Characterization of the spatiotemporal responses of surface and penetrating cerebral arterioles using two-photon microscopy

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Introduction
A mechanistic understanding of the hemodynamic response requires a systematic analysis of single-vessel properties carried out in the framework of realistic vascular microarchitecture and with respect to the underlying neuronal activity. Previously, we used somatosensory stimulation and two-photon microscopy to study neurovascular coupling in rat primary somatosensory cortex [1]. Our results showed that on the level of single surface (pial) arterioles, the response was composed of dilation and constriction phases. Comparison of two-photon data with neuronal measurements demonstrated a correlation of the arteriolar constriction with enhanced neuronal inhibition. Here we extend the two-photon measurements across cortical depth and to the other two vascular compartments, capillaries and veins.

We measure changes in diameter and blood flow velocity of individual arterioles, capillaries, and venules down to 500 μm in response to a somatosensory stimulus in rat primary somatosensory cortex (SI). We ask the following questions: (1) do penetrating arterioles and their branches show biphasic response similar to that of the pial arterioles? (2) does the arteriolar response depend on the cortical depth, and local vascular geometry? (3) are there “hot spots” of arteriolar dilation consistent with anatomical literature [2-3]? (4) do veins/venules swell (dilate) in response to the stimulus? (5) is the velocity profile consistent with the diameter change?

Dilation propagates towards the cortical surface along the main trunks of diving arterioles

Methods
Rats were initially anesthetized with isoﬂurane (2% initially, 1.2-2% during ventilation) and ventilated with a mixture of air and oxygen during surgical procedures. During the surgery cannulas were inserted in the femoral artery and vein. Isoﬂurane was discontinued, and anesthesia was maintained with 50 μg/1 intravenous bolus of α-chloralose followed by continuous intravenous infusion at 40 μg/kg/min. Heart rate, blood pressure and body temperature were continuously monitored. Respiration was aimed to maintain Pco2 between 35 and 45 mmHg.

An area of skull overlying SI was exposed, and the skull and dura mater were removed, and the space between the exposed brain surface and the coverglass was filled with 0.7% (w/v) agarose (Sigma) in ACSF. To avoid hemiation of the exposed brain due to excessive intracranial pressure, dura mater over the fifth cerebral ventricle was punctured and plastic PE50 tube was inserted to allow draining of cerebrospinal fluid (CSF). The draining hole was sealed after sealing of the imaging well.

To visualize the vasculature, −0.3 ml of 5 % (w/v) solution of 2 MDa fluorescein-conjugated dextran (FD-2000, Sigma) in physiological saline was injected intravenously [4]. Images were obtained using Ultima 2-photon microscopy system from Prairie Technologies. We used a 4x air objective (Olympus XLPLfluor/40, NA=0.28) to obtain images of the surface vasculature across the entire cranial window with a field of view of 200 μm (Olympus, NA=0.5) and 40x (Zeiss, NA=0.8) water-immersion objectives were used for high-resolution imaging and line scan measurements. Data analysis was performed in Matlab environment.

Relative peak amplitude of constriction as a function of the distance from the center (left) and as a function of the cortical depth (right). On the right, the three types of arteriolar trunks (blue, green and red) and side branches (black) are superimposed.

Veins do not dilate (swell) at any measured cortical depth irrespective of stimulus duration

Conclusions
1. Penetrating arterioles and their branches can have a biphasic response similar to their parent surface arterioles: the initial dilation followed by constriction. Relative to the initial dilation, a stronger constriction is observed (i) further away from the center of neuronal activity and (ii) deeper down the cortical tissue.

2. Dilation propagates upstream towards the cortical surface along the main trunks of diving arterioles and invades side branches.

3. The largest dilation is observed in the parent surface arterioles of types I and II (deep-dwelling). The “hot spots” are mainly located near arteriolar branching points, both on the surface and throughout the measured depth. Most of them can be recognized by their bottleneck appearance.

4. Veins do not swell but increase the flow velocity. Their velocity change is biphasic indicating that RBCC flux is reduced during arteriolar constriction.

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References

Figure 1. Two-photon image of the surface vasculature.

Figure 2. Measurements of vessel diameter from line-scan data. The diameter changes are captured by repeated line-scans across the vessel that form a space-time image when stacked sequentially (left). Diameters are extracted from profile changes (right: compare blue (baseline) to red (peak dilation)).

Figure 3. Measurements of vessel diameter from frame-scan data. Diameter can also be estimated from image time series of diving vessel obtained in a frame-scan data by counting the number of pixels above a pre-set intensity threshold.

Figure 4. Measurements of velocity of red blood cells (RBCC). The velocity is captured by repeated line-scans along the axis of the vessel that form a space-time image when stacked sequentially and leads to the generation of streaks caused by the motion of RBCCs. The speed is given by the inverse of the slope of these streaks (velocity=linewidth). An algorithm based on singular value decomposition is used to automate the calculation of speed from the line-scan data [5].

Figure 5. Arteriolar dilation and constriction along diving trunks of arteriolar trees before the 1st branching point. A, Schematic representation of three types of vascular geometry. B, Averaged response time-course for each type. Responses from different depths are superimposed. Dilation is plotted upward, constriction downward. For each type, depths are indicated in the inset. C, Time-to-peak of dilation as a function of the cortical depth. The three types of vascular geometry are superimposed in different colors.

Figure 6. Dilation and constriction along branching arteriolar trees. A, The image, calculated as a MIP of an image stack of 0-300 μm, shows a branching diving arteriole. Individual images were acquired every 10 μm. B, Time-courses of arteriolar diameter changes along the branching tree. The locations of diameter measurements are color-coded in A. C, Time-to-peak dilation as a function of the cortical depth. Branches (black squares) are superimposed on type I trunks (blue circles).

Figure 7. Dilation and constriction of arteriolar side branches and capillaries (basal diameter of 5-13 μm), categorized by the branching order (blue, red, and green), in comparison with diving trunks (black).

Figure 8. Peak amplitude of dilation as a function of the cortical depth. The three types of arteriolar trunks (blue, green and red) and side branches (black) are superimposed.

Figure 9. Relative peak amplitude of constriction as a function of the distance from the center (left) and as a function of the cortical depth (right). On the right, the three types of arteriolar trunks (blue, green and red) and side branches (black) are superimposed.

Figure 10. Dilation “hot spots”. A, An example of a “hot spot” on a surface arteriole. B, Another example at 130 μm. Measurement locations are color-coded. C, Hot spots can usually be recognized by their smaller than the average baseline diameter.

Figure 11. An increase in RBCC velocity (red) leads diameter changes (black) at all measures cortical depths. Averaged velocity and diameter response time-courses from the top 100 μm (solid lines) and 100-200 μm (dashed lines) are superimposed. These biphasic time-courses indicate that RBCC flux is reduced during arteriolar constriction.

Figure 12. Veins do not dilate even during long stimulation. A. The image was calculated as a MIP of an image stack of 0-300 μm in depth. Individual images were acquired every 10 μm. B, Top: Time-courses of surface arteriolar (magenta) and veins (blue) diameter changes and venous velocity (black) are superimposed. Bottom: Time-courses of venous diameter changes at different depths (120, 200 and 340 μm) are superimposed: s. surface; d. deep.