Hemodynamic augmentation in response to a paired whisker stimulus as a function of cortical distance

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We used rat barrel cortex as a model system to study neurovascular coupling while varying the evoked hemodynamic response in the temporal and spatial domain. Paired tactile stimuli were delivered to single whiskers at different time delays (0-2000ms). The distance between the centers of the evoked responses was manipulated by stimulating whiskers at different distances on the rat's snout. Consistent with our previous observations (Devor et al, SFN abstract 2005) neuronal activity evoked by the second stimulus was suppressed by the preceding stimulus at intervals <200ms, but was recovered at 200ms. In contrast, the hemodynamic response measured as blood oxygenation and flow exhibited significant augmentation peaking at 500ms. The whiskers situated further apart displayed larger augmentation than neighboring ones. This finding is not consistent with upstream dilation as the mechanism for the observed nonlinearity.

Methods

Rats were initially anesthetized by halothane switched to a-chloralose during data collection. An area of skull overlying the primary somatosensory cortex was thinned. During experiments involving laminar probes, voltage sensitive dyes (VSD) and 2-photon microscopy the thinned skull and dura matter were removed.

Spectral/speckle imaging: Spectral imaging of blood oxygenation was performed simultaneously with laser speckle imaging of blood flow. Detected light was split via a dichroic mirror, filtered and directed towards two dedicated detectors. The filtering was achieved by passing light below 650 nm to spectral detector, and 780 nm light to speckle detector. *Spectral*: Light from tungsten-halogen source was directed through a 6-position rotating filter wheel (560, 570, 580, 590, 600 and 610 nm). Images were acquired by cooled 16 bit CCD camera. The spectral data were converted to percent change maps for oxyhemoglobin (HbO), deoxyhemoglobin (Hb) and total hemoglobin (HbT). *Speckle*: A laser diode (785 nm, 80 mW) was used as a light source for speckle imaging. Raw speckle images were acquired by a high-speed (~120 Hz) 8-bit CMOS detector. A *decrease* in speckle contrast indicates an *increase* in blood flow.

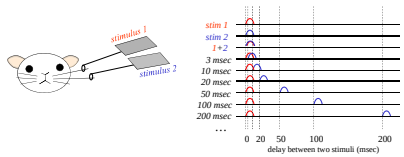
Electrophysiology: We used linear array multielectrodes with 24 contacts spaced at 100 mm depths. The signals were amplified and filtered between 500-5000 Hz to record MUA, and between 0.1-500 Hz to record LFP.

VSD imaging: Voltage sensitive dye 1691 (Optical Imaging Ltd, Mountainside, NJ) was dissolved in the buffered saline. The dye was left for 1.5-2 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).

2-photon microscopy: A chamber consisting of a metal frame and a removable glass coverslip lid was glued to the skull. To visualize the vasculature, ~0.3 ml of 5 % (w/v) solution of 2 MDa fluorescein-conjugated dextran in ACSF was injected iv. Images were obtained using Ultimall 2-photon system from Prairie Technologies. We used a 4x air objective to obtain images of the surface vasculature across the entire cranial window and 20x water-immersion objective for high-resolution imaging and line scan measurements.

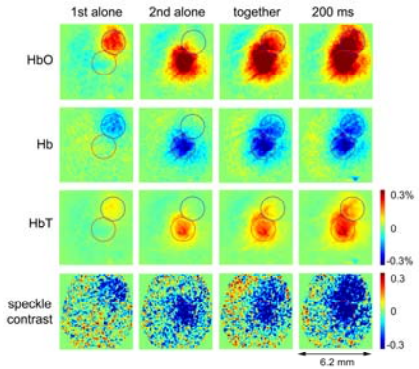
Stimulus: Single whiskers were deflected upward by a wire loop coupled to a computer controlled piezoelectric stimulator. Stimulus conditions were randomized.

Paired whisker stimulus paradigm with a varying delay



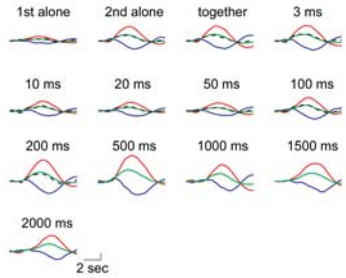
Two single whiskers 1-4 barrels apart were stimulated at different delays. We used the following stimulus conditions: 1st whisker alone, 2nd whisker alone, both whiskers simultaneously, 2nd following 1st after X ms delay where X = [3 10 20 50 100 200 500 1000 1500 2000].

Imaging of blood oxygenation and flow in response to paired whisker stimulus

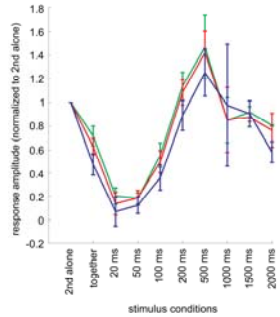


Example HbO, Hb, HbT and speckle images 2.5 s after the stimulus onset for 4 stimulus conditions. Each image represents an individual frame (average of ~??? trials). The signal for Hb and HbO is expressed in percent change relative to its own baseline concentration (40 and 60 mM respectively). HbT was calculated as a sum of Hb and HbO. Decrease in speckle contrast indicates an increase in blood flow.

Augmented blood oxygenation and flow response peaks at 500 ms delay

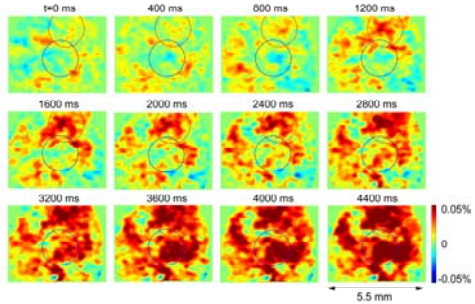


Averaged time courses of HbO (red), Hb (blue), HbT (green), and speckle contrast (black) for each stimulus condition (n=8 subjects, ???trials/subject). Time courses were extracted from 1-mm radius ring centered on the earliest detectable HbT response. Peak HbT and speckle response to stimulation of the 2nd whisker alone was normalized prior to inter-subject averaging (vertical scale bar). Time courses of speckle contrast are inverted to enable comparison with HbT.



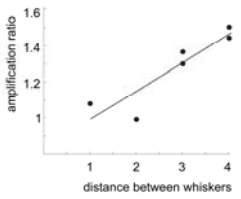
To compare the hemodynamic responses across stimulus conditions we subtracted the response to 1st whisker alone from all conditions where both whiskers were stimulated. The plot on the left shows peak HbO (red), Hb (blue), and HbT (green) response across stimulus conditions. Error bars represent standard inter-subject error.

Augmented response is spatially restricted to the location of the 2nd whisker



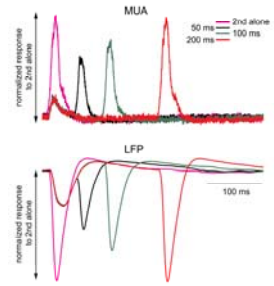
Subtracted images illustrating spatial pattern of augmented response at 500 ms delay. Time series of temporally adjusted sum of responses to 1st and 2nd whisker alone is subtracted from the response to paired stimulus at 500 ms delay. During the initial ~3 s following the stimulus the augmentation is spatially restricted to the location of the 2nd whisker (red circle). Example of a single subject is shown. ??? Trials were averaged for each stimulus condition

Pairs situated further apart display larger augmentation

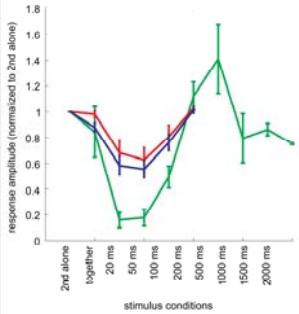


Amplification ratio was defined as the ratio of the response to paired stimulus at 200 ms delay to the time-adjusted sum of responses to 1st and 2nd whisker alone. Peak HbT is shown as a function of distance between whiskers in a pair. Each data point corresponds to one subject, only one inter-whisker distance was tested per subject.

Neuronal activity measured as MUA and LFP returns to baseline at 200 ms

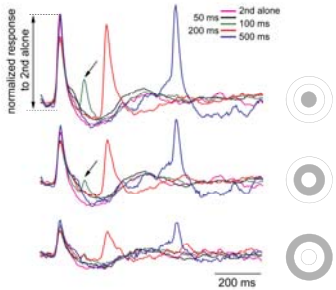


Averaged MUA (top) and LFP (bottom) time courses for 4 stimulus conditions are superimposed (MUA: n=5 subjects, ???trials/subject; LFP: n=8 subjects, ??? trials/subject). Time courses were extracted from contracts corresponding to cortical layer II/III (100-500 μm). Complete recovery occurs at 200 ms delay).

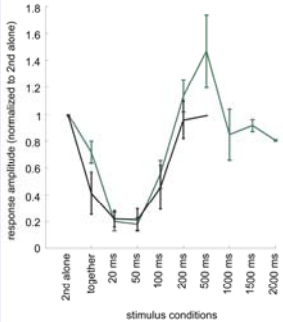


To compare the hemodynamic and electrophysiological data across stimulus conditions we subtracted the response to 1st whisker alone from all conditions where both whiskers were stimulated. Subtraction of 1st whisker alone was applied to both data types. The plot on the left compares peak HbT (green) and peak MUA (red) and LFP (blue) response across stimulus conditions. For delays up to 100 ms HbT followed electrophysiological response. However, augmented HbT response at 200-500 ms was not paralleled in neuronal measurements. Error bars represent standard inter-subject error.

Neuronal activity measured using voltage-sensitive dyes returns to baseline at 500 ms

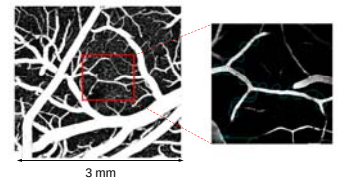


Averaged VSD time courses for 4 stimulus conditions are superimposed (n=4 subjects, ???trials/subject). Time courses were extracted from three 1-mm radius rings centered on the earliest detectable response (schematically shown on the right). In agreement with Civillico and Contreras the recovery occurs from the location of the 2nd stimulated barrel outward (see black arrows indicating partial recovery of the response to 50 ms delay).

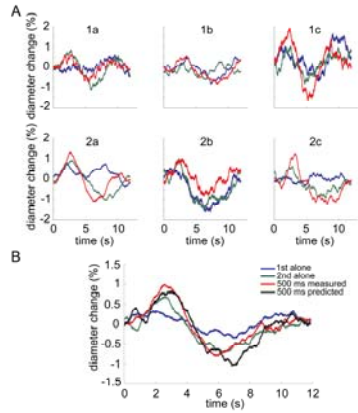


To compare the hemodynamic and VSD data across stimulus conditions we subtracted the response to 1st whisker alone from all conditions where both whiskers were stimulated. Subtraction of 1st whisker alone was applied to both data types. The plot on the left compares peak HbT (green) and peak VSD (black) response across stimulus conditions. For delays up to 100 ms HbT followed VSD response. However, augmented HbT response at 200-500 ms was not paralleled in VSD neuronal measurements. Error bars represent standard inter-subject error.

2-photon measurements from single surface arterioles



Stimulus-evoked diameter changes of individual surface arterioles were measured using 2-photon microscopy. Prior to 2-photon imaging response location was mapped on cortical surface using a ball electrode (not shown). Measurements were made within 500 μm from the maximal response to the 2nd whisker alone. The figure above shows an example of cortical vasculature. The image was calculated as a maximum intensity projection of an image stack of 0-300 μm in depth. Individual images were acquired every 10 μm. The horizontal dimension is 3 mm. The region containing one of the measured arterioles is magnified on the right. The cyan line on the right shows trajectory of the scanning beam.



Arteriolar diameter change in response to three stimulus conditions: 1st whisker alone, 2nd whisker alone and 500 ms delay. A. The traces show percent diameter change relative to the baseline (Δd/d) for 2 subject at three different locations per subject. Dilation is plotted upward, constriction - downward. B. Averaged response. The black trace represents a time-adjusted sum of the first two conditions (the response to 1st alone and 2nd alone).

Conclusions

1. Augmented hemodynamic response at 200-500 ms delay is not reflected in electrophysiological and VSD measures of neuronal activity.
2. Pairs situated further apart display larger augmentation ruling out upstream vasodilation as a potential mechanism.
3. Relatively short time window of augmentation (back to normal at 1 s) also argues against pure vascular phenomena.
4. Potential mechanisms can involve:

- glial Ca^{++} signaling (has been reported to peak 500 ms after stimulus onset by Winship et al.)
- neuronal Ca^{++} signaling (but not likely to be larger for pairs of whiskers situated further apart)
- refractory vasoconstriction (need more averaging across arterioles and across subjects)

Acknowledgements

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References

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