

TRPV1-mediated entry of QX-314 leads to inhibition of nociceptive input as measured by BOLD fMRI in mice using thermal stimulation

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INTRODUCTION: Functional magnetic resonance imaging (fMRI) in animals enables non-invasive studies of brain function, e.g. involving the sensory system. Electrical stimulation of the paws is a commonly used paradigm and has recently been applied to analyze nociceptive processing in mice [1]; however, there are several drawbacks to this stimulation: it is difficult to determine the point at which the input turns from somatosensory to noxious, and the electrical currents may activate neurons directly, instead of the neurons being activated as a result of a peripheral stimulus. Therefore we established a thermal stimulation paradigm inducing noxious stimulation using local heating with an infrared laser source. To demonstrate specificity of the paradigm for assessing nociceptive signaling we applied the quaternary lidocaine derivative QX-314 to the forepaws, which due to its positive charge cannot cross the cell membranes. However, activation of the TRPV1 channel by capsaicin allows QX-314 to enter the cell through the channel, leading to a selective block of the C-fiber nociceptors.

METHODS Animals: Female C57Bl/6 mice were used. All experiments were 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 pulse oximeter for control of oxygen saturation and heart rate. 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, averages NA=3, temporal resolution T_{acq}=7.5s, repetitions NR=152; or TE/TR=8.5ms/1000ms, NA=1, T_{acq}=1s, NR=1140, respectively. In both cases the total scan time was 19min.

Thermal stimulation: The stimulation consisted of sequential bilateral forepaw stimulation using infrared laser diodes (978nm) as a heat source. The skin temperature at the paw was adjusted to 45° or 46°C and controlled by a feedback loop. Stimulation duration was 60s, of which at least 30s had to be within the range of ±0.5°C of the target temperature. The diameter of the laser spot was 2mm in all but one experiment, for which a spot diameter of 1mm was used.

Pharmacological modulation: 10 µL of a mixture of 2% QX-314 and capsaicin (1µg/µL) were injected into the forepaws 90 minute prior to stimulation of the first paw (n=10). Control animals received 2% QX-314 (n=3) or capsaicin (n=3) only. The stimulation was performed at 45°C, using a spot diameter of 2mm and the fMRI protocol with temporal resolution (T_{acq}) of 1s.

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 [2]. 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. Statistical significance was tested using one-way ANOVA and Bonferroni post-hoc test at the 0.05 level.

RESULTS Thermal stimulation of the forepaws led to consistent BOLD responses in the S1 somatosensory cortex and the thalamus. Stimulation at 45°C using the 2mm spot led to a maximal BOLD signal change of 2.75±0.5% in the S1 area contralateral to the stimulated paw. Stimulation at 46°C induced a BOLD change of 4.09±0.6% and 4.37±0.9% for the 2mm and 1mm spot, respectively. The BOLD signal changes correlated well with the stimulation periods as shown in Fig.1. Pretreatment with a mixture of QX-314 and capsaicin led to an abolishment of the BOLD response after stimulation at 45°C (max: 0.61±0.3%; p=0.01). Control experiments with QX-314 or capsaicin only did not lead to a decrease of the BOLD response, but rather showed a trend of enhancing the BOLD response (4.89±0.7% and 5.11±0.8%, respectively).

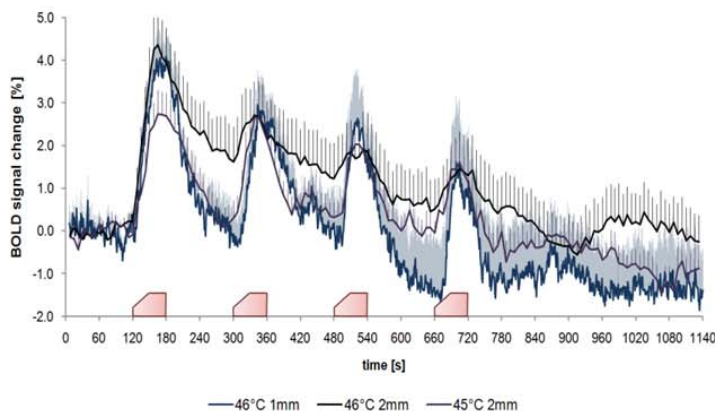


Figure 1: Temporal BOLD profile after thermal stimulation of the forepaws at 46°C (black: 2mm spot, blue: 1mm spot) and 45°C (purple, 2mm spot). Normal temporal resolution of 7.5s (black-purple) versus high temporal resolution of 1s (blue). Red boxes indicate stimulation length and approximate intensity. All values are presented as mean±SEM.

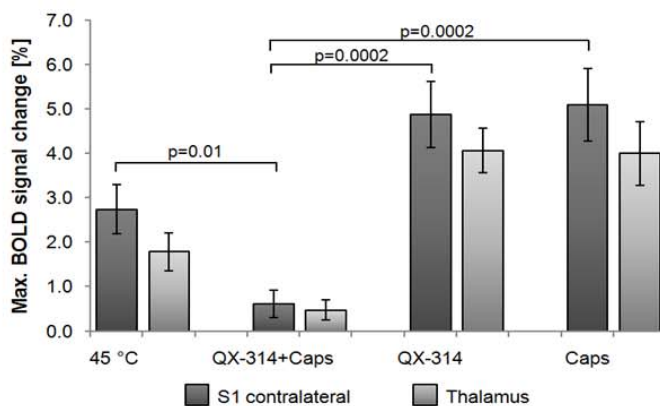


Figure 2: Maximal BOLD signal changes of the somatosensory cortex (S1) and thalamus. Pretreatment with QX-314 and capsaicin led to a significantly decreased BOLD response in both areas. All values are presented as mean±SEM.

DISCUSSION This study showed the feasibility of fMRI using thermal stimulation to study noxious processing in mice with a temporal resolution of 1 second. The amplitudes of the BOLD signal changes correlate well with the different stimulation temperatures and are not influenced by the diameter of the laser spot. Combined pretreatment with QX-314 and capsaicin reduced the BOLD signal changes significantly, indicating a specific block of the nociceptors as described in [3]. Inhibition of nociceptors was only achieved by application of both compounds at the same time, but not by either compound alone. This is due to the inability of QX-314 to enter the cell by diffusing through the cell membrane; only upon opening of the TRPV1 channels QX-314 was able to enter the cells and exert its analgesic effect. The specific presence of the TRPV1 receptors on the C-fiber afferents allows for a strictly nociceptive block without impairing other afferent and efferent neurons, which could for instance lead to a transient loss of somatosensory and motor function. The specificity of the thermal stimulation paradigm in combination with fMRI allows non-invasive monitoring of the nociceptive network at a temporal resolution of seconds.

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