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Summary

Neuroglial activation is accompanied by release of vasoactive mediators that dilate and constrict the surrounding arterioles and capillaries. In our recent study using in vivo 2-photon imaging of single blood vessels in the rat primary somatosensory cortex (SI), we demonstrated a spatial gradient of dilation onset times consistent with upstream propagation of vasodilation towards the cortical surface along the diving arterioles [1]. There, the measurements were performed down to 500 μm (the upper boundary of layer IV in the rat SI) and the deepest locations exhibited the fastest dilation.

To study deeper cortical layers, we translate this experimental paradigm to mouse. With less scattering and thinner cortex in mouse, we can image as deep as 700 μm in mouse SI, spanning layers I-V.

Our results show that 1) the onset of dilation of arterioles and capillaries decreases with depth throughout the entire measured cortical layers; 2) dilation of capillaries is likely delayed relative to pre-capillary arterioles at comparable depth. Using a combination of direct microinjection of glutamate and 2-photon imaging, we provide evidence that local neurovascular coupling mechanism is present in upper layers, including layer I. Hence, both local and propagated signaling are likely to be present in the upper layers, while local neurovascular communication has slower kinetics. While further investigation is needed to address the mechanisms behind the timing differences of local neurovascular coupling across layers, our current data suggest that this mechanism is not immediately related to the level of metabolic activity. This is because the earliest dilation was detected in layer V, whereas the highest metabolism is known to occur in layer IV. The observed gradients of dilation onsets are in agreement with a recent high-resolution fMRI study in humans [2].

Methods

1. 18 mice anesthetized with α -chloralose. $2 \times 2 \text{ mm}^2$ craniotomy over SI with skull and dura removed. Blood plasma labeled with fluorescein-conjugated dextran. In experiments with glutamate injection and calcium imaging, calcium indicator OGB (Oregon Green 488 BAPTA-1 AM) and glutamate were pressure-microinjected into the cortical tissue [3]; sulforhodamine 101 (SR101) applied topically to label astrocytes [4].

- Map neuronal response by recording surface evoked potentials
- Image stimulus-evoked diameter changes of pial and parenchymal arterioles and capillaries in both line- and frame-scan modes using 2-photon microscopy
- Map 2D surface vasculature (Fig. 1); select arterioles and measure their diameter change
- Electrical forepaw stimulus: 1-1.5mA, 0.3ms pulse, 3Hz, 2s, ISI 25s
- Data analysis performed in Matlab

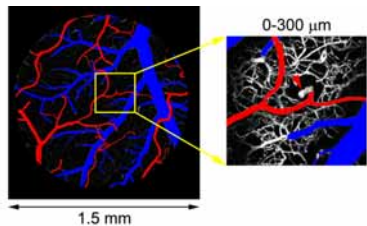


Fig.1 Left: Maximum intensity projection (MIP) of a low-magnification image stack showing surface vasculature. **Right:** MIP of a high-magnification image stack from surface to 300 microns deep into cortical tissue of the region outlined by the yellow rectangle. Red arrow: diving arteriole. Red and blue colors highlight arteries and veins.

Arteriolar dilation indicates an increase in cerebral blood flow (CBF)

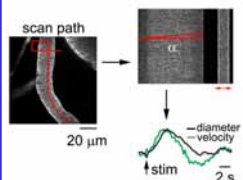


Fig.2 An example of simultaneous measurement of diameter and velocity.

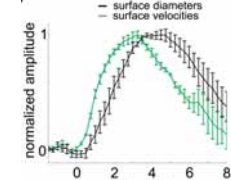


Fig.3 Averaged diameter and velocity time-courses from paired measurements.

Dilation along penetrating arterioles (PA) gets faster with increasing cortical depth down to layer V

Dilation along a single PA

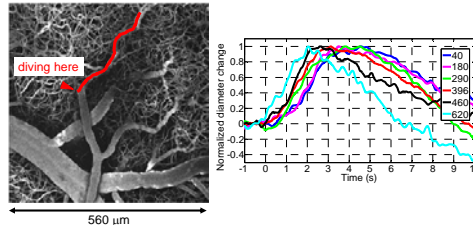
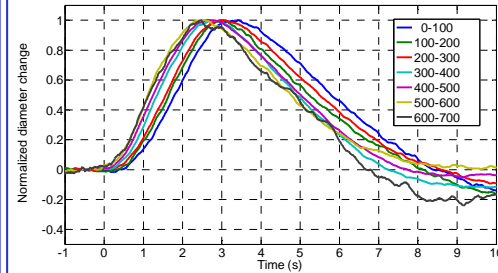


Fig.4 MIP of 0 to 680 μm image stack (2 μm step) Red line and arrow: PA measured and initial diving point.

Fig.5 Normalized diameter changes (2 μm step) measured at different depths along PA shown on left (in microns).



Dilation of pre-capillary arterioles likely precedes that of capillaries at comparable depth

Fig. 7 A pre-capillary arteriole (B1: 1st order branch from PA) dilates earlier than 3rd order branch (B3) at 510 μm

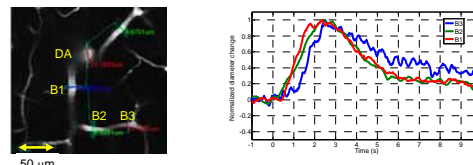
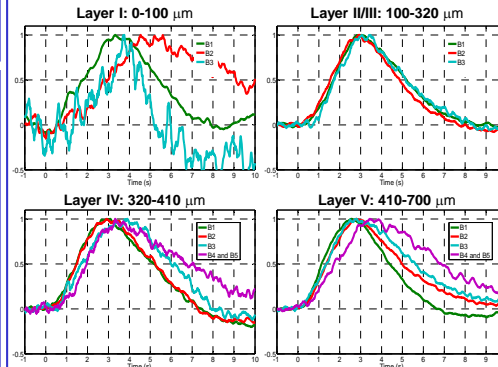


Fig. 8 Averaged diameter changes of 1st-5th order branches (B1-B5) from PA indicate that B1s (pre-capillary arteriole) seem to dilate faster than higher order branches (B3-B5), which are likely to be capillaries



Evidence for local neurovascular coupling in upper layers

Neurons show Ca²⁺ increase to microinjection of glutamate while astrocytes do not show Ca²⁺ increase

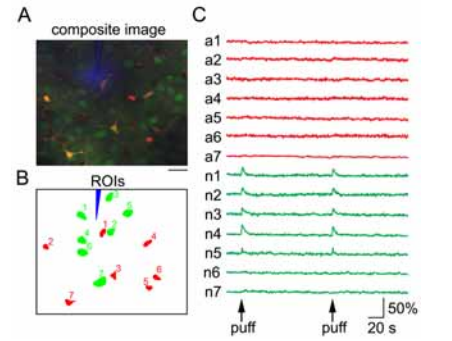


Fig.9 A: Composite image of OGB (green), SR101 (red) and injection micropipette (blue) imaged 115 μm below the cortical surface. B: ROIs for neurons (green) and astrocytes (red). Scale bar = 20 μm . C: Time-courses of percent calcium signal change with puffing of glutamate (1 mM) extracted from astrocytic (a1-a7) and neuronal (n1-n7) ROIs.

A segment of a diving arteriole closer to the plane of glutamate microinjection dilates faster than a segment deeper in the tissue

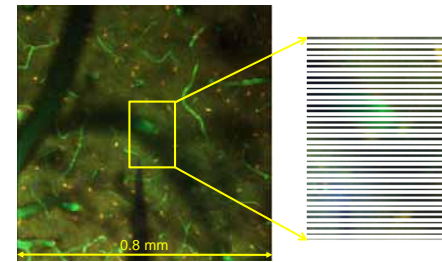


Fig.10 Left: Composite image of vessels (green), astrocytes (yellow), neuropil (green background), and injection micropipette loaded with glutamate (blue) 60 μm below pial surface. Right: High resolution view of the PA measured (green), astrocytic endfeet/astrocytes (yellow), and glutamate released during puff (blue).

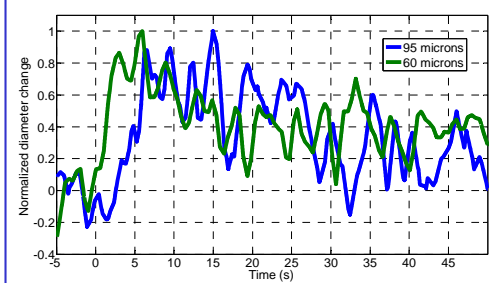


Fig.11 A section of arteriole closer to microinjection (green, located 60 μm below surface) dilates faster than that (blue, located 95 μm below surface) further away from the injection site.

Preliminary data on potential mechanism for the depth-dependence of dilation onset time: the role of cyclooxygenase-2 (COX-2) products

Method

COX-2 inhibitor NS-398 was injected intracranially [5]. To test the drug efficacy, we used intrinsic optical imaging at 570 nm. This largely reflects the change of the total hemoglobin volume (HbT) of vessels on the pial surface and in layer I and upper part of II/III.

Injection of COX-2 inhibitor may affect both amplitude and timing of vascular response

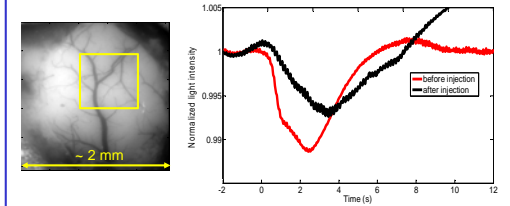


Fig.12 Left: 2D image of the exposure. Region of interest (ROI) is indicated in yellow. Right: Time courses extracted from the ROI. Normalized back-scattered light intensity versus time before (red) and after (black) the injection of NS-398. A decrease in intensity means an increase of HbT. Hence, in this example, COX-2 inhibition led to a smaller and slower HbT response.

Conclusions

- Dilation along PA gets faster with increasing cortical depth from 0-700 μm .
- The earliest dilation detected is in layer V, not in layer IV, so metabolic activity alone cannot explain the onset timing difference observed here.
- Dilation of pre-capillary arterioles likely precedes that of capillaries.
- Local neurovascular coupling is likely to occur in all layers.

Future work

- Further examine local neurovascular coupling in layers I and II/III.
- Investigate the mechanisms responsible for the depth-dependence of the dilation onset time.

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