Optogenetic fMRI Sheds Light on the Neural Basis of the BOLD Signal

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Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) is widely used as a measure of neuronal activity, despite an incomplete understanding of the hemodynamic and neural bases for BOLD signals. Recent work by Lee and colleagues investigated whether activating genetically specified neurons elicits BOLD responses. Integrating optogenetic control of specific cells and fMRI showed that stimulating excitatory neurons triggers a positive BOLD signal with conventional kinetics locally and delayed weaker BOLD signals distally.

Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) is used to noninvasively study brain function, primarily in research, although the technique also has clinical applications such as the preoperative detection of eloquent brain areas, regions of the brain that regulate our senses, movement, and speech. BOLD signals reflect hemodynamic changes in flow and blood volume and exploit intravascular magnetic susceptibility to infer oxygenation and the cerebral metabolic rate of oxygen consumption (Logothetis et al. 2001). To illustrate the increasingly prolific use of fMRI in neuroscientific research, a search on PubMed for “functional magnetic resonance imaging” or related abbreviations in the title or abstract returns 1,666 results within the first 5 months of 2010 alone, more than 11 articles per day. However, the technique has not escaped criticism, particularly from neurophysiologists, since the complexities of the hemodynamic response and its relationship to neuronal activity confound the interpretability of fMRI results. Indeed, it remains unclear which types of neuronal activity are capable of eliciting BOLD signals or even which cell populations contribute (Logothetis 2008). If a cerebral microcircuit receives neuromodulatory input, leading to balanced proportional changes in excitatory and inhibitory activity, it is relatively straightforward to predict the effect on hemodynamic responses in the region and resulting BOLD signal change. However, if cortical excitation and inhibition are driven in opposite directions, resulting in net excitation or net inhibition of our hypothetical cerebral microcircuit, the resulting BOLD signal changes are more difficult to predict (Logothetis 2008), since increased inhibitory activity and reduced spiking may increase local cerebral metabolism (Sokoloff et al. 1977). As well as potentially confusing excitation and inhibition, the interpretability of fMRI activation maps is confounded by an incomplete understanding of the relative contributions of different types of activity to the hemodynamic response, such as local field potentials of various frequencies, multiple unit activity, or spiking of individual neurons. Keep in mind that <3% of the volume of one typical neuroimaging voxel is occupied by vasculature, 97% being neural matter: neurons, synapses, and glia (Logothetis 2008).

The complexities of the functional organization of the brain may limit the value of fMRI; nonetheless, it remains a valuable tool for gaining insights into brain function. Clearly, advancing our understanding of human brain function requires a multimodal approach, combining all available techniques, and promoting our understanding of BOLD signal changes requires answers to the question of which cell types and types of activity are responsible for it.

A recent article published in Nature describes a novel study using optogenetics combined with fMRI to investigate the causal generation of BOLD signals in adult rats (Lee et al. 2010). Optogenetic control of neural activity has significant advantages over traditional electrical techniques for local neuronal stimulation; specific cell types can be targeted and the optical control of neuronal spiking is temporally precise with rapid reversibility (Zhang et al. 2010).

Opsin genes can be introduced using viral vectors, thus driving expression of light-responsive cation channels in target neurons. Lee and colleagues (2010) used Channelrhodopsin-2 (ChR2) from the green algae Chlamydomonas reinhardtii. ChR2 is maximally activated by blue light of 470–475 nm. Light from a high-powered laser was delivered by an optical fiber fixed to the rat’s exposed cranium. The versatility of optical fibers means that recording electrodes can be fused to the light delivery element creating a so-called optrode (Gradinaru et al. 2007), which facilitates simultaneous light stimulation of transfected target neurons and electrical recording of local neuronal spikes.

Lee and colleagues injected the viral vector AAV5-CaMKII::ChR2(H134R)-EYFP into the primary motor cortex (M1) of adult rats. Therefore ChR2 channels were expressed specifically in Ca2+/calmodulin-dependent protein kinase II α (CaMKII α)-expressing principal neurons and not in GABAergic or glial cells. Using an optrode, described earlier, optical stimulation and electrical recordings were taken from M1 and >10 days after the virus injection the fMRI experiment was performed (7.0 Tesla, gradient echo planar imaging, 0.5 × 0.5 × 0.5 mm3, repetition time = 3 s). Importantly, Lee and colleagues demonstrated that 473-nm light stimulation elicited a positive BOLD signal at the virus injection/optical stimulation site, whereas in control animals, injected with saline rather than the opsin viral vector, no BOLD signal occurred (BOLD activation threshold: coherence >0.35; z >4.6, P < 0.2 × 10–5). The dynamics of the optogenetic fMRI (oMRI) BOLD signal and oMRI-hemodynamic response closely matched standard BOLD fMRI responses in the motor cortices of humans and rodents (Buxton et al. 1998; Donahue et al. 2006). It is pertinent to note that neuronal activity in M1 elicited in ChR2 rodents via optical stimulation is sufficient to drive locomotion and has no such effect in a control animal (Deisseroth 2008; video reference).

Statistical methods such as dynamic causal modeling and Granger causal modeling have been developed for the purpose of investigating causal connectivity within the human brain, with respect to how different brain regions communicate with one another (Friston 2009). These techniques are based on...
mathematical models for determining whether one time series predicts another and is likely to have caused it. As with most human brain imaging analysis methods, including fMRI analyses, the techniques are teeming with assumptions and the results are difficult to interpret in the context of the multitude of variables involved in an in vivo system.

In rodents the possibilities are greater, but ofMRI breaks new ground. The specificity of the neuronal stimulation provided by optogenetic stimulation is a major advance, compared with local stimulation by electrodes that will drive all local cell types and antidromically drive nonlocal cells with axons in the area of stimulation. Optogenetic control therefore presents the opportunity to study brain connectivity by monitoring long-range downstream activity in connected brain areas. Lee and colleagues assessed the feasibility of this method in the rat brain using high-resolution fMRI to scan thalamic nuclei during optical stimulation of M1 cortical neurons. Thalamic ofMRI during M1 optical stimulation showed that unidirectional BOLD responses could be observed and measured (Lee et al. 2010). Lee and colleagues observed robust thalamic BOLD signals in response to M1 optical stimulation, although the kinetics of this downstream thalamic positive-BOLD response was delayed compared with the locally stimulated positive-BOLD response they observed intracortically. Their electrode recordings of thalamic spike rate also reflected this nearly 5-s delay. This finding shows that it is indeed possible to globally map the functional outputs of a genetically defined neuron population of a particular brain region using ofMRI. Future advances in specificity are not inconceivable, although they depend on further improvements in the genetic tools available to target and separate different cell types.

Lee and colleagues found that cortical injection of viral vectors resulted in ChR2 expression not only in cortical neurons but also in corticothalamic projection fibers and that photosensitive axons illuminated with blue light will drive both local synaptic output and retrograde propagation to the cell body. A level of specificity is maintained since only targeted fibers is sufficient to trigger both thalamic and cortical BOLD responses, albeit with weaker magnitude and delayed kinetics compared with those observed intracortically, as expected.

An additional experiment was described whereby Lee and colleagues used ofMRI to investigate ipsilateral and contralateral thalamic projections to M1. This time the viral vector CaMKIIα::ChR2 was injected into the thalamus. It should be noted that following each experiment the specificity, sensitivity, and spatial distribution of ChR2 expression were validated using immunohistochemistry and confocal/fluorescent images are presented in the report of that study. Following transduction of ChR2 into the thalamus, optical stimulation was applied to either the anterior or the posterior thalamic nucleus. Posterior thalamic stimulation resulted in BOLD responses locally at the stimulation site and in the ipsilateral somatosensory cortex. Optical stimulation of anterior thalamic nuclei elicited BOLD responses locally and in both the ipsi- and the contralateral motor cortex. These results further support the potential of ofMRI as a tool for globally mapping functional connectivity in rodents. The findings with respect to bilateral versus unilateral projections from the thalamus to the motor and sensory cortices respectively are consistent with the theory that motor control involves bilateral regulation (Alloway et al. 2008).

This technique clearly has huge potential for investigating brain connectivity, although arguably the most interesting aspect of this work is what it reveals about the nature of the BOLD signal. As mentioned, optogenetic fMRI goes a step further than sensory or electrical stimulation methods and shows that activation of a specific subclass of neuron—excitatory cortical neurons—triggers the changes in local blood flow, which result in BOLD signals with a conventional time course. Improved knowledge about differential kinetics of BOLD signals resulting from activation of different classes of neurons or brain regions is useful in itself since, once confirmed, that information can be applied to future fMRI analyses. Electrode recordings showed that the dynamics of increases in spike rate in each region closely matched positive BOLD signals. However, the involvement of subthreshold input cannot be ruled out. Lee and colleagues also present data, within the supplemental information supporting their study, indicating that the local optogenetically driven positive BOLD with corresponding increase in spike rate is flanked by an area of negative BOLD with corresponding reduction spike rate. These data fit well with a hypothesis of lateral inhibition. It would be interesting to further investigate the issue of the contribution of inhibition and excitation to BOLD signals, either by pharmacologically inhibiting certain cells or targeting different cell types step by step optogenetically or, indeed, combining these approaches.

The series of experiments conducted by Lee and colleagues provide support for BOLD fMRI and the potential of ofMRI for mapping causal connectivity in rodents. The technique is
certainly a step forward from current methods in terms of testing specific hypotheses. However, some assumptions are still made and the authors note that the absence of a BOLD signal does not prove a lack of connectivity between regions. As with all research in rodents, it is uncertain how these findings can be related to the human brain, although the versatility of optogenetic techniques suggests that future use in humans is not inconceivable. This new avenue of enquiry may accelerate research to map the major neural circuits in the brain. The technique can be applied to existing animal models to trace connections and help to advance our knowledge of brain dysfunction in mental health disorders and neurological disease. For example, ofMRI could be used to investigate connectivity or therapeutic interventions such as deep brain stimulation in mood disorders. (Fig. 1)

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


