# fMRI at 17.6 T and optical fiber-based Ca2+-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 an implanted optical fiber. We aim for the combination of fiber based optical $Ca^{2+}$ imaging with functional magnetic resonance imaging (fMRI) at 17.6 T in vivo. The 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 and $Ca^{2+}$ imaging. However, it has to be evaluated, whether the fiber results in image distortions such as susceptibility artefacts. Furthermore, we had to rule out that the implanted fiber perturbs brain physiology and thereby BOLD response, e.g. by inducing spreading depression. In addition, to allow for the temporal co-registration of optical-fiber-based imaging and fMRI, the temporal resolution of conventional fMRI sequences ranging at 0.3 to 1 s does not suffice. Therefore we increased the temporal resolution of fMRI by applying k-space segmentation with separate acquisition of individual k-space lines, resulting in a temporal resolution of less than 20 ms. Additionally, we conducted fiber-based $Ca^{2+}$ imaging in mice upon electric forepaw stimulation, to demonstrate the sensitivity and specificity of optical $Ca^{2+}$ imaging in detecting neuronal population activity. ***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). An optical fiber with a diameter of 200 µm was implanted into the somatosensory cortex of the right hemisphere (from bregma: AP: 0 mm, ML: 1 mm, DV: 0.3 mm). 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, first a classical block design for conventional fMRI sequences. One run comprised of 15 s stimulation at 3 mA, 3 Hz and 300 µs pulse duration, followed by 45 s baseline. Each run was repeated three times. For fast fMRI acquisition, one run comprised of 2 s baseline, 4 s electric forepaw stimulation at 2 mA, 3 Hz and 1 ms pulse duration, followed by 19 s baseline. Each run was repeated k-times, k representing the matrix size (64). The 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. First, T2 weighted anatomical reference RARE scans were conducted (TR = 3000 ms, TE 7.4ms, effective TE = 14.8 ms, slice thickness 1.0 mm, spatial resolution 203x195 µm). Second, conventional functional MR images were obtained using a FLASH sequence with a time resolution of 352 ms, TR = 5.5 ms, TE = 3 ms. During each run of the stimulation paradigm for fast fMRI one phase-encoding step was repeatedly acquired with TR = 20 ms and TE = 2.5ms. As a result a set of 1250 images with a temporal resolution of 20 ms were acquired within a total acquisition time of 27 minutes. The spatial resolution of all functional images was 200 x 312 µm. Functional MRI DICOM images were converted into NIFTI image format to allow appropriate mass-univariate statistical analysis using statistical parametric mapping (SPM, Wellcome Department for Neuroimaging, London) for fMRI data. Data were motion-corrected and spatially smoothed with an 1x1x1 mm Gaussian kernel using SPM5. Data analysis was performed using the general linear model (GLM) and modeling "stimulation" as a delta function convolved with a canonical hemodynamic response function (HRF) as implemented in SPM5. Specific effects were tested with a linear contrast of the parameter estimates for the HRF regressor resulting in a t-statistic for each voxel. The overall t-statistics constitutes a statistical parametric map (SPM). Data were analyzed for each session with this animal on a single-case level. For fiber-based $Ca^{2+}$ imaging, mice were anesthetized with 0.8-1.5% isoflurane in pure $O_2$. Upon craniotomy, a glass patch pipette was stereotactically inserted 500 µm into the right somatosensory cortex. 2 µl of calcium sensitive dye Oregon green 488 BAPTA-1 AM (Molecular Probes) were injected into brain parenchyma for multi cell bolus loading. After 30 min, an optical fiber was implanted in the exact location and fixed at the skull with dental cement. A custom made recording setup was used to excite the calcium dye and record changes in fluorescence, mirroring population neuronal activity. ***Results:*** Figure 1 shows an axial 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. Figure 2 shows an overlay of high resolution T2*-weighted coronal MRI of the rat brain in vivo with the activation map upon electric forepaw stimulation, with a temporal resolution of 300 ms. Three runs were analyzed simultaneously in the framework of the general linear model (GLM) as implemented in SMP 5 using an explicit brainmask. Significant results are reported on the level of $p_{FDR}$ <0.05. Brief pulses of 3 mA and 300 µs duration resulted in significant activation patterns both in the primary (S1) and in the secondary (S2) somatosensory cortex. Increasing temporal resolution to 20 ms and applying a single block stimulation design still resulted in activation patterns within the contralateral primary somatosensory cortex (S1)(Figure 3). Red voxels represent results on the level of p<0.01, blue voxels on the level of p<0.001. However, the area of significant voxels is smaller; activation patterns within secondary somatosensory cortex could not be detected. Figure 4 shows traces of fluorescence intensity recorded by optical fiber implanted in somatosensory cortex of adult mice upon staining with $Ca^{2+}$ indicator. Fluorescence intensity is directly proportional to intracellular $Ca^{2+}$-concentration, reflecting neuronal activity on population level. Fig 4 a show population spike upon electric forepaw stimulation, 1 ms duration, 1 mA (red bar). Fig b shows population spikes upon stimulation with 3 pulses at 3 Hz, Fig 4 c 10 pulses at 10 Hz. Latency between first electric pulse and onset of population activity ranges at 300 ms, not depending on number of pulses. Sampling frequency is 2 kHz.

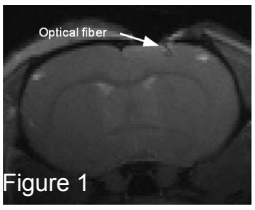

Figure 1

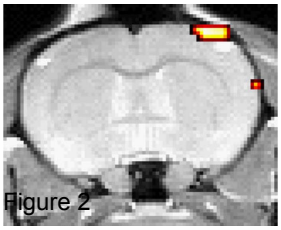
Figure 2

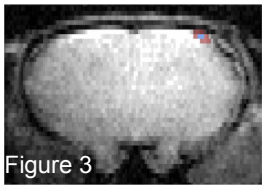
Figure 3

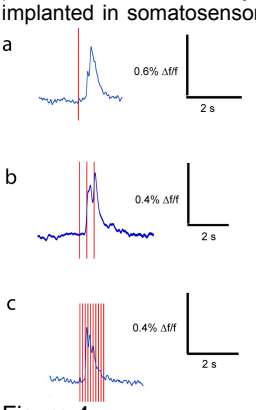

Figure 4

***Conclusions:*** Our data shows the feasibility of fMRI at highest field strength upon electric forepaw stimulation and implantation of optical fiber. 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 with separate acquisition of individual k-space lines. The sensitivity towards BOLD responses was only slightly reduced, even though a single block paradigm was applied. Optical-fiber-based $Ca^{2+}$ imaging in mice 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 $Ca^{2+}$ 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.