The laminar specific neuronal responses to forepaw and optogenetics stimulations

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Introduction:

Studies have demonstrated that cortical plasticity takes place in a laminar specific manner [1, 2]. So far, laminar changes associated with plasticity were studied by invasive electrophysiology methods that provide detailed information however, only from a limited number of neurons [3]. Therefore, establishing a non-invasive *in vivo* strategy to evaluate the laminar specific neuronal activity in respect to larger neuronal networks as can be obtained with fMRI, is of major interest. Recent advances in optical-genetics (optogenetics) approaches enable immediate and reversible manipulations of neuronal firing rate by using light-modulated channels such as channelrhodopsin (ChR2) [4, 5]. These characteristics offer an appealing approach to study neuronal circuits in the normal and the pathological brain. The goal of this work was to determine the laminar specific neuronal responses in the primary somatosensory cortex (S1) of ChR2 engineered rats. Conventional forepaw stimulation and ChR2 activation were applied in ChR2 expressing rats. The laminar neuronal responses detected by BOLD fMRI and by local field potentials (LFP) within S1 were compared between the stimulation methods. Similar to forepaw stimulation, ChR2 stimulation showed a peak in BOLD fMRI and electrophysiology responses in lamina 4, demonstrating the capability of fMRI to resolve laminar communication as a result of optogenetics manipulations.

Material and Methods:

The channelrhodopsin plasmid (Lenti-CaMKIIa-ChR2-EYFP-WPRE) was injected into the right lateral ventricle of 3 day old rat pups. **Animal preparation:** Ten weeks old rats were initially anesthetized with isofluorane. A craniotomy was performed above the right S1. Anesthesia was then maintained by a continuous infusion of dexdomitor (0.1 mg/kg). Respiration rate, PO2 and heart rate were continuously monitored during the experiment. **Forepaw stimulation:** Two mA and 300 µs pulses were repeated at 3 Hz (for electrophysiology recording) and 9Hz (for fMRI). **Light activation of channelrhodopsin (ChR2):** The end of the optic fiber (400 µm in diameter) coupled to a 473nm wavelength laser was placed directly over the right exposed S1. Light pulses (20 Hz, 20 ms) were delivered above the right S1 for 20 s (fMRI) or 30 s (electrophysiology). **Functional MRI:** Images were acquired using a Bruker 9.4 T animal scanner. Rats were placed in a custom-built MRI holder equipped with a dedicated device for positioning the light source coupled MRI compatible optic fiber. A custom-built 1.1 cm diameter surface coil was used to transmit and receive MR signal. A gradient-echo EPI sequence with a 128 × 128 matrix, TE=21 ms, TR=1000 ms, BW=250 kHz, FOV=1.92 × 1.92 cm, and 3, 1-mm thick slices was used. Forty scans were collected during rest and 20 scans were collected during forepaw or ChR2 stimulations. FSL software was used for data analysis. Activation detection was performed using the general linear model (GLM) [6]. Z statistic results were cluster-size thresholded for effective significance of $p<0.05$. **Electrophysiology:** Tungsten electrodes were lowered into right S1. Local field potentials (LFP) were collected in 150 µm increments. Data analysis was performed using Spike2 software. **Immunostaining:** All rats were sacrificed and perfused at the end of the measurements. Free floating 50 µm thick brain sections were immunostained with antibodies targeted towards YFP and CaMKII to confirm ChR2 expression in excitatory neurons.

Results:

BOLD fMRI responses: Three regions of interest (ROI) corresponding to laminae 2+3, 4 and 5+6 were selected within the right S1 (Fig. 1a) according to the Paxinos rat atlas [7]. The normalized average BOLD amplitude of the activated pixels was calculated for each ROI. Consistent with previous studies [8], forepaw stimulation resulted in larger amplitudes of BOLD responses in laminae 2+3 (10.75%) and 4 (10.22%) compared to laminae 5+6 (5.92%) in the contralateral S1. ChR2 activation resulted in BOLD responses within S1 as well. However, ChR2 stimulation resulted in a larger amplitude of BOLD responses only in lamina 4 (3.74%) compared to laminae 2+3 (1.51%) and 5+6 (1.69%). **LFP responses:** The LFP was recorded at 150 µm increments throughout the cortical depth. The LFP responses to forepaw or ChR2 stimulation were averaged at each location. Figure 2 demonstrates that forepaw stimulation resulted in responses in the contralateral S1 with a short (~10 ms) response that peaked at the depths of 550 - 850 µm, corresponding to lamina 4. ChR2 stimulation resulted in a longer (~20 ms) response that matched the length of the light pulses. The peak response observed following ChR2 stimulation was strongest at the depths of 400 – 700 µm. Consistent with the BOLD fMRI responses, the LFP responses to ChR2 stimulation were weaker compared to forepaw stimulation.

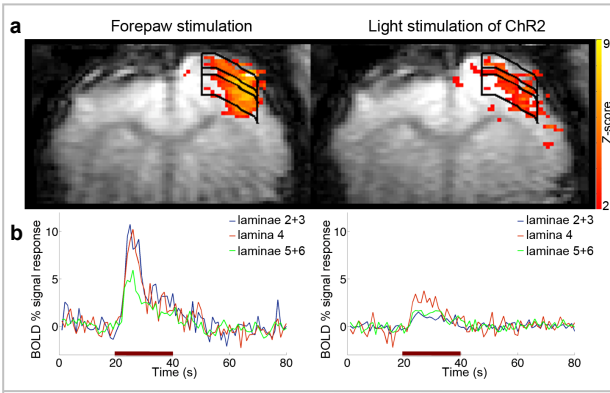

Figure 1 The BOLD fMRI responses to forepaw and ChR2 stimulations. **a,** activation z-maps overlaid on EPI images with the laminae boundaries shown in black. **b,** The time course of the BOLD responses show the differences in relative laminar activation between stimulation methods. Forepaw stimulation resulted in larger amplitudes of BOLD responses in laminae 2+3 and 4 compared to laminae 5+6 in the contralateral S1. ChR2 stimulation resulted in larger amplitude of BOLD responses only in lamina 4 compared to laminae 2+3 and 5+6.

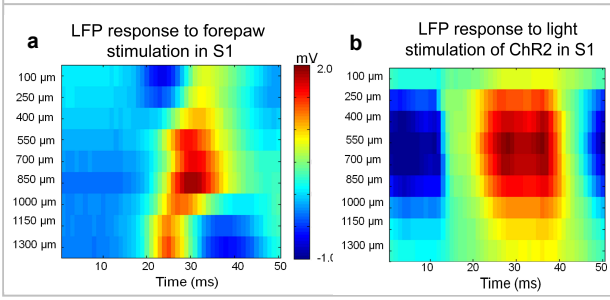

Figure 2 The averaged LFP waveforms time courses for forepaw and ChR2 stimulations across the cortical depth. Both stimulations resulted in principle responses in lamina 4. However, LFP responses to forepaw stimulation were 3 times stronger compared to ChR2 stimulation.

Discussions:

Our findings demonstrate that both fMRI and electrophysiology methods are able to resolve laminar differences in neuronal responses as a result of forepaw and ChR2 stimulations. Forepaw stimulation resulted in increases in BOLD fMRI responses in laminae 2+3 and 4, and increases in LFP responses mainly in lamina 4. ChR2 stimulation resulted in increases in BOLD fMRI and LFP responses specifically in lamina 4. However, both the BOLD fMRI and LFP responses to ChR2 stimulation were smaller compared to conventional forepaw stimulation. These results suggest that fMRI has the capability to determine laminar communication resulting from optogenetics manipulations. This combination of optogenetics with fMRI offers a unique insight into studying changes in laminar communication associated with cortical plasticity.

References:

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