes

- Be careful not to damage any part of brain when taking out of skull after perfusion.
- Sectioning through the whole brain allows for preservation of all brain regions, which is useful later for spatial analysis of tau pathology. Staining every 20th section gives an initial overview of regions with pathology, which can later be identified for further quantification.
- Biogenex system provides better staining and detects more tau-positive pathology. However, it is crucial to keep the timings accurate, particularly on the second day of staining.

3 CONCLUSION

Here we described a method to demonstrate transmission of tau pathologies in a nonTg mouse brain using human AD brain-derived pathological tau. We described in detail our sequential extraction method, which improves upon the purity and yield of insoluble tau in our final fraction. This is necessary to have sufficient quantity of pathological tau to induce pathology in nonTg mice, which we hypothesize is one of the main factors why previous studies have been unsuccessful in inducing significant tau aggregates in nonTg mice. Our method extracts a particular tau strain capable of seeding endogenous mouse tau aggregation in a nonTg mouse brain, thereby developing a sporadic model of tauopathy. The resulting lysate is also capable of seeding in nonTg primary neurons, a tool which can now be used to screen tau-directed therapies. In principle, this method could be applied to other tauopathies to study all human tau strains.

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