BOLD fMRI of Sensory Forepaw Stimulation in Mice Using a Cryogenic RF Probe Operating at 400 MHz

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INTRODUCTION Functional MRI (fMRI) of rodents has become a powerful tool to investigate the functional anatomy of the brain. The ability to monitor changes in brain activity with high spatiotemporal resolution made this non-invasive method a popular tool used in preclinical studies. Despite the extensive use of (genetically engineered) mice in biomedical research both for mechanistic studies and as disease models, most rodent fMRI studies have been performed in rats. This is largely due to the dimensions of the mouse: the small size is challenging for maintaining normal physiology, e.g. keeping blood gas levels stable, which is critical for eliciting robust functional responses. Another difficulty of mouse fMRI studies are the high demands on spatial resolution and thus on the signal-to-noise ratio (SNR). To overcome this limitation, we used a cryogenic RF transceiver probe, which leads to a significant increase in sensitivity compared to room-temperature coils of similar dimension [1,2]. The objective of this study was to develop a robust fMRI protocol at 9.4T based on blood oxygen level dependent (BOLD) contrast for sensory stimulation of the mouse forepaw. The electrical stimulation paradigm is widely used to investigate adaptations and plasticity of sensory function as well as pain sensation after nerve injuries.

METHODS Animals: Female C57Bl/6 mice of 3-4 months of age were used for all experiments. The entire experiment was performed under Isoflurane anesthesia (induction 2.5%, maintenance 1.1%). To keep the blood gas levels in physiological range and prevent any movement artifacts, animals were intubated, artificially ventilated and paralyzed using the neuromuscular blocking agent Pancuronium bromide (1-1.5 mg/kg). Animals were stereotactically fixated to ensure reproducible positioning. Physiologic parameters were monitored using a rectal temperature probe (36±0.5°C) and a transcutaneous electrode on the upper hind limb measuring levels of blood gas (pCO_2, pO_2). All experiments were performed in accordance with the Swiss law of animal protection.

fMRI: Experiments were carried out on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) horizontal bore MR system. A commercially available transceive cryogenic quadrature RF surface coil probe (Bruker BioSpin AG, Fällanden, Switzerland) has been used for all measurements. For the BOLD fMRI experiments a gradient echo – echo planar imaging (GE-EPI) pulse sequence has been used with the following parameters: 5 slices of 0.5mm thickness with 0.7mm interslice distance; in-plane spatial resolution: 200x200µm²; echo/repetition time TE/TR: 8.5ms/2500ms; 3 averages; temporal resolution: 7.5s; 96 repetitions; total scan time: 12min.

Sensory stimulation paradigm: The stimulation consisted of sequential bilateral forepaw stimulations with subcutaneous electrodes following a block design with an amplitude of 1.5mA and a frequency of 3Hz. One stimulation cycle consisted of 60s off- and 40s on-periods, repeated 4 times in one stimulation series (12min). Each forepaw was stimulated twice alternating between left and right with a resting period of 8min.

Data analysis: Data analysis was carried out using Biomap (4th version, M. Rausch, Novartis Institute for Biomedical Research, Basel, Switzerland). To analyze the effect of sensory stimulation on brain activity, parametric maps were calculated using the general linear model (GLM) tool. For GLM analysis the first 32 images of the fMRI data set were discarded to avoid effects of magnetic saturation and eliminate confounding global signal changes caused by systemic adaptations to the first stimulation cycle. For statistical maps, a threshold of p=0.001 and activation cluster size ≥15 voxels have been applied on a selected slice at Bregma -0.10mm [3]. Regions-of-interest (ROIs) were drawn bilaterally on the S1 cortex and on the ventral pallidum (control). Changes in BOLD signal intensity were analyzed for all ROIs. A second control was obtained by acquiring the same sequence without stimulation. A total of 26 scans (n=12 mice) with stimulation and 22 scans (n=10) without stimulation have been analyzed.

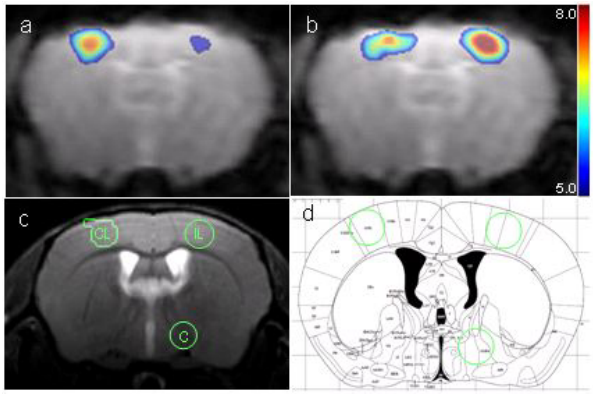

Figure 1: Representative t-maps of one animal (average of the two series for each paw; 2D low pass filtered), overlaid on EPI images showing activation after stimulation of the **(a)** right and **(b)** left forepaw. T-values are indicated at the scale bar. High resolution image with ROIs contralateral to the stimulated paw (CL) obtained through GLM analysis. Ipsilateral (IL) and control (C) ROIs were drawn manually **(c)**. Template of mouse brain atlas at the position Bregma -0.10mm **(d)** [3].

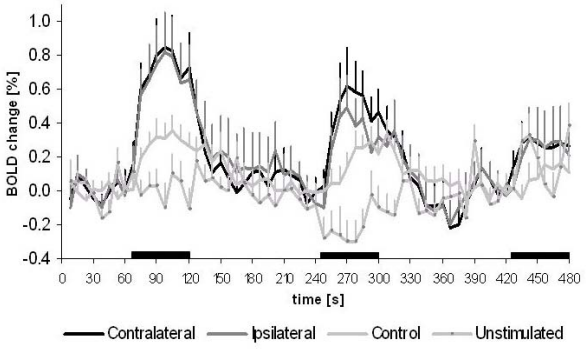

Figure 2: Relative change of the BOLD signal during electrical forepaw stimulation. Black bars indicate stimulation periods. Values presented as mean+SEM.

RESULTS Electrical forepaw stimulation using the previously described block design with current amplitudes of 1.5mA led to statistically significant signal changes in the contralateral primary somatosensory cortex as revealed by the activity maps (t-maps from GLM analysis) overlaid on the corresponding GE-EPI images at a level of -0.10mm relative to Bregma. The activated regions (Fig.1a,b) are consistent with the known topographic representation of the murine forelimb region (S1FL) at this level (Fig.1d). Analysis of the temporal profile in the contralateral S1 (n=26) depicted from the statistically emitted ROI revealed a maximal BOLD signal increase of 0.85±0.21% during the first stimulation cycle, while the second and third stimulation period resulted in a maximal signal increases of 0.62±0.22% and 0.32±0.13%, respectively (Fig.2).

The unilateral stimulation paradigm also led to clear activation of the ipsilateral somatosensory cortex. Analysis of the user-defined ROIs in the ipsilateral somatosensory cortex (n=26) showed maxima in BOLD signal changes of the order of 0.82±0.23%, 0.49±0.19% and 0.30±0.19% for the three respective stimulation cycles. The respective BOLD signal changes for the three stimulation cycles depicted from the control region (n=26) were 0.35±0.09%, 0.33±0.08% and 0.16±0.13%. In both the contralateral and ipsilateral sensory cortex a fast rise of the signal can be observed at the stimulus onset. No difference with regard to the maximum BOLD signal change between the two sides can be observed. A decrease in the BOLD response can be seen over the entire stimulation series. No activated regions were detected in GLM analysis of the control fMRI data sets (no stimulation) and analysis of the temporal profile of the 3 user-defined ROIs revealed an average BOLD signal change of -0.01±0.01% as shown in Figure 2. All values are presented as mean±SEM.

DISCUSSION This study showed the feasibility of BOLD studies of electrical somatosensory stimulation in the mouse forepaw under isoflurane anesthesia using a cryogenic RF probe as a highly sensitive detection device. Average changes in BOLD signal intensity were less than 1% (range 0.06-5.03% for single animals). One reason for these small changes might be attributed to adaptation during the course of the stimulation cycles as revealed by the decreasing BOLD amplitude during subsequent stimulation cycles. Since we excluded the first stimulation cycle from the data analysis to eliminate any systemic response, the first stimulation episode analyzed will be already affected by adaptation. Another factor contributing to the low amplitude is isoflurane, which is known to suppress neuronal activity: it is therefore critical to minimize the isoflurane level necessary to keep the mouse under anesthesia. Constant and well controlled anesthesia depth was achieved by intubating and mechanically ventilating the mouse. The reason of the bilateral activation in the somatosensory cortex might be due to the intensity or the frequency of the electric stimulation. It is possible that the current stimulation paradigm does not only activate sensory but also pain pathways projecting to the ipsilateral hemisphere. This interpretation is supported by the observation of BOLD changes in the thalamus of some animals (data not shown). Further studies are required to address these issues.

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