fMRI and electrophysiology of optogenetic vs. whisker stimulation in the whisker barrel cortex of the awake rabbit

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Introduction. The recently-developed technique of optogenetic control allows precise manipulation of the excitation or inhibition of specific neuronal cell types through the introduction of light-sensitive ion channels (i.e., opsins) by a viral vector. Functional magnetic resonance imaging (fMRI) studies [1] have previously demonstrated the ability to generate robust blood oxygenation level dependent (BOLD) activation by direct optical stimulation of neurons in the cortex and other brain regions. However, the spatial and temporal properties of optogenetic functional activation have not been well characterized as compared to the BOLD signal generated through conventional sensory stimulation. The goal of this study is to compare the BOLD and electrophysiological activity produced by optogenetic stimulation vs. sensory whisker stimulation.

Methods. Dutch-Belted rabbits were chronically implanted with manipulators containing one bundle of 4 microwire gold-silver electrodes aimed at the whisker barrel cortex as well as injection cannula. All fMRI experiments were performed on a 9.4T Bruker BioSpec imaging spectrometer. Imaging data were acquired from the whisker barrel cortex and whisker thalamus from four consecutive slices using a single-shot gradient-echo EPI pulse sequence (TR=2s and TE=13ms). Whisker stimulation consisted of a vibration (75 or 10 Hz) delivered to the whiskers on the left side by means of a nylon band coupled to an oscillating magnetic coil and monitored in real time by an infrared sensor to ensure consistent amplitude and frequency of the vibration [2]. Rabbits were injected with the adeno-associated viral vector pAAV-CaMKIIα::hChR2(H134R)-EYFP. 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 10Hz. Simultaneous electrophysiological recordings and fMRI scans were acquired from each rabbit. 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. Neuronal activity, including single units and local field potentials, was recorded with non-ferrous wires attached to a chronically implanted microdrive. Data were analyzed after removal of blocks of gradient interference.

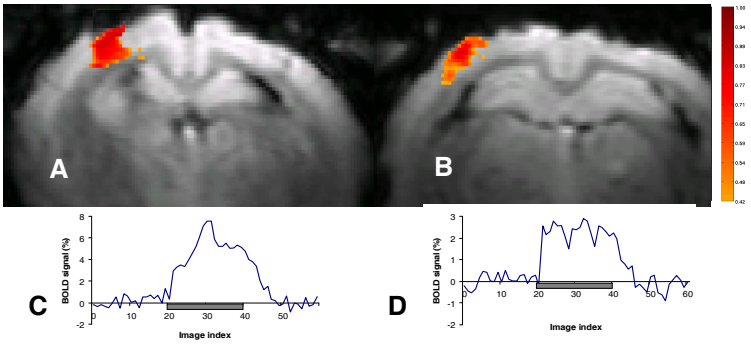

Fig. 1. Functional activation maps and temporal profiles of the whisker barrel cortex during direct 10 Hz optical stimulation (A, C) and 75 Hz whisker stimulation (B, D) The gray bar indicates the timing of the stimulus presentation; the color bar indicates the cross-correlation coefficient.

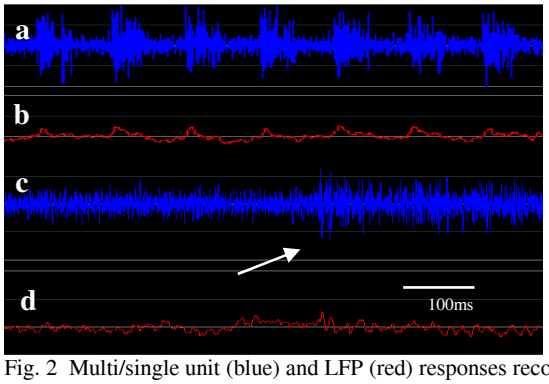

Fig. 2 Multi/single unit (blue) and LFP (red) responses recorded during 10 Hz optical (a,b) and whisker (c,d) stimulation. The arrow shows the onset of the whisker stimulation. Note the bursts of activity produced in the single/multi unit response during each optical pulse.

Results. As shown in Fig. 1, The BOLD response in the cortex produced by direct optical stimulation was similar in area, but noticeably larger in magnitude and duration of the time course, as compared to the response produced by whisker stimulation. Moreover, whereas the response during sensory whisker stimulation is relatively flat, the response during optical stimulation rises to a noticeable peak. Although optical stimulation produced robust responses at relatively low frequencies, higher whisker vibration frequencies were necessary to produce similar levels of BOLD activation. Electrophysiological activity during optical stimulation (Fig. 2) was characterized by noticeable "bursting" in the multi-unit activity at lower stimulation frequencies, in contrast to whisker stimulation at 10 Hz.

Discussion. These results highlight the striking differences observed in cortical BOLD and electrophysiological responses produced by optogenetic stimulation vs whisker stimulation. While it is difficult to compare the BOLD areas considering the vastly different mechanisms of stimulation, the differences in the magnitude, shape, and duration of the time courses could reflect more fundamental differences in the evolution of each signal. Although a wide variety of mechanisms may contribute to shaping these signals, these differences could, in part, reflect the differences observed in the electrophysiology. For this particular channelrhodopsin-2, the relatively fast kinetics likely produce the “bursting” behavior at low frequencies. This unusual profile of neuronal activity, in addition to the more fundamental differences associated with local optical generation of the BOLD signal that circumvents the conventional pathway of thalamic input, could contribute to the differences that manifest in the BOLD signals. Although more work is needed to explore the mechanisms involved in each case, these results indicate the importance of accounting for potential differences in BOLD signal behavior when interpreting fMRI results obtained from optogenetic stimulation.

References.

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