The negative BOLD effect in the rodent barrel cortex model: Investigation using multimodal imaging and electrophysiology.

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Introduction:

Changes in functional magnetic resonance imaging (*f*MRI) signals are based upon neurovascular coupling; that is, the relationship between changes in neural activity and associated hemodynamic changes in blood flow, volume and oxygenation. This study examines the negative blood oxygenation level dependent (BOLD) signal surrounding rodent somatosensory barrel cortex following whisker stimulation.

It is well documented that a positive BOLD signal occurs in response to increases in neural activity. Increased neural activity incurs a metabolic debt that modulates cerebral hemodynamics, decreasing the concentration of paramagnetic deoxy-hemoglobin in the brain parenchyma. However, accompanying the positive BOLD response, sustained negative BOLD changes have been observed in adjacent areas of cortex in human (1), primate (2) and cat (3). Like the positive BOLD response the cause(s) of negative BOLD signals are not known. Several mechanisms have been proposed: (i) a vascular blood 'steal effect' in which active regions of cortex increase their blood flow at the cost of reducing blood flow to surrounding regions. (ii) Direct or indirect inhibitory neural connections to adjacent regions lower neural activity and consequently decrease blood flow to these regions.

The purpose of the present study was to test these hypotheses by taking comparable measures of hemodynamic changes with both *f*MRI and 2D Optical Imaging Spectroscopy (2D-OIS), and neural activity with multi-channel electrophysiology. These measurements allowed direct assessment of neurovascular coupling in regions of both positive and negative BOLD signals.

Methods:

Urethane anaesthetized rats were artificially ventilated and cannulated for monitoring arterial blood pressure and intravenous infusion. The stimulation paradigm entailed 30 trials of 1.2mA electrical stimulation of the entire whisker pad for 16s at 5Hz, with an inter stimulus interval of 70s. For hypercapnic challenges (HCN) following a 60s baseline the animals were subject to 10% increased $FiCO_2$ for 2 minutes and then a 2minute rest period. Rats were used for either a) *fMRI* or b) *2D-OIS and electrophysiology* experiments. The latter experiments required a thinned skull cranial window for direct imaging of the cortex and insertion of the multi-channel probe.

a) fMRI experiments: We utilize a 7 Tesla magnet (Bruker BioSpec^{AVANCE}, 310mm bore, MRI system B/C 70/30), with pre-installed 12 channel RT-shim system (B-S30) and fitted with an actively shielded, 200mm inner diameter, water cooled, 3 coil gradient system (Bruker BioSpin MRI GmbH B-GA20. 200mT/m maximum strength per axis with 200µs ramps). Gradient echo structural images (256*256, TR=1s, TE=6ms, Flip=90°) were taken in both the coronal and topographic (parallel plane to cortical surface) reference frames. Functional data were acquired from a single shot MBEST Gradient Echo - Echo Planar Imaging (GE-EPI) sequence during electrical stimulation and hypercapnic challenges (raw data matrix = 64*64, data sampling interval = 5µs, FOV = 30mm, slice thickness = 1mm, TR/TE=1000/12ms, flip angle 90°, 10 dummy scans). CBV-MRI data was collected after BOLD measurements following infusion of the contrast agent MION: AMI-227 (Guerbet: Sinerem. 10mgFe/kg ~200µmol Fe/kg).

b) 2D-OIS & Electrophysiology: To generate spatial maps of cortical hemodynamic responses, the 2D-OIS technique used a Lambda DG-4 high-speed filter changer (Sutter Instrument Company, Novato, California) using 4 wavelengths (495, 586, 559 and 575nm). The frame rate of the camera was 32Hz, thereby giving an 8Hz effective frame rate for each wavelength. The spectral analysis was based upon the path length scaling algorithm (PLSA) described previously (4). The spectral analysis produced 2D images over time, of oxy, deoxy and total hemoglobin changes (HbO_2, Hbr and Hbt respectively). All electrophysiology experiments were performed with the electrode inserted either into the whisker barrel cortex or surrounding negative region, locations were chosen from analysis of initial 2D-OIS data. Electrophysiological recordings were made using a 16-channel electrode probe (CNCT, University of Michigan) coupled to a data acquisition device (Medusa Bioamp, TDT, Florida) with a custom-written Matlab (The Mathworks Inc., US) interface. The electrode was inserted normal to cortical surface under micromanipulator control to a depth of 1,500µm. The resultant evoked field potential recordings were sampled at 6kHz with 16-bit resolution.

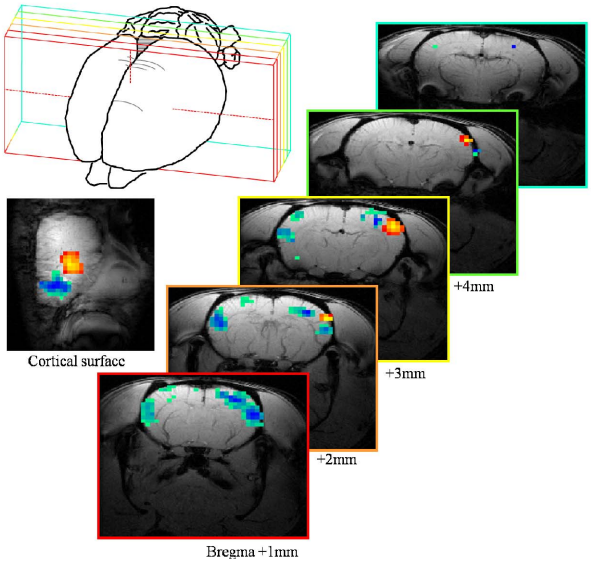

Figure 1: *fMRI statistical maps showing a prolonged negative BOLD effect in areas both surrounding the well documented positive BOLD and the ipsilateral cortex. GE-EPI data is taken in both the coronal and topographic reference frame. The later is parallel to the plane imaged by 2D optical imaging techniques. Results from the imaging modalities were compared and results interpreted with electrophysiological data from regions of interest.*

Summary of Results:

1) *fMRI experiments*: A prolonged negative BOLD response was observed in areas adjacent to the positive BOLD signal in the barrel cortex. Regions of negative BOLD activity were also found in corresponding areas of the opposite cerebral hemisphere (i.e. ipsilateral to stimulation) - figure 1.

2) *2D-OIS & Electrophysiology*: Optical signaling revealed corresponding decreases in blood volume and saturation in regions surrounding the activated cortical barrels. Following cessation of stimulation to the whisker pad, the regions of negative BOLD often showed a sharp rebound increase in blood volume. Simultaneous measures of neural activity in the negative BOLD regions were recorded via a multi-channel electrode (confirmed by histology). At the time of negative BOLD small increases in multi-unit activity evoked by the whisker stimulation were observed from an unaltered baseline state.

Conclusions & Discussion:

This study establishes a model of the negative BOLD signal in rat somatosensory cortex. Our results differ from Shmuel et.al. (2) who reported a decrease in neural activity associated with 'negative' BOLD signals. This inconsistency could be due to many factors including species and procedural differences. Our data will be used to parameterize a forward biophysical model consisting of a dynamic model of oxygen transport to tissue (5), used to predict hemodynamic changes from neural activity, and a Monte Carlo simulation of MR signal attenuation to relate these changes to an observed BOLD signal. The present study confirms that simultaneous measurements of hemodynamic and neural activity is an important pre-requisite to a better understanding of the neurovascular coupling relationships underlying the clinically and experimentally important BOLD signal.

References:

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