asap Aligning Science Across Parkinson's

Collaborative Meeting Series Abstracts

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Team Wood

- Rebecca Andrews (SNCA)
- ***Selected for data blitz:*** **James Evans (SNCA)**
- Melissa Grant-Peters (Sequencing/omics)

Aging & Progression

Dopamine Neuron Subtype-Specific LRRK2 Dysfunction in the Nigrostriatal Synapse

Selected for data blitz

Chuyu Chen[1], Oscar Moreno Ramos[1], Zachary Gaertner[1], Vanessa Promes[1], Natalia Lopez Gonzalez del Rey[1], Rajeshwar Awatramani[1], Loukia Parisiadou[1]

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LRRK2 mutation carriers exhibit pathological and clinical phenotypes largely similar to idiopathic Parkinson's disease (PD) patients, including the loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Thus, understanding LRRK2 dysfunctions in DA neurons will broadly inform the pathophysiological basis of PD. Within the SNc, cell loss in the ventral tier is more prominent than in the dorsal tier in post-mortem PD brains. Despite early recognition of this critical pathological feature, our understanding of the selective link vulnerability of ventral tier neurons and PD is limited. In the present study, we studied DA neuron dysfunction in a cell-type-specific manner to fill this gap, focusing on LRRK2 function in the most vulnerable DA neuron subtype. We used the LRRK2 mutant knock-in (KI) mouse model expressing the most common G2019S mutation, which revealed a decrease in evoked nigrostriatal DA release. Active zones like structures on the DA axon terminals mediate the fast kinetic signaling of DA. We thus assessed DA release site organization in the axons of identified control and mutant LRRK2 DA neurons, leveraging our newly developed intersectional and subtractive strategies to access otherwise indistinguishable and significantly intermingled DA neurons. Employing 3D structured illumination microscopy in the striatal sections of control and mutant LRRK2 KI mice, we demonstrated an increase in the volume of Bassoon clusters (a marker of DA release sites), suggesting disorganization of these sites. Notably, the increase was observed in the vulnerable ventral and not dorsal tier subset of DA neurons. Our findings show an LRRK2-mediated dysfunction in a physiological penetrant mouse model, specifically in the vulnerable PD DA neuron cell type. The precise elucidation of LRRK2 pathological events relevant to PD DA subsets will illuminate disease mechanisms with high specificity and mechanistic insights.

CRN Team:	Team Awatramani
Lab PI:	Loukia Parisiadou
Career Stage:	Postdoc
Abstract Category:	Aging & Progression

Dopamine Neurons Show Distinct Levels of Integrated Stress Response Activation Across Regional and Genetic Subsets in the Healthy Brain

Elana Lockshin[1], Zachary Gaertner[2], Elan Schonfeld[2], Rajeshwar Awatramani[2], Nicole Calakos[1]

[1]Duke University Medical Center, Durham, NC, USA, [2]Northwestern University, Chicago, IL, USA

Objective: The integrated stress response (ISR) is well-known for its proteostasis role in responding to diverse cell stressors. In neurodegenerative diseases, “runaway” ISR activation is thought to contribute to disease progression. Although the ISR has been regarded as a biochemical process that is transiently induced by cell stress or chronically activated in disease, our laboratory recently discovered that some cell subtypes in the healthy mouse brain continuously activate the ISR under steady-state conditions (Helseth et al, 2021). Striatal cholinergic interneurons basally require ISR activation and ISR inhibition in these cells alters learning and memory behaviors similar to effects reported with systemic ISR inhibition in mice. These results highlight a new role for the ISR in neuromodulatory cells. Here we examine whether other neuromodulatory cells share this basal ISR demand characteristic. Methods: ISR activation was quantified in adult C57Bl6 mice using immunohistochemical (phospho-PERK), genetic fluorescent reporter (SPOTlight) and transcriptional (scRNAseq) approaches.
Additionally, human adult scRNAseq database was analyzed. Results: In mice, dopaminergic neurons of the entire substantia nigra pars compacta or ventral tegmental area showed a broader and lower range of ISR activation than cholinergic interneurons. However, anatomically and genetically-defined subsets showed systematic differences. Anatomically, medial-SNc ISR levels were significantly higher compared to lateral-SNc. DANs expressing CALB1 showed higher ISR activation than those expressing ALDH1A1. Using GSEA analysis, we found enrichment of ISR-related transcripts in DAN subsets corresponding to these immunohistochemical results.
Lastly, mouse GSEA findings were further supported by human dataset analyses. Conclusions: In normal brain, DANs show systematic differences in ISR activation across subsets defined by region and/or biochemical/genetic markers. Intriguingly, lower ISR activation, not higher, correlated with DAN subsets that are most vulnerable to degeneration in Parkinson’s disease (PD). Our ongoing studies test the role that basal ISRstate differences play in DAN survival in PD models.

CRN Team:	Team Calakos
Lab PI:	Nicole Calakos
Career Stage:	PhD student
Abstract Category:	Aging & Progression

Cellular Senescence and Brain Aging

Vinal Menon[1], Hector Martinez[1], Mehek Jahan[1], Joseph Robin[1], Alec Wong[1], Joyce Meints[1], Michael Lee[1], Paul Robbins[1], Laura Niedernhofer[1]

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Cellular senescence is well-established to be a driver of aging and age-related diseases, including neurodegenerative disorders. However, there remains controversy as to which cells in the central nervous system (CNS) senesce. To address this gap in knowledge, we deleted Ercc1, encodinf a subunit of the DNA repair endonuclease ERCC1-XPF, in each cell type of the CNS. Lack of ERCC1-XPF causes accelerated accumulation of endogenous DNA damage and thereby accelerated senescence. Current studies in the lab are focused on characterizing these novel models of CNS cell-specific Ercc1 deletion, defining senescent cell burden and neuropathology, along with behavioral tests to determine the impact of senescence on motor and cognitive function. Preliminary qRT- PCR analyses revealed an increase in p16Ink4a expression, a marker of cellular senescence, in several brain regions, including the brainstem, midbrain, thalamus and hippocampus, of mice lacking Ercc1 expression in microglia. This was paralleled by an upregulation of inflammatory markers, which was also seen in mice with pan- neural Ercc1 deletion. Additionally, we are characterizing brain senescence in aged wild-type (WT) mice. By qRT-PCR, we observed the greatest increase in senescence biomarker expression in the thalamus, midbrain and hippocampus of old WT mice relative to young. Furthermore, at three years of age, female mice have a higher senescence burden compared to age-matched male mice. Expression of the cell cycle arrest and senescence marker p21Cip1 was downregulated in several brain regions in old relative to young WT animals; a pattern that was also seen in mice with astrocyte-specific Ercc1 deletion. Preliminary RNAscope data indicated neurons and astrocytes were p16Ink4a positive in the cortex of 2.5-year old WT mice. Ongoing studies in our lab to identify senescent cell types would enable the development of senolytics that are optimally suited for age-related neurodegenerative diseases.

CRN Team:	Team Lee
Lab PI:	Laura Niedernhofer
Career Stage:	Postdoc
Abstract Category:	Aging & Progression

Parkinson Brain Atlas: Elucidating Cell Type Specific Pathway Changes During Disease Progression

Selected for data blitz

Jacob Parker[1, 2], Idil Tuncali[1, 2], Zhixiang Liao[1, 2], Zechuan Lin[1, 2], Jie Yuan[1, 2,], Joshua Levin[1, 3], Su-Chun Zhang[1, 4, 5,], Mel Feany[1, 6], Xianjun Dong[1, 2], Clemens Scherzer[1,2,3]

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During the progression of Parkinson's disease (PD), around when clinical indicators begin to manifest, Lewy body pathology spreads into the temporal cortex. However, PD-associated molecular changes in cortical brain cells remain largely uncharacterized. Specific cell types are preferentially vulnerable to PD, making single nucleus RNA-seq an ideal tool for uncovering key disease related changes in gene expression.To reveal the precise cell-specific pathways that are associated with PD progression, we conducted single nucleus RNA-seq on 100 medial temporal gyrus samples comprising more than 720,000 nuclei across three brains states representing the spectrum from healthy controls to prodromal, PD-associated Lewy body neuropathology to clinically manifest Parkinson's disease as part of our initiative to build a multi-dimensional Parkinson Brain Atlas. For this project, we also developed a bench-to-bioinformatics workflow that optimizes state-of-the-art quality control, processing and analysis of large single nucleus RNA-seq experiments while accounting for potentially confounding variables.Single-nucleus transcriptomics analysis revealed seven cell types and 42 cell subtypes. Initial results from gene set enrichment analyses point to cell type specific changes in pathways relevant to PD. These include systematic upregulation of innate immune system, vesicle transport and heat shock protein pathways specifically in glutamatergic neurons and the perturbation of pathways related to RNA processing in a subset of GABAergic neurons and glial cells. This provides an initial view of transcriptome dynamics at play in specific vulnerable cell types during the onset and progression of PD.

CRN Team:	Team Scherzer
Lab PI:	Clemens Scherzer
Career Stage:	Postdoc
Abstract Category:	Aging & Progression

Dopaminergic Neuron Dysfunction Triggers a PD-Like Sleep Disorder

Selected for data blitz

Patricia Gonzalez Rodriguez[1, 2], Keith Summa[1], Peng Jiang[3], Martha Vitaterna[3], Jose Lopez Barneo[2], Fred Turek[3], James Surmeier[1]

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Parkinson's disease (PD) is frequently associated with sleep disorders that manifest during the prodromal and advanced stages of the disease. These disorders are commonly thought to be mediated by pathology in non-dopaminergic brain circuits. Recently, a mouse model of PD (Gonzalez-Rodriguez et al., 2021) was developed using intersectional genetic strategies that led to a progressive loss of dopaminergic neurons (MCI-Park). In addition to manifesting a progressive motor phenotype resembling human PD, this model unexpectedly exhibited disrupted sleep patterns. Our goal was to rigorously characterize the evolution of the sleep disorder in these mice, allowing it to be compared to that seen in humans. Sleep-wake cycles in MCI-Park mice were studied during prodromal and later Parkinsonian stages. To this end, electroencephalographic (EEG) and electromyographic (EMG) recording electrodes were surgically implanted in younger (5-7 weeks of age) and older (13-17 weeks of age) MCI-Park mice and wild-type littermate controls. EEG and EMG signals were monitored continuously using standard approaches. In prodromal MCI-Park mice, the total amount of time spent sleeping was similar to controls, but sleep was more fragmented with more frequent state shifts between non-REM (NREM) and REM stages. Interestingly, the median duration of periods of wakefulness was increased in prodromal MCI-Park mice and the median duration of NREM sleep was decreased. As MCI-Park mice transitioned to the Parkinsonian stage, deficits in state shifts, bout number, and median bout duration worsened. In addition, Parkinsonian mice exhibited excessive daytime sleepiness, reductions in the total amount of REM sleep, and increased REM fragmentation. In summary, MCI-Park mice – in which there is a selective disruption in dopaminergic neuron function – exhibit a progressive disruption in sleep architecture that resembles that seen in PD patients that do not manifest REM-based sleep disorder (RBD).

CRN Team:	Team Surmeier
Lab PI:	James Surmeier
Career Stage:	Postdoc
Abstract Category:	Aging & Progression

Whole Genome CRISPR/CAS9 Screen Identifies Neddylation as a Regulator of Neuronal Age and Neurodegeneration

Nathalie Saurat[1], Andrew Minotti[1, 2], Alexander Henderson[1], Maliha Rahman[1, 2], Yen Chun Chen[1], Daniela Cornacchia[1, 3], Chao Zhang[2, 4], Doron Betel[2], Lorenz Studer[1]

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Aging is the biggest risk factor for phenotypic penetrance in late onset neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease. However, cellular age is reset during reprogramming to pluripotent stem cells posing a significant challenge to efforts to model late onset neurodegenerative phenotypes in vitro. To date, efforts to address this limitation have focused on direct reprogramming of aged fibroblasts to neurons. However, this approach may result in the generation of neurons with a fibroblast related aging signature rather than true neuronal aging signature. Therefore, we developed a Crispr/Cas9 screening platform that allowed us to identify genes whose loss of function synergized with disease- causing mutations to trigger neurodegeneration in vitro. We overlapped our whole genome screen hits with transcriptomic data from young and old human brain tissue to identify putative regulators of neuronal age. Using this strategy, we identified neddylation as a regulator of age and neurodegeneration in Alzheimer's disease. Blocking neddylation in human cortical neurons induced cellular changes consistent with accelerated aging including increased cellular senescence, loss of proteostasis, loss of heterochromatin, nuclear lamina defects and DNA damage. It also resulted in altered Tau phosphorylation and a selective loss of viability of Alzheimer's Disease neurons. Blocking neddylation in stem cell derived dopaminergic neurons also induced cellular hallmarks of age and experiments to determine whether this induces a selective loss of dopamine neurons derived from PD genetic backgrounds (GBA N370S and LRRK2 G2019S) are ongoing. This study acts as a proof of principle that programming age into stem cell models of disease can enable the study of late onset neurodegenerative disease phenotypes in vitro.

CRN Team:	Team Studer
Lab PI:	Lorenz Studer
Career Stage:	Postdoc
Abstract Category:	Aging & Progression

SNCA

Imaging, for the First Time, Alpha-Synuclein Oligomers in Human Brain Tissue

Rebecca Andrews[1], Bin Fu[1], Joanne Lachica[2], Christina Toomey[2], Jonathan Breiter[1], Ru Tian[1], Lucien Weiss[3], Nick Wood[2], Mina Ryten[2], Michele Vendruscolo[1], Tammaryn Lashley[2], Sonia Gandhi[2, 4], Steven Lee[1]

[1]University of Cambridge, Cambridge, UK, [2]University College London, London, UK, [3]Polytechnique Montreal, Montreal, QC, Canada, [4]The Francis Crick Institute, London, UK

In Parkinson's Disease (PD), Lewy bodies and other large alpha-synuclein aggregates (>5 µm) that occur in the brain are well studied, however, little is known about the precursor small alpha-synuclein aggregates (~100 nm) known as oligomers that are thought to be cytotoxic. To image, for the first time, alpha-synuclein oligomers in human post-mortem brain tissue, we immunofluorescently stained 140 tissue sections for phosphorylated alpha-synuclein in the anterior cingulate gyrus of 6 PD patients and 6 age-matched healthy controls. After treating the sections for autofluorescence, we captured over 100,000 images of the grey matter of the tissue on a custom-built widefield fluorescence microscope. Through image analysis, we detected over 8 million alpha-synuclein oligomers in human post-mortem tissue across both PD and healthy patients. Analysis of the brightness of the oligomer fluorescence showed a distinct population of larger oligomers that were only present in PD brains, therefore, disease specific. This study demonstrates that through the implementation of fluorescence quenching and advanced microscopy alpha-synuclein oligomers can be imaged in human post-mortem brain tissue allowing more information to be known about the role of oligomers in PD.

CRN Team:	Team Wood
Lab PI:	Steven Lee
Career Stage:	Postdoc
Abstract Category:	SNCA

Alpha-Synuclein Induces Oligodendrocyte Dysfunction in Parkinson's

Selected for data blitz

James Evans[1, 2], Gurvir Virdi[1, 2], Emil Gustavsson[3], Anna Wernick[1, 2], Dilan Athauda[1, 2], Mina Ryten[3], Sonia Gandhi[1, 2]

[1]UCL Queen Square Institute of Neurology, London, UK, [2]The Francis Crick Institute, London, UK, [3]University College London, London, UK

The targeted and progressive loss of midbrain dopaminergic (mDA) neurons and the aggregation of alpha-synuclein (a-Syn) are the pathological hallmarks of Parkinson's disease (PD). Genetic data and single cell studies have recently highlighted a significant role for oligodendrocytes in the pathogenesis of PD – first, that the genetic risk for PD is enriched within genes specifically expressed in oligodendrocytes, and second, that oligodendrocyte-related genes are altered early in the disease process, prior to mDA neuronal genes. Despite this, the underlying pathways by which oligodendrocytes contribute to the disease process, and ultimately neuronal degeneration, are unknown. We optimized an experimental paradigm to generate enriched populations of oligodendrocytes from hIPSCs and characterized their molecular and functional identity. We performed targeted long-read RNA sequencing of SNCA in these cells, and in highly enriched iPSC-derived mDA neuronal cultures, to understand how SNCA transcription may be altered in oligodendrocytes compared to mDA neurons, and how this may be impaired in disease. In order to investigate how oligodendrocytes contribute to synucleinopathy, we generated iPSC-derived oligodendrocytes from controls and SNCA mutation/multiplication carriers, and investigated the effect of SNCA mutation on oligodendrocyte protein homeostasis, lipid metabolism, and oligodendrocyte function, and their interaction with mDA neurons. We further investigated how oligodendrocytes handle misfolded protein, and characterized the process and consequences of protein oligomerisation in oligodendrocytes. Taken together, we demonstrate cell specific phenotypes in synucleinopathies. Ultimately, synuclein-induced alterations in oligodendrocyte state and loss of function may impair critical metabolic and trophic support mechanisms, which may cause mDA neuronal dysfunction in disease.

CRN Team:	Team Wood
Lab PI:	Sonia Gandhi
Career Stage:	PhD student
Abstract Category:	SNCA

Alpha-Synuclein Strains in Parkinson's Disease

Selected for data blitz

Kundlik Gadhave[1], Enquan Xu[1], Ning Wang[1], Jacob Deyell[1], Jun Yang[1], Antony Wang[1], Youngjae Cha[1], Ramhari Kumbhar[1], Olga Pletinkova[1, 2], Juan C Troconso[1], Valina L. Dawson[1, 3], Ted M. Dawson[1, 3], Liana S. Rosenthal[1], Xiaobo Mao[1]

[1]Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Jacobs School of Medicine and Biomedical Sciences, Buffalo, NY, USA, [3]Adrienne Helis Malvin Medical Research Foundation, New Oreleans, LA, USA

Objectives: Emerging evidence indicates that pathogenic alpha-synuclein can spread from cell- to-cell and drive the disease progression in Parkinson's disease (PD). These pathogenic alpha- synuclein aggregates act as prion-like seeds that can template endogenous alpha-synuclein monomers to misfold and acquire properties similar to the seed. Different cellular and tissue environments may cause alpha-synuclein to form distinct conformations that may account for different clinical parkinsonian phenotypes. Methods: Using protein misfolding cyclic amplification (PMCA) from clinically well-characterized deidentified cerebral spinal fluid we biochemically characterized alpha-synuclein strains from PD with no cognitive impairment, PD with mild cognitive impairment, and PD with dementia. Results: In this work, we report that pathogenic alpha-synuclein from PD with no cognitive impairment, PD with mild cognitive impairment, and PD with dementia exhibit different biophysical and cell biologic properties. Conclusions:Our findings support the presence of different strains of pathogenic alpha- synuclein in PD versus PD with mild cognitive impairment and PD with dementia. These different strains likely contribute to the clinical heterogeneity of PD.

CRN Team:	Team Kaplitt
Lab PI:	Ted Dawson
Career Stage:	Postdoc
Abstract Category:	SNCA

Cortical Neuronal Vulnerability in Parkinson's Disease

Thomas Goralski[1], Lindsay Meyerdirk[1], Elizabeth Breton[1], Daniella DeWeerd[1], Lisa Turner[1], Katelyn Becker[1], Daniel Newhouse[2], Michael Henderson[1]

[1]Van Andel Institute, Grand Rapids, MI, USA, [2]NanoString Technologies , Seattle, WA USA

Parkinson's disease (PD) is a debilitating neurological disease characterized by accumulation of misfolded α-synuclein (referred to as Lewy pathology) throughout the brain and loss of dopaminergic neurons in the substantia nigra. Lewy pathology progresses to cortical regions as symptoms worsen, with high cortical Lewy pathology associated with progression to dementia. Within the cortex, we lack a basic understanding of which neuron types bear Lewy pathology and to what degree they degenerate in PD. This gap in knowledge prevents insight into mechanisms of disease progression and identification of therapies that can treat the most deleterious symptoms of this progressive disease. In a mouse model with progressive α- synuclein pathology we utilized spatial transcriptomics, cell-type deconvolution, and combined in situ hybridization/ immuno-fluorescence to understand differences between neurons vulnerable or resistant to developing pathology. We identify specific subsets of excitatory neurons that are vulnerable to developing pathology in mice and validate these findings using the same techniques in human PD cortex. We also identify transcriptomic signatures of cell processes disrupted by α-synuclein pathology. Our results demonstrate neuron-intrinsic indicators of vulnerability and response to Lewy pathology in Parkinson's disease.

CRN Team:	Team Biederer
Lab PI:	Michael Henderson
Career Stage:	PhD student
Abstract Category:	SNCA

The Effect of Pathogenic α-Synuclein Mutations on Presynaptic Membrane Association

Akio Mori[1], Haru Yamamoto[1], Gautam Runwal[1], Robert Edwards[1]

[1]University of California, San Francisco, CA, USA

The protein α-synuclein localizes to the axon terminal and plays a central role in the etiology of Parkinson's disease (PD) and other synucleinopathies. α-synuclein interacts with synaptic membranes, but its role in neurotransmitter release and degeneration remain poorly understood. The N-terminal binding domain of α-synuclein to the membrane is highly conserved, in contrast to the less conserved C-terminal region. All missense mutations in a- synuclein linked to the familial PD (A30P, E46K, H50Q, G51D, and A53T) localize to the N- terminal membrane-binding domain. However, their effect on membrane interactions within neurons and in particular on neurotransmitter release remain largely unresolved. In particular, previous work has analyzed the effect of mutations with over-expression in WT neurons containing endogenous synucleins. Using primary cultures from synuclein triple knockout mice lacking all three isoforms, we now examine the membrane association of wildtype and mutant α-synuclein by fluorescence recovery after photobleaching (FRAP). The results show that pathogenic mutations both reduce and increase the membrane association of α--synuclein, suggesting that normal function requires membrane association but that this must not be strong. Future experiments will use synuclein triple knockout neurons to address the role of these pathogenic mutations in neurotransmitter release.

CRN Team:	Team Edwards
Lab PI:	Robert H. Edwards
Career Stage:	Postdoc
Abstract Category:	SNCA

Reverse Engineering Parkinson's Disease in a Dish by Evaluating Gene X Environmental Interactions

Benjamin Nguyen[1, 2], Ariana Kokkinakis[1], Meghan Heer[1], Steve Callaghan[1], Michael Schlossmacher[2, 3], Stephen Baird[4], Maxime Rousseaux[1, 2]

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Objective: Parkinson's disease (PD) is a heterogeneous disorder that likely involves a concoction of genetic risk factors, environmental exposures, and aging. Despite recent advances in understanding the genetics of PD, there remains a gap in our understanding of how genetics interact with environmental factors. >95% of PD patients present with anosmia at time of diagnosis and up to 10 years before motor dysfunction. Given that the olfactory system interfaces the environment and our central nervous system, it is suggested to be a site for pathological α-synuclein (αSyn) metabolism. Understanding how gene-environment (GxE) interactions conspire to regulate αSyn is important to understand the initial steps that lead to pathogenesis. We have developed a fluorescent-based system to test complex GxE interactions in a high-throughput manner to reverse engineer this feature of disease. Methods: We prioritized 26 environmental factors epidemiologically linked to PD. After identifying ideal subtoxic doses, we exposed wild-type primary mouse cortical neurons to binary combinations of each dose (~700 combinations). We will then this "exposome" library under primary neurons of genetically sensitized backgrounds (e.g. mutant Snca, Lrrk2 and Gba alleles) to determine αSyn altering combinations by high-content imaging. Then, we will validate hits by performing both dose-responses and time-courses. We will further explore top hits in increasingly physiologically relevant milieux and explore the mechanisms whereby these GxE interactions influence αSyn. Ultimately, we will confirm these findings in vivo in mouse olfactory circuits to study how GxE converge on PD pathology. Results: We conducted a screen for combinatorial modifiers of αSyn levels and localization in wild-type neurons via high content imaging. Building on the wild-type screen, we are validating some promising early hits, but also retesting this screen on genetically sensitized backgrounds. Conclusion: This project will help uncover complex relationships between GxE interactions, our olfactory system, and PD pathogenesis.

CRN Team:	Team Schlossmacher
Lab PI:	Maxime Rousseaux
Career Stage:	PhD student
Abstract Category:	SNCA

Crosstalk Between Alpha-Synuclein and Neuromelanin Exacerbates Parkinson's Disease Pathology in Melanized Tyrosinase-Expressing Rodents.

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Objectives: Parkinson's disease (PD) is characterized by a preferential loss of neurons containing the pigment neuromelanin (NM), especially dopaminergic neurons of the substantia nigra (SN), and the presence in affected neurons of alpha-synuclein (αSyn)-containing insoluble cytoplasmic aggregates termed Lewy bodies (LB). While αSyn aggregation is considered a central pathogenic event in PD, the mechanisms and significance of LB formation remain unknown. It has been reported that αSyn redistributes to the lipid component of NM at early PD stages and is entrapped within NM granules extracted from PD, but not control, brains. The increased concentration of neuronal αSyn and NM pigment in SN neurons may predispose these neurons to LB formation and cell death. However, it has not yet been possible to experimentally assess in vivo a potential pathological interaction between αSyn and NM because, in contrast to humans, NM is absent in common experimental animals. Methods: We have recently developed the first rodent model of human-like NM production based on the viral vector-mediated nigral expression of melanin-producing enzyme tyrosinase (AAV-TYR). This has revealed that NM can trigger PD pathology when accumulated above a specific pathogenic threshold. Here we aim to assess the potential interaction between αSyn and NM by combining αSyn overexpression with TYR-induced NM production in rodents. Results: AAV-mediated nigral expression of human αSyn in melanized TYRr-expressing rats resulted in an increase of intracellular NM levels, a more sustained formation of LB-like inclusions, an exacerbated presence of αSyn oligomeric species and an enhanced nigrostriatal degeneration. Conclusions: Overall, our results indicate that increased levels of αSyn, as it occurs in PD patients, may accelerate age-dependent NM accumulation and enhance NM-linked PD pathology. Elucidating a potential crosstalk between αSyn and NM might thus be crucial for understanding PD etiopathogenesis and for the development of novel disease-modifying therapeutic strategies.

CRN Team:	Team Vila
Lab PI:	Miquel Vila
Career Stage:	PhD student
Abstract Category:	SNCA

Identifying Cellular and Synaptic Vulnerability Induced by Alpha-Synuclein Pathology in the PFF Mouse Model of Parkinson's Disease

Selected for data blitz

Saroj Sah[1], Viktor Feketa[1], Drake Thrasher, [2], Ilona Kondratiuk[1], Laura Volpicelli-Daley[2], Elena Gracheva[1], Thomas Biederer[1]

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Parkinson's disease (PD) pathology affects multiple neuronal networks, leading to progressive motor and cognitive decline. We hypothesize that PD pathology progressively causes cognitive deficits through damage to vulnerable synapses and disrupting cortical network connectivity. We employ an a-synuclein preformed fibril (PFF) mouse model to understand the impact of pathologic a-synuclein on neuronal dysfunction. Here, injection of PFF into the striatum of wild- type mice acts retrogradely to drive cortical pathology, with injection of a-synuclein monomer serving as control. We validated the approach to obtain consistent PFF quality to drive cortical pathology and identified that the concentration of PFF and duration of sonication affect the fibril size, which is critical in developing synuclein pathology in the cortex. Our team's results in the PFF mouse model support that a considerable amount of pathological synuclein accumulation localizes at synapses in the PFF mouse model. We implement mesoscale 3D SEQUIN super-resolved imaging at twice the resolution of confocal microscopy to identify synapse subtypes vulnerable in alpha-synuclein pathology. This work is complemented by single-cell RNAseq analysis of cellular vulnerability in cortical neurons, which has identified differentially expressed genes in cortices of PFF vs. monomer injected mice. Hits encoding synaptic proteins are prioritized in our studies of synaptic vulnerability.

CRN Team:	Team Biederer
Lab PI:	Thomas Biederer
Career Stage:	Postdoc
Abstract Category:	SNCA

Shining a Light on Parkinson's Disease: Expanding the Tool Box for Parkinson's Research

Erin Scott[1], Nikki Tjahjono[1], Rochelin Dalangin[2], Junqing Sun[1], Kwun Nok Mimi Man[1], Lilian Campos[1], Carly Drzewiecki[1], Miguel Chuapoco[3], Azariah Coblentz[1], Alessio Andreoni[1], Akash Pal[1], Julie Chouinard[1], Viviana Gradinaru[3], Drew Fox[1], Lin Tian[1]

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Advancements in viral and optogenetic tool development have enabled functional measurement of Parkinson's disease (PD)-relevant perturbations and allowed for an unprecedented look into the anatomical and physiological underpinnings of PD. Recently, our group has contributed to developments in viral vectors and genetically encoded fluorescent-based sensors, which can be combined in multiplex imaging to enable measurement of glutamate, dopamine, and calcium release patterns in real-time. Here I will present new advances to this neuroscience toolbox for use in multiplexed neurotransmitter-based detection. These advancements encompass dopamine and glutamate sensor development, including dLight 3.0 and red-shifted Halo GluSnFR. In addition, I will present initial proof of concept studies of a novel genetically encoded fluorescent-based sensor for use in alpha-synuclein fibril detection. Initial studies have demonstrated the potential of this approach and sensitivity of the sensor to preformed fibrils. Finally, we will present initial data aimed at overcoming pre existing limitations associated with extending these cutting-edge approaches for use in non-human primates. Using our newly engineered AAV, AAV.CAP-Mac, we demonstrate our ability to perform systemic injections in the non-human primate. Initial data demonstrates our capacity to deliver genetically encoded cargo for tracing (i.e. XFPs) and functional studies (ie GCaMP8s). Ex-vivo morphometric analysis and slice physiology demonstrate our ability to trace cell circuits across the macaque brain as well as perform two-photon calcium imaging following systemic injection. This presentation will facilitate iterative design of these tools and together with community feedback ensure their widespread dissemination and maximum utility for the Parkinson's disease research community.

CRN Team:	Team Gradinaru
Lab PI:	Dr. Lin Tian and Dr. Drew Fox (co-mentored)
Career Stage:	PhD student
Abstract Category:	SNCA

Exploration of the Role of Neurexins in Cell-to-Cell Transmission of Pathologic Alpha-Synuclein

Ning Wang[1], Xiling Ying[1], Xiaobo Mao[1, 2], Santiago Unda[3], Quan Gan[1], Shiyu Liu[1], Senthilkumar S. Karuppagounder[1, 2], Hu Wang[1], Jingwei Song[1], Dylan Odell[1], Austin Zheng[1], Shigeki Watanabe[1], Michael G. Kaplitt[3], Valina L. Dawson[1, 2, 3, 4], Ted M. Dawson[1, 2, 4]

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Objectives: Cell-to-cell transmission and accumulation of misfolded pathologic α-synuclein (α- syn) underlies Parkinson's disease (PD). The underlying mechanisms of cell-to-cell transmission of pathologic α-syn is poorly understood. Methods: Using in vitro and in vivo approaches we investigated the role of neurexin in cell-to-cell transmission of pathologic α-syn. Results: We report that neurexins bind to α-syn preformed fibrils (α-syn PFF) and deletion of neurexins blocks α-syn PFF propagation in vitro and in vivo. The reduction in the spread of pathologic α- syn leads to less DA neuron loss and neurophysiological and behavioral deficits in mice lacking in neurexins. Conclusions: These results support a role for neurexins in the cell-to-cell transmission of pathologic α-syn and in the pathogenesis of PD. It also provides new opportunities to develop effective approaches to halt the progression of PD.

CRN Team:	Team Kaplitt
Lab PI:	Ted Dawson
Career Stage:	Postdoc
Abstract Category:	SNCA

Cryo-EM Insights in the Proliferation of A-synuclein Fibril-induced Pathology

Arpine Sokratian[1, 2], Ye Zhou[2], Enquan Xu[1, 2], Elizabeth Viverette[3], Yuan Yuan[1, 2], Mario Borgnia[3], Alberto Bartesaghi[2], Andrew West[1, 2]

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a-Synuclein seeded amplification assays (SAAs) are promising emerging diagnostic tools for Lewy body diseases. However, it is not clear what types of a-synuclein assemblies form in these assays and how they may differ from pre-formed fibrils (PFFs) typically used in modeling and therapeutic discovery. In this study, cryo-electron microscopy analysis of SAA products from Lewy body dementia (LBD) cases revealed several novel types of a-synuclein fibrils with β- strand alignments and stacking distinct from pre-formed fibrils. The a-synuclein fibril-specific monoclonal antibody (MJFR-14), but not a-synuclein oligomer-specific antibodies, blocked the amplification of these fibril types from patient CSF, suggesting the originating patient seeds that direct the folding are composed of a-synuclein fibrils. SAA fibrils demonstrate different amyloid dye binding profiles from PFFs and can be stably propagated in vitro. SAA fibrils internalize in neurons similarly to PFFs but are substantially more proliferative in templating new Lewy body- like inclusions from endogenous human a-synuclein. Our results highlight patient-derived recombinant a-synuclein assemblies as a valuable resource for modeling and therapeutic discovery.

CRN Team:	Team Liddle
Lab PI:	Andrew West
Career Stage:	PhD student
Abstract Category:	SNCA

GWAS Functional Validation

Parkinson Brain Atlas: Mapping GWAS Function in Brain Cells With Single-nucleus eQTL

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Genome-wide association studies (GWAS) have unequivocally linked thousands of noncoding variants to Parkinson's disease (PD). However, we do not know how GWAS variants cause neurodegeneration, which genes are causal, and why they impair some brain cells but not others. For many GWAS peaks, cis-regulation of transcription is the most likely determinant of disease susceptibility. The multi-dimensional Parkinson Brain Atlas uses single-nucleus expression Quantitative Trait Locus analyses (sn-eQTL) to uncover the precise proximal mechanisms of GWAS variants in about 1 million human brain single cells from 100 midbrains and 100 temporal cortex samples. Here we evaluated the cis-regulatory effects of 30,503 PD- associated GWAS variants, subthreshold variants, and their proxy variants for the 4,595 genes physically located under 90 GWAS loci associated with susceptibility for PD in the seven major cortical cell types. Seventy sn-eQTLs associated with 24 GWAS loci achieved P values £ 3.38 x 10-7, the Bonferroni-threshold for statistical significance. Of these sn-eQTLs, 37% were cell type-specific, 3% were cell-ubiquitous, and 60% were present in multiple (two to six) cell types. These sn-eQTLs associated multiple novel effect genes with PD GWAS loci. Moreover, we extended gene-regulatory effects for the recently nominated candidate gene GPNMB on chromosome 7 to human brain single cells. Single-nucleus-eQTL analysis in the Parkinson Brain Atlas will help to decode the complex human genetics of PD through a dynamic, multi- dimensional view of proximal cellular mechanisms across brain cells, brain space, and disease progression. These evolving insights will provide a roadmap towards precision medicine.

CRN Team:	Team Scherzer
Lab PI:	Clemens Scherzer
Career Stage:	Postdoc
Abstract Category:	GWAS Functional Validation

The Genetics of Parkinson's Disease Dementia and the Amyloid Pathway

Selected for data blitz

Raquel Real[1], Lesley Wu[1], Zane Jaunmuktane[1], John Hardy[1], Huw Morris[1]

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Objectives: Parkinson's disease (PD) is a common neurodegenerative disorder that characteristically manifests as motor impairment, but cognitive decline and dementia usually develop in a significant proportion of cases. Genetic risk factors contributing to the development of dementia in PD have been described but remain incompletely understood. Methods: To explore the genetic factors associated with rate of progression to PD dementia, we performed a genome-wide survival meta-analysis of 3,923 clinically diagnosed PD cases of European ancestry. In addition, we have analyzed CSF biomarker data from a subset of cases. Results: We have identified the APOE ε4 allele as a major risk factor for the conversion to Parkinson's disease dementia [HR (95% CI) = 2.41 (1.94–3.00), P = 2.32x10-15], as well as a new locus within the ApoE and APP receptor LRP1B gene [HR (95% CI) = 3.23 (2.17–4.81), P = 7.07x10-09]. In a candidate gene analysis, GBA variants were also identified to be associated with higher risk of progression to dementia [HR (95% CI) = 2.02 (1.21–3.32), P = 0.007]. CSF biomarker analysis also implicated the amyloid pathway in PD dementia, with significantly reduced levels of amyloid β42 (P = 0.0012) in PD dementia compared to PD without dementia. Conclusions: We identified a new candidate gene associated with faster conversion to dementia in PD and provide further evidence that the amyloid pathology plays a role in the development of PD dementia, which suggests there is a need to explore anti-amyloid drugs as potential treatments for PD dementia in individuals at risk. We are currently exploring the association between genetics and amyloid pathology in post-mortem PD brains on a large scale, with the aim of better identifying individuals at risk that could benefit from early amyloid-targeting therapies.

CRN Team:	Team Hardy
Lab PI:	Huw Morris
Career Stage:	Postdoc
Abstract Category:	GWAS Functional Validation

Understanding the Functional Effects of Parkinson Disease Associated Mutations in Dopaminergic Neurons and 3D Midbrain Organoids

Khaja Mohieddin Syed[1], Jesse Dunnack[1], Hanqin Li[1], Atehsa Sahagun[1], Oriol Busquets[2], Yeon Lee[1], Yogendra Verma[1], Gabriella R Pangilinan[1], Frank Soldner[1], Donald Rio[1], Helen Bateup[1], Dirk Hockemeyer[1]

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Mutations in several genes (~20) were reported to contribute to familial Parkinson's disease (PD) progression and pathophysiology. But notably, the majority of PD cases are sporadic and through genome-wide association studies(GWAS) it was found that common variants might contribute to PD. However, to date, there is no systematic study to examine the functional consequences of these potential risk genes in relevant cell types. This study focuses on modeling PD to characterize the effects of ~300 possible risk genes implicated in GWAS. To begin with, we have generated human stem cell lines with mutations in familial PD genes (three subclones per genotype: SNCA, PRKN, PINK1, LRRK2, FBOX7, SYNJ1, DNAJC6) and differentiated them into relevant cell types in both monolayer and brain organoid models. We have generated single-cell RNA sequencing (scRNA-seq) transcriptomic maps of in-vitro differentiated monolayer dopaminergic neurons (Day 35) and developing brain organoids models over a period of 3 months (at 1 and 3 months). The transcriptomic signatures ultimately will serve as a comprehensive reference map for how PD disease-relevant mutations affect transcriptional profiles. This reference map will be used to identify potential PD risk genes from the GWAS nominated list following a CRISPRi/a gene perturbation screen (Perturb-seq). Overall, this study will identify the transcriptional signatures of PD disease-risk genes using single-cell RNA-seq in 3D brain organoids and delineate the genotype-to-phenotype relationships using Perturb-seq.

CRN Team:	Team Rio
Lab PI:	Helen Bateup and Dirk Hockemeyer
Career Stage:	Postdoc
Abstract Category:	GWAS Functional Validation

Circuit Physiology and Function

Olfactory Sensory Neuron Environment-to-cns Connection as a Locus for Parkinson's Disease Origin

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Hyposmia is a common early-onset symptom of Parkinson's disease (PD) that frequently precedes classical Parkinsonian symptoms, suggesting a role for the olfactory system towards the defined pathophysiology. The olfactory epithelium is a unique sensory interface where neurons physically interact with the environment and project directly into the CNS. This environment-to-CNS pathway with documented pathology in early PD suggests OSNs may serve as a locus of pathological origin. Here we will test the hypothesis that dysregulation of SNCA in OSNs influences SNCA expression in downstream olfactory circuitry. To test this hypothesis, we will target the nasal epithelium for overexpression of WT and A53T mutant SNCA followed by evaluating the downstream effects on endogenous SNCA expression, transfer and/or propagation of virally-expressed variants, and resulting pathophysiology. We first set out to establish AAV vector expression in the nasal epithelium by infecting WT mice with 3 distinct AAV capsid candidates (DJ/8 n=3, AAV9 n=3, and SCH9 n=2) expressing TdTomato to compare capsid transduction efficiencies. Cryosectioned olfactory bulbs were examined for fluorescent OSN terminals in olfactory glomeruli. We found that DJ/8 exhibited the most robust labeling of OSNs (64.4% of olfactory glomeruli contained fluorescent fibers) with minimal off-target transduction in the olfactory bulb. Our results indicate that nasal lavage of AAV-DJ/8 will be an effective method of transducing OSNs. We have since transduced OSNs of PAC- Tg(SNCAWT);Snca-/- and dbl-PAC-Tg(SNCAA53T);Snca-/- mice with DJ/8 to over-express tagged WT and A53T mutant SNCA. Preliminary immunohistochemistry indicates that tagged SNCA of viral origin is capable of spreading from OSN terminals into the greater olfactory bulb. Using immunohistochemistry, olfactory behavioral assays, and in vivo imaging, we will assess patterns of SNCA spread from OSN terminals throughout the CNS, the effects of SNCA overexpression on endogenous SNCA, and alterations in downstream circuit activity.

CRN Team:	Team Schlossmacher
Lab PI:	Benjamin Arenkiel
Career Stage:	PhD student
Abstract Category:	Circuit Physiology and Function

Motor Cortical Circuit Adaptations in a Progressive Mouse Model of Parkinsonism

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Degeneration of dopaminergic (DA) neurons in the substantia nigra (SN) significantly alters the circuit dynamics of the basal ganglia-thalamocortical network, which causes motor deficits in Parkinson's disease (PD). As a detrimental consequence of basal ganglia dysfunction and/or DA degeneration, the motor cortex develops an aberrant pattern of neuronal activity in parkinsonian state. Yet, the molecular and cellular mechanisms underlying cortical dysfunction remain poorly studied.Using mitoPark mice, a genetic mouse model of parkinsonism that shows progressive SN DA neurodegeneration, we conducted longitudinal studies to define changes in circuit dysfunction of the primary (M1) and secondary motor cortex (M2) at different stages of SN DA neurodegeneration. Combining ex vivo electrophysiology, optogenetics, and retrograde tracing approaches, we quantified alterations in intrinsic excitability and thalamocortical transmission of pyramidal tract neurons (PTNs) in the layer 5 of M1 and M2 at early and late stages of SN DA neurodegeneration.We detected a series of novel intrinsic and synaptic adaptations in PTNs of both M1 and M2 that gradually develop as SN DA neurodegeneration progresses. Our ongoing work focuses on further defining the molecular and biophysical mechanisms underlying the cortical circuit changes developed in mitoPark mice. Our findings provide new insight into the pathophysiology of PD motor deficits.

CRN Team:	Team Wichmann
Lab PI:	Hong-yuan Chu
Career Stage:	Postdoc
Abstract Category:	Circuit Physiology and Function

Unveiling the Synergistic Contributions of Noradrenergic and Dopaminergic Circuit Degeneration in Parkinson Disease in a Neuromelanin Mouse Mode.

Selected for data blitz

Maria P. Contreras[1], Cristian Gonzalez-Cabrera[1], Ernesto Duran[1], Csilla Novak[1], Andres Jaramillo[1], Rafael Parker[1], Tony de Schultz[1], Matthias Prigge[1]

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Neuromelanin is a brownish pigment that accumulates with age in catecholaminergic neurons in humans. High levels of intracellular granules are correlated with an increased neuronal vulnerability and linking neuromelanin accumulation rate with the progressive loss of catecholaminergic neurons seen in Parkinson's disease. In rodents, neuromelanin is not produced in detectable levels within the lifespan of an animal. Team Vila developed a novel rodent mouse model that accumulates neuromelanin in catecholaminergic neurons through the transgenic expression of a tyrosinase enzyme. The new mouse model showed progressive cell loss within the locus coeruleus (LC), substantia nigra (SN) as well as ventral tegmental area (VTA). To dissect the specific contribution of a degenerated noradrenergic and dopaminergic circuit, we developed a viral approach to induce accumulation of neuromelanin in either the LC or the SNC-VTA. Our results confirm that viral-induced expression of the tyrosinase enzyme induces neuromelanin granules in a high number of LC neurons already after 12 weeks. Yet, we did not observe a robust impairment in locomotion, anxiety-like or olfactory functions when compared to control animals. We are now investigating possible compensatory mechanisms that allow the noradrenergic circuit to function despite severe accumulation of neuromelanin. In contrast, induction of neuromelanin in dopaminergic neurons leads to motor deficit observed in a motor behavior (e.g., cylinder test). As noradrenergic neurons in transgenic models as well as in Parkinson are earlier degenerated compared to dopaminergic neurons, our result point to a more effective compensatory mechanism in the LC network than in the dopaminergic circuitry. To test if our behavioral batteries we used to assess early noradrenergic impairment are too insensitive to detect mild cognitive impairments, we develop a complex cognitive flexibility task that depends on switching between explorative and exploitative strategies. Animals with viral-induced LC degeneration and the transgenic mouse line are now investigated.

CRN Team:	Team Vila
Lab PI:	Dr. Matthias Prigge
Career Stage:	Postdoc
Abstract Category:	Circuit Physiology and Function

Open-loop Neural and Cardiac Autonomic Responses to Incentivized Vigor in Humans

Selected for data blitz

Neil Dundon[1], Elizabeth Rizor[1], Joanne Stasiak[1], Jingyi Wang[1], Taylor Li[1], Christina Villaneuva[1], Kiana Sabugo[1], Luna Herschenfeld-Catalan[1], Alexandra Stump[1], Renee Beverly-Aylwin[1], Viktoriya Babenko[1], Regina Lapate[1], Scott Grafton[1]

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Various “closed loop” circuits in basal ganglia, with both inputs from and outputs to primary motor cortices, are affected by Parkinson’s disease (PD). However a putative open-loop circuit (OLC), with inputs to ventral striatum from limbic sites, is hypothesized to be unimpacted by the disease, and may underlie contextually mediated phenomena such as paradoxical kinesia and placebo effects. Separately, disturbances of autonomic nervous system function are common in PD, with increasing evidence in healthy humans suggesting that the sympathetic branch (SNS) is particularly modulated by contextual variables such as reward and cost. The present experiment sought to merge these two areas of inquiry. Specifically, we tested if incentivized motor vigor would engage putative nodes of OLC and further, if contextual factors modulating SNS also predict activations in these brain regions. In a functional magnetic resonance imaging (fMRI) context, 25 healthy human participants performed a computerized task, where infrequent contexts offered opportunities to either yield substantial reward, or avoid substantial costs, by performing time-limited reaches with a joystick. Participants additionally wore recently-developed fMRI-safe wrist electrodes that measure ohmic impedance changes in the radial artery, allowing us to compute fast-timescale estimates of task-driven SNS modulations (cardiac contractility). Results at the behavioral level suggest first that rewarding contexts in particular elicit motor vigor, indexed by credibly faster times to peak movement acceleration. In addition, preliminary fMRI analyses suggest that nodes of the OLC (amygdala and ventral striatum) are preferentially engaged when substantial reward contexts emerge, but these responses diminish by the initiation of movement. Our next set of analyses will take an individual-differences approach. Given that participants vary in terms of task proficiency, we will test if reward history serves as a contextual factor that predicts both SNS modulations (leading up to movements) and any associated recruitment of OLC brain nodes.

CRN Team:	Team Strick
Lab PI:	Scott Grafton
Career Stage:	Staff Scientist
Abstract Category:	Circuit Physiology and Function

Deconstructing Astrocyte-Neuron Connectivity in Health and Parkinson's Disease

Nimrod Elazar[1], Russell Ravenel[1], Kristina Sakers[1], Dhanesh Sivadasan Bindu[1], Lucio Schiapparelli[1], Anket Choudhury[2], Erik J. Soderblom[1], Scott Soderling[1], Mike Tadrooss[1, 2], Cagla Eroglu[1, 2, 3]

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Parkinson's disease (PD) is hallmarked by the loss of dopaminergic neurons of the Substantia nigra pars compacta (SNc), which project to the striatum. Intriguingly, astrocytes, the primary glial cell type that interacts with synapses, also sense and react to dopamine and, in turn, modulate the activity of striatal circuitry. However, astrocyte involvement in PD pathogenesis is primarily studied in the context of astrogliosis, leaving other aspects of astrocyte biology in PD largely unexplored. Here, we utilized astrocyte-specific surface-biotinylation and RNA sequencing to examine surface proteome and transcriptomic changes in response to dopaminergic neuron loss induced by neurotoxin 6-hydroxydopamine (6-OHDA) treatment in vivo. Using this multi-omics approach, we identified novel protein and gene regulatory networks involved in the astrocyte response to the loss of dopaminergic neurons. Proteins involved in glutamate signaling and dynamics were altered in response to 6-OHDA treatment, indicating the novel roles astrocytes play in modulating striatal glutamate in response to the loss of dopaminergic input. Using this data set, we will explore how astrocytes modulate glutamate dynamics in health and PD. This study has the potential to uncover novel roles astrocytes play in modulating neuronal function in PD and provide new therapeutic possibilities for future treatment

CRN Team:	Team Calakos
Lab PI:	Cala Eroglu
Career Stage:	Postdoc
Abstract Category:	Circuit Physiology and Function

Projection-specific Regulation of Nigrostriatal Dopamine by the Subthalamic Nucleus

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Objectives: Nigrostriatal dopamine and the subthalamic nucleus (STN) are key substrates of Parkinson's disease pathology and treatment, but their interactions remain poorly understood. Here we investigated how STN projections to the substantia nigra regulate dopamine neuron activity. Methods: We injected dual-recombinase mice (VGLUT2-Cre x DAT-Flpo) with AAVs to express Cre-dependent ChrimsonR in the STN and Flp-dependent GCaMP6f in substantia nigra pars compacta (SNc) dopamine neurons. STN terminals were optogenetically stimulated while recording SNc dopamine neuron calcium dynamics with fiber photometry. Results: Brief optogenetic stimulation of STN terminals in the nigra increased dopamine neuron calcium signals. Prolonged high-frequency stimulation revealed multiphasic dopamine neuron responses: Transient excitation transitioned to sustained inhibition for the duration of the stimulation, with rebound excitation at stimulation offset. Recording GABA neurons in the substantia nigra pars reticulata (SNr) instead revealed sustained excitation throughout stimulation. Dopamine axons in separate striatal subregions exhibited distinct responses to STN terminal stimulation: Dopamine axons in the tail of the striatum were transiently activated at stimulation onset, whereas those in more rostral dorsolateral striatum were inhibited during stimulation and had prominent rebound excitation, and no consistent effect was observed in the central dorsal striatum. Conclusions: These results are consistent with hypothesized circuitry entailing modest direct excitatory input from STN to SNc, with disynaptic inhibition mediated by canonically dense STN projections to SNr. Distinct activity of dopamine axons in separate striatal subregions suggests that STN and likely SNr may differentially regulate dopamine neuron subpopulations in the dorsal and ventral tiers and lateral SNc, which give rise to these topographically organized nigrostriatal projections. As SNc dopamine subtypes exhibit differential vulnerability in Parkinson's disease, important future work will investigate STN regulation of spared dopamine neurons following selective ablations of preferentially vulnerable dopamine neuron subtypes.

CRN Team:	Team Awatramani
Lab PI:	Thomas Hnasko
Career Stage:	Research Assistant Professor
Abstract Category:	Circuit Physiology and Function

Neural Mechanism of Sleep Deficits During the Progression of Parkinson's Disease

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Sleep deficits are commonly observed in patients with Parkinson's disease (PD). The symptoms, including insomnia (problematic nighttime sleep), hypersomnia (excessive daytime sleepiness), and REM sleep behavioral disorder, seriously affect the quality of life of PD patients and their families, but they are generally refractory to standard anti-parkinsonian treatments. These facts highlight the importance of studying the neural mechanism of sleep impairments in PD, especially how they develop and worsen during disease progression. We characterized a mouse PD model (MCI-Park) developed by the Surmeier group and found sleep deficits similar to those observed in PD patients. The MCI-Park mice manifested insomnia-like behaviors even at the prodromal stage, with sleep loss in the light phase but not the dark phase. Moreover, we observed reduced sleep bout duration, a sign of sleep fragmentation. These sleep deficits worsen as the mice reach the parkinsonian stage. The development of sleep deficits in this model parallels the progressive dopamine depletion in substantia nigra pars reticulata (SNr), which is critical for controlling sleep. We are using a combination of approaches including fiber photometry, optrode recordings, in vivo optogenetics and chemogenetics together with a variety of viral tools to characterize the relationship between dopaminergic signaling, SNr neural activity, and sleep impairments.

CRN Team:	Team Surmeier
Lab PI:	Yang Dan
Career Stage:	Postdoc
Abstract Category:	Circuit Physiology and Function

Factors That May Contribute to Pardoxical Kinesia in Non-human Primates

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Individuals with Parkinson's disease (PD) often show a remarkable yet temporary reduction in motor symptoms in situations that evoke intense positive or negative affective reactions (i.e., emotions). The neuronal mechanisms underlying this phenomenon (termed paradoxical kinesia) remain unclear. To fill that gap in knowledge, we developed a novel behavioral task to evoke positive and negative emotions in non-human primates.The task is a visually-cued instructed delay reaching task. Unlike traditional tasks in which a reward is delivered immediately following each successful reach, our task grants visual reward "tokens" at the end of each successful reach. Those tokens accumulate across trials and are converted to actual rewards after completion of a randomly selected number of trials. In addition to these "normal" trials, we present two emotion-evoking conditions: "jackpot" and "threat" trials. In jackpot trials, an unique visual instruction informs the animal that successful completion of the trial will be followed by delivery of many reward tokens. In threat trials, a visual "robber" stimulus appears on the screen and moves toward the target image soon after go-cue onset. If the animal fails to touch the target before the robber image reaches it, the animal loses all accumulated tokens. Jackpot and threat trials are presented infrequently (10% of trials) and are intermixed randomly with normal trials.For the one animal trained to date, jackpot trials are associated with a slowing of reaction times and reach speeds and increased pupil dilation as compared with normal trials. The threat condition does not influence those parameters.
However, the rate of occurrence of eye blinks following instruction presentation is lower during threat trials than in other trials. Those physiological differences suggest that our task is capable of evoking positive and negative affective reactions. Striatal single-unit recordings will be obtained during this task before and after induction of parkinsonism.

CRN Team:	Team Strick
Lab PI:	Robert Sterling Turner
Career Stage:	Postdoc
Abstract Category:	Circuit Physiology and Function

The Role of Dorsomedial and Dorsolateral Striatal Dopamine Signaling in Cognitive Deficits Associated With Prodromal Parkinson's Disease

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Dopamine neurons in the substantia nigra pars compacta (SNc) that project to the dorsolateral striatum (DLS) are among the earliest dopamine neurons to degenerate in Parkinson's disease (PD), while SNc dopamine neurons that project to the dorsomedial striatum (DMS) degenerate later in disease progression. Yet, early PD patients have difficulty with dynamic probabilistic reward learning, a behavior more closely tied with DMS rather than DLS function. This discrepancy caused us to hypothesize that DMS dopamine dysfunction occurs early in PD, well prior to degeneration of DMS-projecting dopamine neurons, and contributes to the cognitive symptoms of prodromal PD. We tested this hypothesis by recording in vivo dopamine signals in a mouse model of early PD in which alpha-synuclein (α-syn) is virally overexpressed. Mice with α-syn overexpression in SNc dopamine neurons exhibit a cognitive reward learning deficit before overt motor symptoms. We used in vivo fiber photometry to record DMS and DLS dopamine signals during learning, as well as dopamine signals in the midbrain due to somatodendritic dopamine release, and found that overexpression of α-syn caused alterations in learning-related DMS dopamine dynamics. Our findings suggest an important role for DMS- projecting dopamine neurons in prodromal PD that warrants further investigation.

CRN Team:	Team Edwards
Lab PI:	Talia Lerner
Career Stage:	PhD student
Abstract Category:	Circuit Physiology and Function

Pathophysiological Progression of Cortical Cellular- And Circuit-Dysfunction in a Fibrillar Seeding Model of Parkinson's Disease

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Parkinson's disease (PD) is characterized by progressive decline of motor function and tremor primarily resulting from loss of dopaminergic cells in the substantia nigra. However, prodromic and progressive cognitive symptoms also suggest neocortical dysfunction. Indeed, relatively little is known about PD-associated cellular- or circuit-level changes in the cortex. To explore this question, we have adopted a pre-formed a-synuclein fibrillar seeding model to drive pathogenesis in cortical projection neurons. Injection of fibrils into the dorsal striatum robustly drives intracellular accumulation of a-synuclein phosphorylated at serine 129 (pS129) in layer II/III pyramidal cells of somatosensory and motor cortices. To monitor neural activity, we carried out 2-photon calcium imaging of GCaMP6s-expressing somatosensory and motor cortical neurons in awake behaving mice over the course of pathological progression.
Individual neurons were tracked across multiple imaging sessions. At the conclusion of the final imaging session, brains were collected for pS129 immunostaining to identify cells with pathological aggregates. These images were then aligned post-hoc with in vivo imaging data to determine the single-neuron relationship between dysfunction and the presence of inclusions. Our initial results suggest altered spontaneous activity and behavioral state-dependent modulation in pathological cells. To further determine how aggregate burden influences inter- cellular communication, we also imaged axons of single neurons. Finally, we compared spontaneous and sensory-evoked dynamics by analyzing elicited responses from targeted air puffs. In ongoing work, we are extending these results using simultaneous electrophysiological recording of imaged neurons to determine potential alterations in the relationship between spiking and calcium handling for cells with aggregated a-synuclein. Together, these results provide novel insight into the progression of cellular and circuit dysfunction in the neocortex associated with a-synuclein pathology.

CRN Team:	Team Biederer
Lab PI:	Michael Higley
Career Stage:	Postdoc
Abstract Category:	Circuit Physiology and Function

Endolysosomal Pathways

Polyamine Homeostasis as a Developmental Trigger for Parkinson's Disease

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Polyamines are ubiquitous and highly abundant polycations of crucial physiological importance. Polyamine content decreases with aging and abnormal plasma polyamine levels have been reported in Parkinson's disease (PD). Mutations in ATP13A2 - a key lysosomal polyamine transporter - have been implicated in several neurological disorders, including PD. To investigate whether polyamine imbalance can drive and/or contribute to PD-like phenotypes we took advantage of Atp13a2 knockout mice, as a model for polyamine dysfunction in PD. We characterized the polyamine profile, motor and non-motor behavior of Atp13a2 knockout mice at baseline, and after postnatal polyamine supplementation.Loss of Atp13a2 results in early and sustained polyamine imbalance in plasma and brain tissue, characterized by overall polyamine reduction and occurring prior to the onset of gliosis (1 month), motor and non-motor symptoms (2 months). Loss of Atp13a2 also impairs dietary polyamine uptake during polyamine supplementation, resulting in aberrant levels in the brain. Nonetheless, despite the atypical uptake in knockout mice, polyamine supplementation is sufficient to fully rescue motor phenotypes, including akinesia and bradykinesia. Juvenile Atp13a2 knockout mice also display PD-like non-motor symptoms, such as hyposmia and anxiety, which are likewise ameliorated by polyamine supplementation. Interestingly, the rescue effect is strongest with spermidine, and is sustained at least 4 months post-treatment, further highlighting the neurodevelopmental importance of polyamine homeostasis.Our data highlight that juvenile polyamine imbalance contributes to the development and severity of PD-like phenotypes. We show for the first time that loss of Atp13a2 alters the brain polyamine profile, contributing to motor and non-motor symptoms later in life.

CRN Team:	Team Vangheluwe
Lab PI:	Peter Vangheluwe
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Exploring Lysosomal Defects and Rescue Strategies for Alzheimer's and Parkinson's Disease Using Human Transdifferentiated Neurons

Ching-Chieh Chou[1], Ryan Vest[1], Miguel Prado[2], Joshua Wilson-Grady[2], Joao Paulo[2], Yohei Shibuya[1], Patricia Losada[1], Ting-Ting Lee[1], Jian Luo[3], Steven Gygi[2], Jeffery Kelly[4], Michael Greicius[1], Daniel Finley[2], Marius Wernig[1], Tony Wyss-Coray[1], Judith Frydman[1]

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Brain is vulnerable to aging, reflected by increasing incidence of neurodegenerative diseases for individuals after age 60. Protein homeostasis failures including lysosomal dysfunction are extensively involved in protein aggregation and neurodegeneration in Alzheimer's disease (AD) and Parkinson's disease (PD). Lysosomes are regulated by a sophisticated protein network for cellular degradation and nutrient and danger sensing. It is unclear how AD and PD perturbs lysosomal homeostasis of aging neurons, conferring neuropathological changes. One of the hurdles is a limited access to patient living neurons to acquire deep knowledge of disease processes. We developed methods to enable robust transdifferentiation of human fibroblasts into cortical and dopaminergic neurons while retaining epigenetic aging signatures. The objective is to leverage transdifferentiated neurons to understand how proteome-wide remodeling with aging, AD and PD triggers lysosomal dysregulation and activation of death signaling. To explore therapeutic targets, we developed drug discovery platforms and genomic and triaging strategies in human cells to identify novel lysosome-targeting drugs and the cognate mechanism of actions. Transdifferentiated neurons recapitulate protein pathologies in AD and PD. Quantitative proteomics reveal that neurons exhibit unique clusters of protein trajectories changing with AD and PD. Among them, proteins in the endosome-lysosome pathway are the common top hits. The discordant response to lysosomal damage stimuli contributes to lysosomal membrane vulnerability, de-acidification and pro-inflammatory cytokine release, leading to neurotoxicity. The abnormal lysosomal markers correlate with neuropathological changes in patient brain tissue. Coupling phenotypic screening with CRISPR-based genomics enables the detection of underlying mechanisms of drug candidates. One of the novel lysosome activators can restore lysosomal acidification and promote the clearance of pathological proteins and reduction of neuroinflammation in AD and PD cells. The outcomes provide a novel link of proteome-wide remodeling, lysosomal vulnerability, and inflammation in neurons to the neurodegenerative process and therapeutic implication for AD and PD.

CRN Team:	Team Harper
Lab PI:	Judith Frydman
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Dopamine Transporter and Synaptic Vesicle Sorting Defects Underlie Auxilin-linked Parkinson's Disease

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Objectives: Auxilin participates in the uncoating of clathrin-coated vesicles (CCVs), thereby facilitating synaptic vesicle (SV) regeneration at presynapse. Auxilin (DNAJC6/PARK19) loss-of- function mutations cause Parkinson's disease (PD). Here, we utilized auxilin-knockout (KO) mice to elucidate the mechanisms through which auxilin deficiency and clathrin-uncoating deficits lead to PD. Methods: We performed behavioral and histopathological characterization of auxilin KO mice for PD-like phenotypes. To obtain insights into the mechanism, we performed whole brain, synaptosomes, and CCVs proteomics. We performed neurochemical analyses, in-vivo fast-scan cyclic voltammetry and new computational analysis to quantitate striatal dopamine kinetics. Dopamine transporters (DAT) membrane localization was evaluated by immunostaining, novel dichloropane-based ex-vivo imaging, and immuno-electron microscopy (EM). Monoaminergic neurotransmitter transporters, endocytic proteins, SVs, CCVs and autophagic vacuoles were also evaluated. Results: Auxilin KO mice display the cardinal features of PD, including L-DOPA-responsive motor deficits, α-synucleinopathy, nigral dopaminergic neuron loss, decreased striatal dopamine, and gliosis. Unbiased proteomic and neurochemical analyses of auxilin KO brains indicated dopamine dys-homeostasis. We validated these findings by demonstrating slower dopamine reuptake kinetics in-vivo, an effect associated with dopamine transporter misrouting into axonal membrane deformities in the dorsal striatum. Elevated synaptic autophagy and defective SV protein sorting also contribute to ineffective dopamine sequestration and compartmentalization, ultimately leading to neurodegeneration. Conclusions: Auxilin mutations and clathrin-uncoating deficits may lead to PD through; cytoplasmic dopamine accumulation, DAT mis-trafficking, SV sorting deficits and autophagic overload, which collectively lead to dopamine compartmentalization defects. This study advances our knowledge of how presynaptic endocytosis deficits lead to dopaminergic vulnerability and pathogenesis of PD.

CRN Team:	Team Hafler
Lab PI:	Sreeganga S. Chandra
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

GPNMB: Beyond a Biomarker in PD?

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Genome-wide association studies have shed light on the genetic risk landscape of Parkinson's disease (PD), however, defining the biological function of associated risk loci remains challenging. GPNMB, which is considered a secreted biomarker for stressed macrophages, has been nominated as the underlying risk gene for the locus rs199351. GPNMB's nomination is supported by quantitative trait loci mapping which shows increased GPNMB expression in association with the risk allele, however, its biological function in health or PD remains elusive. Therefore, we interrogated the function of GPNMB in macrophages in response to cellular stressors. We show that GPNMB is recruited to lysosomes in response to various cellular stressors but secreted only when lysosomal pH is neutralized. GPNMB KO results in a drastic enlargement of lysosomes but a reduction in stress-induced lysosomal damage. These findings indicate that GPNMB might play a cell-autonomous role in regulating lysosomal homeostasis beyond its secreted function and further highlight dysfunction of the endolysosomal system as an underlying disease cause in Parkinson's Disease.

CRN Team:	Team Hardy
Lab PI:	Patrick Lewis
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Proteomic Landscape of the Retromer and Commander Endosomal Sorting Complexes

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Endosomal sorting of cargo proteins for recycling back to the plasma membrane or for degradation in the lysosome plays a key role in regulating the cell surface levels of roughly 30% of the human proteome, which in turn control diverse fundamental cellular functions such as cell growth, nutrient uptake, cell adhesion, cell motility, and extracellular signaling. Two crucial sorting complexes, called Retromer and Commander, are thought to be responsible for cargo recycling by diverting cargo from lysosomal degradation. Dysregulated endosomal sorting is linked to neurodegeneration and congenital neurological defects, for example via dominant mutation of Retromer subunit VPS35 leading to Parkinson's disease and recessive mutations in Commander subunits linked to Ritscher-Schinzel syndrome and Wilson's disease. Major advances in the field have uncovered dozens of candidate cargos that depend on Retromer for recycling. However, the complete cargo repertoire of Commander is not yet known, and it is not known whether the two sorting complexes have distinct or overlapping sorting roles, the extent to which cargo sorting pathways are cell-type-dependent, and how the system is dysregulated in neurons. Using recently developed methods by our lab and others for selective capture of endosomes, lysosomes, and the Golgi apparatus (termed Endo-IP, Lyso-IP, and Golgi- IP, respectively), as well as plasma membrane proteomics, quantitative mass spectrometry, and in vitro biochemistry, we aim to define the Commander cargo repertoire and determine how mutations in endosomal sorting complex subunits contribute to mis-trafficking and lead to neurodegeneration. Greater understanding of endosomal sorting may enable targeted therapeutics to redirect aggregation-prone species such as a-synuclein to the lysosome and to ameliorate aggregation.

CRN Team: Team Harper
Lab PI: J. Wade Harper
Career Stage: Postdoc
Abstract Category: Endolysosomal Pathways

PGK Activity Rescues Nerve Terminal Bioenergetic Deficits Associated With PD

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Phosphoglycerate kinase 1 (PGK-1), the first ATP producing enzyme in glycolysis, has emerged in the last 5 years as a promising therapeutic target for PD. Its activity has been reported to be enhanced by the serendipitous off-target binding of the a1 adrenergic receptor antagonist Terazosin (TZ) and retrospective clinical analyses have demonstrated that long term treatment with TZ offers significant protection against PD in humans. We show here that that over- expression of this enzyme in the mid-brains of mice offers strong protection against 6- hydroxydopamine-driven dopamine neuron loss. We show that the endogenous enzyme is enriched in nerve terminals and increasing presynaptic PGK expression ~2-fold can completely restore synapse function under hypometabolic conditions that normally leads to massive slowing of the synaptic vesicle cycle. Slow synaptic vesicle recycling is also a hallmark of the impact of several PARK mutations. We show that the synaptic vesicle recycling impairment driven by PARK20 (synaptojanin-1) mutation can be rescued by increasing local synaptic PGK activity. These data indicate that bioenergetic deficits in nerve terminals may be a unifying concept across a spectrum of PD mutations.

CRN Team:	Team De Camilli
Lab PI:	Timothy Ryan
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Loss of Ciliary Hedgehog Signaling in GBA Mutant Cells: Links to LRRK2 Pathways

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Heterozygous mutations in glucocerebrosidase (GBA) are a major risk factor for Parkinson's disease (PD), however, the link between dysfunctional GBA and PD remains elusive.
Homozygous GBA mutations cause the lysosomal storage disorder, Gaucher's disease, and lead to lysosomal accumulation of glucosylceramide and glucosylsphingosine. Accumulation of these sphingolipids is usually accompanied by cholesterol accumulation in lysosomes; conversely, accumulation of cholesterol in cells lacking NPC1 cholesterol transporter function is accompanied by concomitant increase in glycosphingolipids. It is noteworthy that perturbations in cholesterol transport in NPC1-/- cells alter primary cilia morphology, ciliary lipid composition and cholesterol-dependent Sonic Hedgehog (Hh) signaling. We showed previously that pathogenic LRRK2 interferes with primary cilia formation and decreases cilia dependent Hh signaling in cell culture and in cholinergic interneurons and astrocytes of the mouse dorsal striatum. This is important because dopaminergic neurons of the Substantia nigra require Hh for viability and secrete Hh onto cholinergic neurons to promote cholinergic neuron viability and their protective transcriptional responses. Here, we sought to test the hypothesis that GBA mutations also influence Hh signaling in a manner that can explain a link between GBA mutation and PD.When NIH-3T3 cells were treated with the GBA inhibitor, conduritol-β-epoxide (CBE), we observe a 50% reduction in Gli1 levels, a classical Hh signaling readout; ciliation is unchanged. This was confirmed using NIH-3T3 GBA KO cells; Gli1 levels were restored by re-expression of GBA in these cells. GBA mutant MEF cells also show decreased Gli1 expression compared with wild type MEF cells. Collectively, our data suggests that loss of GBA function results in reduced Hh signaling. Similar phenotype are observed in mice with pathogenic LRRK2 mutations, pointing to a common pathway for PD pathogenesis. Experiments are in progress to confirm ciliary and Hh changes in neurons and astrocytes of the dorsal striatum from GBA mutant mice.

CRN Team:	Team Alessi
Lab PI:	Suzanne Pfeffer
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Development of Multi-PTM Enrichment to Study PTM Profile of Lysosomes and Golgi

Selected for data blitz

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The recent development of rapid immunoprecipitation of Organelle (Golgi-IP, Lysosome-IP and mitochondria-IP) enables molecular content of intact Organelles to be analyzed at high- resolution and study how this is linked to disease processes such as Parkinson's. Current methods enable the analysis of protein, metabolite, and lipid content to be assessed in depth using high-resolution mass spectrometry analysis. However, due to the low levels of proteins obtained from organelle IPs typically under 1 microgram it has thus far not been possible to undertake high resolution profiling of post translational modifications (PTMs) such as protein phosphorylation and Ubiquitylation in these fractions. This is important for our research as there is strong evidence that the LRRK2 and other Parkinson's pathways will impact phosphorylation and potentially ubiquitylation pathways in the Golgi, lysosome, and mitochondria. To address this, we have developed an ultra-sensitive protocol that enables phosphorylation and ubiquitinylation sites to be analyzed in submicromolar levels of proteins derived from organelle IPs. This is achieved by combining sequential enrichment of phospho-peptides and ubiquitin-peptides from tryptic digests of organelle immunoprecipitations, followed Ultra-sensitive data independent acquisition and parallel accumulation and serial fragmentation (dia-PASEF) on a timsTOF SCP mass spectrometer. We will present our method and initial data in this area. We are also exploring whether this method will enable us to detect low level proteins that are transiently recruited to lysosomes and Golgi via protein phosphorylation or ubiquitylation that cannot be easily detected by other methods. We will apply our Organelle-PTM approach to study Parkinson's disease linked LRRK2 kinase signalling in Golgi and Lysosomes immunoprecipitations derived from pathogenic LRRK2 G2019S and R1441C cells and mouse tissues. We believe our workflow could open new avenues for studying Organelle specific phosphorylation and ubiquitylation signalling in health and disease.

CRN Team:	Team Alessi
Lab PI:	Dario Alessi
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Membrane Remodeling Properties of LRRK2 and Lipid Transport Properties of VPS13C Cooperate in the Response to Lysosome Damage

Selected for data blitz

Xinbo Wang[1, 2], Peng Xu[1, 2], Shujun Cai[1, 2], Yumei Wu[1, 2], Pietro De Camilli[1, 2]

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Recent studies have raised the possibility that perturbation of endo-lysosome homeostasis may be one of the mechanisms leading to Parkinson's disease (PD). Two genes implicated in lysosome biology and whose gain-of-function and loss-of-function, respectively, cause PD, or increase disease risk, are LRRK2 and VPS13C. LRRK2, which contains a GTPase and a kinase domain and whose kinase activity is enhanced by disease-causing mutations, is recruited to lysosomes in response to lysosome damage. However, the mechanisms and physiological significance of such recruitment remain poorly understood. Here we show that purified LRRK2 directly binds acidic lipid bilayers in vitro and can deform them into narrow tubules in a guanylnucleotide-dependent, but ATP-independent way. Moreover, we show that LRRK2 preferentially binds to highly curved lipid tubules relative to low curvature membrane bilayers. These results reveal that LRRK2 has curvature generating and curvature sensing properties and suggest that these properties may be closely interrelated with its kinase activity in mediating a response to lysosome damage. In preliminary data we found that lysosome damage also results in the recruitment of VPS13C, with faster kinetics than the recruitment of LRRK2. VPS13C is a lipid transfer protein that can bridge the ER to endo-lysosomes, and which is thought to mediate bulk phospholipid transport between these two organelles (most likely from the ER to endo- lysosomes). An attractive possibility, supported by changes in the level of lysosomal proteins and lipids in VPS13C KO cells, is that VPS13C recruitment may help provide lipids for membrane repair. Collectively these findings raise the possibility that the membrane remodeling properties of LRRK2 and the lipid transfer properties of VPS13C may both contribute to the repair of damaged lysosome membranes.

CRN Team:	Team De Camilli
Lab PI:	Pietro De Camilli
Career Stage:	Postdoc
Abstract Category:	Endolysosomal Pathways

Gut/brain, Microbiome, & Clinical Biomarkers

Exploring Blood Gcase/a-synuclein Pattern in a Cohort of Gba Subjects: a Cluster Analysis to Identify a Specific Prodomal Parkinsonian Profile

Selected for data blitz

Micol Avenali[1, 2], Gerardo Ongari[1], Silvia Cerri[1], Chiara Cerami[1, 3], Chiara Crespi[2, 3], Matt Gegg[4], Marco Toffoli[4], Derralynn Hughes[5], Enza Maria Valente[1, 2], Cristina Tassorelli[1, 2], Anthony Schapira[4], Fabio Blandini[2, 6]

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Background and aims: GBA mutations are the most frequent genetic risk factor for Parkinson's disease (PD). Relationship between GBA status and increased risk for GBA-PD is still unclear. We investigated whether glucocerebrosidase activity (GCase) and α-synuclein levels in blood cells in asymptomatic subjects carrying GBA mutations (GBA carriers) are associated with a more severe prodromal PD profile.Methods: 31 GBA carriers, 28 GBA-PD and 38 healthy controls (HC) were enrolled in this study. A cluster analysis was performed to split the subjects into different clusters based on their biochemical profile analyzing GCase and α-synuclein in combination. Motor and non-motor features (UPDRS-III, BDI, SCOPA-AUT, MoCA, RBDsq, PDSS, UPSIT) for prodromal PD were merged in a 7-item cumulative clinical index (CI). One-way ANOVA assessed the effect of cluster analysis groupings on the CI. Finally, we used the clinical data of GBA carriers collected 6 years earlier in order to determine whether the biochemical clusters were able to predict clinical progression over time.Results: Cluster analysis based on combined GCase/a-synuclein levels provided the best performance splitting the sample into a benign (high GCase/mid-low α-synuclein) and malignant (low GCase/high α-synuclein) profile, discriminating HC from both GBA carriers and GBA-PD. Therefore, we found a significant effect of combined GCase/α-synuclein clusters on clinical profile, revealing a significant difference between the malignant and the benign profiles, with the first showing significantly higher values in the CI with dysautonomia, mood and sleep disorders as the most relevant features. In addition, retrospective clinical data analysis showed that GBA carriers in the malignant cluster were those with a lower UPSIT score at baseline and a greater clinical deterioration over time, demonstrated a significant correlation between the biochemical clusters and clinical phenotype.Conclusion: Our study provides novel information about the relationship between biochemical and phenotypic prodromal PD signatures of GBA carriers.

CRN Team:	Team Schapira
Lab PI:	Fabio Blandini
Career Stage:	Postdoc
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Beneficial Effects of Dietary Caffeine on the Dysbiotic Pd Microbiome

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Objectives In Payami_Lab|Team_Liddle, we study the gut metagenomes of persons with Parkinson's disease (PD). We recently published the dysbiotic PD metagenome at high resolution, with functional leads that are being followed experimentally and much untapped potential for data mining (PMID:36376318). We set our next mining priority on caffeine, because PD is growing fast into a pandemic (PMID:30584159), and coffee/caffeine is the only factor, other than smoking, that is robustly associated with reduced risk of developing PD. Caffeine is also reportedly associated with improved motor and GI function in people who have PD. We hypothesized that while reduction in risk is via adenosine A2A receptor antagonism preventing neuronal death in brain, benefits of coffee as symptomatic or disease modifying therapy is via microbiome.Methods We tested association and interaction of dietary caffeine with abundance of microbial species and pathways in metagenomes of 472 PD and 225 neurologically healthy controls. Tests were unbiased, metagenome-wide, and blinded, following the general schema in PMID:36376318.Results Caffeine was associated with elevated levels of one species in both PD and controls, and reduced abundances of several species and pathways in PD only (FDR<0.05). Once analyses were completed, we broke the blind. The species that was enriched by caffeine is Lawsonibacter asaccharolyticus, identified previously in population-based studies as the most robust association with caffeine. Species that are reduced by caffeine are among the dysbiotic features that are elevated in PD (PMID: 36376318). Here, we find that the abnormally high levels are in patients who do not consume caffeine. Results suggest dietary caffeine normalizes some of the dysbiotic features in PD. Conclusions If replicated, these results can help design a microbiome-based biomarker for personalized treatment of PD with caffeine. We have shotgun sequenced our previous 16S datasets and hope to have replication results by the ASAP 2023 meeting.

CRN Team:	Team Liddle
Lab PI:	Haydeh Payami
Career Stage:	Technician
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Microbiome Influences Gut and Brain Pathology During Aging in a Mouse Model of Alpha-synuclein Overexpression

Selected for data blitz

Matheus de Castro Fonseca[1], Anna Valentine[1], Matthew Torres[1], Taren Thron[1], Sarkis Mazmaniam[1]

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Changes to gut microbiome composition have been associated with various immune, metabolic, and neurologic conditions, including Parkinson's disease (PD). The fecal microbiome of PD patients is distinct compared to healthy controls and depleting gut bacteria in mouse models of PD reduces pathology, inflammation, and impacts cognition. The aging process dramatically changes the composition of bacteria in the gut, with altered microbiome profiles linked to poorer health and frailty in the elderly. Fecal transplants from young, healthy donor mice into older animals improves markers of aging, suggesting that microbiomes of younger mice may confer health benefits. Therefore, we plan to explore a role for the microbiome in gastrointestinal function and motor phenotypes during aging in a mouse model of alpha- synuclein overexpression (Thy1-ASO). 16S sequencing of bacterial DNA from fecal pellets of 2- month and 5-month-old mice showed that the microbiome of Thy1-ASO mice “ages” differently than that of wild-type counterparts. Interestingly, both ages already present alpha-synuclein aggregates in the stomach and in the brain at 2 months of age. However, gastrointestinal and motor evaluation showed impairments only in older ASO mice. Fecal transplants from young, healthy donors into ASO recipient mice not only improved PD-related gastrointestinal and motor symptoms, but also decreased alpha-synuclein aggregates in the brain. These data suggest that a combination of genotype plus gut microbiome composition may be important to the establishment of motor and gastrointestinal dysfunctions in this mouse model.

CRN Team: Team Gradinaru
Lab PI: Sarkis Mazmaniam
Career Stage: Postdoc
Abstract Category: Gut/brain, Microbiome, & Clinical Biomarkers

The Gut Microbiome Influences Mitochondrial Function and Oxidative Stress in A- Synuclein Overexpressing Mice

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Gut microbiome-brain interactions have been implicated in a wide range of neurological conditions, including Parkinson's disease (PD). Motor dysfunction in PD is primarily associated with the selective dysfunction and loss of nigrostriatal dopaminergic neurons, potentially due to their relatively high energetic demand in comparison to other neurons. Defects in mitochondrial function may underlie vulnerability to neurodegeneration through impaired cellular respiration and accumulation of reactive oxygen species. While the etiology of PD is incompletely understood, most cases are believed to have a strong environmental contribution. The gut microbiome is altered in PD patients compared to household or population controls. Accordingly, our laboratory and others have demonstrated that the gut microbiome is required for motor deficits, neuroinflammation, and α-synuclein (αSyn) brain pathology in mice. Various metabolites produced by gut bacteria have the potential to modulate host metabolism, but a link between the microbiome and mitochondrial function in the brain remains unknown. Using α-syn overexpressing (ASO), we investigated the influence of the microbiome on mitochondrial function and motor performance. Herein, we reveal that the presence of a microbiome alters mitochondrial morphology and mitochondrial complex I and II respiration in the mouse brain. Furthermore, striatal gene and protein expression patterns suggest a role for the microbiome in regulating mitochondrial protein metabolism and oxidative stress. Motor testing of mice with or without microbiomes uncovered associations with striatal oxidative stress and enhanced progression of PD-like symptoms. These data demonstrate that the microbiome influences mitochondrial functions in the brain of αSyn overexpressing mice, which may impact motor symptoms via effects on neuronal function.

CRN Team:	Team Sulzer
Lab PI:	Sarkis Mazmanian
Career Stage:	Postdoc
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Monsters Under the Bed: the Role of Gut Microbes in REM Behavior Disorder and PD Pathogenesis

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the accumulation of α-synuclein aggregates, neuroinflammation, neuronal loss, and motor deficits. Beyond the CNS, PD patients show systemic immune activation, gut microbiome dysbiosis and gut dysfunction. A sleep disorder, REM-sleep behavior disorder (RBD), has also been implicated as an early marker for PD. In this study, CSF and stool samples were collected from patients diagnosed with RBD, PD, and healthy participants. High-throughput personalized culturomics for each stool sample has been ongoing to identify candidate PD and RBD modulating bacterial strains and communities. These bacterial strains are further characterized using in vitro and in vivo assays.As a proof-of-concept experiment, we carried out FMTs in germ-free mice. Stool from an RBD patient that presented with extensive lymphocytic pleocytosis in the CSF was selected. A fecal microbiota transplant (FMT) was performed, into transgenic alpha-synuclein overexpressing mice. Eight-weeks following FMT, behavioral assessment of the impact of RBD or HC microbiota on PD phenotypes demonstrated that mice with RBD microbiota were more impaired on the beam traversal test, the hindlimb clasping reflex, and the wire mesh hanging test than mice with HC microbiota. Single cell sequencing was performed on various tissues to assess the differential effect of RBD vs HC microbiota on peripheral and CNS immune activation in these mice. These findings provide insight into the role of microbial dysbiosis on peripheral immune activation in RBD patients, which can help in identifying prodromal PD patients decades before motor symptoms arise.

CRN Team:	Team Hafler
Lab PI:	Noah Palm
Career Stage:	Staff Scientist
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Curli Fibrils Cause Injury of Vagal Neurons Independently of Neuronal Alpha-synuclein Expression

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Enterobacteriaceae, such as E. coli, can produce and secrete the amyloidogenic protein CsgA that, in the extracellular space, aggregates into amyloid fibrils called curli. Curli fibrils possess pathogenic properties of potential relevance to Parkinson's disease (PD) that include their ability to induce pro-inflammatory reactions and to interact with other amyloidogenic proteins such as alpha-synuclein. The objective of this study was to determine the effects of curli accumulation on the function and integrity of neuronal projections of the gut-brain axis and, in particular, efferent axons that originate in the dorsal motor nucleus of the vagus nerve (DMnX) and form preganglionic terminals in the intestinal wall. Curli fibrils were produced by incubation of CsgA monomers, and their formation was assessed using thioflavin T fluorescence as well as electron microscopy. They were then injected into the pyloric and duodenal wall of C57BL/6 mice. This treatment damaged vagal preganglionic efferents, resulting in both functional and neurodegenerative consequences. Impairment of vagal parasympathetic function was indicated, for example, by a significant dysregulation of gastrointestinal motility and, histochemically, by a reduction of choline acetyltransferase immunoreactivity within vagal cell bodies in the DMnX. Of note, a small percentage of these cell bodies underwent frank neurodegeneration as indicated by a 5-10% decrease in the number of Nissl-stained DMnX cells. Initial results also indicated that curli-induced injury was independent of neuronal alpha- synuclein content and did not directly involve curli-alpha-synuclein interactions. Symptoms and signs of autonomic failure, including parasympathetic dysfunction, are common in PD, and vagal DMnX neurons are targets of PD pathology. Findings of this study bear therefore important implications as they reveal a potential new mechanism linking the microbiome to PD pathogenetic processes.

CRN Team:	Team Schapira
Lab PI:	Donato A. Di Monte
Career Stage:	Postdoc
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Metagenomics Analysis of the Gut Microbiome in Parkinson Disease Patients With and Without GBA1 Variants and Non-manifesting GBA1 Variants Carriers

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Gilles[3], Benoit Quinquis[2, 3], Christian Morabito[2, 3], Nadine Loefflad[1, 2], Selen Yalkic[1, 2], Naomi Limbachiya[1, 2], Hervé Blottière[2, 3], Stanislav Dusko Ehrlich[1, 2], Mathieu Almeida[2, 3, 4], Anthony HV Schapira[1, 2, 4]

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Objective: To evaluate the gut microbiome composition in patients with Parkinson's disease (PD) with and without variants in the GBA1 gene, and age- and sex-matched asymptomatic GBA1 variants carriers.Background: Gut microbiome alterations might contribute to motor and non- motor dysfunction in PD, however its composition and relationship with clinical phenotype is poorly defined[1]. In animal models, gut microbes could trigger PD pathology, suggesting their causative role[2]. Genetically at-risk cohorts, such as GBA1 variants carriers, provide a unique model to study the contribution of gut microbiome to PD onset and phenotype.Methods: Demographic, medical, and dietary information was collected from four groups of individuals: PD patients, carriers (GBA1-PD) or non-carriers (sporadic PD-sPD) of GBA1 variants, healthy controls (HC)-mainly PD patients' partners, and non-manifesting GBA1 variants carriers (GBA1- NMC). As part of their participation, individuals collected a fecal sample using the OMNIgene®•GUT(OM-200) collection kit. Shotgun metagenomic sequencing was performed on faecal samples and data were analyzed following IHMS standards[3]. An average of 22.27±1.8 million high-quality reads of host DNA filtered were generated and mapped onto an integrated 10.4 million human gut[4] and 8.4 million human oral microbial gene catalog[5] using the METEOR pipeline[6]. Metagenomic Species Pangenomes (MSPs) were used to quantify species associated to the gene integrated reference catalog[7].Results: 151 individuals (15 GBA1-NMC, 23 GBA1-PD, 44 HC, 69 sPD) were included in the current analysis. Demographics and lifestyle habits did not differ across groups. Clinical features analyses revealed worse olfactory function in GBA1-PD compared with sPD. Microbiome analyses showed no differences in MSP species richness across groups. Enterotype Firmicutes/Christensenellales was predominant in sPD whereas enterotype Bacteroides was predominant in HC.Conclusions: Recruitment and analyses for this study are ongoing. Preliminary results suggest no differences in MSP species richness but a difference in enterotype distribution in sPD compared with HC.

CRN Team:	Team Schapira
Lab PI:	Anthony Schapira
Career Stage:	PhD student
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Gut Mucosal Cells Transfer A-synuclein Pathology to the Vagus Nerve

Selected for data blitz

Rodger Liddle[1], **Ian Williamson**[1], Rashmi Chandra[1], Arpine Sokratian[1], Stephanie King[1], Katherine Chavez[1], Sandip Swain[1], Joshua Snyder[1], Senthil Gounder[1], Andrew West[1]

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Experimental models and epidemiological findings in Parkinson's disease (PD) have raised the possibility that misfolded α-synuclein protein in the periphery might spread from the gut to the brain to increase the risk of disease. Enteroendocrine cells (EECs) are sensory cells that reside in the lining of the gut lumen, express α-synuclein, directly connect to the nervous system, and are sensitive to luminal stimuli. Here we test the possibility that pathological α-synuclein might arise in EECs and transfer to connected nerves. In gut organoids, we find that human α- synuclein protein transfers to co-cultured nodose ganglia neurons that otherwise lack α- synuclein expression. In adult mice, conditional overexpression of human α-synuclein isoforms specifically in the gut lining drives α-synuclein pathology in the vagus nerve, increasing the α- synuclein fibril templating activity of the nodose ganglia and the dorsal motor nucleus in sensitive α-synuclein aggregation assays. A subdiaphragmatic vagotomy procedure prior to the induction of human α-synuclein overexpression in the gut lining completely protects the vagus from pathological α-synuclein activity. [IWP1] These findings highlight a novel potential pathological role for gut mucosal cells in the early stages of PD that might be amenable to therapeutic intervention.

CRN Team:	Team Liddle
Lab PI:	Rodger Liddle
Career Stage:	Postdoc
Abstract Category:	Gut/brain, Microbiome, & Clinical Biomarkers

Plasma Metabolomic Analysis Identifies Piperine - a Constituent of Black Pepper With Anti-cancer Properties - as a Novel Biomarker for Parkinson's Disease

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Piperine, an alkaloid isolated from Piper nigrum (black pepper), has shown both anti-cancer and neuroprotective potential in preclinical models. Whether piperine is associated with Parkinson's disease (PD) is undetermined.We analyzed 330 pre-validated metabolites by HPLC-ECD/UV and LC-MS in plasma from 640 subjects from the Parkinson's Progression Markers Initiative (PPMI) who carried a known GBA or LRRK2 PD-pathogenic variant or neither, and who were diagnosed with PD or were unaffected controls (UC). Analytes and pre-specified analyte ratios (including piperine and piperine normalized to curcumin, a constituent of turmeric to control for overall dietary spice intake), were analyzed by ANCOVA adjusting for relevant covariates, including age, sex, and genotype. Nominal and Benjamini-Yekatieli (BY) corrected p-values are reported.The piperine/curcumin ratio was 36% lower in PD vs. UC (p=0.0001; BY-corrected p= 0.032), reflecting a lower piperine level (-32%; p=0.0007, BY-corrected p=0.22) more than a higher curcumin level (+9%; p=0.04, BY-corrected p=1.00) in PD vs. UC. The piperine/curcumin ratio was lower in PD vs. UC across genotypes (GBA+ PD -45%; p=0.01, BY-corrected p=1.00, LRRK2+ PD -32%; p=0.04, BY-corrected p=1.00, GBA-/LRRK- PD -31%; p=0.04, BY-corrected p=1.00).Plasma piperine level normalized to curcumin level is a novel plasma biomarker of PD, irrespective of major PD gene status. Lower peripheral piperine levels in PD may represent an aversion to the ingestion of black pepper (but not of turmeric), reduced gut transport of piperine, or increased metabolism or clearance of piperine in PD. Alternatively, lower plasma levels of piperine in PD may suggest a protective effect against neuronal degeneration. Acknowledgments: Expert and collaborative contributions were provided by Sarah Huntwork-Rodriguez, Romeo Maciuca, and Jung Suh of Denali Therapeutics. Funding by the Michael J Fox Foundation for Parkinson's Research, Aligning Science Across Parkinson's (ASAP), and NIH R01NS110879

CRN Team: Team Chen
Lab PI: Michael Schwarzchild
Career Stage: Postdoc
Abstract Category: Gut/brain, Microbiome, & Clinical Biomarkers

Inflammation & Immune Regulation

Evidence for a Region-specific Neuroinflammatory Response in Parkinson's Disease Using Single-nuclei RNA-seq Analysis

Selected for data blitz

Anita Adami[1], Oliver Tam[2], Raquel Garza[1], Diahann Atacho[1], Annalies Quaegebeur[3], Talitha Forcier[2], Cole Wunderlich[2], Agnete Kirkeby[4], Roger Barker[3], Molly Hammell[2], Johan Jakobsson[1]

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There is growing awareness that neuroinflammation plays an important role in Parkinson's disease (PD) pathology. However, the molecular signature of this inflammatory response remains poorly explored in the human situation and it is unclear if it is variable in different brain regions. To investigate this, we performed single-nuclei RNA-seq (snRNA-Seq) analysis on post- mortem brain tissues from four different brain regions (substantia nigra (SN), prefrontal cortex (PFC), amygdala (AMG), and putamen (PUT)) from 14 PD patients and 8 sex and age matched non-neurodegenerative control individuals (total n = 88). Remarkably, the scRNA-Seq data showed evidence of region specific neuroinflammatory response in the PD samples.We found evidence of activation of an innate immune response including an interferon response in microglia in SN and PUT, characterized by the activation of RNA-sensors such as TLR7 and MAVS as well as the expression of the downstream interferon-response genes IRF3, IRF7 and IRF8. In contrast, in PFC we found evidence for activation of TNF-alpha and NFKB pathways linked to an unfolded protein response in cortical neurons. In AMG, we found limited evidence of neuroinflammatory response.These data demonstrate that the neuroinflammatory response in PD is highly region specific. Our results suggest that an innate immune response linked to interferon activation is predominant in brain regions directly linked to sites of dopaminergic cell and fiber loss (SN and PUT). In other regions where the pathology is less marked despite the accumulation of a-synuclein aggregates, such as PFC, the neuroinflammatory response is different and linked to TNF-alpha-signaling and an unfolded protein response.These results provide a unique insight into the diversity of the neuroinflammatory response across the PD brain and suggests that activation of an interferon response may be directly linked to the dopaminergic pathology. These findings may open new therapeutic opportunities targeting these distinct neuroinflammatory pathways.

CRN Team:	Team Jakobsson
Lab PI:	Johan Jakobsson
Career Stage:	PhD student
Abstract Category:	Inflammation & Immune Regulation

Innate Immune Response to Neurodegenerative Changes in Human Central Olfactory Sensory Areas of COVID19 and LBD/PD Patients

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Spreading of alpha-synucleinopathy as well as tauopathy is known to involve the olfactory system already early on. In this study we investigated central olfactory sensory areas in human autopsy tissue. The piriform cortex is of special interest as it links the primary olfactory nuclei (e.g. anterior olfactory nuclei) to the limbic system involving the amygdaloid complex and the hippocampal regions.Here we report preliminary results in a Lewy body dementia-Parkinson‘s disease (LBD-PD) cohort . The majority of cases analyzed showed synucleinopathy defined by immunhistochemical Lewy-pathology (9/11). Also tauopathy was present in the majority of cases with variable severity. In a COVID19 cohort we interestingly observed an increased rate of synuclein pathology (5/19, local odds ratio >=18) compared to an age-matched control autopsy cohort (0/26). Except for one patient in the COVID19 cohort no one had any record of LBD-PD symptoms. Even though this cohort cannot clarify epidemiological questions like the risk of developing PD after COVID19 we aim at characterizing the role of (aggregated) alpha synuclein in the human olfactory system. This cohort allows us to analyze different microglial activation states and neuronal integrity in human brains challenged by neurodegenerative changes and/or acute viral infection of the olfactory epithelium.

CRN Team:	Team Schlossmacher
Lab PI:	Christine Stadelmann
Career Stage:	Staff Scientist
Abstract Category:	Inflammation & Immune Regulation

A-syn Specific T-cells in Parkinson's Disease as a Monitoring Tool and Potential Therapeutic Target

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The role of adaptive immunity in Parkinson's disease (PD) represents one of the most promising axes of study and offers new therapeutic strategies for patients. For the past few years, several neuroantigens recognized by autoreactive T cells have been identified. Among those neuroantigens, alpha-synuclein (a-syn), a protein part of Lewy bodies and known to be linked to PD development has been more extensively studied. We previously identified a high a-syn- specific T cell reactivity before and close to PD diagnosis, which wanes with time as the disease progresses. These data raise the possibility of monitoring a-syn reactivity in the blood of high- risk populations: REM sleep behavior disorder (RBD) patients and LRRK2 or GBA carriers, and to further investigate the role of these T cells.We aim to confirm a higher T cell response to a-syn before diagnosis and in high-risk population (i.e., RBD) than in PD and healthy controls. We are using PBMCs from individuals with RBD, PD patients and matched healthy controls, as well as a cohort of PD patients with longitudinal samples (before and a few years after diagnosis; from 1.4 year to 11 years). Simultaneously performing multicolor spectral cytometry staining panel and measuring a-syn T cell reactivity by quantifying IFNg, IL-5 and IL-10 following a 2 weeks- culture with a-syn peptides pool, we are phenotypically characterizing our cohorts and correlating T cell frequencies with a-syn reactivity.Altogether, confirming that a-syn-specific T cell reactivity is higher prior to onset and diagnosis of clinical PD will provide a great tool to identify individuals that have high risk of developing PD. Identifying prodromal markers of PD is key to develop therapies to treat patients early on.

CRN Team:	Team Sulzer
Lab PI:	Cecilia S. Lindestam Arlehamn
Career Stage:	Postdoc
Abstract Category:	Inflammation & Immune Regulation

Systematic Activation of Line-1 Elements Causes an Inflammatory Response in Human Neural Progenitor Cells

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Neuroinflammation is a hallmark of Parkinson's disease (PD), but the mechanisms underlying this inflammatory response remains unclear. One possible cause of inflammation involves the transcriptional activation of transposable elements (TEs), such as L1s, the largest family of TEs in the human genome. L1s are viral-like mobile elements that have entered our genome during evolution and have the potential to trigger an innate immune response when transcriptionally dysregulated.To investigate a role for TEs in initiating a neuroinflammatory response in neural cells we have developed a CRISPR-based system to activate L1s. This CRISPRa system is based on a VP64-activation domain fused to catalytically dead Cas9 and we have designed a set of gRNAs targeting consensus sequences of the evolutionary young LINE-1 elements. The L1- CRISPRa vectors have been tested in human neural progenitor cells (hNPCs) and they result in robust transcriptional upregulation of hundreds of L1s as monitored by RNA-seq. Notably, the activation of L1-transcription in hNPCs results in the subsequent upregulation of stress- response and inflammatory-related genes – directly associating the over-expression of L1s to an inflammatory-like response. These results set the stage for our future investigation on the effect in the activation of these elements in cell types related to PD pathology – such as microglia, astrocytes, and dopaminergic neurons. In the end, this research will provide novel knowledge about the role of TEs in neuroinflammation, and could open new research lines for the understanding and treatment of PD.

CRN Team:	Team Jakobsson
Lab PI:	Johan Jakobsson
Career Stage:	PhD student
Abstract Category:	Inflammation & Immune Regulation

Parkin Dysfunction Causes Abnormal Differentiation of Neural Progenitor Cells

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Neural progenitor cells (NPCs) are stem cells of the central nervous system (CNS) that give rise to glial and neuronal cell types that populate the CNS. Mutations in Parkin are the most frequent cause of autosomal recessive Parkinson's disease (PD) and account for 10–20% of early-onset PD. Recent research has reported the loss of dopaminergic neurons from the substantia nigra pars compacta and a motor defect in aged Parkin knockout mice (Sliter et al, Nature, 2018). Here, we examined the effects of Parkin inactivation in mouse NPC cells. Using CRISPR/Cas9, Parkin-knockout NPC cells were generated. Inactivation of Parkin resulted in a deterioration of cell renewal ability and the alteration of morphologic characteristics - loss of cell attachment, irregularity of cell soma shape, and reduction of cell aggregation - in Parkin deficit cells. Inactivation of Parkin caused a decrease in cell proliferation and increase in apoptosis. RNAseq analysis showed that Parkin knockout resulted in 1,481 significantly downregulated genes and 1,417 upregulated genes. The top downregulated genes involved the formation of microtubules (Tuba1A, Tuba1C), cell structure, attachment and connectivity (Actb, Sparc, Zbtb20, Cd81), cell differentiation (Aqp4, Gfap, Ahnak), and inflammation and immune resistance (Ctsb, Lrp1). Gene set enrichment analysis (GSEA) showed that several key pathways, such as coagulation, complement, epithelial mesenchymal transition, IL6/JAK/STAT3 signaling, inflammatory response, IFN-alpha response, INF-gamma response, IL2/STAT5 signaling, were inhibited when Parkin was inactivated NPCs. Analysis of cells expressing mt-Kerma demonstrated that levels of mitophagy in parental cells were considerably more robust than that in Parkin-depleted counterparts. NPC cells were induced to differentiate into different cell types, including dopaminergic neurons, astrocytes, and oligodendrocytes. Parkin loss interfered with normal differentiation. Single cell RNASeq was utilized to explore transcriptional profiles of various differentiated cell populations at scale. Our findings reveal insights into how Parkin inactivation may contribute to Parkinson's disease.

CRN Team: Team Chen
Lab PI: Timothy Chan
Career Stage: Staff Scientist
Abstract Category: Inflammation & sImmune Regulation

Mice with a Parkinson's Disease-associated Mutation in LRRK2 Have Altered Early Responses to Intestinal Infection

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The gut-microbe-brain axis is an area of intense interest in Parkinson's disease (PD) research. Previous data from our group demonstrated that infection of mice with deletion of the PD- associated gene Pink1 triggered the development of motor symptoms later in life. While the overall course of infection was similar in WT and Pink knockout mice, we showed that Pink1 modulates the host response to infection. We are now evaluating whether other PD-associated genes are also implicated in early intestinal immune responses. To this end, male and female wild-type (WT) and LRRK2 knock-in Gly2019Ser (KI) littermate mice were infected with the mouse intestinal pathogen Citrobacter rodentium. Bacterial loads were measured by CFU counts, cell recruitment was analyzed by flow cytometry, lipocalin-2 levels were measured by ELISA, and cells were processed for single cell RNA sequencing. Our results determined that all mice presented similar bacterial loads in feces throughout the course of infection, with complete clearance of the infection after 28 days. In male KI mice, lipocalin-2 levels in both cecum and feces were increased and this correlated with decreased cecal bacterial loads. Flow cytometry analyses of the colonic lamina propria revealed a number of changes in the innate and acquired response to infection in KI mice including increased granulocytes and B cells and decreased macrophages, dendritic cells and NK cells. Our results suggest that the LRRK2 G2019S mutation modulates the intestinal immune response to bacterial infection. We anticipate that the scRNA sequencing results will provide further insights into these changes.

CRN Team:	Team Desjardins
Lab PI:	Samantha Gruenheid
Career Stage:	Postdoc
Abstract Category:	Inflammation & Immune Regulation

Interaction of an Alpha-synuclein Epitope With HLA DRB1*15:01 Initiates Early Enteric Features of Parkinson's Disease in Humanized Mice

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Background: Approximately 40% of Parkinson’s disease (PD) patients possess features of autoimmunity against alpha-synuclein and have anti-a-syn-specific T cells in their peripheral blood. We have previously shown that a-syn autoimmunity in PD patients is linked to the HLA DRB1*15:01 allele, which strongly binds the a-syn32-46 peptide, a neoantigen present in PD patients; however, the role of this interaction in disease pathogenesis remains unclear.Objectives: The primary objective of this study is to test whether a-syn32-46 interactions with the DRB1*15:01 allele are sufficient to drive disease pathogenesis.Methods: We adapted the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, and immunized a humanized mouse strain expressing the DRB1*15:01 allele with an a-syn32-46 peptide followed by administration of B. pertussis toxin to transiently open the blood-brain barrier (BBB).Results: Immunization primed the adaptive immune response to the neo-antigen in the periphery and resulted in inflammation of the gut and a type I interferon response that was associated with weight loss, constipation and death of dopaminergic enteric neurons, which are features associated with early stage PD. In contrast, we did not find any immune cell infiltration into the central nervous system, BBB dysfunction, death of dopaminergic neurons in the brain or defects in motor skills and learning. Upon depletion of CD4+ T cells, there is partial rescue of dopaminergic neuron death, suggesting a role for CD4+ T cells in enteric neuron loss.Conclusions: a-Syn32-46 interactions with HLA DRB1*15:01 induce inflammation and CD4+ T cell-mediated loss of enteric dopaminergic neurons in the humanized mice.

CRN Team:	Team Sulzer
Lab PI:	David Sulzer & Dritan Agalliu
Career Stage:	PhD student
Abstract Category:	Inflammation & Immune Regulation

Dysregulated Intestinal Neuro-immune Axis Underlying Early Parkinson's Disease Symptoms

Selected for data blitz

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Emerging evidence links disrupted immunity to Parkinson's disease (PD) and notably the participation of intestinal inflammation in neurodegenerative processes potentially underlying prodromal pathology. Decades prior to diagnosis, PD patients indeed already present with non- motor symptoms, including constipation. Little is known nonetheless about the mechanisms at play during the evolution of disease originating in the gut. Our aim is to further characterize our previously established model, exhibiting late PD-like symptoms after repeated gut infection of mice deficient in PTEN-induced kinase 1 (Pink1 KO). We assessed colonic immune cells of Pink1 KO mice following acute bacterial infection via single cell RNAsequencing (scRNAseq). We then applied computational methodologies to decipher inflammatory-mediated mechanisms of enteric neuron dysfunction and complemented with in vitro studies. Lastly, we ascertained if acute gut infection of Pink1 KO mice emulates prodromal phenotype in PD, such as constipation. Our findings underscore that infected Pink1 KO mice display enhanced intestinal inflammation pointing to an aberrant myeloid cell lineage as drivers of early disease. The dysregulation in the innate immune response mediated by IL1b instigates a pro-inflammatory milieu conducive to enteric neuronal damage, which presumably underlies gut dysmotility observed in Pink1 KO mice following acute infection. A more exhaustive inquiry of signaling pathways deregulated in enteric neurons will corroborate cell type-specific dysfunction. Intriguingly, scRNAseq analysis of activated CD14+ monocytes from blood of PD patients not only demonstrate higher proportion as compared to controls, but also reveal similarities in gene expression signatures promoting inflammation and interleukin-1 signaling as with intestinal Pink1 KO monocytes after acute infection. PINK1 expression in activated CD14+ monocytes is indeed lower in PD, potentially mimicking a loss-of-function phenotype as seen with Pink1 KO mice. Taken together, we propose that Pink1 KO mice following acute intestinal bacterial infection constitute an optimal model to investigate neuroimmune-related dysregulation underpinning prodromal PD pathogenesis.

CRN Team: Team Desjardins
Lab PI: Jo Anne Stratton
Career Stage: PhD student
Abstract Category: Inflammation & Immune Regulation

Non-cell Autonomous Effects of Neuromelanin on Parkinson's Disease Pathogenesis

Selected for data blitz

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Objectives: Activation of both innate and adaptive immune responses occurs in Parkinson's disease (PD) postmortem brains. PD-linked inflammatory changes are highly localized within neuromelanin (NM)-containing areas, in which extracellular NM released from dying neurons is surrounded by or in contact with activated microglia and cytotoxic T lymphocytes. However, whether NM-linked immune response contributes to the neurodegenerative process remains unknown, partly because in contrast to humans NM is absent in common experimental animals such as rodents. Here we will characterize the relationship between NM-linked immune response and PD-like pathology using novel NM-producing PD rodent models based on the constitutive or viral vector-mediated overexpression of melanin-producing enzyme tyrosinase (TYR). Methods: We first characterized histologically the NM-linked immune response, both innate and adaptive, in human postmortem PD brains and in NM-producing TYR-overexpressing animals, the latter at different time-points post-TYR expression. To determine the potential contribution of NM-linked immune response to PD-like pathology, we next injected AAV-TYR into the substantia nigra of genetically-modified MHC-II KO mice, which lack the capacity of MHCII-mediated antigen presentation, or T-cell deficient athymic nude rats. Results: In both human PD brains and NM-producing rodents, AI-based quantifications of the inflammatory/immune response confirmed increases in microglial/macrophage activation (Iba1/CD68), astrocytic response (GFAP) and T-cell infiltration (mostly CD8) in close association with extracellular NM debris released from dying neurons. In TYR-expressing rodents, both innate and adaptive immune responses occurred at very early stages of the neurodegenerative process, even preceding overt neurodegeneration in this model. We are now assessing whether modulation of the immune response in NM-producing MHC-II KO mice and athymic nude rats influence NM-linked Lewy pathology and/or nigrostriatal degeneration in these animals. Conclusions: Activation of innate and adaptive immune responses by extracellular NM occurs at very early stages of the neurodegenerative process and may thus contribute to PD pathology and progression.

CRN Team:	Team Vila
Lab PI:	Miquel Vila
Career Stage:	PhD student
Abstract Category:	Inflammation & Immune Regulation

The Role of Co-pathologies in Parkinson's Disease

Selected for data blitz

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Objectives: Co-pathologies are a core feature of Parkinson disease (PD). Along with Lewy body formation due to a-synunclein (a-syn) inclusions, ß-amyloid (Aß) and tau aggregates, hallmarks of Alzheimer's disease (AD), are also implicated in the clinical progression of PD. Aß plaques and phosphorylated-tau fibers constitute over 50% of PD cases, with pathology found in the cortex and hippocampus of post-mortem patient brains. Studies have also shown that these three pathologies synergistically interact and may promote the aggregation of each other. As current animal models of PD lack representation of these co-pathologies, this highlights a need for comprehensive models to further explore mechanisms underlying neurodegeneration. Innate and adaptive immune responses, marked by T cell infiltration, gliosis and the increase in pro-inflammatory cytokines in the brain, are prominent progressors of PD and have also been associated with Aβ and tau pathology. However, animal models of these single pathologies do not fully recapitulate the adaptive immune response that is seen to be such an important component of human disease. Methods: To test the hypothesis that Aβ, tau and a-syn co- pathologies converge to drive neuroinflammation, we developed a novel co-pathology model by stereotaxically injecting a-syn pre-formed fibrils (PFFs) and an AAV9-doublemut tau virus into the striatum and entorhinal cortex, respectively, of J20 transgenic mice. Results: 6- month-old co-pathology mice exhibit Aβ, tau and a-syn pathology in the cortex, substantia nigra, hippocampus and striatum. Our results have also shown a robust neuroinflammatory response including increased MHCII, GFAP and Iba-1 positivity along with CD4 and CD8 T cell infiltration into brain regions specific to pathology. Conclusions: With this clinically relevant model of PD, we aim to further explore and understand mechanisms of how these co- pathologies enhance neuroinflammation.

CRN Team:	Team Kordower
Lab PI:	Ashley Harms
Career Stage:	PhD student
Abstract Category:	Inflammation & Immune Regulation

T-cell Dysfunction Associated With LRRK2 Mutation in the Pathogenesis of Parkinson's Disease

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Objectives: Mutations in leucine-rich repeat kinase-2 (LRRK2) are well-recognized genetic risk factors in Parkinson's disease (PD). Among them, the G2019S mutation resulting in aberrantly enhanced kinase activity is the most common pathogenic mutation. Although increased LRRK2 activity was found in immune cells from PD patients, the impact of LRRK2 G2019S mutation on immune functions, particularly T-cell immunity, remains unclear. As previous studies showed that numbers of a-synuclein-specific CD4+ T cells increase in PD patients, we focus on exploring whether LRRK2 G2019S mutation contributes to the pathogenesis of PD via altering CD4+ T-cell functions. Methods: We generated a new T cell receptor (TCR) transgenic mouse strain bearing LRRK2 G2019S knock-in mutation, OT2-LRRK2. As CD4+ T cells from OT2 mice specifically recognize ovalbumin, this new strain enables us to explore the impact of LRRK2 G2019S mutation on T-cell functions in an antigen-specific manner. The molecular and immune profiles of OT2-LRRK2 were characterized by RT-PCR and flow cytometry analyses. CD4+ T cell proliferation and differentiation were determined by cell counting and ELISA, respectively. Results: We found that the percentages and proliferation of immune cells in spleen tissue from OT2-LRRK2 mice are comparable to wild-type controls. However, under Th2 and Th17 polarization conditions, OT2-LRRK2 T cells produce increased levels of IL-4 (Th2) and IL-17 (Th17), respectively. Whereas IL-9 secretion was significantly reduced in OT2-LRRK2 T cells under Th9 polarization condition. Additionally, reduced Treg differentiation was detected in OT2-LRRK2 mice. Conversely, MLi-2, an LRRK2 inhibitor, suppresses the production of IL-4 (Th2), while boosting IL-9 (Th9) secretion in OT2 T cells. Similar alterations induced by MLi-2 treatment in human naive CD4+ T cells were observed.Conclusions: LRRK2 plays a critical role in regulating T-cell differentiation. Our future experiments will determine whether LRRK2 G2019S-induced T-cell dysfunction promotes the damage of dopaminergic neurons in PD models.

CRN Team:	Team Chen
Lab PI:	Weiyi Peng
Career Stage:	Postdoc
Abstract Category:	Inflammation & Immune Regulation

LRRK2 Mutations Are Associated with Altered CD40 Signaling and Immune Responses

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Objectives: LRRK2 mutations can cause Parkinson's disease (PD). LRRK2 may regulate the immune system. CD40, a TNF receptor family member, and its ligand (CD40L) regulate T and B cell collaboration, cytokine production, and B cell survival and differentiation. Although CD40- CD40L interaction has been implicated in neurological diseases, interactions between LRRK2 and CD40 signaling are unclear. This study aims to characterize CD40 signaling pathway in relation to LRRK2 mutations using human samples and animal models.Methods: CSF from people carrying LRRK2 mutations and control subjects without LRRK2 mutations in the LRRK2 Cohort Consortium were used to determine soluble immune modulators by Luminex assay.
Agnostic CD40 antibody was used to treat LRRK2 G2019S knockin (LRRK2 KI) and control WT mice. Splenic immune cell profiling, and locomotor activity and striatal dopamine were assessed by follow cytometry, open field testing, and HPLC, respectively.Results: Levels of soluble CD40 ligand and B-cell activating factor (BAFF), another TNF receptor superfamily member that regulates B cell survival and differentiation, were significantly lower in human CSF samples from LRRK2 mutation carriers compared to non-carriers. LRRK2 KI mice, which demonstrated reduced percentage of Treg cells, exhibited reduced locomotor activity following CD40 stimulation. CD40 did not affect the locomotor activity in WT. In addition, percentages of CD11c and CD11b Gr1 were significantly increased, whereas CD4 effector cells were decreased in LRRK2 KI mice compared to the WT under CD40 stimulation condition. CD40 stimulation induced a significant 32% reduction in striatal dopamine in LRRK2 KI mice while dopamine levels were unaffected in WT controls. Conclusions: LRRK2 mutations are associated with altered CD40 pathway signaling in humans. CD40 stimulation induces immune responses, motor manifestations and dopamine reduction in LRRK2 KI mice. Further studies will contextualize the role of CD40-CD40L and their regulation of T-B cell interactions in LRRK2 PD.

CRN Team:	Team Chen
Lab PI:	Xiqun Chen
Career Stage:	Postdoc
Abstract Category:	Inflammation & Immune Regulation

Mitochondrial Pathways

Interrogation of Lysosomal Content in PINK1/Parkin Mouse Models

Selected for data blitz

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Objectives: The aim of this study is to investigate lysosomal changes in PINK1 knock out and Parkin inactive mutant knockin mice in vivo and in physiological conditions. MethodsFor the quick and precise isolation of lysosomes we availed the Lyso-TAG method, consisting of a C-terminal-3xHA epitope tag on the lysosomal transmembrane protein TMEM192. Lyso-TAG mice were crossed with Parkin S65A and PINK1 KO mice and brain, heart and lungs were isolated from non LysoTAG (Control-IP) and LysoTAG animal. Tissues were homogenized, and lysosomes isolated using magnetic anti-HA beads and, following a series of washings, prepared for proteomics, metabolomics, lipidomics and western blotting. A total of 18 mice were used for each experiment, comprising 6 biological replicates for WT/WT, LysoTAG/ WT and LysoTag/Mutant (either Parkin-S65A or PINK1 KO). Results: Lysosomes were successfully isolated from all three tissues, as demonstrated by the increase in lysosomal markers detected by western blotting and mass spectrometry between Control-IP and Lyso-IP. Proteomic analysis reveals differences in recruitment of several proteins at the organelle level, differences undetectable at the whole tissue scale. In Parkin S65A animals for example, ATRAID, MFSD1, GABARAPL1 where differentially expressed in the lysosome of WT vs mutant animals. Preliminary metabolomics and lipidomics analysis revealed decreased abundance of several species of non-polar metabolites in Parkin mutant animals compared to wild-type controls.4 ConclusionsThe sensitivity of the Lyso-TAG method allowed us to detect differences in the lysosomal content of WT vs mutant mice under physiological conditions. Analysis of the lipidome, metabolome and proteome of the lysosome may help understand the impact of PINK1/Parkin impairment in the context of PD, possibly identifying organelle-specific biomarkers.

CRN Team:	Team Alessi
Lab PI:	Miratul Muqit
Career Stage:	Postdoc
Abstract Category:	Mitochondrial Pathways

Mitochondrial Damage Triggers Degradation of Negative Regulators of Neuronal Autophagy

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Stress-induced damage to mitochondria triggers its clearance by a form of selective autophagy called mitophagy. Mutations in genes that are critical to mitophagy such as Pink1 and Parkin have been reported in patients with Parkinson's disease (PD). Thus, a detailed understanding of the molecular regulation of neuronal mitophagy is key to developing therapeutic strategies against the devastating PD. Autophagy is tightly regulated in cells, including neurons, by a combination of positive and negative regulators. We demonstrate that upon mitochondrial damage, specifically in neurons, there is concerted degradation of the three known negative regulators of autophagy- Rubicon, Myotubularin related protein (MTMR)-5 and MTMR2. We show that the targeted degradation of this class of regulators is mediated specifically by the ubiquitin-proteasomal system (UPS). This degradation is subsequently accompanied by a decrease in the levels of the autophagy receptor - p62/SQSTM1, and a potential increase in autophagosomal acidification, indicating upregulation of autophagic flux. We thus propose a graded neuronal response in the activation of this stress pathway, that connects the UPS to the mitophagy pathway to mediate an increase in clearance of damaged mitochondria. Our results show that this response is independent of the Pink1/Parkin pathway implying the presence of additional ubiquitin E3 ligase(s) which tightly regulate neuronal mitophagy upon mitochondrial stress. Thus, collectively, our work identifies three key regulators of neuronal mitophagy, the levels of which are tightly regulated upon mitochondrial stress to release the brakes on autophagic flux. Future experiments will be aimed towards identifying the E3 ligase, and its potential role in driving this stress response pathway in neurons.

CRN Team:	Team Hurley
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Career Stage:	Postdoc
Abstract Category:	Mitochondrial Pathways

Structural Basis for Parkin-dependent Mitophagy Initiation by the Ulk1 and Pi 3-kinase Complexes

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Mitophagy, a form of selective autophagy, is the process of removing damaged mitochondria. The Parkinson's disease (PD) genes PARK2 and PINK1 encode proteins whose principal function is to mark damaged mitochondria with phosphoubiquitin, and so trigger their lysosomal degradation by mitophagy. Upregulation of mitophagy is considered a potential therapeutic strategy for both PARK2 and PINK1 PD and sporadic PD. The ULK1 and PI3KC3-C1 (C1) complexes are the central players in initiating mitophagy in response to mitochondrial ubiquitination. The human ULK1 complex consists of the ULK1 protein kinase, the FIP200 scaffold protein, and the HORMA domain-containing proteins ATG13 and ATG101. The ULK1 complex provides an assembly site for the mitophagy adaptors such as p62, NDP52, and TAX1BP1, and also binds and phospho-regulates the downstream mitophagy core complex, C1. The C1 complex produces phosphatidylinositol-3-phosphate (PI(3)P), which is the main membrane-resident second messenger in the mitophagy signaling cascade. C1 consists of the lipid kinase VPS34, the scaffold and pseudokinase VPS15, and the regulatory and membrane binding subunits BECN1 and ATG14. Understanding the structure of these assemblies will be central to understanding their regulation in Parkin-dependent mitophagy and targeting them for therapeutic enhancement. The biochemistry and structural biology of these complexes has been very challenging because of their size, complexity, and high content of coiled-coil and intrinsically disordered regions. Our lab and others have gradually improved the resolution and completeness of these structures over the past decade, but atomic resolution has been elusive. Here, we determined the high resolution 3D structures of the ULK1 complex core region and the full length of the C1 complex by cryo-electron microscopy at the best local resolution of 3.35 and 3.26 Å, respectively, provided atomistic insight. New hypotheses for their regulation and concepts for their therapeutic targeting will be discussed.

CRN Team:	Team Hurley
Lab PI:	James Hurley
Career Stage:	Staff Scientist
Abstract Category:	Mitochondrial Pathways

The Pd-risk Gene Fbxo7/Park15 is Dispensable for Mitophagy and Neurogenesis

Selected for data blitz

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One core tenant of neurodegenerative diseases, like Parkinson's disease (PD), is an imbalance of cellular homeostasis, including the turnover of defective mitochondria (mitophagy). FBXO7 (PARK15) is a PD-risk gene previously suggested to modulate mitophagy kinetics (in siRNA models) by interacting with Pink1 and Parkin during mitophagy, proteasome turnover and proteasome activity via a direct interaction with PSMF1/ PI31.Here, we investigate the role of FBXO7 in the early steps of mitophagy. Using HeLa and hESC-derived inducible iNeuron models, combined with several orthogonal proteomic and cell-biological approaches we did not find significant defects in mitophagy kinetics in FBXO7-/- cell lines. Based on super-resolution microscopy and confocal single-cell analysis on thousands of cells, no defect in pUb spreading and clustering or Parkin recruitment after mitophagy induction was observed. In similar vein, mitophagy-flux experiments using both immunoblotting and mtKeima did not indicate a defect in the acidification of mitochondrial fragments, nor was the overall mitochondrial turnover perturbed by the loss of FBXO7. Immunoprecipitation of FBXO7 did reveal the previously interaction with Cul1 and select proteasome subunits but failed to pull-down Pink1 or Parkin.Given that the cellular metabolism shifts from glucose to OXPHOS during neurogenesis, resulting in the cellular dependence on mitochondrial ATP production, we investigate the proteome during differentiation using TMT-multiplexing in FBXO7-/- ESCs, NPCs and iNeurons. No defect in mitochondrial turnover was observed, indicating that the protein is dispensable for both neurogenesis and mitochondrial quality control.Our data suggest that FBXO7 is not involved in modulating mitophagy kinetics, and the associated pathologies will likely be due to other cellular functions of the protein. Further experimental studies to investigate the role of FBXO7 and patient mutations, especially their suggested role in aggregation and proteasome activity, will hopefully shed light into the mechanism of the observed neurotoxicity of FBXO7.

CRN Team:	Team Harper
Lab PI:	J. Wade Harper
Career Stage:	Project Manager
Abstract Category:	Mitochondrial Pathways

Neuromodulator & Neurotransmitter Signaling

Overexpression of Human Alpha-synuclein in a Mouse Model Impairs Striatal Neurotransmitter Release

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Dopaminergic neurons of the substantia nigra pars compacta (SNc) project to the dorsal striatum and, in addition to releasing dopamine (DA), some of these cells can co-release glutamate and GABA in a subregion specific manner. Because the degeneration of SNc DA neurons is a hallmark of Parkinson's disease (PD), the effect of disease development on different modes of neurotransmitter release and the potential differences in vulnerability of DAergic subtypes is of interest. Coincident with DAergic neuron degeneration in PD is the accumulation of a-synuclein (a-syn) and models of PD show that overexpression of a-syn reduces DA release before gross degeneration is observed. To further investigate the effect of wild type human a-syn on neurotransmitter release and co-release, we have developed and begun to characterize a viral overexpression model in mice. Adult DAT-Cre mice were injected with either AAV-DIO-SNCA-mCh (AAV-SNCA) or AAV-DIO-mCh (AAV-mCh) into the SNc. Using immunohistochemistry, we confirmed that injection of AAV-SNCA but not AAV-mCh resulted in expression of human a-syn in SNc within 3 weeks and we start to see degeneration of DAergic neurons starting as early as 8 weeks post injection. Behaviorally, no major motor dysfunction is observed up to 16 weeks post injection, though there is a significant reduction in cognitive performance at 8 weeks. To assess the impact of a-syn before degeneration, we are using fast- scan cyclic voltammetry (FSCV) to measure striatal DA release and whole cell electrophysiology to measure glutamate and GABA co-release at 3-4 weeks post injection. At this time point, there is a significant reduction in DA release in mice injected with AAV-SNCA as compared to the control virus. Further experiments will elucidate the effect of a-syn overexpression on synaptic activity of DAergic terminals and determine the vulnerability of DAergic subtypes.

CRN Team:	Team Edwards
Lab PI:	Chris Ford
Career Stage:	Project Manager
Abstract Category:	Neuromodulator & Neurotransmitter Signaling

Spatiotemporal Relationships Between Dopamine and Acetylcholine Dynamics Across the Striatum During Behavior

Selected for data blitz

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In the striatum, acetylcholine (Ach) and dopamine (DA) are essential for motor and learning functions, and disruption in the DA/Ach balance in the dorsal striatum is believed to contribute to movement deficits in Parkinson's Disease (PD). Recordings in behaving animals indicate that these neuromodulators convey coincident, but opposite phasic signals at rewards and cues, suggesting that they may play opposing roles in regulating learning and motivated behavior.
During spontaneous locomotor movements, however, dynamic DA and Ach relationships in the dorsal striatum can be either positively or negatively correlated, depending on movement state. Moreover, in-vivo and in-vitro evidence suggest that DA and Ach relationships and behavioral dynamics may differ across striatum regions. Although the complex interplay of DA and Ach in the striatum has been heavily studied in brain slices, much less is known about how naturally occurring DA and Ach signals are regulated in-vivo across the entire striatum in healthy and PD states during movement and learning. We used genetically encoded fluorescent sensors in combination with a novel optical approach to chronically measure DA and Ach levels across over 100 striatum locations in head-fixed mice during locomotion. Both neuromodulators displayed a spatial organization to acceleration and deceleration events across distinct striatum locations. DA and Ach signals correlated both positively and negatively depending on striatum region and behavioral event. In healthy mice, levodopa administration differentially impacted behavioral event specific Ach signaling across the striatum. In addition, only specific temporal features of Ach signals in response to unpredicted rewards and reward- predicting cues were altered after levodopa administration. Overall, our findings advance a comprehensive understanding of how striatum-wide DA and Ach dynamics are coordinated during behavior on multiple temporal and spatial scales and provide a foundation for interpreting dynamic changes in neuromodulator signaling in the PD state.

CRN Team:	Team Cragg
Lab PI:	Mark Howe
Career Stage:	Postdoc
Abstract Category:	Neuromodulator & Neurotransmitter Signaling

Defective Dopamine Release from iPSC-derived Dopamine Neurons Harboring Parkinson's Disease-associated SNCA-triplication

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Objectives: Although cell death is classically credited for the loss of dopamine in the dorsal striatum and thus the major motor symptoms associated with Parkinson's disease (PD), evidence from a wide range of animal models of familial PD indicates that defective dopamine release is an early cardinal feature of PD, preceding both neurodegeneration and symptom onset. Less is known about whether or not these defects are present in human dopamine neurons and the mechanisms by which they occur. Therefore, we aimed to address whether dopamine release is dysfunctional in human induced pluripotent stem cell (iPSC)-derived dopamine neurons from PD-affected individuals and the molecular mechanisms by which these occur.Methods: We produced human iPSC-derived dopamine neurons from patients with PD-associated mutation SNCA-triplication using a modified Krik's protocol. KCl-evoked dopamine release and total intracellular dopamine content were measured using high performance liquid chromatography electrochemical detection (HPLC-ECD) and synaptic defects were measured using whole-cell patch-clamp electrophysiology.Results: We observed a substantial decrease in evoked dopamine release from iPSC-DAns harboring SNCA-triplication and found that this coincided with a decrease in total intracellular dopamine content of the same magnitude. Both defective release and content were restored by acute L-DOPA treatment. Further data supports that these defects are not due to defective dopamine synthesis, but rather alterations in its handling. These defects in dopamine release and content coincided with electrophysiological dysfunction.Conclusions: Combined, these data provide support from human models that dopamine release defects are present in PD patients and sheds light on the mechanisms by which these occur. Results from this study will be critical to providing novel targets for the development of effective disease-modifying therapeutics.

CRN Team:	Team Cragg
Lab PI:	Richard Wade-Martins
Career Stage:	PhD student
Abstract Category:	Neuromodulator & Neurotransmitter Signaling

Effects of Optogenetic Manipulations in SNc Dopaminergic Neuron Sub-Types on Motor Behavior

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Optogenetic activation/inhibition of striatal dopaminergic (DA) neurons is hypothesized to bias animals toward/away from movement initiation. However, the effects vary across studies and the loci of stimulation. Our recent studies have identified genetically defined groups of DA neurons in the SNc, and these distinct subtypes of DA neurons exhibit either acceleration- locked (Anxa1+ subtype) or anti-acceleration-locked (Calb1+ subtype and vGlut2+ subtype) signaling patterns during locomotion. We hypothesize that optogenetic activation of the Anxa1+ subtype DA neurons will have pro-motor effects, while activating Calb1+ and vGlut2+ subtypes will have anti-motor effects. We predict that optogenetic inhibition of these cell types would have opposite behavioral effects, and that cues associated with optogenetic manipulations would, over time, induce the same motor effects as the manipulations themselves. Using intersectional-genetic strategies, here we express red-shifted activating/inhibiting rhodopins, ChRmine/Jaws, in isolated subtypes of DA neurons, and we examine their effects in head-fixed mice locomoting on a cylindrical treadmill. The experiments are underway, and the preliminary result indicates that activation of the Anxa1+ DA neurons leads to movement initiation. Since the acceleration-locked DA neuron subtypes appear to be selectively degenerated in post-mortem human PD brains, our preliminary results support the hypothesis that the motor dysfunction in PD results from an imbalance in pro-and anti-motor DA signaling.

CRN Team:	Team Awatramani
Lab PI:	Daniel Dombeck
Career Stage:	PhD student
Abstract Category:	Neuromodulator & Neurotransmitter Signaling

Anatomical Tracing of Circuit Connectivity

Alterations in Macro-Architecture of Cortico-Basal Ganglia Circuits in the Parkinsonian Macaque

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The primary motor cortex (M1) projects to the dorsal putamen (PUTd) and subthalamic nucleus (STNd). These regions form “closed-loop circuits” with M1, by sending multi-synaptic outputs back to M1. Motor signs of Parkinson’s disease (PD) are associated with dopaminergic depletion of PUTd. Using transneuronal tracing in nonhuman primates, we demonstrated that ventral regions of the putamen (PUTv) also send multi-synaptic projections to M1. PUTv does not receive inputs from M1, but receives projections from limbic regions and the amygdala.
Dopaminergic innervation of PUTv is relatively spared in the parkinsonian state. Thus, we proposed that “open-loop circuits” linking the PUTv with M1 may mediate paradoxical kinesia and placebo effects in PD. Here, we investigated the integrity of closed- and open-loop circuits between the basal ganglia and M1 in the parkinsonian state. We injected rabies virus (RV) into the arm region of M1 in a macaque rendered parkinsonian through the administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). We set the survival time to allow for retrograde transneuronal transport of RV from M1 to third-order neurons in the PUT and STN. We observed robust transport of RV to the STNd and to medium spiny neurons (MSNs) in the PUTv, but not to MSNs in the dopamine-depleted PUTd. These preliminary findings raise the possibility that dopamine depletion impairs MSN synaptic output. Previous studies provided evidence that dopamine depletion results in a reduction of spines and increased excitability of MSNs, but have reported no cell loss. Thus, it is generally assumed that synaptic outputs from MSNs in PUTd remain intact in the parkinsonian state. Our results question this assumption and highlight limitations in using PUTd as a target for PD therapies. Instead, our results support our hypothesis that open-loop circuits from PUTv remain relatively intact in the parkinsonian state, and may provide a viable therapeutic target.

CRN Team:	Team Strick
Lab PI:	Peter Strick
Career Stage:	Project Manager
Abstract Category:	Anatomical Tracing of circuit connectivity

Neuroplasticity of Motor Pyramidal Tract Cortical Neurons During the Development of Parkinsonism in Chronically MPTP-Treated Monkeys

Selected for data blitz

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The pathophysiology of motor cortices in parkinsonism remains poorly understood. Previous studies of the primary motor cortex (M1) have shown that the activity of pyramidal tract, but not corticostriatal neurons, is altered in MPTP-treated monkeys. We have recently shown spine loss and reduced thalamocortical innervation of M1 and the supplementary motor area (SMA) in parkinsonian monkeys. Because these findings were obtained from animals with severe dopamine (DA) depletion, it is unclear when these cortical abnormalities develop during the course of DA depletion, and how they relate to the emergence of parkinsonism. In this study, we will use MPTP-treated monkeys at different stages of striatal DA denervation to assess morphological changes in various populations of pyramidal tract corticofugal neurons as identified by injections of the retrogradely transported viruses AAVrg in the lower medulla or the putamen. Following injections in 2 control monkeys, both M1 and SMA were enriched in Golgi-like retrogradely labeled pyramidal neurons allowing for a detailed morphometric analysis of their cell body, dendritic arborization, and spine density using Neurolucida and Neurolucida Explorer (MBF Bioscience,VT-USA). The preliminary data obtained so far indicate: (1) Both corticomedullary and corticostriatal neurons in M1 display a larger soma size and dendritic length and have a more complex and extensive basilar dendritic tree and a larger density of dendritic spines than labeled neurons in SMA and (2) The overall layer distribution and morphology of corticomedullary and corticostriatal neurons in M1 and SMA is significantly different, indicating that they form two distinct populations of corticofugal neurons in the primate motor cortices. Ongoing studies in MPTP-treated monkeys with different stages of striatal DA depletion are in progress. Together, these findings will lead to a better understanding of cortical contributions to the pathophysiology of parkinsonism and, potentially, to the design of antiparkinsonian therapies based on modulating cortical activity.

CRN Team:	Team Wichmann
Lab PI:	Yoland Smith
Career Stage:	Staff Scientist
Abstract Category:	Anatomical Tracing of circuit connectivity

iPSC Models (neurons/glia/2D/3D)

Efficient Generation of an Isogenic HPSC Collection Carrying PD-Linked Familial Mutations Using Optimized Genome Editing Tools and Pipelines

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Parkinson's disease (PD) is associated with many genetic mutations and cell lineages. The lack of a complete understanding of this genetic and cellular context complexity hinders the development of more effective interventions. Genome-edited isogenic human pluripotent stem cells (hPSCs) present an unprecedented opportunity to study the molecular and cellular consequences of mutations in genetically controlled, physiologically relevant cell lineages.
However, the low editing efficiency and labor-intensive and time-consuming genome engineering procedure in hPSCs, especially when introducing heterozygous mutations, prevent a broader adaptation of this powerful platform. The Rio team adopted the recently developed, Cas9 nickase-based editing tool, prime editing (PE), and systematically optimized its delivery methods in hPSCs. We found RNA-based delivery of PE components improved editing efficiencies up to 13-fold compared to plasmid or ribonucleoprotein particle (RNP)-based methods, and when used repeatedly yielded efficiencies exceeding 60%, which allows for the generation of both heterozygous and homozygous familial PD mutations in the same experiment. In addition, we developed an hPSC editing pipeline that simplifies clonal isolation steps and utilizes NGS-based genotyping, with which multiple isogenic hPSC cell lines can be generated within 4 weeks. Using this optimized pipeline, together with PE and classical double-strand break (DSB)-based CRISPR/Cas9 editing methods, the Rio team is generating an isogenic hPSC collection carrying PD-related mutations including \SNCA (A53T/A30P), LRRK2 (G2019S), PRKN (Ex3del), PINK1 (truncation/Q129X), FBXO7 (truncation/R498X), DJ1 (Ex1-5del/L166P), SYNJ1 (truncation/R258Q), DNAJC6 (truncation/c.801-2 A>C), VPS35 (D620N), and GBA (IVS2+1 G>A/N370S/L444P) on multiple genetic backgrounds (currently on WIBR3 and H9). This collection will soon be made available to the ASAP community and beyond. We anticipate this hPSCs collection will greatly facilitate the delineation of genetic and cellular context complexity of PD.

CRN Team:	Team Rio
Lab PI:	Dirk Hockemeyer
Career Stage:	Postdoc
Abstract Category:	iPSC models (neurons/glia/2D/3D)

Bridging Integrator Protein 3 (BIN3) and Its Link to Parkinson's Disease

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Objectives: Genome Wide Association Studies (GWAS) in Parkinson's Disease (PD) patients have identified numerous non-coding variants. It is not clear how these non-coding variants participate in neurodegeneration by targeting specific cell types. Using expression Quantitative Trait Locus (eQTL) analyses of hundreds of thousands of single brain cells in the Parkinson Brain Atlas initiative and in 1,170 human brains, we identified BIN3 (Bridging Integrator Protein 3) as effect gene regulated by a GWAS peak on Chromosome 8. This raises a possibility of BIN3 involvement in PD pathogenesis. Methods and Results: By using CRISPR/cas9 gene editing technology, we generated BIN3 knockout and overexpression human stem cell lines. Loss or overexpression of BIN3 was validated in stem cells and differentiated dopaminergic neurons by immunostaining and western blot. Preliminary study showed a substantial increased alpha-synuclein and serine 129 phosphorylated alpha-synuclein in the BIN3 KO midbrain dopamine neurons. Accumulation of alpha-synuclein may be due to the impaired degradation. Western blotting showed decreased levels of proteins related to the autophagosomal-lysosomal system, including P62, LAMP1 and LC3BII, in BIN3 KO cells. Corresponding to the changes in protein levels, enlarged lysosomes were observed. These results suggest that BIN3 plays a role in regulating vesicular trafficking. Indeed, an endocytosis assay revealed reduced uptake of fluorescent dye by the BIN3 KO cells, especially when the neurons were stimulated. Conclusions: Our preliminary findings suggest that alteration of BIN3 (e.g., KO) impairs the vesicular trafficking in dopamine neurons, including endocytosis. We are currently investigating the detailed mechanism of BIN3 KO/OE in dopamine neuron function and degeneration. This research is funded in whole or in part by Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research (MJFF).

CRN Team:	Team Scherzer
Lab PI:	Prof. Su-Chun Zhang
Career Stage:	Staff Scientist
Abstract Category:	iPSC models (neurons/glia/2D/3D)

A Human iPSC Pipeline for Characterizing Target Genes in Synucleinopathy Patients

Selected for data blitz

Xinyuan Wang[1], Sumaiya Nazeen[1, 2], Isabel Lam[1], Dina Zielinski[1, 3], Ping Xu[1], Erinc Hallacli[1], Jonathan Mitchel[4], Nona Farbehi[5], Renuka Gupta[5], Jose T. Bras[6], Richard H. Myers[7], Clemens R. Scherzer[1], John Q. Trojanowski[8], Vivianna M. Van Deerlin[8], Antony A. Cooper[5], Warren Kaplan[5], Susan Lindquist[9], Shamil R. Sunyaev[1], Yaniv Erlich[3], Joseph Powell[5], Lorenz Studer[10], Jian Peng[11], Vikram Khurana1[2, 12]

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Objectives: We are developing a tractable hiPSC pipeline for functionally validating candidate genes identified by targeted exome sequencing (TES) of synucleinopathy patients. Methods: Previously, we identified networks of alpha-synuclein and beta-amyloid cytotoxicity modifiers. We performed TES of these genes in 499 synucleinopathy patients. Through joint-calling with 2570 control genomes, we identified several gene-level top hits in rare variant (MAF < 1%) association tests. We analyzed cell-type specific differential expression in a case-control setting using publicly available snRNA-seq data. Our hiPSC validation pipeline utilizes CRISPRi-i3N cells, knocked-in with Ngn2 and dCas9-KRAB. Top-hit candidates will be knocked down in cortical neurons in a sgRNA dropout screen, in the presence/absence of Parkinson's disease (PD)-relevant stressors (SNCA over-expression or GBA knock-down, and alpha-synuclein pre-formed fibrils). Positive hits will be followed by RNAseq, cellular assays and further validation in isogenic hiPSC- derived neurons. Results: TES reassuringly recovered known PD risk factors (GBA, LRRK2) as top hits. A rare-variant trend test confirmed the association of top hits with PD in AMP-PD cohort. Like many known familial and common variant PD genes, several top hits show significant differential expression changes in a cell-type specific pattern. We modified cortical neuron differentiation, lentivirus transduction and sgRNA knockdown protocols in CRISPRi-i3N cells. Pooled sgRNA protocols are currently being optimized. Ongoing expression analysis of top-hits is underway. Conclusions: We are developing a pipeline for evaluating novel candidate PD genetic risk factors in hiPSC models. Our current pipeline will be adapted for variant-level analyses based on ongoing genome sequencing and stratification studies.

CRN Team: Team Studer
Lab PI: Vikram Khurana
Career Stage: Postdoc
Abstract Category: iPSC models (neurons/glia/2D/3D)

Advanced Analytical Pipelines for 2-Dimensional and 3-Dimensional Analysis of Grafted Human iPSC Derived Cortical Neurons in the Mouse Brain

Selected for data blitz

Courtney Wright[1], Louise Cottle[1], Benjamin Trist[1], Rain Kwan[1], Alejandra Rangel[1], Asheeta Prasad[1], Stefano Frausin[2], Clare Parish[2], Lachlan Thompson[2], Deniz Kirik[1]

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Accurate visualization, qualification, and quantification of data from immunohistochemistry is critical when reporting on and understanding experimental results. Traditional image analysis may not be appropriate in all cases and can result in bias and misrepresentations of results, particularly when multiplexing is used. The increased need for highly complex image analysis and development of artificial intelligence has promoted refinement in image analysis methods. This project aims to refine the standard analytical methods and provide improved analytical pipelines for more accurate data quantification and acquisition of additional information from standard IHC tissue inputs. Nude mice were grafted with iPSC derived cortical progenitors from PD patients with specific disease related mutations (SNCA, LRRK2, Parkin). After 6 months, 30 µm tissue sections were stained with several different antibody combinations and analyzed for graft size and volume, axonal information, cortical maturation, and phenotypic markers using both chromogenic and fluorescent immunohistochemistry. Characterisation of our grafts for both 2D and 3D information utilizing artificial intelligence and deep neural networks allow us to tailor our analysis to our specific datasets to provide robust, reproducible, and highly accurate analysis of IHC data. These analyses will combine truncated cone shape and Cavalieri's method for volumetric analysis of grafts, advanced tools for axonal analysis, and automated pipelines to reduce the error in IHC analysis common with multiplexing and traditional analytical methods. Utilization of light microscopy paired with unbiased and powerful analytical pipelines for image analysis allow for increased confidence in reporting of results. These methods provide robust, detailed analysis of the graft integration and composition within the host animal, which may reveal important information about how variant PD mutations impact disease development.

CRN Team:	Team Kirik
Lab PI:	Deniz Kirik
Career Stage:	Postdoc
Abstract Category:	iPSC models (neurons/glia/2D/3D)

PD Modeling: PD Rodent & Fly Models

Exploring the Role of the Lysosomal Lipid Flippase ATP10B in the Nigrostriatal Dopaminergic Pathway of Rats

Selected for data blitz

Eduard Bentea[1], María Sanchiz-Calvo[1], Christopher Cawthorne[1], Mirte De Ceuninck[1], Teresa Torre-Muruzabal[1], Chris Van den Haute[1], Peter Vangheluwe[1], Veerle Baekelandt[1]

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Objectives: ATP10B is a lysosomal P-type transport ATPase recently associated with early-onset Parkinson's disease (PD). We demonstrated that ATP10B is a transmembrane lysosomal flippase responsible for the translocation of glucosylceramide and phosphatidylcholine from the inner to the outer lysosomal membrane in cellular systems, although its role in vivo remains poorly understood. In the current study, we aimed to explore the role of ATP10B in vivo by downregulating its expression in neurons of the substantia nigra of rats. Methods: AAV2/7 vectors encoding 2 different shRNAs (miR5 and miR7) targeting distinct regions of rat ATP10B under a neuronal promoter were unilaterally injected in the substantia nigra pars compacta of adult female Wistar rats. A vector with a scrambled sequence was used as control. Longitudinal behavioral evaluation was performed during 12 months using tests for spontaneous locomotion (open-field), motor coordination and balance (rotarod), catalepsy (bar test), and motor asymmetry (cylinder, elevated body swing). In addition, we assessed longitudinal in vivo striatal dopamine transporter binding using 18F-FE-PE2I PET imaging. Results: ATP10B downregulation in nigral neurons led to a time-dependent decrease in striatal dopamine transporter binding in vivo. Behavioral phenotyping revealed significant motor deficits indicative of decreased unilateral dopaminergic neurotransmission, including decreased spontaneous locomotion and rearing, impaired motor coordination, and balance, catalepsy, and motor asymmetry. Similar findings were observed for the two shRNAs. Preliminary data from a pilot experiment revealed increased expression of pathologically Ser129-phosphorylated-α-synuclein and formation of lipid droplets in targeted nigral neurons at 12 months post-injection, together with decreased striatal dopaminergic innervation. Conclusion: Our findings highlight an important role of ATP10B in the nigrostriatal dopaminergic pathway, and show parkinsonian deficits in rats with decreased nigral expression of ATP10B. Ongoing histological and biochemical characterization including changes in α-synuclein, lipid metabolism, and the lysosomal system will provide a better understanding of the model.

CRN Team:	Team Vangheluwe
Lab PI:	Veerle Baekelandt
Career Stage:	Postdoc
Abstract Category:	PD modeling: PD rodent & fly models

Characterizing AAV Vectors for Systemic Gene Delivery to the Brain and Gut of an Emerging Regenerative Rodent Model

Jin Hyung Chung[1], Renée Donahue[2], Changfan Lin[1], Xinhong Chen[1], Mengying Zhang[1], Sayan Dutta[1], Ashley Seifert[2], Viviana Gradinaru[1]

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Spiny mice (genus Acomys) demonstrate a remarkable capability to repair injuries to the central nervous system (CNS) and peripheral organs, and an understanding of this process could inform future therapeutic approaches for Parkinson's disease (PD). Work to date tracing neural circuits in spiny mice has utilized direct injection of recombinant adeno-associated viruses (rAAVs) in the CNS. However, to develop neural disease models and broaden our understanding of nerve regeneration in the spiny mouse, we need systemic gene delivery vectors, similar to those developed for Mus musculus. Here, we characterized the transduction profiles in the CNS and enteric nervous system (ENS) of rAAVs in spiny mice following systemic administration. We administered a pool of AAV9 variants (AAV9, PHP.eB, CAP-B10, CAP-B22, CAP-Mac, X1.1, MaCPNS1, and MaCPNS2), each carrying a unique barcoded cargo, to spiny mice via the intraperitoneal route. Next generation sequencing (NGS) of unique barcode sequences revealed that MaCPNS1 was highly enriched in the CNS, small intestine, and large intestine. We further characterized MaCPNS1 by administering an eGFP cargo driven by the ubiquitous promoter CAG packaged in MaCPNS1 via the retro-orbital sinus of spiny mice. Following administration, we found that MaCPNS1 transfected neurons and astrocytes in the brain of the spiny mouse, transfecting astrocytes more efficiently than neurons. Further, we found robust transfection of the ENS in both the small and large intestine. Together, our results demonstrate that MaCPNS1 can serve as a strong basis for an rAAV toolkit for accessing the nervous system of spiny mice, enabling the development of PD models in the spiny mice and research on the gut-brain axis.

CRN Team:	Team Gradinaru
Lab PI:	Viviana Gradinaru
Career Stage:	PhD student
Abstract Category:	PD modeling: PD rodent & fly models

Phenotypic Analysis of Familial PD Patient iPSC Derived Cortical Neurons Matured in an In Vivo Human-Mouse Xenograft Model

Louise Cottle[1], Courtney Wright[1], Alejandra Rangel[1], Rain Kwan[1], Benjamin Trist[1], Kwaku Dad Abu-Bonsrah[2], Stefano Frausin[2], Niamh Moriarty[2], Chiara Pavan[2], Asheeta Prasad[1], Glenda Halliday[1], Carolyn Sue[1], Jennifer Johnston[3], Clare Parish[2], Lachlan Thompson[2], Deniz Kirik[1, 4]

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Despite the identification of several monogenic causes of Parkinson's disease (PD), including mutations in SNCA, LRRK2, and Parkin, it is currently unclear how/why specific cell types within the brain are susceptible to degeneration in this disease. This project involves studying iPSC derived cortical neurons from familial PD patients in an in vivo xenograft paradigm. We predict that this approach will allow enhanced integration and functional maturation of cells that will allow unique insight into the mechanisms of PD pathobiology. IPSC lines from familial PD patients (with either SNCA, LRRK2, or Parkin mutations) and controls were differentiated to cortical progenitors. Three progenitor lines from each genetic background were generated (total of 12). Each progenitor line was unilaterally transplanted into the cortex of adult immunodeficient BALBc/Nu mice (100,000 cells per mouse), and cells were allowed to mature in vivo for 6 months. Mice were then fixed, brains cryoprotected then sectioned into 12 series' of 30µm sections. Tissue was stained in suspension using chromogenic (DAB) or fluorescent detection for markers of interest.Initial graft characterisation, using NCAM staining, showed that most transplanted cell lines survive in vivo and future analyses will quantify graft size and extent of integration into the host brain. In depth phenotypic analyses of grafts will include assessment of cortical specification including layer subtypes (FoxG1/Ctip2/Brn2) and mature neuronal content (NeuN). These analyses will provide information on graft complexity, maturity, and ability to integrate into the host brain which may reveal mutation specific deficits that contribute to disease.

CRN Team:	Team Kirik
Lab PI:	Deniz Kirik
Career Stage:	Postdoc
Abstract Category:	PD modeling: PD rodent & fly models

Computer Simulation of M1 Pyramidal Tract Neuron Alterations in Parkinson's Disease: Single Cell and Network Studies

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Recent evidence points to Parkinson's disease (PD) pathology resulting in decreased excitability of primary motor cortex (M1) pyramidal tract (PT) neurons of layer 5B (Liqiang et al. 2021). Given that these neurons project to brainstem and spinal cord, PT pathology may be the final domino leading to the direct motor expression of PD pathology – under-activation and abnormal timing in M1 have been associated with PD pathophysiology. We hypothesized that decreased PT excitability would disrupt normal M1 layer 5 output patterns. Using NEURON and NetPyNE, we carried out detailed computer simulations of PT neurons using data collected from healthy and 6-OHDA treated mice. Current-frequency curves for PT neurons from control and 6-OHDA treated mice were matched by single-neuron simulation. Decreased PT excitability in silico was achieved by increasing the contributions of sodium and potassium currents in a balanced manner. Activity in these in-silico PT neurons show similar activity levels to those recorded from brain slices. The simulated control and 6-OHDA PT neurons were next run in a simulated M1 slice. Self-organized and self-sustained activity was initiated in the M1 slices using a brief stimulating current (100 ms; 57 nA) to 7 layer 5B intratelencephalic pyramidal neurons. Stimulation in simulated control M1 slices resulted in sustained PT spiking activity. In contrast, simulated M1 slices using 6-OHDA PT neuron simulations produced bursts of activity in the delta range. Our simulations will be examined further to indicate how relatively small changes in PT neuron excitability alters activity throughout the circuit in a way that might disrupt motor activity in Parkinson's disease.

CRN Team:	Team Wichmann
Lab PI:	William Lytton
Career Stage:	Staff Scientist
Abstract Category:	PD modeling: PD rodent & fly models

LRRK2 Kinase Inhibition in Novel Transgenic Mouse Models with A-Synuclein Spreading from Gut to Brain

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Parkinson's disease (PD) is a progressive neurodegenerative disorder defined by a-synuclein aggregation that has been reported to start in the gut and then spread to the brain. Studies have identified Leucine Rich Repeat Kinase 2 (LRRK2) as a genetic risk factor for PD where mutations in the LRRK2 gene cause familial forms of PD. The interplay between LRRK2 and a-synuclein in the development of PD and spreading of a-synuclein pathology remains unclear. We have developed new transgenic mouse models (Vitras mice) expressing human 1-120 truncated a-synuclein in the epithelial cells of the gut with and without endogenous a-synuclein in the background. In these mice we have observed spreading of gut a-synuclein to the brain. We found that a-synuclein did not alter intestinal structure, LRRK2 was expressed in the small distal intestine (SD) in both control and transgenic mice, and that LRRK2 expression pattern was not altered by age or a-synuclein expression in our models. To assess whether LRRK2 kinase inhibition could affect a-synuclein pathology and its progression, we treated adult mice for 3 months with LRRK2 kinase inhibitor (MLi-2) supplemented-diet. Treatment with MLi-2 did not affect animal survival but prevented animal weight gain in control and transgenic mice. Moreover, LRRK2 inhibition restored gut functionality measured as feces per hour, in the transgenic mice expressing human 1-120 truncated a-synuclein with endogenous mouse a-synuclein. Ongoing studies are investigating whether MLi-2 treatment influences a-synuclein spreading from gut to brain and if overexpression of a-synuclein in the gut affects LRRK2 kinase activity. This work will shed light on LRRK2 and a-synuclein interaction in PD.

CRN Team:	Team Hardy
Lab PI:	Maria Grazia Spillantini
Career Stage:	Postdoc
Abstract Category:	PD modeling: PD rodent & fly models

Dopamine Neuron Vulnerability and Dysfunction in a VPS35 D620N Knock-in Mouse Model of Parkinson's Disease

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Objectives: The mechanisms contributing to the vulnerability of dopaminergic neurons in Parkinson's disease (PD) are not fully understood. To address this knowledge gap, Team Calakos is investigating the role of circuitry in the dysfunction and death of dopamine neurons in a VPS35 D620N knock-in mouse model which recapitulates dopaminergic neuronal degeneration. Here, our subgroup aims to replicate published phenotypes and explore additional measures of dopamine neuron integrity to identify outcome measures that will most robustly support our hypothesis-testing interventions. Our results will inform relevant circuitry perturbations and provide insight into disease progression. Methods: We assessed PD disease hallmarks in 4-, 10-, and 13-month-old wild-type (WT) and VPS35 D620N knock-in littermate mice of both sexes. We measured TH and AT8 levels in the SNc and striatum, outcomes that have been previously described in this mouse model (Chen, Xi et al. 2019). We additionally measured markers for neuroinflammation (GFAP, IBA1), neurite degeneration (Gallyas silver stain), and mitochondrial integrity in the SNc, VTA and striatum. To further understand the impact of the PD-linked VPS35 mutant on gene expression changes in dopamine neurons, spatial transcriptomics using the 10X genomic Visium platform are in progress. Results: In 4 months old mice, no pathological differences between VPS35D620N/WT, VPS35D620N/D620N and WT mice were identified.Preliminary data in aged mice show trends for genotype effects on PD hallmarks of decreased levels of TH in striatal and SNc neurons. Conclusions: Our results are consistent with those of previous studies showing no significant pathology in young VPS35D620N/WT mice compared to WT, but pathology and loss of nigrostriatal integrity observed in aged mice. Aged cohort replication and spatial transcriptomic experiments will be completed in Spring 2023. Our results advance the field by establishing independent replication of published studies and by discovering additional pathophysiology in the VPS35 D620N knockin model.

CRN Team:	Team Calakos
Lab PI:	Nicole Calakos
Career Stage:	Project Manager
Abstract Category:	PD modeling: PD rodent & fly models

Exploring the Implication of Mitochondria-Specific T-Cells and Microglial Inflammation in the Establishment of Parkinson's Disease-like Pathology in PINK1 and Parkin KO Mice

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Growing evidence suggests that PINK1 and Parkin act as negative regulators of innate and adaptive immunity. The presentation of mitochondrial antigens (MitAP) can lead to the establishment of autoreactive mitochondrial specific T-cells in PINK1-KO mice after infections. Such infections can also lead to microglial activation and chronic brain inflammation. However, the specific role of MitAP and microglial activation in neuronal dysfunction in these mouse models of early-onset Parkinson's disease (PD) is presently unknown. To assess the role of MitAP and mitochondrial antigen-specific T-cells, we adoptively transferred activated mitochondria-specific CD8+ T cells or control CD8+ T cells recognizing ovalbumin into wild type or PINK1-KO mice. The frequency and level of activation of these T-cells was assessed using flow-cytometry. In other experiments, microglial cells were isolated from Pink1 or Parkin KO mice and their activation in response to the bacterial endotoxin LPS was compared. LPS was also administered in vivo to mice once a week for four weeks. The integrity of the dopamine system was assessed by immunohistochemistry and PD-like symptoms were assessed using the pole test, the grip strength test, and the open field. Activated mitochondrial antigen specific CD8+ T cells developed into central memory T-cells after adoptive transfer. A subset of the mice died after a delay of 6-7 weeks. Many of the surviving PINK1-KO and WT mice showed impaired motor performance and reduced striatal dopamine innervation. We find that primary microglia from both Pink1 and Parkin KO background show enhanced IL-6 secretion in response to LPS compared to WT cells and that there is an increased level of microglial markers in the brain of Parkin KO mice compared to controls after LPS treatment. The present work supports the hypothesis that following infections in PD-vulnerable mice, the dopamine system may be impaired due to a combination of T-cell attack and microglia-dependent signals.

CRN Team:	Team Desjardins
Lab PI:	Michel Desjardins
Career Stage:	PhD student
Abstract Category:	PD modeling: PD rodent & fly models

PD Modeling: SNCA Models

Use of Novel Mouse Models to Decipher the Role of Sex Dimorphism and Menopause in Parkinson's Disease

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Objectives: Sex dimorphism in Parkinson's disease (PD) incidence, and motor and non-motor symptoms have been reported. Clinical and pre-clinical evidence also support that menopause increases women's susceptibility to PD. Yet, the biological mechanisms underlying the influence of sex and menopause on PD selective neuronal vulnerability in the brain and along the gut- brain axis are unknown. Methods: We are using 3 progressive mouse models of PD (nigral AAV.SNCA, gut or striatal pre-formed alpha- synuclein fibrils – PFF) in combination with the novel accelerated ovarian failure (AOF) model in females, which better mimics the human menopause compared to more traditional models like ovariectomy. To identify the pathogenic mechanisms underlying the menopause influence on PD, we are quantifying the PD motor and non-motor deficits, and nigral neurodegeneration in AOF females compared to intact females and males. Results: In the PD mouse model generated by bilateral nigral injections of AAV expressing human wild type alpha-synuclein, our data show males and AOF females develop motor dysfunction at 1-month post-op compared to age-matched AAV.mCherry-injected controls and to AAV.SNCA-injected intact females. Histological analysis of brain tissue shows that AAV.SNCA-injected AOF females have a significant nigral neuronal loss compared to AAV.mCherry-injected females. Surprisingly, we did not observe significant neurodegeneration in AAV.SNCA-injected males compared to corresponding AAV.mCherry controls, suggesting sex- specific mechanisms regulating motor dysfunction in this PD model. Conclusions: Altogether, our data show that the combination of AOF and AAV.SNCA models has the potential to unravel the sex- and menopause-specific molecular mechanisms in PD pathogenesis. Identification of these mechanisms in glia and neurons of the substantia nigra and dorsal striatum is currently in progress. Significantly, leveraging the use of the striatal-PFF and gut-PFF models, we are studying the biological mechanisms underlying the influence of sex and menopause on the PD pathogenesis, pathology, and progression along the gut-brain axis.

CRN Team:	Team Kaplitt
Lab PI:	Roberta Marongiu
Career Stage:	Postdoc
Abstract Category:	PD modeling: SNCA models

Rapid iPSC Inclusionopathy Models Shed Light on Formation, Consequence, and Molecular Subtype of α-Synuclein Inclusions

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Objectives: Intracellular inclusions accompanying neurodegeneration are histopathologically and ultrastructurally heterogeneous, and poorly correlate with cell loss. iPSC models could provide the human disease context to better understand this heterogeneity, but they do not form mature inclusions in a reasonable experimental timeframe. Here, we aimed to (1) induce rapid inclusion formation in human iPSC-derived neurons, (2) identify the impact of inclusions on neuronal survival, (3) utilize these models to shed light on distinct inclusion subtypes in synucleinopathy brains. Methods: We developed a PiggyBac-based system for rapid, scalable and virus-free conversion of iPSC to CNS cells. aS is over-expressed in this system, either physiologically through altered *SNCA* locus copy-number, or through transgenic over-expression of aS constructs. Inclusion formation can be dramatically accelerated

by exposure to aS fibrils or by expressing aggregation-prone aS mutants. We characterized inclusions in iPSC-derived cortical neurons using dynamic lattice-sheet and correlative light and electron microscopy, and single-cell longitudinal tracking to evaluate their consequences on cell survival. Genetic-modifier and protein-interaction screens were conducted to identify key proteins that are likely to be toxic when sequestered into inclusions in iPSC-derived cortical neurons and postmortem brain. Results: The PiggyBac induced inclusion neuron models form heterogeneous inclusions and recapitulate pathologic aS markers. Furthermore, this model recapitulates advanced pathologies found in postmortem brains and can guide us on novel markers that distinguish inclusion subtypes. Notably, we find that inclusion subtypes in this model differentially impact neuronal survival. Conclusion: Rapid-scale production of patient-specific neurons “in the dish” offers promise for modeling disease biology in an appropriate human cellular context. We envisage these models will be useful for biological and drug discovery in alpha-synucleinopathies.

CRN Team:	Team Studer
Lab PI:	Vikram Khurana
Career Stage:	PhD student
Abstract Category:	PD modeling: SNCA models

Increased Senescence Is Associated with α-Synucleinopathy in the TgA53T Mouse Model and Senolytic Treatment Delays Disease Onset

Selected for data blitz

Indrani Poddar[1], Yue Ma[2], Vinal Menon[1], Rachel Tappe[1], Joyce Meints[1], Paul Robbins[1], Laura Neidernhofer[1], Darren Moore[2], Michael Lee[1]

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Emerging evidence indicates that cellular senescence is a pathological factor in aging and neurodegenerative diseases, including Parkinson's Disease (PD). Because α-synuclein(αS)- pathology and αS-dependent neurodegeneration is mechanistically linked to pathogenesis of PD, we examined the pathological relationship between α-synucleinopathy and cellular senescence. To study the in vivo relevance, we used a transgenic mouse model of α- synucleinopathy (TgA53T), where rapid and reliable onset of disease was induced by intramuscular inoculation with human αS PFF. Analysis of TgA53T mice show that a- synucleinopathy is associated with increased levels of senescence markers including signs of DNA damage response (DDR; γH2Ax, Lamin B1, HMGB1), p16INK4a, p21Cip1, and SASP factors. Cellular localization of senescence markers using Immunohistochemistry and RNAscope analysis show that multiple cell types exhibit increased p16INK4a and/or p21Cip1, including neurons with aS aggregates. Significantly, expression of A53T mutant human αS seem to induce DDR in neurons in absence of overt αS pathology or other signs of senescence. To determine the pathologic significance of senescence, the mice were treated with senolytic cocktail [Dasatinib (12 mg/kg) and Quercetin (50 mg/kg) (D+Q)] or vehicle (DMSO) once weekly orally, starting 21 days post αS PFF inoculation. Analysis of motor behavior (rotarod and open field) show the D+Q treatment attenuated preclinical motor abnormalities in TgA53T mice. More important, D+Q treatment significantly delayed the onset of α-synucleinopathy. While we are currently analyzing the D+Q treated mice for neuropathology and levels of senescence markers, our initial studies indicate that D+Q treatment reduces αS pathology and reduces senescence markers in TgA53T mice. Analysis of transcriptional changes via Nanostring nCounter analysis show that a-synucleinopathy is associated with increases in senescence-associated pathways, including increased neural inflammation and D+Q treatment significantly attenuates these changes. Our data show that cellular senescence is induced by α-synucleinopathy and targeting senescent cells using senolytics may provide neuroprotection from α-synucleinopathy.

CRN Team:	Team Lee
Lab PI:	Dr. Michael lee
Career Stage:	Postdoc
Abstract Category:	PD modeling: SNCA models

Human Alpha-synuclein Overexpression in Midbrain Dopamine Neurons Impairs Cognitive Function in a Mouse Model of Parkinson's Disease

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To elucidate the role of alpha-synuclein in the pathogenesis of Parkinson's disease (PD), human alpha-synuclein has been overexpressed in mouse models using transgenic and viral-based methods. However, many transgenic synuclein overexpression mice lack cell type specificity and produce modest if any behavioral deficits. For this reason, it is difficult to conclude how alpha-synuclein drives the behavioral symptoms of PD through the particularly vulnerable dopaminergic neurons. To fill the gap, we have developed AAVs encoding Cre-dependent wild- type human alpha-synuclein, which can then be targeted to midbrain dopamine neurons. We then have assessed cognitive and motor functions at various time points (three weeks, two months, and four months) following AAV injection. Immunohistochemistry reveals that this viral-based approach results in strong alpha-synuclein overexpression in both dopaminergic cell bodies and axons projecting to the striatum. Interestingly, at early time points, when no overt motor deficits are observed, we see a significant impairment in reward-based learning in mice overexpressing synuclein, as compared to control mice. Based on our preliminary immunohistochemistry and ex vivo cyclic voltammetry results, we hypothesize that cognitive impairment results from reduced striatal dopamine release and resulting changes in patterns of striatal activity. These data suggest that an AAV-based synuclein overexpression mouse model may allow a more mechanistic dissection of cognitive dysfunction in PD and its circuit underpinnings. We hope that this approach will lead to new targets for treatment of cognitive symptoms of PD.

CRN Team:	Team Edwards
Lab PI:	Alexandra Nelson
Career Stage:	Postdoc
Abstract Category:	PD modeling: SNCA models

Sequencing/omics

Data-Driven Experimental Design for Spatial Transcriptomics Experiments

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Spatial transcriptomics technologies are improving our understanding of how the spatial organization of cells influences their cellular identity. Microenvironmental and direct cell-cell interactions play a crucial role in tumor biology, neuroscience, macrophage-driven homeostasis, and many other biological processes. Most targeted spatial transcriptomics technologies require carefully selected gene panels which will determine the successful outcome of such experiments. Here we propose a novel data-driven approach for experimental design of ISH-based spatial transcriptomics assays to maximize their informative potential. To this end we leverage a concrete autoencoder neural network for selecting an optimal set of genes to be assayed in spatial transcriptomics experiments. In multiple real life datasets this concrete autoencoder performs equally to other popular and novel feature selection methods in both cluster identification and neighborhood preservation. The concrete autoencoder minimizes geneset redundancy shown by reduced cross-correlation compared to other methods. Our method is flexible and easily adaptable to additional technological and biological constraints such as dynamic range optimization or the prioritization of specific biological pathways. We also include a Nextflow pipeline for the automated probe design of MERFISH probes after gene selection, including the quality control and filtering on various experimental design constraints. To summarize, we present a flexible data-driven method for optimizing the experimental design of targeted spatial transcriptomics experiments.

CRN Team:	Team Voet
Lab PI:	Thierry Voet
Career Stage:	PhD student
Abstract Category:	Sequencing/omics

Deconstructing Native Molecular Environment of Membrane Proteins with Tunable Nanometer-Scale Spatial Resolution

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Macromolecular associations of proteins and lipids in the cell membrane are fundamental to all membrane-associated signaling events. Capturing these complexes, determining their molecular composition and stoichiometries, and dissecting their spatiotemporal dynamics are pivotal toward deconstructing the molecular choreography that drives any membrane signaling pathway. Addressing this need we have developed a technological platform that can directly excise a target membrane protein in its native complex state from its endogenous membrane, along with its nanometer-scale spatially enriched protein-lipid molecular neighborhood. Next, coupling this with top-down native-mass spectrometry we can quantitatively decipher the protein-lipid distribution around a target membrane protein, along with their formed complex states, with precise nanometer-scale spatial resolution. To achieve this, we have developed a range of membrane active polymers (MAPs). We show that these MAPs can excise out any target membrane protein directly from un-lysed live cells along with its native membrane microenvironment (spatially enriched proteins/lipids) with tunable spatial control between 8- 25nm. Next, we set up a high-throughput fluorescence-based assay and quantitative proteomics workflow that can rapidly screen a MAP-library against any cell type and state. This generates a proteome-wide library that yields the most optimized extraction conditions for each membrane protein present in the cell. Using this platform on HEK293 and HeLa cells we show that we can successfully extract > 95% of integral and peripherally associated membrane proteins present in the proteome. This includes membrane proteins from all organellar membranes such as the plasma membrane, inner/out mitochondria, ER, Golgi, inner/outer nuclear membrane, lysosome, and autophagosomes. Simultaneous EM images of these MAP-scoops confirm our nanoscale spatial control over the extracted membrane. Applying this to VAP-A, an ER-resident membrane protein that is central towards maintaining organellar membrane contact sites and inter-organellar lipid transport, we render a quantitative view of ER-localized spatially enriched lipidome and proteome residing 20nm around VAP-A.

CRN Team:	Team De Camilli
Lab PI:	Kallol Gupta
Career Stage:	PhD student
Abstract Category:	Sequencing/omics

Transcriptional and Anatomical Distribution of MSNS in the Primate Caudal Striatum

Selected for data blitz

Olivia Brull[1], Andreea Bostan[1], Jing He[1], Jianjiao Chen[1], Andreas Pfenning[2], William R. Stauffer[1]

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopamine neurons and dopamine denervation of the striatum. However, striatal dopamine denervation in PD is not uniform, and therefore it is critical to understand the distribution of specific cell types in regions where dopamine denervation is severe, including the sensorimotor putamen, and in regions where dopaminergic function may be preserved, including portions of the ventral striatum. Using single-nucleus RNA sequencing (snRNAseq) we revealed nine different medium spiny neuron (MSN) related subtypes in the rostral non-human primate (NHP) striatum. These MSN subtypes are segregated in different physical locations within the rostral striatum, yet we do not know how MSN subtypes are distributed in caudal regions of the striatum, including the sensorimotor putamen. To gain a complete inventory of MSN subtypes, we set out to catalog MSN subtypes in the caudal striatum. We hypothesized that the caudal ventral putamen, a region that receives dense input from the amygdala, will contain neuron subtypes that resemble those in the rostral ventral striatum, the Nucleus Accumbens (NAc). We performed snRNAseq on four striatal regions from three NHPs, including the head of the caudate, the NAc, the sensorimotor putamen, and the caudal ventral putamen. We identified cell type-specific clusters and used known marker genes to identify major cell classes, including MSNs, interneurons, and glia. We identified a small cluster of MSNs from the caudal putamen that shared key marker genes with NAc ($p < 0.0001$ for all DEGs). We will use fluorescent in situ hybridization (FISH) to confirm these cell types and to build a cell type-specific map of the NHP striatum. These results will reveal the cell-type-specific architecture of the primate striatum, provide a roadmap understanding cell-type-specific contributions to PD, and highlight potential targets for circuit-specific therapeutics.

CRN Team:	Team Strick
Lab PI:	William R. Stauffer
Career Stage:	Technician
Abstract Category:	Sequencing/omics

Whole Genome Sequencing of Induced Pluripotent Stem Cell Lines Reveal Parkinson's Disease Gene Variations, Relatedness, and De-differentiation-induced Genome-wide Changes

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Objectives: The use of induced pluripotent stem cells (iPSCs) is now a standard approach in research, with methods to generate patient-derived, disease-relevant pluripotent stem cell models widely accessible. When reprogramming patient cells to iPSCs and then differentiating to cells of disease relevance, there is generally limited consideration of the genome outside of the primary affected gene locus. Mounting evidence suggests that genetic variation resulting from these processes can be considerable and could impact experimental outcomes and their interpretations. Methods: We used standard Illumina paired-end short-read sequencing to generate whole genome sequences of control, SNCA p.A53T and LRRK2 p.R1441G iPSC lines from PPMI, in addition to other PRKN-mutant and control iPSC lines (including KOLF2.1). We compared sequence variation in curated lists of Parkinson's Disease (PD) and Neurodegenerative genes, as well as looking more broadly at the nuclear and mitochondrial genomes. Sequence variants were called using a standardized nuclear variant pipeline and mityTM for mitochondrial variants. Variant pathogenicity was assessed using Varsome, various tools and databases. Comparisons were made using R and the Illumina Connected Analytics platform. Results: We identified sequence variants of potential disease relevance in PD genes outside the primary causative gene. We also identified potential relatedness between PPMI subjects with the same primary PD variants and found little variation in mitochondrial DNA quality as a function of mutational burden. We found considerable variation between patient primary cells and clonal iPSC lines, in addition to variation between isogenic iPSC clones. Conclusions: Mounting evidence indicates considerable genetic sequence variation can occur during the processes of iPSC generation and differentiation that may have an influence on cellular function and experimental outcomes. Studies using iPSCs, iPSC-differentiated cell lines and CRISPR-altered cell lines should consider both disease-relevant gene variation and genome-wide sequence changes that may impact on pertinent findings.

CRN Team:	Team Kirik
Lab PI:	Carolyn Sue
Career Stage:	Postdoc
Abstract Category:	Sequencing/omics

Transposable Elements Are Elevated in the Substantia Nigra of Parkinson's Disease Patient Tissues

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Transposable elements (TEs) are mobile genetic sequences within genomes that can generate new copies of themselves via insertional mutations. These viral-like sequences comprise nearly half the human genome and are present in any genome-wide sequencing assay. While few TEs have retained their ability to transpose, they can be present in the transcriptome, and aberrant expression of TEs has been implicated in several diseases. TE expression in short-read RNA- sequencing data is difficult to assess, due to their highly repetitive nature, but the introduction of probabilistic mapping strategies, such as those developed by our lab, have enabled accurate estimates of TE expression for bulk sequencing experiments. Bulk sequencing, however, can dilute the signal from differences in expression levels occurring in a subgroup of cells, and preclude the ability to stratify expression by cell type. While several established tools have been developed for single-cell gene expression analysis, few packages exist that can handle TEs in single-cell data. Accurate handling of TE reads becomes especially important when trying to analyze data from single-nucleus RNA sequencing assays where unprocessed intron sequences constitute up to 40% of the transcripts captured – and these intronic sequences are packed with both functional and fragmentary TEs. Here, we present a highly accurate method for single-cell TE and gene expression analysis, CellRanger-TE. We have applied the CellRanger-TE method to 72 postmortem tissue samples from Parkinson's Disease (PD) and control patient samples across 4 brain regions – substantia nigra, prefrontal cortex, amygdala, and putamen. We show that transposable elements are expressed in these tissues in a highly cell-type specific manner. Moreover, we find that TEs are elevated in multiple cell types of the substantia nigra in samples from PD patients. This elevated TE expression also correlates with markers of innate immune activation in these tissues, suggesting a role for TEs in inflammatory pathways.

CRN Team:	Team Jakobsson
Lab PI:	Molly Gale Hammell
Career Stage:	Postdoc
Abstract Category:	Sequencing/omics

Cell Type Susceptibility and Behavioral Progression in a Mouse Model of Parkinson's Disease

Akira Fushiki[1], Joaquim Alves da Silva[2], Tatiana Saraiva[2], Margarida Pinto[2], Vilas Menon[3], Rui
M. Costa[1, 4]

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Parkinson's disease (PD) is a neurodegenerative disease associated with the degeneration of dopaminergic neurons which produces motor symptoms such as tremor, bradykinesia, and rigidity. The differential susceptibility of different midbrain dopaminergic neurons to degeneration has been largely investigated. However, it has not been as established if the vulnerable populations correspond to specific molecularly defined cell types. We characterized the progression of behavioral symptoms and degeneration in MitoPark mice, a well-established PD mouse model, and matched littermate controls. We performed single-nucleus RNA-seq at several stages of progression of the motor symptoms, and correlated the susceptibility of different cell types with the emergence of behavioral phenotype. We identified specific cell types whose loss is closely associated with the development of motor symptoms. We hope these will become valuable resources to systematically dissect the molecular mechanism of PD pathogenesis at single-cell resolution.

CRN Team:	Team Surmeier
Lab PI:	Rui Costa
Career Stage:	Postdoc
Abstract Category:	Sequencing/omics

Elucidating the Role of Ligand-receptor Interactions in the Overlapping Symptomatology of Parkinson's Disease and 24 Other Neurological Disorders

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Although Parkinson's disease (PD) has substantial clinical and pathological overlaps with other CNS disorders, genetic relationships across these disorders are complex and unlikely to fully account for disease similarity. Here we aim to explore whether ligand-receptor (LR) interactions might drive common pathology, and to select candidate LRs which are promising and strategic drug targets. We investigated whether genes listed on Open Targets (https://www.opentargets.org/) as implicated in PD and other 24 neurological and neuropsychiatric diseases encode components of LR pairs listed in OmniPath (https://omnipathdb.org/). We have found that receptors occur more frequently among PD risk genes than would be expected by chance, and 14.1% of genes implicated in PD encode proteins acting as ligands and/or receptors. The majority of these LRs have been identified to be closely connected in the LR network, forming a major interacting hub of risk genes. Within this hub, 7 interactions were identified where both ligand and receptor are associated with PD risk.
Notably, FYN – associated with PD and schizophrenia – was an interactor in 5 of these. FYN also has a significant impact on the PD LR network: It is ranked as the node of highest importance by PageRank algorithm and is involved in 169 LR interactions, 40 of which have signaling partners implicated in other disorders. Using snRNAseq data we have identified that FYN is expressed in astrocytes, microglia and neurons. We are further dissecting FYN expression across PD cases and controls and brain regions. Moreover, we are validating these interactions of interest spatially, scoring their likelihood to be occurring in the CNS. We are also assessing genes and pathways upregulated in the vicinity of ligand-receptor co-expression. Jointly, these data are expected to provide insight into druggability potential of the identified targets aiming to restore homeostasis of the LR network in the brain.

CRN Team:	Team Wood
Lab PI:	Mina Ryten
Career Stage:	Postdoc
Abstract Category:	Sequencing/omics

Molecular Identification of Candidate Modulators Changing in Parkinson's Disease Mouse Models

Selected for data blitz

Marta Graziano[1], Ioannis Mantas[1], Yuvarani Masarapu[2], Solène Frapard[2], Stefania Giacomello[2], Konstantinos Meletis[1]

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Parkinson's disease (PD) is a motor disorder induced by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), but more recently it has been recognised that emotional and cognitive symptoms are present already at the prodromal stage. It is known that striatal neuromodulators, from neurons and non-neuronal cells, can shape the function of the dopamine axons. In this way, striatal neuromodulators can control the dopamine output by modulating axon excitability, conductivity, action potential propagation and DA release.
Nevertheless, little is known about how molecular signals, including neuromodulators, are altered in the prodromal phase and their role at the circuit or behavior level. To address this, we aimed to characterize the transcriptional and concurrent behavioral changes that take place in mouse models of prodromal PD. In this study we used spatial transcriptomics and single-nucleus RNA sequencing in two models of the disease: a transgenic model overexpressing human alpha-synuclein (SNCA-OVX mice) and a model with mild unilateral striatal dopamine depletion induced by injecting a low dose of 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle (MFB). Our findings highlight the dysregulation of activity-dependent genes and candidate markers in striatum that are the basis for further functional investigation in PD models.

CRN Team:	Team Cragg
Lab PI:	Konstantinos Meletis
Career Stage:	PhD student
Abstract Category:	Sequencing/omics

Somatic Copy Number Variations in Synucleinopathies by Single-cell Whole Genome Sequencing: Method Optimization

Selected for data blitz

Ester Kalef-Ezra[1, 2], Sairam Behera[3], Ida Bomann[1], Caoimhe Morley[1], Diego Perez-Rodriguez[1], Marco Toffoli[1], Amy Bowes[4, 5], Zane Jaunmuktane[1, 2], Jonas Demeulemeester[2, 4, 6, 7], Fritz
Sedlazeck[2, 3], Christos Proukakis[1, 2]

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Somatic mutations occur in healthy and diseased human brains, however, their potential role in synucleinopathies is not clear yet. We have detected somatic CNVs of the alpha-synuclein (SNCA) gene using FISH in patients with Parkinson's disease (PD) and Multiple System Atrophy (MSA). Genome-wide Megabase-scale Copy Number Variations (CNVs) are detectable by low- coverage single-cell Whole Genome Sequencing (scWGS) after Whole Genome Amplification (WGA). We have already performed this as proof-of-principle in two MSA brains. Now, we aim to characterize CNV profiles in PD, and to do that more efficiently, we are assessing which WGA methodology yields optimal scWGS from human post-mortem brains. Here, we assessed single-nuclei isolation (manually by CellRaft) and single-cell isolation from slides (Laser Capture Microdissection), as well as 3 scWGA methods (PicoPlex, PTA, dMDA). Furthermore, we are improving our bioinformatics pipeline by assessing different parameters, such as CNV callers (Ginkgo, Copykit), and versions of the human genome. We have employed brain cortex tissues from 4 human donors (1 PD, 2 MSA, 1 Control) and control samples (SNCA triplication fibroblasts, NA12878, sarcoma tissue). We have sequenced 127 single-cells by Illumina of which 11 were also sequenced by Nanopore. We assessed all 3 single-cell amplification methods with CellRaft, and PicoPlex with LCM. For large CNV detection by low-coverage Illumina sequencing, we find that PicoPlex outperforms PTA in 'noise' levels and PTA amplifies more of the genome, but both methods are suitable. Droplet MDA is potentially useful for Nanopore sequencing to define all types of Structural Variants (SVs). Single-cell amplification and preliminary sequencing analysis from synucleinopathies have been successful. Single-cell sequencing in combination with a-synuclein immunostaining and LCM optimization is now ongoing. We are also assessing more single-cells/donors, comparing them with bulk sequencing data, and improving our bioinformatics pipeline.

CRN Team:	Team Voet
Lab PI:	Christos Proukakis
Career Stage:	Postdoc
Abstract Category:	Sequencing/omics

Deciphering the Role of Alternative Splicing Linked to SNCA (A30P) and Prkn (EX3DEL) Mutations in Parkinson's Disease

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Disruption of RNA binding proteins and widespread RNA processing defects are increasingly recognized as critical determinants of neurological diseases. The role of alternative splicing in Parkinson's disease (PD) is understudied. In order to dissect the contribution of familial PD-mutations and its role in PD disease manifestation, PD-associated risk variants, including SNCA (A30P) and PRKN (Ex3del), were generated using CRISPR/Cas9-based genome engineering in WIBR3 hESCs. We performed bioinformatic splicing analyses using RNA-seq data from 35-day differentiated edited wildtype cells and iPSC-derived DA neurons containing SNCA (A30P) and PRKN (Ex3del) using the Junction Usage Model (JUM) software (Wang, 2018). We observed over 1,100 differential splicing events in either SNCA (A30P) and PRKN (Ex3del) expressing DA neurons (FDR q-value < 0.1, DPSI > 5) that are involved in neuromuscular junction development, cytoskeleton organization, and receptor localization to synapse, etc. The finding that that both SNCA (A30P) and PRKN (Ex3del) mutations resulted in differentially splicing transcripts from genes involved in cytoskeletal organization was interesting, as this might lead to reduced cell growth or viability of PD neurons. We also observed 300-480 genes were differentially expressed. There is overlap of these splicing changes with PD patient biopsy samples analyzed in Mina Ryten's lab. Together, dissecting the contribution of the alternative splicing modulation of PD-related genes can provide key points to understand the molecular basis of PD and may illuminate new pathways as potential therapeutic targets.

CRN Team:	Team Rio
Lab PI:	Donald Rio
Career Stage:	Staff Scientist
Abstract Category:	Sequencing/omics

Efficiency and Consistency: Exploring Single-Nuclei RNA-Seq Approaches in Brain Tissue of Parkinson's Disease Patients

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Human brain tissue, especially small regions including the hippocampus and substantia nigra, are difficult to work with when isolating for single-nuclei RNA-sequencing (snRNA-seq). Various reasons include working with frozen tissue and the variability among protocols. Therefore, we tested a combination of different kits to determine which approach would produce an efficient protocol with consistent results. Our homebrew protocol included performing snRNA-seq on 25 samples (15 Parkinson's disease cases and 10 controls) in the middle frontal gyrus, hippocampus, and substantia nigra. Samples were sequenced with a target of 2000-2500 single-nuclei per sample using the Chromium Next GEM Single Cell 5' Kit from 10x Genomics to give 40,000 reads per cell. Using Cell Ranger, Seurat, and Harmony, we preprocessed, clustered, and analyzed this data. A subset of samples were re-isolated with the 10x Genomics Chromium Nuclei Isolation kit, which was estimated to be more time-efficient and tissue-efficient, and were sequenced in the same way as our homebrew protocol. A different subset of samples was rerun with the CRISPRclean Single Cell RNA Boost Kit from Jumpcode Genomics, which removes uninformative reads prior to sequencing to preferentially sequence more biologically relevant and rarer transcripts; nuclei were isolated and sequenced according to our homebrew protocol. The Chromium Nuclei Isolation kit had less inter-sample variability in cell type proportions and less mitochondrial reads; however, there was more variability when using a low quantity of tissue. The CRISPRclean kit increased the number of nuclei and number of transcripts and genes per nuclei indicating that the kit took out uninformative reads. Overall, we determined that use of our homebrew protocol was preferred over the Chromium Nuclei Isolation kit, but use of the CRISPRclean kit might be able to help detect rarer cell types, markers of cellular processes that may be involved in PD, and other minute transcriptional details.

CRN Team:	Team Lee
Lab PI:	Jose Bras
Career Stage:	Technician
Abstract Category:	Sequencing/omics

Identification of Cleft Proteomes from Dopaminergic Striatal Inputs for Discovering Synaptic Molecular Components Involved in Motor Control

Selected for data blitz

Lucio Schiapparelli[1], Daichi Shonai[1], Julie Kent[1], Joey Mao[1], Scott Soderling[1]

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The selective loss of dopamine neurons (DANs) in the substantia nigra pars compacta (SNc) is one of the most remarkable traits of Parkinson's disease (PD). Current research has primarily focused on the DANs-intrinsic mechanisms of neurodegeneration. Recent evidence, however, implies that the deficit of the circuitry of the DANs precedes the degeneration, raising the potential of DANs-extrinsic mechanisms of neurodegeneration. Nevertheless, the molecular profiles mediating the connectivity between DANs and other cell types have not been well characterized. Recently, we developed a novel genetic intersectional proteomics approach, split-surface-TurboID, to identify the "cleft proteome" molecular interactions of two distinct cell types. Our previous research successfully identified the proteomes regulating the intercellular interactions between neurons and astrocytes in vivo in mouse brains, highlighting the usefulness of this split strategy. Here, we further developed an AAV-based strategy to express two halves of the split-TurboID in the specific subsets of cells in the brain by combining Cre/Flpo driver mouse lines. This strategy allows us to examine the molecular profiles at the contact sites between DANs and their connections in a higher-throughput manner. Applying the novel viral approach, we are now depicting the cleft proteomes between DANs and their connections, such as cholinergic interneurons, glutamatergic neurons, and astrocytes in PD mouse models.

CRN Team:	Team Calakos
Lab PI:	Scott Soderling
Career Stage:	Staff Scientist
Abstract Category:	Sequencing/omics

Leveraging Single-Cell Multiomic and Scaled Single-omic Assays to Study Parkinson's Related Changes in Gene Regulation in the Substantia Nigra and Cingulate Cortex

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Unraveling the impact of non-coding genetic variation in the human brain is a key challenge to improve our understanding of Parkinson's Disease. The combination of whole-genome sequencing with donor-matched single-cell multi-omics provides new opportunities to study the effect of cis-regulatory variants in each cell type separately. Our objectives were (1) to optimize protocols for single nuclei isolation from fresh frozen human brain samples in order to be compatible with downstream whole-genome sequencing (WGS) and single-cell sequencing; (2) to investigate cost-effective large-scale atlas-building using a combination of multi-omic and up-scaled single-omic assays; (3) to develop reproducible computational pipelines for data processing. In order to increase throughput, we performed single-cell multiome assays (10x Genomics, Single Cell Multiome ATAC/GEX) on 140 samples, and unimodal single-cell assays on 125 samples. scRNA-seq was performed with a split-pool combinatorial barcoding strategy (Parse Biosciences WT MEGA); while scATAC-seq was performed with a custom barcoded Tn5. The multi-omic dataset is then used as a molecular bridge to integrate the single-omic datasets. We thus far analyzed >200 samples covering substantia nigra and/or cingulate cortex from healthy controls and from patients with PD. WGS was successfully performed on a subset of 60 donors, of which the majority underwent the Fiber-seq procedure, allowing for simultaneous detection of endogenous CpG methylation and chromatin accessibility. We found that the choice of detergents shapes the quality of ATAC data and contributes to the amount of ambient RNA in multi-omic assays. We show that the quality of up-scaled single-omic assays (>1.2 million nuclei) is similar to multi-omic assays (>350 thousand nuclei) and that the datasets from different technologies integrate well. Our results provide a valuable resource for studying genetic variation and its effect on gene regulation in the human brain of healthy controls and patients with Parkinson's disease.

CRN Team:	Team Voet
Lab PI:	Stein Aerts
Career Stage:	Technician
Abstract Category:	Sequencing/omics

Characterization of the Progression of T Cell-Mediated Autoimmunity in Parkinson's Disease

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Rapid eye movement (REM) Sleep Behavior Disorder (RBD) is a parasomnia characterized by dream enactment and loss of normal skeletal muscle REM atonia1, and it is a clinical marker for faster cognitive decline and motor progression in Parkinson's disease (PD), as up to 50% of people with RBD will develop PD2. Here, we sought to characterize immune perturbations in early stages of PD by integration of paired single cell RNA sequencing (scRNA-seq) and single cell T cell receptor (scTCR-seq) data in peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF) cells from healthy controls (HC), RBD patients, PD patients, and PD patients with RBD (PDRBD). Our data showed higher LRRK2 expression in CD14+ and CD16+ monocytes in the blood of RBD patients compared to HC, suggesting an increased probability of developing PD. From CSF samples, we identified five major myeloid cell populations with distinct transcriptional profiles. Specifically, myeloid cell population 1, which had a significantly higher proportion in RBD, PD, and PDRBD, compared with HC, showed higher level of complement genes such as C1QA, C1QB, and C1QC, as well as risk genes for Alzheimer's disease including APOE and TREM2. Further, to investigate T cell clonal expansion, we utilized scTCR- seq data and discovered over 65% of clonally expanded T cells in CSF were CD4 T cells, whereas around 77% of clonally expanded T cells in blood were CD8 T cells. After performing differentially expressed gene analysis between expanded T cells versus unexpanded T cells, we found clonally expanded CD4 T cells expressed higher levels of cytotoxicity genes such as CCL5, CCL4, and CST7 in HC, RBD, and PD. Together, our results provide a high-resolution view of immune landscape across HC, RBD, PD, and PDRBD, and future studies will reveal the immune processes leading to a-synuclein autoimmunity in patients with PD.

CRN Team:	Team Hafler
Lab PI:	Le Zhang
Career Stage:	PhD student
Abstract Category:	Sequencing/omics

Structural Biology

Structures of Full-length LRRK1 and Mechanisms of Autoinhibition

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Leucine Rich Repeat Kinase 1 and 2 (LRRK1 and LRRK2) are homologs in the ROCO family of proteins in humans. Despite their shared domain architecture and involvement in intracellular trafficking through phosphorylation of Rab proteins, their disease associations are strikingly different: mutations in LRRK2 are linked to Parkinson's Disease (PD), while those in LRRK1 are linked to osteopetrosis and osteosclerotic metaphyseal dysplasia. Furthermore, all PD-linked mutations in LRRK2 increase kinase activity and are autosomal dominant gain-of-function, while disease-linked mutations in LRRK1 lead to loss of kinase activity and are autosomal recessive loss-of-function. To understand these differences, we solved cryo-EM structures of LRRK1 in its monomeric and dimeric forms. Both differ from the corresponding LRRK2 structures, and the dimer is particularly dissimilar; unlike LRRK2, which is sterically autoinhibited as a monomer, LRRK1 is sterically autoinhibited in a dimer-dependent manner, a state stabilized by two disordered regions of the structure. Strikingly, there is a second level of autoinhibition that only occurs in LRRK1, where the DYG motif in its kinase is prevented from assuming an active conformation. Indeed, LRRK1 phosphorylation of Rab7a in human cells is markedly increased by mutations in LRRK1 that disrupt either autoinhibition mode and further enhanced by disrupting both modes. Our work suggests that LRRK1 has added layers of regulation that are not present in LRRK2. We also discuss our results in the context of the evolution of the LRRK family of proteins.

CRN Team:	Team Reck-Peterson
Lab PI:	Samara Reck-Peterson
Career Stage:	Postdoc
Abstract Category:	Structural Biology

Functional and Structural Characterization of the F-Box Only Protein 7 and Its Interaction Partners

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Cullin Ring E3 ligases (CRLs) are key regulators of various biological processes and contribute to health and disease. CRLs contain interchangeable substrate receptor/adaptor modules recruiting substrates to a specific Cullin/Ring protein E3 ligase modules. The F-box only protein 7 (Fbxo7/PARK15) is known to assemble in a Cullin1/Rbx1/Fbxo7 E3 ligase complex and mutations within Fbxo7 are associated with Parkinsonian Pyramidial syndrome. We will present our progress in understanding the molecular basis for regulation of Fbxo7 and its interaction partner PI31.

CRN Team:	Team Harper
Lab PI:	Brenda Schulman
Career Stage:	Postdoc
Abstract Category:	Structural Biology

Structural and Morphological Characterization of LRRK2's Association with Lysosomes and Cytoskeleton Using Cryo- Electron Tomography

Tamar Basiashvili[1, 2], Siyu Chen[1, 2], Soojin Kim[1, 2], Robert Abrisch[1, 2], Genevieve Curtin[1, 2], Andres Leschziner[1], Sam Reck-Peterson[1, 2], Elizabeth Villa[1, 2]

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Mutations in leucine-rich repeat kinase 2 (LRRK2) lead to an increased risk of Parkinson's disease (PD). LRRK2 regulates a range of cellular processes to ensure healthy lysosomal functioning, cytoskeletal dynamics, immune response, and autophagy. However, we do not have structural knowledge of how LRRK2 and its pathogenic mutants interact with lysosomal membranes and the cytoskeleton to carry out these functionalities. Here we study how LRRK2 and its pathogenic mutants associate with lysosomal membranes and cytoskeleton using cryo-electron tomography (cryo-ET), the highest resolution imaging modality for the study of macromolecules in their native cellular environment. Cryo-ET has previously allowed us to study LRRK2's association with microtubules when overexpressed in cells. Mapping LRRK2's interaction with lysosomes and cytoskeletal filaments in physiologically relevant systems will provide critical insight into the mechanism of action of the protein. In this study, we use cryo-ET to characterize LRRK2 as it associates with lysosomes and cytoskeletal elements in macrophage and microglial immune cells. Further, we study how LRRK2 and its pathogenic mutants impact the morphology of cellular organelles in response to the lysosomal damaging toxins, LRRK2 targeted inhibitors and inflammatory agents. This study provides a molecular view of LRRK2's cellular interaction network and its impact on cellular architecture and function.

CRN Team:	Team Reck-Peterson
Lab PI:	Elizabeth Villa
Career Stage:	PhD student
Abstract Category:	Structural Biology

Structural and Biochemical Study for the Mitochondrial Tom Translocase and PINK1 Complex

Selected for data blitz

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Mitochondria are essential organelles performing crucial cellular functions such as energy production, metabolism, and cell signaling. Malfunctioning mitochondria cause severe diseases of the nervous system, heart, muscles, and the other tissues. More than 1,500 mitochondrial proteins are coordinated to perform mitochondrial functions. Most mitochondrial proteins are encoded by nuclear DNA and imported into the mitochondria in a post-translational manner. Many studies found that there are intimate interconnections between mitochondrial protein biogenesis and quality control pathways. The most prominent example is mitochondrial damage surveillance by the PINK1 (PTEN-induced kinase 1) kinase, which is activated by an altered interaction with the mitochondrial protein import machine TOM (translocase of outer membrane) complex. By a poorly understood mechanism, PINK1 accumulates on damaged mitochondria, initiating a cascade of signaling events to trigger selective degradation of the damaged mitochondria by a process known as mitophagy. Although the interaction between PINK1 and the TOM complex is the most decisive step for the whole mitophagy process, it is not understood how PINK1 interacts with the TOM complex and how this interaction activates the PINK1. Here, we present the cryo-EM structural study and biochemical study for the PINK1 engaged TOM complex. The outcome of this study will not only advance our understanding of mitochondrial surveillance but also help develop therapeutic interventions for neurodegenerative diseases such as Parkinson's disease.

CRN Team:	Team Hurley
Lab PI:	Eunyong Park
Career Stage:	Postdoc
Abstract Category:	Structural Biology

Structural Investigation of Glucosylceramide Transporters ATP10B and ATP10D

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Dysregulation of the autophagic-lysosomal system is an important factor contributing to PD development[1,2]. In particular, lysosomal accumulation of glucosylceramide (GluCer) through mutations in glucocerebrosidase *(GBA1)*, a hallmark of Gaucher disease, leads to a higher risk of developing PD. Recently, ATP10B was shown to function as an ATP-driven lysosomal exporter of glucosylceramide (GluCer) and phosphatidylcholine[3], while plasma membrane-localized - ATP10D was also shown to have specificity for GluCer[4]. PD-associated mutations impair ATP10B activity and its knockdown leads to the accumulation of fluorescent GluCer in lysosomes and increased expression of *GBA1*, triggering neuronal cell death[3]. A disrupted GluCer cellular homeostasis plays an important role in the development of PD, and approaches modulating the activity of GluCer transporters offer an attractive therapeutic approach. However, the molecular details of regulation and substrate transport by ATP10s are not fully understood. We focus on elucidating the structural and molecular basis of ATP10B and ATP10D function and regulation, as well as their substrate specificity by using cryo-electron microscopy supported by biochemical and cellular assays. We co-express human ATP10B and ATP10D with the CDC50A subunit in HEK293-FS cells. Complex formation with CDC50A has been confirmed and an optimized purification protocol yields pure heterodimer in modest amounts. The protein quality was assessed using FSEC, SEC, negative stain EM and cryo-EM analysis. Initial cryo-EM maps reveal features consistent with autoregulation and are under detailed investigation. The next steps will include acquiring high-resolution cryo-EM data of these transporters at distinct conformations and with different substrates. We will further characterize protein activity in the presence of structural insights using available activity assays.

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CRN Team: Team Vangheluwe
Lab PI: Joseph Lyons
Career Stage: Postdoc
Abstract Category: Tech Track - Structural Biology

The Molecular Mechanisms of LRRK2 Activation and Deactivation

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LRRK2 is a large, multidomain protein with two catalytic activities: a kinase and a GTPase. Structural work on LRRK2 has revealed that the protein is regulated by autoinhibition, where one of its domains the Leucine Rich Repeats (LRR) blocks access to the kinase's active site.
Functional work, both in vitro and in cells, has also shown that LRRK2's activity is regulated by phosphorylation. While many potential phosphorylation sites have been identified, few have been validated, and none are understood at a mechanistic level. My goal is to understand, structurally and mechanistically, how LRRK2's activity is regulated by phosphorylation in two regions: Ser1292 in the LRR, where autophosphorylation is linked to LRRK2 activation, and residues in a disorder loop located between residues 855 and 980, where phosphorylation has been linked to LRRK2 inactivation and interaction with the family of regulatory proteins 14-3-3. Here we expand our understanding of LRRK2's regulation using cryo-EM to obtain a molecular and mechanistic insight into the changes these phosphorylation marks cause in the activation and deactivation of LRRK2. We will use structural information to guide mechanistic hypotheses that we will test with functional assays both in vitro and in cells.

CRN Team:	Team Reck-Peterson
Lab PI:	Andres Leschziner
Career Stage:	PhD student
Abstract Category:	Structural Biology