Whole-brain mRNA imaging unveils the dynamics of neuroinflammation after stroke

Authors
Lea Lydolph Larsen[1,2], Thomas Topilko[1], Bettina Hjelm Clausen[2], Urmas Roostalu[1], Kate Lykke Lambertsen[2,3], Jacob Hecksher-Sørensen[1]

[1]Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark
[2]Department of Molecular Medicine, University of Southern Denmark, Odense, Denmark.
[3]Department of Neurology, Odense University Hospital, Odense, Denmark.

Corresponding author
Lea Lydolph Larsen - lll@gubra.dk

Background & Aim

Microglia respond to brain injury with diverse morphological and gene expression changes, influenced by spatial location, sex, and age. Traditional protein-based staining techniques fail to fully capture these nuanced gene expression dynamics. To address this, we developed a whole-brain three-dimensional (3D) mRNA imaging method. In the current study, *Hexb* is included as a microglial marker, as it has been identified as a homeostatic core gene that is stable in different neuroinflammatory conditions. This platform integrates mRNA and protein imaging to map microglia responses in the context of ischemic stroke. The aim is to elucidate how microglial activation signatures propagate across the brain following stroke.

Methods

Female and male mice were subjected to permanent middle cerebral artery occlusion (pMCAO). Brains were collected at day 1 and 7 post-pMCAO and processed for 1) whole-brain Hexb mRNA imaging using *in situ* hybridization, or 2) whole-brain antibody staining of IBA1, CD31, and SM22 using iDISCO. Upon clearing, brains were scanned using light-sheet fluorescent microscopy.

www.gubra.dk

1 Pipeline for whole-brain mRNA and protein imaging in the pMCAO stroke model

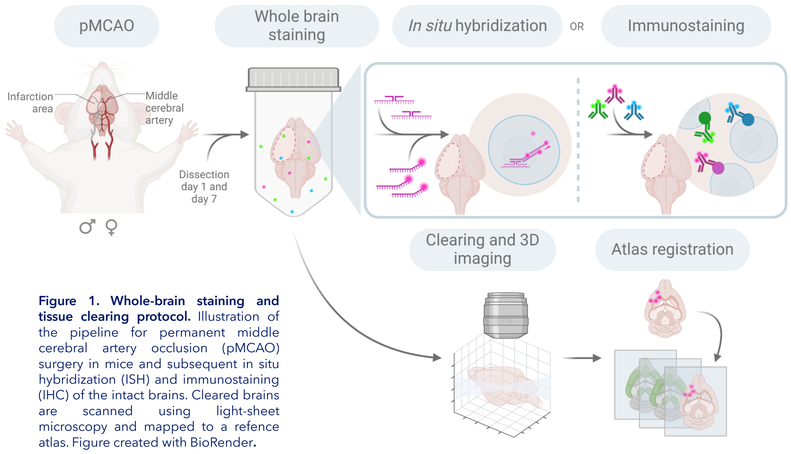

Figure 1. Whole-brain staining and tissue clearing protocol. Illustration of the pipeline for permanent middle cerebral artery occlusion (pMCAO) surgery in mice and subsequent in situ hybridization (ISH) and immunostaining (IHC) of the intact brains. Cleared brains are scanned using light-sheet microscopy and mapped to a refence atlas. Figure created with BioRender.

2 Temporal dynamics of *Hexb* expression in the infarct area of female and male mice

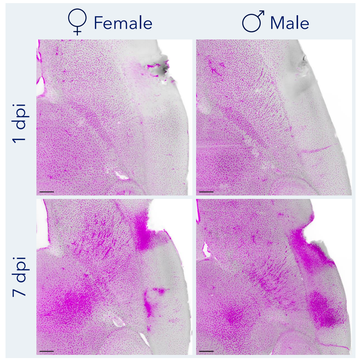

Figure 2. Whole-brain mRNA imaging of Hexb in female and male mice at 1 and 7 dpi (days post-infarct). Magnified horizontal view (50µm projection) show *Hexb* (hexosaminidase subunit beta) (magenta) localization at the infarct area (n=1). Scale bars: 400µm.

3 *Hexb* imaging shows spatial patterns of inflammatory responses driven by microglia post-stroke

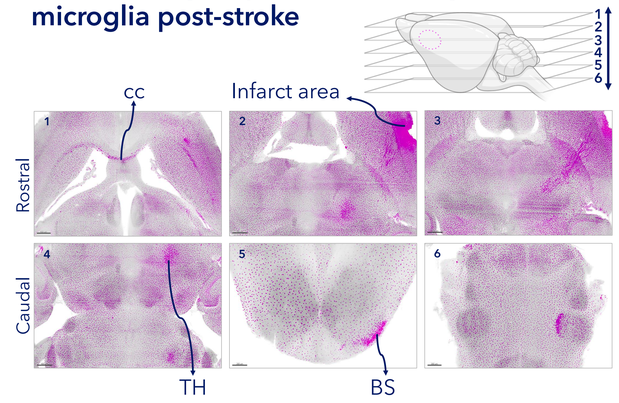

Figure 3. Spatial distribution of *Hexb* expression 7 dpi in female mouse. Magnified horizontal view (50µm) at selected planes show Hexb enrichment around the primary injury site and fiber tracts (n=1). Scale bars: 200-400µm. TH: thalamus, BS: brain stem, CC: corpus callosum.

Scan the QR code to see the poster online

4 By day 7 post-occlusion, microglia accumulate locally with enhanced distribution along the corticospinal tract, accompanied by vascular remodelling

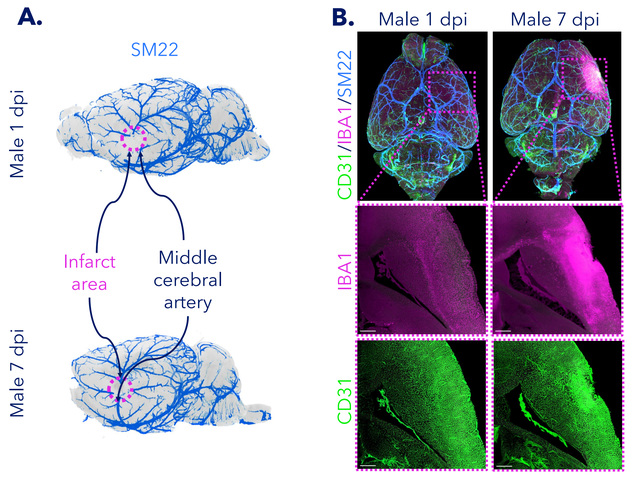

Figure 4. Temporal dynamics of microglial and vascular changes in whole mouse brain post-stroke. (A) SM22 (transgelin) immunostaining in sagittal view displaying the MCA and infarct zone 1 and 7 dpi in male mice (n=1). **(B)** Triplex IBA1 (ionized calcium-binding adapter molecule 1), CD31 (cluster of differentiation 31), and SM22 immunostaining in max projection view (upper) at 1 and 7 dpi. Magnified slice view (50µm) at the level of the infarct area (lower) for IBA1 and CD31. Scale bars: 500µm.

5 Pipeline for analysing microglia response following stroke

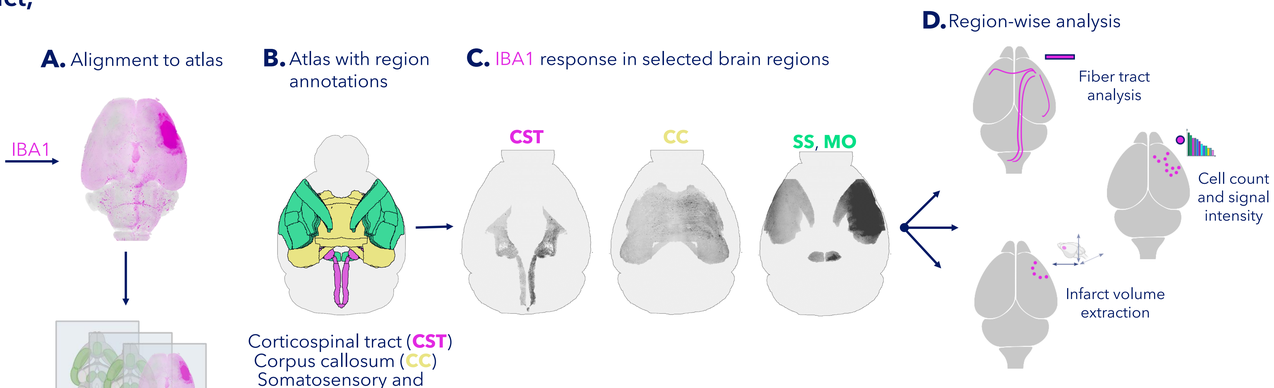

Figure 5. Whole-brain analysis pipeline for microglia. (A) Raw data brain volume with IBA staining is aligned to mouse brain atlas. **(B)** Regions of interest are selected for further analysis. **(C)** Regions Raw data with region masks show IBA+ reactivity in the primary injury site (SS,MO), and along fiber tracts (CST, CC). **(D)** Defined analysis end-point are outlined. The entire brain data is quantified, with results differentiated by specific brain regions.

Conclusion

+ *Hexb* exhibits proximal and distal localization relative to the infarct area in both female and male mice 7 dpi, highlighting an extensive spatial and temporal microglial response
+ SM22 and CD31 staining reveal MCA ligation and vascular reorganization 7 days after stroke
+ IBA1 expression increases seven days post-stroke, with enrichment around fiber tracts.