Quantitative aspects of the microvascular system in macaque visual cortex

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Introduction

Non-invasive functional neuroimaging methods such as functional magnetic resonance imaging (**fMRI**) have become indispensable tools for the neurosciences. The underlying principle of the most frequently used methods is the brain's local **dynamic regulation of blood flow**. The correct interpretation of the neuroimaging results requires an in-depth understanding of the structural and functional **neurovascular coupling** underlying this regulation. The structural coupling, among others, presumes a close match between the **vascular density** and the **steady-state metabolic activity** of a given region (e.g. a tangential or laminar subdivision). Here we report quantitative anatomical data of the microvasculature in the macaque visual cortex.

Methods

Formalin-fixed frozen sections (60 µm) of 4 monkeys (*Macaca mulatta*) were processed for double fluorescence immunohistochemistry. The sections were incubated with **anti-collagen type IV** to stain for vessels (Fig.1C) and **DAPI** to stain for cell nuclei (Fig.1B). Layer and area **specific regions of interest** were determined.In one additional animal, the anti-collagen procedure was combined with cytochrome oxidase staining in V1. Furthermore, synchrotron-based computed tomographies (SRCT) of formalin-fixed and barium sulfate-perfused brain samples from another 2 animals were used to corroborate and extend the histological results.

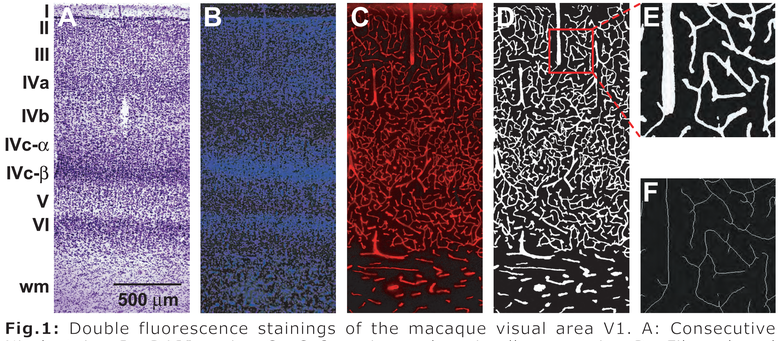

Fig.1: Double fluorescence stainings of the macaque visual area V1. A: Consecutive Nissl stain; B: DAPI stain; C: Cy3-conjugated anti-collagen stain; D: Filtered and thresholded binary vessel image; E: Magnified subarea indicated in D; F: Corresponding digitally eroded trace of vessels. wm=white matter.

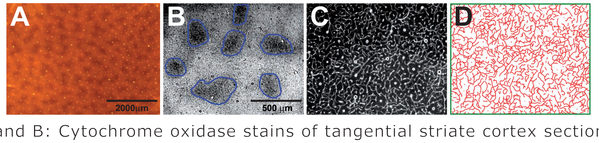

Fig.2: A and B: Cytochrome oxidase stains of tangential striate cortex sections (in B with ROIs); C: Anti-collagen stain; D: Delineation of vessels.

Results

In **V1**, the vascular density was highest in layer **IVc-beta** (LD 674.7 mm/mm³, SD 15.2 mm²/mm³, VF 2.6 %, D 7.2 µm) and lowest in layer I (LD 461.5 mm/mm³, SD 10.9 mm²/mm³, VF 1.9 %, D 7.5 µm). In all **extrastriate** visual areas analyzed (V2, V3, V4, V5), the vascular density was **generally lower**, and the **difference** between layer IV and the remaining layers was **less prominent** when compared to V1. These density values were similar compared to the ones tomographically obtained from SRCT. The vascular density (LD) in cytochrome oxidase rich **blobs** in V1 was **14 % higher** as compared to the interblob region.

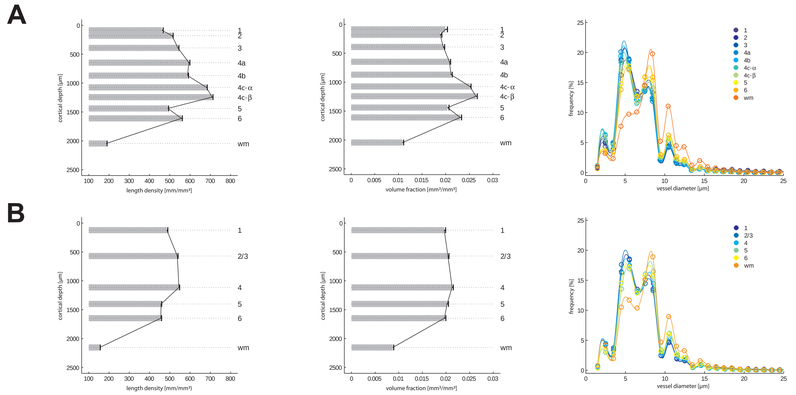

Fig.3: Comparison of the layer specific vascular length densities, volume fractions and vessel diameters in A: V1 and B: extrastriate cortex (each plot is averaged for four animals).

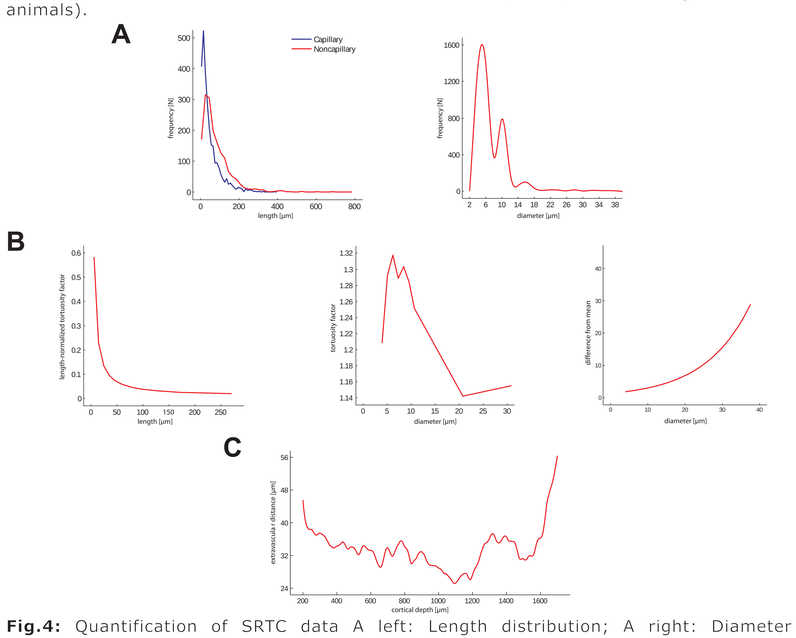

Fig.4: Quantification of SRTC data A left: Length distribution; A right: Diameter distribution; B left: Tortuosity against length; B middle: Tortuosity against diameter; B right: Vessel orientation against diameter; C: Extravascular distance along the cortical depth (black line represents the depth at which white matter starts)

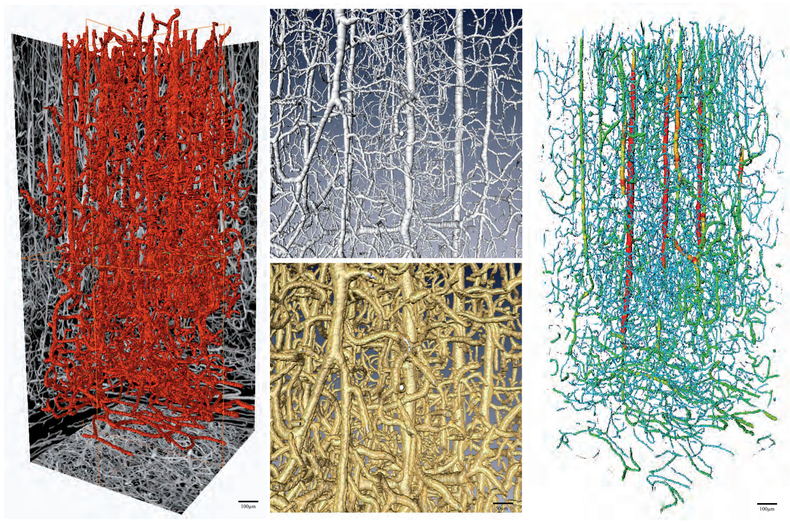

Fig.5: Visualization of vessel topology in the monkey primary visual area V1; left: Segmentation following filtering to show connectivity of vessels (highlighted in red); middle top: 3D reconstruction of the vascular network; middle bottom: 3D visualization of the line set (white cylinders) and the original data (yellow isosurfaces); right: Color coded with respect to thickness.

Discussion

V1 is clearly different from all extrastriate areas analyzed with respect to the **laminar vessel distribution** and the **overall vascular density**. Differences between the extrastriate areas were negligible.

The overall vascular volume fraction in visual cortex derived from immunostaining was approximately 2 %, a value that was well reproduced by the SRCT. Since SRCT provides true volumetric data, important topological vessel parameters can be assessed in 3D with high precision.

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