IMPRiND Research Highlights

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Editorial

Over seven million people in Europe suffer from neurodegenerative diseases and this number is predicted to double by 2040 due to our increasingly ageing population with a dramatic impact on social services and potentially unsustainable financial burden on healthcare providers. Such urgent and currently unmet clinical need requires an unprecedented research effort that can only be achieved through a coordinated approach across leading European laboratories, the pharmaceutical industry and other international initiatives.

A growing body of data indicates that the propagation of pathogenic protein aggregates across neural systems could be mediated by misfolded protein seeds that are released and taken up by anatomically connected neurons causing disruption of their function. Therefore, blocking this process may help arrest the progression of Parkinson's (PD) or Alzheimer's (AD) disease. The IMPRiND consortium – a group of European academic laboratories and members of the European Federation of Pharmaceutical Industries and Associations (EFPIA) – funded by the Innovative Medicines Initiative (IMI), aimed at describing and targeting the critical steps in the spread of α -syn and tau assemblies between neurons.

Within the framework of this collaborative programme, which has mobilised various competences, numerous scientific publications have been produced, of which a subjective selection of highlights is presented here.

Cryo-EM structures of tau filaments from Alzheimer's disease¹

Alzheimer's disease is defined by abundant plaques and tangles in cerebral cortex. Tangles are made of abnormal amyloid filaments of post-translationally modified microtubule-associated protein tau. Paired helical filaments (PHFs) were first identified in Alzheimer's disease brain in 1963. The less abundant straight filaments (SFs) were described later. Between 1985 and 1992, PHFs and SFs were shown to be made of all six human brain tau isoforms. It was suggested that the microtubule-binding repeats of tau form the filament core, with the remainder forming the fuzzy coat. Until the recent resolution revolution in electron cryo-microscopy (cryo-EM), it was not possible to obtain high-resolution structures of non-amplified tau filaments from brain.

In 2017, IMPRiND partner University of Cambridge reported the first high-resolution structures of amyloid filaments from human brain. These findings, cryo-EM structures of tau filaments from Alzheimer's disease, published in Nature, show that the Alzheimer tau fold can form in the absence of extracellular amyloid deposits. Each tauopathy appears to have its own fold, but the same fold can be found in multiple tauopathies.



Schematic representation of full-length tau filaments. Reproduced from Fitzpatrick et al., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5552202; CC BY 4.0.

Tau filament structures differ between neurodegenerative diseases²

Many neurodegenerative diseases, including Alzheimer's disease and Pick's disease, are associated with the ordered assembly of tau protein into abnormal filaments, the abundance of which is strongly correlated with disease symptoms. Tau filaments are found to have disease-specific distributions within the nervous system, as well as distinct biochemical properties. This led to the hypothesis that tau filaments might fold into disease-specific structures.

In this paper published in Nature is explained how tau can adopt distinct folds in the human brain in different diseases. To investigate this, the MRC team extracted tau filaments from the brain of a patient with Pick's disease and collected images of the filaments using cryo-EM. The team was then able to construct a 3D model of the tau filaments using these images and determine the molecular structure of the tau protein within these filaments.

Previously, they had solved the structures of tau filaments from patients with Alzheimer's disease. Comparison of the new structures of filaments from patients with Pick's disease with those from Alzheimer's disease demonstrated that the structures are indeed different. These differences may be responsible for the distinct patterns of accumulation in the brain seen between the two variants and for the different ways in which these diseases affect patients.



Schematic representation of the secondary structure elements in the Pick and Alzheimer folds, depicted as a single rung. The positions of C322 and D348, which differ between the two folds, are highlighted. The symbols mark conserved turns of homologous regions in the two folds. Reproduced from Falcon et al., https://doi.org/10.1101/302216; CC BY 4.0.

The impact of clustering of tau fibrils on α 3-Na+/K+-ATPase and AMPA receptors³

The binding of exogenous tau aggregates to the plasma membrane of naïve neurons is not understood and is a key step in their propagation from cell to cell within the central nervous system. IMPRIND partner CNRS documents this crucial step. In this paper published in the EMBO Journal, they demonstrated that clustering of tau fibrils occurs at the neuron plasma membrane, including the synapses. They identified tau fibrils receptors at the plasma membrane and showed tau fibrils-mediated sequestering and pathogenic redistribution of essential membrane proteins such as the AMPA receptors. These observations suggest that tau fibrils initiate deleterious signaling cascades. A cross-talk between α -syn and tau aggregates at the plasma membrane accounting for the presence of both aggregates in AD and PD is unveiled.



Pathogenic fibrillar tau remodel excitatory synaptic protein composition and imbalance neuronal homeostasis. Reproduced from Shrivastava³; CC BY 4.0

It takes two to tango: α-syn and TPPP/p25α in MSA pathogenesis⁴

Multiple system atrophy (MSA) is characterised by the accumulation of cytoplasmic inclusions filled with the neuronal protein α -syn within oligodendrocytes. Oligodendrocytes are a selective type of glial cells that provide support and insulation to neuronal axons, creating the myelin sheath around them. Accumulation of α -syn together with the oligodendrocyte-specific protein TPPP/p25 α ultimately leads to oligodendroglial degeneration and neuronal demise. Oligodendrocytes in the brain contain p25 α , but very low levels of endogenous α -syn.

In this study published in Acta Neuropathologica IMPRiND partner Biomedical Research Foundation of Athens aimed to identify the mechanisms underlying the abnormal accumulation of α -syn in oligodendrocytes and to uncover the role of p25 α in MSA development and progression. Utilising *in vitro* and *in vivo* experimental models of MSA, this study demonstrates for the first time that endogenous oligodendroglial α -syn is a major component of such insoluble, highly aggregated, pathological assemblies. Interestingly, the over expression of $p25\alpha$ accelerates the recruitment of endogenous protein and the generation of such aberrant species, upon addition of pre-formed fibrils (PFFs). In primary oligodendrocytes and in the mouse brain, the microtubule and myelin networks are disrupted, thus recapitulating a pathological hallmark of MSA, in a manner totally dependent upon the seeding of endogenous α -syn, since these phenomena are not observed in mice lacking α -syn expression.

Such data suggest that endogenous α -syn and oligodendroglial phosphoprotein p25 α form a dangerous dynamic duo that predisposes oligodendrocytes to accumulate intracellular α -syn aggregates reminiscent of the oligodendroglial inclusions in MSA brains. Importantly, the identification of endogenous oligodendroglial α -syn as a major culprit for the development of pathology *in vitro* and *in vivo*, suggests that manipulation of the expression of α -syn and/or p25 α in oligodendrocytes, may provide a rationale approach to combat its accumulation and the progression of MSA.



In the absence of the endogenous oligodendroglial α -syn, PFF addition does not trigger the formation of pathological α -syn assemblies and the disruption of MBP network within oligodendrocytes. By M. Xilouri; CC BY 4.0.

Journey to the centre of pathogenic α-syn aggregates⁵

The protein α -syn aggregates are constituents of Lewy bodies, a histological hallmark of the synuceinopathies Parkinson's disease, Lewy body dementia and multiple system atrophy.

Elucidating the forms α -syn molecules adopt upon staking within aggregates is key for the design of ligands targeting those aggregates. Such ligands could attach along or at the ends of the resulting fibrils and affect their surface properties or their ability to elongate and thus limit the pathological processes involved in synucleinopathies.

IMPRiND Partner CNRS within a collaborative study with experts in cryo-electron microscopy recently determined a new structure for α -syn fibrils, down to an atomic level. Those fibrils, when injected into rodent models, cause the development of symptoms characteristic of Parkinson's disease.

The team obtained their results by combining biochemical to biostructural approaches, specifically solid-state nuclear magnetic resonance and cryo-electron microscopy. Cryo-electron microscopy, abbreviated «cryo-EM», is a method for the preparation of biological samples used in transmission electron microscopy. Developed in the early 1980s, cryo-EM has the advantage of reducing irradiation damages caused by the electron beam. It employs a technique called vitrification to very rapidly freeze samples and thus protect their morphology and molecular structure.

These results, published in eLife, bring new insight into understanding the mechanisms and factors involved in aggregates formation *in vitro* and may furthermore enable the development of ligands targeting α -syn fibrils. Such ligands could be used to differentiate pathogenic aggregates from their normal form of their protein constituent or to prevent aggregates from growing by blocking their ends.



The electron micrograph of fibrillar α -syn protein allows the reconstruction of the fibrils at an atomic-level in three dimensions. The fibrils appear to be made of two protofibrils (blue and green) winding around one another. The tip of the fibril is shown on the right. The image reveals the molecular fold of α -syn within the protofibrils in their fibrillar state. α -syn molecules have the same conformation in the two protofibrils constituting the fibrils. The protofibrils are linked by a pair of symmetric salt bridges running along the fibril axis. By R. Melki; CC BY 4.0.

Detection of α-syn aggregates in gastrointestinal biopsies by protein misfolding cyclic amplification⁶

Lewy bodies and neurites, the pathological signatures found in the central nervous system of Parkinson's disease (PD) patients, are primarily composed of aggregated α -syn. The observation that α -syn aggregates are also found in the enteric nervous system has prompted IMPRiND partner CNRS to develop a diagnostic procedure based on the detection of pathological α -syn in gastrointestinal biopsies. The method, published in the journal Neurobiology of Disease, is based on seeding of monomeric α -syn by aggregates present in affected cells. CNRS shows that the method they implemented is capable of detecting α -syn aggregates in routine gastrointestinal biopsies. Rectum biopsies appear not to contain sufficient amounts of aggregated α -syn to detect seeded assembly while biopsies from antrum and sigmoid do. The study further shows diagnostic potential as seeding is detected in a patient biopsy taken 10 years ahead of PD diagnosis.

Differential membrane binding and seeding of distinct α-syn fibrillar polymorphs⁷

IMPRiND partner CNRS showed in this paper published in Biophysical Journal, that structurally distinct fibrillar α -syn polymorphs trigger either Parkinson's disease or multiple system atrophy hallmarks *in vivo*. This study demonstrated that distinct fibrillar α -syn polymorphs bind to and cluster differentially at the plasma membrane in both primary neuronal cultures and organotypic hippocampal slice cultures from wild-type mice. It demonstrates an α -syn fibrillar polymorphdependent and concentration-dependent seeding within neurons. Altogether, this study demonstrates that distinct α -syn fibrillar polymorphs affect differently neuronal homeostasis.



Quantitative assessment of structurally distinct α -syn fibrillar aggregates obtained under different experimental conditions binding to neurons suggest that the surfaces of the fibrils define their tropism Adapted from Shrivastava⁷; CC BY 4.0.

A novel tau fold in the neurodegenerative disease corticobasal degeneration⁸

Corticobasal degeneration (CBD) is a neurodegenerative disease involving the cerebral cortex and basal ganglia. CBD belongs to a family of diseases called tauopathies in which the protein tau forms abnormal filaments. The MRC team has now solved the first structures of tau filaments from the cerebral cortex of patients with CBD. Importantly, they have also shown that these structures are different from those they previously solved for tau assemblies from Alzheimer's disease, Pick's disease, and chronic traumatic encephalopathy (CTE).

Tau protein is found in six different versions, or isoforms, that are produced by alternative mRNA splicing. These isoforms differ in the presence or absence of a sequence of amino acids near the start of the protein chain and the inclusion or exclusion of a section of the microtubule binding domain nearer the end of the protein. The microtubule binding domain is formed of three or four repeating sequences of amino acids, depending on whether this section is excluded or included. Inclusion of this repeat in three of the tau isoforms results in versions with four repeats (known as 4R), whereas exclusion in the other three isoforms leaves those with three repeats (3R).

There are more than 20 different tauopathies, with Alzheimer's disease being the most common. Tauopathies can be categorised based on which isoforms of tau are incorporated into the filaments: 3R only, 4R only, or 3R and 4R. After Alzheimer's disease, 4R tauopathies are the most common form of this family of diseases. In this Nature publication, the MRC team has now solved the first structures of tau filaments from a 4R tauopathy, CBD.

Every tauopathy for which a tau filament structure has been solved has a unique disease-specific fold. Structures of filaments from different tauopathies may be useful for the development of tracer compounds that are specific for the folds of tau that can be used for diagnosis in live patients. Understanding how and why tau assembles into different disease-specific structures will also be beneficial for the development of new therapies. It might, for example, be possible to design drugs that stop filaments forming in order to stop the progression of neurodegeneration.



Schematic representation of the four known tau filament folds: from Alzheimer's disease, Pick's disease, CTE and CBD. Reproduced from https://doi.org/10.1101/302216; CC BY 4.0.

The structural differences between patient-derived α-syn strains dictate characteristics of Parkinson's disease, multiple system atrophy and dementia with Lewy bodies⁹

In this Acta Neuropathologica publication, IM-PRIND partner CNRS establishes a method where α -syn aggregates from frozen brain tissues can seed the aggregation of pure α -syn. This allowed demonstrating that the resulting aggregates possess distinct biochemical properties *in vitro* and pathological properties in an animal model. The fibrils obtained by amplification of pathogenic α -syn from different PD cases exhibit identical structural properties. The same is true for fibrils obtained from DLB and MSA cases. Comparison of fibrils derived from PD, MSA and PD cases shows they exhibit distinct biochemically and pathological properties.

Thus, each synucleinopathy appears to differ from others by the biochemical and pathological properties of the fibrils.

Seeding propensity and characteristics of pathogenic α-syn assemblies in formalin-fixed human tissue¹⁰

In this paper published in Cells, the IMPRIND team CNRS investigated α -syn seeding activity in tissue from the brain and enteric nervous system. Despite holding their seeding propensity in fixed brain tissues, significant variations in the characteristics of fibrillar aggregates derived from different regions even within the same individual are reported. This finding suggests that fixation stabilises seeds with an otherwise limited seeding propensity, that yield assemblies with different intrinsic structures. This may suggest the existence of different strains within the brain of individuals suffering from PD.



Strain amplification ex vivo: Pathogenic a-syn aggregates in the central or peripheral nervous systems are used to seed and template the aggregation of monomeric a-syn in test tubes, thus reproducing their characteristics. By R. Melki, CC BY 4.0.

Structures of α -syn filaments from multiple system atrophy¹¹

Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) are synucleinopathies. They are characterised by the presence of abundant filamentous inclusions of α -syn in nerve cells and glial cells, in the form of Lewy bodies and Papp-Lantos bodies. The relevance of inclusion formation was established when gene dosage and missense mutations in SNCA, the α -syn gene, were shown to cause inherited forms of PD and DLB, with abundant Lewy pathology. In diseases with Lewy pathology, α -syn inclusions are found mostly in nerve cells, whereas they predominate in glial cells in MSA. Several indirect lines of evidence have suggested that the inclusions in diseases with Lewy pathology and those in MSA are different conformers of assembled α -syn.

Direct evidence requires knowledge of the high-resolution structures of α -syn filaments from the brains of individuals with PD, DLB and MSA. In this Nature publication, IMPRIND partner MRC determined the first near-atomic structures of α -syn filaments from human brain with MSA using cryo-electron microscopy (cryo-EM). Two types of filament (Type I and Type II) were identified, which differed in structure from those of assembled recombinant wild-type and mutant α-syn. Each filament type comprised two asymmetric protofilaments, with a non-proteinaceous, probably hydrophilic, density at protofilament interfaces. Different ratios of Type I and Type II filaments were present in distinct brain regions of the same cases and in the same brain regions of different cases.

 α -syn filaments from the brains of individuals with Parkinson's disease and dementia with Lewy bodies are thinner and less twisted than those from individuals with multiple system atrophy, making it more difficult to determine their high-resolution structures. So far, by using two-dimensional class averaging, we have shown that the structures of α -syn filaments from dementia with Lewy bodies are probably different from those of multiple system atrophy.











MSA case 5



Filamentous α-syn pathology of MSA. Reproduced from Schweighauser et al., https://doi.org/10.1101/2020.02.05.935619; CC BY 4.0.

Distinct α-syn species induced by seeding are selectively cleared by the Lysosome or the Proteasome in neuronally differentiated SH-SY5Y cells¹²

IMPRIND partners Biomedical Research Foundation of Athens and CNRS established a cell model to assess aggregation and turnover of α-syn assemblies. Reporting in Journal of Neurochemistry, they used neuronally differentiated SH-SY5Y neuroblastoma cells with inducible expression of α -syn and induced seeding of endogenous Proteinase K-resistant α-syn species with the addition of α -syn pre-formed fibrils (PFFs). Subsequently, the shutting down of the expression of α-syn in this inducible cell model resulted in the clearance of α -syn aggregates. Using this model, it was demonstrated that macroautophagy seems to serve as the major pathway for clearance of highly aggregated α -syn assemblies whereas the proteasome system is implicated in the degradation of phosphorylated at S129 α -syn oligomers. These findings that different degradation pathways induce the clearance of distinct α -syn aggregated species represent new and important insights into the biology of α -syn aggregation and turnover.

This well established cell model can prove an essential tool to assess aggregation and turnover of α -syn assemblies as well as the role of different post-translational modifications (i.e. phosphorylation, ubiquitylation, truncation, sumoylation) and their effect on oligomerization, and to further screen for modifiers affecting α -syn aggregation, clearance, secretion and cell-to-cell transmission. A deeper understanding of the mechanisms underlying aggregation propensity and clearance may help design novel strategies for regulating the levels of toxic α -syn conformers and eventually develop a treatment for PD and related synucleinopathies.



Different degradation pathways induce the clearance of distinct α -syn aggregated species. Upon incubation with recombinant fibrils (PFFs), endogenous α -syn aggregates are ormed and autophagy serves as the major pathway for clearance. pS129 α -syn oligomers are apparent only upon incubation with high-dose PFFs, and the proteasome system is implicated in the degradation process. By M. Pantazopoulo; CC BY 4.0.

When stealth α -syn fibrils take over¹³

The protein α -syn is involved in the regulation of neurotransmission, and its dysfunction is associated with several neurodegenerative diseases, including Parkinson's disease (PD), dementia with Lewy bodies, and multiple system atrophy (MSA). α -syn is a particularly flexible protein that oscillates between different conformations. But in neurodegenerative synucleinopathies, α -syn ceases oscillating, and get stuck in a particular conformation that automatically piles up and forms fibrillar amyloid aggregates. It is now well accepted that this process is self-sustained since an initial aggregate works as a seed recruiting more monomers into an ever-growing pathological stack that eventually breaks into fragments and disseminates. Similar to prions, this process leads to the spread of the pathology across the brain.

Structural biology approaches have recently shown that several fibril types (called polymorphs) each endowed with a distinctive fold can be experimentally produced *in vitro* using each time a specific medium. Whether the distinctive properties of these different experimental polymorphs could be responsible for the different clinical presentations of synucleinopathies is still matter of intense research.

In this study published in Sciences Advances, the team from the Université de Bordeaux discovered that during fibril formation in a physiological salt solution, α -syn can actually randomly adopt several amyloid folds, but several of the fibril polymorphs that emerge, were, up to now, left unnoticed.

The authors of the study actually made an unexpected discovery: the specific fluorescent dye thioflavin T (ThT), routinely used all over in the labs to monitor α -syn fibrillization, could not detect all of the emerging polymorphs, some of these remaining unseen with this classical and historical tool. They demonstrated that (1) these new α -syn polymorphs show an acute propensity towards self-replication in cultured neurons, and (2) injection in living mice, into the substantia nigra, triggers a synucleinopathy that spreads over long distances towards various anterior brain structures. These "stealth" ThT-invisible polymorphs have no match with any of the polymorph structure families previously characterised, indicating that previously unnoticed polymorphs can silently proliferate, crowd the fibrillar population and be a cause for exacerbated α -syn spread.

This discovery is of particular importance for the research on synucleinopathies: since the primary monitoring of α -syn fibril formation with ThT is a consensus reference method, this has led to ignoring ThT negative polymorphs and focused most research efforts only on the tip of the iceberg, i.e., the sole ThT-positive fibrils.

The authors thus developed a new multiplex assay to rapidly identify both the ThT positive and negative polymorphs, they named it: "Fibrilloscope". This assay could become an essential tool, especially to avoid mistaking the emergence of ThT negative polymorphs for fibrillization inhibitions when seeking drug candidates to prevent α -syn aggregation.



 $[\]alpha$ -syn fibrils invisible to ThT acutely spread in brain. By F. Ichas; CC BY 4.0.

Effects of pharmacological modulators of α-syn and tau aggregation and internalisation¹⁴

Parkinson's disease (PD) and Alzheimer's disease (AD) are common neurodegenerative disorders of the elderly and, therefore, affect a growing number of patients worldwide. Both diseases share, as a common hallmark, the accumulation of characteristic protein aggregates, known as Lewy bodies (LB) in PD, and neurofibrillary tangles in AD. LBs are primarily composed of misfolded α -syn, and neurofibrillary tangles are primarily composed of tau protein. Importantly, upon pathological evaluation, most AD and PD/ Lewy body dementia cases exhibit mixed pathology, with the co-occurrence of both LB and neurofibrillary tangles, among other protein inclusions. Recent studies suggest that both α -syn and tau pathology can spread and propagate through neuronal connections.

The team at University Medical Center Göttingen developed a simple laboratory model system to investigate the mechanisms underlying aggregation and propagation of these proteins, with the goal of informing the development of novel therapeutic strategies. In their study published in Nature Scientific Reports in July 2020, they assessed the effects of molecules on the aggregation and internalisation of tau and α -syn and identified some that can decrease α -syn and/ or tau aggregation. Establishing the effects of small molecules with different chemical properties on the aggregation and spreading of α -syn and tau will be important for the development of future therapeutic interventions.



The initial steps of protein aggregation can be monitored by the bimolecular fluorescence complementation assay (BiFC), based on the reconstitution of the Venus fluorescent protein. This assay enables us to monitor the release and uptake of proteins by cells, and to test the effect of different molecules, such as the ones described in our study. Reproduced from Dominguez-Meijide¹⁴; CC BY 4.0.

Prominent microglial inclusions in transgenic mouse models of α-synucleinopathy that are distinct from neuronal lesions¹⁵

Accumulation of α -syn aggregates is a pathological hallmark of a group of neurodegenerative diseases called α -synucleinopathies. α -syn is the major component of Lewy bodies and Lewy neurites, which are intracellular inclusions found in neurons of patients with Parkinson's disease (PD) and dementia with Lewy bodies (DLB). α -syn also accumulates in oligodendrocytes to form glial cytoplasmic inclusions.

The initial aim of the present work from the team at the Deutsches Zentrum für Neurodegenerative Erkrankungen was to study disease progression and features of α-syn lesions among transgenic (TG) mouse models of α -synucleinopathies and their correlation with α -syn conformers. The results presented in this paper published in Acta Neuropathologica Communication revealed major differences among the mouse lines in age-of-symptom onset and disease progression. Postmortem analysis though revealed an overall very similar appearance and distribution of the α -syn lesions in all the lines. However, strikingly, in addition to neuronal lesions, we found α-syn-positive inclusions in microglia in all four lines.

This unexpected finding of robust inclusions in microglia in α -syn TG mice suggests that there is a link between the neuronal and microglial inclusions in α -syn TG mice.

Classification of human tauopathies based on tau filament folds¹⁶

Abnormal accumulation of misfolded tau protein in filaments characterises numerous neurodegenerative diseases – collectively called tauopathies for this very reason. In this study the MRC team established the structures of tau filaments from a further eight tauopathies. Their findings suggest a hierarchical classification of tauopathies, which holds important implications for future diagnostic and treatment approaches.

Previously, tauopathies have been largely characterised through clinical diagnosis and post mortem neuropathology. When taken alongside their previous work, these cryo-EM findings suggest a new, hierarchical method by which to classify tauopathies on the basis of their filament folds. This approach has also led the group to identify a new disease, named Limbic-predominant Neuronal inclusion body 4R tauopathy (LNT), based on the observation that the structures of tau filaments from an individual diagnosed as progressive supranuclear palsy (PSP) differed from all the others.

Moreover, this method provides new ways for studying the similarities and differences between diseases. For instance, it was once thought that PSP and corticobasal degeneration (CBD) were closely related, as they are both clinically similar 4-repeat tauopathies. However, this paper published in Nature has shown that the tau folds of PSP and CBD are more disparate than was assumed. In fact, solving the structures of tau filaments from PSP has revealed a novel three-layered fold. Cryo-EM analysis has shown that PSP filaments are more similar to those of lobular glial tauopathy (GGT), and that argyrophilic grain disease (AGD filaments), which differ from the previous on account of their four-layered fold, are similar to those from CBD. Filaments with the AGD fold are also found in intron 10 mutation cases. Finally, the structures of tau filaments from cases of (familial British dementia) FBD and familial Danish dementia (FDD) are the same as those from Alzheimer's disease and primary age-related tauopathy (PART).

This new classification complements the previous clinical diagnostic and neuropathological approaches, and allows for the identification of new tauopathies. Even though the different folds are made of the same protein, they give rise to different diseases. The molecular mechanisms by which these different folds are formed remain unknown and are an ongoing topic of research. Deciphering these mechanisms would hold huge implications for the diagnosis and treatment of tauopathies.



A novel way of characterising tauopathies on the basis of their filament folds. Adapted from Shi et al., https://doi.org/10.1101/2021.05.28.446130; CC BY 4.0.

Microglial inclusions and neurofilament light chain release follow neuronal α-syn lesions in long-term brain slice cultures¹⁷

Cerebral proteopathies such as Alzheimer's disease and Parkinson's disease are human age-related disorders that do not naturally arise in animals. Proteopathic lesions develop many years, if not decades before the first symptoms occur, and lesions at death are likely to be different from the ones driving the disease. Moreover, the cell-to-cell spreading of proteopathic lesions is largely a cell-non-autonomous process and largely dependent on the host environment. Thus, cerebral proteopathies are best studied in a living environment that closely mimics the adult or aged brain. While mice have been instrumental in the past to study such induction and propagation of pathogenic seeds, mouse models are time-consuming, costly, and experimental manipulations to mechanistically understand the disease often challenging.

To this end the team from the Deutsches Zentrum für Neurodegenerative Erkrankungen has developed long-term slice cultures from postnatal mouse brain and achieved the seeded induction and propagation of murine and human α -syn inclusions in a complex cellular brain environment. We find that microglia inclusions follow the neuronal α -syn lesions upon seeded induction. We also identified a human anti- α syn antibody that blocks the induction of these lesions and also the spreading along neural pathways. To foster translation, progression of α -synucleinopathy could be monitored by the neurofilament light chain protein release into the culture medium, a biomarker used in preclinical animal studies as well as in clinical settings.

Their paper published in the journal Molecular Neurodegeneration presents this recent milestone finding that brain slices derived from surgical resections are stable *in vitro* for up to three weeks if cultured in human cerebral spinal fluid. Thus, we applied the parameters from the murine cultures to the resection-derived adult human brain tissue cultures and succeeded in inducing α -syn inclusion in a true adult human brain environment.



Distribution of aggregates in transgenic hippocampal slice cultures treated and analysed at 5 weeks post-treatment. Reproduced from Barth¹⁷; CC BY 4.0.

New insights into the behaviour of tau seeded aggregation in neural tissue¹⁸

During neurodegenerative disorders such as Alzheimer's disease, the protein tau forms dense aggregates inside cells of the brain. The question of how this pathology spreads from once cell to another during the progression of disease is a matter of debate. Aggregates could form spontaneously within each cell, or might spread in a prion-like or virus-like manner where aggregated tau 'seeds' normal tau, producing further aggregates. Understanding which of these processes dominates is important as it will inform how best to interfere with the process for medical benefit.

A recent paper from the MRC team in Acta Neuropathologica Communications has shed light on this issue. They developed a new model of tau pathology by culturing thin slices of mouse brain in lab conditions over periods of weeks. The

model allowed precise control over the concentration of tau seeds that the system is exposed to, in contrast to living animals where this is hard to achieve. This meant that the authors could see what happens when slices were exposed to very low levels of tau, similar to those found in the brain. Seeded aggregation of tau occurred readily at high concentrations of tau seed, as expected. Surprisingly however, seeded aggregation entirely disappeared when physiological concentrations of tau were used. The results suggest that healthy brain tissue possesses mechanisms that resist tau seeding at low concentration.

The study suggests that tau aggregates may occur by forming independently in each cell during disease, or that the mechanisms of resistance are lowered in the diseased brain tissue to allow prion-like spread. Understanding how cells resist seeding may therefore uncover new targets to prevent tau pathology spreading during neurodegeneration.



Timeline of organotypic hippocampal slice culture preparation and treatment; schematic of the production of Organotypic hippocampal slice cultures (OHSCs) and resulting imaging. Adapted from Miller¹⁸; CC BY 4.0.

A stem cell-based model offers new insights into the mechanisms of neuronal loss in Parkinson's disease¹⁹

The collaboration between IMPRiND partners University of Oxford and CNRS led to a strong working laboratory model. They used induced pluripotent stem cells (iPSC) derived from both healthy subjects and patients with the α -syn gene defects to generate human dopaminergic neurons that are primarily affected in Parkinson's disease. They found a way of 'amplifying' in a fairly pure form, the main constituent, called fibril, of α -syn clumps directly from post-mortem Parkinson's brains. When they added these brain-derived fibrils onto the human dopaminergic neurons, they successfully triggered the aggregation of α -syn inside the cells and observed progressive neuronal loss. Reporting in Nature Communications, this model was used to show that the two main determinants of neuronal death are: (a) the abundance of α -syn inside nerve cells, and (b) the structure it acquires when it assembles into aggregates. By tracking the molecular interactions of the toxic forms of α -syn aggregates in living cells, they discovered that they cause damage partly by evading the protective effects of PARK7/DJ-1. Deletion of DJ-1 in iPSC-derived neurons increased α -syn aggregation and neuronal death. This could explain why loss of function mutations in DJ-1 in patients causes Parkinson's disease.

These findings are important because they provide a fully human model to decipher how α -syn clumps cause nerve damage. This model will allow us to start targeting the toxic effects of α -syn clumps with novel therapeutics.



Fibrils amplified from post-mortem Parkinson's brain were used to trigger endogenous α -syn aggregation and death in patient-derived neurons. By G. Tofaris; CC BY 4.0.

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