

Two photon microscopy of the vascular response to forepaw stimulation in the rat somatosensory cortex

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Introduction

Since Aristotle, man has attempted to understand the nature of the mind. Modern neuroscience relies heavily on non-invasive functional imaging, much of which that measures the Blood-Oxygen Level-Dependent (BOLD) signal. It is presumed that during brain activation, neurons transiently fire, inducing local hemodynamic or blood flow changes that elicit this BOLD signal.

Determining the neuronal and vascular mechanisms that underlie the observed ensemble hemodynamic response, as recorded from fMRI or Near infra-red spectroscopy (NIRS), is an essential step towards improving the utility of these non-invasive methods of measuring brain activity. Currently in the literature there is widespread disagreement regarding the physical properties and behavior of the vascular compartments during functional stimulation.

Here we attempt to answer the question how does increased neuronal activation correlate with changes in the hemodynamic response. Using high resolution fluorescent optical imaging and microscopy techniques *in vivo* of the somatosensory vascular network during rat fore paw stimulation we endeavor to shed light on this subject area.

This study has two parts:

- (1) fully characterize the structure, specifically the vascular network that fuels the somatosensory regions of the rat cortex and
- (2) dynamically measure changes (dilation and constriction) of these vessels during somatosensory stimulation

Together these data will enable us to determine the transient blood flow changes that occur during cortical activation.

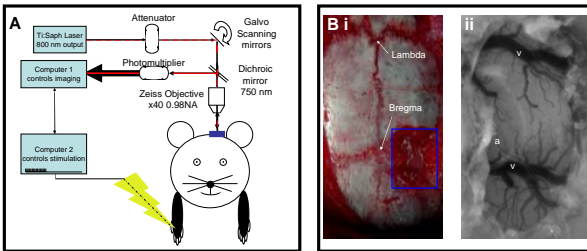
Methods

Prior to imaging rats (250-350g), several preparatory surgical procedures were conducted to ensure the animals' physiological parameters remained constant

Initially animals were anesthetized with Isoflurane (1.5 - 2.5%) mixed with oxygen and air. A tracheal tube was inserted to allow manual ventilation in order to maintain blood pH, CO₂ and O₂ levels within normal parameters (pH 7.35 - 7.45; pCO₂ 35.0 - 45 mmHg; pO₂ 80.0 - 100.0 mmHg). Femoral arterial catheter was inserted to monitor pressure (85 - 110 mmHg) and take blood gas measurements

Femoral venous catheter was inserted allowing us to introduce our fluorophore (FITC 2 MDa ~0.4 mL 500nM) and anesthetic (*α*-chloralose ~2.5%).

Rats were then placed in a stereotaxic frame and skull exposed and cleaned. The Cerebellomedullary cistern was exposed and opened to alleviate intracranial pressure. A craniotomy and duraectomy was done over the Somatosensory cortex and covered with agar (1-2%) and sealed with a glass cover slip



A: Schema of optical imaging paradigm. Imaging of the vascular network was conducted using a two-photon optical system developed in the lab. Stimulation of the fore paw contra-lateral to the cranial window was conducted for experiments for functional activation of the somatosensory cortex

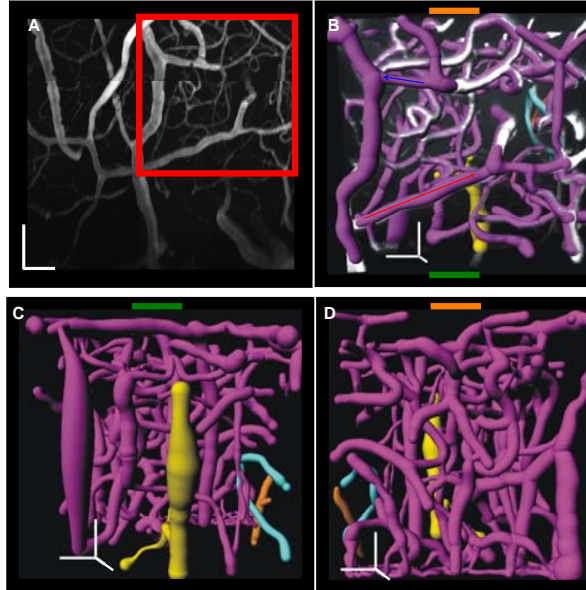
B: Cranial opening over the somatosensory cortex.

(i) Known cranial landmarks were used to identify the region overlying the somatosensory cortex

(ii) After the craniotomy, duraectomy and placement of the cranial window, the cortical vasculature can be clearly seen (v: vein & a: arteries)

Results

Structure

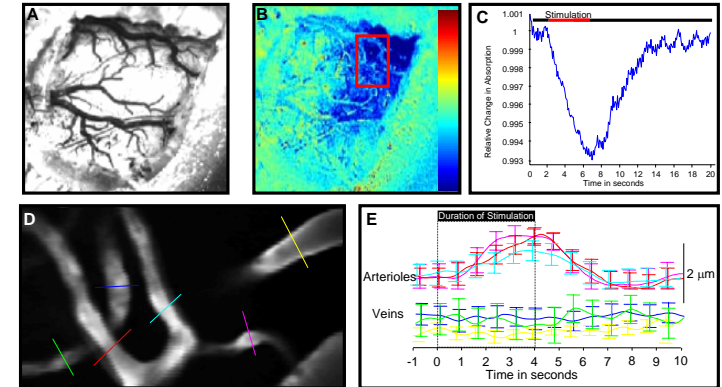


Shortly after the injection of FITC image stacks 1 μ m steps were taken. Several adjacent image stacks were imaged and tiled together using Matlab. **A:** A tiled image of the maximum intensity projection (MIP) for a region from the surface of the cortex to a depth of 250 μ m. **B:** From the inset of **A** is the MIP overlaid on the 3D rendering of created using Matlab and the commercially available software Imaris. This section comprises mainly of one complete vascular loop (Purple) of descending artery (see red arrow) to ascending vein (see blue arrow). **C:** A length view image along the green face. **D:** A length view image along the orange face. Notice the diverse capillary bed at the bottom that connects the arterioles with the venules.

White scale bar = 50 μ m

Filament Colour	Total Length	Volume	# Branch Points	Segments #	Avg. Length
Purple (artery + vein)	10.2 mm	$1.2 \times 10^{-3} \text{ mm}^3$	168	303	33.6 μ m
Yellow (vein)	332.4 μ m	$9.6 \times 10^{-6} \text{ mm}^3$	2	4	83.4 μ m
Orange (unconnected)	117.4 μ m	$9.1 \times 10^{-6} \text{ mm}^3$	1	3	39.1 μ m
Blue (loop)	268.9 μ m	$1.9 \times 10^{-6} \text{ mm}^3$	0	1	268.9 μ m
Green (unconnected)	84.9 μ m	$4.5 \times 10^{-6} \text{ mm}^3$	0	1	84.9 μ m
Total Inset Volume:	$1.7 \times 10^{-2} \text{ mm}^3$	Total Vessel Volume:	$1.3 \times 10^{-3} \text{ mm}^3$		

Function



To correlate structure with function we stimulated to the forepaw contra-lateral to the cranial window. To coarsely identify the somatosensory cortex, we illuminated the region with green light (580nm) that is absorbed by oxygenated blood.

Stimulation Protocol: two pins in the contra-lateral fore paw with 2 μ A @ 3 Hz with a 20% duty cycle for 5 minutes
Optical Recording Protocol: 150 μ m x 100 μ m @ 14 frames / second

A: CCD image of region of the brain under the cranial window that contains part of the somatosensory cortex
B: Region illuminated with green light during forepaw stimulation (darker regions indicate increased absorbance)
C: Relative change in absorbance during stimulation (black line no stimulation, red line during stimulation)
D: Large dip indicates increased absorption in somatosensory cortex.
E: Maximum intensity projection (MIP) of region imaged. Calculations of vessel diameter changes done offline
D: Only arterioles were noticed to show diameter changes during forepaw stimulation.

Discussion

The leading "microscope" into the human mind is the fMRI. Since fMRI uses hemodynamic changes as the metric for neuronal activation, it is equally important to gain a greater understanding of this recorded hemodynamic response. Thus, these data will help us to understand how the BOLD signal correlates to neuronal activation.

Here we have advanced our understanding of the vascular network that supplies oxygenated hemoglobin and washes deoxygenated hemoglobin from the cortex by imaging large arrays of the brain. From these data, we can now calculate changes in volume, flow and oxygen delivery (poster 87.2).

We have imaged changes in vascular diameters but only noticed changes in the arterioles that are covered by smooth muscle cells, a substrate for active diameter control by neurovascular mediators. Continuing with functional imaging we have been able to make spatio-temporal maps of pO₂ and cerebral blood flow during cortical spreading depression (poster 87.9).

Together these results give us a clearer picture of the vascular structure and the dynamic changes that occur to the structure during specific cortical activation.

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References

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