

Phenotyping assay of neuropathic pain models using selective stimulation for peripheral nerve fibers

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Purpose For the development of an effective therapy for neuropathic pain, there is a significant hurdle that achieves objective evaluation methods. As an objective evaluation method of neuropathic pain, functional magnetic resonance imaging can provide quantitative activation map of the brain using tactile stimulus. By considering properties of each fiber, stimuli were given to peripheral fiber ($A\beta$ -, $A\delta$ -, C-fiber) selectively to clarify the location of brain function, which made it possible to detailed evaluation of neuropathic pain. In this study, for evaluation of allodynia, we would conduct fMRI using stimulus depolarized $A\beta$ -fiber to the two sorts of allodynia model mouse that was surgical procedure model and genetical model.

Methods Six intact adult mice (C57BL/6, male, approximately 10-week-old: CLEA Japan Inc., Tokyo, Japan), and same mice for spinal nerve ligation model, six *musashi(msi)*^{2^{-/-}} male mice (C57BL/6 background) and the same number of wild type male littermates [1] were used for this study. Functional MRI was performed using a 7.0 tesla MRI (Biospec; 70/16 Bruker BioSpin, Ettlingen, Germany) with a cryogenic quadrature RF surface probe (CryoProbe; Bruker BioSpin AG, Fällanden, Switzerland). T2WI; TE: 48ms, TR: 6100ms, RARE factor: 8, number of averages: 4, spatial resolution: 75 x 75 x 300 (μm)³, number of slices: 52. BOLD fMRI; TE: 20ms, TR: 1000ms, flip angle: 55°, number of averages: 1, spatial resolution: 200 x 200 x 500 (μm)³, number of slices: 16. Thirty minutes before acquisition of fMRI, the anesthesia was altered from isoflurane to medetomidine (Orion Pharma) with following administration (s.c. 0.3 mg/kg bolus, 0.6 mg/kg/h infusion) [2]. This experiment focused on 2,000Hz electric stimulation: this stimulation selectively depolarizes $A\beta$ fiber specifically, which does not normally provoke pain. To perform a group data analysis of fMRI, a results was created using SPM8 (Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, London, UK) and tailored software in MATLAB. The threshold for this statistics was $p < 0.05$ (corrected by Family wise error rate using Bonferroni's approach).

Results Preoperative and postoperative fMRI were shown as Cyan and Magenta, respectively (Figure 1). The stimulation of 2,000Hz to healthy mice elicited activation only in contralateral S1, whereas the stimulation to neuropathic pain model mice elicited activation in ACC, thalamus besides S1. fMRI in *msi*^{2^{-/-}} and *msi*^{2^{+/+}} mice were shown as Green and Magenta, respectively (Figure 2). For the measurement of normal mice, BOLD activation was observed only in S1. On the other hand, for the measurement of knockout mouse, BOLD activation was observed in ACC and thalamus in addition to S1.

Discussion In accordance with the development of neuropathic pain, functional MRI detected the activation in the ACC, which was not observed preoperatively. Consistently, in functional MRI, We observed activation of ACC in *msi*^{2^{-/-}} mice, which is not activated in wild mice. Decrease of pleiotrophin, neurite outgrowth-promoting factor, which is one of target gene of *msi*², is expressed in *msi*^{2^{-/-}} mouse. Abnormal projection in sensory fibers ($A\beta$ fibers), which input to spinal cord from dorsal root ganglion during development, is associated with allodynic phenotype.

Conclusion Their brain activities with stimulus were observed and difference of activities in ACC was detected. Precise method of stimulus enables to conduct objective evaluation. Establishment of novel pain evaluation tool by fMRI would contribute to realization of regenerative medicine for neuropathic pain.

References [1] Shibata S, Okano H, Neuroscience Meeting. 2010.

[2] Adamczak JM, Farr TD, Seehafer JU, Kalthoff D, Hoehn M. High field BOLD response to forepaw stimulation in the mouse. Neuroimage 2010;51(2):704-712.

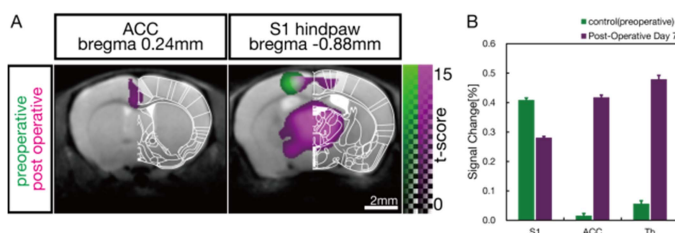


Figure 1. BOLD activation maps (A) and signal change (B) with left hindpaw stimulation of neuropathic pain model. Green: preoperative, Magenta: POD 7 ($P < 0.05$ FWE corrected). Scale bar is 2mm.

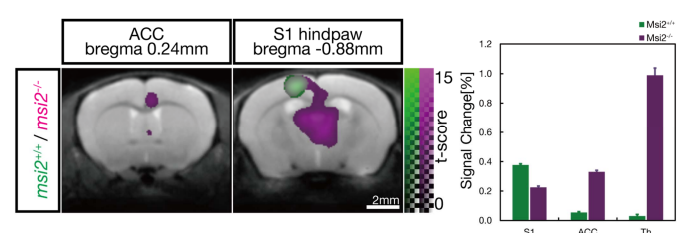


Figure 2. BOLD activations represented by a map (A) and value of BOLD signal change (B) with left hindpaw stimulation of *msi*^{2^{+/+}} and *msi*^{2^{-/-}} ($P < 0.05$ FWE corrected). Green: *msi*^{2^{+/+}}, Magenta: *msi*^{2^{-/-}}.