Bridging the gap: perspectives of combining optogenetics, calcium imaging and fMRI

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Objectives

Advancing our understanding of neuronal network dynamics in neurodegenerative diseases requires the investigation and simultaneous imaging of neuronal populations and their interactions in the intact CNS. The discovery of a rapidly gated light-sensitive cation channel ChR2 suitable for noninvasive control of neuronal activity has made it possible to optically control membrane depolarization on the millisecond timescale in genetically defined neurons, using a non-metallic optical fiber for stimulation. Furthermore, the same optical fiber can be used for recordings of fluorescent calcium indicators, thereby bridging the gap between fMRI and optical imaging. Here, we proved the feasibility of rat fMRI at highest field strength in combination with optical fiber based calcium imaging in vivo, directly mirroring neuronal activity. Also, transduction of mouse adult stem cells with ChR2-YFP and subsequent neuronal differentiation should demonstrate the feasbility of an all-optical physiology, combined with fMRI.

Methods

Rats were anesthetized with an intraperitoneal injection of medetomidine, followed by a continuous i.v. infusion into the tail vein (0.01 mg / h). For Calcium recordings, 2 µl of Calcium sensitive dye Oregon green 488 BAPTA-1 AM (Molecular Probes) were stereotactically injected 500 µm into the left somatosensory cortex upon craniotomy (from bregma: AP: 0 mm, ML: +/-1 mm, DV: 0.3 mm). Subsequently, an optical fiber was implanted in the exact location and fixed at the skull. A custom made recording setup was used to excite the calcium dye, the optogenetic construct ChR2 and record fluorescence emission. For electric stimulation, two needle electrodes were inserted into the left forepaw and connected to a stimulator (Digitimer DS4, Hertfordshire, England). A classical block design was established (15 s stimulation at 3 mA, 3 Hz, 300 µs pulse duration, 45 s baseline). Rat fMRI experiments were performed on a 17.6 T Bruker Avance 750 WB scanner with a rat head coil with an inner diameter of 38mm. The spatial resolution of all functional images was 200 x 312 µm. Functional MRI DICOM images were analyzed using statistical parametric mapping (SPM, Wellcome Department for Neuroimaging, London). Data analysis was performed using the general linear model (GLM).

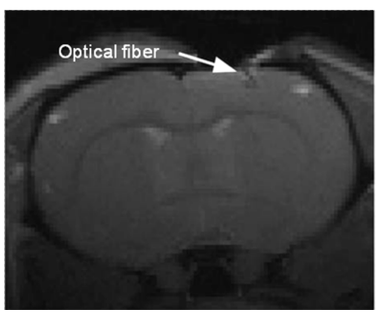

Figure 1

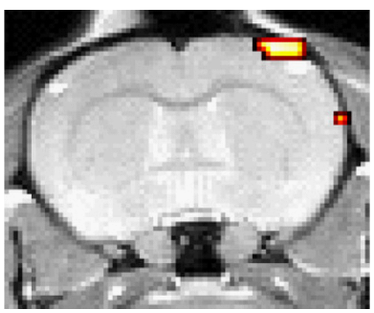

Figure 2

Results

Figure 1: Coronal section of the rat brain upon implantation of optical fiber (arrow). The optical fiber can be delineated as hypointense structure in the T2-weighted image. No significant susceptibility artifacts were observed. (RARE, TR = 3000 ms, TE = 7.4ms, effective TE = 14.8 ms, slice thickness 1.0 mm, spatial resolution 203 x 195 µm)

Figure 2: Overlay of high resolution T2-weighted coronal MRI of the rat brain with the activation map upon electric forepaw stimulation, with a temporal resolution of 300 ms. Significant activation patterns both in the primary (S1) and in the secondary (S2) somatosensory cortex can be observed on the level of pFDR < 0.05. (FLASH, TR = 5.5 ms, TE = 3 ms).

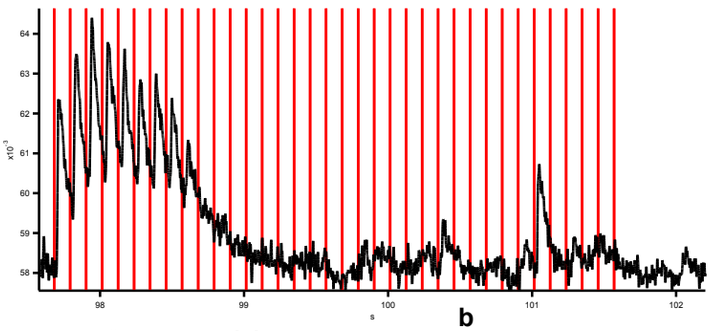

Figure 3: Trace of fluorescence intensity recorded by optical fiber implanted in somatosensory cortex of ipsilateral hemisphere while recording fMRI. Calcium spikes directly related to electric forepaw stimulation at 9 Hz, 1 ms duration, 2 mA (red bars), 4s stimulation. Note, that adaptation of neuronal response to forepax stimulation becomes apparent after the first ~10 pulses.

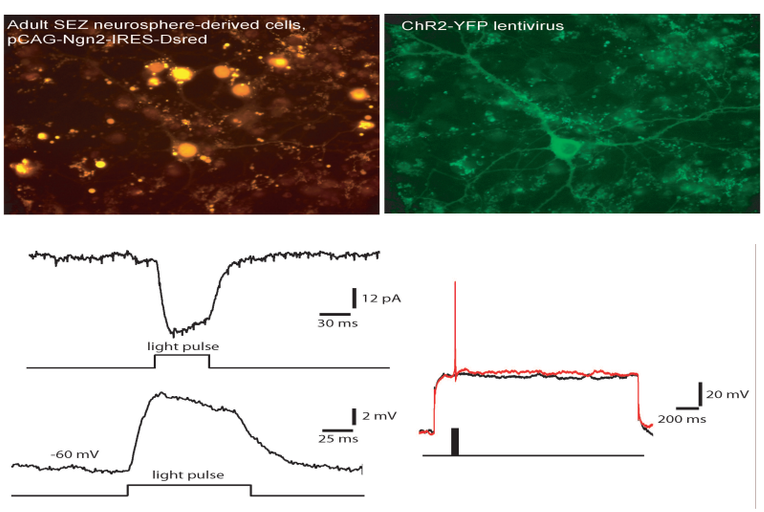

Figure 4: Optogenetic control of adult SEZ-neurosphere derived stem cells. Co-transduction with pCAG-Ngn2-IRES-Dsred and pEF1a-ChR2-EYFP, and withdrawal of EGF and FGF for 10 days leads to neurogenin2 and ChR2 expression indicating neuronal differentiation. Following illumination with blue light (488nm) using the optical fiber system, the membrane protein ChR2 leads to a high-speed depolarization, fast enough to drive precisely timed light-evoked action potentials.

Conclusions

Brain structures known to be activated upon electric stimulation could be identified at 17.6 T. Optical-fiber-based calcium recordings revealed synchronous neuronal activity upon electric forepaw stimulation in somatosensory cortex. Also, using an identical setup, optogenetic stimulation of stem cell derived neurons becomes feasible. Altogether our study indicates that a multimodal approach combining a global method like fMRI with a spatially confined, highly specific methods such as optical calcium recordings and optogenetics becomes amenable. This will allow for the causal assessment of neurovascular coupling and furthering our understanding in the spatio-temporal dynamics of neuronal network activity, e.g. optically controlling anf monitoring the functional integration of stem cells in vivo.

Acknowledgments

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