Westfälische Wilhelms-Universität Münster

fMRI AT 17.6 T AND OPTICAL FIBER-BASED CALCIUM-IMAGING IN RODENTS

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Objectives

In this study we tested the feasibility of rat fMRI at highest field strength in combination with optical fiber based Calcium imaging in vivo, directly mirroring neuronal activity. An optical fiber exhibits significant advantages over conventional metal electrodes, being extremely flexible and small in diameter (200 µm) and most importantly allowing for simultaneous fMRI. However, it has to be evaluated, whether the fiber results in image distortions such as susceptibility artefacts or perturbs brain physiology. In addition, to allow for the temporal co-registration of optical-fiber-based imaging and fMRI, we increased the temporal resolution of fMRI by applying k-space segmentation.

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 imaging, 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 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). Two stimulation paradigms were evaluated, a classical block design (15 s stimulation at 3 mA, 3 Hz, 300 µs pulse duration, 45 s baseline) and a single-run design for fast fMRI acquisition (1 s baseline, 4 s electric forepaw stimulation at 2 mA, 9 Hz and 1 ms pulse duration, 15 s baseline, each run was repeated k-times, k representing the matrix size (64)). 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).

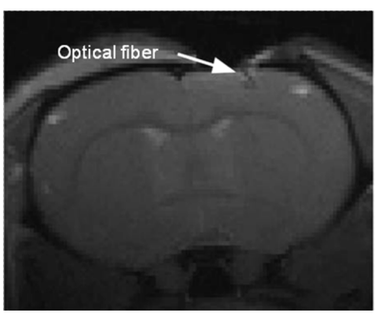


Figure 1

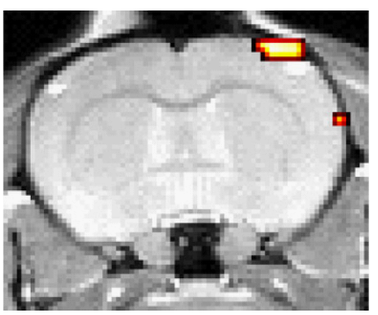


Figure 2

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).

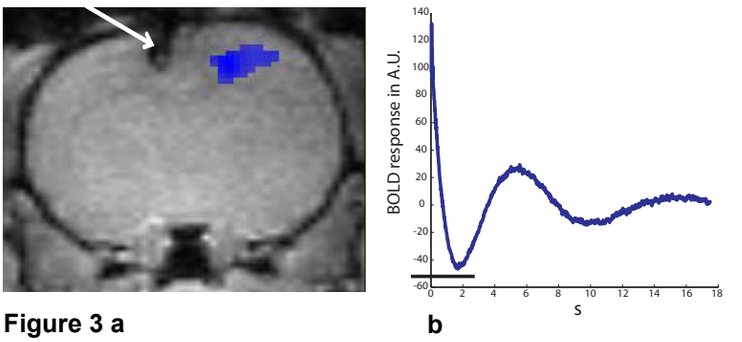
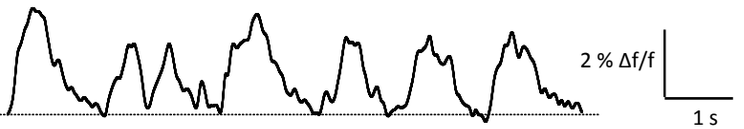


Figure 3 a

b



c

Figure 3 a: Increasing temporal resolution to 20 ms and applying a single block stimulation design resulted in activation patterns within the contralateral primary somatosensory cortex (S1). Blue voxels represent results on the level of $p < 0.05$. During each run of the stimulation paradigm one phase-encoding step was repeatedly acquired with TR = 20 ms and TE = 2.5ms. Optical fiber directly above stained area at ipsilateral hemisphere is visible due to hemorrhage (arrow).

b: Time course of BOLD response averaged over significant voxels. Electrical forepaw stimulation is depicted as black bar. Initial 1.5 s of time course had to be ommited due to incomplete relaxation, still visible as initial BOLD artefact.

c: Excerpt of fluorescence trace simultaneously recorded by optical fiber implanted in somatosensory cortex of ipsilateral hemisphere of same rat, (see figure 3 a, arrow) upon staining with Calcium indicator.

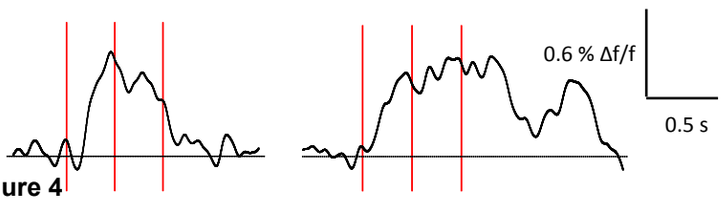


Figure 4

Figure 4: traces of fluorescence intensity recorded by optical fiber implanted in somatosensory cortex of ipsilateral hemisphere in different animal under deeper anesthesia. Population spikes directly related to electric forepaw stimulation at 3 Hz, 1 ms duration, 2 mA (red bar). Latency between first electric pulse and onset of population activity (50% of peak) ranges at 300 ms.

Conclusions

Brain structures known to be activated upon electric stimulation could be identified at 17.6 T. Temporal resolution was increased to 20 ms by applying k-space segmentation. Optical-fiber-based Calcium imaging revealed synchronous neuronal activity upon electric forepaw stimulation in somatosensory cortex. Altogether our study indicates that a multimodal approach combining a global method like fMRI with a spatially confined, highly specific method as optical Calcium imaging 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.

Acknowledgments

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