Elucidating bilaterality of fMRI BOLD signal change in the mouse brain upon unilateral innocuous and noxious paw stimulation

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INTRODUCTION: Functional MRI of rodents has become a widely used tool to investigate brain anatomy and function. So far, most rodent fMRI studies have been performed in rats. When establishing and applying innocuous and noxious sensory stimulation paradigms in isoflurane-anesthetized mice we observed a consistent bilateral BOLD fMRI response involving respective brain areas (S1, S2, insula, thalamus, PAG, motor cortex) of both hemispheres in a symmetric fashion despite of unilateral paw stimulation. Amplitude, spatial extent and temporal pattern were found nearly identical. This stands in contrast to the majority of fMRI studies in healthy rats and mice which report predominantly contralateral responses during unilateral innocuous or mild noxious stimulation [1,2], including our own study on rats using isoflurane anesthesia [3]. We observe a bilateral BOLD signal change in mice independent of whether we apply an electrical [4], thermal or chemical stimulus, of whether we stimulate fore- or hindpaw, independent of stimulus strength, anesthesia depth, mouse strain, gender and age. Although carried out with a small number of animals only, autoradiography and intrinsic optical imaging experiments support our fMRI findings, at least regarding the bilateral activation of secondary cortical and subcortical areas. Assumptions regarding the origin of bilaterality point to a crosstalk between hemispheres via interhemispheric connections, strictly ipsilaterally projecting tracts and type of anesthesia. Besides, the low temporal resolution of the fMRI measurement relative to the fast neuronal response does not allow resolving the temporal pattern and a possible delay between hemisphere activation. This study presents further efforts to characterize the bilaterality of the BOLD fMRI response observed in mice in our laboratory.

METHODS: Animals and physiological conditions: Female mice were intubated, artificially ventilated, paralyzed using Pancuronium bromide (1mg/kg), and stereotactically fixated. Physiological parameters (temperature, blood gas level) were monitored. One set of experiments was performed under isoflurane anesthesia (induction 3%, maintenance 1%) comprising healthy C57Bl/6 mice, the acallosal mouse strain I/LnJ, and C57Bl/6 mice, in which the corpus callosum (CC) and the hippocampal fissure (HF) have been surgically transected. Another experiment was performed under medetomidine anesthesia (for induction isoflurane: 3%, <5min; medetomidine: initial s.c. bolus of 0.3mg/kg, maintenance with s.c. infusion of 0.6mg/kg/h). FMRI: Experiments were carried out on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) horizontal bore MR system using a commercially available transceiver cryogenic quadrature RF surface coil (Bruker BioSpin AG, Fällanden, Switzerland). A GE-EPI sequence with the following parameters was applied: 7 axial slices of 0.5/0.7mm STH/ISD, in-plane spatial resolution=200x200µm^2; TE/TR=8.5/2500ms; NA=3; temporal resolution=7.5s; or TE/TR=8.5/1000ms; NA=1; temporal resolution=1s, respectively. Chemical stimulation: The plantar left hindpaw (lhp) was injected s.c. with a 10µl bolus of either 1µg Capsaicin (CAP) or Saline containing 1% ETOH (vehicle) during the fMRI scan. The timed injection was performed after a 5 to 10min baseline acquisition, the stimulation effect imaged for further 5 to 20min. Data analysis: Data were analyzed using SPM, Biomap (M.Rausch, Novartis, Basel, Switzerland) and Excel. Statistical activation maps were calculated using GLM analysis with a data driven regressor, a threshold of p=0.01 and cluster size of 15 voxels. Regions-of-interests were drawn bilaterally in the S1 cortex and in the above mentioned brain areas, time courses of changing BOLD signal intensity were extracted.

RESULTS: Hindpaw stimulation using CAP injection induced a BOLD signal change in areas commonly termed the pain matrix (S1, S2, insula, thalamus, PAG, motor cortex, Fig.1). The strength of the BOLD response correlated with the CAP dose (data not shown). Consistent bilateral BOLD signal changes in both the vehicle and CAP condition have been observed. In the somatosensory S1 area a coefficient of correlation of R^2=0.85 has been obtained when comparing the BOLD signal change of the contralateral and ipsilateral hemisphere. Similar bilateral BOLD responses have been observed in acallosal I/LnJ mice and mice with a surgical transection of the CC and HF. Also the use of a different anesthetic, medetomidine, did not affect bilaterality; however whereas the thalamic BOLD signal change was very prominent, the cortical BOLD signal change was weaker with the medetomidine dose applied.

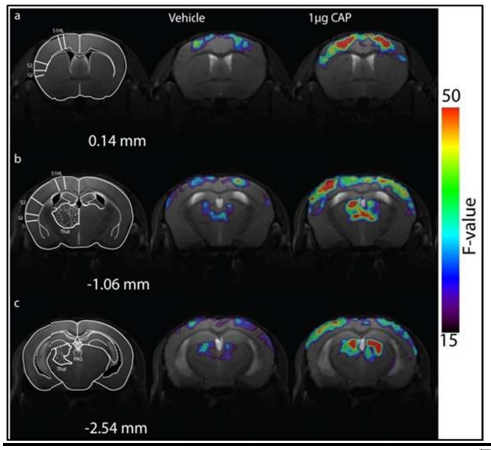

Fig. 1: Activation maps depicting the effect of chemical hindpaw stimulation with 1ug CAP or vehicle (each n=5) on BOLD signal intensity. CAP leads to a stronger, more widespread BOLD signal change, however, both conditions result in a bilateral signal change.

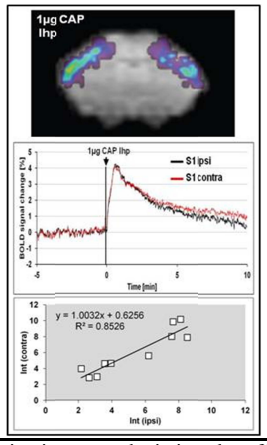

Fig. 2: Activation map depicting the effect of chemical hindpaw stimulation with 1ug CAP on BOLD signal intensity (n=1). Time courses of BOLD signal change extracted from S1 in both hemispheres show a nearly identical pattern. Correlating changes between the hemispheres of n=10 animals yielded in R^2=0.85.

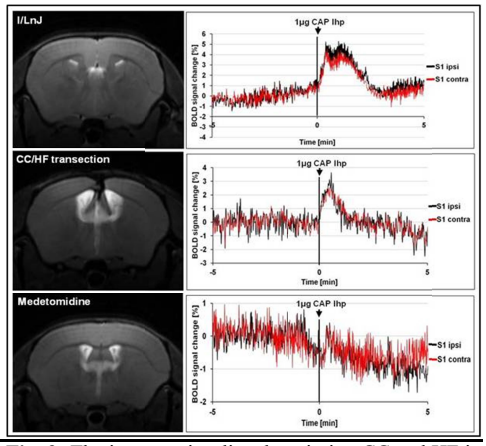

Fig. 3: The images visualize the missing CC and HF in I/LnJ and surgically transected mice, compared to the intact brain of mice studied with medetomidine anesthesia. Time courses of BOLD signal change extracted from S1 in both hemispheres show a nearly identical pattern in all conditions, i.e. bilaterality was not affected.

DISCUSSION: Bilaterality of fMRI BOLD signal change in mice upon unilateral innocuous and mild noxious paw stimulation stands in contrast to the general expectation and majority of results reported in rodent fMRI studies. The conventional organizational principle regarding the sensory projections from periphery to brain comprises predominant processing of afferent input from the body to the contralateral brain hemisphere. However, an increasing number of studies describe a parallel engagement of selected circuits within both hemispheres under specific conditions [5,6,7]. The precise pathway mediating the ipsilateral response is unclear, ranging from transcallosal input, direct uncrossed afferent projections to top-down input from higher-level processing areas. The fMRI data from I/LnJ mice and mice with a surgical CC/HF transection revealed, that this interhemispheric synchrony does not necessarily depend on cross-hemispheric communication via the CC. Currently we cannot assess possible delays between activation onsets in the two hemispheres due to relatively low temporal resolution (1s) of our fMRI data. Ongoing EEG studies should help to get further insight into interhemispheric processing of unilateral peripheral stimuli applied to the mouse paw and should enable adequate correlations of the neuronal with the hemodynamic response measured in BOLD fMRI. Anesthesia is a recurring issue in animal imaging. Isoflurane is easy to administer, yet evokes potent vasodilatory effects: at a dose of 1% used in this study we assume this effects to be rather weak [8]. An increasing number of studies are performed using other agents for anesthesia such as medetomidine [2]. The data of this study did not reveal enhanced lateralization of BOLD signal change when compared to isoflurane data, pointing to other origins of the bilateral signal.

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