THE SELECTIVE ROLE OF CORTICAL INHIBITORY INTERNEURONS IN FUNCTIONAL HYPEREMIA

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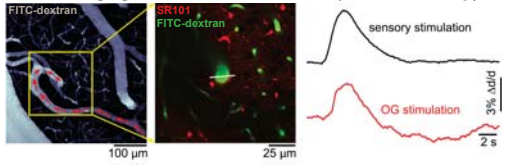
Introduction

- Acute CBF responses driven by fast changes in arteriolar diameter and related to neuronal signaling are mediated by vasoactive messengers released by excitatory and inhibitory neurons.
- CBF responses to sensory stimuli are composed of a combination of dilatory and constrictive phases.
- Inhibitory neurons (INs) release neuropeptides and nitric oxide (NO) and are thought to be able to cause dilation and constriction.
- **Does selective optogenetic (OG) stimulation of INs lead to biphasic dilation and constriction characteristic of sensory-induced responses?**
- **Is the constriction phase specific to IN activation?**
- **Which IN-derived messengers contribute to vasodilation?**

Methods

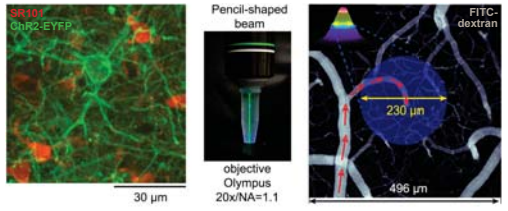
See Uhlirova, Kılıç, et al. for details. Most experiments were performed in the forepaw region of the somatosensory cortex in adult mice under α-Chloralose anesthesia and neuromuscular blockade with pancuronium.

In vivo-imaging of cortical arterioles with 2-photon microscopy (2PM)



Diving arterioles were imaged at different cortical depths. Linescans were used to measure arteriolar diameter changes in response to sensory/OG stimuli.

Optogenetic (OG) stimulation

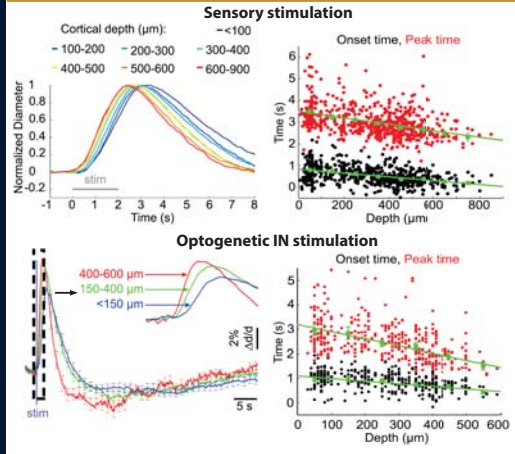


473 nm laser light was used for OG stimulation of INs in VGAT-ChR2-EYFP (Zhao et al.) mice or pyramidal neurons in Thy1-ChR2-YFP (Arenkiel et al.) mice.

References

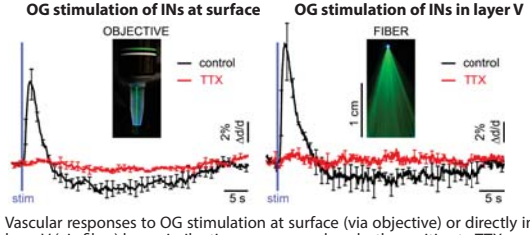
- Arenkiel BR, et al. 2007, Neuron 54:205–218.
- Lindauer U, et al. 1999, Am J Physiol 277: 799-811.
- Uhlirova H, Kılıç K, et al. 2016, eLife 5:1–35.
- Uhlirova H, et al. 2016, Phil Trans R Soc B 371:20150356.
- Zhao S, et al. 2011, Nat Meth 8:745–752.

Sensory vs. Optogenetic Response

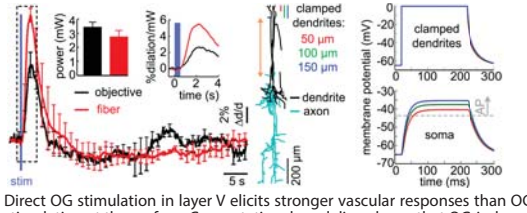


Optogenetic IN stimulation causes a biphasic dilation/constriction response. Responses to sensory and OG stimulation show a depth-dependent onset.

Optogenetic Stimulation at Different Cortical Depths

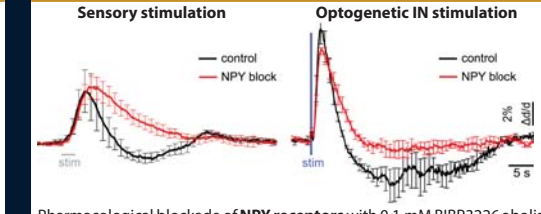


Vascular responses to OG stimulation at surface (via objective) or directly in layer V (via fiber) have similar time courses and are both sensitive to TTX.

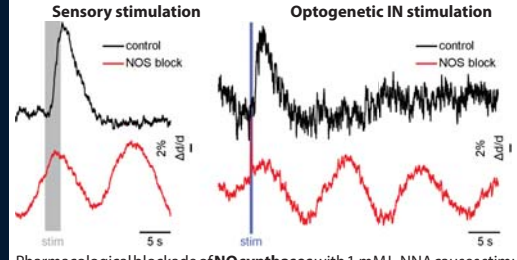


Direct OG stimulation in layer V elicits stronger vascular responses than OG stimulation at the surface. Computational modeling shows that OG-induced membrane depolarization can propagate from superficial dendrites to IN somata in deeper layers to cause action potential (AP) firing.

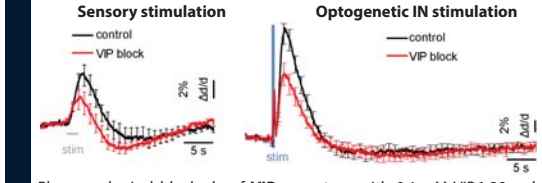
IN-derived Messengers in Constriction and Dilation



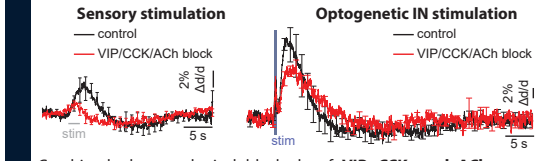
Pharmacological blockade of **NPY receptors** with 0.1 mM BIBP3226 abolishes the constriction phase in response to sensory and OG stimulation.



Pharmacological blockade of **NO synthases** with 1 mM L-NNA causes stimulus-independent vasomotion that has been observed before (Lindauer et al.).

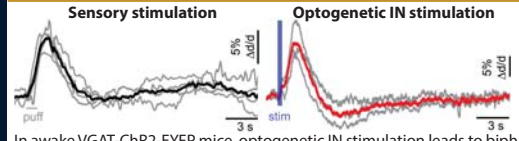


Pharmacological blockade of **VIP receptors** with 0.1 mM VIP6-28 reduces dilation amplitude, but not the onset of vascular responses to sensory and OG stimulation. Constriction is augmented in response to sensory stimulation.



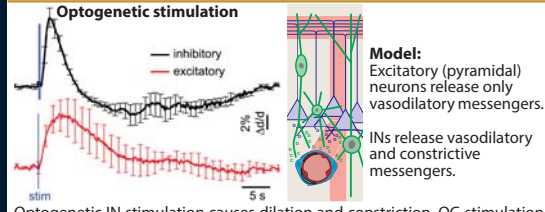
Combined pharmacological blockade of **VIP, CCK, and ACh receptors** (with 0.1 mM VIP6-28, 2 mM proglumide, 0.1 mM scopolamine, 0.1 mM mecamylamine) seems not add to the effects observed upon VIP blockade alone.

Validation in Awake, Head-immobilized Mice



In awake VGAT-ChR2-EYFP mice, optogenetic IN stimulation leads to biphasic responses that are similar to responses to sensory stimulation.

OG Stimulation of Inhibitory vs. Excitatory Neurons



Model:
Excitatory (pyramidal) neurons release only vasodilatory messengers.

INs release vasodilatory and constrictive messengers.

Optogenetic IN stimulation causes dilation and constriction, OG stimulation of excitatory neurons (in presence of AP5 and CNQX) causes only dilation.

Conclusions and Outlook

INs play a major role in shaping functional hyperemia:

- Selective OG stimulation of INs causes biphasic arteriolar responses.
- Dilation in response to sensory and OG stimuli starts in deep cortical layers.
- Release of vasoactive messengers from INs is TTX-sensitive.
- NPY is a major constrictive IN-derived messenger.
- Several messengers including VIP contribute to IN-mediated vasodilation.
- Data aqcuired under anesthesia resembles in awake animals.

Open questions:

- Sites of messenger release (dendrites *vs.* axons)?
- Propagation of dilation and constriction along arteriolar walls?
- Do IN-induced changes in extracellular [K^+] contribute to vasodilation?
- Effect of nNOS blockade/deletion on vascular response to OG IN stimulation?

Outlook:

- Validation of the roles of NPY and VIP in awake animals.
- Analyzing the contribution of different IN subtypes to functional hyperemia.
- Establishment of a modeling framework to predict cell-type-specific neuronal activity from non-invasive multi-modal data acquisition in mice and humans (see Uhlirova et al.).

Acknowledgments

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