Combined optogenetic fMRI and optical Ca2+-recordings for functional mapping of thalamo-cortical circuits in rat

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Target audience

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

Purpose

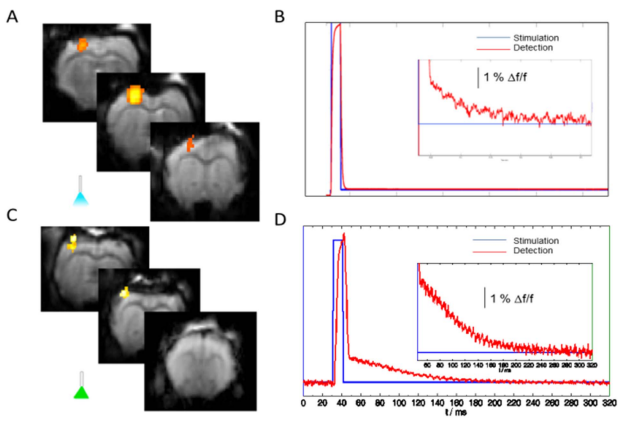
Optogenetics has become a frequently used tool for probing of neuronal networks[1]. To what extent direct optic stimulation resembles endogenous activity of e.g. the sensory network in terms of the spatial and temporal patterns of network recruitment is unclear. Stimulating neurons by activation of Channelrhodopsin (ChR2) with light in combination with fMRI (ofMRI) has recently been demonstrated[2]. We extended this approach and combined it with a neuron-specific readout of suprathreshold spiking, fluorescence detection of a Ca^{2+}-sensitive dye, Oregon Green 488 BAPTA-1 (OGB-1). In this multimodal setup 488 nm laser light at low light intensities is used for constant excitation of OGB-1 and at high light intensities for pulsed excitation of ChR2. To circumvent detector saturation during optogenetic stimulation of ChR2, we selected a second opsin, (C1V1), with an excitation wavelength different from OGB-1. We compared the resulting BOLD responses and Ca recordings with the response to sensory electric forepaw stimulation.

Methods

Opsin expression was achieved by stereotactic injection of adeno-associated viral constructs (rAAV-2) for CHR2 or C1V1 into the forelimb region of the sensory cortex (S1FL, n = 11) or into the thalamus (POm or VPM, n = 6) of female Fisher rats. 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, respectively. Depending on the opsin, either blue pulsed light at 488 nm (ChR2, light intensity 80 mW / mm^2) or green pulsed light at 552 nm (C1V1, light intensity 90 - 150 mW / mm^2) was transmitted for stimulation in a block design consisting of 10 s of stimulation followed by 20 s of rest. Animals with cortical virus injection received stereotactic OGB-1 injection into S1FL. Animals with thalamic viral injection were subjected to functional MRI upon optogenetic stimulation and OGB-1 was subsequently injected into the cortical projection target identified by the BOLD cluster. Both cohorts were additionally subjected to forepaw stimulation with the same paradigm (9 Hz, 1 mA). 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 GE-EPI sequence (TR 1 s, TE 18 ms, spatial resolution 350 x 325 µm², slice thickness 1.2 mm, 9 contiguous slices, total acquisition time 10 min). SPM 8 was used for analysis of fMRI data. BOLD time courses and Ca traces were analyzed with Matlab.

Results

Direct optogenetic stimulation of animals cortically transduced with ChR2 and C1V1 in S1FL led to a similar BOLD response below the fiber tip (representative BOLD maps **A**, **C**). Time course and amplitude of the hemodynamic response as well as cluster size of the activation was comparable to the BOLD response upon forepaw stimulation. Ca^{2+} recordings upon optogenetic stimulation of ChR2 did not allow resolving signal intensity modulations during the first 25 ms after onset of the stimulation pulse (10 ms) because of detector saturation due to the high light intensity of the stimulation pulses (**B**). However, the detector recovery could be fitted with a monoexponential function with a time constant of 0.9954 ± 0.0038 ms (R^2 = 0,9998). When subtracting this decay curve from the raw data, the dead time of the detector was reduced to 16 ms after onset of the stimulation pulse. A monotonic Ca^{2+} signal decay, similar to the response to forepaw stimulation was observed, indicating a primary neural response as origin of the detected signal. No detector saturation occurred when stimulating C1V1 at 552 nm (**D**). Although a residual signal contribution (of much lower amplitude) remained, the Ca response was detectable earlier. Optogenetic stimulation of CHR2 and of C1V1 in thalamus led to a BOLD response in primary and/or secondary sensory cortex. Average BOLD time courses for both opsins followed the expected hemodynamic response function. Onset, peak time and decay characteristics appeared similar to the responses to optogenetic cortical stimulation and also to forepaw stimulation. No activation was detected at the fiber tip in the thalamus. Cortical Ca^{2+} recordings showed strong but delayed neural responses, though similar in amplitude to sensory stimulation.



Discussion

We successfully established stimulation of ChR2 and C1V1 both in cortex and in thalamus. Using different wavelengths for optogenetic stimulation and OGB-1 excitation, we could avoid detector saturation and thereby better exploit the high temporal resolution of our optical readout. We show that by activating opsins in thalamus, we trigger cortical recruitment at the respective projection targets, reflected by BOLD and Ca^{2+} responses in the somatosensory cortex.

Conclusion

With simultaneous global BOLD fMRI and local Ca^{2+} recordings, changes in thalamo-cortical network activity can directly be assessed. The similarities of optogenetic and sensory-driven, endogenous network activation confirm that ofMRI can serve as a model for functional mapping of brain circuits. The methodology can be applied for comparative analysis of thalamo-cortical network activity in models of circuit disorders.

References

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