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Review

A Summary of Phenotypes Observed in the *In Vivo* Rodent Alpha-Synuclein Preformed Fibril Model

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Abstract.The use of wildtype recombinant alpha-synuclein preformed fibrils (aSyn PFFs) to induce endogenous alpha-synuclein to form pathological phosphorylation and trigger neurodegeneration is a popular model for studying Parkinson's disease (PD) biology and testing therapeutic strategies. The strengths of this model lie in its ability to recapitulate the phosphorylation/aggregation of aSyn and nigrostriatal degeneration seen in PD, as well as its suitability for studying the progressive nature of PD and the spread of aSyn pathology. Although the model is commonly used and has been adopted by many labs, variability in observed phenotypes exists. Here we provide summaries of the study design and reported phenotypes from published reports characterizing the aSyn PFF *in vivo* model in rodents following injection into the brain, gut, muscle, vein, peritoneum, and eye. These summaries are designed to facilitate an introduction to the use of aSyn PFFs to generate a rodent model of PD—highlighting phenotypes observed in papers that set out to thoroughly characterize the model. This information will hopefully improve the understanding of this model and clarify when the aSyn PFF model may be an appropriate choice for one's research.

Keywords: Alpha-synuclein, Parkinson disease, preformed fibril, model

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder affecting approximately 1% of the population over the age of 60. Characterized by motor disturbances as well as non-motor symptoms, the pathology of PD involves deposits of aggregated, phosphorylated alpha-synuclein (aSyn) protein in affected tissues and brain structures and degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Given that PD is a human-specific condition, various models have been developed to enable research and therapeutic development for this disease. Common models include injection of neurotoxins to trigger degeneration of the dopaminergic neurons of the SNpc, transgenic rodent models carrying PD-related genetic mutations, and induction of aSyn pathology through viral vector-mediated overexpression of aSyn, among others [1–3]. All models present with advantages and disadvantages, so selection of the model should be based on the desired pathology for the intended research question.

In the last 10 years, a model has arisen that capitalizes on the observations made by Braak and colleagues that aSyn pathology progressively accumulates in different brain regions following a spatiotemporal pattern that suggests spreading [4–7]. This model, dubbed the aSyn preformed fibril (PFF) model, uses injection of recombinant aSyn protein that has been stimulated to form aggregates and son-

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icated to produce short fibrils [8–10]. These aSyn PFFs cause templating of endogenous aSyn into pathological species characterized by phosphorylation at S129 (pS129 aSyn), beta-sheet formation, and aggregation, followed by increases in autophagy and neuronal dysfunction [11]. The flexibility of this model allows injection of different forms of aSyn PFFs (e.g., mouse vs. human aSyn, mutated aSyn), unilateral or bilateral injection, targeting of different brain regions and administration through different peripheral routes to model distinct aspects of the disease. This flexibility is a strength of the model but also serves as a weakness, as the distinct protocols lead to different pathologies which has hampered cross-study comparisons. To better understand the various study designs employed for the aSyn PFF model and the resulting pathologies, a survey of the literature was performed and is summarized within this manuscript.

GUIDE TO READING AND INTERPRETING THE TABLES

As hundreds of studies using the aSyn PFF model have been published, Tables 1–9 herein contain information specifically from publications that sought to phenotype the effects of injection of recombinant wildtype aSyn PFFs into rodents to develop a PD model. As a result, the tables are not comprehensive in nature but do contain reports from a variety of studies across laboratories.

Studies focusing on the uptake of aSyn following injection have been excluded as the study is not designed to thoroughly assess resulting pathology. Studies using the aSyn PFF model to test the effect of an intervention have been excluded as the focus is on the therapeutic intervention tested rather than the characterization of the pathological process and timelines. Studies injecting aSyn PFFs to model another disease (e.g., Multiple System Atrophy) were excluded to focus specifically on PD. Studies injecting aSyn PFFs into non-human primates or using aSyn PFFs in cell culture were excluded for the sake of focus. Studies injecting rodent/patient brain-derived material were excluded due to concerns that the injectate is not homogenous and the concentration of aSyn and other protein components cannot be known or compared across studies. Although a number of studies have been published analyzing the differences in pathogenicity of fibrils of different conformations [12–18], different aSyn mutations [19–22], different aSyn truncations [23–25], and different aSyn post-translational modifications [26], these were excluded from the summary tables as the objective of these experiments is to compare pathogenicity relative to wildtype aSyn PFFs and therefore the nuanced information requires a different venue.

Tables 1–9 are organized by categories such as: injected species (mouse vs. rat), route of administration of aSyn PFFs, and species of aSyn PFF (human vs. mouse). To understand the variation in observed phenotypes within the model, readers should compare only within categories rather than across categories. Please note that there may be differences in study design within categories (e.g., unilateral vs. bilateral injection, wildtype vs. transgenic rodent) that should be taken into account when drawing conclusions on timelines and robustness of phenotypes.

Papers included within the tables are organized chronologically, with high-level information on study design, outcome measures, and notes that may provide additional context for the reader. Information on study design includes the rodent strain used, the injectate, the dose of aSyn PFFs with information on whether this dose was administered bilaterally or unilaterally (for bilateral injections, the total dose noted was for each hemisphere), and the days post-injection (DPI) at which time the model was analyzed. Reported phenotypes are separated by category to facilitate comparisons of common readouts across studies. The time post-injection at which the phenotype was observed is included, with a "+" indicating the phenotype was also observed at the later timepoints. If later timepoints were analyzed within the study but the "+" sign is absent, this indicates that either the phenotype was not analyzed at the later timepoints or was analyzed but not observed. If a phenotype was observed in a particular structure, the structure is included in parentheses. Readouts that were not included in the study are denoted as "N/A". Please note, to fully understand all reported or absent phenotypes in the models, a separate literature review is required.

SUMMARY OF PHENOTYPES REPORTED IN THE ASYN PFF MODEL

The earliest aSyn PFF model studies were performed by injecting aSyn PFFs into the mouse striatum. Table 1 provides a summary of studies that used unilateral or bilateral intrastriatal injection of

Table 1
Injection of mouse aSyn PFFs into the wildtype mouse striatum

	Unilateral Mouse aSyn PFFs								Bilateral Mouse aSyn PFFs		
Paper	**Luk 2012 [27]**	**Masuda-Suzukake 2014 [28]**	**Luk 2016 [19]**	**Fares 2016 [29]**	**Henderson 2019 [30]**	**Izco 2020 [31]**	**Burtscher 2020 [32]**	**Kim 2020 [33]**	**Sorrentino 2017 [34]**	**Stoyka 2020 [35]**	**Ding 2021 [36]**
Rodent Strain	C57Bl6/C3H	C57Bl/6J	C57Bl6/C3H	C57Bl6/C3H	C57Bl/6J	C57BL6/C3H	C57BL/6JRj	C57Bl/6	Unspecified	C57Bl/6J	C57BL/6J
Injectate	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn
Total Dose	5 ug (Unilateral)	10 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	4 ug (Bilateral)	2ul of 300uM (Bilateral)	Unspecified (Bilateral)
DPI	30, 90, 180	30, 90	14, 30, 90, 180	30	30, 90, 180	15, 30, 90	30, 60, 180	30	120	90, 180	30, 90, 180, 270
pS129 aSyn	30+	30	14+	30	30+	15+	30+	30	120	90+	30+
Striatal TH Loss / DA Deficits	90+	N/A	N/A	N/A	N/A	90	N/A	30	N/A	N/A	N/A
SNpc TH+ Cell Loss	90+	N/A	180	N/A	90+	Absent	N/A	N/A	Absent	N/A	N/A
Behavioral Deficits	Motor - 180	•Motor - 90 •Cognitive - Absent	Motor - 180	N/A	90+	Motor - 90	N/A	Absent	N/A	•Motor - Absent •Anxiety - Absent •Memory - 180 •Social - 180	•Motor - 180 •Memory - 180
Immune Response	N/A	N/A	N/A	N/A	N/A	•Microglia Activation (SNpc) - 15, 90 •Astrogliosis (SNpc, STR) - 15+ •Cytokines (SNpc, STR) - 15, 90	N/A	•Microglia Activation - Absent •Astrogliosis - Absent	•Microglia Activation - Absent •Astrogliosis - Absent	N/A	•Macrophages with pS129 aSyn (Meninges) - 180+ •Cytokines (Meninges) - 180+
Other	N/A	•Aggregated aSyn - 90 •Tau Pathology - 30 •TDP-43 Pathology - 30	Aggregated aSyn - 90	Ubiquitinated Inclusions - 30	N/A	N/A	•TOMM20 in Inclusions (AMY) - 60 •VDAC1 Change - Absent •Mitochondrial Respirometry Deficits - Absent •ROS - Absent	N/A	N/A	Cell Loss (AMY) - Absent	•Aggregated aSyn - 180 •Decreased Meningeal Lymphatic Drainage - 180+ •Loss of Meningeal Tight Junctions - 180+
Note	CD1 and Bl6/SJL rodent strains also assessed			Injected human aSyn PFFs and saw little pathology.	Analyzes spread patterns and regional vulnerabilities				Pathology absent with injection of human aSyn PFFs		Pathology worse when meningeal lymphatic drainage blocked

aSyn, alpha-synuclein; PFFs, preformed fibrils; TH, tyrosine hydroxylase; DA, dopamine; N/A, not analyzed; SNpc, substantia nigra pars compacta; STR, striatum; AMY, amygdala; ROS, reactive oxygen species.

Table 2
Unilateral injection of human aSyn PFFs into the wildtype mouse striatum

	Human aSyn PFFs		
Paper	**Luk 2016 [19]**	**Fares 2016 [29]**	**Milanese 2018 [37]**
Rodent Strain	C57Bl6/C3H	C57Bl6/C3H	C57Bl/6
Injectate	Human aSyn	Human aSyn	Human aSyn
Total Dose	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)
DPI	14, 30, 90, 180	30	120
pS129 aSyn	30+	30	120
Striatal TH Loss / DA Deficits	N/A	N/A	120
SNpc TH+ Cell Loss	Absent	N/A	120
Behavioral Deficits	Absent	N/A	N/A
Immune Response	N/A	N/A	N/A
Other	Aggregated aSyn - 90	N/A	DNA Damage (SN) - 120
Note	Also tested chimeric human-mouse aSyn PFFs. Homology to mouse aSyn increased pathology	Injected mouse aSyn PFFs. Pathology with mouse aSyn PFFs greater than human aSyn PFFs.	

aSyn, alpha-synuclein; PFFs, preformed fibrils; TH, tyrosine hydroxylase; DA, dopamine; N/A, not analyzed; SNpc, substantia nigra pars compact.

Table 3
Unilateral and bilateral injection of aSyn PFFs into transgenic mouse striatum

	Synuclein Overexpression				Other			
Paper	**Luk 2012 [23]**	**Sorrentino 2017 [34]**		**Earls 2020 [38]**	**Blumenstock 2017 [39]**	**Henderson 2019 [30]**	**Bieri 2019 [40]**	**Migdalska-Richards 2020 [41]**
Rodent Strain	M83 A53T Hu aSyn	M20 WT Hu aSyn		M83 A53T Hu aSyn	Thy-1 eGFP	BAC LRRK2 G2019S	BAC LRRK2 G2019S	GBA L444P KI
Injectate	Human aSyn	Human aSyn	Mouse aSyn	Human aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn
Total Dose	5 ug (Unilateral)	4 ug (Bilateral)	4 ug (Bilateral)	5 ug (Unilateral)	25 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)
DPI	90	120	120	70	30, 60, 90, 150, 270	30, 90, 180	30, 90, 180	120
pS129 aSyn	90	120	120	70	30+	30+	30+	120
Striatal TH Loss / DA Deficits	N/A	N/A	N/A	Absent	N/A	N/A	N/A	N/A
SNpc DA Cell Loss	N/A	120	120	Absent	N/A	90+	180	N/A
Behavioral Deficits	N/A	N/A	N/A	Motor - 70	N/A	Motor - 90+	Motor - 180	N/A
Immune Response	N/A	•Microglia Activation (CPu injection) - 120 •Astrogliosis (CPu injection) - 120 •pS129 aSyn in Astrocytes - 120	•Microglia Activation - Absent •Astrogliosis - Absent	•Microglia Activation - 70 •NK cell infiltration (SNpc) - 70	Microglial Activation - 150 (variable)	N/A	Microglia Activation - 180	N/A
Other	N/A	N/A	N/A	Aggregated aSyn - 70	•Ubiquitin and ThioS Inclusions - 150 •Dendrite Malformations (CTX) - 150 •Dendritic Spine Loss (CTX) - 150	N/A		N/A
Note	Also injected truncated (AA1-120) human aSyn PFFs	Tested different injection coordinates		NK cell depletion worsened pathology		Pathology in LRRK2 G2019S mouse worse than WT	Pathology in LRRK2 G2019S mouse worse than WT	Pathology in GBA L444P mouse worse than WT

aSyn, alpha-synuclein; PFFs, preformed fibrils; Hu, human; TH, tyrosine hydroxylase; DA, dopamine; N/A, not analyzed; SNpc, substantia nigra pars compacta; CPu, caudate putamen; CTX, cortex.

Table 4
Unilateral and bilateral injection of aSyn PFFs into the wildtype and transgenic mouse olfactory bulb or sublaterodorsal tegmental nucleus

	Olfactory Bulb								Sublaterodorsal Tegmental Nucleus (SLD)
Paper	**Rey 2016 [42]**		**Rey 2018 [43]**		**Graham 2018 [44]**	**Kulkarni 2020 [45]**	**Uemura 2021 [46]**		**Shen 2020 [47]**
Rodent Strain	C57Bl/6	C57Bl/6	C57Bl/6J	C57Bl/6J	C57Bl/6	C57BL/6J	C57BL/6J	BAC Hu A53T aSyn Mouse	C57Bl/6
Injectate	Mouse aSyn	Human aSyn	Mouse aSyn	Human aSyn	Human aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Atto 488-labeled human aSyn
Total Dose	4 ug (Unilateral)	4 ug (Unilateral)	4 ug (Unilateral)	4 ug (Unilateral)	4 ug (Unilateral)	Unclear	2.5 ug (Bilateral)	2.5 ug (Bilateral)	Unclear
DPI	30, 90, 180, 360	30, 90, 180, 360	270, 540, 690	270, 540, 690	90	30, 60, 90	60	60, 180, 210, 240, 300	30, 60, 90, 150, 240
pS129 aSyn	30+	30+	270	270+	N/A	30, 60, 90	60	60+	30+
Cell Loss	Absent (OB)	Absent (OB)	180 (AON) Absent (OB)	180 (AON) Absent (OB)	N/A	N/A	N/A	300 (HPC)	30+ (SLD) 90+ (SNpc)
Behavioral Deficits	•Motor - Absent •Anxiety - Absent •Olfactory - 30	•Motor - Absent •Anxiety - Absent •Olfactory - 30	N/A	N/A	N/A	N/A	N/A	•Memory - 240 •Olfactory - 60 •Anxiety - 210	•Motor - 150+ •Olfactory - 150+ •GI - 150+ •RBD - 30+
Immune Response	•Microglia Activation - Absent	•Microglia Activation - Absent	N/A	N/A	N/A	N/A	N/A	•Microglia Activation - 180+ •Astrogliosis - 180+	N/A
Other	•Aggregated aSyn - 30+ •Tau pathology - Absent	•Aggregated aSyn - 30+ •Tau pathology - Absent	•Tau pathology - Absent •TDP-43 pathology - Absent	•Tau pathology - Absent •TDP-43 pathology - Absent	Phospholipid alterations (brain, serum) - 90	•Spontaneous LFP activity changes - absent •Odor-evoked beta band increase - 30+	Aggregated aSyn - 60	Aggregated aSyn - 60+	•Striatal TH+ fiber loss - 30+ •Striatal DA deficits - 90+
Note	Mouse aSyn PFFs induced more pathology than human aSyn PFFs		pS129 aSyn pathology decreased after 18 months	pS129 aSyn pathology remained static after 12 months	Pooled injected & uninjected hemisphere and all brain structures				

aSyn, alpha-synuclein; PFFs, preformed fibrils; Hu, human; N/A – not analyzed; OB, olfactory bulb; AON, accessory olfactory nucleus; HPC, hippocampus; SLD, subdorsolateral tegmental nucleus; SNpc, substantia nigra pars compacta; GI, gastrointestinal; RBD, REM sleep behavior disorder; LFP, local field potential; TH, tyrosine hydroxylase; DA, dopamine.

Table 5
Unilateral or bilateral injection of aSyn PFFs into the wildtype or transgenic mouse hippocampus, cortex, or substantia nigra

	Hippocampus				Cortex			Substantia Nigra	
Paper	**Rutherford 2017 [20]**	**Luna 2018 [48]**	**Nouraei 2019 [49]**	**Caputo 2020 [50]**	**Masuda-Suzukake 2014 [28]**	**Osterberg 2015 [51]**	**Schaser 2020 [52]**	**Masuda-Suzukake 2013 [53]**	**Masuda-Suzukake 2014 [28]**
Rodent Strain	M20 WT Hu aSyn	C57Bl6/C3H	CD1	GFP-tagged *SNCA* KI	C57Bl/6J	GFP-tagged Hu *SNCA*	GFP-tagged Hu A53T *SNCA*	C57Bl/6J	C57Bl/6J
Injectate	Human aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Human aSyn	Mouse aSyn
Total Dose	4 ug (Bilateral)	5 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	10 ug (Unilateral)	5 ug (Unilateral)	5 ug (Unilateral)	10 ug (Unilateral)	10 ug (Unilateral)
DPI	60, 120	45, 90	90	30	30, 90	30. 90, 210	10, 20, 30, 50	90, 180, 450	30, 90
pS129 aSyn	60+	45+	90	30	30	30+	10+	90+	30
Cell Loss	N/A	45, 90	Absent (HPC)	N/A	N/A	210	30+ (CTX)	Absent (SN)	N/A
Behavioral Deficits	N/A	N/A	•Memory - Absent •Olfactory - Absent	N/A	•Motor - Absent •Cognitive - Absent	N/A	N/A	Motor - Absent	•Motor - 90 •Cognitive - Absent
Immune Response	N/A	N/A	N/A	N/A	N/A	N/A	N/A	•Microglial Activation - Absent •Astrogliosis - Absent	N/A
Other	N/A	N/A	•Synaptophysin increase (CA2/CA3) - 90 •Ubiquitin and ThioS inclusions - 90	N/A	•Tau pathology - 30 •TDP-43 pathology - 90 •Aggregated aSyn - 90	Ubiquitinated inclusions - 90	pS129 aSyn in GFAP+ cells - 50+	•Aggregated aSyn - 90 •Ubiquitin and p62 inclusions - 450	•Tau pathology - Absent •TDP-43 pathology - Absent •Aggregated aSyn - 90
Note	Also used mutant aSyn PFFs	MATH2+ and CTIP2+ cells more susceptible than Prox1+		pS129 aSyn pathology reduced with GFP dosing		Performed in vivo imaging for longitudinal assessments	Performed in vivo imaging for longitudinal assessments	Injection of mouse aSyn PFFs resulted in similar pS129 aSyn	

aSyn, alpha-synuclein; PFFs, preformed fibrils; Hu, human; N/A, not analyzed; KI, knockin; HPC, hippocampus; CTX, cortex; SN, substantia nigra.

mouse aSyn PFFs in wildtype (WT) mice. Table 2 provides a summary of studies that used intrastriatal injection of human aSyn PFFs in WT mice. Table 3 provides a summary of studies that used intrastriatal injection in transgenic mice.

Others have chosen to inject non-striatal brain regions to model prodromal or non-motor features of PD in mice. Table 4 provides a summary of studies injecting aSyn PFFs into the olfactory bulb (OB) or sublaterodorsal tegmental nucleus (SLD) to

Table 6
Unilateral or bilateral injection of aSyn PFFs into the wildtype or knockout rat striatum or substantia nigra

	Striatum						SNpc	
Paper	**Paumier 2015 [54]**	**Duffy 2018 [55]**	**Patterson 2019 [56]**		**Thomsen 2021 [57]**	**Creed 2020 [58]**	**Thakur 2017 [59]**	**Harms 2017 [60]**
Rodent Strain	Sprague Dawley	Fischer 344	Fischer 344	Fischer 344	Sprague Dawley	WT and PINK1 KO Rat	Sprague Dawley	Sprague Dawley
Injectate	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Human aSyn	Mouse aSyn
Total Dose	8 ug (Unilateral)	8ug (Unilateral)	8ug or 16 ug (Unilateral)	16 ug (Bilateral)	8 ug (Unilateral)	20 ug (Unilateral)	Unclear	8 ug (Unilateral)
DPI	30, 60, 90, 180	14, 30, 60, 90, 120, 150, 180	60, 120, 180	180	45, 60, 100, 120	14, 30	10, 20, 90, 180	30, 60, 90, 180
pS129 aSyn	30+	30+	60+	180	45+	14+	10+	30+ (SN) 60+ (STR)
Striatal TH Loss / DA Deficits	•Neurochemistry deficits - 180 •TH increase - 30 •TH decrease - 60+	N/A	TH decrease - 60+	N/A	•TH decrease - 45+ •VMAT decrease - 45+ •DAT decrease - 100+	Absent	TH decrease - 90+	TH decrease - 90+
SNpc DA Cell Loss	180	150+	120+ (16ug) 180 (8ug)	180	100+	WT - Absent PINK1 KO - 30	20+	90+
Behavioral Deficits	•Motor - Absent •Vocalizations - 180	N/A	•Motor (8ug) - N/A •Motor (16ug) - Absent	Motor - 180	Sensorimotor - 60 (absent 120)	N/A	Motor - Absent	N/A
Immune Response	N/A	Microglia Activation - 60+	N/A	N/A	•Microglia Activation (SN) - 45, 100 •Microglia Activation (STR) - 100	N/A	Microgliosis – 10 (absent 90, 180)	•Microglia Activation (SN) - 30+ •Microglia Activation (STR) - 180 •Monocyte infiltration (SN, STR) - 60 •T cell infiltration (SN) - 60
Other		aSyn Aggregates - 30+	aSyn Aggregates - 30+	N/A	aSyn Aggregates - 45+	aSyn Aggregates - 14+	N/A	N/A
Note	TH phenotype in SNpc downregulated before neuronal loss	Reactive microglia associated with pS129 aSyn but reduces during degeneration	More pathology with 16ug vs 8ug. Pathology more severe with bilateral PFF injection.			Pathology in PINK1 KO worse than WT	TH phenotype in SNpc downregulated before neuronal loss; Pathology worse with AAV aSyn addition	

aSyn, alpha-synuclein; PFFs, preformed fibrils; SNpc, substantia nigra pars compacta; WT, wildtype; KO, knockout; TH, tyrosine hydroxylase; DA, dopamine; VMAT, vesicular monoamine transporter; DAT, dopamine transporter; STR, striatum.

model olfactory dysfunction and sleep disturbances, respectively. Table 5 provides a summary of studies injecting aSyn PFFs into the hippocampus, cortex, and SNpc as alternate ways to induce pathology in the mouse.

Although most studies to date have focused on phenotyping mice injected with aSyn PFFs, rats have also been used for this model. Table 6 provides a summary of studies injecting aSyn PFFs into the rat striatum or SNpc.

In addition, both mice and rats have been used for peripheral administration of aSyn PFFs to study the seeding capabilities of aSyn PFFs and peripheral-to-central spread of synuclein pathology. Table 7 provides a summary of studies injecting aSyn PFFs into the gut of rodents to model GI dysfunction and gut-to-brain transmissibility of aSyn pathology. Finally, Table 8 provides a summary of studies performing intramuscular injections of aSyn PFFs into rodents and Table 9 provides a summary of studies performing intraperitoneal, intravenous, intraneural, and intravitreal injection of aSyn PFFs into rodents.

A visual representation of timelines of phenotypes reported in common iterations of the aSyn PFF model is provided in Fig. 1. Replicated phenotypes that have been reported in more than one study are provided along the timeline of the model. Phenotypes that were only investigated in one study are also included but denoted as "underexplored phenotypes". An inset containing phenotypes that were reported as absent is also included.

DISCUSSION

For all studies, one of the earliest phenotypes reported is the presence of pS129 aSyn within brain regions innervating the injected structure. As the model progresses, the density of pS129 aSyn pathology and regions displaying pS129 aSyn pathology increase. This pathology is at times accompanied by cell loss, inflammation, behavioral deficits, and/or other readouts of pathology. Importantly, the phenotypes observed in this model are not always reproducible and their presence/absence varies between studies (Fig. 1). This can be noted

Table 7
Injection of aSyn PFFs into the wildtype or transgenic rodent gut

	Mouse						Rat				
Paper	**Uemura 2018 [61]**	**Kim 2019 [62]**	**Uemura 2020 [63]**	**Challis 2020 [64]**		**Wang 2020 [65]**	**Holmqvist 2014 [66]**	**Manfredsson 2018 [67]**	**Van Den Berge 2019 [68]**		**Van Den Berge 2021 [69]**
Rodent Strain	C57Bl/J	C57Bl/J	BAC Hu A53T aSyn	C57Bl/N (2 month)	C57Bl/N (16 month)	M83 A53T Hu aSyn	Sprague Dawley	Sprague Dawley	Sprague Dawley	BAC Hu WT aSyn	Fischer 344
Injectate	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Human aSyn	Human aSyn	Mouse aSyn	Human aSyn	Human aSyn	Mouse aSyn
Injection Site	Gastric Wall (unclear location)	Pylorus & Duodenum (gastric wall)	Pylorus & Stomach (gastric wall)	Duodenum (gastric wall)	Duodenum (gastric wall)	Stellate ganglia, Celiac ganglia	Stomach & Duodenum (gastric wall)	Descending Colon (gastric wall)	Pylorus & Duodenum (gastric wall)	Pylorus & Duodenum (gastric wall)	Pylorus & Duodenum (gastric wall)
Total Dose	48ug (over 8 sites)	25ug (over 2 sites)	48ug (over 8 sites)	6ug (over 2 sites)	6ug (over 2 sites)	11ug (over 4 sites)	15ug (over 5 sites)	60ug (over 6 sites)	18ug (over 6 sites)	18ug (over 6 sites)	60ug (over 6 sites)
DPI	23, 45, 180	30, 90, 210, 300	30, 60, 120, 180, 240	7, 21, 60, 120	7, 21, 60, 120	30, 60, 90, 120, 150, 180, 210	0.5, 1, 2, 3, 6	30, 180, 360	60, 120	60, 120	60
Peripheral/ Spinal Cord pS129 aSyn	45+ (DMV) 180 (MG)	30+ (DMV)	30, 60 (DMV, MG) Absent in duodenum and SC	60 (MG) Absent (DMV)	120 (DMV)	30+ (SC) 60+ (GI, skin, heart, sweat gland)	2+ (vagal nerve) 6 (DMV)	30+ (MG) 30 (DMV)	Absent	120 (Stomach, DMV, heart)	60 (MG, DMV, SC, heart, muscle, kidney)
CNS pS129 aSyn	Absent	30+ (Brainstem) 90+ (Midbrain) 210+ (Forebrain)	120 (Brainstem) Absent in Midbrain and Forebrain	Absent (Midbrain)	120 (Midbrain)	30+ (Brainstem, Midbrain)	Absent	30 (Brainstem)	Absent	120 (Brainstem, Midbrain)	60 (Brainstem, Midbrain, Forebrain)
Cell Loss	N/A	SNpc DA - 210+	N/A	Absent (MG)	Absent (SN)	N/A	Absent	Absent	N/A	N/A	N.A
Behavioral Deficits	N/A	•Motor - 210+ •Cognitive - 210+ •Psychiatric - 210+ •Olfactory - 300	N/A	•Motor - 60, 90 •GI - 60+	•Motor - 120 •GI - 120	•GI - 90+ •Olfactory - 90+ •Orthostatic hypotension - 90+ •Hypohidrosis - 90+	N/A	N/A	N/A	N/A	N/A
Other	Nitrated aSyn (DMV) - 45		•Nitrated aSyn (DMV, MG) - 30, 60 •Ubiquitin and p62 (DMV, MG) - 30, 60	•GI inflammation - 7+, •GCase Decrease (duodenum) - 7 •Altered GI network - 7+ •Striatal DA deficits - Absent	Striatal DA deficits - 120	Death - 150+	N/A	N/A	N/A	N/A	N/A
Note	Vagotomy prevented pathology in DMV but reduced neurons by 20-40%	No brain pathology with vagotomy or aSyn KO		AAV-PHP.S GCase overexpression reduced pathology				aSyn pathology was equal in PFF & monomer groups			Rats also injected with human S129A aSyn PFFs; pathology worse with age. Pathology with mouse PFFs greater than human PFFs.

aSyn, alpha-synuclein; PFFs, preformed fibrils; Hu, human; DMV, dorsal motor nucleus of the vagus; MG, myenteric ganglia; SC, spinal cord; GI, gastrointestinal system; CNS, central nervous system; SNpc, substantia nigra pars compacta; DA, dopamine; KO, knockout.

Table 8
Unilateral or bilateral injection of aSyn PFFs into the transgenic mouse muscle

	Intramuscular					
Paper	**Rutherford 2017 [20]**	**Ayers 2017 [70]**	**Sorrentino 2018 [71]**	**Schaser 2020 [52]**	**Chu 2020 [72]**	**Ferreira 2021 [73]**
Rodent Strain	M83 A53T Hu aSyn Mouse	M83 A53T Hu aSyn Mouse	M83 A53T Hu aSyn Mouse	GFP-tagged A53T *SNCA* KI Mouse	M83 A53T Hu aSyn Mouse	M83 A53T Hu aSyn Mouse
Injectate	Human aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn
Total Dose	10 ug (Bilateral)	20 ug (Bilateral)	10 ug (Bilateral)	10 ug (Unilateral)	10 ug (Bilateral)	20 ug (Bilateral)
DPI	126-160	134	30, 60, 90	120, 240	30, 90, 117	45
Peripheral/ Spinal Cord pS129 aSyn	126+ (SC)	134 (SC)	60+ (SC)	N/A	N/A	45 (DRG, SC)
CNS pS129 aSyn	126+ (Brainstem, Midbrain, Forebrain)	134 (Brainstem, Midbrain, Forebrain)	60+ (Brainstem, Midbrain) 90 (Forebrain)	120 (Brainstem) 240 (Brainstem, Midbrain, Forebrain)	N/A	45 (Brainstem, Forebrain)
Cell Loss	N/A	N/A	60+ (Motor Neurons)	N/A	N/A	N/A
Behavioral Deficits	Paralysis - 126+	Motor - 134	N/A	N/A	Paralysis - 117	•Motor - 45 •Sensory - 45
Other	Death - 160	N/A	•Ubiquitin and p62 Inclusions - 60+ •Astrogliosis - 90 •Microglial Activation - 90	N/A	•Brain Microstructure Changes - 30+ •Reduced Sensory Activation - 90 •Reduced Spontaneous Brain Activity - 30	Astrogliosis (SC, Brainstem, Forebrain) - 45
Note	Also looked at mutant aSyn PFFs		Tested lower doses as well		dMRI and fMRI performed	

aSyn, alpha-synuclein; PFFs, preformed fibrils; Hu, human; KI, knockin; SC, spinal cord; DRG, dorsal root ganglia; CNS, central nervous system; dMRI, diffusion magnetic resonance imaging; fMRI, functional magnetic resonance imaging.

Table 9
Injection of aSyn PFFs into the wildtype or transgenic rodent peritoneum, vein, nerve, or eye

	Intraperitoneal		Intravenous		Nerve			Intravitreal
Paper	**Ayers 2017 [70]**		**Ayers 2017 [70]**	**Kuan 2021 [74]**	**Ayers 2018 [75]**			**Veys 2020 [76]**
Rodent Strain	M83 A53T Hu aSyn Mouse	M20 WT Hu aSyn Mouse	M83 A53T Hu aSyn Mouse	Sprague Dawley Rats	C57Bl/6 Mouse	M83 A53T Hu aSyn Mouse	M20 WT Hu aSyn Mouse	C57Bl/6 Mouse
Injectate	Mouse aSyn	Mouse aSyn	Mouse aSyn	Human aSyn	Mouse aSyn	Mouse aSyn	Mouse aSyn	Human aSyn
Total Dose	50 ug	50 ug	20 ug	100 ug	4 ug	4 ug	4 ug	5 ug
DPI	180	350	120	180	360	30, 60, 120	60, 120, 240	90, 150
Peripheral/ Spinal Cord pS129 aSyn	180 (SC)	350 (SC)	120 (SC)	Absent	Absent	30+ (DRG, SC)	60+ (SC) 120 (DRG)	Absent
CNS pS129 aSyn	180 (Brainstem, Midbrain, Forebrain)	350 (Brainstem)	120 (Brainstem, Midbrain, Forebrain)	Absent	Absent	60+ (Brainstem) 120 (Forebrain)	120+ (Brainstem) Absent (Forebrain)	Absent
Cell Loss	N/A	N/A	N/A	Absent	N/A	N/A	N/A	N/A
Behavioral Deficits	Motor - 180 (only in half of the cohort)	Motor - Absent	Motor - Absent	•Motor - Absent •GI - Absent •Olfactory - Absent	Absent	Paralysis - 120	Paralysis - 240	N/A
Other	N/A	N/A	N/A	N/A	N/A	Astrogliosis and Microgliosis - 60+ (SC)	Astrogliosis and Microgliosis - 120+ (SC)	N/A
Note								

aSyn, alpha-synuclein; PFFs, preformed fibrils; Hu, human; SC, spinal cord; DRG, dorsal root ganglia; CNS, central nervous system; GI, gastrointestinal.

when analyzing the phenotypes listed in Tables 1–9 when comparing studies of similar designs with regard to injection site, unilateral vs bilateral injection, wildtype vs transgenic rodent, etc.

An example of this can be found in motor deficits observed following intrastriatal injection. Despite using the same dose of aSyn PFFs, some report motor deficits following unilateral intrastriatal injection of mouse aSyn PFFs as early as 90 DPI [28, 30, 31] while others do not observe motor impairments until 180 DPI [19, 27] (Table 1). Others still do not observe motor impairments even at 180 days

Fig. 1. Visual representation of the various phenotypes reported in common iterations of the alpha-synuclein preformed fibril (aSyn PFF) model. Replicated phenotypes (reported in > 1 study) and underexplored phenotypes (observed in only 1 study) are mapped across the timeline of the model. Phenotypes that were investigated but found to be absent are also included in an inset to the right of the table. Italicized phenotypes are those that vary across studies by either their presence/absence (denoted by superscript A) or timing of appearance (denoted with superscript T). For all italicized phenotypes, the most common time at which the phenotype is observed is reported.

following bilateral injection [35]. Some of these differences may be attributed to the behavioral assays employed. For instance, Henderson et al. (2019) used two behavioral tests in the same cohort—grip strength and rotarod—and demonstrated differences in grip strength upon aSyn PFF treatment but no effect of aSyn PFF treatment on rotarod performance [30]. These differences in detecting an effect of aSyn PFF treatment on motor function or non-motor function could be due to the physiology probed within these assays, the sensitivity of the tests, or confounds that may impact the readouts [77].

Another phenotype that greatly varies between studies is pS129 aSyn pathology in the brain following injection of aSyn PFFs to the gut (Table 7). Roughly half of the studies observe pS129 aSyn pathology spread to the midbrain/forebrain [62, 64, 65, 68] whereas the other half observe pathology in the periphery/brainstem that never progresses to the midbrain/forebrain [61, 63, 64, 66, 68]. As mentioned in a recent review by Bindas et al. (2021), the reason for this is unclear but could relate to gastrointestinal conditions, amount of pathology generated, site of pathology, and type of pathology induced by the aSyn PFFs [78].

When attempting to understand the variability within the aSyn PFF model, it is important to understand the various factors that can influence the pathogenicity of the aSyn PFFs. Some factors may be obvious and easily accounted for, such as dose or days post-injection. Other factors are not so clear. The source and method of preparing the aSyn PFFs can greatly influence their pathogenicity. Multiple studies have noted that endotoxin may impact the aSyn

PFF protein [8, 14, 78]. Endotoxin should not only be accounted for due to its ability to generate an immune response that is independent of the aSyn [8, 79], but also for its ability to alter the structure and pathogenicity of the aSyn fibrils themselves [14]. The buffers, temperature, and sonication protocol used to generate aSyn PFFs from monomeric starting material can also lead to variations in the structure of the PFF aggregates that dramatically affect pathogenicity [12–18]. In addition, downstream steps such as storage (duration and temperature) can impact aSyn PFF performance while injection coordinates can impact the pathology observed in the various structures [8].

Taken together, the aSyn PFF model is a popular model due to its ability to recapitulate the pathological hallmarks of PD through the templating of pathology in the endogenous aSyn protein. The model has been used by many to study PD biology and therapeutic interventions targeting aSyn spread, inflammation, neurodegeneration, etc. Although many groups have adopted the model successfully, it is very important to acknowledge the variation in phenotypes between labs. The tables provided in this paper will hopefully assist groups who wish to learn more about the model and clarify which phenotypes are reproducible between labs to prevent issues in adopting the model for one's studies.

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CONFLICT OF INTEREST

The author has no conflicts of interest to report.

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