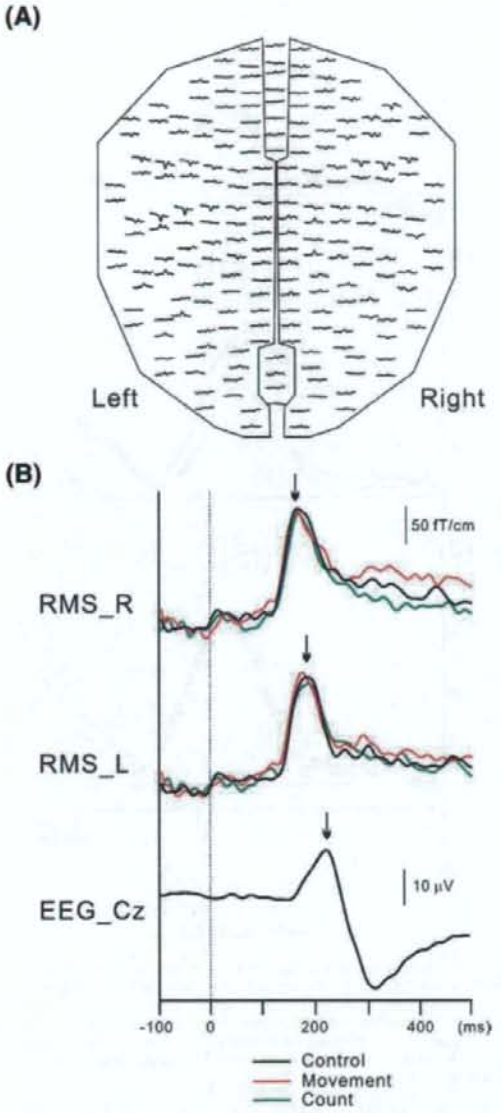

Fig. 5. (A) Sensor layout of the MEG to calculate the root-mean-square (RMS). (B) Change of the grand-averaged RMS waveforms from the right and left hemispheres. The peak amplitudes of RMS in each hemisphere not significantly changed among conditions. Black, red, and green lines indicate waveforms of Control, Movement, and Count, respectively. RMS_R = root-mean-square in the right hemisphere, RMS_L = root-mean-square in the left hemisphere, EEG_Cz = Cz electrode in electroencephalography.

Control, Movement, and Count conditions, respectively (Fig. 9A and Table 6). Fig. 9B indicates the change of the grand-averaged ECD strength waveforms of ACC. In the waveform of Control, two peaks were found at 160 and 210 ms, respectively. In Movement and Count, a single peak was identified around 210 ms, which was consistent with the peak of N2. Thus, we mainly focused on and analyzed this peak. Indeed, since ACC activity was not estimated from all subjects, we could not perform ANOVA, but the average values of the ECD strength was clearly smaller in Movement than Control and Count (Fig. 9C). These results suggest that voluntary movement in a preparatory period reduces the ECD strength of ACC.

Data of a supplementary experiment

Fig. 10 displays grand-averaged LEP waveforms in each condition across all subjects. Clear N2 and P2 components were recorded in all conditions after noxious stimulation.

ANOVAs for the amplitude of N2 revealed a significant main effect of Electrode ($F(4, 28) = 10.371$, $p < 0.01$, $\varepsilon = 0.413$), and a significant effect of Condition–Electrode interaction ($F(8, 56) = 5.288$, $p < 0.001$). Tukey HSD post hoc testing with collapsing Condition revealed that the amplitude was significantly larger at Cz than Fz, Pz, and C4 ($p < 0.05$, $p < 0.001$, and $p < 0.01$, respectively), and C3 than C4 ($p < 0.05$). There was no significant main effect of Condition ($F(2, 14) = 0.991$, $p > 0.05$), and post hoc test did not show differences in values between conditions per electrode (Fig. 11A). These results suggest that voluntary movements with the right hand and left foot did not significantly affect the peak amplitude of the N2.

In ANOVAs for the peak amplitude of P2, there was a significant effect of Condition–Electrode interaction ($F(8, 56) = 9.882$, $p < 0.001$), and a main effect of Condition ($F(2, 14) = 9.058$, $p < 0.01$), and Electrode ($F(4, 28) = 13.240$, $p < 0.01$, $\varepsilon = 0.493$). Post hoc testing showed that the P2 amplitude was significantly larger in Control than in Right Hand, and Left Foot at Fz ($p < 0.05$, and $p < 0.001$, respectively), Cz ($p < 0.001$, and $p < 0.01$, respectively), and C3 ($p < 0.05$, respectively). These findings suggest that both different types of movement, including the right hand and left foot reduced the P2 amplitude (Fig. 11B). In addition, a post hoc test with collapsing Condition indicated that the amplitude was significantly larger at Cz than Fz, and C3 ($p < 0.001$, respectively), Pz than Fz and C3 ($p < 0.001$, and $p < 0.01$, respectively), and C4 than Fz ($p < 0.05$).

ANOVAs for the peak-to-peak amplitude of N2–P2 revealed a significant effect of Condition–Electrode interaction ($F(8, 56) = 6.681$, $p < 0.001$), and main effects of Condition ($F(2, 14) = 10.153$, $p < 0.01$) and Electrode ($F(4, 28) = 11.547$, $p < 0.001$). Post hoc testing showed that the amplitude was significantly larger in Control than Right Hand at Fz, Cz, and C3 ($p < 0.05$, $p < 0.01$, and $p < 0.05$), and Control than Left Foot at Cz, Pz, C3, and C4 ($p < 0.01$, $p < 0.05$, $p < 0.05$, and $p < 0.05$) (Fig. 11C). A post hoc test with collapsing Condition indicated that the amplitude was significantly larger at Cz than Fz, Pz, C3, and C4 ($p < 0.001$, $p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively).

ANOVAs for the peak latency of N2 indicated a significant effect of Electrode ($F(4, 28) = 3.884$, $p < 0.05$, $\varepsilon = 0.528$). Post hoc testing with

Table 5
Results of ANOVAs for the amplitudes of N2, P2, root-mean-square (RMS), and areal mean signals (AMS), and equivalent current dipole (ECD) strength

Component	Factor	*df*	*F*	ε	*P*
N2	C	2, 18	6.092		<0.01
	E	4, 36	9.030	0.542	<0.01
	C×E	8, 72	1.436		
P2	C	2, 18	0.342		
	E	4, 36	17.271	0.483	<0.001
	C×E	8, 72	4.056	0.410	<0.05
N2–P2	C	2, 18	3.818		<0.05
	E	4, 36	18.138		<0.001
	C×E	8, 72	3.704	0.574	<0.05
RMS	C	2, 18	0.918		
	H	1, 9	0.980		
	C×H	2, 18	0.938		
AMS	C	2, 18	0.715		
	A	2, 18	8.959		<0.01
	C×A	4, 36	0.832		
ECD	C	2, 16	0.672		
	S	2, 16	15.401		<0.001
	C×S	4, 32	0.058		

C = Condition, E = Electrode, S = Source, H = Hemisphere, A = Area ε = epsilon.

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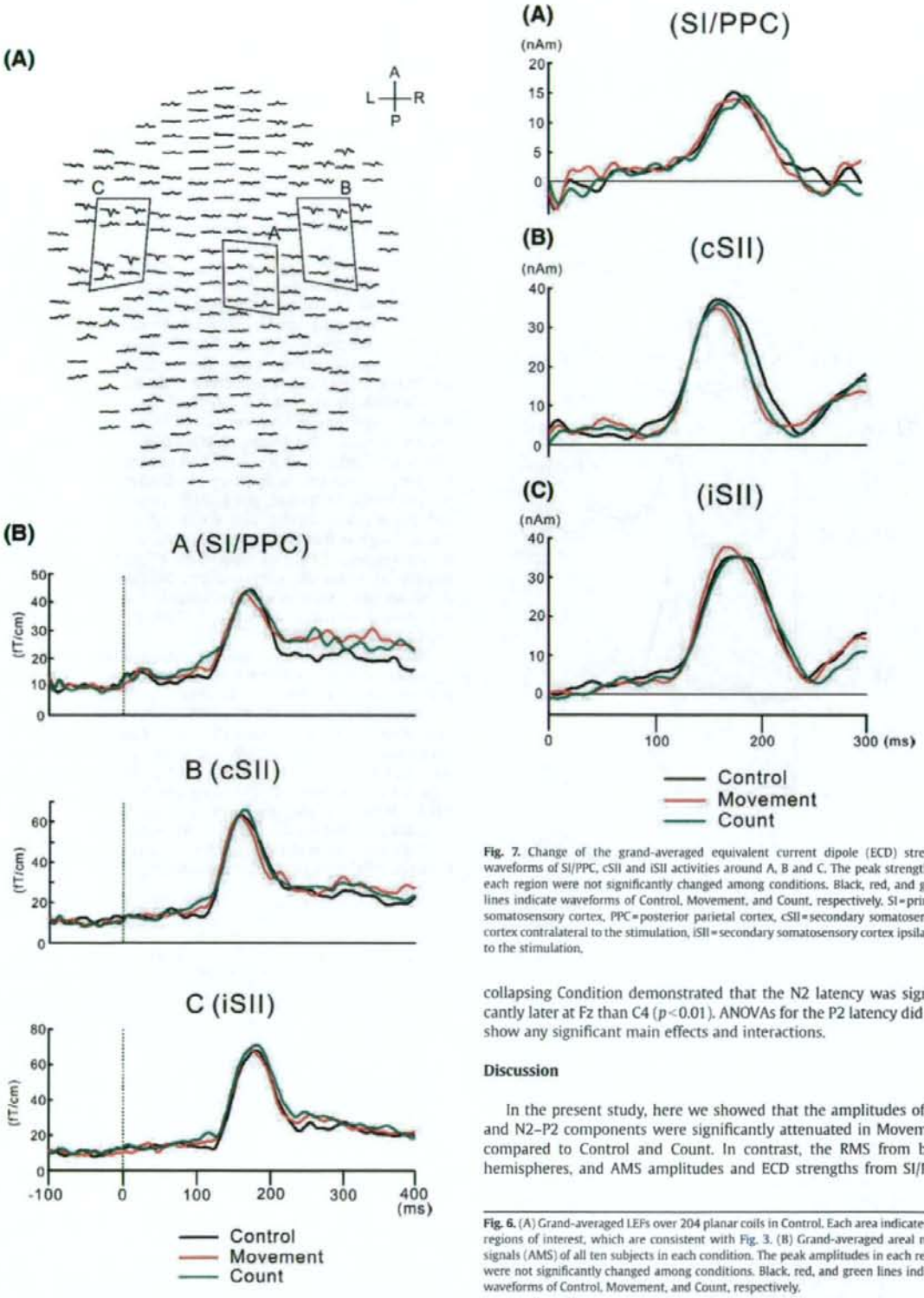

Fig. 7. Change of the grand-averaged equivalent current dipole (ECD) strength waveforms of SI/PPC, cSII and iSII activities around A, B and C. The peak strengths in each region were not significantly changed among conditions. Black, red, and green lines indicate waveforms of Control, Movement, and Count, respectively. SI = primary somatosensory cortex, PPC = posterior parietal cortex, cSII = secondary somatosensory cortex contralateral to the stimulation, iSII = secondary somatosensory cortex ipsilateral to the stimulation.

collapsing Condition demonstrated that the N2 latency was significantly later at Fz than C4 ($p<0.01$). ANOVAs for the P2 latency did not show any significant main effects and interactions.

Discussion

In the present study, here we showed that the amplitudes of N2 and N2–P2 components were significantly attenuated in Movement compared to Control and Count. In contrast, the RMS from both hemispheres, and AMS amplitudes and ECD strengths from SI/PPC,

Fig. 6. (A) Grand-averaged LEFs over 204 planar coils in Control. Each area indicates the regions of interest, which are consistent with Fig. 3. (B) Grand-averaged areal mean signals (AMS) of all ten subjects in each condition. The peak amplitudes in each region were not significantly changed among conditions. Black, red, and green lines indicate waveforms of Control, Movement, and Count, respectively.

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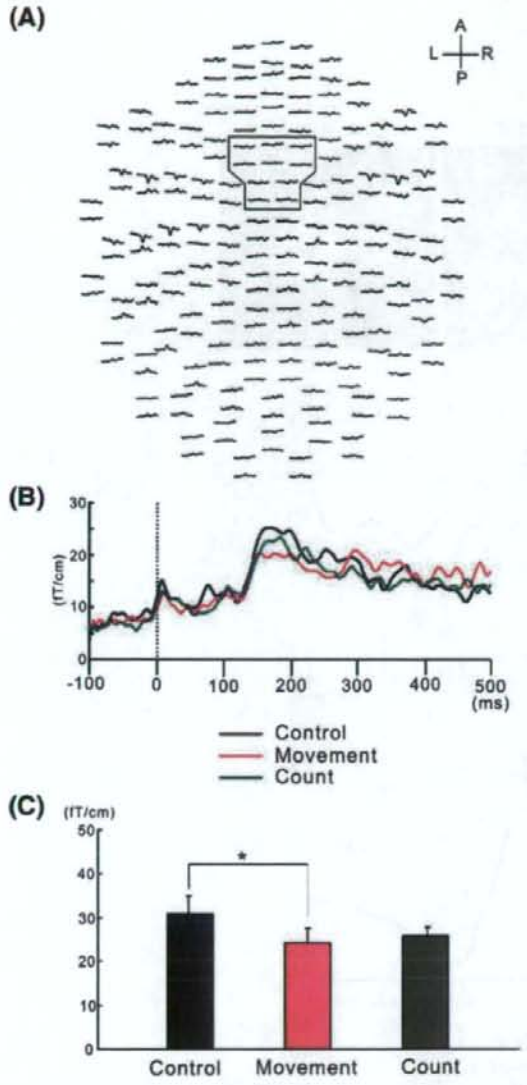

Fig. 8. (A) Grand-averaged LEFs over 204 planar coils in Control. The area indicates the regions of interest to detect activity in the anterior cingulate cortex (ACC). (B) Grand-averaged areal mean signals (AMS) of all ten subjects in each condition. Peaks were found at 160–230 ms after noxious stimulation. Black, red, and green lines indicate waveforms of Control, Movement, and Count, respectively. (C) Mean values of AMS amplitudes. Post hoc test indicated that amplitudes were significantly smaller in Movement than Control ($p<0.05$). Vertical lines indicate standard errors (S.E.). *$p<0.05$.

cSII, and iSII recorded by MEG were not significantly different among the three conditions. The AMS amplitudes and ECD strengths from ACC, which showed a similar peak to the N2 component, were significantly or clearly smaller in Movement than Control and Count. There were no effects of Count on LEPs and LEFs. These results indicated that the attenuation of LEP components and the neural activity of ACC started before voluntary movement, and this attenuation was related to the centrifugal effects caused by movement-related neural activities during a movement preparatory period.

N2 and P2 components

In general, N2 and P2 components are maximal at vertex electrodes, and spread widely after laser stimulation at any of site of the body. The mean peak latencies of N2 and P2 after laser stimulation of the hand are approximately 200 to 240 and 300 to 360 ms, respectively. The early N1 and P1 components are found before N2 and P2, and considered to be generated in the bilateral SII/Insula (reviewed in Kakigi et al., 2000).

Some previous studies reported that the amplitudes of N2 and P2 were attenuated during voluntary movement (Kakigi and Shibasaki, 1992; Kakigi et al., 1993), and pre-movement (Le Pera et al., 2007), which were consistent with our findings. Furthermore, there have been several papers examining the effects of attention/distraction on pain perception. They reported that the amplitudes of N2 and P2 on LEPs were enhanced by attention and attenuated by distraction (Beydoun et al., 1993; Siedenberg and Treede, 1996; García-Larrea et al., 1997; Yamasaki et al., 1999; Legrain et al., 2002, 2003a,b, 2005; Ohara et al., 2004a; Le Pera et al., 2007). In the present experimental design, since we used a task-relevant reaction time paradigm, the subjects were instructed to pay attention to the noxious stimuli due to respond to it or count in Movement and Count, respectively. As a result, modulation of the N2 and N2–P2 amplitudes was found only in Movement, not in Count. Thus, directing attention to the stimulation itself may not be a main factor causing the attenuation of the L2 and N2–P2 amplitudes in Movement, and other mechanisms should be considered.

We discuss the possibility that this finding was caused by overlapping of motor-related potentials. Motor-related activity can be recorded as MRCPs (movement-related cortical potentials) in scalp recordings, showing negative potentials. In particular, MP (motor potential), which consists of a short negative component, appears in the contralateral sensorimotor area (Shibasaki et al., 1980; Hallett, 1994). In the present study, the average values of P2 amplitude were smaller in Movement than Control and Count at Cz (Fig. 2B), and the mean RT across all subjects was 472 ms, and data from some subjects overlapped for the peak latency of P2 (Table 2). Thus, P2 amplitude in Movement may be influenced by this overlapping potential, which distorts the true P2 component. This notion was supported by the data of a supplementary experiment. That is, the amplitude of P2 was significantly smaller in Right Hand and Left Foot than Control (Figs. 10 and 11), indicating that attenuation of the P2 amplitude was affected by overlapping of MP, rather than centrifugal effects during a movement preparatory period.

In contrast, this possibility can be excluded for the N2 component, because, even though the negativity of MP overlapped on LEPs, this phenomenon cannot explain attenuation of the N2 amplitude. In addition, in a supplementary experiment, the N2 amplitude was not influenced by other body movements, such as the right hand and left foot; therefore, we should consider other mechanisms for attenuation of the N2 amplitude.

Our findings suggested that centrifugal effects were found on the N2 amplitude during the movement preparatory period, when the stimulated hand and movement hand were matched. Moreover, based on data of a supplementary experiment, hand movement contralateral to the noxious stimulation (i.e. right hand movement) did not affect the N2 amplitude, and other movement on the same body side to the noxious stimulation (i.e. left foot movement) also did not modulate the N2 amplitude. These results confirmed the result of Le Pera et al. (2007), which showed that hand movement contralateral to the noxious stimulation did not reduce the amplitude of LEPs.

SI/PPC, bilateral SII, ACC activities on LEFs

In the present study, the RMS, AMS, and ECDs peaks of LEFs generated in the contralateral SI/PPC bilateral SII were clearly

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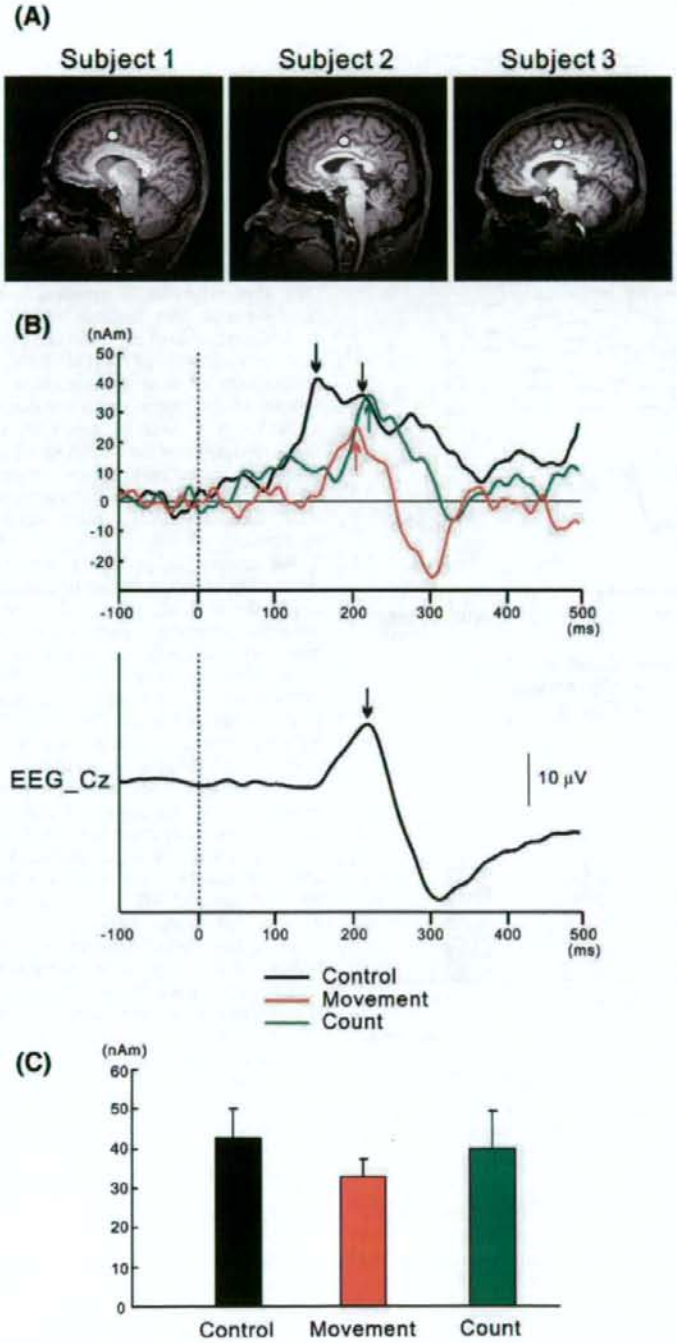

Fig. 9. (A) Location of ECDs for ACC superimposed on a 2D MRI scan in three representative subjects. (B) Change of the grand-averaged ECD strength waveforms of ACC. Black, red, and green lines indicate waveforms of Control, Movement, and Count, respectively. Arrows indicated the peak of the waveform, and peak strengths were smaller in Movement than Control and Count. (C) Mean values of ECD strengths for ACC. Vertical lines indicate standard errors (S.E.).

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Table 6
Mean Talairach coordinates and peak latency of the ACC source for each condition

	n	x (mm)	y (mm)	z (mm)	Latency
Control	6	[8.4] (2.0)	−0.9 (4.9)	38.8 (0.8)	216.5 (7.1)
Movement	5	[7.0] (1.9)	−0.1 (5.4)	38.8 (0.6)	201.6 (8.5)
Count	5	[9.3] (1.4)	−2.6 (4.3)	36.4 (1.2)	222.4 (2.2)

Data are expressed as the mean (SE). n = number of subjects, which was identified in the dipole analysis, | | = absolute value.

identified earlier than N2 and P2 peaks of LEPs (Fig. 5B and Table 1). They peaked between 160–190 ms after noxious stimulation, while N2 component of LEPs peaked about 220 ms. This difference indicated that LEPs and LEFs recorded different neural activities at different time periods. In addition, since the modulations of the RMS and AMS during the three conditions showed similar results to that of ECDs analysis, the RMS and AMS included mainly neural activities from SI/PPC and bilateral SII.

Some MEG studies using axial type coils reported small MEG activities in ACC following strong electrical stimulation (Kitamura et al., 1995), laser stimulation (Bromm et al., 1996) and noxious stimulation caused by specialized intra-epidermal needle electrode (Inui et al., 2003). In the present study, we found ACC activities, estimated by using all 306-channel analysis involving magnetometers, from six, five, and five subjects in Control, Movement, and Count, respectively (Fig. 9 and Table 6). The average values of ECD strength, and AMS amplitudes were clearly or significantly smaller in Movement than Control and Count (Figs. 8 and 9). Judging from the peak latency and modulation of the ECD strengths for ACC, the N2 component should be related to neural activity of ACC. Traditionally, the cingulate cortex has been regarded as part of the limbic system, and was associated with the motivational–affective component of pain (reviewed in Schnitzler and Ploner, 2000). Several LEP studies using dipole modeling reported a close relationship between generator mechanisms of N2 and ACC (Tarkka and Treede, 1993; Valeriani et al., 1996, 2000; Garcia-Larrea et al., 2003; Iannetti et al., 2003; Schlereth et al., 2003; Tsuji et al., 2006). In addition, there is anatomical evidence that motor-related cortical areas, especially the primary motor area (MI), are strongly connected with ACC (Dum and Strick, 1991; Morecraft and Van Hoesen, 1992), and a functional relation between these areas was also demonstrated in neurophysiological (Tamura et al., 2004a,b) and neuroimaging studies (Koski and Paus, 2000; Siebner et al., 2001). Taking these previous studies into consideration, it is likely that the neural activity of ACC was inhibited by movement-related neural activities, mainly in the MI, during a movement preparatory period.

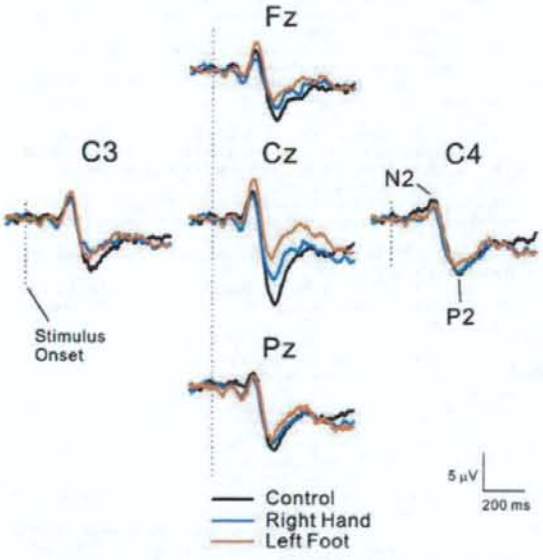

Fig. 10. Grand-averaged LEPs in a supplementary experiment. Noxious YAG laser stimulation was applied to the dorsum of the left hand in each condition. Black, blue, and orange lines indicate waveforms of Control, Right hand, and Left Foot, respectively. Vertical line indicates stimulus onset.

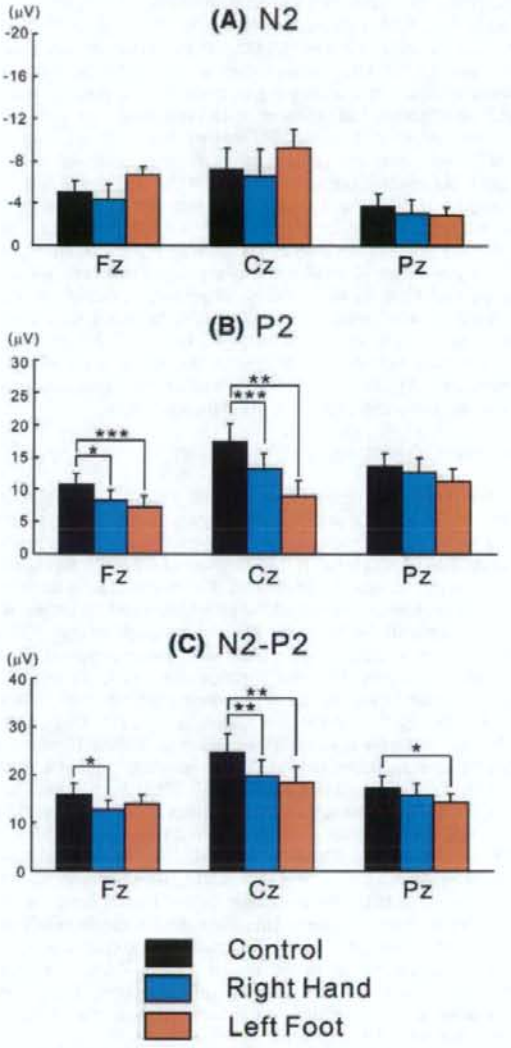

Fig. 11. Mean values of N2, P2, and N2–P2 amplitudes in a supplementary experiment. Vertical lines indicate standard errors (S.E.). $*p<0.05$, $**p<0.01$, $***p<0.001$

However, we should reconsider that the activity of ACC could not be recorded from all subjects, and the number of subjects, identified by ECD analysis, was almost half of the participants (Table 6). Generally, it is difficult for MEG to detect dipoles generated in deep areas compared with EEG (reviewed in Hari et al., 2000), and thus many previous studies using the same Neuromag MEG system mainly

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analyzed ECDs from SI, PPC, and bilateral SII (Ploner et al., 1999, 2000; Kanda et al., 2000; Timmermann et al., 2001; Raij et al., 2003; Nakata et al., 2004, 2008; Forss et al., 2005). Based on the advantages and disadvantages of MEG, further studies are needed to clarify the characteristics of ACC activity in pain processing, for instance, a study with simultaneous MEG and a multi-EEG recording.

Our previous study using MEG showed that neural activities of SI and SII were attenuated during voluntary movement (Nakata et al., 2004), but in the current study, the AMS amplitudes and ECD strengths of SI/PPC and bilateral SII were not modulated before voluntary movement in a forewarned reaction time task. These differences for LEFs may infer that centrifugal modulation on sensory–motor integration is smaller during pre-movement than voluntary movement. In some MEG studies, SII responses elicited by tactile stimulation were enhanced in both hemispheres during voluntary movement (Forss and Jousmäki, 1998; Lin et al., 2000), but the enhancement was significant only in the contralateral SII before movement (Wasaka et al., 2005c). Such small modulation during pre-movement may also apply to pain–motor integration.

Differences of results between LEPs and LEFs

We considered the reason why the N2 and N2–P2 amplitudes of LEPs were modulated during motor preparation processing, but AMS amplitudes and ECD strengths of LEF were not changed. A possible explanation for this result is the difference of generator mechanisms between the N2 and P2 components of LEPs and LEFs. A number of EEG studies have demonstrated the pain-related cortical regions such as SI, bilateral SII, the insula, the ACC, the midcingulate cortex (MCC), the posterior cingulate cortex (PCC), and medial temporal regions including the amygdale and hippocampus using dipole source modeling (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani et al., 1996, 2000; Bentley et al., 2001, 2002, 2003; Garcia-Larrea, 2002; Garcia-Larrea et al., 2003; Iannetti et al., 2003; Schlereth et al., 2003; Tsuji et al., 2006), and intracranial recordings (Lenz et al., 1998a, b, 2000; Ohara et al., 2004a,b,c; Frot et al., 2008). On the other hand, MEG studies have shown that LEF components recorded about 170 ms after noxious laser stimulation of the hand were generated from SI, PPC, and bilateral SII (Ploner et al., 1999, 2000; Kanda et al., 2000; Timmermann et al., 2001; Raij et al., 2003; Nakata et al., 2004, 2008, Forss et al., 2005). Pain processing in the human brain has two different nociceptive systems, lateral and medial (reviewed in Treede et al., 1999). SI and SII cortices belong to the lateral system, while ACC and the insula belong to the medial system. Taking the studies mentioned above into consideration, LEF components from SI, PPC, and bilateral SII are related to the lateral system, and N2 and P2 components on LEPs, the medial system.

In one previous study recording LEPs and LEFs simultaneously, Yamasaki et al. (1999) reported that the N2 and P2 amplitudes were attenuated during calculation and memorization tasks, but SII responses of LEFs were unchanged. Moreover, Le Pera et al. (2007) analyzed both N1–P1 and N2–P2, and found that the N1–P1 amplitude remained unchanged during the pre-movement period, but the N2–P2 amplitude was attenuated. These results were consistent with our findings.

Conclusion

The N2 and N2–P2 components of LEPs following a task-relevant noxious stimulation were modulated through a centrifugal mechanism when the signal triggered a voluntary movement. This effect was not found in Count. In addition, ECD strengths and AMS amplitudes of ACC, which showed a similar peak to the N2 component, were clearly smaller in Movement than Control and Count. We therefore suspect that neural activities related to the generator mechanisms of N2, especially those in the ACC, are inhibited by movement-related neural activities during the preparatory period. In addition, since the N2 amplitude was not affected by the right hand and left foot in a supplementary experiment, we consider that centrifugal effects occur on the N2 amplitude during the movement preparatory period, when the stimulated hand and movement hand are matched.

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Itching-related somatosensory evoked potentials

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Abstract

Electrically evoked itching has the strong potential to be used to investigate the central processing associated with itching at high temporal resolution by employing magnetoencephalography, electroencephalography (EEG), and event-related functional magnetic resonance imaging. However, it has not been investigated whether time-locked brain activity can be measured using this stimulus, and whether the itching sensation induced by electrical stimulation of the skin is associated with C-fibers. Thus, we investigated these problems in this study. Itching sensations were elicited when electrical stimuli were applied to the skin of the right wrist and right forearm. EEG activity was recorded from 5 electrodes (Fz, FCz, Cz, CPz and Pz). When the right wrist was stimulated, the reaction time (RT) and latency of the positive component of somatosensory evoked potentials (P1) were 1215 ms and 963 ms, respectively. When the right forearm was stimulated, the RT and peak latency of the P1 were 1013 ms and 772 ms, respectively. The conduction velocity estimated from the RT and latency of the P1 was 1.04 m/s and 0.92 m/s, respectively. In addition, the itching sensation and P1 were inhibited when the current intensity was increased into the range eliciting pain and touch sensations, implying interaction between C- and A-fibers. These findings demonstrate that time-locked brain activity can be measured using electrically evoked itching and that the itching sensation induced by the electrically evoked itching is associated with C-fibers. Thus, this method is useful for research into the central processing of itching.

Keywords: Electrically evoked itching; Somatosensory evoked potential; C-fibers

1. Introduction

Itching sensations are associated with the excitation of C-fibers induced by histamine [7,22,24,25,29]. Therefore, in most studies of itching, histamine is used to elicit the itching sensation. Recently, functional neuroimaging techniques such as positron emission tomography and functional magnetic resonance imaging (fMRI) have been used to clarify the central mechanism of itching [4,8,15,16]. For example, Mochizuki et al. reported that the posterior cingulate cortex and posterior insula play important roles in itching perception [16]. Laknes et al. suggested that the itch–scratch-cycle, a serious problem among patients with atopic dermatitis, was partly associated with enhanced activity in the striato-thalamo-orbitofrontal circuit [12]. These studies used histamine-induced itching. When histamine is applied to the skin, an itching sensation gradually develops and remains for a long time (5–20 min), then slowly decreases. Therefore, such a chemical stimulus is not useful for measuring time-locked brain activity with magnetoencephalography (MEG), electroencephalography (EEG), and event-related fMRI. However, it has also been reported that a special electrical stimulation

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of the skin can elicit an itching sensation [5,23,28]. Since electrical stimulation has a great advantage over chemical stimulation in terms of time-locked averaging, it can be a powerful tool with which to investigate the brain processing associated with itching at high temporal resolution (ms). Therefore, we considered that electrically evoked itching is useful for basic and clinical research on itching. However, it is still unclear whether time-locked brain activity (i.e., somatosensory evoked potential (SEP)) can be measured using the electrically evoked method. Thus, we investigated this problem. In addition, we also estimated the conduction velocity (CV) of peripheral signals responsible for SEP. This is the first study to measure the CV of itch signals conducted through peripheral nerves following electrical stimulation of the skin.

2. Methods

2.1. Subjects

Nine healthy male volunteers (28 ± 4 years (mean $\pm$ standard deviation (SD))) participated in this study. Subjects with a history of allergy, atopic eczema, or other dermatological diseases were excluded. The study was approved by the Ethics Committee at our Institute. Written informed consent was obtained from each subject, and the study was performed in compliance with the relevant laws and institutional guidelines.

2.2. Stimuli

To evoke itching, a modified version of the method that Ikoma et al. recently developed was used [9]. In brief, the electrode for the electrical stimulus was composed of 4 crossed stainless steel wires (diameter: 0.1 mm, The Nilako Co. Ltd., Tokyo, Japan) and a plastic plate ($1.5 \times 1.5\ cm^2$) (Fig. 1A). The electrode was attached to the right wrist and right forearm in the wrist and forearm stimulus conditions, respectively. A saline-soaked gauze pad ($1.5 \times 1.5\ cm^2$) serving as the reference electrode (cathode) was placed on the right wrist 2.0 cm proximal to the electrode center (Fig. 1B). On the basis of a previous study [9], current-constant square wave pulses (pulse duration, 2 ms; frequency, 50 Hz) were applied to the skin through the electrodes. Twenty pulses were given in one stimulus. Before the experiment, the intensity of the current at which a clear itching sensation was felt was defined for each subject, 0.24 ± 0.06 mA. The electrical stimulus was applied to the right wrist (area: $4 \times 4\ cm^2$) or the right forearm (area: $4 \times 4\ cm^2$). Forty stimuli were given to the subjects in each condition. In addition, before and after the recording of SEP, the electrical stimulus was applied 10 times to obtain the reaction time (RT) and a mean value was used for analysis. In a preliminary study, we observed that repeated stimuli markedly reduced the itching sensation when the inter stimulus interval (ISI) was less than 30 s. Therefore, we considered it better to have a long ISI. Thus, the ISI was over 30 s in this study. Each session included ten stimuli. It took about 7 min in one session. Four sessions were conducted for recording SEP and two sessions for recording RT. The subjects rested for about 10 min between each session. During this time, we changed the site of stimulation within the restricted area ($4 \times 4\ cm^2$) in each condition. In total, it took at least 1.5 h to measure the SEP and RT in one condition (e.g., the wrist stimulus condition). Therefore, it was very tough for subjects to be measured SEP and RT following the stimulation of two different body parts (i.e., the right wrist and right forearm) on the same day. Therefore, the two conditions were performed on different days for each subject.

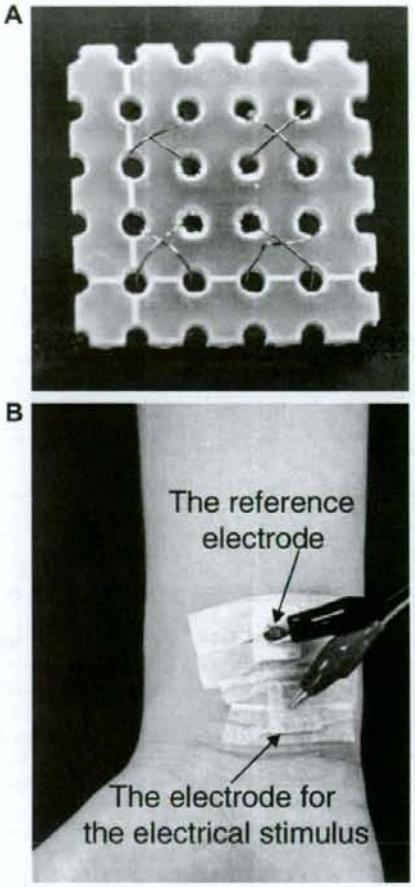

Fig. 1. The electrode used to elicit itching (A) and the right wrist when the electrical stimulus was applied (B).

After each stimulus during the SEP recording, the subjects evaluated itching sensations using a scaling bar whose color gradually changes from white to red (0–20 cm). The left side (0 cm, color: white), middle (10 cm, color: pink), and right side (20 cm, color: red) of the scaling bar indicated no itching sensation, an itching sensation with a strong urge to scratch, and an itching sensation with a very strong urge to scratch, respectively (the itching score).

2.3. Measurements and data analysis

During the electrical stimulation, the subjects' EEG activities were recorded by a Neuropack MEB2200 system (Nihonkohden, Tokyo, Japan) with five electrodes at a sampling rate of 1000 Hz (Fz, FCz, Cz, CPz and Pz according to the international 10/10 system). The linked earlobes were used as the reference and a ground electrode was placed on the forehead. Horizontal and vertical electrooculograms (EOGs) were recorded simultaneously. Impedance was maintained below 5 kΩ. The EEGs and EOGs were divided into 2000-ms segments (−100 to 1900 ms, onset (0 ms): the electrical stimulus). A bandpass filter of 0.1–30 Hz was used. The baseline correction for each epoch was done using the mean activity 100 ms before the presentation of the electrical stimulus. Artifacts including eye movements (amplitude $> \pm 100\ \mu V$) were excluded from the analysis. The epochs were then averaged. The peak latency of the positive component of the SEP (P1), which was the largest and most consistent one, was measured in each subject at each electrode. The amplitude of the P1 was

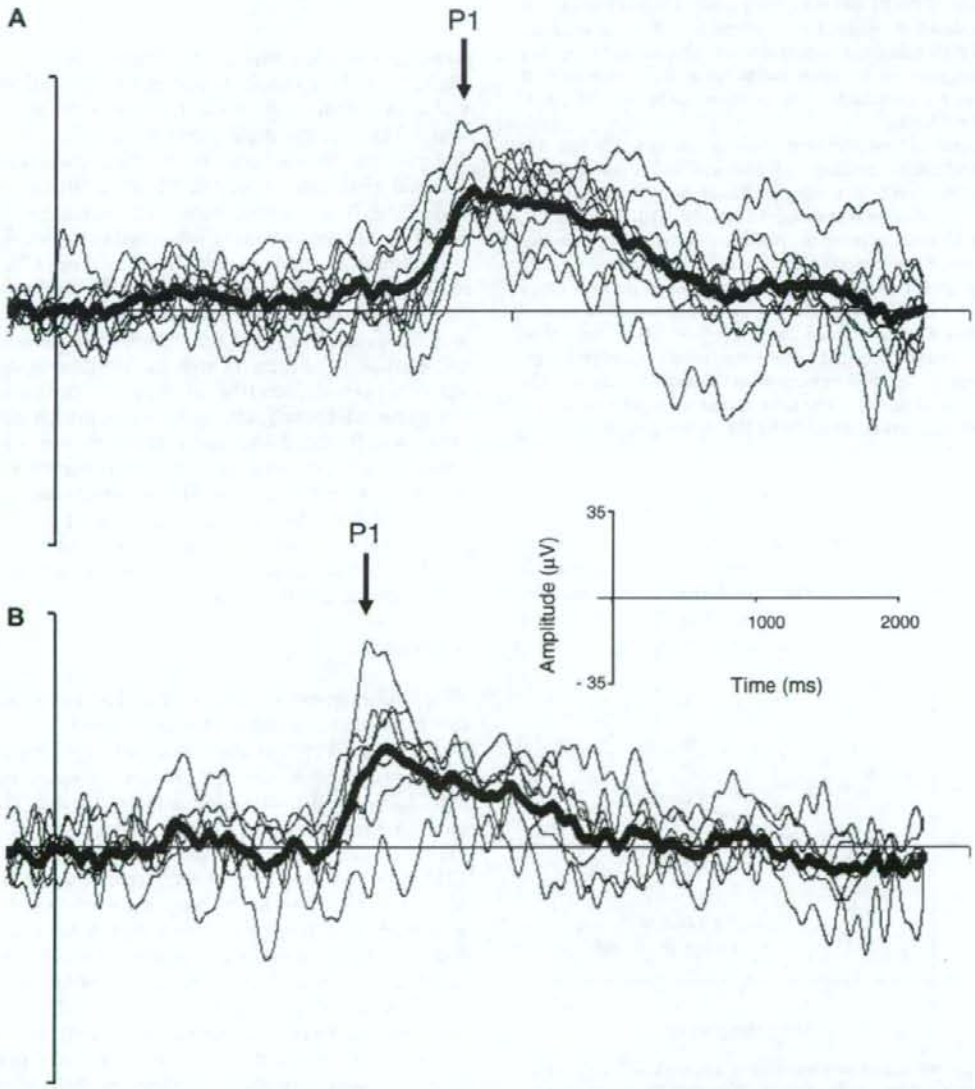

Fig. 2. Itching-related evoked potentials recorded at Cz. Superimposed waveforms of 9 subjects (thin line) and their grand-average (thick line) in the wrist stimulus condition (A) and of 7 subjects (thin line) and their grand-average (thick line) in the forearm stimulus condition (B).

largest at Cz in all subjects. Therefore, we used the SEP recorded from Cz for data analysis. We measured the RT and SEP following stimulation of the right wrist and right forearm. In addition, the CVs were estimated from the differences in the RTs and latencies of the P1 between the wrist and forearm stimulus conditions and the distance between these body parts. Itching sensations were elicited in all subjects in the wrist stimulus condition. However, unfortunately, stimulation of the right forearm did not elicit itching sensations in 2 of 9 subjects. Therefore, SEP and RT data were obtained from only 7 subjects in the forearm stimulus condition. Consequently, the CVs were estimated with the SEP and RT data of seven subjects. We also performed a correlation analysis with the peak amplitude of the P1 and the itching score. The peak amplitude was the mean amplitude from −10 ms to +10 ms of the peak time in each subject in each condition. The itching score was the mean score of the trials except for artifact trials in each subject in each condition. A correlation was considered significant if $p < 0.05$.

A higher current electrical stimulus activates Aβ- and Aδ-fibers and elicits sensations of pain and touch. In addition, the SEP induced by activation of the ascending pathway with a faster CV inhibits that induced later (e.g., [10,17,19]). Thus, as an additional experiment (the high current-wrist stimulus condition), we investigated how the itching sensation and itching-related SEP were modulated when the current intensity increased from the range eliciting an itching sensation to that eliciting sensations of pain and touch (0.6 ± 0.08 mA). Nine subjects who participated in the wrist stimulus condition were employed in the high current-wrist stimulus condition. The stimulus condition was the same as the wrist stimulus condition. We also measured SEP and RT in this condition.

3. Results

The subjects reported that they felt a clear itching sensation with the urge to scratch for short periods (~1 s) after the electrical stimulation (the subjective evaluation of itching: 5.6 ± 2.4). The RT in the wrist stimulus condition was 1215 ± 150 ms. As shown in Fig. 2A, the P1 component emerged at around 700 ms, and peaked around 900 ms in the wrist stimulus condition. The mean peak latency of the P1 was 963 ± 75 ms. In the forearm stimulus condition, the RT and peak latency of the P1 were 1013 ± 154 ms and 772 ± 70 ms, respectively. The waveform in the forearm stimulus condition was similar to that in the wrist stimulus condition (Fig. 2B). The mean CVs estimated with the RT and SEP were 1.04 ± 0.42 m/s and 0.92 ± 0.36 m/s, respectively. In addition, the peak amplitudes of the P1 and the itching score observed in the wrist and the forearm stimulus conditions showed significant correlations (Fig. 3). When the current intensity increased, the subjects reported that the itching sensation was inhibited and that a sharp pain or a mixed sensation of sharp pain and touch was perceived. The RT was 241 ± 49 ms. The SEP corresponded to the behavioral data. As shown in Fig. 4, the P1 component around 900 ms observed in the wrist stimulus condition (thick line) was markedly decreased in the high current-wrist stimulus condition (thin line).

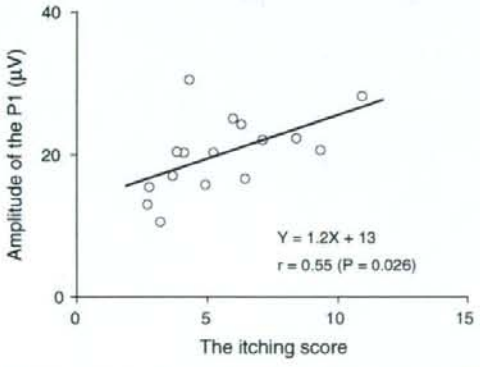

Fig. 3. The correlation between the peak amplitude of the P1 and the itching score. There are16 plots in this graph (9 plots: the wrist stimulus condition, 7 plots: the forearm stimulus condition).

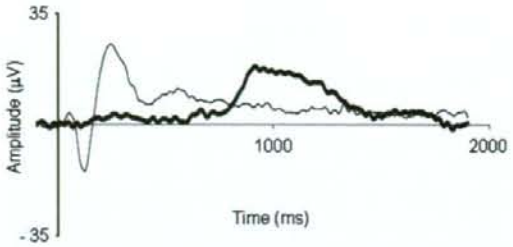

Fig. 4. Mean SEP of 9 subjects in the wrist stimulus condition (thick line) and in the high current-wrist stimulus condition (thin line).

4. Discussion

We investigated whether time-locked brain activity can be measured using electrically evoked itching and whether the itching sensation induced by the stimulation is associated with C-fibers. As in previous studies [5,9,23,28], the electrical stimulation of the skin elicited an itching sensation. The RT and latency of the P1 when the electrical stimuli were applied to the right wrist were 1215 ± 150 ms and 963 ± 75 ms, respectively. In studies of pain, several researchers have observed that the RT and latency of the P1 for pain stimuli were around 1000 ms when C-fibers of the hand were selectively activated [2,18,20,21,26,27]. The stimulated body part mentioned in this study (i.e., wrist) was very close to that mentioned in the previous studies (i.e., hand). Therefore, the RT and latency of the P1 observed in this study would be similar to those observed in those previous studies.

The RT and latency of the P1 when Aδ-fibers of the hand were selectively activated were much shorter than those for C-fiber induced pain and the electrically evoked itching (e.g., [2,11,26]). Therefore, the electrically evoked itching would not be associated with Aδ-fibers. We further estimated the CV of peripheral signals related to the electrically evoked itching. Generally, the CV of C-fibers is 0.4–2.0 m/s [6,26]. The CVs estimated in this study were within that range (see Section 3). On the basis of these findings, it was suggested that the itching sensation elicited by the electrical stimulus was associated with C-fibers.

The P1 component observed at around 900 ms in the wrist stimulus condition was markedly decreased in the high current-wrist stimulus condition (Fig. 4). In the high current-wrist stimulus condition, the subjects reported a sharp pain or a mixed sensation of sharp pain and touch, suggesting that A-fibers (i.e., Aβ- and Aδ-fibers) were activated by the electrical stimulation of the skin with the high current intensity. Actually, the RT was much faster in the high current-wrist stimulus condition than in the wrist stimulus condition. In the study of pain, it is well recognized that the cerebral evoked responses (i.e., SEP) derived from the faster afferent fibers inhibit those derived from the slower ones. For instance, CO_2 laser pulses activate concomitantly Aδ- and C-fibers, and the cerebral evoked responses that are recorded remain limited to Aδ-fibers without any further components at latencies consistent with the activation of C-fibers. It was reported that SEPs associated with the activation of C-fibers could be unmasked by suppressing the activation of Aδ-fibers with a pressure nerve block [2,13]. Since then, SEPs associated with the activation of C-fibers have also been isolated by stimulating tiny skin surfaces, where the probability of finding Aδ afferents is very low [1], or by heating the skin at temperatures below the threshold for the activation of Aδ-fibers [3,14]. These studies demonstrate that SEPs associated with the activation of C-fibers only appear when Aδ afferents are not activated. Considering these previous studies, it was speculated that the current intensity used in the wrist and forearm stimulus conditions did not activate A-fibers. That is, the electrical stimulation induced itching via C-fibers as the threshold sensation. Therefore, we could observe the C-fiber-related P1 component in these conditions.

The peak amplitudes of the P1 and the itching scores observed in the wrist and the forearm stimulus conditions showed a significant correlation (Fig. 3). In addition, as shown in Fig. 4, the P1 component at around 900 ms observed in the wrist stimulus condition was markedly decreased when the subjects did not feel itching sensations (i.e., the high current-wrist stimulus condition). These results indicate the importance of the P1 component in itching perception.

In conclusion, this study showed that electrically evoked itching was associated with C-fibers. As compared to histamine-induced itching, the duration of the itching sensation elicited by the electrical stimulus is very short (~1 s), and, therefore, multiple stimuli can be given to the subjects. The electrical stimulation induced itching via C-fibers as the threshold sensation. Therefore, we could observe the time-locked brain response associated with the excitation of C-fibers. This method is useful for studies of itching using MEG, EEG, and event-related fMRI, and would provide additional information on the central processing of itching.

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Evoked magnetic fields following noxious laser stimulation of the thigh in humans

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ABSTRACT

Primary somatosensory cortex (SI) and posterior parietal cortex (PPC) are activated by noxious stimulation. In neurophysiological studies using magnetoencephalography (MEG), however, it has been difficult to separate the activity in SI from that in PPC following stimulation of the upper limb, since the hand area of SI is very close to PPC. Therefore, we investigated human pain processing using MEG following the application of a thulium-YAG laser to the left thigh to separate the activation of SI and PPC, and to clarify the time course of the activities involved. The results indicated that cortical activities were recorded around SI, contralateral secondary somatosensory cortex (cSII), ipsilateral secondary somatosensory cortex (iSII), and PPC between 150–185 ms. The precise location of PPC was indicated to be the inferior parietal lobule (IPL), corresponding to Brodmann's area 40. The mean peak latencies of SI, cSII, iSII and IPL were 152, 170, 181, and 183 ms, respectively. This is the first study to clarify the time course of the activities of SI, SII, and PPC in human pain processing using MEG.

Introduction

Pain has multiple dimensions, including the sensory-discriminative, affective-motivational, and cognitive-evaluation components (reviewed in Treede et al., 1999), and is processed in multiple cortical areas which are often characterized as a "pain network" or "pain matrix" (Ohara et al., 2006).

Recent neuroimaging studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have shown that the pain network includes primary somatosensory cortex (SI), secondary somatosensory cortex (SII), insula cortex, prefrontal cortex, supplementary motor area (SMA), posterior parietal cortex (PPC), and anterior cingulate cortex (ACC) (Coghill et al., 1994; Svensson et al., 1997; Davis et al., 2002; Qiu et al., 2006; Ogino et al., 2007). The time course of activities of these areas as a neural network has been studied using electroencephalography (EEG) (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani et al., 1996, 2000; Garcia-Larrea et al., 2003; Schlereth et al., 2003; Tsuji et al., 2006) and MEG (Watanabe et al., 1998; Inui et al., 2003a), but the time course and function of PPC in pain perception have yet to be clarified. In fact, Forss et al. using magnetoencephalography (MEG) found that PPC was activated by noxious stimuli (Forss et al., 2005) while others did not (Ploner et al., 1999, 2000, 2002; Kanda et al., 2000; Timmermann et al., 2001; Inui et al., 2002; Raij et al., 2003; Nakata et al., 2004), probably due to problems described later. Therefore, the main objective of this study was to clarify whether PPC is really activated by noxious stimuli, and if so, when and where it was activated.

To solve this problem, we used MEG, which has a high temporal resolution with millisecond order, and measures directly neural responses (reviewed in Hari et al., 2000). Some previous studies indicated a parallel pattern of activation of SI and SII, which peaks at about 170 ms following noxious stimulation of the upper limbs, and shows a similar onset latency between SI and SII (Ploner et al., 1999, 2000, 2002; Kanda et al., 2000; Timmermann et al., 2001; Nakata et al., 2004). These MEG studies mainly have focused on the activities in SI and SII after noxious stimulation, but additional

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activities might be observed in several other regions, especially PPC. As mentioned, neuroimaging studies in humans have shown that PPC is activated after noxious stimulation, and considered to be involved in a pain matrix. PPC includes large regions, such as the superior parietal lobule (SPL; Brodmann's areas 5 and 7) and inferior parietal lobule (IPL; Brodmann's area 40) (Culham and Kanwisher, 2001).

One problem is that most previous MEG studies applied noxious stimulation to only the hand. Thus, it has been difficult to separate SI activities from the PPC activity, because the hand SI and PPC are considered to be located very close together (Forss et al., 1994; Hoshiyama et al., 1997). Concerning tactile stimulation, the region activated in PPC following stimulation of the upper limb was close to that following stimulation of the lower limb, that is, just posterior to the hand area of SI (Hoshiyama et al., 1997). Moreover, an fMRI study showed that the PPC had little or no somatotopic organization, although SI had representations of the hand and foot (Young et al., 2004). If this finding is the same for noxious stimulation, we may be able to separate activity between SI and PPC following stimulation of the lower limb.

The current study was designed to elaborate on whether activities in SI and PPC could be separated or not when the thigh was stimulated. The representation of the thigh in SI is clearly different from that of the hand, located in a medial region of the brain (Penfield and Boldrey, 1937). Therefore, we assumed that the thigh SI and PPC could be easily separated, even if these regions were activated simultaneously after noxious stimulation.

Our results indicated separated activities between SI and PPC in the contaralateral hemisphere to the noxious stimulation, and clarified the time course of the activities of SI, bilateral SII, and PPC in human pain processing.

Materials and methods

Subjects

Ten male volunteers from our department participated in this study. They ranged in age from 24 to 41 (mean±SE: 30.8± 1.5) years, and in height from 165 to 178 (mean±SE: 171.3± 1.2) cm. The subjects had no history of neurological or psychiatric disorders. The protocol was approved by the Institutional Ethics Committee of the National Institute for Physiological Sciences, Okazaki, Japan. Before the experiment, the subjects were informed in detail about the experiment, and gave their written informed consent for the study.

Stimulation

For noxious stimulation, a thulium:YAG laser beam (Neurolaser, BAASEL Lasertech, Germany) was used. The wavelength was 2000 nm, pulse duration was 1 ms, and spot diameter was 6 mm. The laser was applied to the medial side of the left thigh superior to the patella, which is innervated by L3 (Carpenter and Sutin, 1983), in all subjects. Interstimulus intervals (ISI) were randomly varied between 7 and 12 s to avoid habituation of evoked pain-related cortical responses (Raij et al., 2003), and to minimize pain anticipation (Bromm and Lorenz, 1998). To determine the intensity of painful stimulation, we used a visual analogue scale (VAS), in which 0 represented "not painful" and 10 represented "an intensity which subjects could not tolerate". Subjects were asked to rate the intensity of the perceived pain, and a stimulus intensity of VAS 8 was used in each subject for the recording. In addition, subjects were requested to orally provide one adjective from a list of seven descriptors: "not percived", "light touch", "touch", "tingling", "warm", "pricking", and "burning". All subjects reported "pricking" for the intensity of VAS 8. This method for assessing quality of perception followed some previous studies (Opsommer et al., 2001; Mouraux and Plaghki, 2007). There were two reasons why we selected an intensity of VAS 8. First, because a strong stimulus would evoke clear and large cortical responses (Timmermann et al., 2001), we wanted to select the strongest intensity for each subject. Second, if the stimulus intensity was more than VAS 8, it was expected that subjects could not tolerate all noxious stimuli during recordings. The mean intensity was 8.54 mJ/mm^2. Since the laser stimulator caused large magnetic artifacts, it was set outside of the shielded room, and the laser beam was conducted through optical fibers, approximately 6.5 m in length, into the shielded room. In order to maintain the distance between the laser outlet and the skin surface, a cable of the optical fiber was attached to the MEG device, but the irradiated points were slightly moved by an experimenter for each stimulus to avoid tissue damage and habituation of the receptors (Kakigi et al., 1995a). During the recordings, the subjects were instructed to keep their eyes open and look at a small fixation point positioned in front of them at a distance of approximately 1.5 m. The subjects were wearing earplugs to avoid hearing sounds from the stimulator, and non-magnetic goggles for safety. To avoid habituation, only 10 stimuli were applied in one session. The subjects also were asked to report the mean pain level after each session. Eight sessions were conducted for each condition, and subjects were asked to evaluate the pain using a VAS score after each session. A total of 80 stimuli were applied to each subject.

To clarify a principal question of whether there is any somatotopic arrangement in the PPC following noxious stimulation, we performed a supplementary study. We recorded laser-evoked magnetic fields (LEFs) from four subjects following noxious stimulation of the left hand dorsum to compare the dipole location of the hand SI and/or PPC. ISI was randomly varied between 7 and 12 s, and a stimulus intensity of VAS 8 was used for each subject. Subjects were instructed to place the palm of the left hand on the table during the recordings.

Recordings and analysis

LEFs were recorded with a helmet-shaped 306-channel detector array (Vectorview; ELEKTA Neuromag Oy, Helsinki, Finland), which comprises 102 identical triple sensor elements, in a magnetically shielded room. Each sensor element consists of two orthogonal planar gradiometers and one magnetometer coupled to a multi-SQUID (Superconducting Quantum Interference Device) and thus provides three independent measurements of the magnetic fields. In the present study, we analyzed MEG signals from 204-channel planar-type gradiometers, because the data from magnetometers are usually susceptive to global magnetic noise such as changes in geomagnetic field (Hämäläinen et al., 1993) (such noise can be cancelled out in recordings with planar sensors). The signals from these planar sensors are strongest when the sensors are located just above local cerebral sources (Nishitani and Hari, 2002; Noguchi and Kakigi, 2005). The signals were recorded with a bandpass of 0.1–200 Hz and digitized at 990 Hz, rejecting noise, blinks, and eye movements from the analysis automatically. The analysis period of 500 ms included a prestimulus baseline of 100 ms. Before the

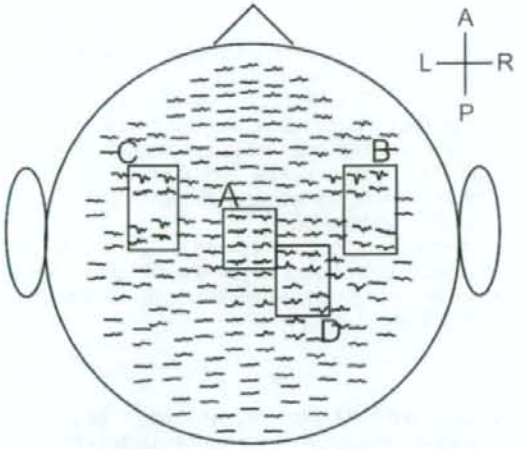

Fig. 1. Grand-averaged laser-evoked magnetic fields (LEFs) over 204 planar coils across all ten subjects. A clear and consistent component was recorded in four scalp areas, A, B, C and D. Each region of interest was shown as a square. All data were digitally filtered (0.1–100 Hz bandpass) for display purposes.

recordings, four head position indicator (HPI) coils were attached to specific sites on the subject's head, and then electric current was fed to the HPI coils to determine the exact location of the head with respect to the MEG sensors. The locations of HPI coils with respect to the three anatomical landmarks (nasion and bilateral PA) were also measured using a three-dimensional digitizer to allow alignment of the MEG and magnetic resonance (MR) images obtained with a 3 tesla MRI system (Allegra scanner, Siemens, Erlangen, Germany). A three-dimensional structural brain image of each subject was obtained using an MP-RAGE sequence with the following parameters: TR=2500 ms, TE=4.38 ms, FA=8°, FOV=230 mm, matrix size=256×256 mm, voxel dimension=0.9×0.9×1.0 mm. The *X*-axis was fixed with the preauricular points, pointing to the right, the positive *Y*-axis traversing the nasion, and the positive *Z*-axis pointing up. We adopted the head-based coordinate system used in our previous studies (Wasaka et al., 2003; Noguchi et al., 2004).

We first calculated vector sums from the longitudinal and latitudinal derivatives of the response recorded on the planar-type gradiometers at each of the 102 sensors' location. This was achieved by squaring MEG signals of gradiometer pairs, summing these signals together, and then recalculating the square root of this sum. We termed this value the "root sum square" (RSS), following our previous studies (Kida et al., 2006, 2007). The calculation was carried out for all 102 sensors' locations to make an isocontour map of RSS amplitude. Then, we analyzed the areal mean signals of four gradiometer pairs that showed the largest response to measure the amplitude and latency of waveforms. Finally, we calculated group averages across subjects. This method of data analysis followed some previous studies using the same MEG system as the present study (Tarkiainen et al., 2003, Nakata et al., 2005; Bonte et al., 2006; Akatsuka et al., 2007a,b).

To identify the sources of the evoked activities, the equivalent current dipole (ECD), which best explains the measured data, was computed by using a least-squares search. A subset of 16–18 channels, including channels which were used for analyzing the areal mean signals, was employed for

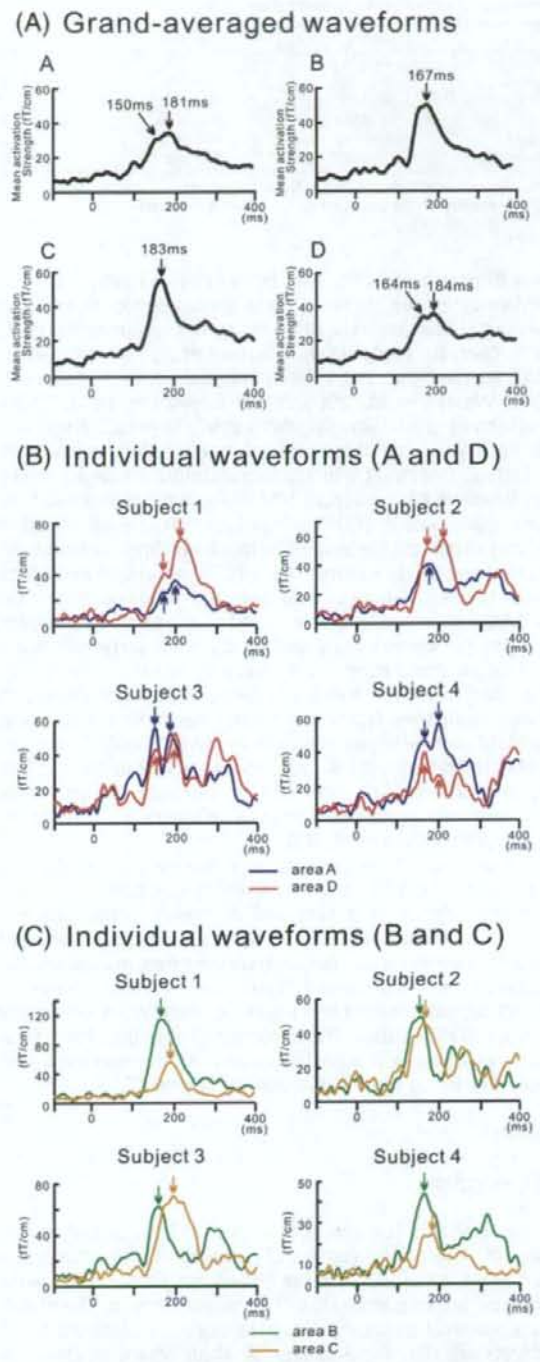

Fig 2. (A) The grand-averaged areal mean signals in each region of interest across all ten subjects. In A and D, two arrows indicated two peaks of the waveform. (B) The areal mean signals at regions A and D in four representative subjects. Blue and red lines indicate the waveforms of A and D, respectively, and arrows demonstrate the peak of the waveforms. (C) The areal mean signals at regions B and C in four representative subjects. Green and orange lines indicate the waveforms of B and C, respectively, and arrows show the peak.

Table 1
The mean peak latencies and amplitudes of areal mean signals for each region

	Latency (ms)	Amplitude (fT/cm)	n
A (first)	164.5 (5.3)	39.0 (5.5)	9
A (second)	199.3 (5.8)	40.4 (6.5)	7
B	165.2 (2.7)	58.7 (6.9)	10
C	173.4 (4.1)	52.8 (5.0)	10
D (first)	164.1 (4.4)	38.3 (4.2)	10
D (second)	211.7 (6.2)	39.9 (6.6)	8

Data are expressed as the mean (SE). A, B, C, and D indicate the same regions as in Figs. 1 and 3. *n* = number of subjects, which was identified in the waveform.

the estimation of ECDs. This number of channels has been used to cover the signal maxima channels over SI or SII in previous studies with the same Neuromag system (Forss et al., 1996, 1999; Lin et al,. 2000; Avikainen et al., 2002; Raij et al., 2003; Nguyen et al., 2004; Möttönen et al., 2005; Nakata et al., 2005; Wasaka et al., 2005, 2007; Nevalainen et al., 2006; Akatsuka et al., 2007b; Sakamoto et al., in press). These calculations gave the three-dimensional location, orientation, and strength of the ECD in a spherical conductor model, which was based on each subject's MRI to show the source location. The goodness-of-fit (GOF) value of an ECD was calculated to indicate in percentage terms how much the dipole accounts for the measured field variance. Only ECDs explaining more than 80% of the field variance during selected periods of time were used for further analysis. The period of analysis was extended to cover the entire period and all channels were taken into account in computing a time-varying multi-dipole model. The strengths of the previously found ECDs were allowed to change while their locations and orientations were kept fixed. The data acquisition and analysis followed Hämäläinen et al. (1993). In addition, the source location was transformed into the Talairach standard brain source (Talairach and Tournoux, 1988), following previous studies (Nishitani et al., 1999; Ploner et al., 2000; Nakata et al., 2005).

For analysis of the peak latency and moment of ECDs, a one-way analysis of variance (ANOVA) was performed with activated region as a repeated measures within-subjects factor. For all repeated measures factors, it was tested whether Mauchly's sphericity assumption was violated. In all cases, the sphericity was maintained. Thus, the Greenhouse–Geisser correction was not used in the present study. When significant effects were identified, the Bonferroni–Dunn post hoc multiple-comparison was adjusted to identify the specific differences. Statistical significance was set at $p<0.05$.

Results

MEG waveform

Fig. 1 shows the group averages of LEFs across all ten subjects. A clear and consistent component was recorded in four scalp areas, A, B, C and D. Large components were observed peaking at about 170 ms in each region. The grand-averaged areal mean signals in the regions of interest for all subjects are illustrated in Fig. 2A. With regard to the components, B and C waveforms peaked at 167 ms and 183 ms after noxious stimulation, respectively. In A and D, it should be noted that complex waveforms containing two peaks were recorded between 150–185 ms, indicating that at least two components were observed during this period. To determine the morphology of the waveforms in more detail, we investigated the individual waveforms in each region, because there was a possibility that the presence of two peaks in A and D was merely due to a latency jitter of only one peak among different subjects. Figs. 2B and C show the individual waveforms from four representative subjects in each region. In Fig. 2B, the waveforms of A and D demonstrated two peaks, while those of B and C showed a single peak in Fig. 2C. Table 1 indicates the mean peak latencies and amplitudes for areal mean signals in each region.

Fig. 3 shows the isocontour maps of RSS signals at this period in four representative subjects. Just like the grand-averaged LEFs, these isocontour maps show distinct neural responses in regions A, B, C, and D, at 150–180 ms.

ECD analysis

We performed an ECD analysis to clarify the generators of each activity in the brain. First, we estimated components that peaked between 150–185 ms after the stimulation. A was estimated to lie between the central sulcus and postcentral sulcus contralateral to the stimulation, corresponding to SI. B and C were estimated to be located in the upper bank of the

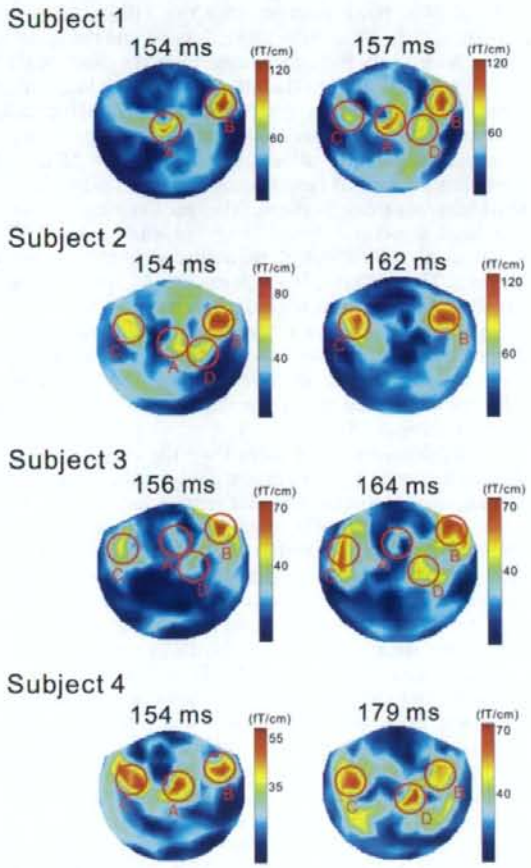

Fig. 3. The isocontour maps of RSS signals at several time points in four representative subjects. Note that these maps show distinct neural responses in four regions, A, B, C and D, between 150–180 ms. The peaks of the responses to represent these maps were chosen. RSS: root sum square.

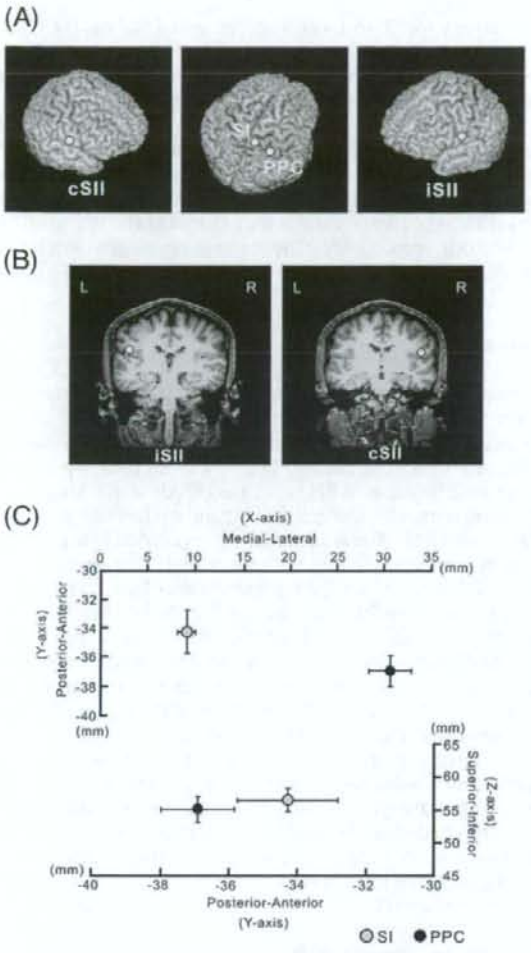

Fig. 4. (A) Results of ECD analysis in a representative subject. The locations of ECDs are superimposed on the same subject's 3D-MRI. (B) Location of ECDs at the level of SII superimposed on a coronal slice of a 2D MRI scan in a same representative subject. (C) The mean Talairach coordinates of ECDs for the SI and PPC responses. Gray and black circles represent the locations of SI and PPC, respectively. Bars indicate standard errors (SE). ECD = equivalent current dipole, SI = primary somatosensory cortex, cSII = secondary somatosensory cortex contralateral to the stimulation, iSII = secondary somatosensory cortex ipsilateral to the stimulation, PPC = posterior parietal cortex, L = left, R = right.

Sylvian fissure, corresponding to SII, in the right and left hemisphere, respectively. We termed them the cSII (contralateral SII) and iSII (ipsilateral SII), respectively. D was estimated to be in the IPL of PPC, contralateral to the stimulation (Figs. 4A and B). Talairach coordinates of PPC indicated Brodmann's area 40. The ECDs of ACC activity were not detected. Group averages of Talairach coordinates (X, Y, and Z) across subjects are listed in Table 2.

We compared the source location between SI and PPC components, using a paired t-test. There were significant differences in X and Y values ($p<0.001$, $p<0.05$, respectively), but not in Z values ($p>0.05$). The SI source was located more medial (21.6 mm) and anterior (2.7 mm) than the PPC source (Fig. 4C).

Fig. 5 indicates the ECD locations following noxious stimulation of the left hand dorsum. Three ECDs were estimated around 160 ms after stimulation, which was consistent with previous studies (Ploner et al., 1999, 2000, 2002; Kanda et al., 2000; Timmermann et al., 2001; Raij et al., 2003; Nakata et al., 2004; Forss et al., 2005). Two ECDs were located in cSII and iSII, respectively, and one ECD was located around SI and PPC. We could not separate the ECDs between hand SI and PPC. Thus, we termed this, hand SI/PPC. The mean Talairach coordinates of the hand SI/PPC were $X=32.2$ mm (SE±1.3), $Y=-34.8$ mm (±1.2), and $Z=49.2$ mm (±3.4), respectively.

Peak latency and strength of MEG response

We analyzed the peak latency and strength of each ECD with ANOVAs using activated region (SI, cSII, iSII, and PPC) as a factor. The ANOVAs for the peak latency revealed a significant main effect of activated region ($F(3, 18)=15.775$, $p<0.001$). Post-hoc analysis indicated that the peak latency of SI was significantly earlier than that of the cSII, iSII, and PPC ($p<0.01$, $p<0.001$, and $p<0.001$, respectively), and the peak latency of

Table 2
Talairach coordinates of components in each region

	X (mm)	Y (mm)	Z (mm)	n
SI	9.1 (1.0)	−34.2 (1.5)	56.5 (1.8)	10
cSII	49.6 (1.4)	−10.0 (1.4)	20.0 (2.9)	10
iSII	−46.1 (2.0)	−11.9 (2.0)	20.5 (1.8)	8
PPC	30.7 (2.3)	−36.9 (1.1)	55.1 (2.0)	9

Data are expressed as the mean (SE). SI = primary somatoensory cortex; cSII = secondary somatosensory cortex contralateral to the stimulation; iSII = secondary somatosensory cortex ipsilateral to the stimulation; PPC = posterior parietal cortex; n = number of subjects, which was identified in the ECD analysis.

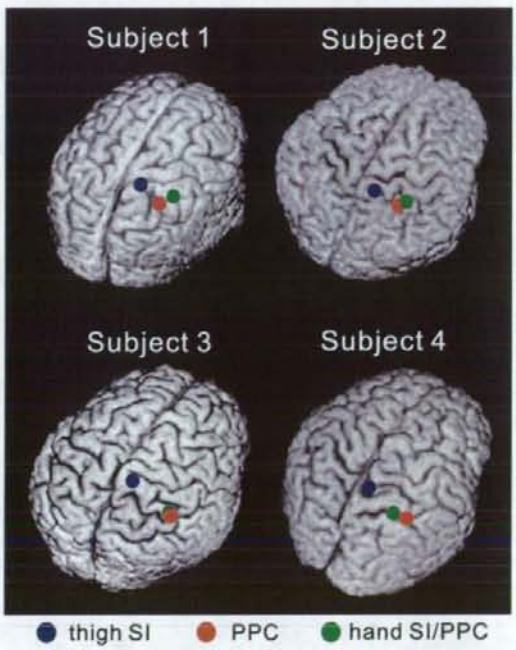

Fig. 5. Results of ECD analysis for the thigh SI, PPC, and hand SI/PPC in four representative subjects. The locations of ECDs are superimposed on own subject's 3D-MRI. Blue and red circles indicate the ECD locations of the thigh SI and PPC, respectively. Green circle shows the ECD location of the hand SI/PPC.

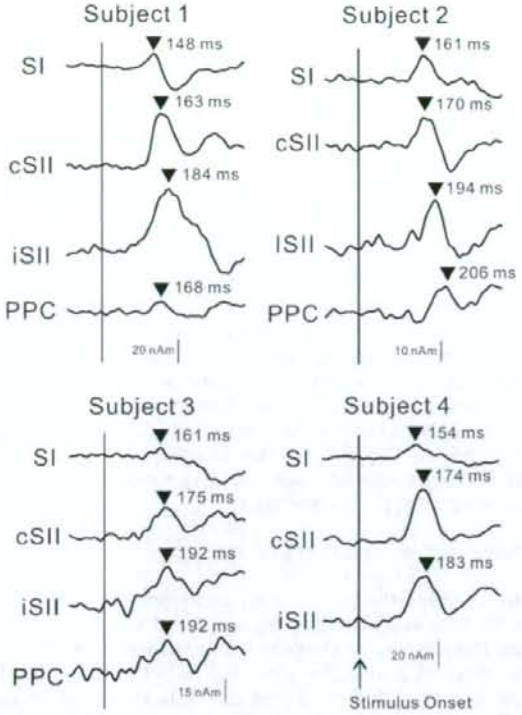

Fig. 6. Time course of the ECD moment waveforms in SI, cSII, iSII and PPC indicated by a multidipole analysis in four representative subjects. The waveform of subject 4 is an example where PPC activity could not be estimated.

the cSII was also significantly earlier than that of the iSII and PPC ($p<0.01$, and $p<0.05$, respectively). These results demonstrated the peak latency of SI activity to be the earliest among the four components. Fig. 6 indicates the time course of ECD moment in four representative subjects. The mean value of peak latency for SI, cSII, iSII, and PPC was 151.7 ms (SE±3.6), 170.2 ms (±2.6), 181.2 ms (±4.1), and 183.3 ms (±7.2), respectively (Fig. 7A). The difference of these results suggests a serial mode of pain processing in a hierarchical structure.

The ANOVAs showed a significant main effect of dipole strength ($F(3, 18)=9.104$, $p<0.01$). Post-hoc analysis indicated that the ECD moment of the cSII was significantly larger than that of SI and PPC ($p<0.01$, respectively), and the moment of the iSII was also significantly larger than that of SI and PPC ($p<0.01$, and $p<0.001$, respectively). The mean of dipole strength for SI, cSII, iSII, and PPC was 12.9 nAm (SE±2.8), 29.4 nAm (±4.1), 34.8 nAm (±4.3), and 9.9 nAm (±1.1), respectively (Fig. 7B).

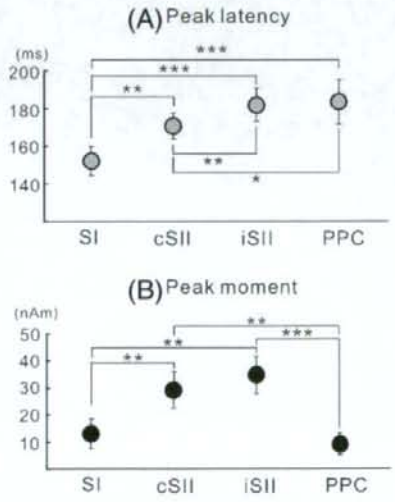

Fig. 7. (A) Peak latency of ECD for each component. (B) Peak moment (nAm) of ECD for each component. Vertical lines indicate standard errors. *** $p<0.001$; ** $p<0.01$; * $p<0.05$.

Discussion

Here, we showed that activities significantly differed between SI and PPC in the contralateral hemisphere to the noxious stimulation of the thigh, and clarified the time course of the activities of SI, SII, and PPC in human pain processing. The precise location of PPC indicated Brodmann's area 40 in IPL, consistent with the results of pain studies using neuroimaging methods. The reason that we succeeded in separating the activities depended on the somatotopic representation in SI. It is well known that the representation of the thigh in SI is located more medial than that of the hand (Penfield and Boldrey, 1937). Our data about the thigh SI was compatible with Penfield's homunculus. Previous MEG studies observed the activities of SI or PPC after noxious stimulation of the hand, but it has been difficult to separate these activities because of the spatial resolution of MEG.

Results of the areal mean signals demonstrated that the waveforms included two peaks in regions, A and D, as shown in Fig. 2. ECDs analysis concerning first and second peaks appeared to be estimated around SI, and PPC, respectively. Since regions, A and D were located closely, the sensor coils might detect both SI and PPC activities in each region. The mean value of ECD peak latency for SI and PPC was 151.7 and 183.3 ms, respectively.

Time course of pain processing

Since our data indicated activation of IPL following the noxious stimulation, we propose three explanations for pain processing. The first is that there is serial pain processing from SI to IPL via SII (Fig. 8A). Anatomical studies have revealed nociceptive projections from lateral thalamic nuclei, particularly from the ventral postrior lateral nucleus (VPL), to SI (Kenshalo et al., 1980; Schnitzler and Ploner, 2000). SI and SII had reciprocal cortico-cortical connections (Jones et al., 1975;

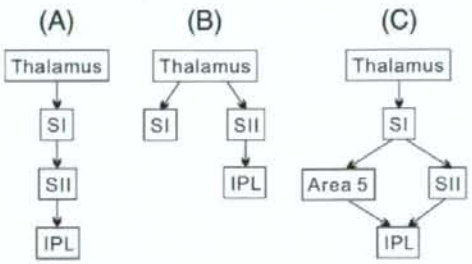

Fig. 8. Schemata of hypotheses for time course of pain processing. Refer to (A), (B), and (C) in the discussion. SI = primary somatosensory cortex; SII = secondary somatosensory cortex; IPL = inferior parietal lobule.