Neurovascular coupling during contra- and ipsilateral somatosensory stimulation in rat SI

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Summary

Previously, using optical spectroscopy, we demonstrated an antagonistic center-surround spatial pattern of the hemodynamic response to a localized somatosensory stimulus [1]. Specifically, the area of stimulus-induced increase in blood oxygenation was surrounded by an area of decreased oxygenation extending over several millimeters from the center of neuronal activity. Stimulus-evoked decrease in blood oxygenation had been also observed by other groups using fMRI [2] [3]. Recently, Shmuel et al. provided experimental evidence that under certain conditions these "negative BOLD" response correlates with a decrease in neuronal firing rate presumably caused by neuronal inhibition [4]. Here using bilateral imaging/recording of neuronal and hemodynamic activity in rat SI we demonstrate that stimulus-evoked decrease in blood oxygenation occurs in areas that are predominantly inhibited. The inhibition was always preceded by excitation and was not associated with a decrease in firing rate (due to the low baseline firing in rat SI [5]). Since both excitatory and inhibitory activity are energy demanding and since the inhibition did not lead to measurable decrease in population firing rate, we conclude that the hemodynamic response is not driven by metabolic/energy demands but rather is a consequence of feed-forward signaling cascade initiated by release of excitatory and inhibitory neurotransmitters or neuropeptides.

1. Devor, A., et al., *Coupling of the cortical hemodynamic response to cortical and thalamic neuronal activity.* Proc Natl Acad Sci U S A, 2005. 102(10): p. 3822-7.
2. Harel, N., et al., *Origin of negative blood oxygenation level-dependent fMRI signals.* J Cereb Blood Flow Metab, 2002. 22(8): p. 908-17.
3. Shmuel, A., et al., *Sustained negative BOLD, blood flow and oxygen consumption response and its coupling to the positive response in the human brain.* Neuron, 2002. 36(6): p. 1195-210.
4. Shmuel, A., et al., *Negative functional MRI response correlates with decreases in neuronal activity in monkey visual area V1.* Nat Neurosci, 2006. 9(4): p. 569-77.
5. Simons, D.J., *Response properties of vibrissa units in rat SI somatosensory neocortex.* J Neurophysiol, 1978. 41(3): p. 798-820.
6. Dunn, A.K., et al., *Dynamic imaging of cerebral blood flow using laser speckle.* J Cereb Blood Flow Metab, 2001. 21(3): p. 195-201.

Methods

Rats were anesthetized by continuous intravenous infusion of α-chloralose at 40 mg*kg^{-1}*h^{-1}. An area of skull overlying SI cortex was exposed and thinned until translucent. During experiments involving laminar array electrodes and voltage sensitive dyes (VSD) the thinned skull and dura matter were removed.

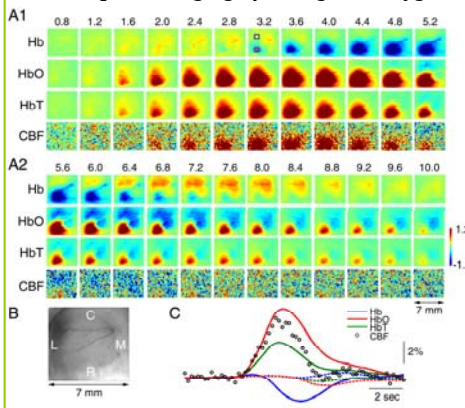
Hemodynamic imaging. *Spectral*: light from a tungsten-halogen source was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm) and coupled into a 12 mm fiber bundle. Images of ~ 6x6 mm were acquired by cooled 12 bit CCD camera at ~15Hz. The spectral data were converted to percent change maps for oxy-, deoxy- and total hemoglobin (HbO, Hb and HbT) using a modified Beer-Lambert law. Differential pathlength correction was applied. *Speckle*: diode laser (785 nm, 40 mW) was expanded to illuminate the cortex at an angle ~ 30°. The laser was coupled into a 600-µm diameter silica optical fiber and a collimating lens. Images were acquired by 8 bit CCD camera at ~200Hz. The raw speckle images were converted to speckle contrast to estimate blood flow [6]. A dichroic beam splitter was used to divert the 780 nm speckle contrast image to the 8 bit CCD camera and pass the 560-610 nm light to the 12 bit CCD camera for simultaneous recording of blood oxygenation and flow.

Electrophysiology. We used linear array multielectrodes with 24 contacts spaced at 100 µm depths. The signals were amplified and filtered between 500-5000 Hz to record MUA, and between 0.1-500 Hz to record LFP.

Voltage sensitive dyes (VSD) imaging. Immediately after the exposure of the cortex the well was filled with a buffered saline containing 135 mM NaCl, 5 mM KCl, 5 mM Hepes, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$. Voltage sensitive dye 1691 (Optical Imaging Ltd, Mountainside, NJ) was dissolved in the buffered saline. The dye was left for 2 hours to impregnate the brain. The unbound dye was washed with fresh buffered saline. The data was acquired using a commercial CCD-based VSD imaging system (Optical Imaging Ltd, Mountainside, NJ).

Neurovascular coupling in contralateral SI

I. Optical imaging of hemoglobin oxygenation and blood flow

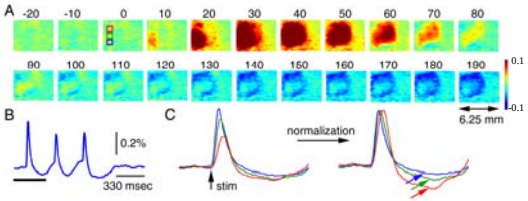


A. Temporal evolution of Hb, HbO, HbT and CBF response to 2-sec electrical forepaw stimulus (3Hz, 0.5 mA). The time series shown in A1 continues in A2. Colorbar: +/- 1.2 % for Hb/HbO/HbT and +/- 2% for CBF. Times relative to the stimulus onset are denoted above the images.

B. Image of the exposure with cortical vasculature corresponding to functional maps in A. The orientation is the same as in A. L, lateral; M, medial; R, rostral; C, caudal.

C. Timecourses extracted from ROI shown in A. The center response (red square in A) is shown by solid lines, the surround (blue square in A) is shown by dotted lines. CBF response timecourse from the center is indicated by black circles. Colorcode: Hb, blue; HbO, red; HbT, green; CBF, black.

II. Voltage-sensitive dyes imaging (of subthreshold intrinsic and synaptic polarization in upper cortical layers)



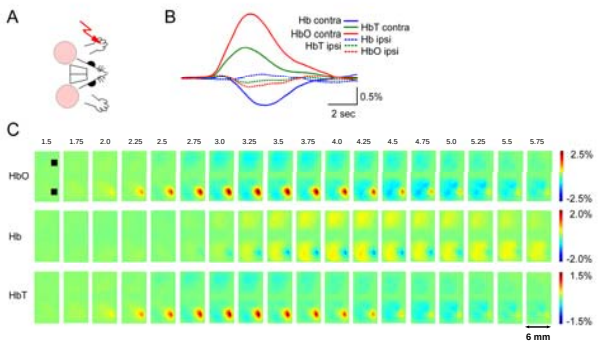
A. Temporal evolution of VSD response to 1-sec electrical forepaw stimulation (3Hz, 0.5 mA). Times relative to the stimulus onset are denoted above the images.

B. Signal timecourse extracted from 1x1 mm ROI at the center of neuronal activity (blue square in frame t=0 in A).

C. Zoom in of the response to the first stimulus. The time period is indicated by a solid black line in B. Left: timecourses extracted from the ROIs shown in A (color-coded). Right: The initial depolarization peak is normalized. Arrows point to the hyperpolarization phase. Relatively to the initial depolarization, the strongest hyperpolarization was observed in the ROI away from the center of neuronal evoked response (red).

Neurovascular coupling in ipsilateral SI

I. Optical imaging of hemoglobin oxygenation

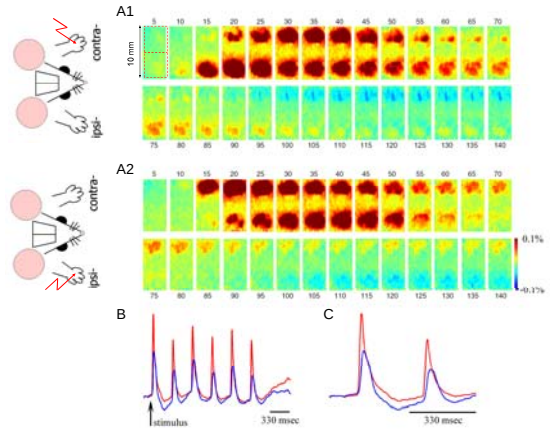


A. A schematic representation of bilateral experimental paradigm. SI is exposed on both sides. The signals are imaged bilaterally in the response to stimulation of one forepaw.

B. Timecourses of contralateral and ipsilateral signal extracted from ROIs shown in C. The contralateral response is shown by solid lines, the ipsilateral response is shown by dotted lines. Colorcode: Hb, blue; HbO, red; HbT, green.

C. Temporal evolution of Hb, HbO and HbT response to 2-sec electrical forepaw stimulus. Times relative to the stimulus onset are denoted above the images.

II. Voltage-sensitive dyes imaging

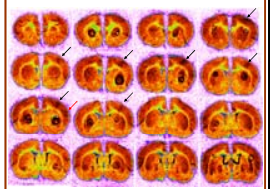


A. Temporal evolution of VSD response to 2-sec electrical forepaw stimulation. Times relative to the stimulus onset are indicated above the images. A1 and A2 show response to stimulation of left and right forepaw respectively.

B. Signal timecourse extracted from each hemisphere. The ROIs are shown by red dotted line in A.

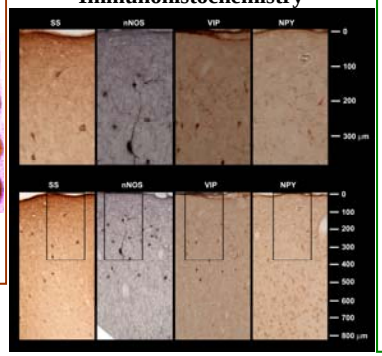
C. Zoom in of the response to the first two stimuli.

2-DG labeling



One single forepaw was stimulated for 45 min (3Hz, 1 mA). Black and red arrows point to labeling of the contralateral SI and SII respectively.

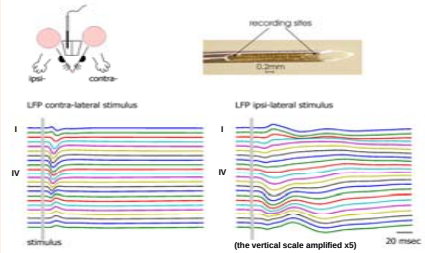
Immunohistochemistry



Inhibitory interneurons expressing SS, nNOS, VIP and NPY are present in upper cortical layers

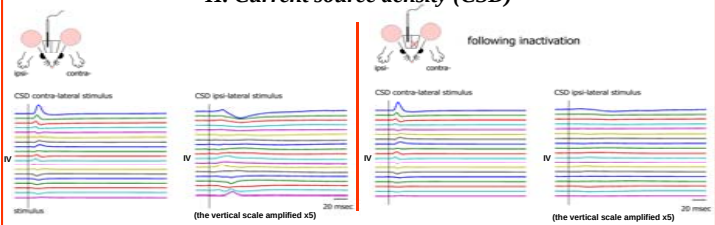
Laminar recordings of local field potentials (LFP) and multiple unit activity (MUA) in response to contra- and ipsilateral stimulus

I. Local field potentials (LFP)



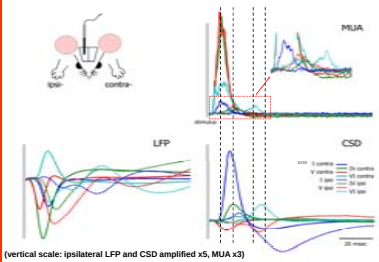
LFP and MUA were recorded simultaneously using a laminar electrode array (shown on top). The array was positioned in one (right) hemisphere. Responses to contralateral stimulus (on the left forepaw) and ipsilateral stimulus (of he right forepaw) are illustrated. The ipsi-stimulus response is amplified x5 comparing to the contralateral one.

II. Current source density (CSD)



CSD was calculated as a second spatial derivative of LPF. Right panel shows the response following inactivation of the hemisphere opposite to the recording array. Note that inactivation abolished the ipsilateral response. This indicates that ipsilateral response is mediated by corpus callosum.

III. Comparison of MUA and CSD



Contralateral stimulus evokes firing in all layers (right top, solid lines). Ipsilateral stimulus evokes firing only below layer IV.

Comparison of MUA to CSD (right bottom) reveals that no firing is associated with the current sink in upper layers during ipsilateral stimulus.

Conclusions from laminar electrophysiology and VSD

I. Contralateral stimulus:

- Activation of cortex through thalamus
- Initial input to layer IV
- Activation propagates throughout the column
- Spiking in all layers
- Field potentials and spikes are tightly correlated

II. Ipsilateral stimulus:

- Input via corpus callosum to upper layers and layer V/VI
- Projections to upper layers cause massive depolarization that *does not propagate throughout the column*
- No spiking except a small increase in layer V
- Subthreshold depolarization and spikes are uncoupled

Conclusions from laminar electrophysiology, VSD and imaging of blood oxygenation and flow

"Positive" hemodynamc response is caused by predominant excitation (release of excitatory neurotransmitters and neuropeptides upon firing of excitatory neurons).

"Negative" hemodynamic response is caused by predominant inhibition (release of inhibitory neurotransmitters and neuropeptides upon firing of inhibitory neurons).

There is no layer-specific hemodynamic response in layer I and II (unless it requires post-synaptic spiking).

The hemodynamic response is not driven by metabolic/energy demands but rather is a consequence of feed-forward signaling cascade.

Further Q: What energy is used to maintain the polarization changes in the upper layers of ipsilateral hemisphere (and spiking of the inhibitory interneurons)? On-going activity might be more dominant in the hemodynamically "negative" regions on trial-to-trial basis.