Changes in resting state networks and biochemistry in a mouse model of inflammatory pain

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Introduction

In a pilot rsfMRI/MRS study we investigated the effects of inflammatory pain, induced by complete Feud's adjuvant (CFA), on resting state network topology in the mouse brain. Networks were defined by interregional correlation of resting state fMRI timecourses. Additionally we measured the influence of pain on the metabolism in prefrontal cortex (PFC) by MRS expecting a hyper-glutamatergic input to nucleus acumbens (NAc)[1].

Methods

Resting state fMRI as well as MR spectroscopy was performed for 16 male, 8 weeks old C57BL6 mice. 8 of them were exposed to inflammatory pain by an intraplantar hindpaw injection of CFA 48 hours prior to the measurement leading to a strong local inflammation with paw edema. Control animals were treated with saline instead. MRS- and fMRI-data were obtained using a 9.4 T MRI-scanner (Bruker BioSpin, Ettlingen,Germany) and a mouse brain cryogenic coil (20K). For fMRI an EPI-sequence was used (TR/TE=1300/18 ms, flip angle=50°, 96x64 imaging matrix, 21 coronal slices and FOV=17.28x11.52 mm², slice thickness=0.4 mm, gap=0.1 mm, 400 acquisitions). Breathing and cardiac rates were recorded with a temporal resolution of 10 ms. Before each functional measurement, a B_0-map was acquired to correct the EPI images. For normalization of the functional data, a structural 3D-image was recorded. The data were further processed by realigning/unwarping (SPM8), movement correction (FSL regfilt), filtering of physiological noise (respiratory and cardiac signal, Aztec), slice timing correction (SPM8), spatial normalization to Lehr-Dorr atlas template (SPM8), filtering of CSF-signal (individual threshold masks), global signal regression (FSL regfilt) and band-pass filtering (0.01-0.3 Hz, AFNI). From the functional data, mean time courses of 27 anatomical regions were extracted and pairwise Pearson's correlation coefficient matrices were calculated and analyzed with the brain connectivity toolbox[2,3] (BCT, toolbox for Matlab). By BCT several graph properties for weighted undirected networks were calculated. To assure robust results the graph analysis was repeated for a range of thresholds (the percentage of strongest connections entering the analysis for each animal) between 10% and 38%. Group differences of graph properties were examined by two samples student's T-test. MRS data were acquired by a PRESS-sequence (TR/TE=4000/10 ms) from a voxel in PFC (1.6x1.2x1.3 mm³).

Results

In the quantified MR-Spectra of the PFC we found a trend for increased glutamate and glutamine concentrations in the CFA-group (p<0.09,Fig1) which is in line with our working hypothesis of a hyper-glutamatergic input to the NAc.

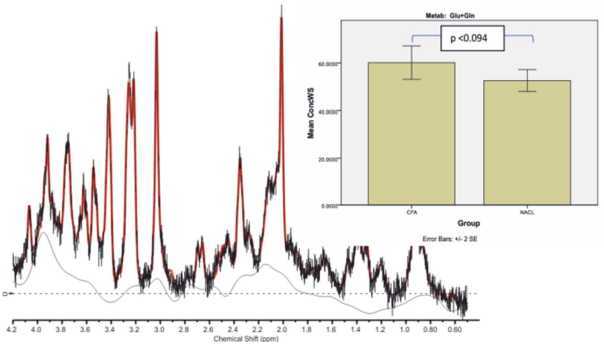

Fig1: Representative spectrum and comparison of average glutamate+glutamine levels for pain and control groups

Group differences of network properties are considered significant, if p<0.05 for a range of threshold values. We found significant differences in 10 brain regions mostly related with mood/stress/reward systems: priaqueductal gray (PAG), pontine nucleus (PN), pre-para subiculum (PS), striatum (St), amygdala, Bed nucleus of stria terminalis (BNST), PFC, interpendencular nucleus (IN), lateral septum (LS) and olfactory bulbs (OB). Fig. 1 shows results for those regions yielding the most significant results. In BNST, LS, PFC and PAG network properties indicating integration with the rest of the network (like degree, strength, betweenness centrality and participation index) show significantly higher values for CFA-treated compared to control group. Correspondingly, properties indicative of segregation (clustering coefficient, local efficiency, path length) are significantly lower for the CFA group in Amygdala, IN, BNST, LS, PFC and PAG. The global network parameters were supporting these findings and showed a significantly lower characteristic path length and clustering coefficient in the CFA-group (data not shown). The results suggest a more global role of these regions, which are associated with mood/stress/reward systems.

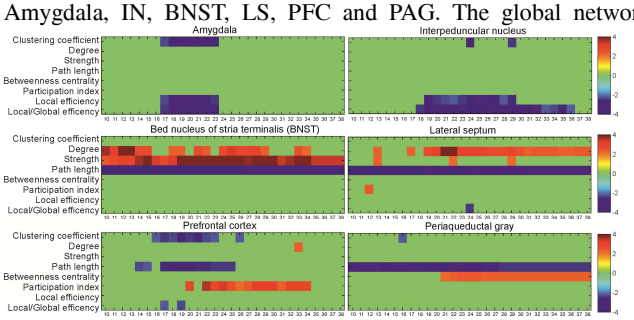

Fig 2: Significant changes in local network features in selected regions (T-value for p<0.05) over a range of cutoffs in CFA-mice vs. controls showing a more global and less local role in the overall network. Cutoffs are the percentage of strongest connections (individually) taken into the graph analysis. Statistical differences are assumed to be robust if they persist over a range of different cutoffs.

Conclusion

The results of this study show that inflammatory pain significantly alters characteristics of brain networks and could also have an influence on brain glutamate and glutamine levels in the prefrontal cortex. Thus, investigating properties of resting state networks and brain biochemistry can be an instrument to further investigate the influence of acute and chronic pain on brain functionality. Furthermore the influence of pain on MRS results lead to MRS as another possible means of investigation.

References

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