From two-photon microscopy to fMRI: BOLD transients as a function of single-vessel dilation and constriction

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Summary and conclusions

We used *in vivo* optical imaging and BOLD fMRI to investigate the transformation of single-vessel diameter and blood flow velocity changes across vascular compartments into depth-resolved BOLD signal change. 2-photon microvascular measurements in cortical layers I-III (down to ~550 μm), CCD-based optical imaging of blood oxygenation/flow and cortical layer-resolved BOLD fMRI were performed under identical conditions in α-chloralose-anesthetized rats responding to forepaw stimulation (~1mA, 300 μsec, 3 Hz, 2 or 20 sec).

Our BOLD results indicate that

1) The earliest rise of the positive BOLD response is observed in middle cortical layers (consistent with Silva&Koretsky PNAS 2002)
2) The initial dip is confined to top layers
3) BOLD amplitude decreases with increasing cortical depth
4) In agreement with our previous optical studies, there is a negative surround response in the contralateral primary somatosensory cortex (SI) accompanied by a negative response in ipsilateral SI
5) There is a post-stimulus undershoot in response to both 2- and 20-sec stimulus

These results are in register with 2-photon measurements of the layers I-III showing that

1) The deepest measured arterioles and capillaries have the fastest dilation onset and time-to-peak
2) Dilation response of the capillary bed in top layers is delayed relative to the deeper layers
3) The largest dilation is observed at the surface arterioles
4) There is more constriction in the surround region of contralateral SI and virtually only constriction in the ipsilateral SI
5) There is a post-stimulus constriction of arterioles with the temporal profile of the diameter change closely resembling that of the BOLD post-stimulus undershoot.

In addition, our results show no evidence for swelling of the venous vessels down to 550 μm.

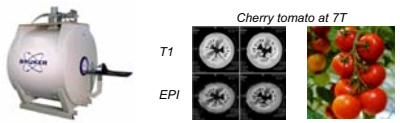
Methods

Animals: Cannulas were inserted in the femoral artery and vein. Anesthesia was maintained with 50 mg*kg^{-1} intravenous bolus of α-chloralose followed by continuous intravenous infusion at 40 mg*kg^{-1}*h^{-1}. HR, BP and body temperature were continuously monitored. Respiration was aimed to maintain pCO_2 between 35 and 45 mmHg. In CCD optical imaging experiments an area of skull overlying SI was exposed and the skull was thinned. In 2-photon experiments the skull and dura mater were removed, and the space between the exposed brain surface and the coverglass was filled with ~1 % (w/v) agarose (Sigma) in ACSF.

Imaging:

BOLD-fMRI:

- 7T/30 cm horizontal bore scanner (BioSpec 70/30 USR, Bruker)
- EPI sequence
  - TE = 10 msec
  - FOV = from 1.6x4 mm to 2.4x4 mm
  - flip angle = 30º
  - in-plane resolution = from 200x500 μm to 300x500 μm
  - matrix = 80x80
  - slice thickness = 1 mm
  - TR = 1 sec
  - 5 adjacent slices
- 10-mm diameter transmit/receive surface RF coil positioned over the SI
- coronal slices



Two-photon imaging is described in [1,2]. ~0.3 ml of 5 % (w/v) solution of 2 MDa fluorescein-conjugated dextran (FD-2000S, Sigma) in saline was injected i.v. Images were obtained using 4-channel Ultima 2-photon microscopy system from Prairie Technologies using 900 nm illumination.

CCD imaging is described in [1,2]. *Spectral*: white light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm). Images were acquired by cooled 12 bit CCD camera at ~15Hz. The spectral data were converted to percent change maps for oxy-, deoxy- and total hemoglobin (HbO, Hb and HbT). *Speckle*: diode laser (785 nm, 40 mW) was used for illumination. Images were acquired by 8 bit CCD camera at ~200Hz.

[1] A. Devor *et al.*, *J Neurosci* **28**, 14347 (Dec 31, 2008).
[2] A. Devor *et al.*, *J Neurosci* **27**, 4452 (Apr 18, 2007).

Upstream propagation of vasodilation along diving arterioles

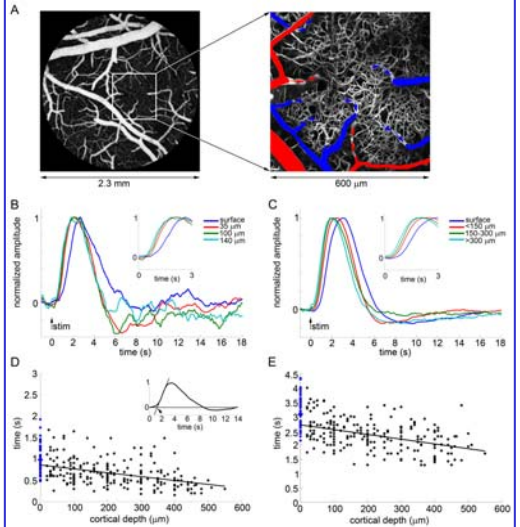


Figure 1. A. MIP of an image stack of 0-300 μm in depth. **B.** An example set of temporal diameter change profiles acquired from an individual arteriolar tree. The inset shows a zoom-in view on the first 3 s following the stimulus. **C.** Population-averaged time-courses from different cortical depths (color-coded, see the inset). **D-E.** Onset time (D) and time-to-peak (E) as a function of the cortical depth.

The capillary dilation in layer I is delayed relative to layer II/III

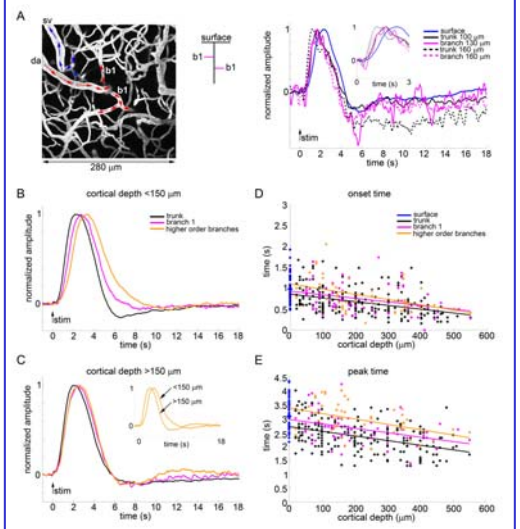


Figure 2. A. An example set of measurements along an individual branching arteriolar tree: MIP of a 0-200 μm image stack, a schematic drawing of branching, and time-courses from the arteriolar trunk and 2 first order branches at different depths. **B.** Population-averaged time-courses of arteriolar trunks and their branches in layer I (<150 μm). **C.** The same as C but for layer II/III (150-550 μm). **D-E.** Onset time (D) and time-to-peak (E) as a function of the cortical depth.

Local neurovascular coupling mechanism in layer I

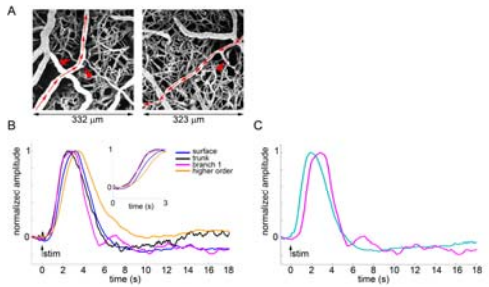


Figure 3. A. Two examples of MIP images (left: 0-400 μm, right: 0-600 μm) showing small diving arterioles branching within layer I (red arrowheads). **B.** Averaged time-courses of different branching order (color-coded, see the inset). **C.** Comparison of the first order branches (magenta) to deep diving arteriolar trunks at >300 μm from Figure 1C (cyan).

The onset and time-to-peak of capillary RBC flux in layer I is delayed

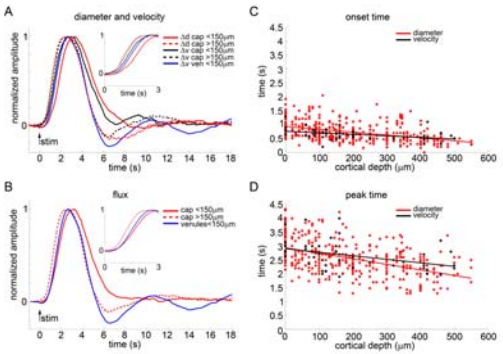


Figure 4. A. Averaged time-courses of diameter and velocity changes in capillaries and venules for different depth categories. **B.** Red Blood Cell (RBC) fluxes. **C-D.** Onset time (C) and time-to-peak (D) of diameter and velocity increase as a function of the cortical depth.

The earliest positive BOLD response is observed in the middle cortical layers

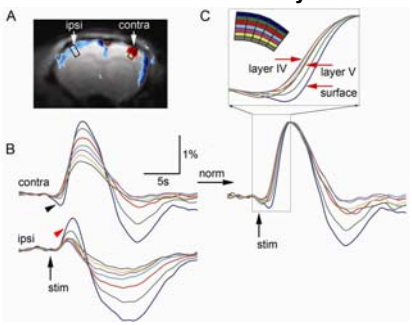


Figure 5. A. Thresholded positive (red) and negative (blue) BOLD fMRI signals at 6 sec post-stimulus superimposed on a corresponding coronal raw EPI image. **B.** BOLD signal time-courses from contra- and ipsilateral ROIs (indicated in A). The depth axis was divided into 7 200-μm width slabs (color-coded; see the inset in C). **C.** Normalized time-courses for the contralateral ROI. Expanded segment on top shows timing differences between the slabs.

Negative BOLD corresponds to a decrease in oxygenation and flow and vasoconstriction

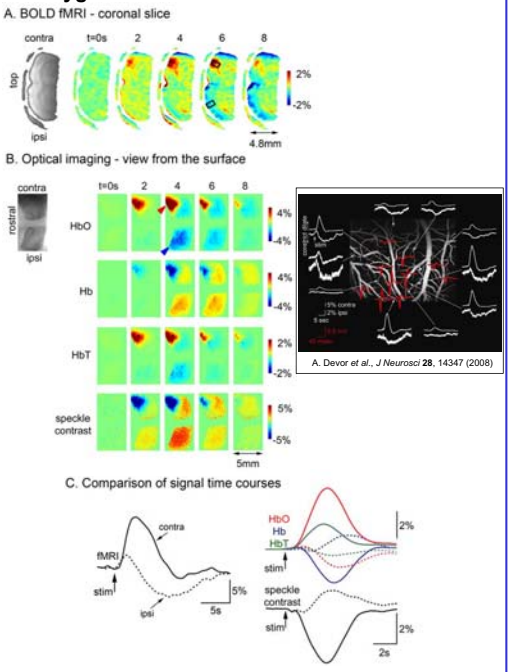


Figure 6. Across-animal comparison of fMRI and spectral/speckle optical data. **A.** Ratio images of BOLD contrast. Raw images were thresholded at 30% of the maximum intensity to reflect the sensitivity of the surface RF coil; a thresholded raw EPI image is shown on the left. **B.** HbO, Hb, HbT and speckle contrast images following stimulus onset. An image of raw vasculature corresponding to functional frames is shown in the upper left corner. **C.** Signal time-courses of BOLD (left) and optical imaging signals (right) extracted from the contralateral (solid lines) and ipsilateral (dashed lines) hemispheres. Bottom: The same for speckle contrast. Note that a *decrease* in speckle contrast indicated an *increase* in blood flow.

BOLD undershoot in response to a long stimulus corresponds to an undershoot in arteriolar diameter

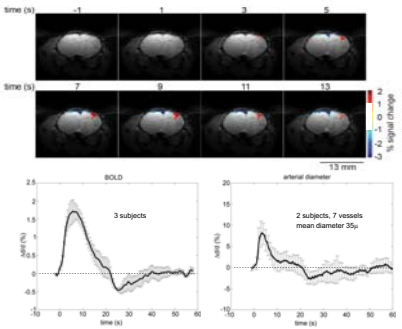


Figure 7. Top: Time series of BOLD response overlaid on the raw EPI image in response to a 20s forepaw stimulus. Bottom: A comparison of averaged time-courses of BOLD signal and arteriolar diameter change.

Acknowledgements

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