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Research Report

Optogenetic drive of neocortical pyramidal neurons generates fMRI signals that are correlated with spiking activity

I. Kahn[a,b,*,1], U. Knoblich[c,1], M. Desai[c], J. Bernstein[c,d], A.M. Graybiel[c], E.S. Boyden[c,d], R.L. Buckner[a,b,e], C.I. Moore[c,2]

[a]Center for Brain Science, Harvard University, Cambridge, MA, USA
[b]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, USA
[c]McGovern Institute for Brain Research and Department of Brain and Cognitive Sciences, MIT, Cambridge, MA, USA
[d]The MIT Media Laboratory, Synthetic Neurobiology Group, and Department of Biological Engineering, MIT, Cambridge, MA, USA
[e]Departments of Psychiatry and Radiology, Massachusetts General Hospital, Boston, MA, USA

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ABSTRACT

Local fluctuations in the blood oxygenation level-dependent (BOLD) signal serve as the basis of functional magnetic resonance imaging (fMRI). Understanding the correlation between distinct aspects of neural activity and the BOLD response is fundamental to the interpretation of this widely used mapping signal. Analysis of this question requires the ability to precisely manipulate the activity of defined neurons. To achieve such control, we combined optogenetic drive of neocortical neurons with high-resolution (9.4 T) rodent fMRI and detailed analysis of neurophysiological data. Light-driven activation of pyramidal neurons resulted in a positive BOLD response at the stimulated site. To help differentiate the neurophysiological correlate(s) of the BOLD response, we employed light trains of the same average frequency, but with periodic and Poisson distributed pulse times. These different types of pulse trains generated dissociable patterns of single-unit, multi-unit and local field potential (LFP) activity, and of BOLD signals. The BOLD activity exhibited the strongest correlation to spiking activity with increasing rates of stimulation, and, to a first approximation, was linear with pulse delivery rate, while LFP activity showed a weaker correlation. These data provide an example of a strong correlation between spike rate and the BOLD response.

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*Corresponding author. Current address: Department of Physiology and Biophysics, Rappaport Faculty of Medicine, Technion—Israel Institute of Technology, Haifa 32000, Israel. Fax: +972 4 829 5461.
E-mail address: kahn@technion.ac.il (I. Kahn).
[1]These authors contributed equally to this report.
[2]Current address: Department of Neuroscience and Brown Institute for Brain Sciences, Brown University, Providence, RI 02912, USA.

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1. Introduction

The blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signal is widely used to study human brain organization. Because this response measures hemodynamic fluctuations, and not the underlying neurophysiological signals that most researchers are mainly interested in, the correlation between neural activity and the BOLD fMRI signal has been studied extensively (Boynton et al., 1996; Dale and Buckner, 1997; Heeger et al., 2000; Logothetis et al., 2001; Miezin et al., 2000; Mukamel et al., 2005; Rees et al., 2000; Shmuel et al., 2006; Viswanathan and Freeman, 2007). Several reports have emphasized the close coupling between local field potential (LFP) activity and BOLD activity (or other measures of the hemodynamic response), de-emphasizing correlations to single-unit and multi-unit action potential (spiking) activity (Logothetis et al., 2001; Sirotin and Das, 2009; Viswanathan and Freeman, 2007).

These reports provide examples of correlations between LFP and BOLD responses when sensory input is used to induce neural activity. However, sensory input drives an ensemble of cell types in the neocortex and connected subcortical regions, and likely recruits neuromodulatory pathways as well (Fournier et al., 2004; Kirifides et al., 2001; Shima et al., 1986), pathways that may not evoke local spikes but are well known to impact local hemodynamics. Even the simplest sensory input will, through feedforward thalamic drive, activate a broad complement of local cell types including pyramidal neurons, fast-spiking interneurons and astrocytes, among others (Schummers et al., 2008; Simons and Carvell, 1989; Swadlow, 1989; Wang et al., 2006). As such, past attempts to understand the neural correlates of the BOLD signal are chiefly observed in the context of a complex barrage of input activity arriving in a neocortical area.

Given the history of such correlation studies, and the current bias to viewing the BOLD signal as best correlated with the LFP, an important unresolved issue is whether, and if so under what conditions, spiking activity might be strongly correlated with the BOLD signal. In support of the possibility that such conditions might exist, Rees et al. (2000) used cross-species correlations to argue that in human area MT+, fMRI responses increase in proportion to the increase in single-neuron spiking in monkey area MT (see Heeger, et al. 2000 for a similar analysis in area V1 of human and monkey; see also Boynton et al., 1996; Dale and Buckner, 1997; Miezin et al., 2000). Similarly, Mukamel et al. (2005) compared activity of individual neurons in the auditory cortex of patients implanted with depth electrodes and fMRI recordings from a separate group of healthy participants, and found that spiking activity was correlated with BOLD responses during presentations of naturalistic stimuli. Thus, in contrast to the widely held interpretation that postsynaptic subthreshold activity, which is a key component of the LFP, is the correlate of the BOLD signal, these additional studies suggest that there are regimes in which spiking activity in the neocortex may be the better correlate of the local BOLD response (for reviews see Heeger and Ress, 2002 and Logothetis, 2008).

To precisely control local neural activity in studying its correlation to the BOLD signal, we employed an 'Opto-fMRI' approach. Similar to recent demonstrations (Desai et al., 2011; Kahn et al., 2011; Lee et al., 2010), we combined activation of the microbial opsin channelrhodopsin-2 (ChR2), a light sensitive nonselective cation channel (Boyden et al., 2005), with high-field functional imaging (9.4 T fMRI). Extracellular recording studies in sensory neocortex of primates—the preparation and cortical area used for most previous studies of BOLD correlates—primarily monitor spike activity of large pyramidal neurons, such as those in layer V. We therefore employed as a model preparation the *Thy1*-ChR2-YFP mouse, as ChR2 is selectively expressed in neocortical pyramidal neurons, primarily those in layer V throughout the neocortex (Arenkiel et al., 2007). Targeting primary somatosensory cortex, we tested whether increased evoked pyramidal cell spike activity would result in proportional increases in the BOLD response. We also manipulated the statistics of these stimulus trains, using periodic vs. Poisson distributed timing that conserved the overall frequency of light stimulation but evoked distinct patterns of neural responses. We reasoned that this manipulation might provide a way to dissociate the neural correlates of the BOLD response, as these optical stimulation regimes induce different patterns of spiking and subthreshold activity, and unlike behavioral stimuli, are associated with precisely controlled activity in a defined cell type.

We found that the BOLD response evoked in this fashion was linear under conditions of increased spike rate. Across stimulus patterns and analyses, the strongest correlation was observed between spiking activity and the BOLD response, with transfer functions generated based on spiking activity providing an accurate estimation of the measured BOLD response. These data provide evidence for a regime, albeit outside natural physiological parameters, of neural activity in the neocortex in which the BOLD signal is predicted best by local spiking activity.

2. Results

2.1. *Optical drive of layer V pyramidal neurons generates a BOLD response*

We first tested whether a BOLD response could be observed in response to optical stimulation in neurons expressing ChR2. Stimuli were presented for 15 s blocks at a frequency of 40 Hz and 8 ms pulse duration, followed by 15 s of no stimulation, and repeated 16 times in each run. Activation was observed in all *Thy1*-ChR2-YFP mice (Fig. 1; 4 animals; $P<0.05$, corrected for multiple comparisons using family-wise error rate method) but not in wild-type mice (ChR2-YFP negative) that received identical stimulation ($n=2$ mice). In all the animals the observed responses were restricted to the area under the fiber optic.

2.2. *The pattern of light-stimulation impacts the amplitude of the BOLD response*

Increasing the frequency of optical stimulation drives an increased number of spikes in neurons expressing ChR2 in the neocortex in vivo (e.g., see Cardin et al., 2009). To test