Cortical inhibitory interneurons shape functional hyperemia

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Hana Uhlirova[1], Kıvılcım Kılıç[2], Peifang Tian[2,3], Payam A. Saisan[2], Qun Cheng[2], Florence Razoux[2], Kimberly L. Weldy[2], Eliezer Masliah[2], David A. Boas[4], Anders M. Dale[1,2] and Anna Devor[1,2,4]

Departments of Radiology[1] and Neurosciences[2], UCSD, La Jolla, CA 92093; [3]Department of Physics, John Carroll University, University Heights, OH 44118;
[4]Martinos Center for Biomedical Imaging, MGH/HMS, Charlestown, MA 02129

INTRODUCTION

Cortical inhibitory interneurons (IN) are known to release a repertoire of neurotransmitters and neuropeptides and pattern activity of cortical circuits. Some of these signaling molecules have vasoactive properties suggesting that IN activity may also regulate neurovascular coupling. Consistent with this idea, experiments in cortical brain slices have demonstrated that stimulation of IN can cause dilation or constriction of arteriolar segments embedded in the sliced tissue with the polarity of the effect depending on the IN cell type. Translation of these results *in vivo*, however, has been hampered by experimental difficulties in isolation of the inhibitory activity without confounding excitatory effects. In the present study we overcome this limitation by using VGAT-ChR2(H134R)-EYFP mice expressing optogenetic (OG) actuator Channelrhodopsin 2 (ChR2) in all GABAergic cortical neurons (Zhao et al., NatMeth 2011). Our results demonstrate that selective activation of IN generated vascular response that shared a number of features with that produced by the sensory stimulus underscoring their role in control of functional hyperemia.

Zhao, S., Ting, J. T., Atallah, H. E., Qiu, L., Tan, J., Gloss, B., Augustine, G. J., Deisseroth, K., Luo, M., Graybiel, A. M., and Feng, G. (2011) Cell type-specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function, Nat Methods 8, 745-752.

METHODS

Animal procedures. VGAT-ChR2(H134R)-EYFP mice expressing ChR2 in all GABAergic cortical neurons (Fig. 1A) were anesthetized with isoflurane 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 round glass coverslip cut straight on one side. The cut side was aligned with the lateral edge of the exposure and 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.

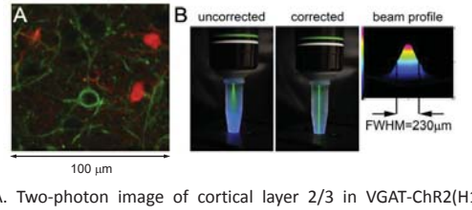

Fig. 1. A. Two-photon image of cortical layer 2/3 in VGAT-ChR2(H134R)-YYFPB mouse. Green – YYFPB, red – SR101. B. Preconditioning of the 473-nm laser beam and the beam profile.

OG stimulation was delivered though the objective using 473-nm (blue) cylinder-shaped laser beam ~230 μm in diameter (FWHM) (Fig. 1B), comparable to the size of a cortical column. 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 two 150-ms light pulses separated by 120 ms.

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, ~0.05 ml of 5 % (w/v) solution of FITC, in physiological saline was injected intravenously. 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.

RESULTS

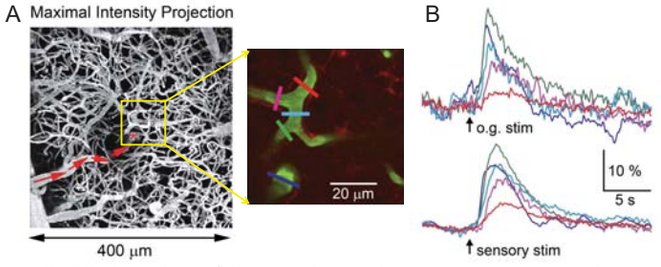

Fig. 2. OG stimulation of the inhibitory cortical neurons produces arteriolar dilation similar to the response to sensory stimulation. A. Maximal intensity projection of a 2-photon image stack throughout the top 290 μm. B. Arteriolar diameter change in response to selective OG activation of inhibition (top) and sensory stimulation (bottom).

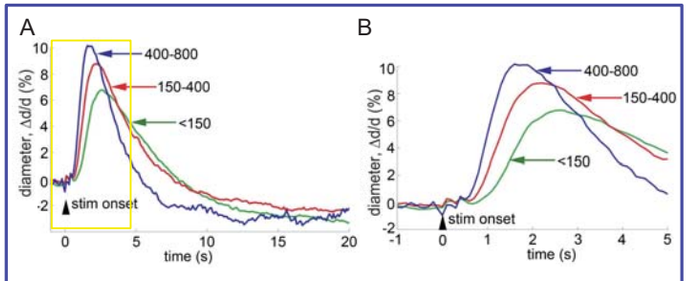

Fig. 3. The onset of OG-induced dilation is depth dependent: the earliest dilation is observed in deep cortical layers. A. Averaged dilation time-courses grouped by depth. Depth in μm is indicated on the right. B. Expanded view of the part outlined by the yellow rectangle in (A).

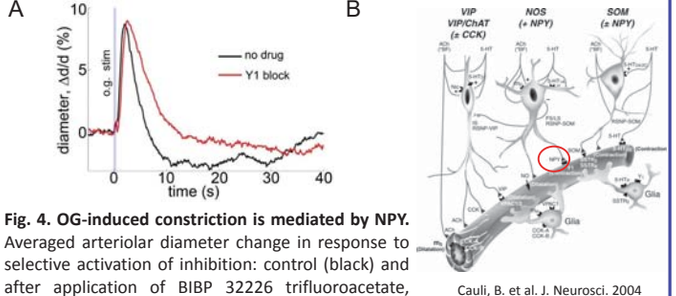

Fig. 4. OG-induced constriction is mediated by NPY. Averaged arteriolar diameter change in response to selective activation of inhibition: control (black) and after application of BIBP 32226 trifluoroacetate, a blocker of Y1 receptors for NPY.

Cauli, B. et al. J. Neurosci. 2004

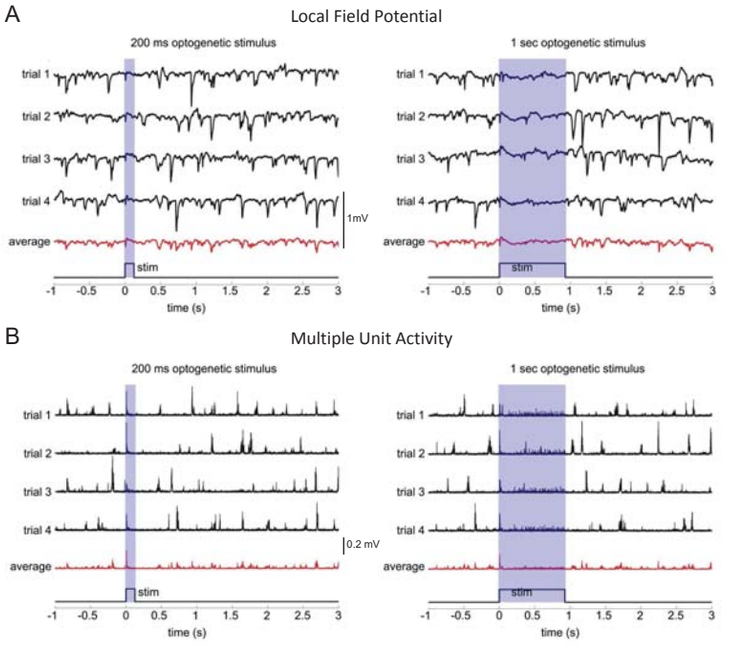

Fig. 5. Lack of evidence for indirect activation of Pyramidal cells. LFP (A) and MUA (B) recorded from layer 2/3 during 200-ms and 1-s OG stimulus.

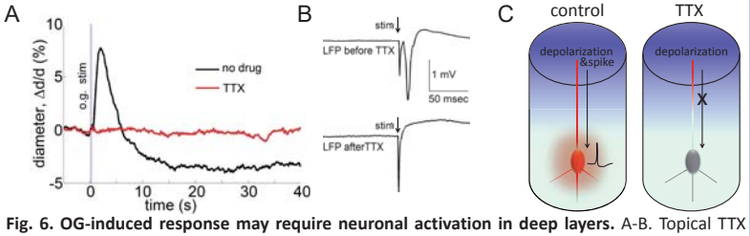

Fig. 6. OG-induced response may require neuronal activation in deep layers. A-B. Topical TTX application blocks OG-induced vascular response in VGAT-ChR2(H134R)-EYFP mice. C. This may indicate that spikes are needed to transmit ChR2-mediated depolarization from upper layers (within penetration of 473-nm light) to deep layers for release of vasoactive messengers.

DISCUSSION & CONCLUSIONS

- Selective activation of inhibitory cortical neurons generate vascular response that shares a number of features with that produced by the sensory stimulus
- Inhibitory neurons play a major role in shaping of the functional hyperemia
- A gradual increase in latency and decrease in amplitude with decreasing depth is consistent with propagated response from deep layers (V, VI)
- Abolishment of the response in the presence of TTX is also consistent with propagated response from deep layers (V, VI)
- The constriction is mediated by microvascular Y1 receptors for NPY

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