

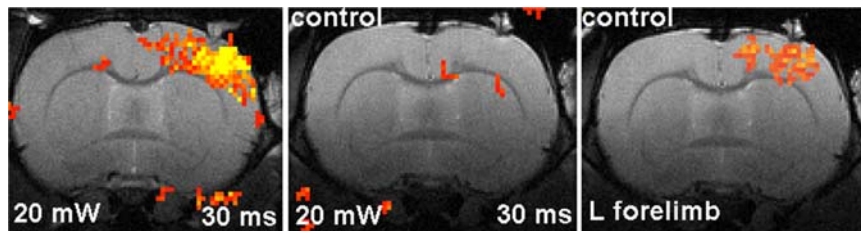
Optogenetic control of hemodynamic response in rat somatosensory cortex - fMRI and optical study

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Introduction Light-activated ion channels are becoming a common neuroscience tool for the exploration of cell-specific cortical circuits [1]. The initial report of optogenetic fMRI [2], surged the interest for hemodynamic-based optogenetic research. However, this approach is still relatively underutilized, possibly due to the complexity of the experimental set up, inhomogeneity artifacts from the optic fiber and concerns about the effects of heat dissipation [3]. In order to use optogenetic fMRI, it is necessary to characterize the hemodynamic response relative to optical stimulation parameters such as laser pulse power, duration and pulse frequency. For this, we performed fMRI and optical imaging studies employing channelrhodopsin (ChR) stimulation in the rat primary somatosensory forelimb cortex (S1FL).

Materials and methods We injected Sprague Dawley rats (5-7 wk old) into right S1FL area with 2.5 μ l of adenoassociated virus expressing ChR under excitatory cell-specific promoter (AAV5-CaMKII.ChR2(H134R)-eYFP/mCherry). For the control group, we used equivalent amount of virus expressing isogenic construct except for the ChR gene. After 3-4 weeks post-inoculation, the animals were anesthetized, skull and dura were removed and a well was built over S1FL. A multi-mode optic fiber (400 μ m core) was positioned to the site of the viral expression. Light stimulations were performed with 473nm laser (OEM) in a block design experiment (4 s stimulus, 40 s inter-stimulus interval, 10 repetitions). We varied the light power (1mW - 20mW), frequency (3 Hz - 20 Hz) and pulse duration (5 ms - 50 ms). We used forepaw stimulation (1.5 mA, 8 Hz, 1 ms duration) as a positive control. Functional responses were readout by 9.4-T MRI and intrinsic optical imaging (OIS). The MRI was obtained with a gradient-echo, echo planar imaging sequence, T_R / T_E = 500/12 ms, 5 slices, 250 μ m in-plane resolution, 1 mm slice thickness. OIS was measured at 570 ± 10 nm and 620 ± 10 nm for CBV and blood oxygenation response. OIS data were acquired at 30 fps using an analog CCD camera and A/D frame-grabbing board.

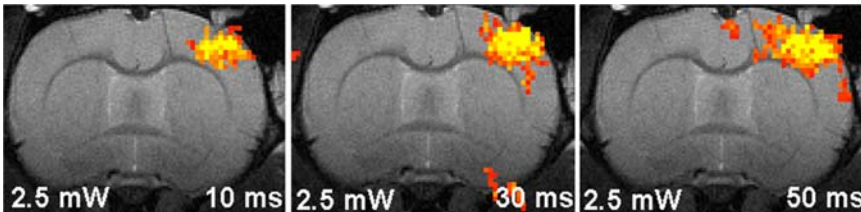


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Results and discussion In order to examine

whether the heating induced by laser stimulation causes fMRI signal change, high power light stimulation was used in ChR and control animals (see Fig. 1). Large BOLD fMRI response was detected in the ChR rat (left) and not in the control rat (middle) even though forepaw stimulation induced robust response (right, Fig 1). This indicates that in our setup, light stimulation does not create measurable heating-induced MRI signal change. The BOLD fMRI response in ChR expressing animals increased with the pulse duration (Fig. 2, and Fig 3 bar graph), and pulse power (not shown). The frequency relationship

Fig. 1. Light stimulus at high power evokes large activation in ChR expressing rats (left). Control rats show no activation under the same conditions (middle) and have robust FLforelimb response(right)



was non-linear with saturation of the activation at higher values (>12 Hz). We observed similar relationships in the CBV measurements at 570 nm (not shown). Fig. 3 shows time series of deoxyhemoglobin-based 620-nm OIS. At 1-3 s after stimulation onset, darkening stimulating hemisphere (top half of hemisphere) was followed by whitening. This biphasic response in 620-nm OIS is commonly observed, while the initial BOLD dip is not detected in fMRI. Generally, BOLD fMRI and OIS responses behaved similarly (see Fig. 3 right). These data in combination with outgoing investigation of neural activity, can provide useful information for determining neurovascular and

Fig. 2. BOLD fMRI response is linear with the length of the light pulse duration

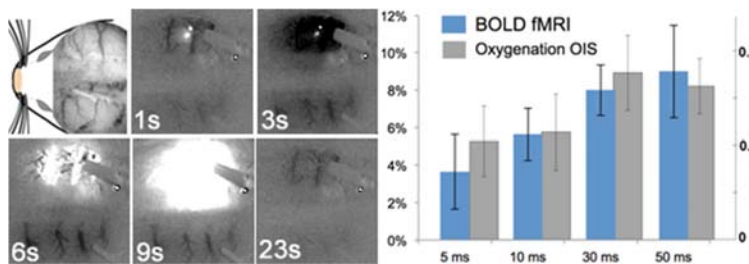


Fig. 3. The amplitude of the OIS correlates well with the size of BOLD fMRI activation.

neurometabolic coupling during light stimulation.

Conclusion Modulation of the light stimulus scales the hemodynamic response, allowing quantitative optogenetic fMRI studies of cell-specific neurovascular and neurometabolic relationships. Under the present paradigm, the heat dissipation is not a significant contributor of the BOLD activation.

References 1.Fenno et al. *Annu Rev Neurosci* 34, 389-412, 2011. 2. Lee et al, *Nature* 465, 788-792, 2010. 3.Christie et al. *Neuroimage* 66, 634-642, 2012.