Optogenetic control of the BOLD response during local suppression of neuronal activity by muscimol

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Introduction. Functional magnetic resonance imaging (fMRI) relies on the coupling between neuronal electrical activity and regional cerebral metabolic and hemodynamic changes to map neuronal activation, but the relationship between these complex processes remains poorly understood.
The selective modulation of cellular activity in specific structures by local injection of pharmacological agents, performed in parallel with fMRI and electrophysiology, offers a powerful tool for helping to disassociate the contributions of different neuronal processes to the BOLD signal. The recently-developed technique of optogenetics enables precise control of the excitation or inhibition of specific neuronal cell types. By combining these techniques we have been able to observe the BOLD response from particular process while abolishing activity due to other neuronal processes. In these experiments we measured the BOLD response before and after local injection of the GABA-A agonist muscimol in the whisker barrel cortex during optogenetic stimulation of cortical pyramidal cells.
Methods. Dutch-Belted rabbits were chronically implanted with a restraining headbolt [1] and manipulator containing one bundle of 4 microwire gold-silver electrodes and guiding cannulae aimed at the whisker barrel cortex. The adeno-associated viral vector pAAV-CaMKIIα::hChR2(H134R)-EYFP was injected into the whisker barrel cortex to drive ChR2 expression in CaMKIIα-expressing pyramidal cortical neurons as well as co-expression of enhanced yellow fluorescent protein. MR imaging experiments were performed on a 9.4T Bruker BioSpec imaging spectrometer. fMRI data were acquired from four consecutive slices using a single-shot gradient-echo EPI pulse sequence (TR=2s and TE=13ms) with a 1 mm slice thickness and a 375x375 μm^2 in-plane resolution. The slices included the whisker barrel cortex and whisker thalamus. Neuronal activity (single units and local field potentials) in the whisker barrel cortex was recorded using the Neuralynx system. Electrophysiological data were analyzed after removal of blocks of gradient interference.
Optical stimulation consisted of blue (473nm) light generated by a laser diode (Optotronics, Inc., Longmont, CO) delivered through an implanted 200 µm multimode optical fiber at 20Hz with a 15ms pulse width (corresponding to a 30% duty cycle). Simultaneous electrophysiological recordings and fMRI scans were acquired from each rabbit before and after local injection of the GABA-A agonist muscimol (1 µl, 3.5 nmol/µl). The stimulus paradigm for each trial consisted of a stimulus-free baseline period (10 images), followed by a stimulation period (10 images) during which the optical stimulation was delivered, and a post-stimulus period to allow recovery of the BOLD signal (20 images). Each experiment consisted of 10 trials. Trials were averaged for each experiment and the averaged fMRI data were analyzed using cross-correlation to detect activated voxels.

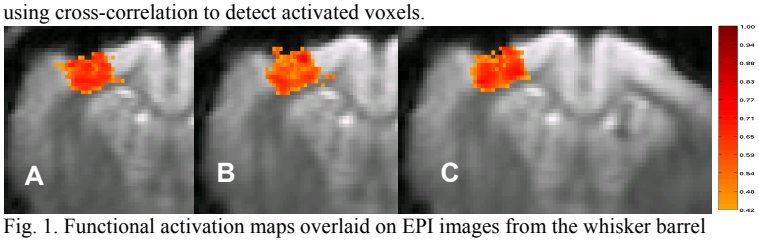

Fig. 1. Functional activation maps overlaid on EPI images from the whisker barrel cortex during direct optical stimulation before (A), immediately after (B) and 30 min after (C) local muscimol injection. The color bar represents the magnitude of the correlation coefficient.

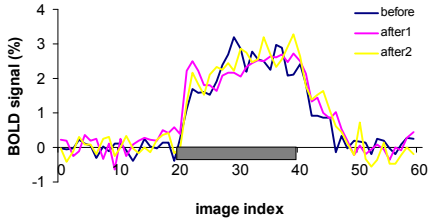

Fig. 2. Averaged BOLD time courses from the whisker barrel cortex during direct optical stimulation before, immediately (after1) and 30 min (after2) after muscimol injection. Grey bar indicates the stimulus.

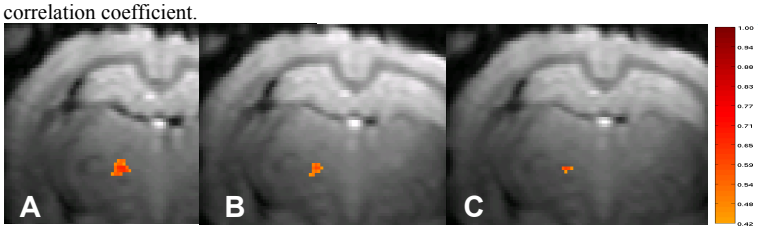

Fig. 3. Functional activation maps from the thalamus during direct optical stimulation of whisker barrel cortex before (A), immediatly after (B) and 30 min after (C) local muscimol injection. The color bar represents magnitude of correlation coefficient.

Results. Muscimol greatly decreased the baseline activity of cells, but no visible change was observed in the BOLD response area (fig. 1) or time course (fig. 2) during optical stimulation. In contrast to the cortex, the thalamus showed a decrease after injection in both BOLD area (fig. 3) and time course magnitude (not shown). Furthermore, the baseline neuronal activity (not shown) decreased in the cortex after muscimol injection.
Discussion. These results highlight the characteristics of cortical BOLD response produced by optogenetic stimulation in the presence of muscimol. The BOLD response to direct optical stimulation was preserved in the cortex even after an increase in cortical inhibition by the GABA agonist muscimol, in contrast to our previous results using whisker stimulation where muscimol decreased cortical BOLD area and magnitude. However, thalamic BOLD area and magnitude decreased. The decrease observed in the baseline of neuronal activity after injection suggests that thalamic BOLD depends on both stimulus-specific thalamocortical input and baseline activity of cortical neurons.

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2. Miller, M. J., L. Li, C. Weiss, J. F. Disterhoft, and A. M. Wyrwicz. 2005. A fiber optic-based system for behavioral eyeblink measurement in a MRI environment. J Neurosci Methods 141:83-87.
Supported by: R01 NS44617, S10 RR15685, S10 RR019920
We thank Dr. Karl Deisseroth from Stanford University for providing the viral construct for our study.