Journal of Neurochemistry

WILEY

SPECIAL ISSUE History, biology and pathobiology of prions: A field of renewed hopes

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry

REVIEW OPEN ACCESS

Limitations and Applications of Rodent Models in Tauopathy and Synucleinopathy Research

Emma Szegvari | Sara A. M. Holec | Amanda L. Woerman

Department of Microbiology, Immunology, & Pathology and Prion Research Center, Colorado State University, Fort Collins, Colorado, USA

Correspondence: Amanda L. Woerman (amanda.woerman@colostate.edu)

Received: 22 December 2024 | **Revised:** 1 February 2025 | **Accepted:** 8 February 2025

Funding: This work was supported by Rainwater Charitable Foundation (23010093) and National Institute of Neurological Disorders and Stroke (R01NS121294, R21NS127002); National Institute on Aging (R01AG071694 and R01AG082349).

Keywords: alpha-synuclein | animal models | neurodegenerative disease | tau

ABSTRACT

Rodent models that accurately recapitulate key aspects of human disease have long been fundamental to the successful development of clinical interventions. This is greatly underscored in the neurodegenerative disease field, where preclinical testing of anti-prion therapeutics against rodent-adapted prions resulted in the development of small molecules effective against rodent-adapted prions but not against human prions. These findings provided critical lessons for ongoing efforts to develop treatments for patients with neurodegenerative diseases caused by misfolding and accumulation of the proteins tau and α-synuclein, or tauopathies and synucleinopathies, respectively. To avoid the potential pitfalls previously identified in the prion field, this review focuses on rodent models currently available to study tau and α-synuclein disease pathogenesis, emphasizing the strengths and limitations of each with the particular goal of better supporting preclinical research.

1 | Introduction

Most neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD), are caused by the misfolding and accumulation of proteins within the brain. Each neurodegenerative disorder is neuropathologically defined both by which protein or proteins have misfolded, as well as the type and distribution of the resulting neuropathological lesions. For example, AD patients develop both extracellular β-amyloid (Aβ) plaques (Glenner and Wong 1984a, 1984b) and intracellular neurofibrillary tangles (NFTs) made of microtubule-associated protein tau (MAPT), or tau (Brion et al. 1985), whereas PD patients develop Lewy bodies (LBs) and Lewy neurites (LNs) made of misfolded α-synuclein (Polymeropoulos et al. 1997; Spillantini et al. 1997). Interestingly, while Alois Alzheimer's seminal report using Bielshowsky silver stain to detect what we now know are NFTs in the brain was published in 1911 (Alzheimer 1911), it was not until 1984 that George Glenner and Colin Masters first isolated Aβ plaques from post-mortem AD patient samples

Abbreviations: 3R, 3-repeat tau; 4R, 4-repeat tau; aa, amino acid; AD, Alzheimer's disease; AD/ALB, Alzheimer's disease with amygdala predominant Lewy bodies; AGD, argyrophilic grain disease; APP, amyloid precursor protein; ARTAG, aging-related tau astrogliopathy; Aβ, β-amyloid; BAC, bacterial artificial chromosome; BM, Barnes maze; CBD, corticobasal degeneration; CJD, Creutzfeldt-Jakob disease; CNP, cyclic nucleotide phosphodiesterase; Cryo-EM, cryo-electron microscopy; CTE, chronic traumatic encephalopathy; DLB, dementia with Lewy bodies; DSAD, Down syndrome Alzheimer's disease; EPM, elevated plus maze; fFTLD-P301L, familial frontotemporal lobe dementia with the P301L mutation; FTLD, frontotemporal lobar degenerative diseases; GCI, glial cytoplasmic inclusion; GGT, globular glial tauopathy; Hemi, hemizygous; HOZ, homozygous; HP, hot plate test; i.c., intracranial; KO, knockout; LB, Lewy body; LBD, Lewy body disease; LN, Lewy neurite; MAPT, microtubule associated protein tau; MBP, myelin basic protein; mpi, months post-inoculation; MSA, multiple system atrophy; MSA-C, multiple system atrophy-cerebellar; MSA-P, multiple system atrophy-parkinsonian; MWM, Morris water maze; NFT, neurofibrillary tangle; NOR, novel object recognition; n.p., not published; OFWM, open field water maze; PAC, P1 artificial chromosome; PAG, periaqueductal gray; PART, primary age-related tauopathy; PD, Parkinson's disease; PDD, Parkinson's disease with dementia; PFF, preformed fibril; PiD, Pick's disease; PLP, proteolipid protein; PPI, prepulse inhibition; PrP^{C}, cellular prion protein; PrP^{Sc}, scrapie prion protein; PSEN1, presenilin-1; PSEN2, presenilin-2; PSP, progressive supranuclear palsy; PVN, paraventricular nucleus of the thalamus; RD, repeat domain; SRM, social recognition memory; TALEN, transcription activator-like effector nuclease; TD, tangle-only dementia; wpi, weeks post-inoculation; WT, wild-type.

Emma Szegvari and Sara A. M. Holec contributed equally to the manuscript.

(Glenner and Wong 1984a, 1984b). One year later, Jean-Pierre Brion showed that the NFTs in AD patients were made of the protein tau (Brion et al. 1985). Similarly, while James Parkinson first described what we now refer to as PD in 1817 (Parkinson 1817), LBs were not described until 1912 (Forster and Lewy 1912). Moreover, the presence of α-synuclein in LBs was not discovered until 1997, through both genetic linkage and immunohistochemistry (Polymeropoulos et al. 1997; Spillantini et al. 1997).

In the intervening time between the first reports of these diseases and our current understanding of the role of misfolded protein aggregates in their etiologies, several clinical strategies were developed and tested with varied success. For example, due to the loss of cholinergic neurons in AD patients (Davies and Maloney 1976), acetylcholinesterase inhibitors—donepezil, rivastigmine, galantamine—emerged as AD therapeutics with the goal of increasing the amount of acetylcholine signaling in the brain. A similar approach for PD patients targeting the lost dopaminergic neurons in the substantia nigra uses levodopa, or L-Dopa, to replace the decreased dopamine signaling. Importantly, these drugs are symptom-alleviating but not disease-modifying. As mounting research has shown that proteins like Aβ, tau, and α-synuclein use the prion mechanism of disease (discussed in (Prusiner 2017)), in which a misfolded protein fibril serves as a template to subsequently induce misfolding of additional proteins, novel therapeutic strategies that target this underlying disease pathogenesis have been the primary focus of drug development. Here, the use of antibodies and small molecules that interfere with the self-templating process, an array of gene therapy approaches that reduce the amount of normal protein available as substrate for misfolding, and proteolysis targeting chimeras (PROTACs) that target fibril degradation via the ubiquitin-proteosome system (Békés et al. 2022) are only some of the strategies currently in preclinical or clinical testing, or in the case of Aβ antibodies, available in the clinic.

Given the current focus of therapeutic strategies for neurodegenerative diseases, it is imperative for the field to learn from key failures identified during efforts to develop small molecules for prion diseases, disorders in which protein misfolding and aggregation are the major, if not sole, drivers of disease. In prion diseases, the cellular form of the prion protein (PrP^{C}) misfolds into the pathogenic conformation (PrP^{Sc}). Subsequent conversion of PrP^{C} into PrP^{Sc} occurs at the catalytic surface of misfolded amyloid fibrils, resulting in the progressive spread of disease throughout the brain. Interestingly, the most common prion disease impacting humans—Creutzfeldt Jakob Disease (CJD) – transmits neurological disease to humanized mice (Telling et al. 1994); however, all attempts to develop robust cell culture systems for CJD prions have failed. As a result, in vitro screens for drug discovery efforts have relied upon animal prions, most notably the mouse-adapted prion RML (isolated by transmitting sheep scrapie to mice at Rocky Mountain Laboratories). For example, in 2010, a small molecule screen for anti-prion compounds against RML led to the discovery of a group of 2-amino-thioazoles, including the molecule IND24, with potent inhibitory effects (Ghaemmaghami et al. 2010). Subsequent studies testing these compounds found that IND24 treatment doubles the lifespan of mice inoculated with RML prions (Berry et al. 2013); however, treatment had no effect in mice inoculated with CJD prions (Giles et al. 2017).

This devastating setback carries with it a critical lesson for therapeutic development for all protein misfolding diseases. While CJD and RML both feature the misfolding of PrP^{C} into PrP^{Sc}, each disease is caused by a distinct misfolded conformation or disease strain. Initial evidence for the presence of distinct disease-causing strains was seen via differences in incubation periods, neuropathological lesion profiles, clinical signs, and biochemical stabilities of PrP^{Sc} fibrils (Bessen et al. 1995; Bessen and Marsh 1992; Caughey et al. 1998; Telling et al. 1996), but more recent breakthroughs in cryo-electron microscopy (cryo-EM) have confirmed that PrP^{C} misfolds into a distinct aggregate conformation in each disease (Alam et al. 2024; Hallinan et al. 2022; Hoyt, Alam, et al. 2022; Hoyt, Standke, et al. 2022; Kraus et al. 2021; Manka et al. 2023; Manka et al. 2022). These structural findings provide definitive support for the strain hypothesis, which posits that the conformation PrP^{C} misfolds into determines which disease a human or animal will develop. In reflecting on the outcomes from the IND24 studies, the lesson becomes quite clear: it is not enough to simply focus on targeting the protein associated with disease, but efforts must also focus on the PrP^{Sc} strain present in the target patient population.

Beginning in 2017, cryo-EM studies have also provided clear support for the role of the strain hypothesis in patients with tau- and α-synuclein-driven diseases, or tauopathies and synucleinopathies, respectively. A series of studies from Michel Goedert and Sjors Scheres have shown that tau adopts distinct fibril conformations in patients with AD (Fitzpatrick et al. 2017), Pick's disease (PiD) (Falcon et al. 2018), chronic traumatic encephalopathy (CTE) (Falcon et al. 2019), corticobasal degeneration (CBD) (Zhang et al. 2020), argyrophilic grain disease (AGD), progressive supranuclear palsy (PSP), globular glial tauopathy (GGT), and GGT-PSP-Tau (Shi et al. 2021). Similarly, distinct α-synuclein conformations were identified in patients with multiple system atrophy (MSA) (Schweighauser et al. 2020; Yan et al. 2024), PD (Yang et al. 2022), and juvenile-onset synucleinopathy (Yang et al. 2023). Recognizing the ever-growing parallels between prion diseases and other protein misfolding disorders, it is critical that ongoing and future drug discovery efforts targeting proteins like tau and α-synuclein recognize and learn from the mistakes made during the discovery of IND24.

With this objective in mind, here we focus on the strengths and limitations of the currently available rodent models for preclinical studies supporting therapeutic development targeting tau and α-synuclein misfolding. The drug discovery process is lengthy and filled with several opportunities for failure to occur. However, a major component of successfully translating a treatment from bench to bedside is the predictive nature of the animal model system used to support in vivo efficacy testing (Figure 1). Programs in which the model system used provides the best link to human biology are undoubtedly more likely to succeed. By building a stronger bridge to the clinical target, drug discovery programs can de-risk that final translational jump by employing model systems that accurately recapitulate the desired aspects of human disease. In the following sections, we will emphasize the strengths that each currently available rodent model for tau and α-synuclein offers, while also

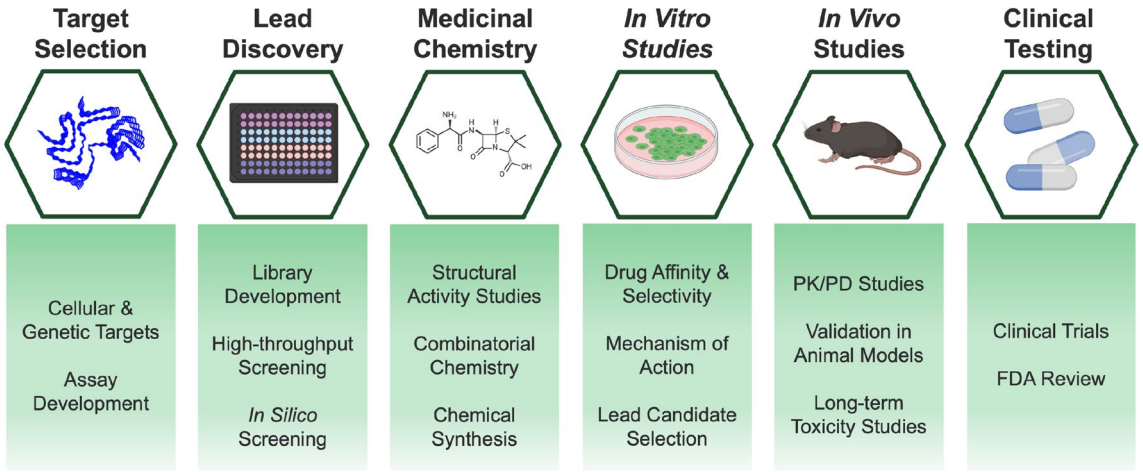

FIGURE 1 | Steps in the drug discovery process. Preclinical testing to support a drug discovery program begins with target selection and assay development, which can either be in a cell-free system or an in vitro cellular model. Lead discovery begins with high-throughput screening of compound libraries and may include *in silico* screening. Data from high-throughput screens is used by medicinal chemists to test structure–activity relationships and test combinatorial chemistry. Additional in vitro studies are then used to determine compound mechanism of action and select lead candidates. Lead candidates are further refined in pharmacokinetic (PK)/pharmacodynamic (PD) studies before in vivo efficacy testing and toxicity studies can proceed. Successful candidate molecules are then advanced into clinical testing prior to submission for FDA review. Made using Biorender.com.

underscoring their major drawbacks as well as opportunities for advancement to better support drug discovery for tauopathy and synucleinopathy patients.

2 | Rodent Models to Investigate Human Tauopathies

2.1 | Variability of Tau Pathology in Human Patients

Tau is an intracellular, soluble protein that is critical for polymerizing and stabilizing microtubules in neurons (Cleveland et al. 1977; Iqbal et al. 1986). Alternative splicing of exons in the tau gene (*MAPT*) gives rise to 6 different isoforms ranging from 352 to 441 amino acids, which can include 0, 1, or 2 N-terminal insertions and either 3 or 4 repeats in the repeat domain (RD) of the protein (Goedert et al. 1992, 1989). Expression of these 6 isoforms varies by age and brain region, with a notable shift from 0N3R expression during infancy to a more balanced 3R:4R expression in adulthood (Goedert and Jakes 1990; Hefti et al. 2018; Majounie et al. 2013; Trabzuni et al. 2012). In the disease state, misfolded tau forms neuropathological inclusions that can be distinguished by type (e.g., NFTs versus coiled bodies), distribution in the brain, and isoform composition.

As previously noted, tau was first associated with dementias after it was isolated from brain tissue from both familial and sporadic AD cases in the 1980s (Brion et al. 1985; Kosik et al. 1986; Wood et al. 1986). The NFTs in AD patients contain a mixture of both 3R and 4R tau isoforms (Goedert et al. 1992) and are found in the presence of Aβ plaques. While *MAPT* mutations are not seen in the subset of AD cases that are familial in etiology, mutations do occur in the genes for the amyloid precursor protein (APP), which is cleaved to form Aβ, and in presenilin-1 and -2 (PSEN1 and PSEN2), which are involved with APP cleavage (Goate et al. 1991; Levy-Lahad et al. 1995; Rogaev et al. 1995; Sherrington et al. 1995). Mutations in these genes are associated with 5%–10% of familial early onset Alzheimer's disease (< 60 years old) and are highly penetrant (Conidi et al. 2015; Cruts et al. 1998; Gatz et al. 2006; Kosik et al. 2015; Lanoiselee et al. 2017; Parker et al. 2019; Rovelet-Lecrux et al. 2006; Sala Frigerio et al. 2015; Wingo et al. 2012). In general, NFT pathology is typically first seen within the hippocampus and entorhinal cortex, with subsequent spread to the fronto-temporal cortices. Along with tau pathology, AD patients also exhibit substantial synapse and neuron loss (Braak and Braak 1991). Notably, familial and sporadic AD patients are neuropathologically similar but differ in the age of disease onset.

NFTs made of both 3R and 4R tau isoforms were also identified in patients with punch drunk syndrome or dementia pugilistica, which results from repeated head trauma (Martland 1928; Millspaugh 1937; Schmidt et al. 2001). Now known as chronic traumatic encephalopathy (CTE), this disease has overlapping pathologies with AD. CTE is defined by both NFTs and astrocytic tau tangles that cluster around blood vessels in the sulcal depths of the cortex. Aβ plaque deposition is seen in the late stages of the disease in 43% of older patients, while TDP-43 neuronal cytoplasmic inclusions are seen in patients several years before death (Bieniek et al. 2021; McKee et al. 2016, 2023, 2013). For example, in a study of American football players in 2017, 91% of players with stage IV CTE had Aβ deposition and 83% had TDP-43 pathology (Mez et al. 2017).

In contrast with AD and CTE, tauopathies known as frontotemporal lobar degenerative diseases (FTLDs) are defined by the presence of only 3R or 4R tau isoforms in the brain. These include the 3R tauopathy PiD, as well as the 4R tauopathies AGD, CBD, GGT, and PSP. Most of these diseases were first identified as tauopathies by linking mutations in *MAPT* to patients with each disease, though GGT was identified as a unique sporadic tauopathy in 2001 (Bigio et al. 2001; Buée et al. 2000; Hutton et al. 1998; Poorkaj et al. 1998). PiD is characterized by distinct tau inclusions

termed Pick bodies and exhibits frontotemporal lobe atrophy, severe neuron loss, and gliosis (Constantinidis et al. 1974). By comparison, AGD is characterized by atrophy of the ambient gyrus in the medial temporal lobe and the presence of tau accumulating into Gallyas-positive argyrophilic grains along with pretangles, coiled bodies within glia, and astrocytic tau inclusions (Tolnay and Clavaguera 2004). CBD patients are differentiated by the presence of astrocytic plaques versus astrocytic tufts in PSP, as well as a greater white matter and forebrain involvement in CBD compared to a more dominant hindbrain and subcortical NFT component in PSP (Dickson 1999; Dickson et al. 2002; Hauw et al. 1994). Finally, there are three neuropathologically distinct subtypes of GGT defined by the region-specific presence of non-argyrophilic 4R tau-immunoreactive globular astroglial inclusions along with argyrophilic 4R tau-immunoreactive globular oligodendroglial inclusions (Ahmed et al. 2013; Kon et al. 2019).

Despite the fact that each tauopathy differs in its associated pathologies, the underlying pathogenic mechanism is thought to be due to misfolding and spreading of tau throughout the central nervous system (Prusiner 2012; Williams 2006). Given this shared disease pathogenesis, animal models used to study tauopathies and support preclinical research efforts have historically relied on overexpression of a single isoform of the human tau protein [either mutant or wild-type (WT)]. However, given the complexity and variability seen in human disease, a major challenge for the field is developing model systems capable of recapitulating the same variability seen in the target patient population. In the following sections, we discuss key lessons the field has learned from frequently used rodent models, as well as their limitations and appropriate uses.

2.2 | *Mapt* Knockout Models

Knockout (KO) models of murine *Mapt* laid the foundation for developing humanized transgenic tauopathy models. While tau plays a critical role in microtubule stabilization in human neurons, most of the reported rodent KO models described to date lack overt cognitive or motor phenotypes (Ayers et al. 2024; Dawson et al. 2001; Fujio et al. 2007; Harada et al. 1994; Tucker et al. 2001). Among the reported models (Table 1), four are functional KOs generated by inserting various targeting cassettes that disrupt Exon 1 of *Mapt*. Harada et al. Dawson et al. and Fujio and colleagues engineered *Mapt* KO models by inserting a PGK-neo cassette, which only allows for the production of short tau fragments that lack microtubule binding capacity (Dawson et al. 2001; Fujio et al. 2007; Harada et al. 1994). Using these mice, Dawson et al. found a delay in the elongation of hippocampal neurons in primary cultures, while Harada et al. observed deficiencies in microtubule number and density in the cerebellum of KO animals (Dawson et al. 2001; Harada et al. 1994). Despite these deficiencies, the mice were histologically normal when compared to littermates that maintained one or both copies of functional tau, which could in part be due to compensatory mechanisms of other neuronal microtubule-associated proteins. In 2001, Tucker and colleagues inserted the enhanced green fluorescent protein (EGFP) gene sequence into the *Mapt* gene, resulting in the expression of the first 31 amino acids of tau fused to EGFP (Tucker et al. 2001). This approach allowed the research team to visualize neurons during axonal elongation using fluorescence microscopy. Consistent with the first three KO models generated, these mice also lack overt cognitive or motor phenotypes. In contrast, the KO mouse model developed by Gumucio and colleagues in 2013 exhibits mild sensorimotor deficits as the mice age (Gumucio et al. 2013). Rather than disrupting exon 1, the neo cassette was used to disrupt exon 10, which may contribute to these differences. In alignment with the likelihood of deficits being linked to targeting a downstream exon in the gene construct, Ayers et al. recently developed a rat KO model using a transcription activator-like effector nuclease (TALEN) approach to target exon 2 of *Mapt*, and again found a lack of deficits when targeting a more 5′ region of the gene (Ayers et al. 2024). Together, the generation of *Mapt* KO models has provided important insight that other microtubule-stabilizing genes may compensate for tau function, which is fundamental to several ongoing gene therapy strategies targeting tau reduction in the brain, though it should be noted that a germline *MAPT* KO may result in compensatory changes in gene expression that would not occur in therapeutic knockdowns. Notably, these findings also suggest that loss of normal tau function alone is likely not a major driver of disease pathogenesis. Instead, these data better align with the gain of toxic function hypothesis, or even a compounding effect in which both the gain of function and loss of function contribute to disease.

2.3 | Spontaneous Models of Tauopathy

Over the last 20 years, several groups created a variety of rodent models that develop a spontaneous tauopathy to both better understand disease pathogenesis in human patients as well as

TABLE 1 | Rodent tau knockout models.

Model	Targeting Vector	Knockout Region	Phenotype	Ref.
TAU$^{-/-}$	PGK-neo cassette	Exon 1	None	(Dawson et al. 2001)
Tau$^{-/-}$	PGK-neo cassette	Exon 1	None	(Fujio et al. 2007)
Tau$^{-/-}$	PGK-neo cassette	Exon 1	None	(Harada et al. 1994)
Tau knockout	EGFP knock-in	Exon 1	None	(Tucker et al. 2001)
E10−/−	Neo cassette	Exon 10	Sensorimotor deficits by 13–17 months	(Gumucio et al. 2013)
Mapt knockout rat [Cy23]	TALEN	Exon 2	None	(Ayers et al. 2024)

TABLE 2 | Spontaneous rodent models of tauopathy.

Model	Isoform/ Mutation(s)	Promoter	Protein level[a]	Deficits		Pathology		Ref(s)
				Cognitive[b]	Motor[b]	Neuronal Loss	Tau Inclusions	
T44 (line 7)	0N3R	*MoPrP.Xho*	5x	n.p.	Progressive motor weakness	Spinal cord by 12 months	Spinal cord by 1 mo; cortex & brainstem by 6 months	(Ishihara et al. 1999)
T44mTKO	0N3R	*MoPrP.Xho* T44 (Ishihara et al. 1999) X $TAU^{-/-}$ (Dawson et al. 2001)	1.5x	n.p.	n.p.	n.p.	None	(He et al. 2020)
mThy-1 3R Tau (line 13)	2N3R L226V, G272V	*mThy-1*	8x	Novel environment by 6–8 months; MWM by 8–10 months	Round beam by 6–8 months	Cortex & hippocampus by 6–8 months	Cortex & hippocampus by 8–10 months	(Rockenstein et al. 2015)
TauRDΔK280	4RD aa 244–372 ΔK280	TetOff *CaMKIIα*	0.7x	MWM and positive avoidance by 10 months	Slightly impaired rotarod by 10 months	Dentate gyrus by 5 months	Entrorhinal cortex & amygdala by 3 months; cortex by 15 months	(Mocanu et al. 2008; Sydow et al. 2011)
TauC3	0N4R Truncated @ aa 421	*BAI1-AP4*	1-2x	Y-maze by 1.3 months; passive avoidance by 2 months	None	None	Hippocampus by 1.3 months	(Kim et al. 2016)
Tg(Prnp-MAPT*P301L) JNPL3Hlmc [JNPL3]	0N4R P301L	*MoPrP.Xho*	HOZ: 2x Hemi: 1x	n.p.	Limb weakness, hunching, impaired righting by 10 months	Spinal cord by 10 months	Throughout brain & spinal cord by 4.5 months Throughout brain & spinal cord by 6.5 months	(Lewis et al. 2000)
rTgTauEC	0N4R P301L	TetOff Neuropsin	n.p.	Fear conditioning by 16 months	n.p.	Entorhinal cortex by 24 months	Entorhinal cortex by 24 months	(de Calignon et al. 2012)

(Continues)

TABLE 2 | (Continued)

Model	Isoform/ Mutation(s)	Promoter	Protein level[a]	Deficits		Pathology		Ref(s)
				Cognitive[b]	Motor[b]	Neuronal Loss	Tau Inclusions	
Tg(Thy1-MAPT*P301S) 2541Godt [Tg2541]	0N4R P301S	*Thy1.2*	HOZ: 2x	n.p.	Hindlimb clasping by 5 months; moribund by 6 months	Spinal cord by 3 months	Throughout brain by 4 months	(Allen et al. 2002; Woerman et al. 2017)
			Hemi: 1x	n.p.	Hindlimb clasping by 12 months	n.p.	None by 6 months	
SPAM mice	0N4R P301S, S320F	*MoPrP.Xho*	1.7x	n.p.	n.p.	Enteric neurons by 6 months	Cortex, hippocampus, amygdala, thalamus, cerebellum, & brainstem by 2 months	(Xia et al. 2022)
B6.Cg-Tg(Thy1-MAPT*) 2652Gds [Tg2652]	1N4R	*Thy1.2*	12x	BM by 3 months	Rotarod by 3 months	n.p.	Cortex, striatum, anterior commissure, corpus callosum, limbic system, & brainstem by 3 months	(Wheeler et al. 2015)
C57BL/6-Tg(tetO-MAPT*A152T)/LiLms/J [hTau-A152T]	1N4R A152T	TetOff *CamKIIα*	3-5x	Nest building by 10–14 months; MWM by 17 months	n.p.	Hippocampus by 20 months	Hippocampus by 8 months	(Maeda et al. 2016)
B6;C3-Tg(Prnp-MAPT*P301S) PS19Vle/j [PS19]	1N4R P301S	*MoPrP.Xho*	5x	HP, PPI, NOR by 6 months	Hindlimb clasping by 3 months; moribund by 9–12 months	Hippocampus by 9 months; cortex by 12 months	Cortex, hippocampus, amygdala, brainstem, & spinal cord by 6 months	(Takeuchi et al. 2011; Yoshiyama et al. 2007)
THY-Tau22	1N4R G272V, P301S	*Thy1.2*	4-6x	EPM by 6 months; MWM by 10 months	None	Hippocampus by 12 months	Cortex & hippocampus by 6 months	(Schindowski et al. 2006)

(Continues)

TABLE 2 | (Continued)

Model	Isoform/ Mutation(s)	Promoter	Protein level[a]	Deficits		Pathology		Ref(s)
				Cognitive[b]	Motor[b]	Neuronal Loss	Tau Inclusions	
ALZ17	2N4R	*Thy1.2*	1.5x	n.p.	Rotarod by 1–3 months; hindlimb clasping by 8–16 months	n.p.	Olfactory bulb, cortex, hippocampus, brainstem, & spinal cord by 11 months	(Probst et al. 2000)
hWT *tau* (line 23)	2N4R	*MoPrP.Xho*	8-10x	n.p.	None	n.p.	None	(Zhang et al. 2004)
hTau^{AT} or hTau40^{AT}	2N4R A152T	*Thy1.2*	1-3x	MWM by 16 months	n.p.	Forebrain by 12 months	Cortex, hippocampus, & spinal cord by 3–5 months	(Decker et al. 2016; Sydow et al. 2016)
hTau40ΔK280 [TauΔK]	2N4R ΔK280	TetOff *CaMKIIα*	1-3x	MWM and passive avoidance by 16 months	None	None	Hippocampus by 3 months	(Eckermann et al. 2007; Van der Jeugd et al. 2012)
Thy1-hTau. P301L [Tau. P301L]	2N4R P301L	*Thy1*	4x	n.p.	Beamwalk and hindlimb clasping by 9–12 months; moribund by 11–13 months	None	Cortex, thalamus, cerebellum, brainstem, & cerebellum by 9 months	(Terwel et al. 2005)
T40PL-GFP	2N4R P301L	*MoPrP.Xho*	2x	n.p.	n.p.	None	None	(Gibbons et al. 2017)
Tg214 [Tau V337M]	2N4R V337M	*PDGF-β*	<0.1x	EPM by 10 months	n.p.	Hippocampus by 10 months	Hippocampus by 11 months	(Tanemura et al. 2001, 2002)
Thy-1 mutated human tau [TMHT]	2N4R V337M, R406W	*Thy1*	n.p.	MWM & buried food test by 5 months	None	n.p.	Hippocampus & amygdala by 3 months	(Flunkert et al. 2013)
RW Tg Mice (line 37)	2N4R R406W	*MoPrP.Xho*	8-10x	n.p.	Hindlimb clasping by 12 months	n.p.	Cortex, hippocampus, cerebellum, & spinal cord by 12 months	(Zhang et al. 2004)

(Continues)

TABLE 2 | (Continued)

Model	Isoform/ Mutation(s)	Promoter	Protein level[a]	Deficits		Pathology		Ref(s)
				Cognitive[b]	Motor[b]	Neuronal Loss	Tau Inclusions	
Tau R406W-CamKII [TAU R406W]	2N4R R406W	*CaMK-II*	0.08–0.18x	Fear conditioning by 16–23 months	None	n.p.	Olfactory bulb, cortex, striatum, & amygdala by 18 months	(Tatebayashi et al. 2002)
8c	6 isoforms 3R > 4R	PAC with *htau*	3.7x	n.p.	None	n.p.	None	(Duff et al. 2000)
Htau	6 isoforms 3R > 4R	8c (Duff et al. 2000) X EGFP knock-in (Tucker et al. 2001)	No *Mapt* to quantify	n.p.	n.p.	n.p.	Cortex & hippocampus by 3 months	(Andorfer et al. 2003)
6hTau	6 isoforms 3R = 4R	PAC with *hTau* (McMillan et al. 2008) X PAC with E10 + 14	2x	n.p.	n.p.	n.p.	None	(He et al. 2020)
Tau 3R/4R (Line 13)	6 isoforms 3R = 4R	*Mapt*	No *Mapt* to quantify	n.p.	n.p.	n.p.	n.p.	(Hosokawa et al. 2022)
Tau 10 + 16 [Tau609]	3R < 4R IVS10 + 16 C>T	*CaMKIIα*	0.66x	MWM by 6 months	n.p.	Hippocampus by 24 months	Hippocampus by 6 months; cortex by 18 months	(Umeda et al. 2013)
Tg12099 rat	0N4R P301S	*RaPrnp*	HOZ: 4.5x	n.p.	Ataxia & bradykinesia by 10 months; moribund by 14 months	Amygdala & piriform cortex in terminal animals	Limbic system by 9 months	(Ayers et al. 2024)
			Hemi: 1.5x	n.p.	None	None	None	

Abbreviations: aa, amino acid; BM, Barnes maze; EPM, elevated plus maze; Hemi, hemizygous; HOZ, homozygous; HP, hot plate test; MWM, Morris water maze; n.p., not published; NOR, novel object recognition; PPI, prepulse inhibition.

[a]Relative to endogenous murine tau as reported in the initial publication.

[b]Tests are only listed if animals displayed a deficit.

perform preclinical studies for drug discovery efforts (Table 2). These models vary in isoform expression (single isoform versus multiple isoforms) and the absence or presence of familial *MAPT* mutations in the human transgene. Two models expressing 3R tau were developed with the goal of recapitulating PiD in a rodent. The T44 model, which expresses the WT 0N3R isoform, develops argyrophilic lesions within the spinal cord and brainstem but lacks robust cortical pathology or cognitive impairment (Ishihara et al. 1999). Moreover, substantial neuron loss in the spinal cord results in progressive motor weakness. Notably, when the T44 model was crossed with the $TAU^{-/-}$ line (Dawson et al. 2010), no tau inclusions were seen, and the same motor weakness was not reported (He et al. 2020). By comparison, the mThy-1 3R Tau model expresses 2N3R tau with the L266V and G272V mutations and develops tau inclusions and neuronal loss predominantly in the forebrain (Rockenstein et al. 2015). This is accompanied by both cognitive and motor deficits by 6–8 months of age, increasing the translatability of the model to human disease phenotypes.

While a limited number of 3R mouse models exist, the list of models expressing 4R tau isoforms is quite extensive (Table 2). Many of these models develop a spontaneous tauopathy, lending to their use in studying the spreading of pathogenic tau throughout the brain. For brevity, we will focus our discussion on two of the most thoroughly characterized and frequently used models, the Tg2541 and PS19 mice (Allen et al. 2002; Yoshiyama et al. 2007). Michel Goedert's laboratory developed the Tg2541 model in 2002 to recapitulate many aspects of human disease. Homozygous $Tg2541^{+/+}$ mice express 0N4R tau with the P301S mutation and develop widespread tau pathology throughout the cortex, hippocampus, midbrain, brainstem, and spinal cord by ~4 months of age (Allen et al. 2002). This results in severe motor impairment and paralysis by 6–7 months of age. By comparison, hemizygous $Tg2541^{+/-}$ mice do not develop any tau pathology by the same age and hindlimb clasping is not seen until ~12 months (Woerman et al. 2017). The PS19 mice, which were developed by Virginia Lee's laboratory in 2007, express 1N4R tau with the same P301S mutation (Yoshiyama et al. 2007). These mice also develop tau inclusions starting around 6 months old in several forebrain regions. While both mouse lines exhibit progressive motor phenotypes, they differ in their transgene expression levels—tau expression is 5x that of endogenous tau in the PS19 mice and 2x in the $Tg2541^{+/+}$ mice (Allen et al. 2002; Yoshiyama et al. 2007). It should also be noted that the PS19 line must be used as hemizygous mice, which may contribute to the extensive variability in disease onset and age of tau pathology formation reported in the model (Woerman et al. 2017). In contrast, the $Tg2541^{+/+}$ mice develop a more uniform disease onset as well as tau fibril and neuropathology formation, though it is important to note that an unexplained increase in transgene expression in female mice around 24 weeks old results in a slightly earlier disease onset in female animals (Woerman et al. 2017). While both models are widely used, the uniformity of the $Tg2541^{+/+}$ model enhances the value of the line for therapeutic efficacy studies. Notably, power analyses using disease onset data from the two models showed that adequately powered efficacy studies capable of detecting an effect size of 10% would require 8 $Tg2541^{+/+}$ mice versus 92 PS19 animals (Woerman et al. 2017).

Other models were engineered to evaluate the role of specific tau fragments in aggregation or use the Tet On/Off system to control tau transgene expression. For example, the TauRDΔK280 mice express the RD of 4R tau using a Tet Off system and have been used to show that the 4RD region of tau is sufficient to induce tau pathology and neuronal loss (Mocanu et al. 2008; Sydow et al. 2011). Notably, this pathology is rescued if expression is abrogated, suggesting the brain has the capacity to clear tau pathology in the absence of continued protein translation. Other models expressing pro-aggregation or disease-causing mutations, such as A152T (Decker et al. 2016; Maeda et al. 2016), G272V, P301L/S (Ayers et al. 2024; de Calignon et al. 2012; Lewis et al. 2000; Terwel et al. 2005), V337M (Tanemura et al. 2001), R406W (Tatebayashi et al. 2002; Zhang et al. 2004), or even a combination of mutations (Flunkert et al. 2013; Schindowski et al. 2006; Xia et al. 2022) were developed to study specific subsets of tauopathy. The variety of these models enables specific targeted expression or patterns of pathological deposition depending on what hypothesis is being investigated. For example, the rTgTauEC model expresses human 0N4R*P301L tau in a subset of neurons in the entorhinal cortex, which may have utility for understanding neuronal damage in AD (de Calignon et al. 2012). By comparison, the SPAM mice, which express 0N4R tau with the P301S and S320F mutations, develop tau pathology in the brain, but neuron loss only occurs in the enteric nervous system, which could support investigation of tau pathogenesis in the periphery (Xia et al. 2022).

Finally, there is an additional subset of models that express various WT tau isoforms to mimic the onset of spontaneous tauopathies in human patients (Andorfer et al. 2003; Duff et al. 2000; Probst et al. 2000; Wheeler et al. 2015; Zhang et al. 2004). These models vary in transgene expression levels from 1x to 12x and rely on multiple promoters, impacting the translatability of findings across model systems. While some of these mice develop tau neuropathology, others fail to develop inclusions or lack the neuronal death seen in human patients. This may seem to be a major detractor; however, the lack of spontaneous disease makes these valuable models that may be useful for establishing injection models. For example, the ALZ17 mice (Probst et al. 2000) were critical for initial studies investigating inducible models of tauopathy using intracranial (i.c.) injection with human patient samples (discussed below).

The spontaneous models of tauopathy have been pivotal in generating key data about tau spreading via connected neuroanatomical tracts, but they remain limited in their ability to study the consequences of tau strain biology. An additional weakness of all spontaneous models described above is the targeted expression of a single tau isoform, given our current lack of understanding about how different isoforms interact in disease. To better address this, the more complicated mouse tauopathy models express all 6 isoforms of WT human tau (Andorfer et al. 2003; Duff et al. 2000; Umeda et al. 2013). Initial efforts to accomplish this goal using an artificial chromosome resulted in the 8c mouse model, which preferentially expresses the 3R tau isoforms over the 4R isoforms and does not develop deficits or pathology (Duff et al. 2000). The 8c model was then crossed with the EGFP knock-in line (Tucker

et al. 2001), which resulted in a similar preference for 3R expression and a lack of deficits, but cortical and hippocampal tau pathology developed by 3 months of age (Andorfer et al. 2003). Subsequently, Umeda et al. generated the Tau 10 + 16 model, which harnesses the intronic mutation in exon 10 that leads to an increase in splicing and expression of the 4R tau isoforms (Umeda et al. 2013). This model develops tau neuropathology that spreads with age in the mice, as well as mild cognitive deficits. Two more recently developed mouse models have achieved the goal of expressing equal ratios of 3R and 4R tau (He et al. 2020; Hosokawa et al. 2022). He et al. achieved this goal by crossing a mouse line that relies on an artificial chromosome to express *MAPT* (McMillan et al. 2008) with a second mouse model harboring an additional artificial chromosome carrying the E10 + 14 mutation to gain the desired ratios of the 6 tau isoforms (He et al. 2020). In an alternative approach, Hosokawa et al. used CRISPR-Cas9 to remove the 5′ splice site of intron 10 in endogenous *Mapt* to prevent the alternative splicing of exon 10 that results in 4R tau production (Hosokawa et al. 2022). The investigators crossed these CRISPR-Cas9 mice to derive a 3R/3R mouse line, which was then bred with WT 4R/4R mice to achieve their final experimental 3R/4R mouse tau model. Interestingly, neither model develops spontaneous tau pathology nor overt phenotypic deficits.

Overall, spontaneous tauopathy rodent models have been largely informative about the underlying role of tau misfolding and spread in disease pathogenesis. However, some models, such as the PS19 line, suffer from high levels of animal-to-animal variability, complicating and limiting their potential use. As an alternative, rodent models with a more consistent onset of disease, like the Tg2541$^{+/+}$ mice, are highly valuable for preclinical applications, such as evaluating the effect of antisense oligonucleotides or small molecule therapeutic candidates on disease. Importantly, research using Tet On/Off animals has not only shed important insight into how pathogenic tau spreads throughout the brain, but has also led to critical findings about the brain's ability to clear mature tau inclusions in the absence of new protein synthesis. The use of various *MAPT* mutations in rodent models has generated key data about the mechanistic consequences of each disease-associated mutation, though it is important to note that the presence of a *MAPT* mutation is not sufficient to induce *bona fide* tauopathy in a rodent. Lastly, successfully recapitulating human expression levels of the 6 isoforms of tau has been challenging, though more recent progress on this front has led to models that can better mimic tau biology in the human brain (He et al. 2020; Hosokawa et al. 2022).

2.4 | Inoculation Models of Induced Tauopathy

Tau inoculation models began in earnest as investigators began to hypothesize that the protein relies on the prion mechanism of disease to cause progressive spread throughout the brain (Table 3). Several studies used WT mice, which predominantly express the 4R isoforms in the adult brain (McMillan et al. 2008). While this is not ideal for studying tau isoform specificity in tauopathies that incorporate 3R tau, it led to a multitude of important discoveries. The pivotal first papers from Clavaguera et al. demonstrated that aged Tg2541$^{+/+}$ mouse brain samples, as well as human patient samples, can induce tau pathology formation following i.c. injection into the hippocampus (Clavaguera et al. 2013, 2009). At the same time, other groups began using recombinant in vitro generated pre-formed fibrils (PFFs) made using 2N4R tau to induce disease (Guo et al. 2016). These initial studies ushered in an era of work focused on transmitting human tauopathy patient samples to WT mice following i.c. injection. Despite a mismatch in species compatibility with endogenous mouse tau, human samples were still able to induce inclusion formation in WT mice with varying levels of spread away from the injection site (Cornblath et al. 2021; Ferrer et al. 2019; Guo et al. 2016; He et al. 2020; Lasagna-Reeves et al. 2012; Narasimhan et al. 2017; Saito et al. 2019; Weitzman et al. 2020). Interestingly, the WT mouse studies highlighted important information about isoform specificity in disease. PiD patient samples induced negligible tau inclusions, likely due to a lack of 3R tau in the adult mouse brain (Ferrer et al. 2019; He et al. 2020), while 4R tauopathies induced mouse tau misfolding and even maintained cellular specificity with CBD, GGT, and PSP causing oligodendrocytic inclusions similar to those seen in human patients (Ferrer et al. 2019, 2020; Narasimhan et al. 2017).

In addition to WT mice, transmission studies have relied upon several humanized mouse models, leading to important discoveries about strain biology. Four inoculation models express all 6 tau isoforms of human or mouse tau: the hTau mice (Andorfer et al. 2003), the 6hTau mice (He et al. 2020), a *MAPT* knock-in model (along with a corresponding *MAPT*/*APP* knock-in) (Saito et al. 2019), and the Tau 3R/4R mice (Hosokawa et al. 2022). Andorfer et al. created the hTau model in 2003, which develops spontaneous tau inclusions around 3 months old (Andorfer et al. 2003). Interestingly, injections using samples from PiD, AD, CBD, and GGT did not accelerate the formation of inclusions, but tau pathology was seen near the hippocampal injection site (Ferrer et al. 2022; Mate de Gerando et al. 2024; Miao et al. 2019; Zareba-Paslawska et al. 2020). Of note, Zareba-Paslawska et al. found oligodendrocytic inclusions in CBD-injected mice, which led them to investigate the cellular distribution of 3R and 4R isoforms in the hTau model. Their work reported that the highest expression level of 4R tau is in oligodendrocytes in these mice, highlighting the importance of understanding the cellular distribution of each isoform in animal models, particularly those that rely on artificial chromosomes (Zareba-Paslawska et al. 2020). Additionally, because this model relies on the EGFP knock-in mouse (Tucker et al. 2001), it is possible that the tau fragment fused to EGFP may interfere with or contribute to pathology formation in these experiments. The most complex of these models is the 6hTau mouse line, which was created by Virginia Lee's laboratory in 2020, expressing equal levels of 3R and 4R tau isoforms (He et al. 2020). Consistent with other PFF studies, they found that PFFs made using either the 1N3R or 1N4R isoforms are inefficient at inducing tau misfolding, whereas i.c. injection of human patient samples successfully induced inclusion formation (He et al. 2020; Weitzman et al. 2020; Xu et al. 2021). Moreover, the 6hTau model was able to recapitulate strain-specific pathologies, with PiD, AD, CBD, and PSP inducing isoform-specific inclusions, and CBD and PSP causing astrocytic and glial pathology (He et al. 2020; Weitzman et al. 2020; Xu et al. 2021). Saito

TABLE 3 | Rodent inoculation models of tauopathy.

Model	Isoform/ Mutation(s)	Promoter	Protein Level[a]	Injection site & age[b]	Inoculum	Tau Pathology	Ref(s)
WT mice	4R > 3R isoforms	None	NA	Hippocampus & overlying cortex @ 2–3 months	2N4R PFFs	Few inclusions at injection site by 9 mpi	(Guo et al. 2016)
				Hippocampus & overlying cortex; age not reported	AD-seeded 2N4R PFFs	Dentate gyrus & entorhinal cortex by 6 mpi	(Xu et al. 2021)
				Hippocampus & overlying cortex @ 3 months	Aged $Tg2541^{+/+}$	Few inclusions at injection site by 6 mpi	(Clavaguera et al. 2009)
				Lateral corpus callosum @ 10–12 months	PiD	Few inclusions at injection site by 6 mpi	(Ferrer et al. 2019)
				Hippocampus @ 3 months	AD	At injection site by 11 mpi	(Lasagna-Reeves et al. 2012)
				Hippocampus & overlying cortex @ 3 months	AGD, PSP, & TD	At hippocampal injection site by 6 mpi; spread to optic tract & thalamus by 15 mpi	(Clavaguera et al. 2013)
				Hippocampus & overlying cortex @ 2–3 months	CBD	Hippocampus & entorhinal cortex by 3 mpi	(Narasimhan et al. 2017)
				Lateral corpus callosum @ 7 months	GGT	Few inclusions at injection site by 6 mpi	(Ferrer et al. 2019)
				Lateral corpus callosum @ 10–12 months	ARTAG, PART, & fFTLD-P301L		
MAPT knock-in	6 isoforms 3R > 4R	*Mapt*	4R is 70% of 3R expression	Hippocampus & overlying cortex @ 19 months	AD tau	Hippocampus & cortex by 3 mpi	(Saito et al. 2019)

(Continues)

TABLE 3 | (Continued)

Model	Isoform/ Mutation(s)	Promoter	Protein Level[a]	Injection site & age[b]	Inoculum	Tau Pathology	Ref(s)
B6.Cg-Mapt$^{tm1(EGFP)Klt}$ Tg(MAPT)8c Pdav/J [hTau]	6 isoforms 3R > 4R	PAC with *htau*	No *Mapt* to quantify	Hippocampus @ 6 months	PiD	Few inclusions near injection site by 3 mpi	(Ferrer et al. 2022)
				Bilateral hippocampus @ 3 months	AD	Hippocampal spread to cortex by 9 mpi	(Hu et al. 2016)
				Striatum @ 3 months	CBD	Striatal spread to corpus callosum in oligodendrocytes by 12 mpi	(Zareba-Paslawska et al. 2020)
				Hippocampus @ 6 months	GGT	Hippocampus & dentate gyrus by 3 mpi	(Ferrer et al. 2022)
6hTau	6 isoforms 3R = 4R	PAC-*hTau* X PAC-E10 + 14	2x	Hippocampus & overlying cortex @ 3–5 months	1N3R and 1N4R PFFs	None by 6 mpi	(He et al. 2020)
				Hippocampus & overlying cortex; age not reported	AD-, CBD-, & PSP-seeded 2N4R PFFs	Hippocampus & entorhinal cortex by 3 mpi with isoform specificity	(Xu et al. 2021)
				Hippocampus & overlying cortex @ 3–5 months	PiD, AD, CBD, & PSP	Hippocampus & entorhinal cortex by 3 mpi	(He et al. 2020)
Tau 3R/4R (Line 13)	6 isoforms 3R = 4R	*Mapt*	No *Mapt* to quantify	Striatum @ 4–6 months	AD & CBD	Striatum & corpus callosum by 6 mpi	(Hosokawa et al. 2022)
					PiD	Striatum & corpus callosum by 12 mpi	
T44mTKO	0N3R	*MoPrP.Xho*	1.5x	Hippocampus & overlying cortex @ 3–5 months	AD & PiD	Hippocampus by 3 mpi	(He et al. 2020)
					CBD & PSP	None	
Tg(Thy1-MAPT*P301S) 2541Godt [Tg2541]	0N4R P301S	*Thy1.2*	2x	Hippocampus & overlying cortex @ 2 months	Aged Tg2541$^{+/+}$	Injection site by 2.5 mpi	(Ahmed et al. 2014)
THY-Tau22	1N4R G272V, P301S	*Thy1.2*	4-6x	Hippocampus @ 3 months	AD	Injection site by 2 mpi	(Mate De Gerando et al. 2023)

(Continues)

TABLE 3 | (Continued)

Model	Isoform/ Mutation(s)	Promoter	Protein Level[a]	Injection site & age[b]	Inoculum	Tau Pathology	Ref(s)
PS19	1N4R P301S	*MoPrP. Xho*	5x	Hippocampus or striatum & overlying cortex @ 2–3 months	K18*P301L & 2N4R*P301S PFFs	Hippocampus & entorhinal cortex by 1 mpi	(Iba et al. 2013)
				Hippocampus; age not reported	Cell-passaged PFFs	Injection site by 4 wpi	(Kaufman et al. 2016)
				Hippocampus & overlying cortex @ 2–5 months	AD & DSAD	Near hippocampal injection site by 1 mpi; cortex by 3 mpi	(Boluda et al. 2015)
					CBD	Near hippocampal injection site by 1 mpi	
ALZ17	2N4R	*Thy1.2*	1.5-10x	Hippocampus & overlying cortex @ 3 months	Aged $Tg2541^{+/+}$	Hippocampus by 12 mpi; cortex by 15 mpi	(Clavaguera et al. 2009)
					AD, AGD, CBD, PSP, & TD	Hippocampus, amygdala, thalamus, entorhinal cortex by 6 mpi	(Clavaguera et al. 2013)
					PiD	Injection sites by 6 mpi	
T40PL-GFP$^{+/-}$	2N4R P301L	*MoPrP. Xho*	2x	Hippocampus @ 2–3 months	2N4R*P301L PFFs	Hippocampus by 3 mpi	(Gibbons et al. 2017)
					AD	Hippocampus & entorhinal cortex by 3 mpi	
Thy1-hTau.P301L [Tau.P301L]	2N4R P301L	*Thy1*	4x	Hippocampus or frontal cortex @ 3 months	K18*P301L PFFs	Injection site by 1 mpi	(Peeraer et al. 2015)

(Continues)

TABLE 3 | (Continued)

Model	Isoform/ Mutation(s)	Promoter	Protein Level[a]	Injection site & age[b]	Inoculum	Tau Pathology	Ref(s)
Tg12099$^{+/-}$ rat	0N4R P301S	*RaPrnp*	1.5x	Thalamus @ 2 months	K18*P301L PFFs	Corticolimbic system by 4 mpi	(Ayers et al. 2024)
					Aged Tg12099$^{+/+}$	Entorhinal cortex by 8 mpi	
					PSP	Sparse in entorhinal cortex by 8 mpi	
					AD	None	
App$^{NL\text{-}G\text{-}F}$/*MAPT* double knock-in [App$^{NL\text{-}G\text{-}F}$/ MAPT dKI]	3R > 4R isoforms	*Mapt*	1x	Hippocampus & overlying cortex @ 19 months	AD tau	Presence of humanized Aβ increased spread from hippocampus to cortex by 3 mpi	(Saito et al. 2019)

Abbreviations: AD, Alzhiemer's disease; AGD, argyrophilic grain disease; ARTAG, aging-related tau astrogliopathy; CBD, corticobasal degeneration; DSAD, Down syndrome AD; fFTLD-P301L, familial frontotemporal lobe dementia with the P301L mutation; GGT, glial globular tauopathy; mpi, months post-inoculation; MWM, Morris water maze; n.p., not published; PART, primary age-related tauopathy; PFFs, pre-formed fibrils; PiD, Pick's disease; PSP, progressive supranuclear palsy; TD, tangle-only dementia; wpi, weeks post-inoculation.

[a]Relative to endogenous murine tau as reported in the initial publication.

[b]Injections were administered unilaterally unless otherwise specified.

et al. created the *MAPT* knock-in model, which also expresses more 3R than 4R tau (Saito et al. 2019). Mice injected with tau isolated from AD patient samples caused minimal pathology 3 months post-injection (mpi). Similar studies in the $App^{NL\text{-}G\text{-}F}$/*MAPT* double knock-in line found that the presence of Aβ augmented the spread of tau pathology, though the inclusions remained soluble, limiting the relevance of the approach to human disease (Saito et al. 2019). Lastly, Hosokawa et al. built the Tau 3R/4R mouse model, which expresses equal ratios of all 6 isoforms of mouse tau rather than human tau (Hosokawa et al. 2022). Mice injected with AD and CBD patient samples developed tau inclusions by 6 mpi, whereas mice injected with PiD patient samples showed inclusion formation by 12 mpi. This model provides an attractive alternative to WT mice for studying the spectrum of 3R and 4R tauopathies, though questions about the human/mouse species barrier still exist.

Several overexpression models have also been used to study i.c. transmission of tauopathy patient samples. The hTau, $Tg2541^{+/+}$, THY-Tau22, PS19, and ALZ17 mouse models all develop spontaneous tau pathology to some extent (Table 2) which is an inherent weakness when studying the induction of tauopathy, as the true cause of the resultant pathology remains unclear (Allen et al. 2002; Andorfer et al. 2003; Probst et al. 2000; Schindowski et al. 2006; Yoshiyama et al. 2007). As discussed above, PiD, AD, CBD, and GGT injections in the hTau model induced tau inclusion formation, but the mice did not show overt differences between each tauopathy strain injected, raising questions about how well the model can recapitulate the varied strain biology seen in human disease (Ferrer et al. 2022; Mate de Gerando et al. 2024; Miao et al. 2019; Zareba-Paslawska et al. 2020). Despite the spontaneous development of tau pathology, the ALZ17, $Tg2541^{+/+}$, PS19, and THY-Tau22 models demonstrated that pathology formation can be accelerated when seeded with various pathogenic tau samples (Table 3). Experiments using the ALZ17 model showed that injections using aged $Tg2541^{+/+}$ mouse samples, as well as 4R tauopathy patient samples, accelerate the formation of tau inclusions (Clavaguera et al. 2013, 2009). Interestingly, despite the expression of 4R tau, Clavaguera et al. showed that PiD patient samples were also able to induce tau pathology (Clavaguera et al. 2013). Similarly, i.c. inoculations in the $Tg2541^{+/+}$ and PS19 mouse models resulted in earlier onset of tau pathology; moreover, strain-specific phenotypes were preserved following transmission to the PS19 model (Ahmed et al. 2014; Boluda et al. 2015; Clavaguera et al. 2014; Iba et al. 2013, 2015; Kaufman et al. 2016, 2017; Liu et al. 2024; Mate De Gerando et al. 2023).

While these initial studies provided important insights into disease pathogenesis in tauopathy patients and even highlighted notable strain differences, each model expresses different tau isoforms and mutations using a variety of promoters. These mix-and-match approaches likely contribute to the variability seen in experimental outcomes, adding challenges to data interpretation for the experimental paradigm being tested. A major consequence of this is the remaining lack of a highly reproducible and successful model system for studying patient-derived tauopathy strains without the background interference from spontaneous disease in the mice.

2.5 | Multi-Gene Models of Alzheimer's Disease

AD is perhaps the most difficult tauopathy to model using a strategy that accurately reflects the co-pathologies of the disease. There are seven commonly used models that incorporate transgenic human tau along with APP, and in some cases PSEN1 or 2 (Table 4). Three of these models incorporate the 0N4R tau isoform under the control of various promoters. Jackson et al. designed a mouse line with Tet Off *MAPT* expression; however, this model only develops tau inclusions and neuronal loss adjacent to the formation of Aβ plaques within the cortex and lacks the widespread tau pathology seen in AD patients (Jackson et al. 2016). The APPSwe-Tau and 3xTg-AD mouse models incorporate the P301L FTLD mutation (Lewis et al. 2001; Oddo et al. 2003). Both models develop Aβ plaques within the cortex and tau inclusions separate from the plaques, suggesting the two form independently rather than the Aβ pathology inducing tau inclusion formation, as is thought to occur in human patients. Interestingly, the APPSwe-Tau mice develop particularly dense inclusions in the spinal cord and pons earlier (3–6 months) than the 3xTg-AD mice, which primarily develop inclusions in the hippocampus by 12 months despite the differences in their tau expression levels (1-2x and ~ 7x, respectively) (Lewis et al. 2001; Oddo et al. 2003). The TauPS2APP and PLB1-triple mouse models express 2N4R tau at similar levels using the *Thy1.2* and *CamKIIα* promoters, respectively (Grueninger et al. 2010; Platt et al. 2011). Both models develop tau inclusions in the hippocampus within a similar timeframe but vary drastically in their formation of Aβ plaques. Huang et al. developed a knock-in model with the P290S mutation in exon 10 of murine *Mapt*, which is the equivalent of the human P301S mutation, resulting in mutant tau expression at physiological levels (Huang et al. 2022). These mice develop plaques within the piriform cortex by 18 months old, along with tau inclusions in the hippocampus and cortex. Notably, these animals also develop neuronal loss in the piriform cortex, which is not consistently seen. Finally, insoluble tau isolated from the terminal $App^{NL\text{-}G\text{-}F}$ KI*x*$Mapt^{P290S}$ KI mice was able to seed aggregation in HEK293T cells expressing human 0N4R*P301S tau (Huang et al. 2022). Of note, none of the previously discussed models fulfill the key criteria of expressing both 3R and 4R tau isoforms to match the isoform specificity of AD. Saito and colleagues developed the first model that expresses the 6 isoforms of tau; however, these mice failed to develop any insoluble tau inclusions, suggesting the role of additional factors necessary for the induction of tau misfolding that are not included in the design of the $App^{NL\text{-}G\text{-}F}$/*MAPT* double knock-in (Hashimoto et al. 2019; Saito et al. 2019).

To date, no models have been able to accurately recapitulate the pathology of AD in the presence of all tau isoforms. Familial AD cases predominantly involve mutations in either the APP gene or the PSEN1/2 genes, leading to the hypothesis that Aβ misfolding somehow precedes and initiates tau misfolding in disease. However, data from currently available animal models suggest that in these mice, Aβ and tau act independently of one another, which may in part be due to the occasional use of FTLD-associated tau mutations to initiate tau misfolding. It is also possible that time or animal age may create a limitation to these models; the effect of Aβ plaques in mediating tau

TABLE 4 | Multi-gene rodent models of Alzheimer's disease.

Model	Isoform/ mutations	Transgene expression[a]	Genes	Tau protein level[b]	Deficits[c]	Pathology			Ref(s)
						Neuronal Loss	Tau inclusions	Aβ plaques	
APP/PS1/ rTg21221	0N4R	TetOff *CamKIIα*	APP PSEN1MAPT	n.p.	n.p.	Near cortical plaques by 8 months	Near cortical plaques by 8 months	Cortex by 8–10 months	(Jackson et al. 2016)
Tg(APPSWE) 2576Kha; Tg(Prnp-MAPT*P301L) JNPL3Hlmc [APPSwe-Tau]	0N4R P301L	*MoPrP.Xho*	APP MAPT	n.p.	Limb weakness, hunching, impaired righting by 10 months	n.p.	Pons & spinal cord by 3 months; limbic system by 6 months, though more predominant in females	Olfactory cortex & limbic system by 8.5 months	(Lewis et al. 2001)
B6;129-Psen1^{tm1Mpm} Tg(APPSwe, tauP301L) 1Lfa/ Mmjax [3xTg-AD]	0N4R P301L	*Thy1.2*	PSEN APP MAPT	~6.8x	n.p.	n.p.	Hippocampus by 12 months	Cortex by 3 months & hippocampus by 6 months	(Oddo et al. 2003)
B6.D2-Tg(Thy1-APPSwe, Prp-PSEN2N141I, Thy1-TauP301L) [TauPS2APP]	2N4R P301L	*Thy1.2*	APP PSEN2MAPT	n.p.	None	None	Hippocampus & amygdala by 4 months	Hippocampus by 8 months	(Grueninger et al. 2010)
PLB1$_{Triple}$	2N4R P301L, R406W	Multi-transgene; *CamKIIa*	APP MAPT PSEN1	2–3x	SRM by 5 months; NOR by 8 months; OFWM by 12 months	Absent	Cortex & hippocampus by 6 months	Limited in cortex & hippocampus by 21 months	(Platt et al. 2011)
App^{NL-G-F} KI*xMapt*^{P290S} KI	4R isoforms P290S	Knock-in; *Mapt*	APP MAPT	1x	n.p.	Piriform cortex by 18 months	Limbic system, PAG, & PVN by 18 months	Neuritic plaques by 6 months	(Huang et al. 2022)
App^{NL-G-F}/*MAPT* double knock-in [App^{NL-G-F}/ MAPT dKI]	3R > 4R isoforms	Knock-in; *Mapt*	APP MAPT	No *Mapt* to quantify	Y-maze by 12 months	None	None	By 2 months	(Hashimoto et al. 2019; Saito et al. 2019)

Abbreviations: BM, Barnes Maze; n.p., not published; NOR, novel object recognition; OFWM, open field water maze; PAG, periaqueductal gray; PVN, paraventricular nucleus of the thalamus; SRM, social recognition memory.
[a]Promoter for tau expression.
[b]Relative to endogenous murine tau as reported in the initial publication.
[c]Tests are only listed if animals displayed a deficit.

misfolding may require longer than the lifespan of a mouse, as this process likely occurs over years to decades in humans. To facilitate the development of new AD rodent models that will enable better clinical translation, the MODEL-AD consortium has generated several mouse lines that will be characterized using the MODEL-AD phenotyping pipeline that was specifically designed to characterize measures relevant for translatable, preclinical testing (Benzow et al. 2024).

2.6 | Limitations of Tauopathy Rodent Models

Despite the extensive knowledge gained from research using tauopathy rodent models, several key limitations remain. First, multiple promoters are used to drive transgene expression, which impacts what brain regions develop pathology, and therefore, determines how neuropathology spreads (Odeh et al. 2011). This complicates the translatability of regional pathology in transgenic animals to human disease, as disease in the rodent may be more influenced by the promoter than the disease itself. As an example, models like the Tg2541$^{+/+}$ animals develop hindbrain-predominant pathology, whereas tauopathies in human patients typically impact more forebrain regions. Notably, apart from knock-in models, the *Mapt* promoter is not used to drive transgene expression, which may offer an important approach for better mimicking tau expression profiles in the human brain. Second, a lack of standard criteria for characterizing new models limits comparisons between these valuable tools. For example, a thorough investigation of when pathogenic tau develops, how it spreads through the brain, and the amount of variability in disease onset are only a handful of the criteria that should be reported to better enable the use of both spontaneous and inoculation model systems in drug discovery studies. In the same vein, a more rigorous approach to using inoculation models is needed. Each lab performs injections at different ages into different brain regions and then investigates the effect of injection at different time points, detracting from the field's ability to draw direct comparisons between studies. Agreement on a more consistent approach would greatly enhance the value of these studies. Third, there is substantial variability in which tau isoform(s) an animal model expresses, with the vast majority expressing a single isoform. While this certainly simplifies work in the rodent system, it lacks the inherent complexity seen in human tauopathies and detracts from the opportunity to better understand isoform specificity in each disease. This is further compounded by the potential role of post-translational modifications to each isoform in mediating tau misfolding and toxicity.

Despite recent advances in tauopathy rodent models, perhaps the most significant limitation is the lack of models that fully recapitulate tau strain biology in human patients. Cryo-EM studies have revolutionized our understanding of how tau misfolds in disease while bolstering support for the strain hypothesis. As shown in Figure 2, a pivotal body of work from Michel Goedert and Sjors Scheres has shown that tau adopts unique fibril conformations in patients with AD, CTE, AGD, CBD, GGT, PSP, and PiD (Falcon et al. 2018, 2019; Fitzpatrick et al. 2017; Zhang et al. 2020). These structures are notably distinct from the tau fibril conformations isolated from both the PS19 and Tg2541$^{+/+}$ mouse models (Schweighauser et al. 2023), which may in part be due to the expression of a single mutant isoform in both transgenic models. Future research efforts should focus on resolving cryo-EM structures of tau fibrils isolated from other tauopathy mouse models to determine if currently available models are better able to recapitulate the known human tau strains. As the field continues to push toward therapeutic development for tauopathy patients, the obvious mismatch in strain biology between preclinical models and clinical targets is reminiscent of the lessons learned from the failures seen with the development of IND24 for prion disease. Given the frequency of failed clinical trials for tauopathy patients, a highly plausible hypothesis explaining the poor translatability of preclinical models is that it is due to their inability to recapitulate the strain biology seen in human disease.

3 | Rodent Models to Investigate Human Synucleinopathies

3.1 | Variability of α-Synuclein Pathology in Human Patients

A-synuclein is a 140-amino acid protein localized to the nucleus and presynaptic terminal of neurons. It is thought to play a role in SNARE complex formation, vesicle recycling, neurotransmitter release (Burré et al. 2010), and potentially in repairing double-stranded DNA breaks (Schaser et al. 2019). Because α-synuclein is an intrinsically disordered protein, it can adopt a myriad of conformations, some of which are pathogenic, leading to protein accumulation throughout the central and sometimes peripheral nervous systems. This disease mechanism underlies several neurological conditions collectively referred to as synucleinopathies, all of which are defined by the ability of misfolded α-synuclein to use the prion mechanism of disease.

Synucleinopathies are a class of movement disorders that are subcategorized into two main groups based on their predominant neuropathological features. In the first, the presence of neuronal LBs and LNs in various brain regions defines Lewy body diseases (LBDs), which include PD, dementia with Lewy bodies (DLB), and Parkinson's disease with dementia (PDD) (Emre et al. 2007; McKeith et al. 2005; Spillantini, Crowther, Jakes, Hasegawa, et al. 1998; Spillantini et al. 1997). Within this subgroup, the distribution of LBs throughout the patient's brain varies by disease. PD patients typically show LB accumulation throughout the brainstem, substantia nigra, and basal ganglia, while in DLB and PDD, LBs are also found in the cerebral cortex (McKeith et al. 2005). In the second category, MSA is defined by glial cytoplasmic inclusions (GCIs) in oligodendrocytes and neuronal cytoplasmic inclusions in neurons (Papp et al. 1989; Spillantini, Crowther, Jakes, Cairns, et al. 1998; Wakabayashi et al. 1998). MSA patients can be further stratified into two subtypes—cerebellar (MSA-C) and parkinsonian (MSA-P)—based on the predominant clinical signs at the time of evaluation.

A critical difference to note between LBD and MSA patients is that no familial mutations have been identified in MSA patients. Alternatively, several genetic mutations are seen in familial LBD cases, a handful of which are in the α-synuclein

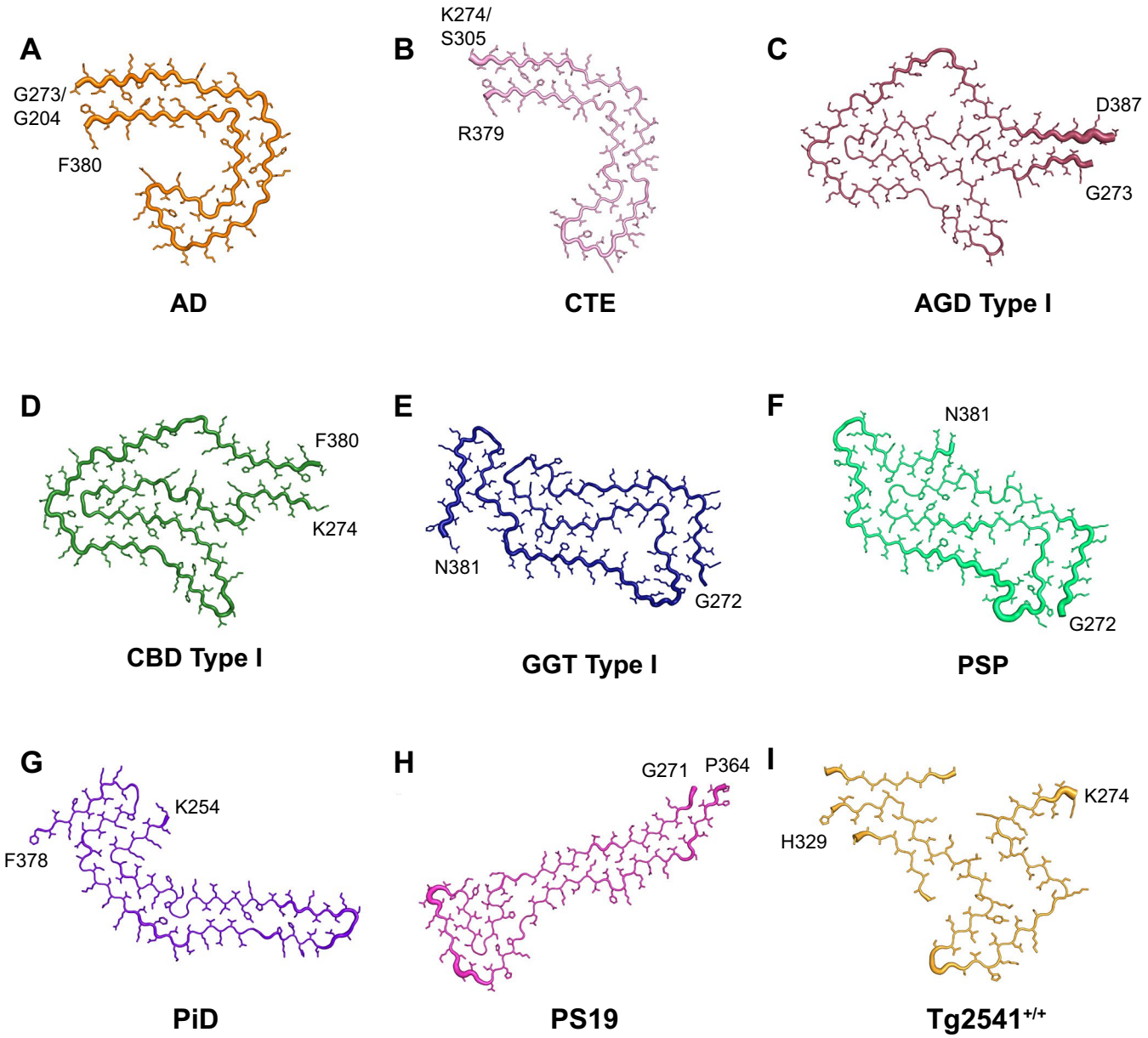

FIGURE 2 | Comparison of tau fibril structures isolated from human tauopathy patient samples and transgenic mouse models of disease. Cryo-electron microscopy evaluation of tau isolated from either human patient samples or terminal transgenic mouse brains shows that transgenic mouse models do not develop tau fibril conformations consistent with those seen in human patients. (A) Alzheimer's disease (AD) shown in orange [PDB: 7UPG; (Seidler et al. 2022); structure (PDB: 5O3L) originally published by (Fitzpatrick et al. 2017)]. (B) Chronic traumatic encephalopathy (CTE) shown in light pink [PDB: 6NWP; (Falcon et al. 2019)]. (C) Argyrphillic grain disease (AGD) type I shown in dark pink [PDB: 7P6D; (Shi et al. 2021)]. (D) Corticobasal degeneration (CBD) type I shown in dark green [PDB: 6TJO; (Zhang et al. 2020)]. (E) Glial globular tauopathy (GGT) type I shown in dark blue [PDB: 7P66; (Shi et al. 2021)]. (F) Progressive supranuclear palsy (PSP) shown in light green [PDB: 7P65; (Shi et al. 2021)]. (G) Pick's disease (PiD) shown in purple [PDB: 6GX5; (Falcon et al. 2018)]. (H) PS19 mouse model shown in fuscia [PDB: 8Q92; (Schweighauser et al. 2023)]. (I) Tg2541$^{+/+}$ mouse model shown in light orange [PDB: 8Q96; (Schweighauser et al. 2023)].

gene, *SNCA*. These mutations, which account for ~2% of PD cases (Bandres-Ciga et al. 2020; Lesage and Brice 2009; Tran et al. 2020), include the A30P/G, E46K, H50Q, G51D, A53E/T/V, and T72M mutations (Appel-Cresswell et al. 2013; Fevga et al. 2021; Kruger et al. 2001; Lesage et al. 2013; Liu et al. 2021; Pasanen et al. 2014; Polymeropoulos et al. 1997; Yoshino et al. 2017; Zarranz et al. 2004). Notably, the heterogeneity in the role of *SNCA* mutations across synucleinopathies adds additional challenges to the development of rodent models capable of recapitulating the α-synuclein strain biology seen in human patients. In the following sections, we will discuss both the appropriate uses and limitations of the currently available rodent model systems based on their behavioral and pathologic disease characteristics.

3.2 | *Snca* Knockout Models

In ongoing efforts to understand the role of the synuclein family in neurophysiology, several groups generated functional KO

TABLE 5 | Rodent α-synuclein knockout models.

Model	Targeting vector	Knockout region	Phenotype	Ref
B6(Cg)-*Snca*$^{tm1.2Vlb}$/J	LoxP	Exon 2	None	(Ninkina et al. 2015)
Snca$^{-/-}$	Neo cassette	Exons 4–5	None	(Cabin et al. 2002)
α-synuclein single KO	Neo cassette	α: Exon 2	None	(Schlüter et al. 2003)
αβ-synuclein double KO	α: (Schlüter et al. 2003) X β: Neo cassette	β: Exon 1	None	(Chandra et al. 2004)
αβγ-synuclein triple KO	αβ: (Chandra et al. 2004) X γ: Neo cassette (Ninkina et al. 2003)	γ: Exons 1–3	Decreased survival starting at 12 months	(Greten-Harrison et al. 2010)

mouse models (Table 5). The synuclein family consists of three proteins—α-synuclein, β-synuclein, and γ-synuclein—though only α-synuclein misfolding is implicated in the disease pathogenesis of synucleinopathies. Interestingly, while α-synuclein is thought to play a role in SNARE complex formation, vesicle recycling, neurotransmitter release (Burré et al. 2010), and double-stranded DNA repair (Schaser et al. 2019), no cognitive or motor deficits are seen in the two functional *Snca* KO models generated using a neo cassette (Cabin et al. 2002; Schlüter et al. 2003). While both mouse models exhibit normal life spans, the *Snca*$^{-/-}$ model does show a reduction in pre-synaptic vesicles, as well as the reserve–resting pool of synaptic vesicles in the hippocampus, though neither appears to alter synaptic transmission (Cabin et al. 2002). Using a Cre-dependent conditional KO approach, Ninkina et al. developed a mouse line with a single ectopic loxP site to facilitate the deletion of α-synuclein in specific cell populations or brain regions (Ninkina et al. 2015). Using this line with a tamoxifen-inducible Cre-ERT2 transgene, the KO mice also show a lack of behavioral effects or changes in synaptic function.

The lack of deficits observed in *Snca* KO models may be explained by compensation from both β- and γ-synuclein. To generate a triple synuclein KO model, Greten-Harrison et al. crossed the α-synuclein functional KO (Schlüter et al. 2003) with the β-synuclein (Chandra et al. 2004) and γ-synuclein functional KO lines, and found that despite having a normal brain architecture through 24 months of age, the mice have an altered synaptic structure and decreased lifespan (Greten-Harrison et al. 2010). Starting as early as 3 months old, the hippocampal synaptic puncta are ~30% smaller in triple KO mice compared to age-matched WT controls, but these effects are rescued by overexpression of human α-synuclein. Notably, these models have served an important role in deciphering the normal role of α-synuclein in the neuron, which has been imperative for many gene therapy strategies currently in development that aim to reduce α-synuclein expression. Moreover, as noted previously regarding tau KO models, these findings also indicate that disease pathogenesis in synucleinopathies is much more likely to arise from a gain of toxic function or a combinatorial effect of gain of function and loss of function, rather than being purely caused by loss of normal α-synuclein function alone.

3.3 | Spontaneous Models of Synucleinopathy

The synucleinopathy field has relied heavily on the use of humanized transgenic mouse models that spontaneously develop clinical signs and α-synuclein pathology to investigate the role of α-synuclein in disease pathogenesis (Table 6). While some of these models are bred onto an endogenous mouse *Snca* knockout background to prevent interference from mouse α-synuclein in disease, this is more the exception than the rule. Instead, studies using *SNCA*-expressing mice typically focus on using different promoters to evaluate the effect of transgene expression in various brain regions and cell types, as well as relying on familial *SNCA* mutations to initiate disease.

A handful of models use the *Thy1* promoter, as this glycoprotein is a terminal marker of differentiation and is therefore expressed in all neurons (Spanopoulou et al. 1991). These include the Thy1-G51D and Thy1-3D mice (Nuber et al. 2024), as well as the 3KL model (Nuber et al. 2018). By comparison, several synucleinopathy mouse models use the *MoPrP.Xho* promoter to achieve neuronal expression, due to high expression of PrP^{C} in neurons (Bendheim et al. 1992). Among these models, the most widely used is the TgM83 line developed by Virginia Lee's laboratory (Giasson et al. 2002), though this group also includes the PrPMtA (Gispert et al. 2003), −/−/A53T (Cabin et al. 2005), TgM20 (Giasson et al. 2002), and TgM47 lines (Emmer et al. 2011), as well. While many of these models develop spontaneous α-synuclein pathology, it is predominantly located in neurons, making these models better suited for investigating disease biology in LBDs rather than MSA. As an alternative, there are three mouse models that use oligodendrocyte-specific promoters to drive *SNCA* expression. These include the proteolipid protein, or PLP, promoter (Kahle et al. 2002), the myelin basic protein, or MBP, promoter (Shults et al. 2005) and the cyclic nucleotide phosphodiesterase, or CNP, promoter (Yazawa et al. 2005). While these models develop oligodendroglial inclusions more consistent with MSA, their major weakness is the forced expression of α-synuclein in oligodendrocytes rather than neurons. Previous studies found that oligodendrocytes typically do not express α-synuclein at easily detectable levels (Asi et al. 2014; Djelloul et al. 2015; Miller et al. 2005; Ozawa et al. 2001; Solano et al. 2000) while more recent work using

TABLE 6 | Rodent models of spontaneous synucleinopathy.

Model	Mutation	Promoter	Protein level[a]	Deficits[b]	Disease Onset	A-synuclein pathology	Ref
B6.Cg-Tg(SNCA)OVX37Rwm *Snca*^{tm1Rosl}/J [*SNCA*-OVX]	None	BAC *SNCA*	1.9x	Increased dry stool weight (males), rotarod, & reduced forepaw stride length by 18 months	n.p.	None	(Janezic et al. 2013)
B6;D2-Tg(Mbp-SNCA) 29Ema/RoriJ [MBP hα-syn Line 29]	None	*Mbp*	n.p.	Rotarod and pole climbing, tremors, ataxia, & seizure by 2–4 months	6 months	Glial pathology in basal ganglia, cerebellum, brainstem, & neocortex by 4 months	(Shults et al. 2005)
B6;D2-Tg(PDGFB-SNCA)4Ema/RoriJ [D Line (PDGFβ-hαsyn)]	None	*PDGFβ*	n.p.	Rotorod by 12 months	n.p.	Neocortex, hippocampus, & substantia nigra by 2 months	(Masliah et al. 2000)
B6;D2-Tg(Thy1-SNCA) 61Ema/RoriJ [Line 61 (mThy1 hα-syn Tg)]	None	*Thy1*	1.5–3.4x	Wire-hang by 1 months, parkinsonian phenotype by 14 months	14 months	Olfactory bulb, thalamus, locus coeruleus, cerebellum, & dorsal nucleus of the vagus by 1 months	(Rockenstein et al. 2002)
Snca^{tm1Rosl} Tg(Cnp-SNCA)M2Vle/KlukJ [CNP-α-Syn M2]	None	*Cnp*	n.p.	Rotorod & wire-hang by 7–9 months	None	Oligodendrocytes in the cerebral cortex & brainstem by 24 months	(Yazawa et al. 2005)
B6;C3-Tg(Prnp-SNCA)20Vle/J [TgM20$^{+/+}$]	None	*MoPrP.Xho*	6.9x	None	None	None	(Giasson et al. 2002)
FVB;129S6-*Snca*^{tm1Nbm} Tg(SNCA)1Nbm/J [Tg(SNCA)1Nbm]	None	PAC *SNCA*	1.3-2x	None	n.p.	None	(Kuo et al. 2010)
B6.Cg-*Snca*^{tm1Rosl} Tg(SNCA)192Rwm/J	None	BAC *SNCA*	< 1x	None	n.p.	None	(Taylor et al. 2014)
PLP-1	None	Proteolipid protein	0.1–1.2X	None	n.p.	Oligodendrocytes in cerebellar white matter by 18 months	(Kahle et al. 2002)
B6;129-*Gt(ROSA)26Sor*$^{tm3(SNCA-119)Djmo}$/TmdJ [ROSA26-Syn-119]	Truncated at residue 119	Gt(*Rosa26*)	< 0.1x	None	None	None	(Daher et al. 2009)
FVB;129S6-*Snca*^{tm1Nbm} Tg(SNCA*A30P)1Nbm Tg(SNCA*A30P)2Nbm/J [Tg(SNCA*A30P)1Nbm]	A30P	PAC *SNCA*	1.3–2x	Prolonged whole gut transit time by 3 months; decreased colonic motility by 6 months (males)	n.p.	Enteric nervous system by 3 months	(Kuo et al. 2010)

(Continues)

TABLE 6 | (Continued)

Model	Mutation	Promoter	Protein level[a]	Deficits[b]	Disease Onset	A-synuclein pathology	Ref
B6.Cg-*Snca*^{tm1Rosl} Tg(SNCA*A30P)192Rwm/J [SNCA-A30P+ Snca−/−]	A30P	BAC *SNCA*	< 1x	Reduced time in open arm by 6 months; increased wheel running	n.p.	None	(Taylor et al. 2014)
B6;C3-Tg(Prnp-SNCA)47Vle/J [TgM47$^{+/+}$]	E46K	*MoPrP.Xho*	3.5x	Kyphosis, limb paralysis, & impaired righting by 16 months	16–29 months	Motor cortex, thalamus, cerebellar nuclei, brainstem, & spinal cord by 18 months	(Emmer et al. 2011)
B6;129-*Gt(ROSA)26Sor*$^{tm3(SNCA*E46K)Djmo}$/TmdJ [ROSA26-Syn-E46K]	E46K	Gt(*Rosa26*)	n.p.	None	None	None	(Daher et al. 2009)
NTac:SD-Tg(SNCA*E46K) 70CJLi	E46K	BAC *SNCA*	2–3x	None unless challenged with rotenone	None	Cortex, striatum, & substantia nigra by 12 months	(Cannon et al. 2013)
C57BL/6J-Tg(Thy1SNCA*)1Dett/J [Thy1-G51D]	G51D	*Thy1*	2.5x	Reduced locomotion & rotarod, increased pole test by 6 months	n.p.	Frontal cortex, midbrain, & caudoputamen by 12 months	(Nuber et al. 2024)
Tg(tetO-SNCA*A53T)E2Cai/J	A53T	*CaMKII* Tet Off	30x	Increased locomotion by 2 months & rearing by 6 months	n.p.	n.p.	(Lin et al. 2009)
B6;C3-Tg(Prnp-SNCA*A53T) 83Vle/J [TgM83$^{+/+}$]	A53T	*MoPrP.Xho*	4.6x	Poor grooming, weight loss, reduced ambulation, partial paralysis of limbs, & periods of hindlimb freezing by 8 months	8–16 months	Striatum, thalamus, cerebellar nuclei, brainstem, & spinal cord by 8 months	(Giasson et al. 2002)
PrPMtA	A53T	*MoPrP*	n.p.	Decreased activity, grip strength, & step length after 12 months	n.p.	Olfactory bulb & cortex by 7 months, substantia nigra by 13 months	(Gispert et al. 2003)
−/−/A53T [PrPMtA (Gispert et al. 2003) X *Snca*$^{-/-}$(Cabin et al. 2002)]	A53T	*MoPrP*	n.p.	Decreased stride length, limb weakness, knuckle walking, & paralysis by 18 months	16–23 months	Spinal cord & sciatic nerve by 19 months	(Cabin et al. 2005)
FVB;129S6-*Snca*^{tm1Nbm} Tg(SNCA*A53T)1Nbm Tg(SNCA*A53T)2Nbm/J [Tg(SNCA*A53T)1Nbm]	A53T	PAC *SNCA*	1.3–2x	Prolonged whole gut transit time by 3 months; decreased colonic motility by 6 months (males); rotarod by 6 months	n.p.	Enteric nervous system by 3 months	(Kuo et al. 2010)
B6;129-*Gt(ROSA)26Sor*$^{tm3(SNCA*A53T)Djmo}$/TmdJ [ROSA26-Syn-A53T]	A53T	Gt(*Rosa26*)	n.p.	None	None	None	(Daher et al. 2009)

(Continues)

TABLE 6 | (Continued)

Model	Mutation	Promoter	Protein level[a]	Deficits[b]	Disease Onset	A-synuclein pathology	Ref
B6.Cg-2310039L15Rik *Tg(Prnp-SNCA*A53T)23Mkle*/J [Line G2-3(A53T)]	A53T	*MoPrP.Xh*	6x	Bradykinesia, mild ataxia, dystonia, loss of righting reflex, & paralysis by 10 months	9–16 months	Midbrain, cerebellum, brainstem, & spinal cord by 10 months	(Lee et al. 2002)
C57BL/6J-Tg(Th-SNCA*A30P*A53T)39Eric/J [HM2]	A30P A53T	Rat Th	0.5x	Increased locomotion by 2 months, which reverses by 7 months; impaired righting reflex by 13 months	n.p.	None	(Richfield et al. 2002)
C57BL/6-Tg(Thy1-SNCA*E35K*E46K*E61K)3798Nuber/J [3KL]	E35K E46K E61K	*Thy1.2*	2x	Abnormal gait, head and body tremor, hind limb clasping, reduced movemet in open field test, rotarod, & pole climbing by 3 months	n.p.	Cortex, striatum, & substantia nigra by 6 months	(Nuber et al. 2018)
C57BL/6J-Tg(Thy1-SNCA*)37Dett/J [Thy1-3D]	V40D G51D V66D	*Thy1*	3x	Reduced locomotion & rotarod, increased pole test by 6 months	n.p.	Frontal cortex, midbrain, & caudoputamen by 12 months	(Nuber et al. 2024)

Abbreviations: BAC, bacterial artificial chromosome; n.p., not published; PAC, P1-derived artificial chromosome; *SNCA*, α-synuclein gene.
[a]Relative to endogenous murine a-synuclein as reported in the initial publication.
[b]Tests are only listed if animals displayed a deficit.

RNAscope to detect α-synuclein transcripts in oligodendrocytes in both control and MSA patient samples found evidence of *SNCA* transcripts in oligodendrocytes, but oligodendroglial expression was substantially lower than was observed in neurons (Kon et al. 2024, 2023). Given this measurable difference in expression level by cell type, it is plausible that the pathology seen in oligodendrocyte-specific overexpressing models is due to altered cellular biology.

Circumventing the shortcoming of using models that rely on the non-endogenous promoter to facilitate transgene expression, other mouse lines rely on artificial chromosomes that incorporate not just the gene, but several upstream and downstream regulatory elements as well. These models tend to result in *SNCA* expression levels that are closer to endogenous protein expression, which is likely a contributor to the later onset of brain pathology, if any develops, as seen in these lines. Moreover, there is often a peripheral component to synucleinopathies, particularly within the gastrointestinal tract. Notably, many of the models that rely on an artificial chromosome develop α-synuclein inclusions in the enteric nervous system concurrent with prolonged gut transit time and reduced colonic motility. This is particularly prominent in the models developed by Robert Nussbaum's laboratory (Kuo et al. 2010).

In addition to differences in how the transgene is expressed, synucleinopathy models also differ in their expression of WT or mutant protein. As mentioned previously, there are no known *SNCA* mutations found in patients with MSA, though a handful of mutations have been identified in LBD patient families. Animal models that rely on the expression of WT *SNCA* typically do not develop α-synuclein inclusions or robust disease. This is seen in mice using artificial chromosomes, such as the *SNCA-OVX* mice (Janezic et al. 2013) and the Tg(SNCA)1Nbm mice (Kuo et al. 2010), as well as models using neuronal promoters for transgene expression, like the TgM20 line (Giasson et al. 2002). As a result, several models rely on the A30P, E46K, and A53T mutations, as these were the first three familial mutations identified in PD patient cohorts. Among these models, the TgM83 mice (Giasson et al. 2002) are likely the most broadly used to investigate disease pathogenesis. Notably, homozygous TgM83$^{+/+}$ mice develop a spontaneous disease around 1 year of age that is characterized by partial paralysis of the hindlimbs, weakness, and ataxia. Symptomatic animals develop neuronal α-synuclein inclusions in corresponding hindbrain regions, including the cerebellar nuclei and brainstem, in addition to extensive spinal cord pathology. By comparison, these same deficits are not seen in hemizygous TgM83$^{+/-}$ mice.

Given that only a small percentage of LBD cases are caused by *SNCA* mutations, and that MSA is not caused by any known mutation, the reliance on mutations to drive disease onset is a weakness of these models. It is unclear how these mutations impact the disease biology under investigation, or if the resulting pathogenic α-synuclein strains are similar to or different from the strains seen in human patient samples. However, as noted previously when discussing the tau mouse models, the lack of spontaneous disease in mice expressing WT α-synuclein, or in asymptomatic hemizygous models with mutations, offers an important opportunity to perform injection studies in the absence of background interference from the model system itself.

TABLE 7 | Rodent inoculation models of synucleinopathy.

Model	Mutation	Promoter	Protein level[a]	Injection site & age[b]	Inoculum	Motor deficits[c]	Incubation period	A-synuclein pathology	Ref(s)
WT	None	None	NA	Dorsal neostriatum @ 2–3 months	WT murine PFFs	Reduced rotoraod & wire hang by 6 mpi	n.p.	Frontal cortex, neocortex, striatum, & amygdala by 6 mpi	(Luk, Kehm, Zhang, et al. 2012)
FVB;129S6-*Snca*^{tm1Nbm} Tg(SNCA)1Nbm/J [Tg(SNCA)1Nbm]	None	PAC *SNCA*	1.3-2x	Striatum @ 1.5–2 months	MSA & LBD	None	n.p.	Throughout the brain by 9 mpi	(Bernis et al. 2015)
Tg(Prnp-SNCA) 20Vle/J [TgM20$^{+/-}$]	None	*MoPrP.Xho*	Hemi: 5.6x	Thalamus @ 2 months	WT PFFs	Hind limb clasping, difficulty rearing, weak grip strength, circling, & bradykinesia by 7 mpi	7 mpi	Hippocampus, thalamus, hypothalamus, midbrain, & pons by 7 mpi	(Holec, Lee, Oehler, Ooi, et al. 2022)
					MSA	Hind limb clasping, difficulty rearing, weak grip strength, circling, & bradykinesia by 9.5 mpi	9.5 months (Females: 8.5 mpi; Males: 10.5 mpi)	Hypothalamus, midbrain, & pons by ~9.5 mpi	
				Hippocampus @ 2mo	WT PFFs	None by 6 mpi	Study ended at 6 mpi	Throughout the brain by 6 mpi	(Lloyd et al. 2022)
					MSA			Limbic system by 6 mpi	
					DLB, AD/ALB			Hippocampus by 6 mpi	
Tg(Prnp-SNCA*E46K) 47Vle/J [TgM47]	E46K	*MoPrP.Xho*	HOZ: 3.5x	Bilateral hippocampus @ 2 months	WT, E46K, & A53T PFFs	None	Study ended at 4 mpi	Hippocampus with modest spread to cortex, midbrain, & brainstem by 4 mpi	(Sacino et al. 2014)
			Hemi: n.p.	Thalamus @ 2 months	E46K PFFs	Hind limb clasping, weak grip strength, & moribund by 9.5 months	9.5 mpi	Hypothalamus, midbrain, & pons by 9.5 mpi	(Holec, Lee, Oehler, Batia, et al. 2022)
					MSA	None	None	None	
FVB;129S6-*Snca*^{tm1Nbm} Tg(SNCA*A53T)1Nbm Tg(SNCA*A53T)2Nbm/J [Tg(SNCA*A53T)1Nbm]	A53T	PAC *Snca*	1.3–2x	Thalamus @ 2 months	MSA	None	None	Hippocampus, piriform cortex and amygdala, & parahippocampal cortex by 11 mpi	(Woerman et al. 2019)

(Continues)

TABLE 7 | (Continued)

Model	Mutation	Promoter	Protein level[a]	Injection site & age[b]	Inoculum	Motor deficits[c]	Incubation period	A-synuclein pathology	Ref(s)
Tg(Prnp-SNCA*A53T)83Vle/J (TgM83)	A53T	*MoPrP.Xho*	HOZ: 4.6x	Intracerebral @ 1.75–2.25 months	$TgM83^{+/+}$	Hindlimb paraysis by 6.3 mpi	6.3 mpi	Hindbrain by 6.3 mpi	(Mougenot et al. 2012)
				Somatosensory cortex & striatum @ 2–4 months	WT PFFs	Hindlimb paraysis by 3.3 mpi	3.3 mpi	Cortex, thalamus, hypothalamus, & brainstem nuclei by 3 mpi	(Luk, Kehm, Zhang, et al. 2012)
			Hemi: 3.3x	Hippocampus and thalamus @ 1.25 months	PFFs A53T—Salt	Hindlimb paralysis, bradykinesia, & moribund by 4.7 mpi	4.7 mpi	Thalamus, hypothalamus, midbrain, cerebellum, & brainstem by 4.7 mpi	(Lau et al. 2020)
					A53T—No Salt	Weight loss, kyphosis, hindlimb shaking, & moribund by 12.5 mpi	12.5 mpi	Throughout the brain by 12.5 mpi	
				Thalamus @ 2 months	MSA	Ataxia, circling, hindlimb paralysis & moribund by 4 mpi	~4 mpi	Hypothalamus, midbrain, & pons by 4 mpi	(Prusiner et al. 2015; Watts et al. 2013)
				Thalamus @ 2 months	$TgM83^{+/+}$	Ataxia, circling, hindlimb paralysis, & moribund by 7 mpi	7 mpi	Motor cortex, striatum, thalamus, hypothalamus, midbrain, & pons by 7 mpi	(Watts et al. 2013)
				Hippocampus & overlying cortex @ 1.25 months	Familial PD with the G51D *SNCA* mutation	None	None	Cortex, parahippacampus, and brainstem by 18 mpi	(Lau et al. 2023)

Abbreviations: AD/ALB, Alzheimer's disease with amygdala predominant Lewy bodies; DLB, dementia with Lewy bodies; Hemi, hemizygous; HOZ, homozygous; mpi, months post-inoculation; MSA, multiple system atrophy; n.p., not published; PAC, P1-derived artificial chromosome, PD, Parkinson's disease, PFFs, pre-formed fibrils; *SNCA*, α-synuclein gene; WT, wildtype.

[a]Relative to endogenous murine α-synuclein as reported in the initial publication.

[b]Injections were administered unilaterally unless otherwise specified.

[c]Tests are only listed if animals displayed a deficit.

3.4 | Inoculation Models of Induced Synucleinopathy

Inoculation studies offer an important benefit over relying on spontaneous disease formation, which is the ability to investigate distinct α-synuclein strains and better understand their corresponding biological consequences and disease pathogenesis (Table 7). Substantial work in the field has relied upon a model system first pioneered by Kelvin Luk in which recombinant α-synuclein PFFs are injected i.c. into WT mice (Luk, Kehm, Carroll, et al. 2012). In these initial studies, injection of mouse PFFs into the dorsal striatum resulted in α-synuclein pathology in the neocortex, ventral striatum, thalamus, and occipital cortex 6 mpi. Similarly, Masuda-Suzukake et al. injected female mice with either murine or human WT PFFs into the substantia nigra and found α-synuclein inclusions in the substantia nigra, amygdala, dentate gyrus, hippocampus, hypothalamus, somatosensory area, visual cortex, cingulate cortex, and corpus callosum at 15 mpi (Masuda-Suzukake et al. 2013). Notably, the human PFFs were only ~90% as efficient as the mouse PFFs, stemming from the known human/mouse species barrier for α-synuclein (Luk et al. 2016). This finding underscores a major weakness of the approach, which is that the most effective transmission studies rely on mouse α-synuclein, impeding the investigation of human synucleinopathy strains. Additionally, PFF transmission to WT mice does not regularly result in the onset of neurological signs, and when mice do develop disease, it occurs > 400 days post-inoculation (Rahayel et al. 2022). As a result, animals are euthanized at set time points to evaluate pathological spread in the brain. However, as previously discussed in detail (Woerman and Bartz 2024), this approach ignores the role of titer differences in determining the rate of spread in the brain. As a result, studies may incorrectly attribute differences in titer to unique strain biologies when varied lesion profiles are seen at study endpoints.

As an alternative, transgenic mouse models are often used for transmission studies, particularly in the case that i.c. injections result in terminal disease. While transmission studies in the tau field have typically relied on accelerating pathology onset in spontaneous disease models (see discussion above), the synucleinopathy field benefits from the availability of several models that do not develop spontaneous disease but do propagate a variety of α-synuclein strains. This work started with the TgM83 mouse model, which expresses the A53T mutation, where initial studies showed that aged TgM83$^{+/+}$ brain homogenates can transmit neurological disease to younger TgM83$^{+/+}$ mice (Luk, Kehm, Zhang, et al. 2012; Mougenot et al. 2012). However, shortly after, studies using the hemizygous TgM83$^{+/-}$ mice, which do not develop spontaneous disease, also showed that aged TgM83$^{+/+}$ brain homogenate can induce neurological disease (Watts et al. 2013), resulting in a shift toward using TgM83$^{+/-}$ mice to avoid the confounding effects of the spontaneous disease background. Since then, substantial work has shown that MSA patient samples can transmit disease to the TgM83$^{+/-}$ mouse model following both i.c. injection (Prusiner et al. 2015; Watts et al. 2013) or peripheral exposure routes (Holec et al. 2024; Woerman, Kazmi, Patel, Freyman, et al. 2018). Moreover, studies from Joel Watts' laboratory have shown that this model is capable of propagating distinct α-synuclein strains by using WT α-synuclein PFFs that were fibrillized either under salt or no salt conditions. The two biochemically distinct fibril strains induce distinct incubation periods, clinical signs, and neuropathological lesions in the brains of terminal TgM83$^{+/-}$ animals (Lau et al. 2020). Notably, however, while LBD patient samples are unable to induce clinical disease in the mice (Prusiner et al. 2015), two groups have shown the presence of subclinical pathogenic α-synuclein replication detectable in inoculated animals (Lau et al. 2023; Thomzig et al. 2021).

A major criticism of the studies performed using the TgM83$^{+/-}$ model is the reliance upon the A53T mutation, particularly because MSA patients do not have *SNCA* mutations and the A53T mutation is only seen in a subset of LBD patients. As an alternative, the TgM20$^{+/-}$ mouse line, which expresses WT α-synuclein, has emerged as an important model based on the ability of both PFFs and MSA patient samples to induce neurological disease in the animals (Holec, Lee, Oehler, Ooi, et al. 2022; Lloyd et al. 2022). Intriguingly, for unknown reasons, disease onset in TgM20$^{+/-}$ mice following MSA transmission occurs ~2 months earlier in female mice compared to male animals (Holec, Lee, Oehler, Ooi, et al. 2022), necessitating the need to increase group size to adequately power studies in this line when considering sex as a biological variable. These sex-specific effects are not typically seen in other models; for example, male and female TgM83$^{+/-}$ mice develop disease onset at similar ages following MSA transmission. In comparison with PFF and MSA studies, it should be noted that LBD patient samples are able to induce α-synuclein inclusions at the injection site by 6 mpi, but the animals fail to develop clinical disease (Lloyd et al. 2022), again limiting the application of the model to compare human-derived strains.

By comparison with the TgM83 and TgM20 models, the TgM47 mice express the E46K mutation, and while the homozygous animals develop spontaneous disease, the hemizygous mice do not (Emmer et al. 2011). TgM47$^{+/+}$ mice develop robust α-synuclein pathology throughout the brain 4 mpi following i.c. injection with a variety of WT and mutant PFFs (Sacino et al. 2014), and TgM47$^{+/-}$ mice develop terminal disease ~9.5 mpi with E46K PFFs (Holec, Lee, Oehler, Batia, et al. 2022). However, this model is resistant to MSA transmission, remaining asymptomatic to ~475 days post-inoculation following i.c. injection (Holec, Lee, Oehler, Batia, et al. 2022) due to the disruption of a salt bridge between residues E46 and K80 that stabilizes the misfolded α-synuclein conformation in MSA patients (Reis et al. 2024; Schweighauser et al. 2020).

Lastly, the PAC models made by Robert Nussbaum's laboratory (Kuo et al. 2010) have been used to study α-synuclein transmission, but do not result in motor deficits. Initial studies using detergent-extracted α-synuclein isolated from either MSA or LBD patient samples induce widespread α-synuclein inclusions 9 mpi into the WT Tg(SNCA)1Nbm/J mice (Bernis et al. 2015). However, inoculation of crude brain homogenates from MSA and LBD patient samples was unable to achieve the same outcome (Woerman et al. 2019), potentially due to differences in sample preparation. Interestingly, MSA patient samples were able to induce α-synuclein pathology in the A53T-expressing Tg(SNCA*A53T)1Nbm mice 1 year post-injection (Woerman et al. 2019). Moreover, brain homogenates prepared from the affected animals were able to induce neurological disease when

transmitted to the TgM83$^{+/-}$ mouse model, demonstrating the model is capable of propagating pathogenic α-synuclein.

Overall, while these models have collectively been incredibly useful for probing the differences between α-synuclein strains and their interactions with mutated protein substrates, a major limitation or caveat to the studies has recently emerged. Previous studies found that both clinical and pathological outcomes are preserved through serial passaging of inoculum (Lau et al. 2020; Woerman, Kazmi, Patel, Aoyagi, et al. 2018; Woerman et al. 2019; Woerman et al. 2015), suggesting that the replication of each strain occurred with high fidelity. However, more recent findings indicate that strain selection and/or adaptation occur during transmission, leading to distinct strain biologies between the inoculum and resulting brain homogenates (Holec et al. 2024; Holec, Lee, Oehler, Batia, et al. 2022; Holec, Lee, Oehler, Ooi, et al. 2022). One potential source for this is the location of the A53T mutation at the protofilament interface in the MSA fibril structure (Schweighauser et al. 2020), which may force deformed templating to occur. However, without cryo-EM structures of the inoculum before and after passage, it is not fully possible to determine the mechanism of adaptation.

3.5 | Limitations of Synucleinopathy Rodent Models

The model systems discussed above have generated highly valuable information about human synucleinopathies; however, there are several limitations to these models that need to be recognized, as they contribute to the appropriate experimental use of each. First, many rodent models rely heavily on *SNCA* mutations to induce α-synuclein misfolding and aggregation, particularly the A53T mutation, but only ~2% of familial LBD cases are caused by *SNCA* mutations (Bandres-Ciga et al. 2020; Lesage and Brice 2009; Tran et al. 2020) and no mutations have been identified in MSA patients. Given the increasing data pointing to the potential for *SNCA* mutations to force strain adaptation in vivo (Holec et al. 2024; Holec, Lee, Oehler, Batia, et al. 2022), the use of WT-expressing models to investigate disease biology is increasingly important. Second, the promoters used to drive transgene expression are typically neuron-specific, which results primarily in neuronal pathology. While LBD pathology is seen in neurons, as mentioned previously, MSA is characterized predominantly by GCIs in oligodendrocytes, though α-synuclein can accumulate in neuronal cytoplasmic inclusions as well. Three mouse models rely on oligodendrocyte-specific promoters to achieve glial inclusions. While this recapitulates the cell-specific pathology in MSA patients, it does not accurately model cell type-specific α-synuclein expression patterns in the human brain. An important outstanding question in the field pertains to how oligodendroglial pathology develops in MSA patients, despite the relatively lower *SNCA* expression in oligodendrocytes compared to neurons. Unfortunately, neither the neuron nor the oligodendrocyte-specific transgenic mouse models are well-suited to answer this question. Mouse models using artificial chromosomes to express the full *SNCA* gene along with its upstream and downstream regulatory elements have also failed to fully recapitulate this aspect of human biology, though glial pathology is observed (Woerman et al. 2019) suggesting knock-in models may be needed to adequately model this process. Notably, it also remains unclear how post-translational modifications may contribute to the cell-to-cell spread or other pathogenic processes in synucleinopathies.

Finally, with the goal of developing therapeutic interventions for synucleinopathy patients, validation that the currently available model systems recapitulate the strain biology in human patients is needed. Cryo-EM studies have been valuable in determining the misfolded conformation of α-synuclein in MSA (Schweighauser et al. 2020; Yan et al. 2024) and LBD patient samples (Yang et al. 2022), but there are currently no reported structures from either spontaneous or inoculated mouse models. This is particularly important in light of the widespread use of PFFs as an inoculum source in synucleinopathy models, given that PFFs have not been shown to adopt the same misfolded conformations seen in human patient samples, as discussed previously (Holec, Liu, et al. 2022). Here, again, there are important lessons to learn from the strain-specific activity of IND24 in the prion field, emphasizing the need to better ascertain translatability between rodent models and the human diseases they are used to study. Expanding the use of cryo-EM to resolve α-synuclein conformations from spontaneous and inoculated mouse models would greatly address this growing need.

4 | Tauopathy and Synucleinopathy Rodent Models Share Several Limitations

The tauopathy and synucleinopathy fields have greatly benefited from the widespread availability of a variety of rodent models, but it is important to remember that regardless of which model system is being used, they each have their individual strengths and weaknesses. No single model can address every scientific question about a disease or group of diseases. Instead, we must operate within the confines of each model, being careful not to over-interpret or draw false conclusions when the wrong model is used to interrogate an important question. It is, therefore, imperative that we understand the strengths and limitations of each model so that we can select the appropriate system for each hypothesis we aim to test. While several overlapping limitations pertain to the currently available rodent models for tauopathy and synucleinopathy, we conclude by focusing on three of the most impactful, while offering suggestions for overcoming these challenges.

First, the varied use of promoters for driving transgene expression in rodent models adds increasing complexity to our understanding of disease biology and data interpretation. For example, as discussed above, the choice between neuron- and oligodendrocyte-specific promoters detracts from our understanding of how GCI pathology forms in MSA patients. Moreover, the variety of neuron-specific promoters creates confusion when discerning which observed effects are driven by transgene expression and which are determined by misfolded protein strain. As an example, are the differences seen in the Tg2541 and PS19 mouse lines caused by using two different neuron-specific promoters, or by the misfolding of tau into two unique conformations? Critically, the lack of *Mapt* or *Snca* promoters in overexpression models means that rodents have

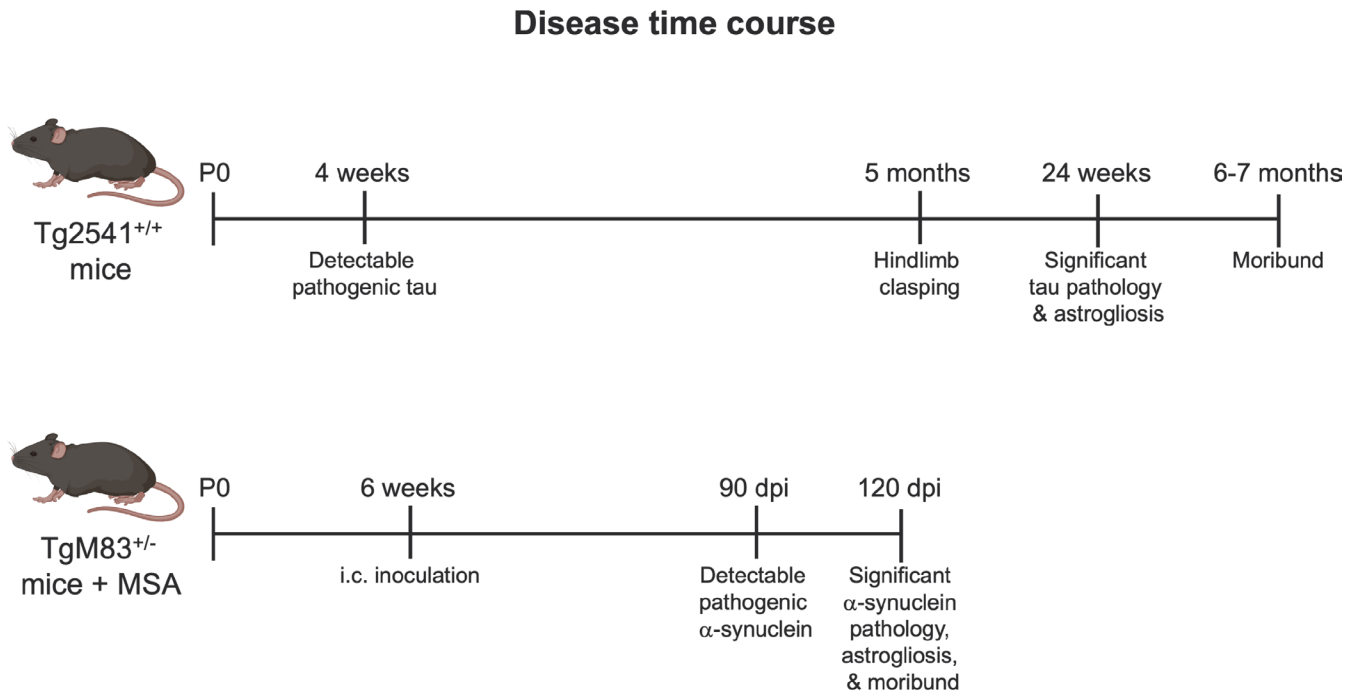

FIGURE 3 | Disease time course in rodent models of tauopathy and synucleinopathy. Previous studies deeply characterized key markers of disease progression in the Tg2541$^{+/+}$ mice (top) and multiple system atrophy (MSA)-inoculated TgM83$^{+/-}$ mice (bottom). (Top) In Tg2541$^{+/+}$ mice, pathogenic tau is detectable in mice ~4 weeks old whereas significant tau pathology and astrogliosis are only quantifiable at 24 weeks (Woerman et al. 2017). Mice develop progressive hindlimb clasping around 5 months old and are moribund by 6 to 7 months old (Allen et al. 2002). (Bottom) By comparison, TgM83$^{+/-}$ mice inoculated with MSA patient samples around 6 weeks old develop pathogenic α-synuclein by 90 days post-inoculation (dpi) (Woerman et al. 2020). Quantifiable α-synuclein pathology (Woerman et al. 2020) and astrogliosis occur shortly before disease onset (Watts et al. 2013), when mice develop progressive hindlimb paralysis and become moribund. Made using Biorender.com.

transgene expression patterns that differ from protein expression in humans. As a result, those model systems fail to mimic the spatial and temporal distribution of lesions in human brains, which may be a major contributor to the inability of rodent systems to fully replicate the complexity of human disease in tauopathies and synucleinopathies.

Second, as we discussed previously, the lack of standard criteria for both characterizing model systems and performing inoculation studies creates challenges when comparing findings across laboratories. Overcoming this obstacle will likely require investing significant time and effort into deep phenotyping across multiple labs to establish measured timelines for the onset of key features of each disease in a highly rigorous and reproducible manner. For example, as discussed above, a careful analysis of the homozygous and hemizygous Tg2541 mice, as well as the PS19 mouse line, established important timelines for the formation of detectable pathogenic tau, the initiation and spread of tau pathology in the brain, the appearance of an astrocytic response, and the onset of progressive motor deficits leading to terminal disease (Figure 3, top) (Woerman et al. 2017). Similar time course studies were also conducted in TgM83$^{+/-}$ mice inoculated with MSA patient samples (Figure 3, bottom) (Woerman et al. 2020). While resource-intensive, these efforts are not only needed to better support the use of an animal model in preclinical studies for therapeutic development, but they are also critical to determining the appropriate use of each model system. For example, given that a robust astrogliosis only occurred around 24 weeks of age in the Tg2541$^{+/+}$ mice, using that specific animal model to interrogate the role of the inflammatory response in the early stages of disease would not generate biologically meaningful results. Alternatively, using this line to determine if a small molecule can interfere with or slow the formation and spread of pathogenic tau, which was detectable by ~4 weeks old, would greatly accelerate the timeline for anti-tau therapeutic development.

Finally, as we strive to achieve better clinical outcomes for patients afflicted with tauopathies and synucleinopathies, it is necessary to focus on how we can build better rodent models that provide a stronger bridge to the clinic. In the age of cryo-EM, evaluating the strain properties of each model system is a necessary endeavor. Using both structural and biological approaches that ensure the strains we are studying in rodent models are consistent with those we aim to diagnose and treat in human patients will greatly strengthen the value and translatability of ongoing research in the field. Learning from the failure of IND24 to translate into a successful therapeutic requires recognizing that the pathogenic strain each potential treatment is tested against is imperative to the overall outcome of a drug discovery program. When model systems fail to capture the strain properties present in the target patient population, we continue to risk the ability to succeed in clinical trials. Prioritizing the development and selection of the right model system to support each experimental goal is critical to making meaningful progress for patients and their families.

Author Contributions

Emma Szegvari: conceptualization, writing – original draft, writing – review and editing. **Sara A. M. Holec:** conceptualization, writing – original draft, writing – review and editing. **Amanda L. Woerman:** conceptualization, funding acquisition, writing – original draft, writing – review and editing, supervision.

Acknowledgements

The Woerman lab is supported by grants from the National Institutes of Health (R01NS121294 and accompanying supplement; R21NS127002; R01AG071694; R01AG082349) and the Rainwater Charitable Foundation (23010093) to Amanda L. Woerman. This work was also supported by Colorado State University. Emma Szegvari is supported by the National Institute of General Medical Sciences of the National Institutes of Health (T32GM136628 "Dual-Degree Medical Scientist Training Program for Veterinarians"). The graphical abstract and Figures 1 and 3 were made using Biorender.com.

Conflicts of Interest

Amanda L. Woerman is a founder of Allagus Therapeutics, which did not contribute financial or any other support to these studies. The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

Ahmed, Z., E. H. Bigio, H. Budka, et al. 2013. "Globular Glial Tauopathies (GGT): Consensus Recommendations." *Acta Neuropathologica* 126, no. 4: 537–544.

Ahmed, Z., J. Cooper, T. K. Murray, et al. 2014. "A Novel In Vivo Model of Tau Propagation With Rapid and Progressive Neurofibrillary Tangle Pathology: The Pattern of Spread Is Determined by Connectivity, Not Proximity." *Acta Neuropathologica* 127: 667–683.

Alam, P., F. Hoyt, E. Artikis, et al. 2024. "Cryo-EM Structure of a Natural Prion: Chronic Wasting Disease Fibrils From Deer." *Acta Neuropathologica* 148, no. 1: 56.

Allen, B., E. Ingram, M. Takao, et al. 2002. "Abundant Tau Filaments and Nonapoptotic Neurodegeneration in Transgenic Mice Expressing Human P301S Tau Protein." *Journal of Neuroscience* 22: 9340–9351.

Alzheimer, A. 1911. "Über eigenartige Krankheitsfälle des späteren Alters." *Zeitschrift für die gesamte Neurologie und Psychiatrie* 4: 356–385.

Andorfer, C., Y. Kress, M. Espinoza, et al. 2003. "Hyperphosphorylation and Aggregation of Tau in Mice Expressing Normal Human Tau Isoforms." *Journal of Neurochemistry* 86: 582–590.

Appel-Cresswell, S., C. Vilarino-Guell, M. Encarnacion, et al. 2013. "Alpha-Synuclein p.H50Q, a Novel Pathogenic Mutation for Parkinson's Disease." *Movement Disorders* 28, no. 6: 811–813.

Asi, Y. T., J. E. Simpson, P. R. Heath, et al. 2014. "Alpha-Synuclein mRNA Expression in Oligodendrocytes in MSA." *Glia* 62: 964–970.

Ayers, J., T. P. Lopez, I. T. Steele, et al. 2024. "Severe Neurodegeneration in Brains of Transgenic Rats Producing Human Tau Prions." *Acta Neuropathologica* 148, no. 1: 25.

Bandres-Ciga, S., M. Diez-Fairen, J. J. Kim, and A. B. Singleton. 2020. "Genetics of Parkinson's Disease: An Introspection of Its Journey Towards Precision Medicine." *Neurobiology of Disease* 137: 104782.

Békés, M., D. R. Langley, and C. M. Crews. 2022. "PROTAC Targeted Protein Degraders: The Past Is Prologue." *Nature Reviews. Drug Discovery* 21, no. 3: 181–200.

Bendheim, P. E., H. R. Brown, R. D. Rudelli, et al. 1992. "Nearly Ubiquitous Tissue Distribution of the Scrapie Agent Precursor Protein." *Neurology* 42: 149–156.

Benzow, K., K. Karanjeet, A. L. Oblak, G. W. Carter, M. Sasner, and M. D. Koob. 2024. "Gene Replacement-Alzheimer's Disease (GR-AD): Modeling the Genetics of Human Dementias in Mice." *Alzheimers Dement* 20, no. 4: 3080–3087.

Bernis, M. E., J. T. Babila, S. Breid, K. A. Wüsten, U. Wüllner, and G. Tamgüney. 2015. "Prion-Like Propagation of Human Brain-Derived Alpha-Synuclein in Transgenic Mice Expressing Human Wild-Type Alpha-Synuclein." *Acta Neuropathologica Communications* 3: 75.

Berry, D. B., D. Lu, M. Geva, et al. 2013. "Drug Resistance Confounding Prion Therapeutics." *Proceedings. National Academy of Sciences. United States of America* 110, no. 44: E4160–E4169.

Bessen, R. A., D. A. Kocisko, G. J. Raymond, S. Nandan, P. T. Lansbury, and B. Caughey. 1995. "Non-genetic Propagation of Strain-Specific Properties of Scrapie Prion Protein." *Nature* 375: 698–700.

Bessen, R. A., and R. F. Marsh. 1992. "Biochemical and Physical Properties of the Prion Protein From Two Strains of the Transmissible Mink Encephalopathy Agent." *Journal of Virology* 66: 2096–2101.

Bieniek, K. F., N. J. Cairns, J. F. Crary, et al. 2021. "The Second NINDS/NIBIB Consensus Meeting to Define Neuropathological Criteria for the Diagnosis of Chronic Traumatic Encephalopathy." *Journal of Neuropathology and Experimental Neurology* 80, no. 3: 210–219.

Bigio, E. H., A. M. Lipton, S. H. Yen, et al. 2001. "Frontal Lobe Dementia With Novel Tauopathy: Sporadic Multiple System Tauopathy With Dementia." *Journal of Neuropathology and Experimental Neurology* 60, no. 4: 328–341.

Boluda, S., M. Iba, B. Zhang, K. M. Raible, V. M.-Y. Lee, and J. Q. Trojanowski. 2015. "Differential Induction and Spread of Tau Pathology in Young PS19 Tau Transgenic Mice Following Intracerebral Injections of Pathological Tau From Alzheimer's Disease or Corticobasal Degeneration Brains." *Acta Neuropathologica* 129, no. 2: 221–237. https://doi.org/10.1007/s00401-014-1373-0.

Braak, H., and E. Braak. 1991. "Neuropathological Staging of Alzheimer-Related Changes." *Acta Neuropathologica* 82: 239–259.

Brion, J.-P., H. Passareiro, J. Nunez, and J. Flament-Durand. 1985. "Mise en évidence immunologique de la protéine tau au niveau des lésions de dégénérescence neurofibrillaire de la maladie d'Alzheimer." *Archives de Biologie* 95: 229–235.

Buée, L., T. Bussière, V. Buée-Scherrer, A. Delacourte, and P. R. Hof. 2000. "Tau Protein Isoforms, Phosphorylation and Role in Neurodegenerative Disorders." *Brain Research. Brain Research Reviews* 33: 95–130.

Burré, J., M. Sharma, T. Tsetsenis, V. Buchman, M. R. Etherton, and T. C. Südhof. 2010. "Alpha-Synuclein Promotes SNARE-Complex Assembly In Vivo and In Vitro." *Science* 329, no. 5999: 1663–1667.

Cabin, D. E., S. Gispert-Sanchez, D. Murphy, G. Auburger, R. R. Myers, and R. L. Nussbaum. 2005. "Exacerbated Synucleinopathy in Mice Expressing A53T SNCA on a Snca Null Background." *Neurobiology of Aging* 26: 25–35.

Cabin, D. E., K. Shimazu, D. Murphy, et al. 2002. "Synaptic Vesicle Depletion Correlates With Attenuated Synaptic Responses to Prolonged Repetitive Stimulation in Mice Lacking Alpha-Synuclein." *Journal of Neuroscience* 22: 8797–8807.

Cannon, J. R., K. D. Geghman, V. Tapias, et al. 2013. “Expression of Human E46K-Mutated α-Synuclein in BAC-Transgenic Rats Replicates Early-Stage Parkinson's Disease Features and Enhances Vulnerability to Mitochondrial Impairment.” *Experimental Neurology* 240: 44–56.

Caughey, B., G. J. Raymond, and R. A. Bessen. 1998. “Strain-Dependent Differences in b-Sheet Conformations of Abnormal Prion Protein.” *Journal of Biological Chemistry* 273, no. 48: 32230–32235.

Chandra, S., F. Fornai, H. B. Kwon, et al. 2004. “Double-Knockout Mice for Alpha- and Beta-Synucleins: Effect on Synaptic Functions.” *Proceedings of the National Academy of Sciences* 101: 14966–14971.

Clavaguera, F., H. Akatsu, G. Fraser, et al. 2013. “Brain Homogenates From Human Tauopathies Induce Tau Inclusions in Mouse Brain.” *Proceedings. National Academy of Sciences. United States of America* 110: 9535–9540.

Clavaguera, F., T. Bolmont, R. A. Crowther, et al. 2009. “Transmission and Spreading of Tauopathy in Transgenic Mouse Brain.” *Nature Cell Biology* 11: 909–913.

Clavaguera, F., J. Hench, I. Lavenir, et al. 2014. “Peripheral Administration of Tau Aggregates Triggers Intracerebral Tauopathy in Transgenic Mice.” *Acta Neuropathologica* 127: 299–301.

Cleveland, D. W., S.-Y. Hwo, and M. W. Kirschner. 1977. “Purification of Tau, a Microtubule-Associated Protein That Induces Assembly of Microtubules From Purified Tubulin.” *Journal of Molecular Biology* 116: 207–225.

Conidi, M. E., L. Bernardi, G. Puccio, et al. 2015. “Homozygous Carriers of APP A713T Mutation in an Autosomal Dominant Alzheimer Disease Family.” *Neurology* 84, no. 22: 2266–2273.

Constantinidis, J., J. Richard, and R. Tissot. 1974. “Pick's Disease. Histological and Clinical Correlations.” *European Neurology* 11, no. 4: 208–217.

Cornblath, E. J., H. L. Li, L. Changolkar, et al. 2021. “Computational Modeling of Tau Pathology Spread Reveals Patterns of Regional Vulnerability and the Impact of a Genetic Risk Factor.” *Science Advances* 7, no. 24: eabg6677. https://doi.org/10.1126/sciadv.abg6677.

Cruts, M., C. M. van Duijn, H. Backhovens, et al. 1998. “Estimation of the Genetic Contribution of Presenilin-1 and -2 Mutations in a Population-Based Study of Presenile Alzheimer Disease.” *Human Molecular Genetics* 7, no. 1: 43–51.

Daher, J. P. L., M. Ying, R. Banerjee, et al. 2009. “Conditional Transgenic Mice Expressing C-Terminally Truncated Human α-Synuclein (αSyn119) Exhibit Reduced Striatal Dopamine Without Loss of Nigrostriatal Pathway Dopaminergic Neurons.” *Molecular Neurodegeneration* 2009: 34.

Davies, P., and A. J. F. Maloney. 1976. “Selective Loss of Central Cholinergic Neurons in Alzheimer's Disease.” *Lancet* 308, no. 8000: 1403. https://doi.org/10.1016/s0140-6736(76)91936-x.

Dawson, H. N., V. Cantillana, M. Jansen, et al. 2010. “Loss of Tau Elicits Axonal Degeneration in a Mouse Model of Alzheimer's Disease.” *Neuroscience* 169: 516–531.

Dawson, H. N., A. Ferreira, M. V. Eyster, N. Ghoshal, L. I. Binder, and M. P. Vitek. 2001. “Inhibition of Neuronal Maturation in Primary Hippocampal Neurons From Tau Deficient Mice.” *Journal of Cell Science* 114: 1179–1187.

de Calignon, A., M. Polydoro, M. Suarez-Calvet, et al. 2012. “Propagation of Tau Pathology in a Model of Early Alzheimer's Disease.” *Neuron* 73: 685–697.

Decker, J. M., L. Kruger, A. Sydow, et al. 2016. “The Tau/A152T Mutation, a Risk Factor for Frontotemporal-Spectrum Disorders, Leads to NR2B Receptor-Mediated Excitotoxicity.” *EMBO Reports* 17, no. 4: 552–569.

Dickson, D. W. 1999. “Neuropathologic Differentiation of Progressive Supranuclear Palsy and Corticobasal Degeneration.” *Journal of Neurology* 246, no. Suppl 2: II6-15.

Dickson, D. W., C. Bergeron, S. S. Chin, et al. 2002. “Office of Rare Diseases Neuropathologic Criteria for Corticobasal Degeneration.” *Journal of Neuropathology and Experimental Neurology* 61, no. 11: 935–946. https://doi.org/10.1093/jnen/61.11.935.

Djelloul, M., S. Holmqvist, A. Boza-Serrano, et al. 2015. “Alpha-Synuclein Expression in the Oligodendrocyte Lineage: An In Vitro and In Vivo Study Using Rodent and Human Models.” *Stem Cell Reports* 5, no. 2: 174–184.

Duff, K., H. Knight, L. M. Refolo, et al. 2000. “Characterization of Pathology in Transgenic Mice Over-Expressing Human Genomic and cDNA Tau Transgenes.” *Neurobiology of Disease* 7: 87–98.

Eckermann, K., M.-M. Mocanu, I. Khlistunova, et al. 2007. “The Beta-Propensity of Tau Determines Aggregation and Synaptic Loss in Inducible Mouse Models of Tauopathy.” *Journal of Biological Chemistry* 282, no. 43: 31755–31765.

Emmer, K. L., E. A. Waxman, J. P. Covy, and B. I. Giasson. 2011. “E46K Human Alpha-Synuclein Transgenic Mice Develop Lewy-Like and Tau Pathology Associated With Age-Dependent, Detrimental Motor Impairment.” *Journal of Biological Chemistry* 286: 35104–35118.

Emre, M., D. Aarsland, R. Brown, et al. 2007. “Clinical Diagnostic Criteria for Dementia Associated With Parkinson's Disease.” *Movement Disorders* 22, no. 12: 1689–1707.

Falcon, B., W. Zhang, A. G. Murzin, et al. 2018. “Structures of Filaments From Pick's Disease Reveal a Novel Tau Protein Fold.” *Nature* 561: 137–140.

Falcon, B., J. Zivanov, W. Zhang, et al. 2019. “Novel Tau Filament Fold in Chronic Traumatic Encephalopathy Encloses Hydrophobic Molecules.” *Nature* 568: 420–423.

Ferrer, I., M. Aguilo Garcia, M. Carmona, et al. 2019. “Involvement of Oligodendrocytes in Tau Seeding and Spreading in Tauopathies.” *Frontiers in Aging Neuroscience* 11: 112.

Ferrer, I., P. Andres-Benito, M. Carmona, and J. A. Del Rio. 2022. “Common and Specific Marks of Different Tau Strains Following Intra-Hippocampal Injection of AD, PiD, and GGT Inoculum in hTau Transgenic Mice.” *International Journal of Molecular Sciences* 23, no. 24: 15940. https://doi.org/10.3390/ijms232415940.

Ferrer, I., M. V. Zelaya, M. Aguilo Garcia, et al. 2020. “Relevance of Host Tau in Tau Seeding and Spreading in Tauopathies.” *Brain Pathology* 30, no. 2: 298–318.

Fevga, C., Y. Park, E. Lohmann, et al. 2021. “A New Alpha-Synuclein Missense Variant (Thr72Met) in Two Turkish Families With Parkinson's Disease.” *Parkinsonism & Related Disorders* 89: 63–72.

Fitzpatrick, A. W. P., B. Falcon, S. He, et al. 2017. “Cryo-EM Structures of Tau Filaments From Alzheimer's Disease.” *Nature* 547: 185–190.

Flunkert, S., M. Hierzer, T. Loffler, et al. 2013. “Elevated Levels of Soluble Total and Hyperphosphorylated Tau Result in Early Behavioral Deficits and Distinct Changes in Brain Pathology in a New Tau Transgenic Mouse Model.” *Neurodegenerative Diseases* 11, no. 4: 194–205.

Forster, E., and F. H. Lewy. 1912. “Paralysis agitans.” In *Pathologische Anatomie. Handbuch der Neurologie*, edited by M. Lewandowsky, 920–933. Springer Verlag.

Fujio, K., M. Sato, T. Uemura, T. Sato, R. Sato-Harada, and A. Harada. 2007. “14-3-3 Proteins and Protein Phosphatases Are Not Reduced in Tau-Deficient Mice.” *Neuroreport* 18, no. 10: 1049–1052.

Gatz, M., C. A. Reynolds, L. Fratiglioni, et al. 2006. “Role of Genes and Environments for Explaining Alzheimer Disease.” *Archives of General Psychiatry* 63: 168–174.

Ghaemmaghami, S., B. C. H. May, A. R. Renslo, and S. B. Prusiner. 2010. "Discovery of 2-Aminothiazoles as Potent Antiprion Compounds." *Journal of Virology* 84: 3408–3412.

Giasson, B. I., J. E. Duda, S. M. Quinn, B. Zhang, J. Q. Trojanowski, and V. M. Lee. 2002. "Neuronal α-Synucleinopathy With Severe Movement Disorder in Mice Expressing A53T Human α-Synuclein." *Neuron* 34, no. 4: 521–533.

Gibbons, G. S., R. A. Banks, B. Kim, et al. 2017. "GFP-Mutant Human Tau Transgenic Mice Develop Tauopathy Following CNS Injections of Alzheimer's Brain-Derived Pathological Tau or Synthetic Mutant Human Tau Fibrils." *Journal of Neuroscience* 37, no. 47: 11485–11494.

Giles, K., S. H. Olson, and S. B. Prusiner. 2017. "Developing Therapeutics for PrP Prion Diseases." In *Prion Diseases*, edited by S. B. Prusiner, 579–597. Cold Spring Harbor Laboratory Press.

Gispert, S., D. Del Turco, L. Garrett, et al. 2003. "Transgenic Mice Expressing Mutant A53T Human Alpha-Synuclein Show Neuronal Dysfunction in the Absence of Aggregate Formation." *Molecular and Cellular Neurosciences* 24, no. 2: 419–429.

Glenner, G. G., and C. W. Wong. 1984a. "Alzheimer's Disease and Down's Syndrome: Sharing of a Unique Cerebrovascular Amyloid Fibril Protein." *Biochemical and Biophysical Research Communications* 122: 1131–1135.

Glenner, G. G., and C. W. Wong. 1984b. "Alzheimer's Disease: Initial Report of the Purification and Characterization of a Novel Cerebrovascular Amyloid Protein." *Biochemical and Biophysical Research Communications* 120: 885–890.

Goate, A., M.-C. Chartier-Harlin, M. Mullan, et al. 1991. "Segregation of a Missense Mutation in the Amyloid Precursor Protein Gene With Familial Alzheimer's Disease." *Nature* 349: 704–706.

Goedert, M., and R. Jakes. 1990. "Expression of Separate Isoforms of Human Tau Protein: Correlation With the Tau Pattern in Brain and Effects on Tubulin Polymerization." *EMBO Journal* 9, no. 13: 4225–4230.

Goedert, M., M. G. Spillantini, N. J. Cairns, and R. A. Crowther. 1992. "Tau Proteins of Alzheimer Paired Helical Filaments: Abnormal Phosphorylation of all Six Brain Isoforms." *Neuron* 8: 159–168.

Goedert, M., M. G. Spillantini, M. C. Potier, J. Ulrich, and R. A. Crowther. 1989. "Cloning and Sequencing of the cDNA Encoding an Isoform of Microtubule-Associated Protein Tau Containing Four Tandem Repeats: Differential Expression of Tau Protein mRNAs in Human Brain." *EMBO Journal* 8: 393–399.

Greten-Harrison, B., M. Polydoro, M. Morimoto-Tomita, et al. 2010. "αβγ-Synuclein Triple Knockout Mice Reveal Age-Dependent Neuronal Dysfunction." *Proceedings of the National Academy of Sciences of the United States of America* 107, no. 45: 19573–19578.

Grueninger, F., B. Bohrmann, C. Czech, et al. 2010. "Phosphorylation of Tau at S422 Is Enhanced by Abeta in TauPS2APP Triple Transgenic Mice." *Neurobiology of Disease* 37, no. 2: 294–306.

Gumucio, A., L. Lannfelt, and L. N. G. Nilsson. 2013. "Lack of Exon 10 in the Murine Tau Gene Results in Mild Sensorimotor Defects With Aging." *BMC Neuroscience* 14, no. 1: 148. https://doi.org/10.1186/1471-2202-14-148.

Guo, J. L., S. Narasimhan, L. Changolkar, et al. 2016. "Unique Pathological Tau Conformers From Alzheimer's Brains Transmit Tau Pathology in Nontransgenic Mice." *Journal of Experimental Medicine* 213: 2635–2654.

Hallinan, G. I., K. A. Ozcan, M. R. Hoq, et al. 2022. "Cryo-EM Structures of Prion Protein Filaments From Gerstmann-Sträussler-Scheinker Disease." *Acta Neuropathologica* 144, no. 3: 509–520.

Harada, A., K. Oguchi, S. Okabe, et al. 1994. "Altered Microtubule Organization in Small-Calibre Axons of Mice Lacking Tau Protein." *Nature* 369: 488–491.

Hashimoto, S., Y. Matsuba, N. Kamano, et al. 2019. "Tau Binding Protein CAPON Induces Tau Aggregation and Neurodegeneration." *Nature Communications* 10, no. 1: 2394.

Hauw, J. J., S. E. Daniel, D. Dickson, et al. 1994. "Preliminary NINDS Neuropathologic Criteria for Steele-Richardson-Olszewski Syndrome (Progressive Supranuclear Palsy)." *Neurology* 44, no. 11: 2015–2019.

He, Z., J. D. McBride, H. Xu, et al. 2020. "Transmission of Tauopathy Strains Is Independent of Their Isoform Composition." *Nature Communications* 11, no. 1: 7.

Hefti, M. M., K. Farrell, S. H. Kim, et al. 2018. "High-Resolution Temporal and Regional Mapping of MAPT Expression and Splicing in Human Brain Development." *PLoS One* 13, no. 4: e0195771.

Holec, S. A. M., C. R. Khedmatgozar, S. J. Schure, T. Pham, and A. L. Woerman. 2024. "A-Synuclein Prion Strains Differentially Adapt After Passage in Mice." *PLoS Pathogens* 20, no. 12: e1012746.

Holec, S. A. M., J. Lee, A. Oehler, et al. 2022. "The E46K Mutation Modulates α-Synuclein Prion Replication in Transgenic Mice." *PLoS Pathogens* 18, no. 12: e1010956.

Holec, S. A. M., J. Lee, A. Oehler, et al. 2022. "Multiple System Atrophy Prions Transmit Neurological Disease to Mice Expressing Wild-Type Human α-Synuclein." *Acta Neuropathologica* 144, no. 4: 677–690.

Holec, S. A. M., S. L. Liu, and A. L. Woerman. 2022. "Consequences of Variability in Alpha-Synuclein Fibril Structure on Strain Biology." *Acta Neuropathologica* 143, no. 3: 311–330.

Hosokawa, M., M. Masuda-Suzukake, H. Shitara, et al. 2022. "Development of a Novel Tau Propagation Mouse Model Endogenously Expressing 3 and 4 Repeat Tau Isoforms." *Brain* 145, no. 1: 349–361.

Hoyt, F., P. Alam, E. Artikis, et al. 2022. "Cryo-EM of Prion Strains From the Same Genotype of Host Identifies Conformational Determinants." *PLoS Pathogens* 18, no. 11: e1010947.

Hoyt, F., H. G. Standke, E. Artikis, et al. 2022. "Cryo-EM Structure of Anchorless RML Prion Reveals Variations in Shared Motifs Between Distinct Strains." *Nature Communications* 13, no. 1: 4005.

Hu, W., X. Zhang, Y. C. Tung, S. Xie, F. Liu, and K. Iqbal. 2016. "Hyperphosphorylation Determines Both the Spread and the Morphology of Tau Pathology." *Alzheimers Dement* 12, no. 10: 1066–1077.

Huang, M., J. Macdonald, I. Lavenir, et al. 2022. "Increase in Tau Pathology in P290S Mapt Knock-In Mice Crossed With app (NL-G-F) Mice." *eNeuro* 9, no. 6: ENEURO.0247-22.2022. https://www.eneuro.org/content/9/6/ENEURO.0247-22.2022.long.

Hutton, M., C. L. Lendon, P. Rizzu, et al. 1998. "Association of Missense and 5′-Splice-Site Mutations in Tau With the Inherited Dementia FTDP-17." *Nature* 393: 702–705.

Iba, M., J. L. Guo, J. D. McBride, B. Zhang, J. Q. Trojanowski, and V. M. Lee. 2013. "Synthetic Tau Fibrils Mediate Transmission of Neurofibrillary Tangles in a Transgenic Mouse Model of Alzheimer's-Like Tauopathy." *Journal of Neuroscience* 33, no. 3: 1024–1037. https://doi.org/10.1523/jneurosci.2642-12.2013.

Iba, M., J. D. McBride, J. L. Guo, B. Zhang, J. Q. Trojanowski, and V. M.-Y. Lee. 2015. "Tau Pathology Spread in PS19 Tau Transgenic Mice Following Locus Coeruleus (LC) Injections of Synthetic Tau Fibrils Is Determined by the LC's Afferent and Efferent Connections." *Acta Neuropathologica* 130: 349–362.

Iqbal, K., I. Grundke-Iqbal, T. Zaidi, et al. 1986. "Defective Brain Microtubule Assembly in Alzheimer's Disease." *Lancet* 2: 421–426.

Ishihara, T., M. Hong, B. Zhang, et al. 1999. "Age-Dependent Emergence and Progression of a Tauopathy in Transgenic Mice Overexpressing the Shortest Human Tau Isoform." *Neuron* 24: 751–762.

Jackson, R. J., N. Rudinskiy, A. G. Herrmann, et al. 2016. "Human Tau Increases Amyloid Beta Plaque Size but Not Amyloid Beta-Mediated

Synapse Loss in a Novel Mouse Model of Alzheimer's Disease." *European Journal of Neuroscience* 44, no. 12: 3056–3066.

Janezic, S., S. Threlfell, P. D. Dodson, et al. 2013. "Deficits in Dopaminergic Transmission Precede Neuron Loss and Dysfunction in a New Parkinson Model." *Proceedings of the National Academy of Sciences of the United States of America* 110, no. 42: E4016–E4025.

Kahle, P. J., M. Neumann, L. Ozmen, et al. 2002. "Hyperphosphorylation and Insolubility of Alpha-Synuclein in Transgenic Mouse Oligodendrocytes." *EMBO Reports* 3, no. 6: 583–588.

Kaufman, S. K., D. W. Sanders, T. L. Thomas, et al. 2016. "Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo." *Neuron* 92: 796–812.

Kaufman, S. K., T. L. Thomas, K. Del Tredici, H. Braak, and M. I. Diamond. 2017. "Characterization of Tau Prion Seeding Activity and Strains From Formaldehyde-Fixed Tissue." *Acta Neuropathologica Communications* 5: 41.

Kim, Y. D., H. Choi, W. J. Lee, et al. 2016. "Caspase-Cleaved Tau Exhibits Rapid Memory Impairment Associated With Tau Oligomers in a Transgenic Mouse Model." *Neurobiology of Disease* 87: 19–28.

Kon, T., S. L. Forrest, S. Lee, et al. 2024. "SNCA and TPPP Transcripts Increase in Oligodendroglial Cytoplasmic Inclusions in Multiple System Atrophy." *Neurobiology of Disease* 198: 106551.

Kon, T., S. L. Forrest, S. Lee, et al. 2023. "Neuronal SNCA Transcription During Lewy Body Formation." *Acta Neuropathologica Communications* 11, no. 1: 185.

Kon, T., F. Mori, A. Arai, et al. 2019. "Atypical Globular Glial Tauopathy With a Combination of Types I and II Pathology." *Neuropathology* 39, no. 2: 127–134.

Kosik, K. S., C. L. Joachim, and D. J. Selkoe. 1986. "Microtubule-Associated Protein Tau Is a Major Antigenic Component of Paired Helical Filaments in Alzheimer Disease." *Proceedings. National Academy of Sciences. United States of America* 83: 4044–4048.

Kosik, K. S., C. Munoz, L. Lopez, et al. 2015. "Homozygosity of the Autosomal Dominant Alzheimer Disease Presenilin 1 E280A Mutation." *Neurology* 84, no. 2: 206–208.

Kraus, A., F. Hoyt, C. L. Schwartz, et al. 2021. "High-Resolution Structure and Strain Comparison of Infectious Mammalian Prions." *Molecular Cell* 81, no. 21: 4540–4551. https://doi.org/10.1016/j.molcel.2021.08.011.

Kruger, R., W. Kuhn, K. L. Leenders, et al. 2001. "Familial Parkinsonism With Synuclein Pathology: Clinical and PET Studies of A30P Mutation Carriers." *Neurology* 56, no. 10: 1355–1362.

Kuo, Y. M., Z. Li, Y. Jiao, et al. 2010. "Extensive Enteric Nervous System Abnormalities in Mice Transgenic for Artificial Chromosomes Containing Parkinson Disease-Associated Alpha-Synuclein Gene Mutations Precede Central Nervous System Changes." *Human Molecular Genetics* 19, no. 9: 1633–1650.

Lanoiselee, H. M., G. Nicolas, D. Wallon, et al. 2017. "APP, PSEN1, and PSEN2 Mutations in Early-Onset Alzheimer Disease: A Genetic Screening Study of Familial and Sporadic Cases." *PLoS Medicine* 14, no. 3: e1002270.

Lasagna-Reeves, C. A., D. L. Castillo-Carranza, U. Sengupta, et al. 2012. "Alzheimer Brain-Derived Tau Oligomers Propagate Pathology From Endogenous Tau." *Scientific Reports* 2: 700.

Lau, A., R. W. L. So, H. H. C. Lau, et al. 2020. "Alpha-Synuclein Strains Target Distinct Brain Regions and Cell Types." *Nature Neuroscience* 23, no. 1: 21–31.

Lau, H. H. C., I. Martinez-Valbuena, R. W. L. So, et al. 2023. "The G51D SNCA Mutation Generates a Slowly Progressive α-Synuclein Strain in Early-Onset Parkinson's Disease." *Acta Neuropathologica Communications* 11, no. 1: 72.

Lee, M. K., W. Stirling, Y. Xu, et al. 2002. "Human α-Synuclein-Harboring Familial Parkinson's Disease-Linked ala-53 —Thr Mutation Causes Neurodegenerative Disease With α-Synuclein Aggregation in Transgenic Mice." *Proceedings. National Academy of Sciences. United States of America* 99: 8968–8973.

Lesage, S., M. Anheim, F. Letournel, et al. 2013. "G51D α-Synuclein Mutation Causes a Novel Parkinsonian–Pyramidal Syndrome." *Annals of Neurology* 73, no. 4: 459–471.

Lesage, S., and A. Brice. 2009. "Parkinson's Disease: From Monogenic Forms to Genetic Susceptibility Factors." *Human Molecular Genetics* 18, no. R1: R48–R59.

Levy-Lahad, E., W. Wasco, P. Poorkaj, et al. 1995. "Candidate Gene for the Chomosome 1 Familial Alzheimer's Disease Locus." *Science* 269: 973–977.

Lewis, J., D. W. Dickson, W. L. Lin, et al. 2001. "Enhanced Neurofibrillary Degeneration in Transgenic Mice Expressing Mutant Tau and APP." *Science* 293: 1487–1491.

Lewis, J., E. McGowan, J. Rockwood, et al. 2000. "Neurofibrillary Tangles, Amyotrophy and Progressive Motor Disturbance in Mice Expressing Mutant (P301L) tau Protein." *Nature Genetics* 25: 402–405.

Lin, X., L. Parisiadou, X.-L. Gu, et al. 2009. "Leucine-Rich Repeat Kinase 2 Regulates the Progression of Neuropathology Induced by Parkinson's-Disease-Related Mutant Alpha-Synuclein." *Neuron* 64, no. 6: 807–827.

Liu, C., L. Meng, Y. Gao, et al. 2024. "PM2.5 Triggers Tau Aggregation in a Mouse Model of Tauopathy." *JCI Insight* 9, no. 14: e176703. https://doi.org/10.1172/jci.insight.176703.

Liu, H., C. Koros, T. Strohaker, et al. 2021. "A Novel SNCA A30G Mutation Causes Familial Parkinson's Disease." *Movement Disorders* 36, no. 7: 1624–1633.

Lloyd, G. M., Z. A. Sorrentino, S. Quintin, et al. 2022. "Unique Seeding Profiles and Prion-Like Propagation of Synucleinopathies Are Highly Dependent on the Host in Human α-Synuclein Transgenic Mice." *Acta Neuropathologica* 143, no. 6: 663–685.

Luk, K. C., D. J. Covell, V. M. Kehm, et al. 2016. "Molecular and Biological Compatibility With Host Alpha-Synuclein Influences Fibril Pathogenicity." *Cell Reports* 16: 3373–3387.

Luk, K. C., V. Kehm, J. Carroll, et al. 2012. "Pathological Alpha-Synuclein Transmission Initiates Parkinson-Like Neurodegeneration in Nontransgenic Mice." *Science* 338: 949–953.

Luk, K. C., V. M. Kehm, B. Zhang, P. O'Brien, J. Q. Trojanowski, and V. M. Y. Lee. 2012. "Intracerebral Inoculation of Pathological α-Synuclein Initiates a Rapidly Progressive Neurodegenerative α-Synucleinopathy in Mice." *Journal of Experimental Medicine* 209, no. 5: 975–986. https://doi.org/10.1084/jem.20112457.

Maeda, S., B. Djukic, P. Taneja, et al. 2016. "Expression of A152T Human Tau Causes Age-Dependent Neuronal Dysfunction and Loss in Transgenic Mice." *EMBO Reports* 17, no. 4: 530–551.

Majounie, E., W. Cross, V. Newsway, et al. 2013. "Tau Expression Varies in Different Brain Regions and Disease State." *Neurobiology of Aging* 34: 1922.e1927–1922.e1912.

Manka, S. W., A. Wenborn, J. Betts, et al. 2023. "A Structural Basis for Prion Strain Diversity." *Nature Chemical Biology* 19, no. 5: 607–613.

Manka, S. W., W. Zhang, A. Wenborn, et al. 2022. "2.7 Å Cryo-EM Structure of Ex Vivo RML Prion Fibrils." *Nature Communications* 13, no. 1: 4004.

Martland, H. S. 1928. "Punch drunk." *Journal of the American Medical Association* 91: 1103–1107.

Masliah, E., E. Rockenstein, I. Veinbergs, et al. 2000. "Dopaminergic Loss and Inclusion Body Formation in a-Synuclein Mice: Implications for Neurodegenerative Disorders." *Science* 287, no. 5456: 1265–1269.

Masuda-Suzukake, M., T. Nonaka, M. Hosokawa, et al. 2013. "Prion-Like Spreading of Pathological Alpha-Synuclein in Brain." *Brain* 136: 1128–1138.

Mate de Gerando, A., A. Khasnavis, L. A. Welikovitch, et al. 2024. "Aqueous Extractable Nonfibrillar and Sarkosyl Extractable Fibrillar Alzheimer's Disease Tau Seeds Have Distinct Properties." *Acta Neuropathologica Communications* 12, no. 1: 145.

Mate De Gerando, A., L. A. Welikovitch, A. Khasnavis, et al. 2023. "Tau Seeding and Spreading In Vivo Is Supported by Both AD-Derived Fibrillar and Oligomeric Tau." *Acta Neuropathologica* 146, no. 2: 191–210.

McKee, A. C., N. J. Cairns, D. W. Dickson, et al. 2016. "The First NINDS/NIBIB Consensus Meeting to Define Neuropathological Criteria for the Diagnosis of Chronic Traumatic Encephalopathy." *Acta Neuropathologica* 131: 75–86.

McKee, A. C., T. D. Stein, B. R. Huber, et al. 2023. "Chronic Traumatic Encephalopathy (CTE): Criteria for Neuropathological Diagnosis and Relationship to Repetitive Head Impacts." *Acta Neuropathologica* 145, no. 4: 371–394.

McKee, A. C., T. D. Stein, C. J. Nowinski, et al. 2013. "The Spectrum of Disease in Chronic Traumatic Encephalopathy." *Brain* 136: 43–64.

McKeith, I. G., D. W. Dickson, J. Lowe, et al. 2005. "Diagnosis and Management of Dementia With Lewy Bodies: Third Report of the DLB Consortium." *Neurology* 65: 1863–1872.

McMillan, P., E. Korvatska, P. Poorkaj, et al. 2008. "Tau Isoform Regulation Is Region- and Cell-Specific in Mouse Brain." *Journal of Comparative Neurology* 511: 788–803.

Mez, J., D. H. Daneshvar, P. T. Kiernan, et al. 2017. "Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football." *JAMA* 318, no. 4: 360–370. https://doi.org/10.1001/jama.2017.8334.

Miao, J., R. Shi, L. Li, et al. 2019. "Pathological Tau From Alzheimer's Brain Induces Site-Specific Hyperphosphorylation and SDS- and Reducing Agent-Resistant Aggregation of Tau In Vivo." *Frontiers in Aging Neuroscience* 11: 34.

Miller, D. W., J. M. Johnson, S. M. Solano, Z. R. Hollingsworth, D. G. Standaert, and A. B. Young. 2005. "Absence of Alpha-Synuclein mRNA Expression in Normal and Multiple System Atrophy Oligodendroglia." *Journal of Neural Transmission* 112: 1613–1624.

Millspaugh, J. A. 1937. "Dementia Pugilistica." *US Naval Medical Bulletin* 35: 297–303.

Mocanu, M. M., A. Nissen, K. Eckermann, et al. 2008. "The Potential for Beta-Structure in the Repeat Domain of Tau Protein Determines Aggregation, Synaptic Decay, Neuronal Loss, and Coassembly With Endogenous Tau in Inducible Mouse Models of Tauopathy." *Journal of Neuroscience* 28, no. 3: 737–748.

Mougenot, A.-L., S. Nicot, A. Bencsik, et al. 2012. "Prion-Like Acceleration of a Synucleinopathy in a Transgenic Mouse Model." *Neurobiology of Aging* 33, no. 9: 2225–2228.

Narasimhan, S., J. L. Guo, L. Changolkar, et al. 2017. "Pathological Tau Strains From Human Brains Recapitulate the Diversity of Tauopathies in Nontransgenic Mouse Brain." *Journal of Neuroscience* 37, no. 47: 11406–11423.

Ninkina, N., N. Connor-Robson, A. A. Ustyugov, T. V. Tarasova, T. A. Shelkovnikova, and V. L. Buchman. 2015. "A Novel Resource for Studying Function and Dysfunction of α-Synuclein: Mouse Lines for Modulation of Endogenous Snca Gene Expression." *Scientific Reports* 5: 16615.

Ninkina, N., K. Papachroni, D. C. Robertson, et al. 2003. "Neurons Expressing the Highest Levels of Gamma-Synuclein Are Unaffected by Targeted Inactivation of the Gene." *Molecular and Cellular Biology* 23, no. 22: 8233–8245.

Nuber, S., M. Rajsombath, G. Minakaki, et al. 2018. "Abrogating Native α-Synuclein Tetramers in Mice Causes a L-DOPA-Responsive Motor Syndrome Closely Resembling Parkinson's Disease." *Neuron* 100, no. 1: 75–90. https://doi.org/10.1016/j.neuron.2018.09.014.

Nuber, S., X. Zhang, T. D. McCaffery, et al. 2024. "Generation of G51D and 3D Mice Reveals Decreased α-Synuclein Tetramer-Monomer Ratios Promote Parkinson's Disease Phenotypes." *npj Parkinson's Disease* 10, no. 1: 47.

Oddo, S., A. Caccamo, J. D. Shepherd, et al. 2003. "Triple-Transgenic Model of Alzheimer's Disease With Plaques and Tangles: Intracellular Abeta and Synaptic Dysfunction." *Neuron* 39: 409–421.

Odeh, F., T. B. Leergaard, J. Boy, T. Schmidt, O. Riess, and J. G. Bjaalie. 2011. "Atlas of Transgenic Tet-Off Ca2+/Calmodulin-Dependent Protein Kinase II and Prion Protein Promoter Activity in the Mouse Brain." *NeuroImage* 54, no. 4: 2603–2611.

Ozawa, T., K. Okuizumi, T. Ikeuchi, K. Wakabayashi, H. Takahashi, and S. Tsuji. 2001. "Analysis of the Expression Level of α-Synuclein mRNA Using Postmortem Brain Samples From Pathologically Confirmed Cases of Multiple System Atrophy." *Acta Neuropathologica* 102: 188–190.

Papp, M. I., J. E. Kahn, and P. L. Lantos. 1989. "Glial Cytoplasmic Inclusions in the CNS of Patients With Multiple System Atrophy (Striatonigral Degeneration, Olivopontocerebellar Atrophy and Shy-Drager Syndrome)." *Journal of the Neurological Sciences* 94, no. 1–3: 79–100.

Parker, J., T. Mozaffar, A. Messmore, J. L. Deignan, V. E. Kimonis, and J. M. Ringman. 2019. "Homozygosity for the A431E Mutation in PSEN1 Presenting With a Relatively Aggressive Phenotype." *Neuroscience Letters* 699: 195–198.

Parkinson, J. 1817. *An Essay on the Shaking Palsy*. Sherwood, Neely, and Jones.

Pasanen, P., L. Myllykangas, M. Siitonen, et al. 2014. "A Novel α-Synuclein Mutation A53E Associated With Atypical Multiple System Atrophy and Parkinson's Disease-Type Pathology." *Neurobiology of Aging* 35: 2180.e2181–2180.e2185.

Peeraer, E., A. Bottelbergs, K. Van Kolen, et al. 2015. "Intracerebral Injection of Preformed Synthetic Tau Fibrils Initiates Widespread Tauopathy and Neuronal Loss in the Brains of Tau Transgenic Mice." *Neurobiology of Disease* 73: 83–95.

Platt, B., B. Drever, D. Koss, et al. 2011. "Abnormal Cognition, Sleep, EEG and Brain Metabolism in a Novel Knock-In Alzheimer Mouse, PLB1." *PLoS One* 6, no. 11: e27068.

Polymeropoulos, M. H., C. Lavedan, E. Leroy, et al. 1997. "Mutation in the α-Synuclein Gene Identified in Families With Parkinson's Disease." *Science* 276, no. 5321: 2045–2047.

Poorkaj, P., T. D. Bird, E. Wijsman, et al. 1998. "Tau Is a Candidate Gene for Chromosome 17 Frontotemporal Dementia." *Annals of Neurology* 43, no. 6: 815–825.

Probst, A., J. Gotz, K. H. Wiederhold, et al. 2000. "Axonopathy and Amyotrophy in Mice Transgenic for Human Four-Repeat Tau Protein." *Acta Neuropathologica* 99: 469–481.

Prusiner, S. B. 2012. "A Unifying Role for Prions in Neurodegenerative Diseases." *Science* 336: 1511–1513.

Prusiner, S. B. 2017. "An Introduction to *Prion Diseases*." In *Prion Diseases*, edited by S. B. Prusiner, 1–29. Cold Spring Harbor Laboratory Press.

Prusiner, S. B., A. L. Woerman, D. A. Mordes, et al. 2015. "Evidence for α-Synuclein Prions Causing Multiple System Atrophy in Humans With Parkinsonism." *Proceedings. National Academy of Sciences. United States of America* 112, no. 38: E5308–E5317.

Rahayel, S., B. Mišić, Y.-Q. Zheng, et al. 2022. "Differentially Targeted Seeding Reveals Unique Pathological Alpha-Synuclein Propagation Patterns." *Brain* 145, no. 5: 1743–1756.

Reis, P. M., S. A. M. Holec, C. Ezeiruaku, et al. 2024. "Structurally Targeted Mutagenesis Identifies Key Residues Supporting α-Synuclein Misfolding in Multiple System Atrophy." *Journal of Parkinson's Disease Online ahead of print* 14: 1543–1558.

Richfield, E. K., M. J. Thiruchelvam, D. A. Cory-Slechta, et al. 2002. "Behavioral and Neurochemical Effects of Wild-Type and Mutated Human Alpha-Synuclein in Transgenic Mice." *Experimental Neurology* 175, no. 1: 35–48.

Rockenstein, E., M. Mallory, M. Hashimoto, et al. 2002. "Differential Neuropathological Alterations in Transgenic Mice Expressing α-Synuclein From the Platelet-Derived Growth Factor and Thy-1 Promoters." *Journal of Neuroscience Research* 68, no. 5: 568–578.

Rockenstein, E., C. R. Overk, K. Ubhi, et al. 2015. "A Novel Triple Repeat Mutant Tau Transgenic Model That Mimics Aspects of pick's Disease and Fronto-Temporal Tauopathies." *PLoS One* 10, no. 3: e0121570.

Rogaev, E. I., R. Sherrington, E. A. Rogaeva, et al. 1995. "Familial Alzheimer's Disease in Kindreds With Missense Mutations in a Gene on Chromosome 1 Related to the Alzheimer's Disease Type 3 Gene." *Nature* 376, no. 6543: 775–778. https://doi.org/10.1038/376775a0.

Rovelet-Lecrux, A., D. Hannequin, G. Raux, et al. 2006. "*APP* Locus Duplication Causes Autosomal Dominant Early-Onset Alzheimer Disease With Cerebral Amyloid Angiopathy." *Nature Genetics* 38: 24–26.

Sacino, A. N., M. Brooks, M. A. Thomas, et al. 2014. "Amyloidogenic Alpha-Synuclein Seeds Do Not Invariably Induce Rapid, Widespread Pathology in Mice." *Acta Neuropathologica* 127, no. 5: 645–665.

Saito, T., N. Mihira, Y. Matsuba, et al. 2019. "Humanization of the Entire Murine Mapt Gene Provides a Murine Model of Pathological Human Tau Propagation." *Journal of Biological Chemistry* 294, no. 34: 12754–12765.

Sala Frigerio, C., P. Lau, C. Troakes, et al. 2015. "On the Identification of Low Allele Frequency Mosaic Mutations in the Brains of Alzheimer's Disease Patients." *Alzheimer's & Dementia* 11, no. 11: 1265–1276.

Schaser, A. J., V. R. Osterberg, S. E. Dent, et al. 2019. "Alpha-Synuclein Is a DNA Binding Protein That Modulates DNA Repair With Implications for Lewy Body Disorders." *Scientific Reports* 9, no. 1: 10919.

Schindowski, K., A. Bretteville, K. Leroy, et al. 2006. "Alzheimer's Disease-Like Tau Neuropathology Leads to Memory Deficits and Loss of Functional Synapses in a Novel Mutated Tau Transgenic Mouse Without any Motor Deficits." *American Journal of Pathology* 169: 599–616.

Schlüter, O. M., F. Fornai, M. G. Alessandrí, et al. 2003. "Role of Alpha-Synuclein in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Induced Parkinsonism in Mice." *Neuroscience* 118, no. 4: 985–1002.

Schmidt, M. L., V. Zhukareva, K. L. Newell, V. M. Lee, and J. Q. Trojanowski. 2001. "Tau Isoform Profile and Phosphorylation State in Dementia Pugilistica Recapitulate Alzheimer's Disease." *Acta Neuropathologica* 101: 518–524.

Schweighauser, M., A. G. Murzin, J. Macdonald, et al. 2023. "Cryo-EM Structures of Tau Filaments From the Brains of Mice Transgenic for Human Mutant P301S Tau." *Acta Neuropathologica Communications* 11, no. 1: 160.

Schweighauser, M., Y. Shi, A. Tarutani, et al. 2020. "Structures of α-Synuclein Filaments From Multiple System Atrophy." *Nature* 585, no. 7825: 464–469.

Seidler, P. M., K. A. Murray, D. R. Boyer, et al. 2022. "Structure-Based Discovery of Small Molecules That Disaggregate Alzheimer's Disease Tissue Derived Tau Fibrils In Vitro." *Nature Communications* 13, no. 1: 5451.

Sherrington, R., E. I. Rogaev, Y. Liang, et al. 1995. "Cloning of a Gene Bearing Missense Mutations in Early-Onset Familial Alzheimer's Disease." *Nature* 375: 754–760.

Shi, Y., W. Zhang, Y. Yang, et al. 2021. "Structure-Based Classification of Tauopathies." *Nature* 598, no. 7880: 359–363.

Shults, C. W., E. Rockenstein, L. Crews, et al. 2005. "Neurological and Neurodegenerative Alterations in a Transgenic Mouse Model Expressing Human α-Synuclein Under Oligodendrocyte Promoter: Implications for Multiple System Atrophy." *Journal of Neuroscience* 25, no. 46: 10689–10699.

Solano, S. M., D. W. Miller, S. J. Augood, A. B. Young, and J. B. Penney Jr. 2000. "Expression of α-Synuclein, Parkin, and Ubiquitin Carboxy-Terminal Hydrolase L1 mRNA in Human Brain: Genes Associated With Familial Parkinson's Disease." *Annals of Neurology* 47: 201–210.

Spanopoulou, E., V. Giguere, and F. Grosveld. 1991. "The Functional Domains of the Murine Thy-1 Gene Promoter." *Molecular and Cellular Biology* 11, no. 4: 2216–2228.

Spillantini, M. G., R. A. Crowther, R. Jakes, N. J. Cairns, P. L. Lantos, and M. Goedert. 1998. "Filamentous α-Synuclein Inclusions Link Multiple System Atrophy With Parkinson's Disease and Dementia With Lewy Bodies." *Neuroscience Letters* 251, no. 3: 205–208.

Spillantini, M. G., R. A. Crowther, R. Jakes, M. Hasegawa, and M. Goedert. 1998. "α-Synuclein in Filamentous Inclusions of Lewy Bodies From Parkinson's Disease and Dementia With Lewy Bodies." *Proceedings. National Academy of Sciences. United States of America* 95, no. 11: 6469–6473.

Spillantini, M. G., M. L. Schmidt, V. M.-Y. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert. 1997. "α-Synuclein in Lewy bodies." *Nature* 388, no. 6645: 839–840.

Sydow, A., K. Hochgrafe, S. Konen, et al. 2016. "Age-Dependent Neuroinflammation and Cognitive Decline in a Novel Ala152Thr-Tau Transgenic Mouse Model of PSP and AD." *Acta Neuropathologica Communications* 4: 17.

Sydow, A., A. Van der Jeugd, F. Zheng, et al. 2011. "Tau-Induced Defects in Synaptic Plasticity, Learning, and Memory Are Reversible in Transgenic Mice After Switching Off the Toxic Tau Mutant." *Journal of Neuroscience* 31, no. 7: 2511–2525.

Takeuchi, H., M. Iba, H. Inoue, et al. 2011. "P301S Mutant Human Tau Transgenic Mice Manifest Early Symptoms of Human Tauopathies With Dementia and Altered Sensorimotor Gating." *PLoS One* 6, no. 6: e21050.

Tanemura, K., T. Akagi, M. Murayama, et al. 2001. "Formation of Filamentous Tau Aggregations in Transgenic Mice Expressing V337M Human Tau." *Neurobiology of Disease* 8, no. 6: 1036–1045.

Tanemura, K., M. Murayama, T. Akagi, et al. 2002. "Neurodegeneration With Tau Accumulation in a Transgenic Mouse Expressing V337M Human Tau." *Journal of Neuroscience* 22, no. 1: 133–141.

Tatebayashi, Y., T. Miyasaka, D. H. Chui, et al. 2002. "Tau Filament Formation and Associative Memory Deficit in Aged Mice Expressing Mutant (R406W) Human Tau." *Proceedings of the National Academy of Sciences of the United States of America* 99, no. 21: 13896–13901.

Taylor, T. N., D. Potgieter, S. Anwar, et al. 2014. "Region-Specific Deficits in Dopamine, but Not Norepinephrine, Signaling in a Novel A30P α-Synuclein BAC Transgenic Mouse." *Neurobiology of Disease* 62: 193–207.

Telling, G. C., P. Parchi, S. J. DeArmond, et al. 1996. "Evidence for the Conformation of the Pathologic Isoform of the Prion Protein Enciphering and Propagating Prion Diversity." *Science* 274: 2079–2082.

Telling, G. C., M. Scott, K. K. Hsiao, et al. 1994. "Transmission of Creutzfeldt-Jakob Disease From Humans to Transgenic Mice Expressing Chimeric Human-Mouse Prion Protein." *Proceedings. National Academy of Sciences. United States of America* 91, no. 21: 9936–9940.

Terwel, D., R. Lasrado, J. Snauwaert, et al. 2005. "Changed Conformation of Mutant Tau-P301L Underlies the Moribund Tauopathy, Absent in

Progressive, Nonlethal Axonopathy of Tau-4R/2N Transgenic Mice." *Journal of Biological Chemistry* 280: 3963–3973.

Thomzig, A., K. Wagnführ, P. Pinder, M. Joncic, W. J. Schulz-Schaeffer, and M. Beekes. 2021. "Transmissible α-Synuclein Seeding Activity in Brain and Stomach of Patients With Parkinson's Disease." *Acta Neuropathologica* 141, no. 6: 861–879.

Tolnay, M., and F. Clavaguera. 2004. "Argyrophilic Grain Disease: A Late-Onset Dementia With Distinctive Features Among Tauopathies." *Neuropathology* 24, no. 4: 269–283.

Trabzuni, D., S. Wray, J. Vandrovcova, et al. 2012. "MAPT Expression and Splicing Is Differentially Regulated by Brain Region: Relation to Genotype and Implication for Tauopathies." *Human Molecular Genetics* 21, no. 18: 4094–4103.

Tran, J., H. Anastacio, and C. Bardy. 2020. "Genetic Predispositions of Parkinson's Disease Revealed in Patient-Derived Brain Cells." *npj Parkinson's Disease* 6: 8.

Tucker, K. L., M. Meyer, and Y. A. Barde. 2001. "Neurotrophins Are Required for Nerve Growth During Development." *Nature Neuroscience* 4, no. 1: 29–37.

Umeda, T., T. Yamashita, T. Kimura, et al. 2013. "Neurodegenerative Disorder FTDP-17-Related Tau Intron 10 +16C → T Mutation Increases Tau Exon 10 Splicing and Causes Tauopathy in Transgenic Mice." *American Journal of Pathology* 183, no. 1: 211–225.

Van der Jeugd, A., K. Hochgrafe, T. Ahmed, et al. 2012. "Cognitive Defects Are Reversible in Inducible Mice Expressing Pro-Aggregant Full-Length Human Tau." *Acta Neuropathologica* 123, no. 6: 787–805.

Wakabayashi, K., M. Yoshimoto, S. Tsuji, and H. Takahashi. 1998. "α-Synuclein Immunoreactivity in Glial Cytoplasmic Inclusions in Multiple System Atrophy." *Neuroscience Letters* 249, no. 2–3: 180–182.

Watts, J. C., K. Giles, A. Oehler, et al. 2013. "Transmission of Multiple System Atrophy Prions to Transgenic Mice." *Proceedings. National Academy of Sciences. United States of America* 110, no. 48: 19555–19560.

Weitzman, S. A., S. Narasimhan, Z. He, et al. 2020. "Insoluble Tau From Human FTDP-17 Cases Exhibit Unique Transmission Properties In Vivo." *Journal of Neuropathology and Experimental Neurology* 79, no. 9: 941–949.

Wheeler, J. M., P. J. McMillan, M. Hawk, et al. 2015. "High Copy Wildtype Human 1N4R Tau Expression Promotes Early Pathological Tauopathy Accompanied by Cognitive Deficits Without Progressive Neurofibrillary Degeneration." *Acta Neuropathologica Communications* 3: 33.

Williams, D. R. 2006. "Tauopathies: Classification and Clinical Update on Neurodegenerative Diseases Associated With Microtubule-Associated Protein Tau." *Internal Medicine Journal* 36: 652–660.

Wingo, T. S., J. J. Lah, A. I. Levey, and D. J. Cutler. 2012. "Autosomal Recessive Causes Likely in Early-Onset Alzheimer Disease." *Archives of Neurology* 69, no. 1: 59–64.

Woerman, A. L., and J. C. Bartz. 2024. "Effect of Host and Strain Factors on α-Synuclein Prion Pathogenesis." *Trends in Neurosciences* 47, no. 7: 538–550.

Woerman, A. L., S. A. Kazmi, S. Patel, et al. 2018. "Familial Parkinson's Point Mutation Abolishes Multiple System Atrophy Prion Replication." *Proceedings of the National Academy of Sciences* 115, no. 2: 409–414.

Woerman, A. L., S. A. Kazmi, S. Patel, et al. 2018. "MSA Prions Exhibit Remarkable Stability and Resistance to Inactivation." *Acta Neuropathologica* 135, no. 1: 49–63.

Woerman, A. L., A. Oehler, S. A. Kazmi, et al. 2019. "Multiple System Atrophy Prions Retain Strain Specificity After Serial Propagation in Two Different Tg(*SNCA**A53T) Mouse Lines." *Acta Neuropathologica* 137, no. 3: 437–454.

Woerman, A. L., S. Patel, S. A. Kazmi, et al. 2017. "Kinetics of Human Mutant Tau Prion Formation in the Brains of Two Transgenic Mouse Lines." *JAMA Neurology* 74: 1464–1472.

Woerman, A. L., S. Patel, S. A. Kazmi, et al. 2020. "Kinetics of α-Synuclein Prions Preceding Neuropathological Inclusions in Multiple System Atrophy." *PLoS Pathogens* 16, no. 2: e1008222.

Woerman, A. L., J. Stöhr, A. Aoyagi, et al. 2015. "Propagation of Prions Causing Synucleinopathies in Cultured Cells." *Proceedings. National Academy of Sciences. United States of America* 112, no. 35: E4949–E4958.

Wood, J. G., S. S. Mirra, N. J. Pollock, and I. I. Binder. 1986. "Neurofibrillary Tangles of Alzheimer's Disease Share Antigenic Determinants With the Axonal Microtubule-Associated Protein Tau." *Proceedings. National Academy of Sciences. United States of America* 83: 4040–4043.

Xia, Y., S. Prokop, B. M. Bell, et al. 2022. "Pathogenic Tau Recruits Wild-Type Tau Into Brain Inclusions and Induces Gut Degeneration in Transgenic SPAM Mice." *Communications Biology* 5, no. 1: 446.

Xu, H., M. O'Reilly, G. S. Gibbons, et al. 2021. "In Vitro Amplification of Pathogenic Tau Conserves Disease-Specific Bioactive Characteristics." *Acta Neuropathologica* 141, no. 2: 193–215.

Yan, N. L., F. Candido, E. Tse, et al. 2024. "Cryo-EM Structure of a Novel α-Synuclein Filament Subtype From Multiple System Atrophy." *FEBS Letters* 599, no. 1: 33–40.

Yang, Y., H. J. Garringer, Y. Shi, et al. 2023. "New SNCA Mutation and Structures of α-Synuclein Filaments From Juvenile-Onset Synucleinopathy." *Acta Neuropathologica* 145, no. 5: 561–572. https://doi.org/10.1007/s00401-023-02550-8.

Yang, Y., Y. Shi, M. Schweighauser, et al. 2022. "Structures of Alpha-Synuclein Filaments From Human Brains With Lewy Pathology." *Nature* 610, no. 7933: 791–795.

Yazawa, I., B. I. Giasson, R. Sasaki, et al. 2005. "Mouse Model of Multiple System Atrophy α-Synuclein Expression in Oligodendrocytes Causes Glial and Neuronal Degeneration." *Neuron* 45, no. 6: 847–859. https://doi.org/10.1016/j.neuron.2005.01.032.

Yoshino, H., M. Hirano, A. J. Stoessl, et al. 2017. "Homozygous Alpha-Synuclein p.A53V in Familial Parkinson's Disease." *Neurobiology of Aging* 57: 248 e247–248 e212.

Yoshiyama, Y., M. Higuchi, B. Zhang, et al. 2007. "Synapse Loss and Microglial Activation Precede Tangles in a P301S Tauopathy Mouse Model." *Neuron* 53: 337–351.

Zareba-Paslawska, J., K. Patra, L. Kluzer, T. Revesz, and P. Svenningsson. 2020. "Tau Isoform-Driven CBD Pathology Transmission in Oligodendrocytes in Humanized Tau Mice." *Frontiers in Neurology* 11: 589471.

Zarranz, J. J., J. Alegre, J. C. Gómez-Esteban, et al. 2004. "The New Mutation, E46K, of α-Synuclein Causes Parkinson and Lewy Body Dementia." *Annals of Neurology* 55, no. 2: 164–173.

Zhang, B., M. Higuchi, Y. Yoshiyama, et al. 2004. "Retarded Axonal Transport of R406W Mutant Tau in Transgenic Mice With a Neurodegenerative Tauopathy." *Journal of Neuroscience* 24: 4657–4667.

Zhang, W., A. Tarutani, K. L. Newell, et al. 2020. "Novel Tau Filament Fold in Corticobasal Degeneration." *Nature* 580, no. 7802: 283–287.