

Krembil Centre for Neuroinformatics

Using big data, artificial intelligence and brain modelling to fundamentally change our understanding of mental illness.



SUMMER SCHOOL 2020

Day 3

Gene expression: DNA to cells

Part 2: single-cell gene expression and integration with bulk

Today's Agenda



Day 3
Genetics and
Transcriptomics

9:00 am -
10:30 am

Basics of Genotype, Central Dogma, GWAS, and Polygenic Risk Scores
Dan Felsky

10:45 am
- 12:15 pm

Transcriptomics at the Single-Cell Level
Shreejoy Tripathy

1:00 pm -
2:30 pm

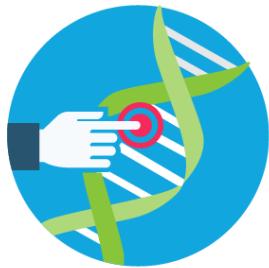
WORKSHOP: Intro to Transcriptomic Data Types, Including Single-Cell and Bulk RNAseq
Sonny Chen

2:45 pm -
4:15 pm

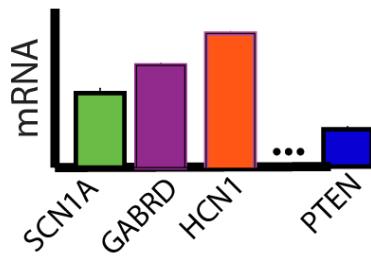
WORKSHOP: Explore Cellular Changes in Major Depression Using Bulk and Single-Cell RNAseq Data
Keon Arbabi

Bridging brain scales

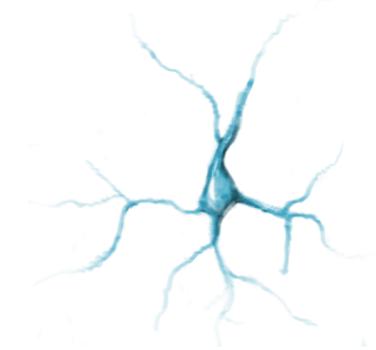
Genetics



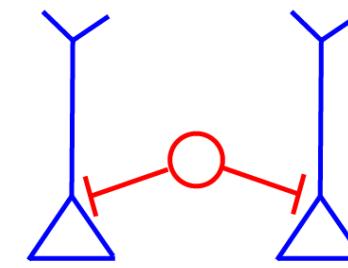
Gene Expression



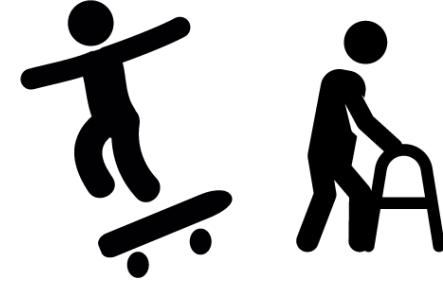
Cellular Physiology



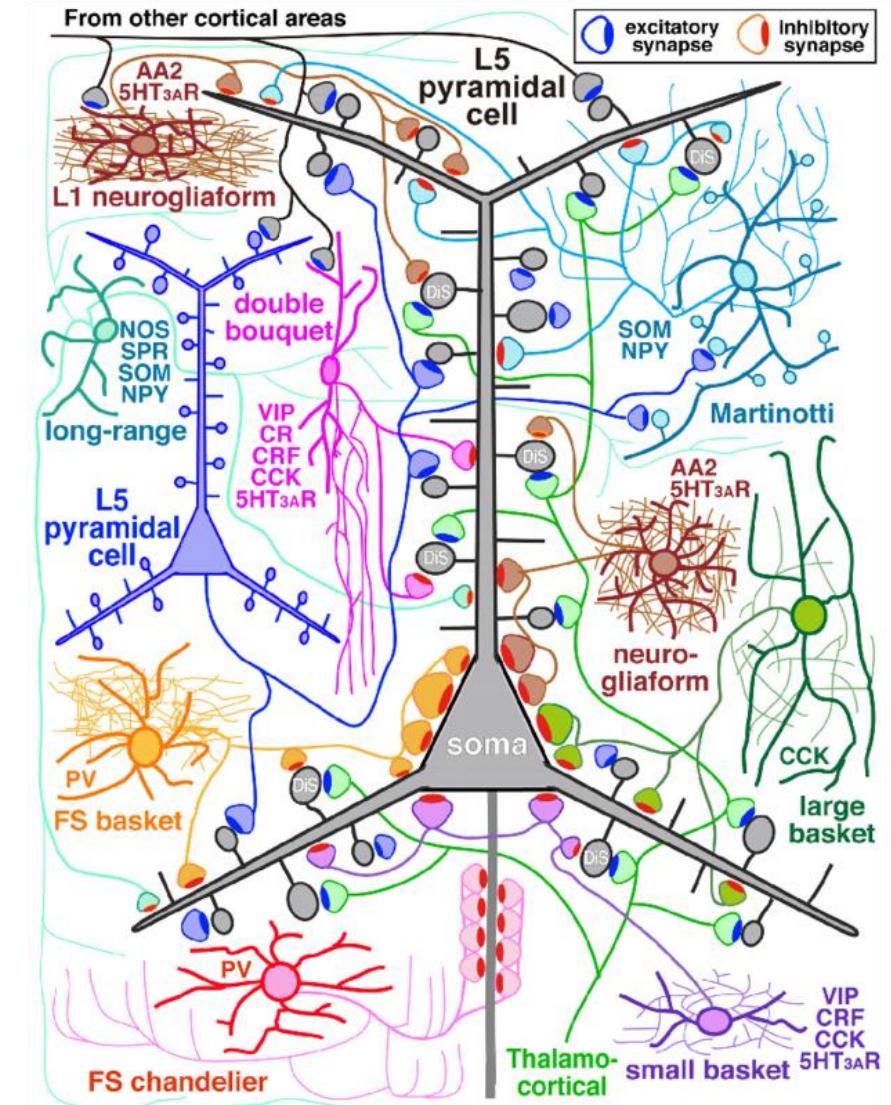
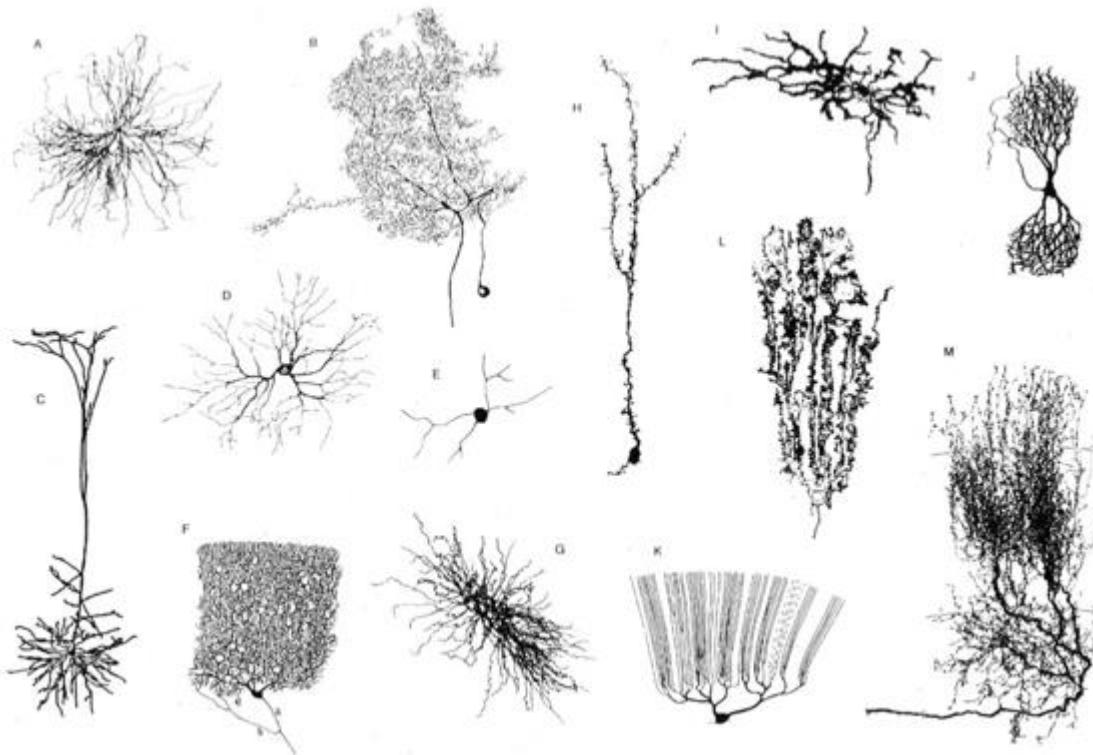
Neural Circuits



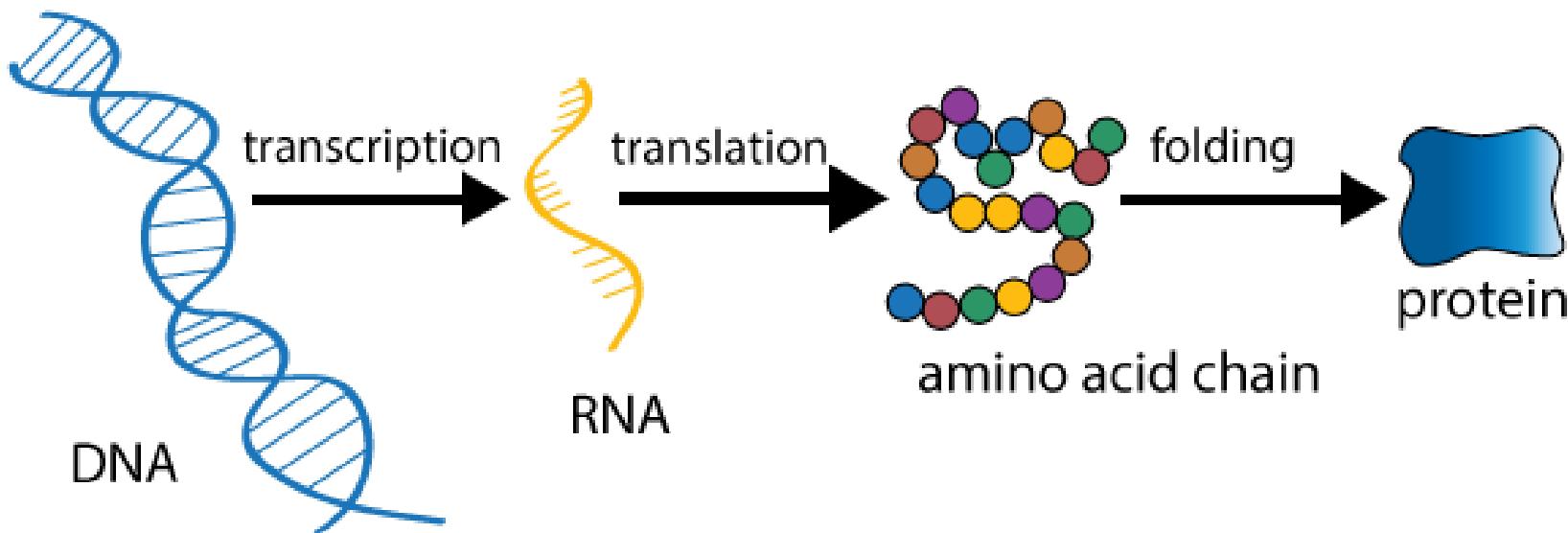
Traits & Behaviors



There are many types of cells in the brain



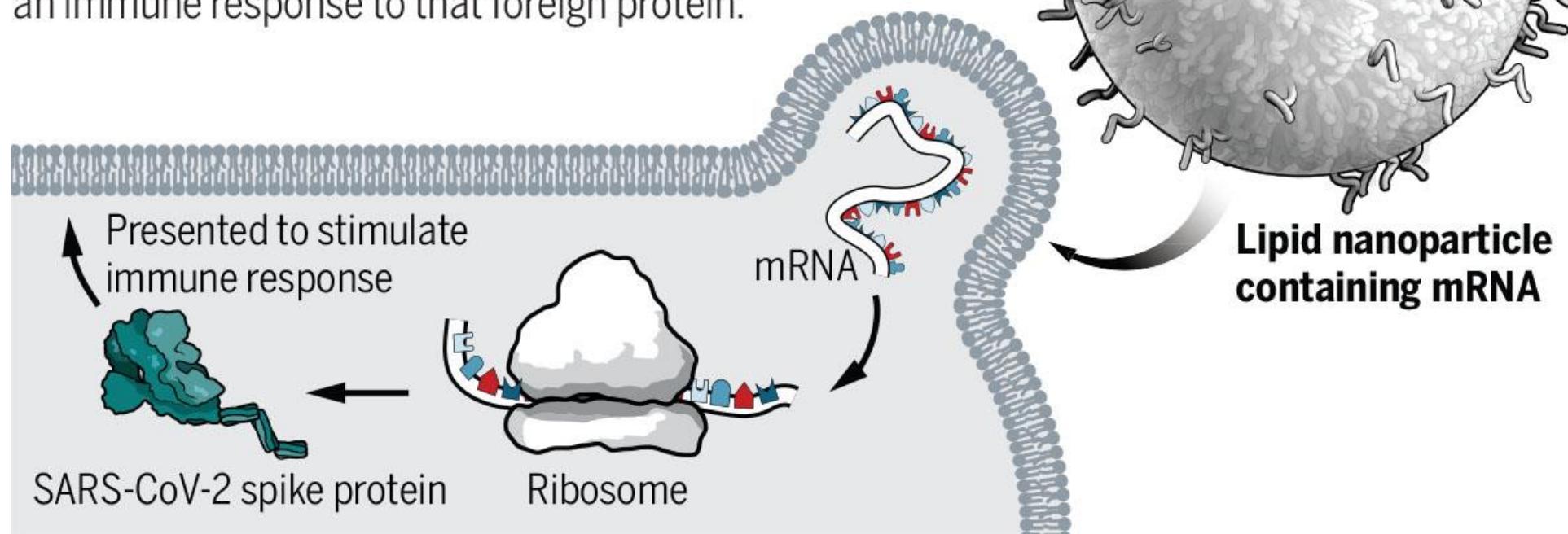
Central dogma of molecular biology



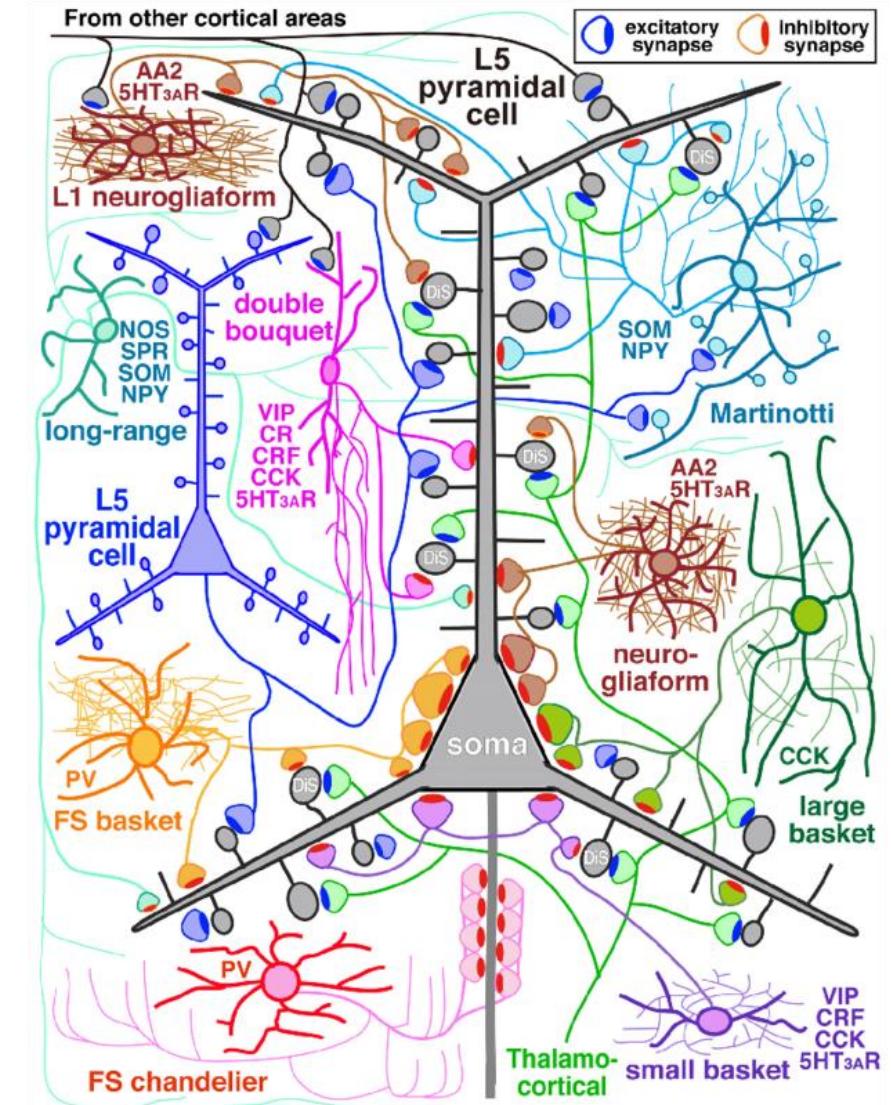
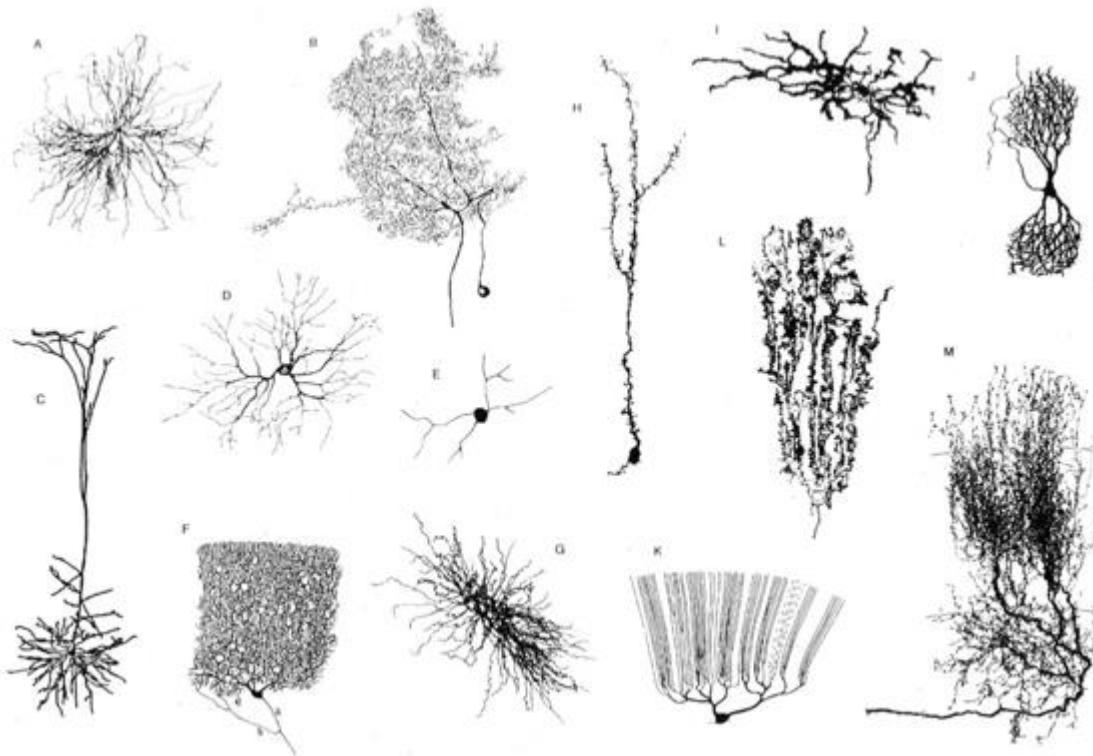
mRNA vaccines illustrate the magic of RNA

Special delivery

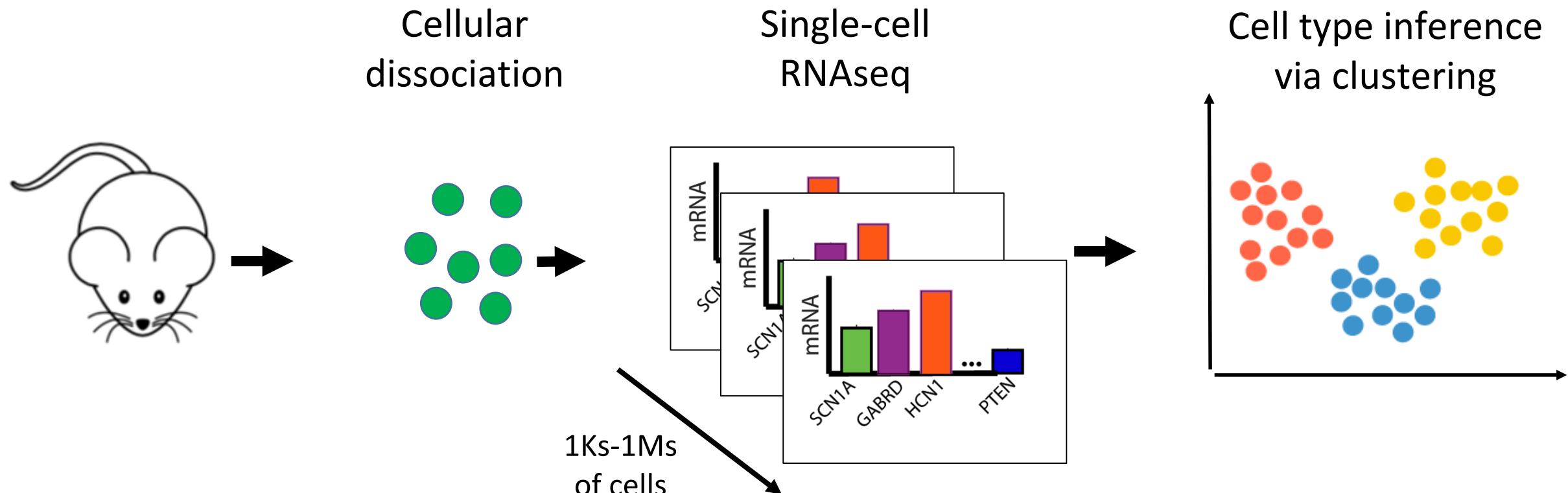
Two apparently successful coronavirus vaccines use fat bubbles called lipid nanoparticles to deliver messenger RNA (mRNA) to cells. Once there, the mRNA directs cells to produce the virus' spike protein, provoking an immune response to that foreign protein.



There are many types of cells in the brain



Single-cell RNA-sequencing



Allen Institute Cell Types Project; Tasic et al., 2016;

2018

Mousebrain.org; Zeisel et al., 2018

Tabula Muris Consortium, many others

RNA sequencing in a nutshell

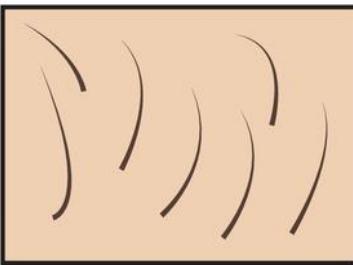
- **RNA-Seq** (named as an abbreviation of "RNA sequencing") is a particular technology-based **sequencing** technique that reveals the presence and quantity of **RNA** in a biological sample at a given moment, analyzing the *continuously changing* cellular transcriptome.

A brief overview of RNA sequencing

Samples of interest

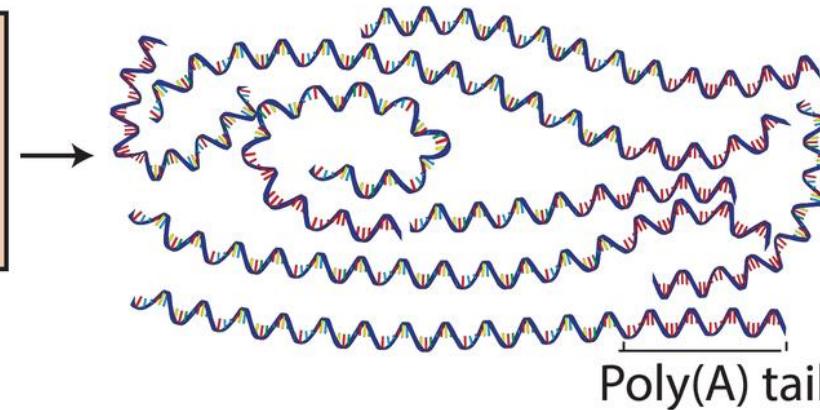


Condition 1
(e.g. tumor)



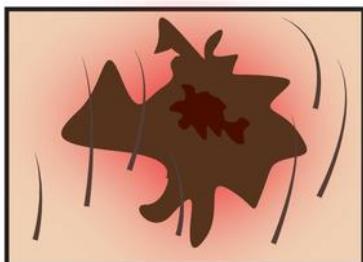
Condition 2
(e.g. normal)

Isolate RNAs

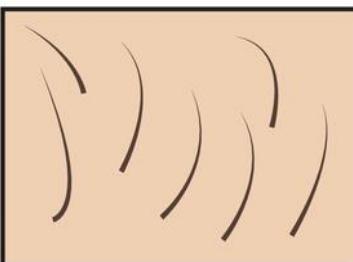


A brief overview of RNA sequencing

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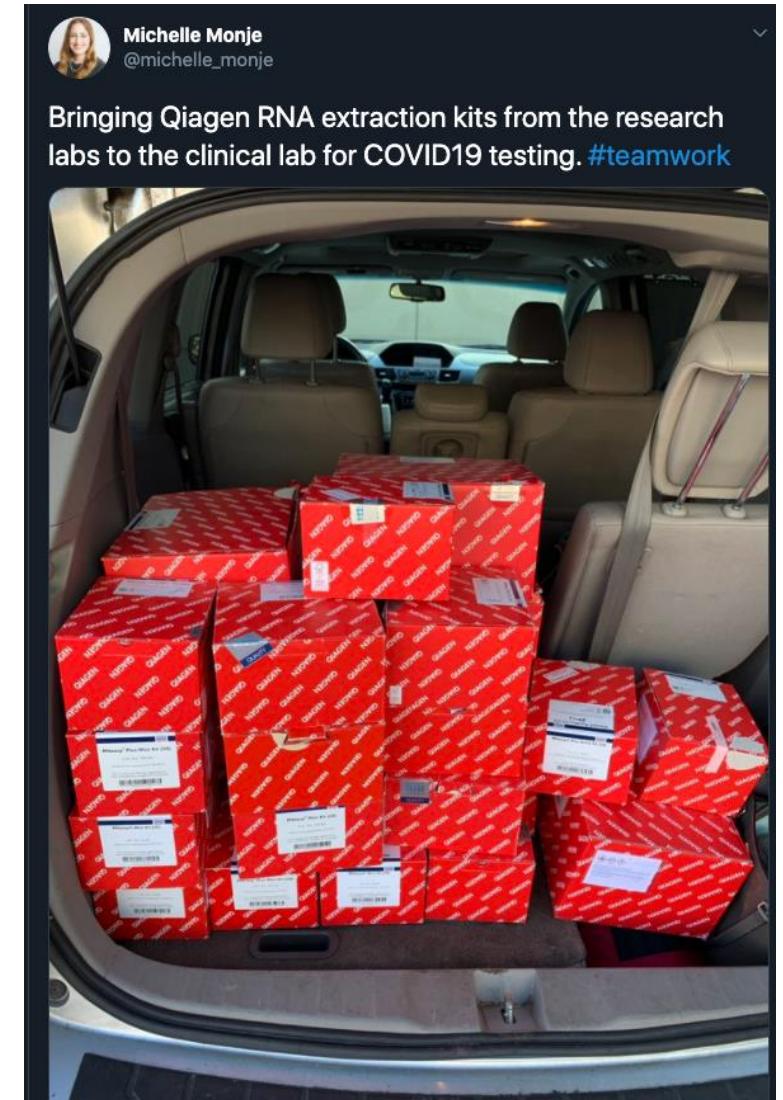
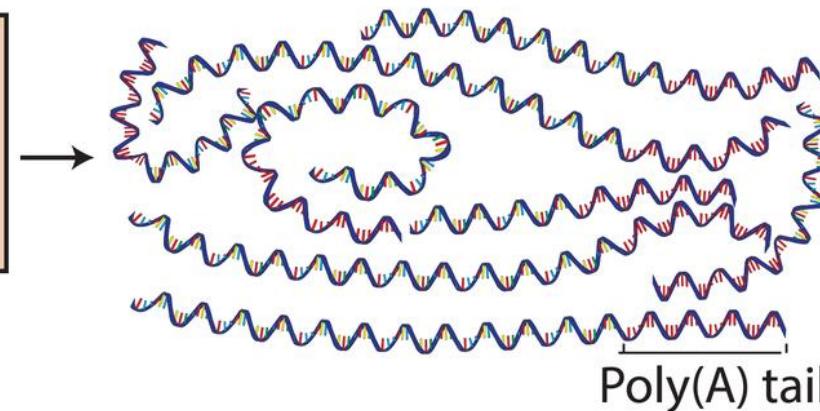


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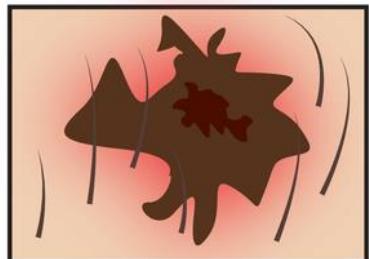


Condition 2
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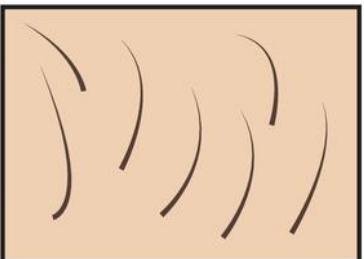
Isolate RNAs



Samples of interest

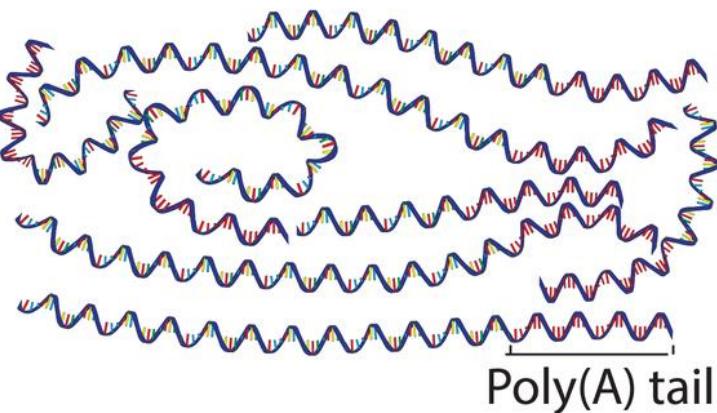


Condition 1
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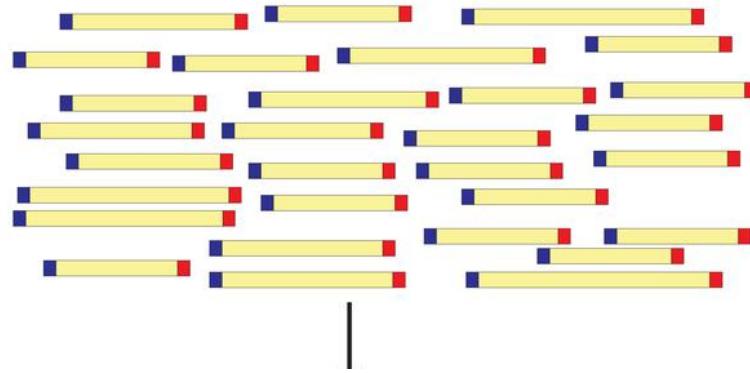
Condition 2
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Isolate RNAs

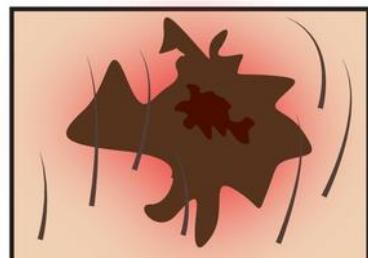


Poly(A) tail

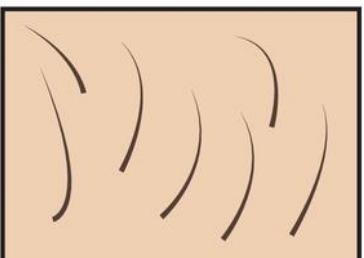
Generate cDNA, fragment, size select, add linkers



Samples of interest

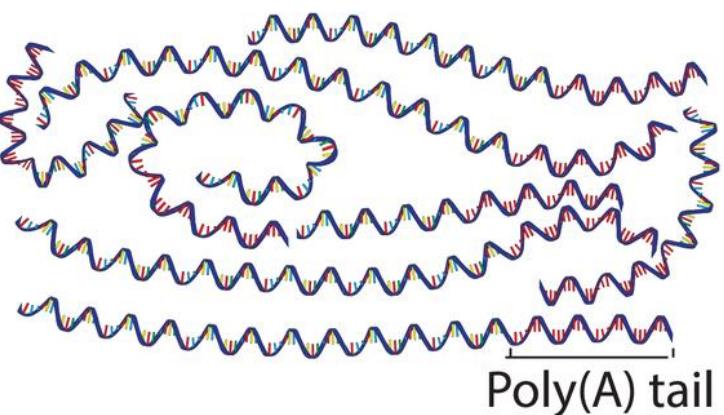


Condition 1
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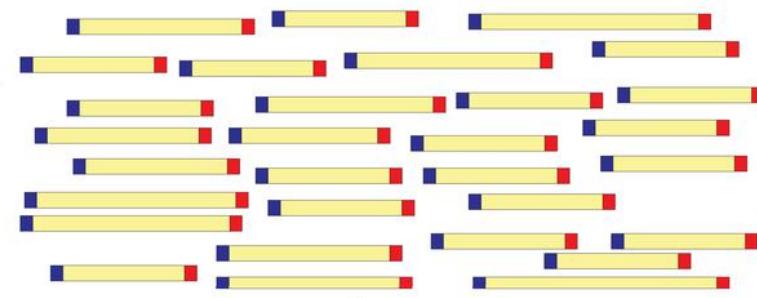


Condition 2
(e.g. normal)

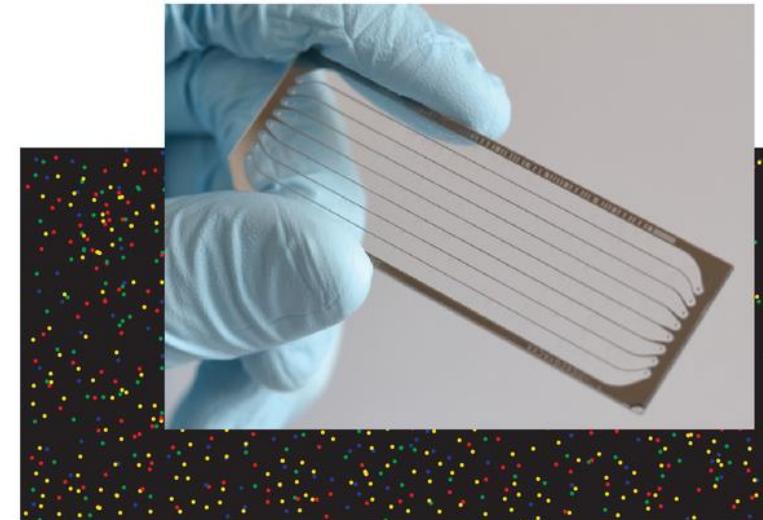
Isolate RNAs



Generate cDNA, fragment, size select, add linkers



Sequence ends

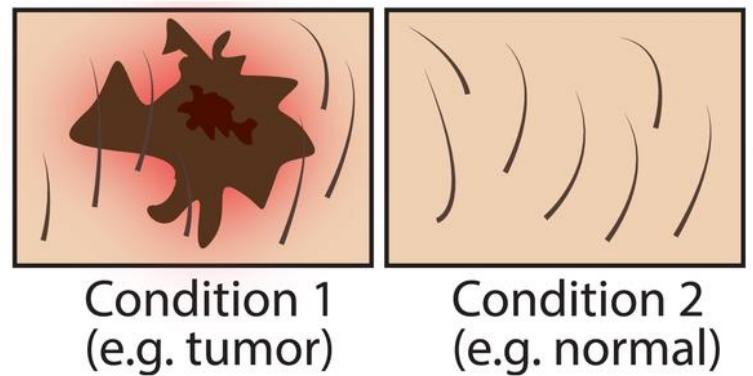


100s of millions of paired reads
10s of billions bases of sequence

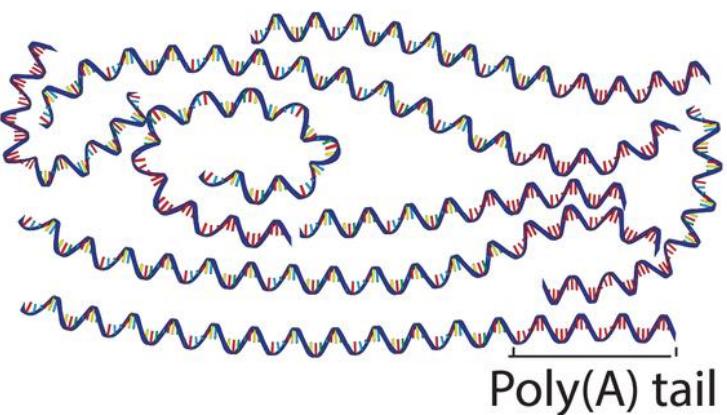
Youtube video illustrating DNA sequencing

https://www.youtube.com/watch?v=2JUu1WqidC4&ab_channel=MayoClinic
(watch from 0:49 to 1:40)

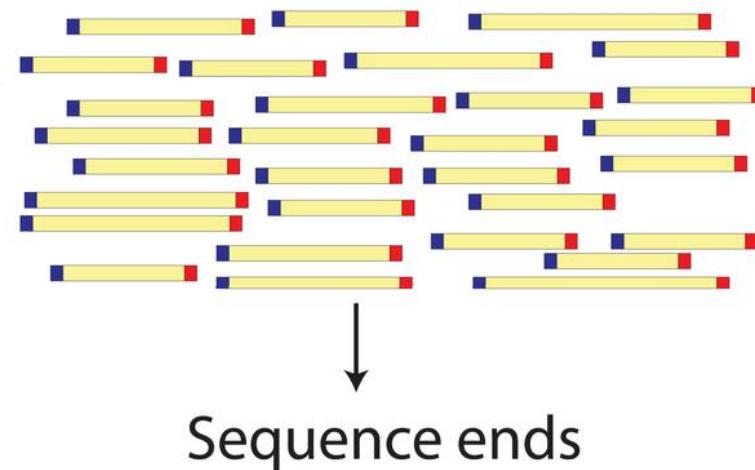
Samples of interest



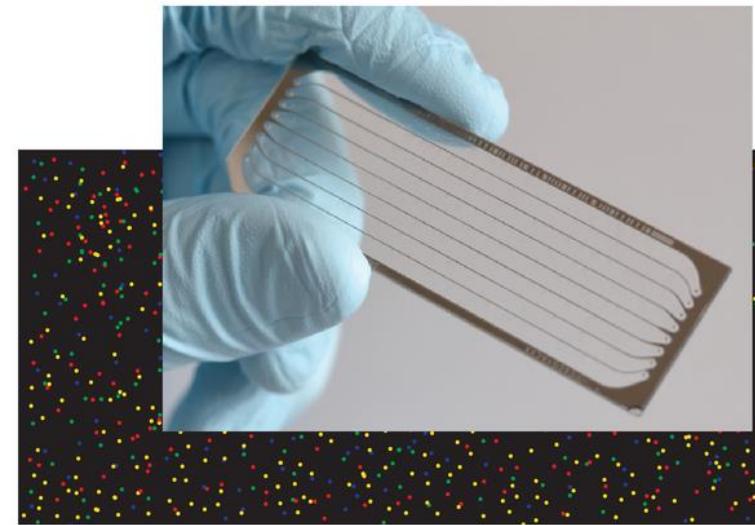
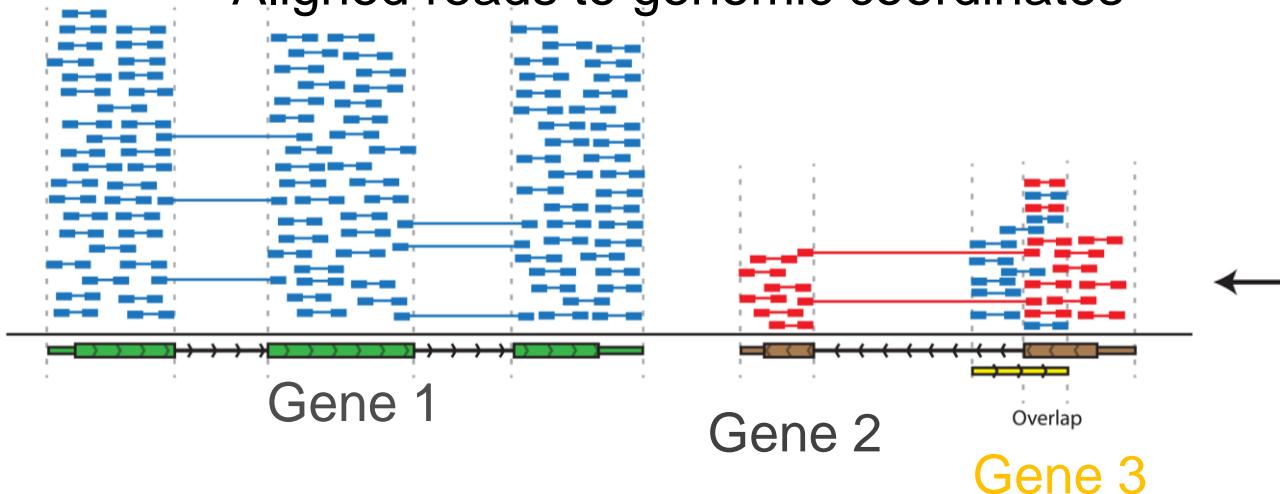
Isolate RNAs



Generate cDNA, fragment, size select, add linkers

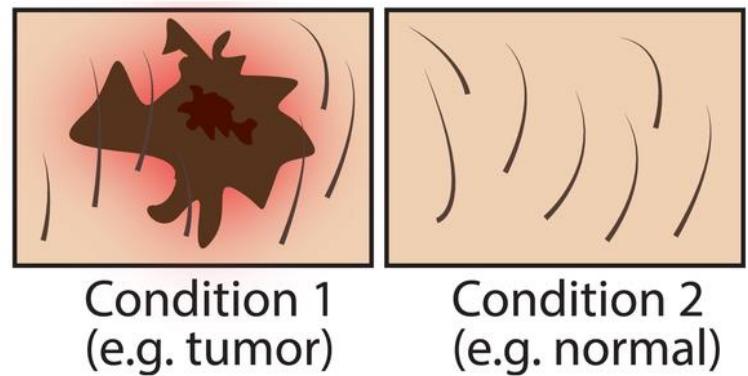


Aligned reads to genomic coordinates

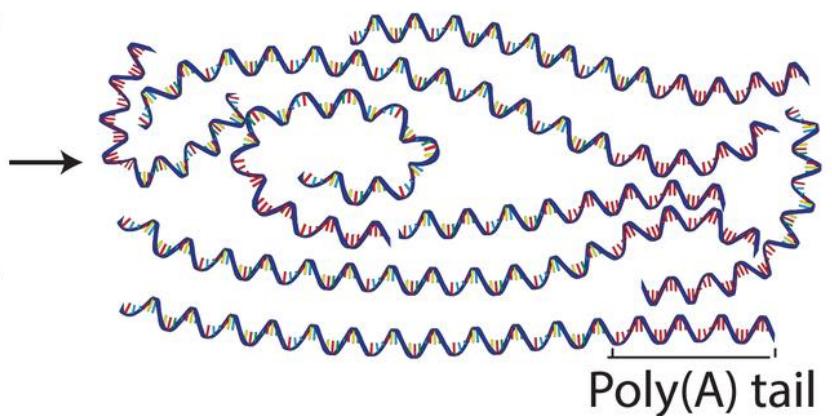


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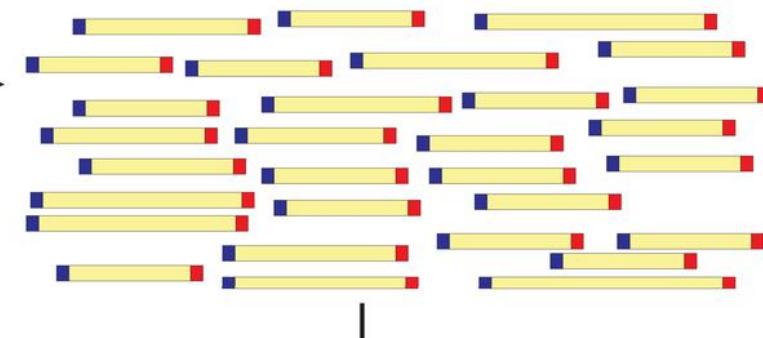
Samples of interest



Isolate RNAs

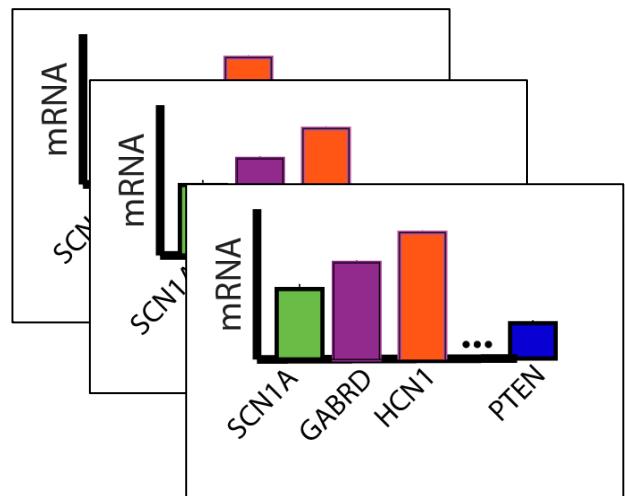


Generate cDNA, fragment, size select, add linkers

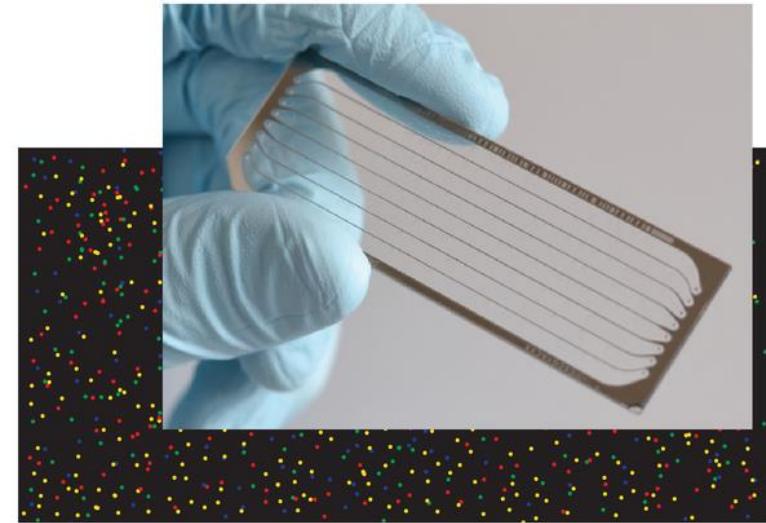
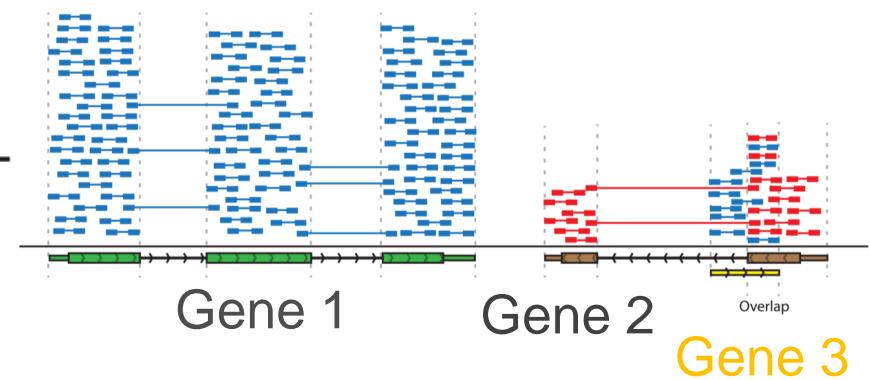


Sequence ends

Quantified mRNA per sample

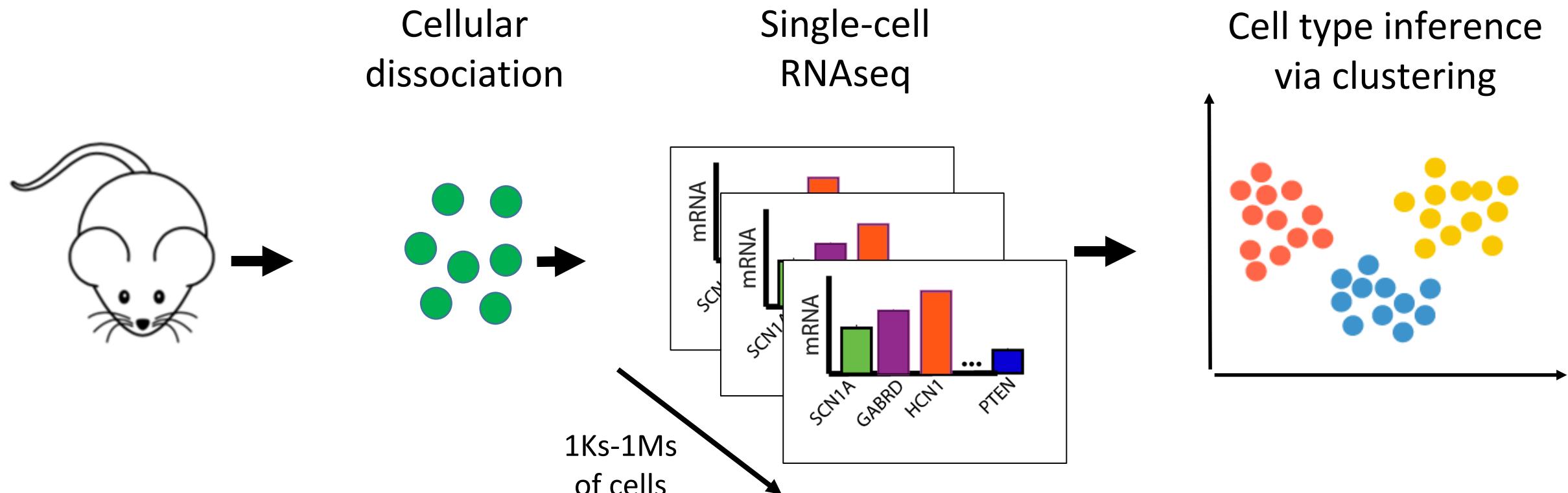


Aligned reads to genomic coordinates



100s of millions of paired reads
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Single-cell RNA-sequencing



Allen Institute Cell Types Project; Tasic et al., 2016;

2018

Mousebrain.org; Zeisel et al., 2018

Tabula Muris Consortium, many others

Gene expression count matrix and metadata

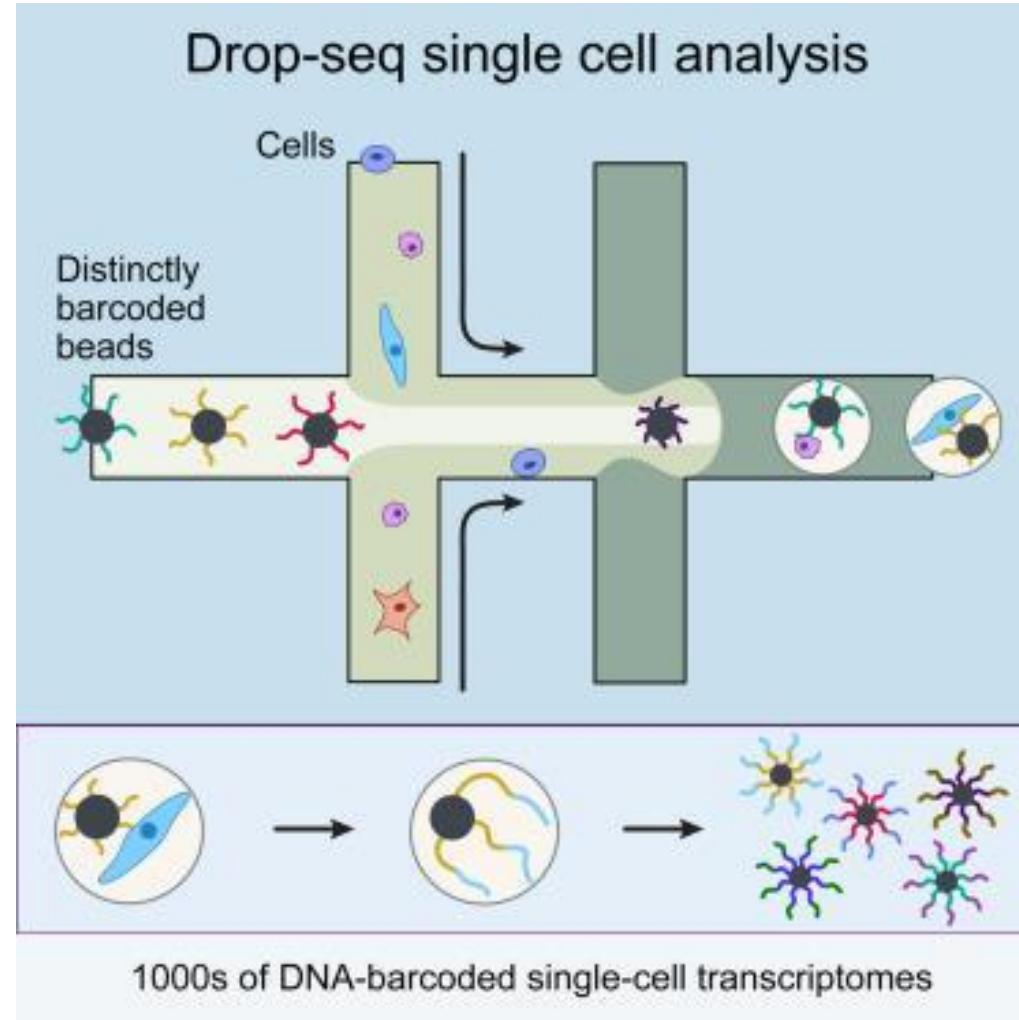
Gene expression “count matrix”

	Cell1	Cell2	...	CellN
Gene1	3	2	.	13
Gene2	2	3	.	1
Gene3	1	14	.	18
...
...
...
GeneM	25	0	.	0

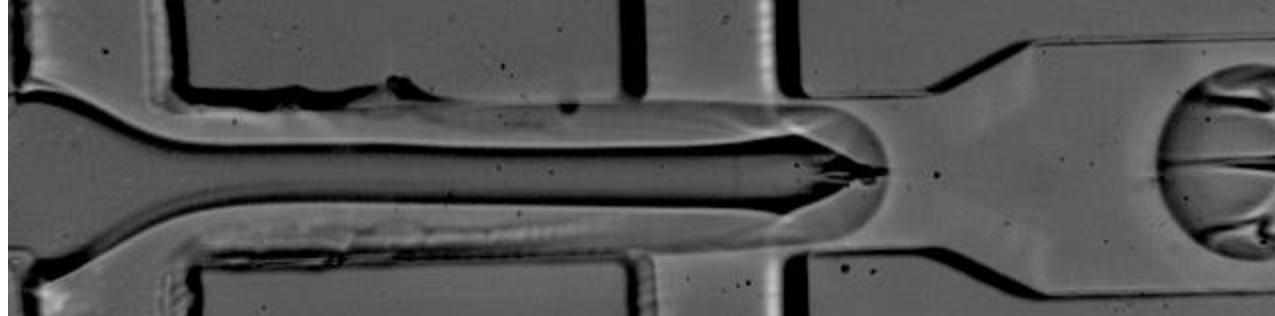
Sample metadata matrix

Cell ID	Subject ID	Expt group	Cell Type	...
Cell 1	Patient 1	Control	Astrocyte	
Cell 2	Patient 1	Control	Neuron	
...				
Cell N	Patient N	Case	Neuron	

Microfluidics enables single-cell omics



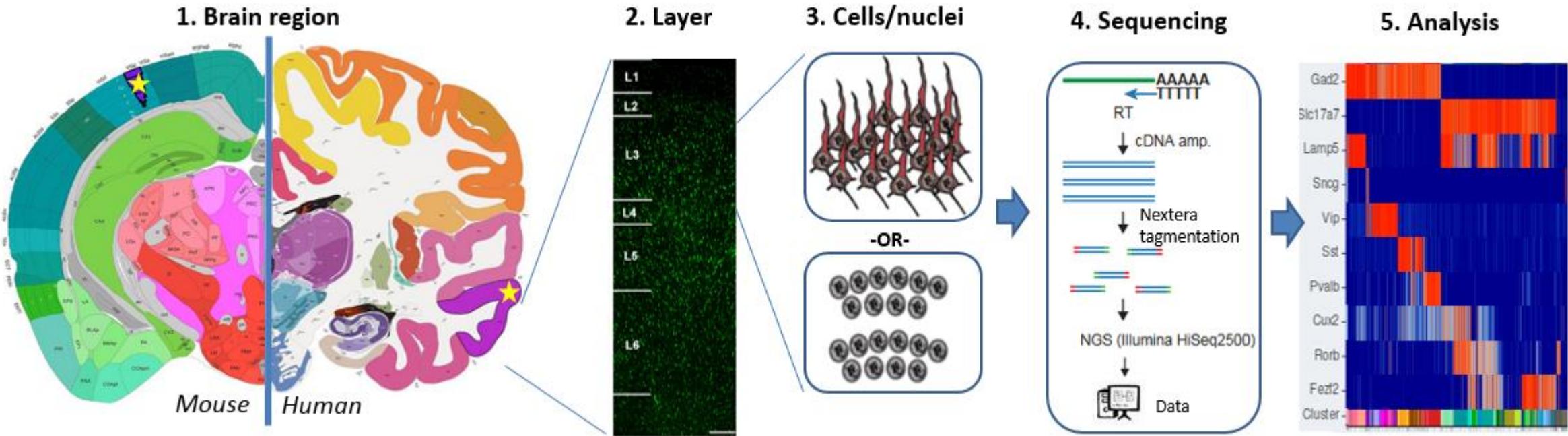
Microfluidics enables single-cell omics



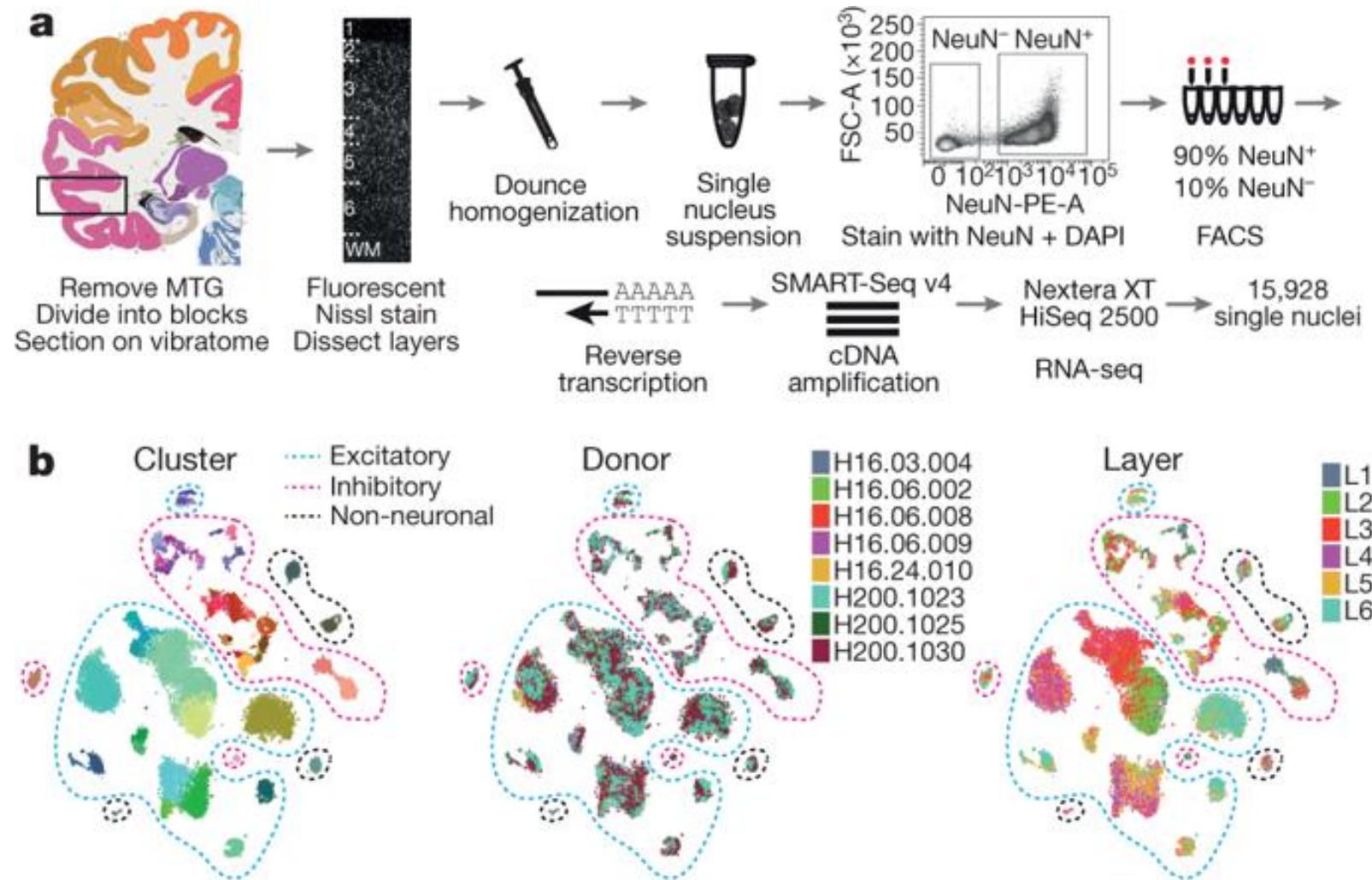
scRNAseq rules of thumb

- Single-cell technologies are *sampling* the transcriptome
 - Most datasets are only sampling a fraction of the most highly expressed genes
- Nuclei are not cells
 - Especially important for large cells, like neurons
- Garbage in, garbage out
 - scRNAseq is less forgiving of degraded mRNA than bulk RNAseq methods

Allen Institute cell type resources

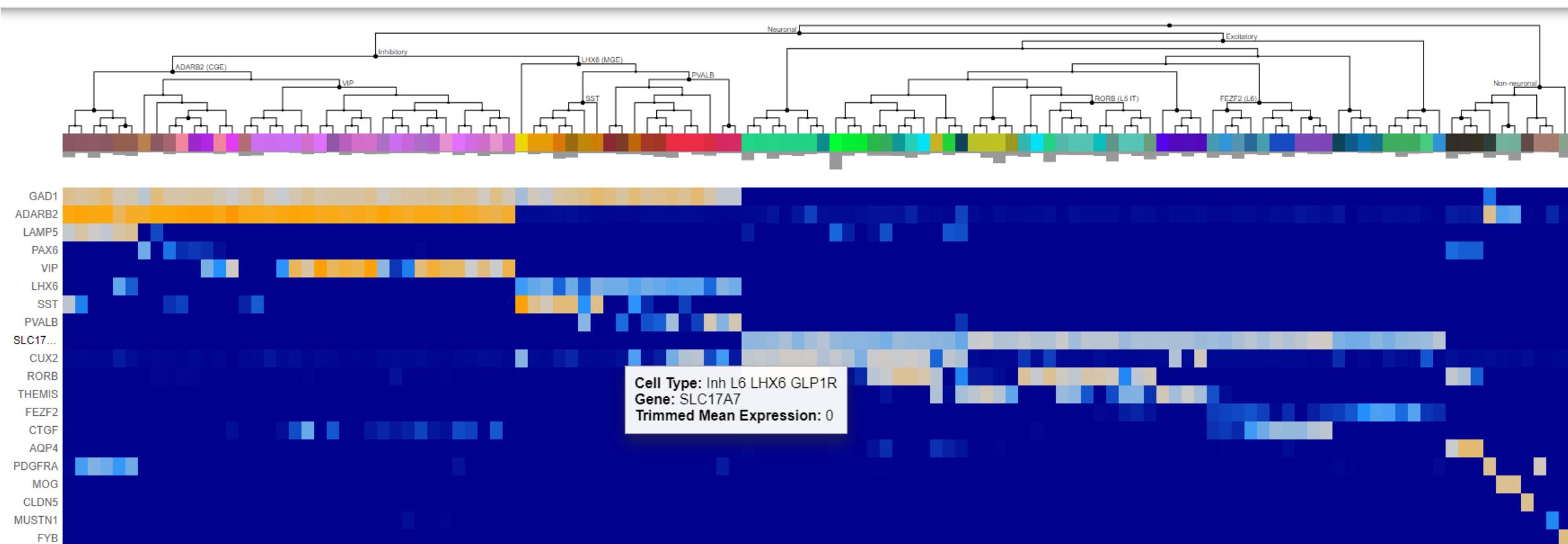


Human cortex single-nucleus RNAseq

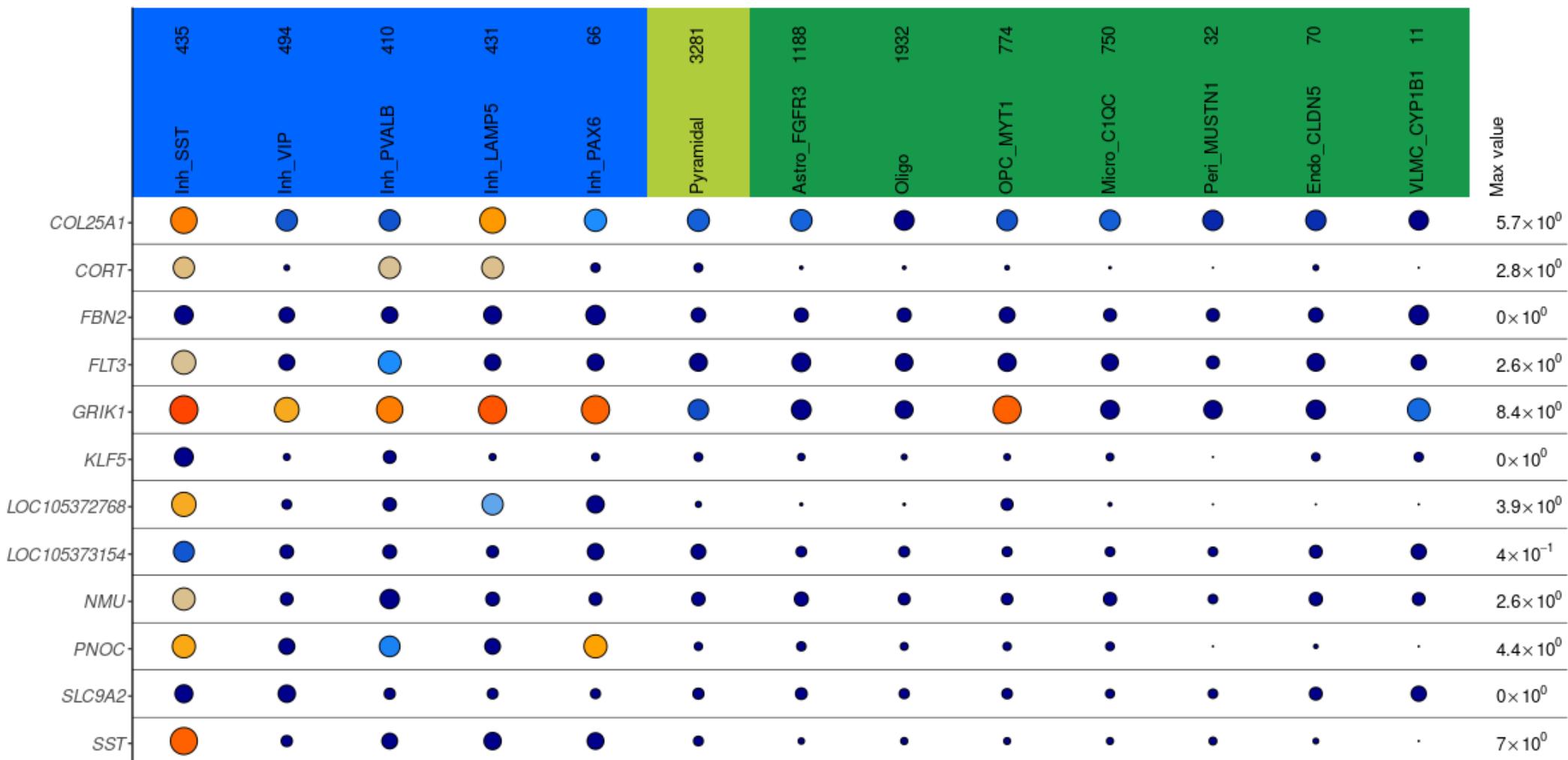


Allen Institute Cell Types Resources

- <http://celltypes.brain-map.org/>



SST cell type markers based on snRNAseq



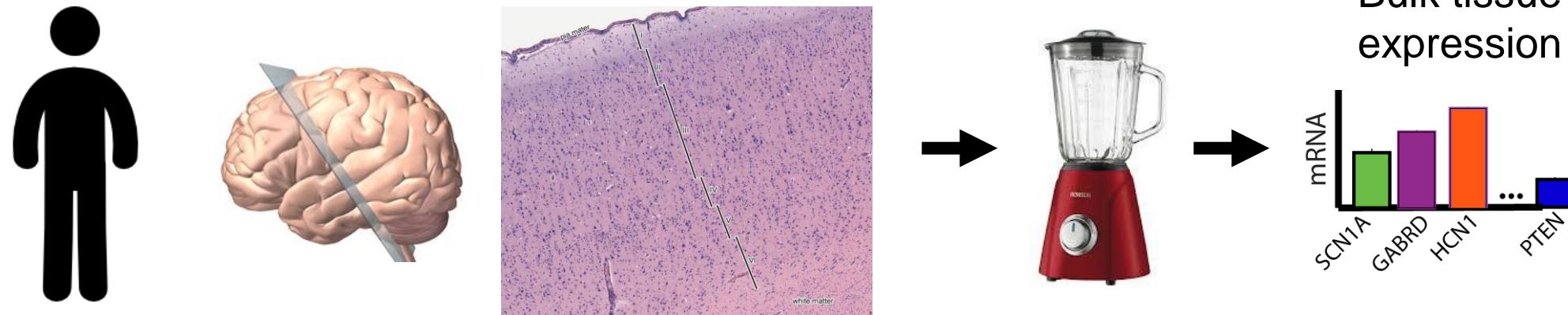
Markers based on Allen Institute human snRNAseq data from ACC
reanalyzed by Sonny Chen, post-doc in Tripathy Lab

Tutorial : Applications of single-cell RNAseq

- Scientific concepts:
 - Become familiar with brain gene expression and brain cell types
 - Learn about cell types and concept of marker genes specific to cell types
- Specific learning objectives
 - Gain familiarity with scRNAseq data, preprocessing, and analysis
 - Gain familiarity with dimensionality reduction

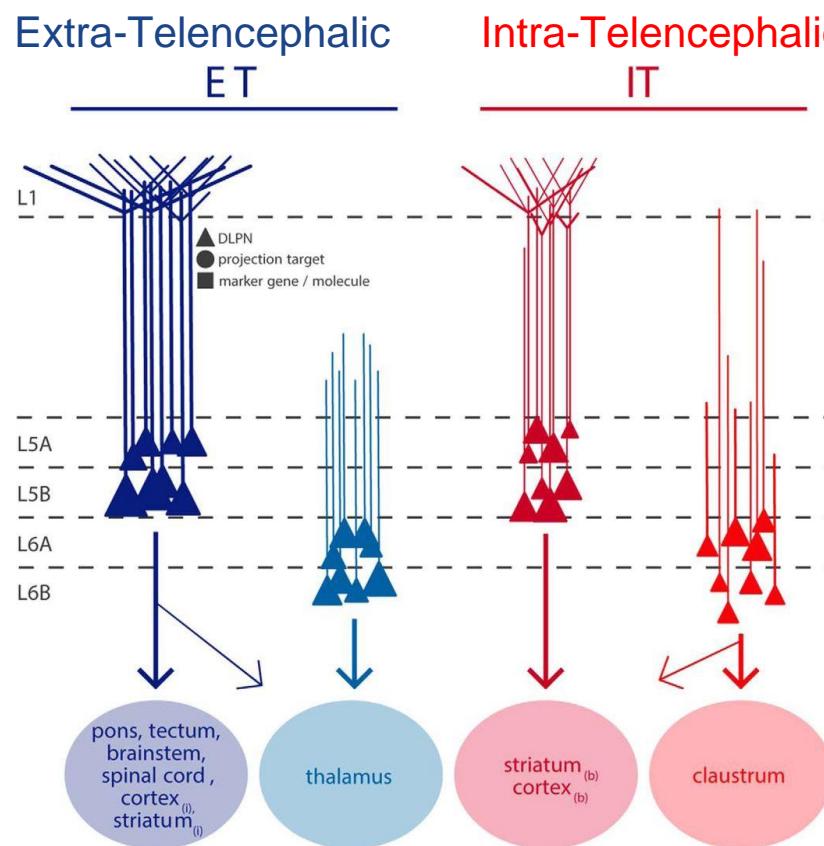
Break for questions

Studying the post-mortem human brain

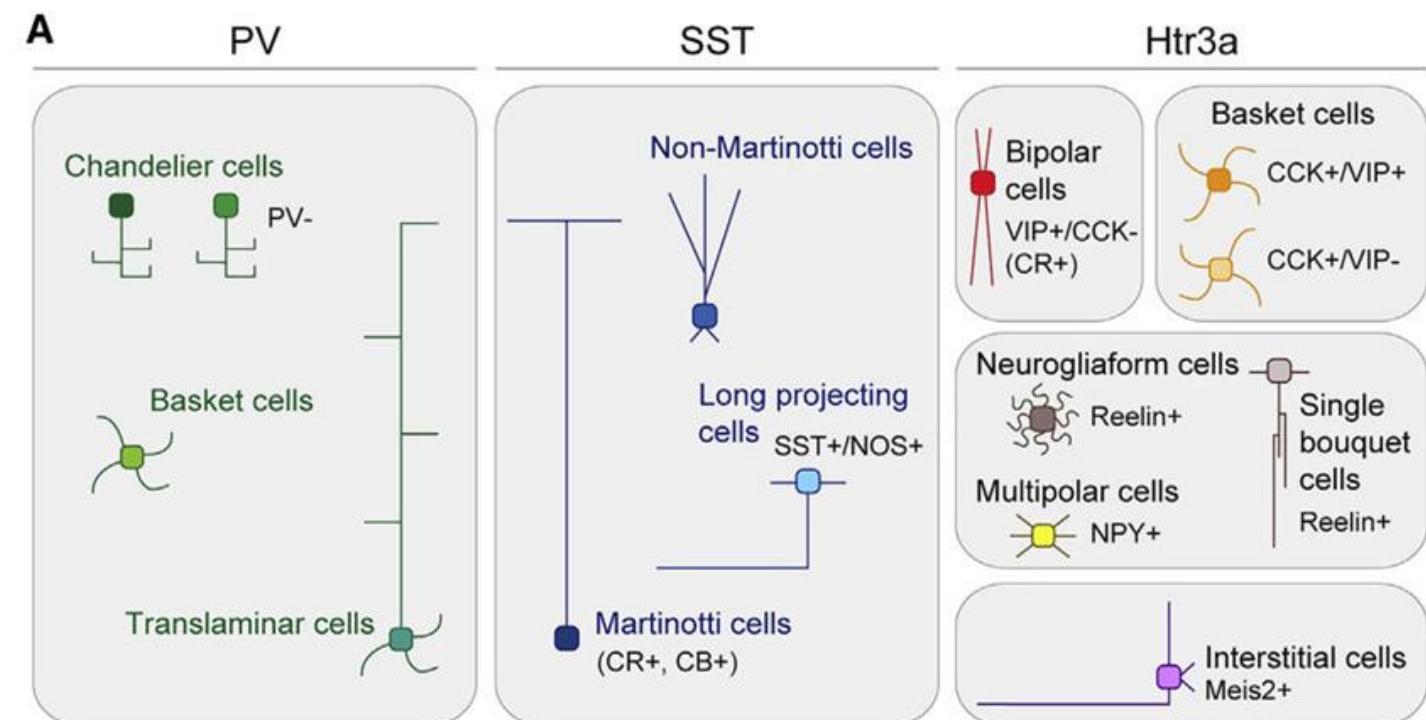


Neocortical cell type diversity

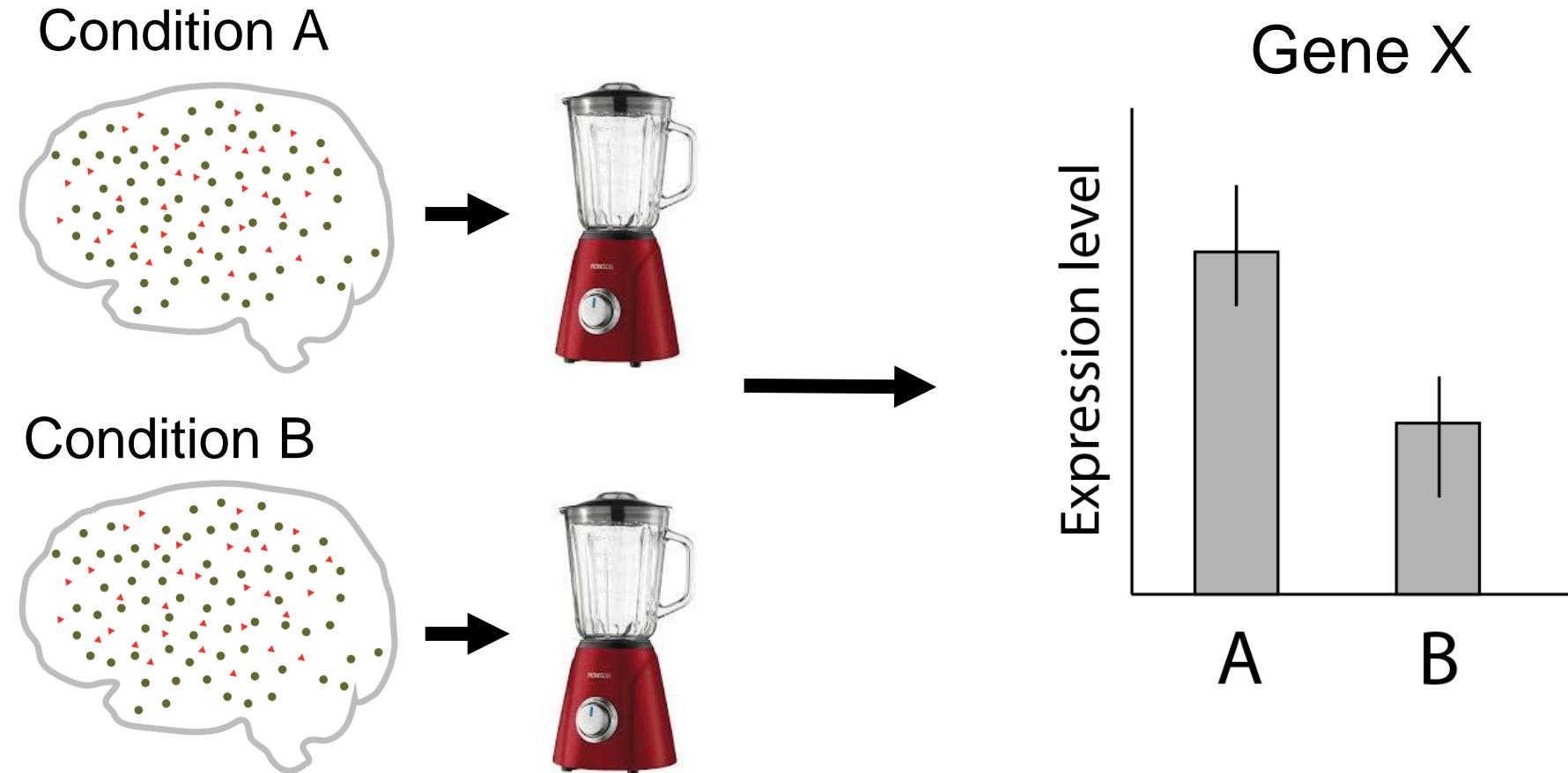
Examples of excitatory neuron types



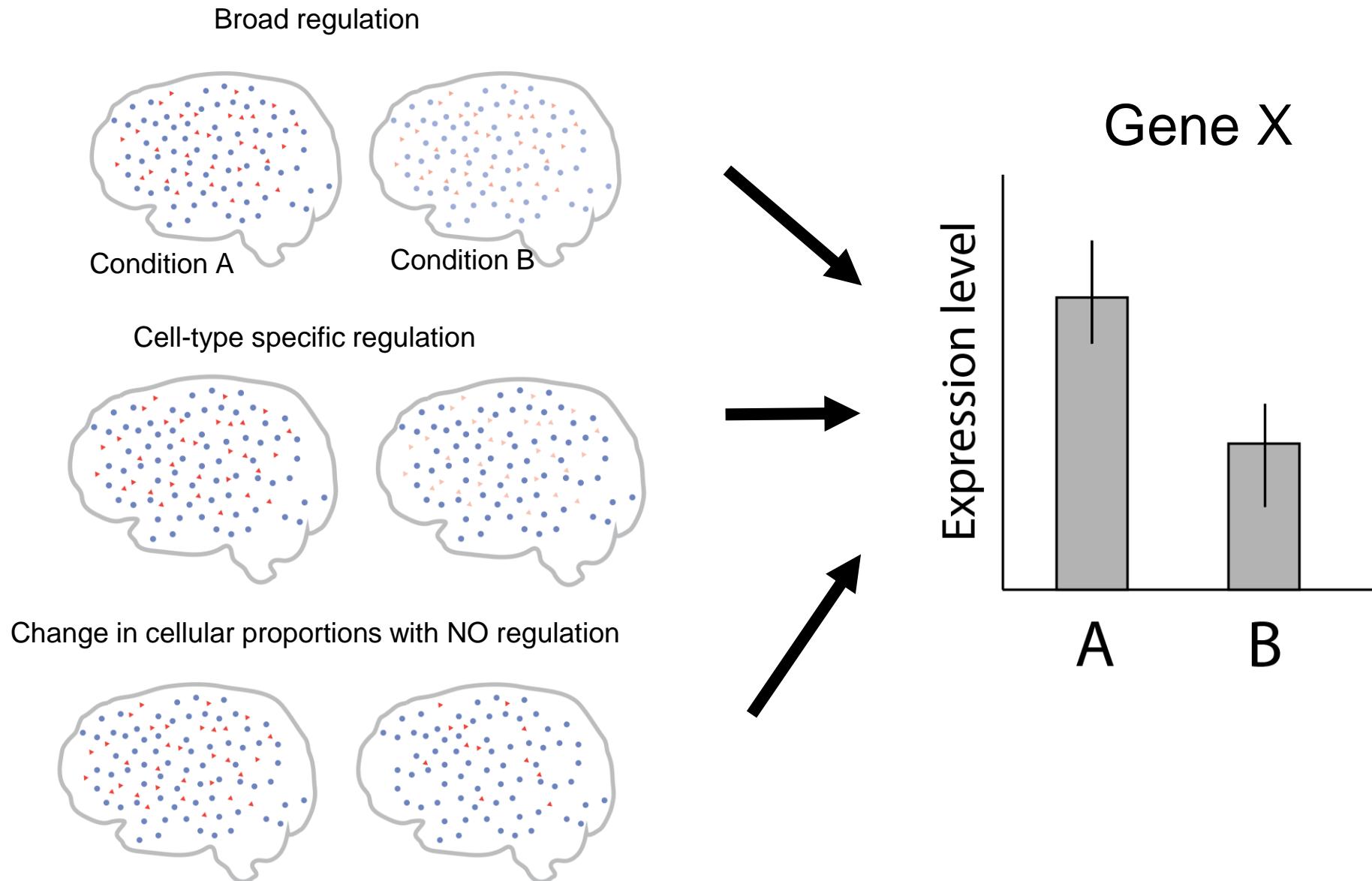
Diversity of major inhibitory neuron types



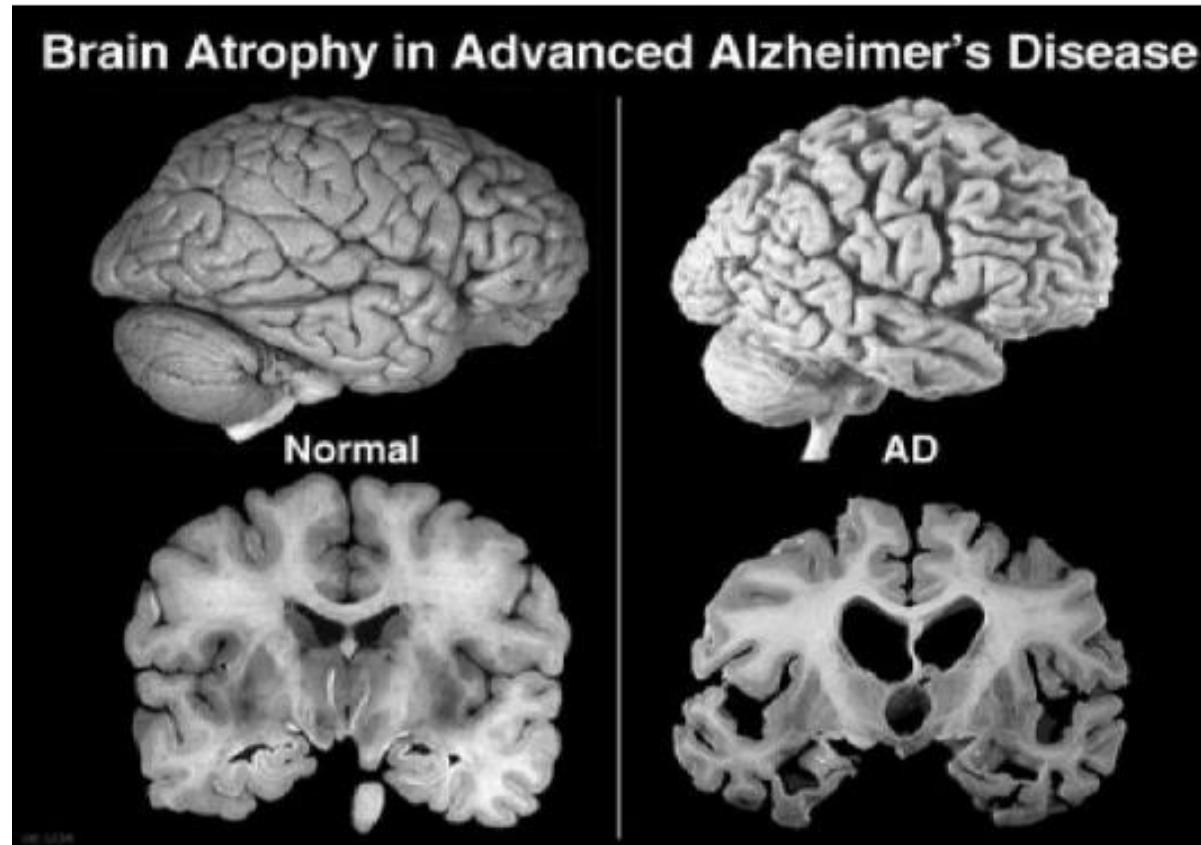
Bulk tissue transcriptomes complicate interpretation



Bulk tissue transcriptomes complicate interpretation



Cell type changes in Alzheimer's disease

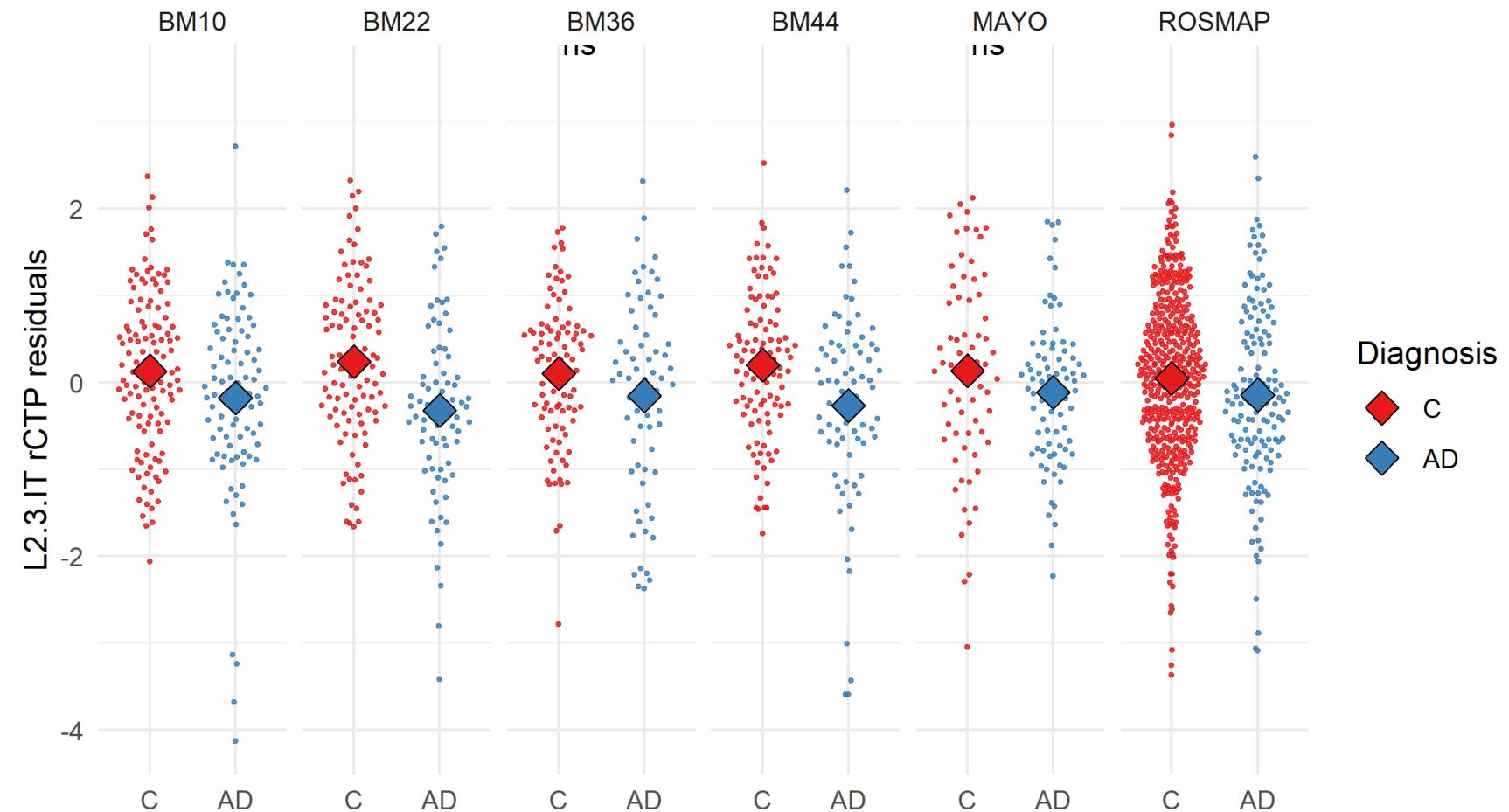
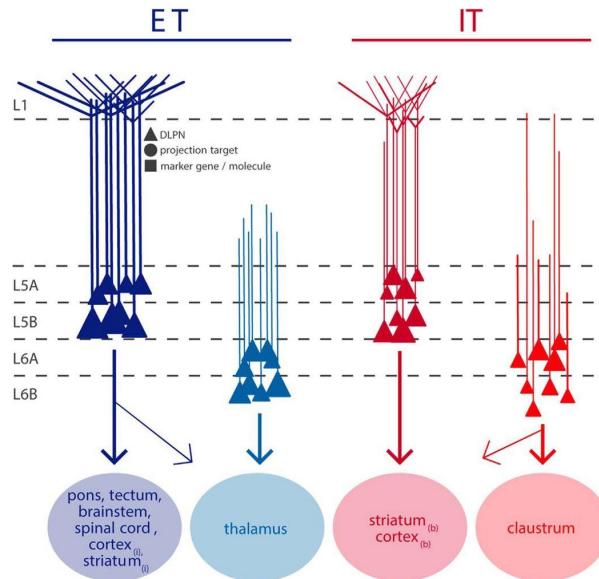


- How does AD pathology impact specific cell types in the neocortex?

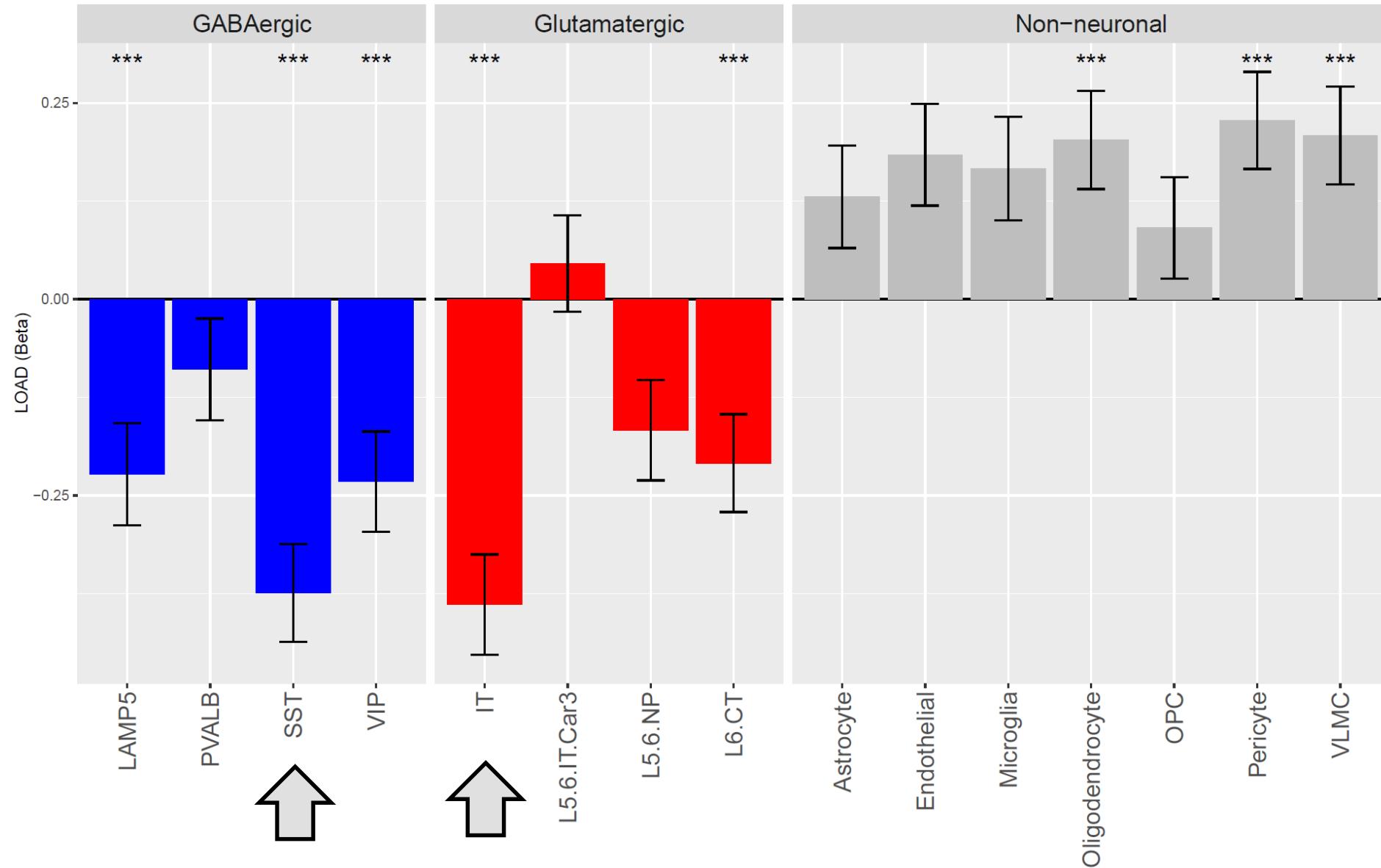


Micaela Consens (co-advised with Dan Felsky)
4th year Undergrad in Bioinformatics and Computer Science

Cell type changes in Alzheimer's disease



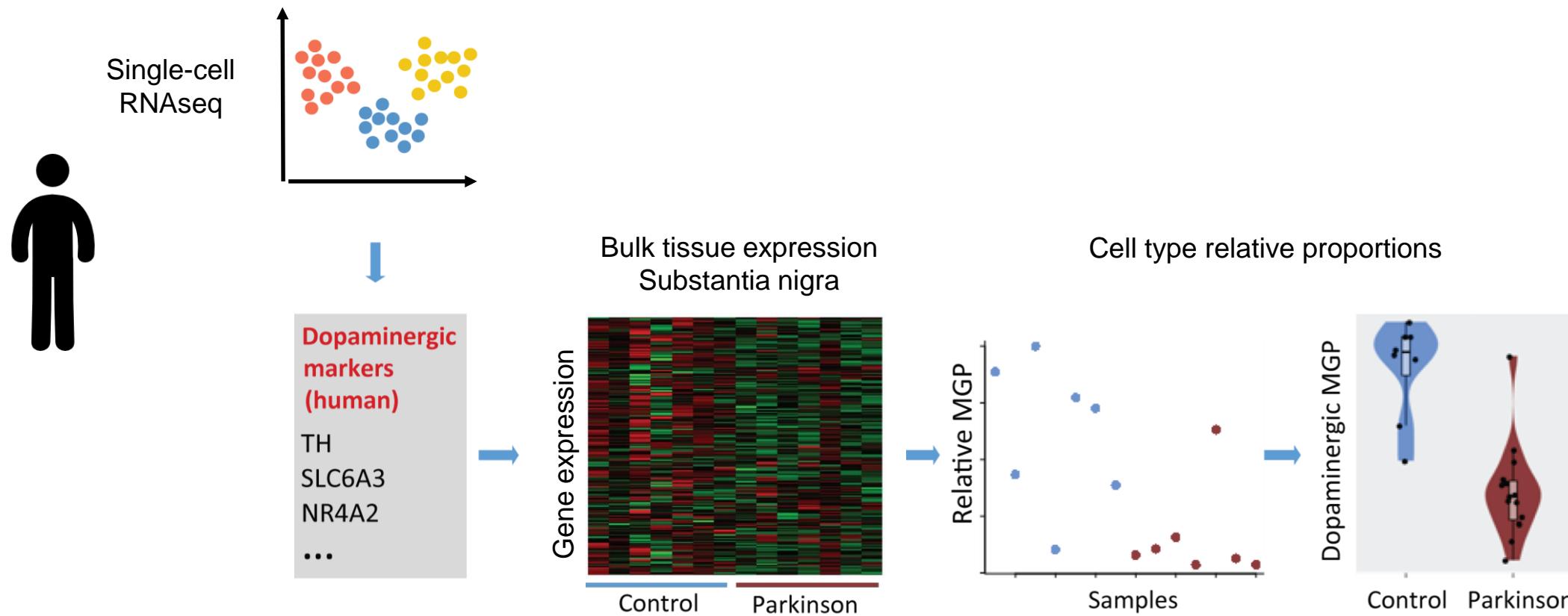
Cell type changes in Alzheimer's disease



Tutorial : Re-interpreting bulk expression profiles for cell type proportion changes

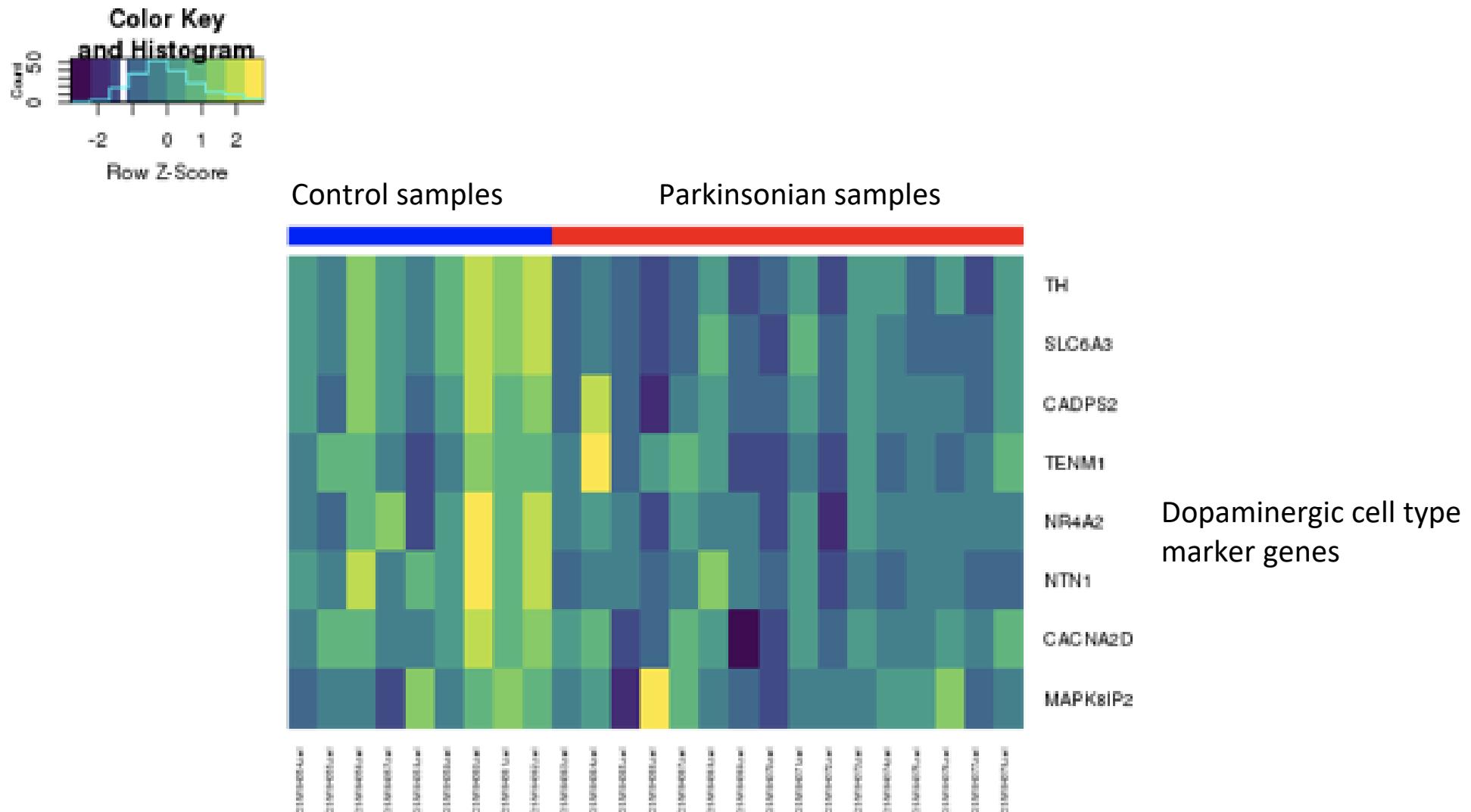
- Scientific concepts:
 - Learn how scientists use post-mortem brain gene expression to infer cellular changes in psychiatric conditions
 - Learn about cell type proportions and how these might differ in neuropsychiatric disease
- Specific Learning objectives:
 - Learn how to analyze brain bulk gene expression data
 - Learn about cell types and concept of marker genes specific to cell types

Cellular abundance estimation using Marker Gene Profiles

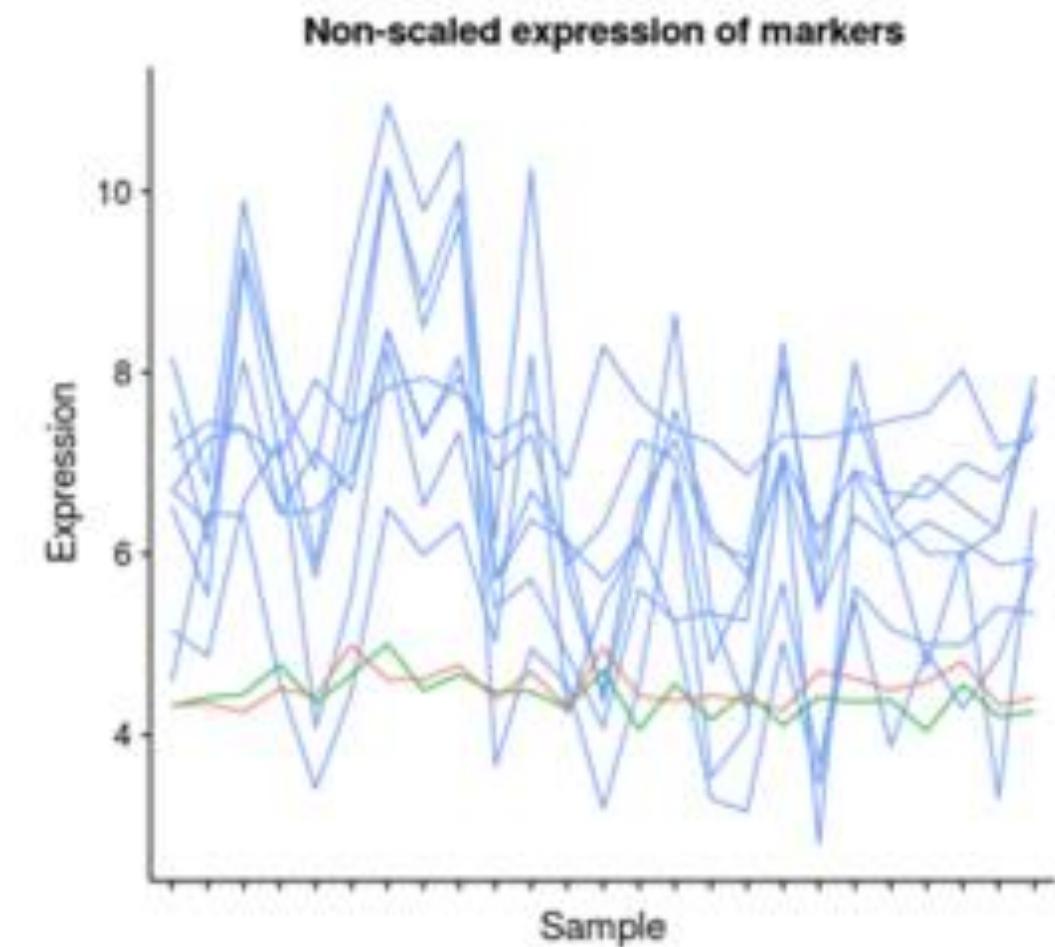
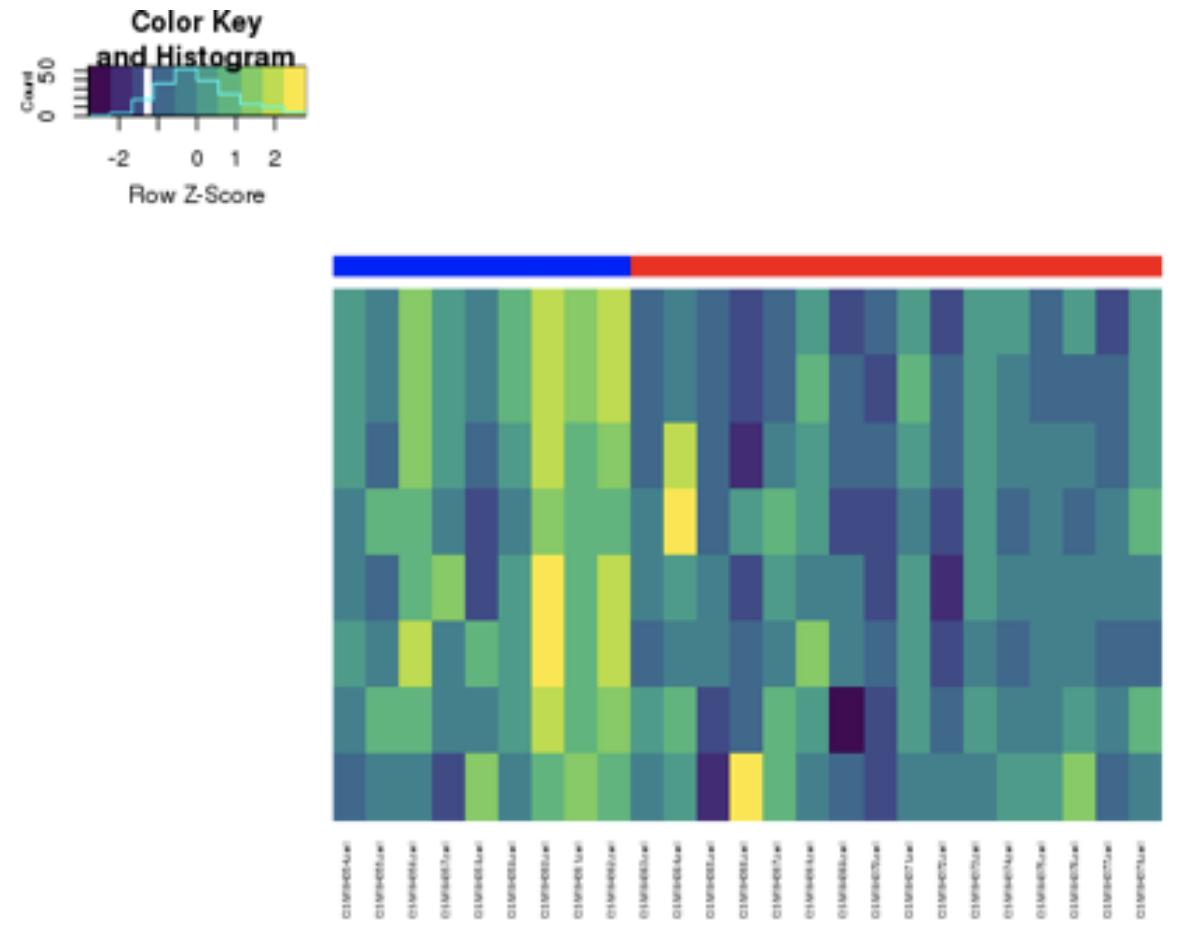


Methodology: Mancarci et al., *eNeuro*, 2017
 Application to SCZ and BD: Toker et al., *Biol. Psych.*, 2018

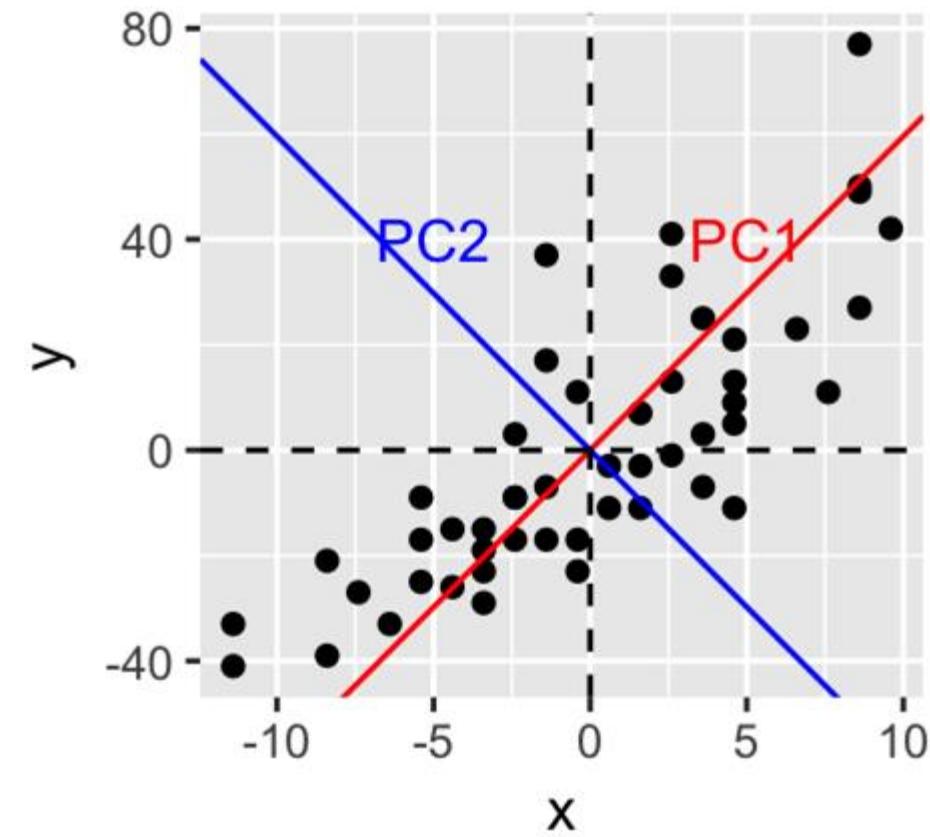
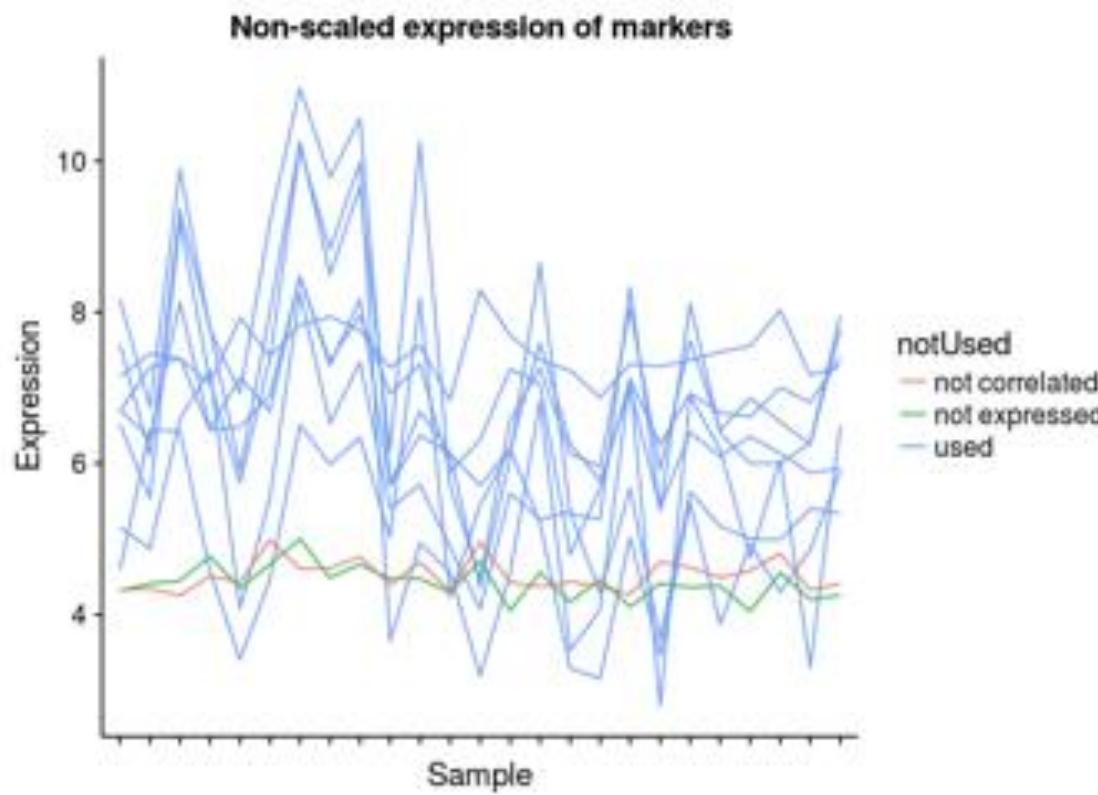
Marker gene profile estimation



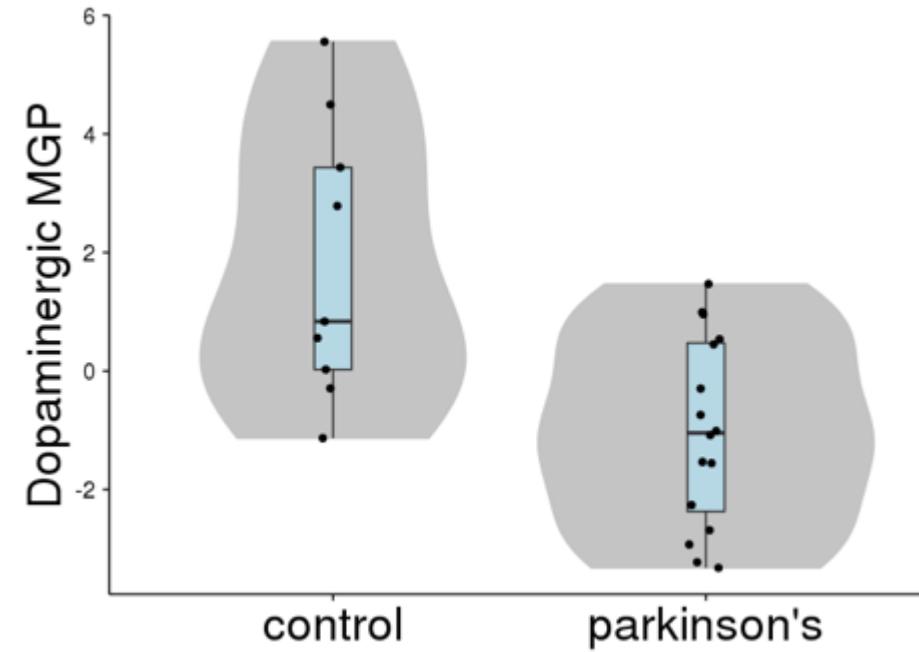
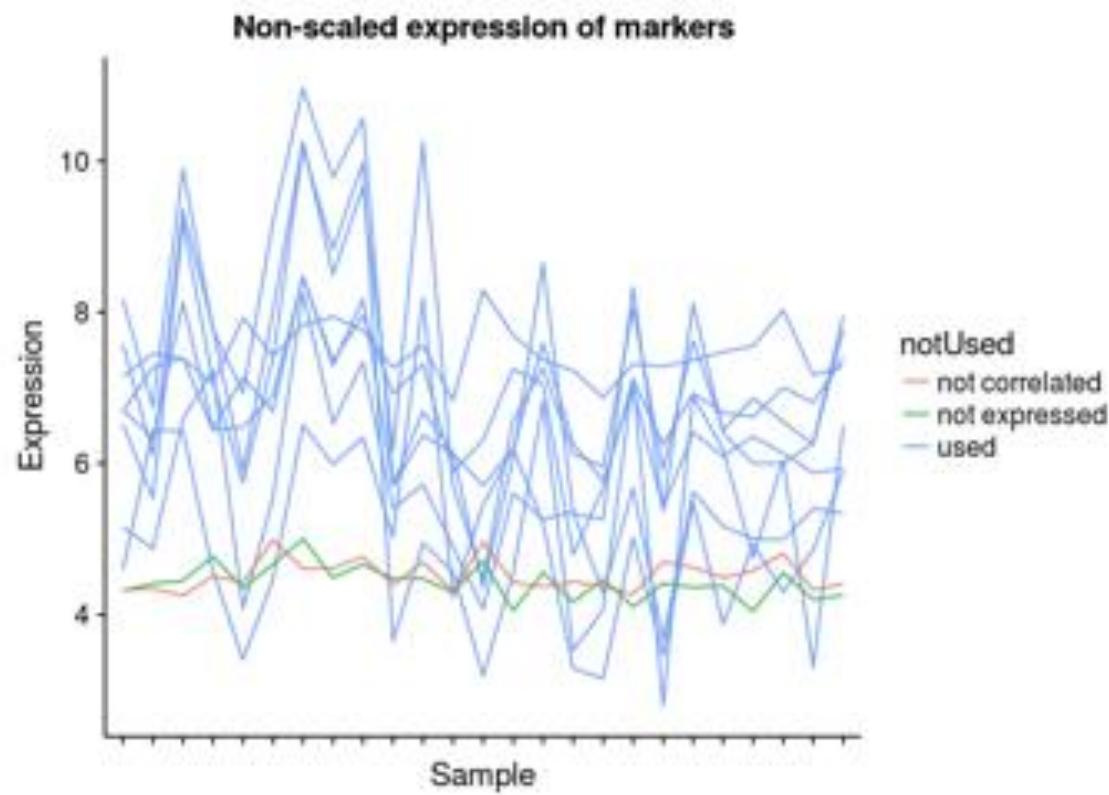
Cell type markers are correlated in bulk samples



Cell type markers are correlated in bulk samples



Marker gene profile estimation



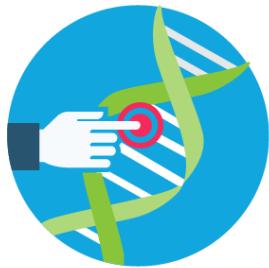
Tutorial : Interpreting bulk expression data for cell type proportion changes

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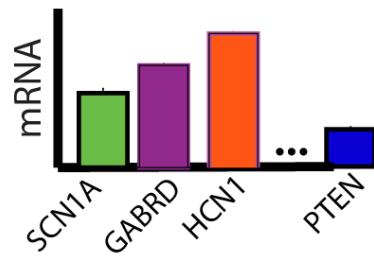
Break for questions

Bridging brain scales

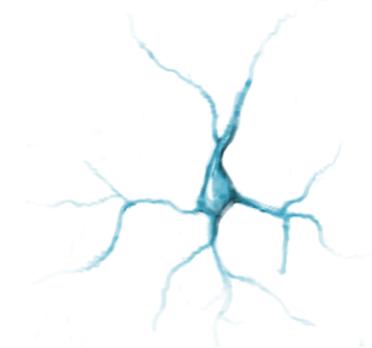
Genetics



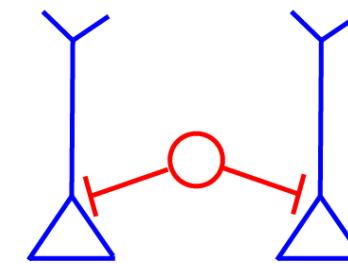
Gene Expression



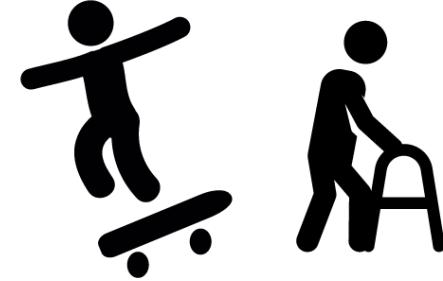
Cellular Physiology



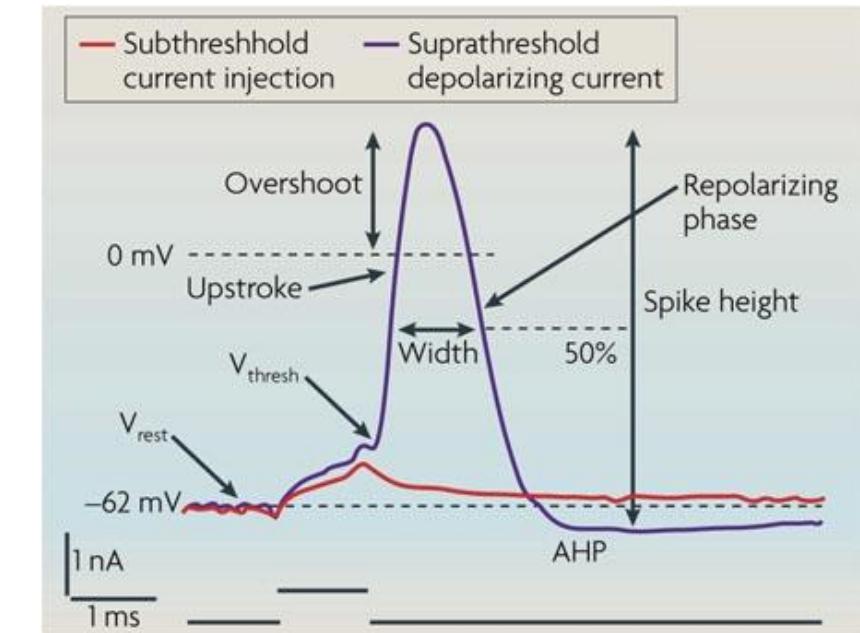
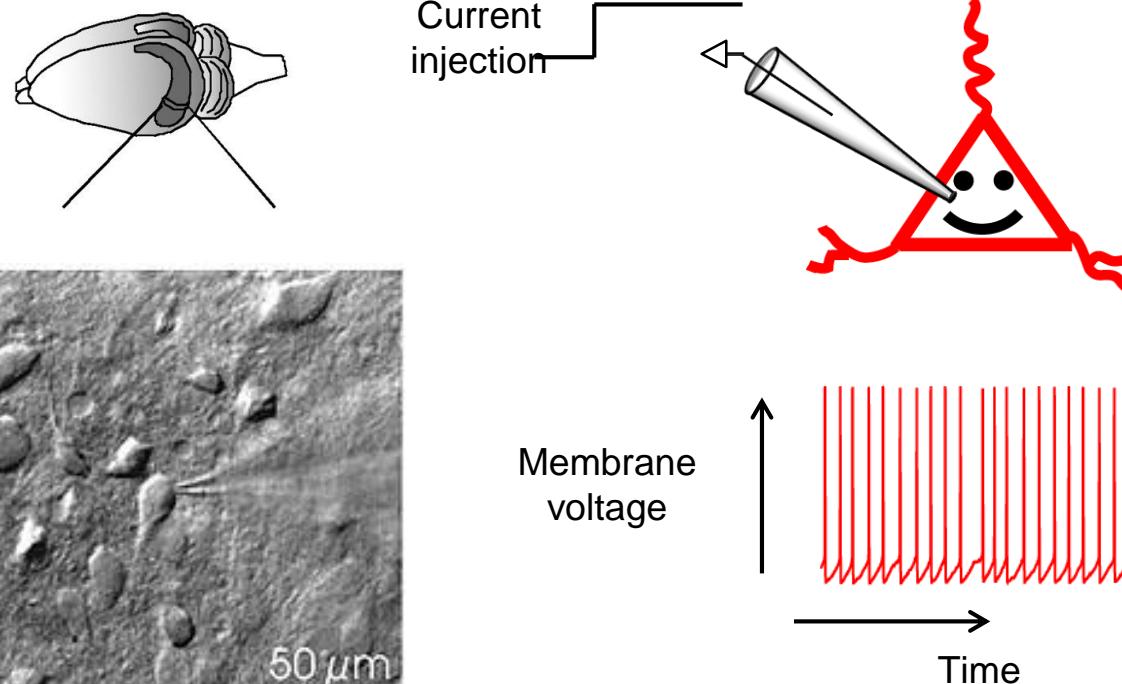
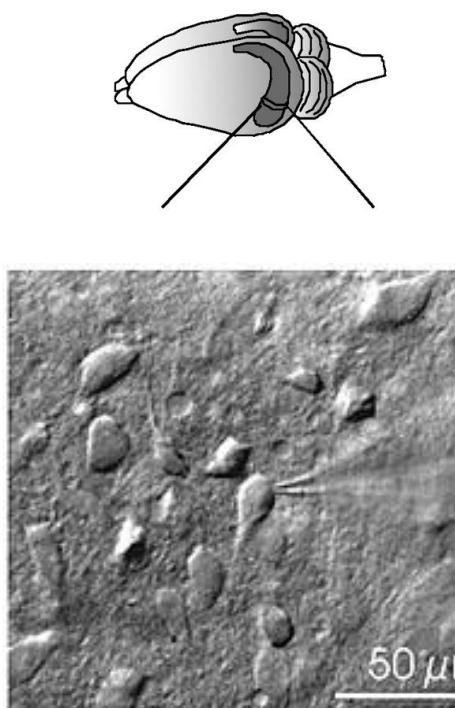
Neural Circuits



Traits & Behaviors

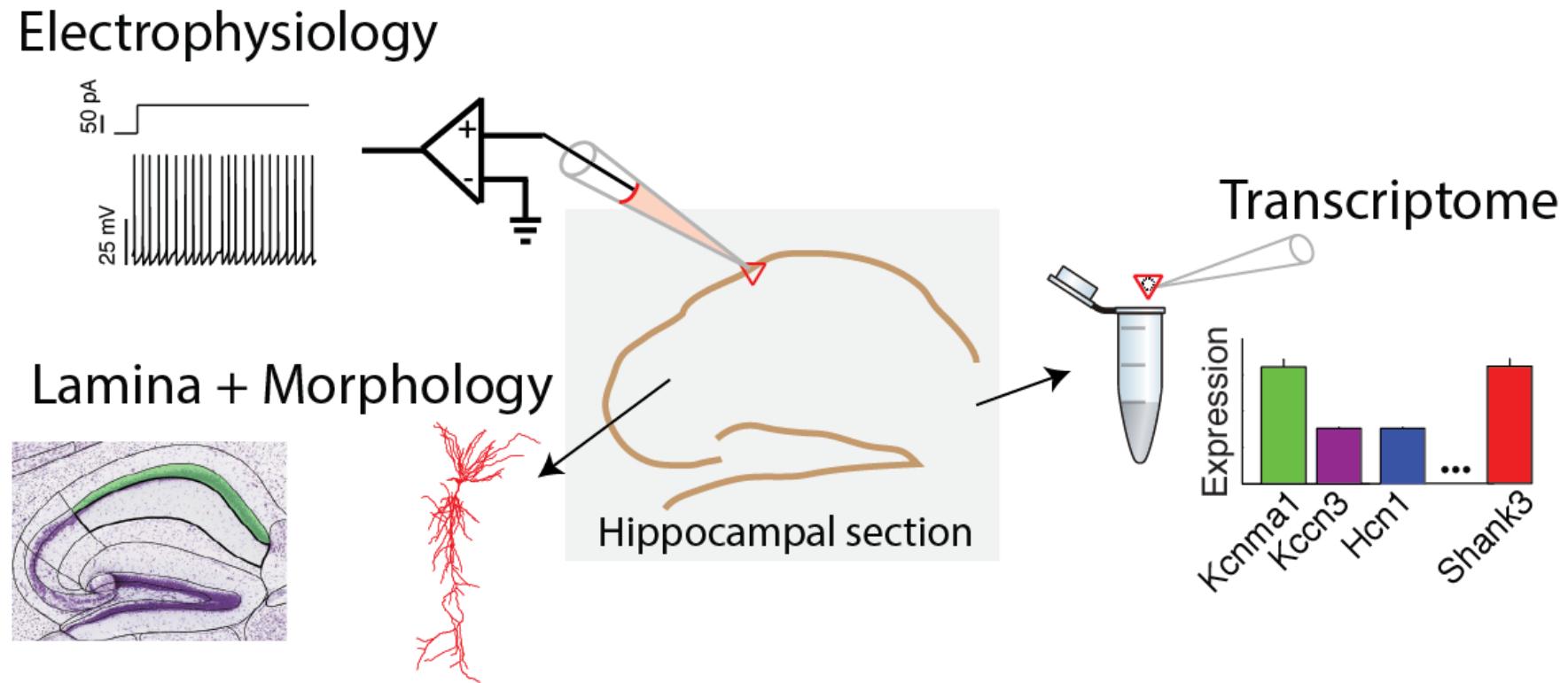


Intracellular electrophysiology

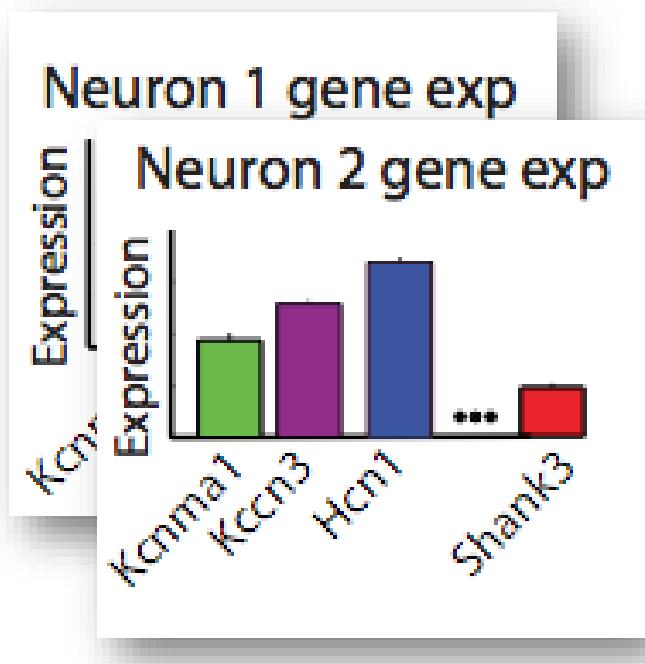


Nature Reviews | Neuroscience

Patch-seq combines scRNAseq, ephys, and morphology

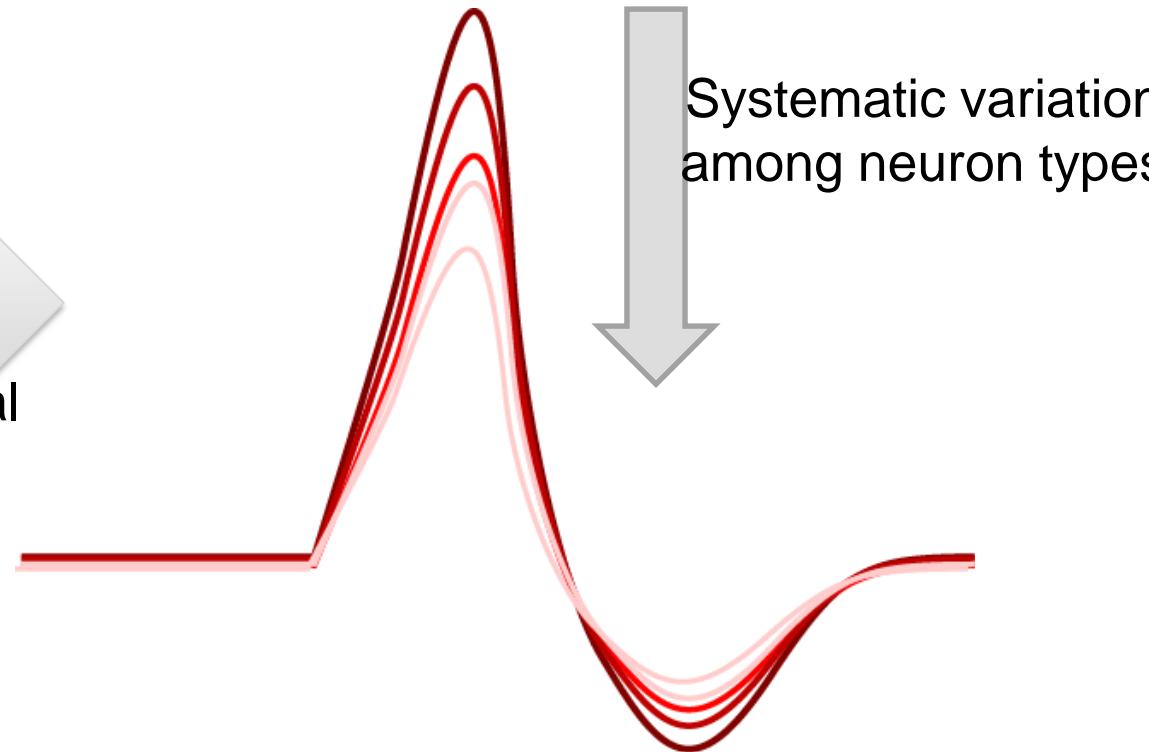


Identifying genes underlying diversity in cell phenotypes



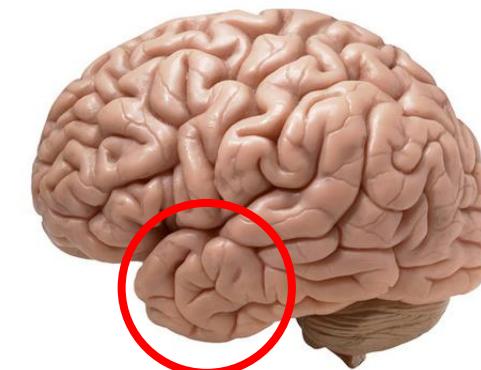
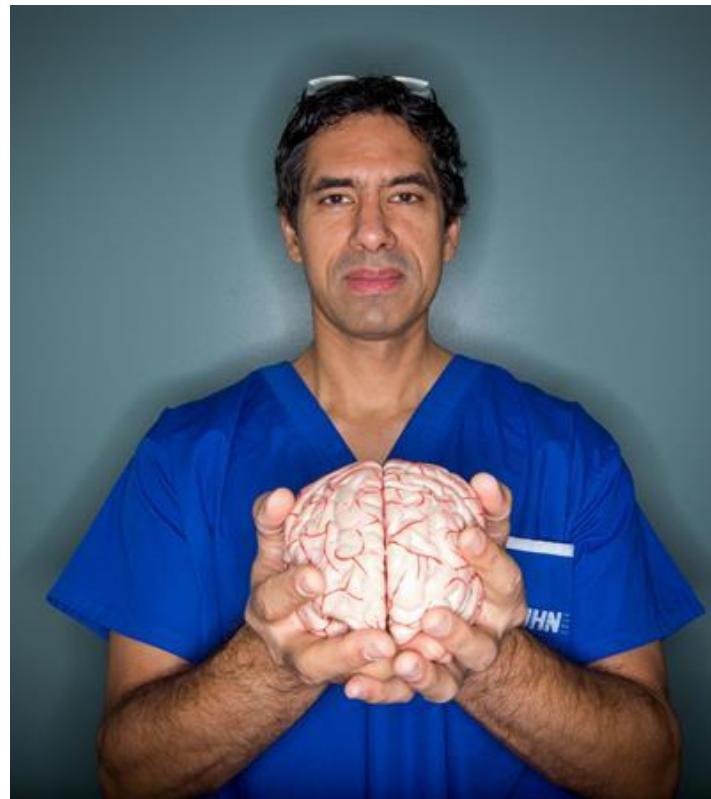
Patterns of gene expression

Electrophysiological phenotypes



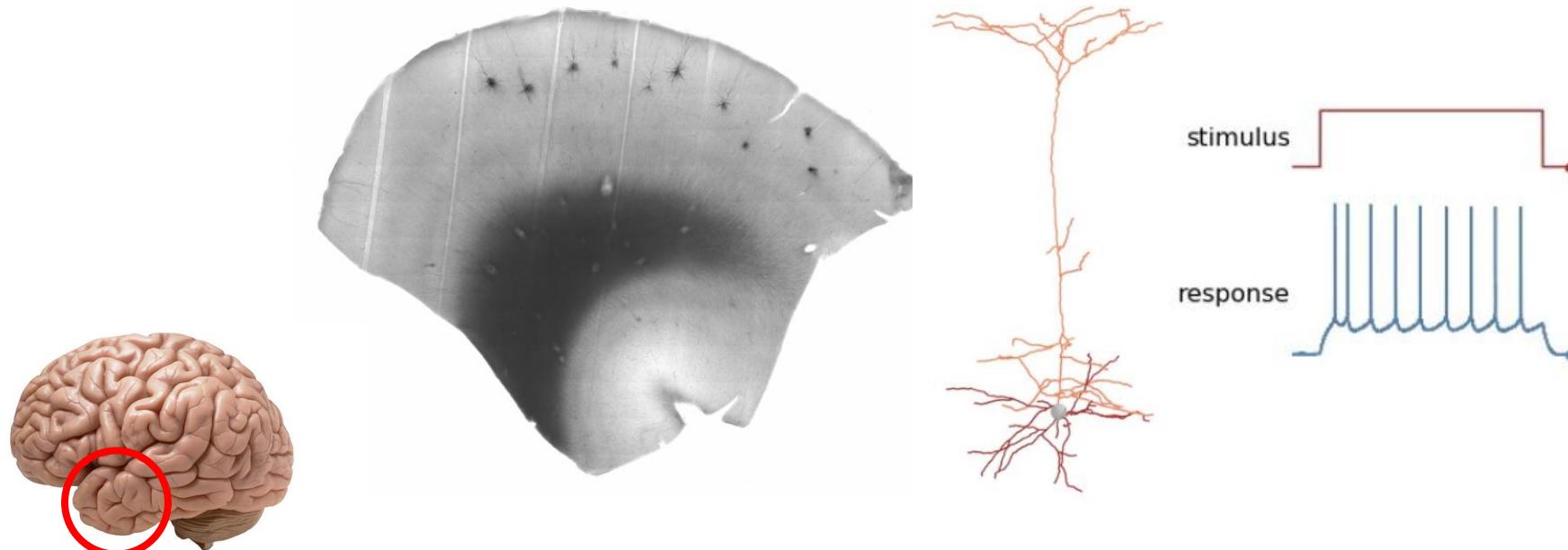
Systematic variation
among neuron types

Neurosurgery enables access to cells in the human brain



Exploring neuron diversity in the human brain

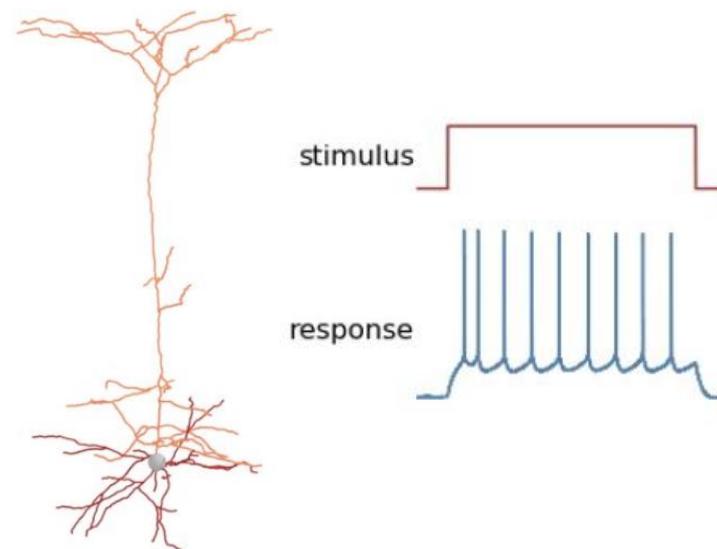
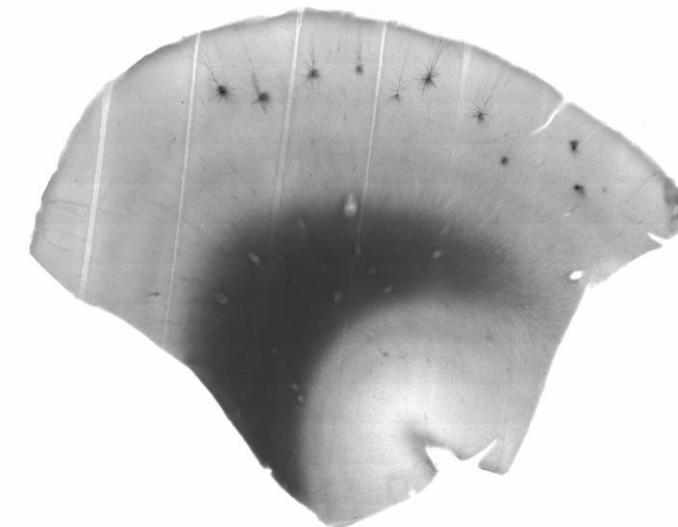
Human neurosurgical tissue



Homeira Moradi, Valiante Lab
Moradi et al, *Nat Comm.* 2021

Exploring neuron diversity in the human brain

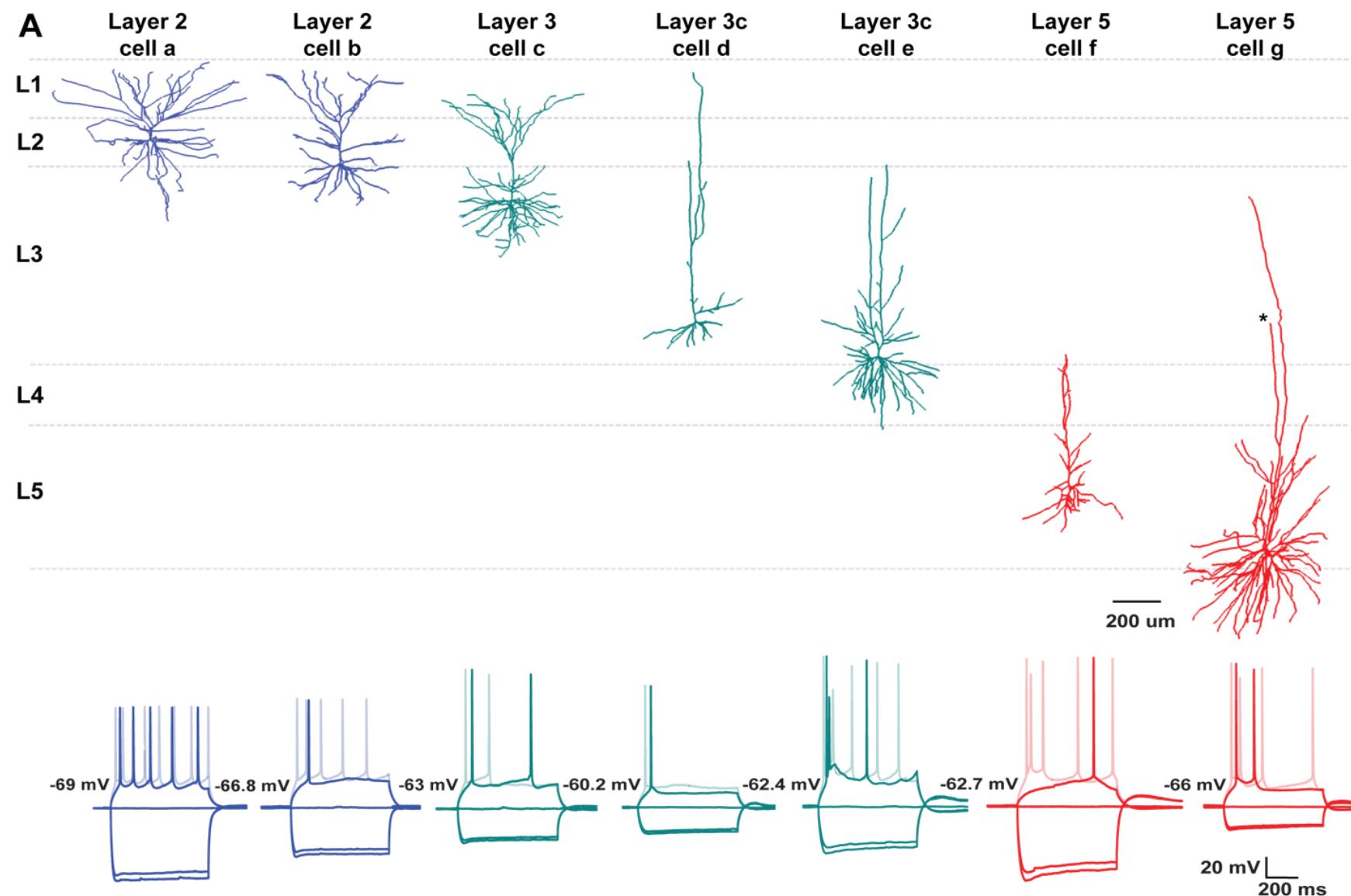
Human neurosurgical tissue



Major Questions

- How are human neurons different...
 - from one another?
 - from those in other species?
 - in aging?
 - because of genetics?
- Can we use these data to build “template” circuits of the human brain in health and in disease?
 - In collaboration with Hay Lab and KCNI

Exploring neuron diversity in the human brain

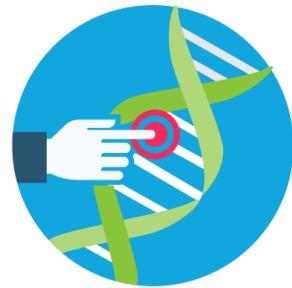


Towards personalized mental health

Clinical populations

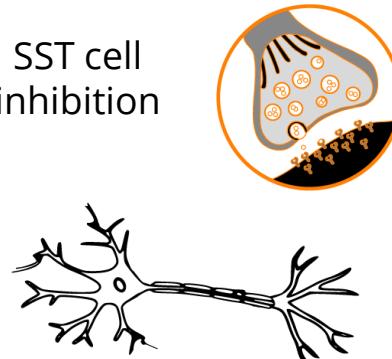


Genomics sampling
(DNA, RNA, etc.)

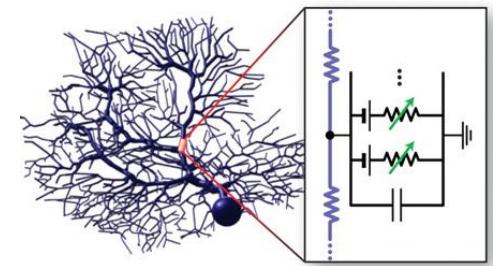


Inference of activity
in brain cells

SST cell inhibition



Personalized brain models



$$\frac{dm}{dt} = \alpha_m(V_m)(1 - m) - \beta_m(V_m)m$$

$$\frac{dh}{dt} = \alpha_h(V_m)(1 - h) - \beta_h(V_m)h$$



Acknowledgements

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