BOLD fMRI of forepaw stimulation at different amplitudes in mice

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INTRODUCTION: fMRI has become an important tool for studies of the functional anatomy of the rodent brain under normal and pathologic conditions as well as for elucidating the mechanism underlying the BOLD response. However, fMRI studies in mice are sparse. This is due to the small size of the mouse, which is challenging both from a MR technological and from a physiological perspective. Eliciting robust BOLD responses requires highly sensitive RF detector systems (e.g. cryogenic surface coils [1,2]) and maintenance of stable physiological conditions, e.g. constant levels of blood gases. Yet, in view of the numerous genetically engineered strains used in biomedical research the relevance of methods enabling noninvasive phenotyping (including mouse fMRI) will increase. The objective of this study was to analyze the robustness and reproducibility of the BOLD fMRI response to electrical forepaw stimulation as a function of the stimulus amplitude. This stimulation paradigm has been widely used in rats and studies have revealed that noxious-evoked activation patterns corresponded well with the structures known to be part of the pain processing pathway.

METHODS Animals: Female C57Bl/6 mice of 3-4 months of age were used. 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. Physiological 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 gases (pCO_2, pO_2). All experiments were performed in strict adherence to 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 (Bruker BioSpin AG, Fällanden, Switzerland) has been used for signal transmission and reception. BOLD fMRI experiments were carried out using a gradient echo-echo planar imaging (GE-EPI) pulse sequence 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 or 112 repetitions; total scan time: 12 or 14min.

Sensory stimulation paradigm: The stimulation consisted of sequential bilateral forepaw stimulations with subcutaneous electrodes following a block design with amplitudes of 0.5 (n=8), 1.0 (n=8), 1.5 (n=7), 2.0mA (n=8), a frequency of 3Hz and a pulse duration of 0.5ms. One stimulation cycle consisted of 120s off- and 60s on-periods, repeated 4 times in one stimulation series followed by a 120s off period (total duration 14min). Each forepaw was stimulated once with a resting period of 8min between left and right forepaw stimulation.

Data analysis: Data analysis was carried out using Biomap (4th version, M. Rausch, Novartis Institute for Biomedical Research, Basel, Switzerland). Parametric maps were calculated using the general linear model (GLM) tool. 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 in the S1 cortical area, the thalamus and the ventral pallidum (control region). Changes in BOLD signal intensity were analyzed for all ROIs. A second control was obtained by acquiring the same sequence without stimulation. Further analysis included separation of the signal into a slow and a fast component, the latter being fitted to a gamma-variate function. For both components, integrals over 12min (120-840s) have been calculated.

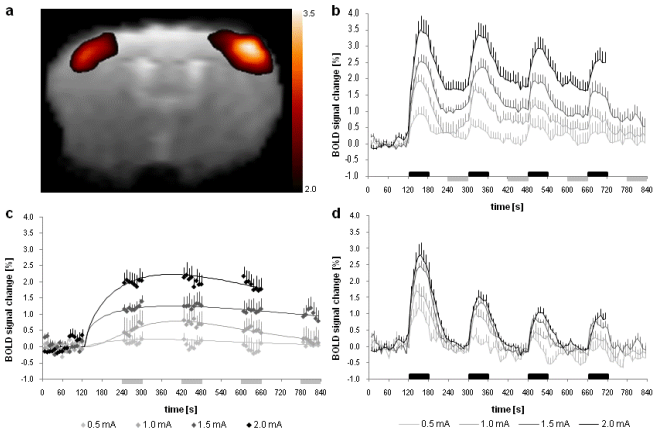

Figure 1: (a) Representative t-map obtained with GLM analysis of one animal (2D low pass filtered), overlaid on the EPI image, showing activation after stimulation of the right forepaw. T-values are indicated at the scale bar. **(b)** Relative change of BOLD signal in contralateral somatosensory S1 area during electrical forepaw stimulation at four different amplitudes. Black bars indicate stimulation periods. Grey bars indicate data points used for fitting the slow component curve **(c). (d)** Fast component obtained by subtraction of slow component fit from experimental data. All values are presented as mean+SEM.

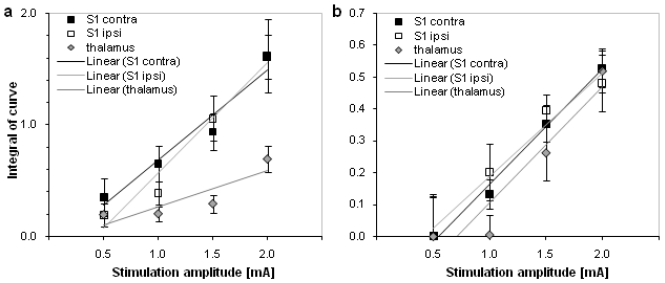

Figure 2: The integrated BOLD signal intensity (integration period 120-840s) as a function of the stimulation amplitude. Integrals of the **(a)** slow and **(b)** fast component for ipsi- and contralateral S1 regions and the thalamus. All values are presented as mean+SEM.

RESULTS Electrical forepaw stimulation led to a statistically significant signal change in the somatosensory cortex and thalamus. The activated regions (t-map Fig.1a) corresponded well to the known topographic murine forelimb representation. The signal changes correlate with the stimulation amplitude (Fig.1b). Stimulation with the lowest amplitude of 0.5mA led to a maximal BOLD change of only 0.91±0.23% in the S1 region contralateral to the stimulated paw. At higher amplitudes of 1.0, 1.5, and 2.0mA, the maximal BOLD signal change of this region amounted to 1.94±0.20%, 2.54±0.22% and 3.52±0.41% respectively. Similar correlations between BOLD signal change and stimulus amplitude were observed for all regions involved in sensory processing. Interestingly, no difference in the maximum BOLD amplitude has been observed between the ipsilateral and contralateral S1 region. The amplitude of the BOLD response of subsequent stimulations in a series decreased for all responsive brain areas analyzed. The control region in the ventral pallidum did not show any change in signal at 0.5-1.5mA amplitude. At 2.0 mA a maximum increase of 0.81±0.12% had been observed. Only background noise but no activation was detected in the GLM analysis of control fMRI data sets acquired without stimulation. The integrals for both the fast and slow component of the two S1 areas correlate linearly with the stimulation amplitude (Fig.2a (fast component): contralateral S1: R^2=0.99, ipsilateral S1: R^2=0.97; Fig. 2b (slow component): contralateral S1: R^2=0.95, ipsilateral S1: R^2=0.96). The corresponding integrals for the thalamic ROI displayed a slightly weaker linear correlation with the stimulation amplitude (R^2(slow)=0.75, R^2(fast)=0.90 , respectively). All values are presented as mean±SEM.

DISCUSSION This study showed that reproducible BOLD activation patterns can be obtained in somatosensory and thalamic ROIs during electrical somatosensory stimulation of the mouse forepaw using different stimulation amplitudes. Strong correlations have been found between the BOLD response and the current amplitude. The segregation of the signal into two components might help to understand the underlying physiological processes. The decrease of the fMRI signal amplitude over the four stimulation cycles might be attributed to adaptation, which could occur either peripherally in the stimulated paw, or centrally in the brain (stimulus dependent inhibitory input [4]). A particular result of this study was the consistent bilateral activation of the somatosensory cortices despite unilateral stimulation. Neither alteration of the anesthesia depths nor unilateral administration of local anesthetics (lidocaine) resolved this bilaterality. The reason for the bilateral activation is currently unclear: There are multiple pathways processing both noxious and innocuous stimuli, which do not have strictly unilateral projections to the brain. Alternatively, the bilateral response might be mouse strain specific. This issue will be investigated in further studies.

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REFERENCES [1] Haueisen R. et al., MAGMA 2006 (19):sup pl. 1, 78; **[2]** Ratering D et al. Magn Res Med 2008, 59: 1440; **[3]** The Mouse Brain; G. Paxinos; K.B.J. Franklin; 2001, **[4]** Buxton et al. Neuroimage 2004, 23: 220.