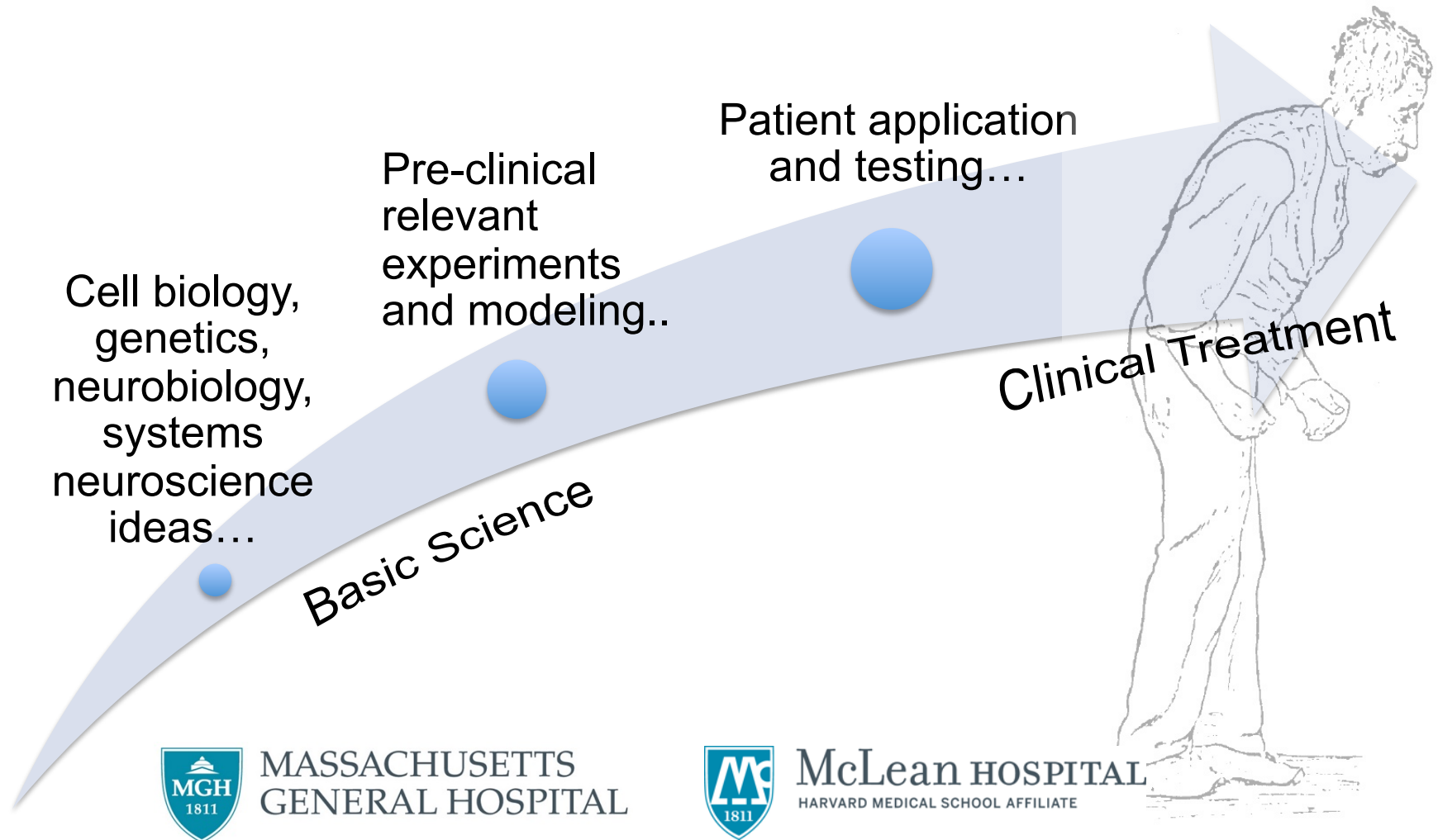


# Novel concepts from human cell biology and genetics for neurodegenerative disease treatments



by Professor Ole Isacson



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McLean HOSPITAL  
HARVARD MEDICAL SCHOOL AFFILIATE

# The clinical observations that precede our scientific understanding...

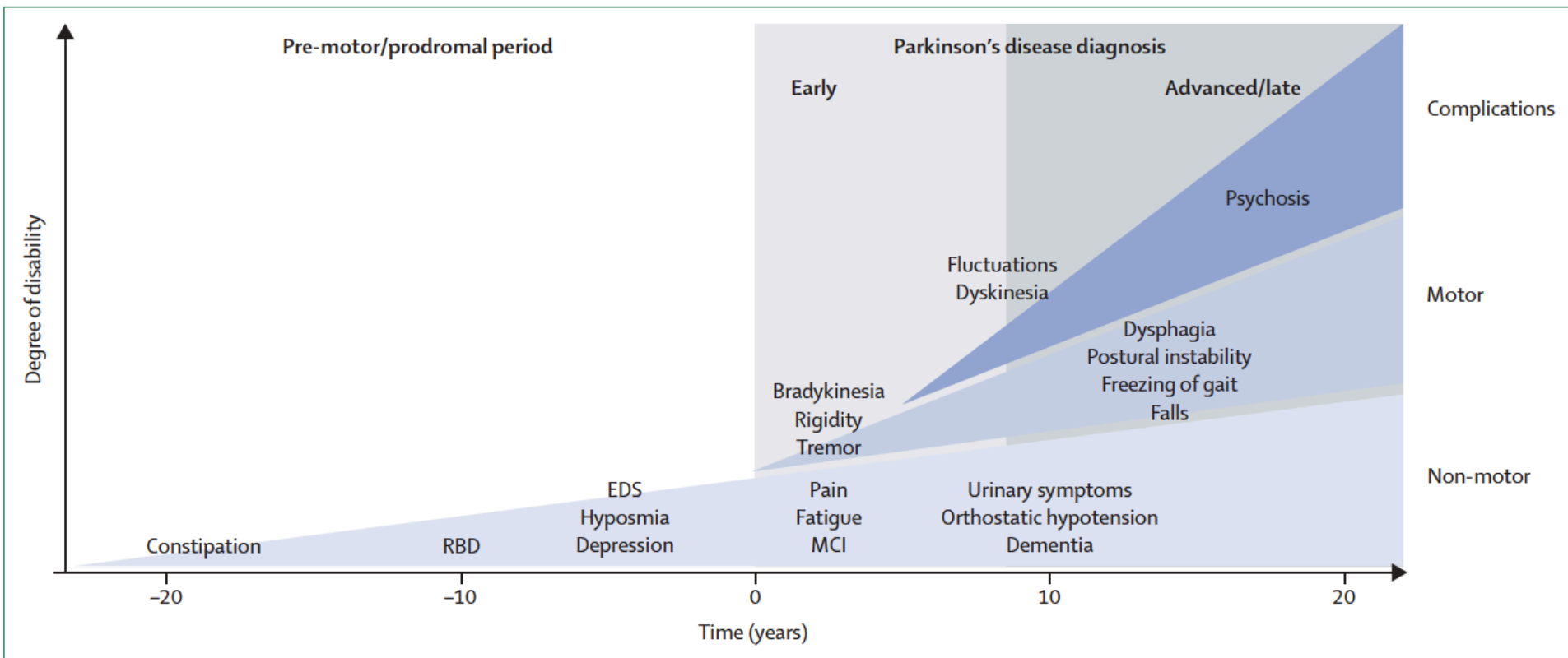
Distinguishing between neurodegenerative disease and disease-free aging:  
correlating neuropsychological evaluations and neuropathological studies in centenarians.

[Silver MH<sup>1</sup>](#), [Newell K](#), [Brady C](#), [Hedley-White ET](#) and [Perls TT](#).

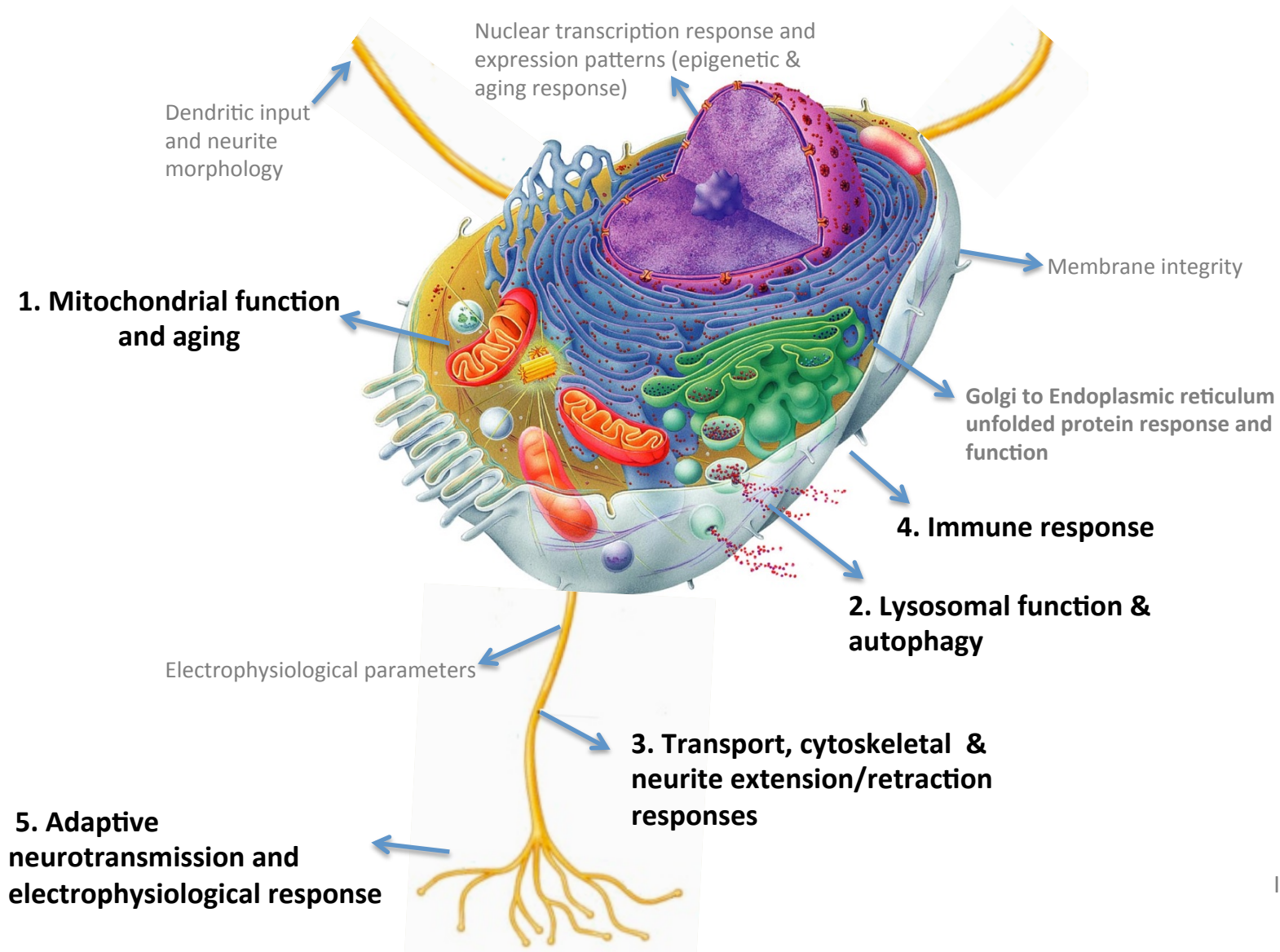
*“Correlation was low for four subjects: two subjects **with no dementia** on neuropsychological Testing met CERAD (neuritic amyloid plaques and Braak and Braak staging of neurofibrillary tangles) neuropathological criteria for possible AD; **two subjects with dementia on testing did not meet CERAD criteria** for definite Alzheimer's disease and had otherwise minimal changes to correlate with the cognitive findings.”*

*“Conclusion: Lack of correlation between level of cognitive functioning and brain pathology in two subjects with no dementia raised the question of **whether a functional reserve delayed the functional expression of pathological changes**. For two subjects with dementia on testing, there appeared to be no sufficient pathological explanation for the extent of the cognitive changes”*

# Unmet Needs in Parkinson's disease and Dementia

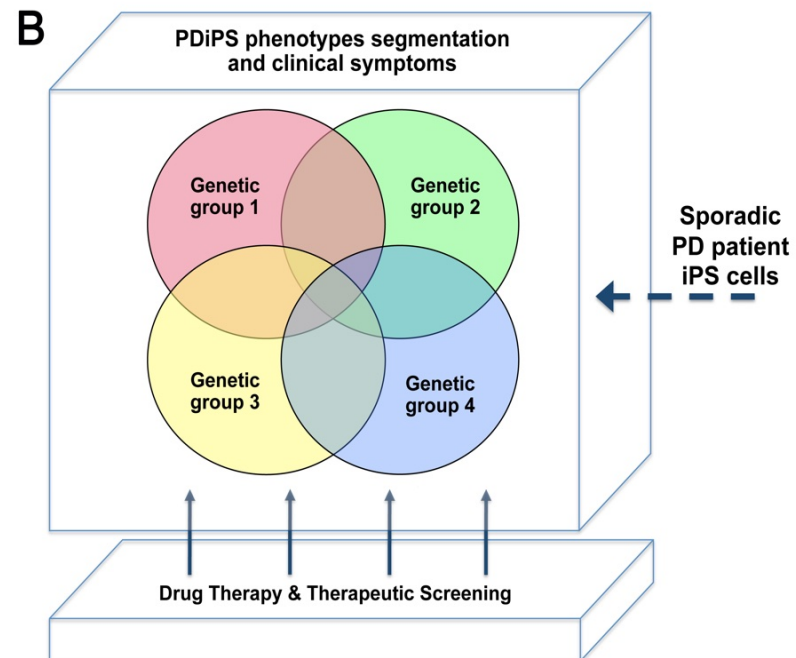
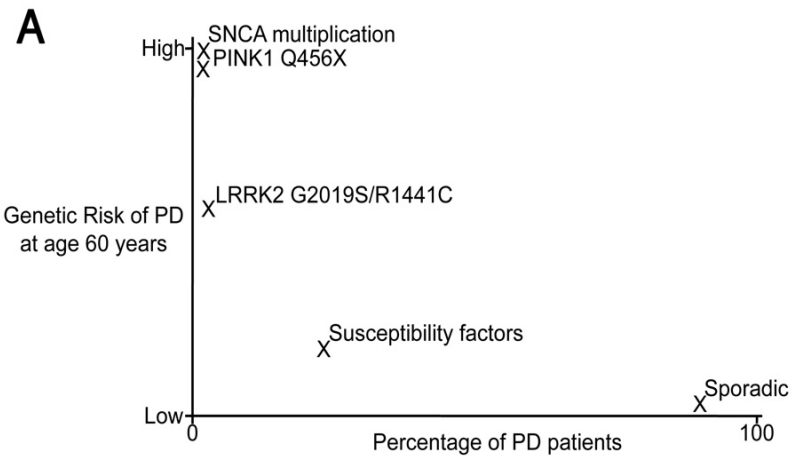


# I. Patient cell and human neurons can be used to reveal gene or disease specific cell responses

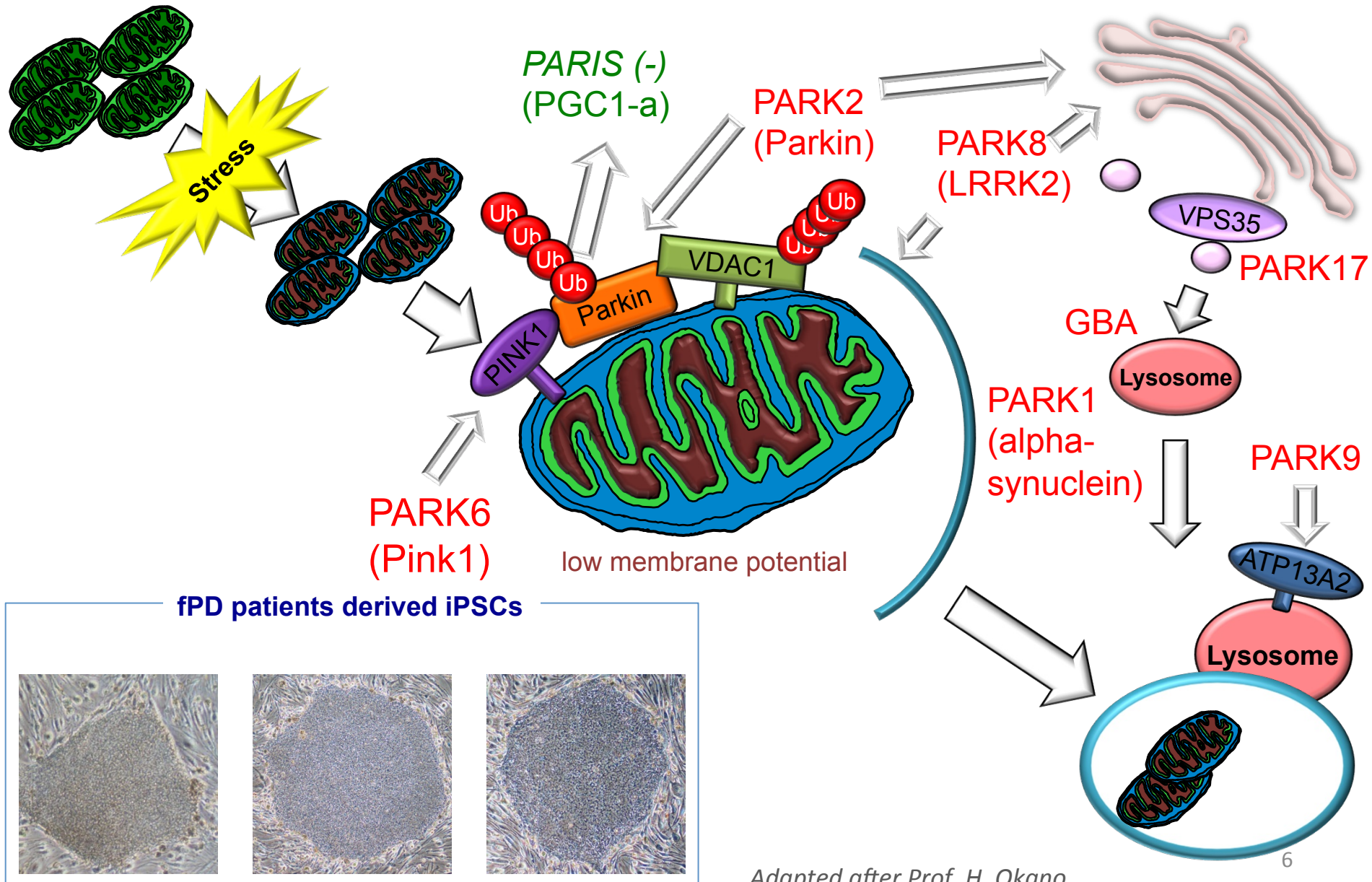




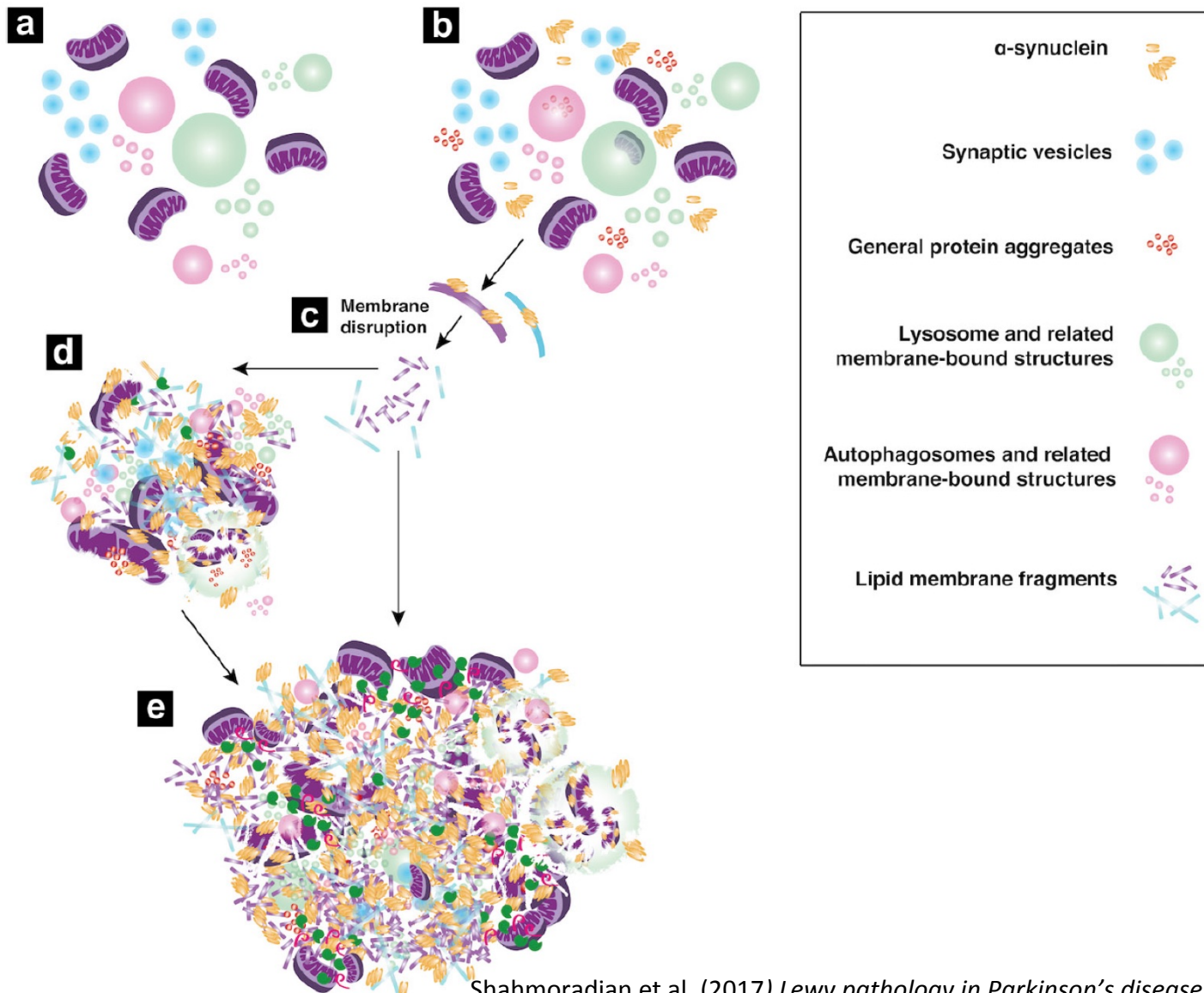
# Cellular and clinical disease phenotypes from familial PD and Lewy body dementia iPS cells



# Discovering genetics and cell biology in Parkinson's disease and Lewy body dementia

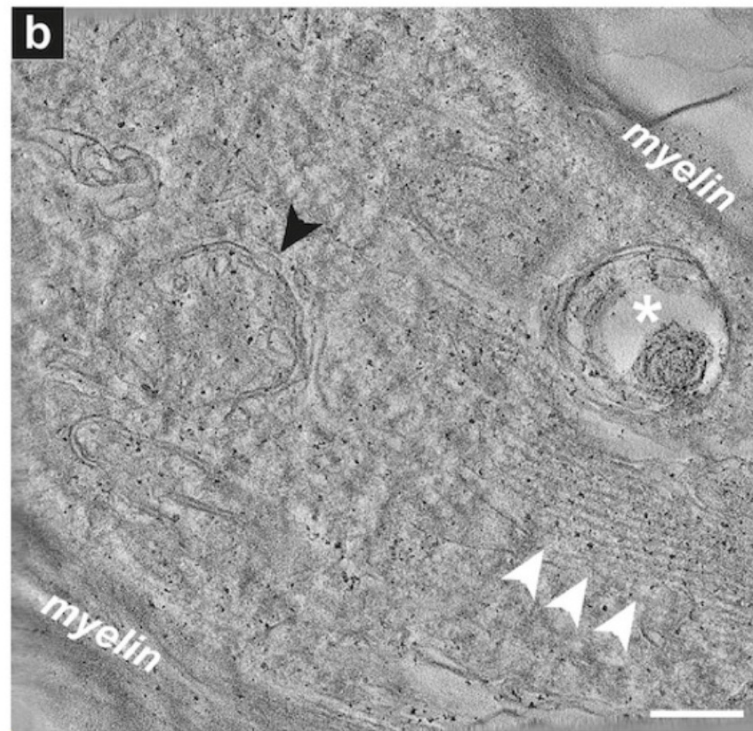
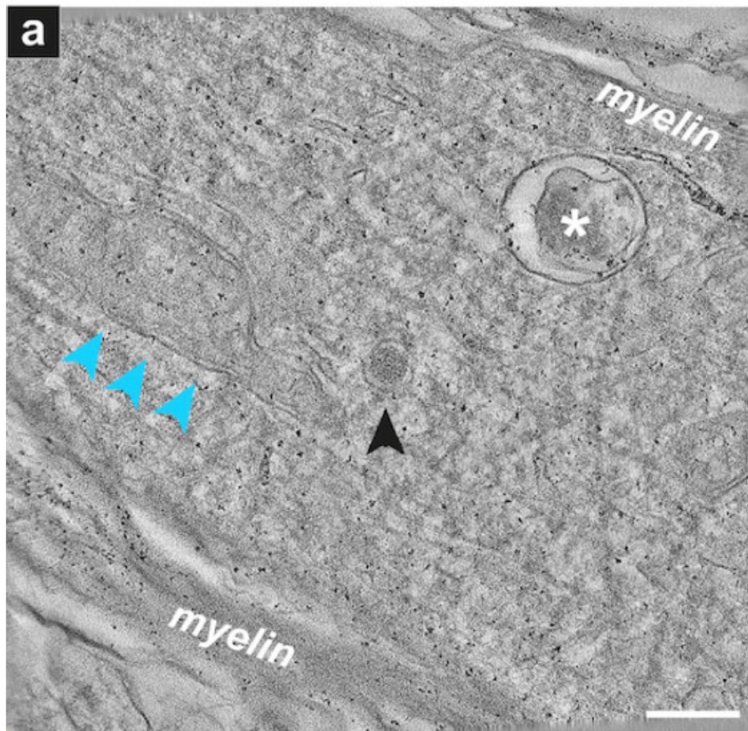


# *Lewy pathology in Parkinson's disease consists of a crowded organellar membranous medley*

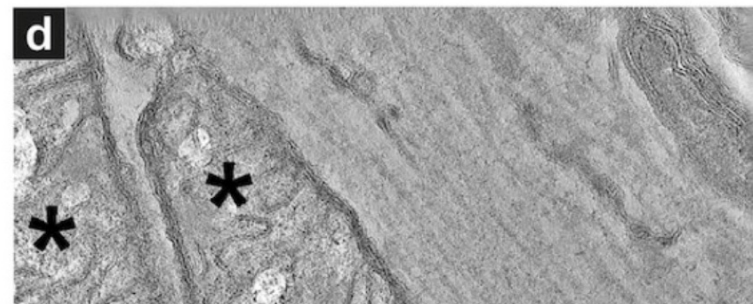
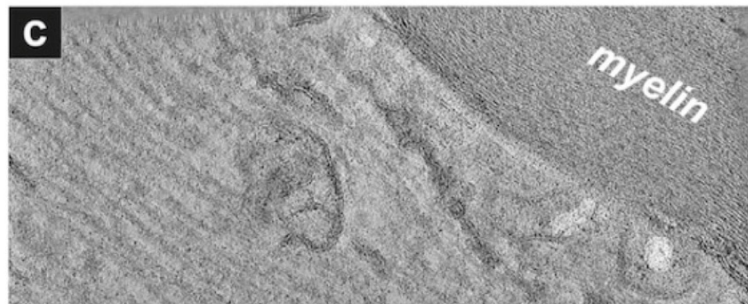




# Lewy Neurite

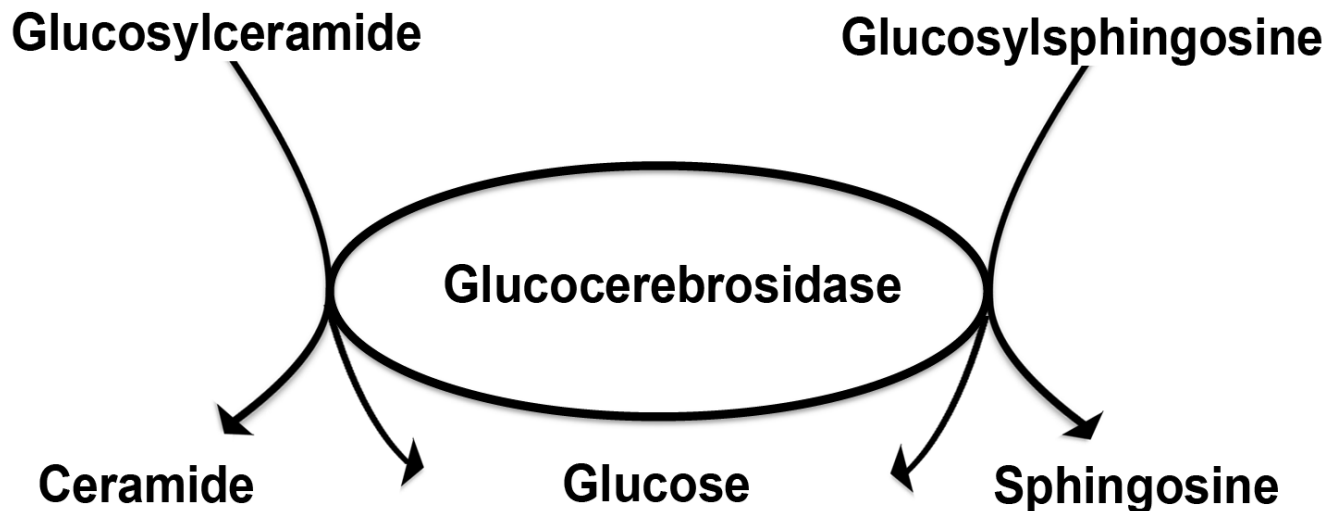


## Control Neurite



## II. The shared biology between Gaucher's disease (GBA1), Parkinson's disease and Lewy body dementia

- Clear risk association and link for GBA1 and  $\alpha$ -synucleinopathies: GBA ↓ then  $\alpha$ -syn ↑

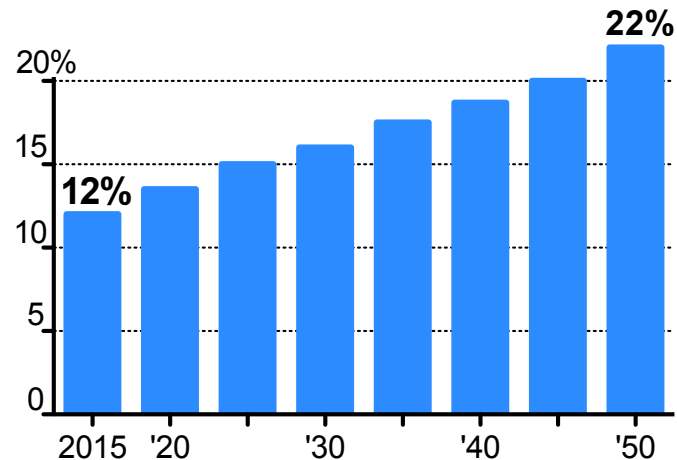


# Aging and rare disease mechanisms

- Could the rare mono-genetic lysosomal disorders have acquired biological phenocopies in the “normal” aging population?

## Aging

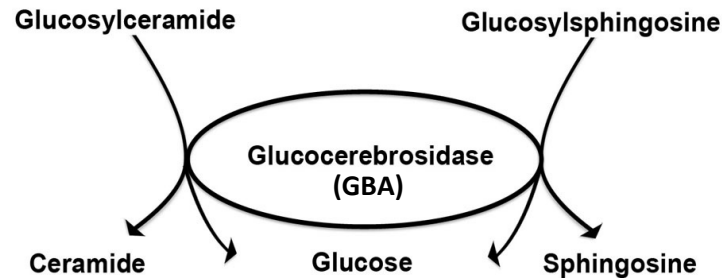
Percent of the global population aged 60 years and older



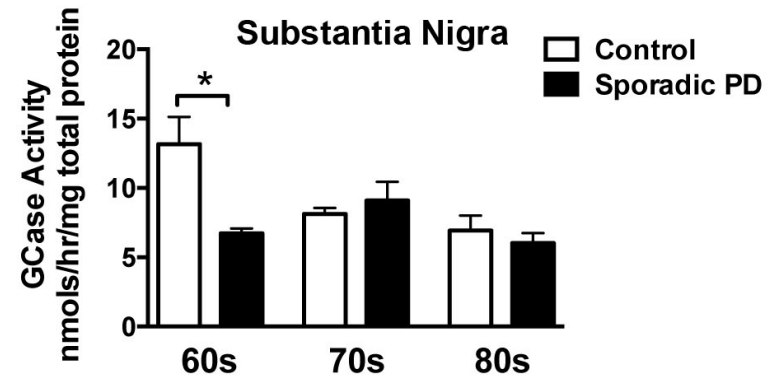
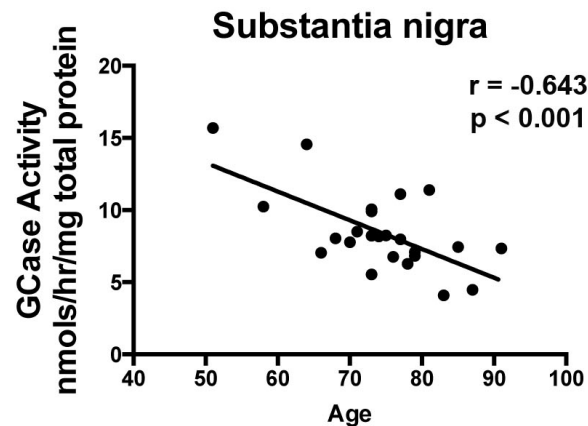
Source: World Population Prospects 2015 revision  
(UN Population Division)

# Linking lysosomal GBA with environmental and adaptive processes with PD and Lewy body dementia

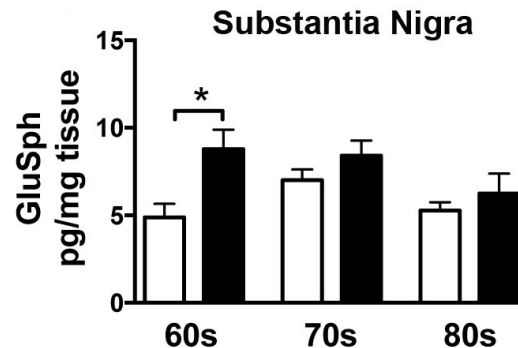
## Clinical studies



## Reduction of GBA activity in Parkinson's disease and normal aging

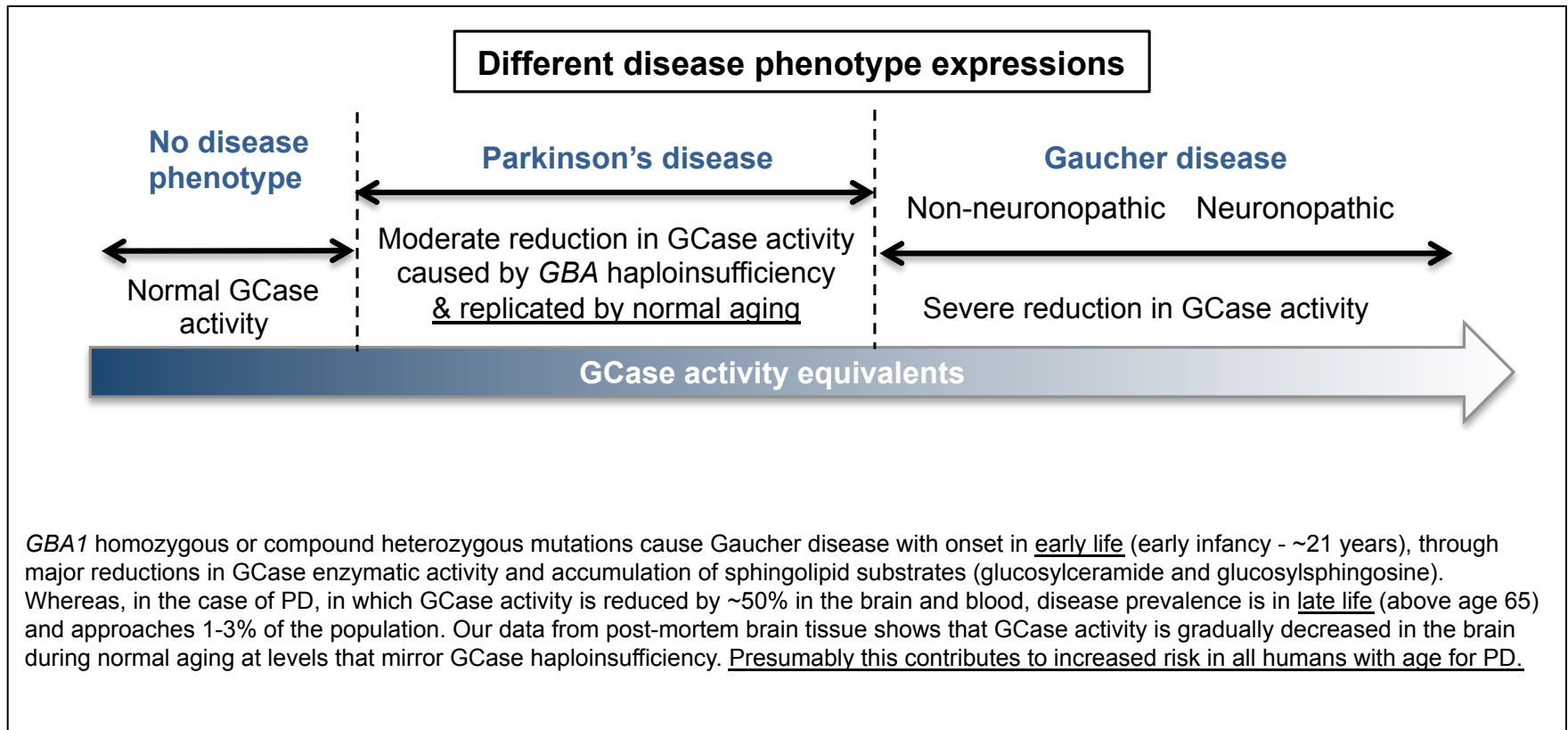


Accumulation of glucosylsphingosine in specific brain regions of Parkinson's disease patients

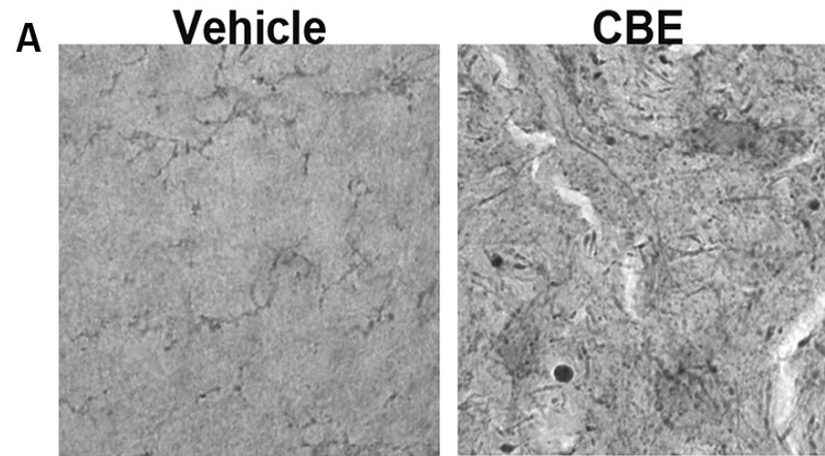
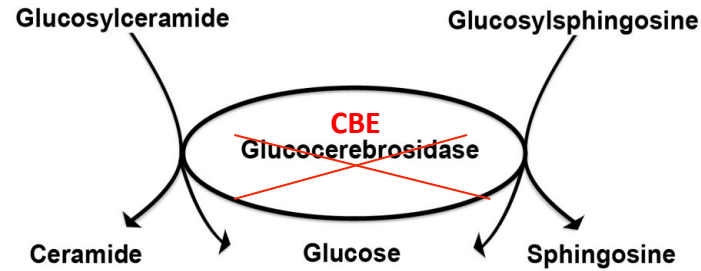




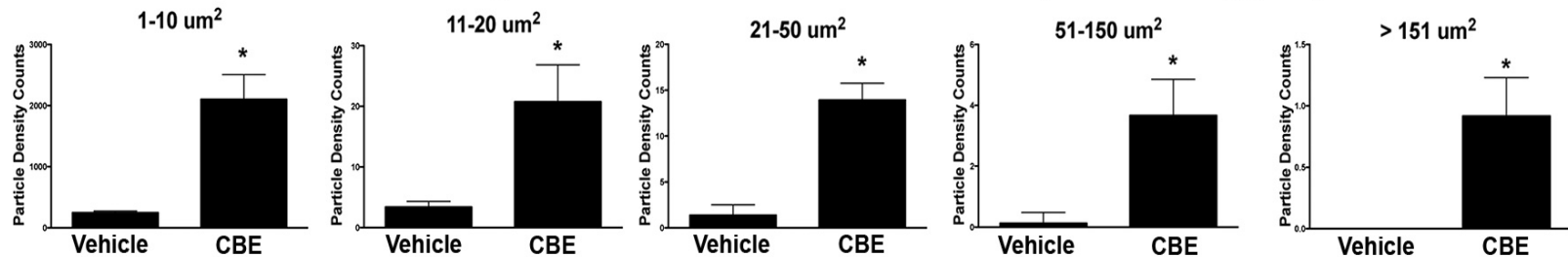
# Different levels of glucocerebrosidase (GBA/Gcase) activity create different diseases



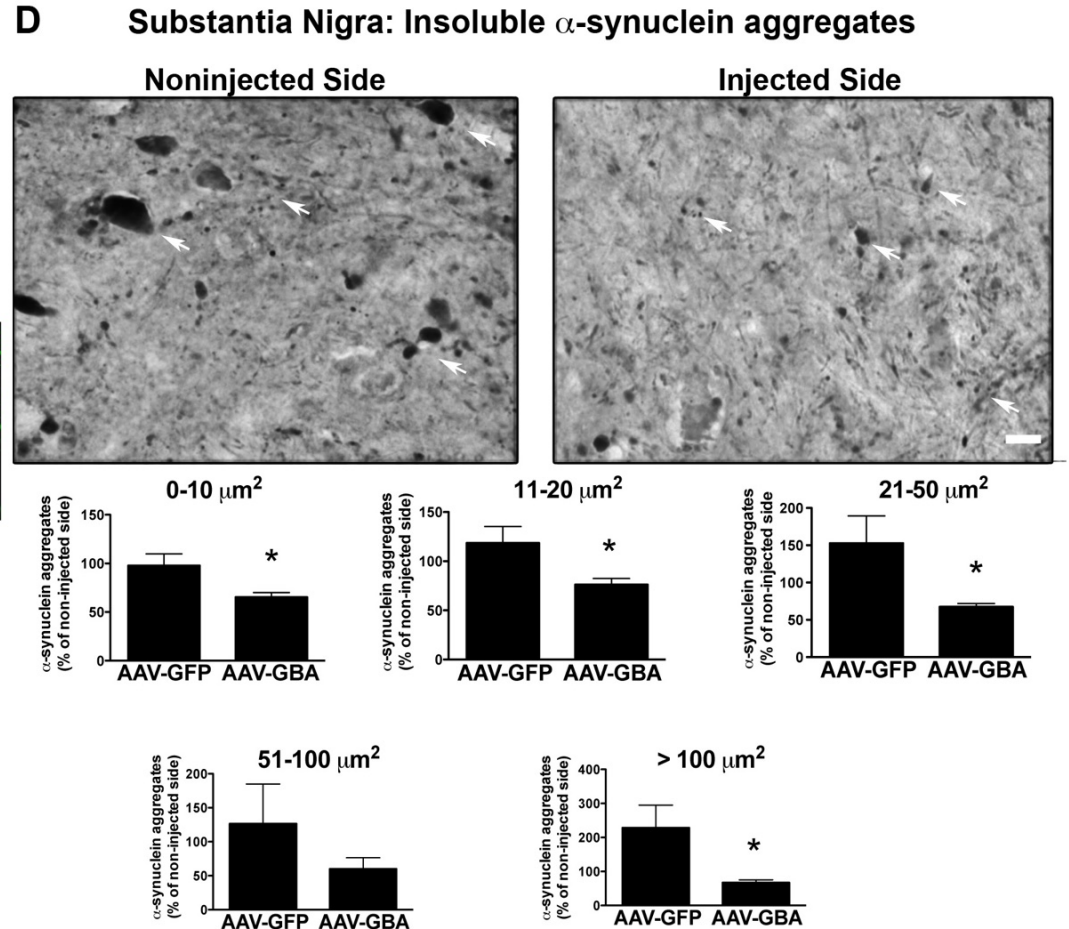
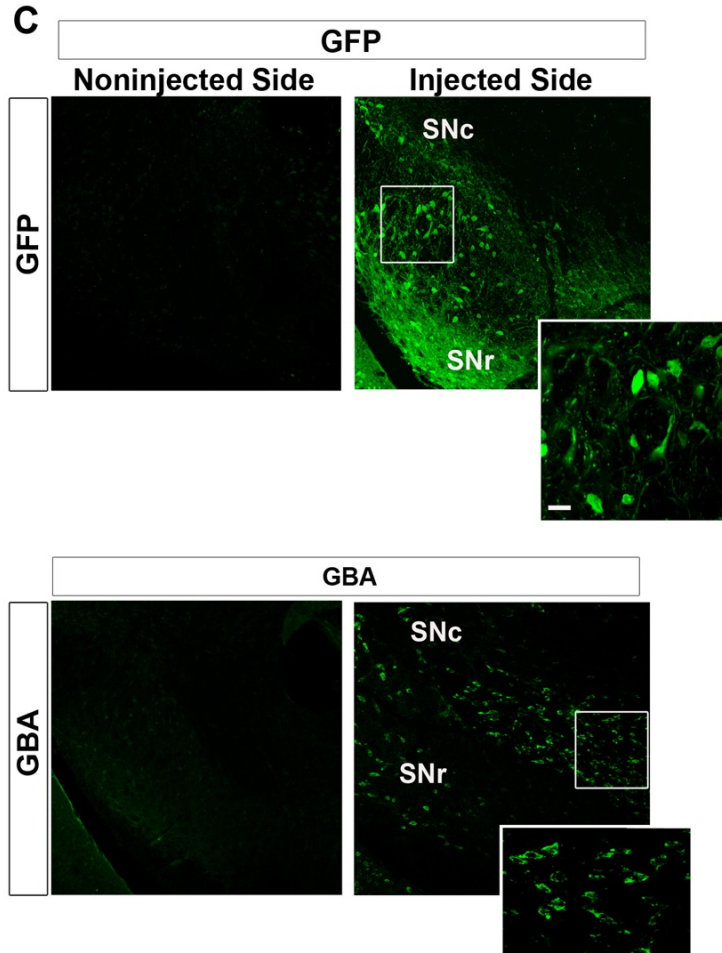
# Inhibition of glucocerebrosidase induces $\alpha$ -synucleinopathy in mice



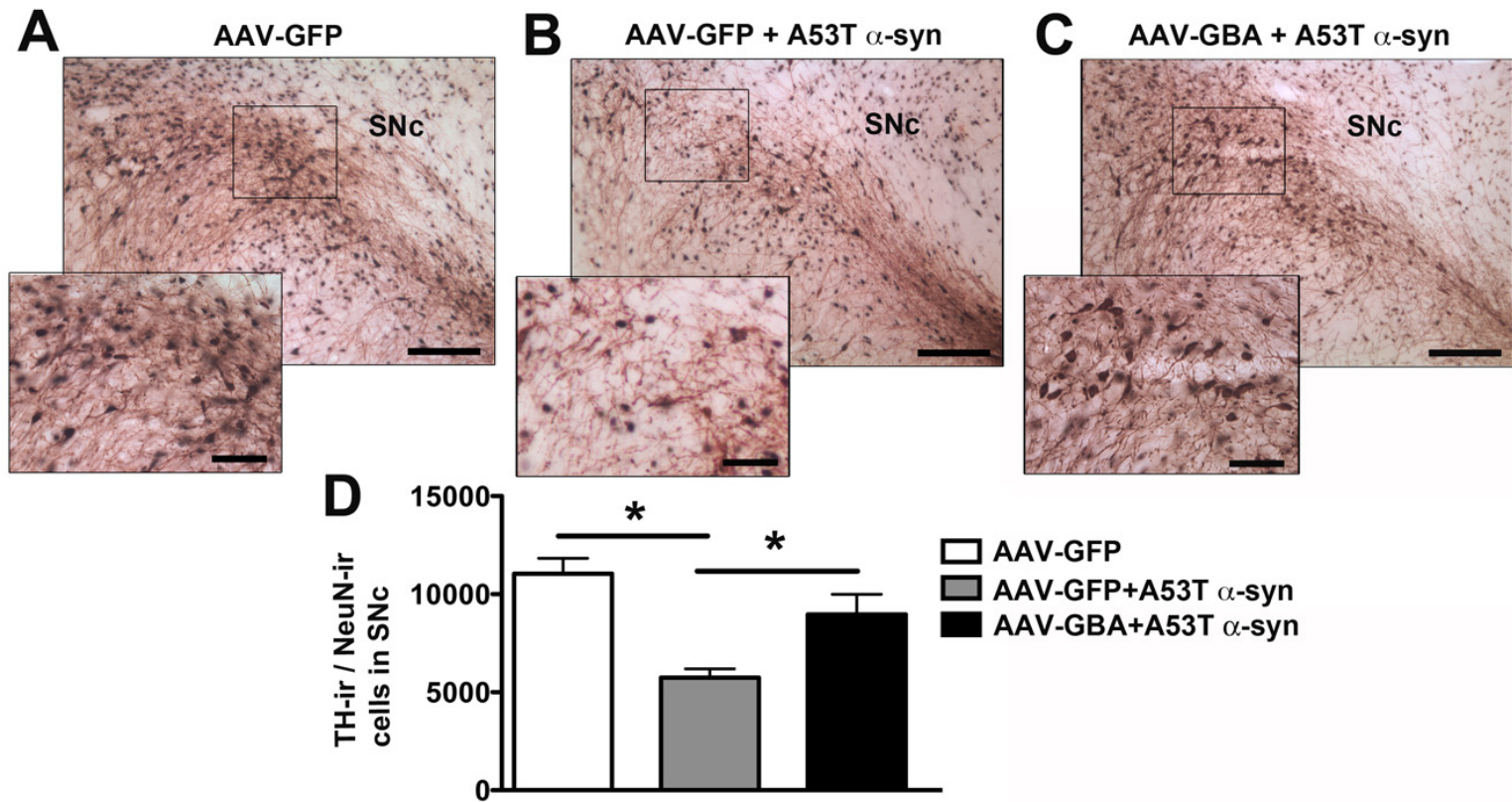
## Substantia Nigra: Proteinase-K insoluble $\alpha$ -synuclein aggregates



# Intra-cerebral injection of AAV2/5-GBA reduced insoluble $\alpha$ -synuclein aggregates in $\alpha$ -syn overexpressing mice, data at 8-months post gene therapy delivery.



# Intra-nigral administration of AAV2/2-GBA1 is neuroprotective against the A53T $\alpha$ -synuclein induced neurodegeneration and cell death

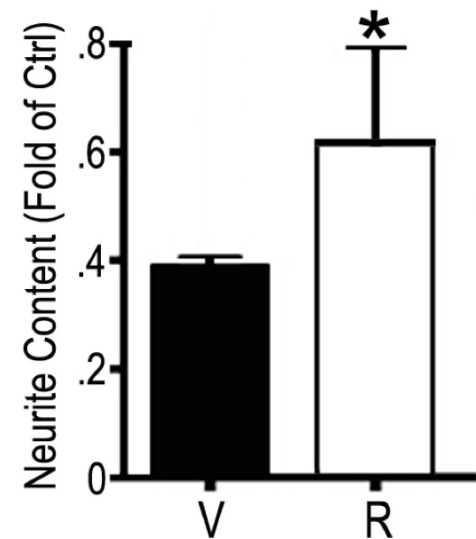
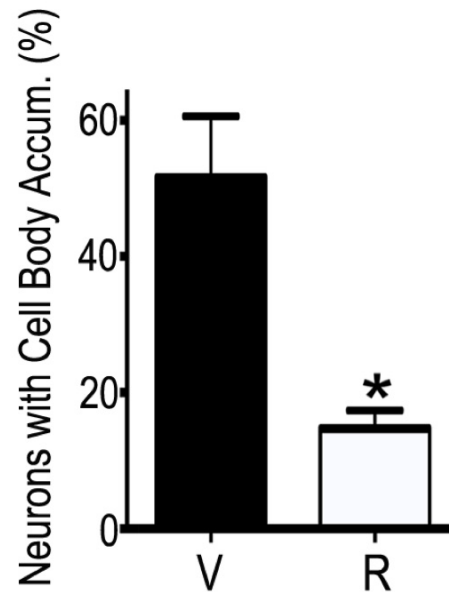
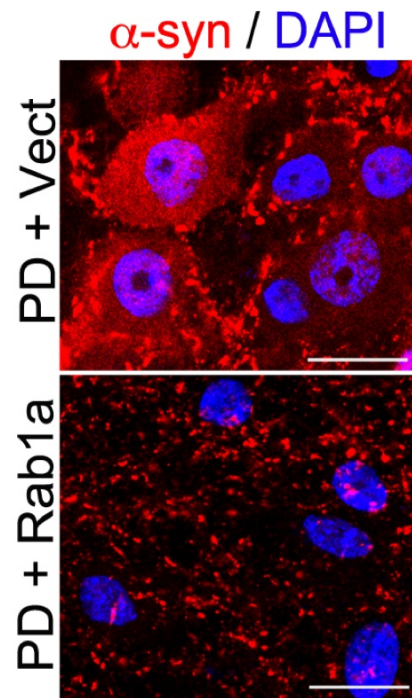




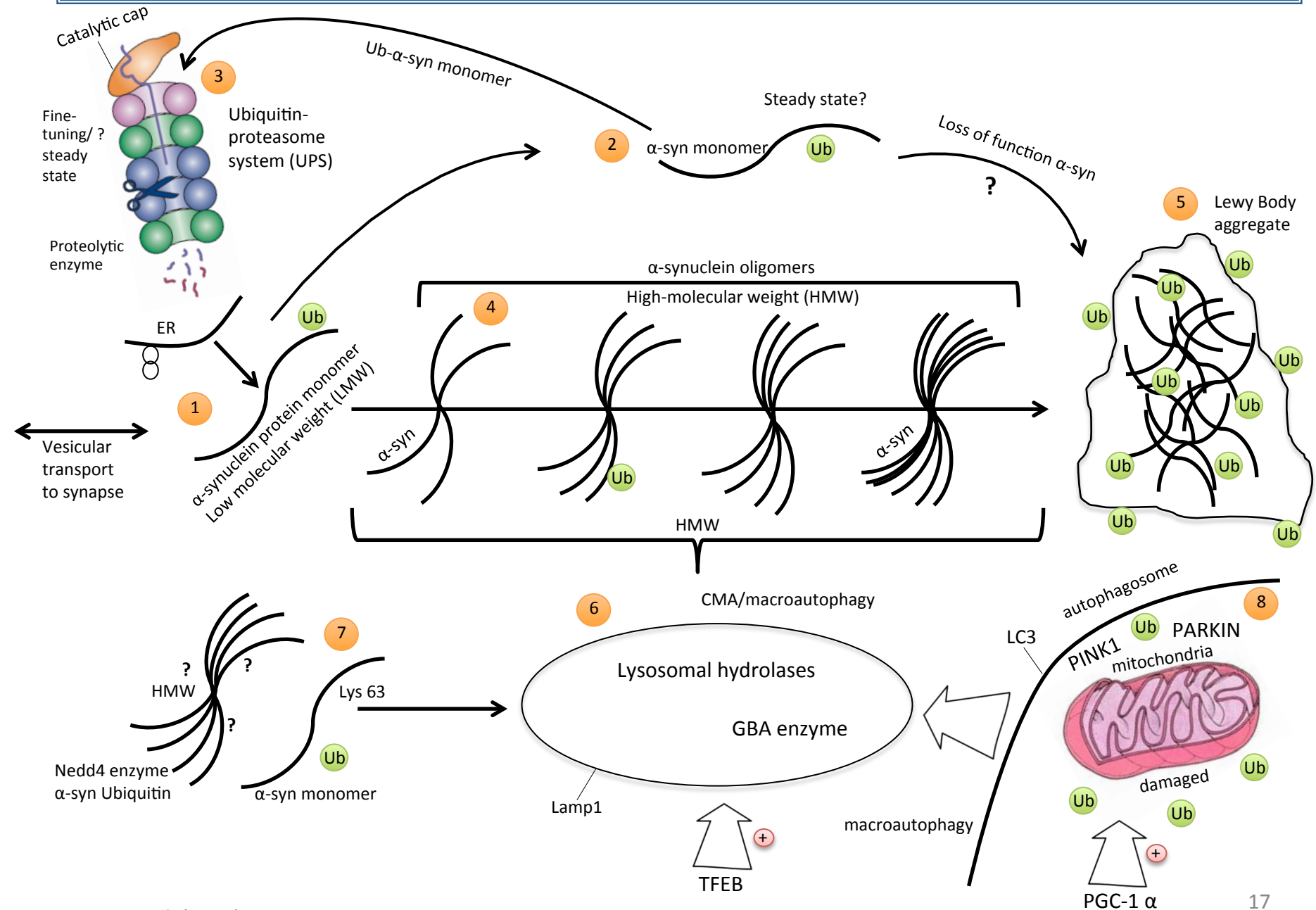
# $\alpha$ -Synuclein–induced lysosomal dysfunction occurs through disruptions in protein trafficking in human midbrain synucleinopathy models

Joseph R. Mazzulli<sup>a,b</sup>, Friederike Zunke<sup>b</sup>, Ole Isacson<sup>c</sup>, Lorenz Studer<sup>d,e</sup>, and Dimitri Krainc<sup>a,b,1</sup>

- Rab1a rescues lysosomal function and reduces pathological  $\alpha$ -syn in human 3x iPSC models.



# Proteasome & lysosomal clearance of intracellular $\alpha$ -synuclein and pathology



## II. Neuroinflammation and neurodegenerative diseases

- Neuroinflammation associated with neurodegenerative diseases traditionally assumed to be a response to pathophysiological events.
- However, new data from preclinical and clinical studies indicate that immune activation contributes to and drives disease pathogenesis.
- Recent genome-wide association studies (GWAS) of sporadic *Alzheimer disease* cases show associations between AD and genes that are involved in innate immunity: TREM2, CD33, complement receptor 1 (CR1).
- Support a role of altered microglial function in AD.
- High incidence of severe *parkinsonism* in people developing encephalopathy after 1918-1919 influenza outbreak.
- Association of increased PD risk with HLA genetic variants.
- Several PD-linked genetic mutations play a role in the regulation of the immune system – genetic vulnerability may predispose to degeneration of vulnerable neurons via inflammatory mechanisms.



# Neuroinflammation and neurodegenerative diseases

**Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging.**

**Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E and Isacson O**

***Eur. J. Neurosci.* 2002 15, 991-998**

**Neuroinflammation mediated by IL-1 beta increases susceptibility of dopamine neurons to degeneration in an animal model of Parkinson's disease**

**Koprich J, Reske-Nielsen C, Mithal P, Isacson O**

***J. Neuroinflammation*, 2008; 5(1): 8**

**Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy.**

**Chung CY, Koprich JB, Siddiqi H, Isacson O.**

**The Journal of Neuroscience; March 18, 2009 • 29(11): 3365- 73**

**The Toll-Like receptor-3 agonist polyinosinic:polycytidylic acid triggers nigrostriatal dopaminergic degeneration.**

**Deleidi M, Hallett PJ, Koprich J, Chung CY, Isacson O.**

**Journal of Neuroscience;, 2010 • 30(48): 16091- 101**

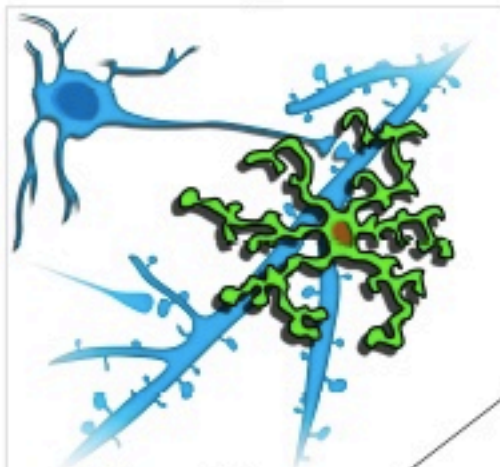
**A Nurr1 agonist causes neuroprotection in a Parkinson's disease lesion model primed with the toll-like receptor 3 dsRNA inflammatory stimulant poly(I:C).**

**Smith et al. *PLoS One*, 2015 • 10(3), Mar. 27, 2015, doi: 10.1371/journal.pone.0121072**

**Sustained Systemic Glucocerebrosidase Inhibition Induces Brain a-Synuclein Aggregation, Microglia and Complement C1q Activation in Mice.**

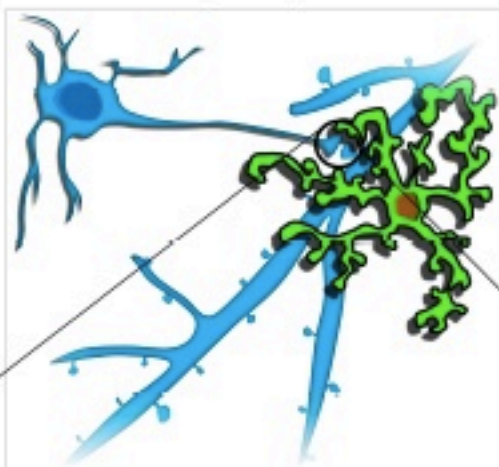
**Rocha et al. *Antioxidants & Redox Signaling*, 2015 • 23(6), doi: 10.1089/ars.2015.6307**

Healthy Brain



Microglial processes  
survey synapses

Early Stage AD

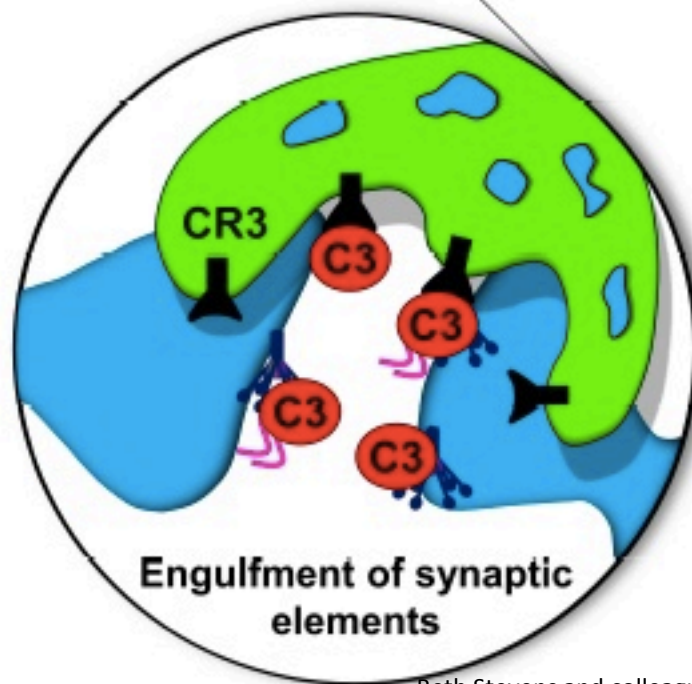
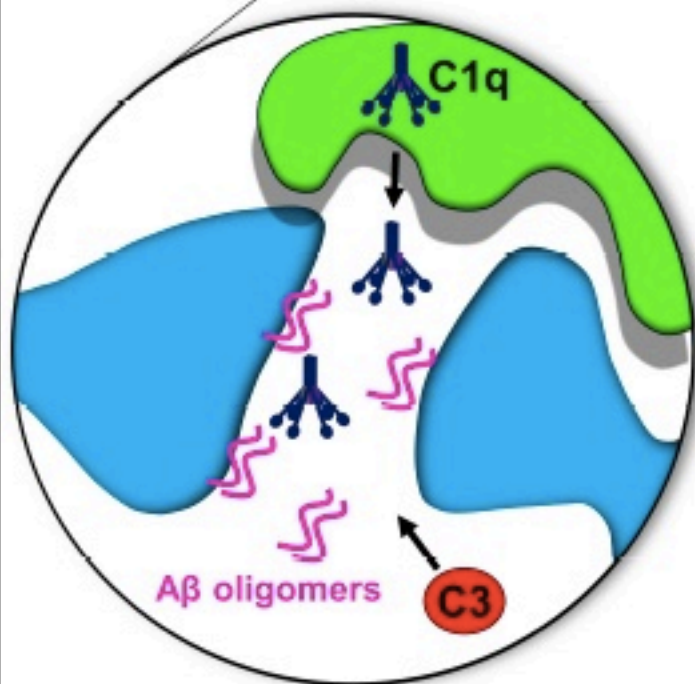


Synapse loss

Late Stage AD



Microgliosis and neuronal  
degeneration

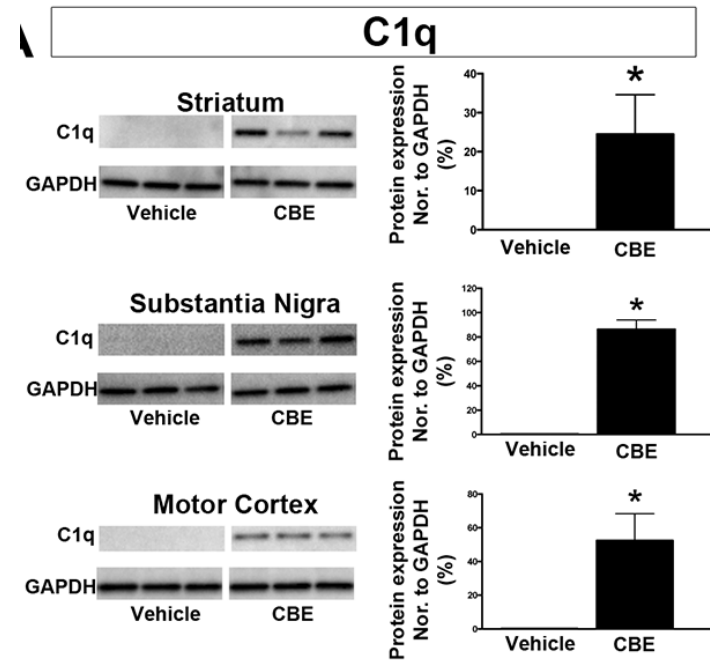
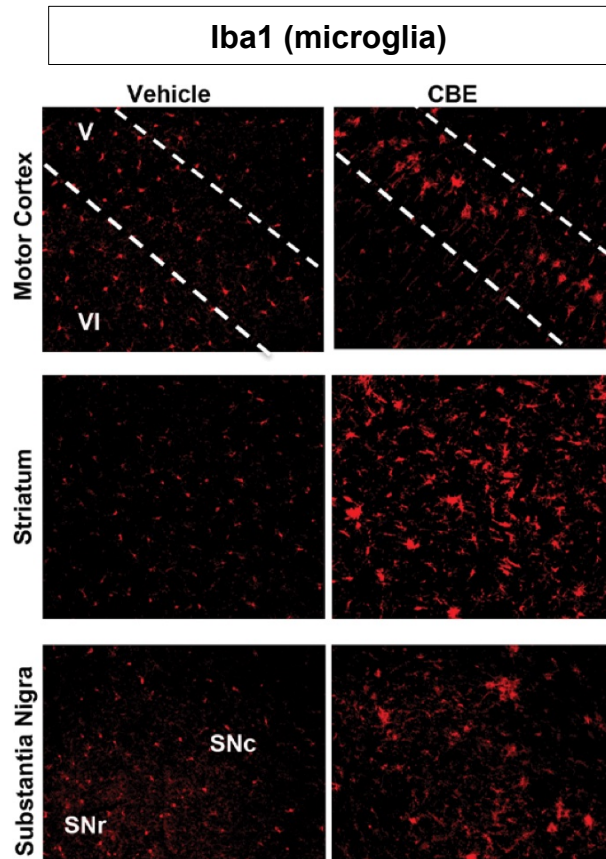
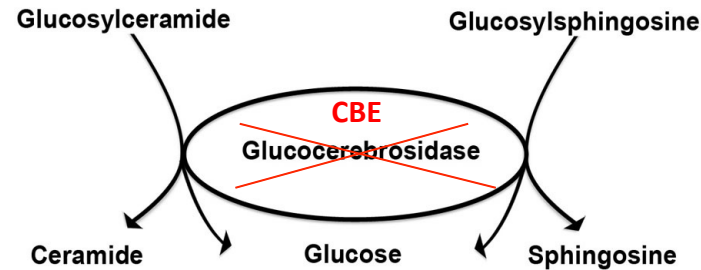


Engulfment of synaptic  
elements

### III. Synaptic pruning and complement pathway activation

- Weak synapses are tagged during normal postnatal development by classical complement pathway for normal synaptic pruning (Schafer et al. 2012; Stevens et al. 2007).
- Microglial recruitment is necessary for removal of unwanted and redundant synapses.
- Synaptic dysfunction and loss precedes degeneration of cell soma, early pathological event in several neurodegenerative diseases.
- C1q implicated in synaptic loss in adult brain during neurodegeneration, and C1q expression is increased in PD, AD, HD, glaucoma.
- C1q expression and neuroinflammation is increased in brain following CBE-induced glucocerebrosidase inhibition, concurrent with  $\alpha$ -synucleinopathy.
- Synapses and axons may be early targets of inflammation-induced neurodegeneration in  $\alpha$ -synucleinopathies via complement pathway.

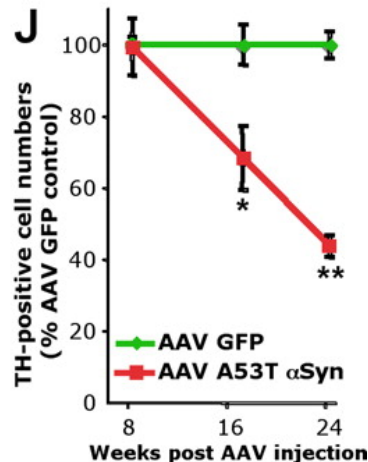
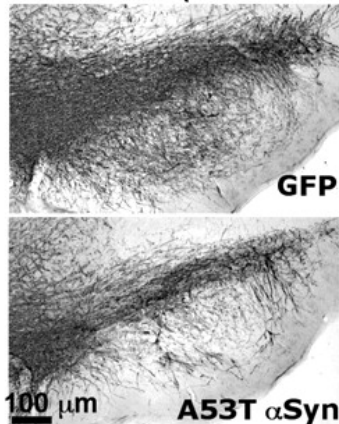
# Inhibition of glucocerebrosidase induces microglial and complement C1q activation in mice



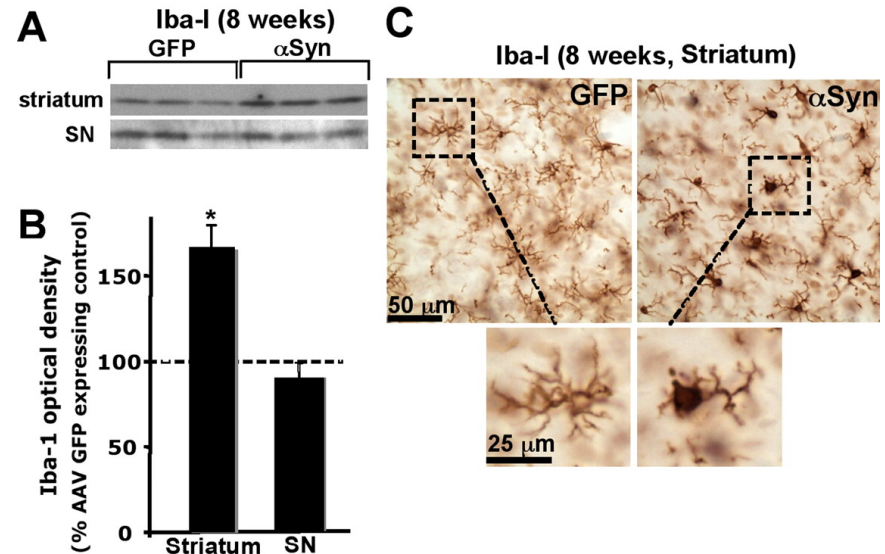
# $\alpha$ -synuclein and immune activation

- Striatal inflammation precedes dopamine neuron degeneration in AAV-A53T  $\alpha$ -synuclein rat model (Chung et al., J. Neurosci. 2009).

24 week SN (TH staining)



$\alpha$ -Synuclein mediated neuroinflammation at 8 weeks

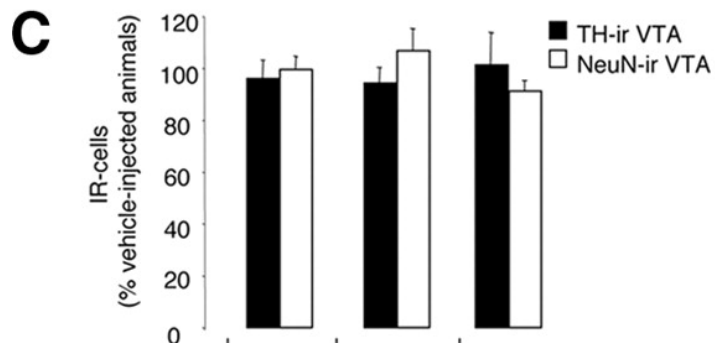
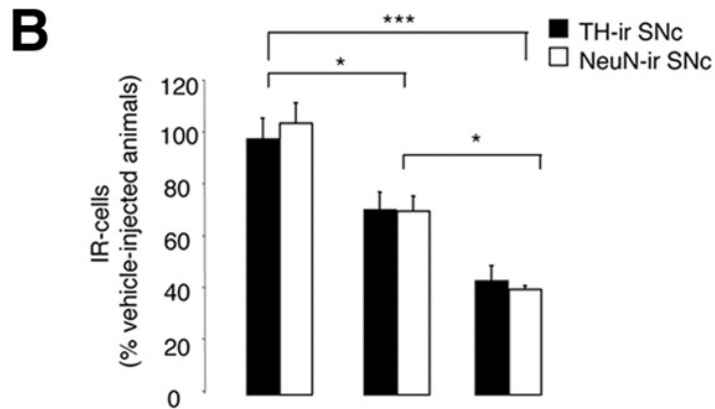
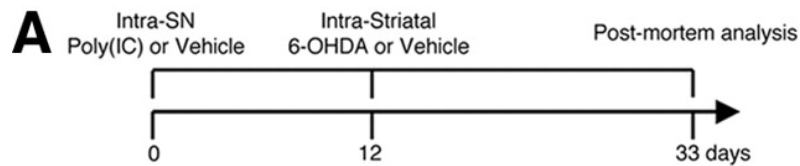


**D**

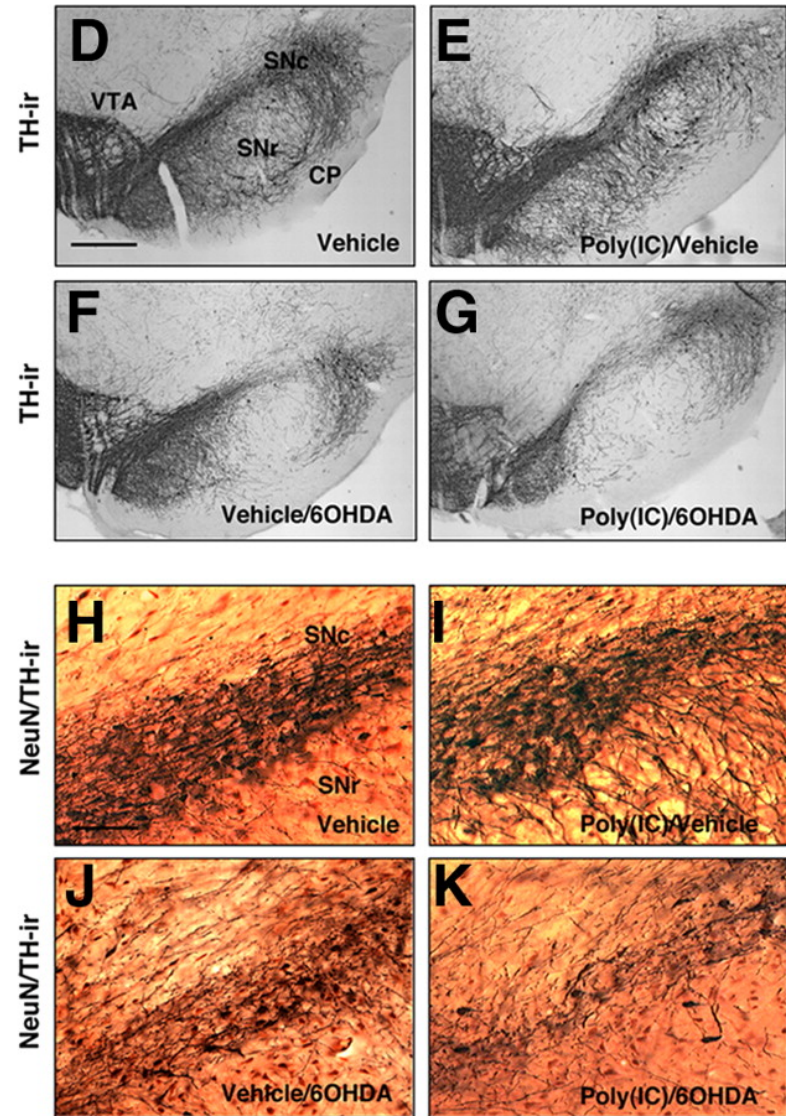
Cytokine	Striatum (pg/mg)		SN (pg/mg)	
	AAV GFP	AAV $\alpha$ Syn	AAV GFP	AAV $\alpha$ Syn
IL-1 $\beta$	3.1( $\pm$ 0.4)	5.0( $\pm$ 0.5)*	3.5( $\pm$ 0.4)	3.9( $\pm$ 0.5)
IL-2	9.2( $\pm$ 1.0)	10.2( $\pm$ 1.4)	8.9( $\pm$ 1.5)	10.3( $\pm$ 1.4)
IL-4	2.2( $\pm$ 0.3)	2.8( $\pm$ 0.2)	1.6( $\pm$ 0.3)	1.6( $\pm$ 0.3)
IL-6	58.3( $\pm$ 7.0)	62.1( $\pm$ 5.9)	50.3( $\pm$ 6.3)	52.3( $\pm$ 5.5)
IL-10	1.9( $\pm$ 0.3)	2.6( $\pm$ 0.2)	2.6( $\pm$ 0.2)	2.9( $\pm$ 0.3)
MCP-1	3.1( $\pm$ 0.2)	2.8( $\pm$ 0.3)	6.0( $\pm$ 0.6)	4.8( $\pm$ 0.5)
IFN- $\gamma$	6.5( $\pm$ 1.5)	13.0( $\pm$ 2.8)*	6.1( $\pm$ 1.1)	6.4( $\pm$ 1.3)
RANTES	2.7( $\pm$ 0.3)	2.7( $\pm$ 0.3)	2.1( $\pm$ 0.1)	2.6( $\pm$ 0.4)
TNF- $\alpha$	3.8( $\pm$ 0.7)	7.8( $\pm$ 1.6)*	3.9( $\pm$ 0.5)	3.5( $\pm$ 0.7)



# Viral-like (TLR3) or LPS (TLR4, Koprach et al 2008) neuroinflammation potentiates *susceptibility* of nigral (A9) dopamine neurons to degeneration, and can be blocked by IL1-beta receptor antagonist



1. SN injection	Poly(IC)	Veh	Poly(IC)
2. STR injection	Veh	6-OHDA	6-OHDA
Animals/group	n=8	n=8	n=8



Alzheimer's disease is a synaptic failure. Science. 2002 Oct 25;298(5594):789-91.



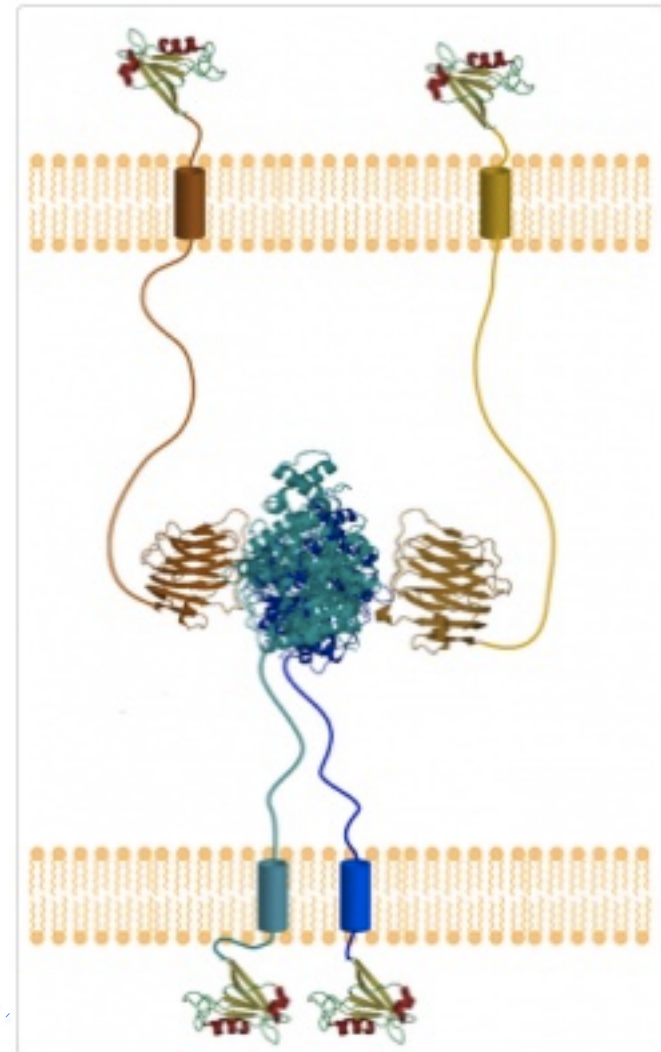
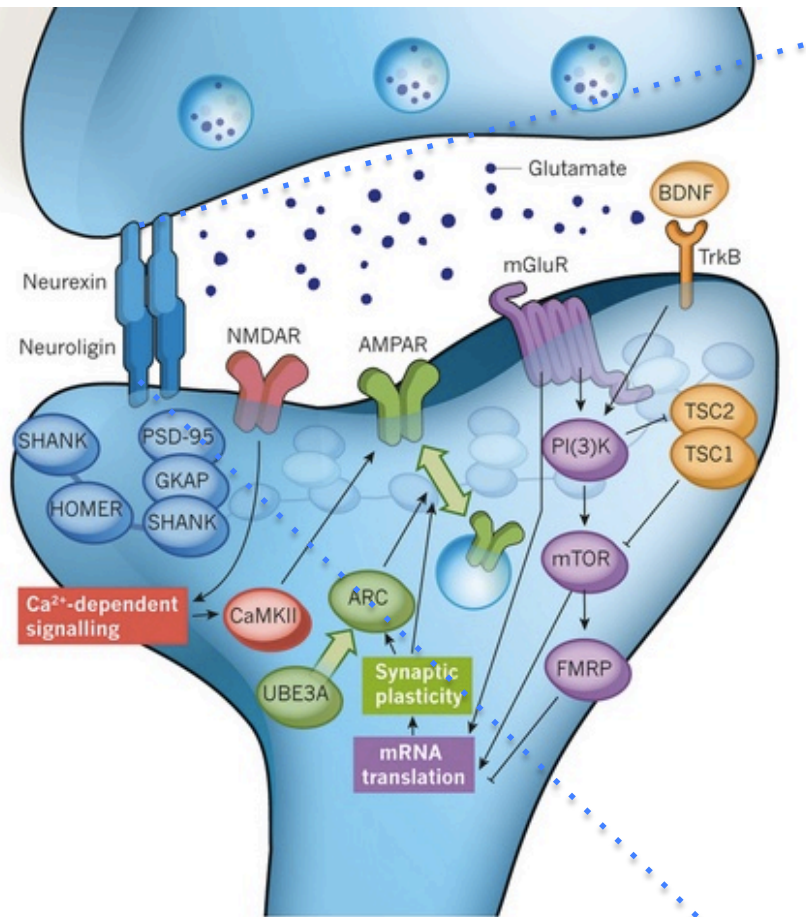
J Neurosci. 2000 Jun 1;20(11):4050-8.

High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation.

Mucke L1, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L.



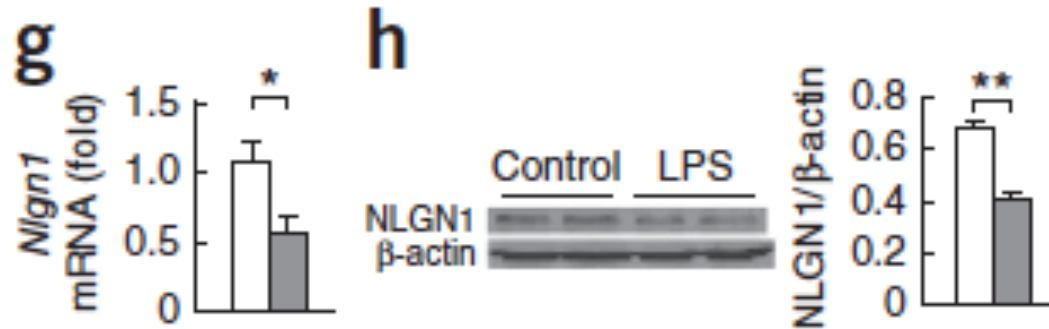
# Evidence for synaptic dysfunction caused by inflammation..



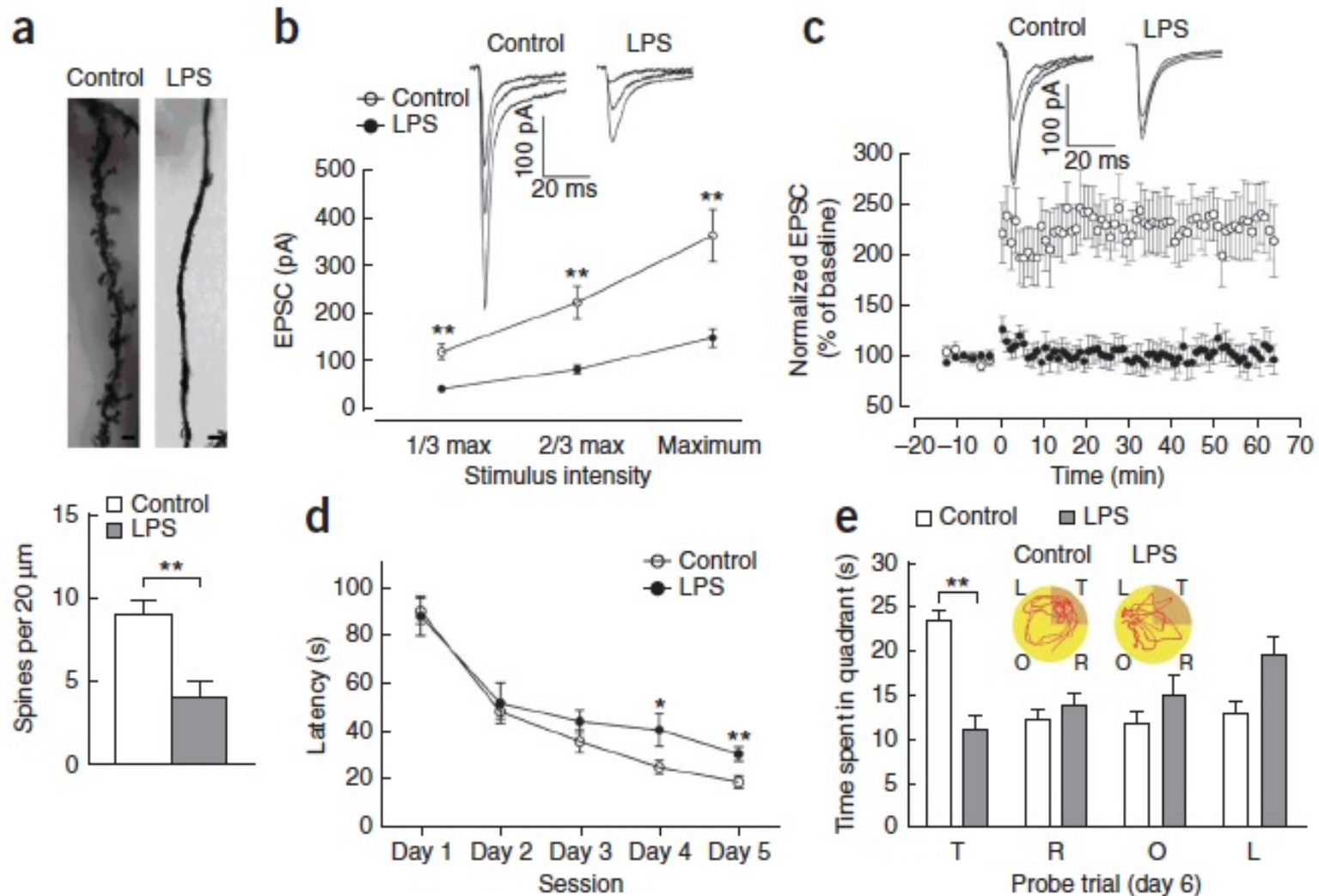
Südhof Nature 2008,  
Bie B et al. Nat Neurosci. 2014 17(2):223-31

Neuroligins connect synapses, but may  
be in short supply in Alzheimer's. Image

# Inflammation (LPS) induces suppression of NLGN1 expression and mirrors the effect of amyloid fibrils



# LPS impairs hippocampal glutamatergic transmission and memory in naive rats

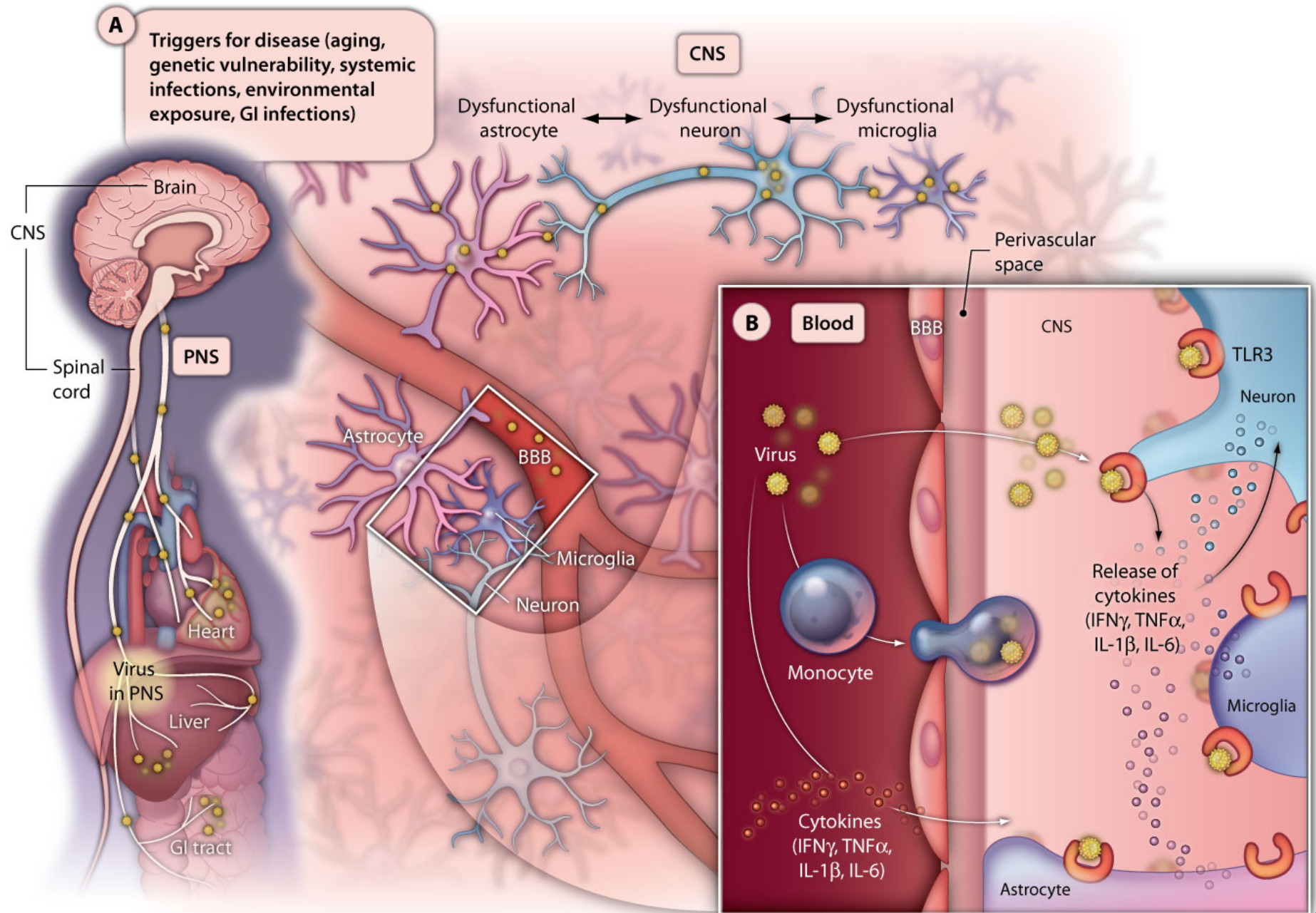


## Parkinson's disease & LBD: LRRK2 and immune activation

- **GWAS studies indicate SNP variants in LRRK2 locus associated with Crohn's disease and leprosy** (*Barrett et al. 2008 Nat. Gen.*; *Zhang et al. 2009 NEJM*).
- **LRRK2 expression is increased in inflamed tissue in Crohn's disease** (*Gardet et al 2011 J. Immunol.*).
- **LRRK2 highly expressed in immune system cells** (inc. PBMCs, macrophages) (*Gardet et al 2011 J. Immunol.*; *Thevenet et al 2011 PLoS ONE*; *Hakimi et al. 2011 J. Neural. Transm.*).
- **Microglial expression of LRRK2 is increased in response to inflammatory challenge in vitro and in vivo** (*Gillardon et al 2012 Neuroscience*; *Moehle et al., 2012 J. Neurosci.*).
- **TLR4 activation in the substantia nigra of mice induces LRRK2 activity** (*Moehle et al., 2012 J. Neurosci.*).
- **LRRK2 knockdown and LRRK2 kinase inhibition in microglia attenuates induction of inflammatory mediators after LPS.** (*Moehle et al. 2012 J. Neurosci.*).
- **LRRK2 deficiency in vivo protects substantia nigra dopamine neurons from LPS-induced degeneration** (*Daher et al. 2014 PNAS*).
- **Enhanced inflammatory response to LPS in R1441G LRRK2 mouse microglia** (*Gillardon et al., 2012 Neuroscience*).
- **Higher levels of peripheral inflammatory cytokines in sera of asymptomatic G2019S carriers and PD patients carrying LRRK2 G2019S** (*Dzamko et al. 2016 Mov. Dis.*). *IL1B up in carriers*



# Inflammation triggers in PD and AD?



## SUMMARY 1: Neuroinflammation, lipids, proteins and neurodegenerative diseases

- **Specific cellular lipid and protein abnormalities in aging and disease** create pre-conditions for the inflammation that leads to the synaptic and dendritic spine loss...
- **Neuroinflammation imbalances are the final triggers for synaptic loss and ultimately cell death in neurodegenerative diseases...**
- The inflammatory signals including **immune cells, cytokines and complements can be monitored as local and peripheral biomarkers...**
- **Drugs and treatments that metabolically correct the patient's cellular lipid and protein abnormalities will reduce inflammatory triggers** and in concert with anti-inflammatory drugs should reduce neurodegeneration.

## **SUMMARY 2 : Human genetics and cells to model neurodegenerative mechanisms, protection and restoration in PD and Lewy body dementia**

- **Cell biological** analyses at **baseline** and with cellular **stressor** reveal **specific or shared** biochemical and pathobiological **phenotypes** from different **human genotypes**
- **Rescue of phenotypes** possible using **drugs, molecular medicine or gene-editing**
- Late stage **development tool for pharmacogenetics and personalized** medicine.
- **Neuronal and glial targeted drugs, cell and gene therapies for PD and LBD can work**, -- and with further **innovations** and tests can reach a broad, safe and **effective applications**.



# Thank you!



## Neuroregeneration Laboratory and Institute

## Collaborations

Penny Hallett  
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Joanna Korecka-Roet  
Gaynor Smith\*  
Elizabeth Moloney  
Emily Rocha\*  
James Schumacher  
Michelle Moore  
Arnar Astradsson  
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CORIELL INSTITUTE  
FOR MEDICAL RESEARCH



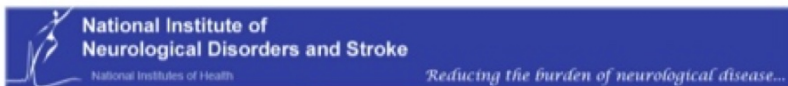
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PARKINSON'S RESEARCH