



# Spatial transcriptomic landscape of experimental ischemic brain injury

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## P 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. With a follow up single-cell transcriptomic experiement, we identified key reactive glial subpopulations, which operate at the lesion border.



(1)

### Assessing the variability of lesioned area volume



Volume and variability of ischemic injury
decrease in time. Using magnetic resonance
imaging (MRI), we measured brain swelling at
three timepoints after pMCAO ischemic injury
(D1, D3, D7). The decrease in volume was
evidenced in separate instances (barplot with SD
error bar, n = 5 mice per condition, Wilcox rank
sum test), but also for a recurring measurement
(3D render with the brain in red, lesion in yellow,
n = 1 mice across the conditions).



Sampled sections originate from a narrow, pre-defined location. Individual sections can be mapped to region ~100 µm wide in sagittal plane. Mapping was performed with Allen Brain Atlas -Mouse Adult coronal atlas (ref. images 63 and 64, respectively). Approximate lesioned area is encircled in red.

### - Č- Impact

### Goal of this study is to:

- Describe the **change in gene expression landscape** after the ischemic stroke, by transcriptome-wide analysis in tissue sections.
- Display similarities in **glial response** between acute brain injury and neurodegenerative disease through the lens of transcriptome response.
- Provide the scientific community with a **high-quality resource** revealing genome-wide transcriptomic changes during acute phase of ischemic brain injury.



Mouse cerebra were collected in a timeseries of no injury (Ctrl), 1 day (D1), 3 days (D3) and 7 days (D7) after permanent middle cerebral artery occlusion (pMCAO) brain injury. Fresh frozen tissues were later cryo-sectioned to 10 µm thickness and processed on 10X Visium platform. Similarly, mouse cerebra were collected and processed for single-cell RNA-Seq in a follow-up experiment. 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





### UMAP clustering divides lesioned area into two zones.

Division of ischemic areas into 'core' and 'periphery' across the sampled time points revisits the current annotation of ischemic injury. The dynamic change is also evidenced in cortical transcriptomic activity, expressed as detected genes per spot (violin plot). Core lesional areas are in light shades of pink, periphereal lesioned areas in dark shades, in every section respectively.

Abbreviations. 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), Iscortex 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)

### The networks of transcriptomic response



## Experimental design

![](_page_0_Picture_32.jpeg)

 Brain anatomy is preserved in the spatial
transcriptomic profile
Analysis of control brain section demonstrated good quality of the data, the anatomic agreement with reference atlases and the power of spatial transcriptomics in characterization of gene expression differences with anatomical precision.

![](_page_0_Figure_34.jpeg)

<u>Regional markers overlap with Allen Brain Atlas marker sets</u>

#### Introduction to the response to ischemic injury.

Characterization of the enriched GO processes, visualized as networks with parent GO term highlighted (Metascape, Zhou et al, 2019). Inflammatory response, leukocyte migration, cell activation, cell death and response to wounding occur predominantly in the earlier timepoints. Extracellular matrix organization, angiogenesis and gliogenesis started to appear later, suggesting an initiation of reparative processes. The zonal contribution to the term (core vs periphery) is color denoted (red vs blue). Selected GO terms are visualized in spatial plots.

### Deciphering of celltype response to injury

(5)

![](_page_0_Figure_39.jpeg)

#### Example of spatial localization for proliferating microglia

![](_page_0_Figure_41.jpeg)

### Dramatic changes in cell type composition revealed by deconvolution.

The lesion was a hotspot for dynamic cell coordination, both spatially and temporally. At first, peripheral immune cells invaded the lesion to contain the damage and establish homeostasis. This was followed by tissue remodelling by phagocytic and vascular cells (macrophages, microglia, fibroblasts, endothelial cells), to allow for regeneration. Some celltypes displayed particularly distinct spatial and temporal localization (i.e. proliferating microglia). *In-house* reanalyzed single-cell dataset of Milich et al. (2021) on spinal cord injury was used as reference for the RCTD deconvolution (Cable et al., 2021).

![](_page_0_Figure_44.jpeg)

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

![](_page_0_Figure_46.jpeg)

![](_page_0_Figure_47.jpeg)

![](_page_0_Figure_48.jpeg)

![](_page_0_Figure_49.jpeg)

#### Validation of DOL presence in the ischemic border

![](_page_0_Picture_51.jpeg)

With immunohistochemistry, we observed *Serpina3n*+ (red) oligodendrocytes (*Plp1*+, green) in the ischemic border (arrowdenoted).

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).

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Miller, B.F., Huang, F., Atta, L. et al. Reference-free cell type deconvolution of multi-cellular pixel-resolution spatially resolved transcriptomics data. Nat Commun 13, 2339 (2022).

![](_page_0_Picture_60.jpeg)

population comprising the profile were DOL-like cells

identifed in Alzheimer disease (Kenigsbuch et al., 2022).

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