Trial of Objective Assessment of Nociceptors-Pain Using Functional MRI in the Brain
— Assessment by the fMRI Signal at Lateral Sulcus —1)

NAGANO Yoshihiko’, NAGANO Michiharu++, TOKUNAGA Fumio‘‘, and SEKI Takaharu‘***

‘ KOBE Co-Medical College, Kobe, Hyogo, Japan
‘‘ Inspection Office of Medical Radiological Technology, Osaka city gov., Osaka, Japan
‘*** Graduate School of Science, Osaka University, Suita, Osaka, Japan
‘**** Research Center for Teacher Education, Osaka Kyoiku University, Kashiwara, Osaka, Japan

(Received March 31, 2014)

An objective measure for assessing pain has yet to be established. We quantitatively applied heat stimulation around the heat pain thresholds (about 43 and 52℃) for two subtypes of a thermal pain nociceptor to the anterior part of the left foot joint, and qualitatively investigated brain regions in which functional magnetic resonance imaging (fMRI) signals occurred based on the intensity and spread of the signals. The intensity of subjective pain to heat pain stimulation compared with that to puncture stimulation at 100 gW was comparable at 53℃ and less than half at 46℃. No subjective pain occurred with heat stimulation at 35℃. In response to heat pain stimulation at 53℃, fMRI signals occurred in the contralateral lateral sulcus and contralateral primary somatosensory area (S1) in all 4 participants. With heat pain stimulation at 46℃, fMRI signals in the contralateral lateral sulcus were observed in all participants, whereas those in the contralateral S1 occurred in 2 of 4 participants. The intensity and spread of fMRI signals in the contralateral lateral sulcus due to heat pain stimulation at 53℃ were comparable to those caused by 100 gW puncture pain stimulation. These results strongly suggest that fMRI signals in the contralateral lateral sulcus can serve as objective images to assess pain.

Key Words: Functional magnetic resonance imaging (fMRI), Pain, nociceptor, lateral sulcus

1) Portions of this manuscript were presented at the 69th Annual Scientific Congress of the Japanese Society of Radiological Technology held in April 2013, Yokohama, Japan.
is being conducted in various directions to clarify the relation between pain stimulation and functional brain responses, by making full use of the methods for testing brain function, which have become possible because of the recent advances in medical technologies [1–3]. Among these technologies, functional magnetic resonance imaging (fMRI) is a promising method for testing brain function because it is non-invasive and characterized by the ability to image the entire brain, including the deep brain regions [4–5]. Here we attempted to capture images of regions with functional brain responses derived from pain stimulation, by using the blood oxygenation level dependent (BOLD) technique. We quantitatively applied heat stimulation or 100 gW puncture stimulation (hereinafter referred to as puncture pain stimulation) to the skin, with the former focused on about 43 and about 52°C [6–8], the response thresholds to heat pain stimulation of the transient receptor potential vanilloid receptor 1 (TRPV1) and the transient receptor potential vanilloid receptor 2 (TRPV2), respectively, which are 2 subtypes of a recently discovered receptor for heat and pain (e.g., nociceptive pain) stimulation. We report results that suggest the possibility of objectively assessing pain states.

II METHODS

1. Apparatus

The MRI scanner used in this study was a Philips Achieva 1.5 T. An 8-channel SENSE head coil was used as the receiver coil. An instrument to apply heat pain stimulation, a PVC container (350 mm × 180 mm), in which 1500 mL of warm water was enclosed, was used; the water temperature was adjusted by a double calibration method involving the use of an infrared digital thermometer and an analog thermometer. For puncture pain stimulation, we fabricated a puncture stimulation device (220 mm × 130 mm) of non-magnetic material, made from lead with a toothpick glued to the tip of its arm. A metal detector to confirm that the participants did not carry any metal was used.

2. Participants

The participants were all males, with 1 individual in his 30s, 2 in their 40s, and 1 in his 50s, and selected excluding individuals with pain, unable to feel pain, or with a brain disease. Regarding the treatment of images, informed consents were obtained from all participants in advance. We thoroughly explained that all personal information would be excluded at the image assessment stage, that the images would not be used for any purpose other than the present study, and that there would be no disadvantageous consequences for the participants. At the time of image assessment, the participants were anonymized so that evaluators could not identify the participants. In addition, a permission for this experiment was obtained from the laboratory chief.

3. Stimulation Techniques

For heat pain stimulation, heat stimuli of 35 and 46°C, below and above, respectively, the response threshold (43°C) for heat nociceptor, TRPV1, and 53°C above the response threshold (52°C) of TRPV2 were applied randomly and without pressure to the anterior part of the left foot joint. For puncture pain stimulation, a toothpick puncture stimulus with a fixed load of 100 gW was applied to the same area. An eye mask and earplugs were used to block visual and auditory, respectively, sensory stimuli, from the surrounding environment. To exclude volitional brain activity (thoughts, visions, hallucinations, etc.), we attempted to unify the participants’ awareness by having them look directly at a “broad sheet of white paper” for 30 s before each fMRI measurement and then image the white paper during the measurement. Furthermore, because fMRI is vulnerable to motion artifacts, this was fully explained to the participants and their bodies were adequately stabilized.
4. Imaging Technique

The BOLD technique (Philips IView Bold) was used for fMRI, and software accompanying the device was used for analysis. Data groups with and without pain stimulation served as the conditions for the task and rest block design, respectively. The number of repetitions was 4 runs at 30-s intervals. For the imaging sequence, the echo planar imaging (EPI) technique was used with the following parameters: slice thickness, 6 mm; gap length, 0 mm; echo time (TE), 50 ms; repetition time (TR), 3000 ms; effective field of view (FOV), 230 mm; flip angle, 90°; matrix size, 64 × 64; and number of slices, 21. The images were obtained in the sagittal direction, and the whole brain was imaged by aligning the central position to the midline sagittal section. For fMRI observation conditions other than those specified by the scanning device (window width, level, and regions of interest [ROIs]), we used settings (window width, 3; level, 2.0; ROIs, 150) with which the motor area — a site where brain function is localized and the appearance of fMRI signals is currently evident [4] — and presupplementary motor area could be clearly observed.

5. Methods to Evaluate Subjective Pain

Pain was subjectively evaluated by Melack’s McGill Pain Questionnaire [9] and the Visual Analog Scale [10]. In the former, words describing the participants’ pain are grouped by 3 aspects (sensory, emotive, and evaluative), each scored on a 5-point scale, and the total score (out of 15 points) is used to evaluate the pain. In the latter, pain is evaluated in 10 stages ranging from no pain to unbearable pain.

6. Slice Planes for Signal Evaluation

The slice planes for signal evaluation were as follows: 6 mm to the right of the midline, which includes the primary somatosensory area (hereinafter referred to as S1) [11-13] contralateral to the stimulation (hereinafter referred to as contralateral), the site where the brain response to pain is thought to be localized; and 48 mm to the right of the midline, which includes the site at which strong signals commonly appeared across participants.

7. Method to Evaluate fMRI Signals

The signal intensity was displayed in terms of the voxel signal level, which ranges from weak (red) to strong (yellow) and is obtained from the BOLD software that accompanied the scanning device. Image processing software (GIMP) was used to determine the spread of the signal by tallying the number of 0.53 mm × 0.53 mm grid squares within a signal targeted for evaluation. The spread was defined as small, medium, or large when this number was 19 or less, 20–39, or 40 or more, respectively.

III RESULTS

1. Subjective Pain

The subjective pain of participants in response to heat stimulation (35, 46, and 53°C) of the anterior part of the left foot joint and to puncture pain stimulation is shown in Table 1. No subjective pain was reported with heat stimulation at 35°C. However, when the temperature exceeded the TRPV1 threshold (43°C), all participants reported moderate pain. When the temperature exceeded the TRPV2 threshold (52°C), the heat stimulation induced a strong pain comparable to that of puncture pain stimulation; the results show that the subjective pain score was at least 2.5 times greater than that due to heat pain stimulation at 46°C.
Table 1 Subjective pain score.

<table>
<thead>
<tr>
<th>Method</th>
<th>Heat stimulation</th>
<th>Puncture Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35°C</td>
<td>46°C</td>
</tr>
<tr>
<td>Subject</td>
<td>MPQ</td>
<td>VAS</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>


2. Identification of Sites Where fMRI signals Appear

The site in the slice plane 48 mm to the right of the midline, in which a strong signal appeared in response to heat pain stimulation at or above threshold in all participants, was identified as the contralateral lateral sulcus by superimposing a Brodmann area map [14] on a standard anatomical chart [15] (Figure 1a, b). Appearance of an fMRI signal in the contralateral lateral sulcus by heat pain stimulation at 53°C in the same participants also confirmed the reproducibility of this result (Figure 1a, 2c). No fMRI signal was observed in the lateral sulcus ipsilateral to the stimulation.

![fMRI images of contralateral lateral sulcus selected for this pain assessment.](image)

Figure 1  fMRI images of contralateral lateral sulcus selected for this pain assessment.

a) The anatomical location of lateral sulcus identified with the place where the signal appeared.

b) The fMRI image piled up with the standard anatomical chart in order to pinpoint a fMRI signal place.

3. Signals in Contralateral S1 and Contralateral Lateral Sulcus

Figure 2 shows fMRI measurement images in the contralateral lateral sulcus (a–d) and the contralateral S1 (e–h) of participant A due to heat pain stimulation (a–c, e–g) and puncture stimulation (d,h). With heat stimulation at 35°C, fMRI signals did not appear in the contralateral lateral sulcus or contralateral S1 (Figure 2a, e). With heat pain stimulation at 46°C, an fMRI signal was clearly observed in the contralateral lateral sulcus (Figure 2b) but not in the contralateral S1 (Figure 2f). With both heat pain stimulation at 53°C and puncture pain stimulation, fMRI signals appeared in both the contralateral lateral sulcus (Figure 2c, d) and the contralateral S1 (Figure 2g, h). The fMRI signal measurements of all 4 participants are summarized in Table 2. In the contralateral lateral sulcus, fMRI signals did not appear with heat stimulation (at 35°C) below the TRPV1 threshold; however, they were confirmed in all participants with heat pain stimulation at 46°C and beyond the TRPV2 threshold at 53°C. In addition, the fMRI signals due to heat pain stimulation at 53°C were comparable in terms of both their intensity and spread to those due to puncture pain stimulation. In contrast, in the contralateral S1, fMRI signals were observed in 2 of the 4 participants with heat pain stimulation at 46°C, and in all participants with heat stimulation at 53°C as well as puncture pain stimulation. However, the signal intensity was weaker and the spread of the signal narrower than in the contralateral...
lateral sulcus.

<table>
<thead>
<tr>
<th>Stimulation Subject</th>
<th>Around the contralateral lateral sulcus</th>
<th>Around the contralateral S1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35°C</td>
<td>46°C</td>
</tr>
<tr>
<td>A</td>
<td>Weak</td>
<td>Weak</td>
</tr>
<tr>
<td>B</td>
<td>Weak</td>
<td>Medium</td>
</tr>
<tr>
<td>C</td>
<td>Medium</td>
<td>Small</td>
</tr>
<tr>
<td>D</td>
<td>Medium</td>
<td>Medium</td>
</tr>
</tbody>
</table>

### Figure 2
Subject A’s fMRI images by which the pain was evaluated.

- a, b, c, d: The fMRI images including contralateral (right) lateral sulcus.
- e, f, g, h: The fMRI images including contralateral (right) primary somatosensory cortex (S1).

### Table 2
Strength and spatial spread of fMRI signals induced by heat-or puncture-pain stimulation.

- "-": No signal.

### IV DISCUSSION

Regarding the localization of brain functions in response to pain, the following observations have been made. By investigations based on positron emission tomography (PET), in response to heat stimulation of the skin, increased blood flow in areas restricted to regions including the contralateral anterior cingulate gyrus, somatosensory area, island rear, secondary somatosensory cortex, and thalamus [11][16]. It is presented that S1 has the ability to distinguish the strength of pain stimulation [13]. The changes in electric potential are observed by electrodes implanted in the lateral sulcus [17], and fMRI can be used to assess pain triggered by noxious heat stimulation in normal healthy individuals [18].

In the present study, we applied heat pain stimulation, around the thresholds for the heat pain responses of 2 subtypes of a thermal nociceptor, to the anterior part of the left foot joint. We found that, similar to our result, the heat pain threshold shown by Wager et al. [18], 47°C, exceeds the TRPV1 response threshold. By the heat stimulation at 46°C, in our experiments, the fMRI signal at the contralateral S1 was observed by 2 of four participants. In addition, the intensity and spread of the fMRI signals at 46°C were poor, compared...
to the intense subjective pain stimulation at 53℃. Therefore, the objective assessment of pain by the fMRI signal at the contralateral S1 was difficult.

In contrast, with heat pain stimulation at 53℃, which exceeds the TRPV2 response threshold, fMRI signals comparable in strength and spread to those with puncture pain stimulation appeared in the contralateral lateral sulcus. In addition, the subjective pain due to heat pain stimulation at 53℃ was comparable to that due to puncture pain stimulation, and fMRI signals in the contralateral lateral sulcus were observed in all 4 participants. These results strongly suggest that the fMRI signals at the contralateral lateral sulcus can serve as objective imaging data to assess pain.

Furthermore, for the two different types of pain stimulation, 53℃ heat and 100 gW puncture, both showed response localization in nearly the same position in the contralateral S1 and contralateral lateral sulcus. These results suggest the possibility that in the brain, both stimuli applied to TRPV2 are processed as the same pain information. To confirm this possibility, future research will be needed with greater numbers of participants and using high static field MRI scanners.

V CONCLUSION

In this study, we showed that heat pain stimulation at 53℃ (in excess of the response threshold for TRPV2) and puncture pain stimulation gave rise to comparable levels of intense subjective pain, and that fMRI signals with comparable intensity and spread appeared in the same position in the contralateral lateral sulcus in response to both stimuli. These fMRI images suggest strongly that the presence and absence of pain can be objectively assessed.

In addition, we found that two different types of pain stimulation, 53℃ heat and 100 gW puncture, both caused fMRI signals to appear in nearly the same position in the contralateral S1 and contralateral lateral sulcus. These results suggest the possibility that the 2 types of stimuli applied to TRPV2 are processed in the brain as the same pain stimulation.

VI ACKNOWLEDGEMENTS

In preparing this report, we received guidance and advice on references and report preparation from Dr. Satoshi Nakano, Head of the Department of Neurology, Osaka City General Hospital, and on editing from Dr. Masatsugu Kimura, Associate Professor, Graduate School of Medicine, Osaka City University, as well as Dr. Nobumichi Nagano, Nippon Bunri University. In addition, for the experiments, we received the cooperation of physicians in charge of MR as well as the radiology technicians at the Central Radiology Department of the said hospital. We express our sincere gratitude and appreciation.

REFERENCES

脳の機能的磁気共鳴撮像（fMRI）による侵害受容器性疼痛の客観的評価の試み
外側溝fMRI信号での評価

我々は、熱痛み刺激受容体の2つのsubtypeの熱痛み反応閾値（約43℃および約52℃）前後の熱刺激を、定量的に左足関節前部に与え、脳の機能的磁気共鳴撮像（fMRI）信号が表出される部位を、信号の強度と広がりにより定性的に調べた。熱痛み刺激での主観的痛みの強さは、100gW穿刺刺激に比べ53℃熱痛み刺激では同程度、46℃熱痛み刺激で1/2以下であった。53℃熱痛み刺激に対し、全対象者（4名）で刺激対側外側溝および対側1次体感野（S1）のfMRI信号が表出された。前者におけるfMRI信号は、100gW穿刺痛み刺激によるfMRI信号と同程度の強さと広がりを示した。以上より、刺激対側外側溝のfMRI信号が疼痛評価の客観的画像となり得る可能性が強く示唆された。

キーワード：機能的磁気共鳴撮像（fMRI）、疼痛、熱痛み刺激受容体、外側溝