Hyperalgesic effects of low dose Lidocaine detected by BOLD fMRI in mice

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

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

INTRODUCTION: Electrical stimulation of the forepaw is a widely used paradigm which revealed that the stimulus-evoked activation patterns correspond well with the structures known to be part of the pain processing pathway. To modulate these activation patterns in mouse brain, forepaw sensitivity of the mouse was altered using the local anesthetic Lidocaine, which blocks the generation and propagation of action potentials in sensory fibers [1]. However, Lidocaine is also able to activate the C-type sensory afferents directly by binding to the transient receptor potential (TRP) channels [2]. As these processes involve different molecular targets, we hypothesized that they could be selectively studied by varying the concentration of the drug. By injecting different concentrations of Lidocaine in the forepaw prior to electrical stimulation, it should therefore be possible to either enhance or inhibit activation in the brain areas involved.

METHODS Animals: Female C57Bl/6 mice of 3-6 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 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 transcutaneous electrode on the upper hind limb measuring levels of blood gases (pCO_2, pO_2). 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: $200x200\mu m^2$; echo/repetition time TE/TR: 8.5ms/2500ms; 3 averages; temporal resolution: 7.5s; 112 repetitions; total scan time: 14min.

Sensory stimulation paradigm: The stimulation consisted of sequential bilateral forepaw stimulations with subcutaneous electrodes following a block design (amplitude: 1.5 mA, frequency: 3Hz, pulse duration: 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 8min between left and right forepaw stimulation.

10µL Lidocaine in low (0.15, 0.3, 0.45, 0.6mM) or high (70mM) concentrations dissolved in 0.9% NaCl was injected into the left forepaw 40 minutes before electrical stimulation (n=8 for each experiment). 10µL of 0.9% NaCl was injected into the right forepaw 40 minutes prior to electrical 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. 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 (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 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 (Fig.1).

Behavioral tests: The dynamic von Frey hair test was used to confirm the fMRI data. Baseline measurements of the forepaw withdrawal resistance were recorded in 6 awake, freely moving mice before injection of 0.3mM Lidocaine under short isoflurane anesthesia. The forepaw withdrawal resistance was continuously measured during 60 minutes following the Lidocaine injection.

RESULTS Electrical stimulation of the left forepaw led to BOLD responses in the S1 somatosensory cortices and in the thalamus. The signal consisted both of a slow and a fast component (Fig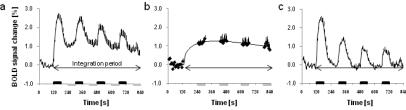.1). Pretreatment with low concentrations of Lidocaine led to a significant increase of the maximum BOLD signal change compared to the untreated forepaw stimulated at the same amplitude. Maximal BOLD signal changes (in % of baseline intensity) in the contralateral S1 region after injection of 0.3mM Lidocaine were 4.8±0.6% as compared to 2.6±0.3% in untreated mice These results corresponded well with the findings of the behavioral test (Fig. 3); the forepaw baseline withdrawal resistance of 0.8±0.07g was reduced significantly to 0.18±0.05g after injection of 0.3mM Lidocaine. The integral of the slow signal component was significantly larger in all three ROIs after pretreatment with 0.3mM Lidocaine when compared to untreated controls (p<0.05, Fig. 2a). A significant increase of the fast component could be observed for both S1 cortices after injection of 0.45mM Lidocaine, as well as for the contralateral S1 cortex after injection of 0.3mM Lidocaine (Fig. 2b). No change of intensity had been observed for the fast component in thalamus. Administration of 70mM Lidocaine, a dose used to induce local anesthesia, abolished the BOLD signal elicited by stimulation of the pretreated paw.

Figure 1: BOLD signal change after stimulation of the untreated forepaw with 1.5mA. The original BOLD signal (a) is decomposed into a slow (b) and a fast (c) component. Black bars indicate stimulation periods; grey bars indicate data points used for curve fitting. All values are presented as mean+SEM.
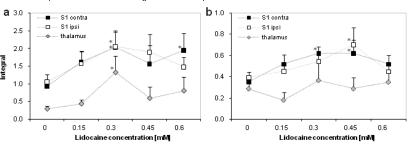

Figure 2: Integrated BOLD intensity of the slow (a) and the fast (b) component after application of different concentrations of Lidocaine. The largest effects can be observed after the injection of 0.3mM and 0.45mM Lidocaine. No difference between ipsilateral and contralateral S1 cortices is apparent. All values are presented as mean+SEM.
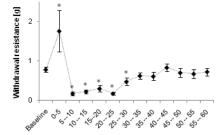

Figure 3: Mean force required to induce withdrawal of the forepaw after mechanical stimulation with dynamic von Frey filaments. Applied force decreased significantly (p<0.05) after injection of 0.3mM Lidocaine. All values are presented as mean±SEM.

DISCUSSION This study revealed that fMRI in mice is sensitive enough to detect changes in the BOLD response due to altered peripheral sensitivity. Furthermore we demonstrated that the local anesthetic Lidocaine has hyperalgesic effects when administered peripherally at low doses. It is known that Lidocaine activates the TRPV1 and TRPA1 receptors of the C-type sensory neurons [2], which might explain the sensitization induced in the forepaw. The fast and slow components of the BOLD signal potentially reflect contributions from different sensory fiber types to the hemodynamic signal. The slow conducting C-fiber is activated only by painful stimuli, while the fast conducting Aβ- and Aδ-fibers are activated by innocuous stimuli. We hypothesize that this might explain the intensity changes observed for the slow component of the thalamus, a prominent structure of in pain processing, but not in the fast component. This could be a first approach to distinguish noxious from innocuous stimuli by non-invasive fMRI.

REFERENCES [1] Nau, C. and Wang, G.K., J.Membr.Biol., 2004 [2] Leffler, A. et al.; J.Clin.Inv.; 2008 [3] The Mouse Brain; G. Paxinos; K.B.J. Franklin; 2001