Vasoconstriction induced by cortical inhibition reduces cerebral blood flow and hemoglobin oxygenation

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INTRODUCTION

• Origin of **positive** BOLD fMRI :

Neuronal activation → CBF, $CMRO_2$ → [HbR] → BOLD

...Q: Origin of **negative** BOLD?

• Activation of **inhibitory** neurons
→ Biphasic arteriolar response [1]
→ Biphasic CBF response

...Q: Effect of **inhibitory** activity on **$CMRO_2$**?
Contribution to positive/negative **BOLD**?

BOLD: Blood Oxgen Level Dependent
fMRI: functional Magnetic Resonance Imaging
CBF: Cerebral Blood Flow
$CMRO_2$: Cerebral Metabolic Rate of Oxygen
[HbR]: Deoxyhemoglobin Concentration

To explore these questions, we performed **simultaneous measurements of CBF and [HbR]** in the cortex of awake, head-fixed mice in response to sensory stimulation as well as optogenetic (OG) stimulation of inhibitory neurons (INs).

METHODS

• Wild type or **VGAT-ChR2(H134R)-EYFP mice** expressing ChR2 in GABAergic cortical neurons (Fig 1A)
• Surgical implantation **of chronic imaging window** (thin skull or craniotomy) and fixation headbar
• Imaging of barrel cortex in **head-fixed, awake mice** (Fig 1E)
• **Sensory stimulation**: 1-3 100 ms air puffs at 3 Hz to contralateral whiskers
• **Optogenetic stimulation**: 80-330 ms, 4-10 mW pulse of 450/473 nm blue light (Fig 1B)
• **Two-photon imaging:** intravenous FITC for vascular diameter measurements by linescans (Fig 2B)
• Simultaneous **intrinsic optical (IOI)** and **speckle contrast (SC) imaging** of [HbR] and CBF (Fig 3A) [2]

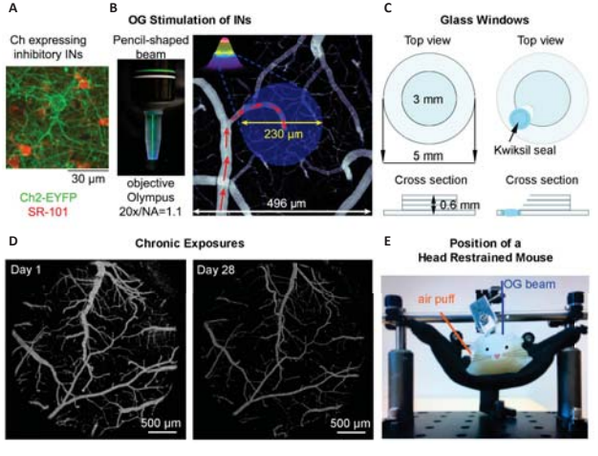

Fig. 1. Awake mouse imaging methods. A) Two-photon image of cortical layer 2/3 in VGAT-ChR2(H134R)-YYFPB mouse. Green: YYFPB, red: SR101. **B)** Blue light beam for OG stimulation. **C)** Cranial window allowing chronic optical imaging of awake mouse cortex [3]. **D)** Chronic exposure 1 and 28 days post surgery. **E)** Setup for awake imaging: head fixation; sensory and OG stimulation.

Table 1 – Measurement of physiological variables in awake mice

Physiological parameter	Measurement modality
Data in this poster	
Arteriolar diameter	Two-photon linescan of vessel section
CBF	Speckle contrast
[HbO],[HbR],[HbT]	Instrinsic optical imaging
Further Directions	
$CMRO_2$	Two-photon phosphorenscence lifetime imaging of pO_2 sensitive dye [6]
BOLD	Functional MRI

RESULTS

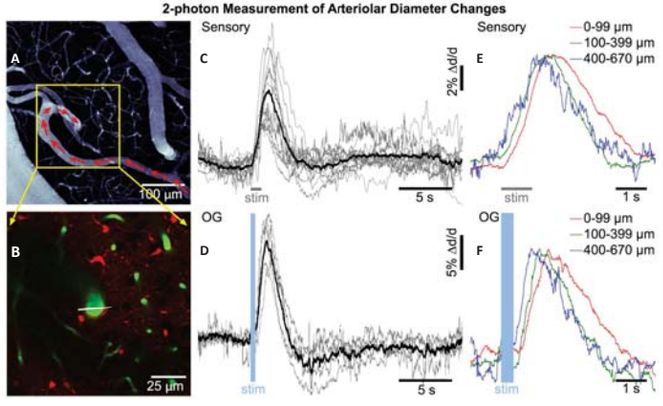

Fig. 2. Two-photon microscopy (TPM). A) TPM image of diving arteriole near the surface and **B)** at 120 µm depth showing linescan pattern. **C-D)** Group-averaged timecourses of arteriole diameter in response to sensory (C, E; N = 9) and OG (D, F; N = 5) stimulation. Each thin gray line represents one animal. E and F show distinct timecourses at different depths from cortical surface.

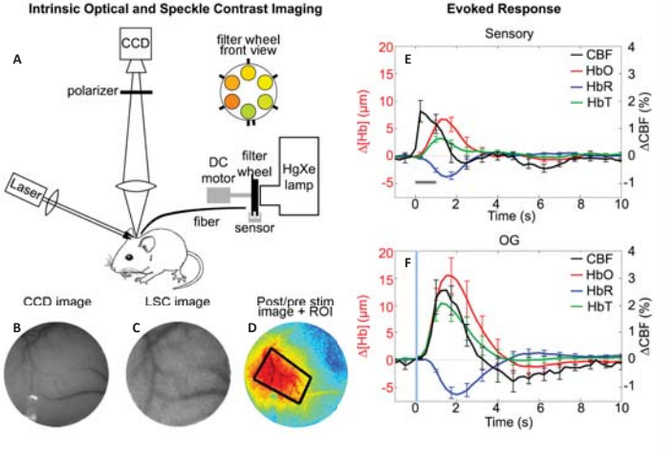

Fig. 3. IOI and SC. A) Custom-built combined imaging system [2]. **B)** CCD image of cortex through a cortical window showing optical fiber used for OG stimulation at the surface. **C)** Speckle contrast image of the same window as in B. **D)** Ratio of post- (1-3 s) to pre (-2-0 s)- stimulus [HbO] map showing the region of activation and ROI used for timecourse extraction. **E-F)** Group- and ROI-averaged timecourses for sensory (E; N = 11) and OG (F; N = 3) stimulation.

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FURTHER DIRECTIONS

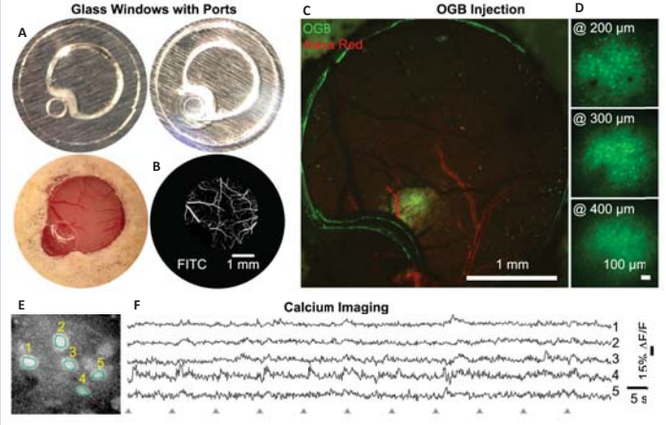

Fig. 4. Dye injection in chronic imaging windows. A) Modified cortical windows with chronic injection port [4]. **B)** Two-photon vascular image showing normal healing 33 days following implantation of a glass window with port. **C-D)** 4x (C) and 20x (D) two-photon images of Oregon Bapta Green Ca+2 indicator injected through the port in B). **F)** Neuronal Ca+2 signal change during sensory stimulation (▲) in 5 different cells (**E**).

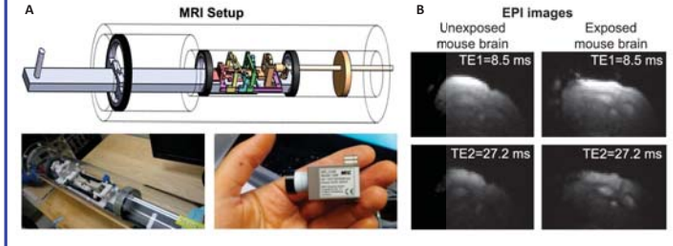

Fig. 5. BOLD MRI imaging in awake mice. A) Custom-built MRI-compatible mouse holder with real-time monitoring, stimulus and reward delivery. **B)** Dual-echo EPI images of a mouse with (right) and without (left) chronic cortical exposure. The cranial window creates susceptibility artifacts.

CHALLENGES

• Movement artifacts in two-photon and MR images
• Magnetic susceptibility artifacts in EPI MR images caused by mismatched susceptibility between glass windows and brain tissue
• Limited timeframe available for each experimental session in awake animals

CONCLUSIONS

• Simultaneous measurements of CBF, [HbO], [HbR] and [HbT], as well as separate two-photon measurements of arteriolar diameter, were obtained during sensory and OG stimulation of inhibitory neurons in awake mice.
• As in anesthetized animals [1,5], in awake mice the arteriolar response to sensory and to OG INs stimulation have a similar shape with an initial dilation followed by constriction. In diving arterioles, dilation starts first in deeper layers, before the surface.
• Following both sensory stimulation and selective stimulation of inhibitory neurons, CBF displays a biphasic response. During the initial CBF increase, [HbO] increases while [HbR] decreases; during the following CBF decrease, [HbO] decreases and [HbR] increases.
• **Therefore, neuronal inhibition drives a CBF response large enough to overcompensate for metabolic demand and cause an overshoot of oxygenation that would generate a positive BOLD response, followed by an undershoot which would be reflected as negative BOLD.**
• Further directions are focusing on quantifying $CMRO_2$ at the microscopic level using two-photon pO_2 sensitive probe PtP-C343 [6], and measuring BOLD in response to sensory and OG stimulation in awake mice with chronic cranial windows.