Quaternary structure of patient-homogenate amplified \( \alpha \)-synuclein fibrils modulates seeding of endogenous \( \alpha \)-synuclein

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Parkinson’s disease (PD) and Multiple System Atrophy (MSA) are progressive and unremitting neurological diseases that are neuropathologically characterized by \( \alpha \)-synuclein inclusions. Increasing evidence supports the aggregation of \( \alpha \)-synuclein in specific brain areas early in the disease course, followed by the spreading of \( \alpha \)-synuclein pathology to multiple brain regions. However, little is known about how the structure of \( \alpha \)-synuclein fibrils influence its ability to seed endogenous \( \alpha \)-synuclein in recipient cells. Here, we aggregated \( \alpha \)-synuclein by seeding with homogenates of PD- and MSA-confirmed brain tissue, determined the resulting \( \alpha \)-synuclein fibril structures by cryo-electron microscopy, and characterized their seeding potential in mouse primary oligodendroglial cultures. The combined analysis shows that the two patient material-amplified \( \alpha \)-synuclein fibrils share a similar protofilament fold but differ in their interprotofilament interface and their ability to recruit endogenous \( \alpha \)-synuclein. Our study indicates that the quaternary structure of \( \alpha \)-synuclein fibrils modulates the seeding of \( \alpha \)-synuclein pathology inside recipient cells. It thus provides an important advance in the quest to understand the connection between the structure of \( \alpha \)-synuclein fibrils, cellular seeding/spreading, and ultimately the clinical manifestations of different synucleinopathies.
α-synucleinopathies are neurodegenerative diseases in which the presynaptic protein α-synuclein (αSyn) misfolds and abnormally aggregates into fibrils. In the case of Parkinson’s disease (PD) and dementia with Lewy bodies, these αSyn fibrils are abundant in Lewy bodies within neurons or Lewy neurites. For multiple system atrophy (MSA), αSyn fibrils are predominantly found in inclusions within oligodendrocytes, with limited pathology in neurons. Remarkably, αSyn directly transmits and self-propagates misfolding when transferred to transgenic mice or cell cultures.

Though sharing a similar general process of seeding and assembly of αSyn, the etiology and clinical manifestations of α-synucleinopathies are different, as are the roles of αSyn as both an effector of neurotoxicity and as a mediator of the pathogenicity and disease progression. These differences are manifest in macroscopic structural differences in the deposited aggregates, ascribed to different conformational polymorphs of fibrillar αSyn. Further suggesting a connection between αSyn fibril structure and disease, in vitro-amplified αSyn fibrils differ in their seeding and self-propagation behavior in vivo, including polymorph-specific pathology and neurotoxic phenotypes.

In addition to the putative structural differences attributed to different α-synucleinopathies, αSyn might adopt at least two structural polymorphs within a single disease reported for MSA, which might further vary between clinical cases. Moreover, there could be more heterogeneity among PD than MSA fibrils, which may explain the greater variety of disease phenotypes in PD. Finally, differences in the structure of αSyn fibrils might arise from different post-translational modifications, in particular phosphorylation and ubiquitination, and thus could account for the differing pathogenesis and disease progression within a single α-synucleinopathy.

Thus, to gain insight into the connection between the molecular structure of αSyn fibrils and the ability to seed endogenous αSyn in recipient cells, we fibrillized αSyn by protein misfolding cyclic amplification (PMCA) using brain extracts of patients pathologically confirmed with PD and MSA, solved their cryo-EM structures, and evaluated their seeding potential in mouse primary oligodendroglial cultures. The structural analysis and cellular experiments were performed on the same sample to enable a direct connection between molecular structure and cellular seeding and avoid the influence of variations in the aggregation process. The combined analysis identifies the quaternary structure of αSyn fibrils as a vital factor in the seeding of αSyn pathology.

**Results**

**MSA-PMCA αSyn fibrils are more active in oligodendroglia than PD-PMCA fibrils.** Recombinant αSyn can directly nucleate and form fibrils in vitro, but the timescales are very long and high concentrations are required unless seeds are used, or aggregation-accelerated mutations are present. On the other hand, PMCA is a well-established procedure that proved to amplify aggregates from blood and cerebrospinal fluid reliably and was also successfully applied for an αSyn anti-aggregating drug screening. We therefore seeded fibril formation of recombinant αSyn through the addition of PMCA-products, which were previously generated from the homogenized brain tissue of a PD and a MSA patient (PD patient #1 and MSA patient #1 in ref. 9). We selected patients PD1 and MSA1 from our previous study (ref. 4) since they had similar disease duration but were most different in their NMR-derived hydrogen-deuterium exchange profile (Fig. Sb in ref. 4). In our previous work, Western blot analysis with an αSyn-specific antibody and fluorescence measurements using the amyloid-binding dye thioflavin-T (ThT) showed that we successfully amplified αSyn aggregates from the brain extracts. Furthermore, control PMCA experiments with brain extract from an individual, in which an α-synucleinopathy was excluded, did not amplify αSyn aggregates. In addition, hydrogen-deuterium exchange coupled to NMR spectroscopy showed that the brain tissue-amplified αSyn fibrils (further termed PD- and MSA-PMCA αSyn fibrils) differ in structural integrity properties.

To gain insight into their differences in the cellular activity of the PD- and MSA-PMCA αSyn fibrils, we added each fibril sample to differentiated murine primary oligodendroglial cultures. The distinct profiles of PD- and MSA-PMCA fibril strains were further validated by the differential pathology-related responses observed in these cultures upon inoculation with PMCA fibrils. In particular, the experiments show that MSA-PMCA fibrils display higher potency in seeding the endogenous oligodendroglial αSyn and promoting the redistribution of the oligodendroglial-specific phosphoprotein TPP2/p25a from the myelin sheath to the cell soma, as compared to PD-PMCA fibrils (Fig. 1). Both events play an essential role in the cascade of events leading to oligodendroglial dysfunction and neuronal demise underlying MSA pathology.

**MSA-PMCA and PD-PMCA αSyn fibrils share a common protofilament fold.** To elucidate why primary oligodendroglia respond differently to PD- and MSA-PMCA αSyn fibrils, we determined their 3D structure by cryo-EM. The 3D structures of the PD- and MSA-PMCA αSyn fibrils were determined using the same samples that induced the differential response in the oligodendroglia. We observed a single dominant fibril type in the micrographs for both fibril samples. The measured crossover distances were ~1000 Å and ~1200 Å for the PD- and MSA-PMCA fibrils, respectively (Fig. 2a, b and Fig. S1). In the case of the MSA-PMCA αSyn fibrils, we also observed non-twisted fibrils (Fig. S1), which were considered preparation artifacts from interactions with the air-water interface.

The 3D structures of PD and MSA-PMCA αSyn fibrils were determined to a resolution of 3.3 Å and 3.0 Å, respectively, based on the Fourier shell correlation 0.143 criterion (Tables 1, 2, Fig. 2, and Fig. S2). In the case of the MSA-PMCA αSyn fibril, the local resolution estimation revealed a pronounced heterogeneity with a resolution of ~2.9 Å in the interprotofilament interface and >4.0 Å at the periphery of the fibril (Fig. S3). In contrast, for the PD-PMCA fibril, the local resolution estimates were more homogeneous and, hence, the reconstructed map revealed precise side-chain densities. This suggests that the overall fold of the PD-PMCA αSyn fibril tends to be more rigid than the MSA-PMCA αSyn fibril.

The reconstructed maps of both αSyn fibril types show a clear β-strand separation along the helical axis and two intertwined protofilaments (Fig. 2a, b). For the PD-PMCA fibrils, the protofilaments are related by C2 symmetry (helical rise = 4.68 Å and twist = −0.78°). In contrast, the protofilaments are related by an approximate pseudo 2 screw symmetry (helical rise = 2.37 Å and twist = 179.66°) in MSA-PMCA fibrils. Therefore, the refined crossover distances are in excellent agreement with the measurements from the micrographs. In both cases, the fibril width is ~100 Å. Due to the lack of twist, the 3D structure of the non-twisted MSA-PMCA αSyn fibrils could not be solved.

The cryo-EM structures reveal that the protofilament folds of the PD- and MSA-PMCA αSyn are highly similar. The PD-PMCA fibril extends from G14 to K96 and is composed of eight β-sheets, from which β2 to β8 are connected by a continuous backbone chain and β2/β3, β4/β5, and β6/β7 form a triple-stacked L-shaped core (Fig. 2c, d). No apparent densities were found for the N-terminus from M1 to E13, the region from V26 to E35, and the C-terminus beyond K96, suggesting that these regions are more flexible.
The MSA-PMCA fold extends from G36 to G93 and comprises seven β-sheets, creating a continuous backbone chain and, similar to the PD-PMCA αSyn fibrils, the β-sheets form the same L-shaped core (Fig. 2a, b). In contrast to the PD-PMCA αSyn fibrils, no backbone densities were found for the first 38 amino acids, similar to other structures of αSyn lacking the same region.\(^\text{19}\) Distinct quaternary arrangement of MSA-PMCA and PD-PMCA αSyn fibrils. While the MSA-PMCA and PD-PMCA αSyn fibrils share a common protofilament fold, they display different quaternary arrangements (Fig. 2a, c). In the PD-PMCA αSyn fibril, two salt-bridges between K45 and E57' and between E57 and K45', harbored on two opposite subunits \(i\) and \(i'\) form the...
PMCA fibrils reveal similarities to in vitro aggregated αSyn fibrils. Next, we compared our αSyn structures to previously solved αSyn structures and used the Ca root mean square deviation (Ca RMSD) to measure structural similarity. Our PD-PMCA αSyn structure is similar to a fibril structure determined for recombinant wild-type αSyn, which was aggregated in the absence of brain homogenates and was named polymorph 2A10 (Fig. 3a). Comparison of the polymorph 2A with the PD-PMCA αSyn fibril structure results in Ca RMSDs of 1.03 Å and 1.33 Å considering one or both protofibrils, respectively (Table S1). Only minor structural deviations were present at the C-terminus from T92 to K96 (Fig. 3a). Although the protofibril fold and inter-protofibril interface are almost identical, the helical rise and twist are different (Table S1), resulting in a lateral displacement of the protofibrils relative to each other (Fig. 3a).

The MSA-PMCA αSyn structure is similar to the in vitro αSyn fibril structure referred to as polymorph 2B20 with Ca RMSDs of 1.47 Å and 1.74 Å considering the resolved amino acids G36 to G93 of one or both protofibrils, respectively (Fig. 3a and Table S2). In contrast to polymorph 2B, however, the N-terminal region from G14 to G25 is not visible in the MSA-PMCA structure, suggesting that the N-terminus tends to be more flexible. In addition, the helical rise and twist differ between the MSA-PMCA and the polymorph 2B structure (Table S2).

Conserved backbone shape of PMCA fibrils and ex vivo αSyn fibrils. Cryo-EM structures of αSyn fibrils extracted using sarkosyl from the brains of MSA-confirmed patients have been previously reported13. Two types of αSyn fibrils were observed in these preparations and their relative abundance varied. For three patients, predominantly, either type I or type II fibrils were detected, while in the other two patients, both fibril types were found. The ex vivo αSyn fibrils differ in their inter-protofibril interfaces but share an identical three-layered L-shaped fold13, which is similar to that observed in the MSA-PMCA αSyn fibril. Indeed, the anti-parallel superposition reveals a similar structural backbone shape in αSyn fibrils purified from MSA patient’s brain and the MSA-PMCA αSyn fibrils seeded from the brain homogenate of an MSA patient: in both a triple-stacked L-shape is present (Fig. 3b), but with opposite sequence direction.

Next, we compared our amplified αSyn structures to other cryo-EM structures from Lövestam et al.18, for which the αSyn was aggregated in the presence of brain extracts of MSA patients but without using PMCA (Table S1, S2). Superposition of one protofibril strand yields Ca RMSDs < 2 Å, but superimposing two opposite protofibril strands yields Ca RMSDs > 10 Å. This indicates that the global symmetry, rise, twist, and, thus, helical organization, but not the core protofibril fold are different between αSyn fibrils, which were amplified from brain homogenates of two different MSA patients using different amplification protocols (Tables S1 and S2). MSA-associated fibrils, either purified by sarkosyl extraction or generated through seeding with brain homogenate, thus share a common protofibril core.

Discussion

The misfolding and aggregation of αSyn is the pathological hallmark of both PD and MSA.21,22 Over recent years, αSyn fibrils prepared in various ways have been suggested as potential toxic species with clinical relevance for PD and MSA.23,24 However, a firm understanding of how αSyn fibrils associated with PD or MSA hamper cellular function has remained elusive. Here, we amplified αSyn fibrils from brain extracts of patients pathologically confirmed with PD or MSA, determined their 3D structures by cryo-EM, and evaluated their potential to seed αSyn-related pathology in oligodendrocytes. Our results show that αSyn fibrils amplified from MSA and PD brain homogenate share a common protofibril fold, but differ in their quaternary structure and their ability to seed endogenous αSyn in mice primary oligodendroglial cultures.

The 3D structures of the PD- and MSA-PMCA αSyn fibrils revealed major differences between both types in their inter-protofibril interfaces, their adopted helical arrangement, and the lacking N-terminal region in the MSA-PMCA αSyn fibril (Fig. 2). Notably, the determined structures are very similar to two previously solved αSyn structures, namely polymorphs 2A and 2B, originating from αSyn aggregated in vitro under continuous-shaking conditions and in the absence of brain homogenate20 (Fig. 3). However, polymorphs 2A and 2B were observed next to each other and originate from an identical preparation20, suggesting that both polymorphs are thermodynamically-stable αSyn aggregates under particular conditions.

In the present study, we also used recombinant αSyn as the substrate for PMCA. However, we obtained a single main conformation in independent experiments, either with seeds from PD
or MSA-diagnosed brains (Fig. 2). PMCA is based on the propensity of prions and prion-like proteins to act like seeds and replicate in an autocatalytic process, thereby converting recombinant protein as a substrate into amyloid fibrils. Indeed, the PMCA technique successfully amplifies and detects misfolded prion proteins\(^{25,26}\), and several lines of evidence suggest prion-like features for αSyn\(^{22,27,28}\). Considering that the identical and extensively validated PMCA procedure was used to amplify αSyn seeds from either PD or MSA\(^6\) yields different αSyn structures, suggests that the brain homogenates must contain structurally different αSyn seeds that served as the starting point for PMCA-induced fibrilization leading to the fibrils described herein.

The key finding from our study is that the PD- and MSA-PMCA αSyn fibrils, with their similar protofilament fold but
Fig. 2 Cryo-EM structures of αSyn PMCA fibrils seeded from brain homogenates. a Cryo-EM structures of PD-PMCA (left) and MSA-PMCA (right) αSyn fibrils. From the outside to the inside, the panels show extracts from representative micrographs, a full cross over (180° turn) of the reconstructed fibril, with two protofibrils colored in different shades of blue (PD-PMCA) or green (MSA-PMCA), and semitransparent surfaces overlaid with their atomic models viewed from different angles. Scale bars, 20 nm. b Amino acid sequence of αSyn (from G14 to K96; based on UniProt: P37840) with a schematic depiction of the secondary structure of the protofibril fold. β-strands are shown as arrows and numbered from β1 to β8 (for PD) or β7 (for MSA), respectively. The region from V26 to E35 was not resolved (indicated by a dashed line). c Top view onto two opposite subunits of the reconstructed PD (left; colored in shades of blue) and MSA (right; colored in shades of green). One protofibril is shown as a mesh-stick model, the other schematically depicted by its secondary structure matching the assignment in b. d Close-up view of the protofibril interface, with interface amino acids shown as stick models. e The calculated electrostatic potential was mapped onto the surface of one protofibril and colored according to the color scale on the left. The central subunits of the other protofibril are shown as cartoon model. Cross-sections are shown as a surface-cartoon model with amino acids forming the central negatively charged cavity in PD-PMCA αSyn (from K45 to E57) labeled explicitly.

### Table 1 Cryo-EM structure determination statistics.

|                | PD-PMCA αSyn | MSA-PMCA αSyn |
|----------------|--------------|---------------|
| Data collection|              |               |
| Microscope     | Titan Krios G2 | Titan Krios G2 |
| Voltage [keV]  | 300          | 300           |
| Detector       | K3           | K3            |
| Magnification  | 81,000       | 81,000        |
| Pixel size [Å] | 1.05         | 1.05          |
| Defocus range [µm] | −0.7 to −2.9 | −0.7 to −2.9 |
| Exposure time [s/frame] | 2.2         | 2.2           |
| Number of frames | 40         | 40            |
| Total dose [e−/Å²] | (1.07 e−/Å²/ frame) | (1.08 e−/Å²/ frame) |
| Reconstruction |              |               |
| Micrographs    | 6,780        | 4,474         |
| Box width [pixels] | 200 (1.05 Å/ pixel) | 250 (1.05 Å/ pixel) |
| Inter-box distance [pixels] | 18         | 50            |
| cYOL0a picked  | 2,223,091    | 1,848,677     |
| segments (no.) |              |               |
| Final segments [no.] | 23,937      | 25,692        |
| Final resolution [Å] | 3.30        | 3.02          |
| (FSC = 0.143)  |              |               |
| Applied map sharpening | −112.5      | −101.8        |
| B-factor [Å²]  |              |               |
| Symmetry imposed | C2           | C1            |
| Helical rise [Å] | 4.68         | 2.37          |
| Helical twist [°] | −0.78        | 179.66        |

### Table 2 Model building statistics.

|                | PD-PMCA αSyn | MSA-PMCA αSyn |
|----------------|--------------|---------------|
| Starting model [PDB code] | 6SSX² | 6SST² |
| Model composition |              |               |
| Non-hydrogen atoms | 497          | 389           |
| Protein residues | 73           | 58            |
| RMS deviations |              |               |
| Bond lengths [Å] | <0.05        | <0.05         |
| Bond angles [°] | 2.01         | 1.68          |
| Validation |              |               |
| MolProbity score | 2.25         | 2.02          |
| Clashscore | 17.17        | 14.41         |
| Poor rotamers [%] | 0.00         | 0.00          |
| Ramachandran plot |              |               |
| Favored [%] | 91.30        | 94.64         |
| Allowed [%] | 8.70         | 5.36          |
| Disallowed [%] | 0.00         | 0.00          |
| Model deposition |              |               |
| PDB code | 7O2ZG        | 7O2H          |
| EMD code | 13123        | 13124         |

*Ref. 61

relationship fully. These observations suggest that some features from the MSA seeds (e.g. the filament interface or the disordered N-terminus) have been transferred to the amplified fibrils, leading to a more MSA-like response in oligodendroglial cells than PD-PMCA fibrils. Alternatively, and beyond the scope of this study, one might also consider an indirect effect of αSyn fibrils on TPPP/p25a redistribution and co-localization.

A fundamental difference between PD and MSA is that in the case of PD αSyn aggregates are predominantly present in dopaminergic neurons, whereas MSA is associated with αSyn inclusions within oligodendrocytes. Peng et al. showed that the intracellular environments of both neurons and oligodendrocytes determine how the same misfolded αSyn seeds develop into different aggregates. While dopaminergic neurons are directly associated with reward-motivated behavior and motor control, oligodendrocytes are essential for the long-distance saltatory conduction of neuronal impulses as they enwrap central nervous system axons with the myelin sheath, a lipid enriched multilayer membrane. Although the lipid content and composition of oligodendrocytes is still unknown, these numbers are well known for the myelin sheath, which is extremely rich in lipids (~80% of its dry weight). Previous studies revealed the αSyn N-terminus is essential for lipid-binding and fatty acid-induced oligomerization. Further biophysical experiments suggest that αSyn-lipid interactions are predominantly driven by electrostatic interactions between charged N-terminal residues and the charged head groups of phospholipids, which comprise ~26% of all lipids found in central nervous system myelin. Thus, the αSyn N-terminus may interact with these phospholipids during distinct quaternary arrangement, have different activity when added to mouse primary oligodendroglial cultures: the MSA-PMCA fibrils are more potent in recruiting the endogenous oligodendrogial αSyn and evoking a redistribution of TPPP/p25a protein when compared to the PD-PMCA fibrils (Fig. 1). Both events are characteristic of MSA. Thus, under physiological conditions, TPPP/p25a is predominant in myelin sheaths but under MSA-related pathological conditions, TPPP/p25a relocates to the oligodendrocyte soma. Notably, TPPP/p25a not only co-localizes with filamentous αSyn but also fosters further aggregation of αSyn into filamentous aggregates. The C-terminus of αSyn fibrils has been identified as the binding epitope for TPPP/p25a, but an atomic picture of how TPPP/p25a binds to filamentous αSyn has not been realized yet. Considering the wide range of αSyn fibril structural polymorphism (Fig. S5) and that neither in our (Fig. 2) nor any structurally related fibril (Fig. 3a; Table S1 and S2) the C-terminus was resolved, clarification of the full-length αSyn structure under pathological conditions might be necessary to elucidate the underlying structure-activity relationship fully. These observations suggest that some features from the MSA seeds (e.g. the filament interface or the disordered N-terminus) have been transferred to the amplified fibrils, leading to a more MSA-like response in oligodendroglial cells than PD-PMCA fibrils. Alternatively, and beyond the scope of this study, one might also consider an indirect effect of αSyn fibrils on TPPP/p25a redistribution and co-localization.
fibril aggregation and seed formation in oligodendrocytes, which may hamper the stabilization of the folded N-terminus in these cells in turn. One could then speculate whether the disordered N-terminus observed in the MSA-PMCA structure is a feature inherited from the seed, although no lipids were present during in vitro fibrilization.

There are two distinct mechanisms by which homogeneous seeds added to monomer solutions can induce aggregation: either the seeds can be elongated through monomer addition, or the seeds can nucleate new fibrils on their surface (secondary nucleation). Elongation mechanisms necessarily perpetuate the structure of the seeds onto monomers, with the lamination of successive cross-beta layers. On the other hand, secondary nucleation of αSyn relies on transient interactions between monomer and fibril surface, with the monomer less closely coordinated with the protofibrils and thus potentially able to adopt a conformation differing from the seeds, though energetic considerations can favor mimicking of the seed packing and morphology. Therefore, when seeds are amplified in vitro, αSyn can sometimes form fibrils of a different structure under different solution conditions. Under conditions conducive to elongation, seeds imprint their structural properties onto fibrils growing by recruiting soluble αSyn molecules. In contrast, it has been shown that in conditions favoring secondary nucleation, the propagation of seed structural properties decreases. Consequently the structure of fibrils, even when formed in the presence of seeds, may be influenced by differing environmental conditions, such as temperature, the concentration of seeds, pH, salt concentration, and the presence of surfaces for nucleation such as air-water interfaces or lipids. We note, however, that although the MSA-PMCA structure determined in the current study does not fully replicate the previously identified structures of αSyn fibrils extracted with sarkosyl from the brain samples of MSA patients, the PMCA-derived structures can be partially aligned in an antiparallel arrangement with the triple-stacked L-shape core region comprising about 40 residues.

Here we establish a connection between the structure and cellular seeding activity of αSyn fibrils amplified from MSA and...
PD brain-derived seeds. Specifically, the quaternary structure of αSyn fibrils influences the seeding of αSyn pathology inside oligodendroglia. Our study thus makes an essential contribution to ongoing research efforts into unraveling the importance of αSyn fibril structure for cellular seeding, the spreading of αSyn pathology in the brain and thus the clinical manifestations in different synucleinopathies.

Methods

Preparation of brain extracts. Ethical approval to access and work on the human brain tissues was given by the Human Research Ethics Committee of the University of New South Wales, under ethics approval numbers one PD1 (ref. 9) and one MSA patient (MSA1 in ref. 9), respectively, were received from the Sydney Brain Bank at Neuroscience Research Australia, which is supported by The University of New South Wales and Neuroscience Research Australia. Patient PD1, male, died at age 79; sample was taken with postmortem delay of 14 h; cause of death was cardiorespiratory failure, and disease duration was 7 years. Human amygdalas were sonicated with Vibra-cells (Sonics, Newton, CT, USA) to 10% weight/volume solution with homogenizing buffer (1% Triton X-100, Proteinase Inhibitor Cocktail in PBS). Sonicated samples were centrifuged at 3000 g for 40 s. Protein concentrations in supernatants were determined by the bicinchoninic acid assay (Pierce, Rockford, IL, USA).

Recombinant αSyn preparation. N-terminally acetylated αSyn was obtained by co-transfection of E. coli BL21 (DE3) cells with plasmid encoding human for αSyn (kindly provided by the Lansbury Laboratory, Harvard Medical School, Cambridge, MA) and S. pombe NatB acylase complex.17 Using pNatB-plasmid (pACYC-Duet-aa20-naa25, Addgene, #53613, kindly provided by Dan Multhoff). The mutant protein αSyn-T54C/A90C was constructed using the QuickChange site-directed mutagenesis kit (Stratagene), and the introduced modifications were verified by DNA sequencing.

For αSyn expression and purification, transformed BL21 (DE3) cells were grown at 37 °C in LB medium to an OD600 of 0.8 and shifted to 25 °C adding 1:1500 dilution of 1:1500.

Immunocytchemistry and confocal microscopy. At 48 h post injection, PMCA fibril addition, cells were fixed with 4% paraformaldehyde for 40 min, blocked in 10% normal goat serum containing 0.4% Triton X-100 for 1 h at room temperature and incubated with anti-human αSyn antibody (ab108085) at 1:1000 (D37A6), or the oxidized/nitrated (Syn303) αSyn and the oligodendroglial phosphophytidylphosphatidylcholine (PtdP/C) (concentration of 0.2 mg/mL and diluted by 1:400, kind gift from Dr. Poul Henning Jensen, Aarhus University, Denmark) overnight at 4 °C. Antibody concentrations of L509 and SYM303 were 1 mg/mL and a dilution of 1:1000 was used for the experiment. The αSyn-D37A6 antibody and L509 were diluted in a 1:400 of the stock solution was used. Images were obtained using a Leica TCS SP5 confocal microscope combined with a dual (tandem) scanner. All confocal images were obtained under equivalent conditions of laser power, pinhole size, gain, and offset settings between the groups. Image (v2.0.0) software was used to quantifiy relative protein levels expressed as % area coverage. normalized to the p25a + cells/field.

Cryo-EM grid preparation and imaging. Sample volumes of 3.5 μL were applied to freshly glow-discharged R3.5/1 holey carbon grids (Quantifoil) and vitrified using a Mark IV Vitrobot (ThermoFischer Scientific) operated at 100% r.h and 20 °C. Micrographs were collected with a Titan Krios transmission-electron microscope operated at 300 keV accelerating voltage at a nominal magnification of 81,000x using the Gatan K Morada camera. Data were acquired in SuperStaR mode, corresponding to a calibrated pixel size of 1.05 Å on the specimen level. In total, 678 PD-PMCA and 474 PD-MSA-PDMA micrographs with defocus values in the range of 0.7 μm to 70 μm were recorded in movie mode with 2.2 second acquisition time. Each movie contained 40 frames with an accumulated dose of ~43 electrons per Å2. The resulting dose-fractionated image stacks, containing all frames 1–40, were subjected to beam-induced motion correction using MotionCor2,15 prior to helical reconstruction. Estimation of contrast transfer function parameters for each micrograph was performed using CFITFIND8. Subsequently, PD-PMCA and PD-MSA-PDMA fibrils were reconstructed using RELION-3.19, following the helical reconstruction scheme.

Helical reconstruction of PD-PMCA fibrils. cryOLO was used for the selection of 74,120 fibrils in the data set, from which 2,223,091 segments were extracted using a 1 Å inter-box distance. Using RELION-3.19, maximum-likelihood two-dimensional (2D) reference-free classification, and 3D classification were performed on an unbinned data set (1.05 Å/pix, 200 px box size); a cylinder with white noise added using EMAN2 was used for the initial reference.

Starting from a featureless cylinder with one helical reconstruction. Estimation of contrast transfer function parameters for each micrograph was performed using CFITFIND8. Subsequently, PD-PMCA and PD-MSA-PDMA fibrils were reconstructed using RELION-3.19, following the helical reconstruction scheme.

Helical reconstruction of MSA-PMCA fibrils. cryOLO was used for the selection of 50,794 fibrils in the data set, from which 1,848,677 segments were extracted using a 50 Å inter-box distance and RELION-3.19 was used for reconstruction. To exclude non-twisted and irregularly twisted segments, we initially performed several rounds of 2D classification on a downsampled data set (2.1 Å/pix, 500 px box size). For 3D classification we (re)extracted 91,352 segments without downsampling (1.05 Å/pix, 250 px box size).

Primary oligodendroglial cultures. Mixed glial cultures generated from P0 to P3 neonatal wild-type mice were maintained in full DMEM for 10 to 14 days until a monolayer of astrocytes on the bottom and primary oligodendroglial progenitor cells (OPCs) with loosely attached microglia on the top, were apparent. The separation of OPCs was achieved initially with the removal of microglia, by shaking in 200 rpm for 1 h in 37 °C and then with continuous shaking under the same conditions for 18 h, as previously described.66 Afterwards, isolated cells were platedted on poly-D-lysine-coated coverslips (P7405, Sigma Aldrich, USA) with a density of 80,000 cells/cm² and maintained in S17 medium (284369) supplemented with 10% FBS and transferrin (500 mg/mL) in MEM solution (4140045; LTS; Gibco, Invitrogen, Carlsbad, CA, USA), 5% penicillin/streptomycin and 1% horse serum (H1138; Sigma-Aldrich, St. Louis, MO, USA) for 4 days. αSyn fibrils (final concentration 0.3 μg/mL culture medium/well) amplified from human MSA and PD brains were added to TPP/P25a-positive mature differentiated oligodendrocytes for 4 days, and then, cells were fixed for immunofluorescence analysis. All experimental procedures were approved by the Ethics Committee for the Use of Laboratory Animals in the Biomedical Research Foundation of Athens.
β-strands along the Z-axis and the approximate 21 screw symmetry between the two protofilaments became visible. From here on, we performed multiple rounds of 3D auto-refinement until no further improvement of the map was observed. Assuming a left-handed twist, the helical twist and rise converged to 179.66° and 2.37 Å, respectively, in agreement with the predominant crossover distances measured on the motion-corrected cryo-EM micrographs. Finally, post-processing with a soft-edged mask and an estimated sharpening factor of ~101 Å yielded post-processing defocus values of -140 Å. The resolution was estimated from the value of the gold curve for two independently refined half-maps at 0.143 Å (Fig. 2b). The helical geometry was then applied to the map, which was then re-sharpened using VIDESEM. Local resolution was determined using RELION-3.1.

Atomc model building and refinement. PDB entries 6SSX and 6ST20 were used for an initial model for PD-PMCA and MSA-PMCA fibrils, respectively. Subsequent refinement in real space was conducted using PHENIX66,67. For PD-PMCA, the final refined protofilament subunit had an RMSD of 0.64 Å to PDB 6SSX. As for MSA-PMCA, the final refined protofilament subunit had an RMSD of 0.95 Å to PDB 6ST5.

Determination of electrostatics for αSyn fibrils. We calculated the electrostatics for PD- and MSA-PMCA fibrils using the APBS/PDEPQ server (https://webserver. poissonboltzmann.org/). Thereupon, we focused on the central slices of the fibrils. APBS calculations for polymorphs A and B26 were conducted analogously.

Statistics and reproducibility. The current study is based on work published previously in which αSyn aggregates were amplified by PMCA from five PD and five MSA patients and extensively characterized by CD, EM, EPR, fluorescence dyes and HD-exchange coupled to NMR spectroscopy. This previous study identified αSyn aggregates amplified from PD patient #1 and MSA patient #1 as most representative of the structural properties of αSyn aggregates amplified from the different PD and MSA patient brains, respectively. αSyn aggregates amplified by PMCA from PD patient #1 and MSA patient #1 were therefore selected and investigated in cells and structurally characterized by cryo-EM.

For cryo-EM structure determination, one data set was collected for each of the MSA and PD samples. For the cell assay (Fig. 1), the aggregates added to the cells were taken from the same sample that was used for cryo-EM.

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**Author contributions**

M.Z. and G.F.S conceived the study; W.S.K provided the brain samples; B.C.J and S.-J.L. amplified αsyn seeds from brain tissue using PMCA; T.S. conducted protein preparation, and fibril amplification; P.M. and M.X. performed the cell experiments, and analyzed the associated data; C.D. conducted cryo-EM grid preparation and data collection; B.F., J.A.G., and G.F.S. performed image processing, reconstruction and model building; B.F., J.A.G., M.Z., and G.F.S. wrote the paper. All authors discussed results and commented on the paper.

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**Competing interests**

The author S.-J.L. declares the following competing interests: S.-J.L. is a founder and co-CEO of Neuramedy Co., Ltd. The other authors declare no competing interests.

**Additional information**

**Supplementary information**

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