

Regional tropism of sporadic CJD subtypes in human brain organoids

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Presenting for Cathryn Haigh, PHD

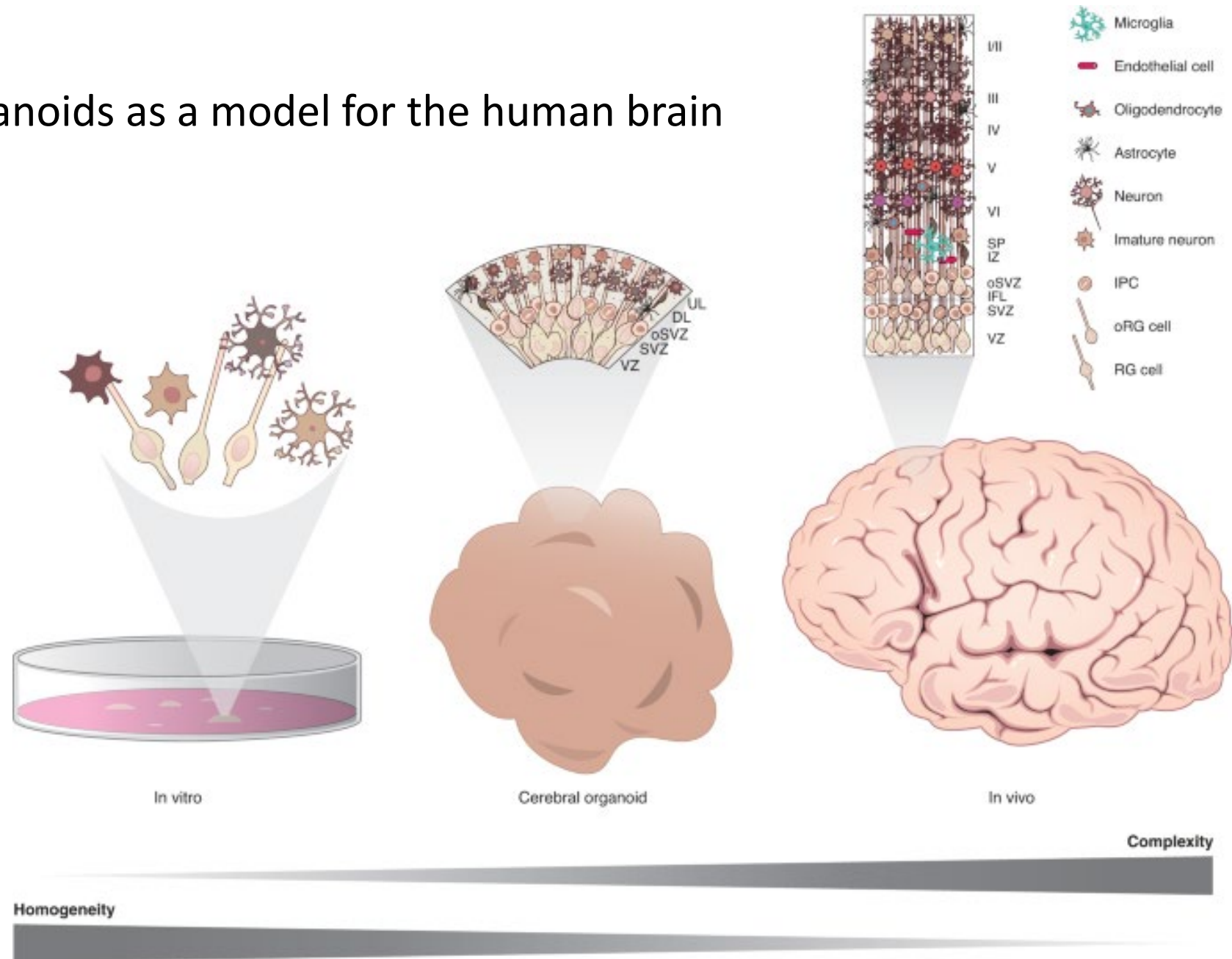
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*Previously the Laboratory of Persistent Viral Diseases (LPVD)

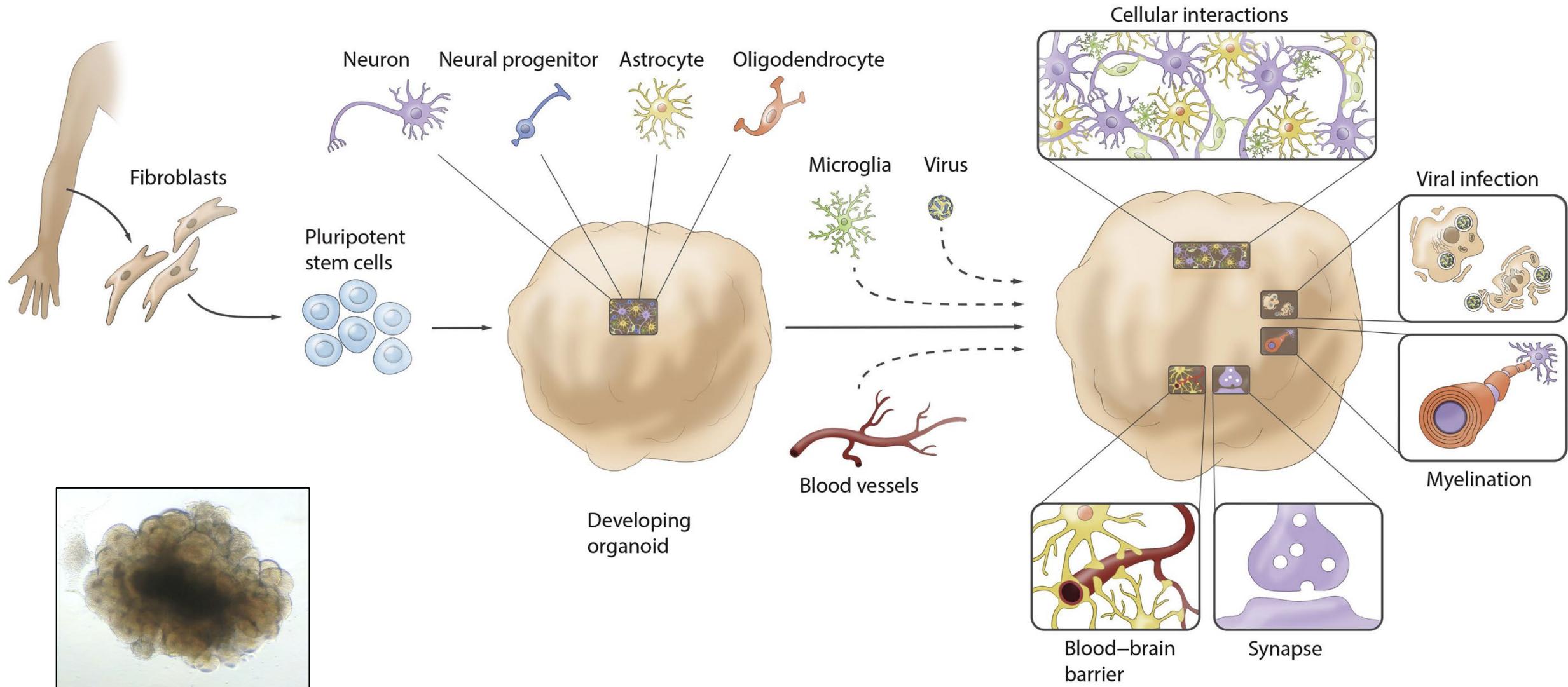
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Organoids as a model for the human brain

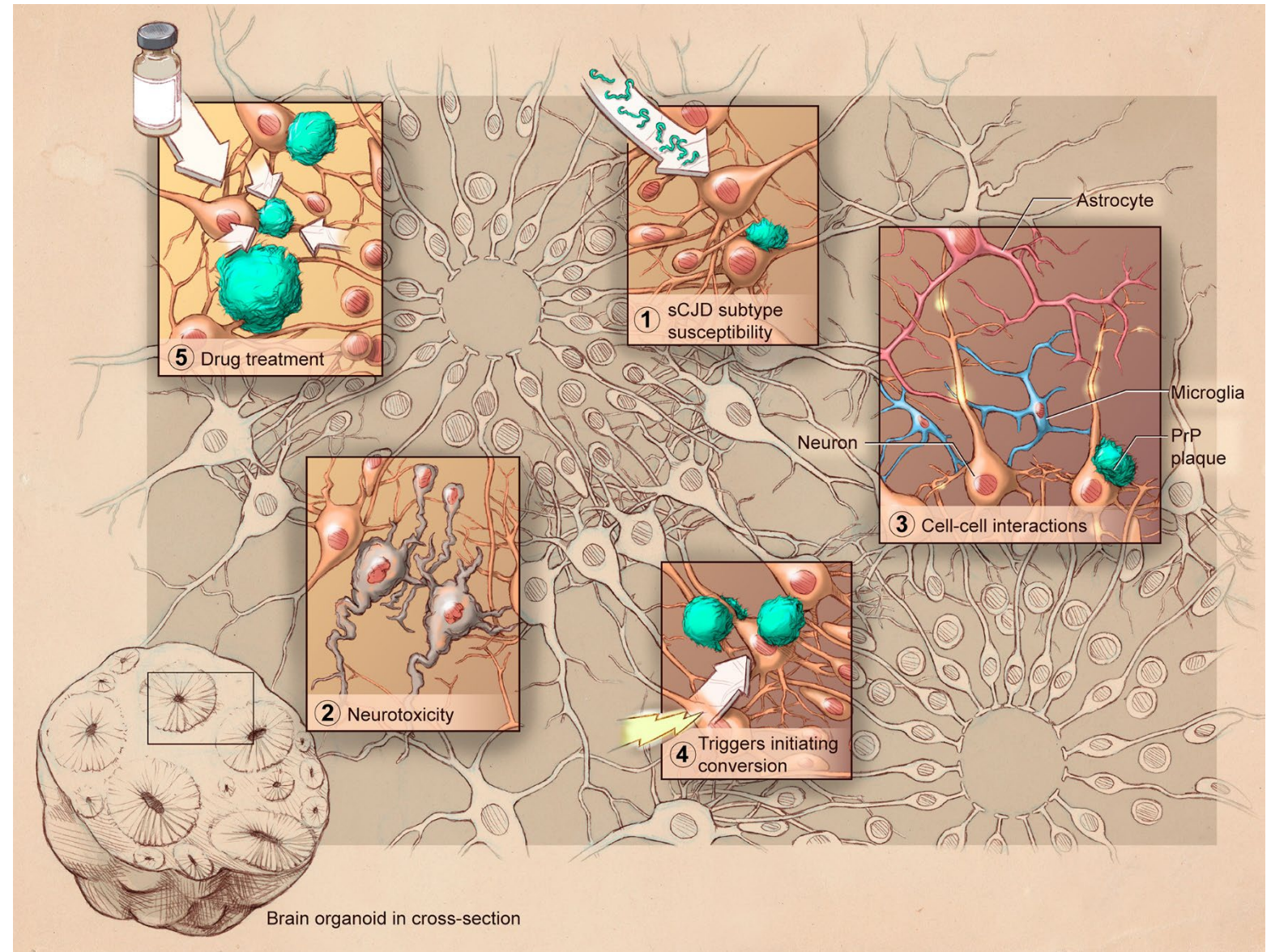


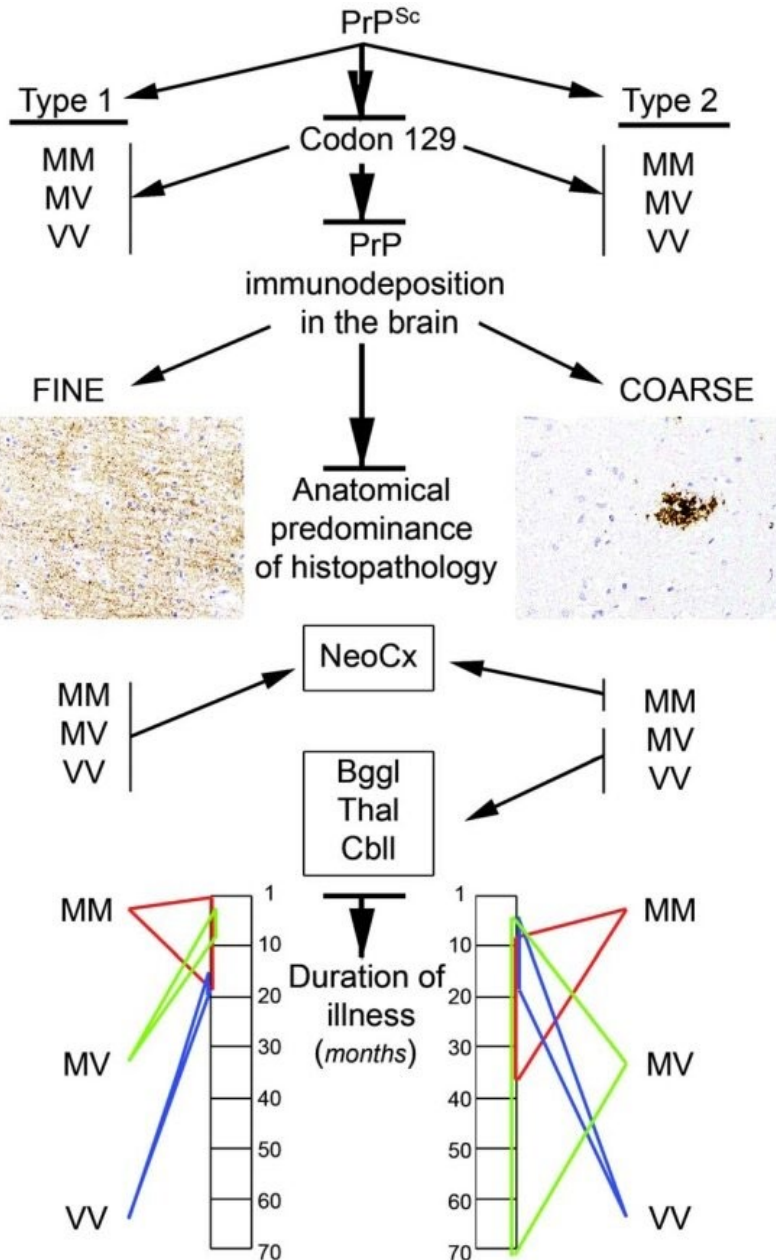
What is a brain organoid?



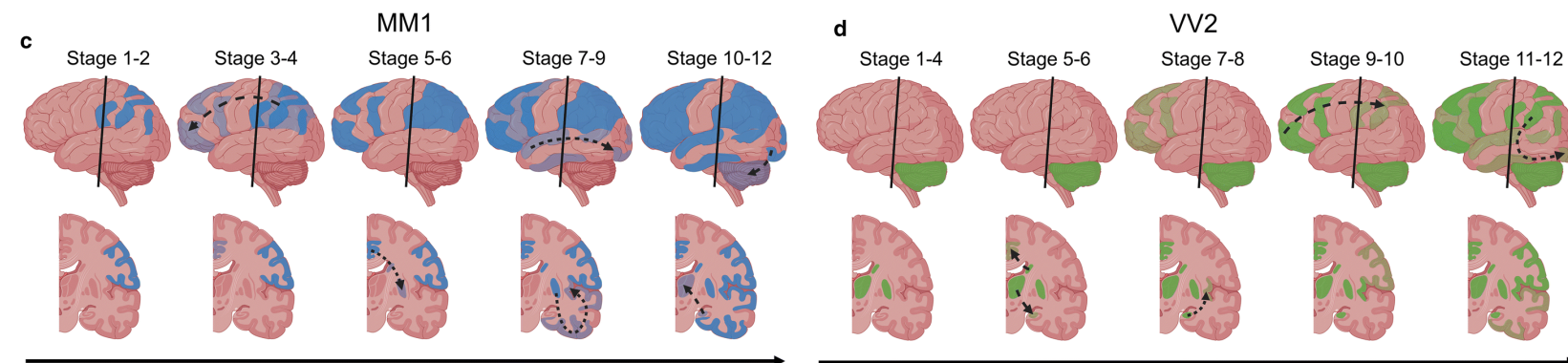
Trends in Cell Biology
Marton and Pasca, 2020

Lessons from cerebral organoid studies of prion disease so far...



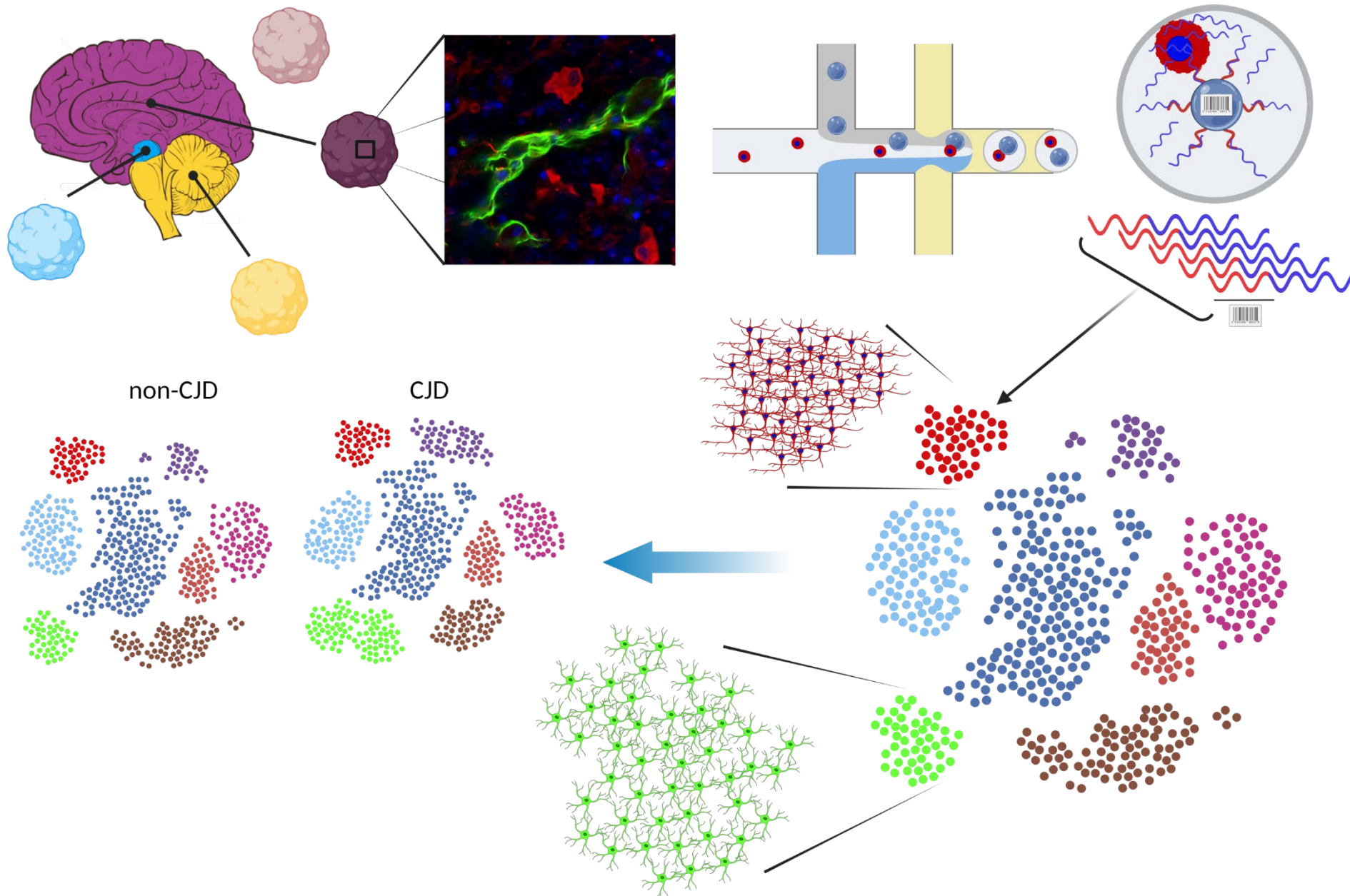


Different subtypes of sCJD affect the brain differently

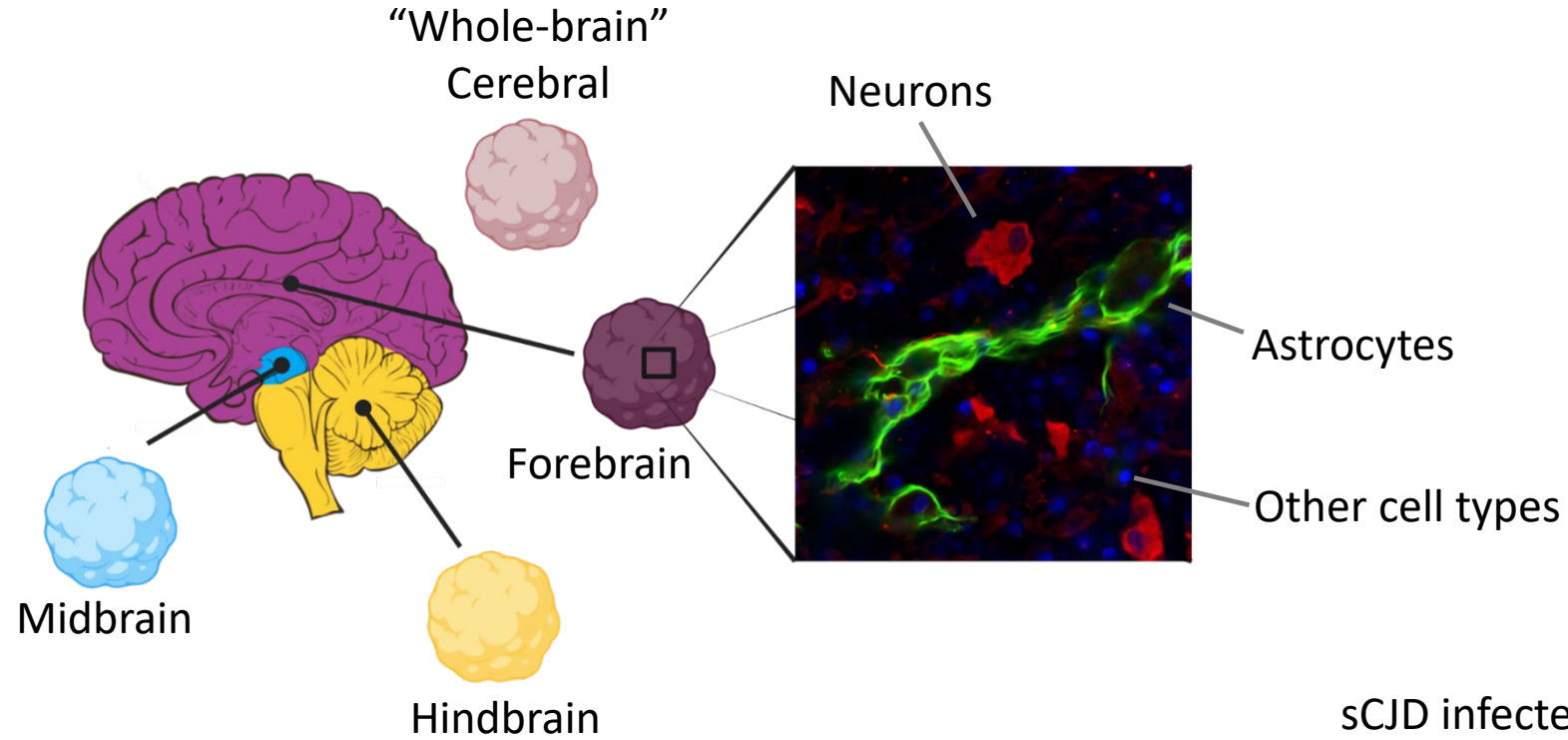


sCJD Group (No. of Cases)	MM1 (199)	MV1 (8)	VV1 (3)	MM2-C (6)	MM2-T (6)	MV2 (27)	VV2 (45)
Cognitive ^a	70	50	100	100	67	74	27
Aphasia	23	25	33	33	0	11	0
Visual ^b	26	12	0	0	0	0	0
Oculomotor	6	12	0	0	17	19	32
Gait or limb ataxia	33	75	0	0	67	81	100
Dysarthria	5	12	0	0	33	11	13
Myoclonus	18	12	0	0	0	0	0
Other dyskinesias	4	0	0	0	0	0	0
Pyramidal	6	0	0	0	0	0	0
Sensory	7	25	0	0	0	7	15
"Psychiatric" ^c	28	12	0	0	50	34	19
Insomnia	8	0	0	0	17	15	9
Unilateral	25	25	0	0	0	7	4

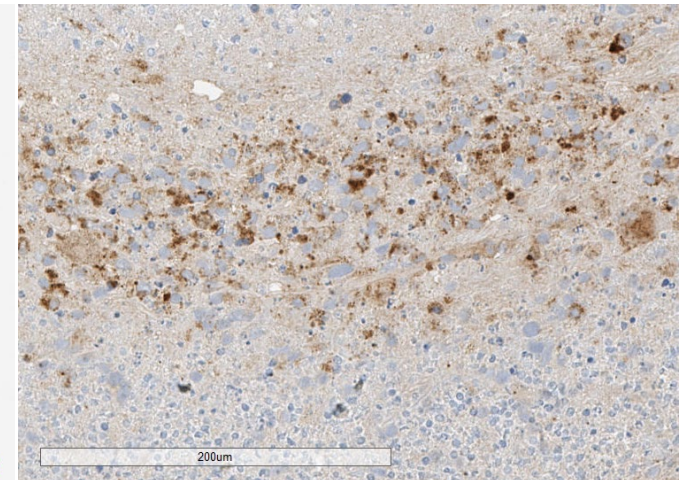
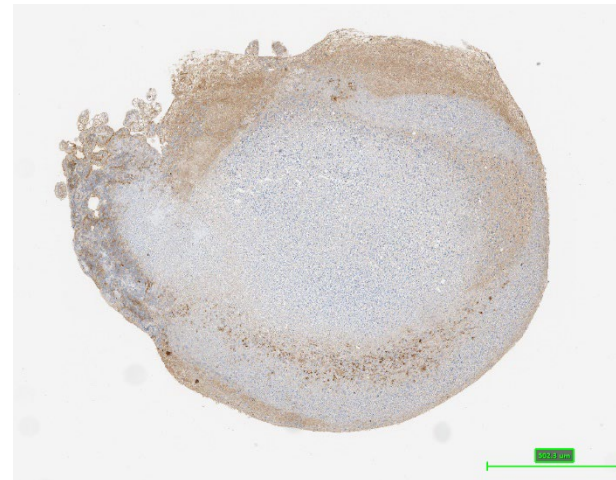
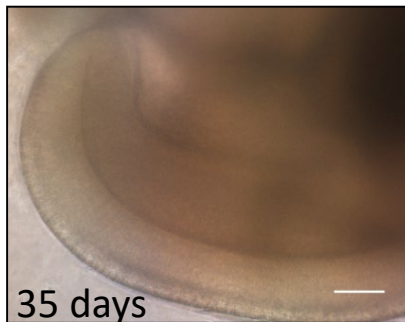
Single cell RNA sequencing outline



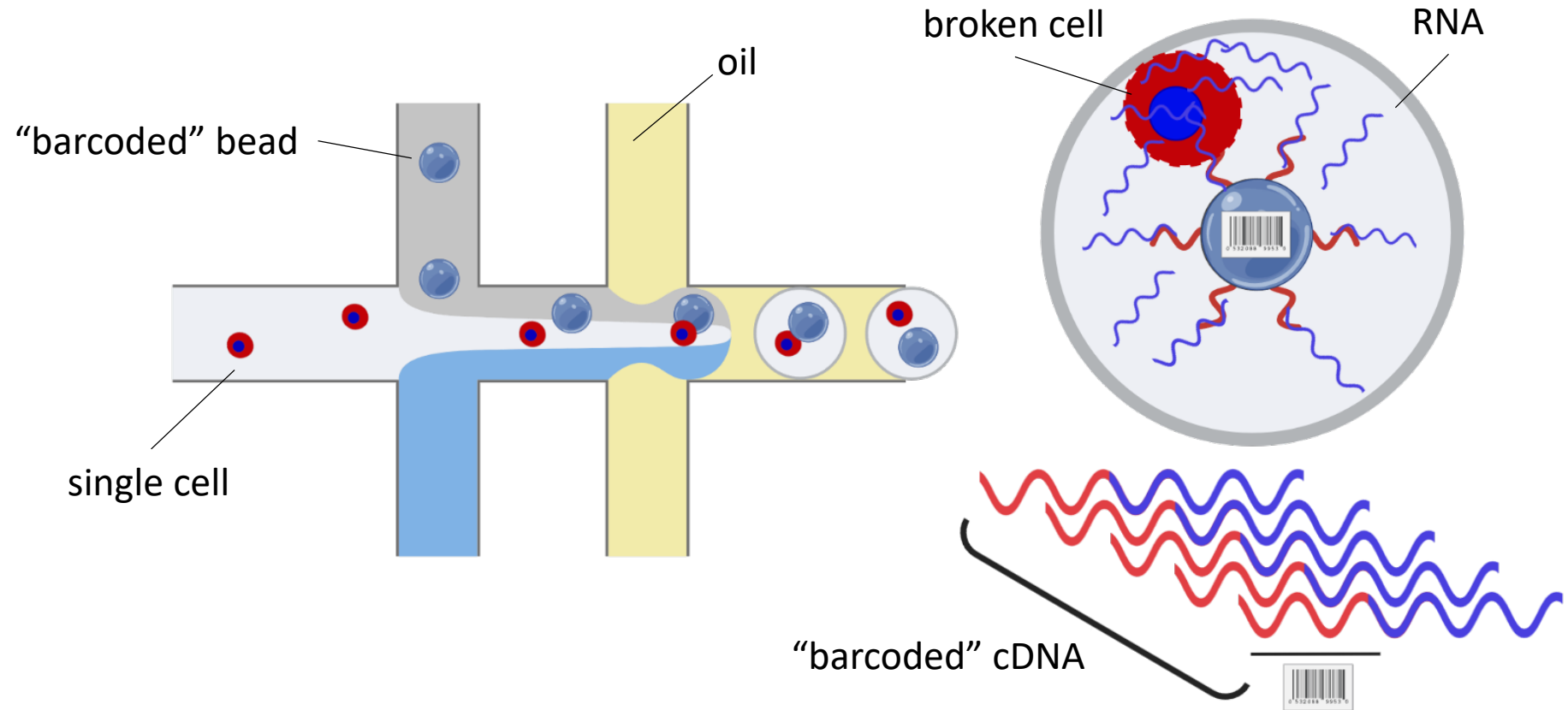
Brain organoids



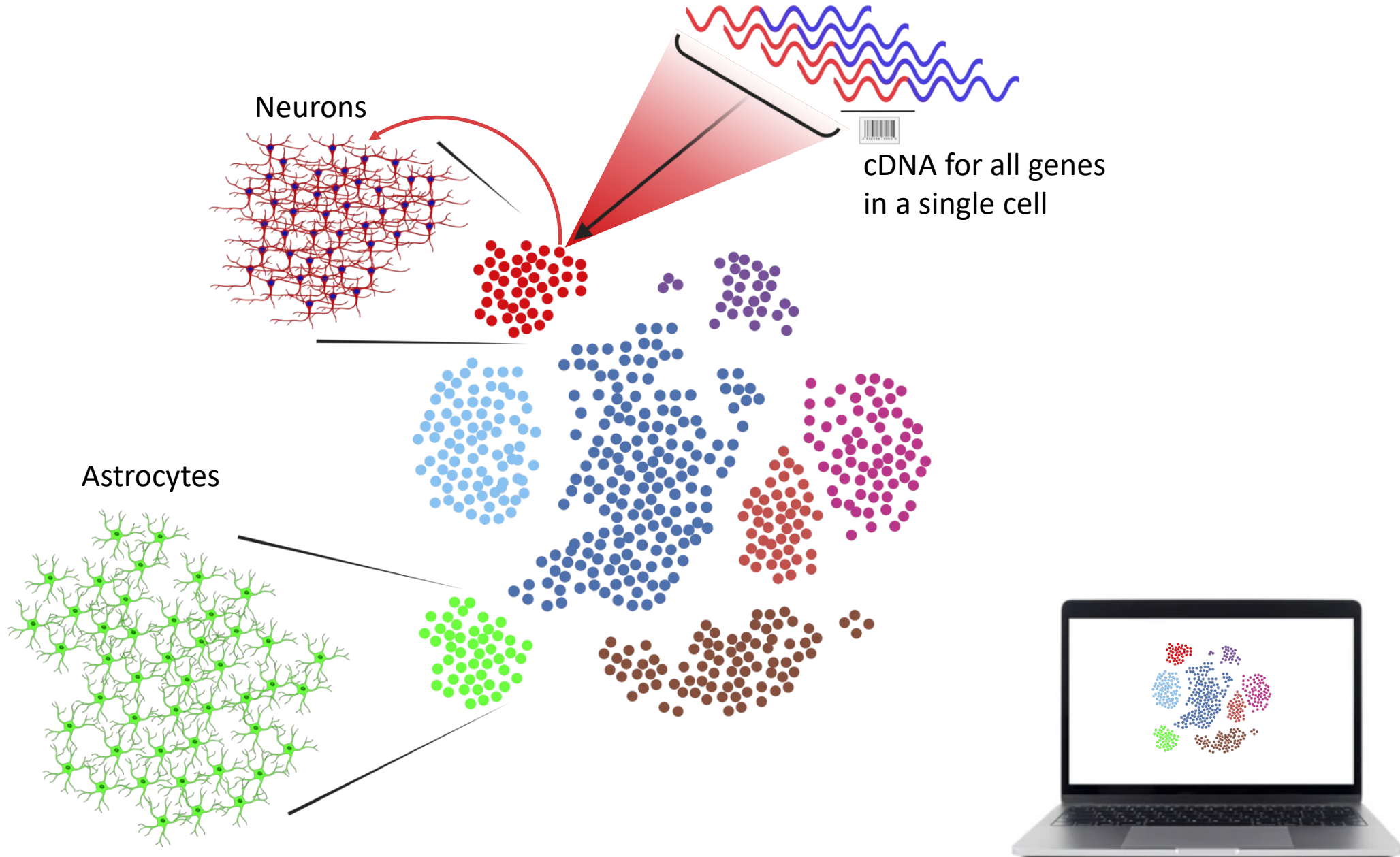
sCJD infected



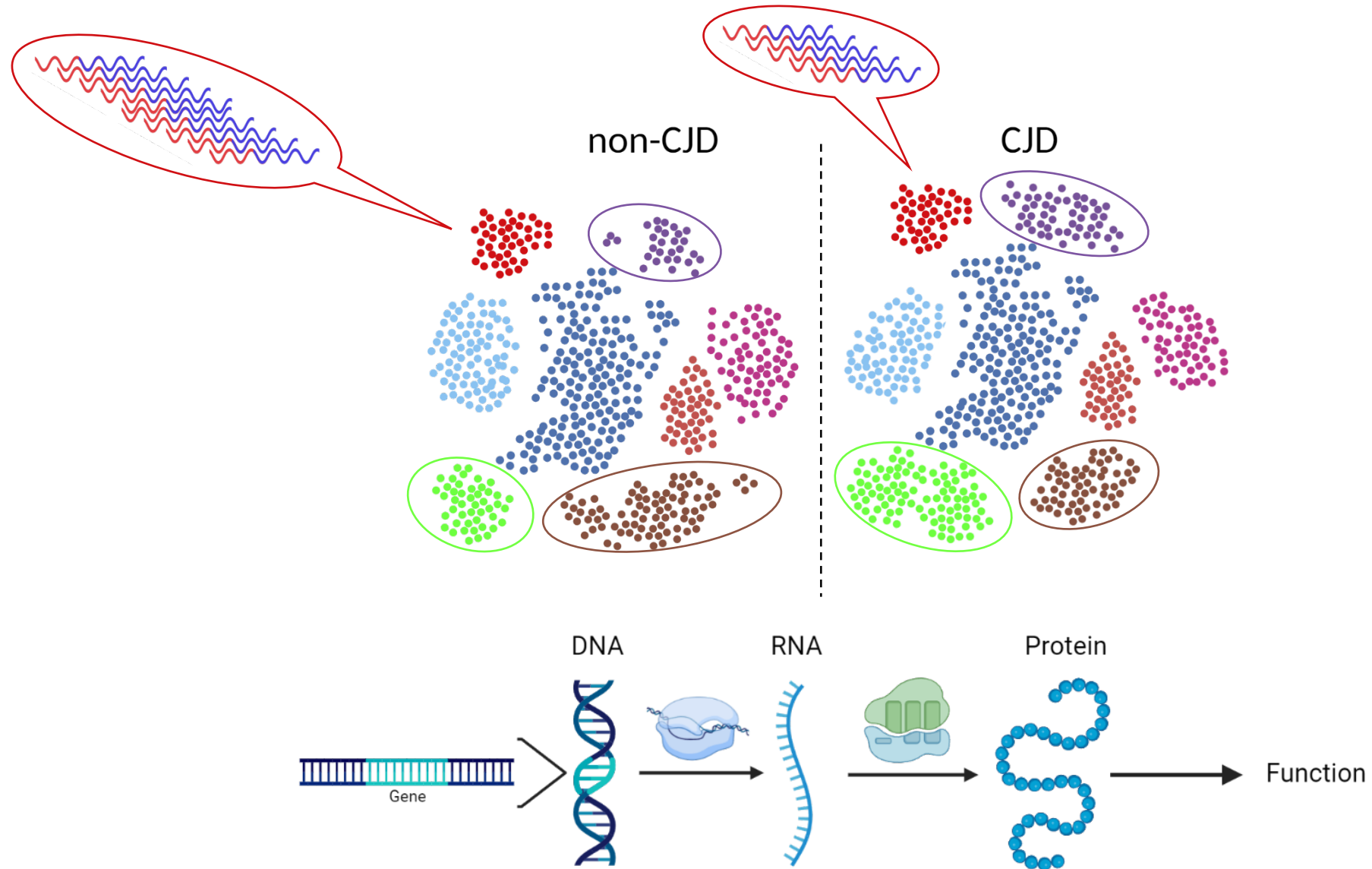
Marking all RNA in an individual cell with a specific identifier



Sorting cells into groups or “clusters” based on present genes



Defining differences based on number of cells in a group or number of genes in a cell



Forebrain organoids show same hallmarks of disease as brain



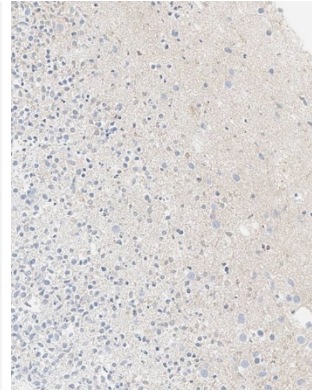
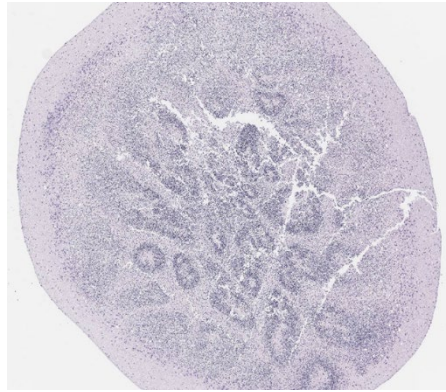
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General tissue stain

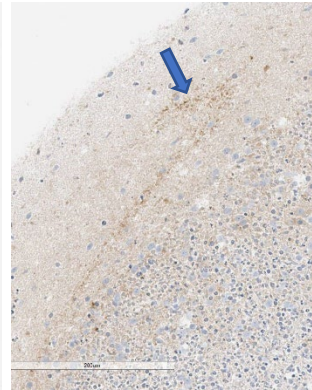
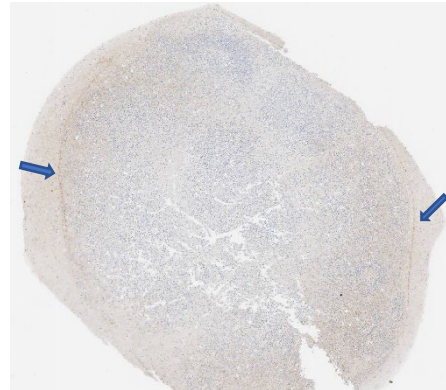
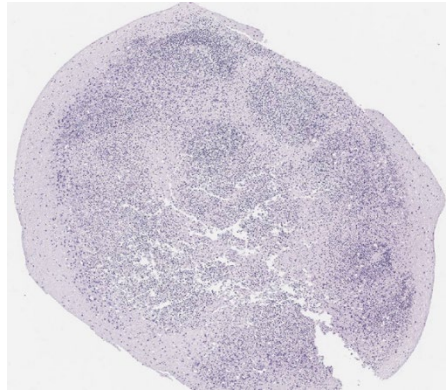
Prion protein deposition

PrP^D seeding activity

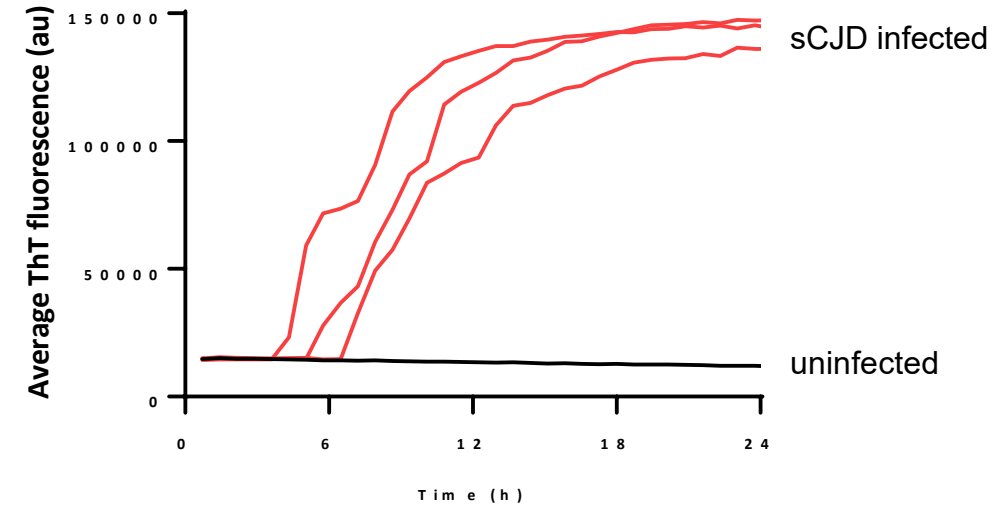
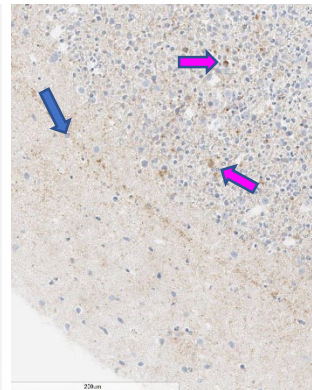
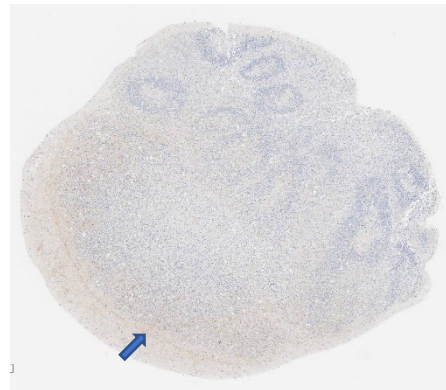
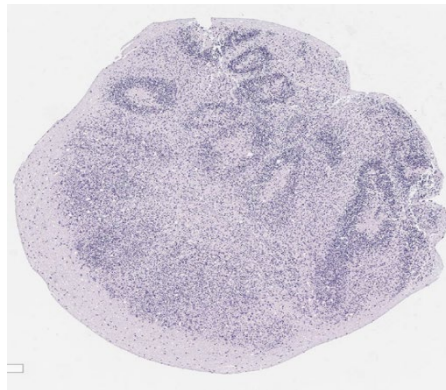
Uninfected
(MV)



MV1



MV2

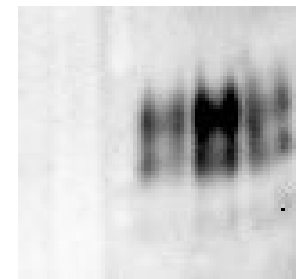


Protease resistant PrP^D

38 -

25 -

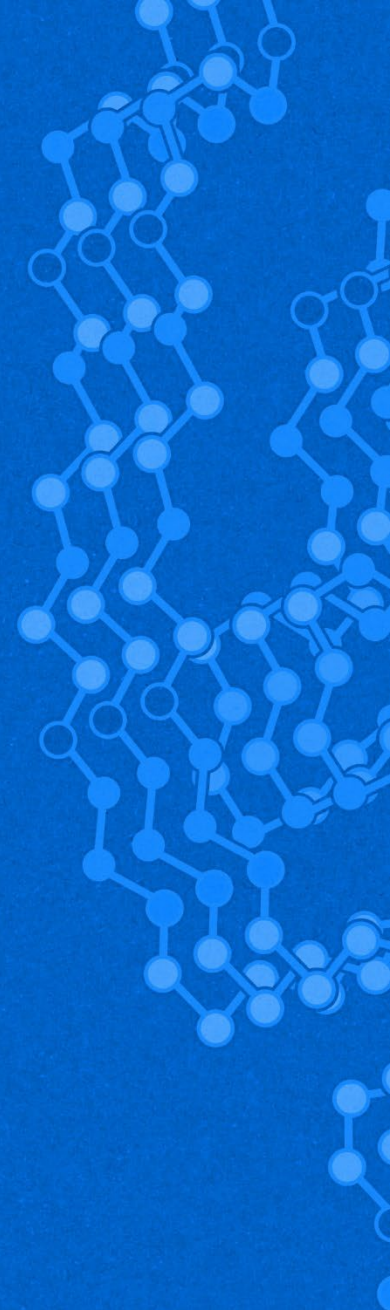
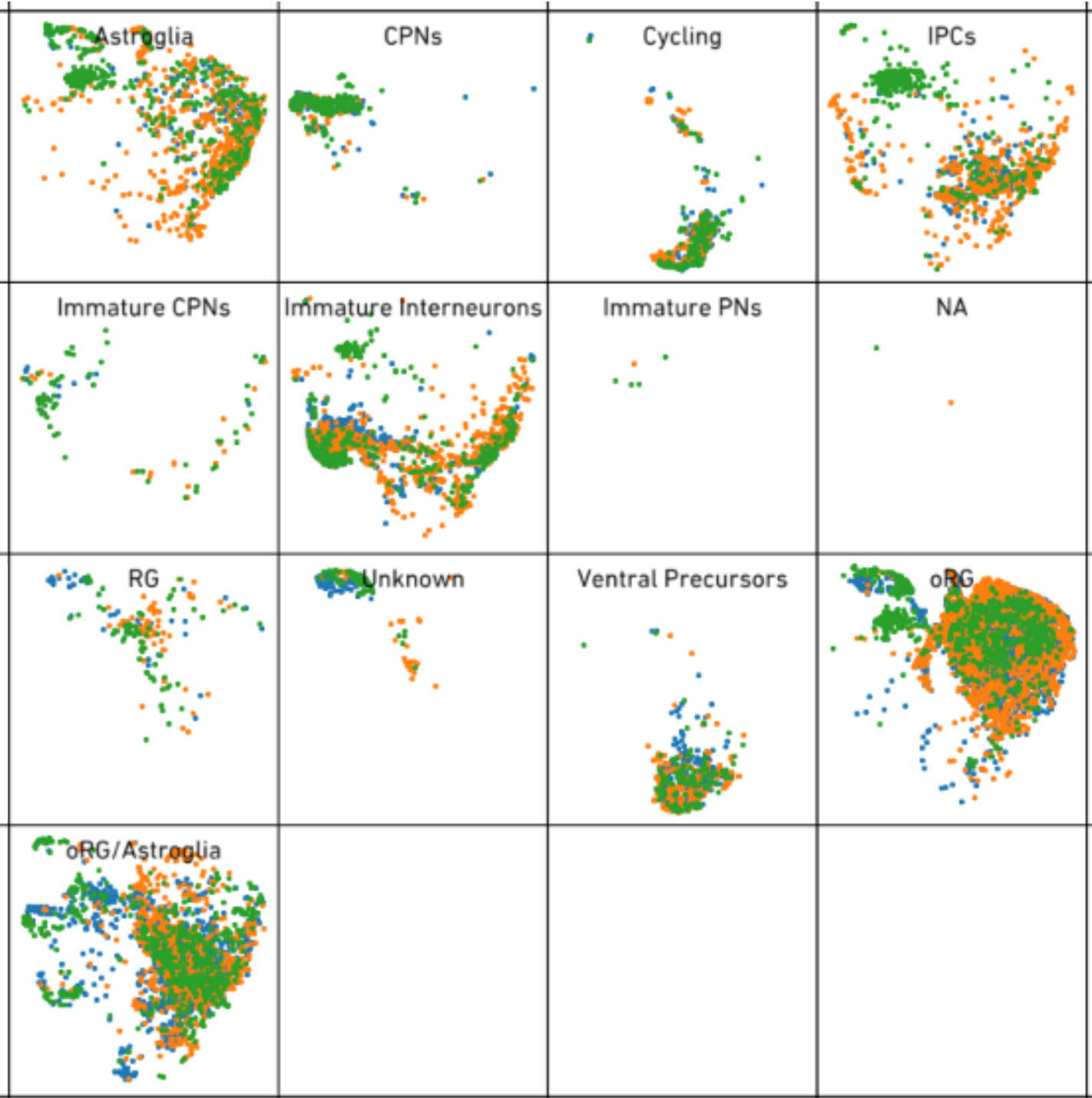
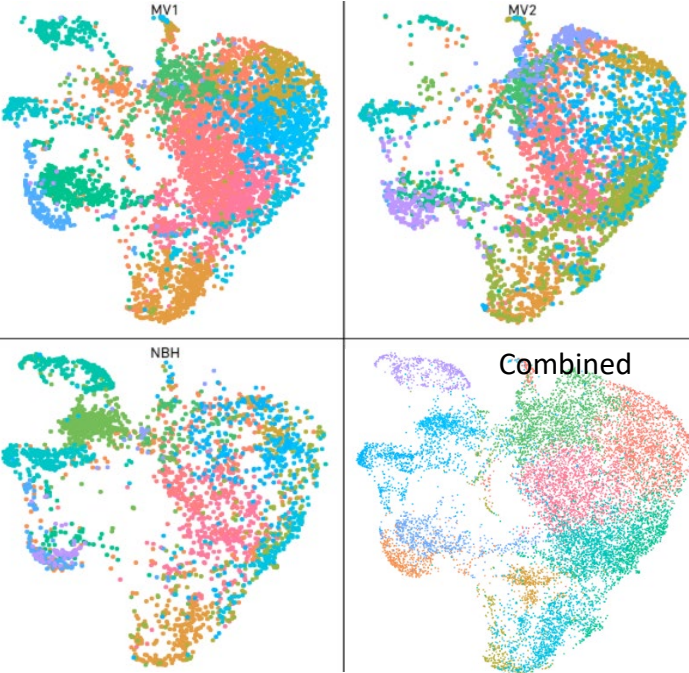
20 -



Uninfected

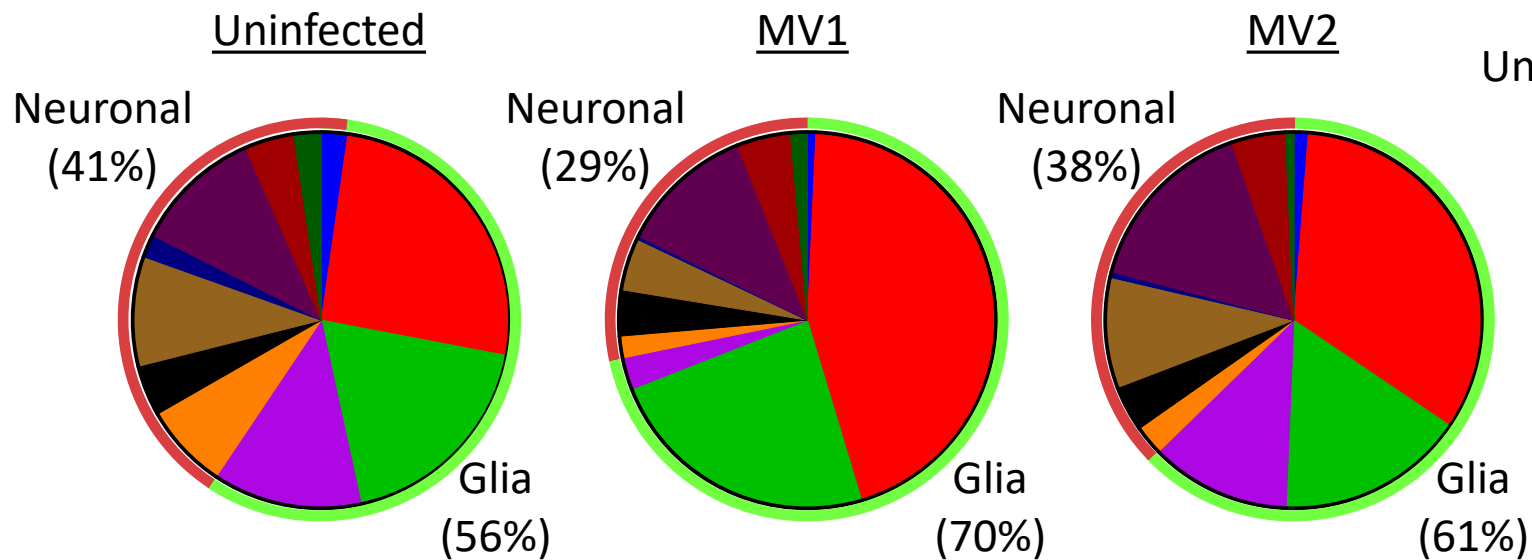
sCJD infected

What cell types and how many of each are present



Cell type abundance

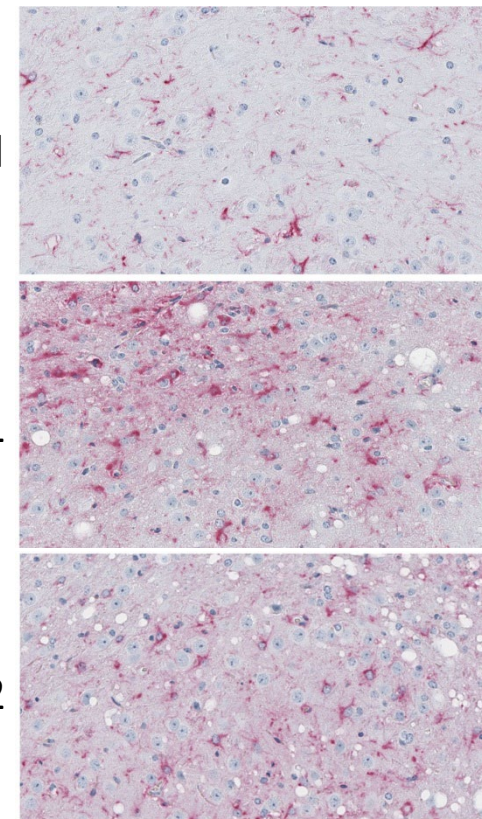
Glial stain



Uninfected

MV1

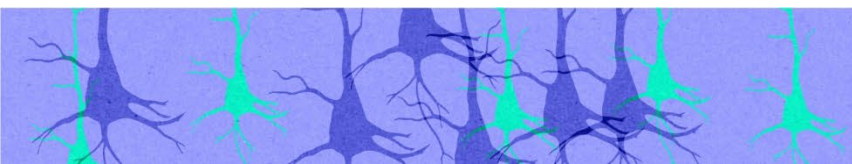
MV2

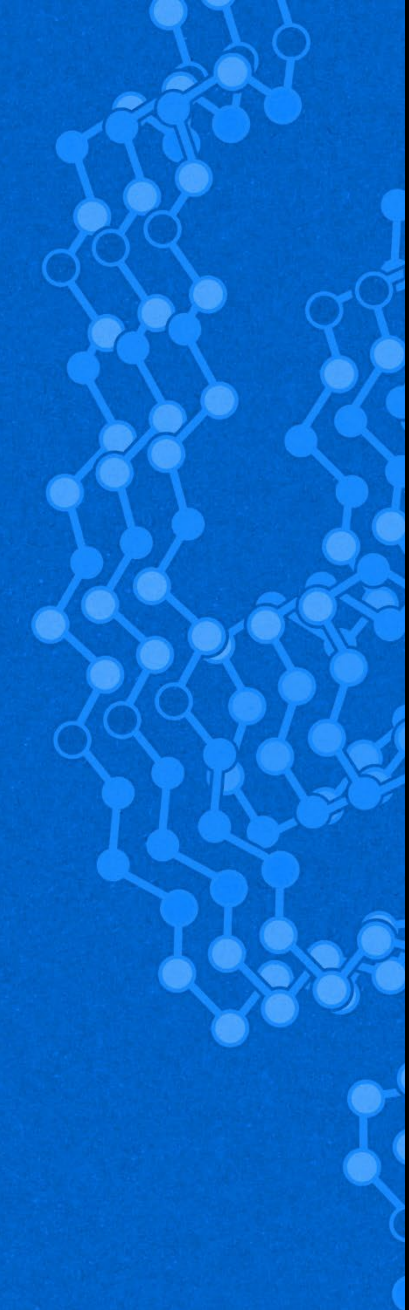


Cells per group: 3,491
Average # genes per cell: 288,738
Total genes analyzed: 1,007,984,358

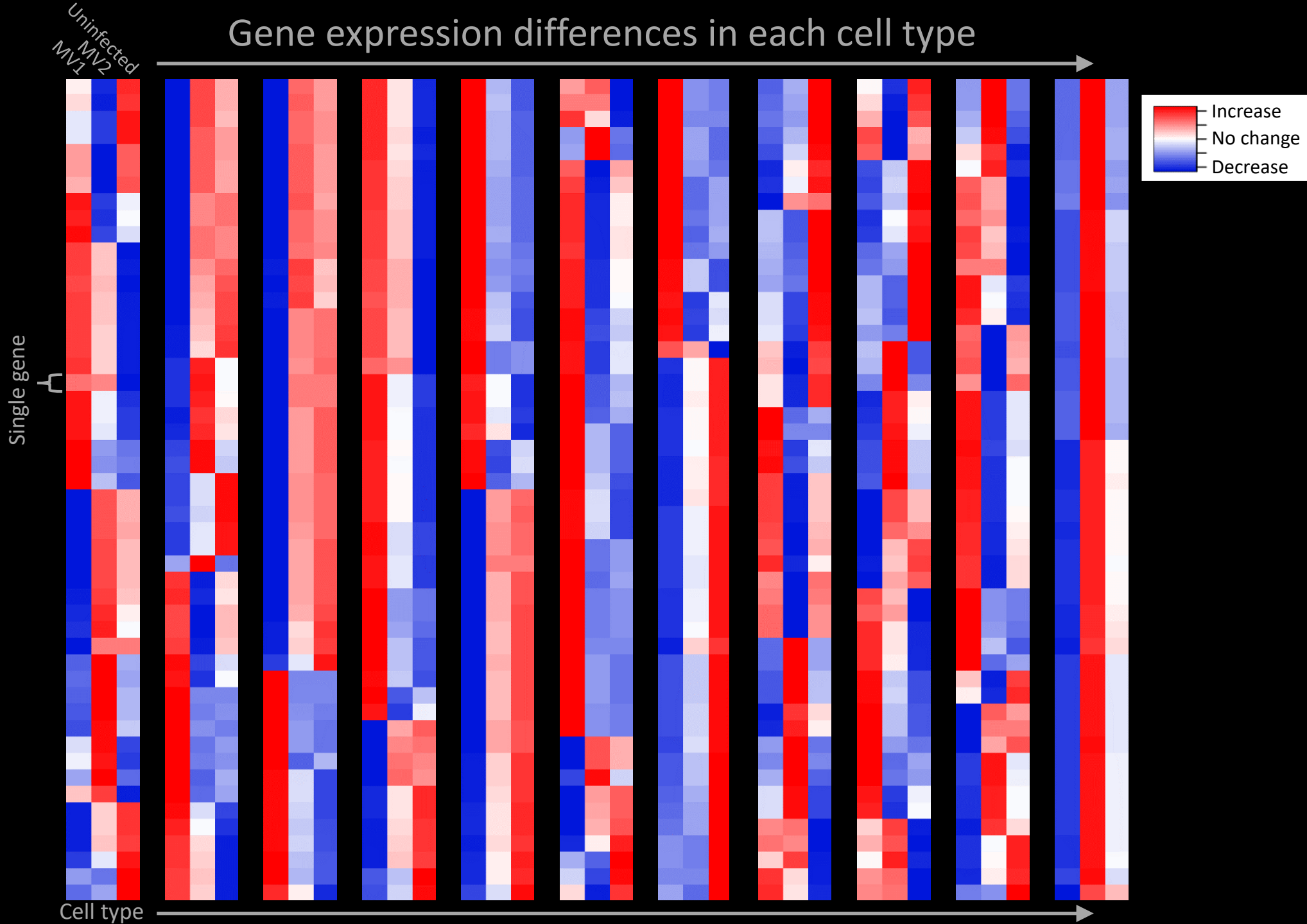
5202
332,506
1,729,696,212

3203
218,918
700,974,369

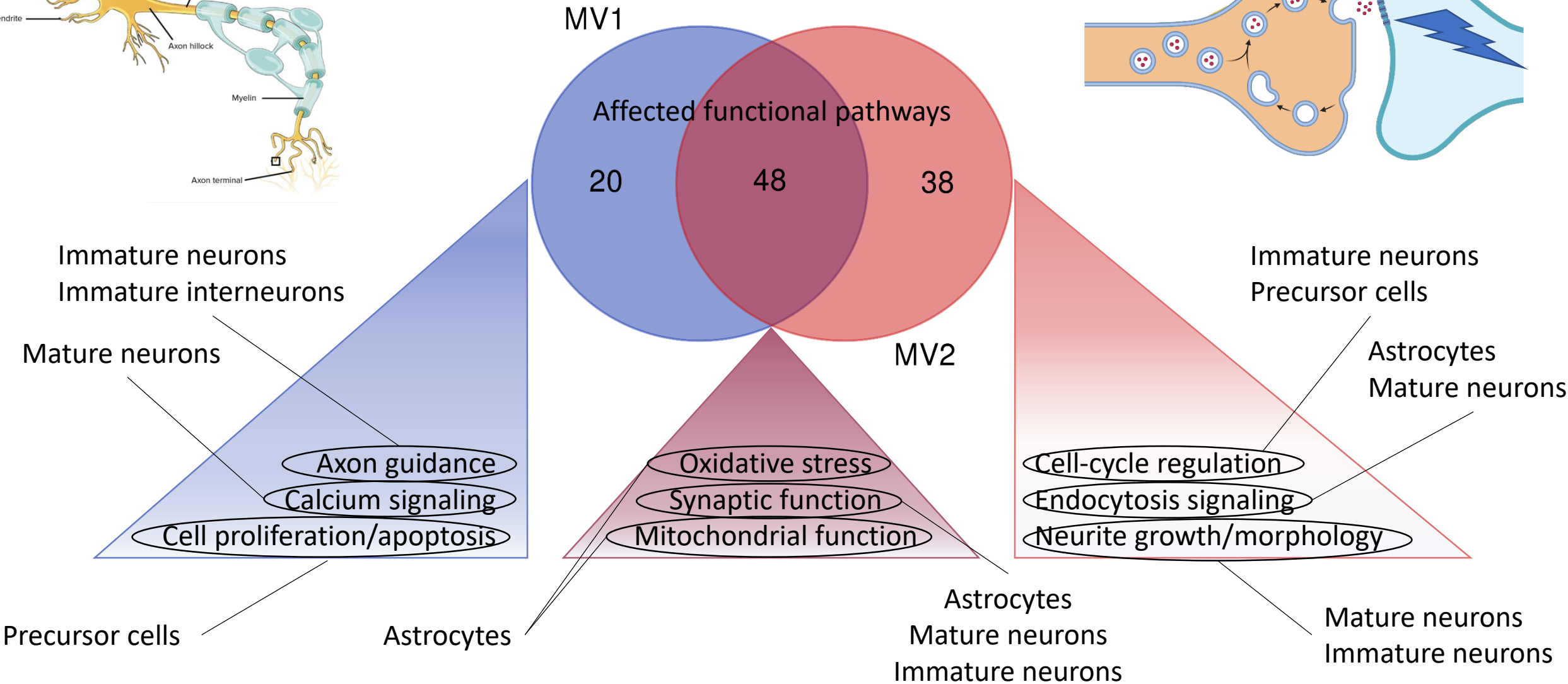
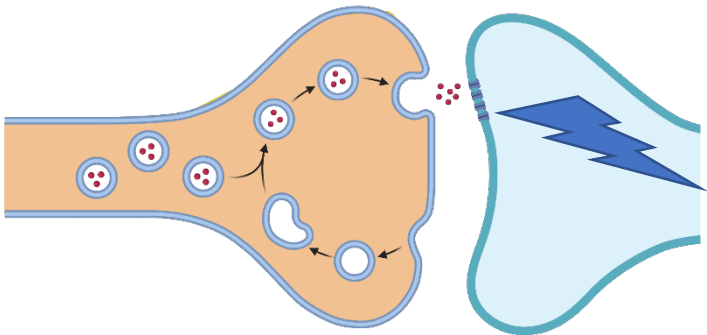
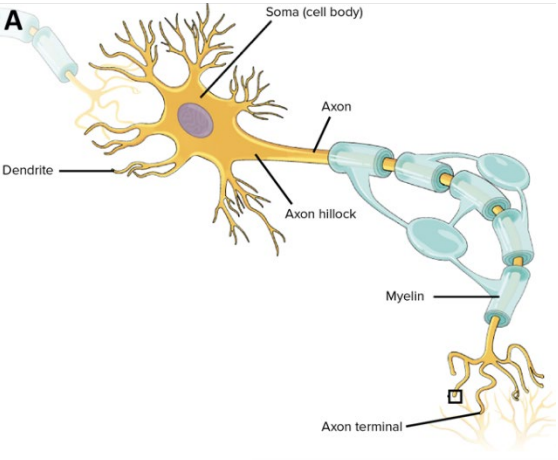




Gene expression differences in each cell type



sCJD affected pathways differ by subtype



Regional tropism of sporadic CJD subtypes in human brain organoids: summary

Human brain organoids are a 3D model of human brain tissue generated from induced pluripotent stem cells (iPSCs), which are derived from skin or blood cells and can be turned into many different types of cells.

We have previously shown that brain organoids are susceptible to infection with CJD.

Single cell RNA sequencing is a new tool to look at the presence and level of all genes in each individual cell of a given tissue.

Pairing human brain organoids with single cell RNA sequencing allowed us to investigate what cellular processes are altered during infection with CJD, and which cells are responsible for those changes.

Brain organoids can be made to recapitulate different brain regions. Our current analysis of cortical organoids infected with MV1 and MV2 subtypes of sCJD has identified disease related changes and subtype specific differences in genetic profiles within different cell types.

Additional data is now being analyzed from whole-brain organoids, and experiments are nearing completion for other brain region specific organoids with similar infections.

By understanding which pathways are targeted in sCJD and in which cells, we can begin to understand the disease processes and, potentially, how to protect those cells and pathways from disease.

Acknowledgments

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