Supplementary online material to: Tau pathology associated with parkinsonism and mutation of mitochondrial DNA helicase gene TWNK

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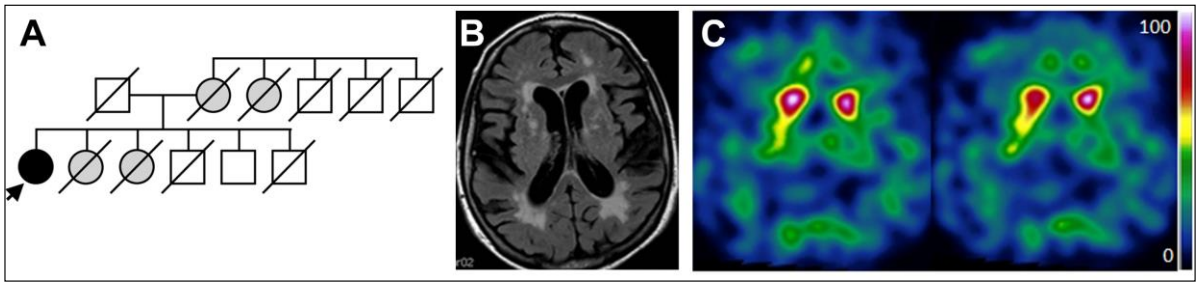
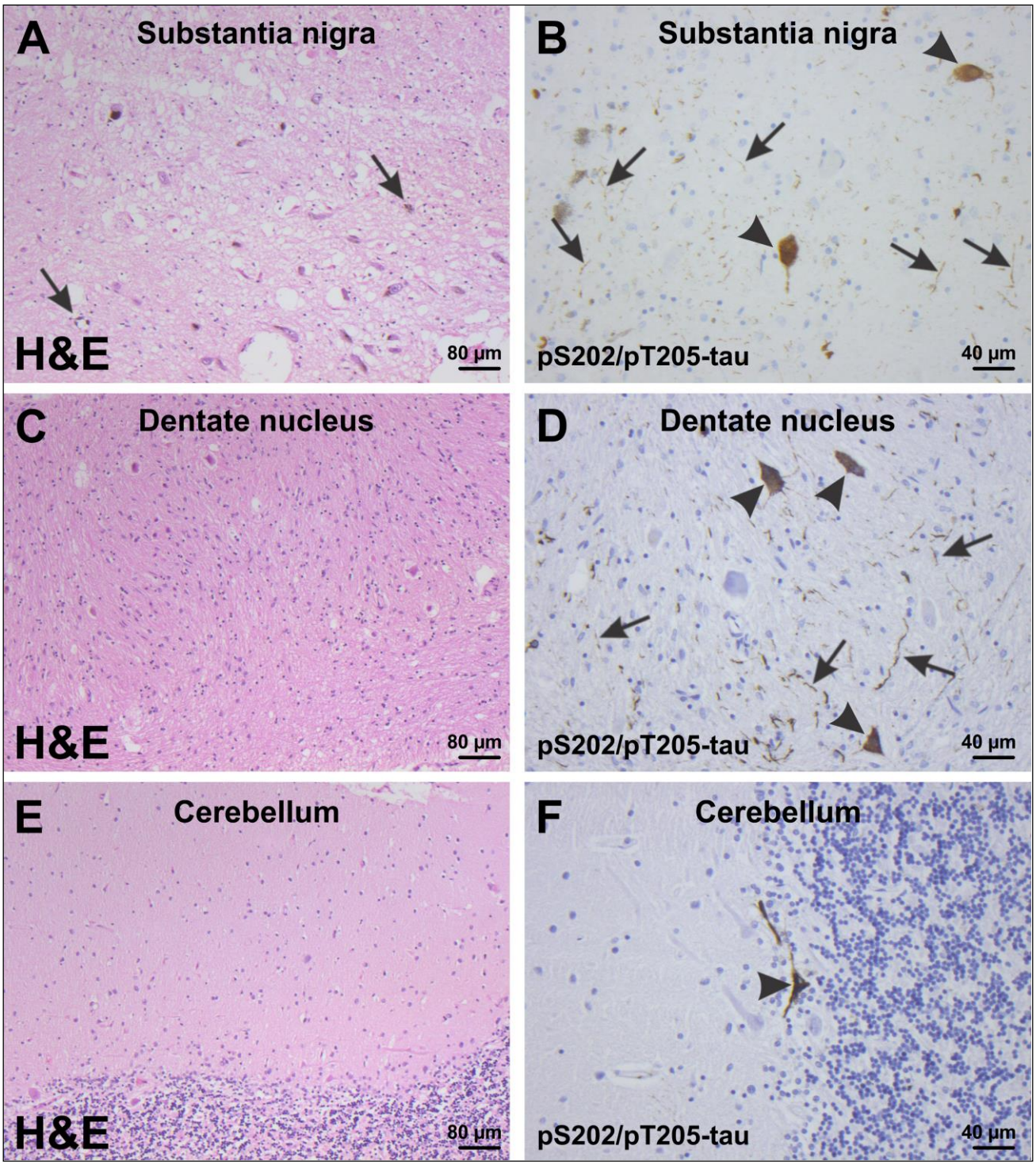
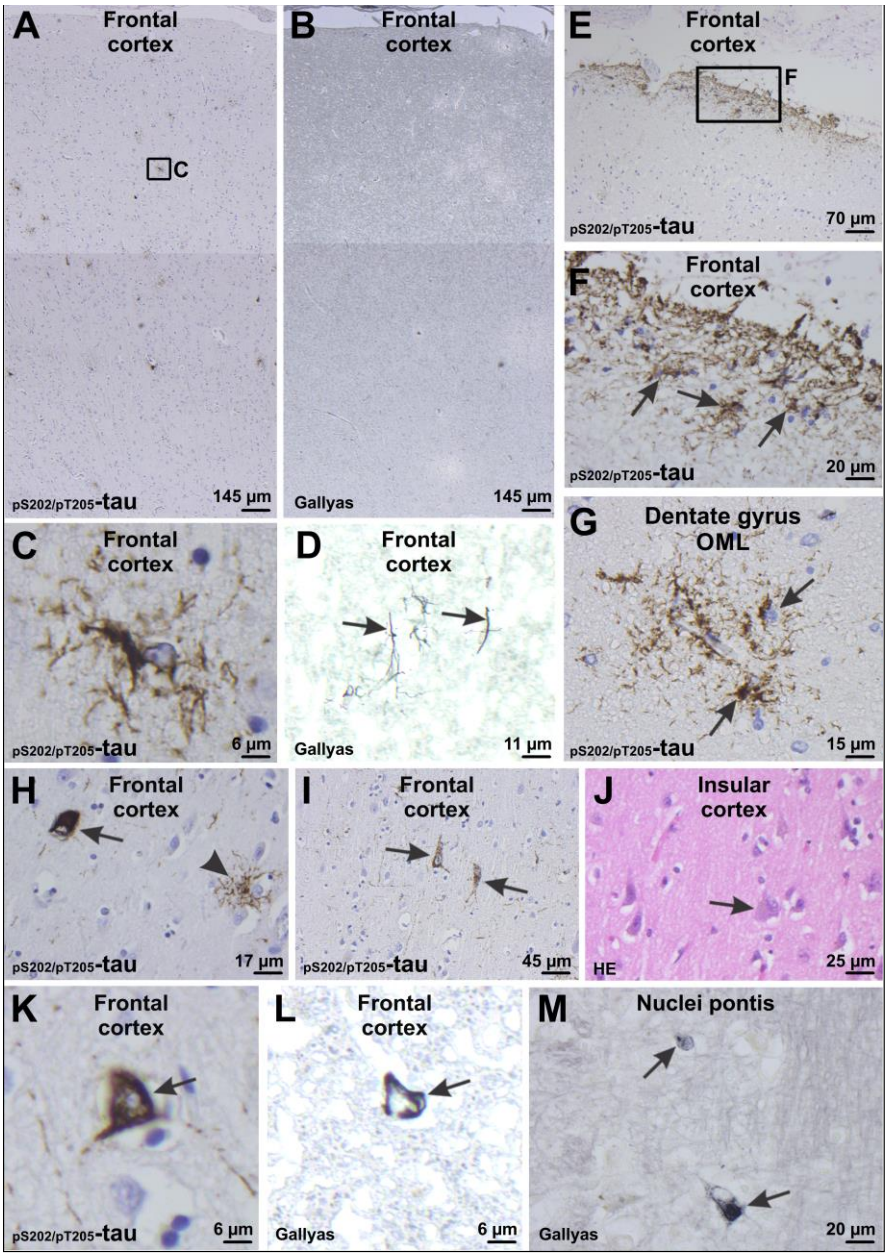


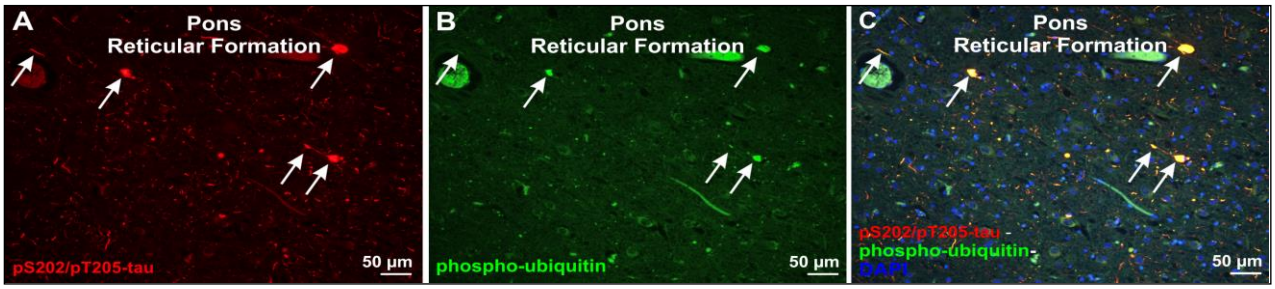
sFigure e-1. Pedigree and brain imaging in the *TWNK* mutation carrier. (A) Pedigree. Squares and circles denote males and females, respectively. Grey symbols indicate individuals with bilateral ptosis, black symbols indicate individuals with both bilateral ptosis and parkinsonism. The arrow indicates the proband. (B) Axial fluid-attenuated inversion recovery (FLAIR) MRI. (C) ^{123}I-FPCIT DAT SPECT. Transverse midstriatal slices with the color bar representing relative uptake.



sFigure e-2. Neuron loss and its associated p-tau pathology. (A) The H&E staining of the substantia nigra indicates a substantially reduced neuronal density, extraneuronal pigment (arrows) and a mild astrogliosis. (B) The anti-pS202/pT205-tau immunostaining shows numerous threads (arrows) and two tau-positive neurons (arrowheads). (C) The dentate nucleus exhibits severe neuronal loss and astrogliosis. (D) Anti-pS202/pT205-tau stained several dentate nucleus neurons (arrowheads) and multiple threads (arrows). (E) Focally, the cerebellum shows a reduced number of Purkinje cells. (F) Single Purkinje cells were pS202/pT205-tau positive (arrowhead) and showed a degenerated dendritic tree. The immunohistochemical staining methods are summarized in sTable e-2.



sFigure e-3. Different types of neuronal and astroglial p-tau pathology. (A) The overview indidates the presence of mild-moderate tau pathology in the frontal neocortex stained with anti-pS202/pT205-tau. (B) In the Gallyas-silver staining most tau lesions were inapparent. (C) In the pS202/pT205-tau staining tufted astrocytes were observed (the frame in (A) indicates which area was enlarged). (D) Astrocytic changes were not convincingly evident in the Gallyas staining. Here only some grouped threads could be observed (indicated by arrows). (E, F) In the subpial zone of the frontal cortex spots of thorn-shaped subpial astrocytes were observed in the pS202/pT205-tau staining. (F) is a high-power view of the framed area in (E). The arrows in (F) indicate thorn-shaped astrocytes. (G) In addition to subpial thorn-shaped astrocytes we also found perivascular thorn-shaped pS202/pT205-tau positive astrocytes in the outer molecular layer (OML) of the dentate gyrus. Here, tau-positive astroglial endfeet were seen attached to the blood vessels. (H-I) pS202/pT205-tau positive neurons with an enlarged, ballooned cytoplasm in the frontal cortex (arrows). These neurons appear also to be bigger than the neighbouring neurons. Therefore, we consider these neurons as most-likely representing ballooned neurons. The Gallyas staining did not show similar morphologies in marked neurons. The arrowhead in (H) indicates a plaque-like lesion, presumably related to a tau-positive astrocyte. (J) In the H&E staining we can identify single neurons that appear to be enlarged compared to their neighbouring cells. However, these neurons do not have the typical appearance of ballooned neurons as expected in cases of corticobasal degeneration. Here, an example (arrow) is shown from the insular cortex. (K, L) Neurofibrillary tangle (NFT, indicated by arrow) of the frontal cortex. The pS202/pT205-tau antibody strongly marked the NFT. The Gallyas silver staining confirms the fibrillar nature of the NFT. (M) NFTs detected with the Gallyas silver staining method (arrows) were also observed in the neurons of the nuclei pontis. The immunohistochemical staining methods are summarized in Table e-2.



sFigure e-4. Partial colocalization of tau and phospho-ubiquitin in neurons in the pontine reticular formation in the *TWNK* mutation carrier. Neurofibrillary tangles and threads exhibiting pS202/pT205-tau (A) colocalized with phospho-ubiquitin (B). Merged images are shown in (C).

Table e-1: Distribution of tau pathology

Pathology Location		Types of inclusions	Severity of tau pathology (anti-pS202/pT205-tau)	Neuron loss
Frontal cortex	*Layers I-VI*	Threads, NFTs, pretangles, plaque-like astrocytic lesions, single tufted astrocytes, single ballooned neurons*, subpial and perivascular thorn-shaped astrocytes	2	no
Parietal cortex	*Layers III, V, VI*	Threads, NFTs, pretangles	1	no
Temporal cortex	*Layers II-VI*	Threads, NFTs, pretangles	1	no
Occipital cortex	-	No τ pathology	0	no
Entorhinal cortex	*Layers pre α - priy*	Threads, NFTs, pretangles	3	mild-moderate (pre-α)
Insular cortex	*Layers I-VI*	Threads, NFTs, pretangles	1	no
Hippocampus - subiculum/CA1	*Mainly CA1*	Threads, NFTs, pretangles	3	no
Hippocampus - CA4	*CA4*	Single threads, NFTs, pretangles	1	no
Dentate Gyrus	*Granule cells, OML*	NFTs, pretangles, threads, perivascular thorn-shaped astrocytes	1	no
Cerebral white matter	*frontal, temporal, fornix*	Coiled bodies	1	n.a.
Amygdala	*Amydalo-hippocampal transition area*	NFTs, pretangles	2	no
Basal nucleus of Meynert	*NBM*	Single NTFs, pretangles, tufted astrocytes, perivascular thorn-shaped astrocytes	1	no
Globus pallidus	*Globus pallidus*	Threads, NFTs, pretangles	1	no
Putamen	*Putamen*	Few threads, NFTs, pretangles	1	no
Caudate nucleus	*Caudate nucleus*	Threads, tufted astrocytes, single ballooned neurons*	2	no
Hypothalamus	*Mammillary body, supraoptic nucleus, paraventricular nucleus, lateral hypothalamic area*	NFTs, pretangles, threads	2	no
Thalamus	*Thalmus at the level of the lateral geniculate body*	Few threads, NFTs, pretangles, tufted astrocytes	1	no
Substantia nigra	*Pars compacta*	Threads, NFTs, pretangles	3	severe
Raphe nuclei	*oral raphe group*	Threads, NFTs, pretangles	1	no
Nucleus NIII		Threads, NFTs, pretangles	2	no
Central gray matter		Threads, NFTs, pretangles	2	no
Inferior colliculus		Threads, NFTs, pretangles	3	no
Locus coeruleus		Threads, NFTs, pretangles	3	mild-moderate
Nuclei pontis		Threads, NFTs, pretangles	3	no
Parabrachial nuclei		Threads, NFTs, pretangles	1	no
Reticular medial longitudinal fasciculus (RMLF)		Threads, coiled bodies	1	n.a.
Nucleus NX		Threads, NFTs, pretangles	2	no
Nucleus NXII		Threads, NFTs, pretangles	1	no
Intermediate reticular zone		Threads, NFTs, pretangles	2	no
Inferior olivary nucleus		Threads, NFTs, pretangles	1	no
Cerebellar cortex	*Granule cell layer, molecular layer*	Few tufted astrocytes, threads	1	no
Cerebellar cortex	*Purkinje cell layer*	Single pretangles	1	mild-moderate
Dentate nucleus	*Dentate nucleus*	Threads, NFTs, pretangles, tufted astrocytes	2	severe
Cerebellar white matter		Coiled bodies, threads	2	n.a.
Spinal cord	*Mainly anterior horn*	Threads, few NFTs, few pretangles, few tufted astrocytes	1	no

Abbreviations: OML = outer molecular layer; NFT = neurofibrillary tangle; HPF = high power field with the 40 x objective; n.a.: fiber tracts and white matter were not assessed for neuron loss.

Semiquantitative rating scale: 0 = no pathology; 1 = mild pathology (no more than 3 lesions/HPF); 2 = moderate pathology (4-10 lesions/HPF); 3 = severe pathology (>10 lesions/HPF); NFTs, tufted astrocytes, plaque-like astrocytic lesions, ballooned neurons, and coiled bodies were considered as separate lesions - the presence of one or more threads was considered as one single lesion in addition to the other lesions.
Neuron loss was determined semiquantitatively at the basis of H&E stained sections: no = no evident neuron loss, mild-moderate = mild-moderately reduced density of neurons with reactive microglia resorbing neuronal debris, severe = only few or no remaining neurons and astrgliosis.

*Ballooned neurons were diagnosed on the basis of pS202/pT205-tau immunostainings indicating tau-positive neurons with a ballooned cytoplasm and a displaced nucleus. The few ballooned neurons were difficult to find in H&E stainined sections. A parallel section stained with the Gallyas silver method did not show these cells (confirmed in the frontal cortex).

Table e-2: Immunohistochemical characterization of pathology

Epitope	Stained neuropathological lesions observed in this case report	Antibody/staining description (host, clone/polyclonal, producer, working dilution, pretreatment if required)/(citation)
pS202/pT205-tau (AT8)	threads, neuronal inclusions (pretangles, NFTs, ballooned neurons), plaque-like astrocytic lesions, tufted astrocytes, perivascular and subpial thorn-shaped astrocytes, oligodendroglial coiled bodies	Mouse; Clone AT-8, Thermo-Scientific – Pierce Biotechnology, Rockford, IL, USA, 1/1,000
pS396/pS404-tau (PHF1)	threads, neuronal inclusions (pretangles, NFTs, ballooned neurons), plaque-like astrocytic lesions, subpial thorn-shaped astrocytes, oligodendroglial coiled bodies	Mouse; clone PHF1, gift from Dr. P. Davies, New York; 1:500
pT231-tau (AT180)	threads, neuronal inclusions (pretangles, NFTs, ballooned neurons), neuronal intracytoplasmic pT231-τ, plaque-like astrocytic lesions, subpial thorn-shaped astrocytes, oligodendroglial coiled bodies	Mouse; Clone AT180; Thermo Fisher cat#MN1040; 1:200
Acetylated tau	single threads, neuronal inclusions (NFTs), coiled bodies	Rabbit; MAB359; Gladstone Institutes; 1/300, formic acid and citrate (pH=6) heat induced epitope retrieval (Tracy et al. 2016)
Conformationally modified tau (MC1)	threads, neuronal inclusions (pretangles), plaque-like astrocytic lesions, oligodendroglial coiled bodies	Mouse; Clone MC1, gift from Dr. P. Davies, New York; 1:100
Gallyas silver stainig	threads, NFTs, frontal plaque-like grouped threads	Braak & Braak 1991
3-repeat tau	single threads and neuronal inclusions (NFTs)	Mouse; 3rp-τ; Clone RD3, 8E6/C11, Millipore, Temecula, CA, USA, 1/500, formic acid and citrate (pH=6) heat induced epitope retrieval
4-repeat tau	threads, neuronal inclusions (pretangles), plaque-like astrocytic lesions, oligodendroglial coiled bodies	Mouse; 4rp-τ; Clone RD4, 1E1/A6, Millipore, Temecula, CA, USA, 1/1000, formic acid and citrate (pH=6) heat induced epitope retrieval
Ubiquitin	frontal no inclusions, in the brainstem single threads and neuronal inclusions	Polyclonal rabbit; Ubiquitin, DAKO, Glostrup, Denmark, 1/100
phospho-ubiquitin	single NFTs and single threads	Polyclonal rabbit; Anti-phospho-ubiquitin (pSer65); Millipore cat#ABS1513-I, Temecula, CA, USA; 1/500, citrate (pH=6) heat induced epitope retrieval
TDP-43	single neuronal inclusion and granulovacuolar degeneration in the CA1/subiculum region	Polyclonal rabbit; pS409/410-2, Cosmo Bio Co., Ltd, Tokyo, Japan, 1/10000, citrate (pH=6) heat induced epitope retrieval
α-synuclein	no inclusions	Mouse, Clone 5G4, Merck-Millipore, Temecula, CA, USA, 1/2000, citrate (pH=6) heat induced epitope retrieval
Aβ	amyloid plaques, CAA-type 2 (Thal et al. 2002)	Mouse; Clone 4G8, Covance, Dedham, USA, 1/5000, citrate (pH=6) heat induced epitope retrieval and formic acid pretreatment

Abbreviations: TDP-43 = transactive response DNA-binding protein TDP-43; Aβ = amyloid-β peptide; NFT = neurofibrillary tangle; CAA = cerebral amyloid angiopathy

References: **Braak, H. and E. Braak** (1991). "Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections." *Brain Pathol* 1(3): 213-216; **Thal, D. R., E. Ghebremedhin, U. Rüb, H. Yamaguchi, K. Del Tredici and H. Braak** (2002). "Two types of sporadic cerebral amyloid angiopathy." *J Neuropathol Exp Neurol* 61(3): 282-293; **Tracy, T. E., P. D. Sohn, S. S. Minami, C. Wang, S. W. Min, Y. Li, Y. Zhou, D. Le, I. Lo, R. Ponnusamy, X. Cong, B. Schilling, L. M. Ellerby, R. L. Huganir and L. Gan** (2016). "Acetylated Tau Obstructs KIBRA-Mediated Signaling in Synaptic Plasticity and Promotes Tauopathy-Related Memory Loss." *Neuron* 90(2): 245-260.