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. Blinding 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 noisier-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 mgkg). Animals were stereotactically fixed 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 (PO2, PCO2). All experiments were performed in strict adherence to the Swiss law of animal protection.

fMRI: Experiments were carried out on a Bruker BioSpec 94/20 (Bruker BioSpin MR, Ettlingen, Germany) horizontal bore MR system. A commercially available transmit/cryogenic quadrature RF surface coil (Bruker BioSpin AG, Filllach, Switzerland) has been used for signal transmission and reception. BOLD fMRI experiments were carried out using a gradient echo-planar imaging (GE-EPI) sequence with the following parameters: 5 slices of 0.5mm thickness with 0.7mm inter-slice distance; in-plane spatial resolution: 200x200μm2; echo/repetition time T/TE/TR: 85ms/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=6), 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 6min between right and left forepaw stimulation.

Data analysis: Data analysis was carried out using Binar Map (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 (ROI) 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.

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 known topographic murine forelimb representation. The signal changes correlate with the known topographic murine forelimb representation.

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 peculiar result of this study was the consistent bilateral activation of the somatosensory cortices despite unilateral stimulation. Neither ablation of the anesthesia depth 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 strict 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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