HOW SPECIFIC IS SPECIFIC? STIMULUS-EVOKED FMRI IN RATS AND MICE

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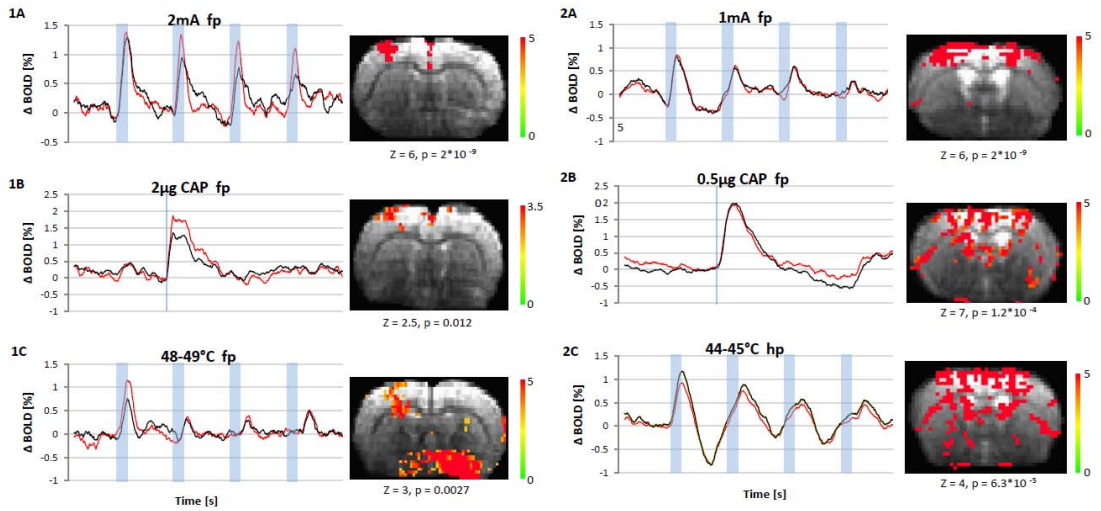
Introduction: Functional MRI (fMRI) has been widely used in rodents to non-invasively visualize brain function and response to innocuous and noxious stimulation. Experiments conducted with mice turned out to be particularly difficult and showed conflicting results. A possible explanation for widespread hemodynamic responses described in mice -observed even when applying innocuous stimuli to the paw- has been proposed by Schroeter et al. (1) attributing missing laterality to a general arousal of the animal independent of the anesthesia used. Strong cardiovascular changes could mask specific fMRI signals to peripheral stimulation. In contrast, fMRI measurements in rats evoke predominantly contralateral activation of the cortical somatosensory area involved in processing of the stimulus. Hence, arousal in rats seems to be milder and data seem less affected by contribution of peripheral cardiovascular changes. To test this hypothesis and to analyze under which conditions specific input-evoked responses might be detected we compared fMRI responses in both rats and mice to our recently established peripheral sensory stimulation paradigms (electrical, chemical, thermal) using innocuous as well as noxious stimuli.

Methods: All MRI experiments were conducted using a Bruker Biospec 94/30 small animal MR system (Bruker BioSpin MRI, Ettlingen, Germany) operating at 400MHz (9.4 T). For rats a room temperature surface phased array coil (Bruker BioSpin AG, Fällanden, Switzerland), and for mice a four-element receive-only cryogenic phased array coil (Bruker BioSpin MRI, Ettlingen, Germany) were used in combination with a linearly polarized room temperature volume resonator for transmission. Throughout the experiment the animals were intubated and mechanically ventilated with a 20% O_2/80% air mixture at a rate of 50 breaths/min (rats) and 80 breaths/min (mice), and isoflurane was adjusted to 1.5% (rats) and 1.2% (mice). Neuromuscular blocking agent pancuronium bromide (Sigma-Aldrich, Steinheim, Germany) was administered i.v.. For the fMRI measurements, data were acquired using a GE-EPI sequence: FOV=18.4x11.3 mm^2 and MD=80x35 (rats), FOV=16.6x7 mm^2 and MD=80x35 (mice), TE/TR=12/1000ms, NA=1, FA=60°. Twelve adjacent coronal slices with a thickness of 0.7mm (rats) and 0.5mm (mice) were acquired. Animals were stimulated on forepaw (fp) and hindpaw (hp) using three different paradigms. Three animals were averaged per stimulus and stimulus strength (Fig. 1, 2). ***Electrical stimulation***: A pair of needle electrodes was inserted s.c. into the paw. Different current amplitudes were applied with a pulse duration of 0.5ms and frequency of 5Hz. The stimulus paradigm consisted of a block design starting with 180s baseline followed by four cycles of 20s stimulus and 120s post-stimulus period. ***Chemical stimulation***: A cannula was inserted s.c. into the paw. Saline and different doses of capsaicin were injected. Injection volumes were 20µl (fp) and 40µl (hp) (rats), and 5µl (fp) and 10µl (hp) (mice). The stimulation paradigm consisted of a 300s baseline and 600s post-injection signal acquisition. ***Thermal stimulation***: A heating plate was attached to the paw. Different temperatures were applied. The stimulus paradigm was identical to the paradigm of the electrical stimulation.

For analysis, spatial preprocessing of MR data and generation of statistical parametric maps (activity maps) was performed in AFNI (http://afni.nimh.nih.gov/). Maps were generated using the canonical SPM hemodynamic response function with time and dispersion derivatives in a general linear model (GLM). The parameter maps that should reflect stimulus-evoked activations in each animal were entered into an ANOVA. The F-statistics of the different experiments were normalized to z scores. Regions-of-interest (ROIs) were defined according to a stereotaxic mouse brain atlas (2, 3) for the contralateral and ipsilateral primary somatosensory cortex (S1) and BOLD signal time courses extracted.

Results: In rats, noxious and innocuous forepaw stimulation led to higher BOLD fMRI responses in the somatosensory S1 region contralateral to the stimulated paw (Fig. 1A-C activity maps). Nevertheless, analysis of the BOLD signal profiles revealed a substantial signal also on the ipsilateral side (at least 60% of contralateral amplitude; Fig. 1A-C BOLD signal time courses). In contrast, in mice, both innocuous and noxious unilateral forepaw and hindpaw stimuli prompted widespread BOLD signal changes of identical amplitude for the two hemispheres (Fig. 2A-C). Interestingly, in rats the response to hindpaw stimulation was typically less specific in comparison to forepaw stimulation, with ipsilateral BOLD signal time courses almost matching those at the contralateral side (data not shown).



Analysis of BOLD signal changes (ΔBOLD) elicited in rats (Fig. 1) and mice (Fig. 2) by electrical (Fig. 1A, 2A), chemical (Fig. 1B, 2B) and thermal (Fig. 1C, 2C) paw stimulation. Activity maps depict BOLD responses from one representative animal per stimulation. Time courses of BOLD signal show significantly higher stimulus-evoked ΔBOLD in the contralateral S1 region for rats when the forepaw was stimulated, whereas stimulation in mice elicited widespread fMRI responses for each stimulus when applied to fore- or hindpaw.

Conclusion: Amplitude, spatial extent and temporal pattern of BOLD signal changes in response to paw stimulation were found nearly identical in both hemispheres for mice. This stands in contrast to fMRI studies in rats, which result in predominantly contralateral responses during unilateral innocuous or mild noxious stimulation, even though also in rats ipsilateral contributions may be substantial. We observe a widespread BOLD response in mice irrespective of the nature of the stimulus (electrical, chemical or thermal), the site of stimulation (fore- or hindpaw), the stimulus strength, type and depth of anesthesia, mouse strain, gender and age (not all data shown). Observations of profound cardiovascular parameter changes indicate a general arousal response. Peripheral hemodynamic changes may overrule cerebral autoregulation and thus mask stimulus specific fMRI signals in mice. Stimulation-related changes in cardiovascular variables in rats are also observed yet are not of sufficient magnitude to confound specific fMRI signal, and cerebral autoregulation seems to be better preserved.

Studying the processing of peripheral input using hemodynamic fMRI readouts in mice constitutes a major challenge and adapted paradigms and/or alternative fMRI readouts should be considered.

References: **1)** Schroeter et al., 2014. Specificity of stimulus-evoked fMRI responses in the mouse: the influence of systemic physiological changes associated with innocuous stimulation under four different anesthetics. NeuroImage. 94: 372-84. **2)** Paxinos, G., 2004. The Mouse Brain in Stereotaxic Coordinates. Gulf Professional Publishing. **3)** Paxinos, G., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press.