Optogenetic Functional Magnetic Resonance Imaging (ofMRI): Genetically Targeted In Vivo Brain Circuit Mapping

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Introduction: BOLD [1] signals reflect complex and incompletely understood changes in cerebral blood flow (CBF), volume (CBV), and metabolic rate of oxygen consumption ($CMRO_2$) following neuronal activity [2]. Candidate circuit elements for triggering BOLD signal include excitatory neurons, mixed neuronal populations, astroglia, and axonal tracts or fibers of passage [3]. Importantly, it is not clear which kinds of activity are capable of triggering BOLD responses, placing limitations on interpretation for both clinical and scientific applications. For example, it is sometimes assumed that positive BOLD signals can be triggered by increased excitatory activity within a structure, but this remains to be formally and causally shown, a challenge, which seriously confounds fMRI interpretation. Moreover, use of MRI-compatible electrodes for local stimulation will drive all local excitatory, inhibitory, and modulatory cell types, as well as antidromically drive nonlocal cells that happen to have axons within the stimulated region, thereby confounding functional circuit mapping using BOLD. We sought to address these challenges by integrating high-field fMRI [4,5] with optogenetics [6-10], in which single-component microbial light-activated transmembrane conductance regulators are introduced into specifically targeted cell types and circuit elements, using cell type-specific promoters to allow millisecond-scale targeted activity modulation *in vivo*.

Methods: In order to selectively stimulate excitatory neurons in the cortex, AAV5-CaMKIIα::ChR2(H134R)-EYFP virus was constructed and injected into the M1 cortex of Female adult (>10 weeks old, 250-350 g) rats. A plastic fiber guide was then implanted at the same location. After allowing animal recovery time and opsin expression time, the animal was scanned using a 7T small animal. Animals were intubated with the tracheal tube connected to a ventilator with 1.3-1.5% isoflurane, 35% O_2, 65% N_2O input gas, and a capnometer. Animal body temperature and endtidal CO_2 was maintained at physiological levels (~3.5%, 34-38 ^oC). fMRI scans were performed using a gradient-echo (GRE) sequence with spiral readout, 750 ms T_R and 12 ms T_E resulting in 3.5 x 3.5 cm^2 FOV, 23 slices covering 1.15 cm in the slice direction, 0.5 x 0.5 x 0.5 mm^3 spatial resolution, and 3 s temporal resolution. During the fMRI scan, 20 Hz, 15 ms pulsewidth light stimulation with 473 nm wavelength was applied for 20 or 30 s in 60 s interval. After the MRI scan, optrode [9-10] recordings were made to compare electrophysiology with fMRI. For opsin expression validation, acute coronal brain slices were fixed, mounted, and examined by a scanning laser microscope.

Results: Robust optically-evoked BOLD signals were observed in cortical gray matter at the virus injection / optical stimulation site. Stimulus-synchronized BOLD hemodynamic responses from activated M1 voxels and optogenetic fMRI hemodynamic response functions (ofMRI-HRF) are displayed in Fig. 1e. Strikingly, the BOLD dynamics observed by optically driving this CaMKIIα-promoted excitatory cell population precisely matched dynamics of conventional stimulus-evoked BOLD-fMRI. In particular, the ofMRI-HRF signal onset occurred after 3 seconds but within 6 seconds of stimulus onset; likewise offset was reflected by a drop in BOLD signal contrast beginning within 6 seconds and returning to baseline in ~20 seconds after optical stimulation. Finally, the pronounced post-stimulus undershoot observed during systemic somatosensory stimulation was preserved in ofMRI-HRFs as well. Slices capturing thalamic nuclei (Fig. 2c) also show robust thalamic BOLD signals in response to M1 stimulation, but with properties quite distinct from the intracortical CaMKIIα+ response. A markedly reduced initial rise and slope for onset kinetics of positive-BOLD downstream thalamic recruitment was observed which matches electrophysiological recording pattern in thalamus, including a commensurate delay in spike-rate increase for thalamic neurons compared to cortical neurons during cortical optogenetic drive (Fig. 2f).

Conclusion: Dynamic properties of ofMRI-HRF correspond well to prior measurements on conventional stimulation-evoked BOLD, consistent with interpretation of positive BOLD signals as representative of local net excitatory activity. Optogenetic unidirectional stimulation of downstream regions (eliminating the antidromic drive confound from which electrical stimulation suffers) also shows distinct, and robust BOLD response enabling precise macro-circuit mapping of the brain. In addition, tight correspondence between positive BOLD and local neuronal excitation is observed.

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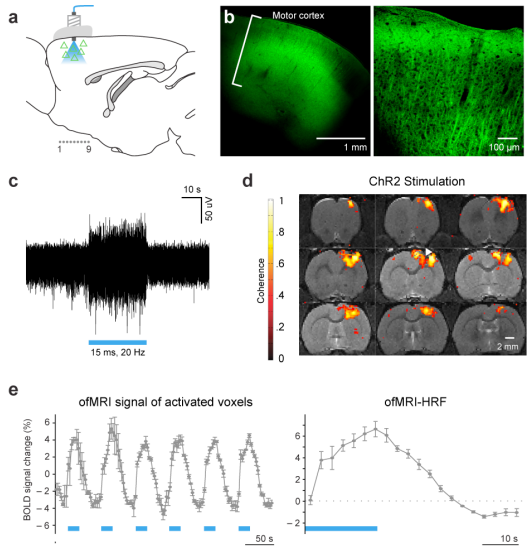

Figure 1 ofMRI: optically-driven excitatory neurons in rodent neocortex drive positive BOLD. **a,** Transduced cells (triangles) and blue light delivery shown in M1 at cannula implantation and stimulation site. Coronal imaging slices shown in (d) marked as "1..9". **b,** Confocal images of ChR2-EYFP expression in M1. **c,** Optrode recording. **d,** BOLD activation near the site of optical stimulation ($p<0.001$). **e,** ofMRI hemodynamic response during 6 consecutive epochs of optical stimulation (left); 20 s of light repeated every 60 s. Mean of all stimulation epochs (right).

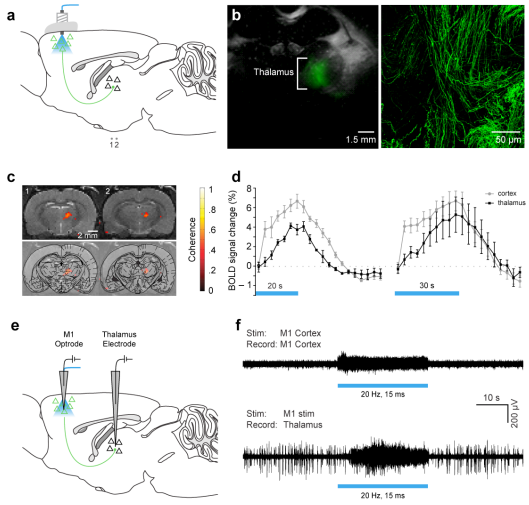

Figure 2 Long-range functional brain mapping with ofMRI. **a,** Schematic shows optical stimulation sites in M1 and coronal imaging slices shown in (c) marked as "1" and "2". **b,** Confocal image of the thalamic area reveals expression limited to axonal fibers. **c,** ofMRI responses in thalamus during optical stimulation of M1. Thalamic activity is centered on VPM, known to be linked with M1 via cortico-thalamic and thalamo-cortical loops. **d,** ofMRI-HRFs obtained from cortical and thalamic BOLD activation areas (gray: cortical; black: thalamic) for 20s (left) or 30s (right) stimulation. **e,** Schematic for two optrodes recording. **f,** Recordings in M1 and thalamus during M1 optical stimulation.