

# Parkinson's Disease Biology: Moving Towards Innovative Treatments

## *Basic Working Group*

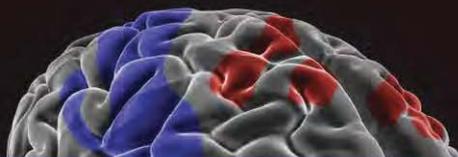
**Co-Chairs:**

Virginia Lee, PhD

Harry Orr, PhD

**Working Group Members (alphabetic):** Ted Dawson MD PhD, Rob Edwards MD, Mel Feany MD PhD, Ann Graybiel PhD, Warren Grill PhD, Hilal Lashuel PhD, Kendall Lee MD PhD, Mark Schnitzer PhD, Andy Singleton PhD, J. Paul Taylor MD PhD

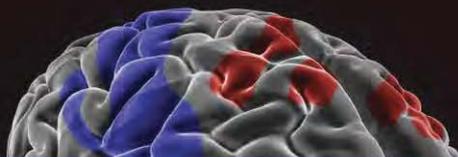
**NINDS Liaisons:** Katrina Gwinn MD, Kip Ludwig PhD, Anna Taylor PhD, Bill Benzing PhD



# Parkinson's Disease Biology: Moving Towards Innovative Treatments

## Recommendation Top Three Priorities

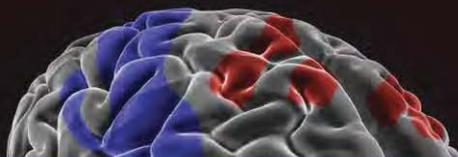
- Develop transmission models of  $\alpha$ -synuclein and tau pathology, and determine the mechanisms of propagation, release and uptake of these misfolded proteins.
- Elucidate the normal and abnormal function of  $\alpha$ -synuclein and its relationship to other PD genes.
- Understand how different cell populations in PD models change their coding properties, firing patterns, and neural circuit dynamics over time, and how these changes relate to behavior and motor control, and how therapeutic interventions may affect such changes.



## Pathways and Signaling

**Presenter: Harry Orr, PhD**

2. Elucidate the normal and abnormal function of  $\alpha$ -synuclein and its relationship to other PD genes (e.g. GBA, LRRK2, ATP13A2, PINK1 and parkin).
4. Generate and characterize a panel of PD-specific iPS cells (sporadic and genetic, including isogenic lines) for 'omic' (RNA seq, proteomics, methylation, etc) pathway analysis and other approaches
5. Integrate comprehensive PD data sets. Perform functional and genetic analyses across large data sets.
7. Develop a more detailed understanding of the genetic basis of PD.



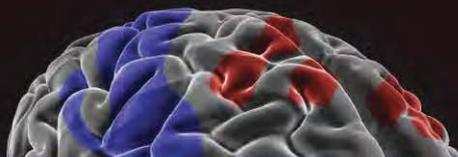
## Pathways and Signaling

**Recommendation 2:** Elucidate the normal and abnormal functions of  $\alpha$ -synuclein and other proteins implicated in PD

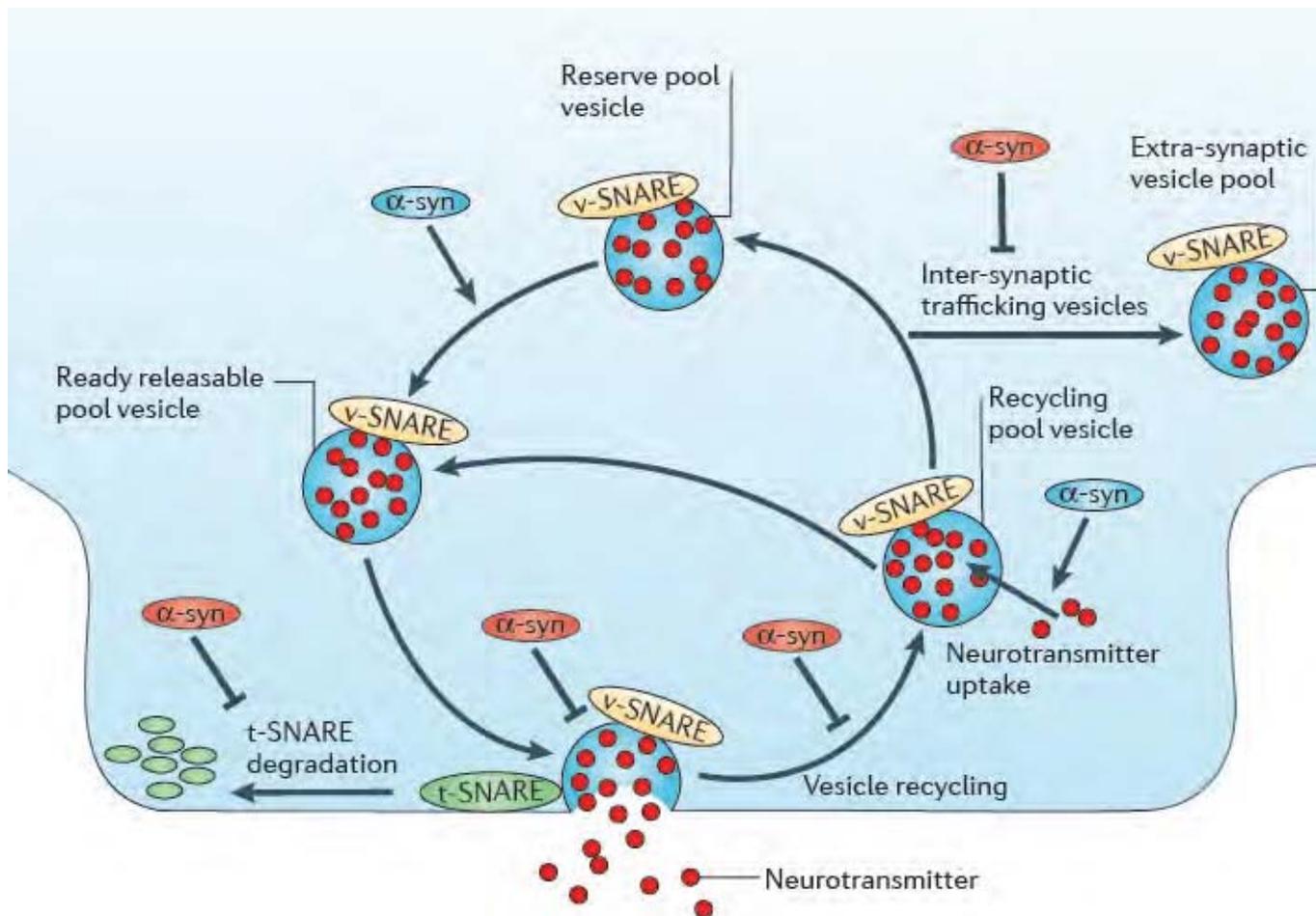
**Need:** The amount of wild type  $\alpha$ -synuclein is a strong predictor of PD risk. Little is known about the role of normal  $\alpha$ -synuclein on neurodegeneration.

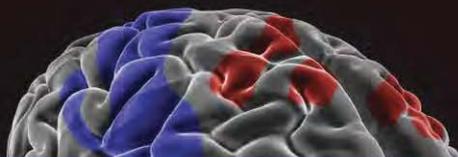
**Approaches:**

- Use  $\alpha$ -synuclein knockout mice and cell based assays to reassess the effect of PD-associated mutations.
- Determine how loss of  $\alpha$ -synuclein function affects basal ganglia circuitry
- Identify mechanisms regulating  $\alpha$ -synuclein expression
- Determine the epistatic relationships between  $\alpha$ -synuclein and other PD genes
- Elucidate relationship between  $\alpha$ -synuclein and organelles implicated in PD
- Determine whether dysfunction precedes or follows protein aggregation in PD patients

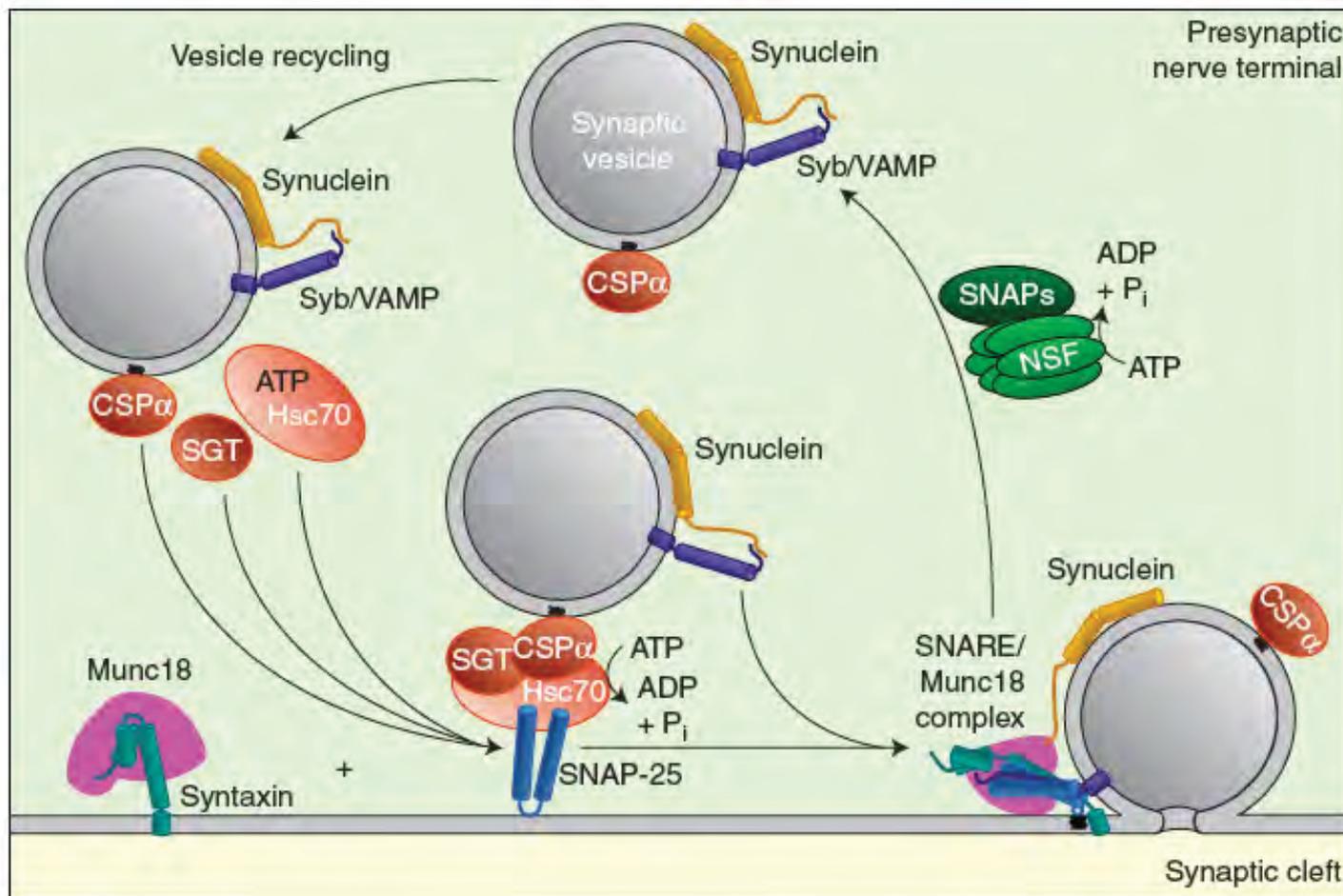


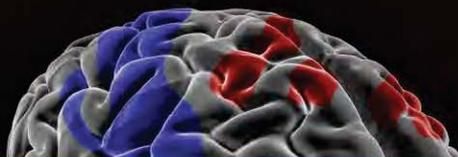
## $\alpha$ -Synuclein: A Regulator of synaptic Vesicle Release





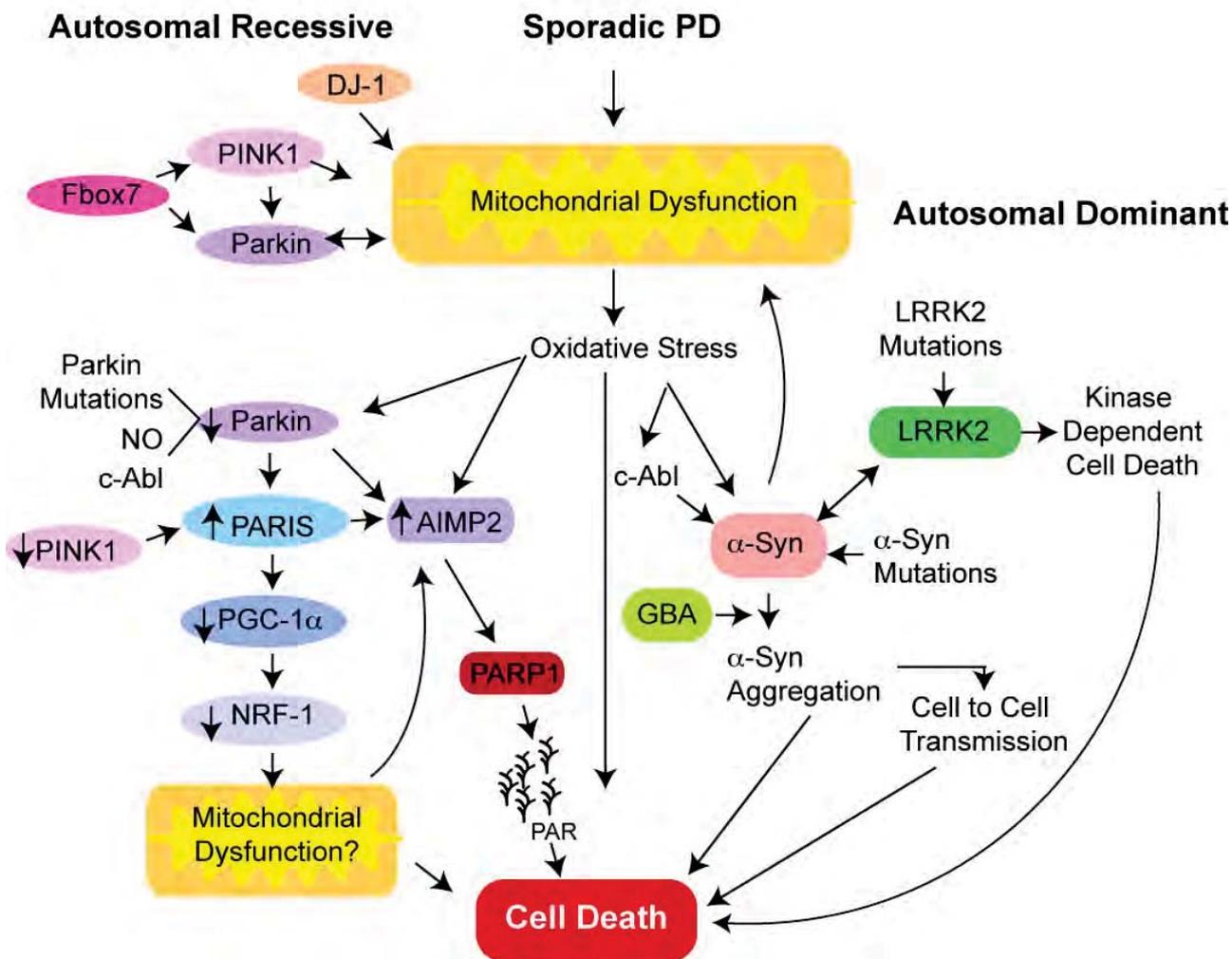
## $\alpha$ -Synuclein: One of Two Chaperones Involved in Sv naptic Function

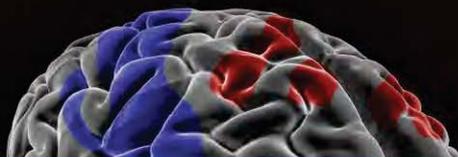




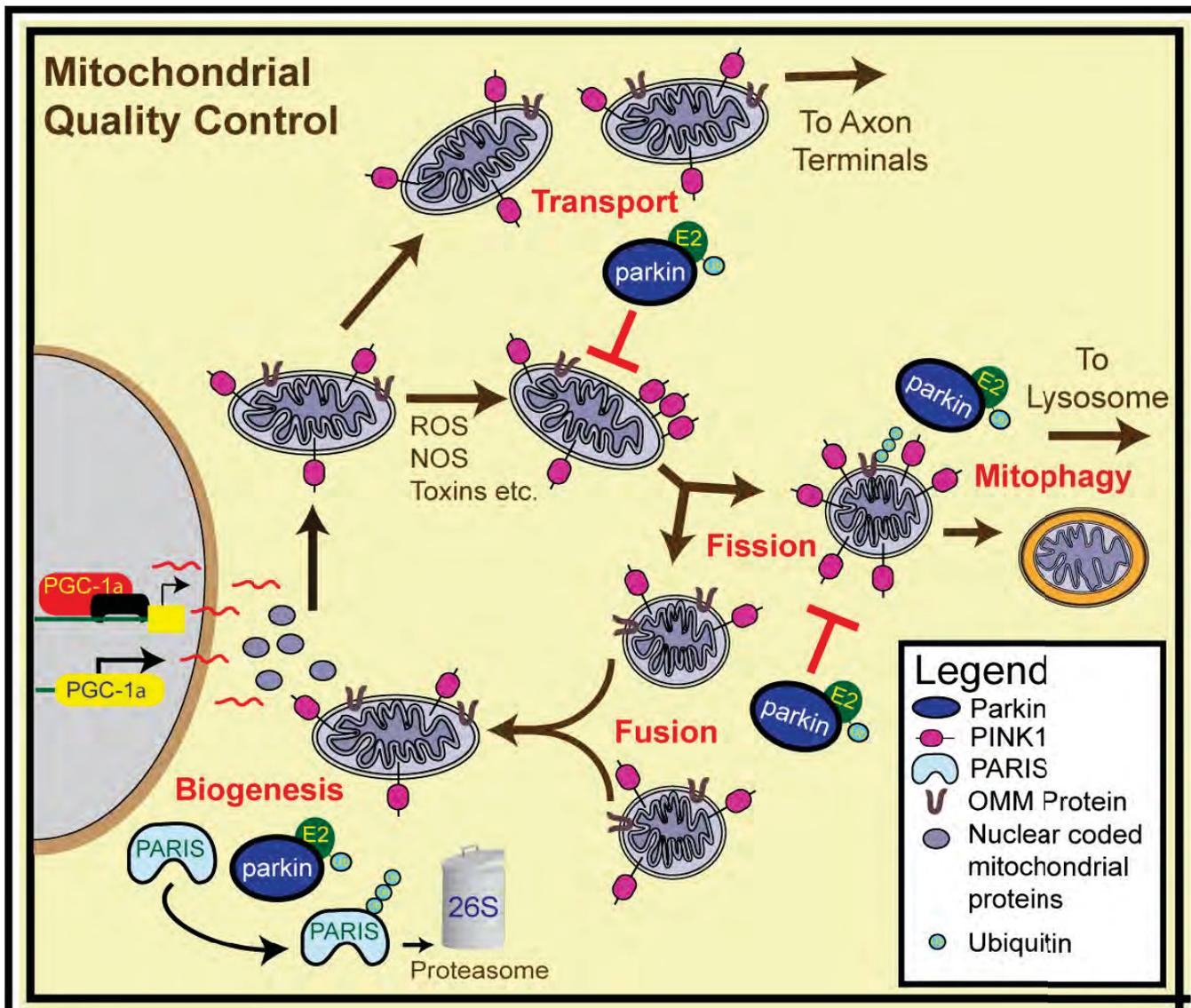
## Overview of Mitochondrial Dysfunction in PD Pathogenesis

### Parkinson's disease





## PINK1, Parkin and Mitochondrial Quality Control

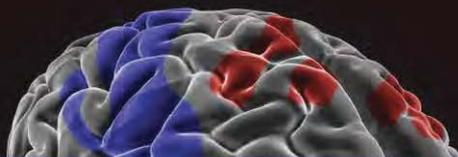


**Biogenesis:** Shin et al., *Cell* 2011

**Fission/Fusion:** Poole et.al., *PNAS* 2008, Deng et. al., *PNAS* 2008, Yang, et. al., 2008

**Mitophagy:** Narendra et.al., *J. Cell Biol*, 2008 *PLoS Biol.* 2010

**Transport:** Wang et.al., *Cell* 2011, Liu et al., *PLoS Genetics* 2012



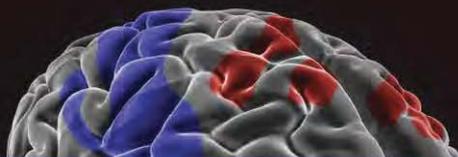
## Pathways and Signaling

**Recommendation 4:** Generate and characterize a panel of PD specific iPS for ‘omic” pathway analysis and other approaches

**Need:** Has the potential to provide new molecular insights into the pathogenesis of PD and for the discovery of biomarkers to monitor disease progression.

**Approaches:**

- Phenotypically characterize PD specific iPS-derived neurons and glia
- Develop methods to differentiate pluripotent stem cells into mature dopamine neurons and tools to interrogate them
- Develop methods to study molecular and physiologic properties of transplanted DA neurons
- Use PD iPS-derived neurons and glia to elucidate mechanisms of neurodegeneration relevant to PD



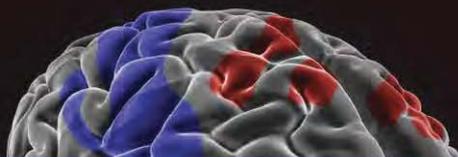
## Pathways and Signaling

**Recommendation 5:** Integrate comprehensive PD data sets. Perform functional and genetic analyses across large data sets.

**Need:** Explosion of large-scale experimental PD data that requires integration for more effective pathway and mechanism discovery

**Approaches:**

- Integrate data from multiple comprehensive data sets. Promote data dissemination from all large scale screens
- Develop analytical tools to interrogate data (e.g. genome browser, PD specific pathway/network)
- Ensure datasets reflect both motor and non-motor manifestations of PD
- Develop system wide approaches to understand the interplay of age, genome, epigenome, splicing, protein modification, etc.
- Systematically validate newly identified targets



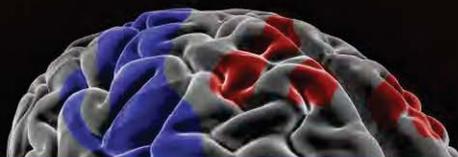
## Pathways and Signaling

**Recommendation 7:** Develop a more detailed understanding of the genetic basis of PD.

**Need:** Essential to understanding the heterogeneity of PD, which is necessary for the prediction of progression and for the application of personalized therapeutics

**Approaches:**

- Understand role of genetic risk variability on gene and protein expression
- Identification of common variant PD risk alleles and rare disease linked variants
- Exome/genome sequencing in 10s of thousands of samples
- Comprehensive genetic analysis in PD cohorts with existing longitudinal data

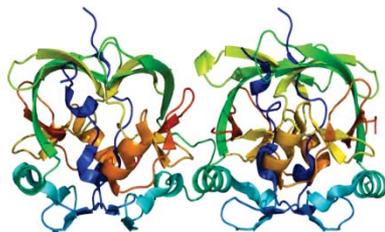


## Pathways and Signaling: PANEL DISCUSSION

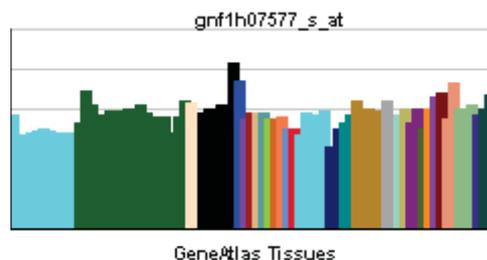
**Panelists: Ted Dawson MD PhD, Harry Orr, PhD, Andrew Singleton PhD**

**Moderator: Mel Feany MD, PhD**

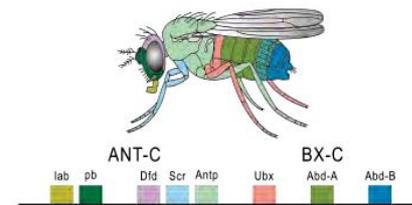
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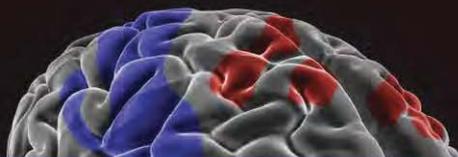
Credit: Wikimedia Commons  
(ATXN1)



Credit: Wikimedia Commons  
(LRRK2 expression pattern)



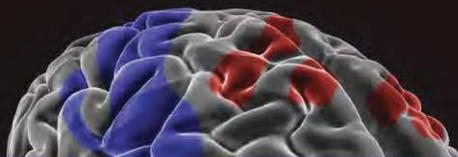
Credit: Wikimedia Commons  
(genes regulated by Hox proteins)



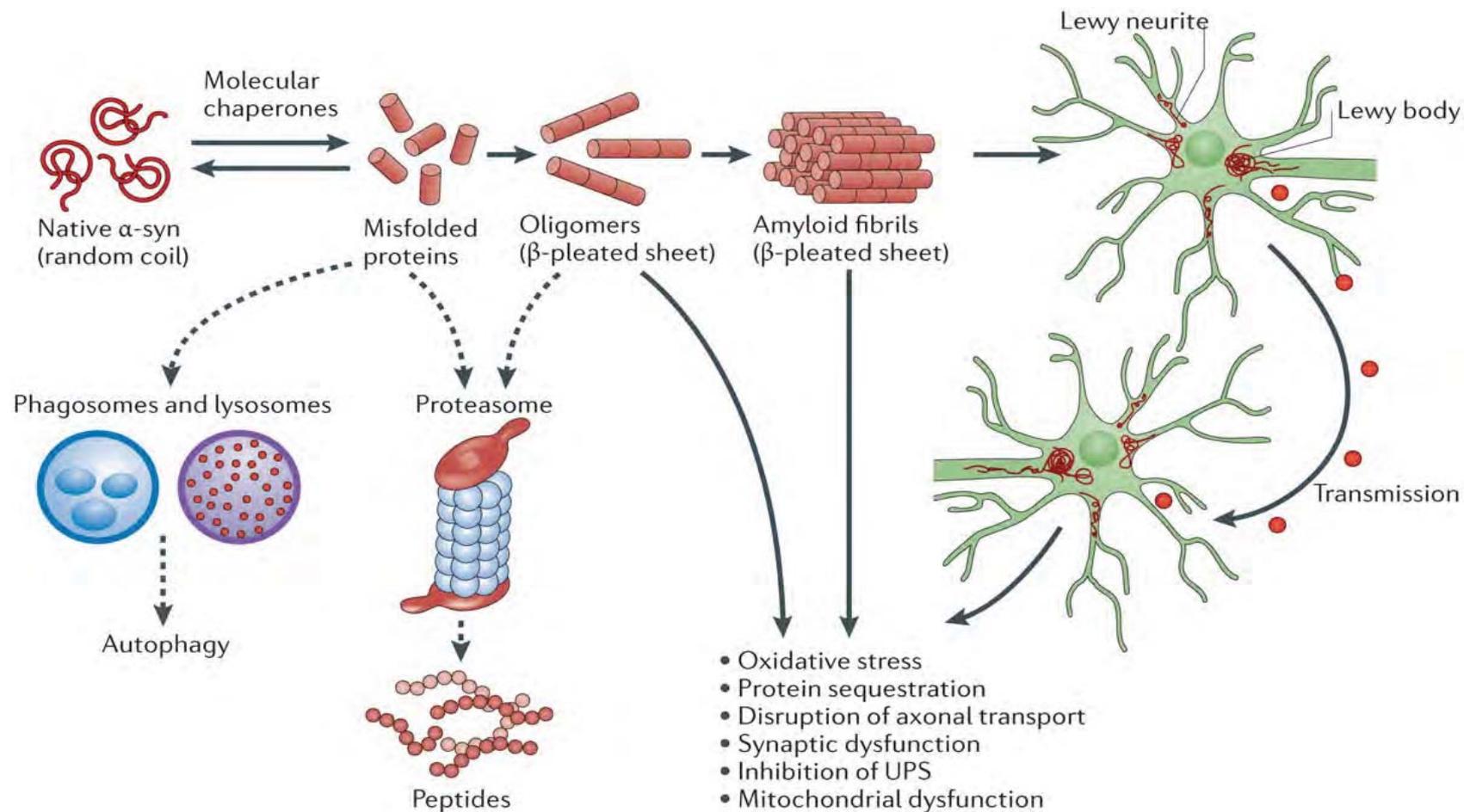
## Protein Processing, Aggregation and Spread

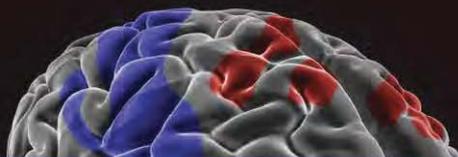
Presenter: Virginia Lee, PhD

1. Develop transmission models of  $\alpha$ -synuclein and tau pathology, and determine the mechanisms of propagation, release and uptake of these misfolded proteins including the role of strains in PD.
2. Elucidate the normal and abnormal function of  $\alpha$ -synuclein and its relationship to other PD genes (e.g., GBA, LRRK2, ATP13A2, PINK1 and parkin).
8. Develop a more detailed understanding of the molecular determinants and mechanisms of  $\alpha$ -synuclein and tau aggregation (oligomer and fibril formation), disaggregation and clearance.
10. Develop more comprehensive understanding of the role of catabolic pathways in PD, including assessment of both the ubiquitin-proteasome and the autophagy-lysosomal systems.



## Overview Of PD Pathobiology





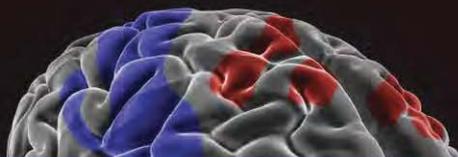
## Protein Processing, Aggregation, Spread

**Recommendation 1:** Develop transmission and propagation models, and mechanisms, of  $\alpha$ -synuclein and tau pathology

**Need:** The newly evolved “transmission hypothesis” for non-prion neurodegenerative diseases provides an explanation for the spread of pathological aggregates in PD. These may identify novel targets for therapeutics.

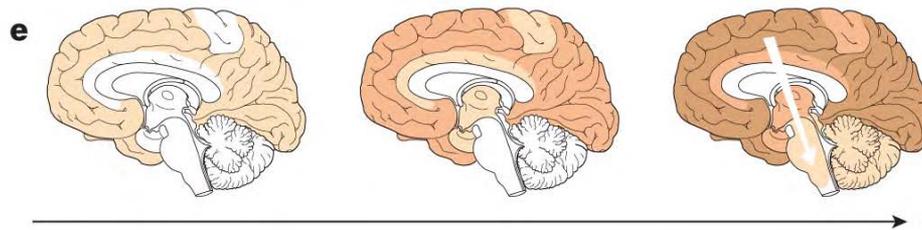
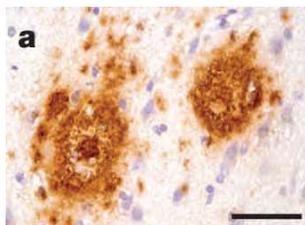
**Approaches:**

- Establish and characterize transmission models of  $\alpha$ -synuclein and tau pathology in animal models
- Determine the sequence and structural determinants of  $\alpha$ -synuclein and tau that are essential for cell-to-cell transmission
- Elucidate the role of  $\alpha$ -synuclein and tau strains in PD, synucleinopathies and tauopathies
- Identify mechanisms for the release and uptake of  $\alpha$ -synuclein and tau that transmit disease in neurons and glia, in vitro and in vivo

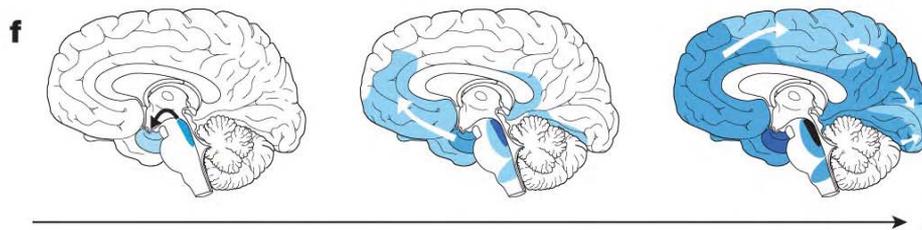


## Stereotypical Spreading of Disease Pathology as a Shared Pathogenic Mechanism Among Age-related Neurodegenerative Diseases

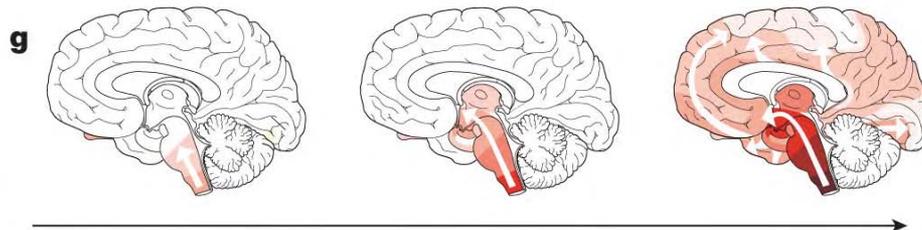
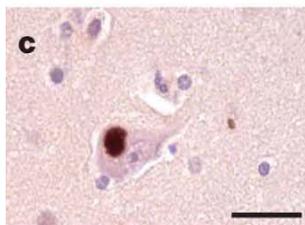
A $\beta$  plaques



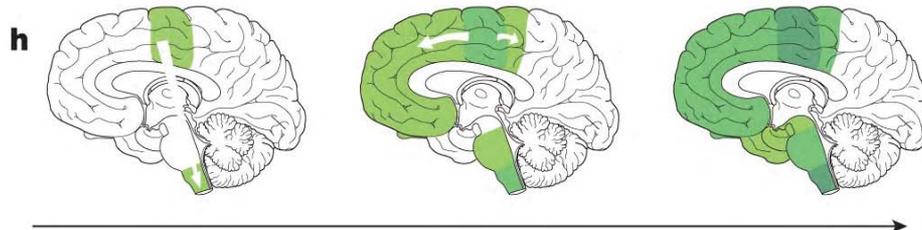
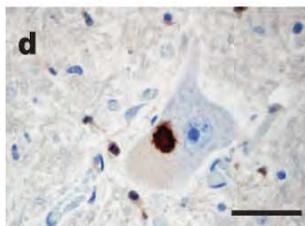
Tau tangles

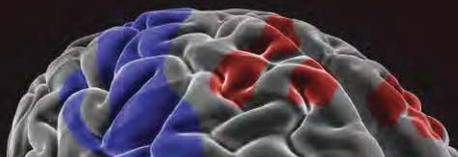


$\alpha$ -syn Lewy bodies

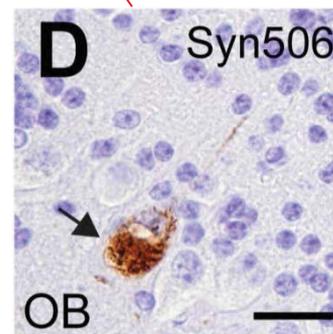
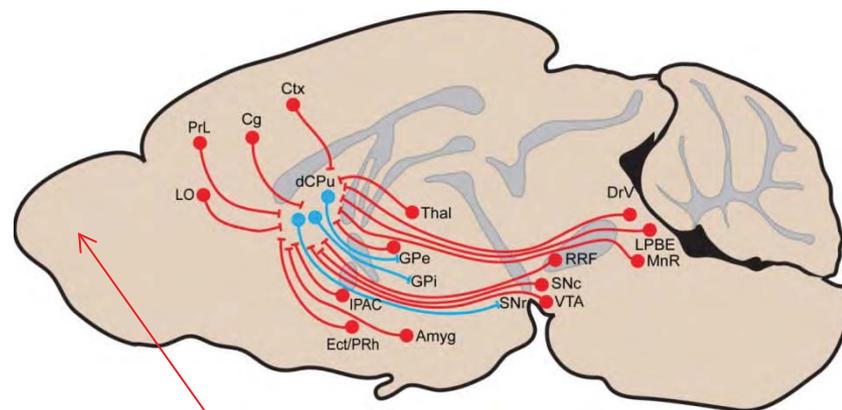
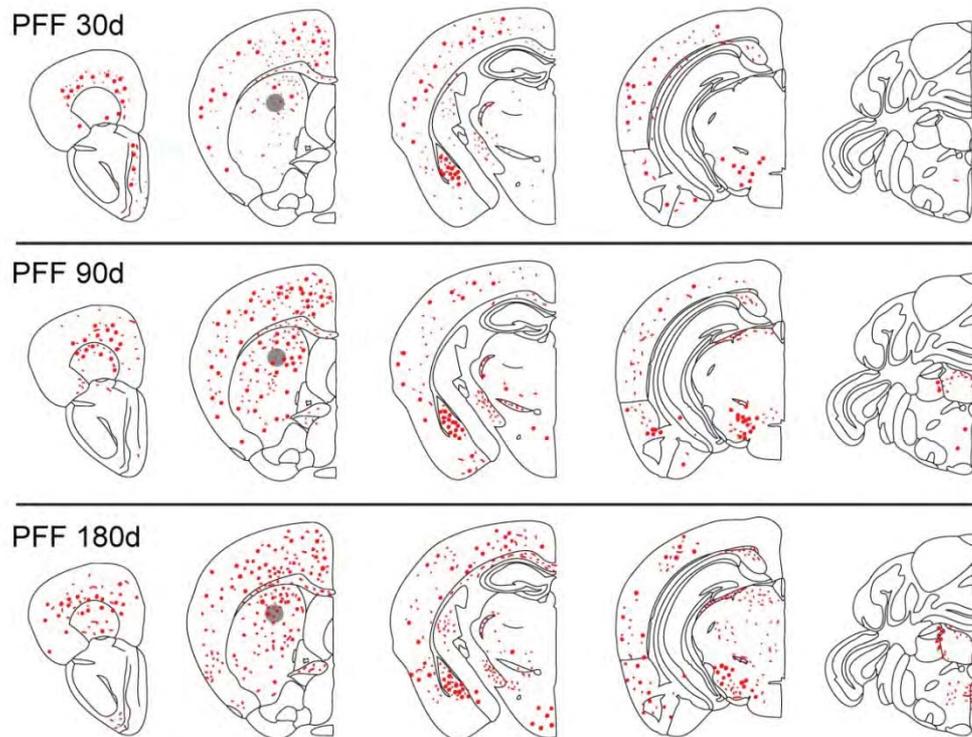


TDP-43 inclusions

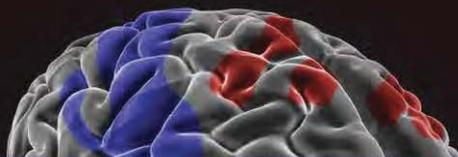




## Progressive Spread of $\alpha$ -Syn Pathology in Non-transgenic Mice Treated with $\alpha$ -Synuclein Fibrils

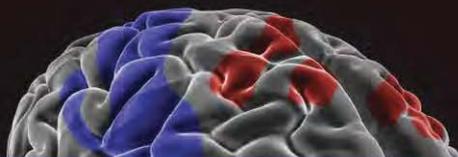


$\alpha$ -syn pathology spreads through neuroanatomic connectome

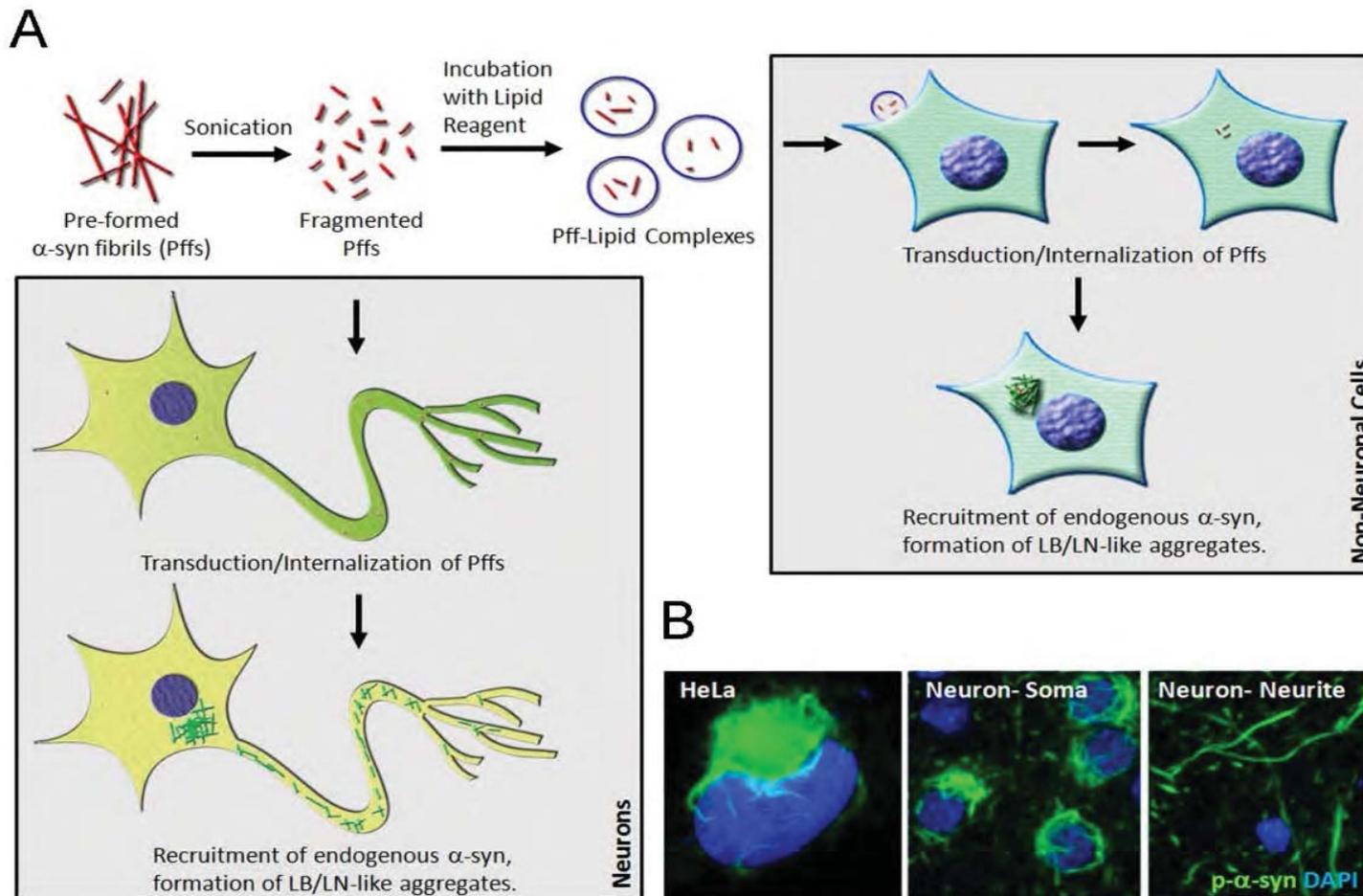


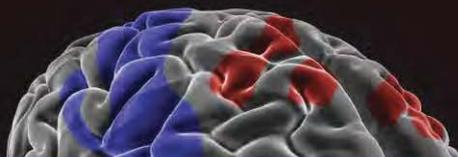
## Establish and Characterize Transmission Models of $\alpha$ -Synuclein and Tau Pathology in Animal Models

- Generate a second generation of animal models through the characterization of  $\alpha$ -synuclein pathology spread after inoculation of  $\alpha$ -synuclein aggregate-containing lysates and/or synthetic  $\alpha$ -synuclein fibrils in non-transgenic mice, rats and non-human primates
- Determine if the patterns of misfolded  $\alpha$ -synuclein anatomical spread is through neuronal networks.
- Use these new animal models of sporadic PD to elucidate how the spreading of  $\alpha$ -synuclein pathology modifies:
  - normal and abnormal function of  $\alpha$ -synuclein
  - Pathways and signaling mechanisms
  - neural circuit dynamics

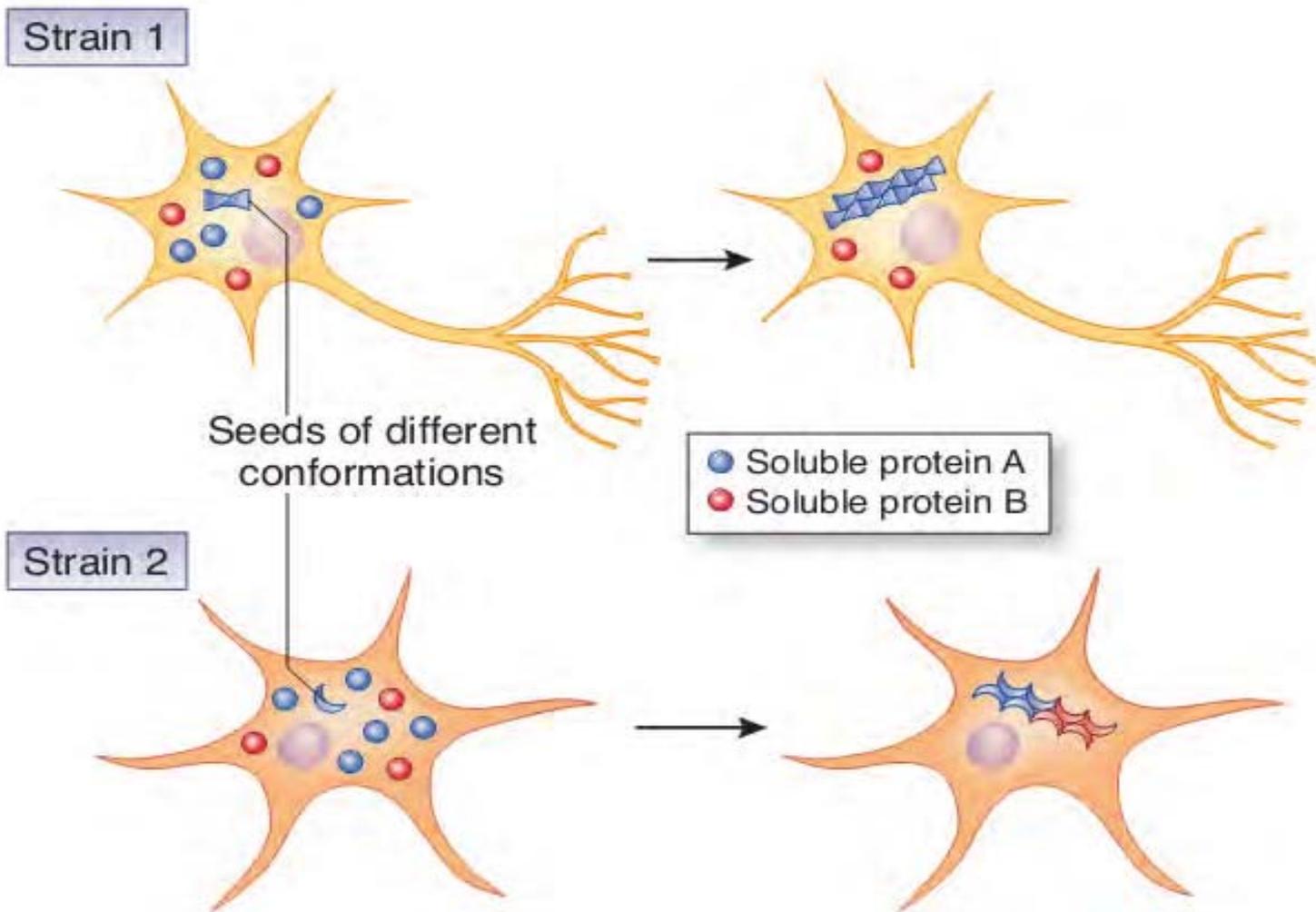


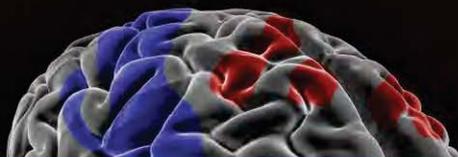
## Transmission of $\alpha$ -Synuclein in Cell-based Models of PD





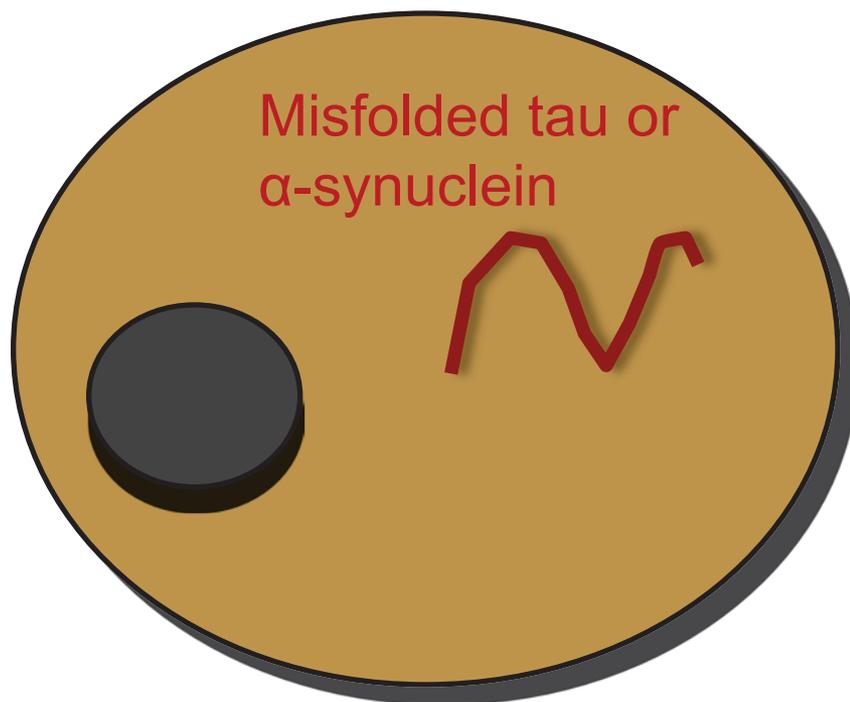
## $\alpha$ -Synuclein Strains in PD and Other Synucleinopathies



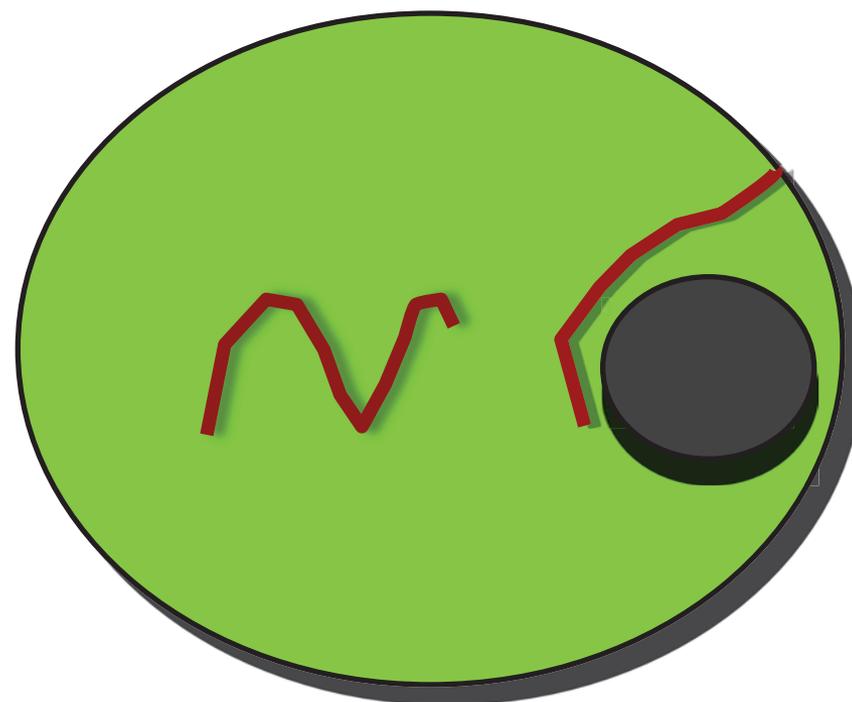


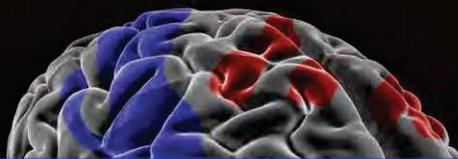
## Targeting Bad Proteins

Affected cell in disease brain



Healthy neighboring cell

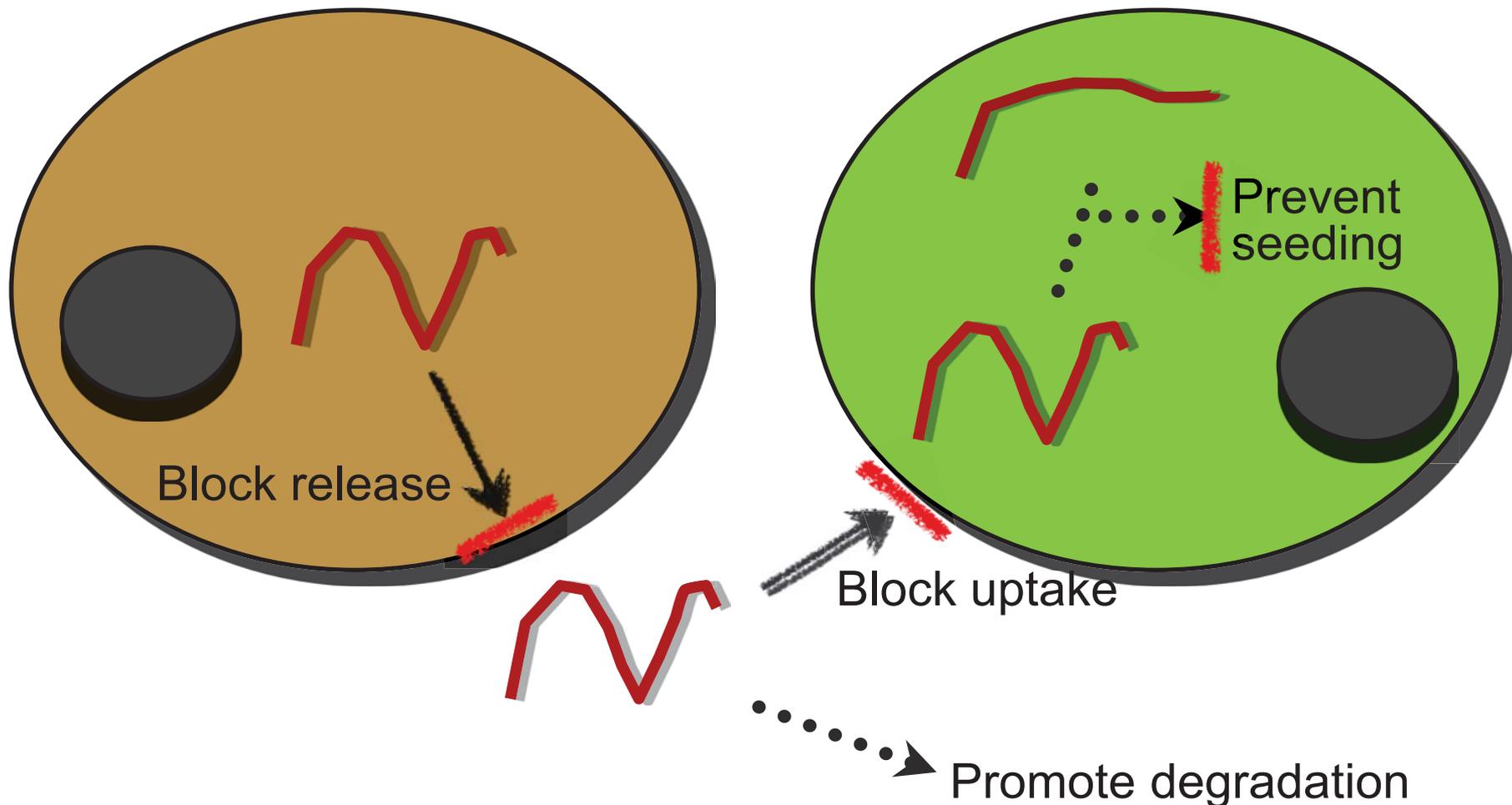


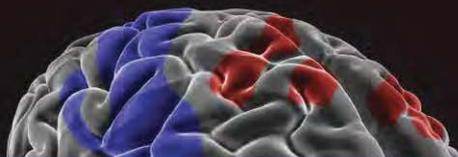


## Targeted Bad Proteins

Affected cell in disease brain

Healthy neighboring cell

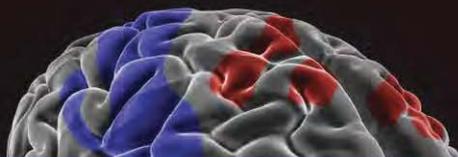




## **Protein Processing, Aggregation, Spread**

**Recommendation 2:** Elucidate the normal and abnormal functions of  $\alpha$ -synuclein and other proteins implicated in PD

*Already discussed in Pathways and Signaling; both sub-groups identified this as a high priority and identified it as a high priority recommendation, and also contributed to its development.*



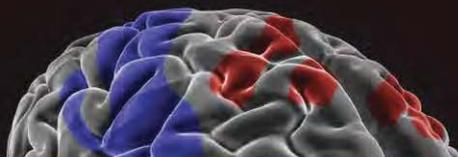
## Protein Processing, Aggregation, Spread

**Recommendation 8:** Understand the molecular mechanisms of  $\alpha$ -synuclein and tau aggregation, disaggregation and clearance

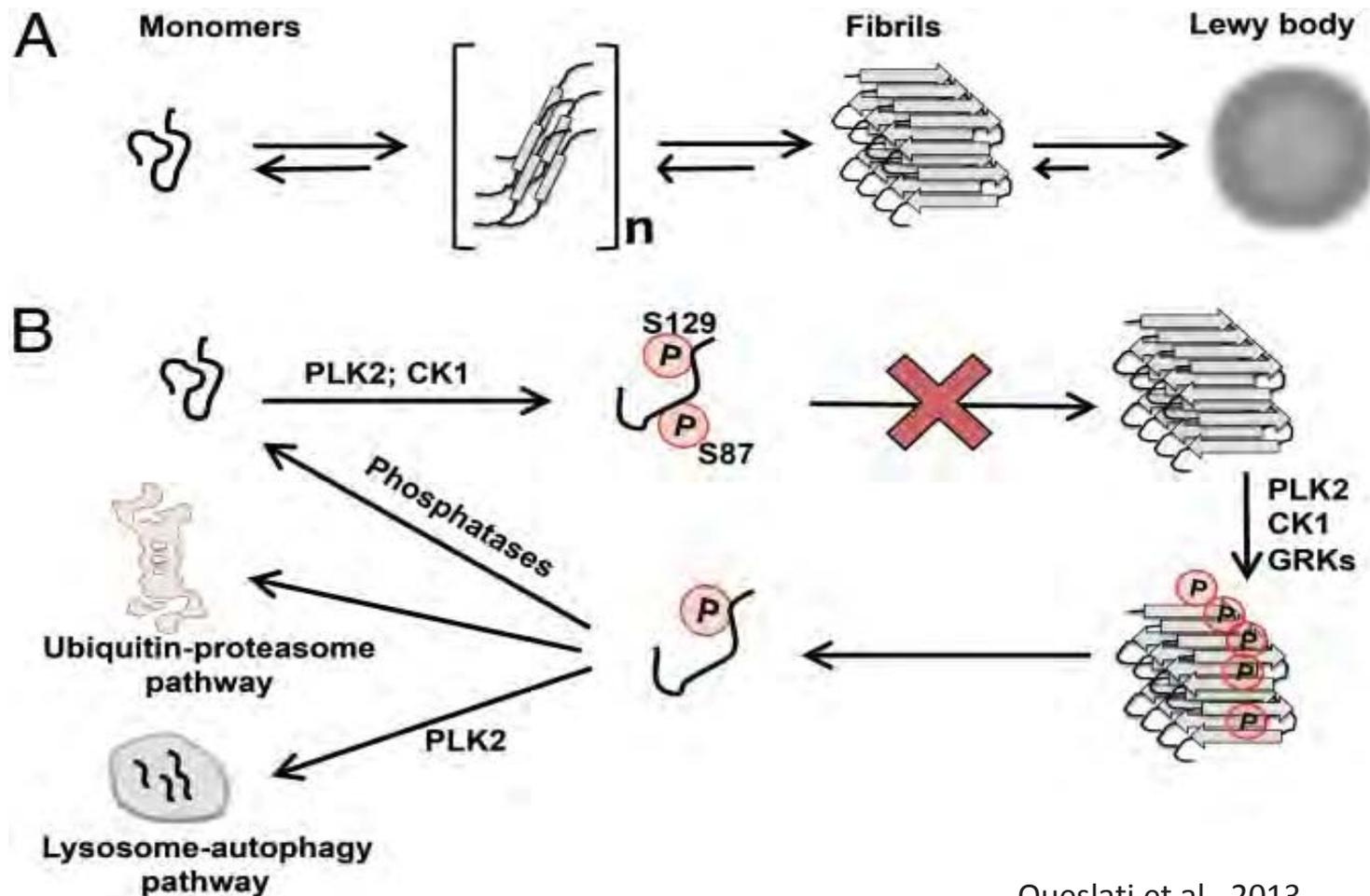
**Need:** Little is known about the structure and dynamic properties of  $\alpha$ -synuclein and tau aggregates and their molecular determinants, cellular mechanisms, pathways, clearance and toxicity.

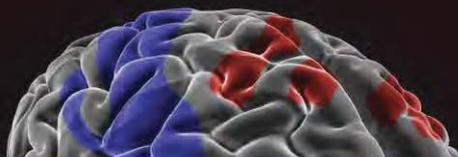
### Approaches:

- Develop and apply super-resolution imaging techniques to achieve characterization and monitoring of the structural properties and dynamics of  $\alpha$ -synuclein and tau
- Determine the role of post-translational modifications of  $\alpha$ -synuclein and tau in regulation, disaggregation and clearance of pathology
- Develop cellular, tissue, and animal models that more faithfully recapitulate human PD pathology
- Determine the role of the cellular environment on these processes
- Determine the influence of tau aggregation on  $\alpha$ -synuclein aggregation and toxicity, and vice versa.



## Mechanisms of $\alpha$ -Synuclein Aggregation, Post-translational Modification, Disaggregation and Clearance





## Protein Processing, Aggregation, Spread

**Recommendation 10:** Understand the role of catabolic pathways in PD, including the ubiquitin-proteasome and the autophagy-lysosomal systems

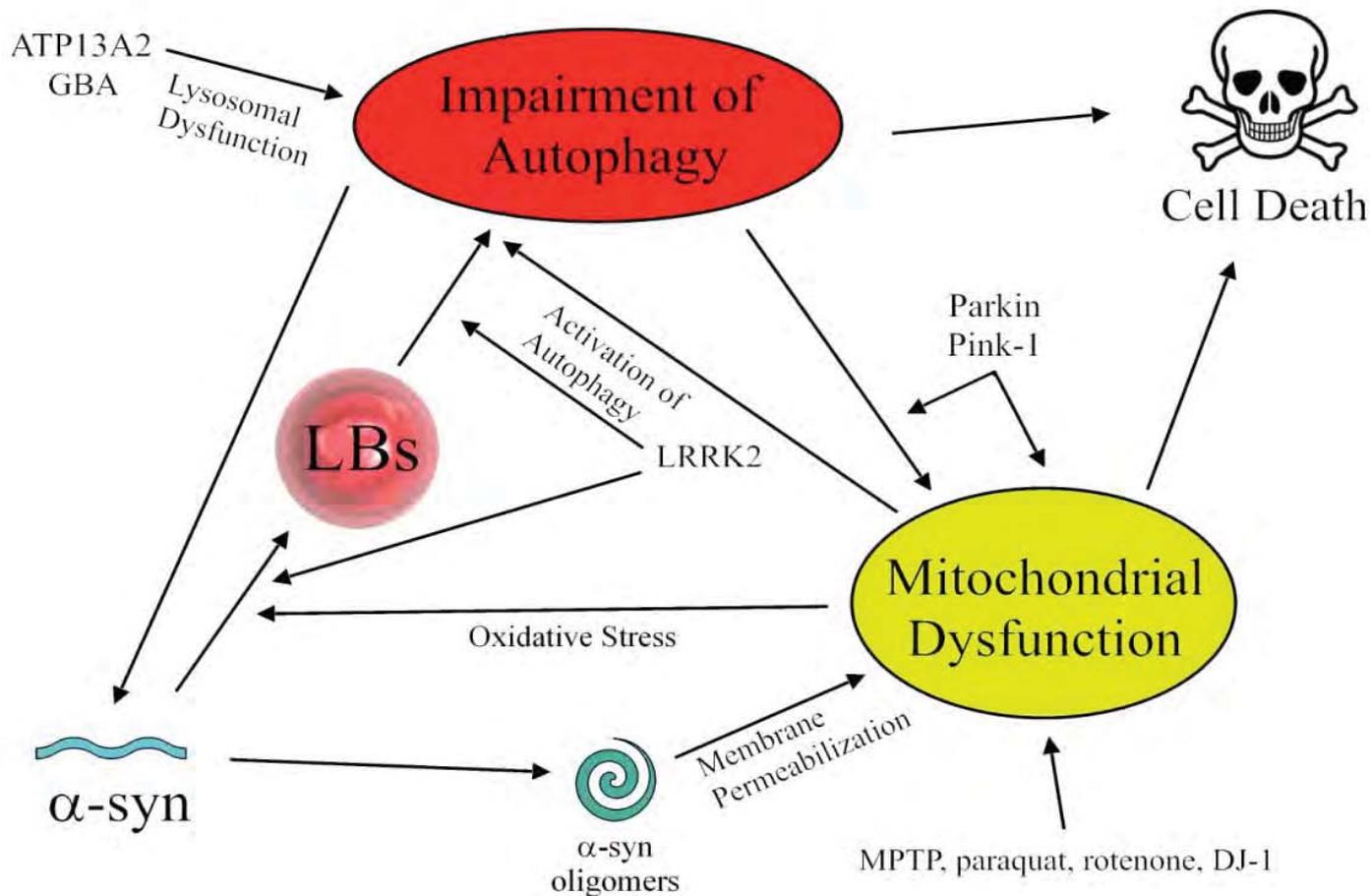
**Need:** Disturbances in the major intracellular catabolic pathways have been identified via a number of types of studies as being implicated in PD including the ubiquitin-proteasome system and the autophagy-lysosomal system. Yet despite compelling evidence implicating catabolic defects in PD, substantial gaps in knowledge remain.

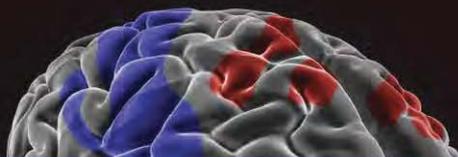
### Approaches:

- Understand the normal function of PD associated genes in these two major catabolic systems
- Understand the downstream consequences of PD-related catabolic pathway defects on cellular physiology
- Assess the contribution of catabolic pathway defects on promoting or limiting other pathogenic mechanisms
- Elucidate the pervasiveness and severity of catabolic pathway defects in different populations of PD patients



## Dysfunctional catabolic pathways in PD



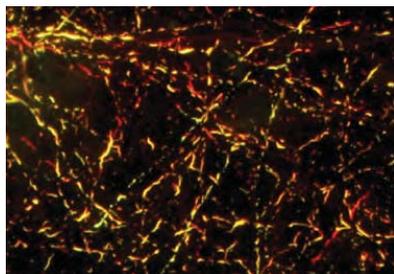


## Protein Processing, Aggregation, Spread: PANEL DISCUSSION

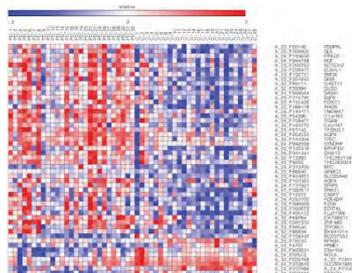
Panelists: Virginia Lee PhD, J. Paul Taylor MD PhD, Hilal Lashuel PhD

Moderator: Rob Edwards MD

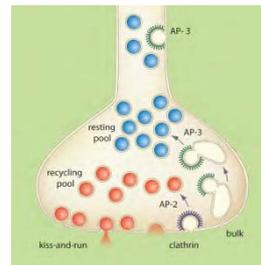
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8. Develop a more detailed understanding of the molecular determinants and mechanisms of  $\alpha$ -synuclein and tau aggregation (oligomer and fibril formation), disaggregation and clearance.
10. Develop more comprehensive understanding of the role of catabolic pathways in PD, including assessment of both the ubiquitin-proteasome and the autophagy-lysosomal systems.



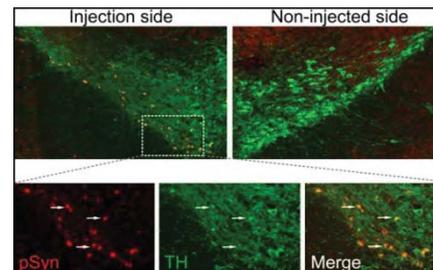
Credit: Virginia Lee, UPenn



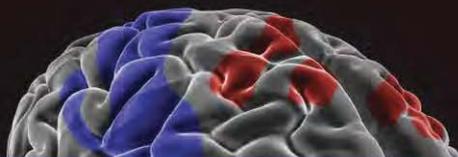
Credit: Andy Singleton NIA-NIH



Credit: Rob Edwards UCSF



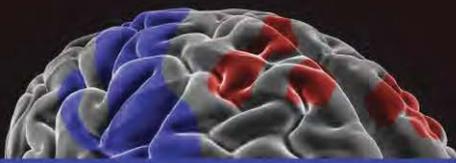
Credit: Virginia Lee, UPenn



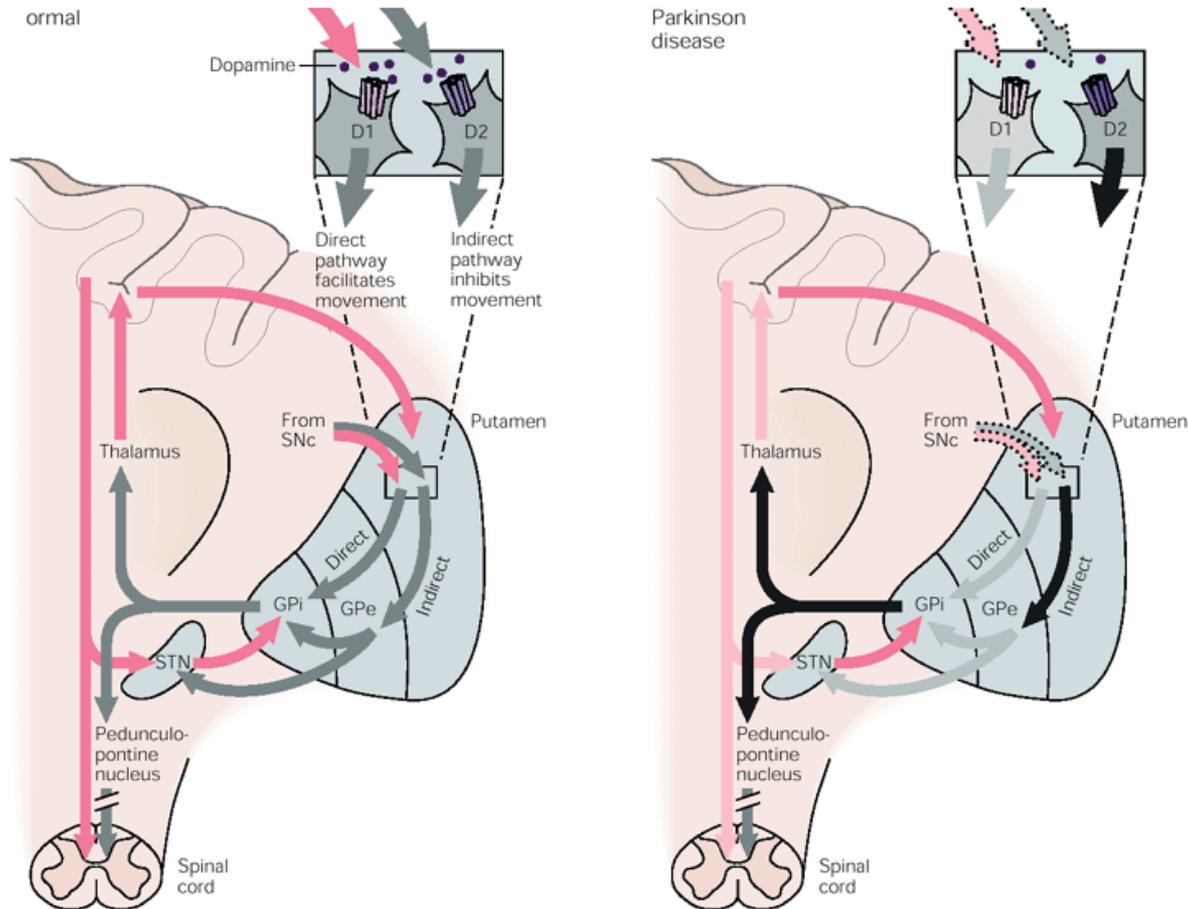
## **Circuit Modification**

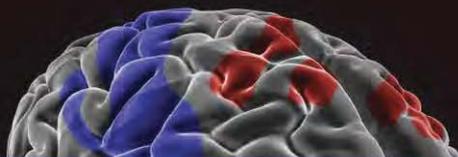
**Presenter: Warren Grill, PhD**

3. Understand how different cell populations change in their coding properties, firing patterns, and neural circuit dynamics over time, how these changes relate to behavior and motor control, and how therapeutic interventions may affect such changes.
6. Develop approaches to exploit direct access to the human brain in persons with PD during neurosurgical procedures such as DBS and using non-invasive imaging technologies such as 7T MRI and HRRT PET.
9. Use a combination of sensor technologies and imaging to develop a more precise understanding of the neural circuit dynamics in PD that enable the development of next-generation therapeutic devices.
11. Advance our understanding of neural circuits, circuit analysis techniques, PD animal models, and optogenetic and related imaging technologies to improve existing therapies and generate next-generation therapies for PD.



## Circuit Modification





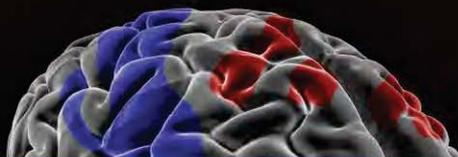
## Circuit Modification

**Recommendation 3:** Understand how cell populations differ in coding, firing, and neural circuit dynamics

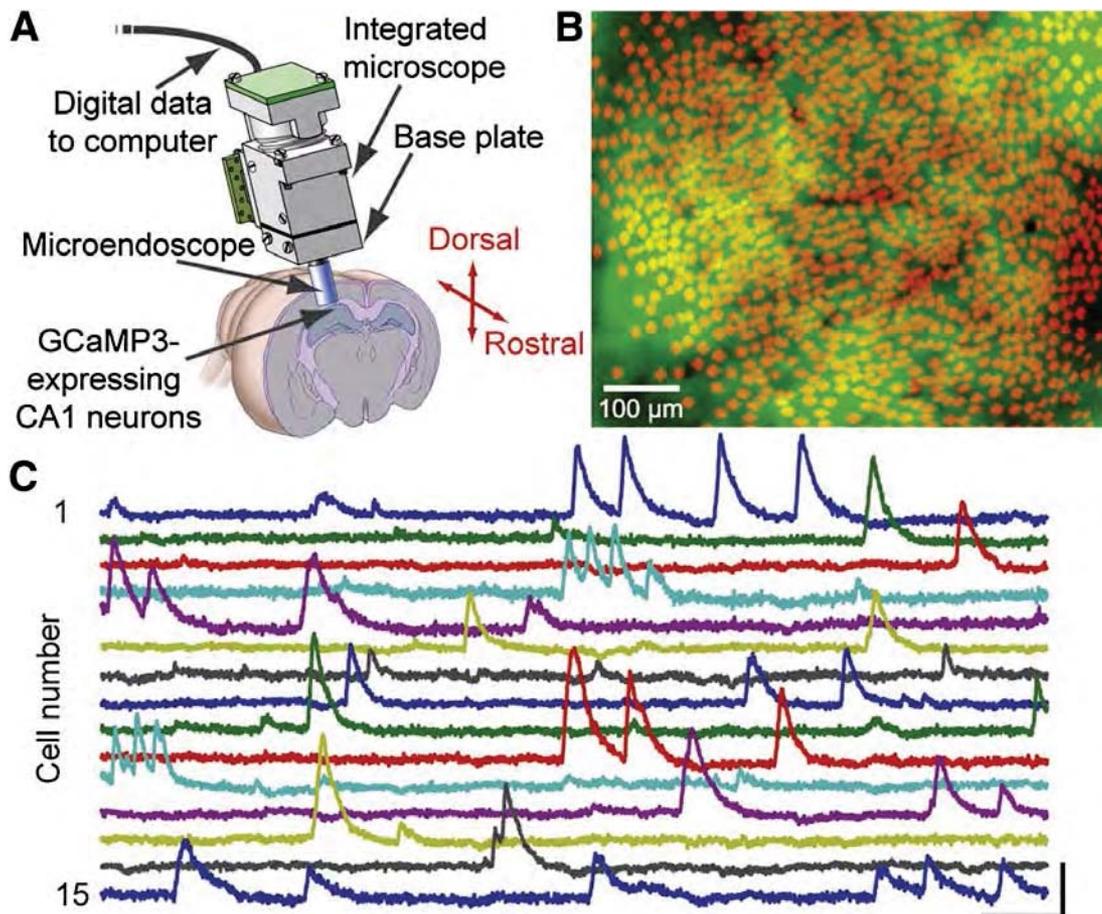
**Need:** Determine how PD related neural circuits encode information, and how these circuits are altered over time due to the genetic, neurochemical, and bioelectrical changes associated with PD.

**Approaches:**

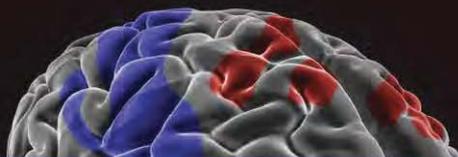
- Circuit mapping with state-of-the-art electrophysiological and anatomical methods to develop circuit plan including connectivity, activity dynamics and micro-circuit interactions.
- Circuit manipulations applied at key mapped nodes by advanced optogenetic and related (e.g. DREADD) manipulations and microfluidic methods as well as improved models of DBS.
- Application of chronic, not only acute, monitoring techniques of electrical activity in Parkinson's circuits combined with neurochemical activity monitoring (e.g., Fast-scan voltammetry and Ca<sup>++</sup> imaging and 2P imaging).
- Development of next-generation neurofeedback techniques for therapeutic use to reconfigure vulnerable circuits.



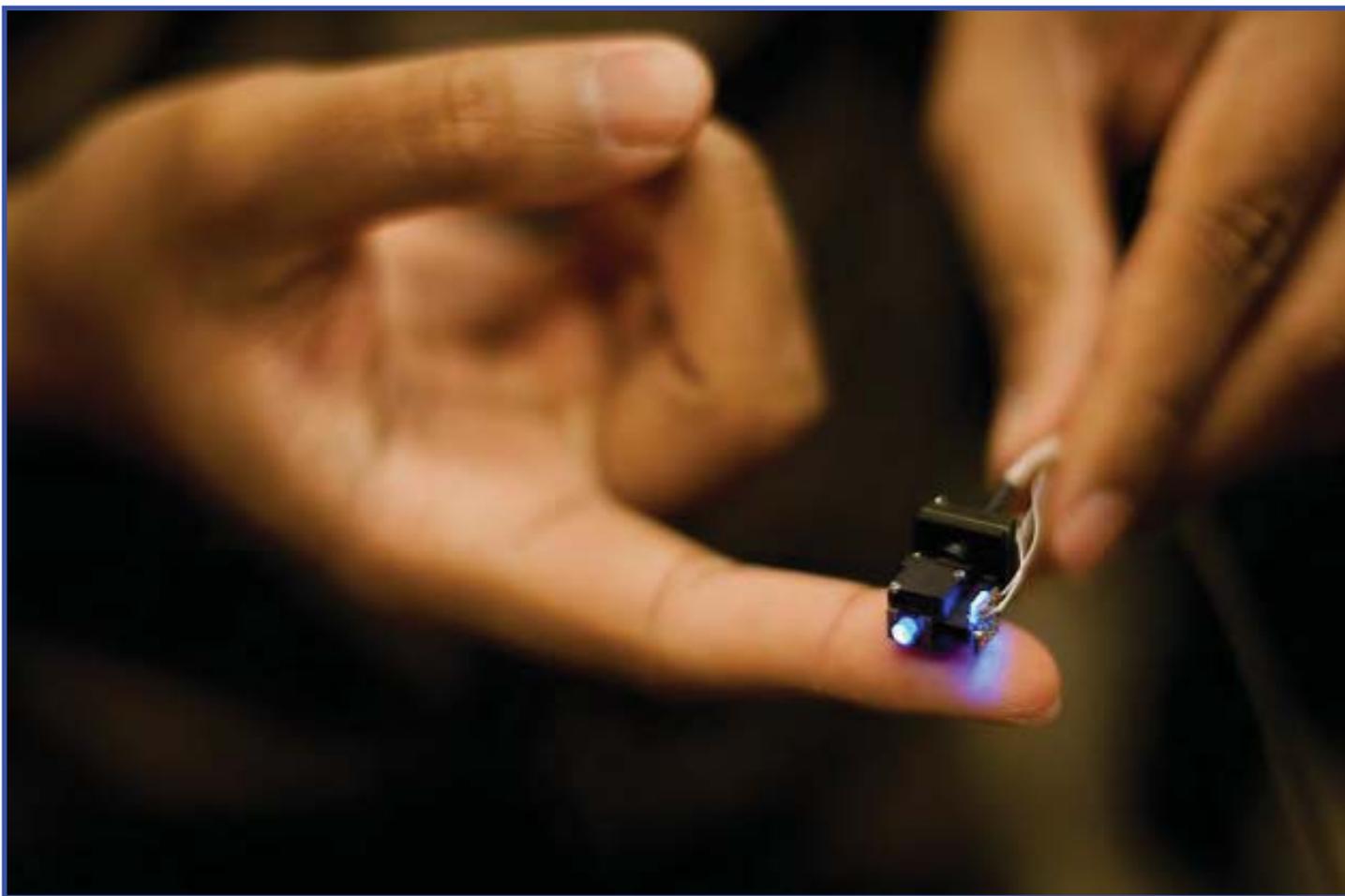
## Circuit Modification

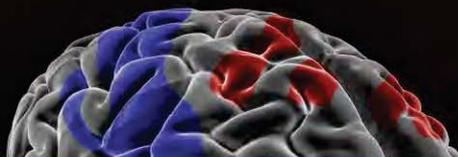


Deiseroth & Schnitzer (2013)



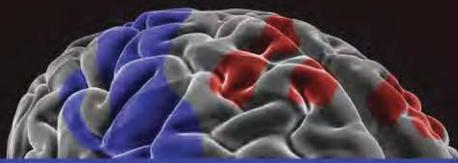
**The integrated microscope is ~2 grams in mass and is made of mass-producible optoelectronics**





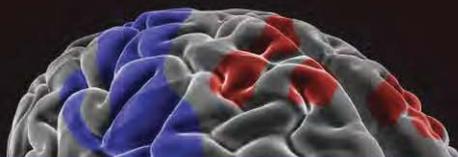
## **Mice behave naturally during digital imaging of activity in large neural ensembles**





# Visualizing $\text{Ca}^{2+}$ dynamics in CA1 pyramidal neurons in behaving mice





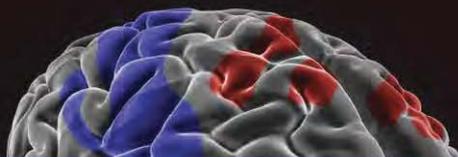
## Circuit Modification

**Recommendation 6:** Develop approaches to study the human brain during neurosurgical and imaging procedures

**Need:** Reverse translation activities are essential parallel adjuncts to studies in animal models to understand, validate and improve the relevance of these animal models.

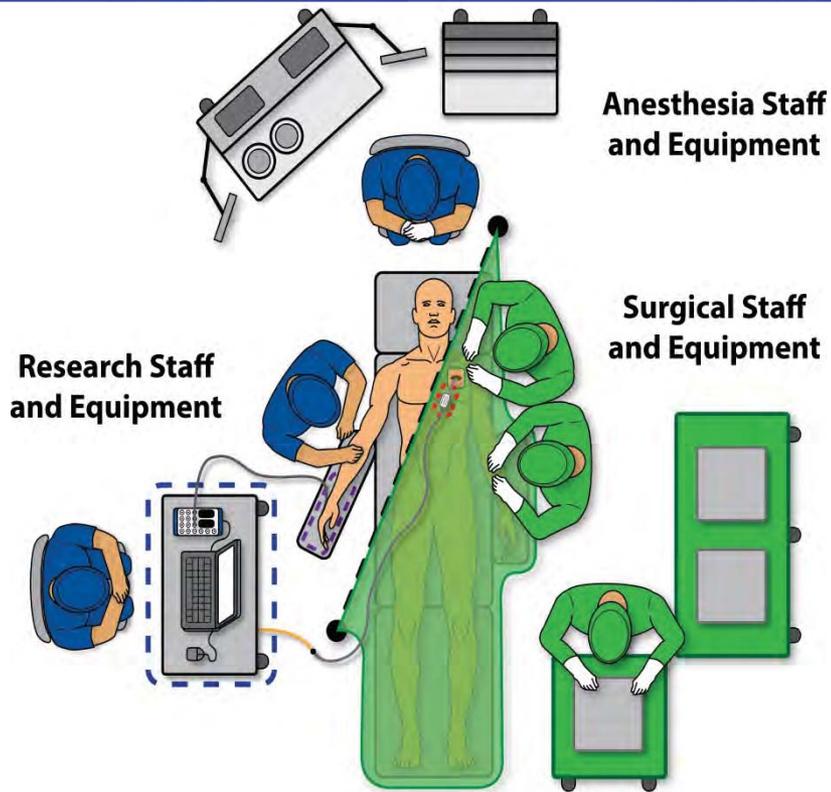
**Approaches:**

- Develop and validate hardware and software tools that enable intraoperative perturbation and recording of relevant variables (electrical, neurochemical) in the human brain, including regulatory strategies to enable application and dissemination of these tools.
- Establish quantitative relationships between patterns of neural activity, including electrical, chemical, and metabolic, and symptoms to track disease progression and establish relevant biomarkers.
- Develop contrast agents / ligands to enable quantitative imaging of structure and biochemistry in the Parkinsonian brain.
- Conduct parallel studies in humans and animals to understand better the limitations of current animal models to provide therapeutic leads.



# PARKINSON'S DISEASE 2014

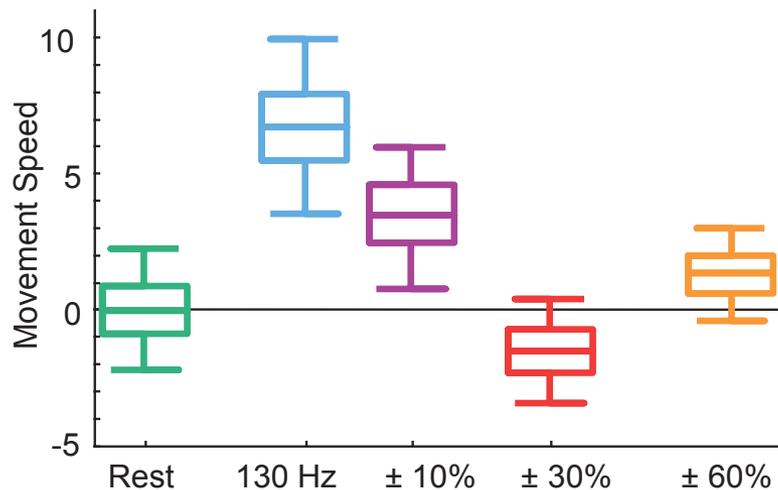
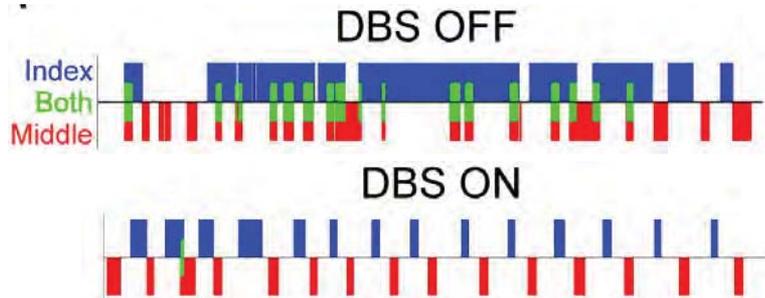
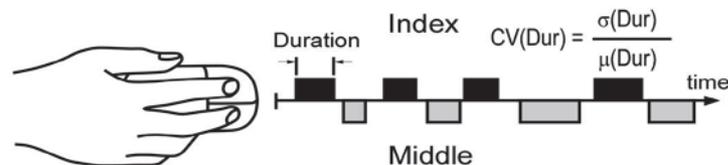
ADVANCING RESEARCH, IMPROVING LIVES

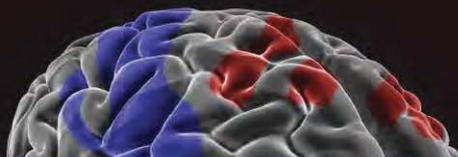


Swan et al. (2013)



Measure speed and regularity of alternating finger tapping





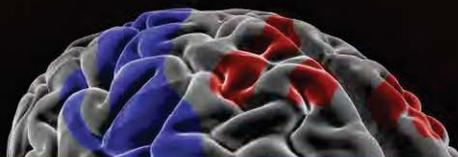
## Circuit Modification

**Recommendation 9:** Use a combination of sensor technologies and imaging to develop a more precise understanding of the neural dynamics in PD.

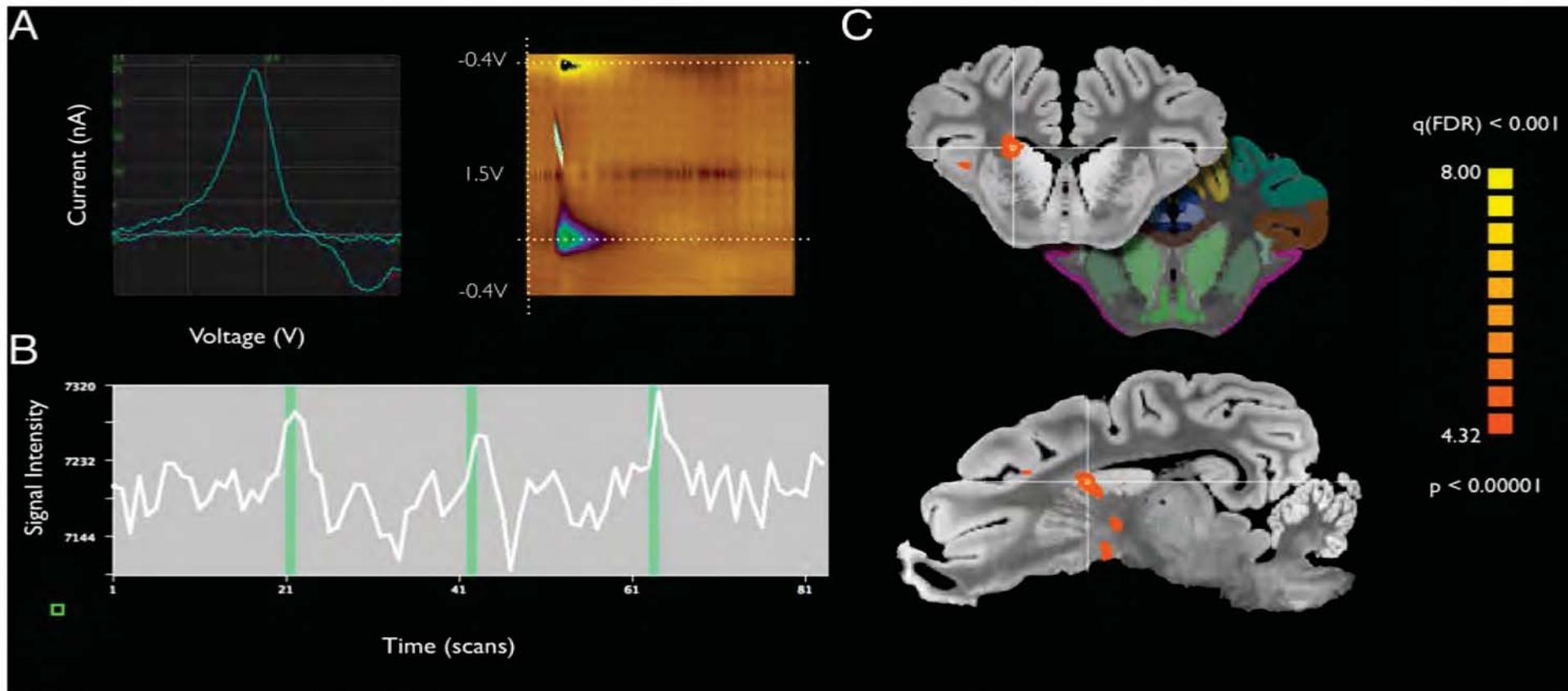
**Need:** Our understanding of the circuit effects of DBS remains far from complete, in large part because of technical difficulties in using imaging and sensor technologies for global assessment of neural activity in animal models and in human patients.

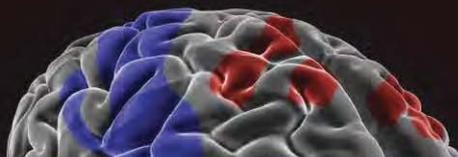
### Approaches:

- Using state of art imaging technologies (such as fMRI or HRRT PET), identify target-specific changes in neural activity in interconnected structures within the basal ganglia complex that ultimately underlie clinical benefit during DBS.
- Using state of art sensor technologies (such as fast scan cyclic voltammetry or amperometry), interrogate the dynamic neurotransmitter and related molecule changes that occur within the DBS target structure as well as their functionally interconnected basal ganglia complex and correlate with clinical outcome measures.
- Develop combination imaging and sensor technologies that can be deployed in human patients and animal models to allow for global network assessment during DBS.
- Develop predictive mechanistic models of neural circuit dynamic changes during DBS using precise target specific activation patterns on imaging and neurotransmitter dynamic changes.
- Using understanding of mechanism of action of DBS, develop next generation of therapeutic devices that utilize closed loop architecture.



## STN DBS results in caudate fMRI BOLD activation and Dopamine release





## Circuit Modification

**Recommendation 11:** Advance understanding of neural circuits, circuit analysis techniques, PD animal models, optogenetic, and related technologies

**Need:** Application of new approaches in freely behaving animal models of PD will yield substantial insights into circuit structure and dynamics.

### Approaches:

- Develop suitable computational methods that can characterize and classify large-scale imaging data sets, so these computational methods can facilitate next-generation, large-scale screens for new pharmacologic or DBS treatments using large colonies of animals and automated computational assessment of treatment efficacy.
- Use imaging techniques in freely behaving animals, in conjunction with fluorescent sensors of calcium, voltage, and transmitter/modulator release dynamics, to evaluate in identified cell types the acute and long-term effects (e.g. circuit plasticity) of existing treatments.
- Extend existing optogenetic and imaging techniques that are chiefly used today in rodent models of PD so that these technologies can be readily used in primate models of the disease.
- To identify novel disease biomarkers, develop tandem methodologies in PD animal models combining measurements that can be performed in human subjects with optogenetic and imaging methods that are normally restricted to animals.

# The Basal Ganglia Modulate a Wide Range of

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Motor

• Par

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• Cog

Neuro

• OCD/Tourette

• Autism/ADHD

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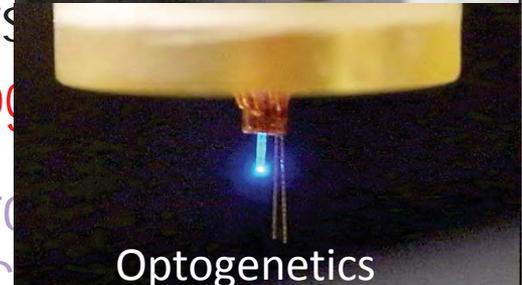
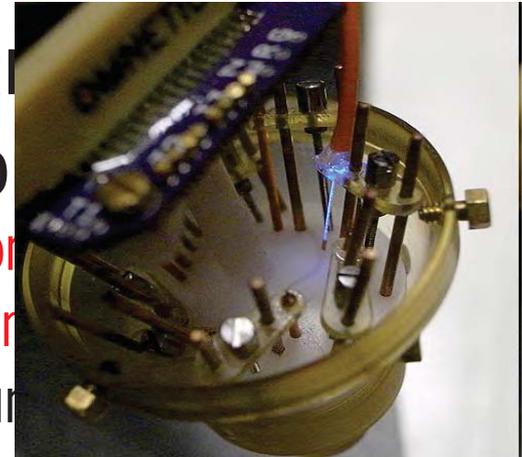
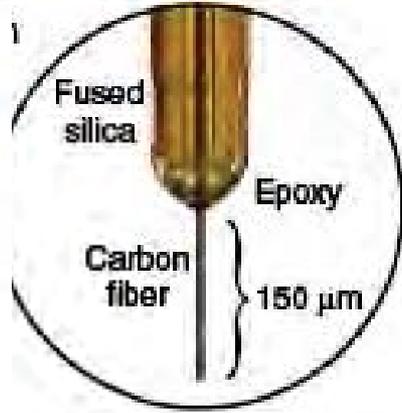
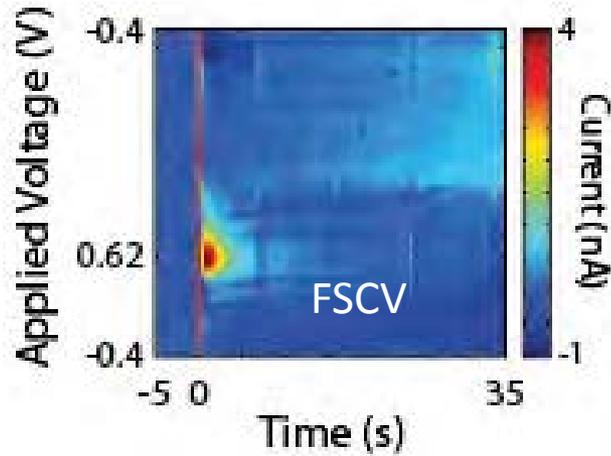
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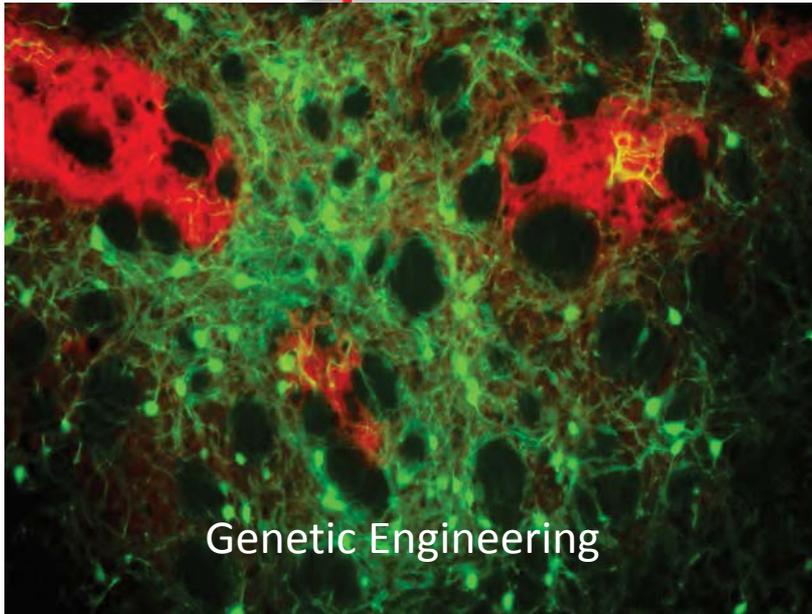
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Optogenetics



Genetic Engineering

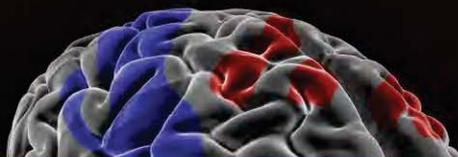


Multi-Electrode Recording



Tetrode Recording

Graybiel and coworkers

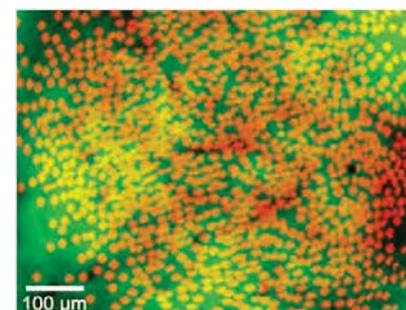
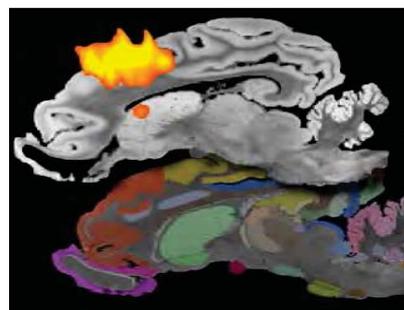
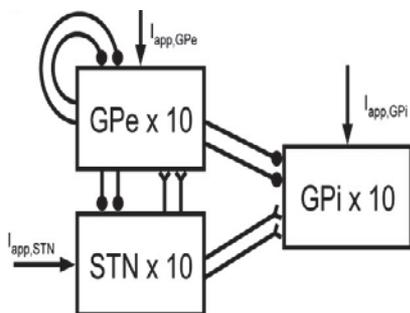
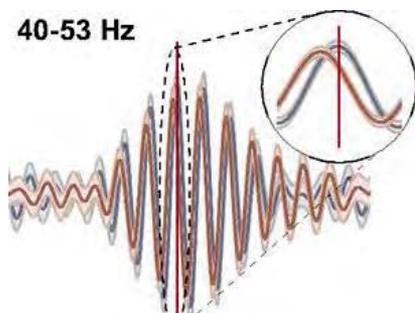


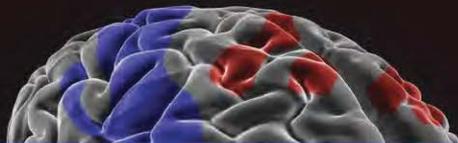
## Circuit Modification: PANEL DISCUSSION

**Panelists: Warren Grill PhD, Ann Graybiel PhD**

**Moderator: Kendall Lee MD, PhD**

3. Understand how cell populations differ in coding, firing, and neural circuit dynamics
6. Develop approaches to further study the human brain during neurosurgical and imaging procedures
9. Use a combination of sensor technologies and imaging to develop a more precise understanding of the neural dynamics in PD.
11. Advance understanding of neural circuits, circuit analysis techniques, PD animal models, optogenetic, and related technologies





# PARKINSON'S DISEASE 2014

ADVANCING RESEARCH, IMPROVING LIVES

| Ranking | Recommendation   |
|---------|--|
| 1       | Develop transmission and propagation models , and mechanisms, of $\alpha$ -synuclein and tau pathology                         |
| 2       | Elucidate the normal and abnormal functions of $\alpha$ -synuclein and others proteins implicated in PD                        |
| 3       | Understand how cell populations differ in coding, firing, and neural circuit dynamics  |
| 4       | Generate and characterize a panel of PD specific iPS for 'omic" pathway analysis and other approaches                          |
| 5       | Integrate comprehensive data sets. Perform functional and genetic analyses across large data sets                              |
| 6       | Develop approaches to further study the human brain in during neurosurgical and imaging procedures                             |
| 7       | Develop a more detailed understanding of the genetic basis of PD   |
| 8       | Understand the molecular mechanisms of $\alpha$ -synuclein and tau aggregation, disaggregation and clearance                   |
| 9       | Use sensor technologies and imaging to develop a more precise understanding of the neural circuit dynamics in PD               |
| 10      | Understand the role of catabolic pathways in PD, including the ubiquitin-proteasome and the autophagy-lysosomal systems        |
| 11      | Advance understanding of neural circuits, circuit analysis techniques, PD animal models, optogenetic, and related technologies |