Light-induced activation of light-sensitive pumps modulates fMRI responses

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Introduction: Recent developments in optical-genetic (optogenetics) approaches enable immediate manipulations of neuronal firing rate by using light-induced activation or silencing of light sensitive channels or pumps (Zhang et al., *Nature*, 2007). These techniques offer precise and transient control of neuronal firing rate making them appealing to study neuronal circuits in the normal and the pathological brain. Here we demonstrate for the first time, the feasibility of fMRI to detect modulations in neuronal firing rate induced by optogenetics techniques. We have engineered the excitatory neurons in rat somatosensory cortex to express halorhodopsin (light-sensitive chloride pump) using direct neuronal infection with lentivirus. Thus, in the presence of light, the chloride pumps open and trigger neuronal hyperpolarization i.e. decreases in neuronal firing rate. Consistent with electrophysiology results, light induced activation of halorhodopsin during forepaw stimulation, decreased the amplitude and the extent of fMRI responses. These results introduce an exciting and novel approach to study neuronal behavior *in vivo*.

Materials and methods: The halorhodopsin plasmid (Lenti-CaMKIIa-eNpHR-EYFP-WPRE) was kindly provided by Dr. Karl Deisseroth. The virus was packed into 293 cells. Direct streotaxic lentivirus injections into the right somatosensory cortex were preformed in adult Sprague-Dawley rats (for electrophysiology) or into the right lateral ventricle of 3 days old rat pups (for fMRI). **Electrophysiology:** Rats (n=4) were anesthetized with urethane and placed in a stereotaxic frame. A craniotomy was performed above the right somatosensory cortex. Tungsten electrodes were lowered into the right somatosensory cortex in a 50 µm increments. Local field potentials (LFP), reflecting the average neuronal activity, were sampled at 1000 Hz and amplified and band pass filtered at 0.1-100 Hz. Two short stimulation electrodes were inserted in the left forepaw and stimulation consisted of 2 mA, 300 µs pulses that were repeated at 3 Hz. **Functional MRI:** Rats (n=3) were initially anesthetized with Isoflurane. A craniotomy was performed above the right somatosensory cortex. Rats were then placed in a home-built MRI holder equipped with a dedicated holder for the light source coupled MRI-compatible optic fiber. A bolus (0.05 mg/kg, S.C.) of dexodormitor (medetomidine) was given and isoflurane was discontinued. During imaging measurement anesthesia was maintained by a continuous infusion of dexodormitore (0.1 mg/kg). Two short stimulation electrodes were inserted in the right and the left forepaws and stimulation consisted of 3 mA, 300 µs pulses that were repeated at 9 Hz. Respiration rate, PO_2 and heart rate were continuously monitored during experiment. **Image acquisition**: All images were acquired using a Bruker 9.4 T animal dedicated scanner. A home-built 1.1 cm diameter surface coil for 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, 120 repetition, and 3, 1-mm thick slices was used. **Data analysis:** FSL software was used for all data analysis. Activation detection was performed using the general linear model (GLM). Z statistic results were cluster-size thresholded for effective significance of $p<0.05$. **Light activation of halorhodopsin:** The end of the optic fiber (400 µm in diameter) was placed directly over the exposed somatosensory cortex for both electrophysiology and fMRI measurements. Light was turned on during forepaw stimulation. **Immunostaining:** Free-floating 30 µm thick brain sections were immunostained with antibodies targeted towards YFP and CaMKII to confirm halorhodopsin expression in excitatory neurons.

Results: **Electrophysiology:** Recording electrodes were lowered into the right somatosensory cortex under the region illuminated by the optic fiber. Marked changes in LFP activity throughout the cortical depth were observed when the contralateral forepaw was stimulated. Figure 1 demonstrates that light induced activation of halorhodopsin caused decreases in the amplitude of LFP responses to contralateral forepaw stimulation. These decreases in LFP responses during light induced halorhodopsin activation were detected as low as 450 µm under the cortical surface.

fMRI: The lentivirus injections were performed in 3 days old pups, thus when the rats reached adulthood the vast majority of excitatory neurons in the somatosensory cortex expressed the halorhodopsin as was confirmed with immunostaining (Figure 2). Figure 3 is a representative data set that demonstrates the result of GLM analysis in the somatosensory cortex contralateral to forepaw stimulation. Forty percent decreases in BOLD responses and 49% decrease in cluster size of significantly activated voxels were observed during light induced activation of halorhodopsin.

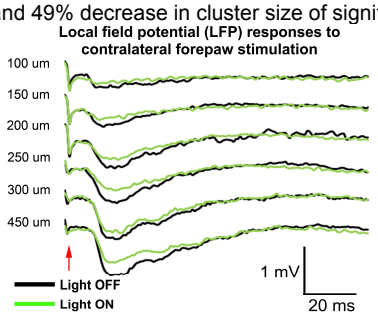

Figure 1 demonstrates attenuation in the amplitude of LFP responses to contralateral forepaw stimulation in different cortical depths of the rat somatosensory cortex under light induced activation of halorhodopsin. Red arrow represent stimulus onset.

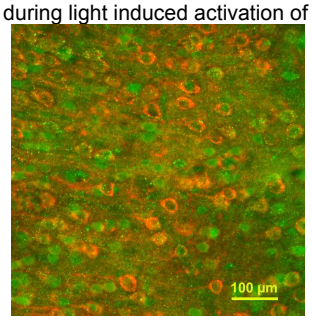

Figure 2 shows that lentivirus injections into the pup's lateral ventricle resulted in halorhodopsin expression (anti-YFP, green) throughout the excitatory neurons (anti-CaMKII, red) located in the rat somatosensory cortex.

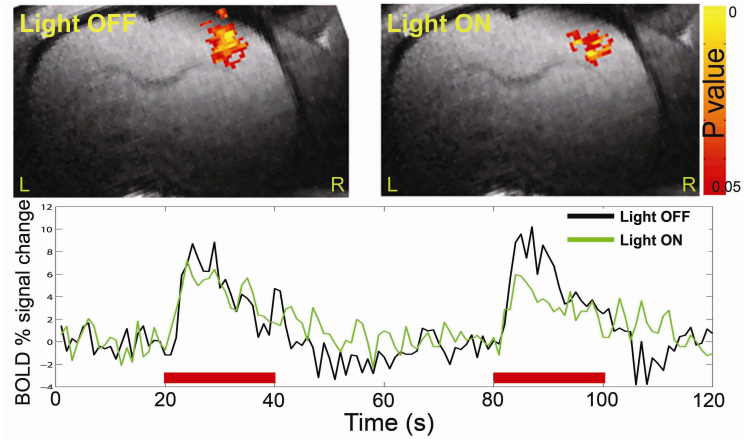

Figure 3 shows the extents and the amplitude of fMRI responses to contralateral forepaw stimulation. The optic fiber was placed directly above the right somatosensory cortex. Light induced activation of halorhodopsin resulted in decreases in both the extent and the amplitude of fMRI responses during forepaw stimulation mainly in the upper cortical laminae. Z statistic activation maps are overlaid on RARE anatomical images. Red bars represent forepaw stimulation.

Discussion: Optogenetics approaches were recently applied to investigate modulations in neuronal behavior in brain slices and *in vivo* and are expected to facilitate developments of new therapeutic strategies. So far, only invasive electrophysiology and optical imaging techniques were used to monitor changes in neuronal activity during optogenetics manipulations. Although these techniques provide superior temporal and spatial resolution, the behavior of only a small population of neurons can be studied at any given time. Here we demonstrate that non-invasive fMRI technique is sensitive enough to detect changes in neuronal activity induced by optogenetics manipulations. This presents a new approach to study the effect of optogenetics manipulations on the behavior of large neuronal networks *in vivo*.