The role of nNOS for hyperemia induced by sensory and optogenetic stimulation *in vivo*

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INTRODUCTION

Multiple vasoactive molecular messengers of different cellular origins have been identified. Not all of these messengers, however, are expected to act at once. Rather, specific pathways are likely to be differentially involved across spatiotemporal scales and physiological conditions providing a means to studying their unique role in neurovascular regulation. In the present study, we coupled microscopic measurements of vascular diameters, pharmacological interventions, and optogenetics to address the question whether vasodilation parameters measured on a microscopic scale can be affected through manipulation of specific vasoactive signaling pathways.

METHODS

Animal procedures. Normal (ICR) , VGAT-ChR2(H134R)-EYFP mice expressing ChR2 in all GABAergic cortical neurons (1), and Thy1-ChR2-YFP mice expressing ChR2 in layer V Pyramidal cells (2) were anesthetized with isofluorane during surgical procedures and α-chloralose during data acquisition. During the surgical procedures, a cannula was inserted into the femoral artery for measurement of blood pressure / blood gas and anesthesia infusion. ~2x2 mm cranial window was covered with a glass coverslip. A gap was left in the seal on the lateral side to allow insertion of the electrode for electrophysiological recordings and diffusion of drugs applied topically.

OG stimulation was delivered though the objective using 473-nm (blue) cylinder-shaped laser beam ~230 μm in diameter (FWHM), comparable to the size of a cortical column (Fig. 1). The beam was positioned using a dedicated set of galvanometer mirrors. Duration of light pulse was controlled by a dedicated shutter and synchronized with imaging. A single OG stimulus trial was presented at each imaging location consisting of one or two 150-ms light pulses separated by 120 ms.

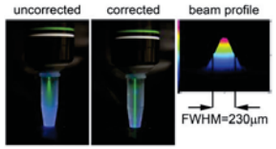

Fig. 1. Preconditioning of the 473-nm laser beam and the beam profile.

Sensory stimulation was delivered to the forepaw contralateral to cortical exposure through a pair of thin needles inserted under the skin using a train of six 100-μs, ~1-mA electrical pulses at 3 Hz.

Vascular diameter measurements. To visualize the vasculature, ~0.03 ml of 5 % (w/v) solution of FITC, in physiological saline was injected intravenously. At depths down to ~600 μm, Ti:sapphire laser @ 800 to 1000 nm was used to excite the intravascular FITC. At depths >600 μm, OPO @ 1360 nm was used to excite intravascular dextran-conjugated Alexa Fluor 680.

Diameter measurements were performed in frame-scan mode at ~10 Hz, or in a "free-hand" line-scan mode using ~1-mm-long line scans across multiple vessels with a scan rate of 80-170 Hz. The scan resolution was 0.5 μm or less.

Estimation of dilation onset. 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 pre-stimulus baseline.

1. Zhao, S. et al. (2011) Nat Methods 8, 745-752.
2. Arenkiel et al. (2006) Neuron 54, 205-18.

ACKNOWLEDGEMENTS

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RESULTS

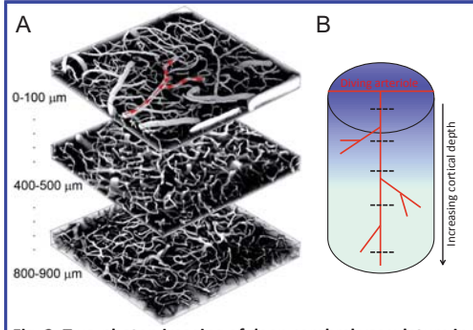

Fig. 2. Two-photon imaging of deep cerebral vasculature in mouse primary somatosensory cortex. A. Red arrows outline one surface arteriole diving at 3 points. **B.** Schematic illustration of the measurement geometry.

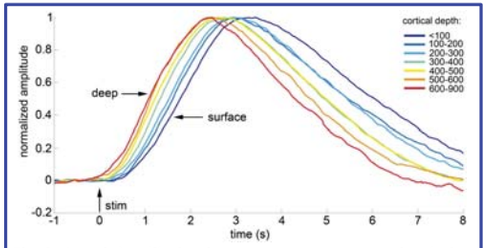

Fig. 3. Arteriolar dilation in response to sensory stimulation as a function of depth. All measurements were obtained along diving arterioles.

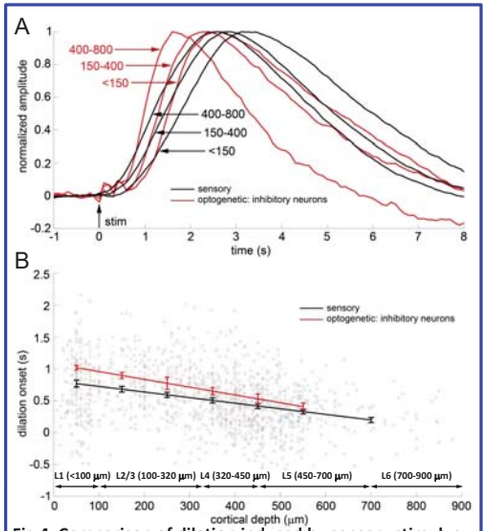

Fig.4. Comparison of dilation induced by sensory stimulus and selective OG activation of inhibitory neurons. A. Averaged and peak-normalized sensory and OG (VGAT-ChR2) time-courses (black and red, respectively). **B.** Onset of the arteriolar dilation (diving trunks) as a function of the cortical depth. Sensory (black) and OG (red) data are overlaid. Error bars show standard error across subjects.

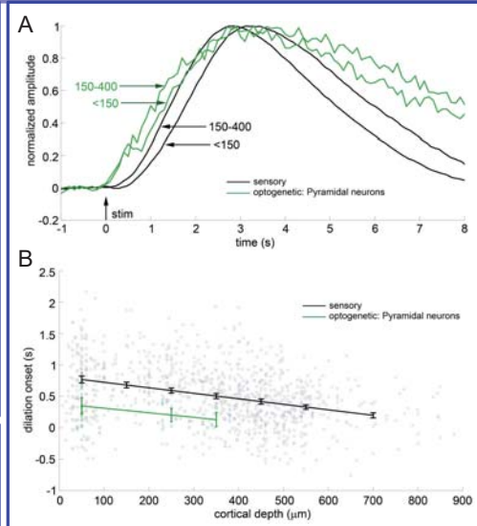

Fig. 5. Excitatory cells provide a "parallel" neurovascular dilatory pathway. A. Averaged and peak-normalized sensory and OG (Thy1-ChR2) time-courses (black and green, respectively). Pharmacological blockers AP5, CNQX, and TTX were applied topically to prevent spread of excitation to other neuronal cell types during OG stimulation. **B.** Onset of the arteriolar dilation (diving trunks) as a function of the cortical depth. Sensory (black) and OG (green) data are overlaid. Error bars show standard error across subjects.

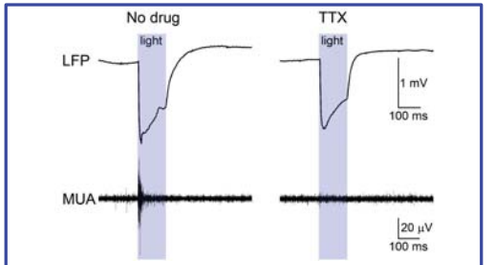

Fig. 6. LFP and MUA in response to OG stimulation of Pyramidal cells (Thy1-ChR2). Single-trial LFP and MUA time-courses before and after TTX: LFP remains and MUA goes away after application of TTX.

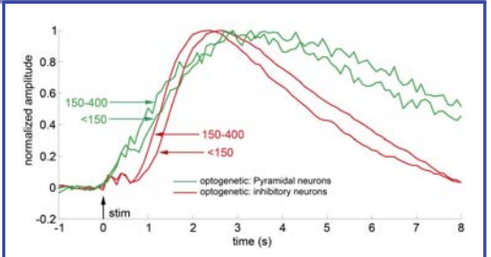

Fig. 7. Averaged and peak-normalized time-courses of arteriolar dilation in response to selective OG stimulation of inhibitory neurons (VGAT-ChR2) and Pyramidal cells (Thy1-ChR2) are superimposed (red and green, respectively).

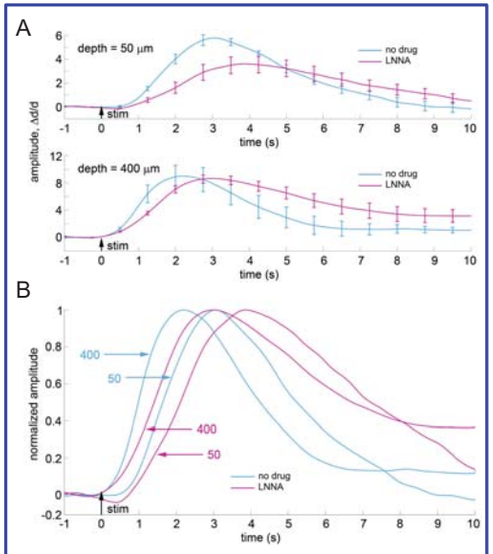

Fig 8. Blocking nNOS reduces dilation amplitude in response to sensory stimulation but does not affect the latency. A. Averaged arteriolar diameter change in response to the sensory stimulus before and after blocking of nNOS (topically applied LNNA, mM). Error bars reflect the standard error across subjects (n=4). **B.** Overlaid peak-normalized curves from (A) demonstrate differences in time-to-peak but similar onset.

DISCUSSION & CONCLUSIONS

- The gradient of dilation onsets along the trunks of diving arterioles – the deepest measurements exhibiting the fastest onset – is a common feature observed with the sensory stimulus and OG stimulation of inhibitory neurons.
- Dilation latency in response to OG stimulation of inhibitory neurons is longer that that in response to sensory stimulus. On the other hand, dilation latency in response to OG stimulation of Pyramidal neurons is less that that of sensory-induced response.
- Virtually instantaneous dilation onset in response to OG stimulation of Pyramidal neurons is consistent with electrical conduction (3) as the initiation mechanism. Electrical conduction is unlikely to play a role in initiation of dilation in response to OG stimulation of inhibitory neurons because of the significant (500+ ms) delay.
- Blocking nNOS decreases dilation amplitude in superficial layers and slows time-to-peak with no effect on the latency. We now attempt blocking nNOS in the VGAT-ChR2; this is still work in progress.

3. Nelson MT and Quayle JM (1995) *Am J Physiol* **268:** C799 – C822.