Vascular density in regions of different levels of oxidative metabolism within the macaque primary visual cortex

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\textbf{Introduction}

As shown in previous studies, the vascularization of the macaque monkey’s striate and extrastriate cortex remarkably differs from each other. Besides having an overall denser vascularization, V1 shows some distinct features: even though both regions have their maximal vascular density in layer IV, the difference between this and the other layers is more prominent in V1 than in the non-striate visual areas. Furthermore, layer VI shows an increased vascular density in V1 but not in the extrastriate cortex (figure 1).

The primate V1 also shows distinct regions of increased cytochrome oxidase activity, so called “blobs”. When visualized in tangential sections the blobs form a pattern of regularly distributed patches which are most prominent in the third cortical layer. Since cytochrome oxidase is an enzyme of the oxidative chain, increased local enzyme activity indicates an increased metabolic activity of a given region. It has been shown in the squirrel monkey that this metabolic difference is reflected in the density of the cortical vascularization. The aim of this study was to replicate and extend this finding with a quantitative and layer specific assessment of the vascular density in blob and interblob regions in the visual cortex of another primate species, the macaque monkey.

\textbf{Methods}

Formalin-fixed frozen 60µm thick tangential sections of 2 animals and horizontal sections of 3 animals (M. mulatta) were first stained with the classical cytochrome oxidase staining method that uses the enzyme activity to precipitate DAB. The sections were then further processed for fluorescence immunohistochemistry. They were incubated with an antibody against collagen type IV, a major constituent of the basal membrane, and a Cy3-conjugated secondary antibody to stain for the blood vessels. Epifluorescence micrographs were taken and the vessels were manually (tangential sections, figure 2) or automatically (horizontal sections, figure 3) delineated. The length density (mm/mm\textsuperscript{3}) and the volume fraction (mm\textsuperscript{3}/mm\textsuperscript{3}) of the blood vessels were taken as a measure for the vascular density. The blobs and the cortical layers were determined in the corresponding brightfield micrograph of the very same section on the basis of the cytochrome oxidase stain and the ROIs drawn upon this information were then transferred to the thresholded, binarized and eroded images to read out the data.

\textbf{Results}

In both the tangential and horizontal sections, the vascular length density and volume fraction was significantly higher for blobs as compared to the interblob regions (paired t-test, p<0.05) (figure 1, figure 3).

In the tangential sections the length density was higher (p<0.001) for blobs (535.2 +/-57.8 mm/mm\textsuperscript{3}, mean +/- SD of 2 animals) than for interblob regions (485.5 +/- 51.2 mm/mm\textsuperscript{3}). In the horizontal sections the mean length density over all layers was 548.4 +/-170.8 mm/mm\textsuperscript{3} (+/- SD of 3 animals) for blobs and 539.3 +/-158.8 mm/mm\textsuperscript{3} for the interblob areas. This difference is less prominent (1.7%), but still significant (p<0.001). The vascular volume fraction was higher (3.37 +/-1.15%) inside the blobs as compared to the interblob regions (3.30 +/-1.02%; p<0.05). The layerwise analysis reveals that the differences in vascular density are restricted to layers I and III.

\textbf{Discussion}

In summary, the blobs’ vascular density is higher as compared to the interblob regions. This probably reflects an adaptation of blood supply in these metabolically and therefore most likely also functionally different regions. However, the differences are considerably smaller than previously reported (Zheng, 1991, J. Neurosci). This discrepancy could be due inter-species or methodological differences and needs further investigations.

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\textbf{Figure 1:} Length density in grape (left) and orange (right) colored sections of the secondary data of 2 animals (3.30 +/-1.02%; p<0.05).

\textbf{Figure 2:} microscopic of a horizontal section of V1 showing the blobs and the interblob region (middle left), the blood vessel image (middle right), the automatic delineation of the vessels (right).

\textbf{Figure 3:} microscopic of a horizontal section of V1 showing the blobs and the interblob region (left), the blood vessel image (middle left), the automatic delineation of the vessels (middle right), and the eroded equivalent (right).

\textbf{Figure 4:} Longitudinal length density (left column) and volume fraction (right column) in blob (top) and interblob regions (model), averaged across 3 animals, gray bars represent standard deviations, * p<0.05. wm=white matter.