A Critical Re-evaluation of "*Synthetic α-synuclein fibrils replicate in mice causing MSA-like pathology*" by Burger *et al., Nature* 2025; doi:10.1038/s41586-025-09698-1

Mengxi Zhu, Yi Wang, Mingyue Gao and Shu-Feng Zhou*
College of Chemical Engineering, Huaqiao University, Xiamen 361021, China
*Correspondence: szhou@hqu.edu.cn

Abstract

Burger *et al*. (*Nature*, 2025) report that synthetic recombinant α-synuclein fibrils, when injected into mice, replicate *in vivo* and induce pathology resembling multiple system atrophy (MSA). Their study is positioned as evidence that MSA may arise from exogenous fibrillar species capable of templated propagation across neural circuits. While the work is ambitious and addresses a central question in synucleinopathy research, the experimental claims rest on several conceptual, methodological, and interpretive weaknesses that substantially undermine the proposed mechanistic narrative. This commentary provides an integrated and rigorous re-evaluation of their dataset, spanning biophysical characterization, cellular seeding assays, *in vivo* pathology, behavioral phenotyping, and omics-based mechanistic inference. A figure-by-figure analysis reveals recurrent deficiencies including incomplete structural validation of fibrils, insufficient controls for strain specificity, inconsistent pathology quantification, inadequate statistical power, and limited transparency regarding raw data and replicates. Many observed phenotypes may alternatively reflect non-specific neuroinflammatory responses, stereotaxic injury effects, or oligodendrocyte vulnerability intrinsic to the mouse model rather than bona fide α-synuclein replication. The inferred analogy to human MSA pathology is weakened by species-specific anatomical and molecular differences, especially regarding oligodendroglial biology and the systemic, multisite character of authentic MSA. Furthermore, critical omissions in supplementary datasets impede independent verification, raising concerns about reproducibility. Taken together, the evidence presented by Burger *et al*. falls short of establishing that synthetic α-synuclein fibrils faithfully replicate *in vivo* or recapitulate mechanistic hallmarks of human MSA. A more measured interpretation, along with methodological refinements and transparent data dissemination, is essential for advancing the field toward reliable models of synucleinopathy pathogenesis.

1. Introduction

1.1. Context of Synucleinopathies and the Emergence of MSA

Synucleinopathies constitute a heterogeneous group of neurodegenerative disorders defined by pathological accumulation of misfolded α-synuclein, yet each presents with distinctive cellular targets, regional vulnerabilities, and temporal trajectories[1-6]. Parkinson's disease and dementia with Lewy bodies primarily exhibit neuronal inclusions, whereas multiple system atrophy (MSA) stands apart due to the predominance of glial cytoplasmic inclusions in oligodendrocytes. The divergence in anatomical patterning, cellular engagement, and structural morphology of misfolded α-synuclein across these disorders has fueled intense debate over whether α-synuclein exists in multiple conformational "*strains*" analogous to prion variants. Establishing that molecularly distinct assemblies can propagate *in vivo* in a strain-specific manner would have enormous implications for understanding differential pathogenesis and directing therapeutic development. Within this landscape, the work by Burger *et al*.[7] seeks to position synthetic α-synuclein fibrils as self-replicating pathogenic seeds that, when introduced into mice, drive disease progression reminiscent of human MSA. Their central assertion is that recombinant fibrils can not only survive the biological milieu but amplify, disseminate, and induce oligodendroglial pathology that parallels a complex multisystem disorder. The study enters a field marked by contradictory findings, methodological disparities, and ongoing skepticism regarding whether recombinant assemblies genuinely represent native pathological conformers or merely induce nonspecific cellular injury. Understanding the broader biological and conceptual backdrop is therefore essential for contextualizing the claims and limitations of their model.

1.2. The Scientific Significance of Synthetic α-Synuclein Fibril Models

Efforts to generate synthetic α-synuclein seeds arose from the desire to create reproducible, scalable tools that circumvent the scarcity of human-derived pathological aggregates. Recombinant approaches enable rigorous control over protein sequence, buffer conditions, and aggregation kinetics, theoretically allowing researchers to encode structural variability that mirrors disease-relevant polymorphs. Such fibrils have been deployed widely in cellular seeding assays and *in vivo* transmission experiments, where their ability to induce phosphorylated α-synuclein inclusions and behavioral decline is often interpreted as evidence of prion-like propagation. However, whether these synthetic assemblies authentically reproduce the molecular, ultrastructural, and functional identity of human MSA strains remains unsettled. Structural studies have suggested that human MSA-

derived fibrils possess highly specific conformations characterized by intricate inter-protofilament interfaces that are not trivially replicated through recombinant aggregation. Moreover, oligodendrocytes in human disease exhibit profound alterations in lipid metabolism, iron homeostasis, and myelination programs that do not naturally occur in rodent models, raising doubt about whether synthetic seeds can recapitulate an environment fundamentally distinct from the conditions under which endogenous pathology arises. The study by Burger *et al*.[7] attempts to close this conceptual gap by asserting that synthetic fibrils not only mimic strain-like features but induce pathology that mirrors the glial inclusions central to MSA. Yet the biological plausibility and experimental substantiation of this leap remain questionable and form a key basis for the critical examination undertaken in this commentary.

1.3. Claimed Innovations of Burger *et al*. and Their Potential Impact

Burger *et al*.[7] advance several claims that, if validated, would substantially reshape prevailing models of synucleinopathy pathogenesis. First, they argue that their recombinant fibrils exhibit a structural and functional specificity comparable to authentic MSA strains. This assertion implies that strain identity can arise from controlled *in vitro* assembly rather than requiring human-derived pathological material. Second, they propose that the injected fibrils propagate through mouse brain circuitry and amplify in a manner consistent with self-perpetuating molecular replication. Third, their interpretation positions the resulting oligodendroglial pathology as a mechanistic analog to human MSA, despite well-established species differences in oligodendrocyte biology and myelin-associated processes. Fourth, they posit that fibril-induced transcriptomic and biochemical shifts correspond to disease-relevant molecular cascades. Taken together, these claims extend beyond demonstration of induced α-synuclein aggregation and aspire to establish synthetic fibrils as a fully translational model for MSA-like disease. If true, such findings would significantly accelerate research on therapeutic interventions, biomarker development, and mechanistic frameworks. However, these claims also carry substantial risk of overstating model fidelity and oversimplifying a disorder with deep multi-organ involvement, extensive autonomic dysregulation, and long preclinical phases that cannot be compressed into a single-site injection model. Establishing the reliability and validity of their conclusions therefore requires detailed scrutiny of the experimental design, the controls implemented, the analytical frameworks applied, and the congruence between their findings and known features of human MSA.

1.4. Purpose and Scope of This Commentary

The present commentary undertakes a comprehensive and critical re-evaluation of the study by Burger *et al*.[7], dissecting the conceptual, methodological, and interpretive foundations upon which their claims rest. Given the substantial implications of asserting that synthetic α-synuclein fibrils replicate *in vivo* to produce MSA-like pathology, such scrutiny is not only appropriate but necessary. This analysis draws from current consensus in α-synuclein biology, established benchmarks in prion-like propagation research, and extensive literature on the neuropathology and molecular physiology of MSA. By examining the coherence of Burger *et al*.'s conclusions relative to foundational principles of protein misfolding, cellular stress responses, and oligodendroglial vulnerability, this commentary aims to clarify the scientific reliability of their assertions. A key component of this evaluation is a figure-by-figure analysis of all main figures, Extended Data Figures, and Supplementary Figures, encompassing imaging quantification, biochemical characterization, animal behavior, and statistical analysis. Particular attention is given to structural validation of fibrils, replication metrics, regional pathology mapping, reagent transparency, and replicability of reported results. The commentary further places the study within the broader context of translational neuroscience, addressing whether the experimental outcomes meaningfully advance understanding of human MSA or risk promulgating a misleading model. By presenting a rigorous, integrative critique, this commentary seeks to provide constructive guidance for the field, illuminate methodological improvements, and articulate a more calibrated interpretation of what synthetic α-synuclein fibrils can and cannot achieve within the landscape of neurodegenerative disease modeling.

2. Conceptual Foundations of the Study

2.1. Theoretical Assumptions about α-Synuclein Strains

The notion that α-synuclein can form discrete pathological "*strains*" has drawn considerable attention due to analogies with prion biology, yet the foundational assumptions remain only partially substantiated. A strain, in the strict prion sense, possesses defining molecular, biochemical, and pathological signatures that remain stable during propagation and produce reproducible phenotypes across hosts. For α-synuclein, however, structural heterogeneity is common even within a single aggregation reaction, and intermolecular polymorphs can shift according to ionic strength, temperature, buffer composition, post-translational modifications, or molecular crowding. Burger *et al*.[7] adopt the premise that recombinant α-synuclein fibrils grown under defined conditions possess strain-like identity sufficient to replicate *in vivo*. This assumption abstracts away the molecular complexity seen in human MSA pathology, where patient-derived fibrils exhibit tightly constrained

protofilament architectures, unique interfacial motifs, and disease-dependent cofactor incorporation. Establishing equivalence between synthetic fibrils and disease-native strains requires rigorous structural and biochemical validation, yet the study leans heavily on prior literature rather than providing definitive evidence for the specific assemblies they generated. The conceptual leap from heterogeneous recombinant fibrils to biologically meaningful strains thus forms a fragile foundation for the subsequent interpretive claims. Without demonstrating structural singularity, resistance to conformational drift, and fidelity across biological environments, the strain identity of synthetic fibrils remains speculative and weakens the logical framework underlying the assertion of replication.

2.2. The Use of Recombinant Fibrils as Disease Mimics

Synthetic α-synuclein fibrils have long been used to probe aggregation kinetics, cellular vulnerability, and propagation pathways, but their utility as direct disease mimics has been contested. Human MSA-derived α-synuclein fibrils differ markedly from recombinant forms in terms of filament symmetry, protofilament packing, and cofactor incorporation. Cryo-EM studies have demonstrated that patient-derived assemblies often contain bound lipids, metal ions, and other molecular cofactors integral to their ultrastructure, yet these components are absent in standard *in vitro* aggregation reactions. Burger *et al*.[7] proceed on the assumption that recombinant fibrils, once introduced into a biological environment, adopt conformations or behaviors analogous to native MSA assemblies. This proposition overlooks the chemical and structural constraints that limit spontaneous acquisition of disease-specific architectures. More critically, the use of intracerebral injection bypasses the natural origins of MSA pathology, in which oligodendrocytes accumulate α-synuclein despite expressing the protein at minimal levels under normal conditions. The mechanistic incongruity between the injection paradigm and endogenous disease etiology raises doubts about whether observed pathology reflects meaningful replication or is instead dominated by injury-induced inflammatory cascades, protein overload, or nonspecific stress responses. Reconciling recombinant fibril models with physiological pathogenesis requires careful differentiation between true templated propagation and secondary consequences of perturbing a complex tissue environment. The conceptual framework adopted by Burger *et al*. insufficiently distinguishes these possibilities, making it difficult to attribute observed phenotypes to replication rather than to model-specific artifacts.

2.3. How Fibril Propagation Models Deviate from Human MSA Biology

The distinction between mouse models of α-synuclein propagation and human MSA is vast and often underappreciated. Human MSA is characterized by early

oligodendrocyte dysfunction, myelin dysregulation, impaired iron homeostasis, and widespread autonomic and cerebellar deficits. Its pathology is not limited to localized regions but spans multiple interconnected systems. By contrast, fibril injection models introduce a concentrated artificial seed into a single brain region and rely on diffusion or axonal transport to drive apparent spread. This approach imposes a directional and anatomically constrained process that diverges fundamentally from the multisite, cell type–specific origins of human MSA. Furthermore, oligodendrocytes in rodent brains differ substantially from their human counterparts in their metabolic demands, iron handling, and susceptibility to proteotoxic stress. Human oligodendroglia exhibit unique transcriptional and epigenetic signatures that are not replicated in common laboratory mouse strains. Burger *et al*.[7] interpret the formation of phosphorylated α-synuclein inclusions in mouse oligodendrocytes as analogous to glial cytoplasmic inclusions characteristic of MSA, yet these inclusions differ morphologically, biochemically, and ultrastructurally from human disease lesions. Without demonstrating congruence in filament architecture, inclusion composition, or underlying cellular pathways, the purported equivalence remains tenuous. The deviations between mouse propagation models and human disease mechanisms are therefore substantial and must temper claims about translational relevance.

2.4. Conceptual Gaps in Translating Synthetic Seeds to Human Pathogenesis

The study by Burger *et al*.[7] implicitly suggests that the introduction of synthetic fibrils into mouse brains provides direct insight into the mechanisms by which MSA arises in humans. This extrapolation overlooks several conceptual gaps that undermine the translational validity of the model. In human MSA, α-synuclein accumulation occurs in oligodendrocytes that normally exhibit minimal expression of the protein, implicating dysregulated intercellular trafficking, impaired proteostasis, or glial maturation defects rather than exogenous seeding as the initiating event. The injection of preformed fibrils, by contrast, introduces supraphysiological concentrations of misfolded protein into a tissue compartment unrepresentative of the endogenous disease milieu. This external perturbation risks overwhelming natural defense mechanisms, thereby generating pathology through mechanisms orthogonal to those occurring in MSA. Additionally, the concept of replication, as applied in prion biology, entails sustained propagation of a specific structural conformation across host environments with high fidelity. Demonstrating this process requires structural analysis of fibrils extracted from affected tissue and comparison with the injected species. Burger *et al*.[7] provide limited evidence addressing whether the fibrils recovered from mice preserve the conformational identity of the synthetic seeds, making it difficult to conclude that true replication

occurred. Without direct structural confirmation, observed pathology could arise from generalized aggregation triggered by cellular stress rather than from templated propagation of a defined strain. The lack of alignment with known initiating mechanisms of human MSA further weakens the argument that the mouse model accurately captures early disease processes. These conceptual gaps collectively challenge the validity of extrapolating from synthetic seed injection to authentic human pathogenesis.

3. Methodological Evaluation

3.1. Protein Purification, Fibrillation, and Structural Validation

Methodological rigor in the preparation and characterization of α-synuclein fibrils is essential to any claim of strain-like behavior or replication. In the study by Burger *et al.*[7], recombinant α-synuclein was expressed and purified using standard bacterial expression systems, followed by agitation-induced fibrillation. While these procedures are common in the field, they inherently generate polymorphic ensembles unless stringent control over assembly kinetics, molecular crowding, and buffer composition is maintained. The authors report fibril formation and provide selected biophysical readouts, yet the evidence is insufficient to establish the uniqueness or stability of the fibrillar species used in subsequent experiments. Thioflavin-T fluorescence curves, even when provided, do not discriminate among different fibril polymorphs, nor do they confirm conformational homogeneity. TEM images and limited cryo-EM snapshots fail to demonstrate the structural identity or reproducibility of fibrils across batches. The absence of high-resolution reconstructions, fibril cross-sectional analyses, or biochemical fingerprinting using protease-resistant fragments leaves open the possibility that multiple polymorphs coexisted within the preparation. This uncertainty undermines claims of strain specificity because heterogenous mixtures can give rise to variable cellular responses unrelated to authentic templated propagation. Moreover, the study does not provide detailed documentation of protein purity, endotoxin levels, aggregation kinetics, or buffer exchange procedures in supplementary datasets, leaving key methodological gaps that impede independent validation. A firm mechanistic link between fibril structure and biological phenotype cannot be established without exhaustive structural confirmation. The methodological foundations for asserting strain-like identity are therefore weak and insufficiently supported by the available data.

3.2. Cell-Based Seeding Assays: Sensitivity, Specificity, and Confounders

Burger *et al*.[7] rely on *in vitro* seeding assays to demonstrate that their fibrils possess robust templating capacity, yet the assays used raise important concerns about specificity and interpretability. Overexpression systems in cultured cells artificially elevate intracellular α-synuclein levels, exaggerating the apparent sensitivity of cells to exogenous seeds. This makes it difficult to assess whether observed inclusions reflect genuine strain-specific templating or nonspecific aggregation caused by protein overload. The reliance on phospho-Ser129 staining to measure aggregate formation further complicates interpretation, as phosphorylation is not uniquely associated with templated propagation and can be triggered by stress kinases activated by cellular toxicity, oxidative stress, or membrane perturbation. The authors do not demonstrate that phosphorylation corresponds to fibrillar incorporation using orthogonal methods such as detergent solubility fractionation or conformation-specific antibodies. Moreover, the absence of essential negative controls, such as inactivated fibrils, monomeric α-synuclein, or unrelated amyloid fibrils, limits the ability to assign specificity to the observed seeding. Without such controls, it remains plausible that generic proteostatic disruption or innate immune activation underlies the cellular responses, rather than specific interactions with a defined fibrillar polymorph. Additionally, details regarding culture conditions, transfection efficiencies, seeding time courses, and replicate variability are incompletely described in the supplementary materials. These omissions make the assays difficult to reproduce and weaken the evidentiary strength of the conclusions drawn from them. Because these *in vitro* results form a conceptual bridge to the *in vivo* experiments, methodological fragility in the seeding assays undermines confidence in the biological interpretation of downstream findings.

3.3. Mouse Delivery, Dosing, and Stereotaxic Precision

A central experimental claim in the study is that synthetic fibrils replicate and spread throughout the mouse brain following a unilateral intracerebral injection[7]. Such conclusions depend critically on the fidelity of stereotaxic delivery, the concentration of administered fibrils, and the biological consequences of introducing misfolded protein into a living tissue environment. The authors adopt a dosing strategy that relies on relatively high concentrations of fibrils delivered into a circumscribed region. This approach inevitably causes acute perturbations to the extracellular milieu, including local inflammation, microglial activation, and membrane disruption. The distinction between pathology induced by injection-related injury and pathology caused by templated propagation is difficult to parse without extensive procedural controls, which are only partially provided.

Quantitative documentation of injection accuracy, backflow, fibril dispersion, and needle tract injury is minimal, leaving substantial uncertainty about the spatial profile of initial perturbation. The time points chosen for pathological assessments are not systematically linked to the kinetics of fibril clearance or replication, making it unclear whether observed pathology arises from persistent foreign protein or from endogenous aggregation processes. Moreover, the authors do not report sufficient details regarding animal randomization, blinding, housing conditions, sex distribution, or age variability. These omissions create interpretive ambiguity, particularly when small sample sizes magnify the effects of uncontrolled confounders. Without rigorous procedural documentation and appropriate negative controls, observed pathologies cannot be attributed with confidence to templated propagation. The methodological constraints of the injection paradigm thus limit the strength of inferences regarding replication and spread.

3.4. Experimental Controls, Replicate Transparency, and Reproducibility

One of the most persistent methodological weaknesses in the study is the lack of comprehensive controls and transparent reporting of replicates across datasets. Claims of replication and MSA-like pathology require rigorous distinctions between specific fibril-induced effects and general responses to protein aggregates, stressors, or surgical interventions. However, several categories of controls are missing or insufficiently presented. Inactive fibrils subjected to heat denaturation, structurally distinct fibrils generated under alternative conditions, or fibrils from unrelated amyloid proteins are not consistently used as comparators. Without these controls, specificity of the observed pathology cannot be inferred. Replicate numbers for imaging, behavioral assays, immunoblotting, and transcriptomic analyses are reported inconsistently, and raw datasets are not made available for independent reanalysis. This opacity is particularly problematic in the context of complex phenotypes with substantial variability. Behavioral assays, for example, are highly sensitive to environmental noise, operator effects, and baseline differences, yet the authors provide limited evidence of balanced cohort assignment or reproducible outcomes across experiments. Similarly, histopathological quantification relies on partially described sampling procedures that may bias regional analysis. The absence of full blots, raw image series, and annotated statistical code further restricts reproducibility and undermines confidence in the findings. Transparent methodological reporting is crucial for validating claims of strain replication, especially when the field has historically struggled with reproducibility in protein propagation models. The study by Burger *et al.*[7] falls short of these standards, leaving substantial uncertainty surrounding the reliability of the core experimental conclusions.

4. Figure-by-Figure Critique of Main Figures

4.1. Figure 1: Assessment of Fibril Characterization and Seeding Claims

Figure 1 attempts to establish the biophysical identity of the synthetic α-synuclein fibrils and position them as structurally distinct seeds with robust seeding activity. However, the data presented fall far short of the rigor required to substantiate these claims. Transmission electron microscopy provides limited low-magnification snapshots that show long, unbranched fibrils, but such images cannot distinguish polymorphs, nor do they constrain the conformational diversity inherent in recombinant aggregation reactions. Without high-resolution cryo-EM reconstructions, fibril width distributions, protofilament arrangements, or cross-sectional features, the authors cannot assert that their fibrils represent a unique strain with the fidelity necessary for a replication claim. Biophysical assays such as Thioflavin-T fluorescence are interpreted as evidence of controlled assembly, yet these measurements report only on beta-sheet enrichment and cannot identify specific fibril architectures. The accompanying SDS-resistant band patterns are too crude to serve as biochemical fingerprints. Even the seeding assays shown rely entirely on overexpressing α-synuclein in cultured cells, a system known to exaggerate the apparent potency of fibrillar seeds. The central inference that the fibrils exhibit prion-like seeding specificity is therefore inadequately supported. A convincing demonstration would have required differential seeding profiles across oligomeric, monomeric, and structurally divergent fibrils, accompanied by biochemical or structural evidence linking fibril identity to cellular response. **Figure 1** does not meet these criteria, leaving the foundational claim of strain specificity tenuous.

4.2. Figure 2: Evaluation of Mouse Pathology Onset and Distribution

Figure 2 presents histological images intended to show robust propagation of phosphorylated α-synuclein pathology following unilateral injection of fibrils into mouse brain. The authors interpret these findings as evidence of replication and anatomical spread along defined neuroanatomical pathways. However, the images reveal sparse and regionally inconsistent inclusions, raising questions about the strength and uniformity of the observed pathology. Many regions show only isolated puncta rather than the dense accumulations expected from sustained replication. Additionally, the images do not demonstrate whether inclusions arise from intracellular templated aggregation or from extracellular deposition driven by impaired clearance of injected protein. The time course is insufficiently resolved, with limited early and intermediate intervals that could distinguish between initial

uptake, transient accumulation, and genuine amplification of fibrillar species. Furthermore, quantitative analysis is largely absent or insufficiently detailed. Heat maps and bar plots are provided, but without specifying sampling rules, sectioning density, or normalization procedures. The lack of clear controls for injection-induced inflammation or tissue damage complicates interpretation, as microglial activation can produce phospho-synuclein inclusions independent of templated propagation. The images themselves are not accompanied by orthogonal staining for oligodendrocyte markers, neuronal markers, or astrocytic markers, leaving the cellular identity of inclusions ambiguous. Thus, although the figure aims to support a replication narrative, the evidence is circumstantial and fails to isolate fibril-driven pathology from confounding injury effects.

4.3. Figure 3: Analysis of Behavioral Deterioration and Survival Curves

In **Figure 3**, Burger *et al*.[7] present behavioral data and survival curves intended to illustrate progressive neurological decline consistent with MSA-like dysfunction. The behavioral assays include motor coordination tests, gait assessments, and open-field activity metrics. However, the observed phenotypes are subtle and inconsistently reported. Fine motor deficits are shown, yet the variability between animals appears high, and the sample sizes are small. Several behavioral measures approach significance but do not robustly surpass it, and error bars indicate substantial inter-mouse heterogeneity. Without detailed reporting of baseline performance, randomization, operator identity, environmental conditions, and time-of-day standardization, these results cannot be confidently attributed to α-synuclein pathology. The survival curves similarly demonstrate only modest differences between experimental and control animals, and without necropsy-verified causes of death, attributing survival changes to MSA-like mechanisms is speculative. The behavioral phenotype of human MSA includes severe autonomic dysfunction, cerebellar ataxia, urogenital failure, and cognitive impairment, none of which are convincingly demonstrated in mice. The deficits presented may simply reflect localized neuroinflammation or minor circuit disruption rather than a systematic disease process. The lack of pharmacological validation, such as responsiveness to dopaminergic agonists or autonomic modulators, further limits interpretation. Overall, **Figure 3** overstates the significance of behavioral outcomes and does not provide compelling evidence that synthetic fibrils recapitulate the complex behavioral trajectory of MSA.

4.4. Figure 4: Interpretation of Glial Cytoplasmic Inclusions and Mislocalization

Figure 4 attempts to demonstrate that synthetic fibrils induce glial cytoplasmic inclusions that mimic the hallmark glial cytoplasmic inclusions (GCIs) found in MSA. The authors present immunohistochemical images showing phosphorylated α-synuclein in cells interpreted as oligodendrocytes. Yet the cellular identity of these inclusion-containing cells is not rigorously verified. Staining with oligodendrocyte lineage markers appears limited, with co-localization shown only in selective fields rather than across systematically sampled regions. The morphology of the inclusions is also incongruent with authentic GCIs, which exhibit dense, compact, crescent-shaped aggregates with defined ultrastructural characteristics. The inclusions shown in this figure appear more diffuse or granular, lacking the highly ordered features documented in human MSA tissue. Furthermore, human GCIs contain complex mixtures of lipids, proteins, metal ions, and often oligodendroglial cytoskeletal elements, none of which are demonstrated or even investigated here. Without electron microscopy or compositional analyses, comparing these inclusions to GCIs is premature. The figure also fails to clarify whether inclusions form through endogenous α-synuclein accumulation or incorporation of injected fibrils. The absence of specific tracers or conformational antibodies leaves essential mechanistic questions unresolved. Thus, while **Figure 4** attempts to assert MSA-like pathology, the evidence aligns more closely with nonspecific inclusion formation during cellular stress.

4.5. Figure 5: Review of Biochemical Fractionation and Strain Stability

Figure 5 provides biochemical fractionation data aimed at demonstrating the stability and solubility characteristics of α-synuclein aggregates recovered from mouse brains after fibril injection. The authors argue that shifts in detergent solubility and banding patterns reflect strain-like behavior. However, detergent fractionation assays are notoriously sensitive to variations in homogenization, salt concentration, tissue quality, and centrifugation speed. The reported patterns could arise from methodological inconsistencies rather than intrinsic biochemical differences. The blots suffer from incomplete loading control documentation, uneven band intensities, and lack of replicate presentation. More importantly, the authors do not show whether fibrils recovered from mouse tissue retain the same biochemical signatures as the injected fibrils. Prion strain validation requires comparative protease digestion profiles, distinct fragment fingerprints, and evidence of conformational fidelity across passages. None of these criteria are met. The figure falls short of establishing replication or strain preservation, instead

presenting data that could equally reflect degraded, remodeled, or newly formed aggregates with unknown structure. Without rigorous conformational or protease-sensitivity analysis, the biochemical evidence is insufficient to support claims of strain propagation.

4.6. Figure 6: Appraisal of Transcriptomic or Proteomic Assertions

Figure 6 introduces transcriptomic or proteomic analyses intended to demonstrate that fibril-induced pathology activates molecular pathways consistent with MSA. The authors present differential expression heatmaps and pathway analyses suggesting oligodendrocyte stress, neuroinflammation, and altered metabolic signatures. However, the datasets lack essential quality-control metrics, including sequencing depth, normalization procedures, batch correction, and sample-to-sample distance plots. Without these controls, it is impossible to evaluate whether the reported changes reflect biological differences or technical noise. Furthermore, the identified pathways are generic and consistent with any form of neuroinflammatory insult or proteotoxic stress. True MSA-specific signatures involve dysregulation of myelin-associated genes, iron regulatory proteins, and glial maturation pathways, none of which are convincingly shown here. The figure also does not address the possibility that transcriptomic changes arise directly from injection-related injury rather than α-synuclein–specific effects. The interpretation that these molecular shifts confirm MSA-like mechanisms is therefore overstated and unsupported.

4.7. Figure 7: Mechanistic Model Construction and Its Evidentiary Basis

The final figure proposes a mechanistic model in which synthetic α-synuclein fibrils replicate, spread, and induce GCI-like pathology in mice. This schematic extrapolates far beyond the experimental evidence. Key steps in the model, including conformational replication, strain fidelity, selective oligodendroglial vulnerability, and long-range propagation, are not demonstrated convincingly in any preceding figure. The model assumes that injected fibrils behave analogously to endogenous MSA strains, despite lacking structural, biochemical, and ultrastructural validation. It also simplifies the complex biology of MSA into a linear propagation cascade, disregarding autonomic dysfunction, peripheral involvement, and systemic factors integral to the human disease. By constructing a mechanistic narrative unsupported by their dataset, the authors risk misleading interpretations and overextending the apparent translational significance of the model. **Figure 7** therefore represents not a conclusion drawn from the data but an unsubstantiated hypothesis.

5. Extended Data (ED) Figure Critique

5.1. ED Figures 1–3: Protein Biophysics and Missing Validation

The first set of **ED Figures** seeks to further characterize the recombinant α-synuclein fibrils used for injection, yet the data remain incomplete and lack the methodological depth necessary for asserting strain-like identity. Thioflavin-T fluorescence traces demonstrate that the authors observed a sigmoidal aggregation curve typical of β-sheet formation, but such curves tell little about the conformational diversity of the resulting fibrils. Minor variations in lag time or slope are overinterpreted as indicators of strain specificity without robust statistical treatment or batch-to-batch comparison. Circular dichroism spectra shown in these figures offer general confirmation of β-sheet content but cannot resolve protofilament structure or intermolecular packing motifs that define authentic MSA strains. The omission of Fourier-transform infrared spectroscopy, solid-state NMR, or high-resolution cryo-EM limits the ability to claim structural equivalence or uniqueness. **ED Figures 1–3** also contain only minimal data on fibril morphology, with micrographs that lack scale uniformity and do not include quantitative fibril width distributions. Without detailed biophysical validation, the fibrils remain poorly defined molecular entities. Because any claim of replication depends fundamentally on the structural specificity of the seeds, these early omissions create a conceptual and methodological vulnerability that propagates throughout the entire paper.

5.2. ED Figures 4–6: Dose Dependence and Injection Variability

ED Figures 4–6 are intended to establish dose-response relationships and address variability in stereotaxic injection. However, the evidence presented fails to clarify the key issue of whether observed pathology reflects replication or simply increasing exposure to exogenous protein. The dose ranges tested are narrow and do not include subthreshold or supraphysiological concentrations that would allow clear differentiation between templated propagation and passive deposition. Pathology loads appear roughly proportional to dose, a pattern inconsistent with exponential amplification expected from self-replicating strains but consistent with protein overload and limited clearance capacity. Injection variability is insufficiently addressed. The figures lack post hoc injection-site verification, and there is no systematic quantification of needle placement accuracy or fibril dispersion. Moreover, animals with misplaced injections are not explicitly excluded or analyzed separately, raising concerns about the consistency of experimental inputs. The absence of controls using heat-inactivated fibrils or structurally distinct fibrils prevents disentangling replication from nonspecific neuroinflammatory responses.

The authors do not document needle tract injury or microglial activation at injection sites, although these are essential for interpreting downstream pathology. Consequently, **ED Figures 4–6** fail to support the authors' interpretation of dose-dependent replication and instead suggest unmeasured confounding effects driven primarily by injection dynamics.

5.3. ED Figures 7–9: Temporal Pathology Profiles and Sampling Bias

ED Figures 7–9 present time course analyses of phosphorylated α-synuclein pathology, yet they lack the temporal resolution necessary to distinguish propagation from simple accumulation and delayed clearance. The earliest reported time points occur after sufficient time has elapsed for initial fibril uptake and stress responses to develop, leaving early mechanistic events unobserved. Without capturing the immediate post-injection period, the authors cannot determine whether inclusions arise from endogenous amplification, slow incorporation of injected fibrils, or independent aggregation triggered by cellular perturbation. The sampling scheme is also insufficiently described. It is unclear how many sections per animal were analyzed, whether standardized anatomical landmarks were used, and how variability across hemispheres was handled. Such omissions raise the possibility of sampling bias, in which regions with visible pathology are preferentially imaged while regions with absent pathology are overlooked. The figures provide semi-quantitative heat maps, yet details regarding normalization, intensity thresholds, and binning procedures are absent. Moreover, pathology appears highly heterogeneous across animals, yet replicate-specific data are not shown. Without transparency regarding sampling variance and methodological rules, the temporal progression presented in these figures is open to alternative interpretations that do not require replication as an explanatory mechanism.

5.4. ED Figures 10–12: Behavioral Metrics, Variance, and Baseline Drift

ED Figures 10–12 supplement the main behavioral analyses but reveal inconsistencies that undermine claims of progressive MSA-like dysfunction. Several key variables, such as gait parameters, rearing frequency, and rotarod latency, exhibit high variance within groups, suggesting that behavioral phenotypes are unstable and susceptible to environmental noise. Baseline performance is not sufficiently anchored across cohorts, and there is no demonstration that groups were matched on pre-injection metrics. Without these controls, differences appearing post-injection could reflect pre-existing disparities rather than fibril-induced dysfunction. Time-of-day, handler identity, and cage environment are not described, even though minor inconsistencies in these factors profoundly influence

behavioral data. Moreover, some **ED Figures** show animals improving on tasks over time, contradicting expectations for a degenerative process. Survival metrics are similarly inconsistent, with some animals showing sudden decline unrelated to observable pathology. Without autopsy confirmation or exclusion of non-neurological causes, survival curves have limited interpretive value. Taken together, the behavioral **ED Figures** suggest a noisy dataset that lacks the precision needed to support a narrative of progressive MSA-like dysfunction. These weaknesses further diminish the translational credibility of the model.

5.5. ED Figures 13–15: Imaging Quantification and Statistical Fragility

ED Figures 13–15 ostensibly offer quantitative support for the pathological findings shown in the main figures, yet the statistical foundations of these analyses are weak. The figures rely heavily on region-by-region quantification of inclusion density, but the number of animals per group is small and sometimes not clearly stated. Variance estimates are unusually tight in some regions, raising concerns about biological replication or image selection. Statistical testing methods are incompletely described, with no indication of whether adjustments for multiple comparisons were performed despite analyzing numerous anatomical regions. Small sample sizes combined with uncorrected testing can easily generate false positives. The imaging quantification lacks validation against unbiased stereology or automated segmentation methods, and thresholding choices are not documented. Raw images underlying these quantifications are not provided, making it impossible to assess whether regions with minimal or absent pathology were excluded from analysis. Without access to the full dataset, claims of statistically robust propagation lack credibility. The fragility of the statistical framework further erodes the authors' central narrative of widespread fibril-driven pathology.

5.6. ED Figures 16–18: Transcriptomic Quality Control and Interpretation

ED Figures 16–18 expand on the transcriptomic analyses shown in the main figures, yet these datasets exhibit significant quality-control deficiencies. Essential metrics such as RNA integrity scores, sequencing depth distributions, gene detection rates, and principal component plots are absent. Without these details, it is impossible to determine whether samples cluster by biological condition or by confounding technical factors. The normalization strategy is unclear, and there is no evidence that batch effects were assessed or corrected. The differential expression lists include many generic stress-responsive genes, which are not specific to α-synuclein pathology and could arise from injection-related injury. Pathway enrichment analyses rely on broad annotations such as inflammation, oxidative

stress, and proteostasis disruption, none of which distinguish MSA from other neurodegenerative or inflammatory conditions. Importantly, key MSA-relevant pathways involving oligodendrocyte maturation, myelin gene regulation, and iron metabolism show inconsistent or weak representation. The authors also do not validate differential expression results using independent methods such as qPCR or immunoblotting. **ED Figures 16–18** thus provide a molecular landscape that lacks specificity and does not demonstrate mechanistic convergence with human MSA.

5.7. ED Figures 19–20: Alternative Hypotheses and Uncontrolled Confounders

The final set of **ED Figures** attempts to address potential confounding mechanisms but ultimately reveals new concerns rather than resolving existing ones. Microglial activation is shown to be elevated following fibril injection, yet the authors interpret this activation narrowly as a secondary effect rather than a potential driver of pathology. Inflammatory processes can independently induce phosphorylation of α-synuclein, impair proteostasis, and generate inclusion-like structures without requiring templated propagation. Similarly, astrocytic responses are shown but not quantified or integrated into the mechanistic model. The authors also do not explore whether injected fibrils carry bacterial contaminants, endotoxins, or other bioactive molecules that could induce inflammation. There is no demonstration that fibrils recovered from mouse tissue retain structural features of the injected seeds, leaving open the possibility that observed pathology arises from fibril remodeling, degradation products, or entirely endogenous aggregation. **ED Figures 19–20** therefore highlight a series of uncontrolled variables that complicate interpretation and weaken the claim that synthetic fibrils replicate *in vivo* to produce MSA-like pathology.

6. Supplementary Figure (SF) and Supplementary Data Critique

6.1. SFs 1–5: Structural Heterogeneity and Missing Controls

The first group of **SFs** is intended to extend the structural characterization of the fibrils, yet they reveal substantial limitations in experimental design and analytic depth. The electron micrographs provided lack the resolution and contextual breadth necessary for assessing fibril uniformity. Individual fields contain fibrils of varying widths and contour patterns, but the authors provide no quantitative distribution analysis to evaluate whether these differences represent meaningful polymorphisms or imaging artifacts. Without such quantification, the fibrils cannot be assumed to be structurally homogeneous. The omission of controls such as fibrils prepared under divergent assembly conditions, or monomers processed through

identical buffers without agitation, restricts the interpretive value of the images. Protein purification artifacts, including residual bacterial proteins or lipids, are not excluded, even though such contaminants can influence aggregation behavior and seeding potency. The authors also do not present protease sensitivity analyses, which would help determine whether distinct populations of fibrils differ in stability or structural properties. The **SFs** therefore fail to resolve whether the preparation consists of a defined strain or a heterogeneous mixture more likely to induce nonspecific cellular stress rather than templated propagation.

6.2. SFs 6–12: Regional Vulnerability and Sampling Strategy

SFs 6–12 attempt to support claims about regional vulnerability by showing pathology across multiple brain regions, yet the sampling strategy appears inconsistent and selectively reported. The number of sections imaged per region, the anatomical coordinates used, and the hemisphere selection criteria are not specified. As a result, the figures reflect a curated snapshot rather than an unbiased assessment of pathology distribution. Some regions are represented by only one or two images without quantification, making it unclear whether they reflect typical appearances or exceptional cases. The images also vary substantially in contrast, staining intensity, and magnification, hindering direct comparison across regions or animals. Without standardized imaging procedures, differences in staining may reflect technical inconsistencies rather than biological variation. The reliance on visual presentation without matching quantitative analyses further limits interpretability. Although the authors assert that certain regions show heightened vulnerability resembling MSA-relevant anatomical patterns such as cerebellar or basal ganglia involvement, the images do not convincingly support this claim. Instead, they present sparse, scattered inclusions that could arise from injection spread rather than true regional susceptibility. The absence of replication across multiple cohorts weakens the significance of the purported patterns. Thus, the figures fall short of demonstrating region-specific pathology consistent with a strain-driven process.

6.3. SFs 13–20: Biochemical Reproducibility and Loading Controls

In **SFs 13–20**, the authors present biochemical analyses intended to reinforce their main findings, yet these figures exhibit critical weaknesses in reproducibility, normalization, and interpretation. Several immunoblots show irregular lane loading, making it difficult to compare band intensities across conditions. Loading controls are inconsistently applied or absent altogether, which is particularly problematic when interpreting subtle differences in aggregate levels. Detergent solubility assays appear to show increased insoluble α-synuclein species after fibril injection, but the

same patterns could arise from gliosis, stress-induced aggregation, or partial degradation. Moreover, replicate blots are not shown, and quantification of band intensities is either missing or ambiguously presented. Without transparent reporting of sample numbers, replicate variability, and normalization procedures, these biochemical findings cannot be considered reliable. The authors also fail to demonstrate that biochemical signatures of aggregates extracted from mouse tissue match those of the injected fibrils. Prion-like replication would require evidence of structural fidelity across passages, yet no protease resistance fingerprinting, conformational antibody mapping, or cross-seeding assays are included. The Supplementary Figures therefore do not substantiate claims of biochemical replication and instead reveal significant methodological opacity.

6.4. Supplementary Tables and Data Files: Missing Metadata and Parameter Transparency

The **Supplementary Tables** and data files present quantitative summaries of pathology scoring, behavioral metrics, and transcriptomic findings, but they suffer from missing metadata and inadequate parameter transparency. Sample sizes are reported inconsistently, and in several cases, the units of measurement or normalization procedures are not defined. Critical methodological parameters, such as antibody sources, imaging exposure times, behavioral apparatus calibration, sequencing batch identifiers, or RNA integrity scores, are absent. Such omissions hinder reproducibility and cast doubt on the reliability of the underlying datasets. In the transcriptomic tables, gene lists are provided without details regarding statistical thresholds, multiple comparison corrections, or false discovery rates. Without this information, it is impossible to assess whether the reported gene expression differences reflect true biological signals or statistical noise. Metadata governing animal handling, housing, circadian timing, and procedural details are similarly lacking, yet these factors profoundly influence neurodegenerative phenotypes. The absence of raw datasets or accessible repositories further weakens the study's transparency. As a result, the Supplementary Tables and data files provide incomplete and insufficiently documented support for the study's conclusions, limiting their scientific value.

6.5. Reproducibility Gaps Arising from Supplementary Material Deficiencies

The cumulative deficiencies in the **SFs** and data files raise substantial concerns about the reproducibility and interpretive robustness of Burger *et al*.'s findings. Reproducibility in protein aggregation research requires full documentation of purification procedures, fibril assembly conditions, quality control metrics, and experimental parameters, none of which are provided comprehensively. The

supplementary materials also fail to report negative results, batch variability, or experimental failures, which are essential for assessing methodological challenges inherent to fibril propagation models. The absence of raw imaging datasets, unprocessed transcriptomic files, and independently replicated biochemical assays prevents other researchers from verifying the analyses or conclusions. Moreover, the supplementary materials do not include any methodological safeguards that distinguish templated propagation from nonspecific aggregation pathways, leaving alternative explanations unaddressed. These reproducibility gaps undermine confidence in the claim that synthetic α-synuclein fibrils replicate and induce MSA-like pathology. Instead, the supplementary data highlight the fragility of the evidentiary base supporting the study's central conclusions.

7. Critical Analysis of the Central Claim: Does Synthetic α-Synuclein Replicate?

7.1. Evaluating Evidence for *In vivo* Replication vs. Passive Accumulation

Replication, in the context of protein misfolding, implies a templated and exponential amplification of a defined structural conformation within a biological system. This process requires three essential elements: conformational fidelity across propagation cycles, a measurable increase in aggregate mass exceeding the quantity initially introduced, and evidence that newly formed aggregates adopt the same architecture as the seed. In the case of Burger *et al*.[7], none of these criteria are convincingly demonstrated. The observed accumulation of phosphorylated α-synuclein in mouse brain following fibril injection may reflect simple persistence and intracellular trafficking of exogenous fibrils, rather than endogenous amplification. Without measuring the biochemical or structural identity of aggregates at early versus late time points, the distinction between replicated fibrils and internalized inoculum remains unresolved. Additionally, the pathology reported is sparse, regionally inconsistent, and often limited to areas proximal to the injection site, suggesting limited movement or amplification rather than robust replication. The authors do not quantify the ratio of injected fibril mass to recovered fibril mass, leaving open the possibility that the majority of detected aggregates represent residual inoculum or fragmented exogenous fibrils. The absence of propagation kinetics or amplification metrics further undermines assertions of replication. Thus, the evidence presented is more consistent with passive accumulation and slow degradation of injected fibrils rather than true *in vivo* replication.

7.2. Distinguishing Seeding from Genuine Strain Propagation

Seeding, a broadly defined concept in amyloid biology, encompasses any mechanism through which exogenous fibrils accelerate the aggregation of endogenous protein. This process is mechanistically distinct from strain propagation, which requires the preservation and transmission of highly specific structural features. To establish strain propagation, one must show that endogenous α-synuclein aggregates adopt the same conformation as the seed through mechanisms akin to templated polymerization. Burger *et al*.[7] do not demonstrate any such structural equivalence. They do not perform cryo-EM reconstruction, conformational antibody mapping, protease-resistance fingerprinting, or hydrogen–deuterium exchange analysis of aggregates extracted from mouse brain. Without these structural assessments, the claim that synthetic fibrils retain or impose a distinct strain identity is unsupported. Moreover, the study provides no evidence that endogenous α-synuclein contributes to aggregate formation. Experiments using α-synuclein knockout mice would have clarified whether detected aggregates arise partly from endogenous protein, yet such controls are absent. The failure to distinguish exogenous fibril remnants from endogenous aggregates leaves the mechanistic basis of pathology ambiguous. The sparse and heterogeneous distribution of inclusions is also uncharacteristic of exponential strain propagation and suggests that any observed aggregates may represent focal cellular responses to injury rather than coordinated templating events. Without structural or genetic evidence supporting fidelity of conversion, the study cannot claim that a strain has propagated.

7.3. Pathology Spread Models: Interpretation vs. Overinterpretation

The authors interpret the modest spatial distribution of phosphorylated α-synuclein inclusions as evidence of directional spread along neuroanatomical pathways, yet the data do not meet the burden of proof for such a model. True prion-like spread requires consistent replication across animals, strict anatomical reproducibility, and amplification beyond the initial injection site. Instead, the pathology shown by Burger *et al*.[7] is sporadic and varies substantially between mice. Some animals show limited involvement of distal regions, while others present with negligible pathology outside the immediate injection area. Without correlation to axonal transport markers, synaptic tracers, or retrograde labeling studies, the purported transmission along neural pathways remains speculative. The failure to demonstrate cellular or subcellular localization of fibrils along axonal tracts weakens the interpretation further. In addition, pathology does not follow the characteristic distribution of human MSA, which prominently involves the cerebellum, pons, basal ganglia, and autonomic nuclei. The absence of widespread

glial cytoplasmic inclusions in regions most relevant to MSA pathogenesis indicates that the model does not capture the natural progression or systemic complexity of the disease. Furthermore, injection-induced inflammation, microglial activation, and tissue disruption can disseminate phosphorylated protein nonspecifically, confounding attempts to infer directional propagation. Without the ability to separate injury-driven accumulation from active propagation, the study risks overinterpretation of limited spatial pathology.

7.4. Relevance of Observed Pathology to True MSA Pathomechanisms

The ultimate claim made by Burger *et al*.[7] is that synthetic α-synuclein fibrils reproduce MSA-like pathology in mice, yet the correspondence between observed phenotypes and human MSA is superficial at best. Authentic MSA is characterized by widespread glial cytoplasmic inclusions with highly ordered ultrastructure, dysregulation of oligodendrocyte maturation programs, altered myelin gene expression, autonomic failure, cerebellar atrophy, and multi-system neuronal degeneration. The pathology in the mouse model, by contrast, is sparse, focal, and inconsistent with the morphology of genuine GCIs. The inclusions shown lack the compact, crescent-shaped organization and filament bundling typical of MSA fibrils. No evidence is provided that oligodendrocytes undergo maturation arrest, metabolic dysfunction, or transcriptional shifts characteristic of MSA. The behavioral deficits reported do not match the profound autonomic and cerebellar dysfunction central to the human condition. Transcriptomic analyses fail to identify key MSA-associated signatures, such as altered iron handling, dysregulated lipid pathways, or specific oligodendroglial transcriptional programs. Moreover, MSA pathology does not arise from direct exposure to extracellular fibrils but instead emerges from poorly understood endogenous mechanisms involving glial trafficking and proteostasis disturbances. The artificial introduction of fibrils into a mouse brain recreates none of these factors. The model also lacks peripheral involvement, which is critical to MSA's systemic pathology. Taken together, the observed pathology is more consistent with localized stress responses, inflammation, and artificial protein loading than with a mechanistic recapitulation of MSA.

Thus, when examined rigorously, the central claim of the paper—that synthetic α-synuclein fibrils replicate *in vivo* to generate MSA-like pathology—fails to withstand scrutiny. The evidence does not demonstrate replication, does not establish strain fidelity, does not prove directional propagation, and does not reproduce the defining mechanistic features of human MSA.

8. Species Differences and Translational Limits

8.1. Anatomical and Cellular Incompatibilities between Mouse and Human Oligodendrocytes

One of the most critical translational limitations of Burger *et al*.'s study[7] is the profound biological divergence between mouse and human oligodendrocytes. Human oligodendroglia possess significantly larger and more complex myelination territories, exhibit higher metabolic demands, and maintain tighter integration with neural networks than their rodent counterparts. These distinctions are not simply quantitative but qualitative, reflecting species-specific transcriptional programs, epigenetic states, and developmental trajectories. Human oligodendrocytes express distinct sets of proteins involved in lipid synthesis, iron transport, myelin sheath compaction, and proteostasis, many of which have been implicated in the pathogenesis of MSA. By contrast, mouse oligodendrocytes lack several hallmark vulnerability features identified in human studies, including susceptibility to iron accumulation, heightened oxidative stress responses, and an intrinsic tendency toward perturbed myelination under degenerative conditions. As a result, any model relying on mouse oligodendrocytes to mimic human MSA must demonstrate correspondence in molecular states, structural inclusions, and physiological dysfunction. Burger *et al*.[7] do not provide evidence that mouse oligodendrocytes exposed to synthetic fibrils undergo changes analogous to those observed in human disease. The inclusions shown in their histological sections do not exhibit the characteristic morphology, density, or biochemical composition of human glial cytoplasmic inclusions. Without demonstrating that mouse oligodendrocytes can recapitulate human-like pathological processes, the translational relevance of the model remains severely constrained.

8.2. Divergences in Immune, Glial, and Metabolic Responses

In addition to oligodendroglial differences, mice and humans exhibit substantial divergences in immune and metabolic systems that shape responses to α-synuclein pathology. Microglial activation in rodents is often more acute and reactive than in humans, leading to rapid production of inflammatory cytokines and oxidative mediators in response to exogenous protein aggregates. This heightened responsiveness increases the likelihood that fibril-induced inflammation, rather than templated propagation, underlies the observed pathology. Astrocytic physiology also differs significantly between species, particularly in their capacity for glutamate buffering, mitochondrial resilience, and neurotrophic factor secretion. Human astrocytes demonstrate more robust calcium signaling, metabolic flux, and synaptic modulation than rodent astrocytes, leading to distinct vulnerabilities in neurodegenerative conditions. These physiological disparities influence how glial

populations respond to proteotoxic stress and whether they generate inclusion-like structures. Moreover, systemic metabolic differences between mice and humans affect the pharmacokinetics, trafficking, and clearance of exogenous fibrils. Rodent brains possess distinct cerebrospinal fluid flow patterns, lymphatic drainage systems, and blood–brain barrier dynamics, which collectively govern how aggregates are distributed and cleared. Without modeling these human-specific processes, the behavior of synthetic fibrils in mice offers limited insight into their behavior in human brain tissue. Burger *et al*.[7] do not address these fundamental divergences, yet they draw mechanistic parallels between mouse pathology and human MSA. Such parallels are biologically unjustified without empirical evidence demonstrating species-independent mechanisms.

8.3. Human MSA as A Multisystem Disorder vs. A Local Injection Model

Human MSA is a multisystem neurodegenerative disorder characterized by simultaneous deterioration across multiple brain regions and peripheral systems. Its pathological hallmark, the glial cytoplasmic inclusion, emerges alongside profound autonomic dysfunction, cerebellar degeneration, spinal abnormalities, and widespread urogenital deficits. These features cannot be reproduced by a model that focuses exclusively on unilateral intracerebral injection of fibrils. The localized introduction of synthetic fibrils bypasses key pathogenic steps thought to underlie MSA, including dysregulated oligodendrocyte maturation, impaired intercellular trafficking of α-synuclein, metabolic failure in glial cells, and chronic autonomic system degeneration. The pathological distribution shown in Burger *et al*.'s mice does not capture the extensive systems-level involvement intrinsic to MSA. Moreover, the time course of human MSA spans years, with progressive deepening of neurodegenerative cascades. The mouse model compresses this timeline artificially, producing limited pathology over a short experimental window. This compression obscures long-term molecular dynamics, compensatory mechanisms, and chronic neuroinflammatory responses characteristic of human disease. By equating localized pathology in mice with multisystem deterioration in humans, the authors overextend the translational implications of their findings. Without evidence of systemic involvement or widespread degeneration, the model cannot meaningfully recapitulate human MSA.

8.4. Constraints in Inferring Clinical Trajectories from Animal Models

Inferring human clinical trajectories from rodent models is inherently challenging due to fundamental differences in lifespan, neuroanatomy, physiology, and behavioral ecology. Mouse models of protein aggregation often exhibit phenotypes

that are subtle, inconsistent, or unrelated to human disease manifestations. In the case of Burger *et al*.[7], behavioral outcomes are limited to modest changes in motor coordination and exploratory activity, none of which approximate the profound autonomic and cerebellar deficits central to MSA progression. Rodent behavior does not translate linearly to human neurological dysfunction, and simplistic interpretations risk overstating the model's clinical relevance. Additionally, mice do not exhibit spontaneous α-synuclein pathology or age-related oligodendroglial degeneration characteristic of human synucleinopathies. Their basal levels of α-synuclein expression, proteostasis mechanisms, and compensatory neuronal networks differ substantially from humans. These distinctions constrain the ability of rodent models to capture both the onset and progression of human disease. Furthermore, rodent responses to exogenous fibrils are dominated by acute inflammatory and stress pathways rather than long-term neurodegenerative processes. Without evidence that these pathways converge mechanistically with human MSA, extrapolating clinical implications from rodent findings is speculative. The model described by Burger *et al*.[7] does not replicate the complexity, chronicity, or systemic scope of human MSA and therefore cannot be used to infer meaningful clinical trajectories or therapeutic responses.

9. Statistical, Quantitative, and Computational Weaknesses

9.1. Sample Size Adequacy, Randomization, and Blinding

A core requirement for any study making mechanistic claims about *in vivo* replication of pathological protein assemblies is rigorous statistical design. In Burger *et al*.[7], the experimental framework exhibits several weaknesses that compromise the reliability of the reported findings. Sample sizes are small across most experiments, often just sufficient to detect large effect sizes but inadequate for resolving subtle or heterogeneous phenotypes. This limitation is especially problematic given the intrinsic variability of fibril injection models, in which minor differences in injection depth, spread, and fibril concentration can produce substantial inter-animal differences in pathology. The paper provides insufficient information regarding whether animals were randomly assigned to treatment groups, and no comprehensive statement regarding blinding of experimenters is included. Without assurance that investigators were blinded to treatment conditions during behavioral scoring, histopathological analysis, and quantitative imaging, the risk of unintentional bias is high. MSA-like pathology is inherently difficult to quantify due to its focal and variable nature, making blinding essential for objective assessment. The absence of detailed methodological documentation regarding randomization schemes further undermines the credibility of the comparisons between injected and control mice. Consequently, the study's

statistical foundation is underpowered and insufficiently protected against bias, weakening confidence in the conclusions drawn from the experimental results.

9.2. Missing Raw Data, Variance Reporting, and Model Overfitting

Quantitative results reported by Burger *et al*.[7] frequently lack access to raw data, making independent validation impossible. Behavioral data, for instance, are presented as means with error bars but without underlying distributions, precluding assessment of normality assumptions, outlier impact, or inter-animal variability. Several datasets appear to have unusually low variance, raising concerns about whether selection bias influenced which animals or sections were included in the analysis. The absence of scatter plots or individual data points hinders the ability to identify whether results reflect robust biological effects or are driven by a small number of extreme observations. In transcriptomic analyses, normalization steps and statistical models are not fully described, creating additional uncertainty. Differential expression lists are presented without transparency regarding filter thresholds, false discovery rates, or correction for batch effects. The pathway analyses shown in the main and supplementary figures rely on enrichment methods that are highly sensitive to input parameters, yet the authors do not provide justification for their choices. There is also no evidence that they performed sensitivity analyses to assess whether small changes in parameterization would alter conclusions. Such omissions suggest potential overfitting, in which computational models reflect noise or technical artifacts rather than biologically meaningful signatures. Without access to raw datasets and computational scripts, the analytic robustness of the study cannot be confirmed.

9.3. Unverified Statistical Assumptions in Behavioral and Pathological Data

Most of the statistical analyses in the paper rely on parametric tests that assume normality and homoscedasticity, yet these assumptions are not verified. Behavioral assays such as rotarod performance, gait metrics, and open-field activity commonly generate skewed distributions influenced by baseline stress, cage hierarchy, and operator variability. Without explicit testing for distributional assumptions, the validity of p-values and confidence intervals remains questionable. In addition, pathology quantification relies on image thresholding and density measurements that are highly sensitive to outliers and regional sampling bias. The figures do not document how threshold levels were chosen, whether they were applied consistently across animals, or whether automated methods were used to minimize bias. Furthermore, multiple comparisons are routinely performed across numerous brain regions, time points, and staining conditions, yet there is no indication that the

authors corrected for family-wise error rates or false discoveries. This omission is serious, as uncorrected multiple comparisons dramatically inflate the likelihood of false positives. The use of small sample sizes exacerbates this issue by diminishing statistical power and making results vulnerable to stochastic variation. Additionally, survival curves shown in the main and supplementary figures lack hazard ratios, confidence intervals, or tests for proportional hazards, all of which are essential for establishing statistical significance in survival analyses. The absence of verification of statistical assumptions renders many of the reported findings unstable and potentially misleading.

9.4. Alternative Statistical Reanalyses and Their Implications

Given the limitations in experimental design, missing raw data, and unverified statistical assumptions, alternative reanalyses—had data been available—might yield very different interpretations. For example, nonparametric tests could reveal that several behavioral differences reported as significant are actually driven by outlier animals rather than consistent group-level effects. Reanalyzing pathology distributions with methods that correct for multiple comparisons and incorporate hierarchical clustering could demonstrate that purported propagation patterns lack statistical robustness. Employing permutation-based enrichment analyses in transcriptomic datasets could show that many pathways highlighted by the authors are indistinguishable from background noise. Bayesian modeling approaches could further indicate that the posterior probability of replication is low given the modest effect sizes, high variability, and lack of structural confirmation. If sensitivity analyses were conducted on thresholding parameters for pathology quantification, it is likely that the observed regional differences would diminish or disappear altogether. Without transparent reporting, these alternative interpretations remain hypothetical, yet they underscore how fragile the authors' conclusions are when viewed through a rigorous quantitative lens. Because the central claim of *in vivo* replication rests on the integration of behavioral, biochemical, histological, and molecular datasets, any statistical instability in these components undermines the coherence of the entire mechanistic narrative. The cumulative weaknesses in statistical rigor therefore challenge not only the reliability of individual datasets but the overall credibility of the study's main conclusions.

10. Alternative Mechanisms and Competing Explanations

10.1. Inflammatory Amplification and Neurotoxic Cascades

One of the most plausible alternative explanations for the observed pathology in Burger *et al*.'s study[7] is inflammation-driven amplification of neurotoxic cascades rather than templated replication of α-synuclein fibrils. Injection of aggregated protein into the brain reliably induces a robust microglial and astrocytic

inflammatory response, irrespective of the specific protein involved. Activated microglia release pro-inflammatory cytokines, reactive oxygen species, and nitric oxide, all of which can stimulate endogenous kinases such as CK2 and PLK2 that phosphorylate α-synuclein at Ser129. These pathways can generate phosphorylated inclusions in neurons and glia even in the absence of fibrillar templating. In parallel, inflammatory stress impairs the ubiquitin-proteasome system and autophagic mechanisms required for clearing misfolded proteins. As a result, intracellular α-synuclein accumulates in a misfolded and phosphorylated state that mimics early inclusion formation. The spatial heterogeneity reported by the authors aligns with regions where inflammatory responses are likely most pronounced, including around the needle tract and along diffusion gradients. Furthermore, the time course of pathology matches the expected progression of inflammation-induced proteostatic failure rather than strain-driven replication. The study does not include pharmacological or genetic inhibition of inflammatory pathways, which would be essential to demonstrate that pathology is independent of inflammation. Taken together, the data more convincingly support an inflammation-centered mechanism than a prion-like propagation model.

10.2. Non-Fibrillar Contaminants as Potential Bioactive Agents

A second competing explanation arises from the possibility that the fibril preparations contain non-fibrillar contaminants that contribute to cellular stress and aggregate formation. Recombinant α-synuclein expressed in bacteria is prone to contamination by lipopolysaccharides and other bacterial components unless stringent purification steps are employed. Even trace amounts of endotoxin can activate microglia and trigger neuroinflammation at levels sufficient to induce phosphorylation and misfolding of endogenous proteins. In addition, partially folded oligomers or soluble aggregates that escape detection by TEM or Thioflavin-T assays can exert potent cellular toxicity distinct from that caused by mature fibrils. These species may penetrate cells more readily and disrupt membrane integrity, mitochondrial function, or vesicular trafficking. The study does not provide detailed endotoxin quantification, oligomer characterization, or mass spectrometry analyses to rule out these possibilities. Moreover, protein purification methods involving size-exclusion chromatography or ion-exchange steps are not described in sufficient detail to evaluate their effectiveness in eliminating contaminants. Without rigorous biochemical profiling, the biological effects observed could be driven not by fibrillar templating but by a mixture of toxic species with diverse bioactive properties. This scenario is consistent with the diffuse and inconsistent pathology patterns reported in the study, as mixed-species preparations produce variable responses across tissues and cell types.

10.3. Oligodendrocyte Stress Responses Independent of α-Synuclein Strains

Another alternative mechanism involves oligodendrocyte-specific stress responses that generate inclusion-like structures independent of α-synuclein strain propagation. Oligodendrocytes are uniquely vulnerable to metabolic and proteostatic disturbances due to their high demand for lipid synthesis, iron regulation, and ATP production. Exposure to exogenous protein aggregates can overload their proteostasis systems, leading to accumulation of endogenous α-synuclein and other misfolded proteins. The inclusions formed under these conditions do not necessarily require templated conversion or strain-specific replication. Instead, they arise through dysregulated chaperone function, impaired autophagy, or ER stress. The study does not demonstrate that the inclusions observed are composed predominantly of endogenous α-synuclein, nor does it show that they adopt the ultrastructural features characteristic of authentic glial cytoplasmic inclusions. Moreover, oligodendrocytes in mice express significantly lower levels of α-synuclein than human oligodendrocytes, making them more likely to generate inclusions through stress-induced accumulation rather than templated conversion. Transcriptomic and biochemical data presented by the authors show signatures of general proteostatic stress rather than strain-specific mechanisms. These findings support the possibility that oligodendrocyte pathology reflects a generic stress response rather than the replication of a pathogenic conformer.

10.4. Preexisting Vulnerabilities in Mouse Strains

A final competing explanation is that the pathology observed reflects preexisting vulnerabilities in the mouse strain used rather than replication of synthetic fibrils. Laboratory mouse strains often harbor genetic or epigenetic configurations that predispose them to heightened inflammatory responses, impaired clearing of exogenous proteins, or exaggerated phosphorylation of endogenous α-synuclein. Some strains exhibit subclinical myelin abnormalities, reduced mitochondrial resilience, or altered iron metabolism, all of which enhance susceptibility to proteostatic disturbances. The authors do not report baseline pathology or proteostasis markers in untreated control mice beyond superficial staining, leaving open the possibility that subtle vulnerabilities contributed to the rapid development of inclusion-like structures following injection. In addition, differences in animal handling, environmental enrichment, and diet can influence neuroinflammatory tone and proteostatic capacity. Without careful control of these factors, the model may amplify strain-independent pathological processes. The absence of knock-in or transgenic controls, including mice lacking α-synuclein or expressing humanized variants, further limits mechanistic interpretation. If endogenous α-synuclein is not

required for inclusion formation, this would strongly argue against templated replication. The study does not conduct such tests, leaving an important alternative explanation unaddressed. Ultimately, the observed pathology could reflect the intersection of fibril exposure with preexisting physiological vulnerabilities rather than a mechanistically coherent replication phenomenon.

11. Impact on α-Synuclein Biology and Neurodegeneration Research

11.1. Implications for the Strain Hypothesis of Synucleinopathies

The strain hypothesis in α-synuclein biology has generated both excitement and controversy, offering a conceptual framework that could explain the divergent phenotypes of Parkinson's disease, dementia with Lewy bodies, and MSA. If distinct conformational variants of α-synuclein are responsible for disease-specific pathologies, then identifying and characterizing these strains becomes central to understanding mechanisms and developing targeted therapeutics. Burger *et al*.[7] position their findings as evidence that synthetic fibrils can adopt and propagate an MSA-like strain, thereby supporting the strain hypothesis. However, the methodological and interpretive weaknesses documented earlier significantly weaken the study's contribution. Without demonstrating structural identity between injected fibrils and aggregates extracted from mouse tissue, the researchers cannot claim replication or strain fidelity. The lack of ultrastructural characterization and biochemical fingerprinting means that key mechanistic criteria remain unmet. As a result, the study risks muddying the conceptual landscape by presenting inconclusive or ambiguous observations as corroboration of strain behavior. Rather than advancing the field, the paper may reinforce misconceptions about how readily recombinant fibrils can mimic human disease conformers, potentially diverting attention away from rigorous structural studies essential for validating or refuting the strain hypothesis. The broader impact, therefore, is cautionary rather than confirmatory.

11.2. Potential Misalignment with Pathological Observations from Human MSA

A central concern in translating findings from mouse models to human synucleinopathies is the substantial divergence in pathological signatures. Human MSA is distinguished by glial cytoplasmic inclusions with highly ordered ultrastructure, widespread involvement of oligodendrocytes, and a multisystem pattern of degeneration. Burger *et al*.'s model, by contrast, yields sparse, focal inclusions lacking the morphology, density, and biochemical hallmarks of authentic

GCIs. The model does not replicate the iron dysregulation, lipid abnormalities, or oligodendroglial maturation defects characteristic of human disease. Furthermore, human MSA exhibits a progressive clinical course with pronounced autonomic dysfunction and cerebellar degeneration, which are not modeled in the reported mouse experiments. Therefore, the claim that synthetic fibrils induce "*MSA-like*" pathology risks conflating superficial similarities with mechanistic equivalence. If the field accepts such incomplete analogies, it may lower the standards for what constitutes a valid disease model, leading to overinterpretation of artificially induced phenotypes and misdirection of therapeutic development efforts. This misalignment between model and disease reinforces the need for careful restraint when extrapolating animal data to human pathology. The paper's impact on the MSA research community could be detrimental if its claims are taken at face value without critical evaluation.

11.3. Consequences for Therapeutic Development and Trial Rationales

Therapeutic strategies targeting α-synuclein aggregation are already being developed for Parkinson's disease and MSA, including monoclonal antibodies, antisense oligonucleotides, and small-molecule inhibitors. The mechanistic premise underlying these interventions depends heavily on the biological nature of pathogenic α-synuclein assemblies. If Burger *et al*.'s conclusions were accepted uncritically, they could encourage the assumption that synthetic fibrils behave similarly to human pathological strains, implying that therapeutic candidates validated in this mouse model would translate effectively to human disease. This is problematic for several reasons. First, the failure to demonstrate strain fidelity means that therapeutic responses in the model may not reflect efficacy against human MSA conformers. Second, the localized pathology and short experimental timelines do not reproduce the chronic, systemic progression of MSA, limiting the ability to test long-term therapeutic impacts. Third, inflammation-driven pathology in the mouse model may dominate therapeutic readouts, meaning that anti-inflammatory effects could be misinterpreted as anti-aggregative efficacy. Fourth, if non-fibrillar contaminants contribute to observed pathology, therapeutic targeting of α-synuclein could yield misleading results. The risk of generating erroneous preclinical conclusions is high. Therefore, prematurely integrating such a model into therapeutic pipelines could lead to costly missteps, wasted resources, and inappropriate prioritization of clinical trial candidates.

11.4. Broader Lessons for Model Construction in Proteinopathy Research

The limitations of Burger *et al*.'s study[7] highlight broader methodological and conceptual challenges in modeling neurodegenerative proteinopathies. The field has increasingly recognized that rapid aggregation induced by exogenous fibrils often reflects acute cellular stress rather than natural disease mechanisms. Without structural validation, careful quantification, and comprehensive controls, such models risk obscuring rather than illuminating pathogenic processes. The study underscores the need for transparent reporting, standardized experimental frameworks, and multi-tiered validation pipelines when constructing models of misfolded protein propagation. It also demonstrates the dangers of relying solely on phenotypic similarities—such as phosphorylated inclusions or subtle behavioral deficits—without verifying mechanistic correspondence. To advance the field, researchers must integrate high-resolution structural techniques, longitudinal multi-omics, and multiscale imaging to connect molecular events to cellular and systemic pathology. The study also serves as a reminder that disease models should emphasize physiological relevance rather than technical convenience. Ultimately, the broader lesson is that robust, mechanistically grounded models require methodological rigor, critical restraint, and a willingness to challenge appealing but unsupported narratives. Burger *et al*.'s paper, despite its limitations, reinforces these principles by illustrating the pitfalls that arise when claims outpace evidence.

12. Ethical, Scientific, and Community Considerations

12.1. Risks of Overstating Translational Significance

Scientific rigor requires that conclusions reflect the strength of the available evidence, yet the study by Burger *et al*.[7] risks overstating the translational significance of its findings by equating fibril-induced pathology in mice with human MSA. This raises ethical concerns because high-impact publications shape research priorities, therapeutic development pipelines, and public understanding of disease mechanisms. Presenting ambiguous or incomplete data as evidence of prion-like replication or strain-specific propagation may encourage researchers, clinicians, and stakeholders to adopt mechanistic frameworks that are insufficiently validated. Such premature conclusions can distort the direction of future research, funneling resources into models that lack predictive validity. For patients and advocacy communities, claims of mechanistic breakthroughs create expectations about imminent therapeutic advances that may not be justified. Ethical responsibility requires precision in describing what the data do and do not demonstrate. By failing to fully acknowledge the model's limitations, the authors risk contributing to hype-

driven narratives that undermine the credibility of translational neuroscience and divert attention away from more rigorous investigations of MSA biology.

12.2. The Importance of Transparency, Data Sharing, and Negative Data

A recurring issue throughout the study is the absence of comprehensive data transparency. The lack of raw imaging datasets, behavioral distributions, proteomic files, and sequencing quality-control metrics restricts the ability of other scientists to validate the findings. Ethical scientific practice requires sharing complete datasets so that claims of replication, propagation, or MSA-like pathology can be independently evaluated. In neurodegenerative disease research, where small effect sizes and biological variability are common, access to raw data is essential for distinguishing true biological signals from noise. Furthermore, the study does not report negative or inconclusive results, such as animals or regions that failed to develop pathology. Suppressing such outcomes hinders community-wide understanding of model limitations and inflates the apparent robustness of the findings. Negative or inconsistent results are crucial for contextualizing variability, refining models, and preventing misinterpretation. Without full transparency, the scientific community cannot properly assess the reproducibility or generalizability of the model. The ethical imperative extends beyond simple data availability: it includes clear documentation of analytic pipelines, thresholding parameters, replicate counts, and sampling rules. The omission of these details in Burger *et al.*'s study limits interpretive clarity and weakens the reliability of its conclusions.

12.3. Field-Wide Implications for Reproducibility and Standardization

The challenges illustrated by this study reflect broader concerns about reproducibility and standardization in protein propagation research. Many laboratories studying amyloidogenic proteins—including α-synuclein, tau, and TDP-43—have recognized that subtle methodological differences can dramatically influence aggregation kinetics, ultrastructure, and bioactivity. Without rigorous harmonization of protocols, claims of strain-specific behavior or prion-like replication are vulnerable to irreproducibility. Burger *et al.*'s study[7] highlights precisely this issue: the fibril preparation is insufficiently characterized, the injection parameters lack the spatial and quantitative precision needed for consistent outcomes, and the analytic frameworks for pathology quantification are opaque. If studies relying on incompletely validated fibrils and unstandardized experimental designs become influential, they risk establishing precedent for weakened methodological norms across the field. This contributes to a reproducibility crisis in which contradictory results arise not from meaningful

biological differences but from technical variability. The community must prioritize standardization of fibril production, endotoxin removal, oligomer quantification, and negative control inclusion. Moreover, adoption of field-wide reporting standards—analogous to CONSORT, ARRIVE, or MIAME guidelines—would improve transparency and foster replicability. Burger *et al*.'s study[7] underscores the need for such frameworks by demonstrating how fragmented or incomplete methodology erodes confidence in mechanistic claims.

12.4. Recommendations for Future Research Practices

The issues raised by this study offer valuable lessons for improving research practices in synucleinopathy and neurodegeneration research more broadly. First, any assertion of replication or strain fidelity must be grounded in high-resolution structural biology. Cryo-EM characterization of injected and recovered fibrils should be considered a minimum requirement for claims of templated propagation. Second, models must incorporate appropriate genetic controls, including α-synuclein knockout mice, humanized α-synuclein lines, and conditional glial expression systems, to disentangle endogenous aggregation from exogenous fibril persistence. Third, reproducible experimental pipelines require systematic documentation of injection accuracy, fibril dosing, endotoxin levels, and batch variability. Without such rigor, models cannot serve as reliable platforms for mechanistic or therapeutic studies. Fourth, the community should adopt more stringent criteria for labeling models as disease-relevant. Superficial similarities, such as the presence of phosphorylated inclusions, should not be equated with mechanistic recapitulation of human disorders. Instead, models must demonstrate multidimensional alignment with human pathology at structural, biochemical, cellular, and systems levels. Finally, collaboration between structural biologists, immunologists, neuroscientists, and clinical researchers is essential to ensure that model development is informed by the complexity of human disease rather than simplified technical assumptions. If implemented, these recommendations would help create more reliable and scientifically grounded models for the study of MSA and other synucleinopathies.

13. Conclusion

13.1. Summary of Core Methodological and Interpretive Weaknesses

The study by Burger *et al*.[7] presents an ambitious attempt to demonstrate *in vivo* replication of synthetic α-synuclein fibrils and to model MSA-like pathology in mice. However, the evidence offered for these claims is limited by significant methodological and interpretive shortcomings. The fibrils themselves are insufficiently characterized, lacking high-resolution structural validation, protease-resistance profiling, and quality-control measures needed to establish them as

distinct conformational strains. The histopathological analyses reveal sparse and inconsistent inclusions that could easily reflect exogenous fibril persistence, injection-related tissue injury, or inflammatory stress responses rather than templated propagation. Behavioral outcomes are modest, variable, and inadequately controlled, making them unreliable indicators of degenerative progression. Transcriptomic findings are dominated by nonspecific stress pathways and fail to identify molecular signatures aligned with human MSA. Most critically, the study does not provide evidence that aggregates recovered from mouse tissue share structural identity with the injected fibrils, an essential criterion for establishing replication. Taken together, the results are more plausibly explained by inflammation-driven proteostasis disruption and stress-induced aggregation of endogenous proteins than by prion-like propagation of synthetic α-synuclein strains.

13.2. Future Directions for Constructive and Rigorous Inquiry

Despite the limitations of the study, the broader area of synucleinopathy research stands to benefit from rigorous clarification of what distinguishes templated propagation from nonspecific aggregation. Future work should prioritize comprehensive structural analyses of both injected and tissue-recovered fibrils using cryo-EM, solid-state NMR, and biochemical fingerprinting. Experimental designs should incorporate genetic controls, including α-synuclein knockout and humanized knock-in mice, to determine whether endogenous α-synuclein is required for inclusion formation. Models must also be improved to reflect the physiological and anatomical contexts of human MSA, which involves complex oligodendroglial biology, iron dysregulation, systemic autonomic failure, and widespread multisystem degeneration. This necessitates longitudinal studies with refined pathology mapping, multi-omics integration, and behavioral assays capable of capturing cerebellar and autonomic dysfunction. Transparent reporting, sharing of raw datasets, and standardized methodologies will be essential for ensuring reproducibility and enabling consensus on mechanistic interpretations. If these improvements are adopted, the field can move toward developing models that truly facilitate mechanistic insight and therapeutic discovery.

13.3. Reframing the Synthetic Fibril Model within Proper Scientific Boundaries

The limitations identified throughout this commentary underscore the importance of framing synthetic fibril injection models within realistic and scientifically grounded boundaries. Such models can serve as useful tools for studying acute responses to proteotoxic stress, understanding glial and microglial signaling cascades, and evaluating general principles of protein uptake and intracellular

trafficking. However, they should not be assumed to recapitulate the full spectrum of human synucleinopathies, nor should their pathology be interpreted as evidence of strain-specific replication without rigorous structural and biochemical validation. The value of these models lies not in their ability to mimic human disease wholesale, but in providing controlled systems for probing defined mechanistic hypotheses under carefully constrained assumptions. Reframing their role in this manner will prevent misinterpretation of incomplete or ambiguous findings and encourage more precise scientific inquiries. By distinguishing what these models can and cannot reveal, the research community can better align expectations, avoid overstated claims, and foster more productive avenues for understanding α-synuclein biology and the pathogenesis of MSA.

Reference

1 Zahra, F. T., Kayani, H., Noreen, S., Noor, A. & Zafar, S. Synuclein proteoforms: Role in health and disease. *Mol Neurobiol* **63**, 87 (2025). https://doi.org/10.1007/s12035-025-05359-6

2 Jeyabalan, J. B. *et al.* Converging pathologies in neurodegeneration: the mechanistic interplay between alpha-Synuclein and Tau in Alzheimer's and Parkinson's. *Neurol Sci* **46**, 4779-4789 (2025). https://doi.org/10.1007/s10072-025-08421-2

3 Uytterhoeven, V., Verstreken, P. & Nachman, E. Synaptic sabotage: How Tau and α-synuclein undermine synaptic health. *J Cell Biol* **224** (2025). https://doi.org/10.1083/jcb.202409104

4 Absalyamova, M. *et al.* Molecular basis of the development of Parkinson's disease. *Neuroscience* **565**, 292-300 (2025). https://doi.org/10.1016/j.neuroscience.2024.12.009

5 Ho, H. H. & Wing, S. S. α-Synuclein ubiquitination - functions in proteostasis and development of Lewy bodies. *Front Mol Neurosci* **17**, 1498459 (2024). https://doi.org/10.3389/fnmol.2024.1498459

6 Zeng, H. *et al.* Interplay of α-synuclein oligomers and endoplasmic reticulum stress in Parkinson's disease: Insights into cellular dysfunctions. *Inflammation* **48**, 1590-1606 (2025). https://doi.org/10.1007/s10753-024-02156-6

7 Burger, D. *et al.* Synthetic α-synuclein fibrils replicate in mice causing MSA-like pathology. *Nature* (2025). https://doi.org/10.1038/s41586-025-09698-1