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RESEARCH ARTICLE

Early cortical activities evoked by noxious stimulation in humans

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Abstract Lasers can selectively activate the nociceptors of A-delta fibers. Since nociceptors in the skin are activated via temperature conduction by the laser beam, a latency jittering of cortical responses among trials would affect results obtained with a conventional averaging (C-AVE) technique. We therefore used a new method, latency-adjusted averaging (L-AVE), to investigate cortical responses to noxious laser stimulation in normal subjects. L-AVE was done by averaging trials after adjusting the latency so that the peak latency of an activity in the temporal region of all trials matched on the time axis. Both in C-AVE and in L-AVE, clear activations were found in the contralateral primary somatosensory cortex (SI) and bilateral parasylvian regions, whose activities peaked 163–181 ms after the stimulation. In addition to these three main activities, weak activities peaking at around 109–119 ms could be identified in only L-AVE in similar cortical regions. Since the direction of the source differed between early and main activities, we considered that the early weak activities were cancelled out by the later main activities with an opposite orientation. The results suggested that early cortical processing of noxious information occurs earlier than previous neurophysiological studies have estimated and that the temporal sequence of activations should be reconsidered.

Keywords Magnetoencephalography · Pain · Somatosensory

Introduction

Functional neuroimaging studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have provided unequivocal evidence of the participation of the cerebral cortex, including the primary somatosensory cortex (SI), secondary somatosensory cortex (SII), and insula, in pain processing (Talbot et al. 1991; Casey et al. 1994; Gelnar et al. 1999; Apkarian et al. 2000; Qiu et al. 2006). In contrast to PET and fMRI, magnetoencephalography (MEG) has excellent temporal resolution, and can be used to investigate the temporal aspect of the processing of information in the cortex. In previous MEG studies, parasylvian regions were consistently activated by noxious stimulation (Huttunen et al. 1986; Kakigi et al. 1995; Hari et al. 1997). In addition, recent studies found activation in SI following laser (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Nakata et al. 2004) and intraepidermal electrical (Inui et al. 2002b, 2003a, b) stimulation. Some studies found a parallel activation pattern of SI and SII (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Inui et al. 2002b; Nakata et al. 2004) showing a similar onset latency between SI and SII activities, while others (Inui et al. 2003a, b) showed an early SI

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activity prior to the main SII activity, implying serial processing through SI and SII. In addition to MEG, laser-evoked potentials (LEPs) have been used for the temporal assessment of cortical pain processing. Although the SII area was the major cortical region responsible for LEPs in most studies (for review, see Apkarian et al. 2005), several studies reported the involvement of the contralateral SI in pain processing (Tarkka and Treede 1993; Schlereth et al. 2003; Ohara et al. 2004). Tarkka and Treede (1993) reported that a N1 component at a latency of 160 ms was generated in SI and SII, whereas others demonstrated an activity in the contralateral SI helped to shape the N2 component (Schlereth et al. 2003; Ohara et al. 2004). Valeriani et al. (2000) reported an early component with a peak latency of 83 ms originating from SII or the insular area, suggesting that the opercular cortex is also involved in early processing. Therefore, the temporal aspect of the processing of noxious information in the cortex still remains to be elucidated.

A laser can activate nociceptors of thinly myelinated A-delta fibers without stimulating tactile afferents, and therefore is a good tool with which to investigate the nociceptive system. However, since the skin's nociceptors are activated via temperature conduction by the laser beam, there is considerable jitter in the latency of the activation of nociceptors among trials (Bromm and Treede 1984), which is problematic for studies using an averaging technique. The main activations in SI and SII reported previously (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Nakata et al. 2004) are less affected by latency jittering because of their long duration. However, the possibility cannot be excluded that some weak and short-lasting activities at an earlier latency were overlooked due to the problem of jittering in conventional averaging (C-AVE). In the present study, we used latency-corrected averaging to test this possibility.

Methods

Subjects

The experiment was performed on nine healthy male volunteers, aged 27–43 years (32.1 ± 5.3). Informed consent was obtained from all participants prior to the study, which was first approved by the Ethics Committee at our Institute.

Laser stimulation

A thulium:YAG laser stimulator (Carl Baasel Lasertech, Starnberg, Germany) was used to elicit noxious stimuli. Laser pulses (1 ms in duration, 2,000 nm in wavelength, and 3 mm in spot diameter) were delivered to the dorsum of the left hand at an interval of between 8 and 12 s. The interstimulus interval of 8–12 s was employed to avoid habituation of evoked cortical responses (Raij et al. 2003). The irradiated points were moved slightly for each stimulus to avoid tissue damage and habituation of the receptors. The mean intensity was 211 mJ, ranging from 200 to 250 mJ, with which a painful sensation having a visual analysis score (VAS) of around 7 was evoked in each subject. Since the laser stimulator caused large magnetic artifacts, it was set outside 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, the optical fiber was attached to the MEG device and subjects were instructed to attach the palm of the left hand to the table during the recording.

MEG recording

Laser-evoked magnetic fields (LEFs) were recorded with a helmet-shaped 306-channel detector array (Vectorview; ELEKTA Neuromag, Helsinki), which comprises 102 identical triple sensor elements, in a magnetically shielded room. Each sensor element consists of two orthogonal planar gradiometers and one gradiometer magnetically coupled to a multi-superconducting quantum interference device (SQUID) and thus provides three independent measurements of the magnetic fields, though in this study, results recorded from 204 planar gradiometers were analyzed. The signals were recorded with a 0.1–100 Hz band-pass filter and digitized at a sampling rate of 900 Hz. The period of analysis was 500 ms including a prestimulus period of 100 ms. Sixty trials following laser stimulation were recorded.

Prior to the recording, the exact location of the head with respect to the sensors was found by measuring the magnetic signals produced by currents leading to four indicator coils placed at known sites on the scalp. The four indicator coils attached to the subject's head were measured with respect to the three anatomical landmarks using a 3D digitizer to allow alignment of the MEG and magnetic resonance (MR) image coordinate systems (3.0-T Siemens Allegra). The x-axis was fixed with the preauricular points, the positive direction being to the right. The positive y-axis passed through the nasion and the z-axis thus pointed upward. Current was then fed to the indicator coils and the resulting magnetic fields were measured with the magnetometer, which allowed for aligning the individual head coordinate system with the magnetometer coordinate system.

Averaging of trials

First, C-AVE using the onset of the noxious stimulation was done. In C-AVE waveforms, the largest response was

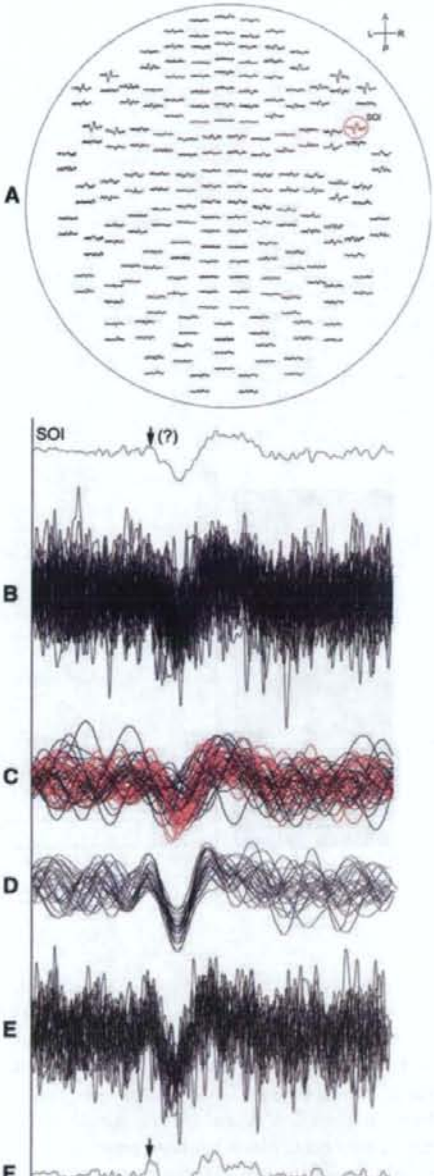


Fig. 1 Procedures followed for latency-adjusted averaging (L-AVE) in a single subject. **a** Laser-evoked magnetic fields recorded from 204 planar coils and the SOI with the largest amplitude in the right temporal area. **b**, **c** Superimposed waveforms of 60 trials of the SOI obtained with a low-pass filter of 100 and 15 Hz, respectively. **c** *Waveforms in red* show the trials selected for L-AVE. **d** Selected trials were latency-corrected. **e** Latency-adjusted trials with a bandpass of 0.1–100 Hz. **f** Averaged waveform of selected trials after latency-adjustment. *SOI* sensor of interest. *Arrows* indicate the early activity that appeared after L-AVE

usually recorded in the right (hemisphere contralateral to the stimulated side) temporal area (Fig. 1A) at around 150–200 ms after stimulation consistent with previous studies (Kakigi et al. 1995; Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Nakata et al. 2004). We selected the channel with the largest amplitude around the temporal region as a sensor of interest (SOI, Fig. 1A). The peak latency of the SOI was determined in each subject and was used for latency-adjusted averaging (L-AVE).

Second, L-AVE was done, in which each trial had been latency-adjusted before the averaging. One problem with a single-trial analysis is that the signal-to-noise ratio (S/N) is very low for single epochs. Notably, high frequency noises superimposed on the evoked response were problematic when determining the peak of the response. After several attempts, we found that a cutoff frequency of 15 Hz is appropriate for determination of the peak latency of the main component. Therefore as a first step, MEG signals of each trial were filtered with a low-pass of 15 Hz. Then we used the SOI to select trials to include averaging (Fig. 1C). That is, only the trials whose SOI had an unambiguous peak within the range of the peak latency of the C-AVE waveform ±20 ms were selected by visual inspection (red traces in Fig. 1C). Such a procedure has been shown to improve S/N ratio of LEP components (Iannetti et al. 2005). Once trials to be included for L-AVE were determined, the original 0.1–100 Hz waveforms of the selected trials were then latency-corrected (Fig. 1E), so that the peak of the SOI matched on the time axis and averaged (Fig. 1F).

Data analysis

First, the source of the main components in C-AVE and L-AVE was estimated in order to know whether the quality of L-AVE was changed as compared with C-AVE. 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 the local signal maxima was used for the estimation of ECDs. These calculations gave the 3D location, orientation, and strength of the ECD in a spherical conductor model, which was based on each subject's MR images 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 85% of the field variance at selected periods of time were used for further analysis. Finally, all channels

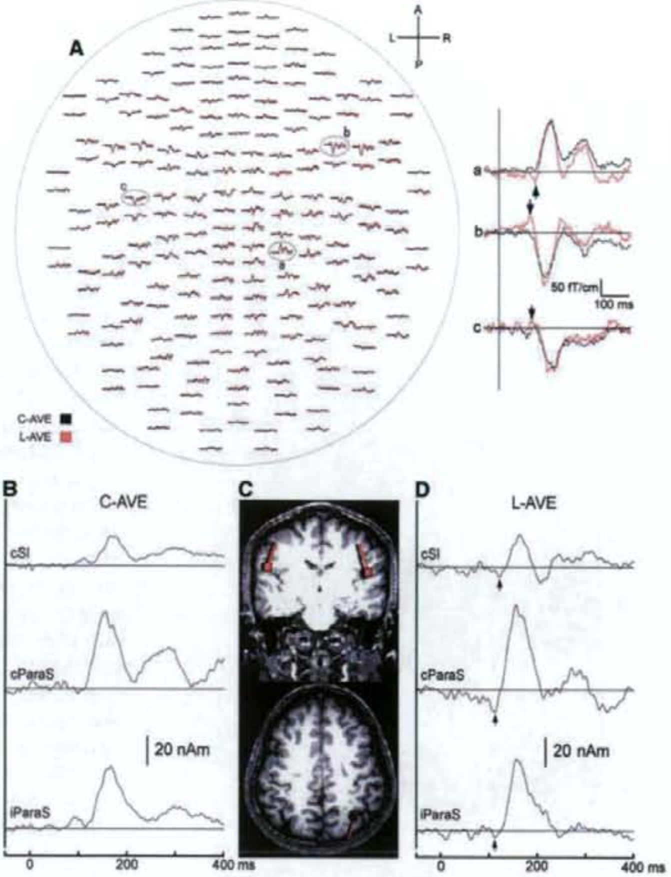


Fig. 2 Magnetic fields following noxious laser stimulation applied to the dorsum of the left hand. **a** Waveforms of evoked magnetic fields obtained in conventional averaging (C-AVE) and latency-adjusted averaging (L-AVE) were superimposed. The *upper right figures* show enlarged waveforms recorded from **a**, **b**, and **c**. *Arrows* show the early activities. **b**, **d** The time-varying source strength of cSI, cParaS, and iParaS in C-AVE (*lower left*) and L-AVE (*lower right*), respectively. **c** The location and orientation of each source are superimposed on the MRI scans. *cSI* contralateral primary somatosensory cortex, *cParaS* contralateral parasylvian region, *iParaS* ipsilateral parasylvian region

were used to compute the time-varying multidipole model allowing the strengths of the previously found ECDs to change over the entire period of analysis while the source locations and orientations were kept fixed. The data acquisition and analysis followed Hamalainen et al. (1993).

Second, the possibility that there emerge additional components at an early latency in L-AVE was examined. When a new deflection had a peak amplitude larger than the baseline + 3 times the standard deviation (SD), we accepted it as a significant component. In the present study, the onset latency of a component was defined as a latency point where the amplitude first exceeded the baseline + 2 SD.

Data were expressed as the mean ± SD. A paired *t*-test was used to compare the source's location and peak amplitude between the C-AVE and L-AVE. A one-way analysis of variance (ANOVA) was used to compare the latency among cortical sources. *P* values less than 0.05 were considered to be significant.

Results

After the application of our criteria, 27–35 (mean 30.7) trials were included for L-AVE in each subject, which corresponded to 45–58% of the 60 trials used for C-AVE.

Figure 2a shows evoked magnetic fields recorded from 204 planar gradiometers in C-AVE (black lines) and L-AVE (red lines). Both in C-AVE and L-AVE, a clear and consistent main component, which has been reported in previous studies, was recorded in three cortical areas; the left (contralateral) parietal region and bilateral temporal regions. An ECD analysis and subsequent superposition of sources on individual MR images revealed that ECDs responsible for these three main components were located around the postcentral gyrus of the contralateral hemisphere and around the upper bank of the Sylvian fissure or near the insular circular sulcus of both hemispheres, corresponding to the contralateral SI (cSI), contralateral parasylvian region (cParaS), and ipsilateral parasylvian region (iParaS), respectively. This three-source model is compatible with previous laser-evoked MEG studies (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Nakata et al. 2004). The location of each cortical activity is shown in Table 1. The location of each source in L-AVE did not differ significantly from that in C-AVE (Fig. 2C). The GOF for the cSI (94.7 ± 3.7) and cParaS (97.1 ± 2.7) sources was significantly larger in L-AVE than in C-AVE (91.1 ± 5.0 and 94.9 ± 4.6, respectively). The GOF for the iParaS showed no significant difference between C-AVE (95.8 ± 2.9) and L-AVE (94.6 ± 1.6) ($P = 0.25$). The onset and peak latency of the main deflection in the three cortical areas did not differ significantly between C-AVE and L-AVE (Table 2). In both C-AVE and L-AVE, the onset or peak latency for iParaS was significantly longer than that for cSI or cParaS. The onset latency did not differ between cSI and cParaS (Table 2). The time-varying source strength in each region is shown in Fig. 2B, D. The peak amplitude of the three main activities was significantly greater in L-AVE than in C-AVE (Table 3). ECD locations of these three regions showed no significant difference between C-AVE and L-AVE, indicating that the new method, L-AVE, was reliable.

In addition to the main activities, early deflections were identified in the contralateral parietal region and both temporal regions in both C-AVE and L-AVE (Fig. 2A). However, early deflections in C-AVE were very weak and usually did not meet our criteria for a significant deflection. By contrast, such deflections were identified more clearly in L-AVE. In C-AVE, significant early deflections were identified in four subjects for cSI, four subjects for cParaS, and three subjects for iParaS. After the L-AVE, significant early deflections were identified in seven subjects for cSI, in seven subjects for cParaS, and in five subjects for iParaS. Usually significant deflections at early latencies were detected in three distinct areas; the contralateral parietal region and both temporal regions, which were almost identical to the locations for the three main components in C-AVE (Fig. 3).

Table 1 The mean location of each source for C-AVE and L-AVE

	x (mm)	*y* (mm)	*z* (mm)
C-AVE			
cSI	27.8 ± 9.7	11.9 ± 18.3	107.4 ± 11.7
cParaS	52.2 ± 7.5	33.4 ± 6.5	64.0 ± 10.2
iParaS	−53.3 ± 4.4	21.3 ± 4.1	71.3 ± 8.1
L-AVE			
cSI	27.6 ± 12.3	14.0 ± 17.5	107.7 ± 11.3
cParaS	54.0 ± 9.7	31.6 ± 8.1	63.6 ± 7.0
iParaS	−53.0 ± 4.7	21.2 ± 5.0	72.1 ± 7.7
Early-cSI ($n = 3$)	30.0 ± 11.4	10.8 ± 19.3	110.7 ± 6.6
Early-cParaS ($n = 5$)	52.9 ± 9.4	24.1 ± 13.7	62.9 ± 5.4
Early-iParaS ($n = 4$)	−53.3 ± 5.3	13.6 ± 11.8	67.8 ± 10.2

The *x*-axis passed through the preauricular points, the positive direction pointing to the right. The positive *y*-axis traversed the nasion. The positive *z*-axis pointed up. The location of each source did not differ between C-AVE and L-AVE

Figure 4 shows L-AVE waveforms of three channels respectively selected from the contralateral parietal region and bilateral temporal regions, in which the early and major deflections had the largest amplitude in all subjects (a) and ground-averaged waveforms (b). Figure 5 shows the waveform of the SOI and root mean square (RMS) of all subjects. For early deflections, a one-way ANOVA showed no significant difference in the onset and peak latencies among the three activities ($P = 0.48$), although the onset latency of cParaS tended to be shorter than that for cSI or iParaS (Table 2). The ECD of the early deflections could be estimated in three subjects for cSI, in five subjects for cParaS, and in four subjects for iParaS. In these samples, there was no consistent difference in the location of the source between the early and main activities (Table 1 and Fig. 6).

Discussion

In the present study, we found that three main activities originating from the contralateral SI and bilateral parasylvian regions and peaking at around 160–180 ms were responsible for laser-evoked magnetic fields. Both the locations and response latencies of the activities were consistent with previous MEG studies (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Nakata et al. 2004), in which these activities have usually been considered the primary cortical response. However, in addition to these main activities, L-AVE in the present study revealed the presence of early activities in these three cortical areas peaking at 110–120 ms, indicating that the cortical processing of information on pain took place earlier than previously considered. Since the early component had an opposite direction to that of the main component, the early component is considered to be a discrete component but is not a part of the main component. The onset latencies (88–105 ms) of the early activities appear to be appropriate for the earliest cortical activity given a peripheral conduction velocity of 15 ms/s (Inui et al. 2002a, b) in A-delta fibers and 10–20 m/s in the spinal cord (Kakigi and Shibasaki 1991; Cruccu et al. 2000; Tsuji et al. 2006). Traveling at 15 m/s, it would take roughly 80 ms to move from the hand to the cortex (120 cm).

Methodological considerations

Before discussing the findings of L-AVE, we should consider the possibility that the early activities detected

Table 2 The onset and peak latency of early and main deflections in L-AVE and C-AVE (ms)

	L-AVE				C-AVE			
	Early deflection		Main deflection		Early deflection		Main deflection	
	Onset	Peak	Onset	Peak	Onset	Peak	Onset	Peak
cSI	104.7 ± 16.8	118.7 ± 19.0	136.7 ± 13.5*	169.3 ± 16.4	104.0 ± 16.1	114.3 ± 15.4	134.6 ± 16.1*	168.1 ± 17.8
cParaS	88.1 ± 20.4	109.0 ± 12.9	136.0 ± 11.8*	163.0 ± 11.8*	84.3 ± 13.0	98.3 ± 4.9	131.1 ± 11.2*	163.9 ± 14.3*
iParaS	94.0 ± 17.1	111.8 ± 11.6	152.1 ± 14.4	180.7 ± 13.3	89.0 ± 23.5	115.0 ± 11.1	153.0 ± 14.5	181.3 ± 13.3

The number of subjects who showed a significant early deflection was seven, seven, and five in L-AVE, and four, four and three in C-AVE for cSI, cParaS, and iParaS, respectively

cSI contralateral primary somatosensory cortex, *cParaS* and *iParaS* contralateral and ipsilateral parasylvian regions, respectively

* $P < 0.05$, compared with iParaS (Fisher's PLSD procedure)

Table 3 The peak amplitude of early and main deflections in L-AVE and C-AVE (nAm)

	L-AVE		C-AVE	
	Early deflection	Main deflection	Early deflection	Main deflection
cSI	−35.3 ± 9.8	93.7 ± 31.7*	−29.6 ± 3.2	70.4 ± 23.1
cParaS	51.6 ± 21.8	−139.8 ± 33.9*	38.2 ± 17.6	−89.5 ± 19.8
iParaS	43.4 ± 18.1	−107.2 ± 17.2*	40.9 ± 8.5	−83.3 ± 15.3

The number of subjects who showed a significant early deflection was seven, seven, and five in L-AVE, and four, four, and three in C-AVE for cSI, cParaS, and iParaS, respectively

cSI contralateral primary somatosensory cortex, *cParaS*and *iParaS* contralateral and ipsilateral parasylvian regions, respectively

* $P < 0.01$, compared with the main deflection in C-AVE (paired *t*-test)

with L-AVE were artificial. We could exclude this possibility based on the following. (1) In a few subjects, there were significant early deflections prior to the main deflections even in the C-AVE waveforms though they were low in amplitude as compared to the main deflections. (2) Although we used the peak latency of the main component for the adjusting, the L-AVE technique made both the early and main deflections clearer as compared to C-AVE. (3) Although we selected the sensors with the largest amplitude around the temporal area as SOIs and used them for adjusting the latency of each trial, the quality of the data from SI as well as the cParaS region was improved. Since the early activity was low in amplitude and had the opposite orientation to that of the main activity, we considered that it was easily cancelled out by the main activity in the C-AVE process.

Cortical activations in SI and the parasylvian regions

In C-AVE, main activities were found to originate from SI and the parasylvian regions, confirming previous findings (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Inui et al. 2002b; Nakata et al. 2004). The peak latency of the activity, 160–180 ms, was consistent with results of previous MEG studies using laser stimulation (Ploner et al. 1999; Kanda et al. 2000; Timmermann et al. 2001; Nakata et al. 2004). In addition, the simultaneous activation of SI and cParaS and significantly later activation of iParaS were consistent with a recent MEG study (Ploner et al. 1999). The involvement of these cortical areas in pain processing has also been demonstrated in PET (Talbot et al. 1991; Casey et al. 1994) and fMRI studies (Gelnar et al. 1999; Apkarian et al. 2000).

However, only two papers have described the early activity prior to the main activity in SI (Inui et al. 2003a, b). The early activity identified in the contralateral SI area in the present study seems to correspond to that described by Inui et al. (2003a), who showed that the onset latency of the early SI activity (80 ms) following a noxious epidermal electrical stimulation was shorter by 29 ms than that for the main parasylvian activity (109 ms). Therefore, the temporal relationship between the early SI and the main parasylvian activities was very similar to the present results showing a latency delay of 31 ms. For the latter, the slightly longer onset latency (105 ms) of the early SI activity in the present study might be due to the temperature conduction time for laser stimulation.

As for the early activity in the parasylvian region, there are two studies that reported its presence. Valeriani et al. (2000) reported an early positive component (eP) in the contralateral parasylvian region with a peak latency of 83 ms that preceded the N1 negativity. Since they used a

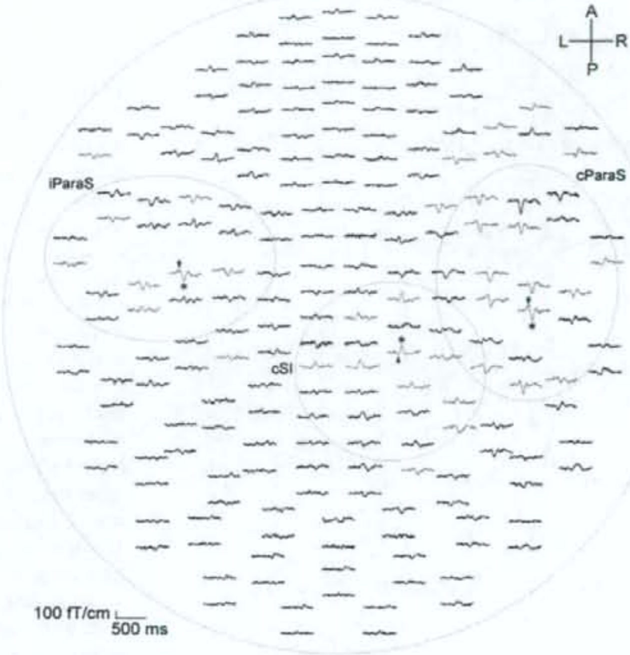


Fig. 3 Laser-evoked magnetic fields recorded from 204 planar coils in L-AVE in a single subject. The *waveform in grey* denotes that a significant early deflection prior to the main component is detected in this channel. *Arrows* and *asterisks* indicate the early and main deflection, respectively, with the largest amplitude in three areas around the contralateral parietal region and bilateral parasylvian regions. Significant early deflections are detected in the three cortical areas indicated by *circles*

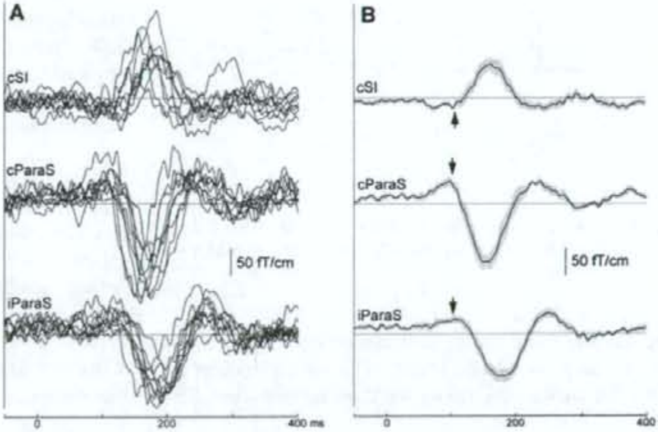


Fig. 4 **a** Superimposed waveforms of evoked magnetic fields of all subjects recorded form three channels, in which the main components showed the largest amplitude in the contralateral parietal region and bilateral temporal regions, respectively. **b** Group-averaged waveforms. *Shaded areas* depict ±SE. *Arrows* show the mean peak latency of the early responses

CO_2 laser and we used a YAG laser to elicit pain-related potentials, it is difficult to directly compare the early component between their study and the present study. However, the early deflection in the parasylvian region in the present study might correspond to the eP of Valeriani et al. (2000). In both studies, the early component preceding the N1 component had a small amplitude and an opposite orientation to that of the N1 component. In another study using intracranial recordings, Frot et al. (1999) demonstrated an early negative response at a latency of 135 ms in the parasylvian region, followed by a positive response peaking around 170 ms, which seems to correspond to the present early and main polarity-reversed activities.

As for the latency difference of the parasylvian activity between hemispheres, the 17-ms delay for the ipsilateral response of the main component in the present study was consistent with results of the intracranial recording study by Frot et al. (1999). In the study by Frot et al. (1999) a similar time

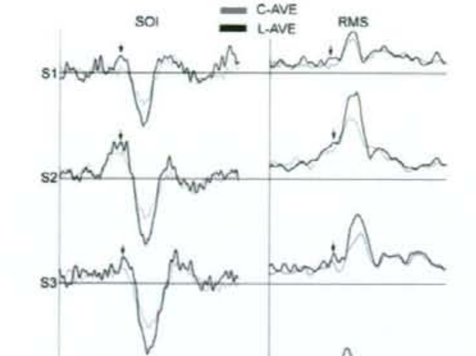


Fig. 5 The waveform of the SOI and RMS of nine subjects in C-AVE and L-AVE. *SOI* sensor of interest. *RMS* root mean square. *Arrows* indicate the early activity

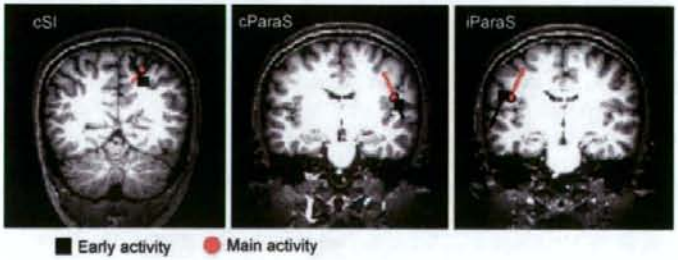
lag was also found for the early component (15 ms). In the present study however, the latency of the early component did not differ significantly among the three cortical areas. This discrepancy was probably due to the low S/N ratio of the early component or the small sample of data in the present study.

The precise anatomical location of the early parasylvian activity was not clear like the main activity in this region. The location of nociceptive cortical areas around the sylvian fissure is still a matter of controversy. It has been difficult to determine whether the nociceptive area is situated within the classic SII (parietal operculum) or within an adjacent somatosensory area such as the frontoparietal operculum or insula. Many previous studies have shown that noxious stimuli activate at least one cortical area around the sylvian region other than SII. For example, fMRI (Bingel et al. 2003; Brooks et al. 2005; Iannetti et al. 2005) and intracranial EEG (Lenz et al. 2000; Frot and Mauguiere 2003) studies found activation in the posterior insula following noxious stimulation. Our previous studies also showed that activity from the insula may contribute to the major MEG signals evoked by noxious stimuli (Inui et al. 2003a; Wang et al. 2004). In the present study, the dipole was estimated to be located in the upper bank of the sylvian fissure in some cases but deeper around the circular sulcus in others. Therefore, we consider that activation in the sylvian region in this study may be a summation of activities from SII and adjacent areas. With regard to the early parasylvian activity, a reliable estimation of its source could not be obtained in some subjects because of the low S/N ratio. However, the sources of the early deflections were estimated to lie around the bilateral parasylvian region in the other subjects with a GOF of more than 85%. These findings suggested that the early components originated from similar regions to the main activities.

Temporal sequence of activation

The present results showing the simultaneous onset of the main SI and contralateral parasylvian activities are consistent with recent MEG studies (Ploner et al. 1999). These findings support the notion of a parallel mode of pain processing between the SI and parasylvian region. However, the temporal sequence of cortical activation should be reconsidered because of the presence of earlier activities. Our results suggested that early cortical processing of noxious information occurs earlier than previous neurophysiological studies

Fig. 6 Source locations of early and main activities in a representative subject. The locations were almost the same, but the dipole's direction was opposite



have estimated. As for the early component, our results did not find a significant difference in latency among the three cortical areas. However, this could be due to the small number of subjects, or due to the low S/N ratio of these activities. The slightly shorter latency of the contralateral parasylvian source compared to the other two sources might suggest the dominance of the contralateral parasylvian region in the early processing of noxious information.

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Multiple pathways for noxious information in the human spinal cord

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Abstract

To investigate the pathways of noxious information in the spinal cord in humans, we recorded cortical potentials following the stimulation of A-delta fibers using a YAG laser applied to two cutaneous points on the back at the C7 and Th10 level, 4 cm to the right of the vertebral spinous process. A multiple source analysis showed that four sources were activated; the primary somatosensory cortex (SI), bilateral parasylvian region (Parasylvian), and cingulate cortex. The activity of the cingulate cortex had two components (N2/P2). The mean peak latencies of the activities obtained by C7 and Th10 stimulation were 166.9 and 186.0 ms (SI), 144.3 and 176.8 ms (contralateral Parasylvian), 152.7 and 185.5 ms (ipsilateral Parasylvian), 186.2 and 215.8 ms (N2), and 303.0 and 332.3 ms (P2). Estimated spinal conduction velocities (CVs) of the respective activities were 16.8, 9.3, 8.7, 10.1 and 10.7 m/s. CV of SI was significantly faster than the others ($P < 0.05$). Therefore, our results suggested that noxious signals were conveyed through at least two distinct pathways of the spinal cord probably reaching distinct groups of thalamic nuclei. Further studies are required to clarify the functional significance of these two pathways.

Keywords: Pain; Spinothalamic tracts; Conduction velocity; Electroencephalography; Laser evoked potential

1. Introduction

Noxious stimuli applied to the skin surface are detected by nociceptors in the epidermis (Burgess and Perl, 1967; Beitel and Dubner, 1976). The signals are conveyed through thinly myelinated A-delta-fibers and unmyelinated C-fibers to reach the dorsal horn of the spinal cord (Light and Perl, 1979; Sugiura et al., 1986). In the spinal cord, two different groups of neurons receive inputs from the periphery, that is, neurons in the superficial lamina (mainly lamina I) and deep lamina (mainly lamina V). Therefore, the processing of noxious signals in separate systems starts at the spinal level (Craig, 2003; Price et al., 2003). Nociceptive-specific (NS) neurons in lamina I respond to cold and noxious stimuli (Christensen and Perl, 1970; Kumazawa et al., 1975; Dostrovsky and Craig, 1996), while wide dynamic range (WDR) neurons in lamina V respond to both noxious and innocuous stimuli (Wall, 1960; Mendell, 1966; Willis et al., 1974). Then they project to distinct thalamic regions, although their terminations partly overlap (Apkarian and Hodge, 1989a). Spinothalamic neurons in lamina I mainly project to the medial thalamic nuclei and possibly to posterior part of the ventral medial nucleus (VMpo), while those in lamina V mainly project to the ventral posterior lateral nucleus (VPL) (Kenshalo et al., 1980; Ralston and Ralston, 1992; Craig et al., 1994). Furthermore, these thalamic nuclei have distinct projection sites. That is, VPL, VMpo, and medial thalamic nuclei mainly project to the primary somatosensory cortex (SI), the insula, and the anterior cingulate cortex (ACC), respectively (for review, see Treede

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et al., 1999). Considering the different response properties of neurons and different anatomical pathways, these two streams of nociceptive processing appear to differ in function. In spite of these findings in animal studies, the functional and anatomical segregation in humans is still a subject of controversy, since less is known about segregated nociceptive processing largely due to the limitations of experimental methods. Notably, whether there are distinct pathways in the spinal cord itself is not well understood.

Therefore in the present study, we sought to clarify multiple spinal pathways for nociceptive processing in humans. We recorded cerebral evoked potentials (EPs) following Tm:YAG laser stimulation applied to two different levels of the right side of the back to measure the conduction velocity (CV) of signals in the spinothalamic tracts (STT). Previous studies in animals showed that pathways from the dorsal horn neurons in lamina I and lamina V to the thalamus had different CVs (Ferrington et al., 1987). Therefore, we considered that different pathways would have distinct CVs also in humans.

2. Methods

The experiment was performed on 10 healthy right-handed male volunteers, aged 22–36 years (mean, 29.3 ± 4.0). The study was approved in advance by the Ethics Committee of the National Institute for Physiological Sciences and written consent was obtained from all the subjects.

2.1. Noxious stimulation

For noxious stimulation, we used a Tm:YAG laser (Neurolaser, Baasel Lasertech, Germany). The wavelength was 2000 nm, pulse duration was 1 ms, and spot diameter was 6 mm. Laser stimuli were applied to two sites: the right side of the back 4 cm lateral to the 7th cervical vertebral spinous process (C7) and 10th thoracic vertebral spinous process (Th10). We chose these sites for stimulation to minimize the peripheral conduction distance of primary neurons, and stimulate the peripheral nerve of one side. In our previous report (Inui et al., 2006), we confirmed that the back is one of the cutaneous areas at which noxious stimulation evokes clear brain responses. Also, Cruccu et al. (2000) and Iannetti et al. (2003) obtained good results after noxious stimulation of the back. Histologically, the intraepidermal nerve fiber density of the trunk is higher than that of the distal leg (Lauria et al., 1999). The threshold for a pinprick sensation in the trunk is lower than that in the extremities (Agostino et al., 2000). The stimuli were delivered randomly at an interval of 10–15 s. The irradiated points were moved slightly within a transverse 4 cm area centered on the points after each stimulus to avoid tissue damage and habituation of the receptors. Subjects were asked to rate the intensity of the perceived pricking pain on a visual analogue scale (VAS, 0–10) prior to the experiment, and the stimulus intensity was adjusted to the level eliciting a VAS score of around seven.

2.2. EP recordings

Subjects lay prone on a bed and were asked to relax their muscles and keep their eyes open. The room temperature was 25 °C and sound and light were regulated. Skin temperature was kept above 30 °C (Kakigi and Shibasaki, 1991). A simultaneously recorded electro-oculogram (EOG) was used for artifact rejection. Signals were recorded with a band-pass filter of 0.1–100 Hz. The window of analysis was 600 ms including 100 ms of a prestimulus period, and the sampling rate was 1000 Hz. Laser stimuli were applied to C7 and Th10 randomly. For each site of stimulation, over 25 artifact-free trials were selected and averaged off-line. To minimize endogenous factors, subjects were asked not to predict which site would be stimulated and not to pay attention to the stimulated sites.

2.3. Source modeling

We recorded EPs using the standard 19 electrodes of the 10–20 system (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, T3, T4, T5, T6, P3, P4, Pz, O1, O2) and an additional 13 electrodes including FC1, FC2, C1, C2, C5, C6, T9, T10, CP1, CP2, P1, P2 and CPz (Fig. 1). The electrode impedance was always kept below 5 kΩ. We used the balanced non-cephalic reference (BNP, Stephenson and Gibbs, 1951), to reduce problems due to activities of the reference. We linked two electrodes, one from over the right sternoclavicular junction and the other from over the tip of the 7th cervical spine, and incorporated a variable resistor. By adjusting the resistance, the pickup of cardiac potentials was minimized.

Since several cortical activities following noxious stimulation overlapped temporally, we analyzed theoretical multiple source generators of EPs using the brain electric source analysis (BESA) software package (NeuroScan, Inc, Mclean, VA, USA). Model adequacy was assessed by examining: (i) percent variance; (ii) F-ratio (ratio of reduced χ^2 values before and after adding a new source); and (iii) residual waveforms

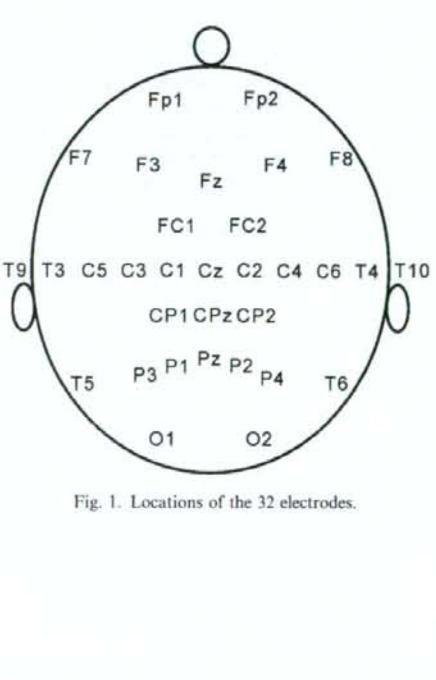

Fig. 1. Locations of the 32 electrodes.

(i.e., the difference between the recorded data and the model) as described elsewhere (Inui et al., 2004). Percent variance measures the goodness of fit (GOF) of the model comparing the recorded data and the model. The integral probability of obtaining a *F*-ratio value equal to or greater than the obtained value is calculated to evaluate whether a model with a larger number of dipoles represents a statistical improvement of the fit over a model with a smaller number of dipoles. When a P value was <0.05, we considered the new dipole as significant. BESA uses a spherical 4 shell model with an 85 mm radius. The spatial position of each dipole is defined by reference points on the head known as fiducials. The reference points are nasion, the left preauricular point (T9), and the right preauricular point (T10). The x axis is defined by the line joining T9 and T10, positive towards T10. The y axis is defined by the line through nasion that is perpendicular to the x axis (positive towards nasion). The z axis is perpendicular to the x and y axes, and goes up out of the head in the vicinity of Cz.

2.4. Measuring conduction velocities

The CV of a given nerve fiber can be calculated by measuring the difference in response latency of cerebral potentials between two different stimulation sites. For example, by dividing the distance between hand and arm stimulation sites by the latency difference of evoked potentials following hand and arm stimulations, one can estimate the CV of the peripheral nerve. By use of laser-evoked potentials (LEPs), the CV of A-delta fibers can be measured (Kenton et al., 1980; Bromm and Treede, 1987). By use of similar methods, CVs in STT were also measured (Kakigi and Shibasaki, 1991; Cruccu et al., 2000; Rossi et al., 2000; Qiu et al., 2001; Iannetti et al., 2003).

In the present study, the spinal conduction time was taken from the difference in the peak latency of each cortical activity between C7 and Th10 stimulation. Although the onset latency is desirable when examining the timing of the arrival of nociceptive signals in a cortical area, its determination was difficult. Therefore, we used the peak latency as in previous reports (Kakigi and Shibasaki, 1991; Cruccu et al., 2000; Rossi et al., 2000; Qiu et al., 2001; Iannetti et al., 2003). The peak latency was the latency point with the maximal amplitude. The CV was calculated by dividing the distance between C7 and Th10 by the difference in latency of each activity.

In the present study, we had an interest in the spinal conduction time, not in other conduction times. So, even though the peak latency does not directly reflect the conduction time calculated with the CV, it did not matter. For example, although the latency of P2 is too late for the CV, it would not result from the spinal cause (Kakigi and Shibasaki, 1991; Rossi et al., 2000; Iannetti et al., 2003). The latency difference could reflect the spinal conduction time.

2.5. Analysis

Data were expressed as means ± SD. The statistical significance of the peak latencies of each stimulated site and CVs was assessed with a one-way analysis of variance (ANOVA). When the P value was less than 0.05, a post hoc analysis with the Bonferroni and Dunn method was performed.

3. Results

Fig. 2 shows results for a representative subject. Laser stimuli applied to C7 produced an activity at around 127 ms in the parasylvian region of both hemispheres. The topography also indicated a negativity around the parietal region contralateral to the stimulated side at around 149 ms. At later latencies, a large negativity at around 175 ms followed by a larger positivity at around 320 ms was evident and corresponded to well-known N2/P2 components (Kakigi et al., 2000). These topographic findings indicated that at least four distinct sources were active during the period of analysis. Therefore, we analyzed the data using BESA to differentiate each source activity.

Isocontour maps at 127 ms in Fig. 2B show that bilateral sources around the sylvian region were active. To explain the data at this latency point, two sources were estimated to be located in the bilateral parasylvian region (Fig. 2E, a). Fig. 2C, a shows the theoretical distribution of these sources. This two-dipole model could explain 74.2% of the recorded EPs at 127 ms. Then we subtracted the theoretical waveforms of these sources from the recorded EPs (Fig. 2A, b). To explain the subtracted waveform, the best source was estimated in the midcingulate cortex (MCC) based on the recent cytoarchitectonic subdivision by Vogt (2005) (Fig. 2E, b), which appears to correspond to a region described as the anterior cingulate cortex in many previous reports. At a latency range from 175 to 450 ms, the addition of the MCC source markedly improved the fit (for example, the GOF value at 320 ms increased from 0.8% to 91.5%). We considered that this activity was compatible with the well-known N2/P2. This three-dipole model could explain 87.2% of the recorded EPs, but weak dipolar fields around the parietal region remained to be explained (Fig. 2A, c and D, a). To explain the residual waveform, the best source was estimated to be located in a parietal region slightly posterior to the central sulcus around the midline (Fig. 2E, c), which probably corresponds to the medial part of the postcentral gyrus of the contralateral hemisphere. With the addition of this source (SI source), the residual waveforms in Fig. 2A, c were almost abolished. This four-source model provided a GOF value of 90.7%.

Similar results were obtained in the remaining subjects. The mean coordinates of each dipole are shown in Table 1. The SI source was located around the midline, which was compatible with the trunk area of SI. These results were similar to the four-dipole model after stimulation of the hand reported by Tarkka and Treede (1993) and Schlereth et al. (2003). The MCC source as the major contributor to EPs following stimulation of the back was also consistent with the results reported by Iannetti et al. (2003). Accordingly, five distinct activities, SI, bilateral parasylvian activities (Pc and Pi) and MCC (N2/P2), were used to measure CVs.

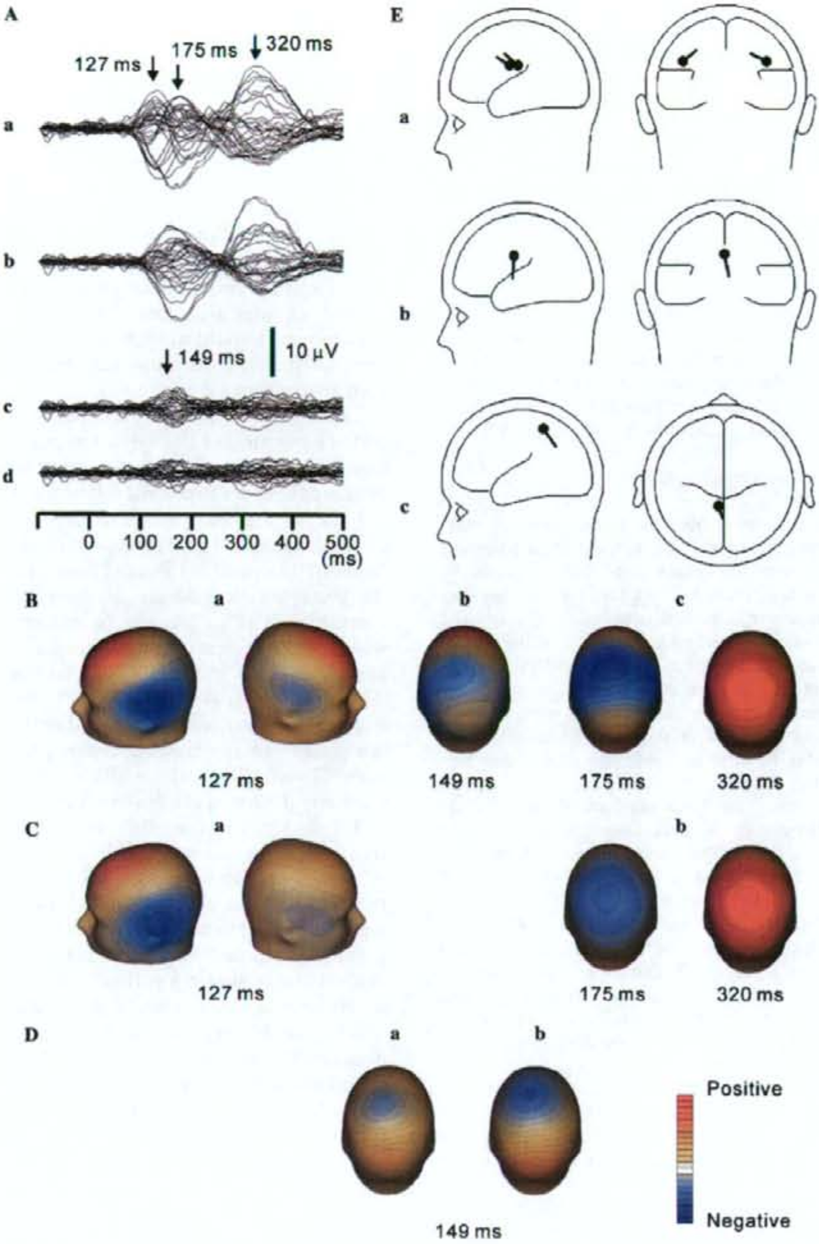

Fig. 2. Procedures of the multiple source analysis in a representative subject. First, two dipoles around the sylvian region (E, a) were estimated to explain the waveform recorded around 127 ms. B, a and C, a show isocontour maps of the recorded data and the two-dipole model at 127 ms, respectively. A, b shows the residual waveform obtained by the subtraction of the theoretical waveform due to these two sources from the recorded waveform (A, a). To explain the residual waveform (A, b), the best third source was estimated to be located in the cingulate cortex (E, b). B, c and C, b show isocontour maps of the recorded data and the third source model, respectively. A, c shows the residual waveform that remains to be explained by the three-dipole model. To explain the distribution of the residual waveform (D, a), the fourth source was estimated to lie around the medial part of the postcentral gyrus (E, c). D, b shows the theoretical field distribution of this source. After the fitting of these four sources, the residual waveform (A, d) showed no clear components.

Table 1
Locations of dipoles

	C7			Th10		
	x	y	z	x	y	z
SI	−4.8 ± 6.8	−16.6 ± 20.7	86.0 ± 8.4	−3.9 ± 6.5	−15.1 ± 19.1	87.4 ± 7.4
Pc	−45.3 ± 10.6	15.4 ± 4.8	56.3 ± 4.6	−45.1 ± 10.5	15.6 ± 7.2	55.6 ± 4.9
Pi	47.1 ± 12.2	15.0 ± 7.3	56.3 ± 5.7	47.6 ± 12.1	15.3 ± 7.2	56.0 ± 4.9
MCC	−3.1 ± 7.4	10.1 ± 12.9	56.8 ± 18.3	−0.6 ± 5.6	12.3 ± 15.8	59.5 ± 13.9

SI, primary somatosensory cortex; Pc, parasylvian source in the hemisphere contralateral to the stimulated side; Pi, parasylvian source in the hemisphere ipsilateral to the stimulated side; MCC, midcingulate cortex.

The peak latencies of each activity are shown in Table 2. An ANOVA showed that there was a significant difference in peak latency among the five activities following C7 stimulation ($F(1,4) = 218.8$, $P < 0.01$). The peak latency of P2 was significantly later than that of any other activities (post hoc test: $P < 0.01$), and the peak latency of N2 was also significantly later than that of SI, Pc or Pi (post hoc test: $P < 0.05$). On average, the peak latency of P2 was later than that of N2 by 116.8 ms, and in turn, the peak latency of N2 was later than that of SI, Pc and Pi by 19.3, 41.9 and 33.5 ms, respectively. The temporal relationship of each activity following Th10 stimulation was similar to that following C7 stimulation.

The estimated CVs of each activity are shown in Table 2. An ANOVA showed that there was a significant difference in CVs among the five activities ($F(1,4) = 6.4$, $P < 0.01$). The CV of SI was significantly greater than that of any other activities (post hoc test: $P < 0.05$). There was no significant difference among CVs for Pc, Pi, N2 and P2 (see Fig. 3).

4. Discussion

The present results clearly showed that at least two distinct pathways in the spinal cord transmitted noxious signals. This is the first report to confirm in the human spinal cord that nociceptive signals are processed by both the faster spinal CV pathway projecting to SI and the slower spinal CV pathway projecting to the parasylvian region and MCC.

4.1. Cortical activities

We identified activities in four cortical areas, SI, bilateral parasylvian regions and MCC, which is consistent with previous LEP studies (Tarkka and Treede, 1993; Schlereth et al., 2003). Previous LEP studies (Table 3) consistently found activities in the sylvian region. However, the locations of the parasylvian source were variable. Dipoles in electroencephalographic (EEG) and magnetoencephalographic (MEG) studies tend to be estimated in the upper bank of the sylvian fissure, while dipoles identified using subcortical and intracerebral recordings tended to be in deeper areas. MCC is an essential source in EEG studies, but not in MEG studies.

In previous studies, a somatotopic arrangement of SI activities after noxious stimulation to the hand and foot compatible with the well-known somatosensory homunculus has been reported in monkeys (Kenshalo et al., 2000) and humans (Penfield and Boldrey, 1937; Tarkka

Table 2
The peak latency of each source activity and estimated conduction velocity in the spinothalamic tract

Subject	Peak latency (ms)										Distance (cm)	Conduction velocity (m/s)				
	SI		Pc		Pi		N2		P2							
	C7	Th10	C7	Th10	C7	Th10	C7	Th10	C7	Th10		SI	Pc	Pi	N2	P2
1	157	178	146	181	153	192	187	221	285	321	28.5	13.6	8.1	7.3	8.4	7.9
2	184	207	128	149	157	184	175	194	324	350	27.5	12.0	13.1	10.2	14.5	10.6
3	180	198	144	169	152	180	171	204	327	345	26.5	14.7	10.6	9.5	8.0	14.7
4	148	173	134	170	153	183	194	216	331	354	27.2	10.9	7.6	9.1	12.4	11.8
5	161	171	150	170	155	183	194	219	283	302	26.8	26.8	13.4	9.6	10.7	14.1
6	178	190	159	184	159	187	185	214	315	336	26.0	21.7	10.4	9.3	9.0	12.4
7	171	202	139	199	149	180	188	239	293	338	27.5	8.9	4.6	8.9	5.4	6.1
8	174	187	149	186	146	199	186	205	287	322	28.5	21.9	7.7	5.4	15.0	8.1
9	144	172	140	179	136	174	165	191	278	330	27.5	9.8	7.1	7.2	10.6	5.3
10	172	182	154	181	167	193	217	255	307	325	27.8	27.8	10.3	10.7	7.3	15.4
Mean	166.9	186.0	144.3	176.8	152.7	185.5	186.2	215.8	303.0	332.3	27.4	16.8	9.3	8.7	10.1	10.7
SD	13.7	13.0	9.3	13.3	8.2	7.4	14.5	19.6	20.2	15.6	0.8	7.1	2.8	1.6	3.1	3.6

SI, primary somatosensory cortex; Pc, parasylvian source in the hemisphere contralateral to the stimulated side; Pi, parasylvian source in the hemisphere ipsilateral to the stimulated side.

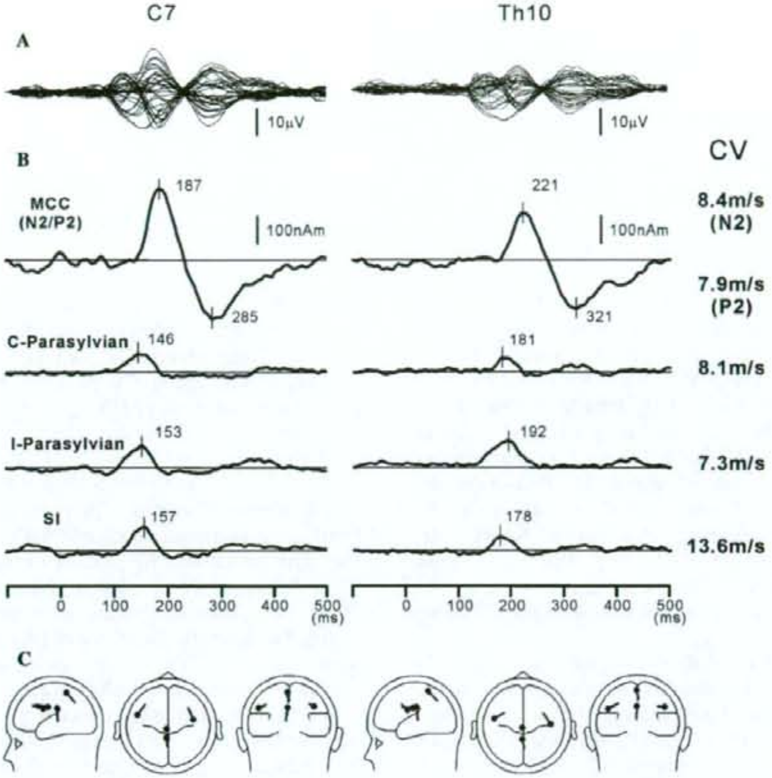

Fig. 3. Estimation of conduction velocity of each source activity in the spinal cord in a single subject. (A) Superimposed waveforms of evoked potentials following C7 (left) and Th10 (right) stimulation. (B) Time course of each cortical activity obtained by a multiple source analysis. (C) Schematic drawings of the location and orientation of each source. Estimated conduction velocity (CV) of each activity is shown. Note the similar latency difference between C7 and Th10 stimulation with the exception that the difference is smaller for the SI activity than the other source activities.

and Treede, 1993; Bingel et al., 2004; Ogino et al., 2005). These studies showed that the hand was represented in the lateral part of the postcentral gyrus and the foot, near the midline. These results imply that the pain system employs a body surface map in SI that is similar to the somatosensory homunculus. Since the representation of the trunk in SI in the somatosensory system is located around the midline, our results seem to support this idea. However, the precise location of nociceptive neurons in SI was slightly posterior to area 3b and probably in area 1, area 2 or the posterior parietal cortex in animals (Kenshalo and Isensee, 1983) and human studies (Kanda et al., 2000; Ploner et al., 2000; Inui et al., 2003; Ohara et al., 2004; Valeriani et al., 2004). The timing of SI activation in the present study was consistent with previous EEG and MEG studies (Tarkka and Treede, 1993; Ploner et al., 1999; Inui et al., 2003).

Our multiple source analysis showed that bilateral parasylvian EP components come from the sylvian region. Although previous LEP studies agreed that this activity was generated in the secondary somatosensory cortex (SII) area (Tarkka and Treede, 1993; Bromm and Chen, 1995; Kakigi et al., 1995; Valeriani et al., 1996), recent subdural LEP studies showed that it was generated in the frontoparietal operculum overlying insula, not in the parietal operculum overlying SII (Lenz et al., 2000; Vogel et al., 2003). In functional magnetic resonance imaging (fMRI) studies, both SII and the insula were activated by noxious stimuli (Brooks et al., 2002; Bingel et al., 2003). Our previous MEG study using noxious intraepidermal electrical stimulation (Inui et al., 2003) showed that the insula was activated almost simultaneously with SII, although the results could not be directly compared with those of the present study because of different stimulus and recording conditions. The precise anatomical area responsible for this activity has not yet been determined. Therefore, we considered that parasylvian activity in the present study might be a summation of several temporally overlapping activities from the sylvian region.

Table 3
Source locations in previous studies (scalp EEG, MEG, subdural, and intracerebral)

Reference	Recording	Laser	Activated cortical area
Tarkka and Treede (1993)	EEG	CO_2	SI, SII, ACC
Bromm and Chen (1995)	EEG	CO_2	SII, ACC, frontal cortex
Kakigi et al. (1995)	MEG	CO_2	SII
Valeriani et al. (1996)	EEG	CO_2	SII, CC, mesial-temporal cortex
Watanabe et al. (1998)	MEG	CO_2	SII, amygdala-hippocampal formation
Lenz et al. (1998a)	Subdural	CO_2	Parietal operculum and/or insula
Lenz et al. (1998b)	Subdural	CO_2	ACC
Yamasaki et al. (1999)	MEG	CO_2	SII
Ploner et al. (1999)	MEG	YAG	SI, SII
Frot et al. (1999)	Intracerebral	CO_2	Frontoparietal operculum
Valeriani et al. (2000)	EEG	CO_2	SII, CC, insular-temporal cortex
Ploner et al. (2000)	MEG	YAG	SI (area 1), SII
Kanda et al. (2000)	MEG	CO_2	SI (area 1), SII
Lenz et al. (2000)	Subdural	CO_2	Parietal operculum–insula
Timmermann et al. (2001)	MEG	YAG	SI, SII
Frot et al. (2001)	Intracerebral	CO_2	Frontoparietal operculum
Ploner et al. (2002)	MEG	YAG	SI, SII, ACC
Iannetti et al. (2003)	EEG	CO_2	ACC
Schlereth et al. (2003)	EEG	YAG	SI, ACC, operculum
Vogel et al. (2003)	Subdural	CO_2	Frontoparietal operculum
Frot and Mauguiere (2003)	Intracerebral	CO_2	Operculum–insula
Valeriani et al. (2004)	Subdural	CO_2	SI (area 1, 2 or posterior parietal cortex)
Ohara et al. (2004)	Subdural	YAG	SI (not area 3b or 1), parasylvian area, ACC, SMA

SI, primary somatosensory cortex; SII, secondary somatosensory cortex; ACC, anterior cingulate cortex; SMA, supplementary motor area.

The main source generator was located in MCC, also in accordance with previous reports (Iannetti et al., 2003). Activation in these areas following noxious stimuli is consistent with fMRI studies (Bornhovd et al., 2002). Therefore, these five activities (SI, Pc, Pi, N2 and P2) were consistent with previous studies. We measured CVs of the STT using these five activities.

4.2. Methodological considerations when measuring CV

CVs of noxious signals in the human spinal cord were reported for the first time by Kakigi and Shibasaki (1991). They estimated CV by comparing the latencies of P2 following laser stimulation to the hand and foot. Although their method was confirmed later by Rossi et al. (2000), its peripheral component was not negligible. Recently, Cruccu et al. (2000) measured CV using EPs after stimulation of the dorsal midline. Since the back midline has the shortest conduction distance from the stimulated point to the spinal cord, this method has the advantage of reducing the peripheral components. In the present study, we stimulated two different levels of the right side of the back 4 cm lateral to the midline. This method had an additional advantage over previous studies in that it activated nociceptors belonging to the peripheral nerve of one side. Since noxious stimuli activate several cortical areas bilaterally with a 15–20 ms delay for the ipsilateral activity (Inui et al., 2003), the evoked response should be very complicated when stimuli are applied to the midline. That is, responses in one hemisphere contain both contralateral and ipsilateral responses due to the stimulation of peripheral nerves of both sides. Another methodological advantage of this study was the random stimulation paradigm. The EP waveform is substantially affected by level of arousal, attention and expectancy (Kakigi et al., 2000). Our method could minimize such effects.

4.3. CV in the spinal cord

The estimated CVs in the spinal cord following noxious stimulation in the present study were 8.7–16.8 m/s. The CVs calculated from the peak latency of N2 (Cruccu et al., 2000), P2 (Kakigi and Shibasaki, 1991; Rossi et al., 2000) and N1/P1 (Rossi et al., 2000) were 21, 10 (8–12) and 10.0 m/s, respectively. Therefore, values in the present study were approximately consistent with those in other studies. The CV for the SI activity has not been reported previously.

4.4. Two pathways in the STT

The STT neurons are functionally separated into NS and WDR neurons. The locations are also different, namely, NS cells are mainly located in lamina I and WDR cells are mainly present in lamina V. Axons of NS and WDR cells ascend in different parts of the STT (Apkarian and Hodge, 1989b; Craig, 2003). Furthermore, NS and WDR cells have distinct projection targets in the thalamus, although their terminations partly overlap (Apkarian and Hodge, 1989a; Willis and Westlund, 1997). As for the CV, signals of WDR cells conduct in the STT significantly faster than those of NS cells in monkeys (Ferrington et al., 1987). In this study, the CV for SI was significantly faster than that of any other activity, implying that activation in SI came from WDR cells. This notion is consistent with the fact that lamina V WDR neurons predominantly project to VPL and in turn, VPL predominantly projects to SI (Kenshalo et al., 1980), and that the majority of nociceptive neurons both in VPL (Kenshalo et al., 1980) and in SI (Kenshalo and Isensee, 1983) are of the WDR type. Based on comparisons of neurophysiological data between humans and monkeys (Mayer et al., 1975; Price and Mayer, 1975), it has been demonstrated that WDR cells are responsible for the sensory aspect of pain.

On the other hand, signals from lamina I NS neurons ascend through the STT with a slower CV to reach the insula (Friedman and Murray, 1986) or cingulate cortex (Vogt et al., 1979) via medial nuclei of the thalamus and possibly VMpo (Ralston and Ralston, 1992; Craig et al., 1994; Craig, 2004). Therefore, MCC with a slower CV in the spinal cord in this study appeared to come from lamina I NS neurons. This idea is supported by the finding in a unitary recording study in animals (Koyama et al., 1998) and humans (Hutchison et al., 1999) that the cingulate cortex contains neurons that respond to noxious stimuli exclusively.

As for the parasylvian activity, various areas around the sylvian fissure have been reported as responsible (Table 3). If parasylvian activities reflect SII activity, our finding that the parasylvian activity had a slower spinal CV than the SI activity is congruent with the findings that VPI receives input from neurons in both lamina I and lamina V (Apkarian and Shi, 1994), and that the nociceptive neurons in VPI (Apkarian and Shi, 1994) and SII (Dong et al., 1989) are of both WDR and NS types. If parasylvian activities reflect the insular activity, the present findings are consistent with the lamina I NS cells-VMpo-insula pathway proposed by Craig et al. (1994). In the thalamus, intralaminar nuclei also relay noxious information from STT neurons to the sensory cortex. For example, the centrolateral nucleus (CL) receives inputs from STT neurons and projects to SI (Gindold et al., 1991) and SII (Stevens et al., 1993). Since a large percentage of SII-projecting thalamocortical neurons do not receive direct inputs from the spinal cord (Stevens et al., 1993), polysynaptic pathways to the thalamus, such as spinoreticulothalamic projections to the intralaminar nuclei, are possible. The present results could not clarify which spinal pathway with a slow CV is responsible for the parasylvian activity.

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