Reduced BOLD response in mice lacking nociceptor specific sodium channels (Nav1.7) indicates altered pain processing

S. C. Bosshard[1], C. Baltes[1], and M. Rudin[1,2]

[1]Institute for Biomedical Engineering, ETH Zürich, Zurich, Zurich, Switzerland, [2]Institute of Pharmacology and Toxicology, University of Zurich, Zurich, 8057, Switzerland

INTRODUCTION Functional magnetic resonance imaging (fMRI) has been widely used to study brain function in humans and in animals, especially in rats. In view of the increasing number of transgenic mouse lines used in research, we developed a robust stimulation paradigm to study pain processing in mice [1]. Studies in rats using electrical forepaw stimulation have revealed that noxious-evoked activation patterns corresponded well with the structures known to be part of the pain processing pathway. To test for its specificity we applied this paradigm to transgenic mice lacking the voltage-gated sodium channel Na_v 1.7 specifically at the nociceptors ($Na_v1.7R^{-/-}$)[2]. The Na_v 1.7 channel is located at the terminal of small-diameter sensory neurons and plays a critical role in generating and conducting action potentials. These nociceptor-specific knock out animals show increased mechanical and thermal pain thresholds [2]. Our hypothesis is that knocking Na_v 1.7 receptors will lead to a reduced fMRI signal in response to electrical stimulation of the forepaws.

METHODS Animals: Three groups of animals were examined: (i) The wildtype (WT) group consisted of 8 female C57Bl/6 mice of 3-6 months of age. (ii) The $Na_v1.7R^{-/-}$ group consisted of 6 animals, 4 males and 2 females, aged 2-8 months. (iii) The WT littermates of the $Na_v1.7R^{-/-}$ mice consisted of 2 females and 3 males, aged 2-8 months. The WT littermates were used to control for any differences due to the different origin of the mice (London and Zurich). 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. Body temperature was controlled using a rectal temperature probe (36±0.5°C) and blood gases levels (pCO_2, pO_2) were monitored using a transcutaneous electrode on the upper hind limb. 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 0.5 mm thickness with 0.7 mm interslice distance; in-plane spatial resolution: 200x200 μm^2; echo/repetition time TE/TR: 8.5ms/2500ms; 3 averages; temporal resolution: 7.5 s; 112 repetitions; total scan time: 14 min.

Sensory stimulation paradigm: The stimulation consisted of sequential bilateral forepaw stimulations with subcutaneous electrodes following a block design with the following parameters: 1.5 mA stimulation amplitude, 3 Hz frequency and 0.5 ms pulse duration. One stimulation cycle consisted of 120 s off- and 60 s on-periods, repeated 4 times in one stimulation series followed by a 120 s off period (total duration 14min). Each forepaw was stimulated once with a resting period of 8 min 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. 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 signal was obtained in the S1 area after acquiring the same sequence without stimulation. For statistical evaluation we compared the integrated values of the four stimulation periods (indicated by black bars in Fig. 2) for all groups.

RESULTS Electrical forepaw stimulation led to a statistically significant BOLD signal change in all experimental groups. Distinct activation was detected in the somatosensory cortex ipsi- and contralateral to the stimulated paw (Fig. 1) and the thalamus. While the temporal profiles of WT and WT littermates are virtually identical, $Na_v1.7R^{-/-}$ mice display a significantly reduced response (Fig. 2, see also Fig.1). The maximal BOLD signal amplitude differed significantly between the $Na_v1.7R^{-/-}$ and the WT and WT littermate mice ($p<0.05$). The values of the integrated BOLD profiles of the contralateral somatosensory cortex yielded 111.2±28.8 in the $Na_v1.7R^{-/-}$, 224.1±43.6 in the WT littermates and 232.2±21.0 in the WT animals as compared to the baseline signal (Fig. 3).

DISCUSSION Electrical stimulation of forepaws in mice using a current amplitude of 1.5mA leads to robust and reproducible BOLD signal changes in brain areas associated with pain processing (somatosensory cortex S1 and S2, thalamus). For WT animals the BOLD amplitude displayed a coefficient of variation of 0.25. As expected, the cortical and thalamic BOLD response was significantly reduced in $Na_v1.7R^{-/-}$ mice. This is in line with behavioral data reported for these mice, which show a dramatic reduction in mechanosensation [2]. However, electrical stimulation of the forepaw might not be the ideal paradigm to test for pain and pain pathologies: it is impossible using this paradigm to discriminate whether the detected signal in the brain is due to the induced pain or due to direct electrical activation of the nociceptors. This might explain why we still detect a BOLD signal change of more than 1% in the $Na_v1.7R^{-/-}$ mice, an effect which could be due to direct stimulation of the large diameter sensory neurons. Therefore it is important to establish additional, more physiological stimulation paradigms such as thermal stimulation.

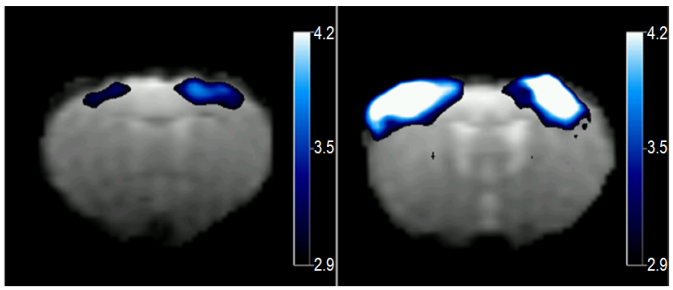

Figure 1: Statistical t-maps (2D low pass filtered) obtained with GLM analysis of two representative animals: **(a)** $Na_v1.7R^{-/-}$; **(b)** WT. Statistical maps are overlaid on the EPI image, showing activation after stimulation of the left forepaw. T-values are indicated at the scale bar.

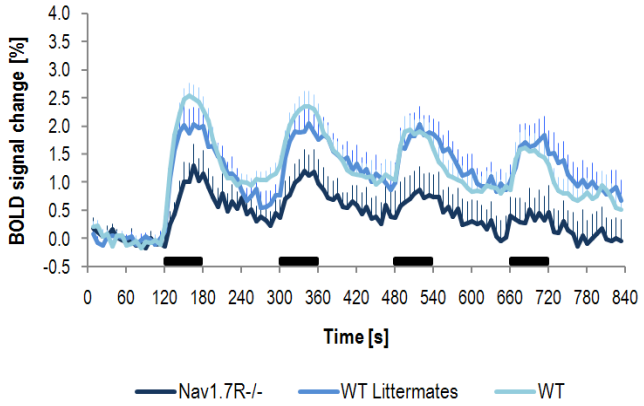

Figure 2: Relative change of BOLD signal in contralateral somatosensory S1 area during electrical forepaw stimulation at 1.5 mA for the $Na_v1.7R^{-/-}$ (dark blue, n=6), WT littermates (blue, n=5) and the WT animals (light blue, n=8). Black bars indicate stimulation periods. All values are presented as mean+SEM.

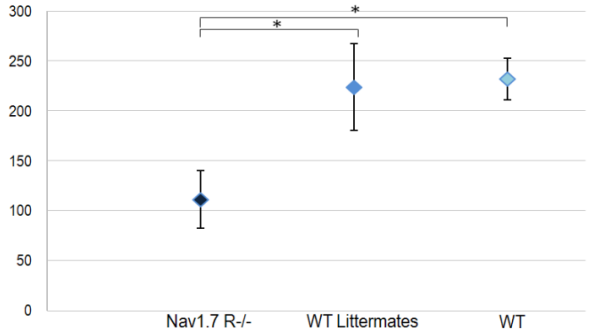

Figure 3: Areas under the curve integrated during the 4 stimulation periods of the somatosensory cortex contralateral to the stimulated paw. $Na_v1.7R^{-/-}$ are indicated in dark blue, WT littermates in blue, and WT in light blue. All values are presented as mean±SEM. Asterisks indicate a significant difference at the 0.05 level.

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