Neuroscience 344 (2017) 133–147

CENTRAL SENSITIZATION-RELATED CHANGES OF EFFECTIVE AND FUNCTIONAL CONNECTIVITY IN THE RAT INFLAMMATORY TRIGEMINAL PAIN MODEL

TAMÁS SPISÁK,[a]* ZSÓFIA POZSGAY,[a] CSABA ARANYI,[b] SZABOLCS DÁVID,[a] PÁL KOCSIS,[a] GABRIELLA NYITRAI,[a] DÁVID GAJÁRI,[a] MIKLÓS EMRI,[b] ANDRÁS CZURKÓ[a] AND ZSIGMOND TAMÁS KINCSES[a,c,d]

[a] *Preclinical Imaging Center, Gedeon Richter Plc., Budapest, Hungary*

[b] *Department of Nuclear Medicine, University of Debrecen, Debrecen, Hungary*

[c] *Department of Neurology, University of Szeged, Hungary*

[d] *International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic*

Abstract—Central sensitization is a key mechanism in the pathology of several neuropathic pain disorders. We aimed to investigate the underlying brain connectivity changes in a rat model of chronic pain. Non-noxious whisker stimulation was used to evoke blood-oxygen-level-dependent (BOLD) responses in a block-design functional Magnetic Resonance Imaging (fMRI) experiment on 9.4 T. Measurements were repeated two days and one week after injecting complete Freund's adjuvant into the rats' whisker pad. We found that acute pain reduced activation in the barrel cortex, most probably due to a plateau effect. After one week, increased activation of the anterior cingulate cortex was found. Analyses of effective connectivity driven by stimulus-related activation revealed that chronic pain-related central sensitization manifested as a widespread alteration in the activity of the somatosensory network. Changes were mainly mediated by the anterior cingulate cortex and the striatum and affected the somatosensory and motor cortices and the superior colliculus. Functional connectivity analysis of nested BOLD oscillations justified that the anterior cingular–somatosensory interplay is a key element of network changes. Additionally, a decreased cingulo-motor functional connectivity implies that alterations also involve the output tract of the network. Our results extend the knowledge about the role of the cingulate cortex in the chronification of pain and indicate that integration of multiple connectivity analysis could be fruitful in studying the central sensitization in the pain matrix.

Key words: rodent pain model, fMRI, effective connectivity, functional connectivity, central sensitization.

INTRODUCTION

Repeated or long-lasting, strong nociceptive stimulation is able to sensitize the neurons processing sensory information. These neurons can be at any level of the information processing pathway or in centers of the pain matrix. Central sensitization may be present in a variety of painful conditions such as fibromyalgia, irritable bowel syndrome, endometriosis and primary headache disorders (Bigal et al., 2008; Lipton et al., 2008). The precise neural mechanisms of the development of sensitization are not entirely clear yet, however, sustained activation of the involved neural structures with altered signaling outlasting tissue injury and repair may play a crucial role. Maladaptive plasticity and altered synaptic strengths were suggested in the pathomechanism modulated by multiple intracellular signaling pathways and in the long-term by transcription-dependent mechanisms (Ji et al., 2003). While considerable amount of information is available about the role of the lower level (first-, second- and third-order neurons) centers in sensitization, little is known about the role of higher cortical centers (Treede et al., 1999; Cohen and Mao, 2014). Magnetic Resonance Imaging (MRI) of the brain provided novel insights into the structural and functional characteristics of these regions (Davis and Moayedi, 2013). However, cortical key regions of chorionic pain typically subserve multiple functions, including sensory-discrimination, motivation-affect, motor, attention arousal and response selection functions (Davis and Moayedi, 2013), suggesting that chronic pain-related central sensitization can be considered as maladaptive interactions in the activity of these regions.

In the present study, we hypothesized that chronic pain-related central sensitization can be depicted as altered connectivity mediated by key regions of the nociceptive network. We aimed to identify these key regions involved in central sensitization and to

*Corresponding author. Address: Pharmacology and Drug Safety Research, Gedeon Richter Plc., POB: 27, Budapest 10 H-1475, Hungary. Fax: +36-1-505-7275.
E-mail address: spisakta@richter.hu (T. Spisák).

Abbreviations: BMS, Bayesian Model Selection; BOLD, blood-oxygen-level dependent; CFA, complete Freund's adjuvant; DCM, Dynamic Causal Modeling; FDR, False Discovery Rate; fMRI, functional Magnetic Resonance Imaging; MRI, Magnetic Resonance Imaging; ROI, Region-of-Interest.

http://dx.doi.org/10.1016/j.neuroscience.2016.12.018
0306-4522/

characterize their network-level influence on sensory processing and general network activity in a chronic rodent inflammatory trigeminal pain model.

In our animal model, complete Freund's adjuvant (CFA) was injected into the rat's whisker pad to evoke long-lasting pain. CFA is known to be an immunopotentiator and it is frequently used to study sterile inflammatory reaction-associated pain (Lee et al., 2010). Air-puff stimulation of the rat's whisker pad was used as a non-noxious stimulus to evoke blood-oxygen-level-dependent (BOLD) response in a block-design functional Magnetic Resonance Imaging (fMRI) experiment on 9.4 T. The fMRI measurements were repeated two days (acute effect) and one week (chronification of pain) after injecting CFA into the rats' whisker pad and compared to the baseline measurements. Changes in activation pattern were analyzed in order to identify regions with altered BOLD responses in chronic pain conditions.

To investigate how these areas influence information processing in the somatosensory circuit, we examined network-level activation changes with two complementary connectivity analysis methods. Network structure, dynamics and effective connectivity directly driven by response to stimuli were investigated by Dynamic Causal Modeling (DCM), a technique which is able to infer the causal architecture of coupled or distributed systems of brain regions based on e.g. their response to stimuli (Friston et al., 2003). We also hypothesized that central sensitization not only modulates the propagation of responses to sensory stimuli but also affects subtle "nested" fluctuations not directly time-locked to the stimuli (Lohmann et al., 2010; Baliki et al., 2012). For this purpose, we applied band-pass filtered partial correlation analysis, which rules out signal components directly time-locked to stimuli and shared among network nodes. This method provided an alternative data-driven approach to infer conditional interdependencies altered by chronic pain.

EXPERIMENTAL PROCEDURES

Animals and procedure

Twenty-six drug-naïve adult male Sprague–Dawley rats were used for the experiments. Initial weights of the rats were between 188 and 214 g, and between 289 and 302 g by the end of the study. The mean (± standard deviation) weight was 258(±32) g over all experiments.

The animals were kept in polycarbonate cages in thermostatically controlled room at 21 ± 1 °C. The room was artificially illuminated from 6 a.m. to 6 p.m. The rats were fed with conventional laboratory rat food (sniff R/M + H Spezieldiäten GmbH D-59494 Soest). All of the procedures conformed to the guidelines of the National Institutes of Health for the care and use of laboratory animals and were approved by the local Ethics Committee (Scientific and Research Ethics Committee of the Medical Research Council, Ministry of Health, Medical Research Council, Budapest, Hungary). Experiments were performed and reported according to the ARRIVE guidelines on animal research.

The rats were anesthetized before transferring them to the magnet room. The anesthesia was introduced with 5% isoflurane and then 1.25% during scanning. During the experiment, the body temperature was maintained at 38 ± 1 °C with thermostatically controlled air flow around the rat. The respiration of the animal was monitored continuously with a small pneumatic pillow sensor during the experiment (SA Instruments, Inc., NY, USA).

Each animal was scanned five times. Baseline activation patterns were mapped with three baseline fMRI scans for each animal (5–8 days between scans of the same animal). To induce a local painful reaction (Lee et al., 2010), 100 μl CFA was injected into the rat's left whisker pad. The CFA injection was performed just after the third baseline scan, while the rat was still in anesthesia. The short- and long-term effects of CFA-treatment were investigated by longitudinal fMRI scans performed 48 h and 7–8 days after the treatment.

Image acquisition

The fMRI experiments were performed by a 9.4 T Varian MRI system with a free bore of 210 mm, containing a 120-mm inner size gradient coil (minimum rise time 140 μs, 200 μs were used). For excitation a two-channel volume coil system with inner size 72 mm was used and a fix tuned receive-only phase array rat brain coil (RAPID Biomedical GmbH, Rimpar, Germany) located directly above the dorsal surface of the rat's head to maximize the signal-to-noise ratio.

Proton density-weighted anatomical scans were acquired using gradient echo multi slice (echo time: 3.83 ms, repetition time: 200 ms, flip angle: 45°, averages: 1, dummy scans: 4, data matrix 256 × 256, total scan time 45 s) sequence with a field of view of 40 mm × 40 mm and a slice thickness of 0.6 mm without inter-slice gap (resolution: $0.156 \times 0.156 \times 0.6 mm^3$). Twenty-four slices were acquired in interleaved order. The default horizontal orientation of the scanner was slightly changed to get standard anatomically horizontal sections. Anatomical images were acquired in independent measurements after functional scans to minimize movement-induced blurring. To increase signal-to-noise ratio, anatomical scans were repeated 8 times then averaged into a single image.

An interleaved triple-shot gradient-echo echo planar imaging (echo time: 10 ms, repetition time: 2030 ms, flip angle: 90°, averages: 1, dummy scans: 4, data matrix: 50 × 50, 450 repetitions, filed-of-view: 40 mm × 40 mm, slice thickness: 0.8 mm, no inter-slice gap, 16 horizontal slices) sequence with compressed segments and ramp sampling was used for T2*-weighted MR images (resolution: $0.8 \times 0.8 \times 0.8 mm^3$). Slice orientation was the same as in the anatomical setup, but surpassed with 1–1 slices in the superior and inferior directions to assist the non-linear co-registration during analysis. In order to reduce phase delay errors, functional images were acquired with two opposite gradient polarity, which results in an effective 4060-ms repetition time. Although this correction doubles the effective repetition time (4060 ms), it results in a significant reduction of EPI