Mapping Structure-Function Relationships in the Brain

Supplementary Information

Using optogenetics to study neurovascular coupling

BOLD-fMRI and optical intrinsic signal imaging (OISI) have been extensively used to indirectly examine neural activity in the mammalian brain. Because OIS and BOLD responses are used to indirectly measure neural activity, understanding how underlying electrophysiological activity, either in single or multiple cell populations, relates to the local hemodynamic response is essential for understanding the spatiotemporal capabilities of hemodynamic mapping.

Brain imaging using blood-based contrast assumes a linear relationship between local changes in neural activity and the hemodynamic response (1). Generally, OIS and BOLD measures are most highly correlated with the local field potential (2). However, local spiking and/or multi-unit activity (MUA) can account for a non-negligible fraction of hemodynamic signaling variance, and in certain regimes, be more highly correlated with local hemodynamics (2, 3). The first study to examine the linearity between optogenetically-evoked neural activity and hemodynamic responses was in 2011 by Kahn et al. (4). In mice expressing ChR2 in layer 5 excitatory neurons, the authors used photostimulation for controlling the timing of cortical pyramidal neural activity while recording single-unit activity (SUA), MUA, and the local field potential (LFP) in relation to the BOLD response. For short stimulus trains, a linear sum of the BOLD response was proportional to the cumulative neural activity recorded as SUA, MUA and LPF

signals. In a separate study, the same group employed light trains with periodic or Poisson distributed pulses to generate dissociable patterns in BOLD signaling associated with SUA, MUA, and LFP activity. BOLD activity exhibited the strongest correlation to spiking activity with increasing rates of stimulation, while LFP activity showed a weaker correlation, challenging the common assumption that postsynaptic activity (as reflected by the LFP) is the dominant contributor to the BOLD signal. It is also important to note that photostimulation with sufficiently high power can produce measurable arteriolar dilations even in naïve (wild type) animals, possibly due to local tissue heating (5, 6). Researchers should therefore include adequate control experiments when using optogenetics to study the hemodynamic response in relation to neuronal activity.

Photostimulating different cell-types can provide insights into which populations might contribute to local neurovascular coupling. For example, activity in pyramidal neurons does not directly result in local vasodilation and increased oxygenation, but instead drives activity in other cells (e.g. interneurons (7) or astrocytes (8, 9) that recruit a local hemodynamic response (10) (the role of astrocytes in neurovascular coupling is still under debate (11, 12)). Supporting this finding was a study performed by Scott and Murphy (13) where they showed that blocking ionotropic glutamate receptors resulted in appreciable reduction in hemodynamic responses to peripheral stimulation, but not from photostimulus-evoked responses from excitatory neurons (Thy1-ChR2 mice) (14). In mice expressing ChR2 in GABAergic neurons (VGAT-ChR2 mice), the same group showed that photostimulation of sensorimotor cortex significantly attenuated spontaneous spiking while increasing local cerebral blood flow (CBF) (15). Thus,

activation of inhibitory neurons can increase regional CBF in a manner that does not depend on ongoing neuronal activity. Photostimulation of parvalbumin cells expressing ChR2 (PV-ChR2 viral vector) results in contraction of penetrating arterioles (16) and increased BOLD signaling at the site of activation (17), but reductions in the BOLD signal in surrounding areas (17), commensurate with spike rate. Thus, local and distant neural activity can differentially influence regional hemodynamics. Because inhibitory and excitatory activity can contribute to short and long range hemodynamic signaling, stimulation of these different cell populations can be used for mapping local and distant connections within these circuits.

The spatial resolution of hemodynamic mapping

To place an upper limit on the spatial resolution achievable with blood-based mapping, it is important to understand the spatial extent of the hemodynamic responses resulting from a point-like neural stimulus (i.e., the hemodynamic point spread function (PSF)). Hemodynamic responses following peripheral stimulation have been shown to extend spatially farther than confined neurally-active tissue (18-21) (i.e., beyond neuroanatomical boundaries of a single barrel column), or dictated by vascular architecture (22) and can be driven by neural activity distant from the locus of activity (20). However, this spread does not necessarily prevent accurate spatial estimates of focal activity. Targeted blood flow and blood volume increases within neurally-active regions can resolve functional organization within a single barrel column (23), ocular dominance columns(24, 25) or iso-orientation columns within the visual cortex (26-28). From these studies, estimates of the width of the hemodynamic PSF are approximately

500 µm.

Generally, photostimulation of ChR2-expressing neurons produces local patterns hemodynamic activity comparable to those following deflection of the vibrissa (4, 13), or peripheral stimulation of the forepaw (15, 29, 30). Because light can be focused to a diameter smaller than a single functional column, optogenetics can be used to establish an upper limit on the spatial resolution achievable with hemodynamic mapping. Vazquez et al., investigated this possibility in transgenic Thy1-ChR2 mice (31). Using fluorescence signaling from direct YFP excitation (fused to the membrane-bound ChR2) along with the measured LFP and MUA, the authors estimated an upper limit of the hemodynamic PSF between ~100 and 175 µm. Functional structures separated by a few hundred micrometers might be resolved with blood-based imaging.

It's important to note that optogenetically-evoked hemodynamic responses are highly dependent on the photostimulus used. The number of photostimulus-evoked action potentials is proportional to the stimulus intensity (32) (i.e., higher optical power produces larger hemodynamic responses (29, 31)), as well as the type of cell population stimulated. The expression profile of the promotor and light scattering within biological tissue will also play a role. The majority of optogenetic studies in transgenic mice are performed in Thy1-ChR2 mice where ChR2 is predominantly expressed in cortical layer 5 (14, 33). However, given the strong scattering of blue light in biological tissue, superficial dendritic processes primarily are activated, along with orthodromic (downstream) targets. In the study by Vazquez et al., photo-stimulated neural activity and subthreshold depolarizations were confined to the activated area (31). However using depth electrodes, they found that activity distant from the site of stimulation

(>0.5mm) was mostly restricted to cortical layers 2/3. While layer 2/3 pyramidal neurons receive input and project back to layer 5, they also exhibit strong ipsilateral projections over the cortex, all of which could contribute to the spatial spread of hemodynamic activity. Together, these and other studies (29, 30, 34) demonstrate that optogenetic photostimulation of opsin-expressing neurons reliably evokes robust neuronal signaling that is detectable using blood- based imaging.

Supplemental References

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