are part of the limbic system, the center of autonomic function and emotion. It goes in accordance with the fact that various smells can induce autonomic changes compatible with autonomic relaxation [1,12] or excitation [1,2], as well as changes compatible with basic emotions [19]. Such autonomic changes following treatments with perfumed fragrances (aroma-therapy) can be detected by power spectral analysis (PSA) of heart rate variability (HRV). A few studies have demonstrated usefulness of HRV measurement for evaluation of autonomic changes induced by various odorants such as lavender [8,16].

It has been said that "lavender", one of the most popular flower fragrances used in aroma-therapy, usually does not induce strong emotions but does comfortable feeling and relaxation. The effects of these fragrances on brain functions do not seem to be simple. It is expected that many brain regions, including sensory and limbic regions, are involved in olfaction. It is already recognized that the primary olfactory regions include the anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala, periamygdaloid regions, and entorhinal cortex. The secondary olfactory regions include the main composition of the limbic system: i.e. the hippocampus, ventral striatum and pallidum, thalamus, hypothalamus, orbitofrontal cortex, insular cortex, and cingulate gyrus. So far, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have been used to investigate regional brain activation due to various sensory and emotional stimuli. However, functional neuroanatomy of autonomic changes induced by aroma-therapy has not been studied well using PET.

The aim of the present study is to investigate the effects of lavender fragrance on autonomic nervous functions in terms of HRV and to observe brain responses in terms of brain glucose metabolic changes measured by PET.

2. Materials and methods

2.1. Subjects and materials

Ten healthy female volunteers, ranging from 20 to 27 years old (*mean* ± *S.D.*: 23 ± 3.0 years old), were recruited for the present experiment after obtaining their written informed consent. The present study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. The subjects were non-smokers, and none of them had any olfactory problems nor were they on any types of medication at the time of the experiment. Subjects were requested to refrain from foods and drinks that would affect olfactory functions for 24 h (alcohol, coffee, chewing gum, etc.), and to take adequate rest and sleep during the night before the experiment day.

Before starting the study, each subject was interviewed for her preferences to the lavender fragrance, and was also requested to rate her own stress using the Stress Response Scale (SRS-18). The subjects were requested to be seated on a comfortable chair with the eyes open in the PET operation room. Holter ECG recording in the NASA leads (FM-300, Fukuda Denshi, Tokyo, Japan) was performed for evaluation of the HRV. Respiratory rate was also monitored in order to ensure the absence of respiratory changes after lavender administration, but the data were lost because of technical problem. Then, the respiratory data were collected again from other 15 subjects (ranging from 20 to 23 years old; *mean age* ± *S.D.*: 21.0 ± 1.0 years old).

To supply the lavender fragrance, a plaster prepared for aroma-therapy ("Lavender girl", Teikoku Pharmaceuticals, Tokyo, Japan) was attached on the subjects' right shoulder shortly before administration of [^{18}F]labeled fluorodeoxyglucose (FDG) injected through the right cubital vein (37 MBq). After 40-min-long uptake phase of FDG, all the electrodes and the lavender plaster were removed and soon PET scanning procedure was initiated using SET2400W scanner (Shimadzu Inc., Kyoto, Japan). PET

scanning covered each subject's whole brain taking 12 min in total (6 min for emission scan and 6 min for transmission scan). Tissue attenuation of positron annihilation photons was corrected using post-injection transmission data. Measurement was done twice within 3 months period but with an interval of at least 1 week: lavender administration and control conditions. For the control condition, exactly the same experimental procedure was repeated for each subject except for the absence of the lavender plaster. An order of the 2 conditions was balanced for each subject in order to remove an order effect. Five randomly chosen subjects were studied with a "lavender-control" order while the other 5 subjects were studied in the order of "control-lavender".

Additionally, the whole measurements were done within 10 days after the first day of menstruation. The reasons were to match their hormonal environment and to avoid radiation exposures during an early stage of pregnancy. Subjects were requested to rate their own stress using SRS-18 after PET scanning.

2.2. *Frequency-domain analysis of HRV*

Analyzing HRV in the frequency domain is a valuable method to determine quantitatively the sympathetic and parasympathetic modulations of heart rate (HR) [18]. The autonomic modulation of sinus rates has turned out to be a significant prognostic determinant in many cardiovascular diseases [9,18]. Two main spectral components are most commonly distinguished in the HRV spectrum: low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.4 Hz) components, respectively [9,18] (Fig. 1).

For analysis, segments of 10-minute-long records (before and after lavender administration: 10, 20 and 30 min post administration) were used. Beat to beat (R-R) intervals of heart rates were measured by detecting a peak of QRS waves of the ECG, and HR was estimated from the R-R intervals. Power spectral analysis of variability of these cardiovascular parameters was performed with the fast Fourier transformation technique (FFT; 512 points) through a Hamming window. LF and HF components were distinguished by frequency bands [9,18]. For further evaluation, normalized LF and HF (nLF and nHF, respectively) and low-high frequency ratio (LF/HF) were used. nLF and nHF were calculated as follows: $nLF = LF \times 100/(LF + HF)$, $nHF = HF \times 100/[LF + HF])$, respectively, where [LF + HF] in termed as total power (TP) [18].

2.3. *FDG-PET*

PET brain images were analyzed to identify regional changes of glucose metabolic rate using a widely-used software package, statistical parametric mapping (SPM2 [6,7], Functional Imaging Laboratory, London, UK). An FDG brain template distributed by Montreal Neurological Institute, McGill University, Canada, [6] was used for anatomical standardization (spatial normalization) of the PET images by applying Affine and non-linear transformations. Gaussian smoothing of 12 mm full width at half maximum was applied to compensate for errors in spatial normalization. Voxel-wised paired t-statistics was conducted based on the general linear model to find differences in the regional brain activity in the control and lavender fragrance conditions. Because the number of subjects was small, the threshold for the significance was set at $p < 0.001$ without corrections for multiple comparisons.

3. Results

Representative data of HRV measurement taken from a subject are demonstrated in Fig. 1. R-R intervals tended to be longer later in the experiment compared to the initial resting condition. The R-R

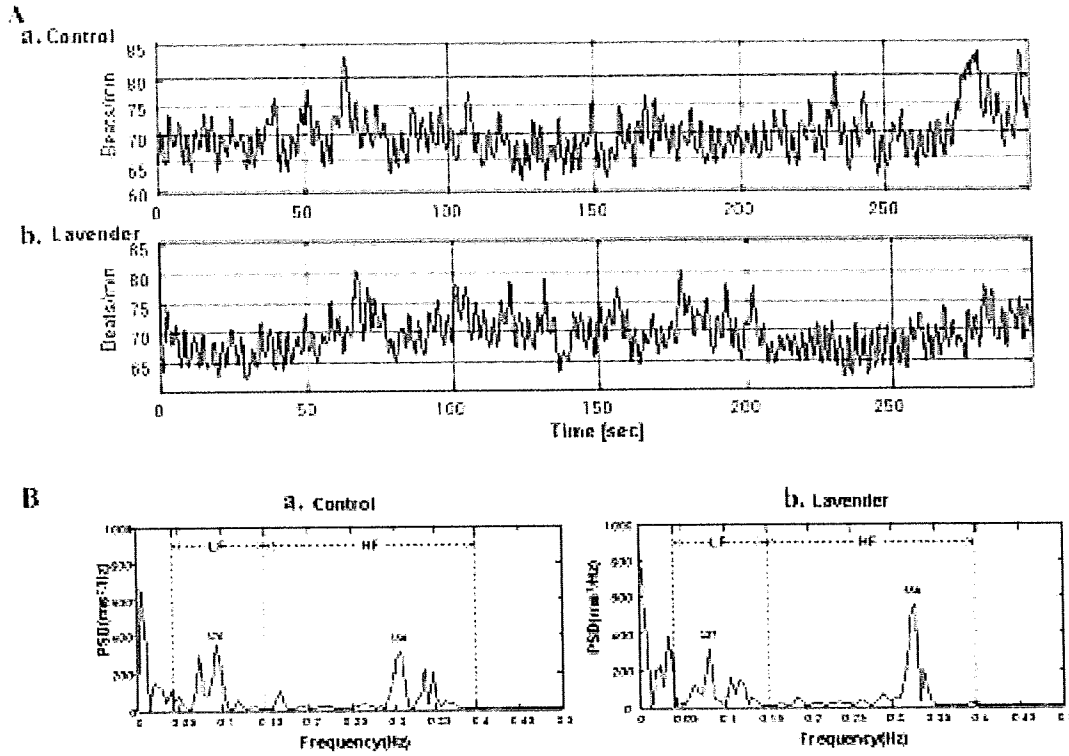

Fig. 1. Representative data of heart rate (HR) and HR variability (HRV) during exposure to control and lavender fragrance (A). Time course of HR changes during control (Aa) and lavender fragrance (Ab) conditions, respectively. Note that the HR gradually decreases and fluctuates more slowly during the lavender fragrance condition (Aa,b). Results of HRV spectral analysis (B) are shown during exposure to the control (Ba) and lavender fragrance (Bb) conditions, respectively. Note that HF component of HRV increased from 350 to 558 $(\text{beats/min})^2$/Hz while LF/HF ratio decreased from 1.04 to 0.51, following administration of a lavender fragrance plaster. Abbreviations: LF = low frequency; HF = high frequency.

intervals were somewhat shorter in the lavender administration condition in comparison to the control (Fig. 1A). The fraction of HF was higher and the LF/HF ratio was lower in the lavender condition (Fig. 1B). Similar tendency was observed also in averaged data of 10 subjects as demonstrated in Fig. 2 and Table 1. nHF tended to increase and nLF tended to decrease in the first 10 min following lavender plaster administration, resulting in decreased LF/HF (Fig. 2).

Summary of physiology data obtained from the 10 subjects are shown in Table 2. None of HR, and systolic, diastolic and mean blood pressure (SBP, DBP and MBP, respectively) manifested significant difference between the control and lavender administration conditions. Additional respiration data also indicated no change after lavender administration as follows: 16.9 ± 1.9 (rest), 16.6 ± 1.2 (10 min post-administration of lavender), 16.6 ± 1.3 (20 min), 16.3 ± 1.8 (30 min). However, nHF was significantly higher (*mean* ± *S.D.*: 0.51 ± 0.21 Hz) with lavender use than that in the control condition (0.44 ± 0.11 Hz) ($p < 0.05$). In contrast, changes in LF/HF were significantly lower (1.41 ± 0.67) with lavender use in comparison to the control (1.91 ± 0.82) ($p < 0.05$). SRS-18 scores were significantly lower (14.1 ± 13.2) in the lavender fragrance condition compared to the control (18.8 ± 14.1) ($p < 0.05$). Scores of SRS-18 were also lower in the lavender administration condition (Table 2).

Table 1
Measures of heart rate variability between the control and lavender fragrance condition

Subject	Control				Lavender			
No.	rest	5 min	10 min	15 min	rest	5 min	10 min	15 min
nLF								
1	0.33	0.37	1.04	0.28	1.69	0.72	1.05	0.15
2	1.71	0.27	0.46	0.96	0.87	0.27	0.38	1.06
3	1.39	0.59	0.97	0.75	1.37	0.37	0.99	0.39
4	2.11	1.02	0.95	0.81	0.68	0.29	0.76	0.38
5	0.75	0.27	0.43	0.37	0.52	0.46	0.63	0.84
6	0.36	1.25	0.59	0.69	0.85	0.28	0.87	0.44
7	0.61	0.80	0.93	0.40	0.91	0.63	0.73	0.25
8	0.66	0.35	0.53	0.71	0.51	0.16	0.37	0.24
9	1.40	0.75	1.28	0.63	1.98	1.02	0.95	1.00
10	0.95	1.24	1.17	0.72	0.51	0.26	0.33	1.22
mean (s.d.)	1.03 (0.61)	0.69 (0.38)	0.84 (0.31)	0.63 (0.22)	1.10 (0.52)	0.45 (0.27)	0.71 (0.27)	0.60 (0.39)
nHF								
1	0.25	0.49	0.45	0.32	0.41	0.81	0.85	0.76
2	0.38	0.35	0.36	0.39	0.35	0.34	0.39	0.35
3	0.55	0.74	0.75	0.61	0.39	0.58	0.73	0.41
4	0.42	0.49	0.41	0.54	0.24	0.53	0.30	0.42
5	0.32	0.42	0.35	0.53	0.18	0.45	0.32	0.35
6	0.13	0.59	0.43	0.31	0.36	0.75	0.56	0.47
7	0.59	0.34	0.39	0.27	0.31	0.59	0.53	0.56
8	0.21	0.35	0.48	0.41	0.22	0.45	0.42	0.38
9	0.24	0.53	0.42	0.45	0.38	0.89	0.73	0.75
10	0.34	0.42	0.39	0.36	0.21	0.45	0.25	0.49
mean (s.d.)	0.34 (0.15)	0.47 (0.13)	0.44 (0.11)	0.42 (0.11)	0.31 (0.08)	0.58 (0.18)	0.51 (0.21)	0.49 (0.15)
LF/HF								
1	1.32	0.65	3.37	0.89	2.55	0.59	1.23	1.01
2	3.15	0.76	1.21	3.01	4.01	0.81	0.99	0.85
3	2.76	0.81	0.94	0.48	3.11	1.15	1.35	0.98
4	4.71	0.58	2.33	2.14	2.65	0.98	0.83	0.65
5	2.15	1.95	1.91	0.73	2.74	0.73	1.96	1.25
6	2.74	3.37	1.95	1.53	2.23	0.63	1.26	1.35
7	1.46	2.37	1.53	2.01	5.12	0.44	1.95	2.01
8	3.15	0.85	0.99	1.25	2.31	0.37	0.52	0.57
9	6.11	1.21	3.06	1.46	5.12	1.46	2.85	2.31
10	2.83	1.91	1.85	1.76	2.36	0.58	1.15	1.22
mean (s.d.)	3.04 (1.44)	1.45 (0.92)	1.91 (0.82)	1.53 (0.75)	3.22 (1.13)	0.77 (0.34)	1.41 (0.67)	1.22 (0.56)

Abbreviations: nLF = low frequency in normalized units; nHF = high frequency in normalized units; LF/HF = low frequency/high frequency ratio.

PET analysis revealed changes in cerebral metabolism between the conditions ($p < 0.001$). Administration of lavender administration was associated with activation in the orbitofrontal cortex, posterior cingulate cortex, brainstem (mainly pons), thalamus and cerebellum while deactivation was observed mainly in the pre/post-central gyrus and frontal eye field (Fig. 3).

4. Discussion

So far, psychological and physiological effects of various odorants have been investigated, and it has been reported that lavender elicited mostly "relaxation" and "happiness" [19]. Their results demon-

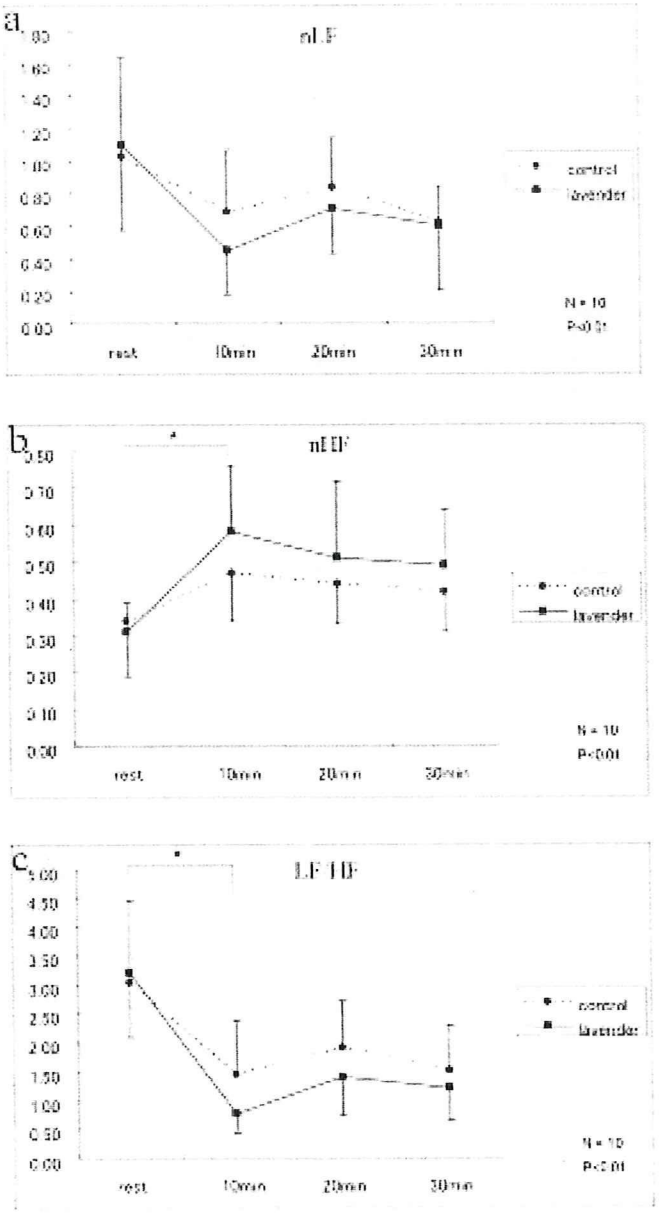

Fig. 2. Time-related changes of the nHF, nLF and LF/HF of heart rate variability (HRV) in lavender condition (closed squares; $n = 10$) and control condition (closed triangle; $n = 10$). Changes of nLF in the course of experiment (a). Changes of nHF in the course of experiment (b). Changes of LF/HF in the course of experiment (c).

strated no significant reduction in SBP, DBP, MBP and HR during lavender stimulation, but they did not investigate HRV. In the present study, spectral analysis of HRV indicated a significant increase in HF component and reduction in LF/HF ratios during the lavender use. These changes in the cardiovascular parameters were consistent with peripheral autonomic nervous activity, suggesting that lavender

Table 2
Autonomic functions in the control and lavender fragrance conditions ($n = 10$)

	Control	Lavender administration	P value
SBP (mmHg)	109 ± 8	107 ± 6	NS
DBP (mmHg)	69 ± 6	68 ± 5	NS
HR (beats/min)	73 ± 9	65 ± 11	NS
nLF	0.84 ± 0.31	0.71 ± 0.27	$p < 0.05$
nHF	0.44 ± 0.11	0.51 ± 0.21	$p < 0.05$
LF/HF (ratio)	1.91 ± 0.82	1.41 ± 0.67	$p < 0.05$
SRS-18 score	18.8 ± 14.07	14.1 ± 13.2	$p < 0.05$

Data are reported as mean ± SD. Abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; MBP = mean blood pressure; nLF = low frequency in normalized units; nHF = high frequency in normalized units; LF/HF = low frequency/high frequency ratio; SRS = stress response scale; NS = non significant.

administration increased parasympathetic nervous activity while it reduced sympathetic activity. These results go in accordance with the previous study by Saeki and colleagues [16] where 10 healthy female subjects were studied. They demonstrated that lavender fragrance induced HRV changes associated with relaxation. Kuroda and colleagues [8] conducted a study using 12 subjects to find the similar results to those by Saeki et al. Additionally, they demonstrated that (R)-(-)-linalool, one of the major odor components of lavender, induced the same autonomic responses. These results suggested that certain fragrances can induce a feeling of calmness or relaxation accompanied by increased parasympathetic nervous activity.

Heart rate rhythm is under an influence of the autonomic nervous system. Tension of the autonomic nervous system is kept by opposing tones of sympathetic and parasympathetic systems. The variability in heart rate is known to reflect the balance of the autonomic system. Frequency analysis of oscillation in R-R intervals of electrocardiography (ECG) has been widely applied to assess HRV as a marker of autonomic nervous activity [13]. LF component (0.04–0.15 Hz) mainly reflects both sympathetic and parasympathetic nervous activities, while HF (0.15–0.40 Hz) reflects parasympathetic nervous activities [13,18]. In addition, LF/HF is widely used as a relative marker of sympathetic nervous activities or sympathovagal balance [11,18]. It has been established that various cardiovascular disease states are associated with typical changes in HRV [18]. The present study observed that the lavender plaster increased HF with simultaneous reductions in the LF/HF ratios. This implied that lavender augmented parasympathetic tones. It is known that HRV results can be affected by respiratory changes. At least 3 previous reports, however, demonstrated no significant changes in respiratory rates before and after lavender administration [5,16,17]. Though the data were obtained from different subjects, the present results also served supporting data for lavender's lack of respiratory effect. Thus, it can be postulated that the effect of respiratory changes on the HRV was very small in the present study.

As for the brain PET study, it was expected that various olfactory regions would be activated. Olfactory nerves are sending inputs to the primary olfactory regions such as the anterior cingulate, olfactory tubercle and entorhinal cortex, from which the olfactory information is further transmitted to the olfactory association regions such as the orbitofrontal cortex, hippocampus, striatum, thalamus, and insula. A previous fMRI study demonstrated activation of these regions induced by olfactory stimulations [4]. A similar study was conducted using PET as well [14]. Another fMRI study demonstrated that the pleasant smell activated the medial part of orbitofrontal cortex and the unpleasant smell did the lateral part, respectively [15]. Metabolic reduction in the olfactory regions was reported in patients with disturbed olfaction [10].

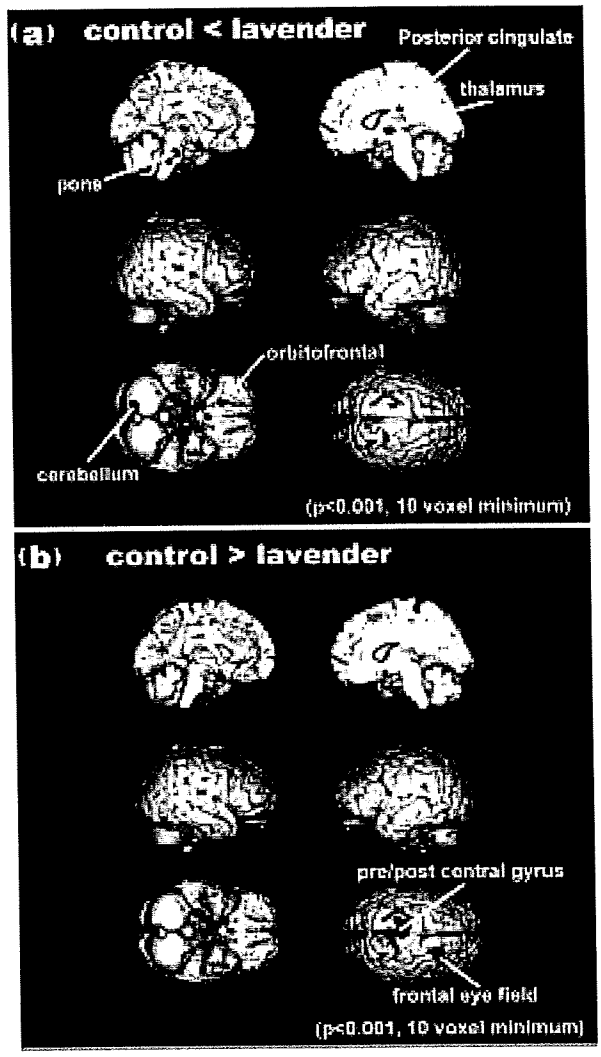

Fig. 3. Results of voxel-by-voxel comparison of brain glucose metabolic images ($p < 0.001$, 10 voxel minimum). Brain regions with metabolic increase due to lavender administration (a) and regions with metabolic reduction (b).

Contrary to the authors' expectation, the present study did not find involvement of any primary olfactory regions but involvement of a few association regions such as the orbitofrontal cortex, thalamus and cerebellum. A possible explanation for this discrepancy may be the difference of time window because of methodological difference. FDG accumulation in the brain tissue is a rather slow process while olfaction is a relatively instantaneous phenomenon. Other methods measuring cerebral blood flow (perfusion) changes, such as fMRI and [^{15}O] H_2O PET, would be more suitable to record brain activities lasting for a few seconds $\sim$ a few minutes. Since the FDG PET method tends to average brain activities of longer time span (approximately 30 to 40 min), it does not detect brain responses which may last temporally. Therefore, it seems that an interpretation of the present results should be done based on the concept of "identifying brain regions influenced by environmental changes due to lavender fragrance lasting 40 minutes" that would make the interpretation easier.

Based on such a standpoint, lack of activation in the anterior cingulate gyrus in the present study would be reasonable because this region is regarded as one of the centers of sympathetic nervous activities that were possibly suppressed after lavender administration. Metabolic increase in the brainstem and posterior cingulate gyrus, though not usually included in the olfactory regions, seems to be associated with improved mental functions during relaxation rather than olfaction itself. In human brain, the reticular formation located in the brainstem is associated with maintenance of arousal level, and the posterior cingulate gyrus is associated with cognition. Metabolic increase in these regions might suggest increased arousal level and cognitive ability in the subjects possibly induced by lavender. In addition, the present study demonstrated metabolic reduction as well in the pre/post-central gyrus (mainly including the primary sensorimotor area), premotor area and frontal eye field. These findings might be associated with decreased sensory inputs from the body trunk and extremities, decreased motor outputs and muscle movement, and decreased eye movement. Thus, the combined finding of a reduced sensorimotor activity and an improved arousal/cognitive function seem to be a good brain representation of "physical relaxation" and "improved mental functions" induced by lavender, as recognized as a calming or relaxing agent [3].

5. Conclusion

In conclusion, the authors have demonstrated that lavender fragrance can promote relaxation by depressing sympathetic activity while augmenting parasympathetic activity in normal adults. Our findings suggest a possible use of lavender fragrance to treat patients with various types of autonomic dysfunctions.

Acknowledgements

The authors thank Miss Sayuri Seto for experimental preparation and kind cooperation, Miss Kazuko Takeda for her excellent technical assistance and kind cooperation in some medical procedures. This work was partly supported by a 21 COE program of Biomedical Engineering based on Bio-Nano Technology at Tohoku University, Health and Labour Sciences Research.

References

[1] Alaoui-Ismaili, E. Vernet-Maury, A. Dittmar, G. Delhomme and J. Chanel, Odor hedonics: connection with emotional response estimated by autonomic parameters, *Chem Senses* **22** (1997), 237–248.
[2] P. Brauchli, P.B. Ruegg, F. Etzweiler and H. Zeier, Electrocortical and autonomic alteration by administration of a pleasant and unpleasant odor, *Chem Senses* **20** (1995), 505–515.
[3] G. Buchbauer, L. Jirovetz, W. Jager, H. Dietrich and C. Plank, Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation, *Journal of Biosciences* **46** (1991), 1067–1072.
[4] I.E. De Araujo, E.T. Rolls, M.L. Kringelbach, F. McGlone and N. Phillips, Taste-olfactory convergence, and the representation of the pleasantness of flavour, in the human brain, *Eur J Neurosci* **18** (2003), 2059–2068.
[5] C. Dunn, J. Sleep and D. Collett, Sensing an improvement: an experimental study to evaluate the use of aromatherapy, massage and periods of rest in an intensive care unit, *Journal of Advanced Nursing* **21** (1995), 34–40.
[6] K. Friston, J. Ashburner, C. Frith et al., Spatial registration and normalization of images, *Human Brain Mapping* **2** (1995), 165–189.
[7] K.J. Friston, A. Holmes, J.B. Poline et al., Detecting activations in PET and fMRI: levels of inference and power, *Neuroimage* **4** (1996), 223–235.
[8] K. Kuroda, N. Inoue, Y. Ito, K. Kubota, A. Sugimoto, T. Kakuda and T. Fushiki, Sedative effects of the jasmine tea odor and (R)-(-)-linalool, one of its major odor components, on autonomic nerve activity and mood states, *Eur J Appl Physiol* **95** (2005), 107–114.

[9] M.T. La Rovere, J.T. Bigger Jr., F.I. Marcus, A. Mortara and P.J. Schwartz, Baroreflex sensitivity and heart rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators, *Lancet* **351** (1998), 478–484.
[10] L.M. Levy, R.I. Henkin, C.S. Lin and A. Finley, Rapid imaging of olfaction by functional MRI (fMRI): identification of presence and type of hyposmia, *J Comput Assist Tomogr* **23** (1999), 767–775.
[11] A. Malliani, F. Lombardi and M. Pagani, Power spectrum analysis of heart rate variability: a tool to explore neural regulatory mechanisms, *Br Heart J* **71** (1994), 1–2.
[12] M. Nagai, M. Wada, N. Usui, A. Tanaka and Y. Hasebe, Pleasant odors attenuate the blood pressure increase during rhythmic handgrip in humans, *Neurosci Lett* **289** (2000), 227–229.
[13] B. Pomeranz, R.J. Macaulay, M.A. Caudill et al., Assessment of autonomic function in humans by heart rate spectral analysis, *Am J Physiol* **248** (1985), 151–153.
[14] A. Qureshy, R. Kawashima, M.B. Imran, M. Sugiura, R. Goto, K. Okada, K. Inoue, M. Itoh, T. Schormann, K. Zilles and H. Fukuda, Functional mapping of human brain in olfactory processing: a PET study, *J Neurophysmol* **84** (2000), 1656–1666.
[15] E.T. Rolls, M.L. Kringelbach and I.E. de Araujo, Different representations of pleasant and unpleasant odours in the human brain. Eur. 1, *Neurosci* **26** (2002), 695–703.
[16] Y. Saeki, The effect of foot-bath with or without the essential oil of lavender on the autonomic nervous system: a randomized trial, *Complement Ther Med* **8** (2000), 2–7.
[17] C.J. Stevensen, The psychophysiological effects of aromatherapy massage following cardiac surgery, *Complementary Therapies in Medicine* **2** (1994), 27–35.
[18] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology,. Standards of measurement, physiological interpretation, and clinical use, *Circulation Heart Rate Variability* **93** (1996), 1043–1065.
[19] E. Vernet-Maury, O. Alaoui-Ismaili, A. Dittmar, G. Delhomme and J. Chanel, Basic emotions induced by odorants: a new approach based on autonomic pattern results, *J Auton Nerv Syst* **75** (1999), 176–183.

ELSEVIER

NeuroImage

www.elsevier.com/locate/ynimg
NeuroImage 36 (2007) 706–717

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

Hideki Mochizuki,[a,b,c,*] Norihiro Sadato,[d,e] Daisuke N. Saito,[d] Hiroshi Toyoda,[d] Manabu Tashiro,[c] Nobuyuki Okamura,[c] and Kazuhiko Yanai[c]

[a]*Department of Sensory-Motor Integration, National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki, Aichi, Japan*
[b]*Department of Animal Models for Human Disease, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira-shi, Tokyo, Japan*
[c]*Department of Pharmacology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Japan*
[d]*Division of Cerebral Integration, National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki, Aichi, Japan*
[e]*JST/RISTEX, Kawaguchi, Japan*

Received 21 December 2006; revised 26 March 2007; accepted 2 April 2007
Available online 10 April 2007

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.

Keywords: Itching; Pain; PCC; Posterior insula; Thalamus; S2

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.

* Corresponding author. Department of Animal Models for Human Disease, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi-cho, Kodaira-shi, Tokyo, 187-8502, Japan. Fax: +81 3 346 1724.
E-mail address: motiz@ncnp.go.jp (H. Mochizuki).
Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2007.04.003

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; Darsow 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±SD, 26±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×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, Uniflows, 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×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/3.0×3.0×3.0 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

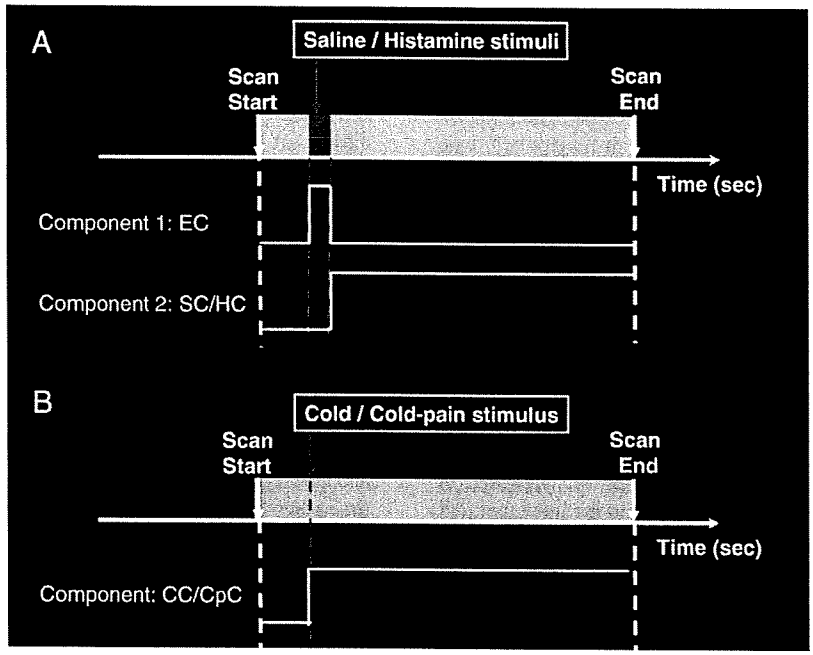

Fig. 1. Time course of neural activity in each condition. (A) Itching session. We hypothesized that the time course of neural activity in the histamine stimulus condition can be divided into two components. One is iontophoretic stimulus-related neural activity (electric component: EC), and the other 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

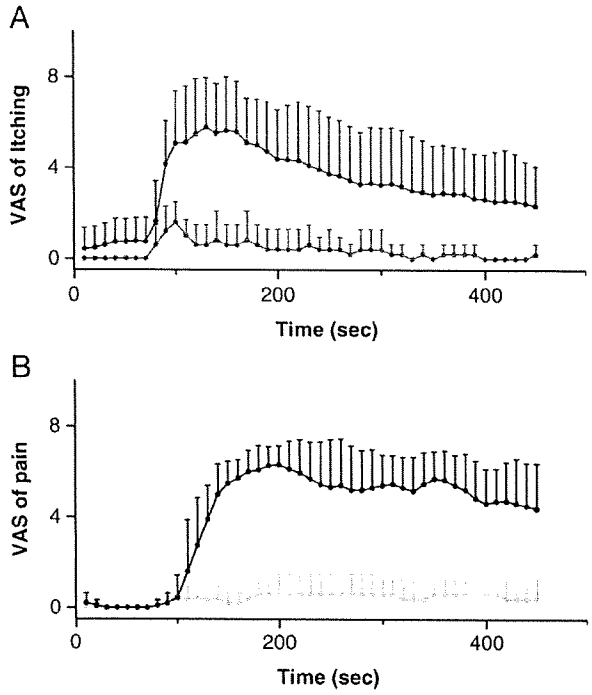

Fig. 2. Subjective evaluation of itching and pain sensations. (A) VAS score of itching was recorded in the histamine and the saline stimuli conditions, and (B) that of pain was recorded in the cold-pain and the cold stimuli conditions. Each line is the mean (SD) of VAS score of 14 subjects in each condition.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

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.

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

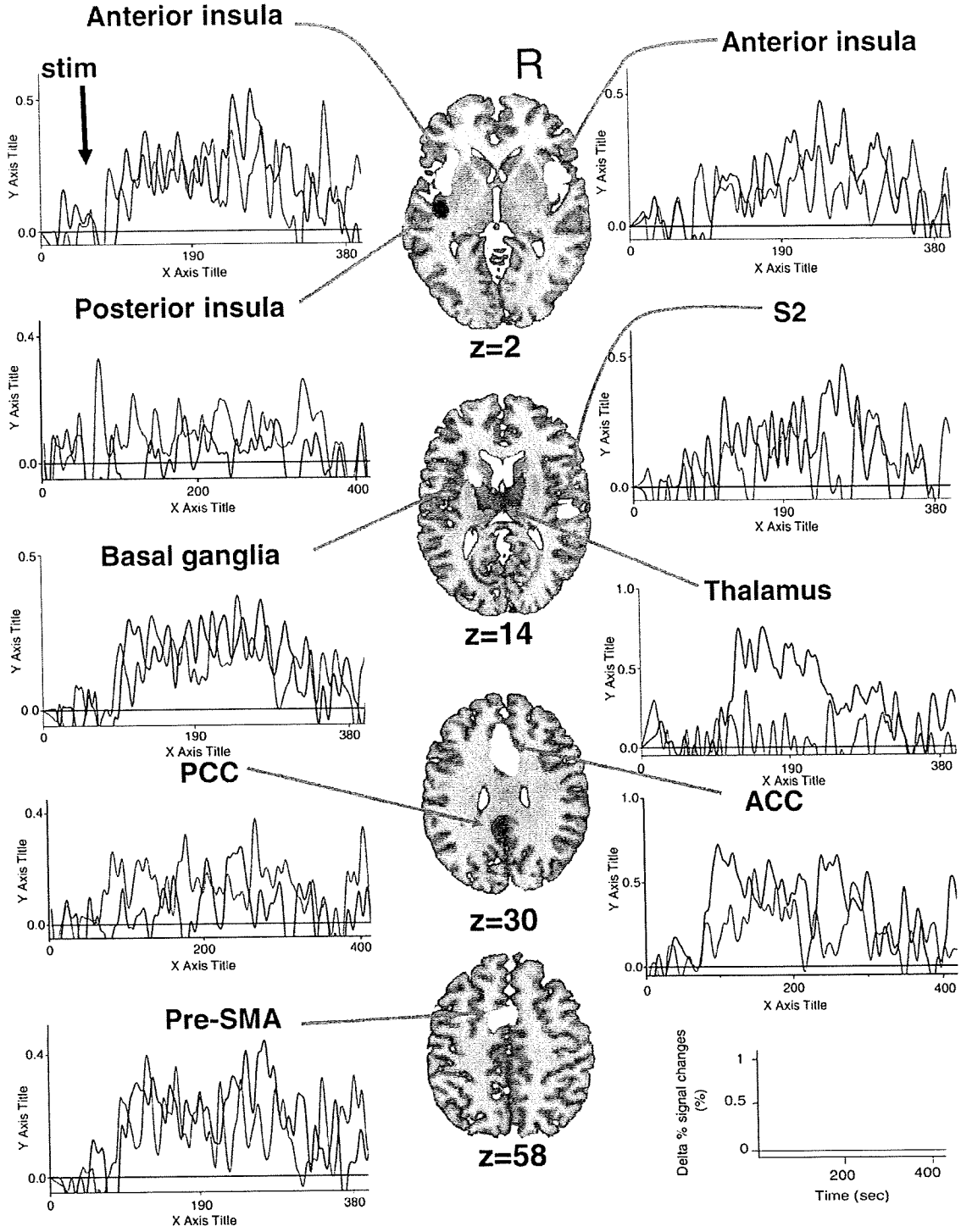

Fig. 3. Distribution of itching- and pain-related brain regions and the time course of their signal changes. In this study, we categorized itching- and pain-related brain regions into three types: common brain regions (yellow), brain regions more sensitive to itching (itching > pain) (red), the brain 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

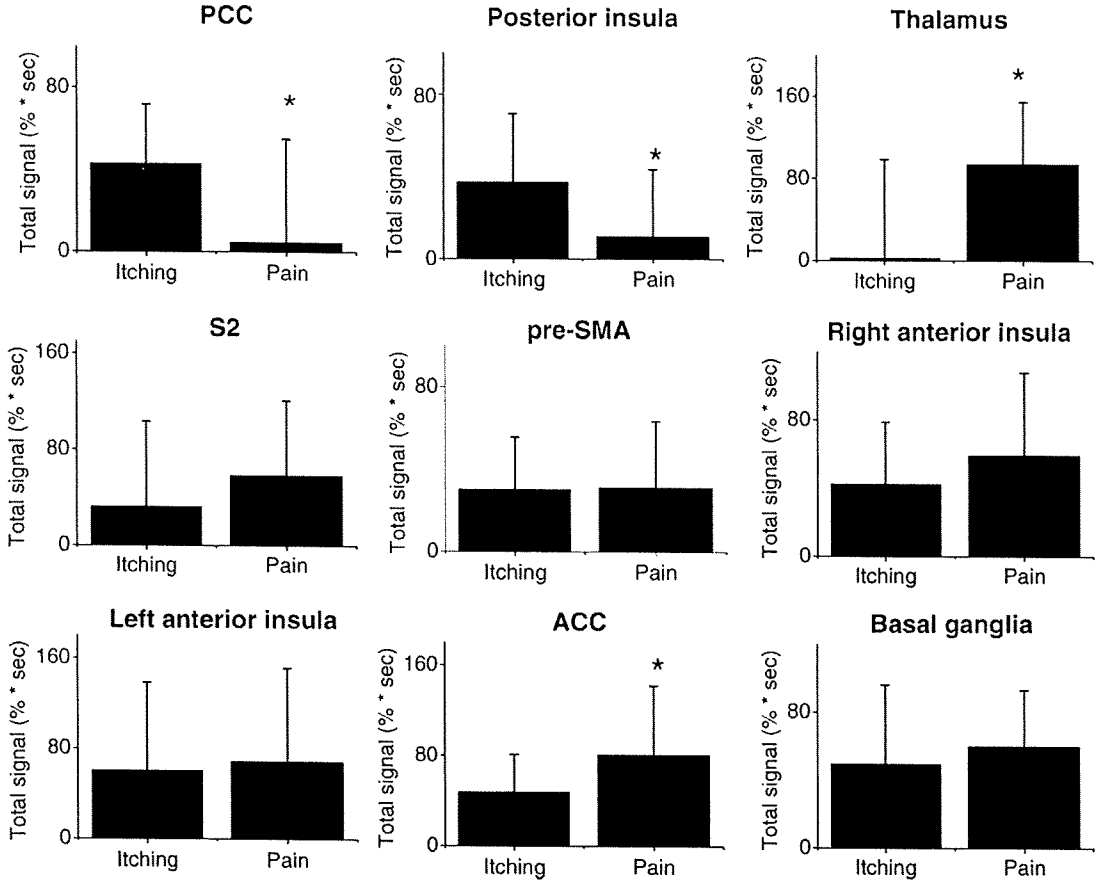

Fig. 4. Comparison of total signal between itching and pain. Total activity associated with itching and that associated with pain were compared between itching and pain. *$p<0.05$.

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

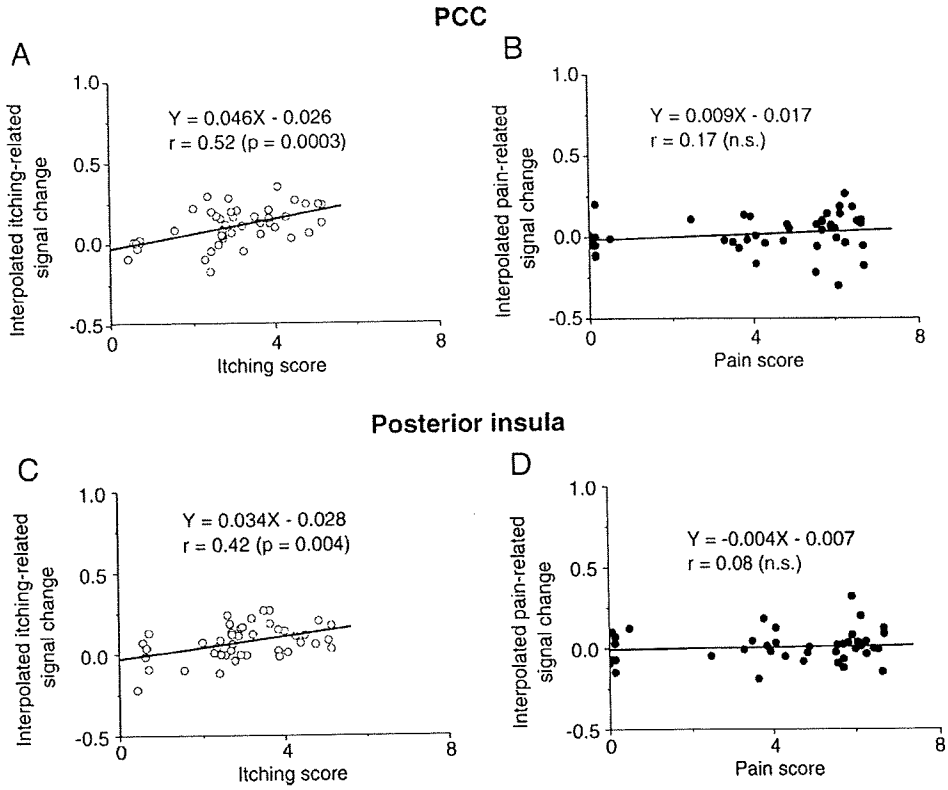

Fig. 5. Brain regions whose neural activity significantly correlated to itching sensation but did not to pain sensation (mean data of 14 subjects). (A, B) Brain region: PCC, (A) correlation between the interpolated itching-related signal change and the itching score, and (B) that between the interpolated 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

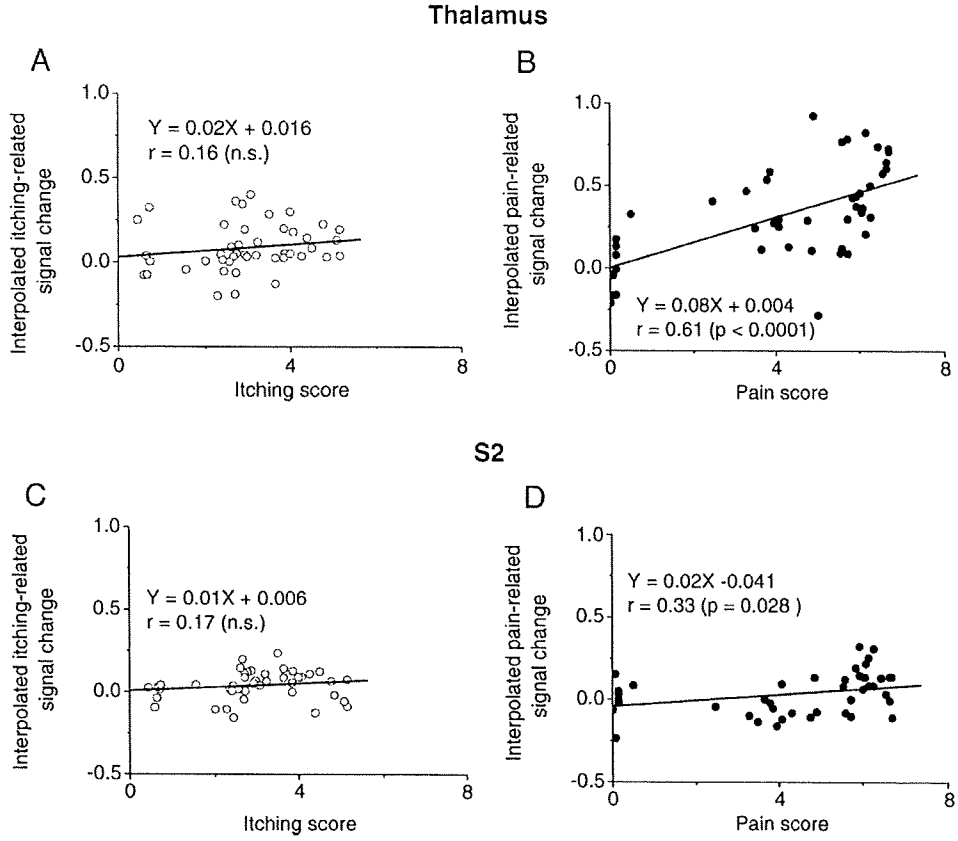

Fig. 6. Brain regions whose neural activity significantly correlated to pain sensation but did not to itching sensation (mean data of 14 subjects). (A, B) Brain region: thalamus, (A) correlation between the interpolated itching-related signal change and the itching score, and (B) that between the interpolated 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 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 week 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

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

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.

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.

Acknowledgments

The authors thank the volunteers in the fMRI measurement. This work was supported in part by Grants-in-Aid (#14370027, #1569126) for scientific research from the Japan Society of Promotion of Science (JSPS) and a 21st Century COE program (Bio-nano-technology) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Andreasen, N.C., O'Leary, D.S., Flaum, M., Nopoulos, P., Watkins, G.L., Ponto, L.L., Hichwa, R.D., 1997. Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. Lancet 349, 1730–1734.

Andersson, J.L., Lilja, A., Hartvig, P., Langstrom, B., Gordh, T., Handwerker, H., Torebjork, E., 1997. Somatotopic organization along the central sulcus for pain localization in humans, as revealed by positron emission tomography. Exp. Brain Res. 117, 192–199.

Andrew, D., Craig, D.C., 2001. Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. Nat. Neurosci. 4, 72–77.

Apkarian, A.V., Hodge, C.J., 1989. Primate spinothalamic pathways: III. Thalamic terminations of the dorsolateral and ventral spinothalamic pathways. J. Comp. Neurol. 288, 493–511.

Apkarian, A.V., Shi, T., 1997. Thalamocortical connections of the cingulate and insula in relation to nociceptive inputs to the cortex. In: Ayrapetian, S., Apkarian, A.V. (Eds.), Pain Mechanisms and Management. IOS Press, Amsterdam, pp. 212–220.

Bear, M.F., Connors, B.W., Paradiso, M.A., 2001. The somatic sensory system. Neuroscience Exploring the Brain. Lippincott Williams and Wilkins, Baltimore, pp. 396–435.

Britton, J.C., Phan, K.L., Taylor, S.F., Welsh, R.C., Berridge, K.C., Liberzon, I., 2006. Neural correlates of social and nonsocial emotions: an fMRI study. NeuroImage 31, 397–409.

Bremner, J.D., Vythilingam, M., Vermetten, E., Southwick, S.M., McGlashan, T., Staib, L.H., Soufer, R., Charney, D.S., 2003. Neural correlates of declarative memory for emotionally valenced words in women with posttraumatic stress disorder related to early childhood sexual abuse. Biol. Psychiatry 53, 879–889.

Bromm, B., 2004. The involvement of the posterior cingulate gyrus in phasic pain processing of humans. Neurosci. Lett. 361, 245–249.

Bush, G., Luu, P., Posner, M.I., 2000. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn. Sci. 4, 215–222.

Calder, A.J., Keane, J., Manes, F., Antoun, N., Young, A.W., 2000. Impaired recognition and experience of disgust following brain injury. Nat. Neurosci. 3, 1077–1078.

Casey, K.L., Minoshima, S., Morrow, T.J., Koeppe, R.A., 1996. Comparison of human cerebral activation patterns during cutaneous warmth, heat pain and deep cold pain. J. Neurophysiol. 76, 571–581.

Coghill, R.C., Talbot, J.D., Evans, A.C., Meyer, E., Gjedde, A., Bushnell, M.C., Duncan, G.H., 1994. Distributed processing of pain and vibration by the human brain. J. Neurosci. 14, 4095–4108.

Darsow, U., Drzezga, A., Frisch, M., Munz, F., Weilke, F., Bartenstein, P., Schwaiger, M., Ring, J., 2000. Processing of histamine-induced itch in the human cerebral cortex: a correlation analysis with dermal reactions. J. Invest. Dermatol. 115, 1029–1033.

Davis, K.D., Kwan, C.L., Crawley, A.P., Mikulis, D.J., 1998. Functional MRI study of thalamic and cortical activations evoked by cutaneous heat, cold, and tactile stimuli. J. Neurophysiol. 80, 1533–1546.

Derbyshire, S.W., Jones, A.K., 1998. Cerebral responses to a continual tonic pain stimulus measured using positron emission tomography. Pain 76, 127–135.

Derbyshire, S.W., Jones, A.K., Gyulai, F., Clark, S., Townsend, D., Firestone, L.L., 1997. Pain processing during three levels of noxious stimulation produces differential patterns of central activity. Pain 73, 431–445.

Dong, W.K., Salonen, L.D., Kawakami, Y., Shiwaku, T., Kaukoranta, M., Martin, R.F., 1989. Nociceptive responses of trigeminal neurons in SII-7b cortex of awake monkeys. Brain Res. 484, 314–324.

Drzezga, A., Darsow, U., Treede, R.D., Siebner, H., Frisch, M., Munz, F., Weilke, F., Ring, J., Schwaiger, M., Bartenstein, P., 2001. Central activation by histamine-induced itch: analogies to pain processing: a correlational analysis of O-15 H_2O positron emission tomography studies. Pain 92, 295–305.

Friston, K.J., Ashburner, J., Frith, C.D., Poline, J.B., Heather, J.D., Frackowiak, R.S.J., 1995a. Spatial registration and normalization of images. Hum. Brain Mapp. 2, 165–189.

Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J.B., Frith, C.D., Frackowiak, R.S.J., 1995b. Statistical parametric maps in functional imaging: a general linear approach. Hum. Brain Mapp. 2, 189–210.

Forss, N., Raij, T.T., Seppa, M., Hari, R., 2005. Common cortical network for first and second pain. NeuroImage 24, 132–142.

Genovese, C.R., Lazar, N.A., Nichols, T., 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. NeuroImage 15, 870–878.

Guilbaud, G., Bernard, J.F., Besson, J.M., 1994. Brain areas involved in nociception and pain. In: Melzack, W.R. (Ed.), Textbook of Pain. Churchill-Livingstone, Edinburgh, pp. 113–128.

Handwerker, H.O., Forster, C., Kirchhoff, C., 1991. Discharge patterns of human C-fibers induced by itching and burning stimuli. J. Neurophysiol. 66, 307–315.

Hansen, N., Klein, T., Magerl, W., Treede, R.D., 2007. Psychophysical evidence for long-term potentiation of C-fiber and Aδ-fiber pathways in humans by analysis of pain descriptors. J Neurophysiol. 97, 2559–2563.

Ho, A.P., Gillin, J.C., Buchsbaum, M.S., Wu, J.C., Abel, L., Bunney Jr., W.E., 1996. Brain glucose metabolism during non-rapid eye movement sleep in major depression. A positron emission tomography study. Arch. Gen. Psychiatry 53, 645–652.

Hsieh, J.C., Hagermark, O., Stahle-Backdahl, M., Ericson, K., Eriksson, L., Stone-Elander, S., Ingvar, M., 1994. Urge to scratch represented in the human cerebral cortex during itch. J. Neurophysiol. 72, 3004–3008.

Jinks, S.L., Carstens, E., 2000. Superficial dorsal horn neurons identified by intracutaneous histamine: chemonociceptive responses and modulation by morphine. J. Neurophysiol. 84, 616–627.

Kakigi, R., Hoshiyama, M., Shimojo, M., Naka, D., Yamasaki, H., Watanabe, S., Xiang, J., Maeda, K., Lam, K., Itomi, K., Nakamura, A., 2000. The somatosensory evoked magnetic fields. Prog. Neurobiol. 61, 495–523.

Maddock, R.J., Buonocore, M.H., Kile, S.J., Garrett, A.S., 2003. Brain regions showing increased activation by threat-related words in panic disorder. NeuroReport 14, 325–328.

Miyata, M., Kashiwadani, H., Fukaya, M., Hayashi, T., Wu, D., Suzuki, T., Watanabe, M., Kawakami, Y., 2003. Role of thalamic phospholipase C [beta]4 mediated by metabotropic glutamate receptor type 1 in inflammatory pain. J. Neurosci. 23, 8098–8108.