A BOLD-fMRI study of allodynic pain evoked by green laser stimuli of rats

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Introduction: Fibromyalgia and neuropathic disorders characterized by chronic pain induce the pathological condition "allodynia", in which a stimulus that is normally not painful causes pain sensations. The quality of life for patients is severely impaired by allodynia, and thus the elucidation of the pathogenic mechanism underlying the chronic pain and the development of therapeutic agents are eagerly sought. The establishment of an experimental system applicable for both clinical and preclinical studies is required, from the viewpoint of translational science. In current pain research, mechanical forces, chemical reagents such as formalin, or electrical pulses are used for stimulation, but the fine-tuning of pain strength and local stimulation is difficult. A 532 nm green laser is transparent to water molecules and easy to use for local stimulation without burn injury and direct contact, and thus is ideal for allodynia studies. Recently, a "reserpine-induced myalgia (RIM) rat", an animal model of fibromyalgia manifested by chronic muscular pain together with tactile and heat allodynia, was developed, and is expected to facilitate research on fibromyalgia [1]. The aim of this study is to reveal the brain activities responding to allodynic pain evoked by green laser stimuli on RIM or normal rats, based on Blood Oxygenation Level Dependent (BOLD) signal changes, by a non-invasive procedure at high spatial resolution.

Methods: Sprague Dawley rats (8 weeks old) were converted to RIM rats according to the reported method [1]. The tactile allodynia thresholds for all rats were determined by von Frey hair tests. To determine the 532 nm laser output power and duration time for the laser-evoked BOLD experiments, laser-evoked behavior tests were performed (Fig. 1). Three RIM and three control rats were anesthetized with urethane 1.25 g/kg i.p. in a N_2/O_2 70/30 mixture. The left hind paws of the rats were irradiated with the green laser under various laser output power conditions (200–500 mW by 50 mW steps) and duration times (1, 2 or 3 s). The escape behaviors of the irradiated left hind paws were monitored visually. Five RIM and five control rats were used for laser-evoked BOLD experiments. The rats were first anesthetized with isoflurane (5% for induction and 2% formaintenance during surgery) for femoral arterial and venous cannulations to monitor the arterial blood pressure and to administer gallamine, respectively.The rats were tracheotomized, artificially ventilated, and treated with gallamine.After surgery, the anesthesia was switched to urethane 1.25 g/kg. MRI experiments were performed with a 7.0 Tesla Bruker Biospec 70/20 scanner and a rat brain 4-channel phased array surface coil. Functional data were acquired with a 4-shot GRE-EPI sequence (TR 500 ms, TE 15 ms, FA 45°, 13 slices, 0.6 mm slice thickness, matrix 64 x 64, FOV 2.56 x 2.56 cm). After 150 images were obtained, the green laser (350 mW, 2 s) was used to irradiate the left hind paws, and the scan was continued for 300 images. Blood samples were obtained before and after the measurement, and the arterial blood gas values were within the normal physiological range. The BOLD signal intensity and the t-map were analyzed with the SPM8 software with Matlab, to identify the activated brain regions.

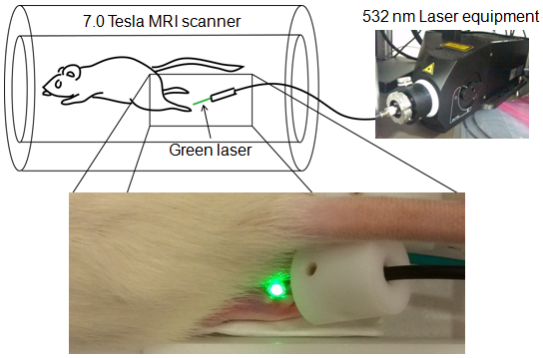

Fig. 1 Schematic diagram of laser-evoked BOLD experiments. An optical fiber was set in an MRI scanner for laser irradiation.

Results: In the von Frey hair tests, the tactile allodynia thresholds were decreased for all tested RIM rats, but not for the control rats. In the laser-evoked behavior tests, a 2-s laser irradiation triggered escape behaviors of the irradiated left hind paws of the RIM rats, under the conditions of 350 mW and more output power, while the escape behaviors of the control rats were triggered under the conditions of 450 mW and more output power. Burn injury was not observed for both the RIM and control rats under the conditions of 350 and 400 mW output powers (2 s duration time). In the laser-evoked BOLD experiments for the RIM rats under the conditions of 350 mW output power and 2-s duration, positive BOLD responses were observed in the cingulated cortex (Cg) (4% signal enhancement), the primary somatosensory cortex (S1) (3%), the secondary somatosensory cortex (S2) (3%), the insula cortex (IC) (3%), and the thalamus (TH) (2%) (Fig. 2). In contrast, no positive BOLD responses were observed in the control rats.

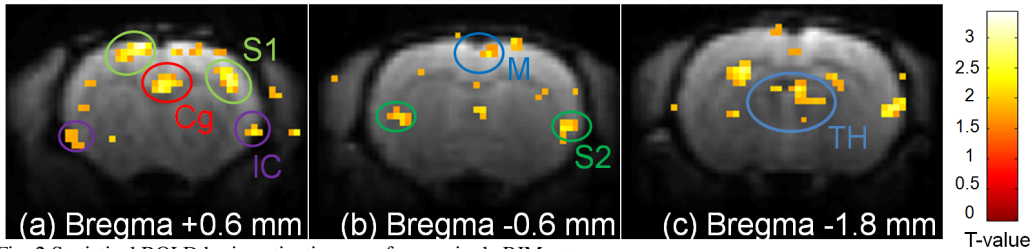

Fig. 2 Statistical BOLD brain activation map from a single RIM rat. Brain activation regions evoked by the green laser (350 mW, 2 s), mapped on the anatomical image (Cg, cingulate cortex; S1, primary somatosensory cortex; S2, secondary somatosensory cortex; M, motor cortex; IC, insula cortex; TH, thalamus).

Discussion: A group of brain structures including Cg, S1, S2, and IC, referred to as the "Pain Matrix" [2], was jointly activated by painful stimuli. The activation of these brain regions was observed in our laser-evoked BOLD experiments, which revealed that the laser-evoked pain was detectable by our BOLD-fMRI procedure, without burn injury or direct contact. Positive BOLD responses were observed for the RIM rats, but not for the control rats, thus demonstrating that allodynia-specific responses were induced by the laser stimulation.

Conclusions: We successfully observed allodynia in an animal model of fibromyalgia, by the combined use of the 532 nm green laser and BOLD-fMRI. The green laser greatly facilitates the elucidation of the allodynia-specific neural circuits, since it can execute non-contact stimulation without neural activation by direct contact, unlike other existing stimulation methods. Our experimental system is designed to be applicable for clinical use, and thus is expected to provide a robust clinical and preclinical evaluation system for new analgesic agents by combined use with connectivity analyses applying resting state fMRI.

References: [1] Nagakura, Y. *et al.*, *PAIN*, **146**, 26–33 (2009), [2] Garcia-Larrea L. & Peyron R., *PAIN*, in press (2013)