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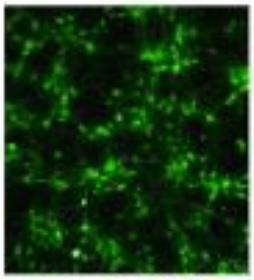

Oxford Parkinson's Disease Centre

Welcome to the Oxford Parkinson's Research Day 2025

Tuesday 3rd June

Programme and abstracts

wellcome

Medical Research Council

asap Aligning Science Across Parkinson's

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Biotechnology and Biological Sciences Research Council

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UNIVERSITY OF OXFORD

The event has been kindly supported by the Christ Church College Research Centre

OXFORD PARKINSON'S DISEASE CENTRE

Welcome

Welcome to the Oxford Parkinson's Research Day 2025 showcasing the breadth and diversity of research in Parkinson's underway across the University of Oxford. We are pleased to present a packed day of research talks and poster presentations with an expected attendance of over 180 participants and 38 posters from across the pre-clinical and clinical sciences.

Since its launch in 2010 originally funded by the Monument Trust Discovery Award from Parkinson's UK, the Oxford Parkinson's Disease Centre (OPDC) has established itself as a world-leading multi-disciplinary translational research program. Work at the Centre spans multiple pre-clinical and clinical Departments applying cutting-edge research techniques to drive translational work towards a better understanding of disease mechanisms for disease prediction, earlier diagnosis, better biomarkers and new treatments. We would like to thank our current major funders: Parkinson's UK, the Medical Research Council (MRC), the Michael J Fox Foundation, the Oxford/GSK Institute for Molecular and Computational Medicine (IMCM), the Wellcome Trust and the Aligning Science Across Parkinson's (ASAP) Collaborative Research Network for supporting Parkinson's research at the University of Oxford.

Today, we are delighted to welcome four keynote speakers: -

1. Günter Höglinger, University of Munich: *"Towards a Biological Definition of Parkinson's disease'.*
2. David Rubinsztein, Dementia Research Institute, University of Cambridge: *'Autophagy and Alpha-synuclein'*.
3. Stephanie Cragg, Dept of Physiology, Anatomy & Genetics, University of Oxford: *'Modulating dopamine transmission in health and disease: old friends and new stars'*.
4. Michael Johnson, Imperial College London: '*The Landmark study in Parkinson's'.*

Today's programme is arranged around four sessions covering a wide range of translational work into Parkinson's: "Clinical Research into Parkinson's"; "Cellular models and target discovery"; "Neuronal circuits underlying Parkinson's"; and "Molecular mechanisms and related biomarkers". In addition to the talks, there will be poster presentations on display during the refreshment breaks, during lunch, and at the drinks reception.

We would like to thank our special guests; David Dexter, Director of Research, Parkinson's UK, who will open the proceedings, and James Ferguson, Consultant Hepatologist, Honorary Professor University of Birmingham, who will say a few words about living with Parkinson's. James has also kindly agreed to present the Best Poster Award voted for by all attendees from the many we have on display today. We are also joined by Chris Kobylecki, Manchester Centre for Clinical Neurosciences, who will discuss "Translation into clinical practice" in the first morning session.

We hope you have an enjoyable and stimulating day and invite you to stay and attend a drinks reception after the talks at 5.30pm.

Richard Wade-Martins
Principal Investigator

Michele Hu
Co- Principal Investigator

Programme Talks held in Seminar room 20-138 & Seminar room 20-026

0830-0900 Registration and refreshments in foyer	
09:00	Welcome: **Richard Wade-Martins, Dept of Physiology, Anatomy & Genetics, University of Oxford**
09:05	Introduction: **David Dexter, Director of Research, Parkinson's UK**
Session 1: Clinical Research into Parkinson's. Chair: Michele Hu, Nuffield Dept of Clinical Neurosciences, University of Oxford	
09:10	**James Ferguson, Consultant Hepatologist, Honorary Professor University of Birmingham** Living with Parkinson's
09:15	**Michele Hu, Nuffield Dept of Clinical Neurosciences, University of Oxford** Audience vote: Do you support the new Biological Definition of Parkinson's disease?
09:20	**KEYNOTE LECTURE: Günter Höglinger, Dept of Neurology, Ludwig-Maximilians-University Hospital, Munich** Towards a Biological Definition of Parkinson's disease
09:50	**Chris Kobylecki, Manchester Centre for Clinical Neurosciences, Salford** Translation into Clinical Practice
10:10	**Karolien Groenewald, Nuffield Dept of Clinical Neurosciences, University of Oxford** Translation into Clinical Trials-Discovery Cohort
10:30	**Panel discussion, Q&As, repeat audience vote**
1045-1115 Refreshments and Posters in the foyer & lower atrium	
Session 2: Cellular models and target discovery. Chair: Ashwini Oswal, Nuffield Dept of Clinical Neurosciences, University of Oxford	
1115	**Sally Cowley, James and Lillian Martin Centre for Stem Cell Research, University of Oxford** Microglia handling of aggregation-prone proteins
1135	**Rachel Heon-Roberts, Dept of Physiology, Anatomy & Genetics, University of Oxford** CRISPR interference screening of Parkinson's disease risk genes in human neurons reveals modulators of □-synuclein aggregation
1155	**KEYNOTE LECTURE: David Rubinsztein, Dementia Research Institute, University of Cambridge** Autophagy and Alpha-synuclein
1225	**Flash talk (No. 29 poster): Pietro Luca Ratti, Nuffield Department of Clinical Neurosciences, University of Oxford** Sleep benefit in Parkinson's disease: a reappraisal based on video analysis.
1230	**Flash talk (No. 1 poster) Kaitlyn Cramb, Dept of Physiology, Anatomy & Genetics, University of Oxford** Dopamine but not glutamate release is disrupted in Parkinson's patient-derived dopaminergic neurons due to impaired synaptic vesicle loading
1235	**Flash talk (No. 7 poster) Katherine Brimblecombe, Dept of Physiology, Anatomy & Genetics, University of Oxford** Exploiting our understanding of selective vulnerability of dopamine axonal subpopulations to identify a2δ as a promising target for Parkinson's
1240-1340 Lunch and Posters in the foyer & lower atrium	

Session 3: Neuronal circuits underlying Parkinson's. Chair: Charmaine Lang, Nuffield Dept of Medicine, University of Oxford	
1340	**Camille Loiseau, MRC Brain Network Dynamics Unit, University of Oxford** Interplay between Lewy pathology and macroautophagy in midbrain dopaminergic neurons *in vivo*
1400	**Shenghong He, Parkinson's UK Senior Research Fellow Elect, Nuffield Dept of Clinical Neurosciences, University of Oxford** Neural dynamics and neuromodulation in Parkinson's disease
1420	**Oliver Curry, Dept of Physiology, Anatomy & Genetics, University of Oxford** 3D Droplet Printing of a Human Cortico-striatal-dopamine Microcircuit to Model Parkinson's Disease
1440	**KEYNOTE LECTURE: Stephanie Cragg, Dept of Physiology, Anatomy & Genetics, University of Oxford** Modulating dopamine transmission in health and disease: old friends and new stars
1510-1540 Refreshments and Posters in the foyer & lower atrium (Voting deadline 1540)	
Session 4: Molecular Mechanisms and related biomarkers. Chair: Laura Parkkinen, Nuffield Dept of Clinical Neurosciences, University of Oxford	
1540	**Isar Nassiri, Nuffield Dept of Clinical Neurosciences, University of Oxford** Studying Selective Vulnerability in Parkinson's disease using Spatial Transcriptomics
1600	**Nick Gatford & Chor Lai Lam, Nuffield Dept of Clinical Neurosciences, University of Oxford** Spatio-temporal resolution of a-synuclein pathology in human dopaminergic neurons
1620	**Sophie Farrow, Parkinson's UK Senior Research Fellow, Dept of Physiology, Anatomy & Genetics, University of Oxford** Integrating 3D genomic and epigenomic data to enhance gene-drug target discovery in Parkinson's
1640	**Ira Milosevic, Nuffield Department of Medicine, University of Oxford** Targeting endocytic pathways in Parkinson's disease
1700	**KEYNOTE LECTURE: Michael Johnson, Imperial College London** Molecular causal inference and the Landmark Parkinson's Project
1730	**Poster Prize Awards** presented by James Ferguson
1735-1830 Formal programme ends. Drinks Reception and Posters in the lower atrium	
1830	Research Day ends

Session 1 Talk abstracts:
Clinical Research into Parkinson's
Chair: Professor Michele Hu

KEYNOTE LECTURE

Professor
Günter Höglinger, MD

Department of Neurology, Ludwig-Maximilians-University (LMU) Hospital, Munich, Germany

Towards a Biological Definition of Parkinson's disease

Günter Höglinger is the Director of the Department of Neurology at the Ludwig-Maximilians-Universität (LMU), and an expert in the design and conduct of clinical trials, in the evaluation of safety and efficacy of molecular interventions with disease-modifying intent. He is also the Senior Scientist at the German Center for Neurodegenerative Diseases (DZNE) and Principal Investigator at the Cluster of Excellence for Systems Neurology (SyNergy), Munich, Germany. His previous experiences include Deputy Director of the Department of Neurology at the Philipps University of Marburg, and Chair of Translational Neurodegeneration at both the Technical University of Munich and DZNE Munich. His scientific work is dedicated to the neurobiology of synucleinopathies and tauopathies

Clinical Research into Parkinson's

Dr Christopher Kobylecki

Department of Neurology,
Manchester Centre for Clinical
Neurosciences,
Northern Care Alliance
NHS Foundation Trust, Salford

Biological definition of Parkinson's: translation into clinical practice

[1] Department of Neurology, Manchester Centre for Clinical Neurosciences, Northern Care Alliance NHS Foundation Trust, Salford, UK

[2] Division of Neuroscience, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

Recent developments in Parkinson's include a biological definition of the condition. Two differing yet complementary definitions have been proposed, namely the SynNeurGe and Neuronal alpha-synuclein disease (NSD) criteria. Both systems are based around the detection of pathological alpha-synuclein deposition using cerebrospinal fluid seed amplification assays (SAA), but have important differences in methods used to detect neurodegeneration in Parkinson's and the role of genetics in the definition of the condition. In addition, the NSD criteria propose biological staging of Parkinson's, whereas the SynNeurGe criteria do not cover this aspect. While alpha-synuclein SAA is a promising diagnostic tool for Parkinson's and related conditions, it is important to consider potential issues around the future implementation of these criteria in clinical practice.

In this talk I will review the applicability of the SynNeurGe and NSD criteria to clinical practice. I will discuss potential issues, including: problems of co-pathology; the accuracy and reproducibility of alpha-synuclein SAA; equity and ethical issues; and the potential pitfalls of biological staging of Parkinson's. The aim of the talk is to give a balanced understanding of the potential implications of proposed biological definition of Parkinson's for clinicians managing the condition.

Clinical Research into Parkinson's

Dr Karolien Groenewald

Nuffield Department of Clinical Neurosciences, University of Oxford

Translation into Clinical Trials-Discovery Cohort

The Syntara trial ("A Phase 2a, Multi Centre, Double-Blind, Randomized, Placebo-Controlled, Parallel-Group Study to Assess the effect of 12 Weeks Treatment with Oral PXS-4728A on Microglia Activation, as Measured by Positron Emission Tomography, in Participants with Isolated Rapid Eye Movement Sleep Behavior Disorder") is a world-first therapeutic trial in prodromal Parkinson's Disease.

In this talk, we will discuss insights from the Oxford Discovery Cohort, explore the role of neuro-inflammation in Parkinson's Disease and illustrate how the combination of animal models, clinical data, wet biomarkers, imaging and postmortem studies can inform therapeutic trials, aiming to change the course of neurodegeneration.

Session 2 Talk abstracts:
Cellular models and target discovery
Chair: Dr Ashwini Oswal

Cellular models and target discovery

Dr Sally A. Cowley

James and Lillian Martin Centre for Stem Cell Research, Sir William Dunn School of Pathology, University of Oxford

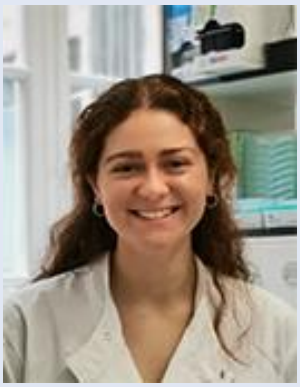
Microglia handling of aggregation-prone proteins

[1] Sir William Dunn School of Pathology

Microglia are increasingly implicated in the pathophysiology of neurodegenerative disease, including Parkinson's. We have pioneered methods for the efficient differentiation of microglia from human induced Pluripotent Stem Cells. We use these iPS-cell models to examine the role of neurodegenerative disease-associated genes in microglia, especially in relation to inflammatory pathways, and the processing of aggregation-prone proteins, including alpha-synuclein and tau. As a recent exemplar, using Cryo-EM we have observed that fibrillar tau aggregates that are taken up by microglia can be packaged into extracellular vesicles, and we have demonstrated that these EVs can seed aggregation in downstream neurons.

Cellular models and target discovery

Ms Rachel Heon-Roberts



Department of Physiology, Anatomy and Genetics / Kavli Institute for Nanoscience Discovery, University of Oxford.

CRISPR interference screening of Parkinson's disease risk genes in human neurons reveals modulators of α-synuclein aggregation

Rachel Heon-Roberts[1], Sarah NJ Franks[1], Camille Loiseau[2], Elly Robles Rodriguez[1], Stewart W. Humble[1], Peter Holderrieth[1], Martha Lavelle[1], Maria Claudia Caiazza[1], Amelia Smith[1], Peter Magill[2], Richard Wade-Martins[1], Brent J Ryan[1]

[1] Department of Physiology, Anatomy and Genetics, University of Oxford

[2] MRC Brain Network Dynamics Unit, Nuffield Department of Clinical Neurosciences, University of Oxford

Parkinson's disease is the second most common age-related neurodegenerative disease and is characterised by the death of neurons in the substantia nigra as well as other brain areas, such as the cortex, over the course of the disease. The main pathophysiological finding in the affected brain regions at autopsy is aggregates of the pre-synaptic protein α-synuclein, which forms Lewy bodies and Lewy neurites. This aggregation process is thought to cause cellular dysfunction, which contributes to neuronal death, in substantial part through impacting the function and degradation of mitochondria. Likewise, environmental toxins targeting mitochondrial respiration or mutations in the mitophagy-associated proteins PINK1 and PRKN are both causative of PD.

Genome-wide association studies have identified over 90 loci which have been linked to sporadic PD genetic risk, to which they contribute by undefined mechanisms. We hypothesised that these genes may alter the rate of α-synuclein aggregation, or impair effective clearance of mitochondria targeted for degradation. We screened a library of 71 candidate genes sourced from identified risk loci in a human iPSC-derived neuronal model where α-synuclein aggregation was seeded by in vitro-generated α-synuclein pre-formed fibrils. Measuring α-synuclein aggregation and mitophagy by immunostaining revealed modulators of α-synuclein aggregate formation, indicating that several genes contribute to PD aetiology through this pathway. Many of the genes that alter α-synuclein levels were also found to modulate levels of the mitophagy marker phospho-serine 65 ubiquitin, and there was mild correlation between the readouts, suggesting a pathological cascade where α-synuclein aggregation contributes to mitochondrial dysfunction.

KEYNOTE LECTURE

Professor David Rubinsztein

Cambridge Institute for Medical Research and UK Dementia Research Institute, University of Cambridge

Autophagy and Alpha-synuclein

David Rubinsztein is Professor of Molecular Neurogenetics and a UK Dementia Research Institute Group Leader at the University of Cambridge. His laboratory is based in the Cambridge Institute for Medical Research. Dr. Rubinsztein earned his MB ChB, BSc (Med)Hons, and PhD degrees from University of Cape Town. He came to Cambridge in 1993 as a Senior Registrar in genetic pathology and was the first person to complete formal training in this field in the UK. His research is focused in the field of autophagy, particularly in the context of neurodegenerative diseases. His laboratory pioneered the strategy of autophagy upregulation as a possible therapeutic approach in various neurodegenerative diseases, and has identified drugs and novel pathways that may be exploited for this objective. He has made contributions that reveal the relevance of autophagy defects as a disease mechanism and to the basic cell biology of this important catabolic process.

Professor Rubinsztein was elected Fellow of the Academy of Medical Sciences (2004), EMBO member (2011), Fellow of the Royal Society (2017) and membership of Academia Europaea (2022). He was awarded the Graham Bull Prize (2007), Thudichum Medal (2017), Roger de Spoelberch prize (2017), the Goudie Medal (2020) and The Movement Disorders Research Award from American Academy of Neurology (2024).

Session 3 Talk abstracts:
Neuronal circuits underlying Parkinson's
Chair: Dr Charmaine Lang

Neuronal circuits underlying Parkinson's

Dr Camille Loiseau

MRC Brain Network Dynamics Unit, University of Oxford

Interplay between Lewy pathology and macroautophagy in midbrain dopaminergic neurons *in vivo*

Camille Loiseau[1], Morgane Storey[1], Ben Edwards[1], Natalie Doig[1], Benjamin Vallin[2], Richard Wade-Martins[2] and Peter J. Magill[1]

[1] MRC Brain Network Dynamics Unit, Nuffield Department of Clinical Neurosciences, University of Oxford

[2] Department of Physiology, Anatomy and Genetics, University of Oxford

Several motor symptoms of Parkinson's arise from the degeneration of midbrain dopaminergic neurons (DaNs) of the substantia nigra pars compacta (SNc). This degenerative process correlates with the accumulation of misfolded and aggregated α-synuclein (α-Syn), a major constituent of Lewy Bodies (LBs), in SNc DANs.

Macroautophagy, a key intracellular degradation pathway, is thought to counteract LB formation and might be impaired in Parkinson's. Evidence suggests a 'vicious interplay' wherein disrupted proteostasis leads to α-Syn accumulation, which impairs macroautophagy, thence further reducing clearance of α-Syn aggregates. However, the role of macroautophagy in the emergence and progression of α-Syn pathology in SNc DaNs in vivo is unclear.

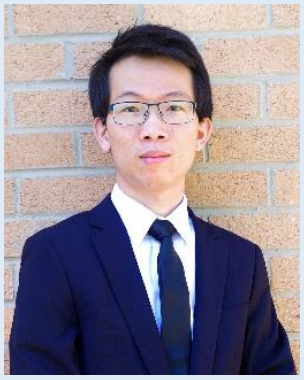
To address this, we compared SNc DaN phenotypes in control mice and macroautophagy-deficient (Atg7 KO) mice following dorsal striatal injections of pre-formed α-Syn fibrils (PFFs) to trigger LB-like pathology. Brains were analysed at 1, 3, and 6 months post-injection (mpi) and processed for unbiased stereology and single-cell image analysis to assess SNc DaN survival, phosphorylated α-Syn (pSyn) burden, and markers of macroautophagy machinery.

Stereological analysis revealed a detrimental combined effect of macroautophagy deficiency and PFF injection on DaN survival at early timepoints. However, loss of Atg7-dependant macroautophagy in SNc DaNs did not induce the de novo formation of LB-like (pSyn positive) aggregates in the absence of PFFs. Single-cell imaging data showed a rise in pSyn burden from 1 to 3 mpi across genotypes, followed by a decline by 6 mpi. Importantly, SNc DaNs from control mice showed LAMP1+ lysosome recruitment to pSyn aggregates, a response diminished in macroautophagy-deficient mice.

These findings suggest that, at early time points after exposure to misfolded/fibrillar α-Syn, SNc DaNs engage their macroautophagic machinery to cope with α-Syn pathology, which may confer transient resilience to degeneration. Impaired macroautophagy hampers this protective response, accelerating disease progression.

Neuronal circuits underlying Parkinson's

Dr Shenghong He



Nuffield Department of Clinical Neurosciences, University of Oxford

Neural dynamics and neuromodulation in Parkinson's disease

[1] MRC Brain Network Dynamics Unit, Nuffield Department of Clinical Neurosciences, University of Oxford

Parkinson's is a progressive neurological disorder characterised by the degeneration of dopaminergic neurons, leading to motor symptoms such as tremor, rigidity, bradykinesia, and gait disturbances. These symptoms respond variably to dopaminergic medication and deep brain stimulation (DBS). Additionally, attempts to improve parkinsonian rigidity and bradykinesia with DBS can sometimes deteriorate tremor or gait. This variability may be due to the distinct underlying neural mechanisms associated with different parkinsonian symptoms and their unique interactions with DBS treatments.

In my Parkinson's UK Senior Research Fellowship project, I will combine the well-established DBS treatment with an emerging and promising technology called low-intensity transcranial focused ultrasound stimulation, to gain a deeper understanding of the brain network dynamics responsible for different parkinsonian symptoms, and to use this knowledge to improve brain stimulation techniques for better clinical outcomes in treating Parkinson's.

Neuronal circuits underlying Parkinson's

Oliver Curry

Dept of Physiology, Anatomy and Genetics, University of Oxford / Kavli Institute for Nanoscience Discovery / Department of Chemistry, University of Oxford

3D Droplet Printing of a Human Cortico-striatal-dopamine Microcircuit to Model Parkinson's Disease

Oliver Curry[1, 2, 3], Ricardo Marquez Gomez[1, 2], Linna Zhou[2,3], Hagan Bayley[2,3], and Richard Wade-Martins[1,2]

[1] Department of Physiology, Anatomy and Genetics, University of Oxford

[2] Kavli Institute for Nanoscience Discovery, University of Oxford

[3] Department of Chemistry, University of Oxford

Damage to the cortico-striatal-dopamine circuit is highly conserved across Parkinson's Disease (PD). In this circuit, neurodegeneration of dopamine neurons disrupts the communication between the cortex and striatum, leading to the distinctive PD symptomatology. With a steadily increasing prevalence of PD globally there has never been a more urgent time for in-vitro model development, specifically a model able to target the under-studied area of synaptic deficits.

Here we show that the 3D printing method, and derivatives of the method, successfully print droplets in an organised manner. Immunocytochemistry of our droplet circuits validates the differentiation of cortical, striatal, and dopaminergic cultures. It further demonstrates that cell droplets remain compartmentalised over time, and bridging regions confine and direct axonal growth. Triculture circuits remain healthy for over 100 days and calcium recordings demonstrate functional longevity, with highly active populations recorded over day 175.

Our results demonstrate a characterised and functional cortico-striatal-dopamine circuit. The cells grow together in a triculture circuit, surpassing the viability of similar 2D cultures. The project aims to include disease lines to probe inter-patient variability, and there will be a specific focus on neuromodulation of dopamine by localised receptors and quantification of changes across lines and conditions.

KEYNOTE LECTURE

Professor
Stephanie Cragg

Dept of Physiology, Anatomy and Genetics,
University of Oxford

Modulating dopamine transmission in health and disease: old friends and new stars

Stephanie Cragg is Professor of Neuroscience in the Department of Physiology, Anatomy and Genetics, at the University of Oxford, in association with a Tutorial Fellowship at Christ Church College. She is a co-founder of the Oxford Parkinson's Disease Centre, of the international Dopamine Society, and of the inaugural Editorial Board for npj Parkinson's Disease.

She is currently serving as President of the International Society for Monitoring Molecules in Neuroscience, as Council Member of the International Basal Ganglia Society, and on advisory boards for the journals Addiction Neuroscience and ACS Chemical Neuroscience.

Her laboratory focuses on understanding the control of dopamine neurotransmission and related circuits within the basal ganglia, and their dysfunction in disease. She heads an international collaborative team in the Collaborative Research Network launched by the Aligning Science Across Parkinson's Initiative through the Michael J. Fox Foundation in 2020, through which Team Cragg are advancing knowledge of neuromodulation in striatal circuits. Her work has helped to define key mechanisms that regulate dopamine transmission, including long-debated reciprocal interactions with striatal cholinergic interneurons. Her group's findings are also adding to growing appreciation that non-neuronal astrocytes, act as extensions of these neural circuits and shape striatal function, including for cholinergic interneurons and dopamine.

Today she will highlight some of these findings in her talk.

Session 4 Talk abstracts:
Molecular Mechanisms and related biomarkers
Chair: Professor Laura Parkkinen

Mechanisms and related biomarkers

Dr Isar Nassiri

Nuffield Department of Clinical Sciences, University of Oxford

The systematic investigation of selective neuronal vulnerability in Parkinson's disease using spatial and single-nuclei transcriptomics integration

Isar Nassiri[1,2], Annie Ziyi Zhao[2], Laura Parkkinen[1,2]

[1] Oxford-GSK Institute of Molecular and Computational Medicine (IMCM), Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford

[2] Nuffield Department of Clinical Neurosciences, Oxford Parkinson's Disease Centre, University of Oxford

In Parkinson's disease, dopaminergic neurons of the substantia nigra (SN) show selective cell loss and build-up of α-synuclein aggregates in the remaining cells. Our study aims to investigate the molecular mechanisms that contribute to this vulnerability by integrating spatial and single-nuclei transcriptomics (ST).

We used GeoMx ST platform together with immunofluorescence staining with C-terminus α-synuclein antibody, LB509 which is less susceptible to Protein Kinase that is required for GeoMx workflow. The pathologist marked manually SN neurons with and without α-synuclein-positive Lewy bodies (LB) in segmented areas. GeoMx enabled us to collect expression information only from these segmented areas and sequence them. We integrated our ST results with a single-nuclei in-house and published datasets that included 8 donors with PD. After evaluating various tools extensively, we created and implemented a computational framework to manage the quality, integrate, and analyse spatial and single-nuclei transcriptomics datasets.

We projected 1303 snRNA-seq neurons onto the vulnerable (with LBs) and resistant (without LBs) neurons that were identified during the ST experiment. Integration resulted in an increase of the number of neurons in the ST profile from 70 to 564 cells. There were 801 genes that differentially expressed, with 61 being upregulated and 740 being downregulated ($P < 0.05$). The organization of microtubule cytoskeleton was impacted by the differentially regulated genes such as *NEFM, SOD1 and KLC1*. This indicates that there may be a problem with the assembly or degradation of microtubules in neurons that are vulnerable.

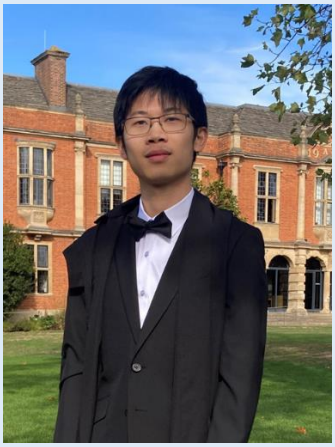
Our study showed that there are variations in the gene expression between neurons that are vulnerable and resilient, which can lead to significant insights. This knowledge can lead to targeted therapies that protect vulnerable neurons and slow disease progression.

Mechanisms and related biomarkers

Nick Gatford

Nuffield Department of Clinical Sciences, University of Oxford

Chor Lai Lam



Nuffield Department of Clinical Sciences, University of Oxford

Spatio-temporal resolution of α-synuclein pathology in human dopaminergic neurons

Nicholas J. F. Gatford[1,2], Chor Lai Lam[1,2] George K Tofaris[1,2]

[1] Nuffield Department of Clinical Neuroscience, University of Oxford

[2] Kavli Institute of Nanoscience Discovery, University of Oxford

The primary mechanism and subcellular localisation of α-synuclein toxicity in Parkinson's pathogenesis remain unknown. We spatially and temporally resolved proteomic and transcriptomic changes in human iPSC-derived dopaminergic neurons with increasing burden of pathological α-synuclein. We show that microscale α-synuclein aggregates are biochemically inert whereas misfolded α-synuclein proteoforms, signified by the formation of nanoscale intraneuronal puncta, are associated with impaired Sec61A translocon function at the endoplasmic reticulum (ER). α-Synuclein blocks the translational translocation of ER-processed proteins including the vacuolar-type ATPase V0a1 subunit, glucocerebrosidase, and Cathepsin B, causing defective organelle function, such as impaired lysosomal acidification and proteasomal recruitment. Reduction of pathological α-synuclein by pharmacological activation of proteasomal degradation mitigates the ER defect.

Our study offers a unifying mechanistic link between α-synuclein pathology and dysregulation of diverse organelle-associated proteins that are both translocon substrates and genetic modifiers as well as a therapeutic rationale for proteasomal activation with repurposed drugs in early Parkinson's disease.

Mechanisms and related biomarkers

Dr Sophie Farrow

Department of Physiology, Anatomy and Genetics, Oxford Parkinson's Disease Centre, Kavli Institute for Nanoscience Discovery, University of Oxford

Integrating 3D genomic and epigenomic data to enhance gene-drug target discovery in Parkinson's disease

Sophie L. Farrow[1,2,3], Elizabeth Cowan[1,2], Michele Hu[1,4], Richard Wade-Martins[1,2]

[1] Oxford Parkinson's Disease Centre (OPDC), Department of Physiology, Anatomy and Genetics, University of Oxford

[2] Kavli Institute for Nanoscience Discovery, University of Oxford, Dorothy Crowfoot Hodgkin Building, South Parks Road, Oxford

[3] Liggins Institute, University of Auckland, Auckland, 1023, New Zealand

[4] Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford

Introduction

Parkinson's disease (PD) is often described as a sporadic disorder, indicating that the precise cause(s) remain unknown. Despite this, genome wide association studies (GWAS) have identified >100 genetic variants associated with an increased risk of PD, implicating underlying molecular mechanisms yet to be fully understood. Pinpointing *how* and *why* these genetic variants increase disease risk remains a critical step for elucidating disease pathogenesis and therapeutic translation.

Methods

To establish function for PD-associated variants, we generated high-resolution multi-omic profiles (transcriptomic (RNAseq); chromatin accessibility (ATACseq); and chromatin conformation (Micro-C)) of iPSC-derived microglia (and dopamine neurons – not discussed here) from people with sporadic Parkinson's (PwP) and healthy controls (HC). We used integrative computational pipelines to analyse the resulting datasets, aiming to identify cell-type specific gene targets for PD risk loci.

Results

Transcriptomic analysis identified 32 differentially expressed genes (log2FC>2) in PwP-derived microglia, compared to HC, including *PAK6* and *GABRG2*. However, consistent with previous findings, global expression patterns showed limited clustering by disease status. Integrating chromatin profiles (Micro-C and ATACseq) enabled identification of both established and novel target genes at PD risk loci. One notable example is rs34504234, which resides in an accessible chromatin region and forms a significant chromatin interaction with *FDFT1,* a gene which is also upregulated in microglia from PwP. Recent studies have also highlighted *FDFT1*, over the previously assumed target gene (*CTSB*) at this locus.

Conclusion

The integration of multi-omic datasets is a critical step for resolving the functional consequences of PD-associated variants in disease-relevant cell-types. The findings presented here highlight the first stage of a larger integrative pipeline aimed to prioritise gene-drug targets for PD. Integration with whole genome sequencing will clarify whether certain gene targets/pathways are more likely to be affected in certain individuals, ultimately informing patient stratification and a more targeted approach for treating PD.

Mechanisms and related biomarkers

Associate Professor
Ira Milosevic

Nuffield Department of Medicine,
Centre for Human Genetics,
University of Oxford

Targeting endocytic pathways in Parkinson's disease

Ana Bura[1], Ana Caulino[2], Christine Rostosky[3], Nuno Raimundo[2,4], Ira Milosevic[1,2]

[1] Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford
[2] Multidisciplinary Institute for Ageing, University of Coimbra, Portugal
[3] European Neuroscience Institute, Georg August University Göttingen, Germany
[4] Penn State College of Medicine, PA, USA

Membrane trafficking at the synapse is among the most complex, rapid and tightly regulated processes in cell biology, and it is linked to several brain diseases including Parkinson's disease (PD), the second most common neurodegenerative disorder. Specifically, neuronal cells rely on endocytosis and synaptic vesicles (SV) recycling to sustain high rates of activity. The main pathway of SV recycling, clathrin-mediated endocytosis, builds on a clathrin coat formation and dissociation. Mutations in two uncoating factors, auxilin and synaptojanin-1, were found to cause early-onset PD. Auxilin is recruited to the clathrin coats due to the action of synaptojanin-1, which is itself brought to clathrin-coated pits by the key endocytic adaptor endophilin-A. Endophilin-A is directly linked to PD and neurodegeneration – its levels are altered in the cortex of PD patients, and it interacts with two hallmark PD proteins, the E3 ubiquitin ligase Parkin and the leucine-rich repeat kinase LRRK2, the most commonly disrupted gene in familial PD. We have reported that a partial loss of endophilin-A in mice results in neurodegeneration, ataxia, altered gait and motor coordination, defective SV recycling, impaired autophagy and shorter lifespan. Building on data generated by a comprehensive cell biology tool kit including biochemistry, microscopy at different scales and genome editing tools, we found that the impaired synaptic membrane trafficking results in selective neurodegeneration of dopaminergic neurons and/or neurons with long axons in PD. Further work on the potential synergistic function of several PD-linked proteins on synaptic endocytic pathway will also be presented. By defining the molecular and cellular networks in which these proteins operate, we aim to identify strategies for reversing the cellular vulnerabilities that cause PD or increase disease risk.

KEYNOTE LECTURE

Professor
Michael Johnson

Department of Brain Sciences,
Imperial College London

Molecular causal inference and the Landmark Parkinson's Project

Michael is Professor of Neurology and Genomic Medicine at Imperial College London, and Honorary Consultant Neurologist at Imperial College Healthcare. His research focuses on systems-level data integration to identify causal genes and predictive biomarkers which has resulted in drug target and therapeutic use patent applications and enduring pharma collaborations (for a detailed description of our current approach please see Haglund et al., ***Nature Genetics*** 2025:57:358-368).

Michael is on the Executive Management Committee for the Department of Brain Sciences at Imperial College London, and has previously served as Deputy Head of the Division of Clinical Translation and Interim Head of the Division of Neurology.

In collaboration with Parkinson's UK, he recently established a large multimillion public-private partnership for causal inference in Parkinson's disease (The Landmark Project). He co-leads the Advanced Therapeutics and Disease Modification Theme in the recently established UK Epilepsy Research Institute and is an advisor to the UK Parliamentary Health Ombudsman.

Poster List (presenting author underlined)

*Posters marked with a red asterisk are accompanied by an oral presentation during the day

1*

Dopamine but not glutamate release is disrupted in Parkinson's patient-derived dopaminergic neurons due to impaired synaptic vesicle loading

Kaitlyn M L Cramb, Iona Thomas-Wright, Humaira Noor, Sandor Szunyogh, Maria Claudia Caiazza, Ira Milosevic, Dayne Beccano-Kelly, Stephanie J Cragg, Richard Wade-Martins

Dopamine release defects are an early and consistently-observed pathological feature of Parkinson's disease, however the underlying molecular mechanisms have not yet been elucidated. Here we demonstrate dopamine release is impaired in patient-derived human dopamine neurons with *SNCA* triplication despite reaching equivalent maturations as assessed by whole-cell patch clamp electrophysiology. We find that this synaptic dysfunction is due to impaired vesicle loading as assessed by VMAT2 substrate, fluorescent false neurotransmitter (FFN) 206, which leads to reduction in synaptic vesicle pool size. This disruption in dopamine loading coincides with reduced VMAT2 levels, thus resulting in reduced dopamine content and increased dopamine degradation while dopamine synthesis remains intact. Importantly, supplementation with 100 µm L-DOPA allows for the rescue of these observed dopamine content and release defects. In contrast, neither glutamate release nor VGLUT2 levels are impaired in Parkinson's patient's cells, revealing a specificity of dysfunction towards dopaminergic neurons. Combined, these data reveal dopamine synaptic vesicle loading and specifically, VMAT2, as a critical target in Parkinson's disease therapies.

2

Modelling Lewy Pathology in Differentially Vulnerable Cortical and Dopaminergic Neurons from Patients with Alzheimer's and Parkinson's Disease

Ajantha Abey, Eden Mellor-Davis, Bryan Ng, Rachel Heon-Roberts, Becky Carlyle, Nora Bengoa-Vergniory, Richard Wade-Martins

Alzheimer's (AD) and Parkinson's disease (PD) feature progressive aggregate pathology and neurodegeneration in a regionally selective manner. Neuropathological studies show that alpha-synuclein deposits, while typically associated with PD, are also remarkably common in AD. However, their origin and role in AD is poorly understood and underappreciated. Therefore, we modelled synucleinopathy in induced pluripotent stem cell (iPSC)-derived neurons. We aimed to determine cell-type and disease specific determinants of cellular vulnerability, and the effects of pathological aggregates on live cells.

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iPSCs from patients with AD-related presenilin-1 mutations (n=6), PD-related leucine rich repeat kinase 2 mutations (n=6), and isogenic corrected (n=4) and healthy controls (n=4) were differentiated into both cortical and midbrain dopaminergic neurons. This enabled comparison of pathology in different neuronal subtypes from the same patient. We utilised a pre-formed fibril (PFF) model to induce Lewy pathology in neurons, and multielectrode arrays, high-content live imaging, and immuno-labelling to assess the drivers and effects of pathology.

PFF insult seeded time and dose-dependent synucleinopathy in iPSC-derived neurons which was phospho-alpha-synuclein+, TOM20+, P62+, and ubiquitin+, and strikingly morphologically akin to brain pathology, including Lewy body structures. TH+ cells in midbrain dopaminergic cultures were highly susceptible to aggregate formation, while cortical neurons were relatively resilient, except with PSEN1-Intron-4-deletion mutations. This differential vulnerability pattern was reversed for tau PFFs. We found cell-intrinsic deficits in calcium and autophagic flux that led to heightened vulnerability to aggregation in a cell-type and genotype-dependent manner. Furthermore, PFFs led to disease-relevant, time-dependent functional perturbations, including on neurite outgrowth, synaptic density, and nuclear morphology.

These results are the first demonstration of synucleinopathy in an AD cell model, an overlooked aspect of the disease. These also demonstrate that iPSC-derived neurons exhibit cell-type and pathology-dependent differences in vulnerability that reflect selective vulnerability patterns in the brain, allowing for insights into disease mechanisms and therapeutic targets.

3 Investigating BIN1 involvement in tau handling and extracellular vesicle secretion in human iPSC-microglia

Anne Hedegaard, Maria K Karabova, Emma Mead, Theresa A Day, Basavaraj Hooli, Yaming Wang, William S James, Sally A Cowley

Background: BIN1 has emerged as a very attractive genetic target for sporadic Alzheimer's Disease (AD), being the next-highest risk factor after APOE. Certain BIN1 isoforms have been shown in mouse microglia to contribute to increased incorporation of tau into extracellular vesicles (EVs), and conversely, knocking out BIN1 reduced tau spread via EVs (Crotti et al., 2019). This project utilises human microglia derived from induced Pluripotent Stem Cells (iPSC) to investigate the function of BIN1 in the context of tau processing by microglia, secretion via EVs, and potential implications for neuronal tau pathology.

Methods: We use human induced Pluripotent Stem Cell (iPSC)-derived microglia and manipulate their level of BIN1 expression through knockdown or overexpression of relevant isoforms. Exposing these isogenic microglia with low/endogenous/high BIN1 levels to tau protein (both generated recombinantly and isolated from AD brains) allows assessment of microglial processing of tau, along with incorporation of tau into secreted EVs, visualised with cryoET.

Results: Human iPSC-derived microglia express several of the expected BIN1 isoforms and we can achieve 60% BIN1 reduction using shRNAs. EVs purified from endogenous BIN1 microglia supernatants display canonical characteristics and proteomes. We tracked iPSC-microglial handling of tau after a period of uptake, followed by enzymatic wash and 24h of processing. Monomeric tau was mostly dealt with but fibrillar tau was still detected in the microglial cell lysate, released into the supernatant and importantly, associated with the EVs. Our cryo-tomography suggests that microglia processing of tau involves chopping up µm long fibrils into fragments for packaging inside nm-sized EVs.

Conclusions: The BIN1 expression of human iPSC-derived microglia resembles that of primary microglia, and we can manipulate the protein levels. While microglia take up and digest tau, they also appear to participate in a form of tau release, partly via EVs, and we are investigating the seeding capacity of the tau-containing EVs.

4 The neuropathological signature of REM sleep behaviour disorder in selectively vulnerable brainstem nuclei and the association with Parkinson's disease

Nita Alpin, David A Menassa, Laura Parkkinen

Background: REM sleep behaviour disorder (RBD), characterized by loss of muscle atonia and dream enactment during REM sleep, has gained significant attention for its association with Parkinson's disease (PD). The majority of RBD patients convert to PD or another type of synucleinopathy, implying that these patients may exhibit an early clinical manifestation of a neurodegenerative process. Partially due to limited material availability, no systematic post-mortem studies on RBD pathophysiology have been conducted.

Methods: Using comprehensive clinico-pathological profiling, we constructed a well-matched cohort of 32 PD patients with RBD and 32 PD patients without RBD from the Oxford and Imperial Brain Banks. Immunohistochemistry was utilised to assess alpha-synuclein pathology and cell densities in the pontine nuclei (e.g., locus coeruleus, LC) implicated in REM sleep regulation and PD pathogenesis. LC norepinephrinergic neuronal loss and orexinergic modulatory profile were investigated utilising a QuPath-trained artificial neural network classifier.

Results: We identify greater alpha-synuclein burden within the LC of PD patients compared to those with comorbid RBD. The proportion of neurons affected by pathology (~43%) does not differ between groups. While LC norepinephrinergic cell densities are statistically equivalent between the groups, a marked reduction in orexinergic puncta density was observed in PD patients with RBD.

Conclusions: The difference in LC alpha-synuclein burden may indicate differential distribution and progression of pathology between the patient groups. As the proportion of diseased neurons was comparable between groups, the higher alpha-synuclein burden in the PD-only group may be related to extracellular pathology (i.e., Lewy neurites, ghost bodies). The observed reduction in orexinergic puncta in PD patients with comorbid RBD implicates the orexinergic system in the pathophysiology of RBD, independent of neuronal loss.

By characterising the topographical course of degenerative changes in selectively vulnerable nuclei, our research aims to provide critical insights into the underlying disease mechanisms of RBD and PD.

5 Development of pre-clinical models for patient stratification in Parkinson's disease

Sarah H Ellwood, Anna Lavayssiere, Sally A Cowley, Brent J Ryan, Michele T M Hu and Richard Wade-Martins

Objectives: Parkinson's Disease (PD) is the second most common neurodegenerative disease, with the majority of cases being idiopathic (iPD). iPD presentation varies between patients and the drivers behind this heterogeneity are unknown. To investigate this Lawton et al (2018) have clustered two PD cohorts into four subtypes revealing extremes of phenotype. Our aim is to test the hypothesis that pre-clinical iPSC models reflect clinical subgroups in iPD. We are generating 40 new idiopathic

PD induced pluripotent stem cell (iPSC) lines for differentiation into dopaminergic neurons to explore the mechanisms behind these phenotypic differences.

Methods: Fibroblasts from iPD patients were selected for reprogramming from two clusters displaying fast motor progression and slow motor progression respectively. Fibroblasts were reprogrammed into iPSCs using Sendai virus vectors. After infection fibroblasts were plated on mouse embryonic fibroblasts to support emerging clones. Clones were picked and grown to p10 where cells should be virus free and self-sustaining. Once free of virus each cell line is expanded, banked and put through quality control steps.

Results: We have generated and banked 40 new sporadic PD lines that have passed quality control (QC) steps. The cells were shown to be self-maintaining iPSC via FACs of pluripotency markers Nanog and Tra-1-60. All lines were tested for the presence mycoplasma and were found to be negative. SNP analysis was carried out and lines did not have any karyotypic abnormalities.

Conclusions: We have successfully reprogrammed >20 sPD fibroblasts into new iPSC lines which have been fully QC'd. These can now be utilized to investigate the mechanisms driving the phenotypic extremes of iPD, focussing on mitochondrial and lysosomal dysfunction which are common to PD.

6 Monitoring Expression and Function of Voltage-Gated Calcium Channels in Stem Cell-Derived Neuronal Models of Parkinson's

Parnaz Sharifi, Maria-Claudia Caiazza, Johanna Hoffman, Sophie Gibson, Fangjia Yang, Akansha Mehta, Elena Britti, Elliot Mock, Richard Wade-Martins

Parkinson's disease (PD) is characterised by the loss of dopaminergic neurons (DaNs) in the substantia nigra pars compacta (SNpc), while DaNs in the neighbouring ventral tegmental area (VTA) remain spared. The mechanisms underpinning this region-specific degeneration remain elusive. The sustained use of voltage-gated Ca^{2+} channels (VGCCs) by SNpc neurons during their pacemaking activity and associated Ca^{2+} oscillations has been implicated in the pathogenesis of PD. Using human induced pluripotent stem cells (iPSCs), we have generated midbrain DaNs from PD patients and healthy controls to investigate how disease burden alters activity-dependent Ca^{2+} handling.
Our profiling revealed dynamic VGCC expression during DaN maturation, with notable upregulation of R-type VGCCs. Electrophysiological recordings confirmed intrinsic pacemaking activity in healthy iPSC-derived DaNs, and pharmacological blockade of VGCCs paired with Ca^{2+} imaging demonstrated that R-type channels contribute significantly to Ca^{2+} oscillations. New data shows that these oscillatory patterns are altered in disease-derived lines, both in frequency and amplitude. Moreover, genetic and pharmacological manipulation of R-type channels further implicates the channels in modulating these activity-dependent signals. Together, these findings support a model where dysregulated Ca^{2+} influx via R-types contributes to altered neuronal excitability in PD. This iPSC-based platform enables mechanistic interrogation of human midbrain vulnerability and highlights R-type channels as a potential therapeutic target in PD.

7*

Exploiting our understanding of selective vulnerability of dopamine axonal subpopulations to identify α2δ as a promising target for Parkinson's

Katherine R Brimblecombe, Adam Harris, Bethan O'Connor, Lucille Duquenoy, Rishi Anand, Emanuel Lopes, Bradley M Roberts, Lauren Burgeno, Mark Walton, Stephanie J Cragg

We aimed to exploit known differences in the roles of voltage gated Ca^{2+} channels (VGCCs) in axonal dopamine release between neuronal populations that are differently vulnerable to Parkinsonian degeneration and investigate their intersection between population risk factors to Parkinson's (e.g. sex and α-synuclein load) to identify potential intervention strategies to limit Parkinsonian degeneration. We targeted $\alpha_2\delta$ subunits of VGCCs with gabapentinoids, administered either acutely on *ex-vivo* striatal slices (50 µM >30 mins) or chronically administered to *SNCA*-OVX mice for 3 days, twice daily (30 mg/kg). Using fast-scan cyclic voltammetry (FCV) to measure electrically evoked axonal dopamine release from *ex-vivo* striatal slices, we compared dorsal and ventral territories, to elucidate mechanisms that may contribute to their differing vulnerabilities to Parkinsonian degeneration. To disentangle the roles of $\alpha_2\delta$-1 and $\alpha_2\delta$-2, we performed FCV in male and female transgenic mice with disrupted gabapentinoid binding sites.

Following *in-vivo* administration of pregabalin, we performed open-field locomotion tasks and measured electrically evoked dopamine release with FCV on acutely prepared striatal sections. Gabapentinoids increased dopamine release, selectively in the (vulnerable) dorsal striatum, via sexually dimorphic mechanisms. In female mice, gabapentinoids increase dopamine release by disinhibition via GABA, whereas in males, dopamine is increased due to a reconfiguration of VGCCs, limiting the role of LTCC and subsequent coupling to RyRs. Furthermore, pregabalin lead to improvements in behavioural deficits in a mouse model of early Parkinson's.

These findings indicate the repurposing potential of gabapentinoids in early Parkinson's, whereby gabapentinoids modify dopamine axonal function from vulnerable territories and also affect striatal circuit function in a manner that is favourable to supporting dopamine release. We hope to exploit our understanding of regional vulnerability of dopamine neurons to approach an inflection point where we can move beyond pre-clinical understanding towards tangible help for people with Parkinson's.

8

Targeting ATP13A2 to treat alpha-synuclein cellular pathology in iPSC-dopamine neuron models of Parkinson's Disease

Elena Britti, Nicole Li, Akansha Mehta, Rachel L Heon-Roberts, Nancy Ahuja, Jess Mark, Floriana Licitra, Joanna Wolak, William McGuinness, Brent J Ryan, Charmaine Lang, Richard Wade-Martins

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to reduced dopamine levels. Point mutations, duplications and triplications of the α-synuclein (SNCA) gene, as well as mutations in GBA and ATP13A2, which encode for the lysosomal enzyme glucocerebrosidase (GCase) and a lysosomal ATPase, respectively, have been associated with familial PD. The asparagine-to-serine substitution at residue 370 (N370S) in GCase induces loss of enzymatic activity, increases α-synuclein release and is associated with an increased risk of PD. Unpublished data from our laboratory in GBA-N370S induced pluripotent stem cell (iPSC) derived dopaminergic neurons (DAns) reveals decreased

ATP13A2 mRNA and protein levels, together with lysosomal dysfunction. Given the central role of lysosomal and mitochondrial dysfunction in PD, as well as α-synuclein aggregation into fibrillary structures and α-synuclein secretion, understanding the interplay between these mechanisms is crucial.
We have developed PD models using pre-formed fibrils (PFFs) of α-synuclein in iPSC-derived DAns from patients carrying the GBA-N370S or SNCA-Triplication mutations.
Our findings demonstrate that PFFs treatment or increasing the endogenous levels of SNCA progressively increase phosphorylated SNCA (pS129), a marker of SNCA aggregation, and induces lysosomal dysfunctions in iPSC-derived DAns. We are now investigating the potential of this model to be used in drug discovery for PD.
Overall, our data support ATP13A2 activation as a promising therapeutic strategy for improving lysosomal dysfunction and ameliorating PD-related neurodegeneration in GBA-N370S DAns.

9 Characterisation and high-throughput quantification of alpha-synuclein pathology in Alzheimer's disease and dementia with Lewy bodies

Dominik Domanski, Robbie Allen, David A Menassa, Laura Parkkinen

Objectives: It is increasingly recognized that a majority of Alzheimer's disease (AD) cases present with mixed pathologies, such as alpha-synuclein pathology (aSyn) also seen in dementia with Lewy bodies (DLB) and Parkinson's disease, which may influence the disease development and therefore treatment. The misfolded aSyn accumulates in neurons and glial cells, especially in the limbic regions in AD. However, the degree to which this co-occuring aSyn pathology and different pathophysiological domains (e.g. inflammation) contribute to an individual's disease progression is not well understood.

Methods: The project aims to analyse selected cases from the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort (n~500), that include cases with pure AD, pure DLB, mixed AD-DLB and healthy controls. We have developed deep learning algorithms that recognise different aSyn pathologies, neuronal Lewy bodies and Lewy neurites and astroglial accumulation using Aiforia platform. Similarly, we have also established algorithms that classify Iba-1 immunopositive microglia according to three morphological classes: proliferative, homeostatic/ramified and phagocytic/amoeboid. Here, we will employ both of these algorithms in four different disease groups.

Results: This project will examine if the quantitative aSyn load and pattern is different in mixed AD-DLB versus pure DLB and how does it contribute to the progression of dementia. Furthermore, we will examine how the neuroinflammatory landscape will differ between pure AD/DLB and mixed AD/DLB and if this has clinical implications.

Conclusions: Deep learning and spatial statistics are powerful tools that reduce the complexity of multidimensional data to allow fast and efficient processing of multiple disease features. We are currently deploying these algorithms to study both concomitant aSyn pathology and inflammation to reveal the role they play in type and progression of dementia.

10 Over-expression of ATP13A2 rescues lysosomal dysfunction in Parkinson's induced pluripotent stem cell-derived dopamine neurons

Akansha Mehta, Nancy Ahuja, Elena Britti, Joanna Wolak, Charmaine Lang & Richard Wade-Martins

Parkinson's disease (PD) is characterised by the progressive loss of dopamine neurons in the brain, but the mechanism of this neurodegeneration remains to be elucidated. Mutation in the GBA gene encoding lysosomal enzyme β-glucocerebrosidase (GCase) has been implicated as an important genetic risk factor in PD of which GBA-N370S mutation contributes to the strongest risk variant resembling idiopathic PD. GBA-N370S mutation is linked to lysosomal dysfunction by loss of GCase activity, enlarged lysosomes, and alkalinised lysosomes. Similarly, mutations in ATP13A2, a lysosomal transmembrane protein, has been implicated in mediating lysosomal dysfunction in Kufor-Rakeb Syndrome (KRS), a rare form of PD.

In this study we investigated the potential role of ATP13A2 in context of GBA-N370S PD related lysosomal abnormalities. We first looked at ATP13A2 expression levels in control and GBA-N370S PD patients iPSC-derived dopamine neurons as well as PD idiopathic postmortem mid brain tissue and observed decreased ATP13A2 levels in both GBA-N370S PD and idiopathic PD patients. Subsequently, we knocked down ATP13A2 in control dopamine neurons which resulted in enlargement and accumulation of lysosomes by DQ-BSA assay and alkalinisation of lysosomes by lysosomal pH assay. Consistent with this, we also observed enlargement and accumulation of lysosomes in GBA-N370S PD dopamine neurons. Overexpression of ATP13A2 in both Control and GBA-N370 PD dopamine neurons led to the rescue of lysosomal number, size along with reacidification of lysosomes.

Overall, our findings show that reduced levels of ATP13A2 leads to disruption of lysosomal homeostasis, a key feature also in GBA-N370S PD. The ability of ATP13A2 overexpression to rescue these defects highlights the therapeutic potential of ATP13A2 for GBA PD.

11 Investigating the role of lysosomal calcium channels in Parkinson's Disease iPSC-derived Dopaminergic Neurons

Johanna L Hoffmann, Maria Claudia Caiazza, William McGuiness, Parnaz Sharifi, Stewart W Humble, Richard Wade-Martins

Parkinson's Disease (PD) is the second most common neurodegenerative disorder worldwide, characterised by the progressive loss of substantia nigra dopaminergic neurons and the accumulation of intracellular α-synuclein aggregates. Defective protein clearance - particularly through autophagy - is a hallmark of PD, with disrupted lysosomal function contributing to α-synuclein accumulation. At the same time, growing evidence implicates calcium signalling in disease progression, with lysosomes emerging as key regulators of this process.

My research focuses on ligand-gated Ca^{2+} channels on the lysosomal membrane—specifically, two-pore channels 1 and 2 and TRPML1—and their involvement in PD pathophysiology. Comparing iPSC-derived dopaminergic neurons from PD patients and healthy controls, I examine how these channels influence lysosomal function and calcium homeostasis. To better define their role, I complemented these studies with CRISPRi-mediated knockdown.

Using a combination of Ca^{2+} imaging techniques (FURA-2 AM and genetically encoded calcium indicators) and lysosomal functional assays to assess pH and glucocerebrosidase activity, I aim to uncover how lysosomal Ca^{2+} channel dysfunction contributes to PD and its potential as a therapeutic target.

12

Using CRISPRi Screens in Human iPSC-Derived Dopamine Neurons to Probe Endolysosomal Pathway Dysfunction in Parkinson's Disease

Gizem Onal, Hugo Fernandes, Victoria Lievens, Benedetta Carbone, Dehua Zhao, Lisa Mohamet, Brent J Ryan, Alastair Reith, and Richard Wade-Martins

Parkinson's disease (PD) remains a challenging neurodegenerative disorder characterized by the progressive loss of dopamine neurons in the substantia nigra, leading to motor and cognitive impairments. Despite significant advancements, understanding the precise molecular mechanisms underlying PD pathogenesis and identifying effective therapeutic targets remain elusive. Here, we present a high-throughput approach utilizing CRISPR-interference (CRISPRi) gene knockdown technology in induced pluripotent stem cell (iPSC)-derived dopamine neurons (DaNs) to elucidate the role of endolysosomal biology in PD, with the ultimate aim of identifying potential therapeutic targets.

Our methodology employs integrated catalytically inactive Cas9 (dCas9) machinery in control and PD patient-derived DaNs, enabling precise modulation of gene expression. Utilizing a 384-well automated format, we deliver a dual single-guide RNA(sgRNA) lentiviral library targeting over 100 individual lysosomal pathway-related genes of interest. By systematically perturbing gene expression, we aim to identify genes critical for endolysosomal function in DaNs. To assess lysosomal function, we use the DQ-BSA assay as the primary readout, allowing us to evaluate lysosomal activity, morphology, and endolysosomal trafficking dynamics as a multiparameter phenotypic readout.

We achieve a high efficiency of DaN transduction, with transduction rates reaching up to 90% by Day35 of DaN maturation. Through qRT-PCR analysis, we assessed the knockdown efficiency of selected guides in pilot studies, revealing significantly decreased gene expression with knockdown levels ranging from 50% to 95%. Our multiparameter DQ-BSA phenotypic assay identified candidate genes that either enhance or suppress lysosomal number, size, and activity, offering valuable insights into potential targets for modulating endolysosomal pathways in PD.

Next, we plan to validate the candidate results obtained from the CRISPRi screen using complementary functional assays (e.g., GCase activity, lysosomal pH) to further unravel the molecular mechanisms of specific candidate genes in neuronal homeostasis and/or pathogenesis. These efforts in control and patient-derived DaNs aim to uncover new avenues for therapeutic intervention.

13

Stools and stool-derived extracellular vesicles from patients with Parkinson´s disease show alpha-Synuclein species with seeding capacity

Livia Civitelli, Poppy Dorlandt-Stafford, Selene Lee, Elisabeth Dellar, Filip Scheperjans, Laura Parkkinen

Background: Parkinson's disease (PD) is a neurodegenerative disorder with no current cure or reliable biomarkers for early detection. PD pathology is triggered by misfolding and subsequent accumulation of alpha-Synuclein (aSyn) protein into pathological aggregates in the neurons and glial cells. Seed amplification assay (SAA) is a highly sensitive and specific diagnostic tool developed to detect pathological aSyn species in the cerebrospinal fluid (CSF) of PD patients. However, aSyn aggregates are found in multiple tissues and biosamples, including stools. In this study, we aimed to investigate the potential diagnostic value of SAA using stool samples from PD patients and healthy controls.

Methods: Stool samples from PD patients (n=45) and healthy controls (HC, n=35) were analysed for the presence of aSyn species using slot-blot with a panel of different aSyn antibodies and ELISA assays. Samples were subjected to SAA and the end-point products (SAA EP) were characterised using transmission electron microscopy (TEM). Extracellular vesicles (EVs) (n=5, per group) were isolated by using size exclusion chromatography and subsequently characterized by TEM. Seeding activity of isolated EVs was also tested using SAA, followed by TEM analysis of SAA EP.

Results: Protein extracts from both PD and HC stool samples showed the presence of pathological aSyn species in the slot-blot assay using the aSyn pS129 and MJFR-14 antibodies. ELISA assays showed statistically increased levels of total aSyn in PD samples compared to HC, but no differences in oligomeric aSyn levels were detected. SAA assay in stool protein extracts showed 55% sensitivity and 60% specificity, whilst this was increased to 100% sensitivity and 60% specificity using stool-isolated EVs from PD patients and HCs.

Conclusion: Our findings suggest that stool samples, particularly stool-isolated EVs, may serve as a valuable, non-invasive screening tool aiding to diagnose PD. However, further optimisation is required to improve specificity.

14

CRISPRi-Mediated Downregulation of α-Synuclein in human iPSC-Derived Dopaminergic Neurons to Investigate Parkinson's Disease Mechanisms

Ana Aragón-González, Chor Lai Lam, Hung-Ju Chueh, Benedict Tanudjojo and George K Tofaris

α-Synuclein accumulation is causatively linked to the degeneration of midbrain dopaminergic neurons (DANs). This is supported by the identification of familial Parkinson's disease (PD) patients with α synuclein gene (SNCA) multiplications, where increased gene dosage is associated with disease severity. Our objective is to suppress SNCA gene expression in mature iPSC-derived DANs with SNCA triplication in order to understand the reversibility of α-synuclein proteotoxicity.

To achieve this, we generated a doxycycline inducible dCas9-KRAB SNCATRIP human inducible pluripotent stem cell (hiPSC) line. HiPSCs were transduced with lentiviruses expressing SNCA gRNAs followed by puromycin selection. HiPSC-derived DANs (iDANs) were generated and maintained in culture. Doxycycline was added at different time points and cellular phenotypes in DANs were monitored up to day 80.

We confirmed the induction of dCas9-KRAB with doxycycline and gRNA mediated silencing of SNCA. Early activation during floor-plate induction was remarkably effective, fully silencing SNCA gene expression. α Synuclein levels were normalised when dCas9 was induced during the dopaminergic maturation phase. The reversibility of aggregation and aggregate-induced phenotypes will be presented.
Our study shows that an inducible CRISPRi can effectively reduce α-synuclein levels in mature hiPSC derived DANs from PD patients, providing a valuable model for investigating molecular mechanisms underlying synucleinopathies.

15

Establishing mitochondrial function and mitophagy assays in iPSC-derived dopaminergic neurons

Amelia Smith, Rachel Heon-Roberts, Elena Britti, Sarah NJ Franks, Becky Carlyle, Brent J Ryan

Parkinson's Disease (PD), the second most common neurodegenerative disease, is characterised by dopaminergic neuron loss in the substantia nigra pars compacta. Mitochondrial dysfunction has been implicated in PD pathogenesis. For example, environmental toxins targeting mitochondria are causative of PD. Additionally, mitophagy deficits are evident in monogenic forms of PD where mutations are present in mitophagy-regulating genes such as PINK1 and Parkin. While monogenic PD cases are described, these only account for a minority of patients (~10%). The majority of PD cases are idiopathic, where the exact cause is unknown. In these idiopathic cases, common genetic variants and environmental exposure are likely to both contribute. Therefore, we hypothesise that the interplay between genetic and environmental factors (toxicogenomic interactions) may explain a proportion of idiopathic PD cases.

This study assesses how dopaminergic neurons harbouring mutations in PINK1 perform mitophagy and respond to PD-causing toxins. To investigate the effect of loss of PINK1 function we have integrated patient/isogenic and CRISPR models in iPSC-derived dopaminergic neurons. We have differentiated iPSCs into dopaminergic neurons from a patient with a PINK1 mutation and its' isogenic control. Additionally, we have done this in iPSCs with CRISPR-mediated knockout of PINK1 and CRISPRi-mediated knockdown of PINK1 to phenocopy the loss of function PINK1 patient mutation.

Utilising these dopaminergic neurons we have assessed global mitophagy using the mitoSRAI reporter under a dox-inducible system with lentiviral delivery. To further elucidate active mitophagy pathways, we have explored levels of P65Ub, BNIP3 and NIX as read-outs of PINK1/Parkin-dependent and independent mitophagy under basal and toxin-induced conditions. In addition to mitophagy, mitochondrial oxidation has been investigated using mito-roGFP and mitoSOX assays.

We have established several assays to assess mitophagy and mitochondrial dysfunction in iPSC-derived dopaminergic neurons, the cell-type preferentially lost in PD. This will act as proof-of-concept work to understand toxicognomic interactions in idiopathic PD.

16 Optimisation of the protocol for the isolation of extracellular vesicles from stools in Parkinson's disease patients

Annabelle Bath, Darragh O'Brien, Michele T M Hu, Stephanie Fowler, Livia Civitelli

Extracellular vesicles (EVs) are secreted by almost all cell types, including neurons. In Parkinson's disease (PD), EVs are associated with the pathological species of the protein alpha-synuclein (aSyn), aiding its prion-like spread. Abnormal accumulation of aSyn is found primarily in the substantia nigra, but aggregates are also found in the periphery, such as the gastrointestinal tract (GI). Despite this, the mechanism underlying their presence in the GI tract remains debated.

In this study we refined a density gradient protocol to isolate EVs from stools of PD patients and healthy controls (HC) with the ultimate goal of investigating the presence of EVs of both human and bacterial origin. Immunoblotting of human and bacterial EVs, using CD63 and OmpF respectively, revealed successful separation of the two populations by our protocol, with human EVs found in the 10-30% fractions and bacterial EVs found in 30-40% fractions. Proteomic analysis of EVs from 3 PD and 3HC samples confirmed successful enrichment of EVs and demonstrated a downregulatory trend in the immune system in PD patients compared to HCs.

We also demonstrated a second immunoprecipitation-based method for isolating EVs from stools. We specifically isolated human EVs through an antibody-based approach, using CD63, a human EV marker, as bait. Through immunoblotting we demonstrated that this was successful in the extraction of human EVs.

Optimised EV isolation from stools will support future research into the gastrointestinal origins of PD and help uncover biomarkers for early detection. These improvements could lead to earlier diagnosis and a better understanding of PD progression.

17 Investigating the differential response of human iPSC-microglia to monomeric and fibrillary tau

Anna del Ser-Badia, Zeynep Baykam, Maria Kreger Karabova, Anne Hedegaard, William S. James, Sally A. Cowley

Introduction: Tau pathology progression in Alzheimer's disease (AD) and other tauopathies follows a characteristic spatiotemporal pattern that correlates with disease severity. Tau transmission occurs in a prion-like manner, where misfolded tau protein induces the fibrilization of native tau. There is evidence that rodent microglia can engulf, degrade, and release tau. However, the precise pathways of uptake, process, and spread of tau by human microglia remain largely unexplored.

Methods: We use an *in vitro* model of human iPSC-derived microglia to examine the microglial response to human recombinant 2N4R tau monomers or fibrils, and tau seeds purified from postmortem human brains. Tau uptake was evaluated using imaging techniques, and the seeding capacity of unprocessed tau species was assessed using a fluorescence resonance energy transfer (FRET) biosensor cell line.

Results: Here, we show a differential ability of human microglia to uptake and process recombinant 2N4R tau monomers, fibrils, and human tau. Indeed, fibrillar tau is partially internalized via heparan sulfate proteoglycans and can escape lysosomal degradation. The resulting undigested and seeding-competent tau species can be released to the conditioned medium and inside extracellular vesicles.

Conclusions: Human iPSC-microglia internalize tau monomers and fibrils through different but partially overlapping routes of entry but are unable to fully degrade internalized tau fibrils, which have seeding competency and are secreted to the extracellular medium. This human *in vitro* model will be useful to better understand the pathways linking microglial phagocytic alterations in AD and the spread of proteopathic seeds.

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Identifying mechanisms regulating α-synuclein degradation by CRISPR knockout screening

Andrew Castle, Rahel Lewin, Sarubini Kananathan, Fiona Menzies, Suchira Bose, Gopuraja Dharmalingam, and George K Tofaris

Genetic studies of families with *SNCA* multiplications or expression-enhancing polymorphisms indicate that increased intraneuronal α-synuclein (αSyn) levels are causatively linked to the pathogenesis of Parkinson's disease. Therefore, it is vital to define the regulators of αSyn degradation under physiological and pathological conditions. To this end, we have performed fluorescence-associated cell sorting-based pooled CRISPR knockout screens using a guide RNA library focused on proteostasis-related genes and Parkinson's disease GWAS hits. Through these screens, we aimed to identify modulators of the following: 1) expression level of an αSyn-mCherry fusion protein in SH-SY5Y cells; 2) seeded aggregation of αSyn in HEK293 cells overexpressing an αSyn-Venus fusion protein; and 3) αSyn ubiquitination in HEK293 cells detected by bimolecular fluorescence complementation.

By focusing on shared hits across our genetic screens and cross referencing with candidate αSyn interactors identified in a separate study, we have assembled a list of candidate E2 ubiquitin-conjugating enzymes and E3 ubiquitin-ligases that directly or indirectly regulate αSyn degradation.

Based on STRING analysis and other parameters, we have prioritized these hits for targeted knockout or knockdown experiments in cell lines and iPSC-derived neuronal models. Thus, we are gaining new insights into the mechanisms regulating αSyn turnover with a view to determining which targets are most relevant to neuronal αSyn homeostasis.

19

Defining Proteomic Subtypes to understand The Heterogeneity of Parkinson's Pathophysiology

Rashmi Maurya, Sacha Gandhi, Alejo Nevado-Holgado, Donald Grosset, Laura Winchester

Aims

To identify proteomic biomarkers of change during Parkinson's disease (PD) progression related to cognitive and motor decline and to define proteomic subtypes to understand PD pathophysiology heterogeneity.

Methods

We analyzed longitudinal proteomic data from the Tracking Parkinson's Cohort, consisting of 4530 samples from 1918 recent-onset PD patients across 72 UK sites. Samples were measured on the Somalogic platform (7596 proteins) at up to five timepoints, but for robust analysis, we focused on

the first three timepoints for 794 patients. Key clinical measures included MoCA (cognition), MDS-UPDRS-III (motor scores) and levodopa equivalent daily dose. Using Weighted Gene Co-Expression Network Analysis (WGCNA), we generated protein co-expression clusters and performed pathway enrichment analysis. Cluster preservation across timepoints was also assessed.

Results

Cross-sectional clustering revealed consistent protein co-expression modules across timepoints. The largest module, strongly preserved across all three timepoints, was enriched for cytokine-cytokine receptor interaction pathways and showed the strongest negative correlation with age (r = -0.12, -0.14, -0.17 and p-value = 5.51×10^{-4} , 5.92×10^{-5} , 7.77×10^{-7} across timepoints). Module preservation analysis indicated high preservation for most modules, with the largest having the highest preservation (Zsummary > 30). Pathway enrichment analysis also uncovered novel metabolite pathways in modules with dynamic protein expression changes.

Conclusions

Our analysis identifies potential proteomic subtypes that enhance our understanding of PD heterogeneity. The largest preserved module, enriched for cytokine interactions, along with another module highlighting novel metabolite pathways, may reveal insights into disease progression and clinical variability. Detailed characterisation of these proteomic clusters will provide a more comprehensive view of PD progression offering deeper insights into PD pathophysiology.

20 Synthesis and validation of PROTACs for degradation of □-synuclein aggregates in cellular models of Parkinson's disease

Taniya Bhardwaj, Hung-Ju Chueh, Dmitry Zenko, Alexander Ignatyev, Massimiliano Travagli, George K Tofaris

: PROteolysis Targeting Chimeras (PROTACs) are bifunctional hybrid molecules that bind to both an E3 ligase and an unwanted protein, promoting its degradation by ubiquitination. Parkinson's disease is causatively linked to α-synuclein accumulation and we previously showed that the E3 ligase NEDD4 ubiquitinates α-synuclein. Our objective was to design and test whether PROTACs that promote NEDD4-mediated ubiquitination of α-synuclein fibrils accelerate the clearance of intracellular aggregates.

Methodology: We have utilised a combination of *in silico* screening and structural biology to identify small molecules that bind to NEDD4. Prioritised compounds were linked to warheads that bind to α-synuclein fibrils and tested biochemically *in vitro* and in cellular models of seeded aggregation.

Results: From virtual HTS, 300 compounds were selected and screened using SPR binding assay; two series were identified, one of which was further developed. More than 150 ligands were synthesised. A position to introduce the linker was identified in the ligand and 40 PROTACs were generated. Crystallography confirmed that the ligands bind to NEDD4. Using a dynamic *in vitro* ubiquitination assay with NEDD4 as the E3 we confirmed that the PROTACs accelerate the ubiquitination of α-synuclein fibrils. We developed a flow cytometry assay to measure the effect of PROTACs on seeded aggregation in cell expressing fluorescently tagged □-synuclein. Dose-response

curves and estimated EC_{50} values indicated that PROTACs were effective in the nanoM range and these findings were further corroborated by measuring Ser129 phosphorylated and total α-synuclein in cell lysates using immunoblotting. Lastly, we confirmed their effect in a primary neuronal model of seeded aggregation at baseline and demonstrated using siRNA that NEDD4 is required for their action.

Conclusions: PROTACs directing the E3 ligase NEDD4 to misfolded α-synuclein offer an effective means of clearing intracellular α-synuclein aggregates and could be considered for the development of therapeutics in Parkinson's disease.

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Dopamine replacement therapy modulates dopa decarboxylase, prolactin and AOC3 levels in Parkinson's disease

Ludo van Hillegondsberg, Shahzad Ahmad, Tanja Zerenner, Michael Lawton, Yoav Ben-Shlomo, Micah Fletcher, Karolien Groenewald, Avigail Taylor, Alexander Thompson, Michele T M Hu

Introduction and objectives: New high-throughput technologies have allowed for the interrogation of the PD proteome and has led to the identification of multiple candidate biomarkers. In this study we investigate the impact of dopamine replacement therapy (DRT) on the serum and CSF proteome of people with Parkinson's disease (PwPD).

Methods: Using a proximity-extension assay proteomics platform, we tested the serum of 722 PwPD on DRT, 122 treatment-naïve PwPD, 256 people with iRBD (PwRBD) and 218 healthy controls (HC) and the CSF of 41 PwPD on DRT, 19 treatment-naïve PwPD, 14 PwRBD and 19 HC enrolled in the Oxford Parkinson's Disease Centre (OPDC) Discovery cohort. A simple linear model was used to identify proteins associated with DRT at baseline. Linear mixed-effects models were used to associate proteins with drug dose and assess differences in serum protein levels over time.

Results: In serum, 3 proteins were associated with DRT at baseline, namely dopa decarboxylase (DDC), prolactin and amine oxidase copper-containing 3 (AOC3). In CSF no proteins were associated with DRT. In serum, DDC was higher and prolactin and AOC3 lower in PwPD on DRT. In CSF, DDC was higher in PwPD regardless of treatment status, and prolactin and AOC3 were lower in PwPD on treatment. Changes in serum DDC and AOC3 levels were related to those on peripheral decarboxylase inhibitors, while prolactin was altered in those on dopamine agonists. DDC levels increased and prolactin levels decreased over time only in PwPD where DRT was started or increased.

Conclusions: DRT modulates DDC, PRL, and AOC3 levels in PD, with distinct patterns in serum and CSF likely driven by specific drug classes. These results emphasize the importance of understanding the impact of DRT on the PD proteome, and its potential to obscure the separation of treatment effects from disease biology in proteomic studies.

22 Correlating mitochondria phenotypic profiling with proteomics in a simulated drug screen to reveal drug-protein-functional axes

Sarah NJ Franks, Arkadiusz Nawrocki, Andrey Kormilitzin, Martin Larsen, Helle Bogetofte Barnkob, Brent J Ryan

A wealth of epidemiological and genetic evidence implicates mitochondrial dysfunction in the aetiology and progression of multiple neurodegenerative diseases, such as Parkinson's disease. To understand the multifaceted consequences of mitochondria dysfunction, this project established a novel platform for multidimensional functional phenotyping from cell microscopy images, dubbed "MitoPaint". This assay involves the simultaneous live-cell fluorescent visualisation of mitophagy, mitochondria membrane potential (MMP) and reactive oxygen species (ROS). High content images are then analysed to generate a multiparametric phenotypic "fingerprint", a profile of several thousand features describing the morphology, texture and intensity of mitochondria functional fluorophores within the cell.

Experiments in SH-SY5Y demonstrate "MitoPaint" can robustly capture drug-induced mitochondria phenotypes, which cluster in a dose-dependent and mechanism of action (MOA) dependent manner. In a simulated drug screen of 38 drugs and mitochondrial toxicants, shared nearest-neighbour clustering was used to group like-for-like "MitoPaint" profiles, identifying similarity between drugs of similar MOA. Armed with this evidence from "MitoPaint" profiling, it was explored whether the molecular mechanisms underpinning these phenotypic profiles could be rationalised at the protein level. Data-independent acquisition (DIA) was used as an approach for high-throughput proteomics of 192 total samples of SH-SY5Y treated with different drugs and mitochondrial toxicants.

Future efforts will focus on employing appropriate data imputation and batch correction strategies, before differences in protein abundance can be meaningfully interpreted. Additionally, preliminary experiments are optimising how "MitoPaint" can be translated to a stem cell derived neuronal model, to investigate mechanisms of mitochondria dysfunction in a model relevant to neurodegeneration.

This novel experimental paradigm has broad applications for understanding mitochondria function in both drug screening, and disease biology contexts. Furthermore, the coupling of multi-modal data should offer richer insight into novel cellular mechanisms modulating mitochondria function.

23 Extended follow-up of baseline PD subtypes

Michael Lawton, Yoav Ben-Shlomo, Donald G Grosset, Michele T M Hu

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by a wide range of motor and non-motor features. There is considerable heterogeneity within the severity and presentation of these motor and non-motor symptoms. This heterogeneity has led some to believe that there may be different PD subtypes. In 2018 we published a paper that developed and validated four PD subtypes within the Tracking Parkinson's and Oxford Discovery cohorts using a data-driven approach. These subtypes were developed using the comprehensive baseline phenotype data from these two cohorts in over 2,500 people with Parkinson's. We subsequently found that these subtypes were associated with rate of progression in the MDS-UPDRS III over time.

However, at the time we published our original paper we only had a median follow-up time of 3 years in both cohorts with a maximum follow-up of 6 years. Tracking Parkinson's has now finished follow-up and Oxford Discovery has up to 12 years of follow-up in some participants.
In this poster I will present results showing the association between our subtypes and rate of progression in different clinical scales using this extended follow-up.

24 Investigating Dysfunction in iPSC-Derived Medium Spiny Neurons from Parkinson's Patients with SNCA Triplication

Humaira Noor, Kaitlyn M L Cramb, Lahiru Handunnetthi, Richard Wade-Martins

Aims: Parkinson's disease (PD) is a neurodegenerative disorder marked by motor dysfunction due to the degeneration of dopaminergic neurons (DaNs) in the Substantia Nigra pars compacta, which heavily innervates the striatum. Medium spiny neurons (MSNs), making up 90–95% of the striatum, are the main postsynaptic targets of DaNs and play a key role in motor control. Despite this, their direct contribution to PD pathophysiology remains poorly understood. This study investigates intrinsic defects in induced pluripotent stem cell (iPSC)-derived MSNs from PD patients and examines how reduced dopamine input alters their function. This research aims to explore how disrupted dopamine signalling contributes to striatal network dysfunction, advancing our understanding of PD at cellular and circuit levels.

Methods: iPSCs from patients with a triplication of the SNCA gene and healthy controls were differentiated into GABAergic MSNs and characterised by immunocytochemistry and RT-qPCR. Cytosolic calcium dynamics were assessed using ratiometric fluorescent dye, Fura-2 imaging post-stimulation. GABA release was measured using ELISA and the iGABASnFR fluorescent reporter. A triculture model comprising DaNs, MSNs, and cortical neurons was developed using microfluidic devices to recapitulate in vivo anatomical connections, validated through immunocytochemistry using cell-type-specific markers.

Results: iPSC-derived MSNs expressed hallmark markers DARPP32 and GAD67 by Day 40. MSNs from SNCA triplication lines showed reduced intracellular calcium responses to stimulation. Preliminary data also point to a defect in GABA release following depolarisation. Additionally, the neurons in the triculture system demonstrated successful expression of synaptic and cell-type-specific markers, establishing functional synaptic connections and validating its potential for further exploration.

Conclusion: ipsc-derived MSNs with SNCA triplication exhibit calcium dysregulation, which may compromise their functional output, such as neurotransmitter release, potentially contributing to PD pathophysiology. Ongoing work using advanced fluorescent sensors and the triculture platform will further elucidate the interplay between dopamine deficiency and MSN dysregulation, advancing our understanding of PD pathogenesis at both cellular and network levels.

25 Investigating adenosine signalling in iPSC and mouse models of Parkinson's disease

Aishwarya Vedula, Ricardo Marquez Gomez, Kathryn Todd, Richard Wade-Martins, Joseph Morgan and Stephanie J Cragg

Adenosine is an activity-dependent neurotransmitter uniquely placed to translate bioenergetic abnormalities into alterations in neuronal activity and transmitter release. In the dorsolateral striatum (DLS) adenosine, acting through A_1 receptors, modulates network activity and dopamine release. Dysregulated adenosine signalling contributes to motor symptom progression in patients with Parkinson's disease (PD), and A_{2A} receptor antagonism ameliorates neuronal cell death induced by alpha synuclein oligomers. Low-affinity A_{2B} receptors, on the other hand, have been shown to play crucial roles in neuron-astrocyte metabolic coupling. However, it is not known whether aberrant adenosine signalling through A_1 and A_{2B} receptors contributes to early abnormalities in neuronal activity, dopamine release and organellar dysfunction in PD models. Here, we show that iPSC-derived dopaminergic neurons from healthy controls and Parkinson's patients with alpha synuclein triplication express adenosine receptors, including A1, at the mRNA and protein level. iPSC-dopamine neurons also release ATP (the precursor for extracellular adenosine) in response to KCl-induced depolarization, as measured by a luciferase assay.

To detect real-time modulation of dopamine neuron activity and transmitter release by adenosine, we optimized a fluid-walled dumbbell system for compartmentalizing dopamine neuron axons and somata. iPSC-dopamine neurons grown in these dumbbells differentiate appropriately and show spontaneous activity on calcium imaging. They can be transduced with lentivirus-based channelrhodopsin, GRAB-dopamine and GRAB-adenosine. Preliminary results also indicate that A2B receptors are expressed in the mouse DLS, and A_{2B}R antagonism appears to impair the adaptation of dopamine release to low-glucose, low-calcium conditions, measured using fast-scan cyclic voltammetry.

These results suggest potential roles for the A1 and A2B receptors in modulating dopamine neuron function in human and rodent models, which we will continue to investigate using the optimized iPSC microcircuit model as well as a human synuclein- overexpressing mouse model of PD.

26 The Levodopa Paradox in Parkinson's Disease: Causal Inference Analysis in the OPDC Cohort

Anahita Nodehi, Jorik Nonnekes, Tanja Zerenner, Michael Lawton, Lotte van de Venis, Bas Bloem, Michele T M Hu, Sirwan Darweesh, Yoav Ben-Shlomo

Freezing of gait (FOG) is a disabling symptom that affects people with moderate to advanced Parkinson's disease (PD). PD levodopa medication is given to alleviate FOG but this complication was rarely before the drug was prescribed (known as the "levodopa paradox"). One hypothesis suggests that the pulsatile nature of standard levodopa may prime FOG, whereas slow-release formulations could reduce the risk of developing FOG.

To explore this hypothesis, we analysed data from the OPDC Discovery cohort, a prospective longitudinal study involving approximately 1000 individuals with PD, followed for up to 12 years. Participants are assessed every 18 months, meaning the exact onset of FOG is not known but occurs within the intervals between clinic visits (interval censoring). Both medication dosages (standard and slow-release levodopa) and disease severity (a potential confounder) are time-varying variables recorded only at each visit.

To address these complexities, we employed joint marginal structural models to estimate the causal effect of both medication types (in terms of dosage) on the development of FOG. This modelling allows for adjustment of time-varying confounders and selection bias due to informative censoring. We applied inverse probability of treatment weighting (IPTW) to account for time-dependent confounding and inverse probability of censoring weighting (IPCW) to correct for potential bias due to informative censoring. These weights were incorporated into a marginal structural model to yield an unbiased estimate of the causal effect, under standard assumptions. Our findings provide a clearer understanding of the levodopa paradox in PD and highlight the potential role of slow-release levodopa in reducing the risk of FOG. Results will be presented at the meeting.

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3D Droplet Printing of a Human Cortico-striatal-dopamine Microcircuit to Model Parkinson's Disease

Oliver Curry, Ricardo Marquez Gomez, Linna Zhou, Hagan Bayley, and Richard Wade-Martins

Damage to the cortico-striatal-dopamine circuit is highly conserved across Parkinson's Disease (PD). In this circuit, neurodegeneration of dopamine neurons disrupts the communication between the cortex and striatum, leading to the distinctive PD symptomatology. With a steadily increasing prevalence of PD globally there has never been a more urgent time for in-vitro model development, specifically a model able to target the under-studied area of synaptic deficits.

Animal and cellular models to study PD circuitry have limitations in recapitulating the cortico-striatal-dopamine circuit anatomy and biology. More specifically, the use of patient cells is a more genetically relevant scenario than animal models, and 3D cultures promote self-organisation and push longevity for a more mature neuronal environment than in 2D. In this project, we apply an automated 3D bio-printing technique to generate droplets containing hiPSC derived cortical, striatal, and dopamine neurons for the creation of a physiologically relevant and reproducible model of PD circuitry. The droplet printing technology allows control of compartmentalisation and directionality, whilst granting a far higher throughput than in-vivo experiments.

Here we show that the 3D printing method, and derivatives of the method, successfully print droplets in an organised manner. Immunocytochemistry of our droplet circuits validates the differentiation of cortical, striatal, and dopaminergic cultures. It further demonstrates that cell droplets remain compartmentalised over time, and bridging regions confine and direct axonal growth. Triculture circuits remain healthy for over 100 days and calcium recordings demonstrate functional longevity, with highly active populations recorded over day 175.

Our results demonstrate a characterised and functional cortico-striatal-dopamine circuit. The cells grow together in a triculture circuit, surpassing the viability of similar 2D cultures. The project aims to include disease lines to probe inter-patient variability, and there will be a specific focus on neuromodulation of dopamine by localised receptors and quantification of changes across lines and conditions.

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Pharmacological correction of excessive basal mitophagy by pathological levels of α-synuclein restores neuronal function in human dopaminergic neurons

Elliot D Mock, Benjamin Vallin, William McGuinness, Raman van Wee, Martha Lavelle, Benjamin Jenkins, Brent J Ryan, Richard Wade-Martins

Objectives

α-Synuclein accumulation in Lewy bodies is one of the hallmarks of Parkinson's disease, which predominantly affects dopaminergic neurons. It is well-established that elevated α-synuclein results in mitochondrial dysfunction and fragmentation, however the exact mechanism is poorly understood. Moreover, α-synuclein binds anionic lipids which regulate mitochondrial fission and fusion via DRP1. We hypothesised that pathological levels of α-synuclein may interfere with mitochondrial dynamics leading to mitochondrial and neuronal dysfunction.

Methods

We characterised iPSC-derived dopaminergic neurons from a patient with an α-synuclein gene triplication mutation (*SNCA-Trp*), which causes early-onset familial PD with dementia, and compared these to neurons from healthy donors. The mt-Keima mitophagy assay was used to measure basal and PINK1/Parkin-dependent mitophagy using the mitochondrial uncoupler CCCP. Neuronal activity was assessed between DIV30-DIV90 using a multi-electrode array (MEA) system. We investigated cellular lipid perturbations by untargeted lipidomics.

Results

We found that under basal conditions *SNCA-Trp* neurons showed an increase of mitolysosomes compared to controls between DIV35-50. This could be explained by increased expression of BNIP3/NIX and loss of proteasome activity in the *SNCA-Trp* mutation, while PINK1/Parkin-dependent mitophagy was not affected. The MEA assay revealed significantly reduced firing and bursting events in the *SNCA-Trp* neurons from DIV60, suggesting that mitochondrial dysfunction may precede neuronal dysfunction. Next, we assessed global lipid changes and found significantly elevated levels of cardiolipin and reduced phosphatidic acid in the *SNCA-Trp* neurons, which respectively restrains or activates DRP1, suggesting that elevated α-synuclein may drive DRP1 overactivation. Indeed, we found that pharmacological inhibition of DRP1 corrected the excessive mitophagy phenotype in *SNCA-Trp* neurons. Finally, DRP1 blockade restored intracellular calcium release and the neuronal activity deficit to healthy control levels.

Conclusions

These combined results indicate that pathological levels of α-synuclein interfere with mitochondrial dynamics governed by anionic lipids and DRP1, thereby causing excessive mitophagy and mitochondrial and neuronal dysfunction.

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Sleep benefit in Parkinson's disease: a video analysis based reappraisal

Pietro-Luca Ratti, Nushara Wedasingha, Paulo Nunes-Ferreira, Stefano Scafa, Alessandro Puiatti

Background

Forty percent of Parkinson's disease patients report prominent, transient, spontaneous improvement in mobility upon morning awakening, before taking dopaminergic medications, which seems to be highly variable from day to day. This intriguing phenomenon, named 'Sleep Benefit', remains controversial as current assessment methods have failed to quantify it objectively.

Methods

Twenty Parkinson's disease patients underwent in-lab video-polysomnography for one or two non-consecutive nights. Patients completed a validated electronic finger tapping test and the Movement Disorders Society Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS-III) assessment at bedtime and 30 minutes after awakening. Video recordings of the clinical examinations were analysed using AI-based techniques to extract four motor parameters: power spectral density, speed, amplitude, and smoothness during finger tapping tasks. Additionally, overnight slow wave activity decay rate was calculated from scalp EEG (C3/C4) as a measure of sleep-dependent homeostatic pressure dissipation.

Results

Whilst conventional MDS-UPDRS-III scores and electronic finger tapping tests showed no significant bedtime-to-morning variation, the AI-based video analysis revealed significant improvements in all four motor features ($p<0.001$) across all patients (31 observations). This improvement exceeded 20% in at least one night for 13 out of 20 patients across all features. The morning motor improvement strongly correlated with overnight slow wave activity decay rate (amplitude $r=0.81$, $p<0.001$; speed $r=0.66$, $p<0.001$; smoothness $r=0.75$, $p<0.001$).

Conclusion

This research provides preliminary evidence that Sleep Benefit is an objective, measurable phenomenon in Parkinson's disease. Our AI-based video analysis approach offers a novel perspective that may facilitate future studies and potentially lead to new therapeutic strategies for Parkinson's Disease.

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Predicting level-2 cognitive outcomes and research clinic diagnosis of MCI and dementia in Parkinson's Disease from the MoCA

Tanja Zerenner, Sanjay G Manohar, Michael Lawton, Jamil Razzaque, Falah Al Hajraf, Karolien Groenewald, Ludo van Hillegondsberg, Tamir Eisenstein, Johannes C Klein, Yoav Ben-Shlomo, Michele T M Hu

Background and Objectives

The Montreal Cognitive Assessment (MOCA) is frequently used in cohort studies into Parkinson's disease (PD) as it is a simple and inexpensive tool for assessing the cognitive state of patients. However, cut-off values for distinguishing between normal cognition, mild cognitive impairment (MCI) and dementia differ across the literature. We comprehensively evaluate the accuracy of the MoCA for stratifying patients, investigate whether stratification can be improved by including additional routinely collected information and provide guidance on the selection of suitable cut-points.

Methods

We analysed longitudinal data from 1094 PD patients and of 267 healthy controls of the PPMI cohort in which detailed level-2 cognitive testing is conducted and research clinic diagnoses of MCI and dementia recorded. Multilevel logistic regression was used to predict level-2 outcomes and diagnoses from MoCA scores in conjunction with other routinely collected information on basic demographics, functional impairment (MDS-UPDRS 1.1) and/or MoCA subscores. Model performance was compared using the AUC. Optimal cut-offs for patient stratification with respect to Youden's J, equal proportions, screening and diagnosis were derived.

Results

MoCA total scores predicted level-2 cognitive impairment in PD (2 impaired domains or more) with an AUC of 0.86 (95% CI 0.84–0.88). Youden's J was maximized at cut-off ≤24 with sensitivity 74.7 (70.5-79.3) and specificity 83.1 (82.0-84.2); cut-off ≤21 equated proportions. Prediction of level-2 cognitive impairment was not substantially improved by additional covariates. Functional impairment/MDS-UPDRS 1.1 was found to be superior over the MoCA for predicting research clinic diagnosis of PD-MCI or PDD. A combination of MDS-UPDRS 1.1 ≥ 1 and MoCA ≤ 26 equated proportions for discriminating any impairment from no impairment; A combination of MDS-UPDRS1.1 ≥ 2 and MoCA ≤ 20 equated proportions for discriminating dementia from no dementia.

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Central cholinergic degeneration in prodromal and early Lewy body disease: a link to present and future disease states

Tamir Eisenstein, Karolien Groenewald, Ludo van Hillegondsberg, Falah Al Hajraf, Tanja Zerenner, Michael Lawton, Yoav Ben-Shlomo, Ludovica Griffanti, Michele T M Hu, Johannes C Klein

The neuropathological process in Lewy body disorders has been shown to extend well beyond the degeneration of the dopaminergic system—affecting other neuromodulatory systems in the brain which play crucial roles in the clinical expression and progression of these disorders.
Here, we investigate the role of the macrostructural integrity of the nucleus basalis of Meynert (NbM), the main source of cholinergic input to the cerebral cortex, in cognitive function, clinical

manifestation, and disease progression in non-demented subjects with Parkinson's disease (PD) and individuals with isolated REM sleep behaviour disorder (iRBD).
Using structural MRI data from 393 early PD patients, 128 iRBD patients, and 186 controls from two longitudinal cohorts (i.e., PPMI and Oxford Discovery), we found significantly lower NbM grey matter volume in both PD (β=-12.56, *p*=0.003) and iRBD (β=-16.41, *p*=0.004) compared to controls. In PD, higher NbM volume was associated with better higher-order cognitive function (β=0.10, *p*=0.045), decreased non-motor (β=-0.66, *p*=0.026) and motor (β=-1.44, *p*=0.023) symptom burden, and lower risk of future conversion to dementia (Hazard ratio (HR)<0.400, *p*<0.004). Higher NbM volume in iRBD was associated with decreased future risk of phenoconversion to PD or dementia with Lewy bodies (DLB) (HR<0.490, *p*<0.016). However, despite similar NbM volume deficits to those seen in PD, associations between NbM structural deficits and current disease burden or clinical state were less pronounced in iRBD.
These findings identify NbM volume as a potential imaging-derived biomarker with dual utility: predicting cognitive decline and disease progression in early PD, while also serving as an early indicator of phenoconversion risk in prodromal disease. The presence of structural deficits before clear clinical correlates in iRBD suggests complex compensatory mechanisms may initially mask cholinergic dysfunction, with subsequent failure of these mechanisms potentially contributing to clinical conversion.

32 Constraints of a genome-first approach in the diagnosis of Parkinson's Disease

Dianne F Newbury, Alistair Pagnamenta, Ira Milosevic, Jenny C Taylor

Precision medicine can leverage genomic data to improve diagnosis and risk stratification of genetic disorders. The ACMG/ACGS guidelines highlight characteristics of pathogenic variants enabling a genome-first approach with diagnostic yields of up to 70% for some genetic conditions. However, this approach remains challenging for adult-onset neurological disorders, which are characterised by variable penetrance, comorbidities and a higher likelihood of non-genetic causes. Currently, the genomic diagnostic yield for Parkinson's Disease (PD) is under 10%.

This study investigated genetic causes of Parkinson's Disease in the 100,000 Genomes Project dataset which includes 755 probands (311 male, 444 female) with a recruitment phenotype of "Early onset and familial Parkinson's disease" or "complex Parkinsonism (includes pallido-pyrimidal syndromes)". The average age of onset was 56.4 years and the majority of cases were recruited as singletons with only 21% reporting a positive family history.

Thirty eight cases were considered "solved", representing a diagnostic yield of 5%. These probands carried 32 distinct pathogenic variants across 18 different genes. Twenty-eight percent of variants were loss-of-function and only one fell in a gene not included in adult-onset neurodegenerative disorder gene panels.

The remaining 717 unsolved Parkinson's cases were screened for 128 ClinVar-listed PD pathogenic variants identifying 11 distinct pathogenic variants in an additional 15 Parkinson's patients across 7 genes. These variants primarily conferred missense changes, occurred in singleton cases and had a population frequency of greater than 1 in 1000 (0.1%).

These findings indicate that causative variants in PD often do not align with ACMG criteria, which prioritise loss-of-function variants that are rare in the general population. In addition, late-onset diseases like PD complicate family-based interpretation due to the limited availability of samples. Understanding these constraints may help to guide refinement of guidelines for adult-onset disorders and improve diagnostic yield in PD.

33 Differentiating Lewy body dementias through quantitative clinicopathology

Kristijan D Jovanoski, David A Menassa, Laura Parkkinen

Lewy body dementias (LBDs) are typically categorized into dementia with Lewy bodies (DLB) and Parkinson's disease with dementia (PDD). Clinically, DLB and PDD are differentiated by the relative onsets of dementia and motor symptoms, but whether they are distinct diseases with different underlying mechanisms remains unclear.

Here, we demonstrate our progress towards linking clinical data from patients diagnosed with DLB or PDD with quantifications of their Lewy body pathology in different brain regions from the Oxford and Imperial Brain Bank collections.

Using state-of-the-art machine learning algorithms, we reveal novel relationships between clinical diagnoses and quantitative neuropathology.

34 Extracellular vesicles as carriers of pathogenic α-synuclein in neurodegenerative diseases

Suman Dutta, Stelios Chatzimichail, Achillefs N Kapanidis and George K Tofaris

Introduction

Pathogenic alpha-synuclein (α-syn) protein aggregation is a hallmark of neurodegenerative disorders, including Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Given the potential of extracellular vesicles (EVs) as transport vehicles for misfolded proteins within the central nervous system (CNS), we investigated α-syn in brain-derived EVs to assess their role in disease propagation and as biomarkers for distinguishing PD/DLB from MSA.

Methods

EVs were isolated from post-mortem brain tissues of healthy controls (HC) and patients with PD, DLB, and MSA. A combination of ultracentrifugation and size-exclusion chromatography was used for EV enrichment, followed by nanoparticle tracking analysis (NTA) and western blotting for characterization. Single-molecule localization studies with direct stochastic optical reconstruction microscopy (dSTORM) were employed to visualize EV-associated α-syn and the expression of various CNS cell-type-specific markers.

Results

The composition of neuronal and glial marker-positive EVs in the brain was determined and found to be similar in healthy brains and those with α-synucleinopathies. Neuron-originating EVs (L1CAM+) in PD and DLB samples exhibited increased association with α-syn compared to MSA or healthy controls. In MSA samples, α-syn showed a slightly higher association with oligodendrocyte-originating EVs (MOG+) than with neuron-originating EVs, suggesting a potential disease-specific EV signature.

Summary/Conclusion

There is a distinct pattern of α-syn trafficking via EVs across neurodegenerative diseases, highlighting the potential of EVs as biomarkers for early diagnosis and differentiation between PD/DLB and MSA.

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Investigating Neuron–Microglia Interactions using a Human iPSC-based model for Parkinson's Disease

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Microglia are the primary immune cells of the brain but their role in Parkinson's disease is not fully understood. Chronic microglial activation is toxic to neurons, but in the early stages of neuronal pathology they also exert beneficial functions. We used iPSC modelling to investigate how human microglia respond to α-synuclein aggregates that form *de novo* inside human dopaminergic neurons with α-synuclein gene triplication or are triggered by fibrils.

We found that microglia cleared Ser129 phosphorylated α-synuclein aggregates without exacerbating neurotoxicity, using a contact-dependent mechanism. In the presence of intraneuronal α-synuclein aggregates, microglia exhibited morphological changes and transcriptomic profiles indicative of activation. Using single cell sequencing, we defined the molecular profile of a sub-cluster of disease-associated microglia (DAM) responsible for this phenotype and identified targets potentially as a key effector in α-synuclein aggregate clearance by microglial lysosomes. Additionally, our secretome analysis suggests that the identified microglial sub-cluster is negatively regulated by an autocrine loop mechanism involving IL-10 and blocking this pathway using antibodies promoted the phagocytic response.

Taken together, our study identifies a subtype of human microglia mediating the removal of intraneuronal aggregates and targets for disease modification. More broadly, our data suggest that stabilising specific microglial states during the neurodegenerative process could be a useful therapeutic strategy.

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Investigating LRRK2 PD-related lysosomal dysfunction in iPSC-microglia

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Mutations in LRRK2 account for 5-13% of familial and 1-5% of sporadic Parkinson's Disease (PD), with the G2019S mutation being the most common genetic cause of PD. A recent study of PD-associated common noncoding variant (rs6581593) found its effect on LRRK2 expression to be specifically propagated through microglia rather than other brain cells. In addition, LRRK2 protein levels are increased 10-fold in microglia upon proinflammatory stimuli, which makes them of particular interest when studying PD. As LRRK2 has been linked to lysosomal function and stress response, we aimed to investigate how PD-associated LRRK2 affects lysosomal function in microglia.

To do this, we differentiated iPSCs from 3 healthy controls and 6 PD patients carrying the LRRK2 G2019S or R1441C mutation into microglia. These cells were subjected to a range of assays to determine how the PD risk mutations affect lysosomal function, including lysosomal proteolysis, lysosomal pH, lysosomal Ca^{2+} and glucocerebrosidase activity.

Our findings support a link between LRRK2 and lysosomal function and highlight the role of microglia in PD. iPSC-derived microglia from LRRK2 G2019S and R1441C PD patients showed a mildly perturbed lysosomal function, with particularly prominent changes in lysosomal Ca^{2+}.

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Neural activity motifs and dopamine-acetylcholine dynamics in the striatum: insights from a mouse delayed-go reaching task

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The striatum is a key brain region involved in coordinating motor and cognitive functions, with the neuromodulators dopamine (DA) and acetylcholine (ACh) playing central roles in organising striatal activity through their intricate interactions. These neuromodulator dynamics are essential for normal brain function and are strongly implicated in neurological disorders such as Parkinson's disease. However, the ways in which DA and ACh relate in time and influence each other, as well as how their interplay shapes striatal activity in both healthy and diseased states, are still not fully understood.

In this study, we set out to investigate this complexity by examining the relationship between DA, ACh, and striatal neuronal activity during sensorimotor behaviour. We employed a Delayed-Go reaching task designed for head-fixed mice, which allowed us to temporally separate cue, movement, and reward components. During task performance, we recorded real-time fluctuations in DA and ACh using fibre photometry, while simultaneously capturing striatal spiking activity with high-density Neuropixels probes.

This approach enabled us to track how neuromodulator signals and neural activity co-evolve across distinct behavioural epochs. Using dynamic time-warping and hierarchical clustering, we identified a diverse set of neuronal activity response motifs within the striatum. These motifs exhibited spatial localization, suggesting region-specific processing dynamics. Furthermore, their correlations with DA and ACh signals varied across cue, movement, and reward phases, suggesting the involvement of distinct underlying neural circuits during different behavioural events.

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Subsecond Striatal Dopamine and Acetylcholine Signalling Dynamics during a Delayed-Go Reaching Task in Dopamine-Intact and Parkinsonian Mice

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Dopamine and acetylcholine are key neuromodulators in the striatum, which serves as a critical hub for motor and cognitive processing in the basal ganglia. Understanding their release dynamics on short timescales is crucial for elucidating their impact on striatal function in health and in parkinsonian states.

Aims: This study aimed to define the subsecond DA and ACh dynamics in the dorsal striatum across cue, movement and reward phases of a Delayed-Go reaching task in both dopamine-intact and parkinsonian mice.

Methods: During a Delayed-Go reaching task performed by head-fixed mice, we recorded fluctuations of DA and ACh, readout by fluorescence changes of genetically-encoded sensors via fibre photometry, before and after 6-OHDA lesion of dopamine neurons.

Results: DA and ACh signals exhibited distinct, phasic changes around cue, movement, and reward delivery. These transients were modulated by different behavioural factors. In Parkinsonian mice, partial DA depletion disrupted the normal DA and ACh signalling, with phase-specific alterations observed across the task structure.

Conclusion: Our findings show that DA and ACh signals rapidly fluctuate in the dorsal striatum during goal-directed movement, and reveal how these signalling dynamics are altered in parkinsonian states. These insights deepen our understanding of striatal neuromodulation in both health and disease.

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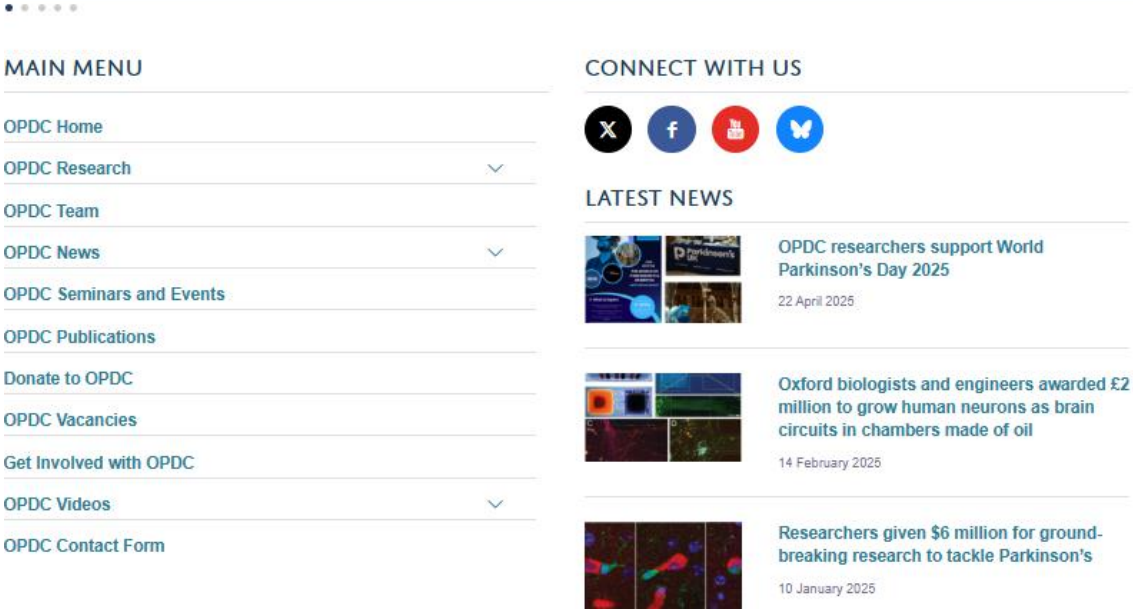

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