

Neural correlates of perceptual difference between itching and pain: A human fMRI study[☆]

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It has been wondered why we can discriminate between itching and pain as different sensations. Several researchers have investigated neural mechanisms underlying their perceptual differences, and found that some C fibers and spinothalamic tract neurons had different sensitivity between itching and pain. These findings suggest that such differences in ascending pathways are partly associated with perceptual difference between itching and pain. However, it was still unclear how our brains distinguish itching from pain. Thus, by functional magnetic resonance imaging (fMRI) time series analysis, we investigated the neural substrates of perceptual differences between itching and pain. The anterior cingulate cortex, the anterior insula, the basal ganglia and the pre-supplementary motor area were commonly activated by itching and pain. Neural activity in the posterior cingulate cortex (PCC) and the posterior insula associated with itching was significantly higher than that associated with pain and significantly proportional to itching sensation. Pain, but not itching, induced an activation of the thalamus for several minutes, and neural activity of this brain region significantly correlated to pain sensation. These findings demonstrate that the difference in the sensitivity of PCC, the posterior insula and the thalamus between itching and pain would be responsible for the perceptual difference between these sensations. The previous itching studies did not observe an activation of the secondary somatosensory cortex (S2) by itching. However, we observed that an activation of S2 by pain was

not significantly different from that by itching, indicating that S2 was associated with not only pain but also itching.

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Introduction

Itching and pain are unpleasant sensations with clearly different perceptions. Although the perceptive discrimination between itching and pain is clear for us, its neural mechanism is little understood. In 1922, von Frey proposed that an itching sensation is induced by a low-frequency excitation of nociceptors that also mediate a pain sensation (von Frey, 1922). Thereafter, several studies have been conducted to clarify the neural mechanisms of itching and pain sensations. An itching sensation is associated with the excitation of peripheral C fibers induced by histamine (Tuckett and Wei, 1987; Simone et al., 1987; Torebjörk, 1974). Because peripheral C fibers also mediate nociceptive input, researchers have assumed that an itching sensation is conveyed by specific peripheral C fibers. Handwerker et al. (1991) investigated the differences in the peripheral neural mechanism between itching and pain by recording peripheral C fibers using microneurography. They, however, did not find any difference in discharge patterns encoding itching and burning pain sensations evoked by histamine and mustard oil, respectively. Thereafter, Schmelz et al. (1997, 2003) found that there were some C fibers selective for pruritogens (e.g. histamine). In addition, electrophysiological studies have shown that there are spinothalamic tracts (STTs) selectively sensitive to pruritogens and

[☆] Investigation on how our brains discriminate between itching and pain as different sensations.

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those sensitive to both pruritogens and algogens (Andrew and Craig, 2001; Simone et al., 2003; Jinks and Carstens, 2000). It is suggested that those different sensitivities in C fibers and STT neurons are associated with perceptual difference between itching and pain. On the other hand, recently, functional neuroimaging techniques, such as positron emission tomography (PET), have been used for clarifying the neural mechanism of itching in the brain. PET studies of itching showed that itching does not activate the thalamus and the secondary somatosensory cortex (S2) (Drzezga et al., 2001; Darso et al., 2000; Hsieh et al., 1994), whereas previous pain studies demonstrated that these brain regions are involved in pain processing (Peyron et al., 2000). These findings support the notion that the thalamus and S2 are important components for pain perception. Unfortunately, previous itching studies failed to show clear evidence for the central mechanism of itching distinct from that of pain. Therefore, it is also uncertain how the perceptual difference between itching and pain is represented in the brain. Previous itching studies simply evaluated brain activation induced by a pruritic stimulus. Such an experimental design would be too simple to identify the brain regions playing important roles in itching perception. One possibility is that the brain regions associated with itching might be identified by directly comparing between brain activities under the pruritic and the painful stimulus conditions. Another possibility is a time course analysis. An itching sensation lasts for several minutes and its intensity changes with time. The brain regions reflecting such phenomena as neural activity would be important for itching perception. Unlike PET, functional magnetic resonance imaging (fMRI) enables the visualization of the time course of brain activity. Therefore, in this study, by 3 T fMRI, we compared brain activities under the pruritic and the painful stimulus conditions to clarify the neural substrates of itching and pain.

Methods

Subjects

Fourteen healthy male volunteers (mean age \pm SD, 26 \pm 5 years old) participated in this study. Subjects with a history of allergy, atopic eczema or other dermatological diseases were excluded from the study. None of the subjects were under any medication nor had any history of psychiatric disorders. All of the subjects were evaluated as right-handed based on the Edinburgh Inventory (Oldfield, 1971). A written informed consent was obtained from each subject, and the study was performed in compliance with the relevant laws and institutional guidelines. This study was approved by the institutional review boards of Tohoku University and National Institute for Physiological Sciences.

Stimuli

Itching session: 2 ml of histamine dissolved in saline (0.1%) (histamine solution) was infiltrated into a square electrode pad (2 cm \times 2 cm) and attached to the subject's left wrist. An itching sensation was induced by electrical subcutaneous infusion of the histamine solution with an iontophoresis system (UI-2060, Uni-flows, Japan; histamine-iontophoresis stimulus) (current: 0.5 mA; duration: 20 s). As a baseline, saline was also administered similarly using the square electrode pad infiltrated with saline and iontophoresis.

Pain session: A water pack (25 °C) and an ice pack (0 °C) (2 cm \times 2 cm) were used in the cold and the cold-pain stimulus

conditions, respectively. These stimuli were given on the subject's left wrist.

fMRI measurement

The fMRI experiment was conducted using a 3.0 T MRI scanner (MAGNETOM Allegra, Siemens, Erlangen, Germany). Functional images (fMRI data) were acquired using a T2*-weighted echo planar imaging sequence (repetition time/echo time/flip angle/field of view/voxel size/slice number = 3000 ms/30 ms/80°/192 mm/3.0 \times 3.0 \times 3.0 mm/36 axial slices). A high-resolution structural image was acquired using a magnetization-prepared rapid acquisition in gradient echo (MPRAGE) sequence.

Data acquisition

fMRI scan was started 1 min and 18 s before applying each stimulus. In our pilot study, an itching sensation lasted about 6 min after giving the histamine-iontophoresis stimulus using 0.1% histamine solution. Therefore, the duration of fMRI scanning was 6 min and 12 s. In total, fMRI scans were performed for 7 min and 30 s in each stimulus condition. The sequence of the sessions was randomized and the time interval between sessions was more than 2 h in order to eliminate the effect of the previous stimuli. The subjects took a rest outside of the fMRI room between the sessions. In each session, the baseline stimulus condition (the saline and the cold stimuli) was performed before the target stimulus condition (the histamine and the cold-pain stimuli) with over a half-hour interval. During fMRI scanning, the subjects were instructed to look at a digital watch projected through a liquid crystal display projector (DLA-M200L; Victor, Yokohama, Japan) and score the itching (in the histamine and the saline stimulus conditions) and the pain (in the cold-pain and the cold stimulus conditions) sensations every 10 s on a 0-to-10 scale with their right fingers. To eliminate brain activity associated with continuous scoring of the itching and pain sensations, the subjects were asked not to score the itching and the pain sensations in the mind continuously during fMRI scans. A score of "0" indicated no itching or pain sensation. When the itching and pain sensations were most severe, the score was "10".

Functional image processing

The first 3 volumes of each fMRI session were discarded due to unsteady magnetization, and the remaining 146 volumes per subject were used for the analysis. The data were analyzed using statistical parametric mapping (SPM99; Wellcome Department of Cognitive Neurology, London, UK) (Friston et al., 1995a,b). Following the slice-timing correction and realignment of the fMRI data, the high-resolution structural image was coregistered to the fMRI data. The parameters for affine and nonlinear transformation into the standard stereotaxic space (Montreal Neurological Institute [MNI] template) were estimated using the high-resolution structural image with least squares means. The parameters were then applied to the realigned fMRI data. The anatomically normalized fMRI data were filtered using a Gaussian kernel of 8 mm (full-width at half-maximum) in the *x*, *y*, and *z* axes.

Brain regions activated by itching

In the histamine stimulus condition, we applied two boxcar functions. One was electrical stimulus-related neural activity, for

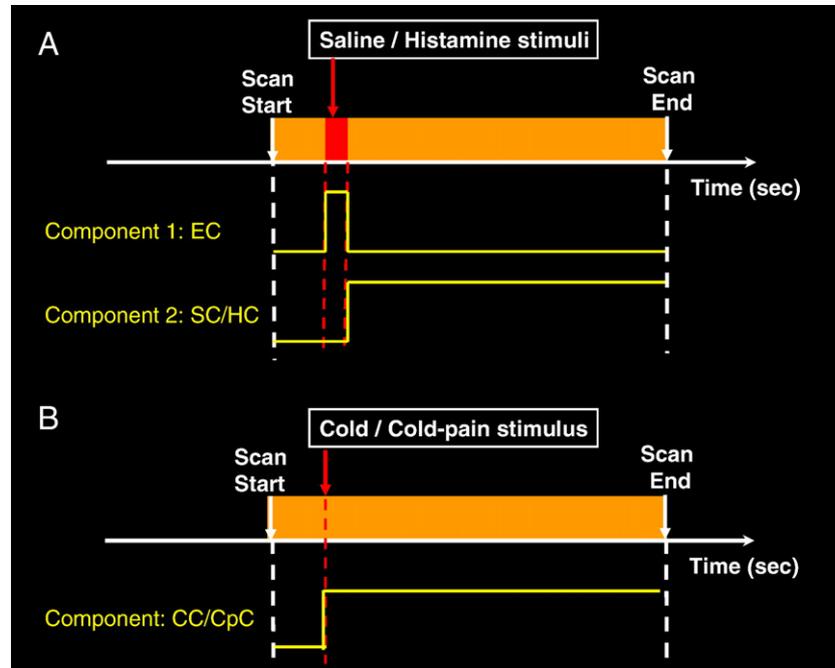


Fig. 1. Time course of neural activity in each condition. (A) Itching session. We hypothesized that the time course of neural activity in the histamine stimulus condition can be divided into two components. One is iontophoretic stimulus-related neural activity (electric component: EC), and the other is histamine-related neural activity (histamine component: HC). The same functions (EC and SC: saline component) were used for the saline stimulus condition. (B) Pain session. Cold and cold-pain components (CC and CpC, respectively) reflect the time courses of neural activity using the cold and the cold-pain stimuli, respectively.

which the onset of the boxcar of the electrical component (EC) was at 1 min and 18 s from the beginning of fMRI scans and its duration was 20 s (corresponding to the duration of iontophoretic stimulus) (Fig. 1A). The other was the histamine stimulus-related activity, for which the onset of the boxcar of the histamine component (HC) was at 1 min and 38 s from the beginning of fMRI scans (immediately after the iontophoretic stimulus) and its duration was 6 min and 12 s (Fig. 1A). The same functions were also used for the saline stimulus condition (EC and saline component (SC)) (Fig. 1A). First, we made a statistical parametric map related to HC in each subject (first-level analysis). In first-level analysis, in order to eliminate baseline drifts of fMRI signals within a condition, we applied a high pass filter (cutoff period: 512 s). After that, we performed group data analysis (one-sample t -test). The threshold was false discovery rate (FDR) $p=0.05$ ($Z=2.79$). With this analysis, we identified the brain regions continuously activated after giving the histamine stimulus. Subsequently, images of parameter estimates for the contrast [HC – SC] were created for each subject (first-level analysis). In this first-level analysis, we applied a high pass filter (cutoff period: 512 s) to eliminate baseline drifts of fMRI signals within each condition. In addition, we also scaled voxel values for that a mean voxel value in a whole brain was 50 in each condition (grand mean scaling). We corrected baseline differences of fMRI signals between conditions by grand mean scaling. Then, we performed group data analysis (one-sample t -test). The brain areas for this analysis were limited to those associated with HC, because we would like to identify brain regions significantly activated by itching. The threshold was FDR $p=0.05$ ($Z=1.75$) (Genovese et al., 2002).

Brain regions activated by pain

In the cold-pain stimulus condition, the onset of the boxcar was at 1 min and 18 s from the beginning of fMRI scans and its duration was 6 min and 22 s (cold-pain component (CpC)). The same function was also used for the cold stimulus condition (cold component (CC)) (Fig. 1B). First, we constructed a statistical parametric map related to CpC in each subject (first-level analysis). In first-level analysis, in order to eliminate baseline drifts of fMRI signals within a condition, we applied a high pass filter (cutoff period: 512 s). After that, we performed group data analysis (one-sample t -test). The threshold was FDR $p=0.05$ ($Z=2.79$). With this analysis, we identified the brain regions continuously activated after giving the cold-pain stimulus. Thereafter, images of parameter estimates for the contrast [CpC – CC] were created for each subject (first-level analysis). In this first-level analysis, we applied a high pass filter (cutoff period: 512 s) to eliminate baseline drifts of fMRI signals within each condition. In addition, we also scaled voxel values for that a mean voxel value in a whole brain was 50 in each condition (grand mean scaling). We corrected baseline differences of fMRI signals between conditions by grand mean scaling. Then, we performed group data analysis (one-sample t -test). The brain areas for this analysis were limited to those associated with CpC to identify brain regions significantly activated by pain. The threshold was FDR $p=0.05$ ($Z=1.75$).

Difference in brain activity between itching and pain

In this analysis, we tried to identify differences in brain activity between itching and pain. First, images of parameter estimates for the contrast [HC – CpC] were created for each subject (first-level

analysis) and then entered into a second-level analysis using the one-sample *t*-test across the subjects. The brain areas for this analysis were limited to those activated in the histamine stimulus as compared to the saline stimulus conditions (see Brain regions activated by itching). With this analysis, we identified brain regions more sensitive to the histamine than the cold-pain stimuli in the brain regions associated with itching (itching > pain). On the other hand, images of parameter estimates for the contrast [CpC – HC] were created for each subject (first-level analysis) and then entered into a second-level analysis using the one-sample *t*-test across the subjects. The brain areas for this analysis were limited to those activated in the cold-pain stimulus condition as compared to the cold stimulus condition (see Brain regions activated by pain). With this analysis, we identified brain regions more sensitive to the cold-pain than the histamine stimuli in the brain regions associated with pain (pain > itching). The threshold was FDR $p=0.05$ ($Z=1.75$). On the basis of these comparisons, we classified the brain regions activated by itching and pain into the three types: (1) Commonly activated areas: no significant difference in activity between the histamine and the cold-pain stimulus conditions. (2) More sensitive to itching areas: significantly higher activity in the histamine than the cold-pain stimulus conditions (itching > pain). (3) More sensitive to pain areas: significantly higher activity in the cold-pain than the histamine stimulus conditions (pain > itching). In first-level analysis of each contrast (i.e. [HC – CpC] and [CpC – HC]), we applied a high pass filter (cutoff period: 512 s) to eliminate baseline drifts of fMRI signals within each condition. In addition, we also scaled voxel values for that a mean voxel value in a whole brain was 50 in each condition (grand mean scaling). We corrected baseline differences of fMRI signals between conditions by grand mean scaling.

Time course of neural activity associated with itching and pain and comparison of total signal between itching and pain

We also performed volume of interest (VOI) analysis to obtain the time course of neural activity in the brain regions activated by itching and pain. VOI was placed on the brain regions activated by itching and pain (see Brain regions activated by itching and Brain regions activated by pain). VOI was a sphere with a 5-mm radius centered on the peak (local maximum of *t* statistics) in each brain region.

The average of voxel values within VOI was used as the adjusted blood oxygenation level dependent (BOLD) signal. The % change of the adjusted BOLD signal relative to the mean adjusted BOLD signal before applying each stimulus (saline, histamine, cold and cold-pain) was used as the % signal change in this study. Itching-related signal change was determined as the difference in the % signal change between the histamine and the saline stimulus conditions. Pain-related signal change was obtained by subtracting the % signal change in the cold stimulus condition from that in the cold-pain stimulus condition. The time courses of itching- and pain-related neural activities in each brain region are shown in Fig. 3. In addition, we also compared total signal during fMRI scans to confirm the reliability of the results obtained in the above analysis (Difference in brain activity between itching and pain). The integral of % signal change from 1 min and 38 s after the beginning of fMRI scans (time corresponding to the end of iontophoretic stimulus in the itching session) to the end of fMRI scans in the saline, the histamine, the cold and the cold-pain stimulus conditions were used as total

signal associated with the saline, the histamine, the cold and the cold-pain stimulus conditions, respectively. Differences of total activity between the histamine and the saline stimulus conditions were used as total activity associated with itching. In addition, differences of total activity between the cold-pain and the cold stimulus conditions were used as total activity associated with pain. Total activity associated with itching and that associated with pain were compared for each brain region using the paired *t*-test across the subjects. Statistical significance was defined as $p<0.05$.

Correlation analysis

We further performed a correlation analysis between itching- and pain-related signal change and the subjective evaluation of itching and pain, respectively. The time courses data of itching- and pain-related signal changes were taken at 3-s intervals (total time points: 146), because the sampling rate in fMRI scans was 3 s. On the other hand, time courses data of the visual analog scale (VAS) score was taken at 10-s intervals (Total time points: 45). The time courses of itching- and pain-related neural activities did not correspond to those of the VAS score. Thus, we interpolated the time courses of itching- and pain-related neural activities so that they corresponded to the time course of VAS score for each subject using linear interpolation function supplied in Matlab (total time points: 45). The difference in VAS score for itching between the histamine and the saline stimulus conditions (itching score) and that for pain between the cold-pain and the cold stimulus conditions (pain score) were used for the correlation analysis. We calculated the mean time course of the itching score and that of the interpolated itching-related signal change of 14 subjects and performed correlation analysis. We also calculated the mean time course of the pain score and that of the interpolated pain-related signal change of 14 subjects and performed correlation analysis. A significant correlation was defined as $p<0.05$. In addition, in individual subject, we performed correlation analysis with the time courses of the interpolated itching-related signal change and the itching score. We also performed correlation analysis with the time courses of the interpolated pain-related signal change and the pain score in each subject. After that, the slopes of linear regression obtained from these correlation analyses were then compared between itching and pain (paired *t*-test). A statistically significant difference in the slope was defined as $p<0.05$.

Results

Behavioral data

The subjective evaluation of itching showed a score of almost 0 in the saline stimulus condition (Fig. 2A). A slight itching sensation was elicited around 90 s in the saline stimulus condition. That was due to electric stimulation by iontophoresis given from 78 to 98 s. In the histamine stimulus condition, the itching sensation was elicited by the histamine-iontophoresis stimulus and lasted for several minutes (Fig. 2A). The itching sensation weakened with time. However, even at the last recording point (450 s after the beginning of fMRI scans), all the subjects felt the itching sensation in the histamine stimulus condition whereas most of the subjects reported no itching sensation in the saline stimulus condition. VAS score of itching in the histamine stimulus condition was significantly larger than that in the saline stimulus condition at

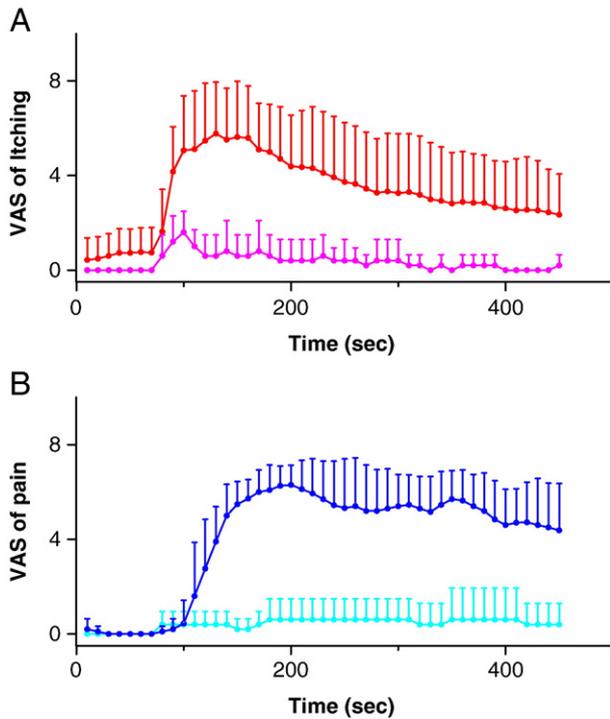


Fig. 2. Subjective evaluation of itching and pain sensations. (A) VAS score of itching was recorded in the histamine and the saline stimuli conditions, and (B) that of pain was recorded in the cold-pain and the cold stimuli conditions. Each line is the mean (SD) of VAS score of 14 subjects in each condition. Red, pink, blue and cyan represent the histamine, the saline, the cold-pain and the cold stimuli conditions, respectively.

the last recording point [p value (paired t -test)=0.0001; histamine (mean (SD)): 2.2 (1.7); saline: 0.2 (0.4)], indicating that the histamine stimulus elicited itching sensation during fMRI scans. The cold-pain stimulus also induced a strong pain sensation during fMRI scans whereas the score of the pain sensation was almost 0 in the cold stimulus condition (Fig. 2B).

Brain regions activated by itching and pain

Neural activity in the pre-supplementary motor area (pre-SMA), the bilateral anterior insula, the posterior insula, the anterior

Table 1
Brain regions activated by itching

Brain region	Coordinates				HC v.s. Cpc	
	x	y	z	Z score	Activity	Z score
Pre-SMA	-6	-4	64	4.89	n.s.	
Right anterior insula	48	8	8	4.35	n.s.	
Left anterior insula	-48	10	4	4.20	n.s.	
Posterior insula	-38	-14	-4	3.47	itching>pain	2.84
Anterior cingulate cortex	8	16	36	3.59	n.s.	
Posterior cingulate cortex	2	-38	26	3.51	itching>pain	3.62
Basal ganglia	-16	4	6	4.02	n.s.	

The first three columns show the brain regions activated by itching and their coordinates and Z scores.

Fourth column: neural activity in the brain regions activated by itching was compared between the histamine and the cold pain stimulus conditions. Itching > pain: neural activity related to itching was significantly higher than that related to pain.

Table 2
Brain regions activated by pain

Brain region	Coordinates				HC v.s. CpC	
	x	y	z	Z score	Activity	Z score
Pre-SMA	-4	-2	58	5.21	n.s.	
Right anterior insula	36	10	6	3.15	n.s.	
Left anterior insula	-42	10	-4	2.70	n.s.	
Anterior cingulate cortex	-8	10	40	5.12	n.s.	
Basal ganglia	-18	6	4	4.45	n.s.	
S2	50	-22	12	2.71	n.s.	
Thalamus	-2	-4	6	4.84	pain>itching	5.00

The first three columns show the brain regions activated by pain and their coordinates and Z scores.

Fourth column: neural activity in the brain regions activated by pain was compared between the histamine and the cold pain stimulus conditions.

Pain > itching: neural activity related to pain was significantly higher than that related to itching.

cingulate cortex (ACC), the posterior cingulate cortex (PCC) and the basal ganglia was significantly higher in the histamine condition than the saline condition (Table 1). On the other hand, pre-SMA, the bilateral anterior insula, ACC, the basal ganglia, the thalamus and S2 were more significantly activated in the cold-pain stimulus condition than in the cold stimulus condition (Table 2).

Comparison of brain activity between itching and pain

A direct comparison of brain activity between itching and pain showed that the neural responses in PCC and the posterior insula to itching were significantly higher than those to pain (Fig. 3, red-colored brain regions, Table 1) and that neural activity in the thalamus associated with pain was significantly higher than that associated with itching (Fig. 3, blue-colored brain regions, Table 2). These results were also confirmed by the comparison of total signal between itching and pain (Fig. 4). We observed a significant activation of S2 by pain but not by itching (Table 2). However, there was no significant difference in neural activity in S2 between itching and pain (Fig. 3). Total activity in S2 did not show a significant difference between itching and pain (Fig. 4). We did not observe significant difference in activity between itching and pain in pre-SMA, the bilateral anterior insula, ACC and the basal ganglia (Fig. 3). However, total signal associated with pain was significantly higher than that associated with itching in ACC (Fig. 4).

Correlation analysis

In PCC and the posterior insula, a significant correlation was observed between the mean time courses of the interpolated itching-related signal change and the mean time courses of the itching score (Figs. 5A and C), whereas no significant correlation was found between the mean time courses of the interpolated pain-related signal change and mean time courses of the pain score in these brain regions (Figs. 5B and D). The slopes of linear regression obtained from correlation analysis for each subject tended to be higher in itching than in pain in PCC [itching (mean (SD)): 0.023 (0.040); pain: 0.005 (0.027); n.s.] and the posterior insula [itching: 0.021 (0.027); pain: 0.005 (0.019); n.s.]. The thalamus showed a significant correlation between the mean time course of the interpolated pain-related

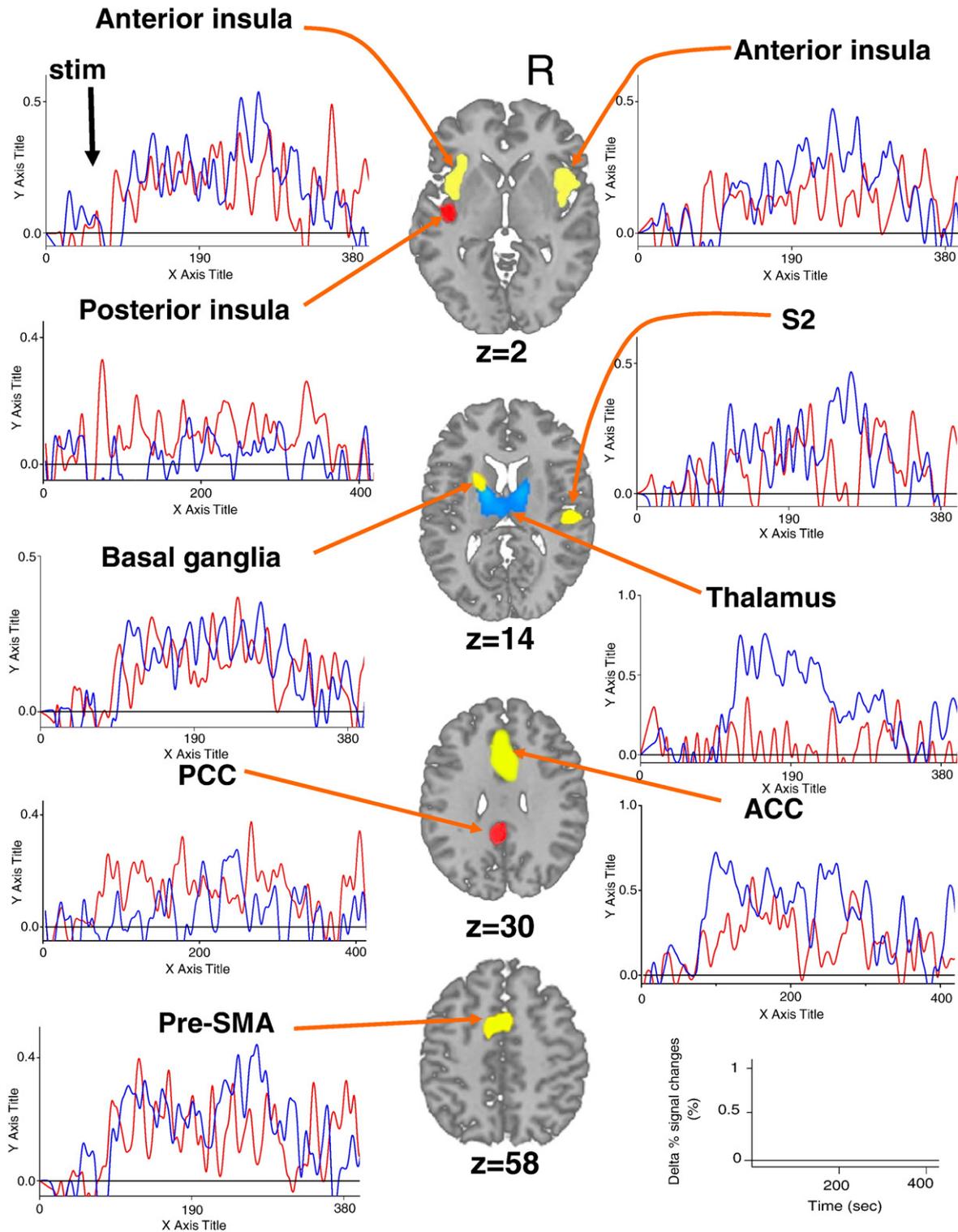


Fig. 3. Distribution of itching- and pain-related brain regions and the time course of their signal changes. In this study, we categorized itching- and pain-related brain regions into three types: common brain regions (yellow), brain regions more sensitive to itching (itching > pain) (red), the brain regions more sensitive to pain (pain > itching) (blue). Graphs represent the mean time courses of itching- (Red lines) and pain- (Blue lines) related neural activities of 14 subjects. The black arrow in the graph shows the time when the stimulus was applied. Stim: stimulus. R: right hemisphere.

signal change and the mean time course of the pain score (Fig. 6B) but did not between the mean time course of the interpolated itching-related signal change and the mean time course of the itching score (Fig. 6A). The slopes of linear regression were

significantly higher in pain than itching in the thalamus [pain (mean (SD)): 0.081 (0.092); itching (mean (SD)): 0.015 (0.057); $p=0.04$]. In S2, the mean time course of the interpolated pain-related signal change was significantly proportional to the mean

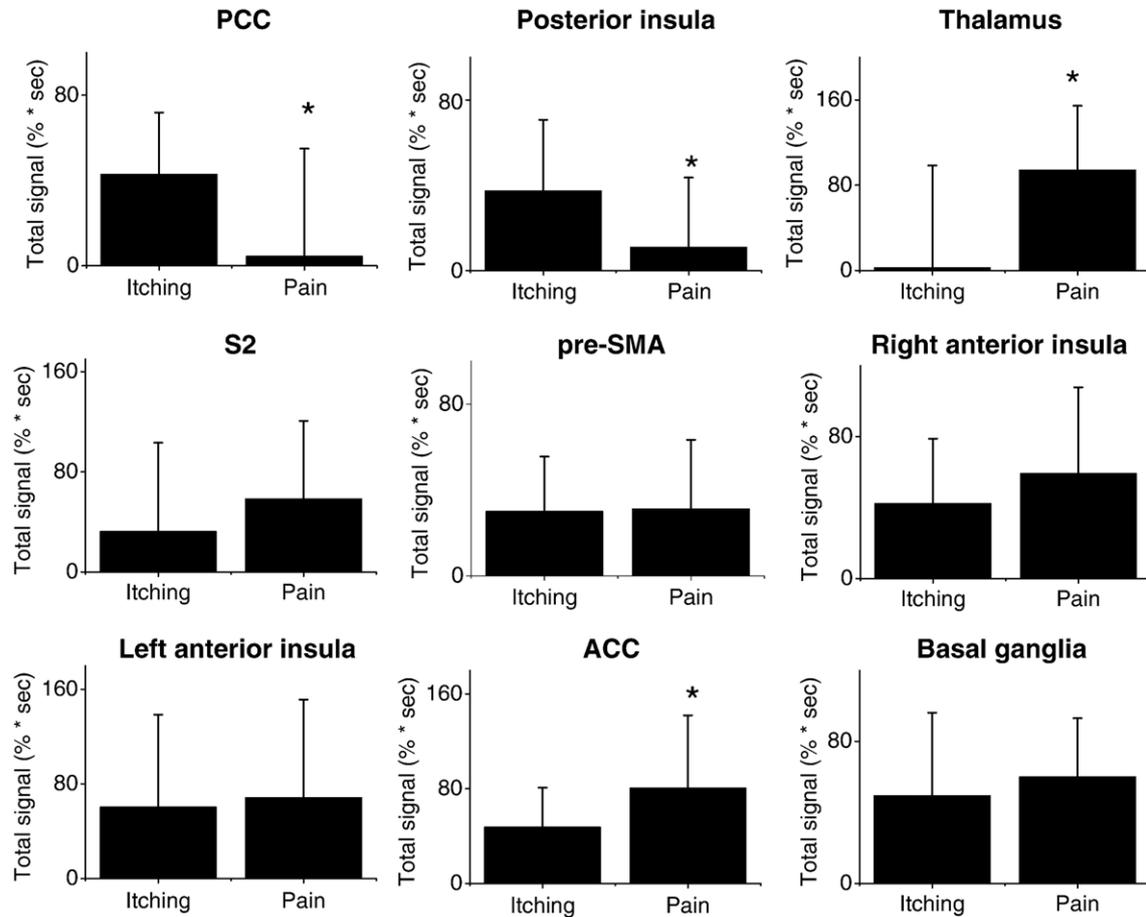


Fig. 4. Comparison of total signal between itching and pain. Total activity associated with itching and that associated with pain were compared between itching and pain. * $p < 0.05$.

time course of the pain score, but that of the interpolated itching-related signal change did not show a significant correlation to the mean time course of the itching score (Figs. 6C and D). The slopes of linear regression was slightly higher in pain than itching in S2 [pain: 0.017 (0.038); itching: 0.006 (0.038); n.s.]. The mean time courses of the interpolated itching- and pain-related signal change in pre-SMA, the bilateral anterior insula, ACC and the basal ganglia significantly correlated to those of the itching and pain scores, respectively (Table 3). The slopes of linear regression were significantly higher in pain than in itching in pre-SMA [pain: 0.04 (0.04), itching: 0.01 (0.04), $p = 0.03$], whereas they were almost the same between itching and pain in ACC [itching: 0.041 (0.045), pain: 0.044 (0.051), n.s.], the basal ganglia [itching: 0.033 (0.034), pain: 0.032 (0.029), n.s.], the right anterior insula [itching: 0.025 (0.027), pain: 0.028 (0.027), n.s.], and the left anterior insula [itching: 0.022 (0.034), pain: 0.028 (0.029)].

Discussions

Recently, functional neuroimaging techniques have been applied to clarifying the central itching mechanism. However, in previous studies, brain activity was not compared between itching and pain. Thus, it was still unclear how our brains

distinguish itching from pain. In the present study, we tried to identify the neural substrates associated with itching and pain by comparing itching- and pain-related brain activities by fMRI.

Common areas for itching and pain

In the histamine stimulus condition, histamine was induced to infiltrate into the skin by the iontophoretic stimulus and it excited peripheral C fibers. Thus, the itching sensation lasted for several minutes (Fig. 2A). The cold-pain also elicited pain sensation for several minutes (Fig. 2B). On the other hand, most of the subjects did not report any itching and pain sensations during the saline and the cold stimuli conditions, respectively (Figs. 2A and B). On the basis of the behavioral data, we identified the brain regions continuously activated during fMRI scans (Fig. 1). Pre-SMA, the bilateral anterior insula, ACC and the basal ganglia were commonly activated by itching and pain (Fig. 3). The activation of these brain regions was also observed in previous itching and pain studies (Drzezga et al., 2001; Mochizuki et al., 2003; Hsieh et al., 1994; Peyron et al., 2000). ACC is associated with emotion (Bush et al., 2000). It was reported that the anterior insula was activated by pain, disgust facial expression and offensive tastes (Peyron et al., 2000; Phillips et al., 1997; Yaxley et al., 1988), indicating that this region was also related to the emotional

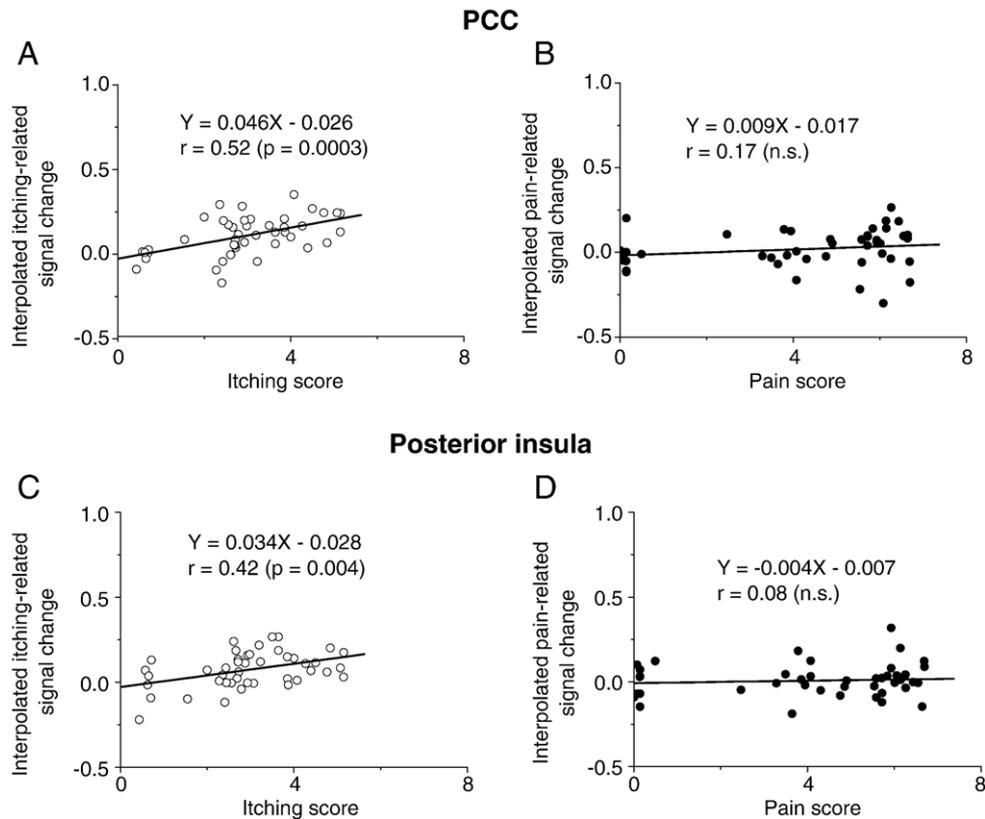


Fig. 5. Brain regions whose neural activity significantly correlated to itching sensation but did not to pain sensation (mean data of 14 subjects). (A, B) Brain region: PCC, (A) correlation between the interpolated itching-related signal change and the itching score, and (B) that between the interpolated pain-related signal change and the pain score. (C, D) Brain region: posterior insula, (C) correlation between the interpolated itching-related signal change and the itching score, and (D) that between the interpolated pain-related signal change and the pain score. Itching score: difference of VAS of itching between the histamine stimulus and the saline stimulus conditions. Pain score: difference of VAS of pain between the cold-pain stimulus and the cold stimulus conditions. Interpolated itching-related signal change: the difference of the % signal change between the histamine and the saline stimulus conditions. Interpolated pain-related signal change: the difference of the % signal change between the cold-pain and the cold stimulus conditions.

aspect. Pre-SMA and the basal ganglia are related to movement and motor programming (Tanji, 1999). If neural activity in pre-SMA and the basal ganglia were only related to finger movements carrying out to evaluate the itching and the pain sensations, their activity should be the same between the target (the histamine and the cold-pain conditions) and the baseline conditions (the saline and the cold conditions), because the subjects moved their fingers in all conditions. However, pre-SMA and the basal ganglia were more significantly activated in the target condition than in the baseline condition (Tables 1 and 2 and Fig. 3), suggesting that these brain regions would be associated with not only the finger movement for VAS scaling but also itching and pain. In addition, neural activity in ACC, the bilateral anterior insula, pre-SMA and the basal ganglia significantly correlated to both the itching and pain scores (Table 3). Concerning these points, it was suggested that areas commonly activated carry out similar processings between itching and pain, such as the motor reaction (pre-SMA and the basal ganglia) to withdraw from unpleasantness (ACC and anterior insula). Total activity in ACC related to pain was significantly higher than that related to itching (Fig. 4). This difference might partly be attributed to the different intensities of the pruritic and the painful stimuli (Figs. 2A and B).

Brain regions more sensitive to itching than to pain

PCC and the posterior insula were significantly activated by the pruritic stimulus but not by the painful stimulus (Figs. 3 and 4). Drzezga et al. (2000) also observed activity in PCC (lower than the significant level) during the pruritic stimulus. These results were also supported by the comparisons of total signal associated with itching to that associated with pain (Fig. 4). But we do not think that these brain regions are specific for itching because several previous pain studies observed activations in PCC and the posterior insula by the painful stimulus (e.g. Bromm, 2004; Derbyshire and Jones, 1998). Especially, the posterior insula is one of important structures in pain perception. On the other hand, we observed significant activations of ACC, the anterior insula and the thalamus in the comparison between the cold-pain and the cold stimulus conditions. Some neuroimaging studies also failed to observe a significant activation of the posterior insula by pain, although these studies and other neuroimaging studies of pain observed significant activations of the anterior insula, ACC and the thalamus (Andersson et al., 1997; Derbyshire et al., 1997; Peyron et al., 1999; Tracey et al., 2000, Qiu et al., 2006; Peyron et al., 2000). Davis et al. (1998) investigated an individual difference of neural response during

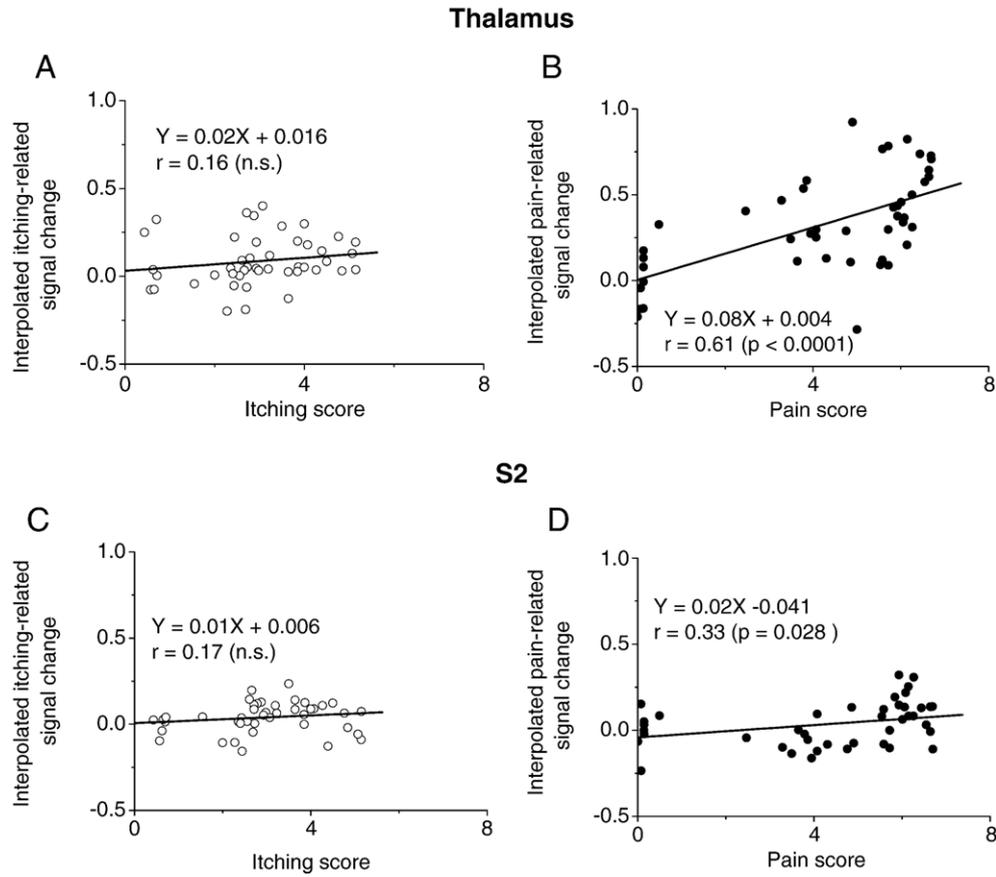


Fig. 6. Brain regions whose neural activity significantly correlated to pain sensation but did not to itching sensation (mean data of 14 subjects). (A, B) Brain region: thalamus, (A) correlation between the interpolated itching-related signal change and the itching score, and (B) that between the interpolated pain-related signal change and the pain score. (C, D) Brain region: S2, (C) correlation between the interpolated itching-related signal change and the itching score, and (D) that between the interpolated pain-related signal change and the pain score. Itching score: differences of VAS of itching between the histamine stimulus and the saline stimulus conditions. Pain score: difference of VAS of pain between the cold-pain stimulus and the cold stimulus conditions. Interpolated itching-related signal change: the difference of the % signal change between the histamine and the saline stimulus conditions. Interpolated pain-related signal change: the difference of the % signal change between the cold-pain and the cold stimulus conditions.

pain. Interestingly, in their experiment, only a few subjects (total 12 subjects) showed significant activations of the posterior insula by pain, whereas over half of 12 subjects showed significant activations in other brain regions such as the anterior insula and the thalamus. Considering these previous studies, a neural response of the posterior insula to pain might be weak as

Table 3

Correlation coefficient between the interpolated signal changes and VAS scores in the pre-SMA, the bilateral anterior insula, ACC and the basal ganglia

Brain region	Correlation coefficient (<i>p</i> value)	
	Itching	Pain
Pre-SMA	0.61 (<i>p</i> < 0.0001)	0.30 (<i>p</i> = 0.05)
Right anterior insula	0.53 (<i>p</i> = 0.0002)	0.50 (<i>p</i> = 0.0005)
Left anterior insula	0.53 (<i>p</i> = 0.0002)	0.50 (<i>p</i> = 0.0005)
Anterior cingulate cortex	0.65 (<i>p</i> < 0.0001)	0.68 (<i>p</i> < 0.0001)
Basal ganglia	0.64 (<i>p</i> < 0.0001)	0.54 (<i>p</i> = 0.0001)

Itching: correlation analysis with the time course of the interpolated itching-related signal change and that of itching score.

Pain: correlation analysis with the time course of the interpolated pain-related signal change and that of pain score.

compared to the anterior insula, ACC and the thalamus. This speculation was partly supported by our total signal data. In this study, the number of subjects whose total signal associated with the cold-pain stimulus was larger than that associated with the cold stimulus was 10 of 14 subjects in the posterior insula. On the other hand, for example, those were also 10 of 14 subjects in the right anterior insula. Thus, the number of subjects was the same. However, differences of total signal between the cold-pain and the cold stimuli (i.e. total signal associated with pain, see also Fig. 4) were largely different [the posterior insula (mean (SD)): 11 (32); the right anterior insula: 68 (82)]. As compared to the anterior insula, ACC and the thalamus, a response of the posterior insula to pain might be too weak to be detected as a significant activation in this study. Another possibility was signal to noise ratio (S/N ratio) of fMRI signals. In most of prior pain studies, painful stimuli were repeatedly given in each subject to increase S/N ratio of stimulus-related fMRI signals. On the other hand, we gave a painful stimulus once in each subject. Low S/N ratio due to single pain stimulation might also be associated with that we did not observe significant activation of the posterior insula. Each stimulus was given once in each subject in this study. Thus, S/N ratio would not be so different between itching and pain. Considering this point, at least, it could be saying that

that PCC and the posterior insula are more sensitive to itching than to pain (Fig. 4).

Interestingly, we observed that itching-related signal change in PCC and the posterior insula significantly correlated to the itching score, whereas such correlations were not observed in pain (Figs. 5A–D). Darsow et al. (2000) also observed that the subjective evaluation of itching showed significant correlations to neural activity in the posterior insula. In this study, VAS of itching and pain increased after giving the histamine and the cold-pain stimuli, respectively, and kept high scores until the end of fMRI scans (Fig. 2). Such a trend was seen in the time course of itching-related signal change in PCC and the posterior insula (Fig. 3). For example, we could see that itching-related signal changes continuously kept high signal change (over 0%) after giving a stimulus as compared to pre-stimulus (Fig. 3). On the other hand, pain-related signal change in PCC and the posterior insula fluctuated around 0% (Fig. 3). Therefore, VAS of itching and itching-related signal change would show a significant correlation in PCC and the posterior insula (Fig. 5). Anatomical studies have shown that PCC and the posterior insula receive nociceptive input from STT via the thalamus and process noxious somatosensory information (Apkarian and Hodge, 1989; Shi and Apkarian, 1995; Apkarian and Shi, 1997). Interestingly, Andrew and Craig (2001) reported that there were some STTs selectively sensitive to itching. Itching-specific information might be sent to PCC and the posterior insula via itching-specific STT. Several studies suggest that PCC and the posterior insula are associated with the negative emotional aspect. For example, an animal study demonstrated that PCC was activated when stress and anxiety were given (Park et al., 2003). The activation of PCC is commonly observed in patients with psychological diseases such as schizophrenia, depression, panic disorder and posttraumatic stress disorder and its activity is proportional to the severity of the disorder (Ho et al., 1996; Andreasen et al., 1997; Bremner et al., 2003; Maddock et al., 2003). It was reported that a patient with lesions in the posterior part of the insula had impairments in recognition and experience of disgust (Calder et al., 2000). It was also reported that the posterior insula responded to physically bodily disgusting stimuli such as footages of wounded bodies, amputation procedures and burn victims (Britton et al., 2006). In this study, we observed that the sensitivities of PCC and the posterior insula were different between itching and pain (Figs. 3–5). Such differences might be associated with the distinct unpleasant sensations of itching and pain. It was reported that ascending pathways in the peripheral C fibers were different depending on how pain sensation was induced (Schmelz et al., 1997, 2003). In this study, we used cooling-induced pain. Further study will be needed to confirm whether the similar results were also observed when other painful stimuli such as capsaicin and mustard oil were used.

Brain regions more sensitive to pain than itching

Pain, but not itching, induced a significant activation of the thalamus (Figs. 3 and 4). On the other hand, in our previous study, we found the activation of the thalamus by itching (Mochizuki et al., 2003). The discrepancy between the previous and the present studies may be attributed to a difference in the methodology. That is, in the previous study, we measured brain activity by PET only during the histamine-iontophoresis stimulation, whereas in this study, we identified the brain regions continuously activated after the histamine-iontophoresis stimulation (Fig. 2). It was reported

that the peripheral C fibers were activated more than 10 min after the injection of histamine into the skin (Schmelz et al., 1997). Therefore, it was difficult to consider that the thalamus did not activate after finishing the histamine-iontophoresis stimulation. As shown in Fig. 2, the score in the subjective evaluation of pain sensation was larger than that of itching sensation. Some might point out that such differences were due to the different activity in the thalamus between itching and pain. Partly, this is correct. If neural activity in the thalamus represented only the intensity of the stimulus from the periphery, neural activity related to itching should increase after giving the iontophoretic stimulus, because all the subjects reported the itching sensation in the histamine stimulus condition (Fig. 2A). However, we did not observe such a tendency (Fig. 3). Several neuroimaging studies of itching also reported that an pruritic stimulus did not activate the thalamus (Drzezga et al., 2001; Darsow et al., 2000; Hsieh et al., 1994). The thalamus is considered to play a remarkable role in pain processing (Guilbaud et al., 1994; Silva et al., 2001; Miyata et al., 2003). Our results also supported the importance of the thalamus in pain processing, because pain-related signal change significantly correlated to the score of the subjective evaluation of pain, whereas such a correlation was not observed in itching (Figs. 6A and B). Considering the results of previous pain and itching studies and our results, it can be considered that the thalamus is one of key structures in pain perception. Generally, a pain sensation is transmitted by A-delta and C fibers with the first sharp pain transmitted by A-delta fibers and the following long-lasting chronic pain transmitted by C fibers (Bear et al., 2001). However, it could not be ruled out that continuous painful stimulus used in this study activated some of A-delta fibers (e.g. Hansen et al., 2007). Therefore, a significant difference of neural activity in the thalamus between itching and pain might include some effects of the excitation of A-delta fibers by the cold-pain stimulus.

A number of studies have shown that a painful stimulus activates the S2 region of the brain (Forss et al., 2005; Kakigi et al., 2000; Casey et al., 1996; Coghill et al., 1994). It is suggested that noxious information is relayed to S2 via a connection between spinothalamic neurons and thalamocortical neurons (Stevens et al., 1993) and processed in S2 (Whitsel et al., 1969; Robinson and Burton, 1980; Dong et al., 1989). Previous itching studies showed that S2 is not significantly activated by itching (Mochizuki et al., 2003; Drzezga et al., 2001; Hsieh et al., 1994). However, as shown in Figs. 3 and 4, neural activity in S2 was not significantly different between itching and pain. The previous itching studies did not compare neural activity in S2 between itching and pain (Drzezga et al., 2001; Hsieh et al., 1994). It might be that an activation of S2 by itching did not reach statistical significance in the previous itching studies. Therefore, it was suggested that S2 was associated with not only pain but also itching. In the correlation analysis, we observed that neural activity in S2 significantly correlated to the score of the subjective evaluation of pain but did not to that of itching (Figs. 6C and D), suggesting that S2 was less sensitive to itching than pain.

Conclusion

In conclusion, using fMRI time series analysis, we found in this study several differences in neural substrates between itching and pain sensations. We observed that the signal changes in PCC and the posterior insula by itching were significantly higher than those

by pain and significantly correlated to the itching scores, whereas the signal changes in the thalamus by pain were significantly higher than those by itching and were significantly proportional to the pain scores. These observations indicate that neural activity in PCC and the posterior insula represent itching sensation better than pain sensation, whereas that in the thalamus represent pain sensation better than itching sensation. Such distinct sensitivities in PCC, the posterior insula and the thalamus for itching and pain would be responsible for the perceptual difference in these sensations. In contrast to the previous itching studies, our result indicated that S2 was associated with not only pain but also itching.

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