Combining bimodal optogenetic control with BOLD fMRI for causal analysis of the cortico-thalamic circuitry

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Target audience

Researchers and clinicians with an interest in neuroimaging, BOLD fMRI, brain connectivity or optogenetics

Purpose

Imbalances of neuronal network activity may be causative for neuronal disorders such as e.g. epilepsy. We utilize optogenetic tools for a temporally and spatially defined modulation of components of the cortico-thalamic and the thalamo-cortical neuronal network in naïve rats and use BOLD fMRI as a global, noninvasive readout. Stimulating neurons by activation of Channelrhodopsin (ChR2) by light in combination with fMRI has recently been demonstrated by Lee et al[1]. We extend this approach and inhibit neuronal network components by activating the hyperpolarizing proton pump ArchT in the sensory cortex in a combined fMRI experiment.

Methods

Expression of opsins in neurons was achieved by stereotactic injections of adeno-associated viruses (rAAV-2). We performed three different experiments: A: ChR2 was injected into the forelimb region of the sensory cortex (S1FL, n=11); B: ChR2 was injected to the posterior thalamic nuclei group (PO, n=3); C: ArchT was injected into S1FL (n=7). Functional imaging was performed at least 14 days after virus injection. For optic stimulation a multimode optical fiber with a diameter of 200 µm was led through a custom-built radiofrequency coil and implanted dorsal to the virus-injected area. Depending on the opsin, either blue pulsed light at 488 nm (ChR2) or green continuous light at 552 nm (ArchT) was transmitted for stimulation in a block design consisting of 10 s of stimulation followed by 20 s of rest. Experiments were carried out under medetomidine sedation in a 9.4 T small animal scanner. T_2^*-weighted images were acquired with a single-shot EPI sequence (TR 1 s, TE 18 ms. spatial resolution 350 x 325 µm², slice thickness 1.2 mm, total acquisition time 10 min). BOLD response to optic activation of ChR2 in S1FL (A) or PO (B) and optic activation of ArchT in S1FL (C) was compared to BOLD response to sensory electric forepaw stimulation (9 Hz, 1 mA), a well-established model to study rodent brain function.

Results

A: Blue light pulses of 10 ms duration at 9 Hz (light intensity 80 mW/mm^2) resulted in a positive BOLD response in ChR2-transduced S1FL spanning over up to 4 mm in anterior-posterior direction. Optogenetically induced activation areas overlapped with sensory evoked activation areas. Cluster sizes did not differ significantly (paired t-test, p= 0.607). B: Optic stimulation of ChR2-transduced PO resulted in a positive BOLD response in the primary and secondary sensory cortex. Notably, no activation was detected in the thalamus. C: Optical stimulation with continuous green light resulted in a negative BOLD response in ArchT-transduced S1FL. When combining optic and forepaw stimulation, activation clusters represented a negative Bold response directly at the fiber tip and a positive Bold response in the adjacent sensory cortex (Fig. 1). MR intensity disturbances at the site of fiber implantation were occasionally seen in cortex and regularly seen in the thalamus in anatomical T2 weighted and corresponding EPI MR images. However, BOLD activation maps were not affected by these image artifacts. Confocal imaging of histological slices confirmed strong, yet sparse expression of ChR2-YFP, ChR2-mCherry, and ArchT-GFP at virus injection sites. At cellular resolution, membrane-bound expression with no cytotoxicity was observed.

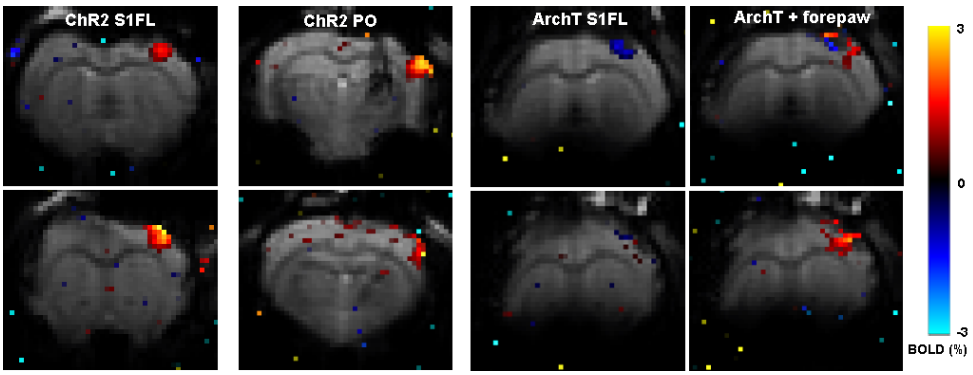

Fig. 1: Two consecutive slices showing BOLD activation in S1FL for each optic stimulation: ChR2 activation in S1FL and PO and inhibition using ArchT without and with forepaw stimulation.

Discussion

We successfully established stimulation of ChR2 both in the cortex and in the thalamus as well as inhibition using ArchT alongside fMRI acquisition, resulting in positive or negative BOLD responses, respectively. We show that by activating ChR2 in PO, we trigger cortical recruitment, represented by a BOLD response in the somatosensory cortex.

Conclusion

Our data suggest that information flow from the thalamus to the cortex upon forepaw stimulation can be manipulated by optogenetic tools. Simultaneous BOLD fMRI can directly assess changes in thalamo-cortical network activity. Consequently, this methodology can be applied for comparative analysis of thalamo-cortical network activity in models of circuit disorders.

References

[1]Lee JH, Durand R, Gradinaru V, Zhang F, Goshen I, Kim DS, Fenno LE, Ramakrishnan C, Deisseroth K: Global and local fMRI signals driven by neurons defined optogenetically by type and wiring. Nature 2010;465(7299):788-92