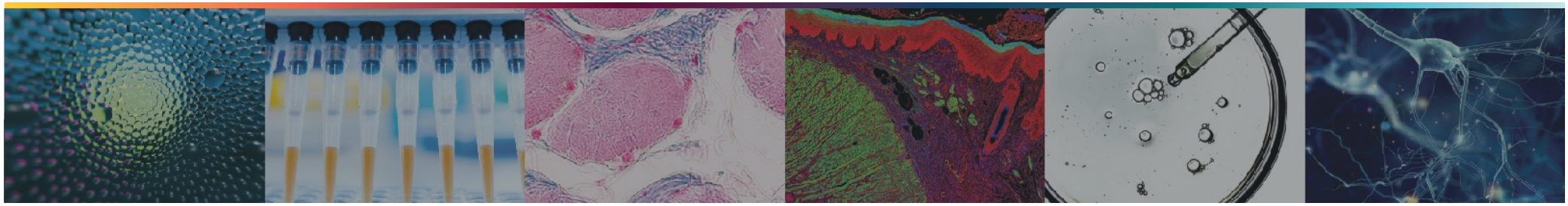
THE FRONT ROW
at Scripps Research

Paving the Way for New Therapies for Neurodegenerative Diseases

Sandra E. Encalada, PhD
Associate Professor, Department of Molecular Medicine
Dorris Neuroscience Center Investigator
The Scripps Research Institute

Wednesday, February 15, 2023 | 1:00 pm PT/4:00 pm ET



My Background and Interests of The Encalada Laboratory at Scripps Research

Sandra's Academic Journey

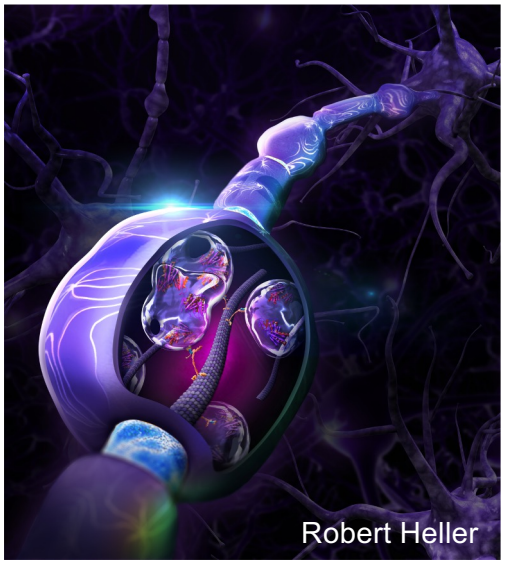
- Earlham College (Indiana): BA Physics
- University of Florida: MS Population Genetics
- University of Oregon: PhD Molecular Genetics
- UCSD: Postdoctoral studies
- Scripps Research: Assistant Professor
  Associate Professor

Encalada Lab 2022

The Encalada lab studies mechanisms of neurodegeneration by building models of Alzheimer's and prion diseases by focusing on cell biological studies inside neurons that inform us on the development of therapies to treat these fatal disorders

OUTLINE

- **Intro to Aging/Neurodegeneration Connection: some stats**
- **Alzheimer's Disease (AD) and Prion Diseases**
- **Prion Disease: inside neurons**
  - •• Active transport of proteins inside neurons
  - •• Prion protein aggregates form inside fluid-filled sacks called endosomes
- **Towards therapies to ameliorate prion disease toxicity**



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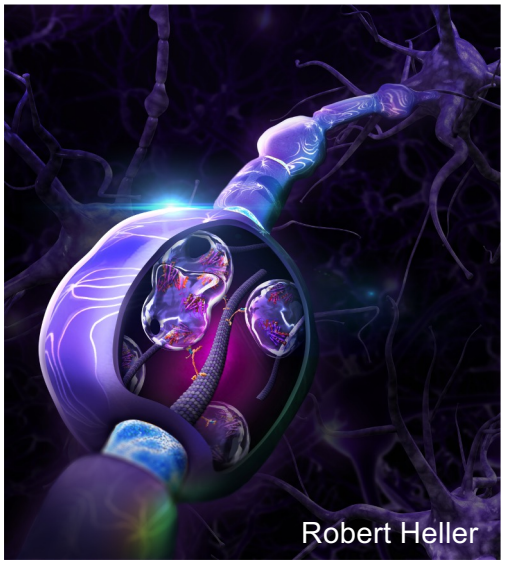
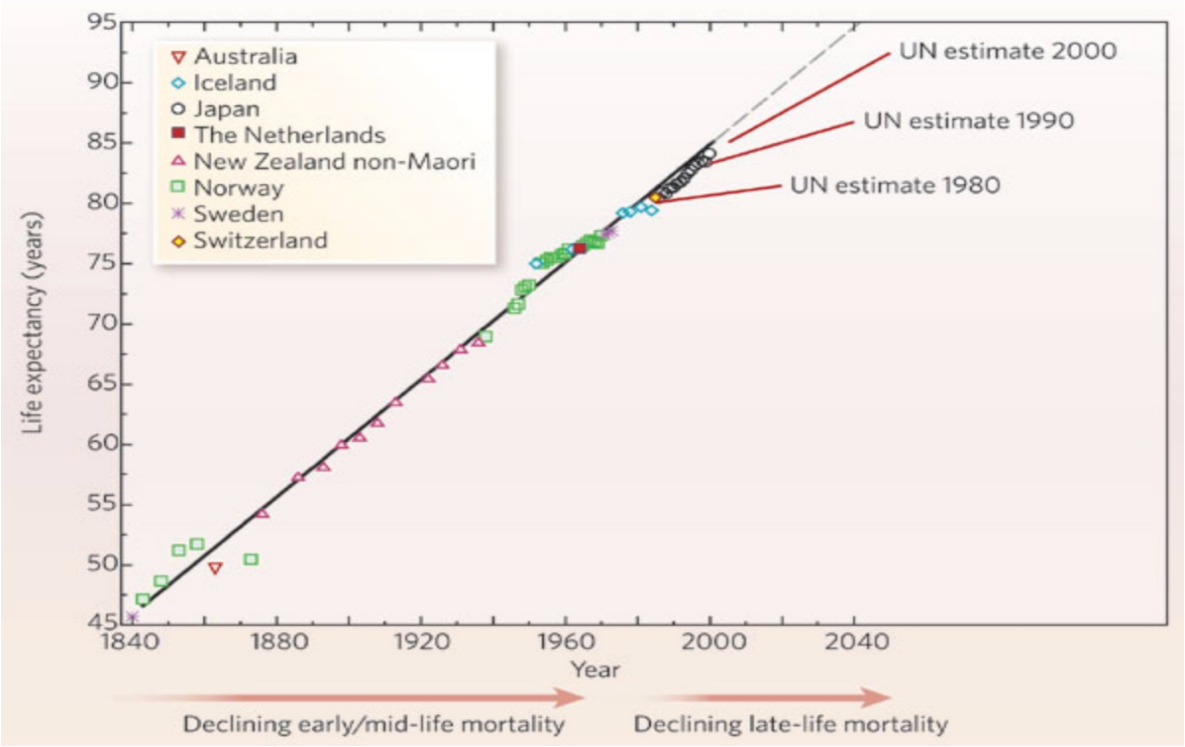


Six Generations of Daughters – From Baby to 111-Year-Old Great, Great, Great Grandmother

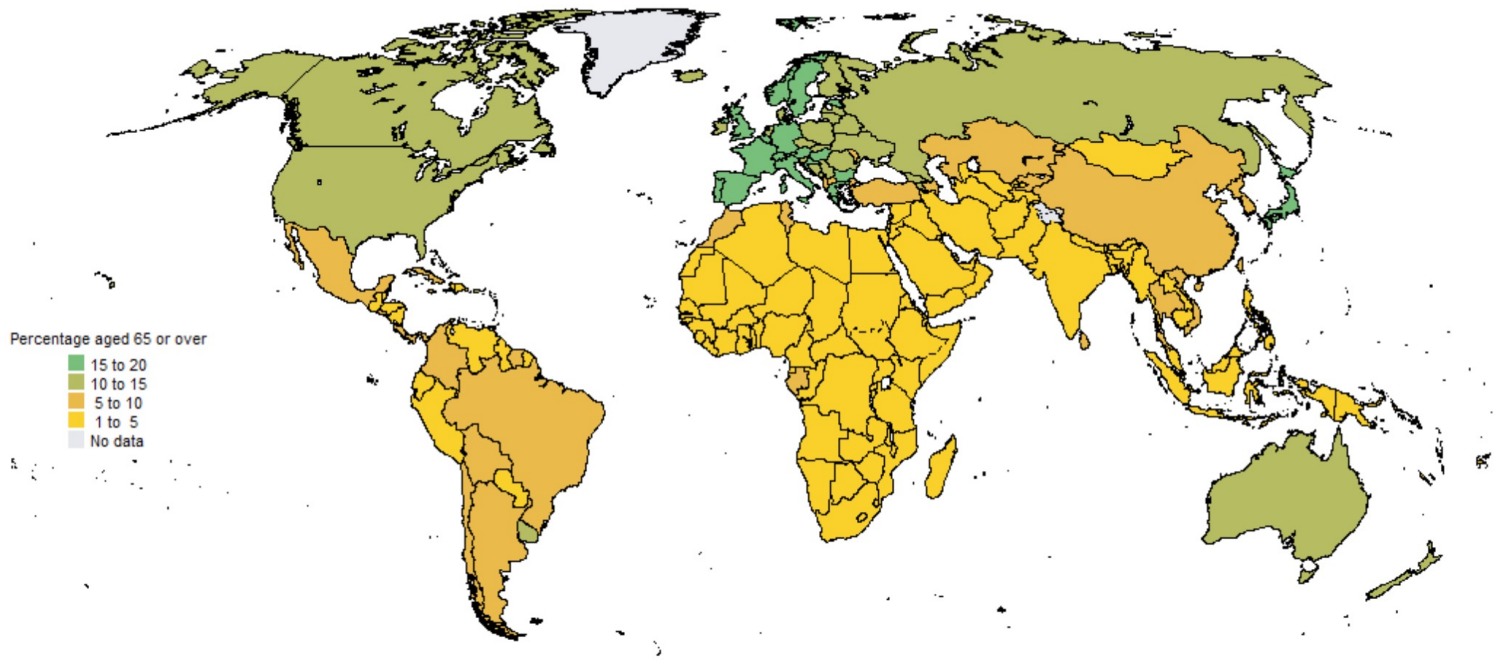
https://abcnews.go.com/blogs/headlines/2012/05/six-generations-of-daughters-from-baby-to-111-year-old-great-great-great-grandmother

Life Expectancy Has Increased in the Last 200 Years



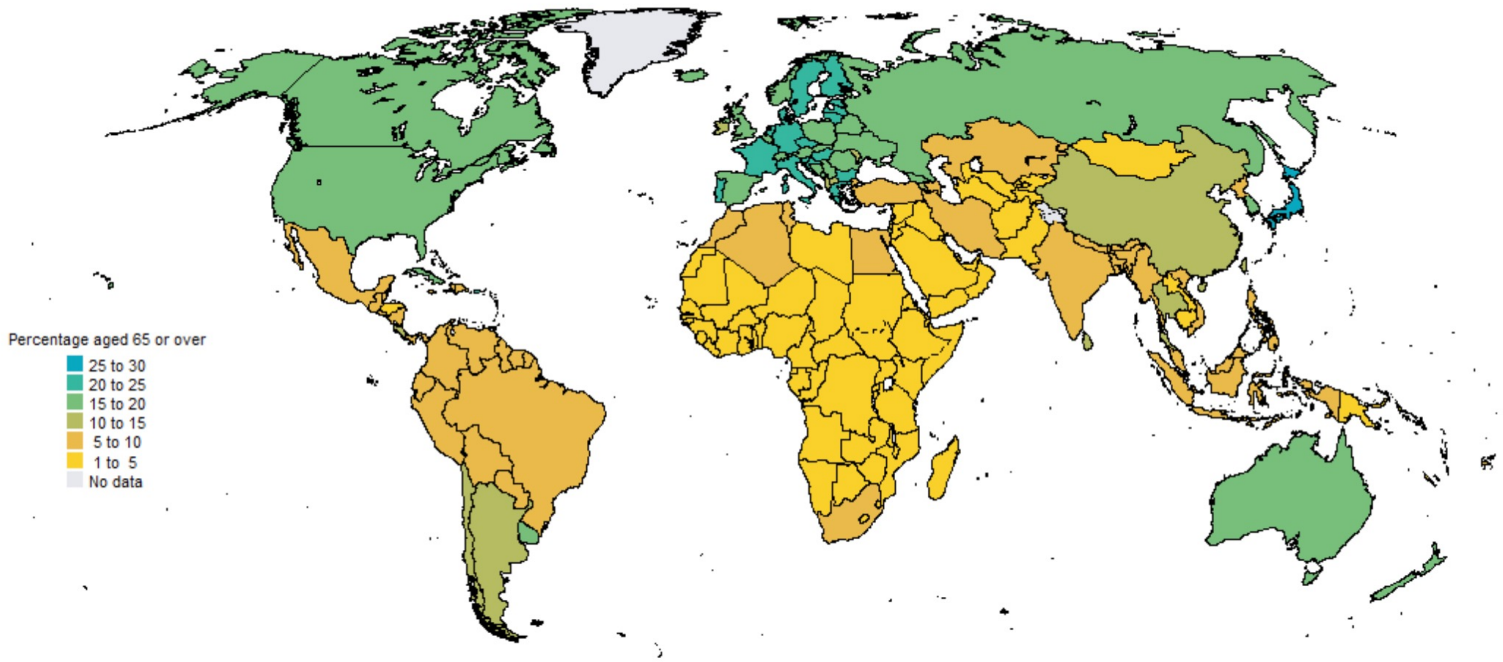
Zhaurova *Nature Education 2008*

Global Population > 65 Years Old in 2000



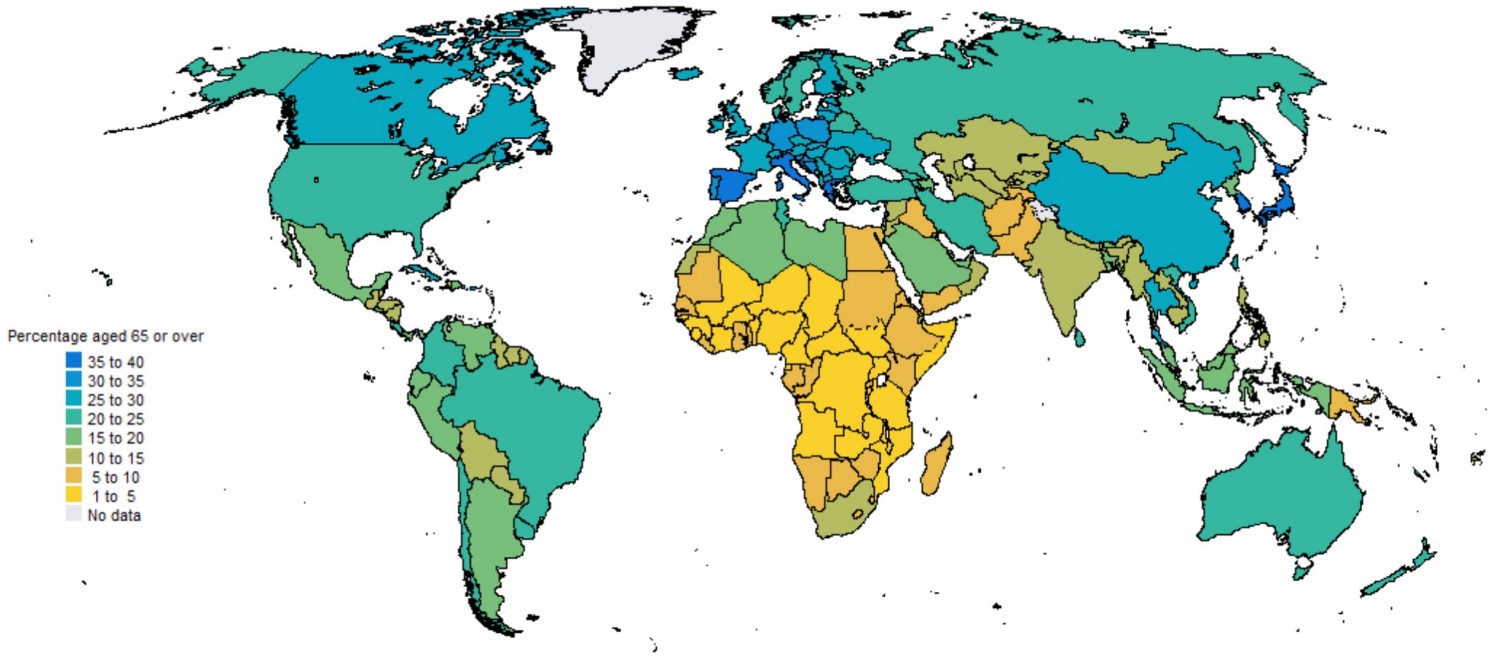
United Nations, DESA/Population Division
https://population.un.org/

Global Population > 65 Years Old in 2020



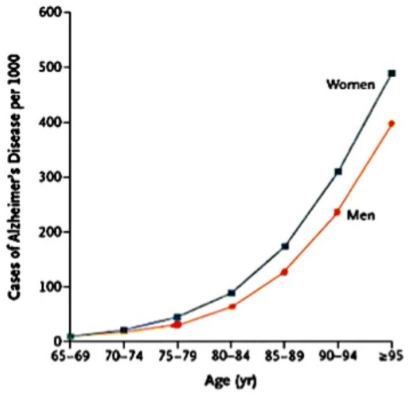
United Nations, DESA/Population Division
https://population.un.org/

Global Population > 65 Years Old in 2050

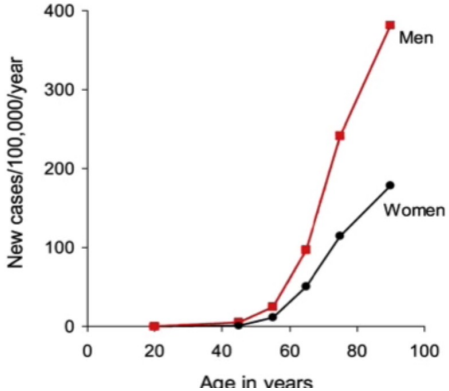


United Nations, DESA/Population Division
https://population.un.org/

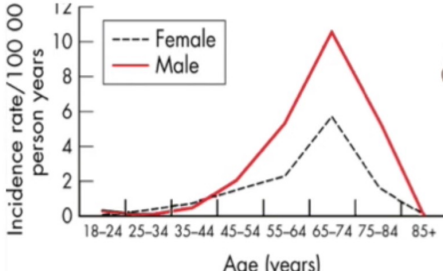
Age is the Biggest Single Risk Factor for Developing Dementias and other Neurodegenerative Disorders



Alzheimer's Disease



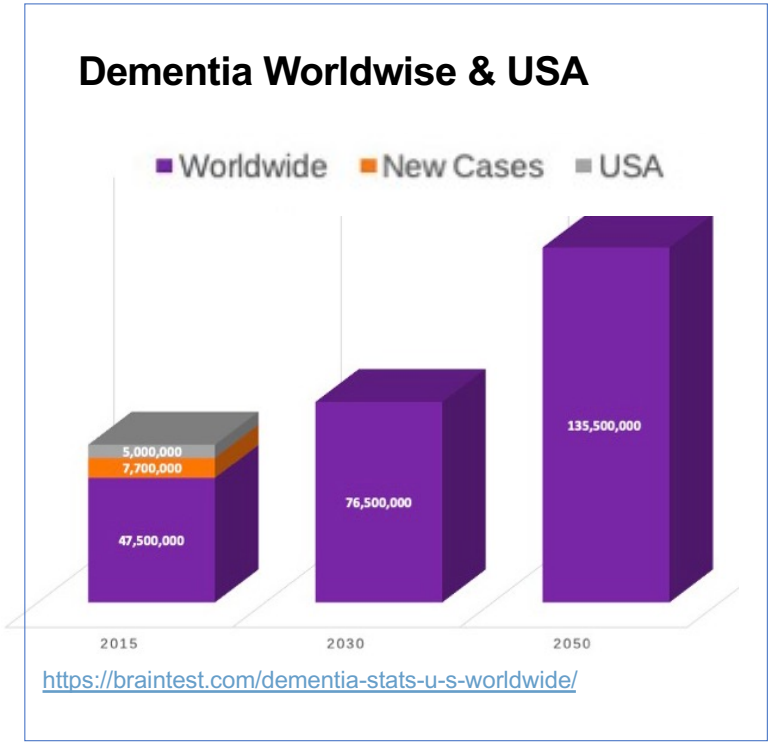
Parkinson's Disease



ALS

https://www.ibiology.org/neuroscience/neurodegenerative-disease/

Dementias are a HUGE and Growing Global Epidemic



https://braintest.com/dementia-stats-u-s-worldwide/

FACTS:

- Someone in the world develops dementia every 3 seconds.
- **Prevalence:** ~ 50 million people living with dementias worldwide.
- **Yearly:** 10 million new cases of dementia.
- **Economic Impact:** total estimated worldwide cost by 2018 > **US$ trillion.**

World Health Organization, September 2019

https://www.who.int/news-room/fact-sheets/detail/dementia

OUTLINE

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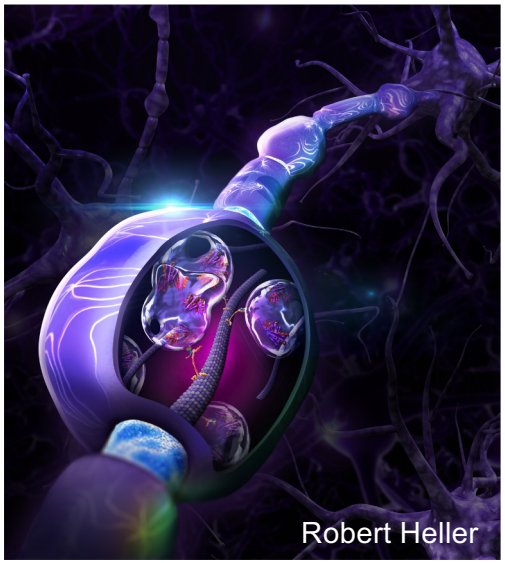
- **Alzheimer's Disease (AD) and Prion Diseases**

- Prion disease: inside neurons
  - Active transport of proteins inside neurons
  - Prion protein aggregates form inside fluid-filled sacks called endosomes

- Towards therapies to ameliorate prion disease toxicity



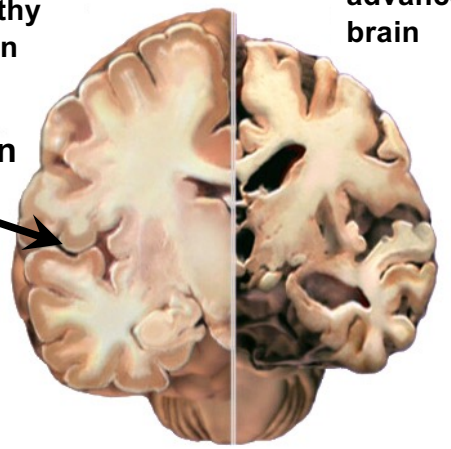
Neuronal Degeneration and Cell Death are Hallmarks of Neurodegenerative Disorders

Brain degeneration

heathy brain

advanced AD brain

~ one hundred billion neurons



http://www.alz.org/braintour/healthy_vs_alzheimers.asp

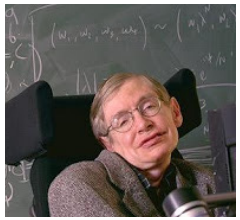
Alzheimer's Disease

Ronald Reagan

Parkinson's Disease

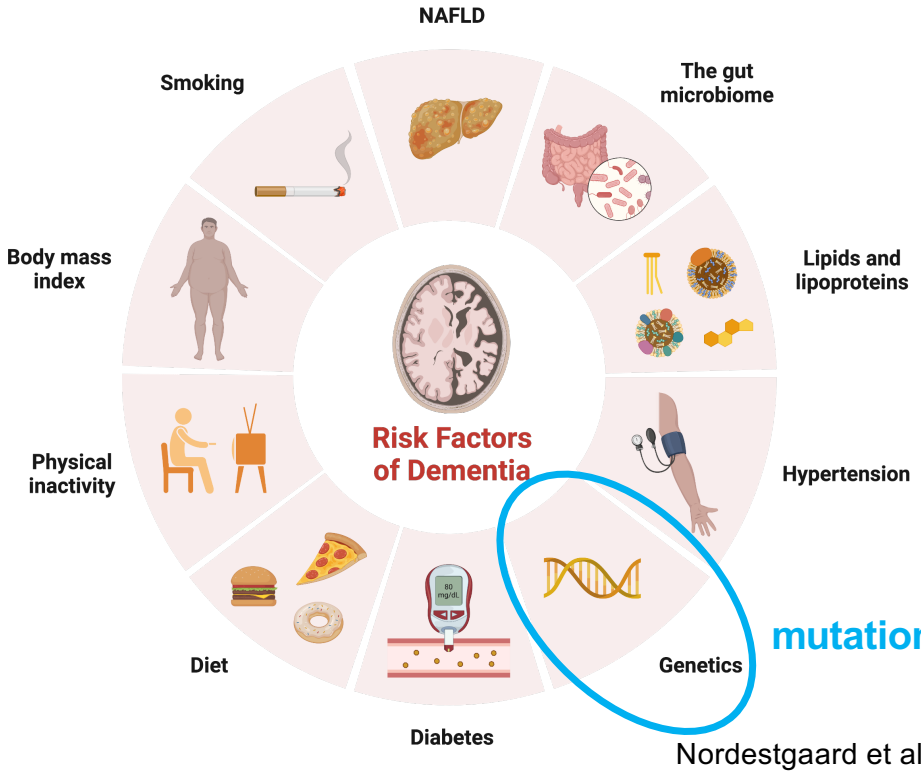
Michael J. Fox

Lou Gehrig's Disease



Stephen Hawking

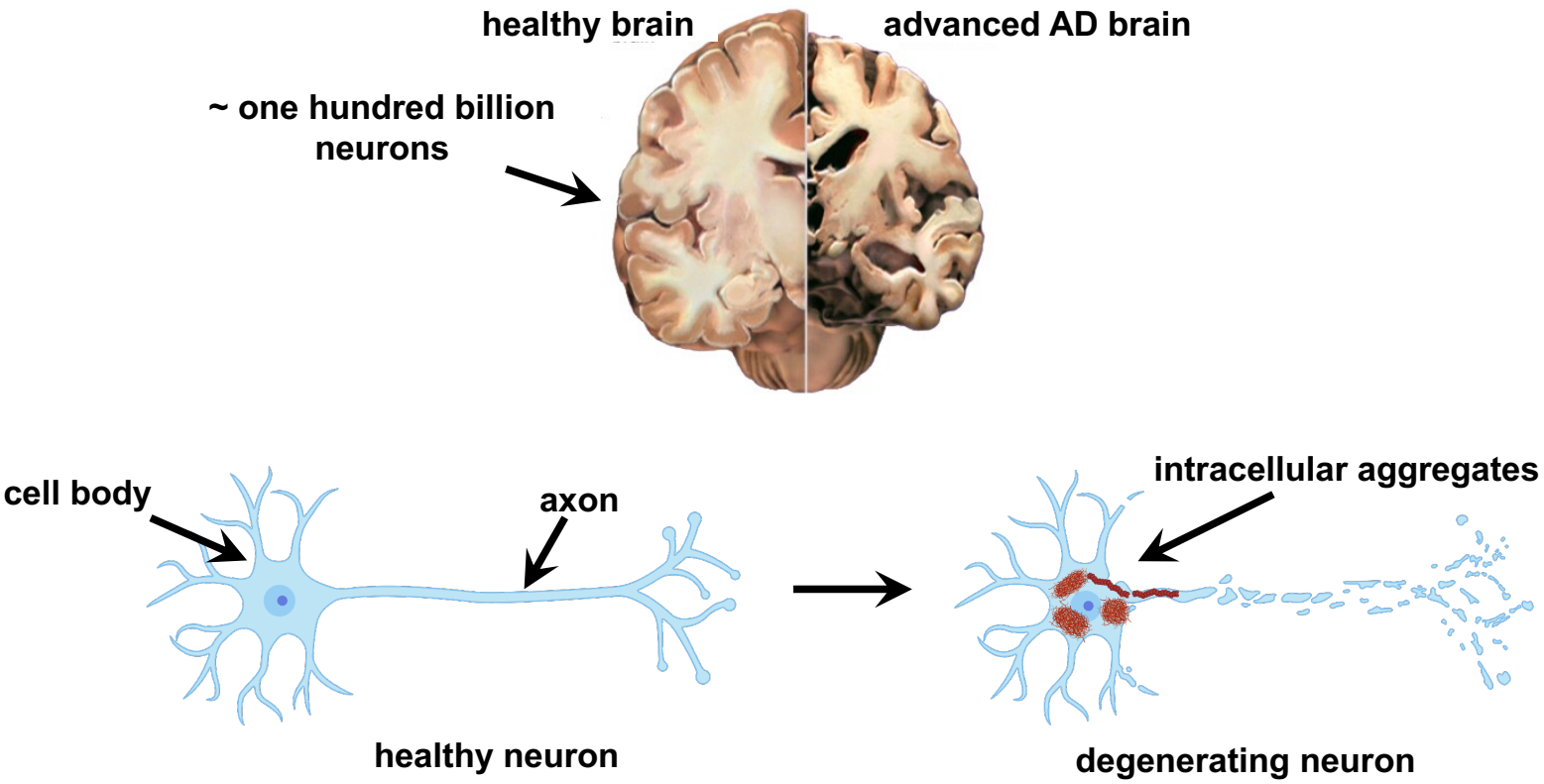
Risk Factors of Dementia



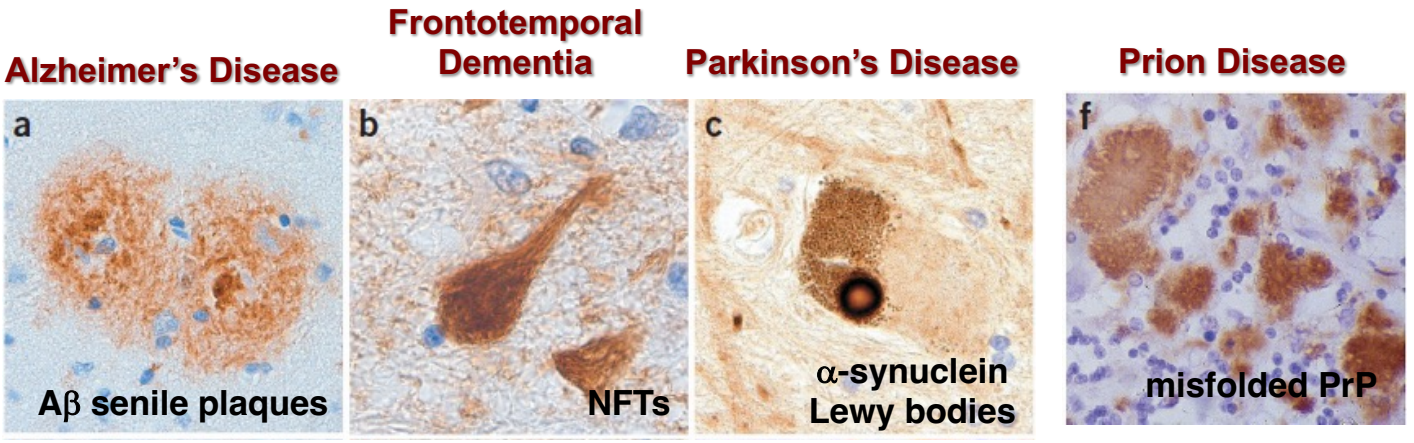
mutations in inherited genes

Nordestgaard et al *Int. J. Mol. Sci.* **2022**

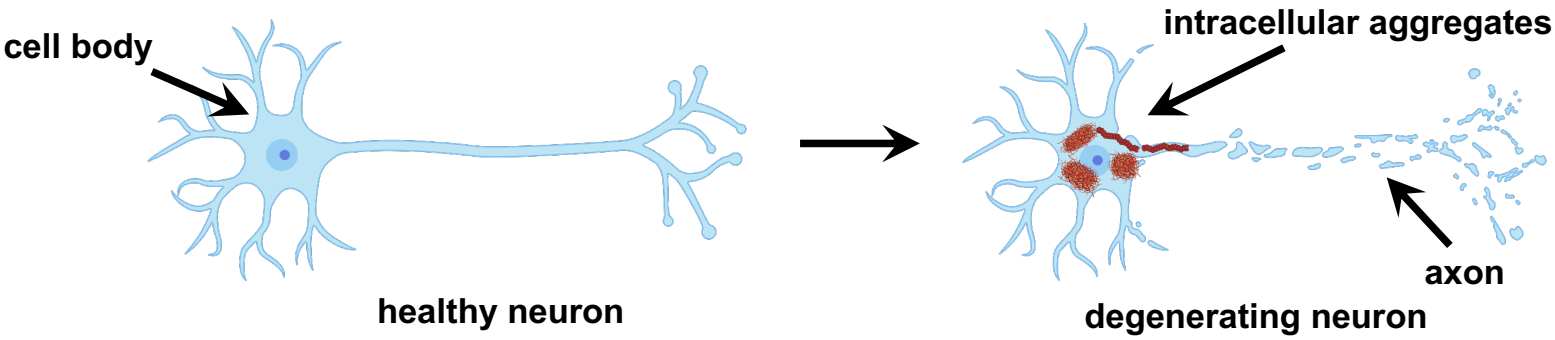
Misfolded Proteins Form Aggregates Inside Neurons



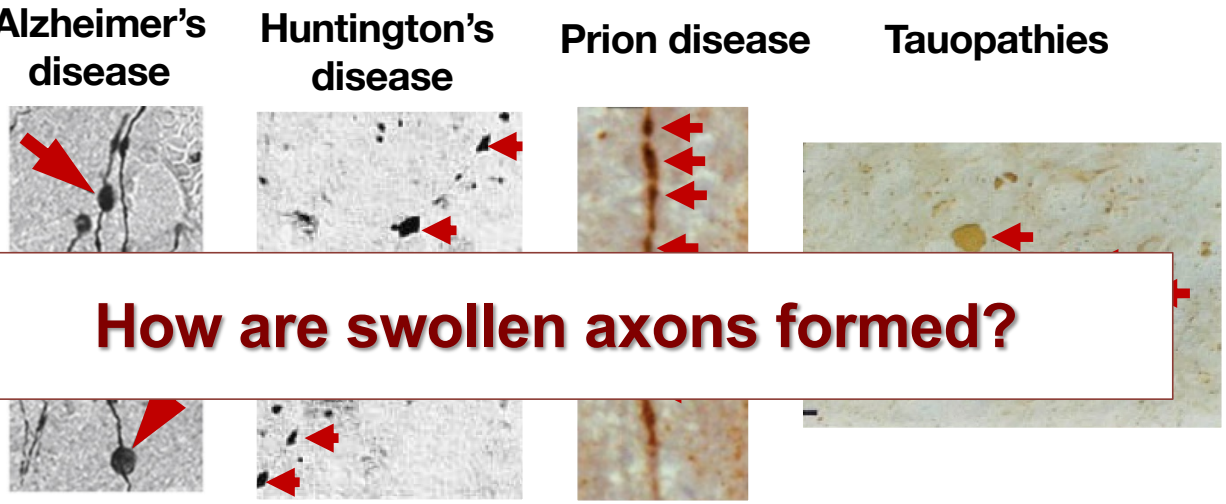
Misfolded Proteins Form Aggregates Inside Neurons



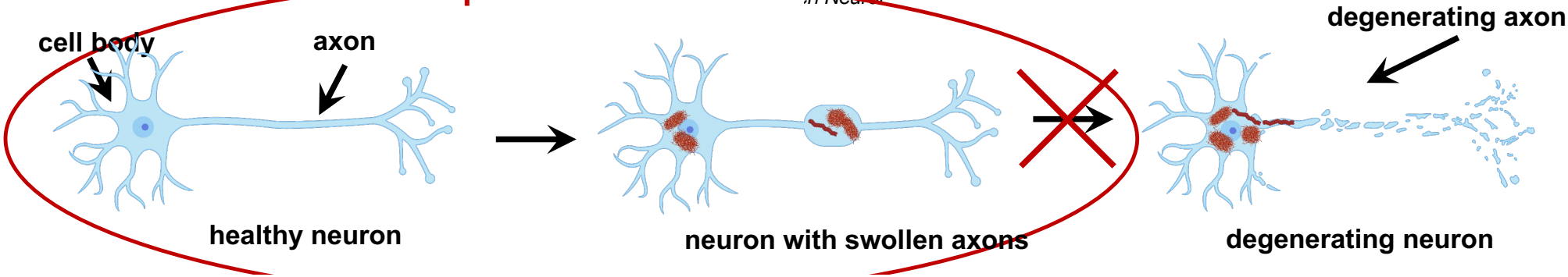
Modified from Forman *et al.* (2004) *Nat Med*



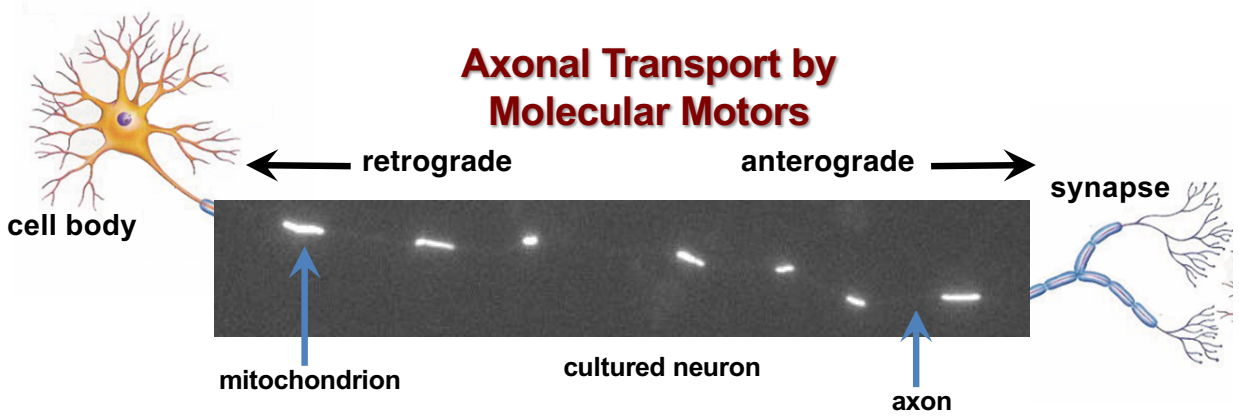
Protein Aggregate Inclusions in Axons are Common in Neurodegeneration



therapeutic intervention?



Regulation of Microtubule-based Transport in Neurons



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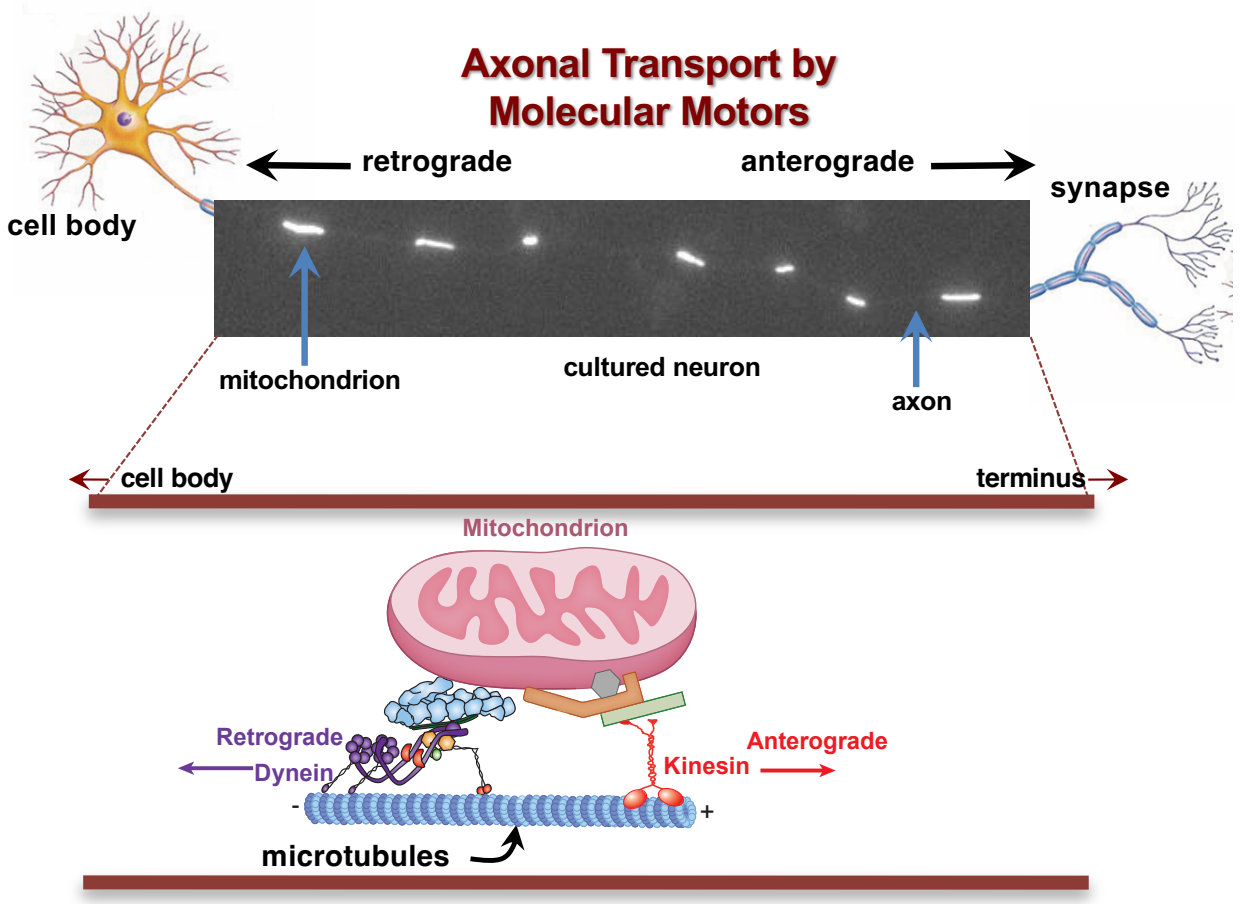
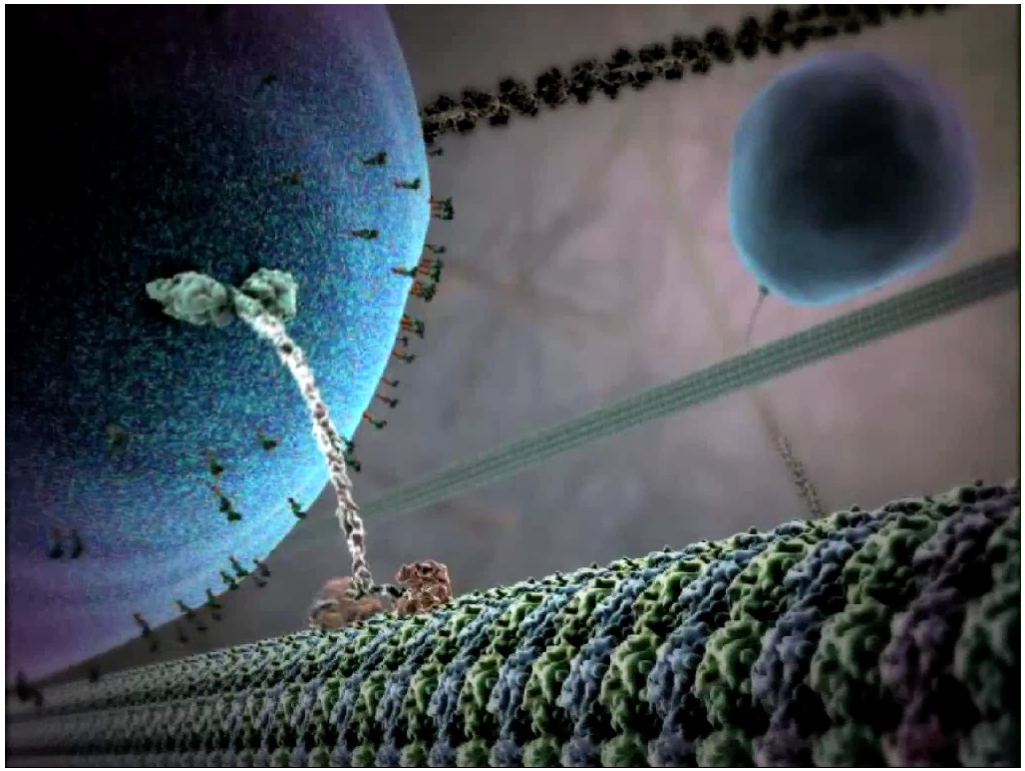


Kinesin Motor Protein Carrying a Vesicle Along Microtubules

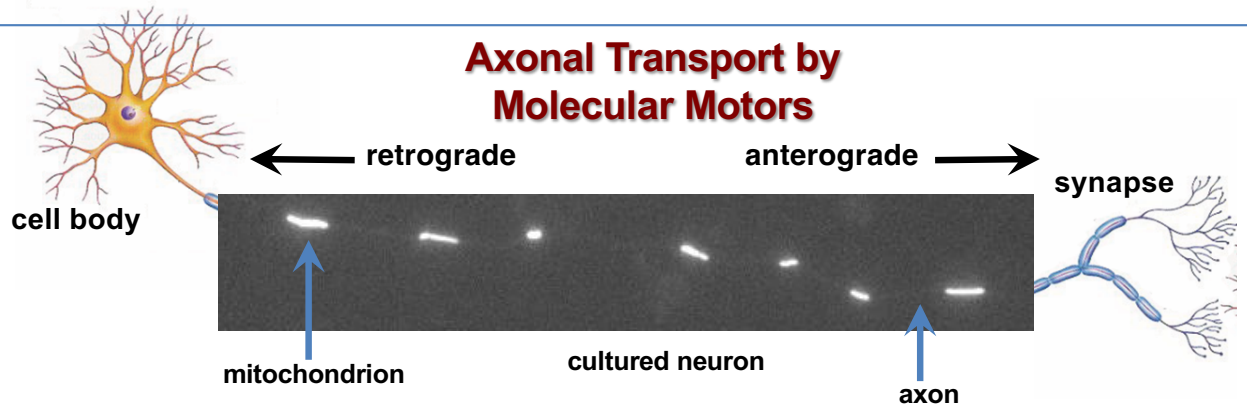
Model based in part on work by Ron Milligan (Scripps Research)

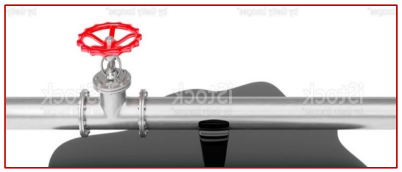
Rice *et al.* Nature 1999

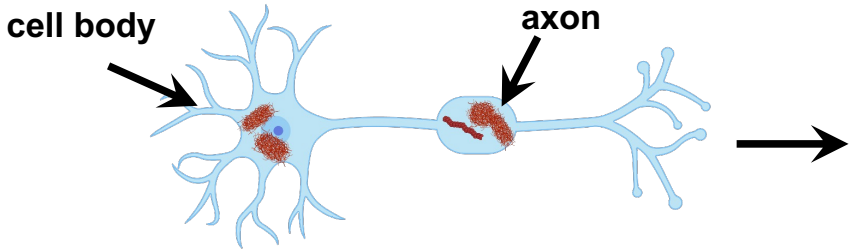


BioVisions at Harvard. The Inner Life of the Cell animation conception and scientific content by Alain Viel and Robert A. Lue. Animation by John Liebler/XVIVO

Regulation of Microtubule-based Transport in Neurons

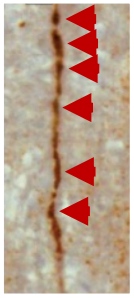






neuron with swollen axons

Prion disease



Zanusso *et al.* (2007) *Arch Neurol*

OUTLINE

- Intro to Aging/Neurodegeneration Connection: some stats

- Alzheimer's Disease and Prion Diseases:
  - Intra-neuronal misfolded protein aggregates

- **Prion Disease: inside neurons**
  - Active transport of proteins (including the prion protein – PrP) inside axons
  - Prion protein aggregates form inside endosome

- Towards therapies to ameliorate prion disease pathologies

Prion Diseases

Prion Diseases can be:

- **Sporadic (85%)** → age and specific genetic polymorphisms
- **Familial (15%)** → hereditary mutations in *PRNP* gene that encodes for the prion protein (PrP)
- **Transmissible (1%)** → contamination with tissue from infected individual

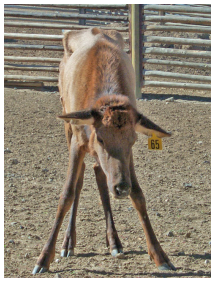
Human Prion Disease

-Creutzfeldt-Jacob disease (CJD; 10-15% familial; 85% sporadic)

-New-variant CJD (transmitted from cows to humans)

Sheep Scrapie

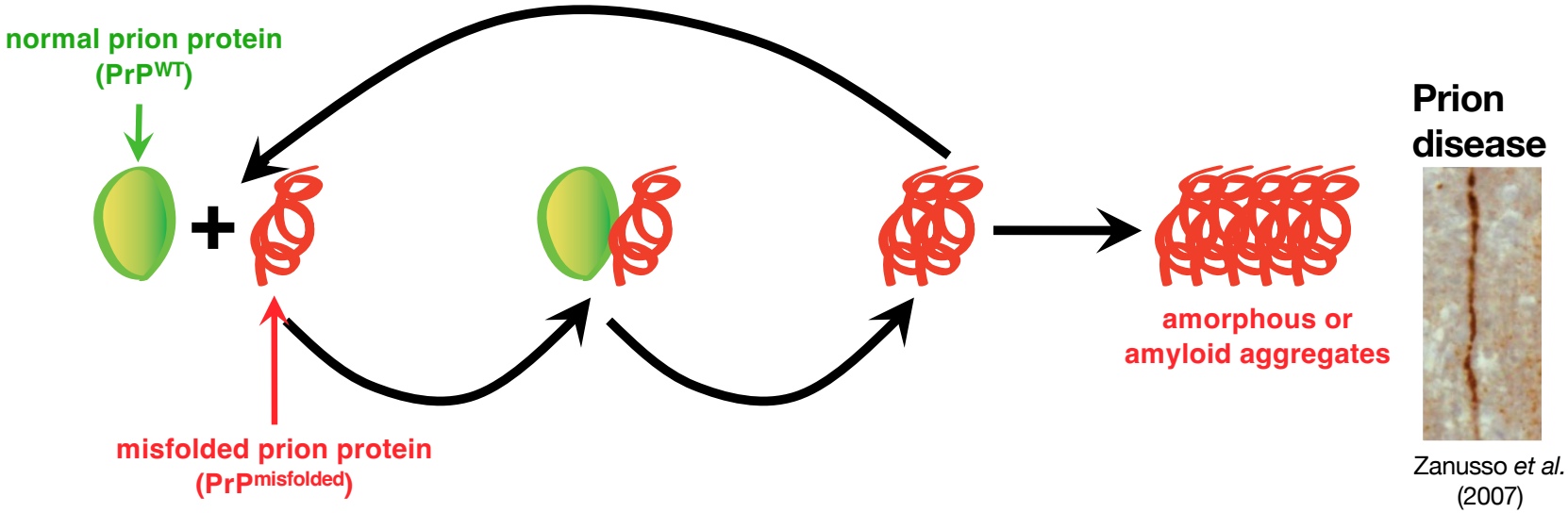
Mad Cow Disease



Chronic Wasting Disease

- **Prion diseases manifest as ataxias, behavioral changes, and dementia**
- **Prion diseases are invariably fatal. Death occurs often within a year of symptomatic onset**

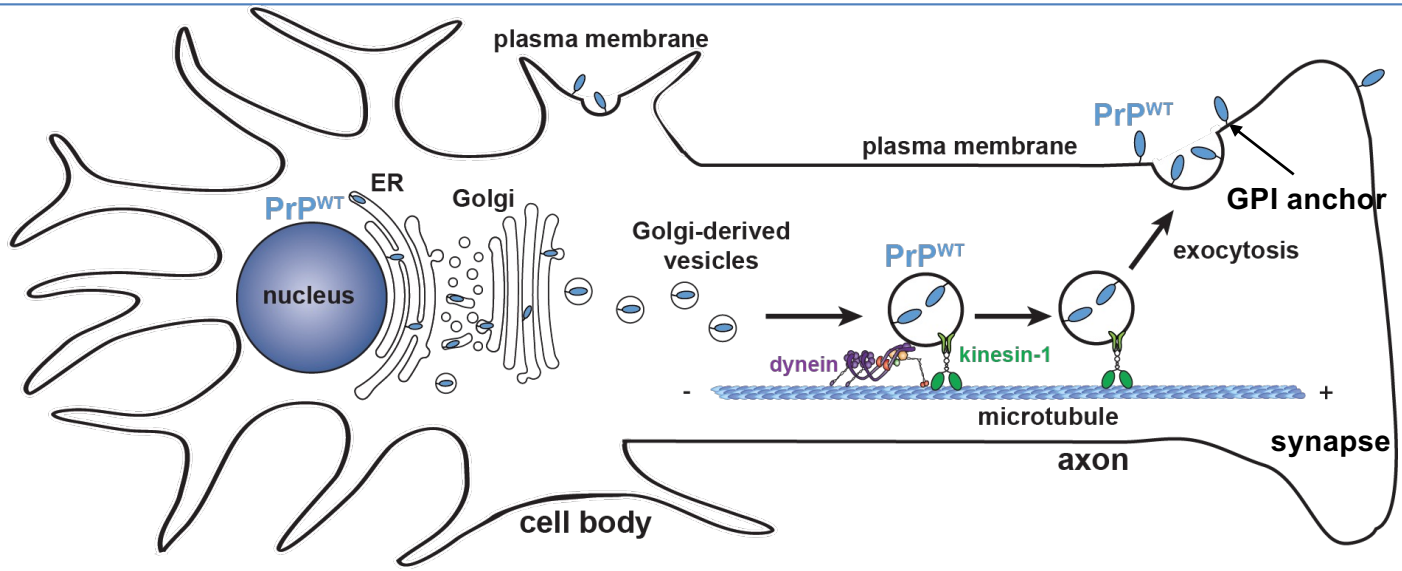
The Normal (Wild-Type "WT") Prion Protein Converts Into a Misfolded Toxic Prion Protein



Zanusso *et al.*
(2007)
Arch Neurol

The wild-type prion protein (PrP^{WT}) is required for pathogenesis: PrP^{WT} -/- mice do not get prion disease

PrP^{WT} is Transported in the Secretory/Endomembrane System to the Cell Surface by Molecular Motors



Encalada *et al Cell* (2011)

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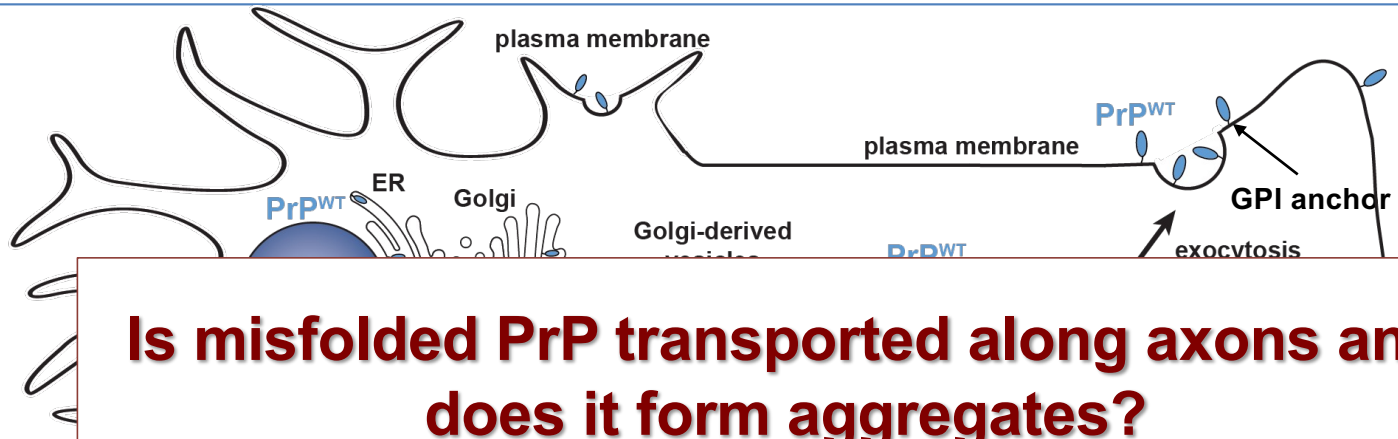


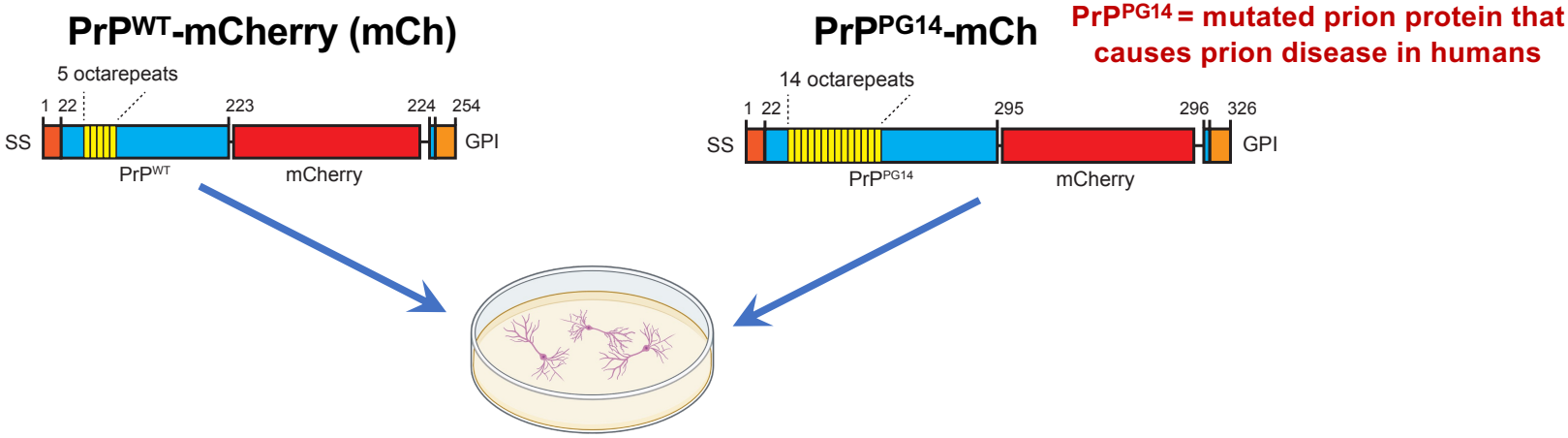
Function of PrP^{WT}:
- cell adhesion
- circadian rhythm
- signaling
- urogenesis and
- erentiation

Is misfolded PrP transported along axons and does it form aggregates?

What's the role of trafficking in misfolded prion protein aggregate formation?

Encalada *et al Cell* (2011)

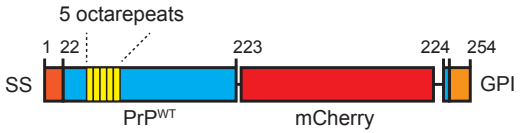
Expression of Disease-Causing PrP^{PG14} Mutant Results in Prion Aggregate Formation in Neurons



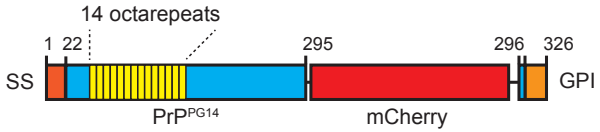
PrP^{PG14} = mutated prion protein that causes prion disease in humans

Expression of Disease-Causing PrP^{PG14} Mutant Results in Prion Aggregate Formation in Neurons

PrP^{WT}-mCherry (mCh)



PrP^{PG14}-mCh



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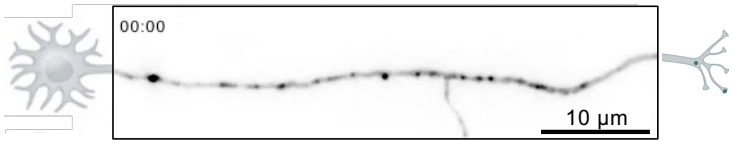
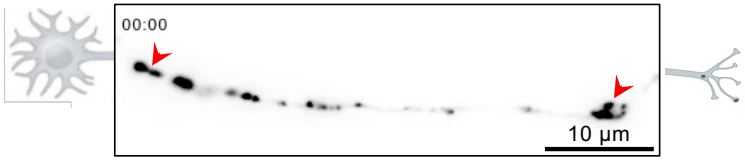
Hippocampal neurons in culture

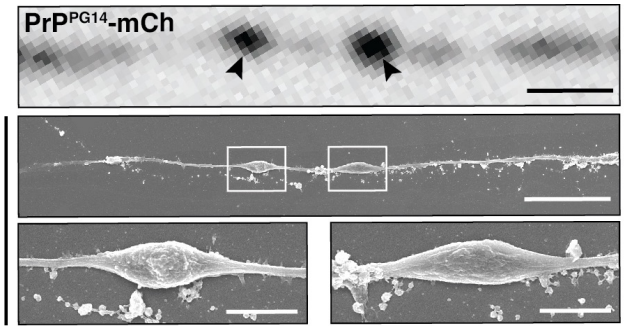




How are swollen axons formed?

Widefield

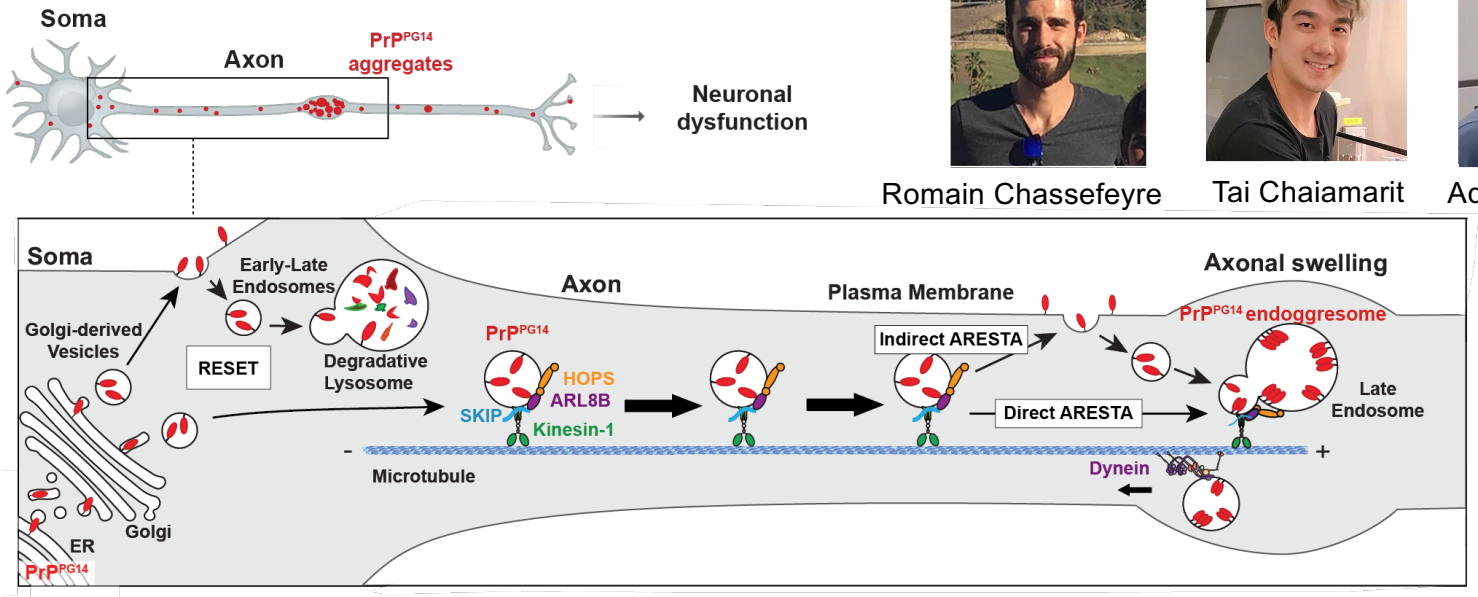
Scanning Electron Microscopy (SEM)



Axonal swellings in PrP^{PG14}-mCh axons

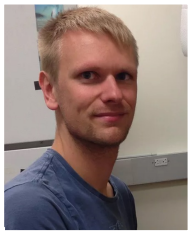
Endolysosomal Trafficking Promotes the Aggregation of misfolded PrP in Axons

PrP^{PG14} forms aggregates *inside* fluid-filled sacks called **endoggresomes**



Romain Chassefeyre

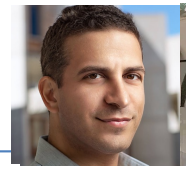
Tai Chaiamarit



Adriaan Verhelle

Chassefeyre*, Chaiamarit*, Verhelle* *et al. Sci. Adv.* 2021

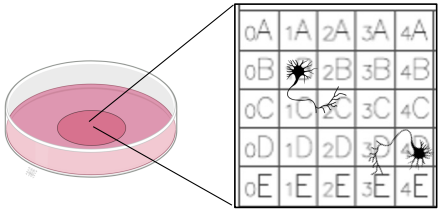
Ultrastructure of Axonal Prion Aggregates

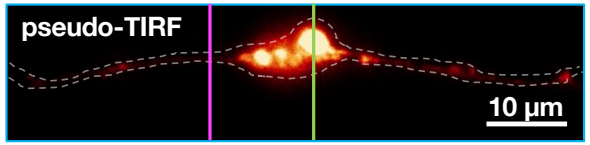

Sammy Weiser Novak Uri Manor Tai Chaiamarit

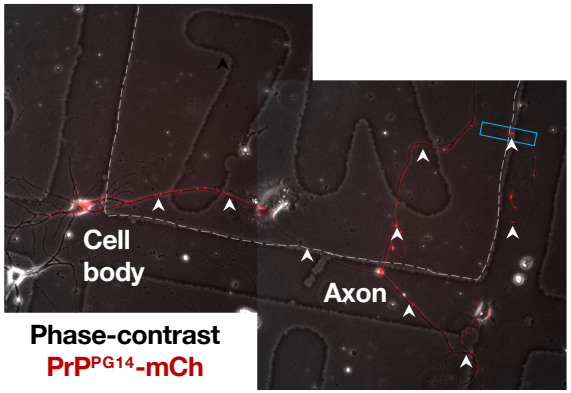
Correlative Light and Electron Microscopy (CLEM) to test at the ultrastructural level whether PrP^{PG14} aggregates are formed in late endosomes

1. Gridded coverslip to locate the cell



2. Live fluorescence imaging to locate the PrP^{PG14}-mCh aggregates

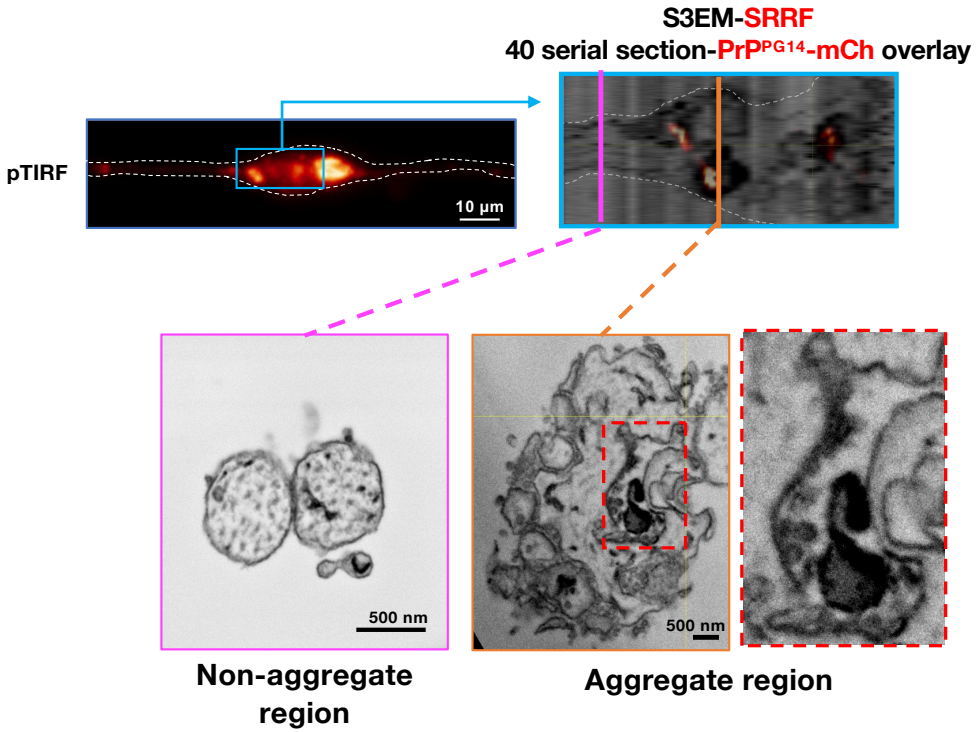




3. Ultrathin Serial Sectioning
SEM (S3EM) and 3D Volumetric Reconstruction

Endoggresomes: Prion Aggregates Form within Endo-lysosomes in Axons

Reconstructed CLEM image through the aggregates

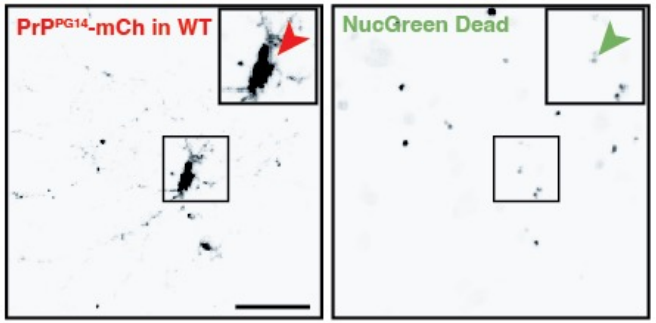


Chassefeyre*, Chaiamarit*, Verhelle* *et al. Sci Adv* 2021

PrP^{PG14}-mCh aggregates form within endosome compartments.

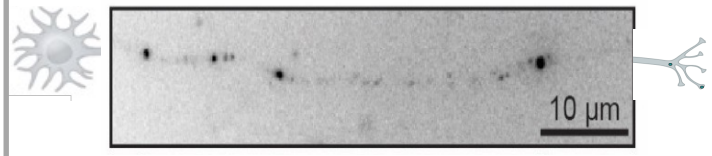
PrP^{PG14} Aggregates are Neurotoxic and this Toxicity Depends on Kinesin-1 Function

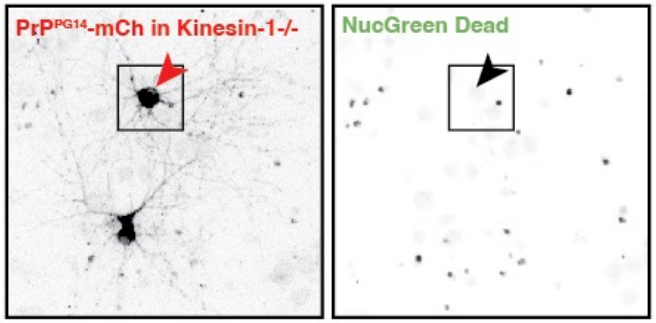
PrP^{PG14}-mCh in WT neurons



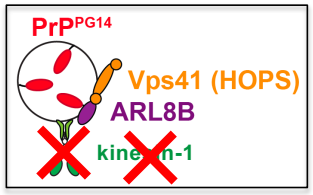
Normal (WT) neurons with PrP^{PG14} aggregates don't survive

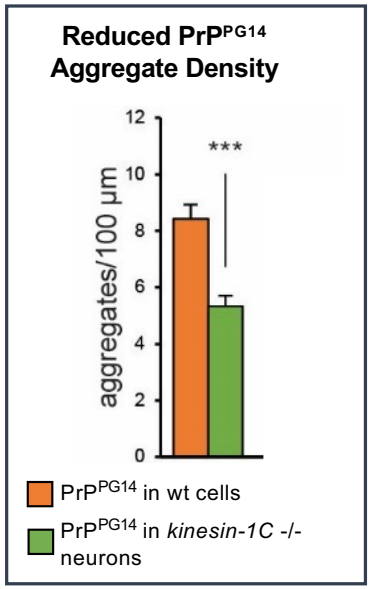
PrP^{PG14}-mCh in *kinesin-1 -/-* neurons





Kinesin-1-/- neurons with PrP^{PG14} aggregates survive longer

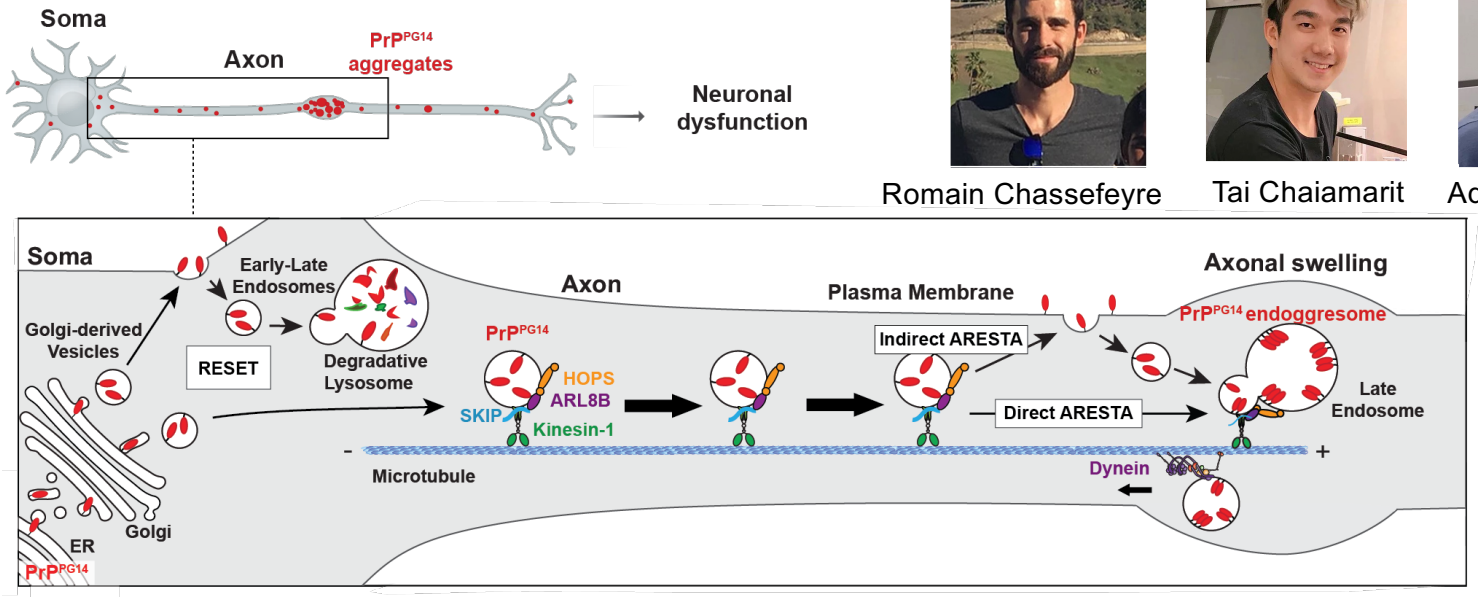




Chassefeyre*, Chaiamarit*, Verhelle* *et al. Sci. Adv.* 2021

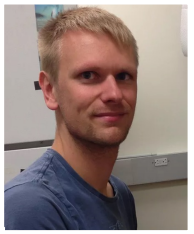
Endolysosomal Trafficking Promotes the Aggregation of misfolded PrP in Axons

PrP^{PG14} forms aggregates *inside* fluid-filled sacks called **endoggresomes**



Romain Chassefeyre

Tai Chaiamarit



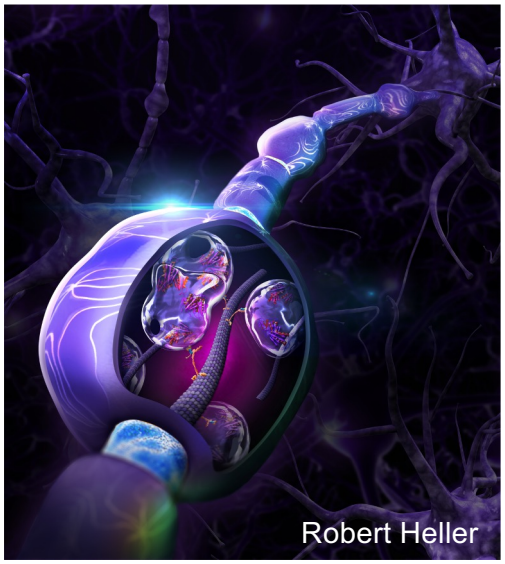
Adriaan Verhelle

Chassefeyre*, Chaiamarit*, Verhelle* *et al. Sci. Adv.* 2021

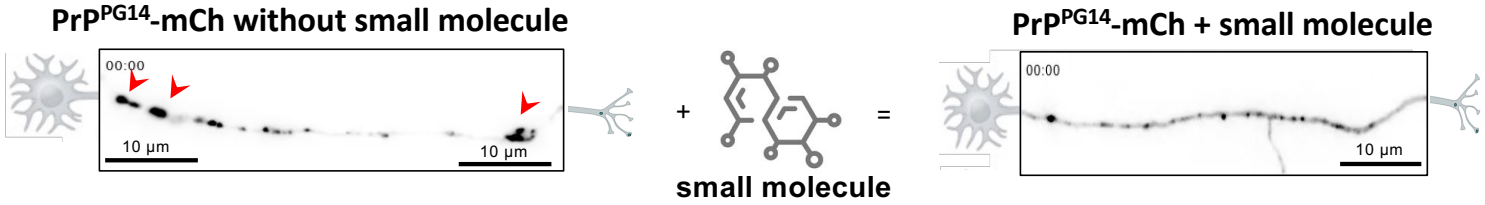
Genetic removal of kinesin-1 is not a feasible therapeutic strategy to clear misfolded PrP aggregates and treat prion disease

OUTLINE

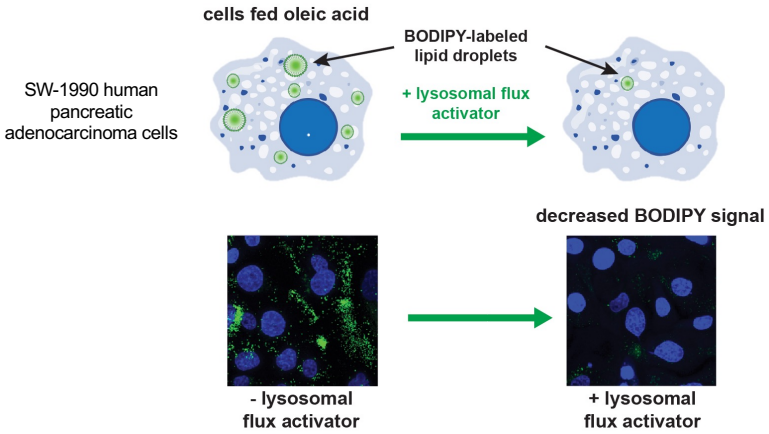
- Intro to Aging/Neurodegeneration Connection: some stats
- Alzheimer's Disease (AD) and Prion Diseases
- Prion disease: inside neurons
  - Active transport of proteins inside neurons
  - Prion protein aggregates form inside fluid-filled sacks called endosomes
- **Towards therapies to ameliorate prion disease toxicity**



Pharmacological Enhancement of Degradation of PrP^{PG14} Aggregates in Axons



Lipid Droplet Degradation High-Throughput Screen (collaboration with Jeff Kelly's lab - Scripps)

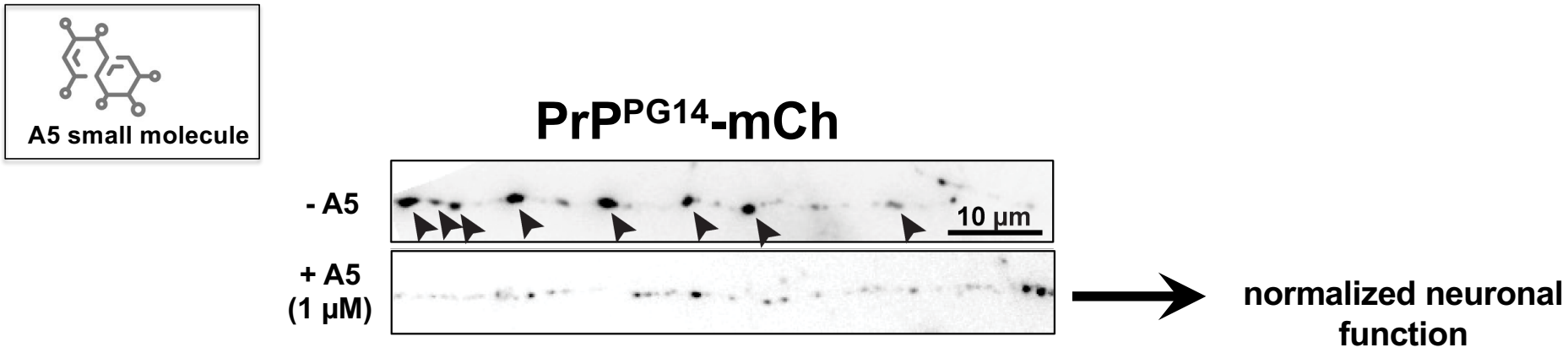


Rachel Botham Leonard Yoon Jeff Kelly Adriaan Verhelle

From ~940,000 small molecules, identified 77 small-molecule LFAs that reduced lipid droplets

Botham, Yoon, Verhelle *et al. bioRxiv* doi.org/10.1101/2022.09.29.509997 *2022*

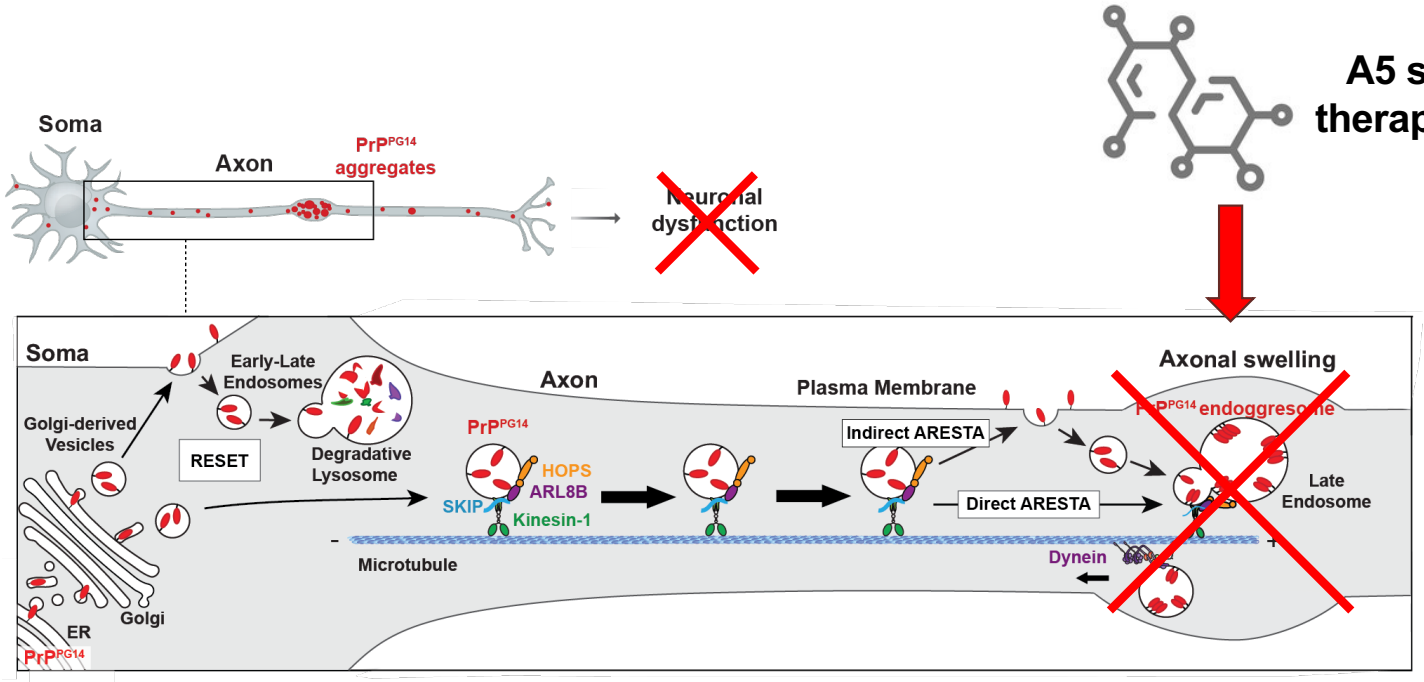
Small Molecules Can Clear PrP^{PG14} Aggregates from Axons



Botham, Yoon, Verhelle *et al. bioRxiv* doi.org/10.1101/2022.09.29.509997 *2022*

A5 enhances the degradative capacity of neurons

IN SUMMARY: Pharmacological Modulation of Endolysosomal Pathways to Degrade Mutant PrP Aggregates in Axons

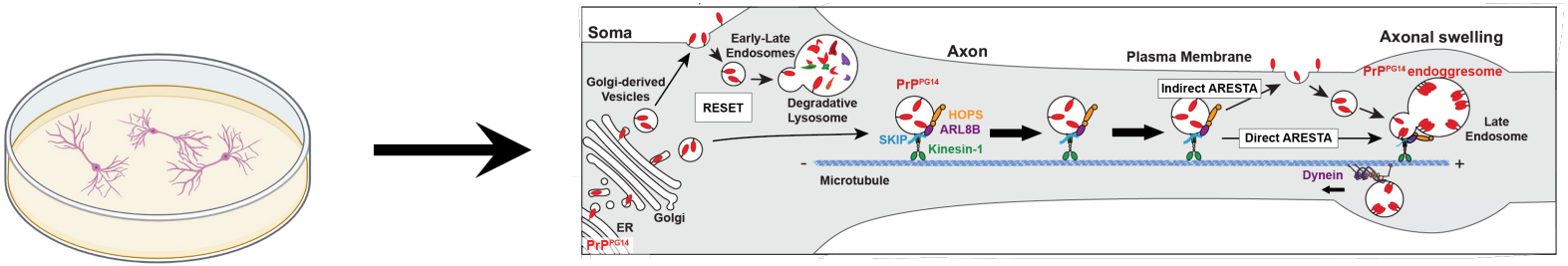


Chassefeyre*, Chaiamarit*, Verhelle* *et al. Science Advances* 2021
Botham, Yoon, Verhelle *et al.* In prep

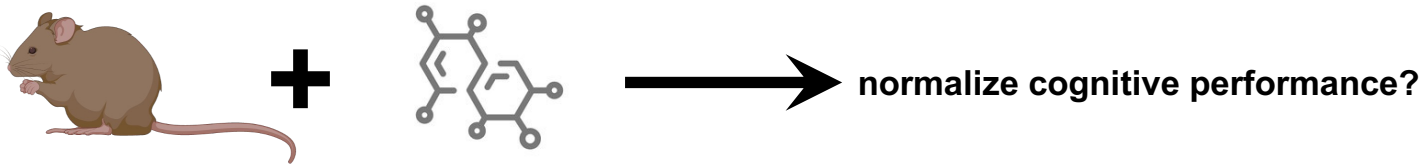
A5 enhances the degradative capacity of neurons

IN PROGRESS AND FUTURE STUDIES

1. Continued characterization of basic mechanisms of aggregate formation in neuronal cell models of prion disease and in Alzheimer's disease: tau? proteins misfolded in Parkinson's and Huntington's disease?



2. Pre-clinical trials in mouse models of familial mutant prion disease and Alzheimer's disease: testing small molecules for amelioration of toxicity (collaboration with Jeff Kelly and Michael Petrascheck at Scripps):



normalize cognitive performance?

3. Medicinal chemistry (collaboration with Jeff Kelly's and Michael Petrascheck's labs at Scripps):

- **Develop and test modified compounds for better performance in a living organism**

Scripps Research

Thank You

Encalada Lab:

Tai Chaiamarit
Yin Wu
Keishla Sanchez-Ortiz
Kiley Hughes
Subhalakshmi Guha
Kiera Fleck
Anna Crie

Previous Lab Members:

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Sylvia Neumann

Tai Chaiamarit

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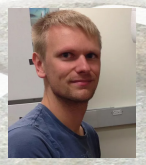

Adriaan Verhelle, PhD

Collaborators:

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Leonardo Andrade (Salk)
Sammy Weiser Novak (Salk)
Jeff Kelly (Scripps)
Rachel Botham
Dan Garza
Leonard Yoon
Malcolm Wood (Scripps)
Jesse Aaron, Satya Khyon, Teng-Leong Chew (Advanced Imaging Center, Janelia)
Marc Diamond (UT Southwestern)
Todd Golde (UFlorida)
Malene Hansen (SBP)
Michael Petrascheck (Scripps)
Massimo Hilliard (University of Queensland)
Eric Vitriol (August College of Medicine)

Support:

The Ellison Medical Foundation

GLENN FOUNDATION FOR MEDICAL RESEARCH

NIH Aging

DPST. The Presidential Policy Directive on Investment and Promotion of Science and Technology Talent

Skaggs Graduate School at Scripps Research

DORRIS NEUROSCIENCE CENTER

NIH National Institute on Alcohol Abuse and Alcoholism

- Arnold and Arlene Goldstein
- Baxter Family Foundation
- Hewitt Foundation
- CTW Foundation, Inc.

For questions/comments, please contact me at encalada@scripps.edu

THE FRONT ROW at Scripps Research

THE FRONT ROW
at Scripps Research

Paving the Way for New Therapies for Neurodegenerative Diseases

Sandra E. Encalada, PhD
Associate Professor, Department of Molecular Medicine
Dorris Neuroscience Center Investigator
Scripps Research Institute

Wednesday, February 15, 2023 | 1:00 pm PT/4:00 pm ET