Delayed and infrequent astrocytic calcium response does not support the hypothesis of calcium-dependent astrocytic triggering of vasodilation

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Summary

Understanding the mechanisms that control cerebral blood flow is crucial for development of new strategies for treatment of cerebrovascular disease and for better interpretation of noninvasive neuroimaging data. Recently, it has been proposed that vascular dilation in response to an increase in neuronal activity is mediated by calcium-dependent release of vasoactive gliotransmitters [Iadecola&Nedergaard, Nat Neurosci 2007]. However, it remains unclear whether the onset of astrocytic calcium response precedes the onset of vasodilation, as would be expected if the former triggers the latter.

To investigate the temporal relationship between astrocytic increase in intracellular calcium and microvascular dilation, we used 2-photon microscopy to simultaneously image neuroglial calcium activity and diameter changes in nearby arterioles and capillaries. Our results indicate that, despite the robust dilation response, astrocytic calcium increase was observed only infrequently and was considerably delayed relative to the onset of the arteriolar/capillary dilation.

Methods

Animals: Mice were anesthetized with α-chloralose. HR, BP, expired CO_2 and temperature were continuously monitored. Respiration was aimed to maintain pCO_2 between 35 and 45 mmHg.

Cortical exposure: An area of skull overlying SI was exposed, 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.

Dyes: After mapping the neuronal response using surface potentials, calcium indicator OGB1 was microinjected in the center of the responsive area. SR101 was applied to the surface. To visualize the vasculature, ~5 % (w/v) solution of fluorescein- or rhodamine-conjugated dextran in saline was injected IV.

Imaging: Images were obtained using 4-channel Ultima 2-photon microscopy system from Prairie Technologies. We used a 4x objective (Olympus XLFluor4x/340, NA=0.28) to obtain images of the surface vasculature across the entire cranial window to aid in navigating and a 20x water-immersion objective (Olympus, XLUMPlanFI20x NA=0.95) for high-resolution and functional imaging. Data analysis was performed in Matlab.

Timing of microvascular (arteriolar and capillary) dilation in response to sensory stimulation depends on the cortical depth

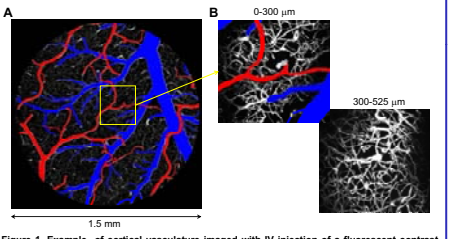

Figure 1. Example of cortical vasculature imaged with IV injection of a fluorescent contrast agent.
(A) Maximal intensity projection (MIP) of a low magnification image stack showing the surface vasculature.
(B) Two consecutive in depth high-resolution MIPs of 0-300 μm (top) and 300-525 μm (bottom) of the region of interest (ROI, outlined on A by a rectangle).

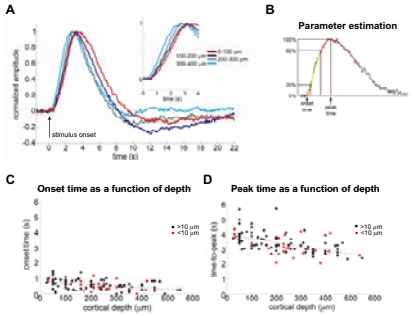

Figure 2. The onset and time-to-peak of arteriolar/capillary dilation decreases with increasing cortical depth.
(A) Superimposed time-courses of diameter change across the depth categories.
(B) An example illustrating estimation of the onset time and time-to-peak. The onset was estimated by fitting a line to the rising slope between 20 and 80% to the peak and calculating an intercept with the prestimulus baseline.
(C) Onset time as a function of the cortical depth.
(D) Time-to-peak as a function of the cortical depth. Red dots indicate measurements from vessels with baseline diameter <10 μm.

Astrocytic calcium response to a brief sensory stimulation, known to cause vasodilation, is infrequent and has slow kinetics

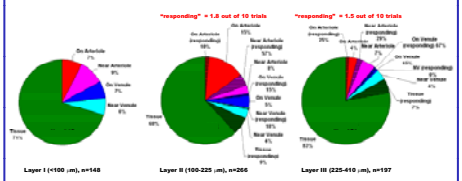

Figure 3. Astrocytic increase in intracellular calcium ("astrocytic calcium response") following sensory stimulation is a rare event in SI. "Responding" is defined as an increase in ΔF/F within a 25-s window following the stimulus onset for at least one out of 10 trials. "Near" is defined as within a 15 μm radius from a diving artery or surfacing venule.

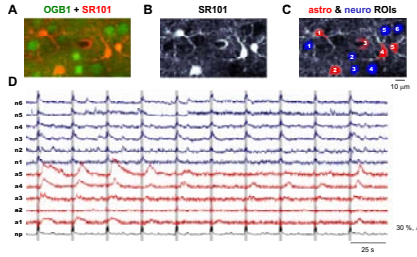

Figure 4. The best example of astrocytic calcium response.
(A) Composite two-photon image of OGB1 and SR101 fluorescence at the baseline.
(B) SR101 image used to define astrocytic regions-of-interests (ROIs).
(C) Astrocytic and neuronal ROIs.
(D) Calcium signal time-courses extracted from the ROIs in C, expressed as % change relative to the pre-stimulus baseline. Gray bars indicate stimulus duration (2 s).

Simultaneous measurements demonstrate vasodilation in the absence of measurable astrocytic calcium response

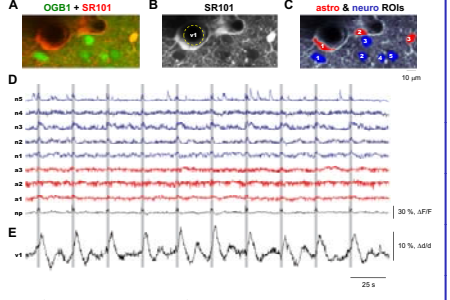

Figure 5. Simultaneous measurements of vascular diameters and calcium imaging.
(A) Composite two-photon image of OGB1 and SR101 fluorescence at the baseline.
(B) SR101 image used to define astrocytic regions-of-interests (ROIs).
(C) Astrocytic and neuronal ROIs.
(D) Calcium signal time-courses extracted from the ROIs in C, expressed as % change relative to the pre-stimulus baseline.
(E) Dilation time-course expressed as % change relative to the pre-stimulus baseline.

Acknowledgements

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Calcium increase limited to the endfoot is infrequent and has slow kinetics

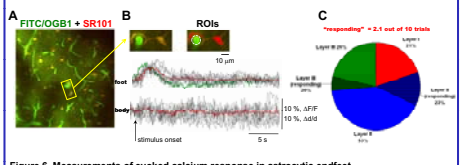

Figure 6. Measurements of evoked calcium response in astrocytic endfeet.
(A) Composite two-photon image of OGB1/FITC and SR101 fluorescence at the baseline.
(B) Top: a zoomed image of an astrocyte with endfoot surrounding a diving arteriole (left) and ROIs corresponding to the cell body and endfoot. Bottom: Extracted time-courses; individual stimulus trials are overlaid in black. The red curves show trial-averaged time-course. Arteriolar dilation is superimposed on the endfoot time-course in green.
(C) Layer distribution of endfoot calcium responses (total number of measured endfeet =57).

Astrocytic calcium response is too slow to account for the onset of vasodilation

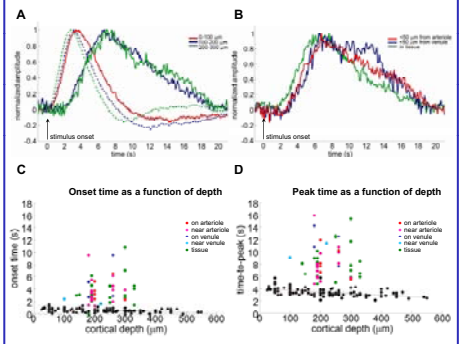

Figure 7. Comparison of the onset and time-to-peak of vasodilation and astrocytic calcium response.
(A-B) All measured astrocytic calcium responses grouped by the cortical depth. Vasodilation time-courses from the corresponding depths are overlaid as dashed lines.
(C) Onset time as a function of the cortical depth. Vascular and astrocytic measurements are overlaid.
(D) Time-to-peak as a function of the cortical depth. Vascular and astrocytic measurements are overlaid.

Conclusions and outlook

- *Within the center of cortical forepaw region in SI, small arterioles and their branches had a robust dilatory response to a brief (2-s) stimulus. The onset of dilation following the stimulus onset varied as a function of the cortical depth: from ~0.5 s in layer IV to ~1 s close to the cortical surface. Similar behavior of dilation has been observed previously in the rat SI [Tian et al., PNAS 2010].*
- *Only 9% of the imaged astrocytes exhibited calcium transients temporally locked to the stimulus; these 9% of astrocytes responded on average to16 % of the stimulus trials.*
- *When observed, the onset of astrocytic calcium response was delayed by >2 s relative to the onset of arteriolar/capillary dilation.*
- *Responding astrocytes were observed also around venules despite the fact that venous diameters cannot be actively controlled.*
- *Among 45 cases where both the cell body and endfoot were imaged, only one had a calcium response in the endfoot in the absence of that in the cell body (Figure 6). The endfoot calcium response had a delayed onset relative to the simultaneously measured arteriolar dilation.*
- *Among the 45 cases where both the cell body and endfoot were imaged, two cases had a calcium response in the endfoot preceding that in the cell body (Figure 4). However, in all cases the endfoot calcium increase was as infrequent as the somatic one.*
- *Slow and delayed astrocytic increase in intracellular calcium does not support the hypothesis that astrocytes trigger vasodilation and the associated blood flow response through calcium-dependent mechanisms.*
- *Astrocytes are unlikely to play a role in generation of the dilatory response to a brief stimulus in healthy subjects and, therefore, might not be relevant for interpretation of fMRI signals using event-related stimulus paradigms.*
- *As an alternative hypothesis, the observed dependence of dilation on the cortical depth might be related to potential laminar differences in neuronal response. In particular, dilation onset might reflect a possible laminar gradient in release of vasoactive neurotransmitters reaching the threshold for dilation sooner in deeper cortical layers.*