

Spatiotemporal Correlation of Optical Neuronal Calcium Recordings with BOLD fMRI in Rats

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Objectives: Furthering our understanding of the spatiotemporal dynamics of neurovascular coupling requires the simultaneous and unperturbed recording of neuronal spiking and BOLD fMRI. Here, we demonstrate the feasibility of optical recording of neuronal activity with sub-millisecond temporal precision within a 9.4 T small animal scanner and simultaneously recording BOLD fMRI. Using fluorescent indicators for intracellular Ca^{2+} concentrations, we achieve a direct optical readout of super-threshold neuronal spiking activity, representing three major advantages compared to conventional electric recordings: First, optical recordings using a flexible optical fiber are not distorted by the magnetic field of the scanner; Second, intracellular Ca^{2+} concentrations are only depending on neuronal spiking, supra-threshold synaptic potentials do not perturb the signal; Third, the cortical or sub-cortical area of integrating neuronal spiking activity is spatially well defined and can be precisely controlled by the stereotactic injection of Ca^{2+} indicators as well as the positioning of the optical fiber.

Methods: Rats were initially anesthetized with isoflurane for surgical procedures (craniotomy, dye injection, fiber implantation) followed by a continuous subcutaneous infusion of medetomidine (bolus 0.04 mg/kg, continuous infusion 0.05 mg/kg/h) for subsequent fMRI / Ca^{2+} measurements. For optic fiber-based Ca^{2+} recordings, rats were placed in a stereotactic frame and a craniotomy was conducted at the level of the somatosensory cortex of the right hemisphere (from bregma: AP: +1 mm, ML: 2.5 mm). 2 μl of Ca^{2+} sensitive dye Oregon green 488 BAPTA-1 AM (OGB-1) were injected at cortical depths of 300 and 700 μm . An optical fiber with a diameter of 200 μm was implanted at the exact location and fixed at the skull with UV glue (Polytec). A custom made optical laser setup was used to excite the Ca^{2+} dye and record changes in fluorescence at a sampling rate of 2 kHz. For electric stimulation, two needle electrodes were inserted into the left forepaw and connected to a stimulator (Digitimer DS5). Two stimulation paradigms were evaluated, comprising of either 4 s or 10 s stimulation at 1 mA, 9 Hz and 1 ms pulse duration, followed by 16 and 20 s baseline, respectively. Simultaneous functional MRI was performed at 60 to 90 min after dye injection, using a 9.4 T Biospec (Bruker) with an in-house built RF coil with lead-through for the fiber. Images were acquired with a single-shot EPI sequence with TR = 1 s and TE = 18 ms. The spatial resolution of functional images was 350 x 325 μm , slice thickness 1.2 mm. fMRI data analysis was performed with SPM8.

Results: Injection of Ca^{2+} indicator resulted in a columnar staining with a diameter of about 300 μm . Figure 1 displays a micrograph of a coronal brain slice at the level of the somatosensory cortex, overlay of OGB-1 fluorescence and transmitted light image. Morphological MRI acquisition upon implantation of optical fiber did not reveal any image artifacts caused by staining or fiber. 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 1 s. Significant results are reported at the level of $p_{\text{FDR}} < 0.05$ in the primary (S1) and secondary (S2) somatosensory cortex. Neuronal activity was recorded simultaneously, revealing short latency (12-15 ms) neuronal population spikes directly related to electric forepaw stimulation (Figure 3, red trace stimulation pulses, black trace fluorescence intensity). Note, that adaptation of neuronal response to forepaw stimulation becomes apparent after the first ~10 pulses. Figure 4 displays the corresponding 20 BOLD time courses for a 10 min fMRI experiment using the 10s stimulation paradigm.

Conclusions: Our study demonstrates the feasibility of combining fMRI, where the BOLD signal indirectly indicates brain activity of the respective imaging voxel with the spatially confined, highly specific method of optical Ca^{2+} recording mirroring neuronal spiking. This multimodal approach allows for the causal assessment of neurovascular coupling and opens the door for further studies on the spatio-temporal dynamics of neuronal network activity.

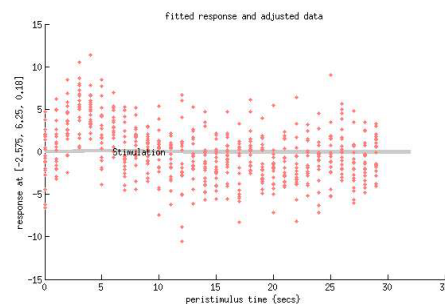
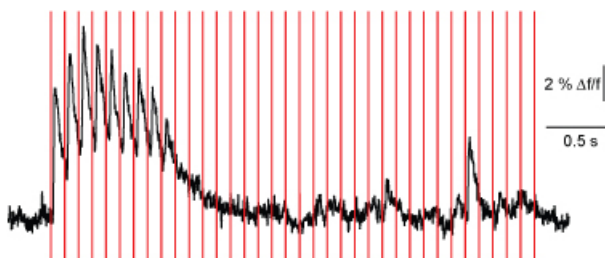
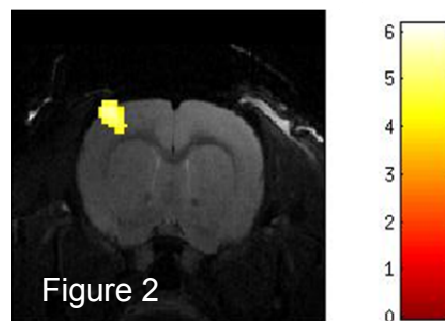
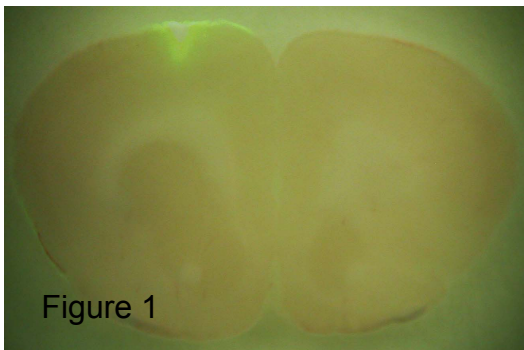


Figure 3

Figure 4