Microgenomics 2021 Webinar Series

LABORATORY OF GENE EXPRESSION

Spatiotemporal dynamics of ischemic brain injury resolved at single-cell level

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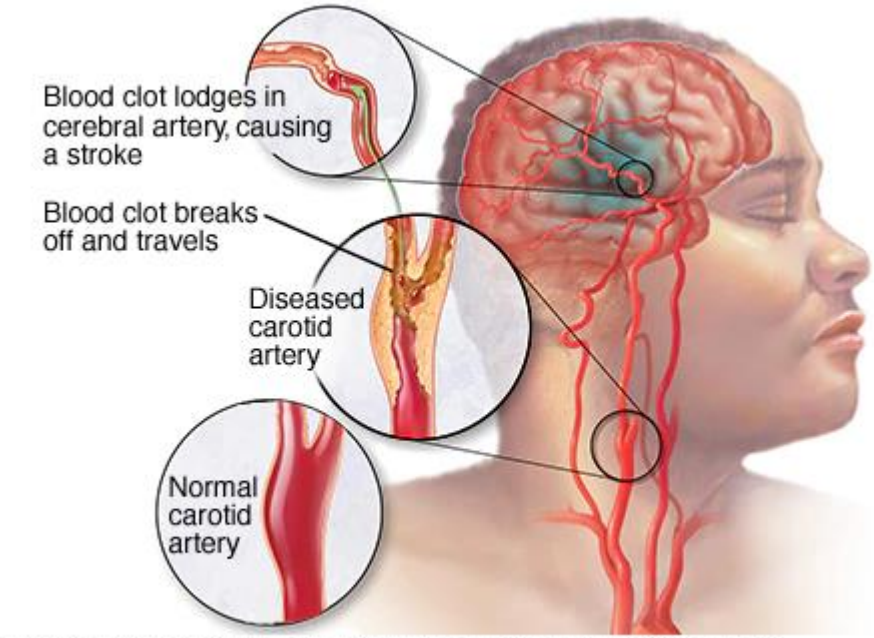
Laboratory of Gene Expression
Institute of Biotechnology, Czech Academy of Sciences

Ischemic brain injury (stroke)

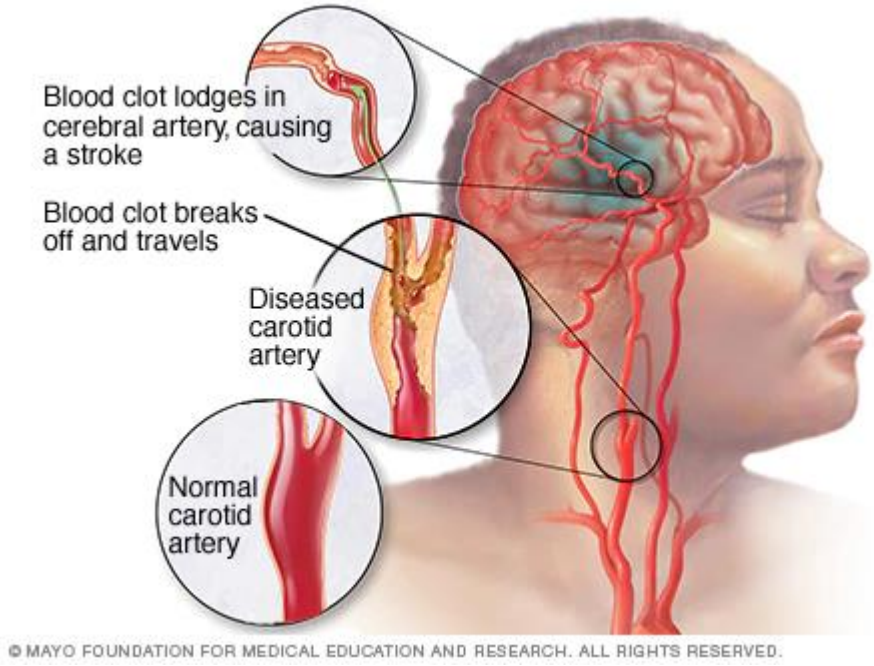
- Critical reduction in blood flow caused by either sudden or gradual occlusion of cerebral arteries
- Blockage of blood circulation causes neurologic deficits



Ischemic brain injury (stroke)

- Critical reduction in blood flow caused by either sudden or gradual occlusion of cerebral arteries
- Blockage of blood circulation causes neurologic deficits

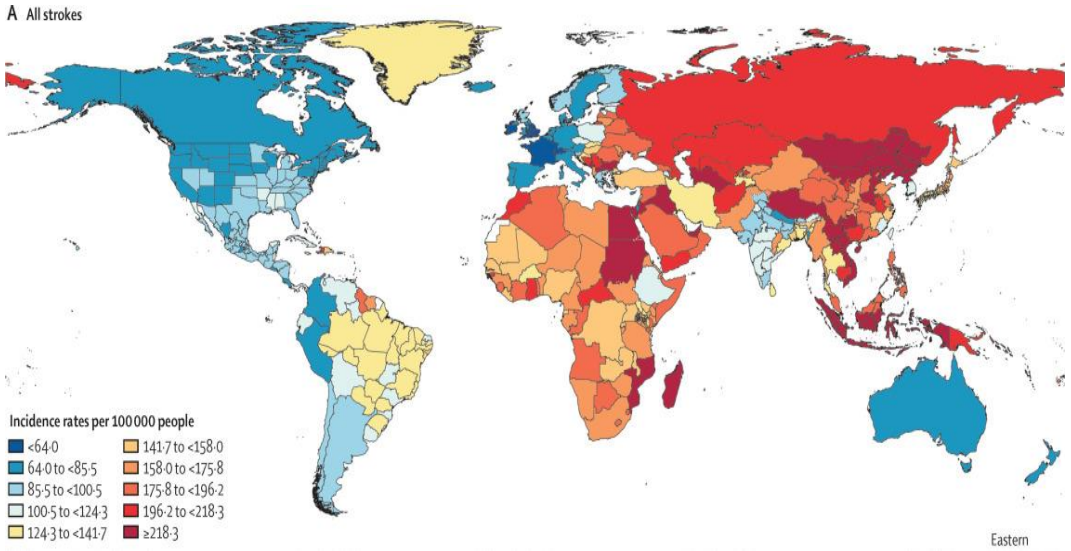
- Main pathologic changes include
  - Energy depletion, calcium overload, reactive oxygen species generation, inflammatory response, and ion imbalance
- Changes detrimental to basic cell functions leading to cell death



Ischemic brain injury (stroke)

- Affects over 12 millions people per year world-wide
  - Second leading cause of death (6.5 millions)
  - Third leading cause of death and disability combined

-> Major health care and economic burden



GBD 2019 Stroke Collaborators, 2021

Ischemic brain injury (stroke)

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  - Second leading cause of death (6.5 millions)
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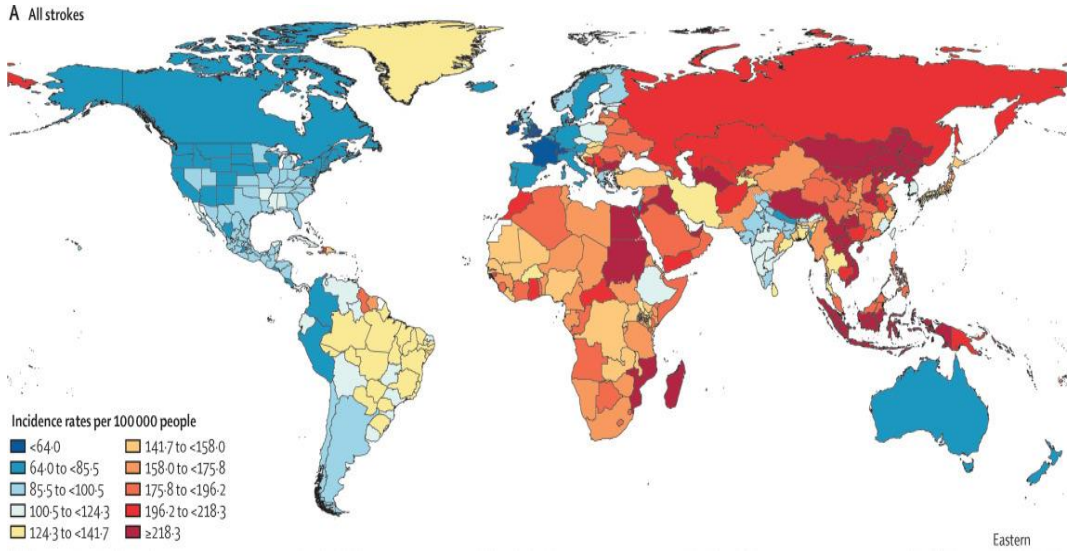
-> Major health care and economic burden

- Demand on development of new neuroprotective strategies
  - >1000 drugs investigated
  - >100 tested in clinical trials
- Early clot lysis remains the sole approved therapy



GBD 2019 Stroke Collaborators, 2021

Ischemic brain injury (stroke)

- The major reasons causing the lack of new therapeutics
  a) Complexity of the disease involving interactions of large number cell types
  b) Cell-type heterogeneity further exaggerating the complexness of the disease
  c) Temporal and spatial factors defining the role of cell types in the disease
  d) Inappropriate experimental models

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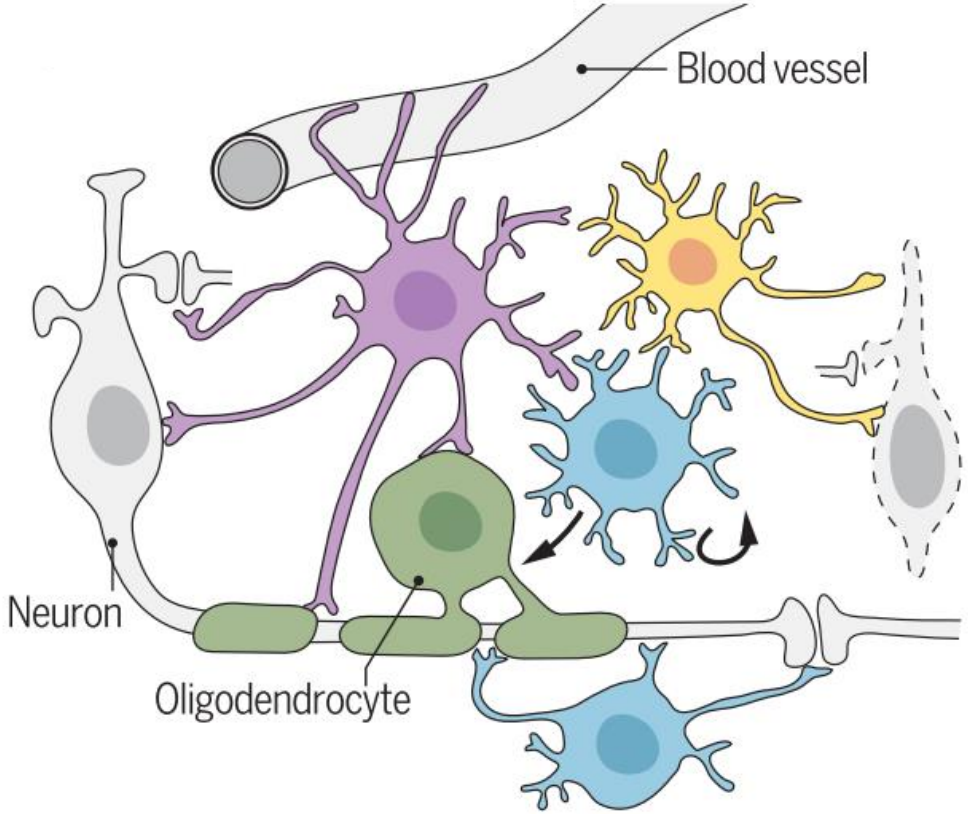
  d) Inappropriate experimental models

- Unique opportunity to apply recent technologies for transcriptomic analysis
  - Single-cell RNA-sequencing
  - Spatial transcriptomics
  - Integrative analysis

Glial cells

- Major players in response to ischemic brain injury
- Contribute to neuroinflammation, restriction of the injury site and recovery processes
- Beneficial as well as detrimental effects during ischemic stroke

- High level of heterogeneity

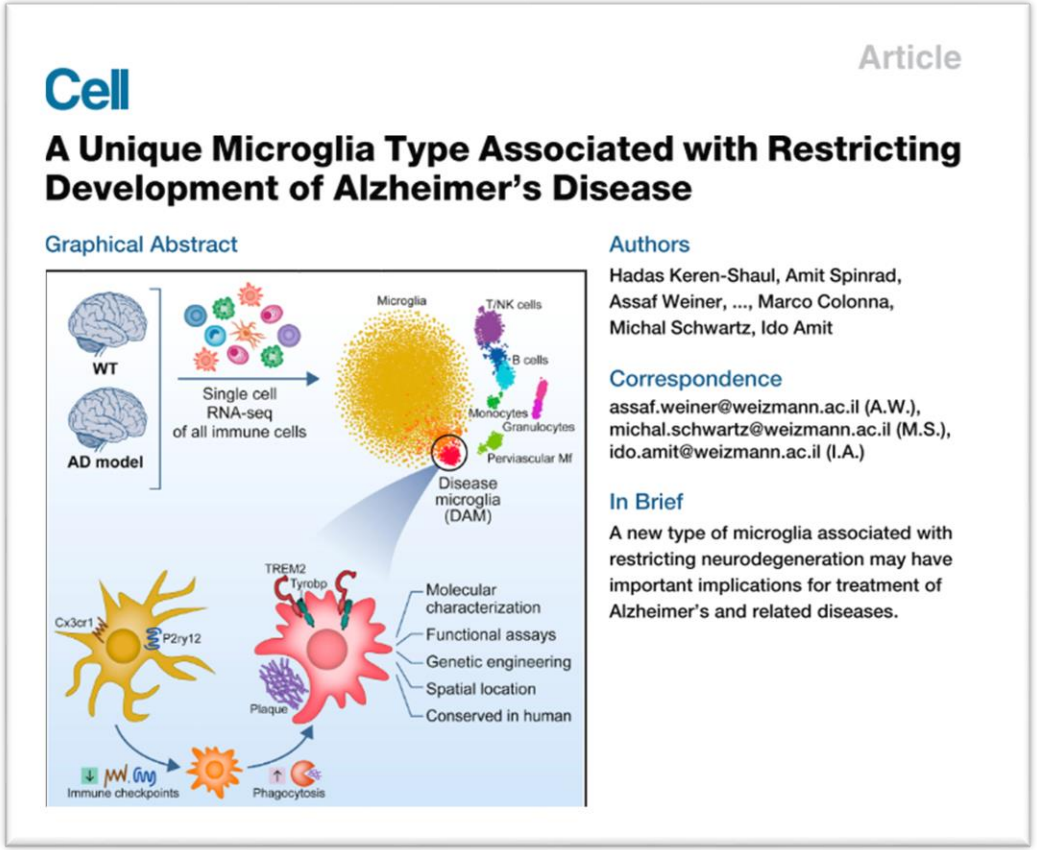


Allen & Lyons, 2018

Microglia

- Resident immune cell of CNS
- Active and dual role in neuropathologies

- Heterogeneous cell type
  - M1/M2 microglia (pro-inflammatory vs anti-inflammatory)
  - Disease-associated microglia (DAM)



Article

Cell

A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease

Graphical Abstract

Authors

Hadas Keren-Shaul, Amit Spinrad, Assaf Weiner, ..., Marco Colonna, Michal Schwartz, Ido Amit

Correspondence

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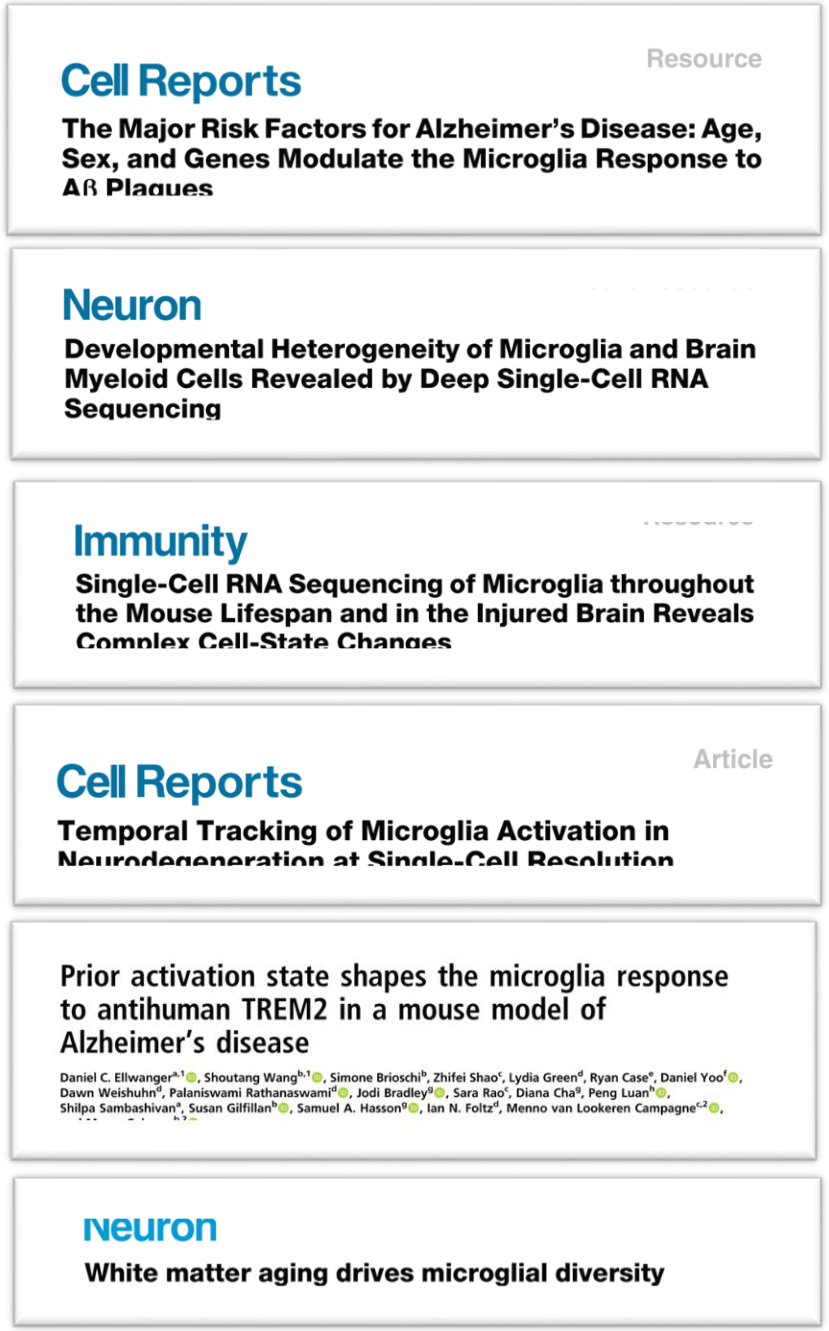
In Brief

A new type of microglia associated with restricting neurodegeneration may have important implications for treatment of Alzheimer's and related diseases.

Microglia

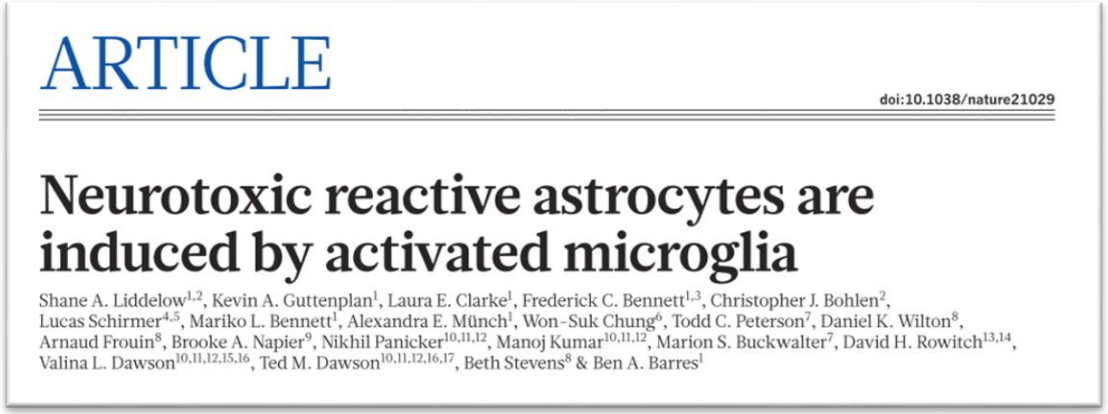
- Resident immune cell of CNS
- Active and dual role in neuropathologies

- Heterogeneous cell type
  - M1/M2 microglia (pro-inflammatory vs anti-inflammatory)
  - Disease-associated microglia (DAM)
  - Activated response microglia (ARM)
  - Interferon-responsive microglia (IRM)
  - MHC-II microglia
  - Proliferating microglia
  - ATM, PAM, WAM etc.



Astrocytes

- Traditional role - restriction of the injury site
- New roles
  - Modulators of immune response
  - Neurogenic capacity
- Heterogeneous cell type
  - A1/A2 astrocytes (neurotoxic vs neuroprotective)



ARTICLE

doi:10.1038/nature21029

Neurotoxic reactive astrocytes are induced by activated microglia

Shane A. Liddelow[1,2], Kevin A. Guttenplan[1], Laura E. Clarke[1], Frederick C. Bennett[1,3], Christopher J. Bohlen[2], Lucas Schirmer[4,5], Mariko L. Bennett[1], Alexandra E. Münch[1], Won-Suk Chung[6], Todd C. Peterson[7], Daniel K. Wilton[8], Arnaud Frouin[8], Brooke A. Napier[9], Nikhil Panicker[10,11,12], Manoj Kumar[10,11,12], Marion S. Buckwalter[7], David H. Rowitch[13,14], Valina L. Dawson[10,11,12,15,16], Ted M. Dawson[10,11,12,16,17], Beth Stevens[8] & Ben A. Barres[1]

Astrocytes

- Traditional role - restriction of the injury site
- New roles
  - Modulators of immune response
  - Neurogenic capacity

- Heterogeneous cell type
  - A1/A2 astrocytes (neurotoxic vs neuroprotective)
  - Disease-associated astrocytes (DAA)
  - Inflammatory reactive astrocytes 1 and 2 (IRAS1, IRAS2)


nature neuroscience
BRIEF COMMUNICATION
https://doi.org/10.1038/s41593-020-0624-8
Check for updates

Disease-associated astrocytes in Alzheimer's disease and aging

Naomi Habib[1,2]✉, Cristin McCabe[2,8], Sedi Medina[3,8], Miriam Varshavsky[1,8], Daniel Kitsberg[1], Raz Dvir-Szternfeld[3], Gilad Green[1], Danielle Dionne[2], Lan Nguyen[2], Jamie L. Marshall[2], Fei Chen[2], Feng Zhang[2,4,5], Tommy Kaplan[6], Aviv Regev[2,7]✉ and Michal Schwartz[2,3]✉

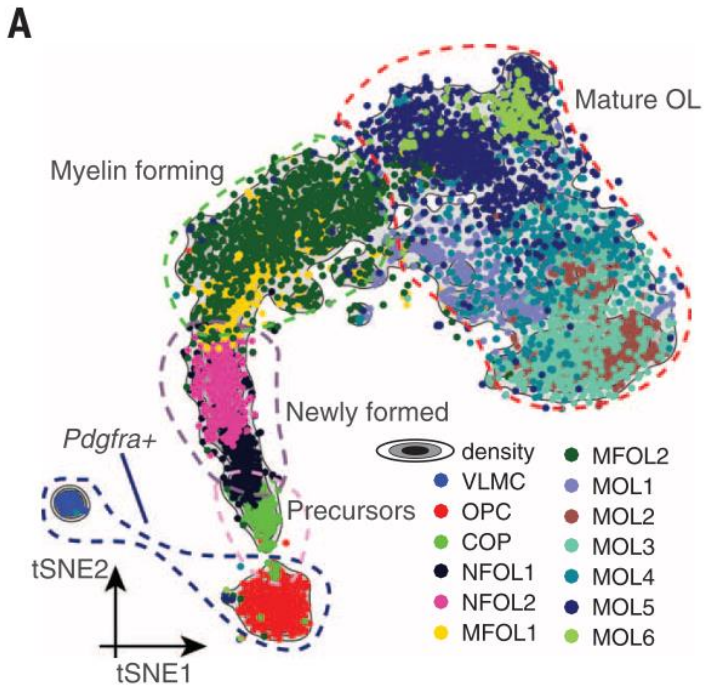
CRISPRi screens in human astrocytes elucidate regulators of distinct inflammatory reactive states

Kun Leng[1,2,3,*], Brendan Rooney[1], Hyosung Kim[4], Wenlong Xia[5], Mark Koontz[6], Mitchell Krawczyk[7,8], Ye Zhang[8], Erik M. Ullian[6], Stephen P.J. Fancy[5], Matthew S. Schrag[9,10,11], Ethan S. Lippmann[4, 9,10,11], Martin Kampmann[1,12,13,14,*]

Oligodendroglial lineage

- Oligodendrocytes and their progeny
- Not only passive players, but also active modulators of immune response
- De-differentiation potential

- Heterogeneous cell type
  - Various maturation phases



Marques et al., 2016

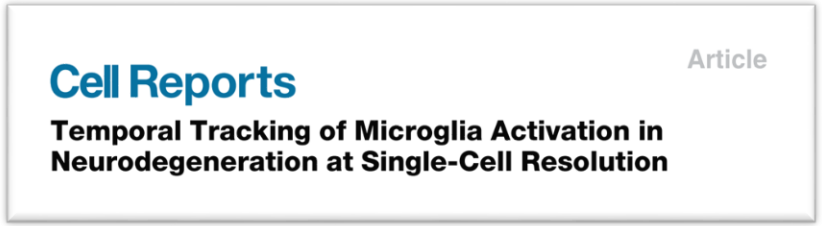
Oligodendroglial lineage

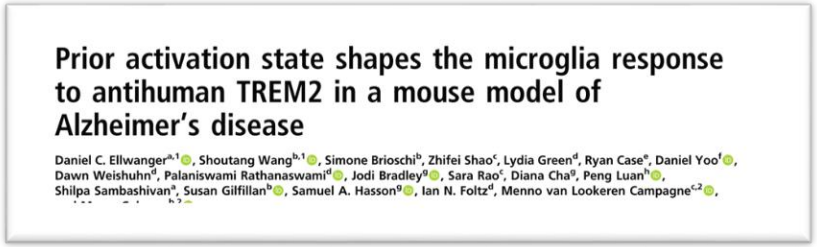
- Oligodendrocytes and their progeny
- Not only passive players, but also active modulators of immune response
- De-differentiation potential

- Heterogeneous cell type
  - Various maturation phases
  - Disease-exclusive oligodendrocyte lineage cells in MS
  - OLs with spatial preference and different response to injury

Glial cells

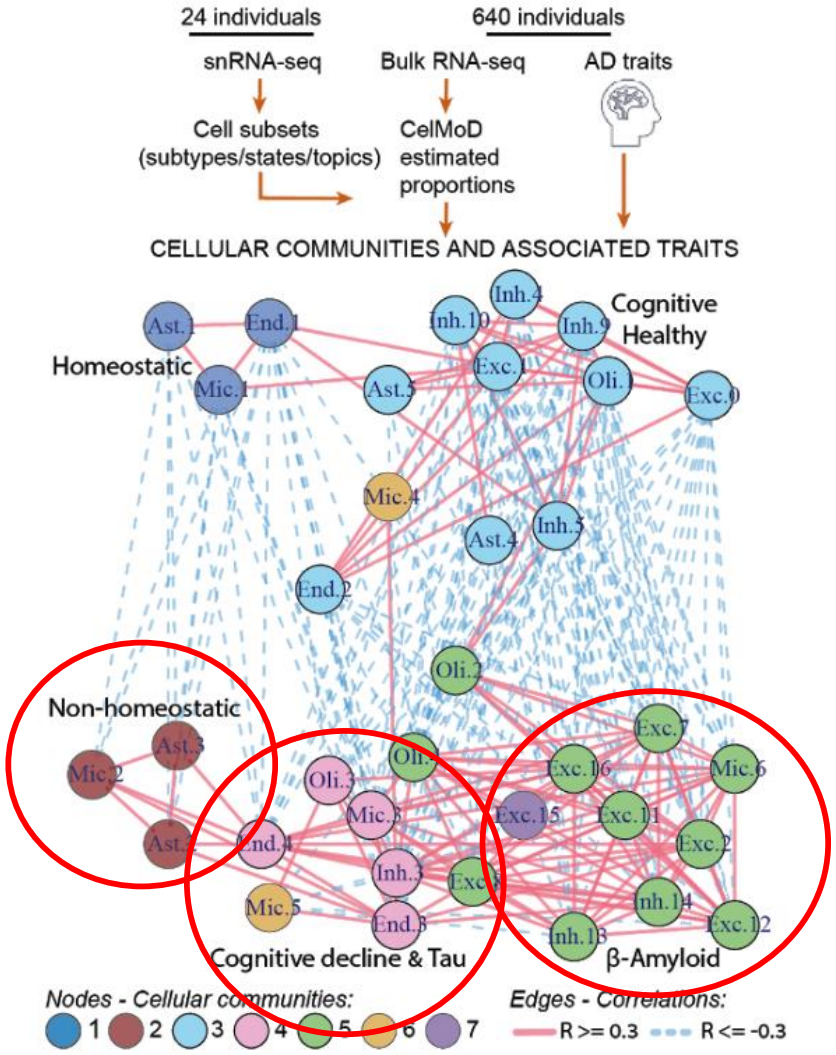
- Heterogeneity of glial cells mostly studied in neurodegenerative context





Glial cells

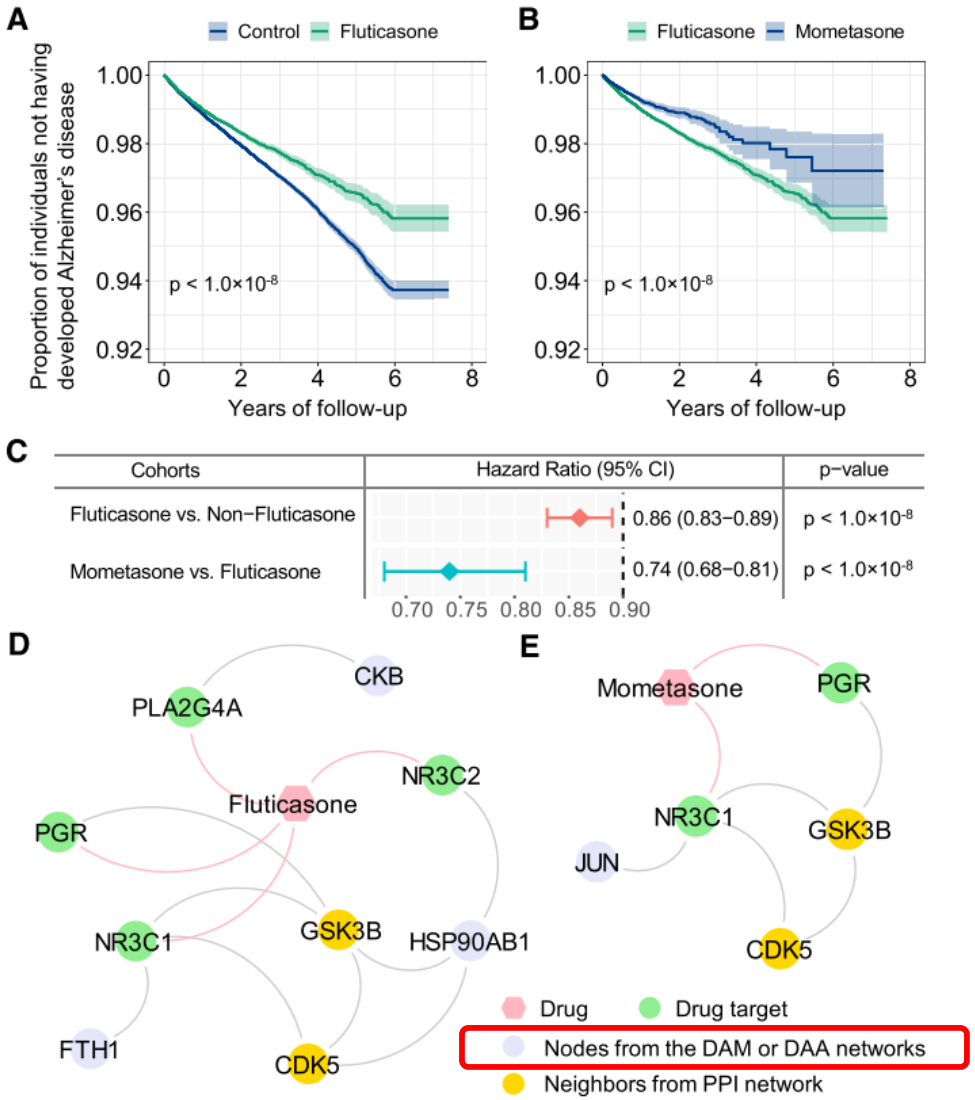
- Heterogeneity of glial cells mostly studied in neurodegenerative context
- Disease-associated subpopulations share transcriptional program and actively communicate



White et al., 2020

Glial cells

- Heterogeneity of glial cells mostly studied in neurodegenerative context
- Disease-associated subpopulations share transcriptional program and actively communicate
- Shared transcriptional program may be therapeutically targeted



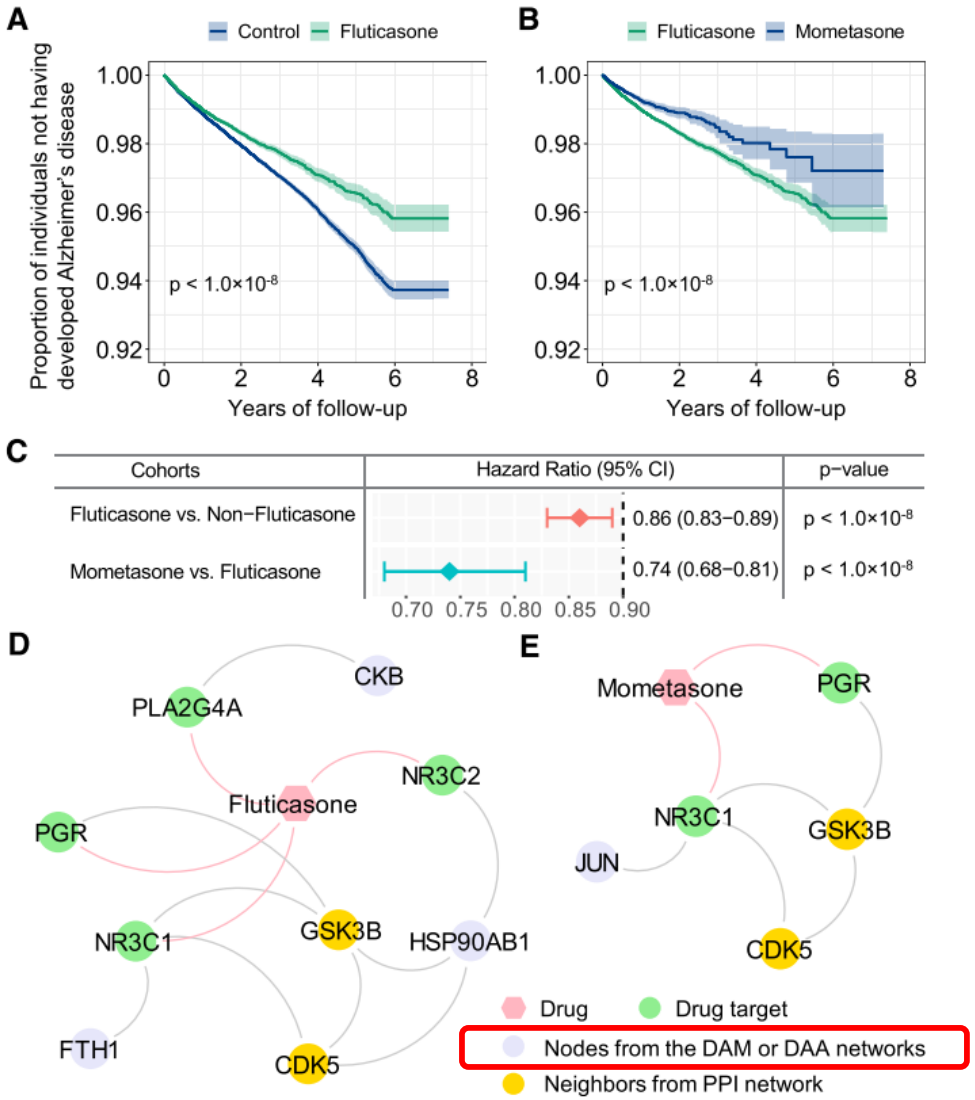
Xu et al., 2021

Glial cells

- Heterogeneity of glial cells mostly studied in neurodegenerative context
- Disease-associated subpopulations share transcriptional program and actively communicate
- Shared transcriptional program may be therapeutically targeted

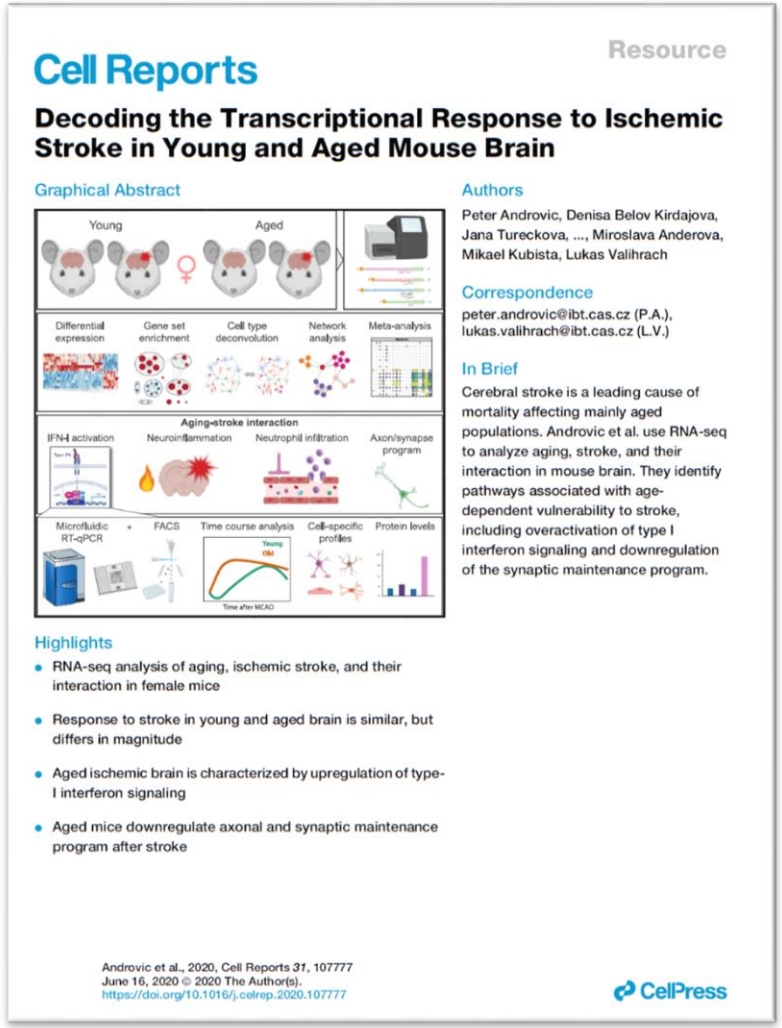
-> Little is known about reaction of disease-associated subpopulations in ischemic brain injury

-> Promising target for new glia-oriented therapy



Xu et al., 2021

Premises and promises



Cell Reports

Resource

Decoding the Transcriptional Response to Ischemic Stroke in Young and Aged Mouse Brain

Graphical Abstract

Authors

Peter Androvic, Denisa Belov Kirdajova, Jana Tureckova, ..., Miroslava Anderova, Mikael Kubista, Lukas Valihrach

Correspondence

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In Brief

Cerebral stroke is a leading cause of mortality affecting mainly aged populations. Androvic et al. use RNA-seq to analyze aging, stroke, and their interaction in mouse brain. They identify pathways associated with age-dependent vulnerability to stroke, including overactivation of type I interferon signaling and downregulation of the synaptic maintenance program.

Highlights

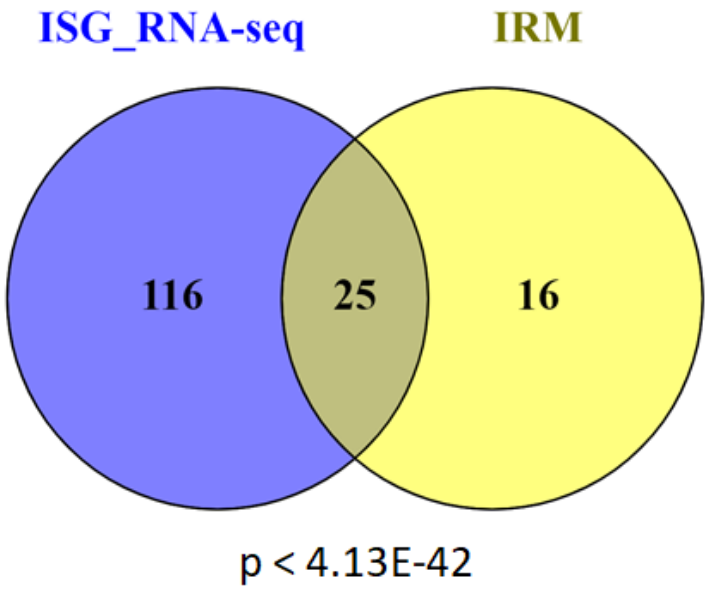
- RNA-seq analysis of aging, ischemic stroke, and their interaction in female mice
- Response to stroke in young and aged brain is similar, but differs in magnitude
- Aged ischemic brain is characterized by upregulation of type-I interferon signaling
- Aged mice downregulate axonal and synaptic maintenance program after stroke

Androvic et al., 2020, Cell Reports *31*, 107777
June 16, 2020 © 2020 The Author(s).
https://doi.org/10.1016/j.celrep.2020.107777

CellPress

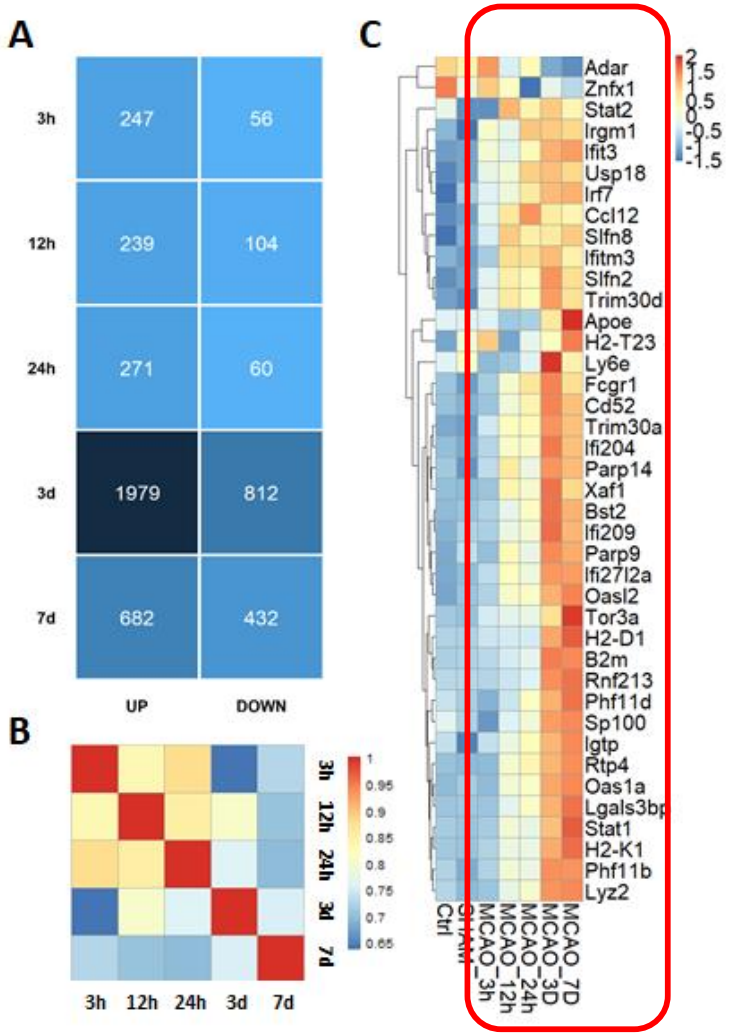
- Response to stroke in young and aged brain is similar, but differs in magnitude
- Aged ischemic brain is characterized by upregulation of type-I interferon signaling
- Glial cells main contributors

Premises and promises



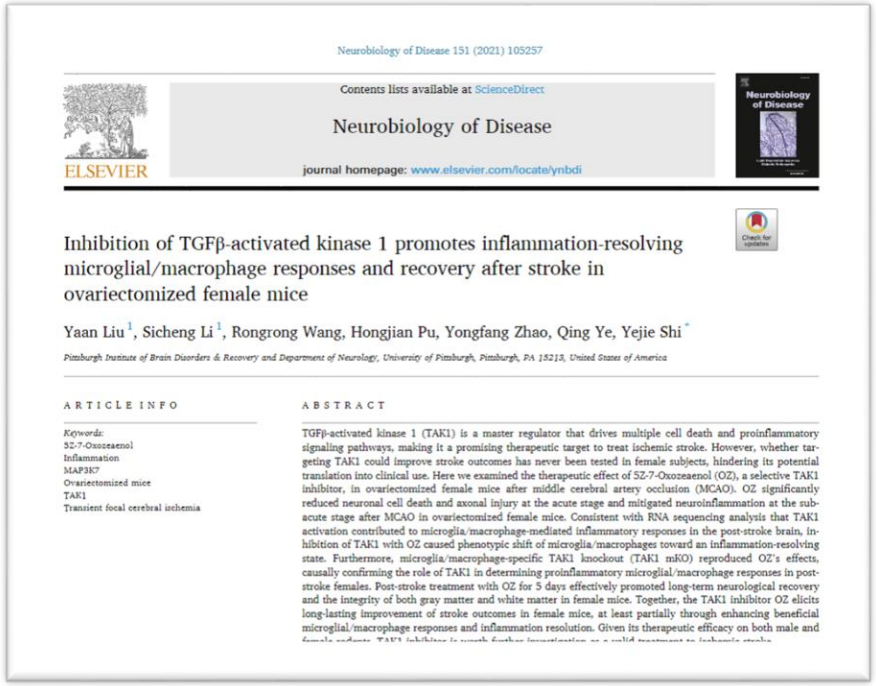
- Stroke-induced interferon response showed strong overlap with markers of interferon response microglia (IRM)

Premises and promises



- Stroke-induced interferon response showed strong overlap with markers of interferon response microglia (IRM)
- Activation of IRM signature observed in time-series bulk RNA-seq data

Premises and promises



Neurobiology of Disease 151 (2021) 105257

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Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Inhibition of TGFβ-activated kinase 1 promotes inflammation-resolving microglial/macrophage responses and recovery after stroke in ovariectomized female mice

Yaan Liu [1], Sicheng Li [1], Rongrong Wang, Hongjian Pu, Yongfang Zhao, Qing Ye, Yejie Shi *

Pittsburgh Institute of Brain Disorders & Recovery and Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15213, United States of America

ARTICLE INFO

Keywords:
5Z-7-Oxozeaenol
Inflammation
MAP3K7
Ovariectomized mice
TAK1
Transient focal cerebral ischemia

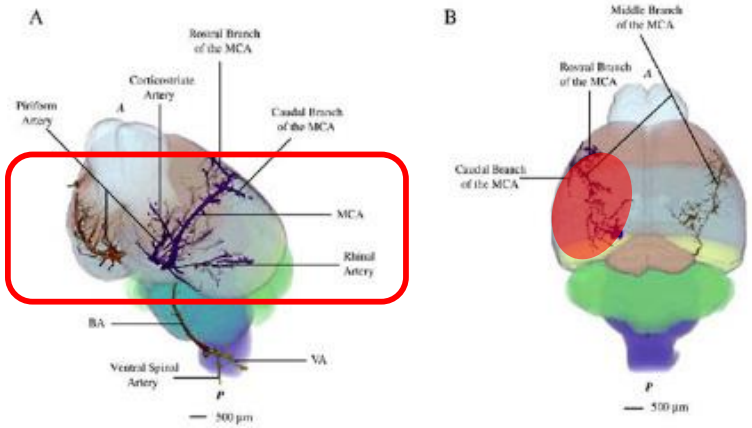
ABSTRACT

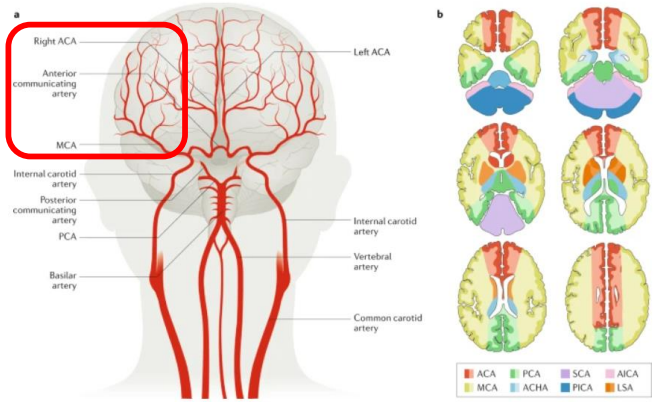
TGFβ-activated kinase 1 (TAK1) is a master regulator that drives multiple cell death and proinflammatory signaling pathways, making it a promising therapeutic target to treat ischemic stroke. However, whether targeting TAK1 could improve stroke outcomes has never been tested in female subjects, hindering its potential translation into clinical use. Here we examined the therapeutic effect of 5Z-7-Oxozeaenol (OZ), a selective TAK1 inhibitor, in ovariectomized female mice after middle cerebral artery occlusion (MCAO). OZ significantly reduced neuronal cell death and axonal injury at the acute stage and mitigated neuroinflammation at the subacute stage after MCAO in ovariectomized female mice. Consistent with RNA sequencing analysis that TAK1 activation contributed to microglia/macrophage-mediated inflammatory responses in the post-stroke brain, inhibition of TAK1 with OZ caused phenotypic shift of microglia/macrophages toward an inflammation-resolving state. Furthermore, microglia/macrophage-specific TAK1 knockout (TAK1 mKO) reproduced OZ's effects, causally confirming the role of TAK1 in determining proinflammatory microglial/macrophage responses in post-stroke females. Post-stroke treatment with OZ for 5 days effectively promoted long-term neurological recovery and the integrity of both gray matter and white matter in female mice. Together, the TAK1 inhibitor OZ elicits long-lasting improvement of stroke outcomes in female mice, at least partially through enhancing beneficial microglial/macrophage responses and inflammation resolution. Given its therapeutic efficacy on both male and

- Manipulation of inflammatory response governed by microglia improved regeneration after transient MCAO

Experimental design

- Permanent middle-cerebral artery occlusion (pMCAO)
  - Representing majority of clinical stroke cases





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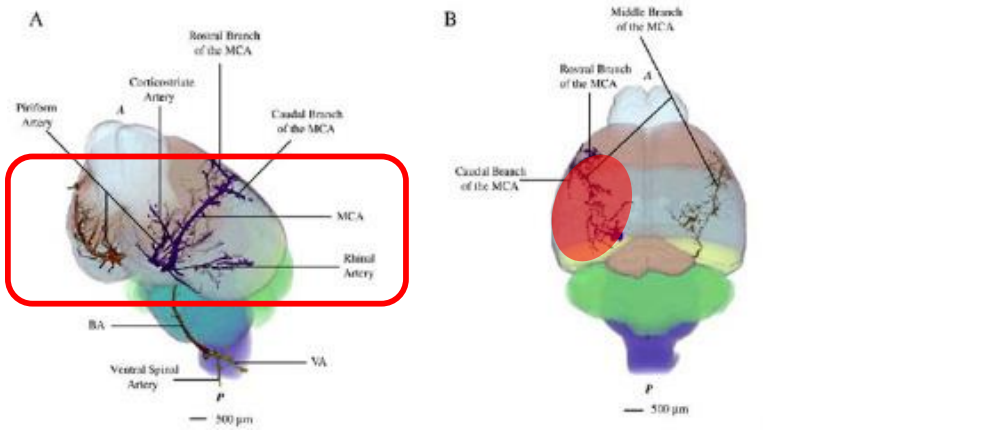
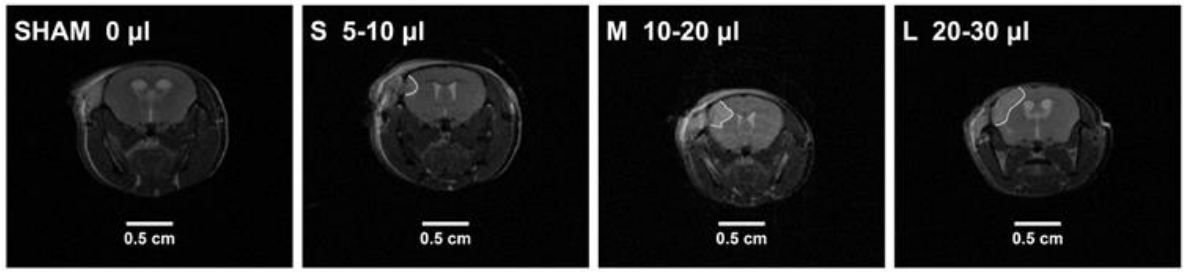
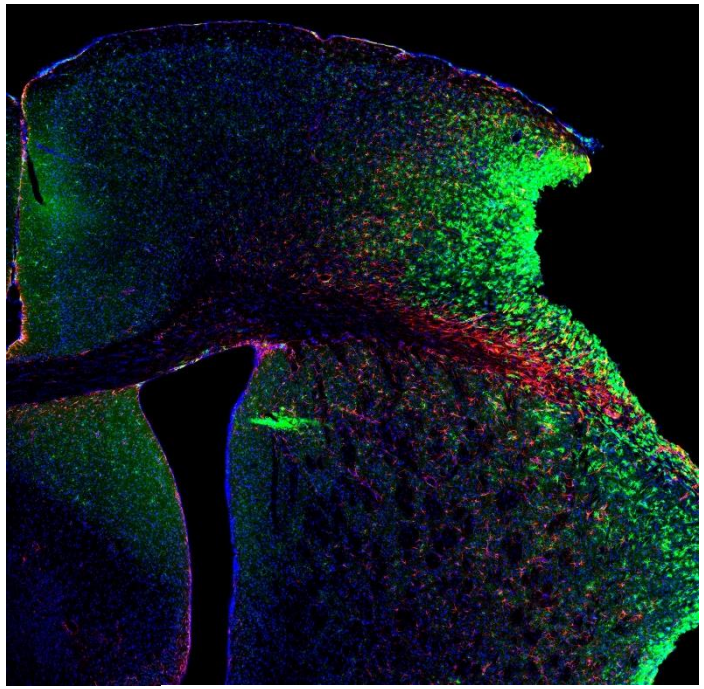
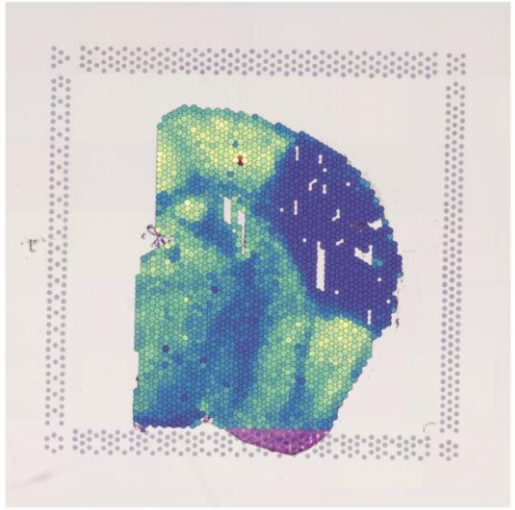




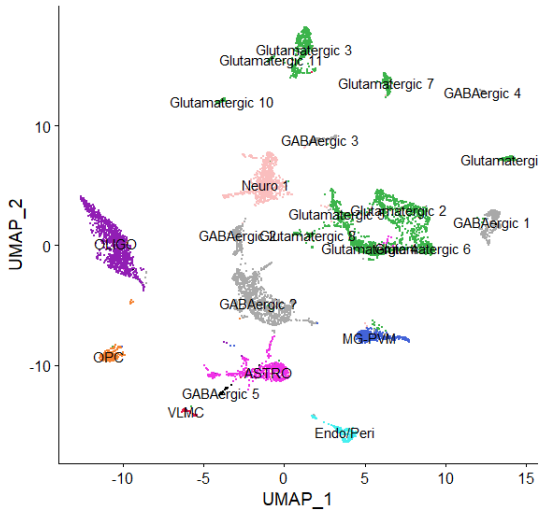


Microglia Astrocytes

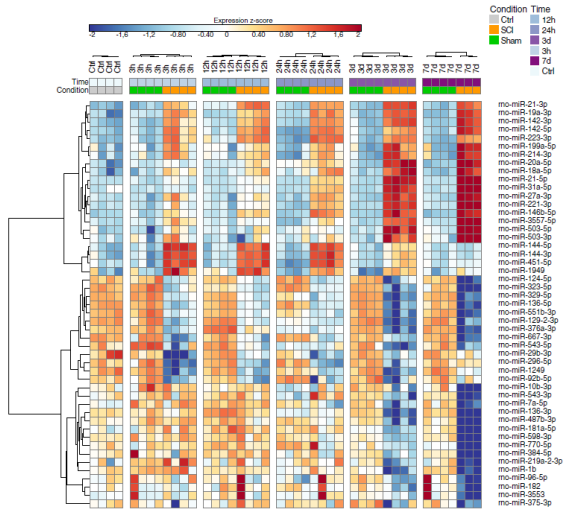
Experimental design



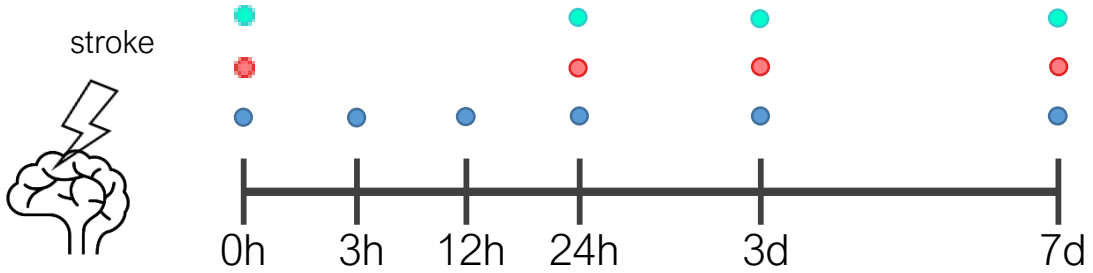
Spatial Transcriptomics (Visium)



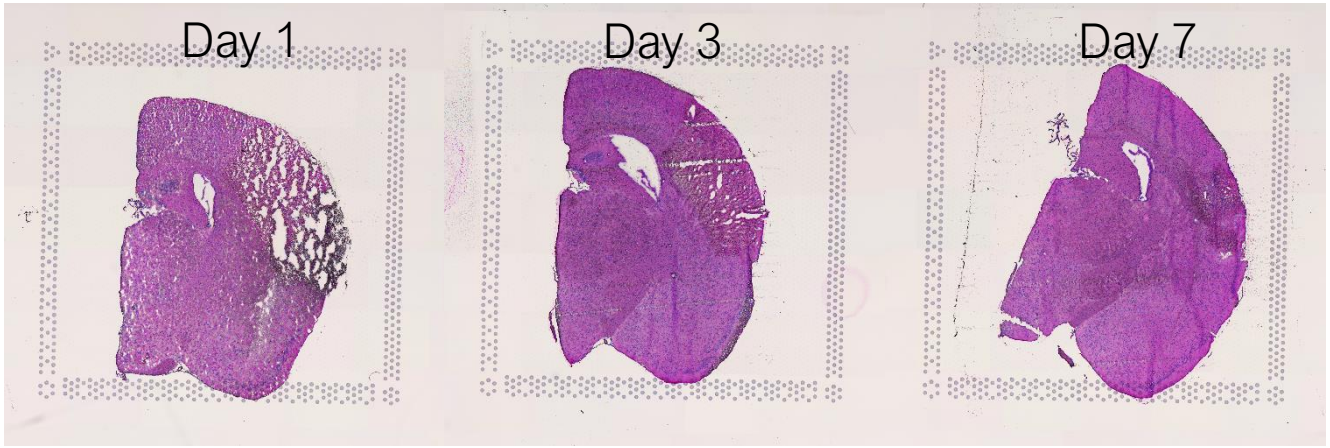
Single-nucleus RNA-Seq



Bulk RNA-Seq



Spatial Transcriptomics



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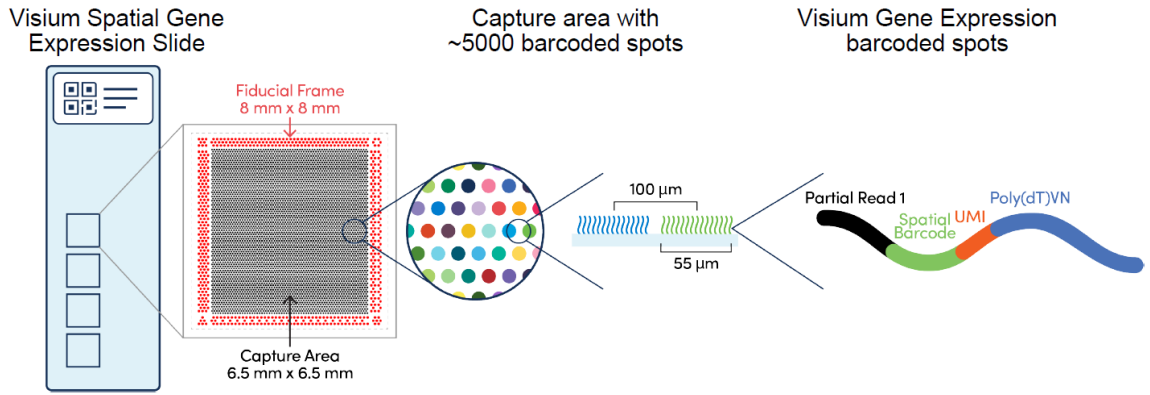
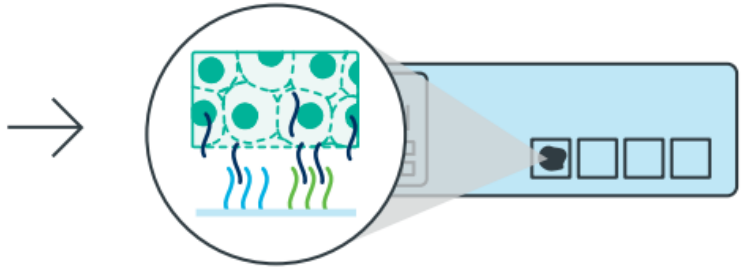
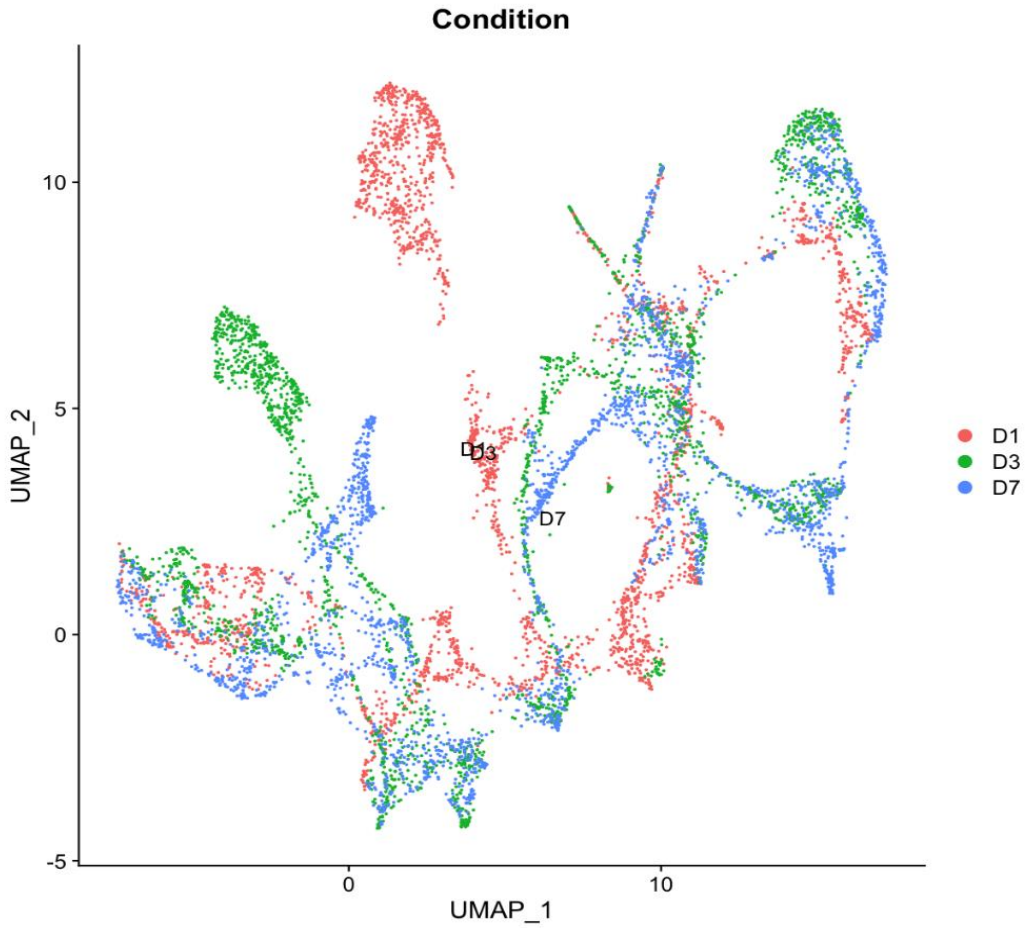


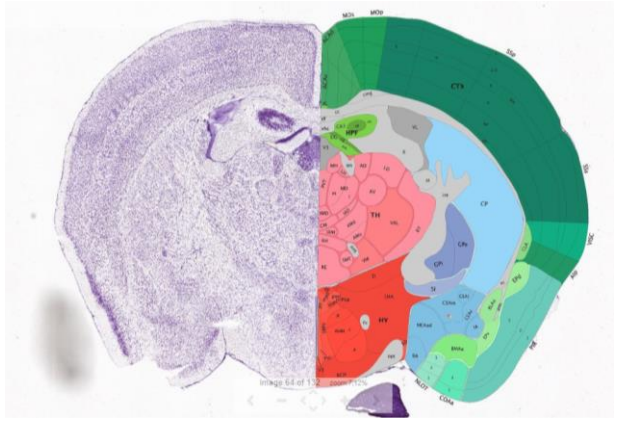


Whole transcriptome
and
its spatial localization

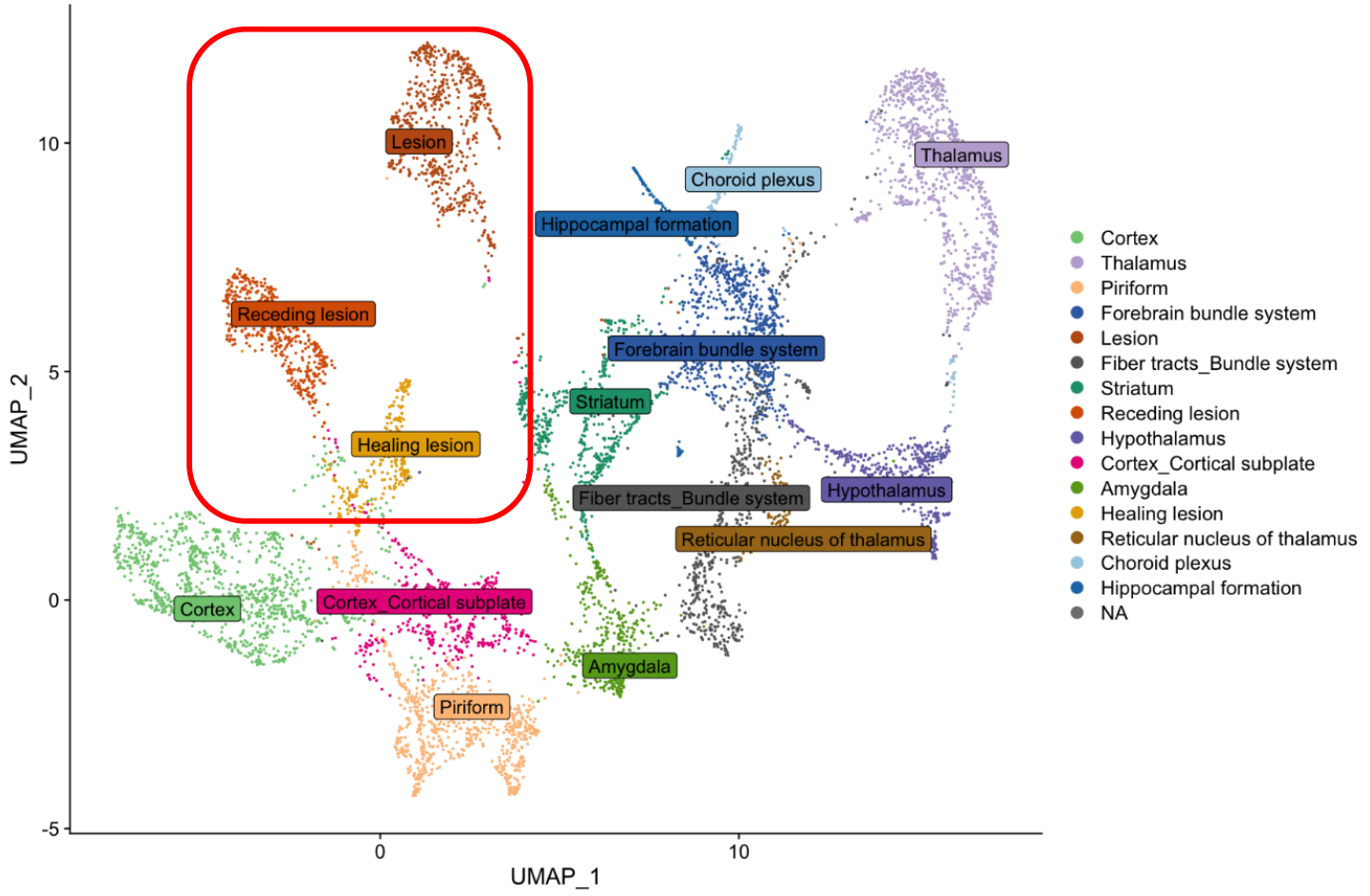
Early look – whole brain



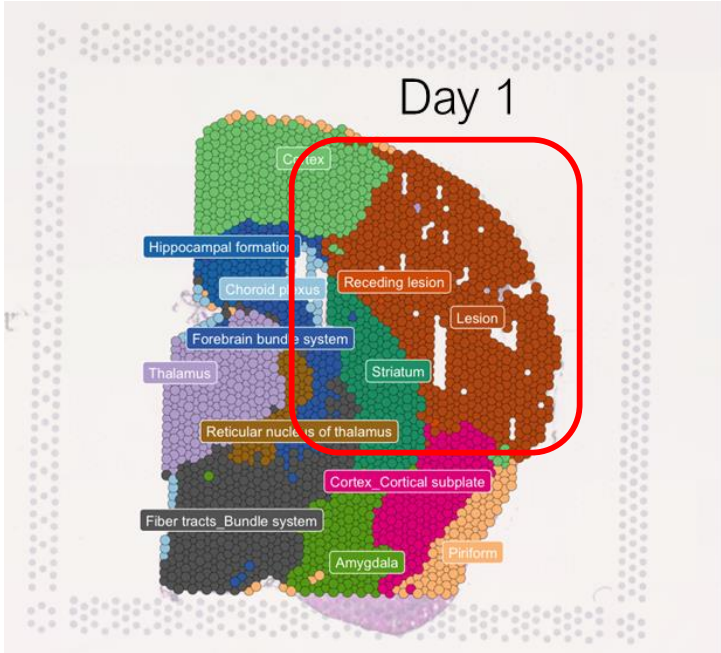
Early look – whole brain

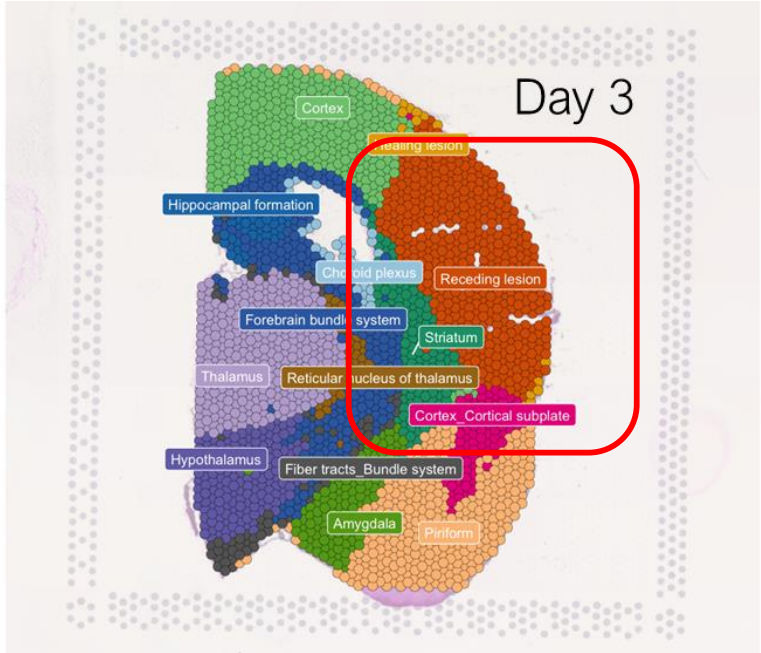


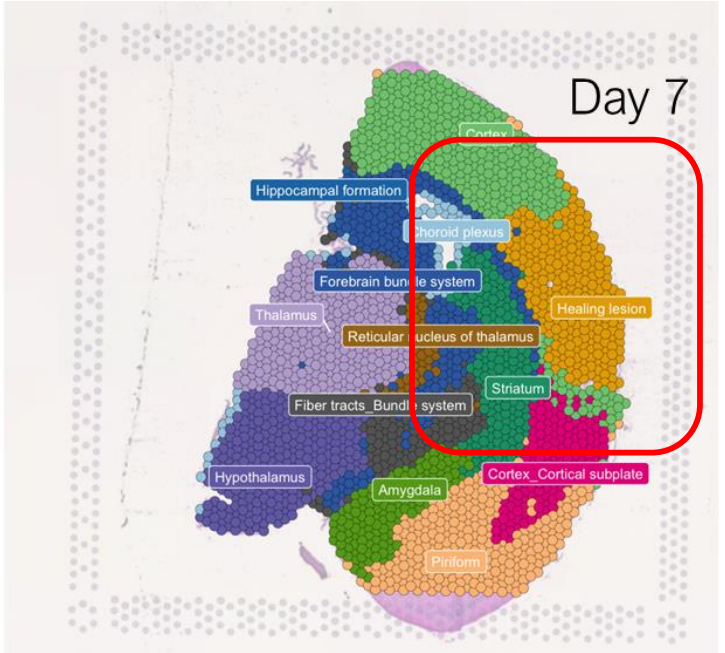
Allen Brain Atlas: Mouse Brain



Early look – whole brain

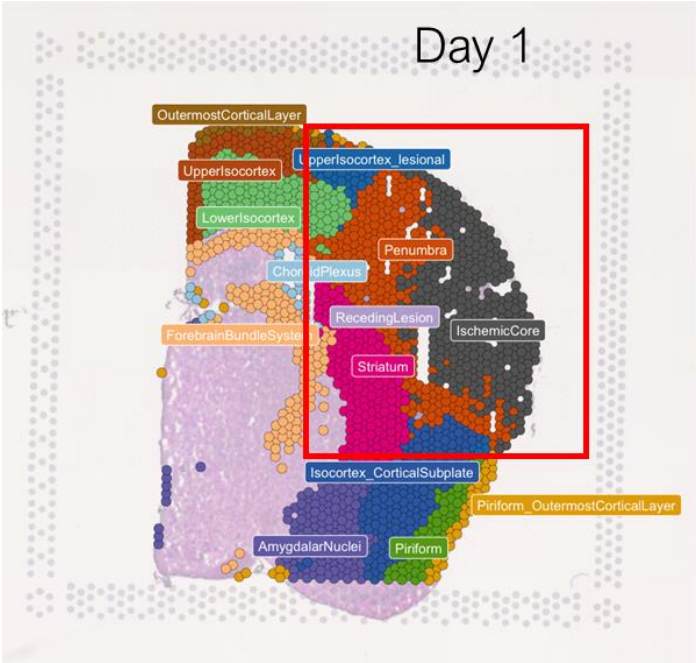


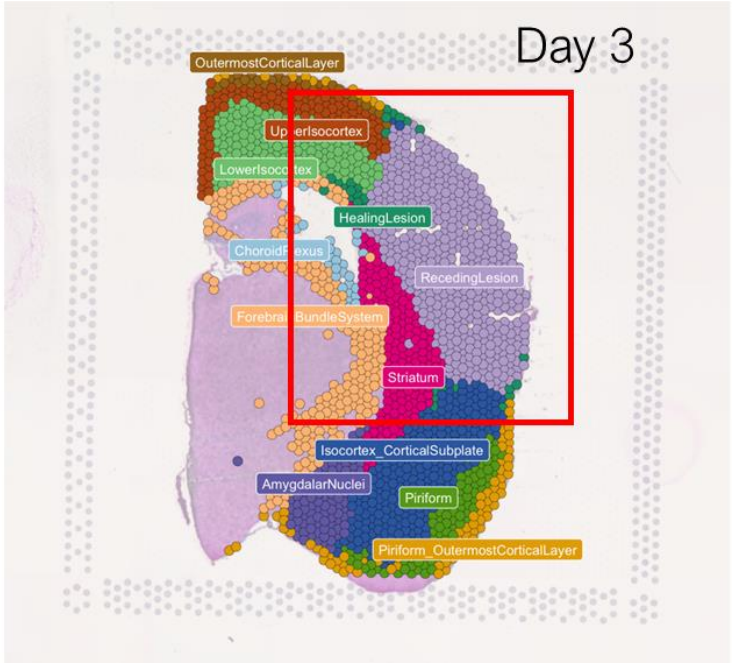


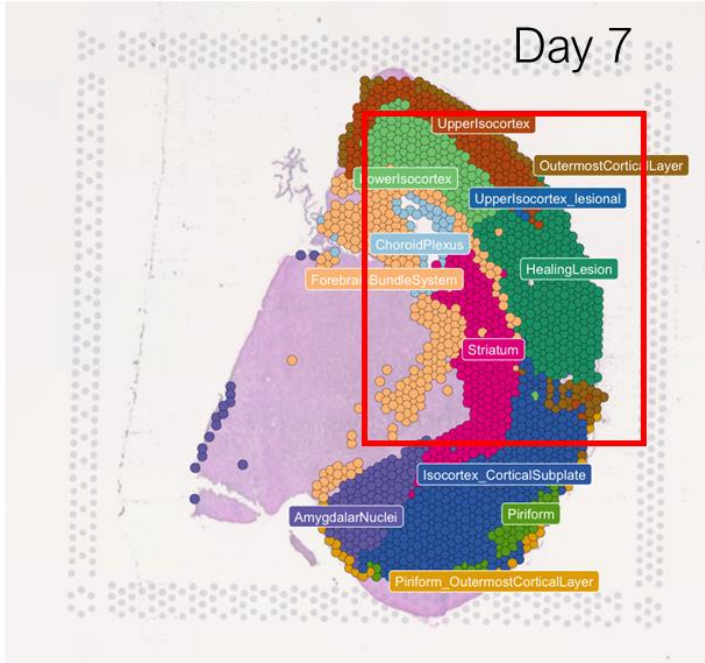


LABORATORY OF
GENE EXPRESSION

Zoomed in: Outer Layers







What is the spatial gene expression profile?

How unique/distinct are the cellular states?

LABORATORY OF
GENE EXPRESSION

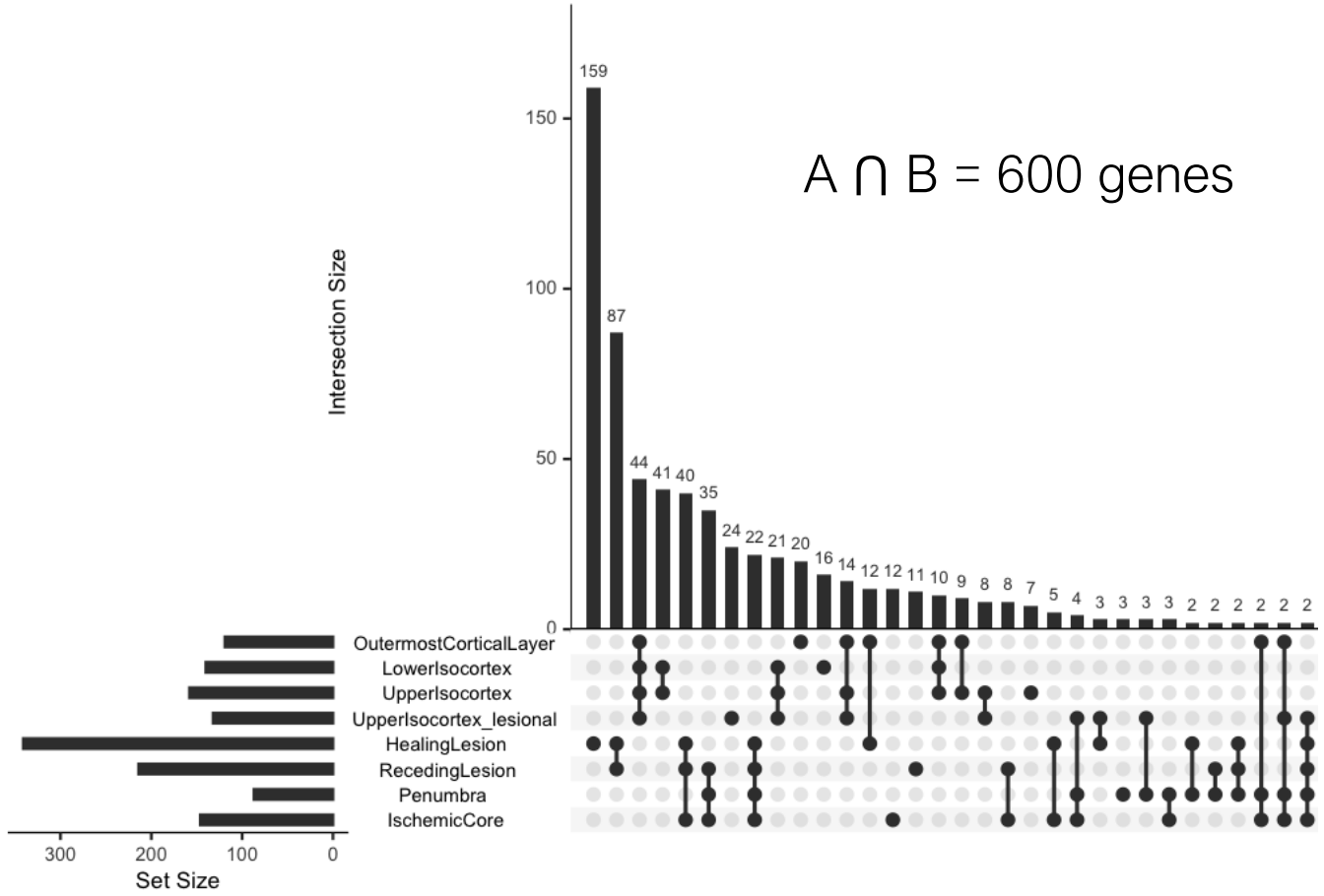
Marker discovery

A. Cluster characterization
B. Spatial profile

Marker discovery

A. Cluster characterization
B. Spatial profile

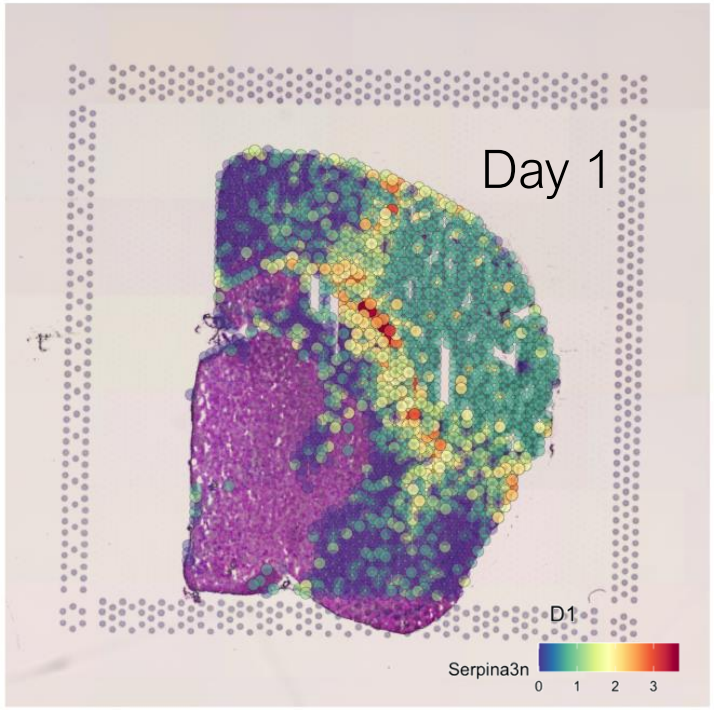
A ∩ B = 600 genes

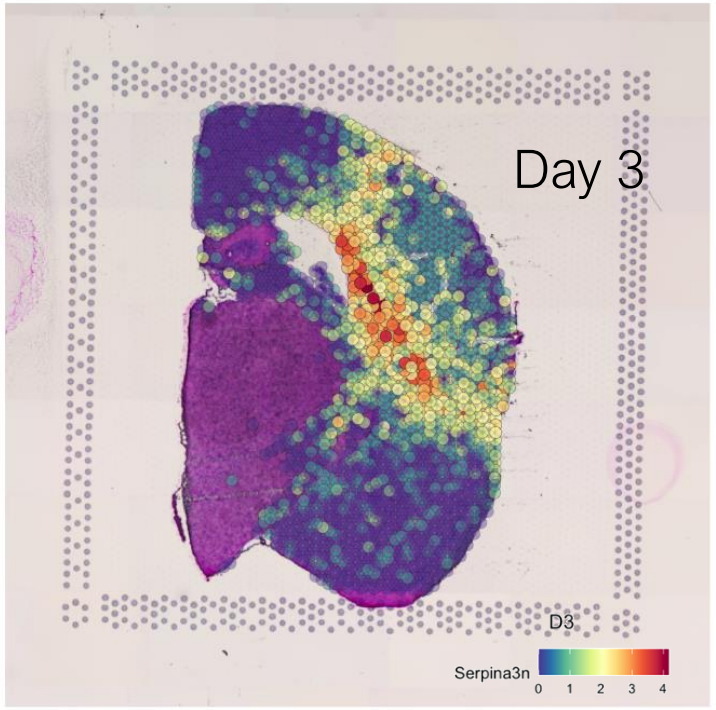


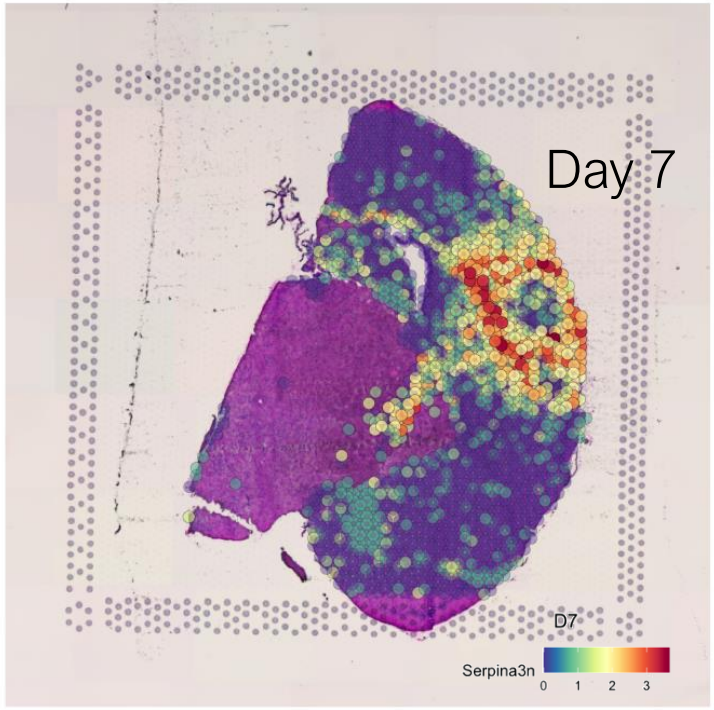
Markers are expressed in zones, with respect to the lesional site

Zonality

- Astrocytes: Enclosing the lesion

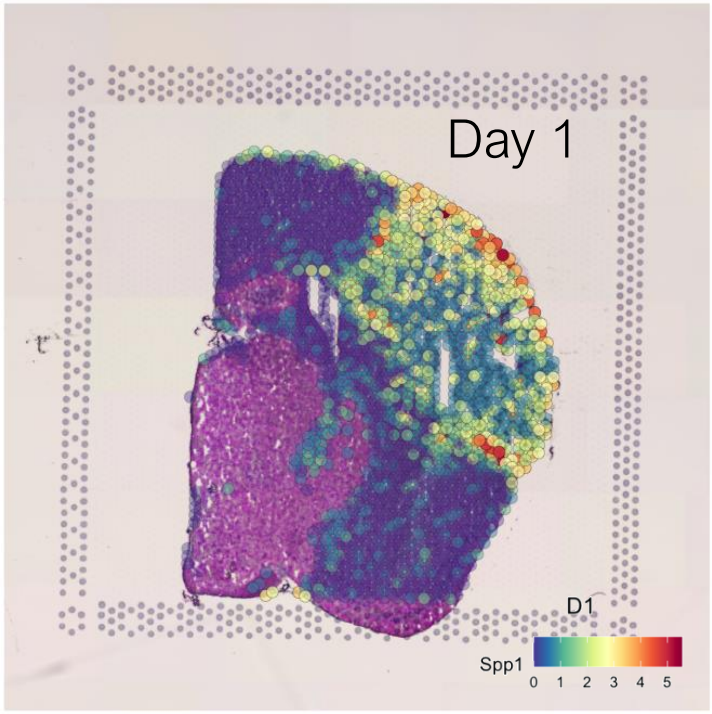


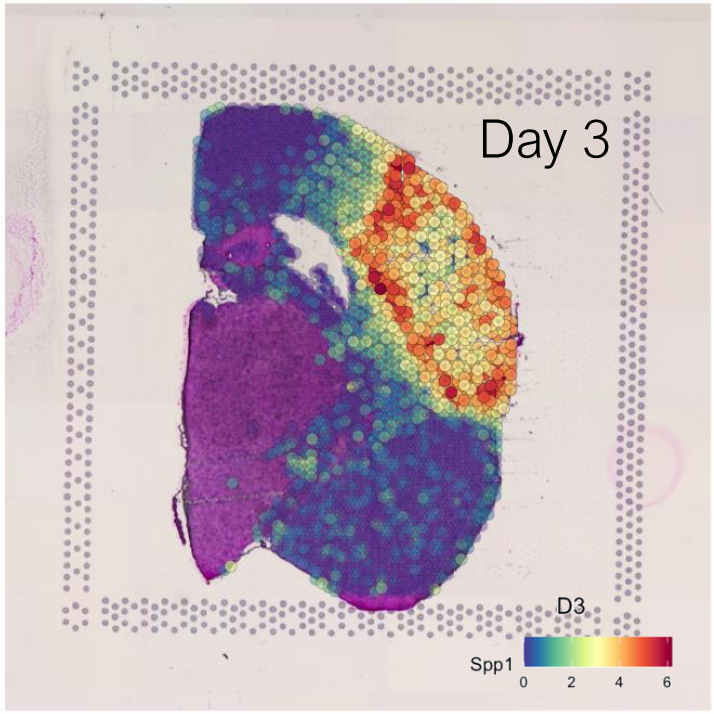


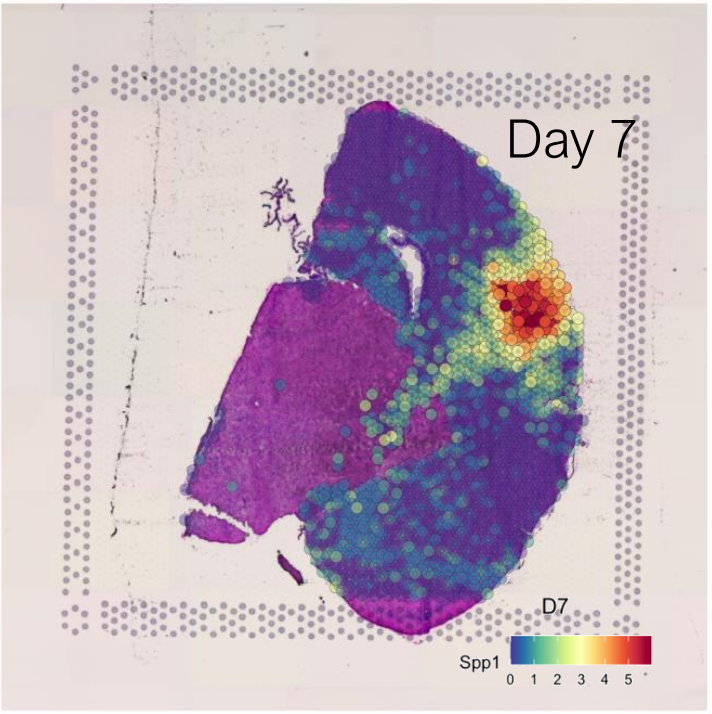


Zonality

- Microglia: Forming active immune defense

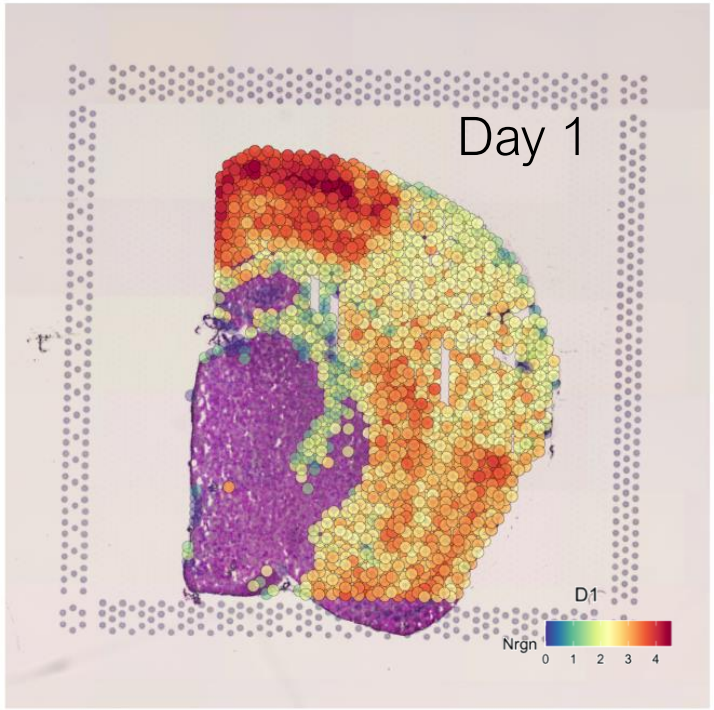


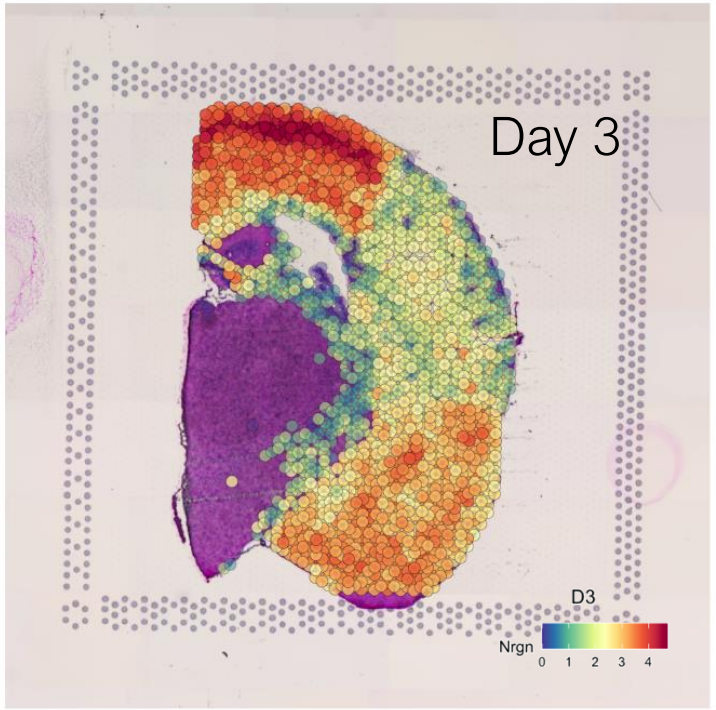


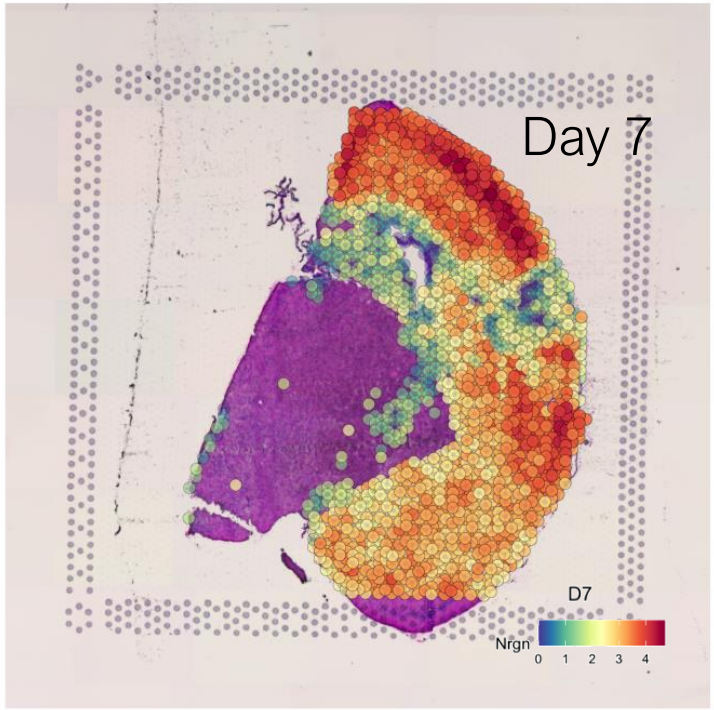


Zonality

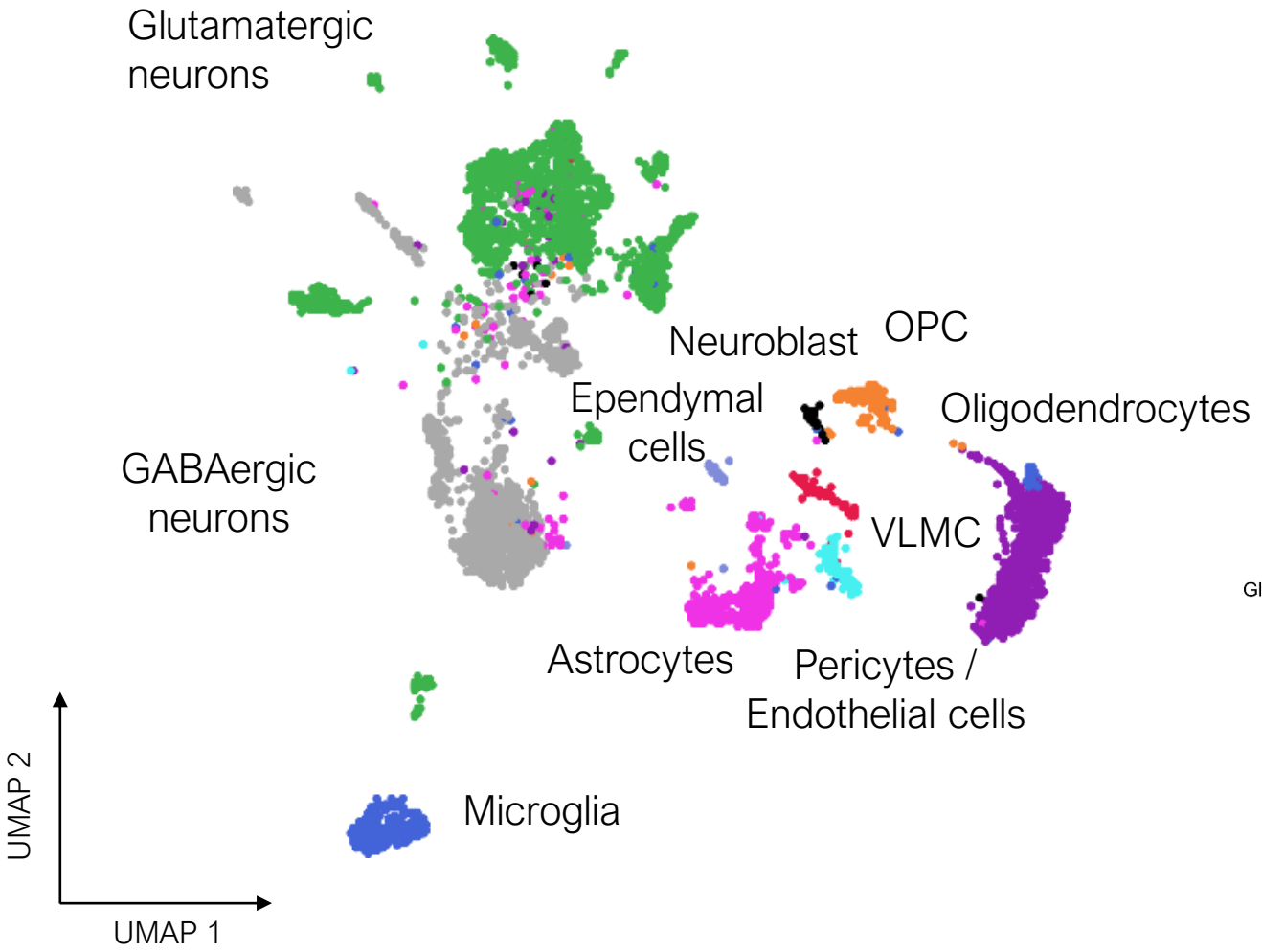
- Excitatory Neurons: Survive



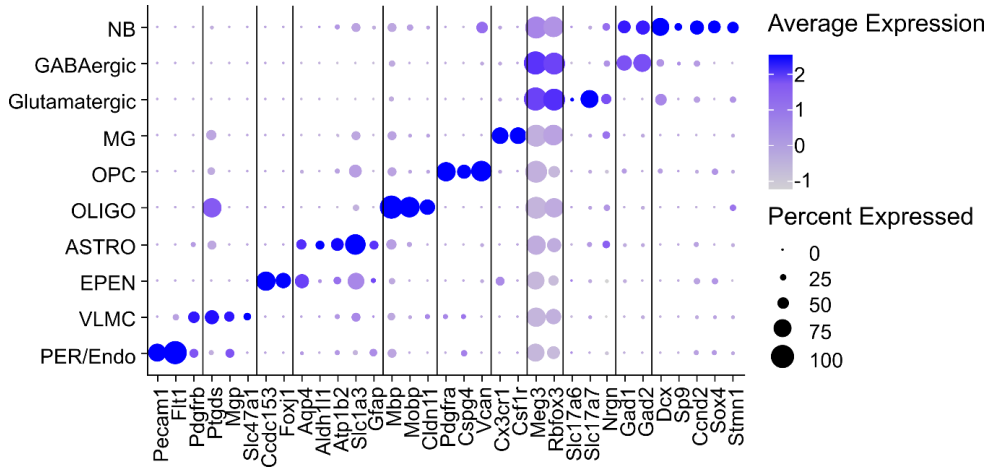




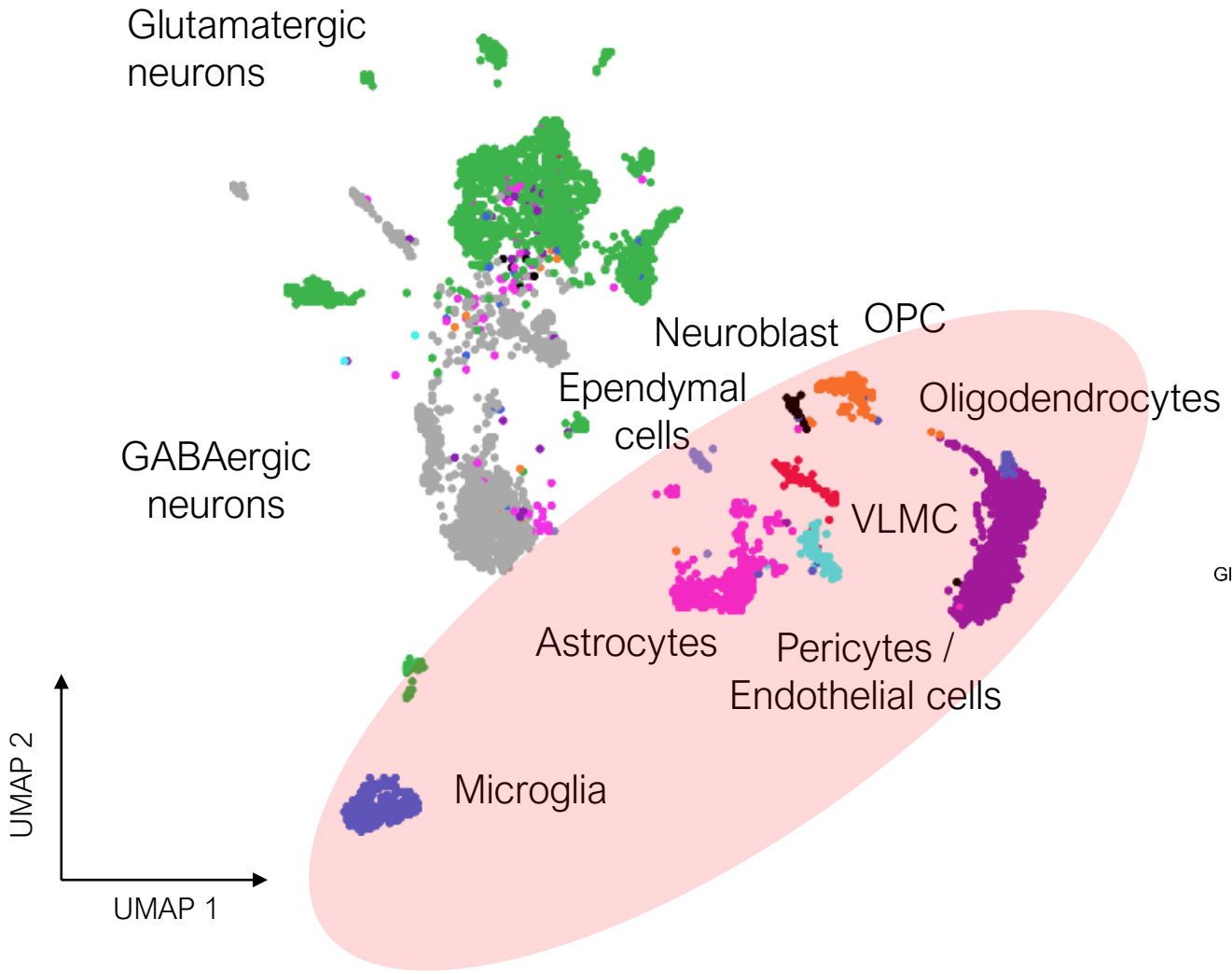
snRNA-seq



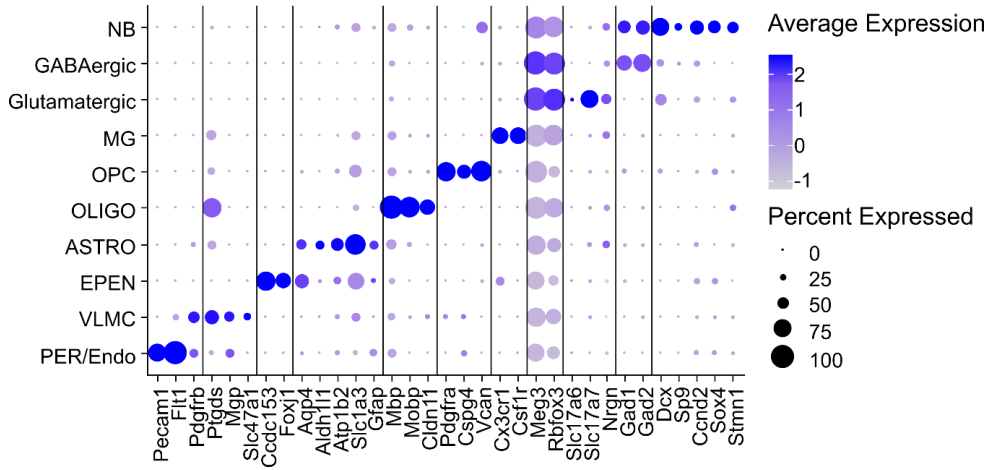
- Sham, D1, D3, D7
- Captured major cell populations



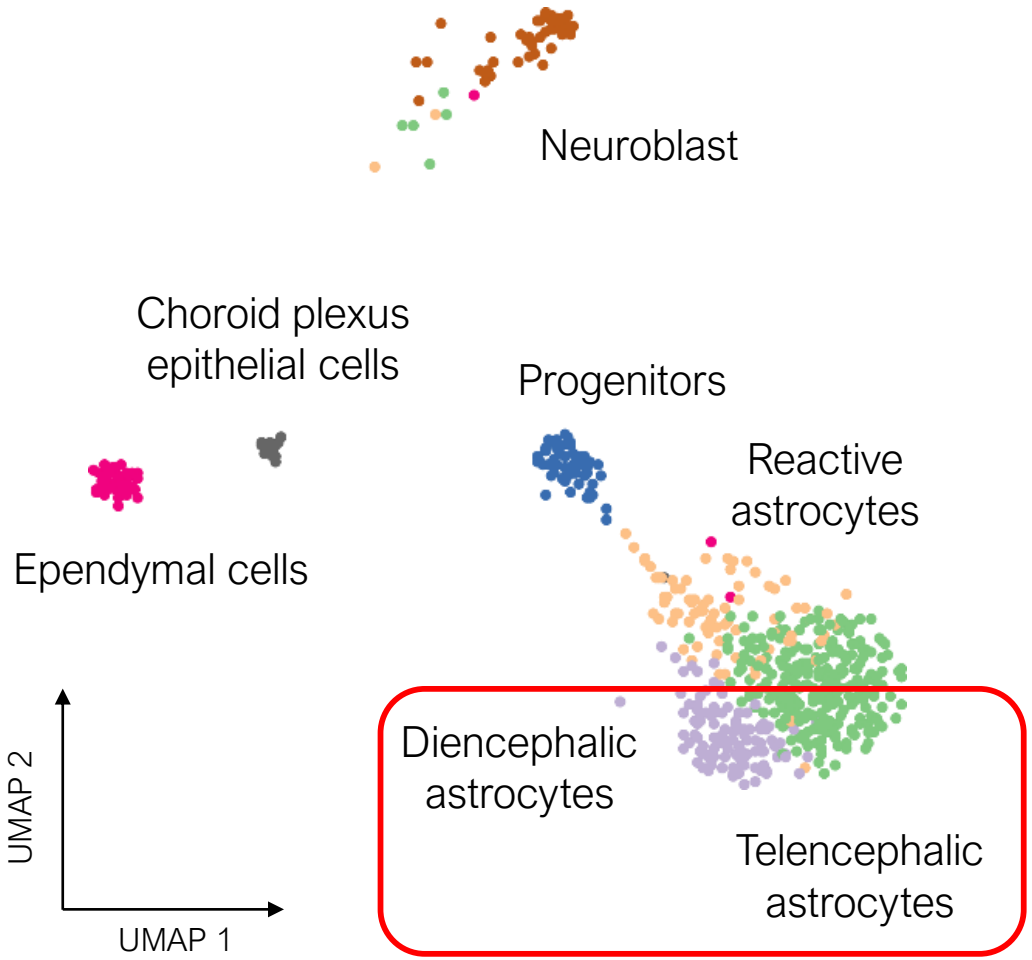
snRNA-seq

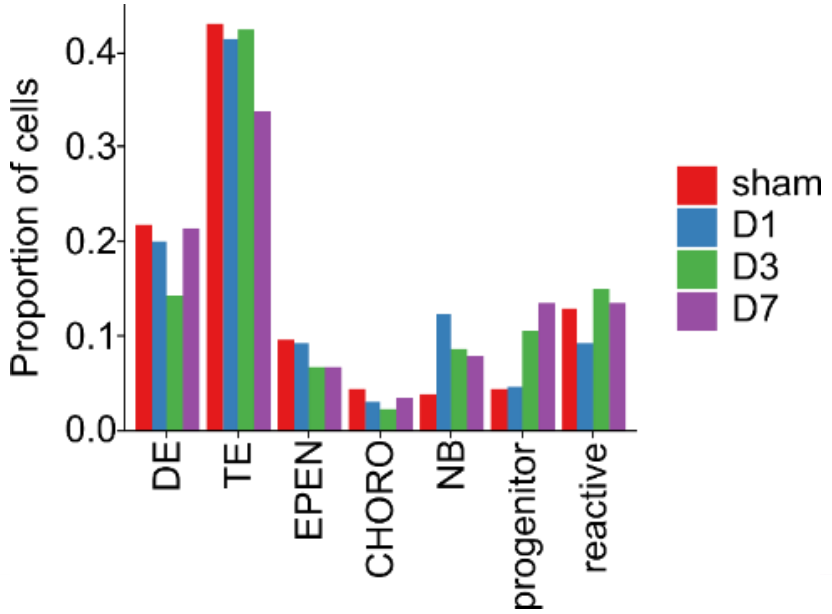


- Sham, D1, D3, D7
- Captured major cell populations
- Glial cells analyzed in detail



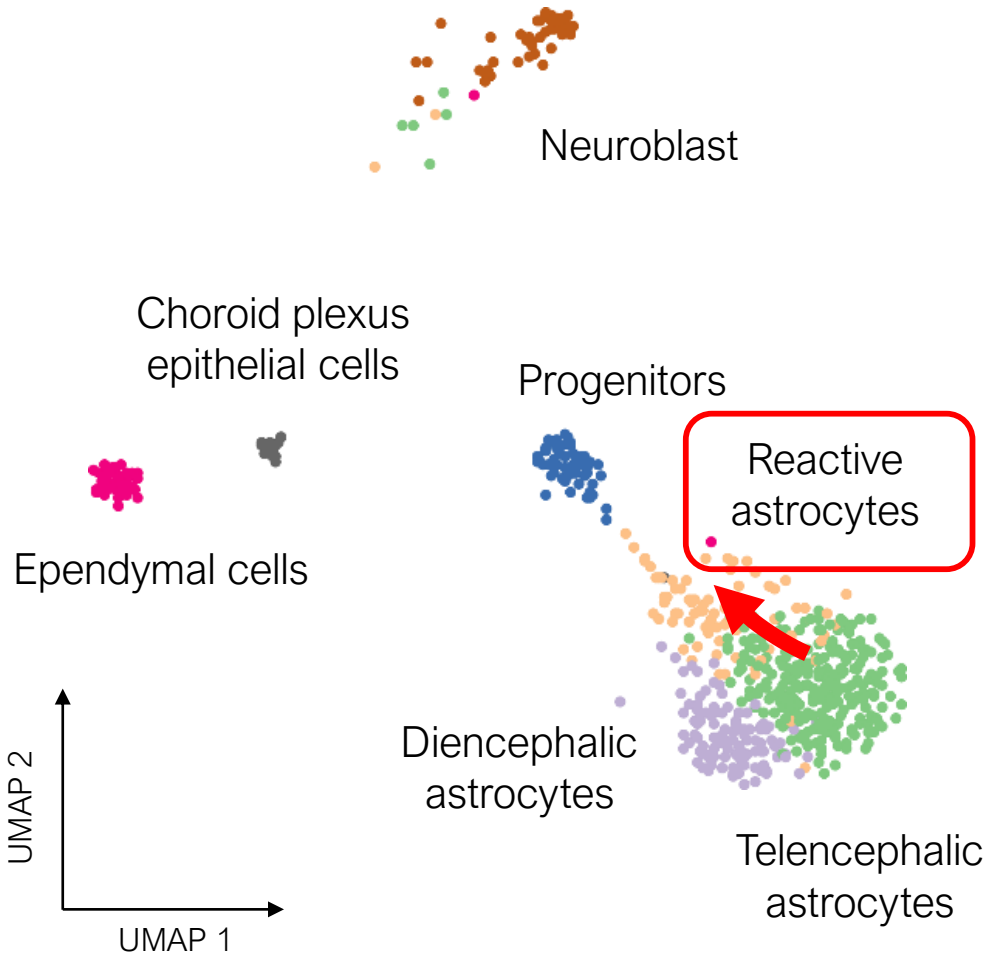
Astrocytes

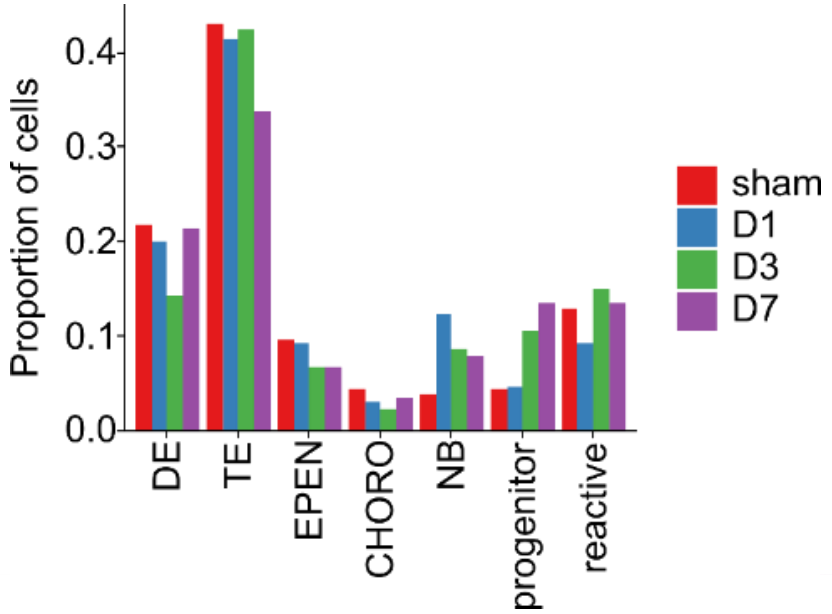




- Regional heterogeneity of astrocytes

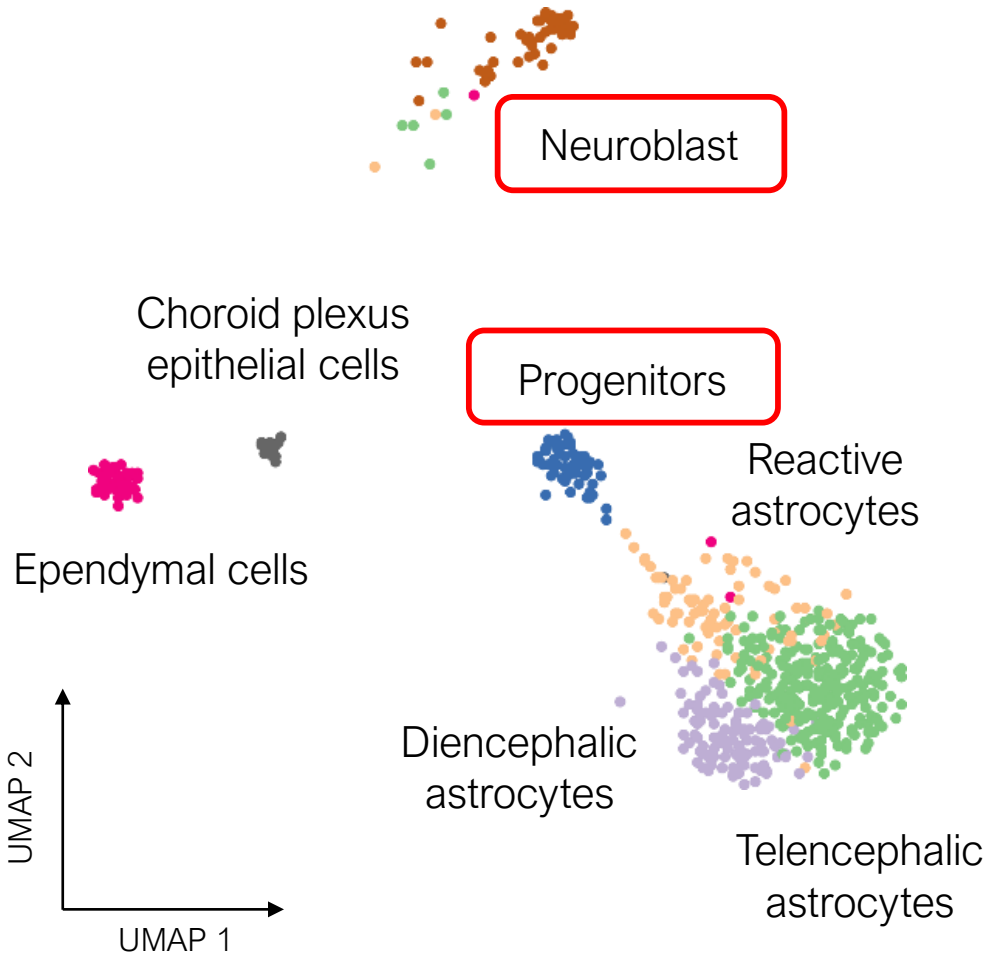
Astrocytes

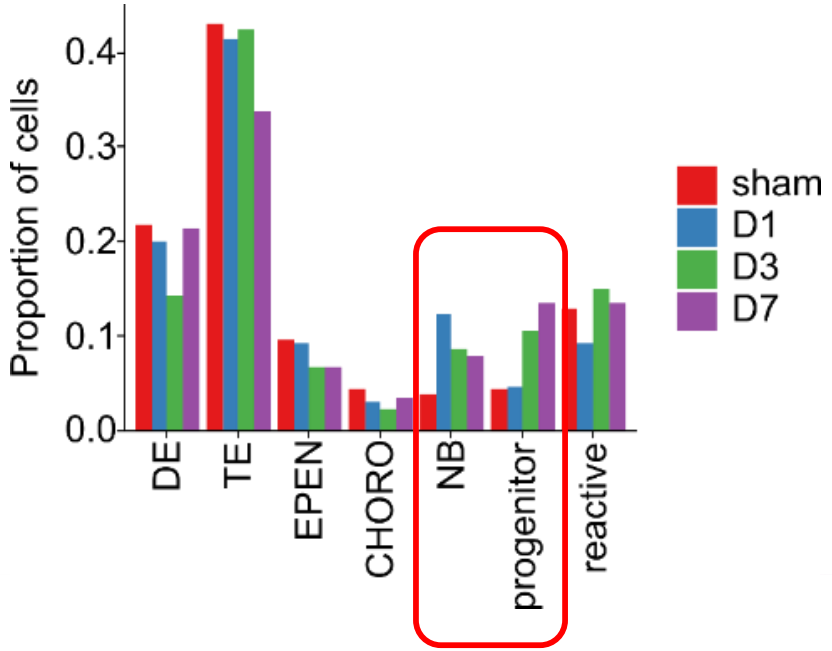




- Regional heterogeneity of astrocytes
- Reactive astrocytes generated primarily from telencephalic astrocytes

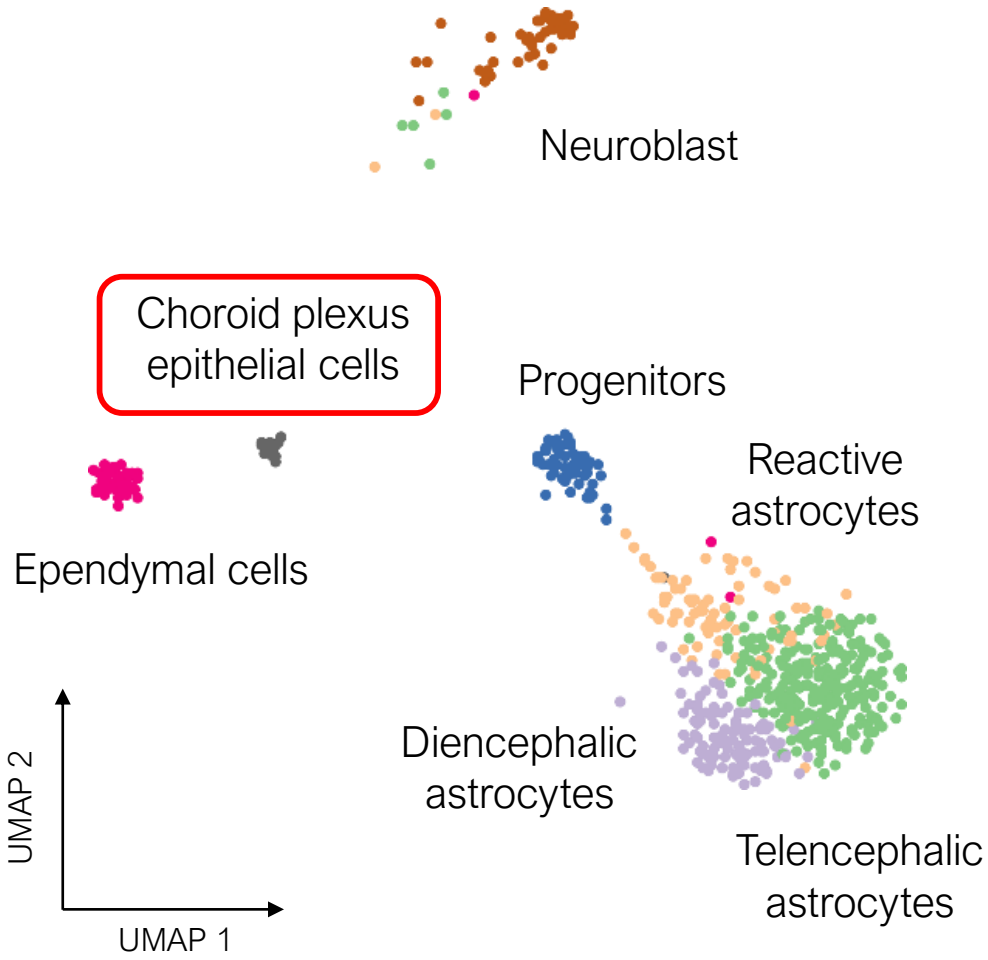
Astrocytes

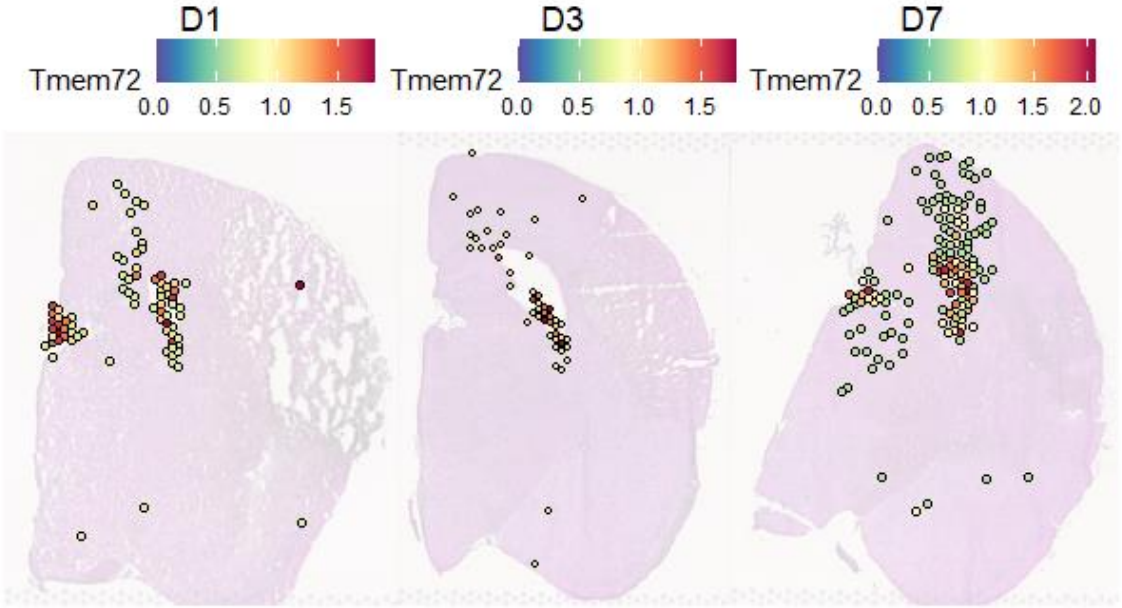




- Regional heterogeneity of astrocytes
- Reactive astrocytes generated primarily from telencephalic astrocytes
- Neurogenic potential of striatal astrocytes

Astrocytes





- Regional heterogeneity of astrocytes
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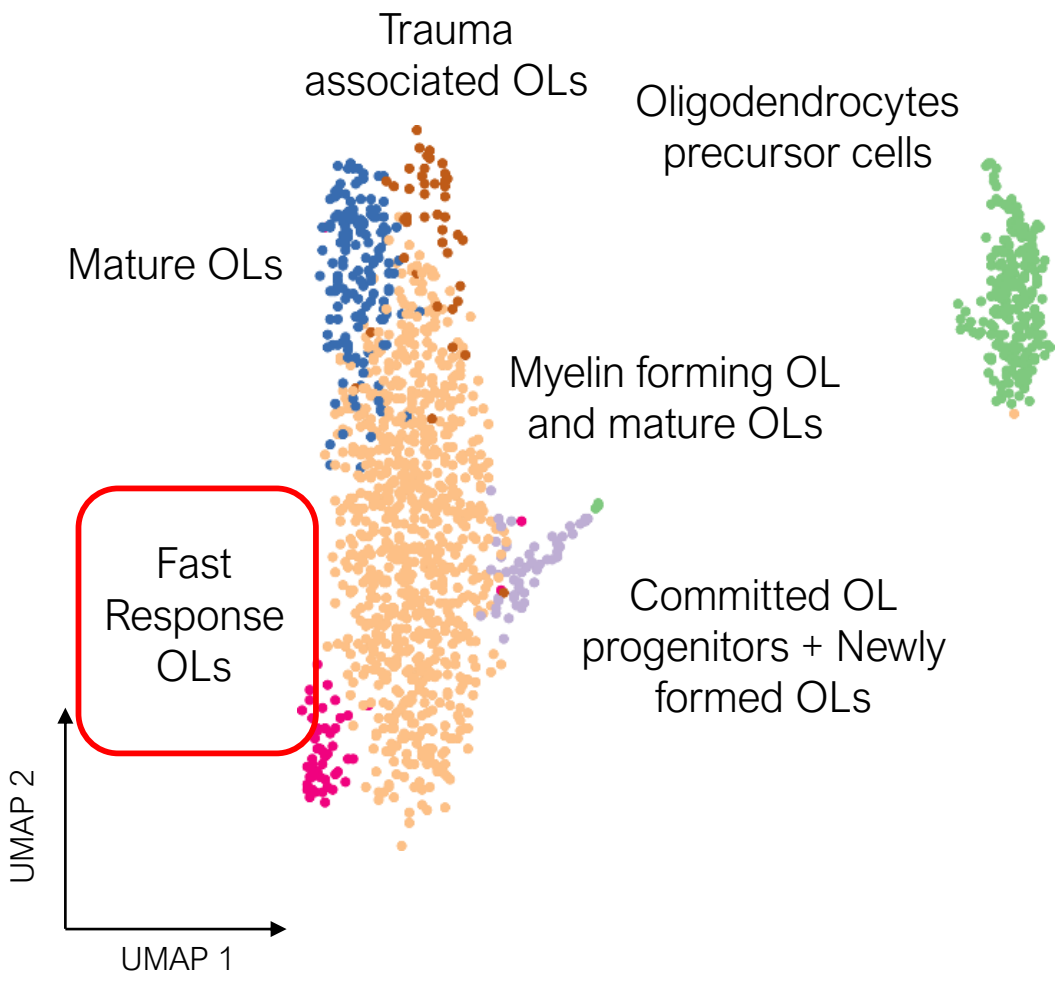
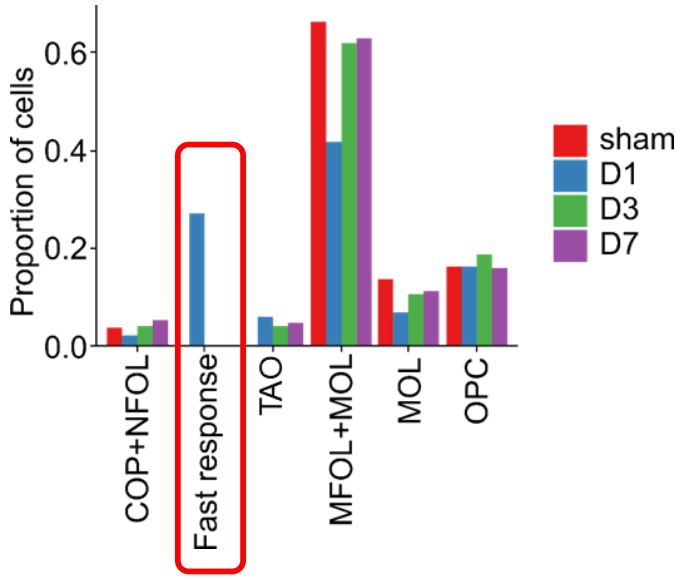
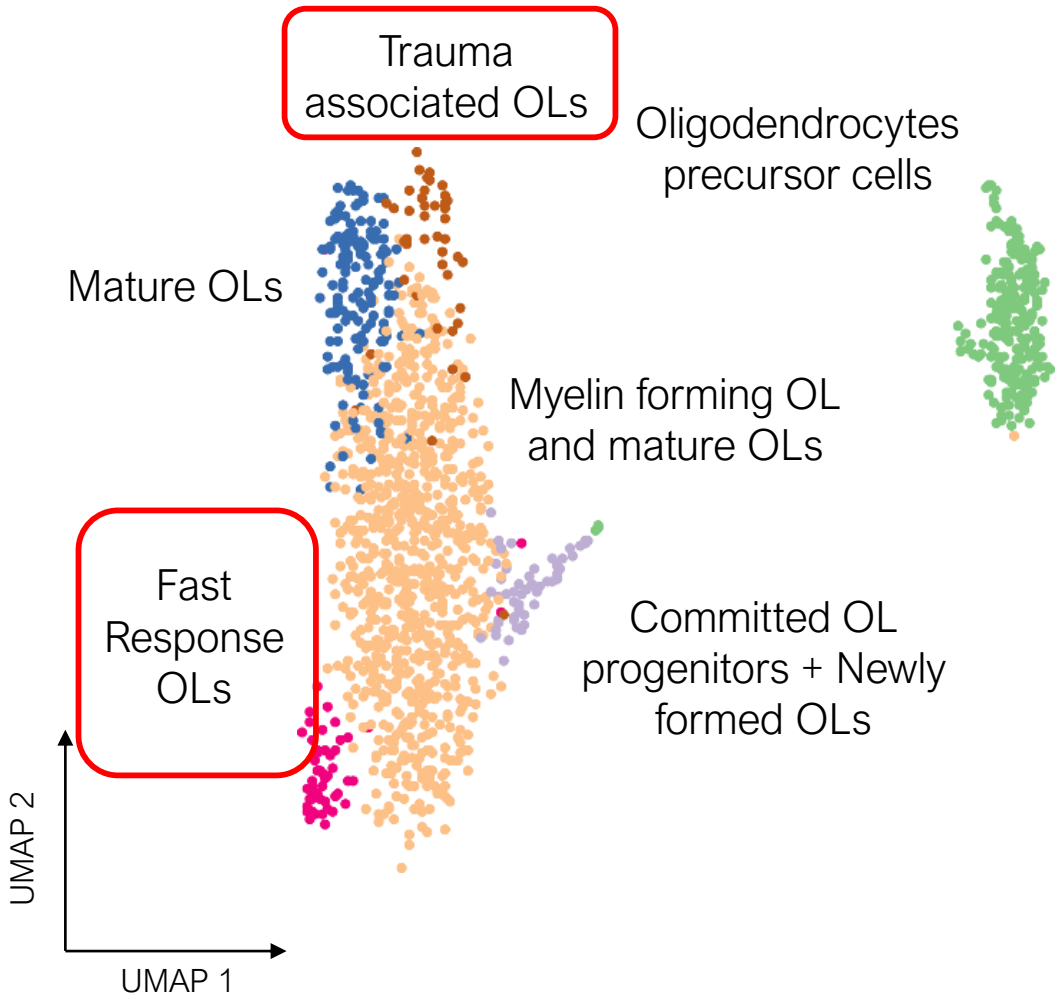
Oligodendrocytes



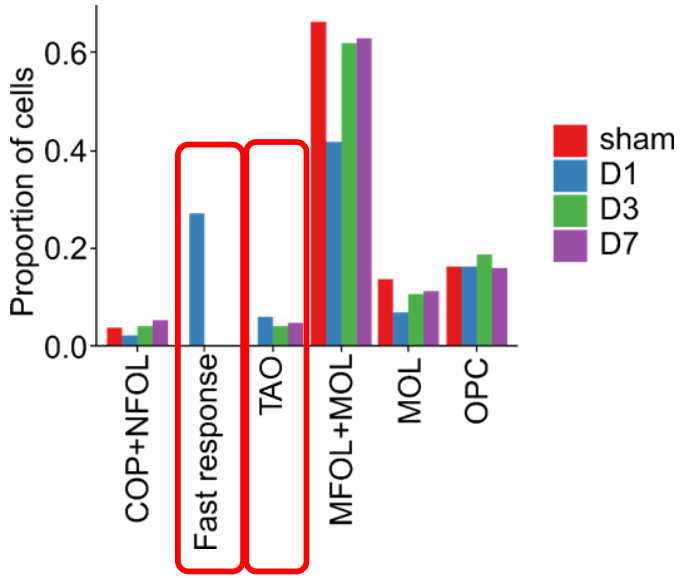
- Two new subpopulations
  - Fast response OLs



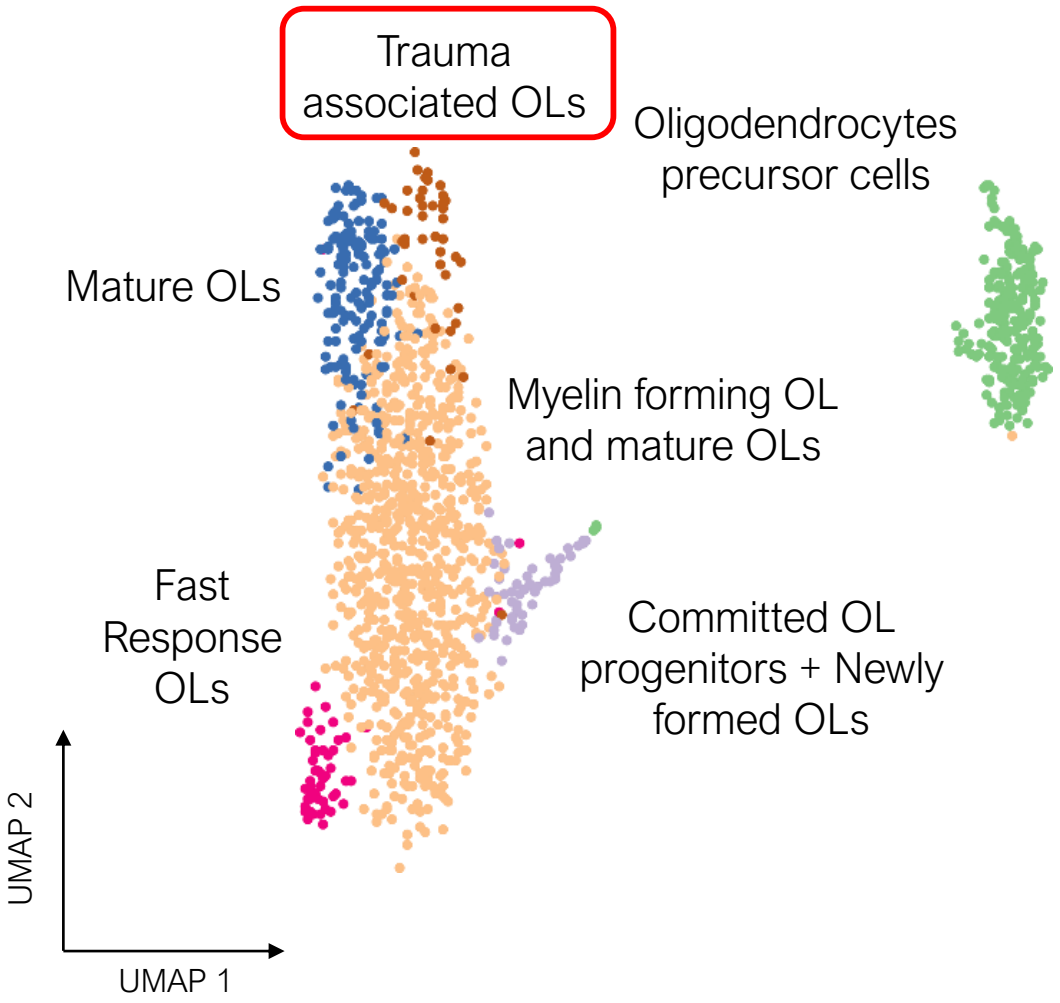
Oligodendrocytes



- Two new subpopulations
  - Fast response OLs
  - Trauma associated OLs



Oligodendrocytes



- Two new subpopulations
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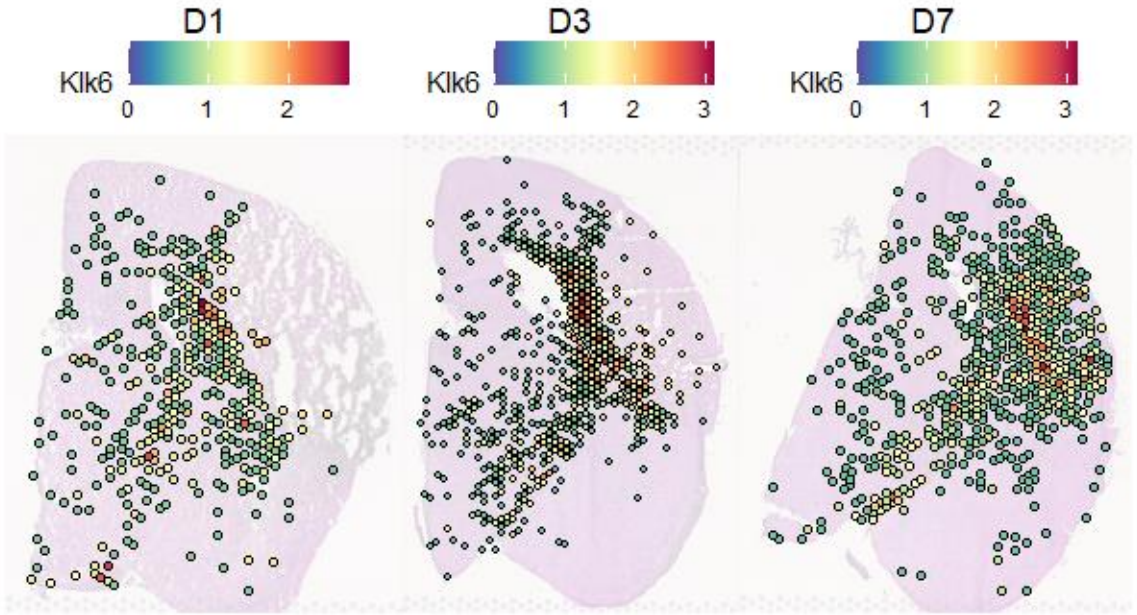
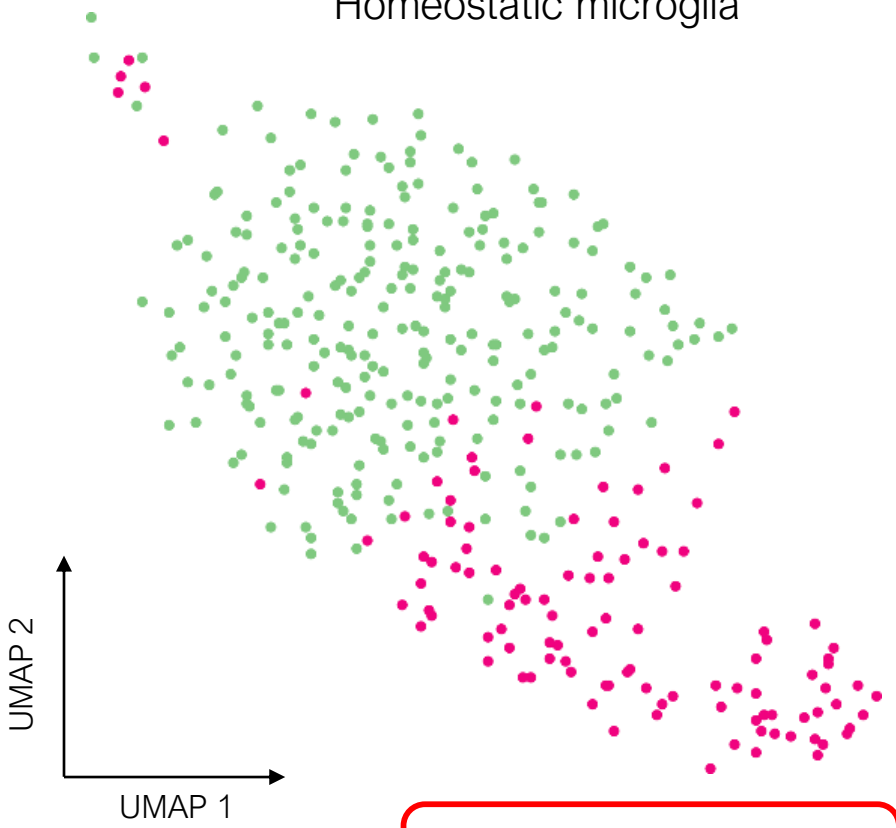
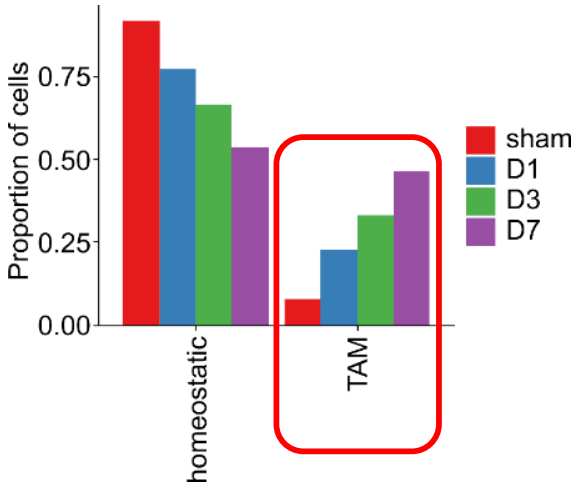
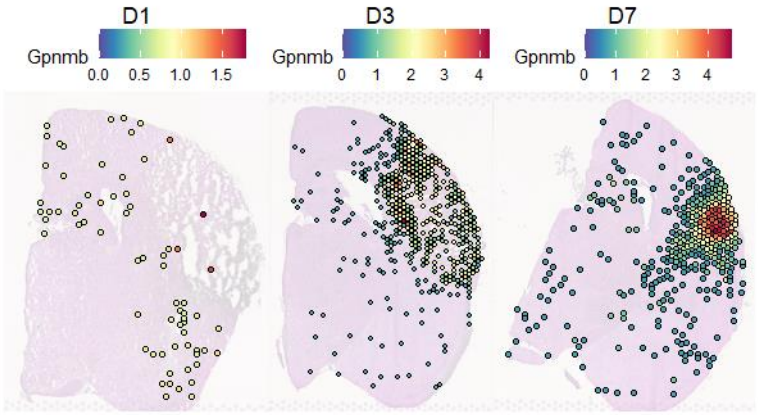


Microglia



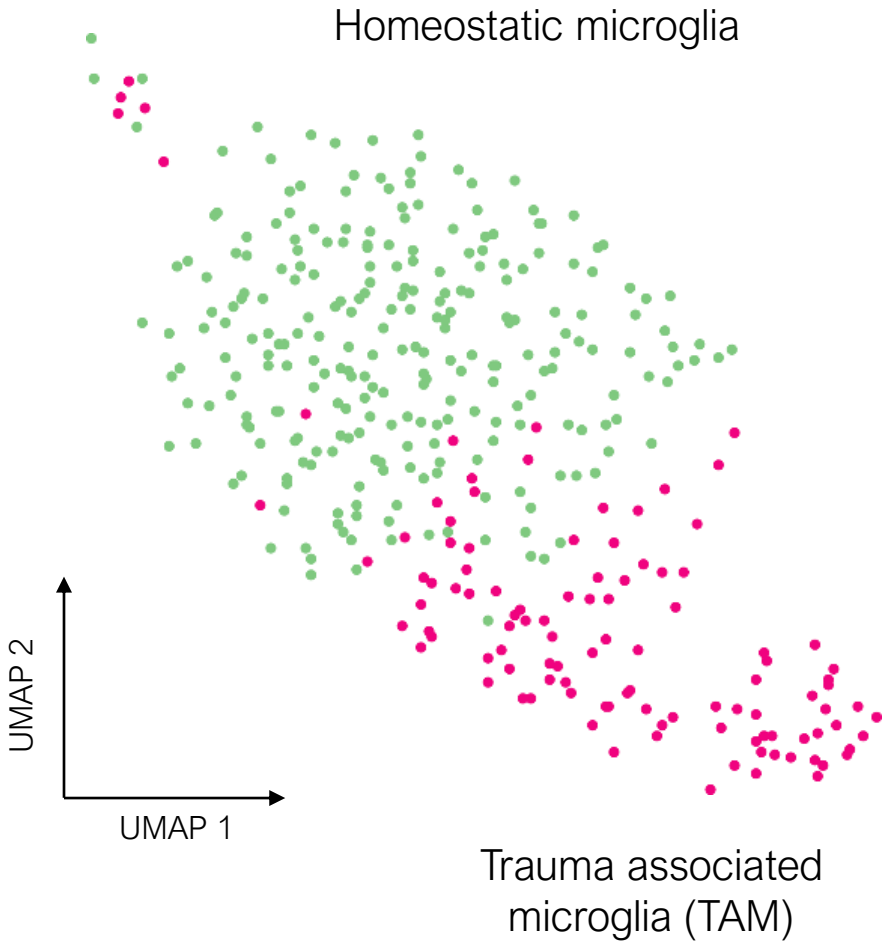
Trauma associated microglia (TAM)

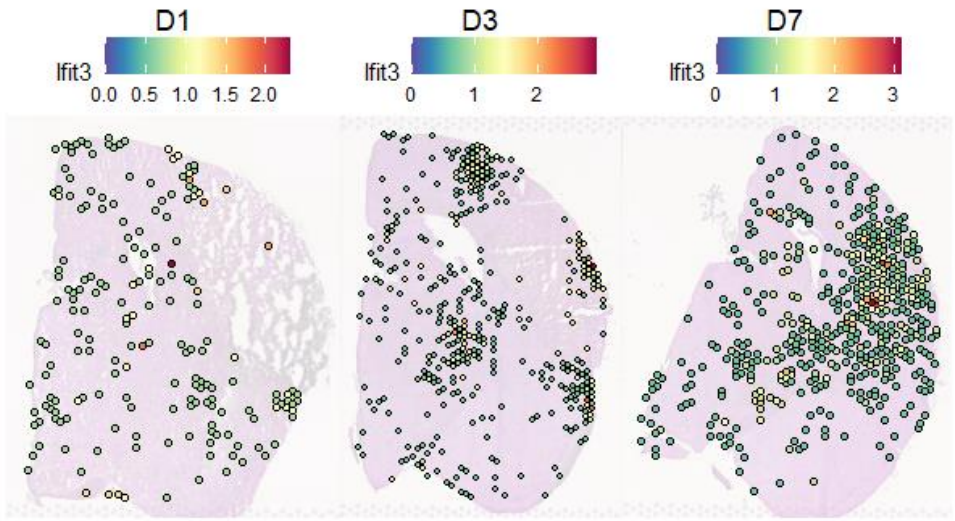




- TAM counts increased over time

Microglia

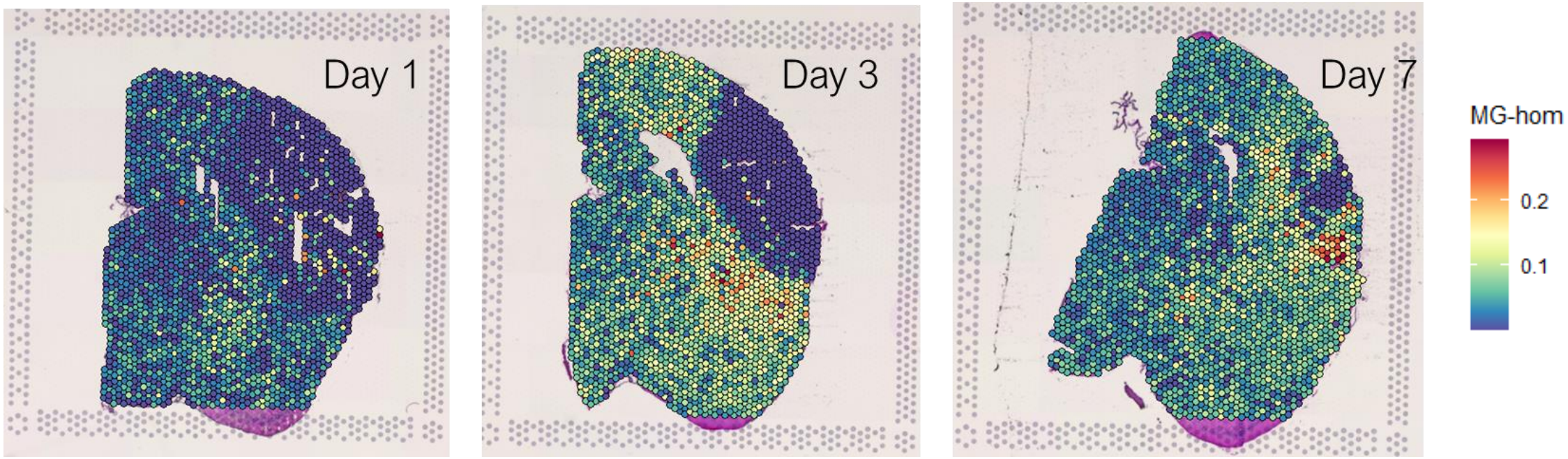




- TAM counts increased over time
- IRM not identified in snRNA-seq, but IRM markers detected in ST

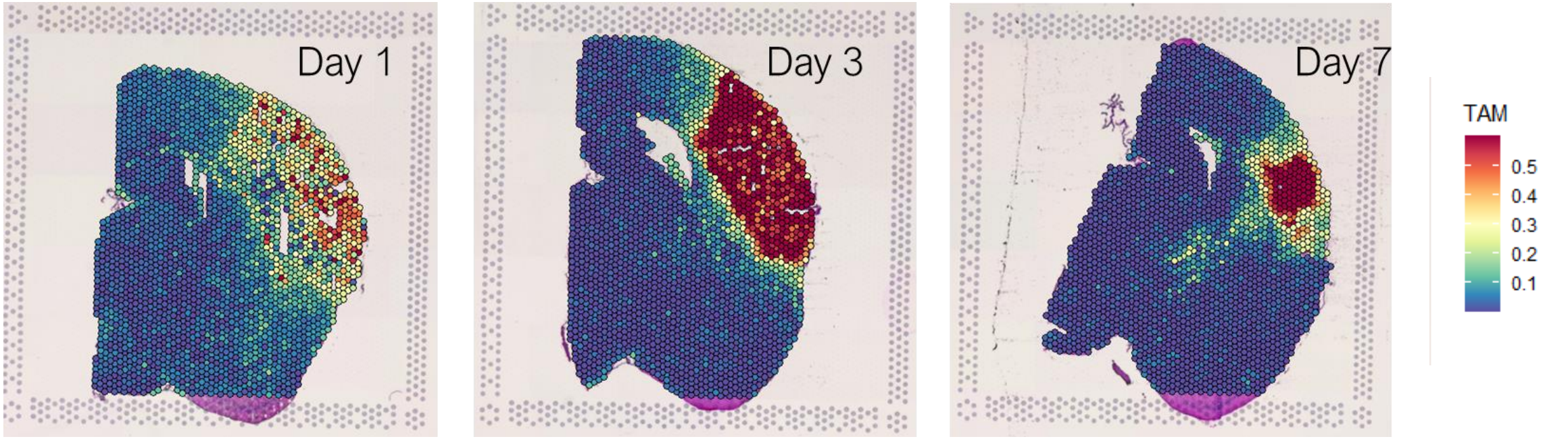
Integration of snRNA-seq and ST

- Homeostatic microglia



Integration of snRNA-seq and ST

- Trauma associated microglia (TAM)



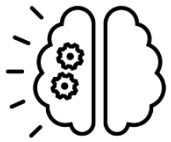
Perspectives

- Additional scRNA-seq of astrocytes, microglia and oligodendrocytes
- Deeper spatial and temporal analysis
- Integrative analysis
  - Cell-cell communication analysis
  - Deconvolution
  - Meta-analysis
- Validation
- Network analysis

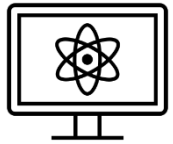
-> Identification of target for therapeutic intervention

Summary

Encompassing look at the transcriptomic heterogeneity



Dynamics of ischemic brain injury



Descriptive → functional annotation -> therapeutic intervention

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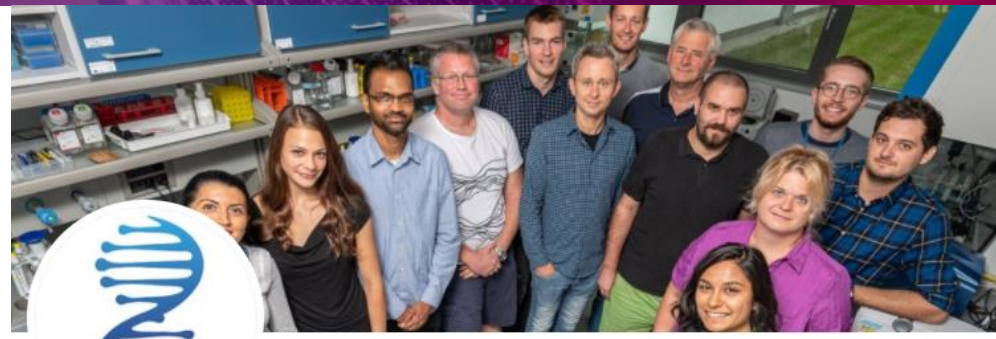

Upravit profil

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