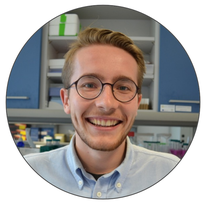
Ischemic Stroke Vis(ium)alized



Zucha Daniel[1,2], **Abaffy** Pavel[1], **Kirdajova** Denisa[3], **Anderova** Miroslava[3], **Valihrach** Lukas[1]

[1] Laboratory of Gene Expression, Institute of Biotechnology CAS, 252 50 Vestec, Czech Republic; [2] Department of Informatics and Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology, 166 28 Prague, Czech Republic; [3] Department of Cellular Neurophysiology, Institute of Experimental Medicine CAS, 142 20 Prague, Czech Republic

Introduction

Ischemic stroke is a manifestation of reduced nutrition delivery to the brain due to **impairment of the cerebral blood flow**. It is a life-threatning neurological disease, with its risks underlying in the **lack of efficient medication procedures and mechanistical understanding** of the processes following the injury. In this study, **we inspect the time-course of up to 7 days** after the ischemic stroke in the mouse model of permanent middle-cerebral artery occlusion (**pMCAO**), mimicking the permanent clogging of major cerebral artery. The information from whole-transcriptome combined with the spatial component provides valuable insights into the **anatomy of the ischemic injury, functional annotation of its processes and cell-type composition**.

Impact

Goal of this study is to provide the ischemic brain injury scientific community with:

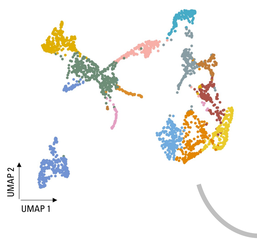
- Publicly accessible **browsing tool**, where experts can explore the spatial context for individual genes, biological processes and cell types,
- Broadened knowledge on the **ongoing molecular changes** after the ischemic stroke, by providing the transcriptome-wide analysis with spatial context.

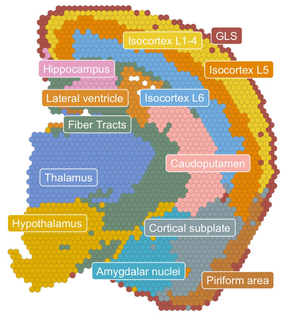
Experimental design

Ischemic injury
(tissue biopsy)

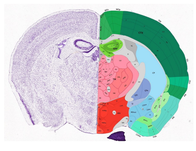
Mouse brains were collected at 1 day (**D1**), 3 days (**D3**) and 7 days (**D7**) after craniotomy followed by permanent middle cerebral artery occlusion (**pMCAO**). Fresh frozen tissues were later cryo-sectioned to 10 µm thickness and processed on 10X Visium platform. Data were processed using R package Seurat v4 (Hao et al, 2021). Brain regions were annotated with the help of Allen Brain Atlas – Mouse, P56.

Control Mouse Brain Section



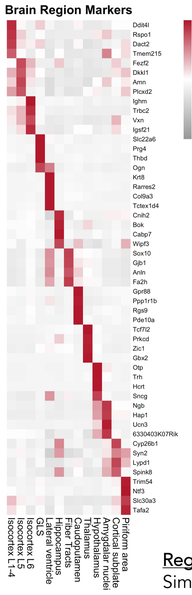


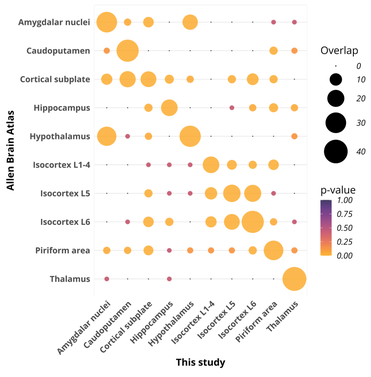
Brain anatomy is preserved in the UMAP clustering
UMAP clustering replicated separation into anatomically distinct brain regions, which are distinguishable by sets of marker genes.



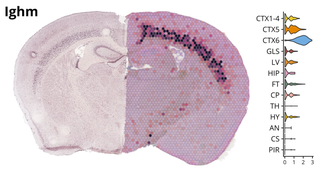
Allen Brain Atlas Reference
P56, Adult, Image 65

Brain Region Markers

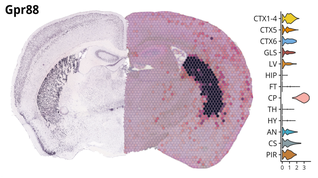




Ighm



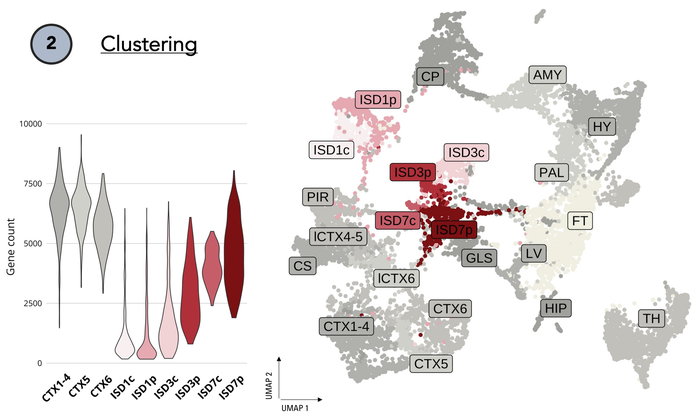
Gpr88



Regional markers overlap with Allen Brain Atlas marker sets
Similarity of spatial localization is shown in the stitched images of Allen Brain Atlas' *in-situ hybridization* atlas and our spatial feature plots (with violin plots depicting signal strength). Dotplot summarizes the overlap between the top 100 regional markers.

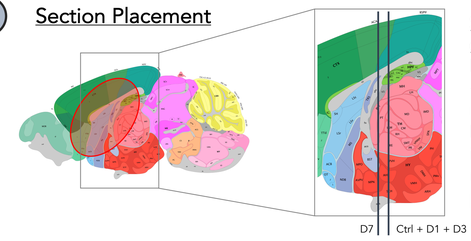
Ischemic stroke

2 Clustering

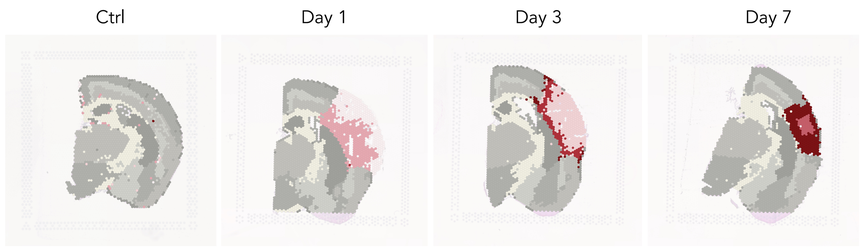


Ischemic lesion gene content varies in time.
Violin plot shows number of detected genes per spot for selected UMAP clusters. 'ISD' refers to ischemic area followed by time and location (c = core, p = periphery, CTX = isocortical layers).

1 Section Placement



Sampled sections originate from a narrow, pre-defined location.
Individual sections can be mapped to region ~100 µm wide in sagittal plane. Manual mapping was performed with the help of Allen Brain Atlas - Mouse Adult coronal atlas (ref. images 63 and 64, respectively). Encircled in red is the approximated lesion area after pMCAO.

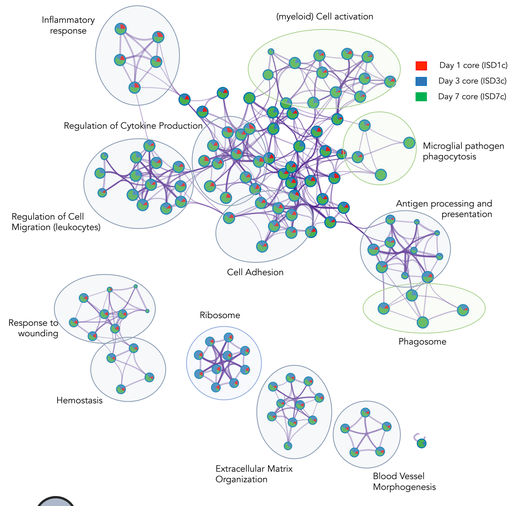


UMAP clustering divides ischemic areas into zones.
Division of ischemic areas into '**core**' and '**periphery**' across the sampled time points revisits the current annotation of ischemic injury, and provides resolution to functional biological annotation. Core lesional areas are depicted in lighter shades of pink, peripheral lesional areas in darker, for every section respectively.

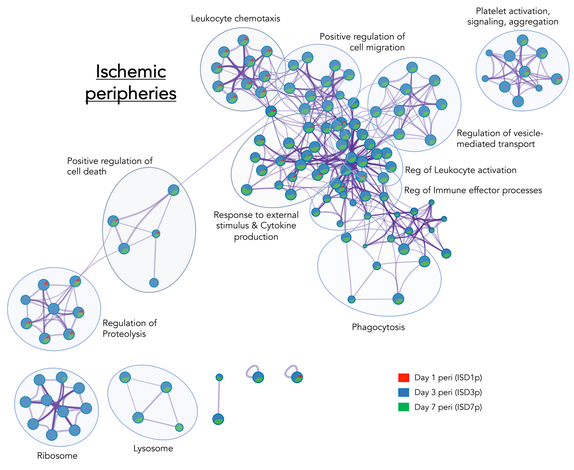
Shortcuts. Amygdalar area (AMY), Caudoputamen (CP), Cortical subplate (CS), Fiber tracts (FT), Glia limitans superficialis (GLS), Hippocampus (HIP), Hypothalamus (HY), Ischemic area D1 core (**ISD1c**), Ischemic area D1 periphery (**ISD1p**), Ischemic area D3 core (**ISD3c**), Ischemic area D3 periphery (**ISD3p**), Ischemic area D7 core (**ISD7c**), Ischemic area D7 periphery (**ISD7p**), Isocortex Layers 1-4 (CTX1-4), Isocortex Layer 5 (CTX5), Isocortex Layer 6 (CTX6), Lateral Isocortex layer 4/5 (ICTX4-5), Lateral Isocortex layer 6 (ICTX6), Lateral Ventricle (LV), Pallidum (PAL), Piriform area (PIR), Thalamus (TH)

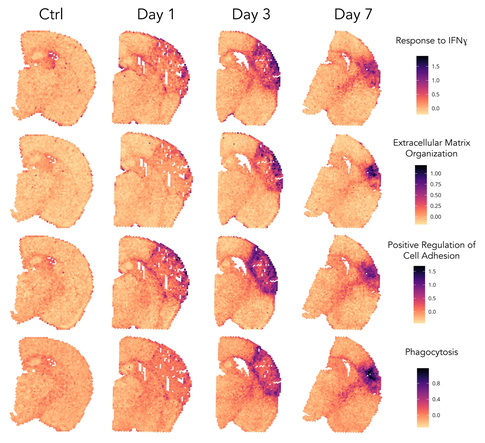
3 Functional Annotation

Ischemic cores



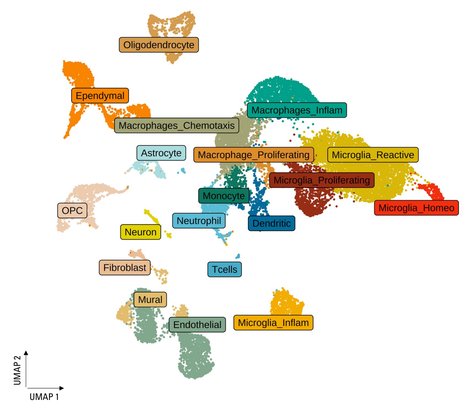
Ischemic peripheries





Gene Ontology clustering and process visualization
Clustering of enriched GO terms with highlighted parent GO terms. Pie charts display contribution of time-specific markers to the enriched term. Plots were generated with Metascape (Zhou et al, 2019). Spatial plots show spatial localization of representative processes.

4 Celltype Deconvolution



Identification of celltype proportions using the RCTD algorithm
Study of Milich et al., (2021) on spinal cord injury was used for single-cell reference. Their experimental design closely matches ours (ctrl, D1 - D3 - D7) with no filters for cell types were applied. The dataset was *in-house* re-analyzed. The RCTD deconvolution algorithm was developed by Cable et al. (2021). Spatial plots depict relative proportions.

References

Allen Brain Atlas, portal.brain-map.org
Cable, D.M., Murray, E., Zou, L.S. *et al.* Robust decomposition of cell type mixtures in spatial transcriptomics. *Nat Biotechnol* **40,** 517–526 (2022).
Hao, Y., Hao, S., Andersen-Nissen, E., *et al.* Integrated analysis of multimodal single-cell data. *Cell* **184,** 3573-3587 (2021).
Zhou, Y., Zhou, B., Pache, L. *et al.* Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* **10,** 1523 (2019).
Milich, L., Choi, J., Ryan, C. *et al.* Single-cell analysis of the cellular heterogeneity and interactions in the injured mouse spinal cord. *Journal of Experimental Medicine* **218,** 1-25 (2021).

Funding

This study was supported by Czech Science Foundation (20-05770S) and institutional support (RVO 86652036).

More from us

Scan me!

labgenexp.eu / @labgenex / @DanielZucha