UCSD

In vivo two-photon measurement of single vessel diameter changes using astrocytic indicator SR101

286.3

Ivan C. Teng[1], Peifang Tian[1], Anders M. Dale[1,2] and Anna Devor[1,2,3]

[1]Departments of Neurosciences and [2]Radiology, University of California, La Jolla, San Diego, CA, [3]Martinos Center for Biomedical Imaging, MGH, Harvard Medical School, Charlestown, MA,

National Institute of Neurological Disorders and Stroke | NIBIB

Introduction

Neurons, astrocytes, and blood vessels form a metabolic and homeostatic network, the neurovascular unit (NVU), to maintain an adequate cerebral perfusion [1]. Therefore, it is important to examine the neuronal, astrocytic, and vascular activity simultaneously *in vivo*.

This can be achieved by employing two-photon microscopy and applying exogenous fluorescent dyes to label the three components of the NVU, neurons, astrocytes, and blood plasma. Note that the complexity of the fluorescent light detection goes up if we use three channels to delineate the contribution of each component. It may result in less fluorescent signal and thus reducing the penetration depth. Therefore, it is advantageous to measure the activity of the NVU with fewer dyes.

In general, calcium sensitive dyes are employed to study the activities of neurons and astrocytes while a second dye, sulforhodamine 101 (SR101) is used to specifically label astrocytes, thus differentiating them from neurons [2,3]. On the other hand, astrocytic end-feet wrap around blood vessels [1]. Hence, we investigate whether we can extract the blood vessel diameter change without staining the blood plasma, but with SR101. This may provide a method to measure all three components of the NVU with only two dyes.

In particular, we examine whether SR101 staining can reveal the contour of the vasculature, and then reflect the diameter change of single arterioles and capillaries. Veins are not studied because our work indicate that they do not swell using various sensory stimuli [4,5].

1. Hamel E, Perivascular nerves and the regulation of cerebrovascular tone. J Appl Physiol 100: 1059-1064 (2006).
2. Stosiek et al, In vivo two-photon calcium imaging of neuronal networks. Proc Natl Acad Sci USA 100:7319–7324 (2003).
3. Nimmerjahn, et al, Sulforhodamine 101 as a specific marker of astroglia in the neocortex in vivo. Nat. Methods 1, 31–37 (2004).
4. Devor et al, Suppressed neuronal activity and concurrent arteriolar vasoconstriction may explain (the) negative blood oxygenation level-dependent signal. Journal of Neuroscience 27:4452-4459 (2003).
5. Tian et al, SFN 2008 poster 286.5

Method

Animal preparation: Rats were initially anesthetized with isofluorane (3% initially, 1-2% 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 50mg/kg intravenous bolus of α-chloralose followed by continuous intravenous infusion at 40mg/kg/h. Heart rate, blood pressure and body temperature were continuously monitored. Respiration was aimed to maintain pCO_2 between 35 and 45 mmHg.

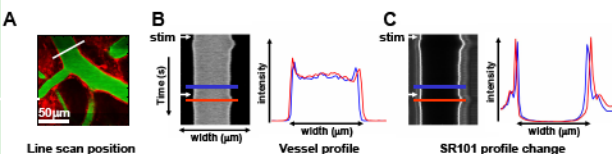
An area of skull overlying the primary somatosensory cortex was exposed, the skull and dura mater were removed, and the space between the exposed brain surface and the coverglass was filled with 0.7 % (w/v) agarose (Sigma) in artificial cerebro-spinal fluid (ACSF). To avoid herniation, dura mater over the IVth cerebral ventricle was punctured and plastic PE50 tube was inserted to drain cerebro-spinal fluid (CSF). The draining hole was sealed after sealing of the imaging well.

Staining procedure: To visualize the vasculature, ~0.3 ml of 5% (w/v) solution of 2 MDa fluorescein-conjugated dextran (FD-2000S, Sigma) in physiological saline was injected intravenously [6]. SR101 (1 mg in 4 ml of ACSF) was either pressure-injected at the depth of 300 µm or applied topically.

Data acquisition: Images were obtained using Ultima 2-photon microscopy system from Prairie Technologies. We used a 4x air objective (Olympus XLFluor4x/340, NA=0.28) to obtain images of the surface vasculature across the entire cranial window to aid in navigating around the cortical vasculature. 20x (Olympus, NA=0.5) and 40x (Zeiss, NA=0.8) water-immersion objectives were used for high-resolution frame scan and line scan measurements. Data analysis was performed in Matlab environment.

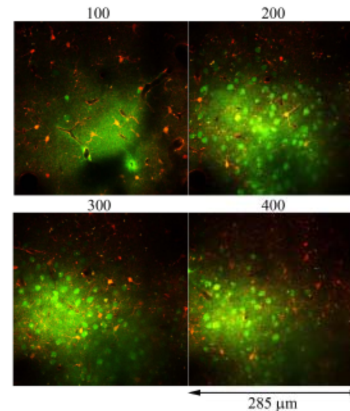
6. Nishimura et al, Penetrating arterioles are a bottleneck in the perfusion of neocortex. Proceedings of the National Academy of Sciences USA 104:365-370 (2007)

Method to extract the vessel diameter change from line scan



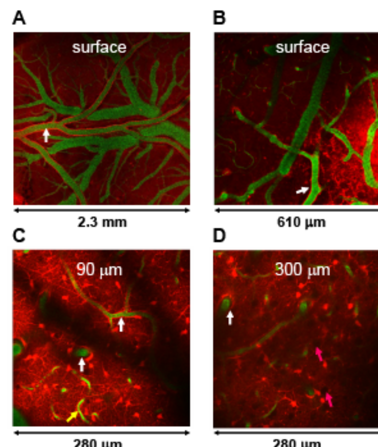
A: Displacement of vessel (in green) and the accompanying SR101 staining profile (in red) is captured by repeated line-scans (position indicated by white bar).
B, C: A space-time image is formed when line scans are stacked sequentially and diameters are extracted from profile changes (before stimulus: blue; peak dilation: red).

Dark bands or holes with bright boundaries of S101 staining appear to be vessels



Calcium sensitive dye Oregon Green 488 BAPTA-1 AM (OGB) and SR101 were pressured injected into the cortex. Neurons and astrocytes are show in green and yellow. The dark bands/holes with bright boundaries formed by astrocytic endfeet appear to be vessels. All four images were taken using two-photon microscope at different depths (indicated above images).

Perivascular SR101 labeling varies with vessel types and cortical depth



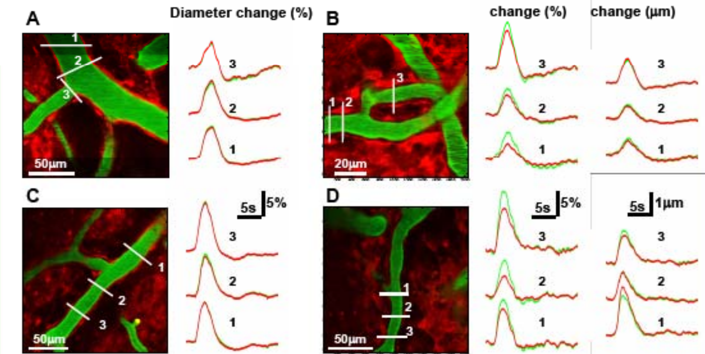
Co-staining the cortex and blood plasma with SR101 and fluorescein-conjugated dextran confirms that the dark holes/bands seen earlier are vessels. In particular, many arterioles (white arrows) and capillaries (yellow arrow) have SR101 envelope that may be used to infer the vessel diameter change. This is because astrocytes are present throughout the cortical depth and their end-feet wrap around parenchymal arterioles and capillaries[7]. Note that neurons are not labeled and appear black (D: magenta arrows).
In addition, surface arteries and some arterioles are accompanied by a very bright SR101 band, whose origin is not clear. This will be employed to extract the vessel diameter.

7. Takano et al, Astrocyte-mediated control of cerebral blood flow, Nature Neuroscience 9, 260 - 267 (2005).

Estimating diameter change of surface arterioles

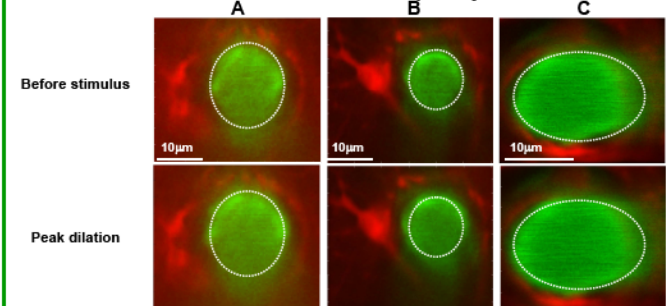
No visible spatial gap between SR101 staining and blood plasma

There is a visible spatial gap between SR101 staining and blood plasma



On the pial surface, very bright SR101 staining accompanies many arteries/arterioles. In some cases (A and C), there is no visible gap between them, thus, the diameter change extracted from SR101 is identical to that obtained from labeling the blood plasma. In other cases, there is a visible gap between SR101 staining and blood lumen (B and D), hence, the percentage diameter changes obtained from these two methods are not equal, however, their absolute diameter changes in microns are the same.

Astrocytic end-feet movement does not necessarily follow that of the vessel in deeper layers



Response of three penetrating arterioles are shown here. Vessels (green) and astrocytes (red) were stained with fluorescein-conjugated dextran and SR101, respectively. Top row shows the condition before the stimulus. Bottom row shows the vessels and astrocytes when the vessels dilate most. As seen here, the astrocytic end-feet move little (A, C) or substantially (B). Therefore, SR101 staining profile may not reflect the accurate diameter change. Note that the circle in each figure illustrate the boundaries of the blood lumen before stimulus.

Conclusions

1. **Many arteries and capillaries appear as dark bands/holes surrounded by SR101 staining when SR101 is injected or topically applied to the cortex.**
2. **Surface arteries/arterioles have very bright SR101 labeling. This can be used for quantitative estimation of vessel diameter changes on the cortical surface.**
3. **For penetrating arterioles and capillaries in deeper layers, the displacement of the astrocytic end-feet does not necessarily match the changes in vessel diameter. Hence, such change may not reflect the accurate vessel diameter change.**

Acknowledgements

We gratefully acknowledge support from the NINDS (NS-051188 to AD and NIBIB (EB00790 to AMD)