Unveiling the third dimension: whole-brain imaging in neuroinflammation after stroke

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

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

Conclusion

- *Hexb* localizes to the infarct area in female and male mice 7 days after stroke
- SM22 and IBA1 staining reveal MCA ligation and vascular reorganization 7 days after stroke
- IBA1 is increased 7 days after stroke, with enrichment around the corticospinal tract in the male mouse

Background & Aim

Microglia, as frontline responders in ischemic stroke, exhibit diverse morphology, gene expression patterns, and functions influenced by spatial location, sex, and age[1]. Traditional techniques, reliant on protein-based staining, often fall short in capturing the nuanced gene expression dynamics of these cells[2]. In response, we introduce a three-dimensional imaging platform, integrating both mRNA and protein, tailored specifically for the study of neuroinflammation. The aim is to build a robust package of markers that can unravel the complexities of microglial heterogeneity and gene expression dynamics. *Hexb* has been identified as a homeostatic core gene that is stable in different neuroinflammatory conditions, in contrast to microglia core genes (*Tmem119* and *P2ry12*)[3]. In the current study, *Hexb* was included to study the expression in male and female mice in the context of stroke. Additionally, arterial and microglial immunostaining were included to provide insights into vascular dynamics and microglial recruitment post-stroke.

Methods

Female and male mice were subjected to permanent middle cerebral artery occlusion (pMCAO) as previously described[4]. 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[5]. Upon clearing, brains were scanned using light-sheet fluorescent microscopy.

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

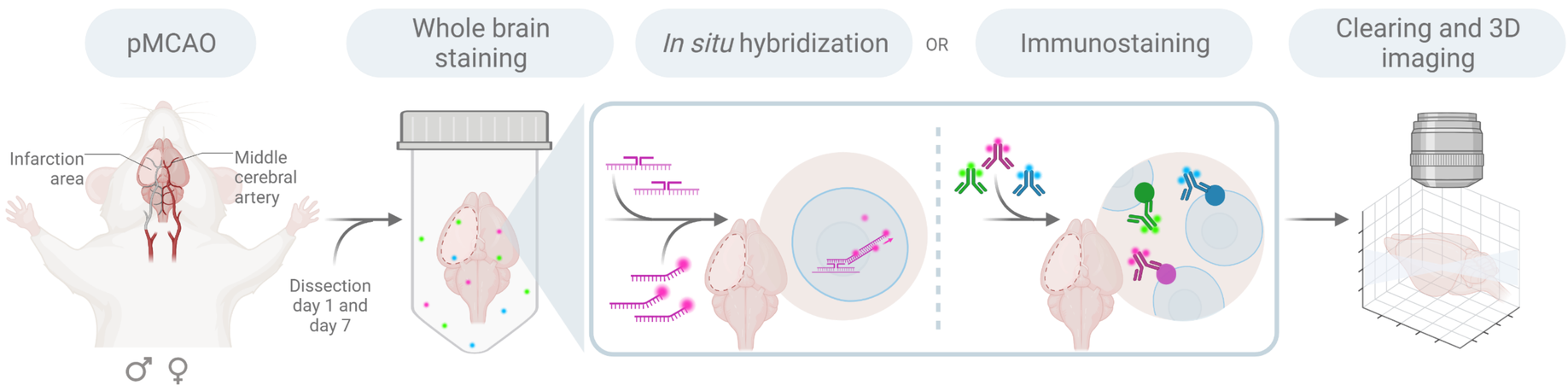

Figure 1. Whole-brain staining and tissue clearing protocol. Illustration of the pipeline for permanent middle cerebral artery occlusion (pMCAO) surgery in mice and subsequent *in situ* hybridization (ISH) and immunostaining (IHC) of the intact brains. Figure created with BioRender.

2 mRNA imaging reveals temporal dynamics of *Hexb* localization to the infarct area in female and male mice following ischemic stroke

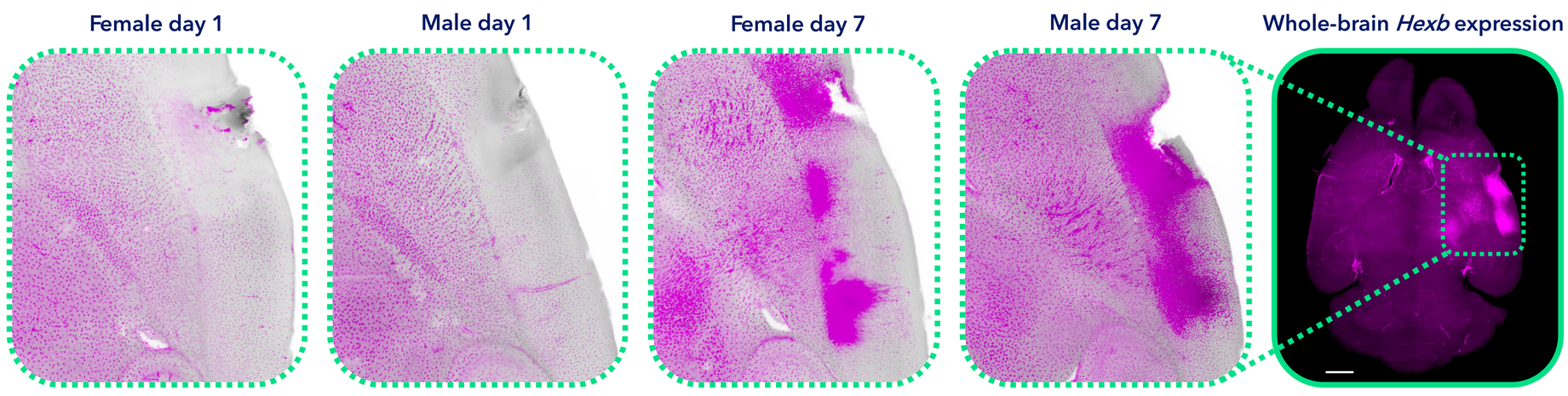

Figure 1. Whole-brain mRNA imaging of *Hexb* at day 1 and 7 post-pMCAO in female and male mice. To the right, the raw data max projection image of *Hexb* (hexosaminidase subunit beta) expression is shown for male day 7. Scalebar 1000µm. To the left, the *Hexb* staining is shown in magnified horizontal slices (50µm) at day 1 and 7 post-pMCAO for female and male mice (n=1).

3 At day 7 post stroke, microglia are enriched along the corticospinal tract in male mouse, and differential vascular reorganization and infarct size between sexes shows

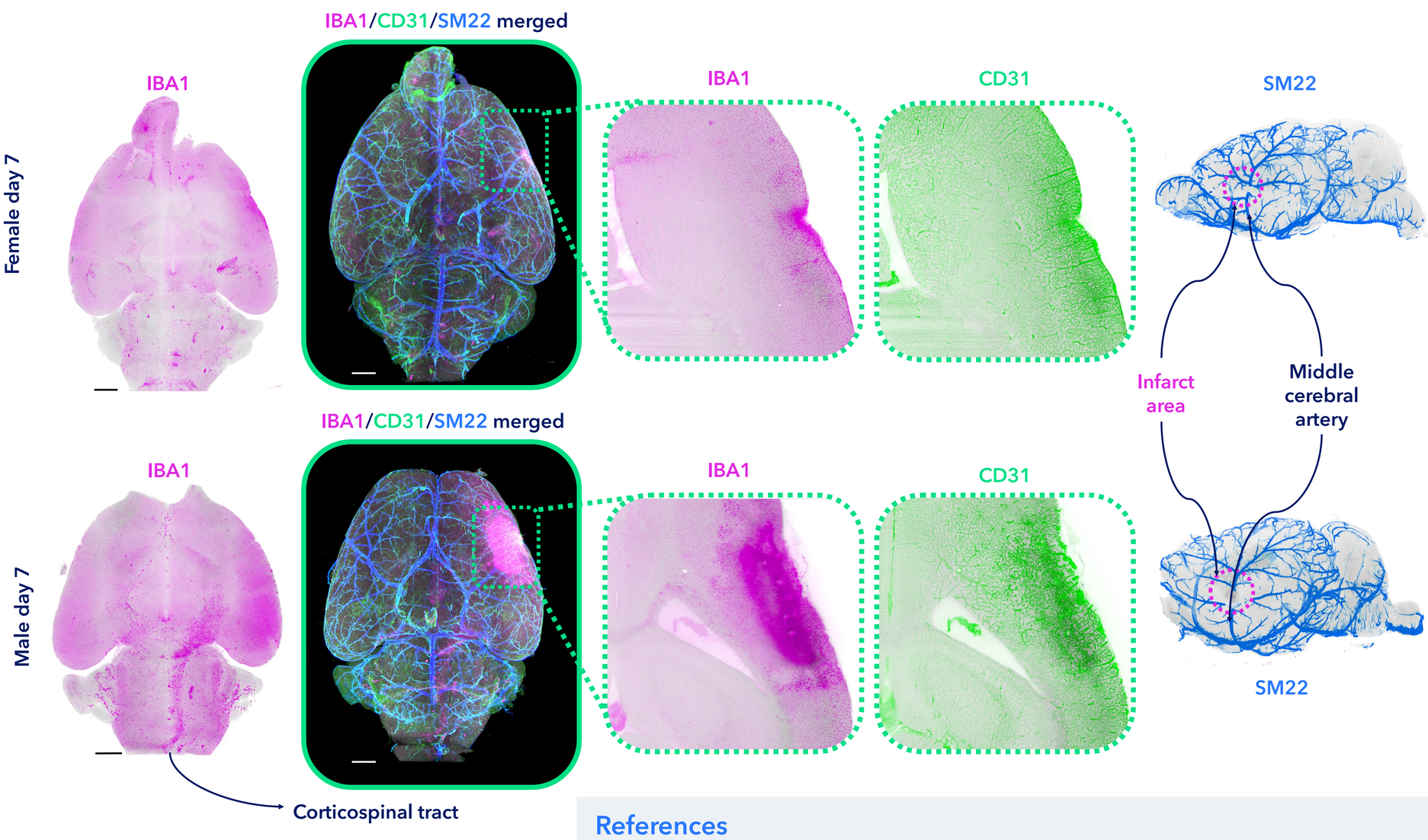

Figure 1. Whole-brain microglial and vascular staining in female and male mice 7 post-stroke. Raw data max projection images show IBA1 (ionized calcium-binding adapter molecule 1), CD31 (cluster of differentiation 31), and SM22 (transgelin) in female (n=1) and male mice (n=1). A magnified slice view (50µm) at the level of the infarct area is shown to the right for IBA1 and CD31 staining. SM22 staining is shown in sagittal view displaying the MCA and infarct zone. To the left of the triplex staining, a thick section of IBA1 staining is shown. Tissue autofluorescence is grey. Scalebars 1000µm.

References

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