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A 3D Reconstruction of Whole-Brain Vascular immunoreactivity

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Background and aim: The brain's vasculature is critical for sustaining neural function and shapes in vivo neuroimaging signals such as BOLD fMRI. The microvasculature, including capillaries and arterioles, is closely tied to sites of neural activation, while macrovasculature supplies and drains broader regions. Classical ink-injection studies suffered from limited penetration into finer vessels[1],[2], while in vivo MRI methods remain limited in spatial resolution. To improve vascular visualization, we developed a whole-brain atlas combining post-mortem (immuno)histochemistry with high-resolution MRI. We quantified vascular scales across 31 subcortical structures, providing a reference framework for vascular architecture.

Methods: A healthy donor brain (male, 76y) was obtained through a whole-body donation program (Amsterdam UMC; Fig. 1A). After 10 % formalin perfusion fixation, quantitative MRI maps of longitudinal relaxation rate R1, transverse relaxation rate R2* and proton density were acquired at 400-µm and 200-250-µm isotropic resolution on a Magnetom 7T scanner (Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany; Leiden UMC)[3],[4]. The brain was then cryoprotected in sucrose, frozen in TissueTek, and coronally sectioned into 811 slices at 200 µm using a cryomacrotome (-16 ℃). Blockface images were captured for MRI alignment and histology registration (Fig. 1B). Sections were stained alternately with CD31 (PECAM- 1), SMA (Smooth Muscle Actin), and Bielschowsky Silver to visualize the vascular bed and scanned at 21-µm in-plane resolution (Fig. 1C–E). Color deconvolution was performed in Python using scikit-image's Hematoxylin+DAB matrix (Fig. 1F), followed by vessel extraction and 3D mapping using Nighres filtering (Fig. 1G).The MASSP2.0 algorithm was applied to parcellate 31 subcortical structures on the 3D blockface reconstruction[3],[5]. Labels were projected onto the aligned histology stack to identify anatomical structures (Fig. 1H). Within each MASSP2.0-defined region, vessel extractions were performed and densities quantified (Fig. 1I). Additional microscopic assessments characterized vessel morphology in SMA- and CD31-stained sections.

Results: We developed a comprehensive dataset of vessel densities across all MASSP2.0-defined structures (Fig. 1J), along with regional vessel morphology and orientation. In general, grey matter regions like the accumbens and thalamus showed denser capillary (CD31) perfusion than white matter structures, which are dominated by larger vessels (arteries). CD31 densities varied greatly across regions, ranging from 33% in the accumbens to 5% in the posterior commissure. SMA density percentages varied less, with 16% in the globus pallidus pars externa to 4% in the CA1.

Discussion: The integration of high-resolution post-mortem MRI with (immuno)histochemistry creates a framework that captures the broader anatomical context and fine-scale vascular profiles across (sub)cortical structures, addressing current gaps in vascular mapping. These data will form the basis for future studies aimed to ultimately improve interpretation of BOLD fMRI signals and account for regional vascular variability. The dataset will be made freely available upon publication of the full paper to support its use as a benchmark in future studies.

[1] Duvernoy HM et al. Brain Res Bull. 1981;7(5):519-79.

[2] Lauwers F et al. Neuroimage. 2008;39:936-48.

[3] Alkemade A et al. Sci Adv. 2022;8(17):eabj7892.

[4] Alkemade A et al. Front Neuroanat. 2020;14:536838.

[5] Bazin P et al. Imaging Neurosci. 2025;doi:10.1162/imag_a_00560.

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