Neurological Disorders and the Brain

Bioinformatics tools and resources to study healthy and diseased brains

Bioinformatics and Research Computing
Whitehead Institute

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Bioinformatics & Research Computing

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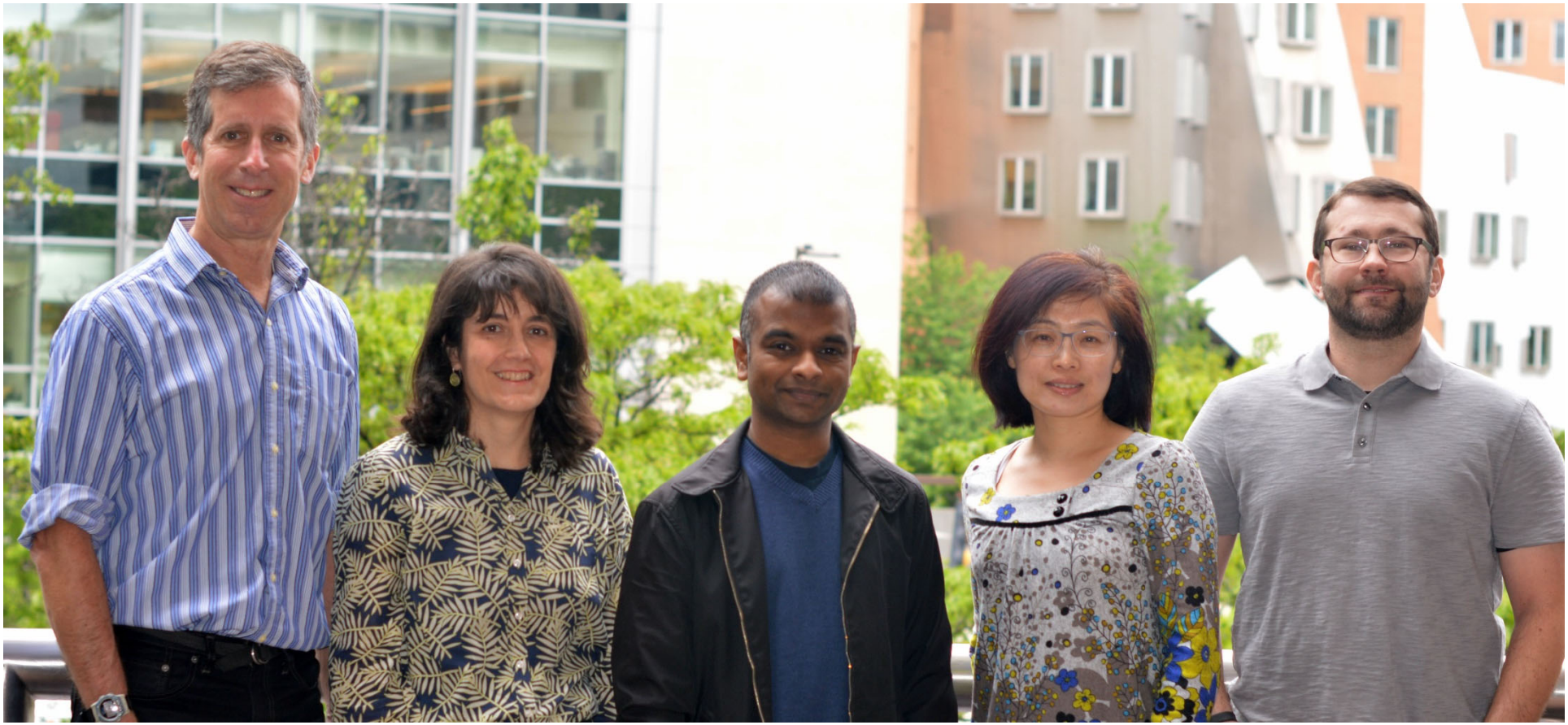


George Bell	Inma Barrasa	Prat Thiru	Bingbing Yuan	Kris Richardson

What is bioinformatics?

Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data and interpreting experiments.

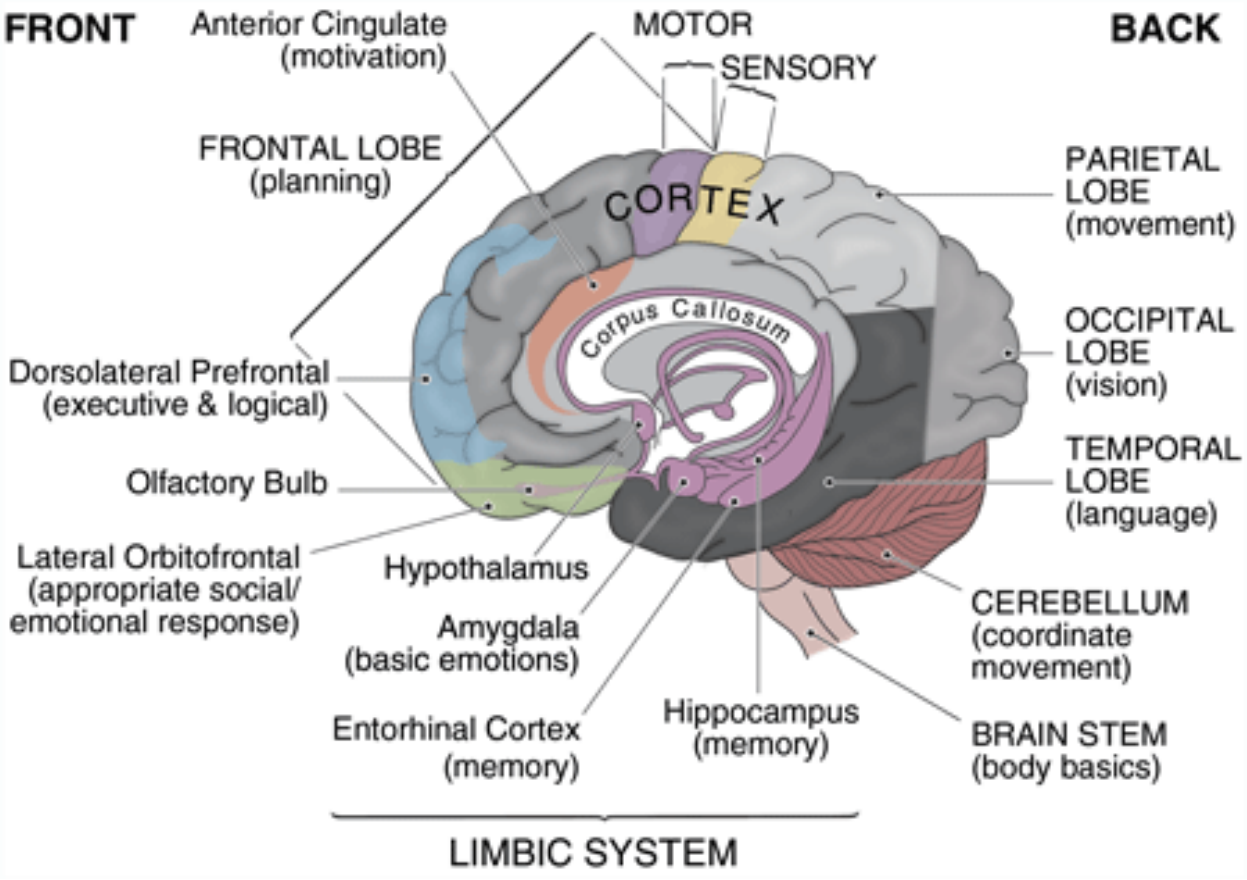
It combines

- biology
- computer science
- mathematics
- statistics

Overview of today's session

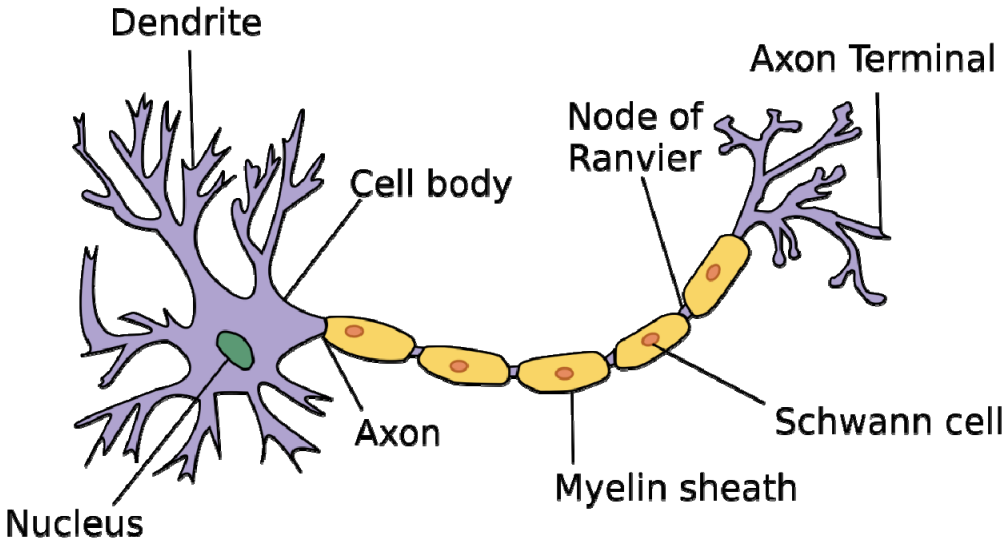
- Introduction: brain anatomy and cell types
- Studying gene expression in the brain
  - Allen Brain Atlas. **Ex1** browsing the Aging, Dementia and Traumatic Brain Injury Study.
  - Tabula Muris: Gene expression of individual cells **Ex2**
  - Protein expression: Histology staining **Ex3**
- Brain genetics and brain disease
  - Brain genetics **Ex4**
    - Tay–Sachs disease
    - SNPedia, OMIM
- Models to learn about the human brain
  - Studying human proteins in yeast (Parkinson's Disease)
  - Benefits of iPS cell - derived human cells (Parkinson's Disease)
  - Seizure experiments in zebrafish

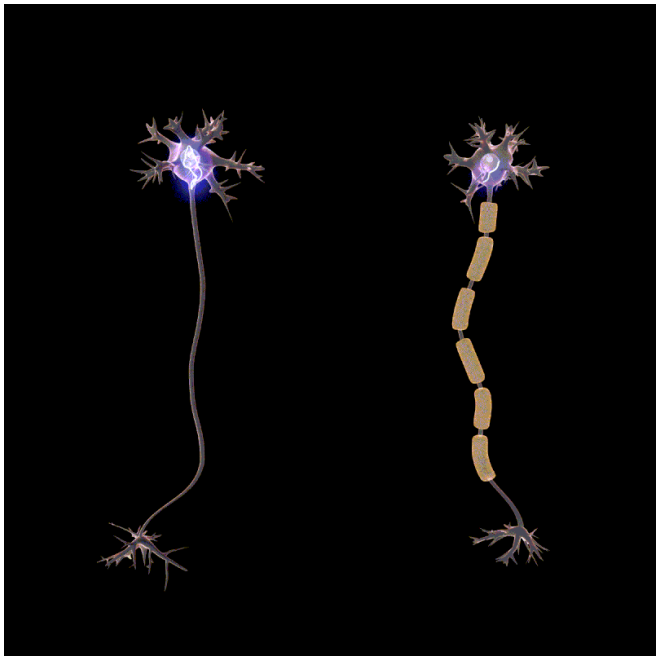
Brain anatomy and function



http://www.brainwaves.com/
http://www.brainfacts.org/3d-brain

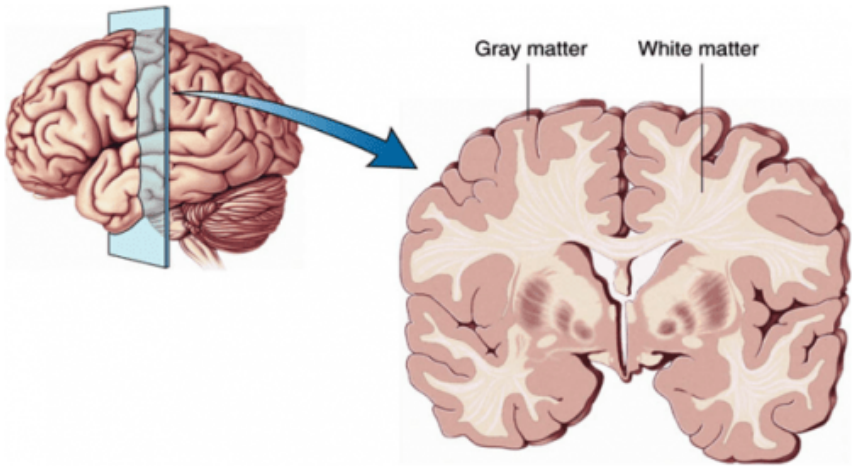
Myelin function





https://en.wikipedia.org/wiki/Myelin

White vs grey matter



https://difference.guru/

- **Grey matter:**
  - high densities of neuron cell bodies
  - Grey matter undergoes development and growth throughout childhood and adolescence
  - High alcohol consumption has been correlated with significant reductions in grey matter volume
- **White matter:**
  - light color: due to fiber from myelin.
  - continues to develop, and peaks in middle age
  - Alzheirmer's disease: amyloid plaques in white matter

Brain Myeloid Cells

- Microglial cells
- Macrophage

- Brain myeloid cells provide a form of continuous immune surveillance within the brain.
- The population of myeloid cells play an essential role in both health and disease with regard to the brain.

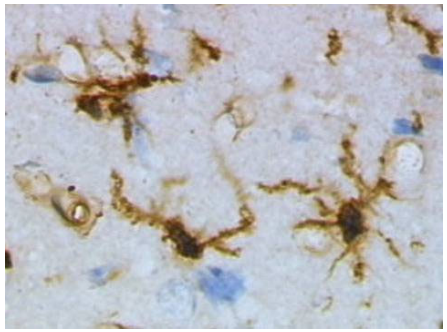
Brain Non Myeloid Cells

- Astrocytes
- Oligodendrocytes
- Neurons
- Others…

- Non brain myeloid cells are non-immune cells.
- The mammalian brain contains between 100 million and 100 billion neurons, depending on the species.
- These cells carry out essentials roles of the brain – structure, signaling, protection, etc…

Brain Myeloid Cells

- Microglial cells
- Macrophages

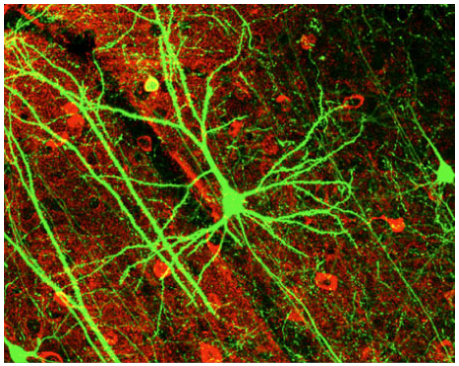


Microglial

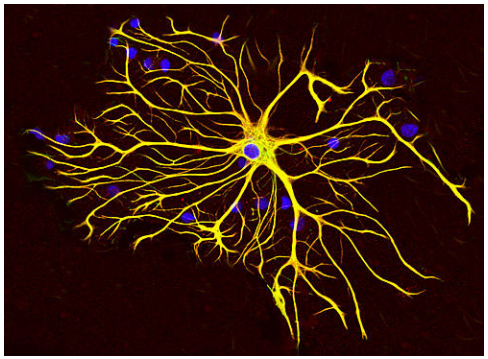
Macrophage

Brain Non Myeloid Cells

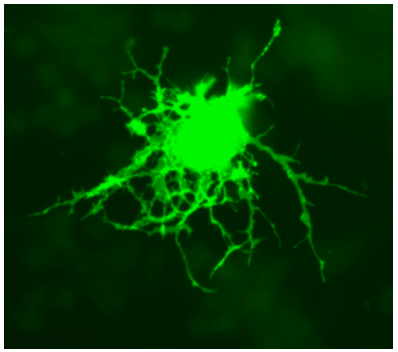
- Astrocytes
- Oligodendrocytes
- Neurons
- Others…



Neuron



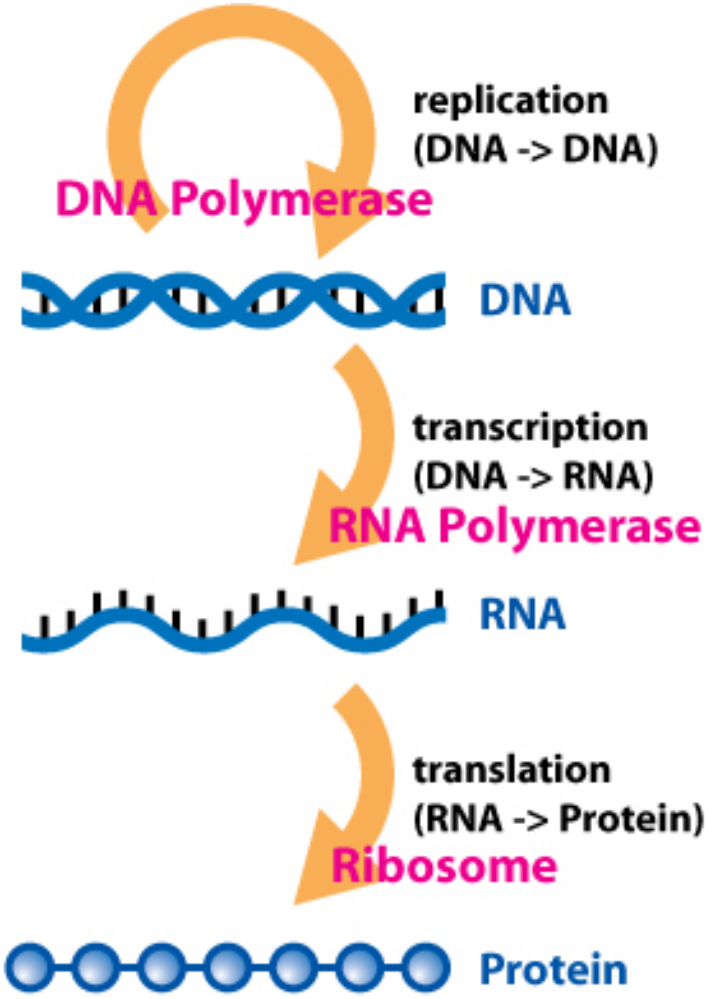
Astrocyte



Oligodendrocyte

The central dogma of biology

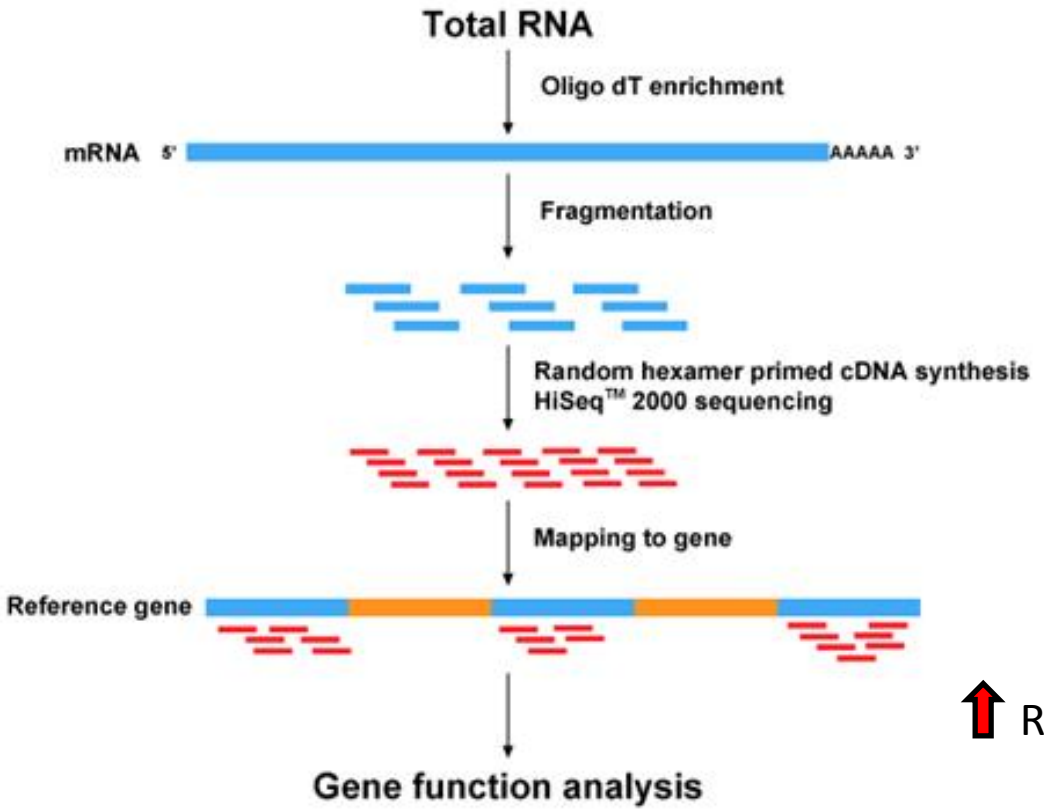
"DNA makes RNA and RNA makes protein"



- For most activities in the cell, proteins are what do the actual work
- Looking at changes in amounts of a protein could reveal a lot about changes in cell state
- Problem: It's hard to measure levels of all proteins at once
- Partial solution:
  - Measure RNA levels instead
  - Assumption: Changes in RNA levels tend to match changes in protein levels

Image: https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology

Measuring the RNA level of every gene at once



- The technique of **RNA-seq** can measure the amount of RNA from every gene in the genome in any collection of cells

Assumption: For every gene,

↑ RNA ⇒ ↑ protein ⇒ ↑ metabolic activity

↓ RNA ⇒ ↓ protein ⇒ ↓ metabolic activity

Image: http://bio.lundberg.gu.se/courses/vt13/rnaseq.html

Allen Brain Atlas

- http://portal.brain-map.org/
- The Allen Brain Atlas is a resource containing information about
  - Human and mouse brain
  - Brain cell types
  - Brain development
  - Aging, dementia and traumatic brain
  - Glioblastoma

Ex1.a Browsing the Aging, Dementia and Traumatic Brain

- On go to http://aging.brain-map.org/
- Click on "Data Overview" on the top right of the page
- Click on "Brain regions" and wait for the figures to display.

- Sequentially click on the Brain Region circles and the CABP7(calcium binding protein 7), CUX2(cut-like homeobox 2) and RORB (RAR-related orphan receptor beta) to see genes expressed in hippocampus versus genes expressed in cortex.
- Click on "15 most region-enriched genes in cortex" to see a heatmap of gene expression.

Ex1.b Browsing the Aging, Dementia and Traumatic Brain

- Repeat the exercise by looking at Grey versus White matter and genes markers for excitatory, **NEFH** (neurofilament, heavy polypeptide), or inhibitory, **PVALB** (parvalbumin) neuronal populations.

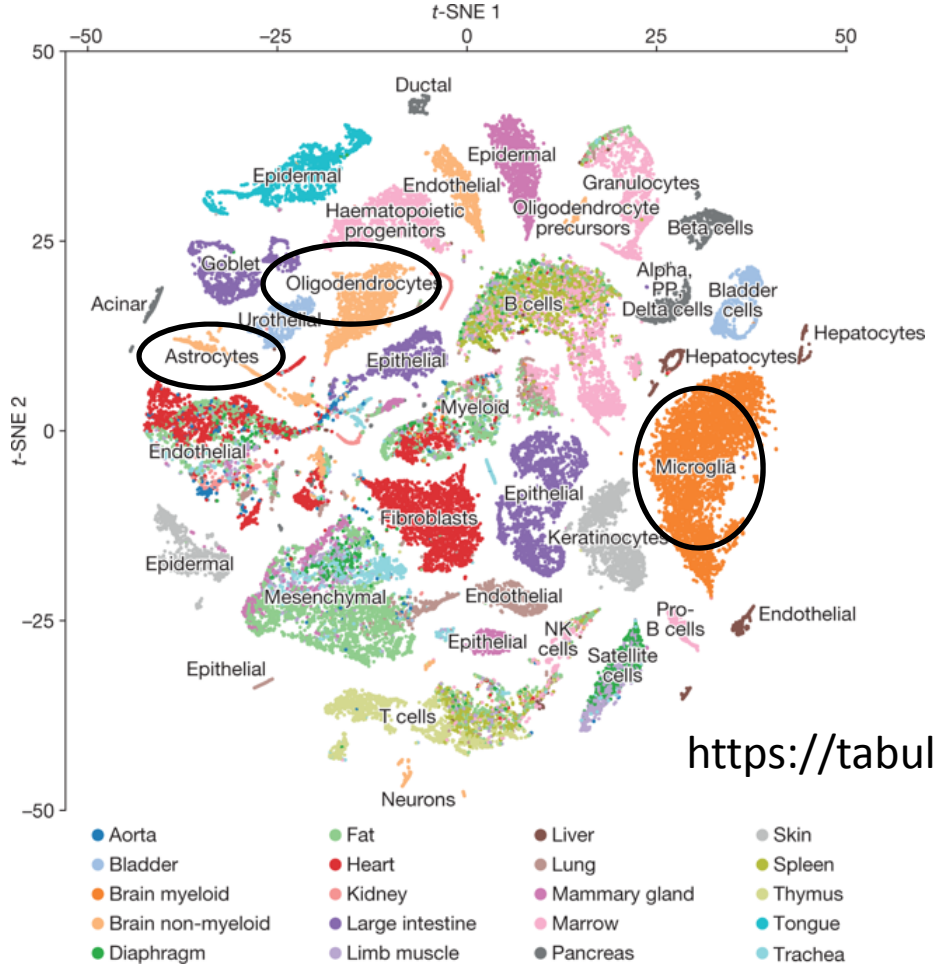
GREY VS. WHITE MATTER

- Explore the Dementia tab 2 of 2

DEMENTIA

Tabula Muris

- Sequenced 100,000 cells from 20 tissues/organs from 3 female and 4 male mice.

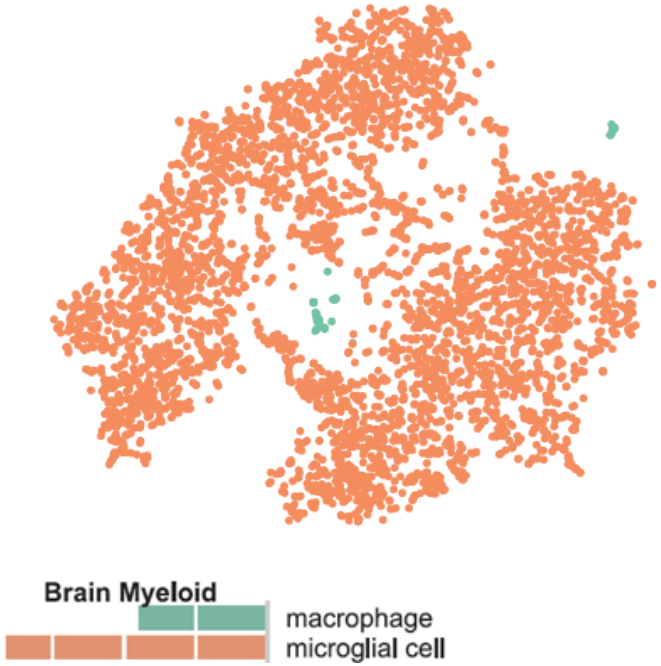


https://tabula-muris.ds.czbiohub.org/

The Tabula Muris Consortium et al. *Single –cell transcriptomics of 20 mouse organs creates a Tabula Muris* Nature 562:362-367 (2018)

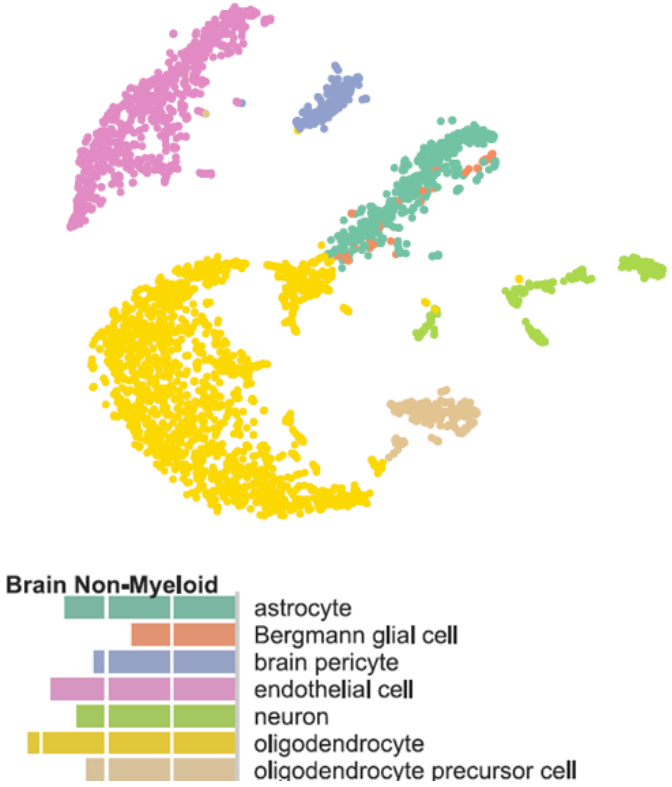
Tabula Muris:
Cell Type Markers

Brain Myeloid*



**Myeloid: immune cells found in the brain*

Brain Non–Myeloid



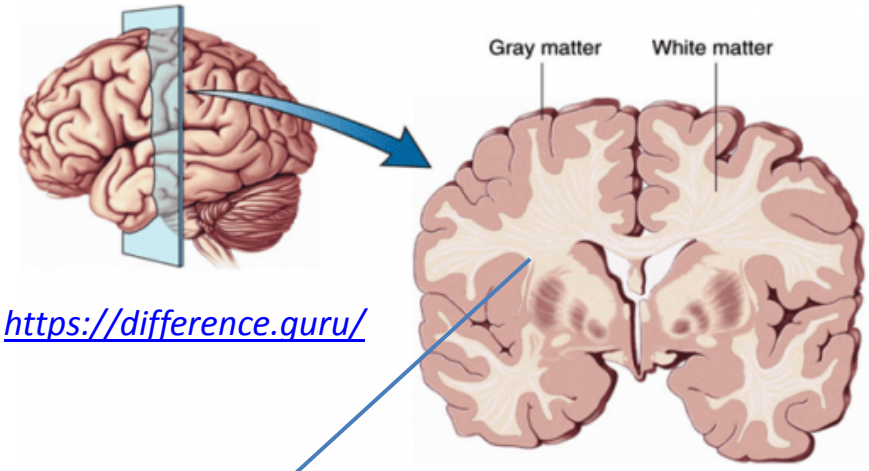
Ex2 Browsing Tabula Muris to see gene expression in single cells (scRNAseq)

1. Tabula Muris site

   https://tabula-muris.ds.czbiohub.org/
2. Select FACS and Brain Non-Myeloid
3. Type each of the gene names in the blank table, one at a time, and see where they are expressed
4. Does this agree with what we saw before?
5. Are some genes expressed in

   all cells? no cells?

Measuring the protein level of a gene

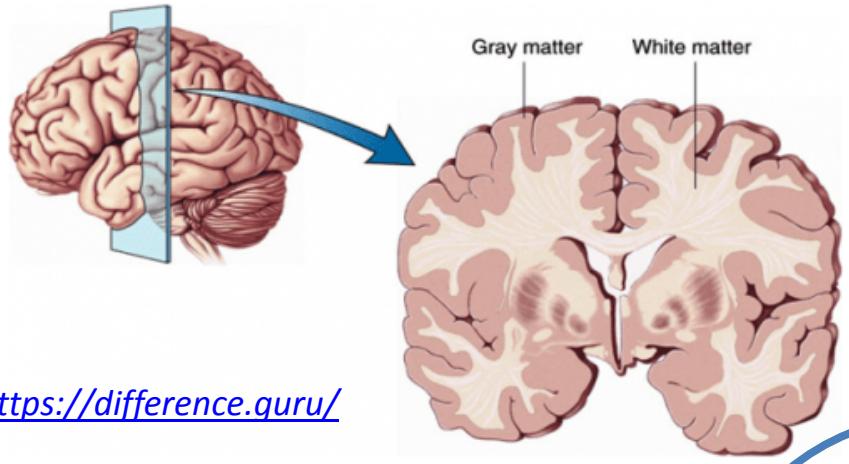


https://difference.guru/

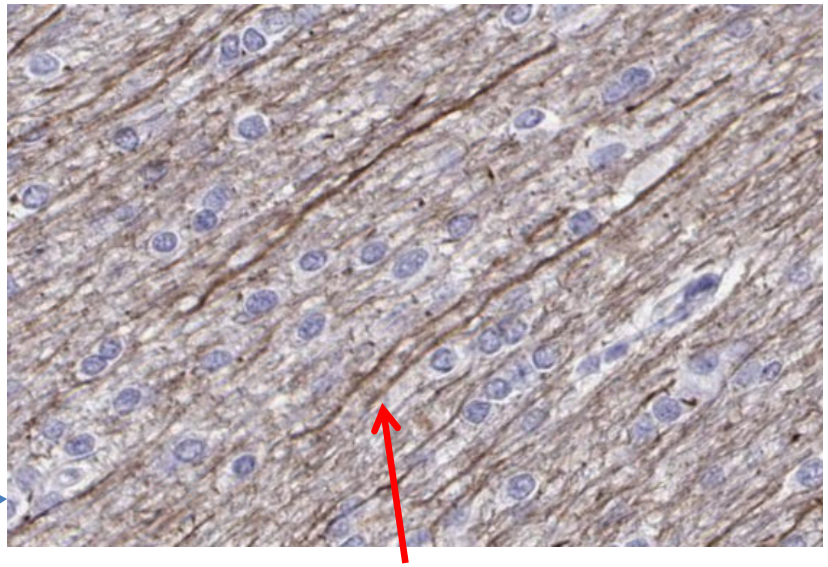
Cell seeding | Fixation & Immunostaining | Imaging | Annotation / Image analysis

https://www.proteinatlas.org/learn/method/immunocytochemistry

NEFL (Neurofilament light) located in white matter



https://difference.guru/

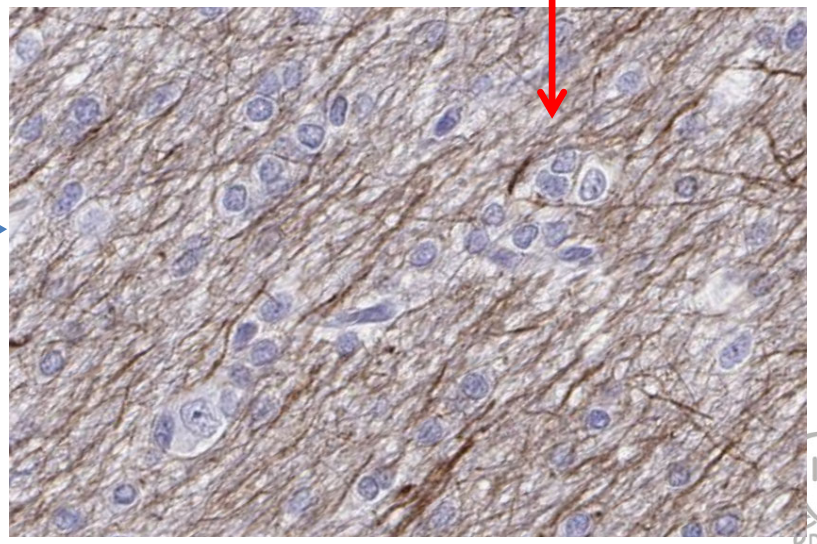


Neuronal projections, stained in brown color

Grey matter

White matter

Zoom in

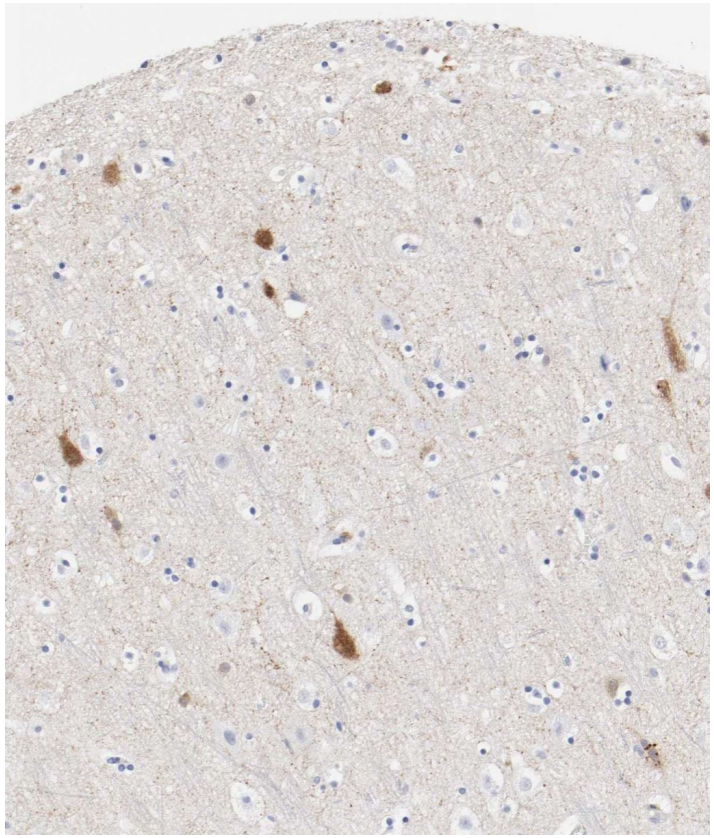


https://www.proteinatlas.org/

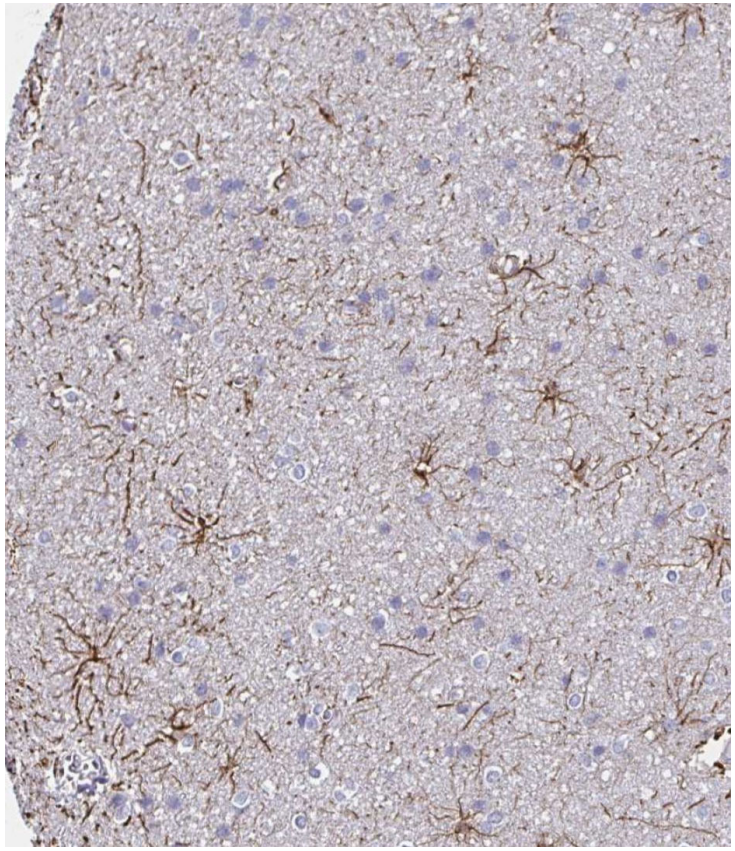
Ex3: Identify Brain neural cells by antibody staining

- Go to Human Protein Atlas website:
  - https://www.proteinatlas.org/humanproteome/tissue/brain
  - Search for either GAD1 (inhibitory GABAergic interneurons glutamate decarboxylase 1) or GFAP (Glial fibrillary acidic protein)
  - Click on **TISSUE** from top right
  - Under **RNA and Protein Expression Summary**, click on **Brain** and choose **Cerebral cortex**.
  - Find out the cells express this gene under **Antibody staining** section. Cells expressed this gene were stained with brown color. You can view the cells by clicking on images and zooming in.

Ex3: GAD1 and GFAP in cortex



GAD1



GFAP

Brain genetics and brain disease

Tay-Sachs disease (TSD)

- Rare disease affects central nervous system
- Variant forms: infantile, juvenile, adult onset
- Loss of learned skills, smile, crawl
- Paralysis, dementia, death
- No treatment, no way to prevent/reduce the progression of this disorder.

Ex4: Tay-Sachs disease

- **Ex4.a** Which gene is responsible for the disease?
  - Go to OMIM (https://www.omim.org) and search for "Tay-Sachs disease" [Hint: Look under Phenotype-Gene Relationships]
- **Ex4.b** How does the mutation of this gene result in Tay-Sachs disease?
  - Hint: look under "Biochemical features".
- **Ex4.c** What mutation(s) in this gene cause Tay-Sachs disease?
  - Hint: look under "Molecular Genetics".

Ex4c: Mutations causing Tay-Sachs

To look at these mutations in more detail…

- Go to the UCSC Genome Browser: https://genome.ucsc.edu/cgi-bin/hgGateway
- Select human (hg38)
- Under Position/Search Term, type HEXA and then any of the “HEXA” links
- Under "Phenotype and literature", set "ClinVar Variants" to “pack“ and click a “refresh” button.
- The red variants are pathogenic (harmful).

Models to learn about the human brain

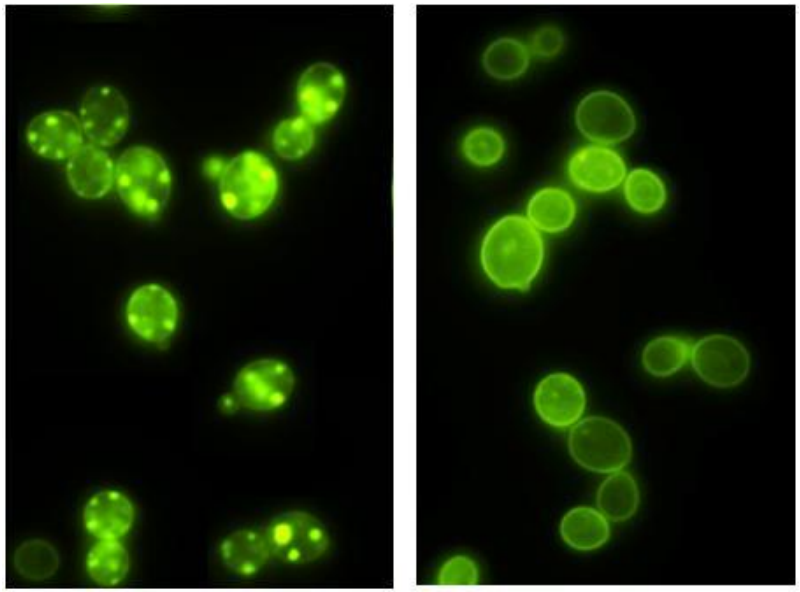
- **Yeast**
- Mouse
- **Zebrafish**
- Induced pluripotent stem cells (also known as iPS cells or **iPSCs**) derived from patients.

Parkinson’s disease

- Parkinson's disease occurs when neurons in an area of the brain that controls movement become impaired and/or die. Normally, these neurons produce an important brain chemical known as dopamine.
- Many brain cells of people with Parkinson's contain Lewy bodies, unusual clumps of the protein **alpha-synuclein**.
- Scientists are trying to better understand the normal and abnormal functions of alpha-synuclein and its relationship to genetic mutations that impact Parkinson’s disease and Lewy body dementia.

Studying Parkinson's disease in yeast

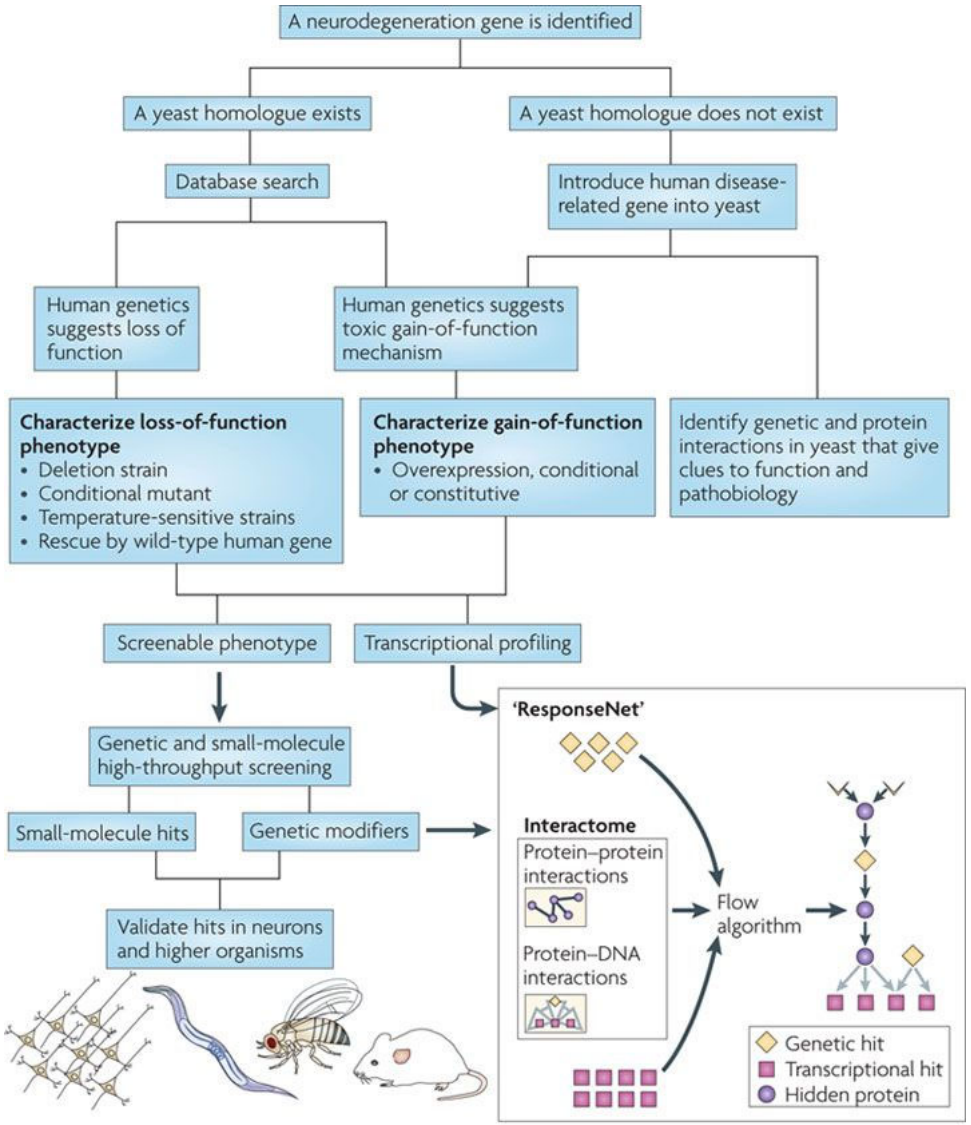
- Many people with Parkinson's disease have clumps of a protein (alpha-synuclein) in their brain
- What do these clumps do?
- Can we prevent or treat these clumps?
- One solution: genetically modify yeast cells so they make clumps of this same human protein



Yeast cells with alpha-synuclein attached to a fluorescent marker (bright spots on left) change after treatment with chemical called NAB (on right).

Tardiff et al., 2013

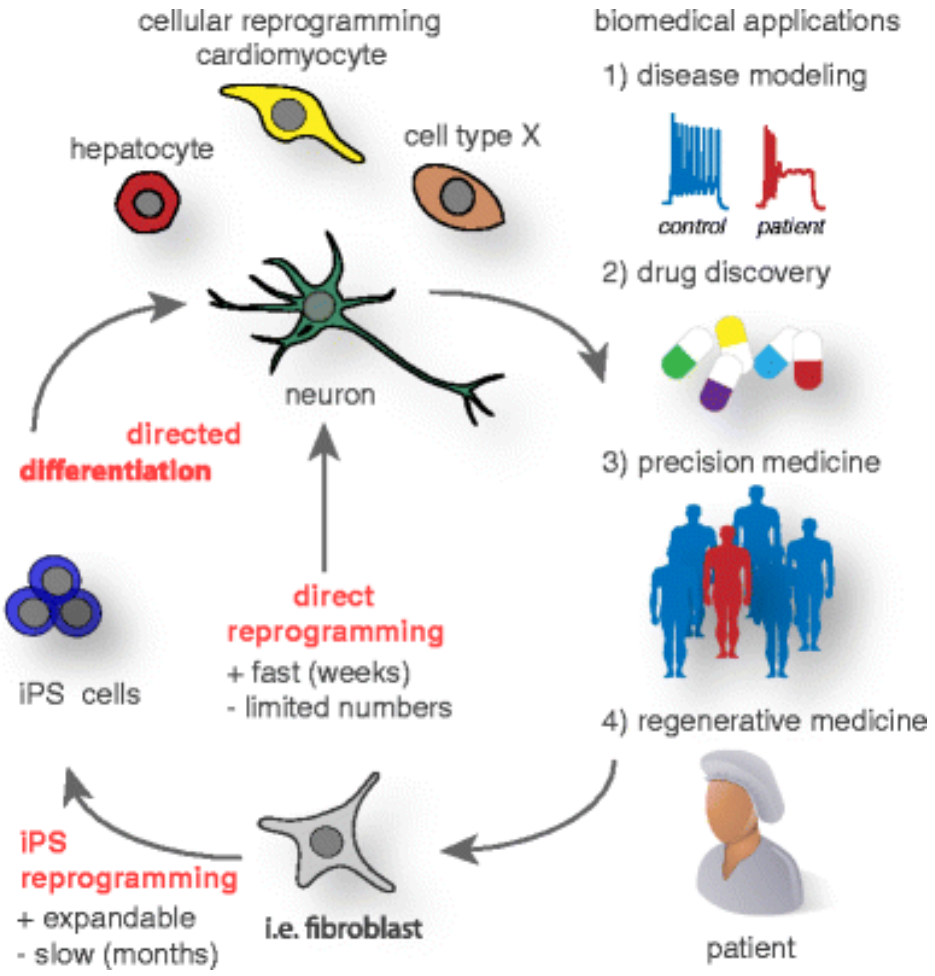
Modelling neurodegeneration in yeast



Creating, characterizing and screening a yeast model of neurodegenerative disease

Khurana V, Lindquist S. 2010

Studying Parkinson's disease in human neurons

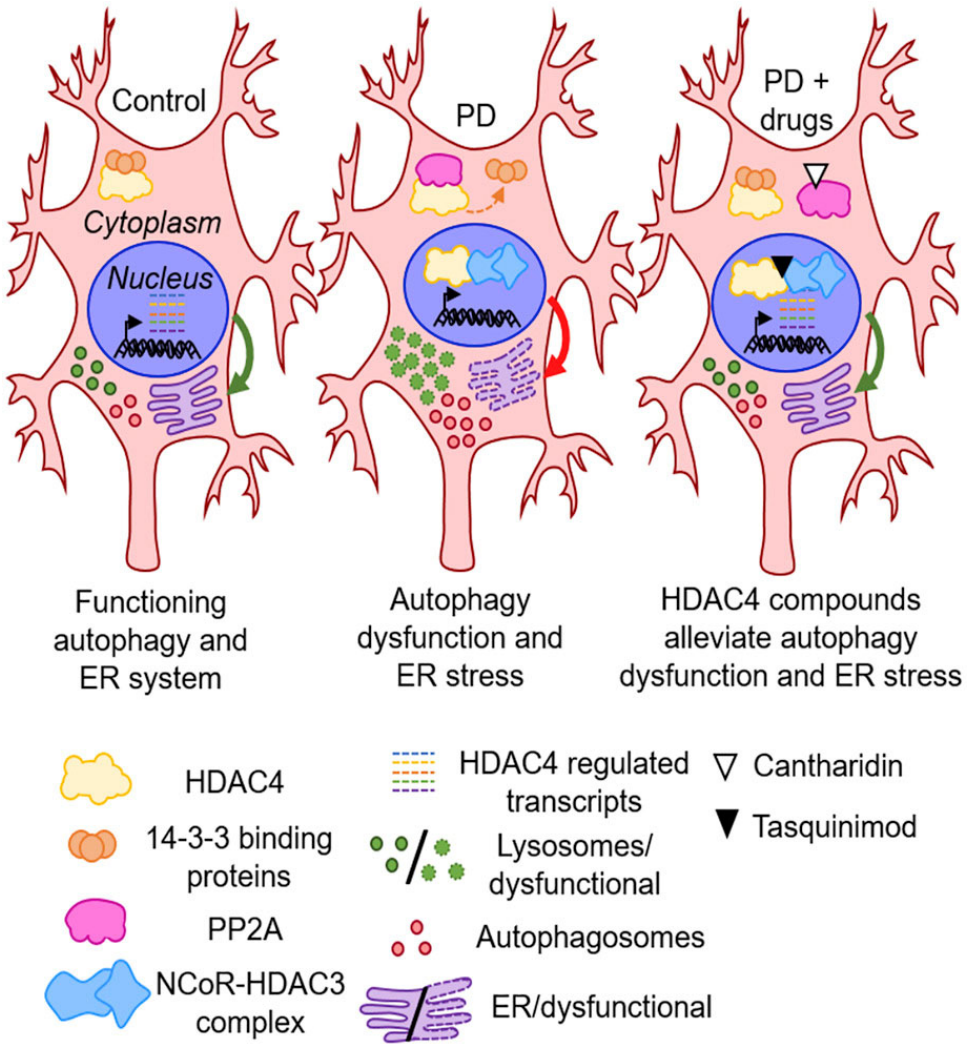


Mall, M. & Wernig, M. J Mol Med (2017) 95: 695. https://doi.org/10.1007/s00109-017-1550-4

Single-Cell Sequencing of iPSC-Dopamine Neurons Reconstructs Disease Progression and Identifies HDAC4 (histone deacetylase 4) as a Regulator of Parkinson Cell Phenotypes

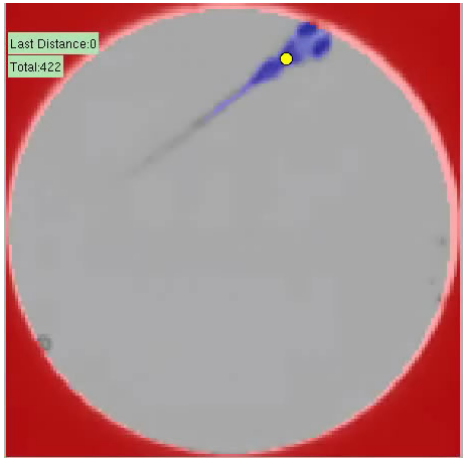
Charmaine Lang et al.

Cell Stem Cell
Volume 24, Issue 1,
Pages 93-106.e6
(January 2019)
DOI:
10.1016/j.stem.2018.10.023



Studying seizures and the brain in zebrafish

- What happens in the brain when one has a seizure?
- Can we do anything to change the severity of the seizure?
- One solution: Study zebrafish that have been treated with PTZ to cause seizures



Tracking a normal fish (video)

Studying seizures and the brain in zebrafish