Near-Physiological Mouse fMRI of Nociception

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Target Audience: MR scientists, clinicians and clinical scientists with interest in preclinical fMRI and pain research.

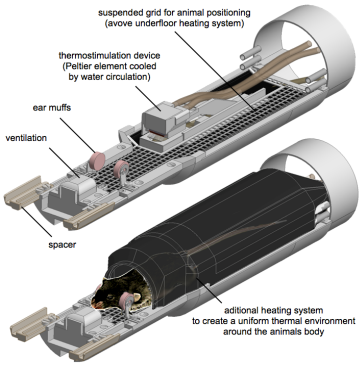
Purpose: Chronic pain is still a major clinical issue with limited treatment options. The combination of fMRI and mouse genetics provides great potential to unravel the underlying mechanisms by investigating the influence of pain transducer molecules on central nervous processing. Thermal stimulation is key to study distinct somatosensory transduction pathways by specifically activating peripheral receptor proteins with respect to their intrinsic thermal thresholds. Pioneering studies of heat stimulation demonstrated the proof-of-principle for mouse fMRI with physiological nociceptive stimuli [1,2]. Yet, BOLD sensitivity was shown to be an order of magnitude below that reported for electrostimulation [3,4], which puts it at the borderline of the detection level. This challenge constitutes an obstacle for functional brain mapping under thermal stimulation and presents a roadblock for detailing specific effects of target molecules on pain processing in transgenic mice. To advance the capabilities of fMRI of thermal stimulation we present enabling methodology tailored for probing of nociception in mice by near-physiological functional MRI. Our developments include (i) a dedicated mouse cradle (*rodents convective environment for thermostimulation*, *RoCET*), that affords uniform physiological body temperatures in anesthetized mice and (ii) improved data processing including mouse brain atlas registration and independent component analysis based signal filtering.

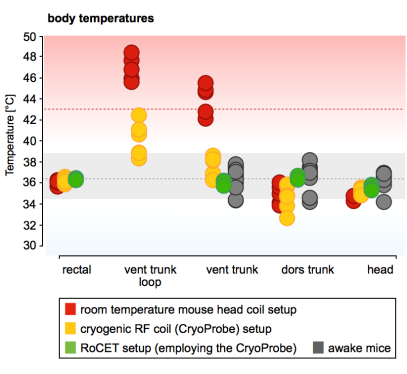
Materials and Methods: ***RoCET.*** The customized mouse cradle RoCET (Fig.1) was designed using CAD software. RoCET comprises a suspended grid for animal placement surrounded by a chamber-like heating system allowing for non-contact warming predominantly by convection/radiation. ***Thermal measurements.*** Skin/body/ambient temperatures were measured using fibre-optic temperature probes. Two conventional experimental MRI setups were used as a reference: i) setup customized for a *room temperature* mouse head RF coil array (RT), and ii) a setup tailored for a cryogenically cooled mouse head coil (*CryoProbe*, CP) ***Thermostimulation device.*** The stimulation system comprises a (14x14x2)mm^3 Peltier element, a fiber-optic temperature sensor, and a feedback control software (Fig.1). The Peltier‘s heating/cooling performance was enhanced by water circulation. The driving current was adapted/corrected in real-time (every 200ms) in a P-control algorithm. Paradigm used for heat stimulation was: 35.5°C baseline, 4°C/s, 48°C peak, 20s, 4x, interstimulus interval 90s, error <0.2°C. Stimulation was carried out for the plantar hindpaw. ***Animal experiments.*** Six male C57BL/6N mice (weight 25-28 g) were studied using thermostimulation (48°C) under 1% isoflurane anesthesia. The animals were intubated, artificially ventilated and paralyzed using the neuromuscular blocking agent pancuronium bromide (1mg/kg) in order to avoid motion artifacts [3]. Experiments complied fully with local institutional ethical and legal requirements. ***MR Imaging.*** High- resolution sagittal T_2 weighted imaging were used to position 11 axial slices used for T_2*-weighted fMRI (GE-EPI, TR/TE/FA = 2500ms/11.0ms/80°, FOV/mtx/res = 24x12x5mm / 90x60x11 / 188x188x500µm), TA = 12min. All images were acquired on a 9.4T Bruker Biospec (Ettlingen, Germany) using a transceive cryogenic quadrature RF surface coil (Bruker, Ettlingen, Germany). ***Analysis.*** FMRI data were motion corrected, smoothed, statistically analyzed and inference corrected by family wise error (FWE), $p < 0.0001$ (FSL). Scanner drifts and physiological noise were detected by independent component analysis (ICA) and removed prior to analysis. Resulting z-statistical maps were registered to a mouse brain atlas (ANTs) and group conjunction analysis was performed.

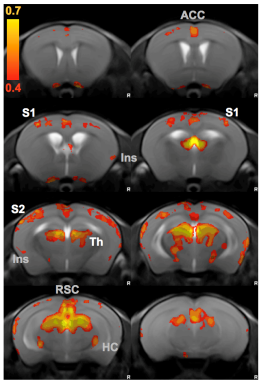
Results: Comparing the body surface temperatures of anesthetized mice in two conventionally available mouse cradles and RoCET showed dramatic differences (Fig.2): In conventional mouse cradles floor temperatures of up to 42°C (RT) and 48°C (CP) are required above the floor heating loops to maintain physiological rectal temperatures of 36°C. The measured body temperatures within RoCET are fully in the physiological range (Fig.2, gray shading; temporal mean (gray dotted line)) [5]) and equal those of awake, freely moving mice. The application of heat stimuli revealed highly reproducible activation patterns in brain areas associated with the central processing of heat stimuli [6,7]: anterior cingulate cortex (ACC), hippocampal area CA3 (HC), insular cortex (Ins), primary and secondary somatosensory cortices (S1, S2), retrosplenial cortex (RSC), and thalamus (Th), which are preserved in whole brain conjunction maps (Fig.3). Signal-time-plots of significant areas over time exhibited BOLD signal changes of 3% (S1/S2) to 5% (Th) comparable to human studies of noxious heat stimulation (Fig.4).

Discussion and Conclusions: Mouse cradles used in today’s mouse fMRI require unphysiologically high floor temperatures, which might effect thermal sensation and physiological processes [5,8]. The proposed RoCET setup maintains physiological rectal temperatures with physiological whole body tempering, which is a major step towards preserving physiological conditions within anesthetized mice. *In vivo* application of our thermostimulation fMRI methodology for mice yielded spatially discrete BOLD effects of up to 5% magnitude for mild noxious stimuli of 46°C, demonstrating greatly improved BOLD sensitivity of this approach versus previous reports on thermostimulation mouse fMRI [1,2]. This is the first report on BOLD activation patterns or BOLD effects of this significance and magnitude in mouse fMRI with thermostimulation. Based upon this progress we anticipate further improvements of our mouse fMRI protocols to allow for translational studies.

References: [1] Hess et al., 2011, PNAS 108:3731-3736, [2] Neely et al., 2010, Cell 143:628-638, [3] Bosshard et al., 2010, Pain 151:655-663, [4] Baltes et al., 2011, NMR in biomedicine 24:439-446, [5] Gordon, 2012, J Therm Biol 37:654-685, [6] Lanz et al., 2011, J Neural Transm 118:1139-1154, [7] Becerra et al., 2011, NeuroImage 54:1355-1366, [8] Lautenbacher et al., 2007, Somatosens Mot Res 24:189-201, [9] Newsom et al., 2004, J Am Assoc Lab Anim Sci 43:13-18, [10] Yarmolenko et al., 2011, Int J Hyperthermia 27(4):320-343.







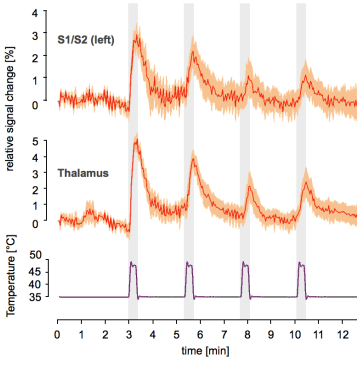


Fig.1 Schematic of the RoCET setup. The CryoCoil, which covers the mouse’s head exhibits an adjustable surface temperature [4] thus creating an enclosed uniform thermal ambient together with the RoCET heating system. **Fig.2** Body temperatures of isoflurane anesthetized mice in three fMRI set-ups. Rectal temperature and body surface temperatures – dorsal and ventral referenced against measured trunk temperatures of awake mice. Positions above heating loops of floor heating systems were monitored. The red dotted line indicates potential tissue damage above 43°C [10]. **Fig.3** Conjunction analysis of axial z statistical maps from 6 mice (overlap of 85%) projected onto mean structural reference scans. Significant structures are described in the results section. **Fig.4** Relative BOLD signal change over time for thalamus and ipsilateral S1/S2. ROIs were thresholded from activity maps.