

# Translational BOLD fMRI in mice and man: preclinical and clinical treatment approaches

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**Introduction:** Pain is the key symptom in patients with arthritis. We hypothesized that in arthritis hypernociception due to chronic TNF overexpression leads to an altered pain processing in the brain. Using functional magnetic resonance imaging (fMRI) we demonstrated that mice overexpressing human tumor necrosis factor (hTNF), as well as rheumatoid arthritis patients exhibit more intensive, widespread and prolonged brain activity upon nociceptive stimuli. Modern antibody therapies like the clinically approved Infliximab should lead to a reduction of this pain sensitization.

## Material and Methods: Functional MRI in mice

Male WT and hTNFtg mice (n = 10 per group, 10 weeks) were anesthetized with isoflurane and placed on a cradle inside the magnetic resonance tomograph (Bruker BioSpec 47/40, quadrature head coil) with extensive physiological monitoring. The contact heat stimuli sequences (40°C, 45°C, 50°C, and 55°C, 20 seconds) were presented at the right hind paw with 3 minutes and 25 seconds interval, 3 times. A series of 750 sets of functional images (matrix 64 x 64, FOV 15 x 15 mm, slice thickness 0.5 mm, axial, 22 slices) were sampled using GE EPI (single shot: TR = 4000 ms, TE<sub>eff</sub> = 24,38 ms, NEX = 2) within 50 min. Finally, 22 corresponding anatomical T2 reference images (RARE, slice thickness 0.5 mm, field of view 15 x 15 mm, matrix 256 x 128, TR = 2000 ms, TE<sub>eff</sub> = 56 ms, RARE) were taken.

## Functional MRI in humans

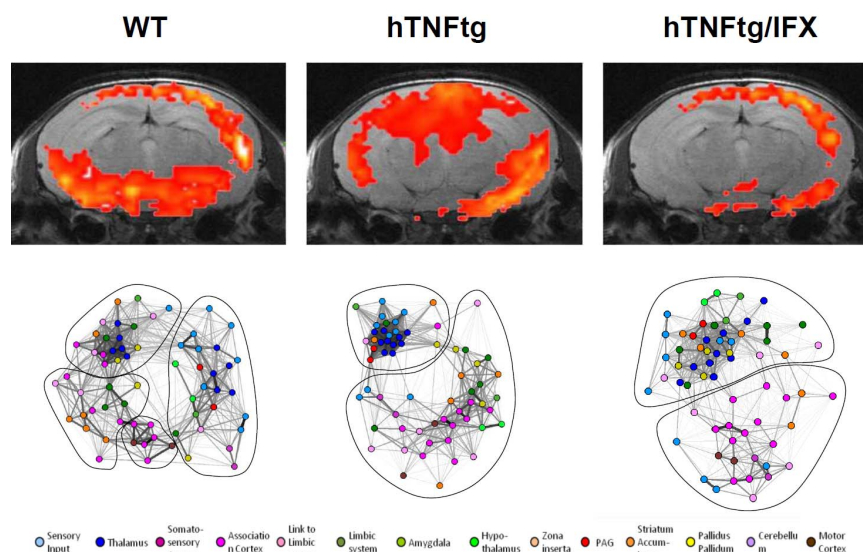
All anatomical and fMRI data were acquired on a 3 T scanner (Magnetom Trio, Siemens, Erlangen, Germany) using a standard 8-channel phased array head coil. For anatomic data sets we used a T1 weighted MPRAGE-sequence (FOV = 256 mm, matrix size = 256 x 256, voxel size = 1.0 x 1.0 x 1.0 mm<sup>3</sup>, slices = 176, slice thickness = 1 mm, TR = 1900 ms, TE = 1.13 ms). This anatomical dataset was recorded in the same session as the functional measurements. It was acquired before the fMRI scans to allow adaptation of the patient to the scanning procedure since for all patients this was the first MR scanning session. For each subject, two experiments with different stimulation conditions (first, finger-tapping and second, compression of the metacarpophalangeal joints) were performed. In each of them 93 whole-brain images were obtained with a gradient-echo, echo-planar scanning sequence (EPI; TR 3000 ms, TE 30 ms, flip angle 90°; FOV 220 mm<sup>2</sup>, acquisition matrix 64 x 64, 36 axial slices, slice thickness 3 mm, gap 0.75 mm). The first two volumes were discarded to account for spin saturation effects. fMRI was performed immediately before infusion of IFX as well as 24 hours after receiving the first infusion. All patients received a second infusion after 4 weeks, when clinical response to IFX is typically seen. BOLD signals were measured again 6 weeks (42 days) after the first infusion.

## Functional MRI analysis

Functional analysis was performed for mice and humans using Brain Voyager QX (Version 10.3) and our own software MagnAn. In summary, after pre-processing (motion-corrected using sinc interpolation Gaussian spatial (human: FWHM = 4 mm, mouse: 0.469 mm) and temporal (FWHM = 3 volumes) smoothing), GLM analysis with separate predictors for each stimulus was performed. The SPM's obtained were FDR thresholded around ZS-level of 3.3 and different groups of activated voxels were labeled as belonging to certain brain structures based on (i) the mouse atlas from Paxinos or (ii) the Mai atlas of the human brain. The mean corresponding peak activity was determined for each stimulation temperature for mice and for tapping and compression in humans before and after Infliximab treatment to provide a global group comparison. Next a Graph-theoretical analysis was performed: Functional connectivity patterns were computed as cross-correlations of the residuals after global signal regression for all brain structures and represented as correlation matrices. The networks are visualized using a force-based algorithm after Kamada-Kawai for achieving that all edges are of more or less equal length and there exist as few crossing edges as possible.

## Results:

Classical BOLD analysis revealed increased activity in the classical regions of the pain system for I) hTNF mice compared to WT mice as well as to hTNF mice after treatment and II) for the patients before compared to after the treatment. Graph theoretical connectivity analysis of the fMRI data showed rewiring within the pain matrix under chronic pain conditions i.e. tight clustering of brain activity in thalamus and periaqueductal grey. Neutralization of TNF by antibodies rapidly reversed this hypernociception in mice and men. This was reflected by an overall decrease of the functional activity in the brain pain matrix and by dissociation of the tight clustering long before anti-inflammatory effects were evident. These results suggest profound functional changes of nociceptive brain activity during arthritis in mice and men, which normalize upon hTNF blockade. This similarity of pain related effects in mouse and man facilitates a translational approach for searching novel analgesics and by validation therapeutic success by non-invasive fMRI.



**Acknowledgement:** BMBF (Immunopain and 0314102), and by the Doerenkamp Foundation for Innovations in Animal and Consumer Protection, ELAN grant from FAU.