Spatial Neurovascular Coupling as Revealed by Full-Field Neuronal and Hemodynamic Imaging

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Introduction

Accurate interpretation of functional magnetic resonance imaging (fMRI) signals requires knowledge of the relationship between changes in the hemodynamic response and the neuronal activity that underlies it. Previously, using a brief and localized somatosensory stimulus (deflection of a single whisker), we have demonstrated a nonlinearity in the relationship between "point measures" of neuronal and vascular signals recorded from the same cortical column (Devor et al., 2003). The hemodynamic signals increased supra-linearly with an increase in neuronal activity. We further demonstrated that a point hemodynamic measure is influenced by neuronal activity across multiple cortical columns (Devor et al., 2005). This finding potentially explains the supra-linear relationship. In addition, we showed that the hemodynamic response to deflection of a single whisker has an antagonistic center-surround spatial pattern extending over several millimeters (Devor et al., 2005). In the present study we use a more complex stimulus: deflections of two different whiskers at varying delays, to manipulate the location and timing of neuronal activity. Our data show that neuronal activity cannot be uniquely determined from the the hemodynamic response recorded at the same location w/o apriori knowledge of the nature of the stimulus. Interestingly, we find that the hemodynamic response is amplified at 200 msec delay. This hemodynamic behavior is uncoupled from the measurements of neuronal activity.

Methods

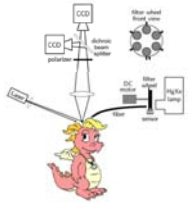
Rats were initially anesthetized with 1.5% halothane and ventilated with ~ 1% halothane in mixture of air and oxygen. Halothane was discontinued, and anesthesia was maintained with 50 mg*kg^{-1} intravenous bolus of α-chloralose followed by continuous intravenous infusion at 40 mg*kg^{-1}*h^{-1}. An area of skull overlying the primary somatosensory cortex was exposed and thinned until translucent. During experiments involving laminar probes and voltage sensitive dyes (VSD) the thinned skull and dura matter were removed.

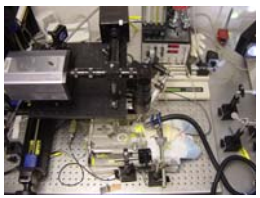
Hemodynamic imaging. *Spectral*: light from a mercury xenon arc lamp was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm) and coupled into a 12 mm fiber bundle. Images of about 6 mm^2 area were acquired by cooled 12 bit CCD camera at ~15Hz. The spectral data were converted to percent change maps for oxy-, deoxy- and total hemoglobin using the modified Beer-Lambert law. Differential pathlength correction was applied to adjust for the differential optical pathlength through the tissue at different wavelengths. *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 (Dunn et al., 2003). 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 blood flow.

Electrophysiology. We used linear array multielectrodes with 24 contacts spaced at 100 μm depths (Ulbert et al., 2001). The signals were amplified and filtered between 500-5000 Hz to record MUA, and between 0.1-500 Hz to record LFP.

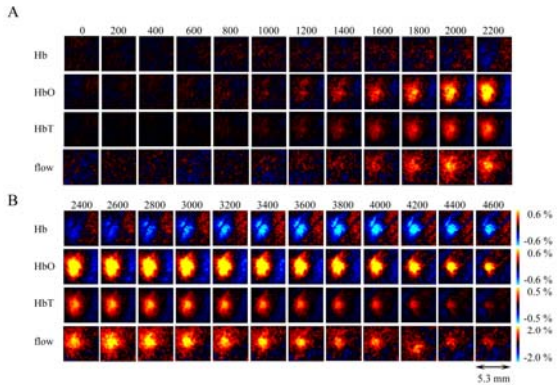
Voltage sensitive dyes imaging. Immediately after the exposure of the cortex the well was filled with a buffered saline containing 135 mM NaCl, 5 mM KCl, 5 mM Hepes, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$. Voltage sensitive dye 1691 (Optical Imaging Ltd, Mountainside, NJ) was dissolved in the buffered saline. 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).

Stimulus. Single whiskers were deflected upward by a wire loop coupled to a computer controlled piezoelectric stimulator. We employed a fast, randomized event-related stimulus presentation paradigm analogous to that used in event-related fMRI studies. The stimulus sequence was optimized for event-related response (Dale 1999).



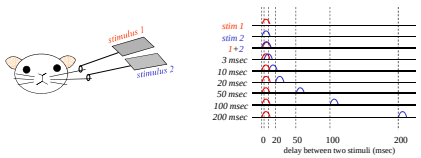


Spatiotemporal evolution of blood oxygenation, volume and flow



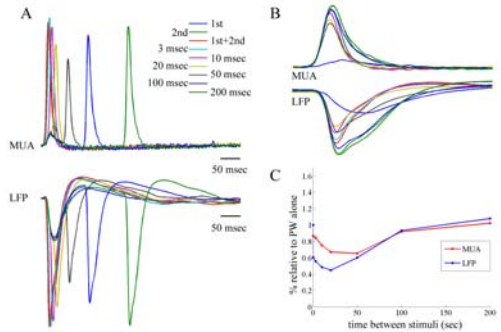
Full-field time series of changes in deoxyhemoglobin (Hb), oxyhemoglobin (HbO), total hemoglobin (HbT) and blood flow. Hb, HbO and HbT were calculated from 6 wavelength data. Flow was calculated from laser speckle contrast (see Methods). Each image represents an individual frame (average of ~360 trials). Time between consecutive images is 200 msec. Stimulus was delivered at time 0. This is the only figure showing 2 sec-long repetitive stimulus of a single whisker delivered at 8 Hz (rather than a single deflection). 2-sec stimulus was used to gain additional SNR for the blood flow data. The stimulus was delivered at 9 different amplitudes. All 9 amplitudes are averaged. Panel B is a continuation of the time series shown in panel A. The signal for Hb and HbO is expressed in percent change relative to its own baseline concentration (40 and 60 mM respectively). HbT was calculated as a sum of Hb and HbO.

2-whisker stimulus paradigm with a varying delay



Two single whiskers three barrels apart were stimulated at different delays. We used the following 9 stimulus conditions: 1st whisker alone, 2nd whisker alone, both whiskers simultaneously, 2nd following 1st after X msec delay where X = [3 10 20 50 100 200].

Multiple unit activity (MUA) and local field potential (LFP) return to the baseline at 200 msec delay

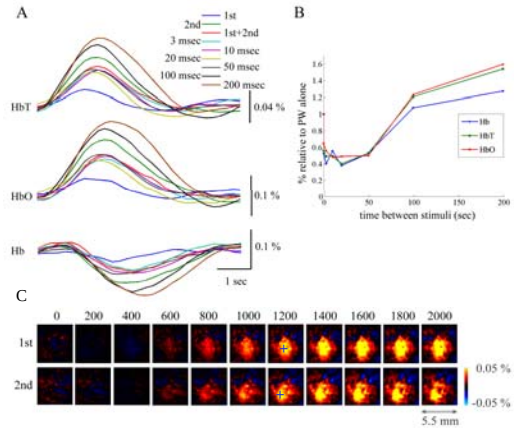


MUA and LPF were recorded using two laminar electrode arrays inserted three barrels apart in Barrel cortex. A. Timecourse of the response recorded by a laminar array inserted into the barrel corresponding to the 2nd whisker (blue in the diagram above). Responses to different stimulus conditions (inset) recorded from layer IV are superimposed. Data from 4 animals were averaged. Note that a prior stimulation of 1st whisker causes a reduction in the response to the 2nd whisker deflection at 10-100 msec delays.

B. Estimates of the incremental MUA and LFP response to 2nd whisker deflection for each stimulus condition (adjusted for the stimulus delay).

C. Incremental MUA and LFP response to the 2nd whisker deflection recorded at the 2nd whisker barrel as a function of delay from the deflection of the 1st whisker. The response was normalized to that of 2nd whisker deflection alone (green in A).

Blood oxygenation and volume responses are amplified at 200 msec delay

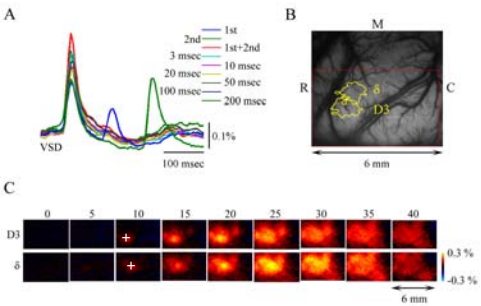


A. Timecourse of Hb, HbO and HbT response recorded from 300x300 μm ROI corresponding to the 2nd whisker barrel. The location of the ROI is denoted by + on C. Responses to different stimulus conditions (inset) are superimposed. Data from 4 animals were averaged (~1000 trials).

B. Incremental Hb, HbO and HbT response to the 2nd whisker deflection as a function of the delay from the 1st. The response was normalized to that of the 2nd alone (green in A). Note an amplification of the hemodynamic response at 200 msec delay.

C. An example of full-field time series of changes in HbT from one of the 4 animals. The location of the ROIs corresponding to the 1st and the 2nd whiskers are indicated by blue crosses.

Voltage sensitive dyes (VSD) show no evidence of amplification

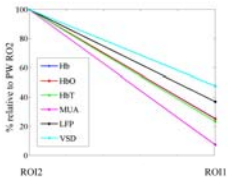


A. Response timecourse recorded from ROI corresponding to the 2nd whisker barrel. The location of the ROI is denoted by + on C. Responses to different stimulus conditions (inset) are superimposed. Data from 2 animals were averaged.

B. An image of the exposed cortex with cortical vasculature from one subject. 50% contour of the response to 1st whisker (D3) and 2nd whisker (δ) 25 msec following the stimulus onset are superimposed (yellow).

C. An example of full-field time series of VSD signal change from the subject shown in B. The location of the barrels corresponding to the 1st (D3) and the 2nd (δ) whiskers are indicated by white crosses. ~100 trials were averaged.

The hemodynamic spatial fall-off is intermediate of MUA and LFP

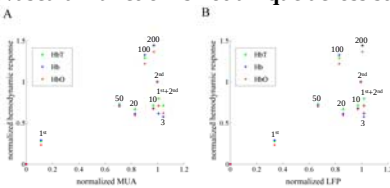


Hb (blue), HbO (red), HbT (green), MUA (magenta), LFP (black) and VSD (cyan) response to stimulation of the 2nd whisker in the corresponding barrel (300x300 μm region of interest, ROI2) and in the 1st whisker barrel (ROI1). The response was normalized to that of the principal barrel (ROI2).

MUA sharply decreases by >90% from the principal barrel to three barrels away. LFP has a broader spatial tuning and decreases only by ~60%. All hemodynamic parameters are in between MUA and LFP. VSD has even broader spatial tuning, probably reflecting broad lateral connections in layer I/II.

Note that a sharper fall-off of the hemodynamic response is consistent with center-surround spatial structure (Devor et al., 2005), and might imply "negative" hemodynamic response in areas of weak subthreshold neuronal activity.

The neurovascular function is not unique across stimulus conditions



Hb (blue), HbO (red) and HbT (green) as a function of MUA (A) and LFP (B) across all stimulus conditions. For each parameter all values are normalized to stimulation of the 2nd whisker alone. Onditions are indicated above datapoints. In contrast to our previous study with varying stimulus amplitude (Devor et al., 2003), these datapoints cannot be fitted by one function.

Conclusions

1. **Hemodynamic response is amplified within a specific time-window. This amplification cannot be explained by the measured electrophysiological activity (MUA, LFP, VSD).**

   Speculation: this phenomenon might indicate a cooperative interaction of two signaling pathways, for example a fast neurotransmitter-mediated pathway and a slow second-messenger-mediated pathway.

2. **Neuronal response as measured in our study cannot be uniquely determined from the hemodynamic response recorded at the same location without apriori knowledge of the nature of the stimulus. A unique neurovascular coupling function might exist in respect with a different neuronal-activity-related parameter not measured in the current study such as a specific ion or neurotransmitter concentration.**

3. **An incremental hemodynamic response to the second stimulus as a function of inter-stimulus intervals is consistent with that of LFP at short intervals (<50 msec), *but uncouples from all neuronal measures at 200 msec.***

4. **MUA shows the sharpest spatial fall-off followed by the hemodynamic parameters, LFP and VSD. The spatial fall-off of the hemodynamic response is sharper than the spatial falloff of LFP. This observation is not sufficient to determine relative contributions of spiking vs synaptic activity to the hemodynamic response, but is consistent with our previous observation of a nonlinear neurovascular relationship (Devor et al., 2003).**

5. **Blood flow response is qualitatively similar to that of blood volume (HbT) and peaks at the same time as HbT.**

References

Dale, A. M. (1999) *Hum Brain Mapp* 8, 109-14.
Devor, A., Dunn, A. K., Andermann, M. L., Ulbert, I., Boas, D. A. & Dale, A. M. (2003) *Neuron* 39, 353-9.
Devor, A., Ulbert, I., Dunn, A. K., Narayanan, S. N., Jones, S. R., Andermann, M. L., Boas, D. A., Dale, A. M. (2005) PNAS 102:3822-7.
Dunn, A. K., Devor, A., Bolay, H., Andermann, M. L., Moskowitz, M. A., Dale, A. M. & Boas, D. A. (2003) *Opt Lett* 28, 28-30.
Ulbert, I., Halgren, E., Heit, G. & Karmos, G. (2001) *J Neurosci Methods* 106, 69-79.