Spatiotemporal profile of stimulus-evoked diameter changes of single surface vessels over a large cortical area in relation to the underlying neuronal activity

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Introduction

- The spatiotemporal characteristics of dynamic responses of individual cerebral vessels to sensory-stimuli is crucial to form a mechanistic understanding of vascular based functional imaging technologies, such as fMRI, as well as for understanding neurovascular dysfunction, as occurs in stroke and dementia.
- Currently, the cerebral hemodynamics obtained from macroscopic full-field imaging technologies is largely based on spatially averaged responses over multiple vessels and sometimes even of multiple vessel types (arteries, veins, and capillaries).
- Hence, we have characterized the cerebral vascular response (diameter changes) in relation to the underlying neuronal activity with single surface vessel (down to diameter of ~ 5 mm) spatial resolution and over a large cortical area 4.5x3.5mm^2 in the rat somatosensory cortex *in vivo* by employing two-photon laser scanning microscopy (TPLSM). This is a first step to obtain a 3D map of the stimulus-evoked cerebral hemodynamics on the level of individual vessels across multiple cortical columns.

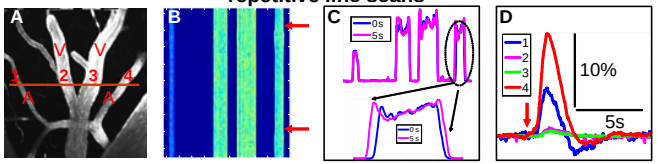
Previous *in vivo* imaging of cerebral blood flow using TPLSM

- TPLSM is well suited for imaging cerebral blood flow because it can image 600 μm below pia with micron resolution in cortical tissues.
- TPLSM has imaged
  - blood flow changes of capillaries in response to odor stimulus in the rat dorsal olfactory bulb, as well as to sensory stimuli and hypercapnia in the rat somatosensory cortex.
  - flow velocity changes of vessels (from pia to ~400 μm below pia) to local occlusions of targeted vessels.
  - diameter changes of arteries, veins, and capillaries in response to increase of Ca^{2+} in astrocytes.
- There is a lack of systematic study of the stimulus-evoked vascular diameter changes in relation to underlying neuronal activity over a large population of surface vessels

Methods

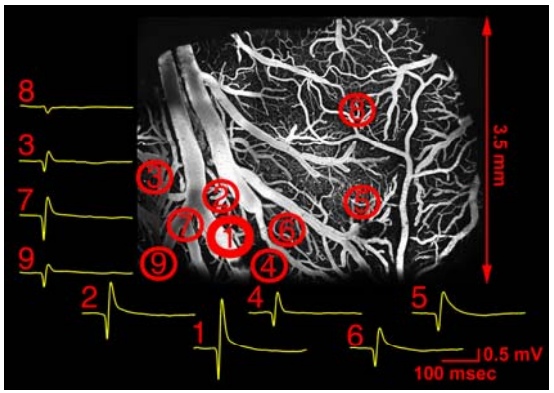
- Animal surgery
  - Rats (female Sprague–Dawley, *n=7*, 220–260g) were anesthetized by continuous intravenous (i.v.) infusion of a-chloralose at 40 mg/kg/h. An area of skull and dura ~ 5x5 mm^2 overlying primary somatosensory cortex were removed. 0.4 ml bolus of 5% fluorescein-conjugated-dextran in physiological saline was injected i.v. to label the blood plasma
- Map the neuronal activity prior to imaging with TPLSM
  - Surface evoked potentials (EPs) recorded using a ball electrode. Center of mass of the neuronal activity corresponds to largest surface EP
- Image stimulus-evoked cerebral vascular diameter changes of surface vessels with TPLSM
  - Stimulus paradigm: electrical forepaw stimulus (1mA, 0.3ms square pulse, 3Hz, 1s, ISI 20s)
  - Map 2D surface vasculature; select vessels and measure their diameter changes
- Data analysis
  - Edge detection program to obtain vessel diameter
  - Analyze diameter changes versus vessel distance from the center of neuronal activity

Simultaneously measure diameter changes of multiple vessels by repetitive line scans



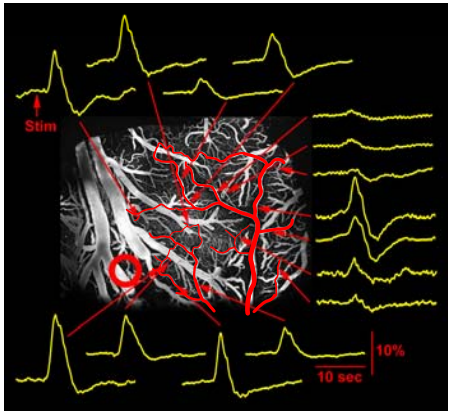
Blood plasma is labeled with dyes and imaged by TPLSM. Vessel diameter refers to the intraluminal diameter.
A) Frame image shows line scan (red line) intercepting with 2 arterioles and 2 veins (marked with A and V);
B) Each line scan is displayed below the previous one, forming a space–time image.
C) Profile of two scans at 0 and 5s after the stimulus started. Those of vessel 4 at the bottom show vasodilation.
D) Temporal dynamics of fractional diameter changes of all vessels. (Red arrows in B and D mark the start of the stimulus.)

Map of the neuronal response overlaid on 2D surface vasculature in the rat somatosensory cortex



Neuronal responses at 9 different locations were revealed by the evoked surface potentials (yellow trances) measured with a ball electrode. The center of the neuronal activity is situated where trace 1 is. The neuronal activity map is overlaid on the 2D surface vasculature (~ 4.5x3.5mm^2), generated by maximal intensity projection of an image stack of 0-300 mm along the cortical axis.

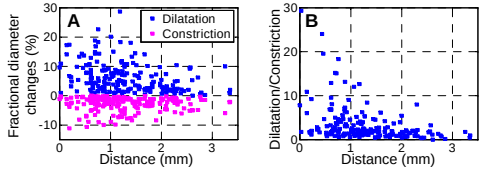
Stimulus-evoked diameter changes of surface arteries in relation to the neuronal activity over a large cortical area



Major surface arterial network is traced with red lines. Yellow traces show the fractional diameter changes of single vessels in response to electrical forepaw stimuli at different locations (indicated by red arrows).

Note that on the level of single vessels, 1) most arteries/arterioles show a biphasic behavior, vasodilatation followed by substantial vasoconstriction; 2) arterial diameter responses depend on the distance between the vessel and the center of the neuronal response (marked by red circle). There is a trend for the fractional peak dilatation to decrease and vasoconstriction to be more dominant with the increase of the distance.

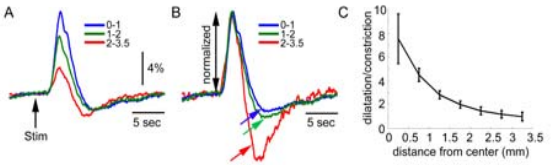
Distance dependence of the arteriolar diameter changes of a large population of single vessels



A) Scatter plot of the fractional peak dilatation and constriction versus the distance between individual vessels and the center of the neuronal response. B) Scatter plot of the ratio of the fractional peak dilatation to constriction versus the same distance. (Data are from 7 animals.)

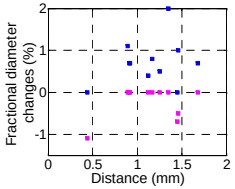
Note that 1) there is a trend for the fractional peak dilatation to decrease and for the vasoconstriction to be more dominant as vessels move from the center to surrounding cortical areas; 2) there exists a large variation on the level of single vessels on top of this trend.

Distance dependence of the averaged arteriolar diameter changes



A) The average of fractional arteriolar diameter changes within 1 mm (blue) from, in 1-2 mm (green) and 2-3.5 mm (red) annuli around the center of neuronal response, respectively. B) The same as A with the peak dilatation normalized to 1. C) The ratio of the fractional peak dilatation to constriction versus vessel distance from the center of the neuronal response. (Data from 7 animals are averaged.) A-C show the distance dependence of the average (macroscopic) arterial diameter responses: the fractional peak dilatation decreases monotonically while vasoconstriction becomes more dominant as vessels move from the center to surrounding cortical areas.

Stimulus-evoked diameter changes of surface veins



Scatter plot of the fractional peak dilatation and constriction versus the distance between individual veins and the center of the neuronal response. (Data are from 7 animals.)

Note that 1) only 13% (13 out of 97 measured veins) shows observable responses; 2) 1 vein shows biphasic behavior; 3) the amplitude of both the fractional peak dilatation and constriction is smaller than that of the arteries; 4) the distance dependence of the diameter changes of surface veins is not clear due to lack of data points.

Summary and future work

- The stimulus-evoked diameter changes of individual surface vessels over a large cortical area (4.5x3.5mm^2) have been characterized in relation to the underlying neuronal activity map. 434 vessels of 7 rats were measured. We have observed:
  - Response of arteries versus veins
    - 54% (183 out of 337) arteries/arterioles and 13% (13 out of 97) veins/venues show observable diameter changes.
    - Most arteries and only 1 vein show biphasic behavior.
    - The amplitude of diameter changes of veins are smaller than those of the arteries;
  - Distance dependence of diameter changes observed on the level of single arteries and the macroscopic scale (~1mm)
    - Fractional peak dilatation tends to decrease and the contribution of vasoconstriction increases as vessels move from the center to surrounding cortical areas
    - There exists a large variation on the level of single vessels
- Future work
  - Measure blood flow of surface vessels to study
    - The characteristics, *e.g.*, biphasic versus monophasic, of the temporal dynamics of the blood flow changes of individual surface vessels.
    - The dependence of the flow velocity change of a vessel on its distance from the center of the neuronal activity.