SECOND PAIN PROCESSING TRACED BY ELECTRO- AND MAGNETO- ENCEPHALOGRAPHY IN HUAMANS

秋 云海 **(QIU, Yunhai)**

Doctor of Philosophy

Department of Physiological Sciences,
School of life Sciences,
The Graduate University for Advanced Studies,

and

Department of Integrative Physiology,
National Institute for Physiological Sciences,
Myodaiji, Okazaki, 444-8585,
JAPAN

2004

Contents

1. Keywords

Sharp, pricking pain associated with rapidly conducting Aδ-fibers (first pain)

Dull, burning pain associated with slowly conducting C-fibers (second pain)

Small myelinated fiber (Aδ-fiber)

Unmyelinated fiber (C-fiber)

CO_2 laser

Laser-evoked potentials (LEP)

Laser-evoked potentials relating to Aδ-fibers (late LEP)

Laser-evoked potentials relating to C-fibers (ultra-late LEP)

Laser-evoked magnetic field (LEF)

Laser-evoked magnetic fields relating to C-fibers (ultra-late LEF)

Electroencephalography (EEG)

Magnetoencephalography (MEG)

Microelectroneurography

Conduction velocity (CV)

Spinothalamic tract (STT)

Attention

Brain electric source analysis (BESA)

2. Abstract

It is known that there are two kinds of pain sensation, a sharp or pricking pain associated with rapidly conducting Aδ-fibers (first pain), and a dull or burning pain associated with slowly conducting C-fibers (second pain). We activated C-fibers selectively by stimulating a tiny area of the skin with a CO_2 laser beam using a thin aluminum plate with numerous tiny holes as a spatial filter. Using this new method, we successfully recorded C-fiber discharges with a microneurographic study and cortical responses evoked by C-fiber stimulation using electroencephalography (EEG) and magnetoencephalography (MEG) in humans.

First, we investigated C-fiber discharges and cerebral potentials evoked by weak CO_2 laser beams applied to a tiny skin area in five healthy subjects. Microneurography was recorded from the peroneal nerve in the right popliteal area. Cerebral potentials were recorded from the Cz electrode (vertex, based on the international 10-20 system) referred to linked earlobes. The mean conduction velocity (CV) of the five stable single units was 1.1±0.3 m/s. The mean latency of the positive peak of the cerebral potentials was 1327.4±46.2 ms, which was markedly longer than that from Aδ-fiber stimulation. In contrast, this stimulation method did not activate the Aδ units at all. These findings indicate that this new stimulation method selectively activates C-fiber nociceptors of the skin (Chapter 5 and Qiu et al., 2003).

Second, we measured the CV of C-fibers in the spinothalamic tract (STT) using this method. We delivered CO_2 laser pulses to tiny areas of the skin overlying the vertebral spinous processes at different levels from the 7th cervical (C7) to the 12th thoracic (T12), and recorded the cerebral evoked potentials in 11 healthy men. The mean CV of C-fibers in the STT was 2.2±0.6 m/s, which was significantly slower than that of the Aδ-fibers (10.0±4.5 m/s). This technique is novel and simple, and should be useful as a diagnostic tool for assessing the level of spinal cord lesions (Chapter 6 and Qiu et al., 2001).

Third, we evaluated the effects of attention, distraction and sleep on CO_2 laser-evoked potentials (LEP) related to C-fibers (ultra-late LEP), since the degree of perceived pain sensations is known to be influenced by the subjects' attention levels. CO_2 laser pulses were delivered to a tiny skin area of the

dorsum of the right hand. Ultra-late LEP were recorded from 10 normal subjects in 5 different conditions: control (wakefulness), attention, distraction, drowsiness and sleep (stage 2). The amplitude of ultra-late LEP was slightly increased during attention and significantly decreased during distraction, relative to the control. The ultra-late LEP greatly decreased in amplitude or almost disappeared during sleep. We confirmed that the brain responses related to signals ascending through C-fibers were greatly affected by the level of consciousness, being consistent with the findings of late LEP related to Aδ-fibers. This is the first study indicating the important characteristics of ultra-late LEP related to consciousness, suggesting that they include cognitive function, and reporting that one has to be aware of changes in alertness when recording (Chapter 7 and Qiu et al., 2002).

Fourth, using MEG, we evaluated the cerebral regions related to second pain perception ascending through C-fibers and investigated the effect of distraction on each region. Thirteen normal subjects participated in this study. CO_2 laser pulses were delivered to the dorsum of the left hand to selectively activate C-fibers. The MEG responses were analyzed using a multi-dipole model. Results showed that (1) the primary somatosensory cortex (SI), and (2) the secondary somatosensory cortex (SII) - insula were the main generators for the primary component, 1M, the mean peak latency of which was 744 ms. In addition to (1) and (2), (3) the cingulate cortex and (4) the medial temporal area (MT) were also activated for the subsequent component, 2M, the mean peak latency of which was 947 ms. During a mental calculation task (distraction), all six sources were significantly reduced in amplitude, but the SII-insula ($P<0.01$) and cingulate cortex ($P<0.001$) were more sensitive than the SI ($P<0.05$) and MT ($P<0.05$). We confirmed that the SI in the contralateral hemisphere and the SII-insula, cingulate cortex and MT in the bilateral hemispheres play a major role in second pain perception, and all sites were greatly affected by changes in the attention levels, indicating that these regions are related to the cognitive aspect of second pain perception, and particularly to activities in the cingulate cortex (Chapter 8 and Qiu et al., 2004).

3. Introduction

There are two systems for nociceptive perception, one ascending through small-myelinated fibers (Aδ- fibers) related to first or sharp pain, and one ascending through unmyelinated fibers (C-fibers) related to second or burning pain. Since Mor and Carmon demonstrated that a brief CO_2 laser pulse can produce brain potentials related to Aδ-fiber activities (Mor and Carmon, 1975), a CO_2 laser beam is frequently used to record pain-related brain potentials (laser-evoked potentials, LEP) and magnetic fields (LEF) in humans, specifically activating nociceptors without stimulating mechanoreceptors related to tactile sensation (see reviews by Bromm and Lorenz, 1998; Chen et al., 1998a,b,c; Kakigi et al., 2000; Treede et al., 2000). Although a laser pulse can activate both Aδ- (late LEP) and C-fibers (ultra-late LEP) nociceptors (Bromm and Treede, 1984), it is very difficult to stimulate the latter selectively using the conventional laser stimulation method. Recently, Belgium group developed a new method for selectively stimulating C-fiber nociceptors, and recorded ultra-late LEP by the selective activation of C-afferent sensory terminals in the skin using a CO_2 laser to stimulate a tiny surface area (Bragard et al., 1996; Opsommer et al., 1999b). The physiological background of this method is the C-afferent sensory terminals in the skin have a higher density and lower activation threshold than Aδ-terminals (Ochoa and Mair, 1969; Schmidt et al., 1994; Treede et al., 1994).

A few recent studies have confirmed that similar methods can produce very late cortical responses recorded by electroencephalography (EEG) (Tran et al., 2001,2002a) and magnetoencephalography (MEG) (Tran et al., 2002b). These studies recorded clear ultra-late LEP and LEF related to C-fibers (ultra-late LEF) by modifying the method using a thin aluminum plate with numerous tiny holes as a spatial filter, and reported that the conduction velocity (CV) of the peripheral nerve and spinal cord following this specific stimulation was approximately 1-4 m/s, which is within the CV range for C-fibers (Tran et al., 2001, 2002a). However, there is no direct evidence for the selective C-nociceptor activation. Therefore, the first objective of the present study was to clarify whether this method actually activates C-nociceptors selectively. In addition, we measured the CV of C-fibers in the

spinal cord by a more accurate and reliable method.

Pain caused by injury or disease is reduced when attention is directed elsewhere (distraction), such as by playing sports, studying or reading. In contrast, subjective pain feeling is increased when we pay a close attention to the painful stimulus (attention). This clearly indicates that pain can be modified by attention/distraction. There have been several papers studying the effects of attention/distraction on pain perception related to first pain ascending through Aδ-fibers (Beydoun et al., 1993; Towell and Boyd, 1993; Siedenberg and Treede, 1996; Kanda et al., 1996; Zaslansky et al., 1996; Garcia-Larrea et al., 1997; Yamasaki et al., 1999; Legrain et al., 2002; Spence et al., 2002), but th effects related to second pain ascending through C-fibers were not clarified. Opsommer (1999a) examined changes in ultra-late LEP using the oddball paradigm, and found that the amplitude was enhanced when the subject's attention was given to the stimulus. However, there has been no systematic study focusing on changes in ultra-late LEP caused by a change in attention or the level of consciousness. Therefore, we studied the effects of attention, distraction and sleep on ultra-late LEP to elucidate the characteristics of brain activities related to second pain.

Our previous study showed that the 3-dipole model of primary somatosensory cortex (SI) and bilateral secondary somatosensory cortex (SII) sources could explain the primary component (1M) of MEG responses following stimulation of C-fibers (Tran et al., 2002b), but the generating mechanisms for later magnetic fields (second component, 2M) are still unclear, and, as well as, which regions are responsible for a subjective change in painful feeling by attention/distraction. Therefore, we first determined an appropriate source model, and then examined how each cortical activity was affected by a change in attention.

4. Methods for selective stimulation of C-fibers

The CO_2 laser stimulator was specially designed by Nippon Infrared Industries Co. Ltd. (Tokyo, JAPAN) to activate cutaneous C-fiber nociceptors. The laser beam was 10.6 μm in wavelength, 2 mm in diameter, and 20 msec in duration. For recording ultra-late LEP and LEF, we developed a new method using a thin aluminum plate. It was 0.1 mm in depth, 40 mm in length and 60 mm in width. In a 25×25 mm^2 on this plate, parallel lines were drawn every 1 mm, giving 26×26 intersections. A total of 676 (26×26) thin holes were drilled at these intersections, each with a diameter of 0.4 mm, corresponding to an area of 0.125 mm^2 for each hole. This thin plate was used as a spatial filter and placed on the skin at the site to be stimulated. The array of holes allowed the 2 mm laser beam to pass through 1–4 holes to reach the skin (Fig. 1A).

The principle of this method was based on previous studies reported by Belgium group (Bragard et al., 1996; Opsommer et al., 1999a,b, 20001), but was slightly modified due to some technical problems (Tran et al., 2001). We used a plate with many holes attached directly to the skin, but the Belgium group used a plate with one hole attached at the top of the stimulus probe. The principle was the same, and similar waveforms were recorded using either method. The physiological background for this method is that skin has a higher innervation density and lower activation threshold of C-fiber terminals. In humans, they are 6–8 times more numerous than Aδ-fiber terminals, and the activation threshold of C-fiber and Aδ-fiber were 40 °C and 45 °C, respectively (Ochoa and Mair, 1969; Schmidt et al., 1994; Treede et al., 1994). Accordingly, the selectivity for C-fibers with this method may result from a higher probability of hitting C-fiber than Aδ-fiber terminals.

The laser pulses was delivered at a slightly different site to avoid habituation of the receptors, within a transverse 25×25 mm^2 area of skin corresponding to the area of the array of holes. To avoid habituation as well as expectancy, we used random interstimulus intervals (3–10 s). In addition, we changed the stimulus site by moving the stimulus probe at least 5 mm for each stimulus. Therefore, the same receptor field was not stimulated sequentially, since each hole is only 0.4 mm in diameter, and the stimuli were delivered in a random sequence to different sites. The stimulus intensity was

approximately 2–4 W, and we used the minimum intensity that elicited clear ultra-late LEP in each subject. The stimulus intensity was stable during the experiment in each subject. The subjects felt pressure, touch or a slight burning pain.

Actually, when a painful stimulus is applied to the skin in our daily lives, both Aδ- and C-fibers are activated simultaneously, and we feel both first and second pain or only first pain. However, we do not normally experience a situation where C-fibers are stimulated selectively, and never feel only the second pain after skin stimulation in daily life. Therefore, we may feel a sensation of touch or pressure when only C-fibers are activated as shown in the present study.

5. Microneurographic study of C-fiber discharges induced by CO_2 laser stimulation in humans

5.1 Methods

Five healthy male volunteers participated in the study. Their ages ranged from 28 to 39 (mean±SD: 35±5) years and their heights from 165 to 172 (mean±SD: 169±3) cm. None of the subjects had diseases that might affect normal somesthetic perception. All participants gave informed consent and the research protocol was approved by the Ethics Committee of our Institute.

To activate cutaneous Aδ- and C-fiber nociceptors, a CO_2 laser stimulator and a thin aluminum plate were used (see details in Chapter 4 and Fig. 1A). Laser pulses were delivered with interstimulus interval of 20–40 s on the dorsal foot.

Single C- and Aδ-unit activity was recorded from the peroneal nerve. A tungsten microelectrode (tip diameter, 1 μm; shaft diameter, 100 μm; impedance, 10–12 MΩ) (Frederick Haer & Co, Bowdoinham, ME) was inserted percutaneously into the peroneal nerve in the popliteal area without local anesthesia (Fig. 1B). Neural signal were amplified and filtered (500–5000 Hz) and the responses were displayed on an oscilloscope. Data were collected at a sampling frequency of 10 kHz using an analogue to digital converter recorded on digital audio tapes (DAT) with a DAT recorder (PC216Ax; Sony Precision Technology, Tokyo, Japan). Data were analyzed off-line using published software (Spike 2; Cambridge Electronic Design Limited, Cambridge, UK). Mechanical search stimuli were applied to the dorsum of the foot. When a single unit was found, its CV was measured by transcutaneous electrical stimulation (0.25 Hz, 120–150 V) within the receptive field to classify the recorded fibers, namely, Aβ-, Aδ-and C-fibers. When Aδ-or C-fiber unit was found, we stimulated the receptive fields with CO_2 laser pulses and recorded nerve discharges. We analyzed the present results based on previous microneurographic study that CV of C- and Aδ-fibers was 0.4–1.8 and 4–30 m/s, respectively (Vallbo et al., 1979).

Evoked EEG was recorded simultaneously. Exploring electrodes were placed at the Cz, C_3 and C_4 (according to the international 10–20 system) and were referenced to linked earlobes (A_1+A_2). Impedance was maintained below 5 KΩ. Amplifier frequency response ranged from 0.1 to 50 Hz with 10

μV/cm sensitivity. The analysis time was 2 s and the sampling rate was 512 Hz. The room temperature was 25°C and sound and light were regulated. Skin temperature was kept above 30°C.

5.2 Results

Five individual afferent C-fibers by electrical stimulation were identified in four subjects (Fig. 2, Table 1). The mean CV was 1.1±0.3 m/s. All these five C-units were also activated by CO_2 laser pulses. The latency difference between electrical and laser stimulations ranged from 25 to 88 ms with a mean of 56.6±44.6 ms. As for EEG responses, the main positive deflection peaked at 1248–1360 ms.

CV of the two afferent Aδ-fibers observed by electrical stimulation was 11.7 and 14.1 m/s, respectively. However, CO_2 laser pulses through the thin aluminum plate could not produce spikes in these two fibers (Fig. 3). This finding indicated that our C-fiber stimulation method with a plate selectively activated C-fibers.

The CV of C-fibers measured in the present study was 1.1±0.3 m/s, which was similar to the CV reported in earlier microneurographic studies (0.4–1.8 m/s) (Vallbo et al., 1979) and CV estimated by averaged EEG (0.8–2.6 m/s) (Opsommer et al., 1999b; Tran et al., 2001). The CV of Aδ-fibers in the present study, 11.7 and 14.1 m/s, were similar to the CVs measured by microneurography: 4–30 (Vallbo et al., 1979) and 19.2±9.4 m/s (Adriaensen et al., 1983). The CV was also similar to the values estimated on averaged EEG studies (Kakigi et al., 1991a, 2000). In the present study, all C-units were activated by CO_2 pulses to a tiny skin area while Aδ-fibers were activated only by conventional laser stimulation, indicating that our method using a special spatial filter could selectively stimulate C-fiber sensory terminals. Results of simultaneously recorded EEG responses also supported this view. CO_2 laser pulses through an aluminum plate evoked EEG response components (1327 ms). Since the signals of C-fibers ascend through the spinothalamic tract with a CV of 2.2 m/s (Qiu et al., 2001), a gross calculation indicates that it takes about 1318 ms to reach the cortex from the foot (115 cm/1.1 m/s plus 60 cm/2.2 m/s), which is compatible with the present EEG findings.

5.3 Discussion

Since laser beams activate skin receptors via temperature conduction, there is a latency difference between the onset of laser stimulation and actual activation of receptors. The latency difference between electrical and laser stimulation-evoked spikes of C-fibers in the present study was 56.6±44.6 ms. With similar methods, the value was reported to be 50 ms in monkeys (Campbell and Lamotte, 1983) and 37 ms in humans (Bromm and Treede, 1984). Such a period is necessary to activate C-fibers by CO_2 laser stimulation after discharge.

In conclusion, the present microneurographic study provided direct evidence that C afferent fibers are selectively activated by CO_2 laser stimulation of a tiny area of skin. In addition, the present study showed that the latency of EEG responses well matched the presence of nerve fiber discharges in C-fibers.

6. Conduction velocity of the spinothalamic tract in humans as assessed by CO_2 laser stimulation of C-fibers

6.1 Methods

Eleven healthy male volunteers participated in the study. Their ages ranged from 27 to 43 (mean±SD: 33.3±4.6) years and their heights from 165 to 180 (mean±SD: 171.4±4.4) cm. None of the subjects suffered from diseases that might affect normal somesthetic perception. All participants gave their informed consent and the research was approved by the Ethical Committee at our Institute.

We used two different types of stimulation: (1) non-painful CO_2 laser stimulation of the skin overlying the C7, T4, T8, and T12 vertebral spinous processes to elicit ultra-late LEP (see details in Chapter 4, Fig. 1A and Fig. 4); and (2) conventional painful CO_2 laser stimulation of the C7 and T12 to elicit late LEP. Both were applied to each subject.

Subjects lay prone and were asked to relax their muscles and stay awake. The room temperature was 25°C and sound and light were regulated. Skin temperature was kept above 30°C. Exploring electrodes were placed at the Cz, C3 and C4 (according to the international 10–20 system) and were referenced to linked earlobes (A1+A2). Impedance was maintained below 5 KΩ. Amplifier frequency response was from 0.1 to 50 Hz with 10 μV/cm sensitivity. The analysis time was 1 s and the sampling rate was 512 Hz.

We delivered laser pulses to the skin overlying the C7, T4, T8, and T12 vertebral spinous processes for recording ultra-late LEP. We delivered each pulse at a slightly different site, within a transverse 2×1 cm area centered over the spinous process. To avoid habituation, sensitization and tissue damage, we used random interstimulus intervals (3–10 s), and the stimuli were delivered in a random sequence to different dorsal skin sites. For recording late LEP, to reduce the experimental time and the subjects' fatigue and discomfort (it was very painful), we stimulated only C7 and T12.

For each site of stimulation, two series of 20 artifact-free trials were selected and averaged off-line. We measured the peak latency of the main positive (P) waves. To estimate the CV in the STT, we used different methods for the ultra-late LEP and late LEP, since four sites were stimulated for ultra-late LEP but only two sites for late LEP. For measuring the CV of

C-fibers (ultra-late LEP), we made calculations based on the regression line between peak latencies of ultra-late LEP following the stimulation of four different sites and the distance from C7 to each stimulus site. For measuring the CV of Aδ-fibers (late LEP), we measured the differences of peak latency following stimulation of T12 and C7 and the distance between two stimulus sites (T12 and C7).

6.2 Results

Both ultra-late LEP and late LEP following each stimulation were clearly recorded in all subjects. P-wave latencies gradually and significantly increased from C7 to T12 (Fig. 5). The mean, standard deviation (SD), range of the peak latencies of the P-wave, and calculated CV are shown in Table 2.

On measuring the CV of the C-fibers, a regression line calculated from the four P-wave latencies following stimulation of the four sites along the spine indicated a significant linear relationship between distance and time (Fig. 6). The resulting CV (reciprocal of the slope) was 2.2±0.6 m/s. For measuring the CV of the Aδ-fibers, we divided the distance between C7 and T12 by the latency difference of the P-waves at the two sites, to obtain 10.0±4.5 m/s, a significantly higher value than that for C-fibers ($P<0.0001$, paired t-test).

6.3 Discussion

This is the first systematic attempt to measure the CV in STT related to C-fibers by direct stimulation of the skin overlying the spinous processes. Since clear components were recorded following each stimulation in all subjects, this method is probably applicable not only to research but also to clinical situations.

The CV of C-fibers in the STT measured in the present study was 2.2±0.6 m/s. Tran et al. (2002a) also calculated the CV related to C-fibers, by measuring ultra-late LEP following stimulation of the upper and lower limbs, and obtained a value of 2.9±0.8 m/s. The difference in CV between these two studies was probably due to a difference of methods and subjects, although it was small, only 0.7 m/s. The method used in the present study is more direct and may be able to detect the level in the spinal cord that the lesions occur. In contrast, the method reported by Tran et al. (2002a) is simpler and easier

for recording and the subjects do not have to undress. Our method was based on Cruccu et al. (2000), whereas they measured the CV related to Aδ-fibers. Very recently, they reported similar results (Iannetti et al., 2003), and the value for the CV of C-fibers, about 2.5 m/s, was very similar to ours.

The CV of Aδ-fibers in the STT, 10.0±4.5 m/s, was very similar to that in previous reports (Kakigi and Shibasaki, 1991b; Rossi et al., 2000). The present findings that the CV in the STT has two different values depending on the ascending fibers of the peripheral nerve indicated at least two different pathways in the STT. This is compatible with animal studies. In cats, most axons in the STT were unmyelinated in the dorsal funiculi (Chung et al., 1985). Therefore, we speculate that the signals ascending through C-fibers are conveyed by unmyelinated axons in the STT.

A fundamental question is why late LEP were recorded but ultra-late LEPs were not following painful CO_2 laser stimulation. Previous papers (Bjerring and Arendt-Nielsen, 1988; Price et al., 1997) reported the inhibition of C-fiber inputs by Aδ-fiber afferents at the spinal level as proposed by the gate control theory, based on quantitative or qualitative aspects of perception.

In conclusion, we recorded clear ultra-late LEP following stimulation of C-fibers using a novel method, and then measured the CV of C-fibers in the STT. Since ultra-late LEP could be easily recorded, this technique should be useful as a diagnostic tool for assessing the level of spinal cord lesions, and provide reliable diagnostic information on patients with myelopathy, particularly when combined with conventional somatosensory evoked potentials (SEP) following electrical stimulation and with late LEP following painful laser stimulation to elucidate the physiological functions of the dorsal column and STT.

7. Effects of attention, distraction and sleep on CO_2 laser evoked potentials related to C-fibers in humans

7.1 Methods

Ten healthy volunteers participated in this study (8 males and two females). Their ages ranged from 26 to 49 (mean±SD: 37.7±6.2) years and height from 160 to 180 cm (mean±SD: 170.4±6.8). All 10 subjects were colleagues in our department and medical doctors. Therefore, they understood the aim of this study and declared that they were free from pain and sleep disturbance. All participants gave their informed consent and this research was approved by the Ethical Committee at our Institute.

For recording ultra-late LEP, we used a CO_2 laser with a thin aluminum plate (see details in Chapter 4 and Fig. 1A). We delivered laser pulses to the dorsum of the right hand. Subjects were seated in a comfortable reclined chair and asked to relax their muscles. The room temperature was 25°C and sound and light were regulated. Skin temperature was kept above 30°C. Exploring electrodes were placed at the Cz, C3, C4 and Oz (according to the international 10-20 system) and were referenced to linked earlobes (A1+A2). The Oz electrode was used to determine different states of arousal in Experiment 2 of this study. Impedance was maintained below 5 kohm. The amplifier frequency response was from 0.1 to 50 Hz with a sensitivity of 10 μV/cm. The analysis time was 2 s and the sampling rate was 512 Hz. Electrooculography was simultaneously performed for artifact rejection.

7.1.1 Experiment 1 (effects of attention and distraction on ultra-late LEP)

In the attentive condition, subjects were instructed to mentally count the number of laser pulses paying close attention to the stimuli. In the distractive condition, the subjects were asked to calculate. In front of the subjects, a piece of paper with 25 random two-digit numbers (5×5 lines) was presented at a distance of 1.5 m. The paper was replaced with a different set of numbers after each session. The calculation task was to add the 5 numbers on each line as many times as possible. While the laser pulse was delivered, the subjects had to try their best to calculate mentally. The subjects were then asked to provide the answer to each line just after

finishing the addition, and the experimenter checked the answer. The accuracy of the response was recorded and the test accepted only when at least 60% of the responses were accurate. Since only one trial was performed for each subject, we did not take into account the training effect. We tentatively used 60% as the standard to confirm whether a subject paid close attention to the calculation task. Actually, all the subjects achieved this. In the control condition, a paper with a small black point was presented instead of the paper with numbers, and the subject was asked to look at the point without paying close attention to it. Recordings of ultra-late LEP under different conditions were made randomly, and the stimulus intensity was kept constant.

7.1.2 Experiment 2 (effects of sleep on ultra-late LEP)

Ultra-late LEP were obtained from each subject while awake, drowsy and in stage 2 sleep. The stimulus conditions were the same as in Experiment 1. Sleep stage was decided by using criteria proposed by Rechtschaffen and Kales (1968). The subjects fell asleep in the reclining arm-chair.

The experiment was performed either in the morning or afternoon, depending on the subject, but mainly in the afternoon after lunch, when sleep comes easily. Most subjects could fall asleep naturally, but some had had slightly less sleep the night before the experiment.

Experiment 1 was performed first, followed by Experiment 2, in all subjects. During Experiment 1, we changed the order of each condition (control, distraction and attention) at random in each subject. We did not change the stimulus intensity through Experiment 1 or Experiment 2. It took approximately 30–40 min to complete Experiment 1, but the time taken for Experiment 2 varied depending on how long it took for each subject to fall asleep.

Twenty-five stimulus trials were averaged for each recording. We recorded at least twice for each condition (control, attention, distraction, drowsiness and sleep) to confirm the reproducibility. Therefore, at least 10 recordings were made for each subject. We performed Experiments 1 and 2 only once for each subject, since results were clear and simple, and were very consistent in all subjects.

After confirming the reproducibility of waveforms among different conditions (control, attention, distraction, drowsiness and stage 2 sleep) for each subject, two series of 25 artifact-free trials were selected and averaged off-line. The peak amplitudes were measured from baseline. Statistical analysis was performed using an analysis of variance (ANOVA) followed by the Bonferroni–Dunn test (Experiment 1) or using the paired *t* test (Experiment 2). In addition, the amplitude in each condition was standardized with the control set at 100%. Wilcoxon's test was used for comparison of the normalized data.

7.2 Results

The ultra-late LEP, small negative component (N1) and a large positive component (P1), were recorded. We placed 3 electrodes at C3, C4 and Cz. Since the peak amplitude of N1–P1 was largest at the Cz in all subjects in the control condition, we mainly analyzed the results recorded there. We found no consistent new additional component at the C3 or C4 electrode. Since N1 was recorded in only 5 subjects in the control condition, we statistically analyzed the amplitude and latency of only P1 in this study (Table 3).

7.2.1 Experiment 1 (control, attention and distraction conditions)

Results of ANOVA indicated a significant relationship between the P1 amplitude and attention level (P=0.0011). The P1 amplitude was slightly increased during the attention task (P=0.19) but significantly decreased during the distraction task (P=0.0068), relative to the control by post-hoc comparison. The P1 amplitude was significantly larger during the attention task than the distraction task (P=0.0003) (Fig. 7and Fig. 8). In contrast, the attention level was not a significant factor for the P1 latency by ANOVA.

7.2. 2 Experiment 2 (effect of drowsiness and sleep)

The P1 amplitude was very less in 5 subjects and was low or absent in the other 5 subjects during drowsiness. The mean amplitude decrease during drowsiness was significantly relative to the control condition ((P=0.0044,

n=5) when analyzing 5 subjects who showed P, and (P=0.0001, n=10) when analyzing 10 subjects (Fig. 9 and Fig. 10). Conversely, the change in peak latency did not reach a level of significance. In all subjects, ultra-late LEP were completely abolished during stage 2 sleep (Fig. 9). Even in a visual inspection of a single trial average, none of the subjects showed detectable ultra-late LEP.

We also analyzed the amplitude difference after normalization to control data (100%) by using Wilcoxon's test. The P1 amplitude was slightly increased (131%) during the attention task, but it was not significant (P=0.20). The P1 amplitude was significantly decreased (43%) during the distraction task (P=0.008). The P1 amplitude decrease during drowsiness was significantly relative to the control condition (58%, P=0.0431) when analyzing 5 subjects who showed P1, and (39%, P=0.0051) when analyzing all 10 subjects.

7.3 Discussion

A small negative component (N1) and a large consistent component (P1), which was largest at the Cz electrode, was identified in this study. We found no consistent additional new component at the C3 or C4 electrode. Opsommer et al. (2001) analyzed the scalp topography of ultra-late LEP by placing many electrodes on the scalp, but did not find new components except for those corresponding to our N1 and P1.

A clear P1 component of ultra-late LEP was found during the control session in all subjects, but was significantly decreased in amplitude during the distraction task and much reduced or absent during sleep. In addition, since it was slightly but clearly enhanced during the attention task, the P1 component should include cognitive function. Opsommer (1999a) examined changes of ultra-late LEP using the oddball paradigm, and found that the amplitude was enhanced when the subject's attention was given to the stimulus. Our study supported their finding, and this is the first systematic study to clarify this important characteristic of ultra-late LEP relating to the level of arousal.

Most previous reports, which focused on the effects of attention and distraction, analyzed late LEP relating to Aδ-fibers (first pain), and found that the late LEP were changed to a large extent by the effect of

attention/distraction (Beydoun et al., 1993; Towell and Boyd, 1993; Siedenberg and Treede, 1996; Kanda et al., 1996; Zaslansky et al., 1996; Garcia-Larrea et al., 1997; Yamasaki et al., 1999). In particular, Beydoun et al. (1993) clarified that the late LEP was much reduced in amplitude by distraction and almost disappeared during sleep. The present findings were fundamentally very similar to their results, although we studied ultra-late LEP relating to C-fibers.

We have to speculate as to the regions responsible for such large effects of a change of attention on ultra-late LEP. Opsommer et al. (2001) recorded the scalp topography of ultra-late LEP by placing many electrodes on the scalp, and reported that the secondary somatosensory cortex (SII) and the cingulate cortex of the bilateral hemispheres were generators for the ultra-late LEP. Therefore, the bilateral SII and cingulate cortex seem to play an important role in the change of ultra-late LEP induced by a change in the level of attention and sleep. With regard to the studies of positron emission tomography (PET) (Andersson et al., 1997; Iadarola et al., 1998) and functional magnetic resonance imaging (fMRI) (Baron et al., 1999; Malisza and Docherty, 2001) relating to C-fiber functions (second pain) using capsaicin, blood flow was increased in the cingulate cortex, insula and SII.

The state of arousal had a significant effect on the amplitude of ultra-late LEP. The responses were significantly reduced in 5 subjects and were absent or flat in 5 subjects during drowsiness compared to the state of wakefulness (control). Indeed, the responses were undetectable during stage 2 sleep. This result demonstrates that the responsiveness to C-fiber activation is modulated during drowsiness and especially during sleep. A similar reduction in amplitude during sleep has been reported in the late LEP (Beydoun et al., 1993) and pain-related SEP following painful electrical stimulation (Naka and Kakigi, 1998), both of which were related to Aδ-fibers. Although the ascending fibers differed between those studies and ours, the basic mechanisms are considered to be similar. As discussed for the effects of attention/distraction, neuronal activities following C-fiber stimulation in several probable generators such as the SII and cingulate cortex were reduced to a large extent or absent during sleep.

In conclusion, we confirmed that the brain responses relating to signals ascending through C-fibers were much affected by the level of consciousness, being consistent with the findings of late LEP relating to Aδ-fibers. This is

the first study to indicate the important characteristics of ultra-late LEP relating to consciousness, suggesting that they include cognitive function. Finally, we would like to note the following for clinical application. When applying this new method in a clinical setting, one has to be very careful of the change in the patients' level of alertness and, if possible, ask the patients to pay close attention to the stimuli, to record clear and consistent components.

8. Effects of distraction on magnetoencephalographic responses ascending through C-fibers in humans

8.1 Methods

Seventeen healthy male volunteers participated in this study. They ranged in age from 25 to 43 (mean±SD: 33.4±4.9) years, and in height from 165 to 180 (mean±SD: 170±4.7) cm. All participants gave their informed consent and this research was approved by the Ethical Committee at our Institute. None of the subjects suffered from diseases that might affect normal somatosensory and pain perception. All subjects were staff in our department and were well trained in MEG.

For recording ultra-late LEF and LEP, we used a CO_2 laser with a thin aluminum plate (see details in Chapter 4 and Fig. 1A). To avoid magnetic noise caused by the stimulator, the stimulator was located outside a shielded room, and the laser beam was carried through specially developed optical fibers approximately 3.5 m in length, which penetrated the wall of the shielded room. The laser power was attenuated to approximately 40% of the original power by passage through the fibers. We used the smallest intensity that generated a clear ultra-late LEP in each subject in our previous study (Tran et al., 2001, 2002a,b; Qiu et al., 2001, 2002, 2003; Kakigi et al., 2003). Stimulation was given between the first and second metacarpal bones of the dorsum of the left hand. The room temperature was kept at approximately 22–25 °C, and sound and light were regulated. The subjects were calm, vigilant, attentive and relaxed, with eyes opened. Their eyes were protected from the CO_2 laser beam by goggles. In our previous studies, we found that 10 trials were sufficient to elicit clear LEP responses (Tran et al., 2001, 2002; Qiu et al., 2001, 2002). More than 10 trials would attenuate the responses due to habituation. Thus, 10 trials were averaged in each session and 8 artifact-free sessions were grand averaged. Reproducibility was confirmed from these different sessions.

8.1.1 Experimental paradigm

Ultra-late LEF and ultra-late LEP were recorded under two different sets

of conditions. (i) Control in which the subjects were instructed to mentally count the number of laser pulses. (ii) Distraction in which the subjects were asked to calculate (see Chapter 7.1.1).

We also asked subjects to rate the subjective stimulus strength during Distraction between 0 and 10, if that in Control was 10. If the subject felt the stimulus was the same strength during Distraction as that in Control, the rate was 10, if they felt it to be about half the strength, it was 5, and if they did not feel it at all, it was 0.

8.1.2 MEG recording

The ultra-late LEF was measured with a dual 37-channel biomagnetometer (Magnes, Biomagnetic Technologies Inc., San Diego, CA). The detection coils of the biomagnetometer were arranged in a uniformly distributed array in concentric circles over a spherically concave surface. The device was 144 mm in the diameter and 122 mm in the radius of its curvature. The outer coils were 72.5° apart. Each coil was 20 mm in diameter, and the centers of the coils were 22 mm apart. Each coil was connected to a superconducting quantum interference device (SQUID). When the left and right hemispheres were recorded simultaneously, the two probes were centered at C3 and C4 (International 10–20 system) in each subject. The C3 and C4 positions covered the primary and secondary somatosensory cortices, SI and SII, of the left and right hemisphere, respectively. The magnetic fields were recorded with a 0.1–50 Hz bandpass filter, and digitized at a sampling rate of 1041.7 Hz. The analysis window was 2 s after the stimuli, and a pre-stimulus period of 300 ms was used as the DC baseline. The bandpass filter was further set at 0.1–30 Hz at the analyzing step. There were a total of 10 sessions in each condition, and one session included 10 trials. Thus, 10 trials were averaged in each session and 8 artifact-free sessions were grand-averaged. Therefore, 80 trials in total were averaged for each condition, which, based on our experience was enough to record a clear LEP/LEF. Each session (10 trials) took 2.5 min including a 1 min interval between sessions. So, the entire experiment lasted for about 50 min. The subject's head was fixed to the lower device by tape to avoid movement. The location of the head was checked after the experiment, and did not show significant change.

8.1.2 EEG recordings

The ultra-late LEP was also recorded simultaneously with the ultra-late LEF to know the difference between them. The placement of electrodes was based on the International 10–20 system. Three exploring electrodes were placed at Cz (vertex), C3 (around the hand area of SI in the left hemisphere), and C4 (around the hand area of SI in the right hemisphere) referred to linked earlobes. Impedance was maintained below 5 kΩ. The ground electrode was placed on the forehead. The same filters, sampling rate, and analysis window were used as for the ultra-late LEF recordings. The potentials were recorded with a sensitivity of 10 μV/cm. The latency of each component was measured at the peak, and amplitudes were measured from the baseline. Electrooculography (EOG) was also simultaneously recorded for monitoring eye movements, and trials contaminated with large artifacts caused by eye movements were not averaged.

8.1.3 Multi-dipole source analysis

We used the brain electric source analysis (BESA) software package made by Scherg (BESA 2000, NeuroScan Inc., McLean, VA) for the computation of theoretical source generators in a 3-layer spherical head model, since at least two sources in primary and secondary somatosensory cortices (SI and SII) were activated simultaneously following laser stimulation (Tran et al., 2002b). The BESA was modified for the use of our 2×37-channel magnetometers, one each placed on the two hemispheres. This method allows the spatio-temporal modeling of multiple simultaneous sources over defined intervals. Before starting the modeling, a principal component analysis (PCA) was applied to identify how many principal components explain the significant variance. The number of components sufficient to explain more than 95% of the power was determined as the number of dipoles. The location and orientation of the dipoles were calculated by an iterative least-squares fit. The residual variance (%RV) indicated the percentage of data, which could not be explained by the model. The goodness of fit (GOF) was expressed as (100−%RV). A GOF larger than 90% is considered to be indicative of a good multiple dipole model.

The differences in latency between components of the contralateral and

ipsilateral hemispheres, between components of MEG recordings and EEG recordings, and between dipoles in BESA analyses were analyzed using the two-tailed Wilcoxon paired-sample test, with P values less than 0.05 considered to be significant in all the analyses.

8.1.4 MRI overlaying

Magnetic resonance imaging (MRI) scans (Shimadzu Magnes 150 XT 1.5 T, Kyoto, Japan) were obtained for all subjects. T1-weighted coronal, axial and sagittal images with continuous slices 1.5 mm in thickness were used for overlays with ECD sources detected by MEG. The same anatomical landmarks used to create the MEG head-based three-dimensional (3-D) coordinate system (the nasion and bilateral preauricular points) were visualized in the MRI images by affixing to these points high-contrast cod liver oil capsules (3 mm in diameter), whose short relaxation time provides a high-intensity signal in T1-weighted images. The common MEG and MRI anatomical landmarks allowed easy transformation of the head-based 3-D coordinate system used for the MEG source analysis in MRI. The head coordinate system in BESA is almost the same, rotated by 90°, as the BTi system, and therefore can be used to overlay individual MRI scans with a minor adjustment.

8.2 Results

8.2.1 Subjective rate of stimulus strength during Distraction

All subjects felt that stimulus strength was much reduced during Distraction, and the mean rate was 5.1±1.6. Since each subject had a slightly different response to our C-fiber activation in the Control, that is, some felt a touch, some a pressure, and others a slight burning pain, it is not easy to evaluate this result, but subjects who felt a slight burning pain in the Control declared that the feeling was abolished or much reduced during Distraction.

8.2.2 Effects of Distraction on ultra-late LEF

In 4 out of 17 subjects, we could not clearly identify the ultra-late LEF component due to artifacts or a small signal-to-noise ratio. Therefore, we only analyzed results obtained from the other 13 subjects.

The laser stimulus was applied to the left hand, so we used the terms 'contralateral hemisphere' and 'ipsilateral hemisphere' for the right and left hemisphere, respectively. In the Control condition, the first component (1M) and second component (2M) were clearly identified in both hemispheres (Fig. 11). Peak latencies of 1M and 2M were from 590 to 834 ms (mean±SD: 744±72 ms) and from 799 to 1031 ms (926±85 ms), respectively in the contralateral hemisphere, and from 606 to 851 ms (765±77 ms) and from 818 to 1103 ms (mean±SD: 958±96 ms), respectively, in the ipsilateral hemisphere. The 1M and 2M recorded from the ipsilateral hemisphere was significantly longer in latency in all the subjects, and the inter-hemispheric difference in latency ranged from 16 to 43 ms (21±26 ms) (P=0.01) and from 19 to 94 ms (32±26 ms) (P=0.002) (Fig. 11 and Fig. 12). As for the amplitude, root mean square (RMS), which is a measure of the magnetic signal strength of the data collected from 37 channels, was calculated. The RMS of 1M and 2M was from 32 to 130 fT (71±30 fT) and from 25 to 82 fT (54±22 fT), respectively, in the contralateral hemisphere, and from 26 to 115 fT (65±29 fT) and from 25 to 87 fT (51±24 fT), respectively, in the ipsilateral hemisphere (Fig. 11 and Fig. 12). There were no significant inter-hemispheric differences in RMS.

During Distraction, the 1M and 2M were also identifiable in all 13 subjects (Fig. 11). The latency of 1M and 2M did not show a significant change from the Control in either hemisphere (Fig. 11 and Fig. 12). The RMS of 1M and 2M was significantly smaller than in the Control, P=0.01 for 1M and P=0.005 for 2M, in the contralateral hemisphere (Fig. 11 and Fig. 12). In the ipsilateral hemisphere, the RMS of 1M and 2M was significantly smaller than the Control value, P=0.005 for 1M and P=0.002 for 2M (Fig. 11 and Fig. 12).

8.2.3 Effects of Distraction on ultra-late LEP

During Control recordings, a small negative (N1) and a major positive component (P1) were recorded in the ultra-late LEP. P1 was recorded in all 13 subjects, but N1 was not clearly identified in two subjects. Since both N1

and P1 were consistent and maximal at the Cz (vertex) electrode, we analyzed those recorded at Cz (Fig. 11). The mean peak latency of the N1 and P1 components was 770±89 and 931±78 ms, respectively, while the amplitude was 7.4±5.6 and 13.1±7.4 μV, respectively. Comparison between LEF and LEP revealed that the peak latency of N1 was always and significantly longer than that of 1M recorded from the contralateral hemisphere, 26 ms on average (P=0.0022, n=11) (Fig. 11 and Fig. 12). The peak latency of P1 was longer than that of 2M recorded from the contralateral hemisphere, by 5 ms on average, but the difference was not significant (Fig. 11 and Fig. 12).

During Distraction, the amplitude of both N1 and P1 was significantly decreased compared to the Control, P=0.037 (n=11) for N1 and P=0.0029 (n=13) for P1. The latencies of both N1 and P1 did not show a significant change from the Control (Fig. 11 and Fig. 12).

8.2.4 Results using BESA

At first, we compared BESA results between a 3-dipole model of SI and bilateral SII sources and a two-dipole model with only SII sources during the 1M period, approximately 100 ms before and after the peak of 1M, since our previous study (Tran et al., 2002b) showed that these 3 sources during this period well explain the recorded data. We found that the 3-dipole model could explain 91.8% of the data whereas the two-dipole model only 68.4%. We considered that this result suggested a significant contribution of the SI source. In the next step, we expanded the analysis window to a period lasting from the onset of 1M to the offset of 2M, approximately 450 ms. The GOF value with the 3-dipole model was not reliably high, 64.7±7.2%, suggesting that some other sources had to be necessary in order to explain the mechanisms generating the 2M component. In previous studies on pain perception for the signals ascending through Aδ-fibers using MEG (Inui et al., 2003; Kitamura et al., 1995, 1997; Watanabe et al., 1998) and EEG (Valeriani et al., 1996, 2000), and also an EEG study for the signals ascending through C-fibers (Opsommer et al., 2001), as well as neuroimaging studies using positron emission tomography (PET) and fMRI for signals ascending through C-fibers using capsaicin (Andersson et al., 1997; Iadarola et al., 1998; Baron1999; Malisza and Docherty, 2001), 3 regions were candidates for

additional sources, the cingulate cortex, the anterior site of the medial temporal area (MT) and the insula.

Therefore, we added sources in the cingulate cortex and MT to the 3-dipole model, namely, we made a 6-source model by locating Source 1 in SI of the contralateral hemisphere, Source 2 and 3 in SII-insula of bilateral hemispheres, Source 4 in the cingulate cortex and Source 5 and 6 in MT of bilateral hemispheres (Fig. 13; Table 4 and Table 5). The GOF value for this 6-dipole model in the Control was much increased as compared with that for the 3-source model, from 64.7±7.2% to 92.9±1.1% (Table 5). We tried to make other models by adding or changing sources, but this 6-dipole model was consistently most appropriate in all subjects. The waveform of each source and their locations on MRI scans, are shown for one representative subject in Fig. 13. The mean location of each source is shown in Fig. 14. The current strength of each source appeared to overlap temporally, but activities in SI and SII appeared to be the main factors generating the 1M component. In contrast, activities in the cingulate cortex and MT appeared to be the main contributors to the generation of the 2M component. SII activities were still present for generating 2M, but SI activity was much diminished for the 2M period. This tendency was consistent in all subjects. To evaluate the contribution of each source to the overall GOF value, we examined the change in GOF values on adding each source in each subject (Table 5).

It is known that the cingulate cortex is activated in both hemispheres, even when only one side of the body is stimulated (Hutchison et al., 1999). However, the cingulate cortices of the two hemispheres are located in a deep area and very close to one another, so it is difficult to separate them clearly using BESA. Therefore, we placed one source there. Garcia-Larrea, (1998) Bentley et al.(2001) and Inui et al. (2003), following stimulation of Aδ-fibers, placed a source in the insula in addition to the SII source. Therefore, it is also highly possible for the insula to be activated following C-fiber stimulation. However, we could not successfully separate activities from SII and insula in some subjects, probably because the activated regions in insula and SII were located very close to each other, particularly in the Distraction task, since their activities were much reduced. Therefore, we placed one source there as SII-insula, in this study.

Then, we analyzed the change of each source during Distraction. The current strength of SI and SII was significantly smaller in the Distraction

than Control condition, $P<0.05$ for SI and $P<0.01$ for SII-insula (Fig. 13 and Fig. 15). The current strength of the cingulate cortex and MT was significantly smaller in Control than in Distraction, $P<0.001$ for the cingulate and $P<0.05$ for the MT (Fig. 13 and Fig. 15). The SII-insula and cingulate were more effectively attenuated than SI and MT. Since the inter-individual difference for MT activities was large (see the large standard deviation in Fig. 15), the level of statistical significance was not as high as in the cingulate cortex, though the amplitude reduction of MT in some subjects was very large. The peak latency of each source did not show a significant change between the two conditions.

In the ipsilateral hemisphere, activity in SI was absent. This finding was consistent with a previous report (Tran et al., 2002b), and finally, source 3 in SII-insula and Source 6 in MT were considered the most appropriate (Fig. 13, Table 5). We also tried to make other models by adding or changing sources, but this 3-dipole model was consistently most appropriate in all subjects. The current strength of SII-insula and MT was significantly smaller in Distraction than Control, $P<0.01$ for SII-insula and $P<0.05$ for MT (Fig. 13 and Fig. 15). The peak latency of each source did not show a significant change between the two conditions.

Concerning the inter-hemispheric differences, peak latencies of the SII and MT sources recorded from the contralateral hemisphere in the Control were significantly shorter than those recoded in the ipsilateral hemisphere ($P<0.05$ for both SII-insula and MT) (Fig. 15).

Finally, we calculated the correlation coefficient (r) for the change in subjective stimulus strength and change of RMS or current strength of each component. The two parameters showed a positive correlation, since both the subjective rate and component amplitude were reduced in all subjects, but r^2 values were not significant, 0.32 between subjective strength and RMS and 0.59 between subjective strength and current strength, probably because the subjective rate showed a large inter-individual difference.

8.3 Discussion

The 1M latency was the shortest, then N1 appeared with a slightly but significantly longer latency than 1M, and the latencies of 2M and P1 were almost the same. Therefore, we can speculate that 1M reflects the primary

activities generated in SI and SII-insula in the contralateral hemisphere and only SII-insula in the ipsilateral hemisphere, and both 2M and P1 reflect the secondary activities generated in the cingulate cortex, MT and SII-insula (probably mainly insula).

Most important findings in this study is that we found activated regions following the stimulation of C-fibers and clarified their changes during the Distraction condition. Only one study has reported ultra-late LEF following C-fiber stimulation using the same stimulus methods as the present study (Tran et al., 2002b). They focused on the primary activities corresponding to our 1M component, and found that SI and SII were simultaneously activated in the contralateral hemisphere, and only SII was activated in the ipsilateral hemisphere. These findings were compatible with our results, and we added activities in the cingulate cortex, the MT, and probably the insula as mainly later activities in the present study.

Activities in the cingulate cortex and/or SII-insula following C-fiber stimulation have been found in almost all electrophysiological (Opsommer et al., 2001; Tran et al., 2002b; Valeriani et al., 2002) and neuroimaging studies (Andersson et al., 1997; Baron et al., 1999; Iadarola et al., 1998; Malisza and Docherty, 2001). Activities in the cingulate cortex and/or SII-insula following Aδ-fiber stimulation were also reported in previous MEG studies (Hari et al., 1983, 1997; Huttunen et al., 1986; Kakigi et al., 1995, 1996; Kitamura et al., 1995, 1997; Bromm et al., 1995; Watanabe et al., 1998; Yamasaki et al., 1999, 2000; Arendt-Nielsen et al., 1999; Ploner et al., 1999, 2000; Kanda et al., 2000; Hoshiyama and Kakigi, 2000; Wang et al., 2003; Inui et al., 2003) or intracranial recordings (Lenz et al., 1998; Treede et al., 2000; Frot et al., 2001). These findings clearly indicated that both the SII-insula and cingulate cortex play a major role in pain perception following the stimulation of both Aδ-fibers and C-fibers.

Although several LEP (EEG) and MEG studies have found activation of the medial temporal area around the amygdala nuclei and hippocampal formation following Aδ-fiber stimulation using CO_2 laser beams (Valeriani et al., 1996, 2000; Watanabe et al., 1998) and noxious intra-epidermal stimulation (Inui et al., 2003), only a few imaging studies (Derbyshire et al., 1997; Bornhovd et al., 2002; Schneider et al., 2001) have reported pain-related activation of this area. Regarding C-fiber-related pain, no study has detected activation in this area. With PET (Andersson et al., 1997;

Iadarola et al., 1998) or fMRI (Baron et al., 1999; Malisza and Docherty, 2001) imaging, the blood flow in the medial temporal area was not found to increase in response to C-fiber activation (second pain) using capsaicin. We speculate that the blood flow in this area is not significantly increased by continuous stimulation such as capsaicin injection, or a large inter-individual difference might make its significance level low after grand-averaging results obtained in all subjects. In addition, such a deep source is usually difficult to detect by MEG. However, MT activation was constantly found in this study. We considered that the activation in MT found in this study corresponded to Aδ-fiber-related activation in this area. It is difficult to explain why few imaging studies have detected activation in the MT region unlike MEG/EEG studies, but it may be due to a difference of methods. That is, short-duration or shock pain is used in MEG/EEG studies whereas long-duration or continuous pain is used in imaging studies. Also, neuronal activities are measured in EEG/MEG studies whereas blood flow or metabolic changes are measured in imaging studies.

The recorded activity changed during the Distraction condition. Peak latencies of SII-insula and MT were significantly shorter in the contralateral than ipsilateral hemisphere. This finding seemed to indicate that the signals were transferred from the contralateral to ipsilateral hemisphere through the corpus callosum, although we have no definite evidence to support this hypothesis. The RMS of 2M in the contralateral hemisphere was more affected by the Distraction task than that of 1M (Fig 12). Therefore, the late component, 2M, might be more affected by a change of attention than the early component, 1M, indicating that cognitive processing for pain was mainly performed in the period of 2M.

During the Distraction condition, the current strength of all 6 sources, SI, SII-insula, cingulate cortex and MT, was significantly reduced, but the change in SI was relatively small. Therefore, we speculate that SI is concerned with the cognitive function for pain perception to some degree, but its main role is at the peripheral site where the stimulus is given. The finding that SI in the ipsilateral hemisphere was not activated also supported our hypothesis. Activity was reduced more in the SII-insula and cingulate cortex was more reduced than the SI and MT. This finding indicated that the SII-insula and cingulate cortex might be more important for pain recognition.

Activities in the SII-insula, cingulate cortex, and MT were much reduced in amplitude during Distraction. This finding strongly indicates that such regions in the limbic system are responsible for cognitive function in pain perception. Treede et al. (1999), in their review, suggested that there are two main systems for pain perception, a lateral system with a sensory-discriminative component involving SI and SII, and a medial system with an affective-motivational component involving the insula and cingulate cortex. Our findings were fundamentally consistent with their hypothesis. SII activities were also reduced during Distraction in the present study, and SII activities following somatosensory stimulation of Aβ-fibers stimulation were also much affected by a change of attention (Mima et al., 1998) and during sleep (Kitamura et al., 1996). Therefore, SII may be involved in both lateral and medial systems. The role of MT in pain perception is not established, since neuroimaging .studies did not find blood flow changes there following painful stimulation, and the review by Treede et al. (1999) made no mention of the role. It seems natural that amygdala or hippocampal bodies are activated by noxious stimulation and are affected by a change of attention, but this is still a matter of some controversy.

In conclusion, we confirmed that the cerebral response relating to signals ascending through C-fibers was much affected by the level of consciousness, possibly corresponding to second pain perception. Moreover, since the activities in the SI, SII-insula, cingulate cortex and MT were significantly reduced when the subject was distracted from the stimuli, the level of attention should be strictly controlled when the ultra-late LEP and LEF recording technique is used for clinical purposes.

9. General discussion and conclusion

By using a novel method, the application of a CO_2 laser beam to a tiny area of skin using a very thin aluminum plate with numerous tiny holes as a spatial filter, we could stimulate C-nociceptors selectively and record consistent and clear brain responses using EEG and MEG. The CV of the C fibers of the peripheral nerve and spinal cord, probably STT, is approximately 1–4 m/s. In addition to these studies, a more accurate or direct method for measuring the CV of C-fibers using microneurography indicated that this new method selectively activated C-fiber nociceptors of the skin.

There are three types of C-fibers: (1) polymodal or mechano-heat responsive C-fibers; (2) warm or heat responsive C-fibers; and (3) silent or mechano- and heat-insensitive C-fibers. Second pain is transmitted by (1) (Konietzy et al., 1981; Ochoa and Torebjork, 1989). In normal subjects, CO_2 laser stimulation can activate (1) and (2) (Bromm and Treede, 1984). However, it was found that (2) have a higher heat threshold (48°C) than (1) (40°C) (Weinder et al., 1999). In the present study, we used a CO_2 laser to stimulate a tiny area of skin at weak intensity. Therefore, this stimulation probably activated polymodal C-fibers. The activity level in nociceptors may or may not be associated with pain, depending on whether the stimulus intensity is above of below the pain threshold. However, there is a clear causal relationship between the two (William, 1995). There is probably no difference between cerebral activation of C-fiber responses in the present study and second pain-related cerebral activation.

Following C-fibers stimulation, as for the primary component (1M), in the hemisphere contralateral to the stimulation, two regions in the hand area of the SI and SII-insula were activated. The onset and peak latency of the two sources in SI and SII-insula were not significantly different. In the hemisphere ipsilateral to the stimulation, only one source was estimated in SII-insula, and its peak latency was significantly longer than that of the SII-insula source in the contralateral hemisphere, probably indicating the transmisson through the corpus callosum. Our findings suggest that parallel activation of SI and SII-insula contralateral to the stimulation represents the first step in the cortical processing of C-fiber-related activities. In addition to SI and SII-insula, the cingulate cortex and MT around amygdala

and hippocampus in bilateral hemispheres were also activated for the subsequent component, 2M. All components of EEG and MEG responses were significantly reduced in amplitude during distraction and diminished during sleep, particularly 2M component. These findings indicate that these regions are related to the cognitive aspect of second pain perception, particularly activities in cingulate cortex.

Finally, human studies of cerebral pain mechanisms have concentrated largely on cutaneous pain and have identified several cortical structures important for cutaneous nociception of second pain. Like the cutaneous second pain, C-fibers are involved in the visceral pain. However, the visceral pain has not been clarified especially in humans. It is clear that researches of the cutaneous as well as visceral pain have important clinical significance. Our findings for the cutaneous second pain might stimulate further studies of the visceral pain mechanisms.

10. Acknowledgements

It is a great pleasure for me to express my sincere gratitude to my supervisor, Professor Ryusuke Kakigi for all of his help, science guidance and constant encouragement over the years and for providing a chance of studying in The Graduate University for advanced Studies and National Institute for Physiological Sciences.

I would like to give special thanks to Dr. Koji Inui, Dr. Xiaohong Wang, Dr. Tuan Diep Tran, Dr. Qi Fu and Dr. Satoshi Iwase for invaluable advice for planning experiments, analyzing experimental results and discussion. Without their help, this thesis would not have been possible.

I wish to address my gratitude to Mr. Osamu Nagata and Mr. Yasuyuki Takeshima for their technical help.

My thanks are also extended to all the stuff of the Department of Integrative Physiology, Division of Sensory-Motor Integration, National Institute for Physiological Sciences for their help during my stay in Japan.

11. References

Adriaensen, H., Gybels, J., Handwerker, H.O., and Van Hees, J., Response properties of thin myelinated (A-delta) fibers in human skin nerves. *J Neurophysiol* 1983;49:111–122.

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

Arendt-Nielsen, L., Yamasaki, H., Nielsen, J., Naka, D. and Kakigi, R., Magnetoencephalographic responses to painful impact stimulation. *Brain Res* 1999;839:203–208.

Baron, R., Baron, Y., Disbrow, E. and Roberts, T.P., Brain processing of capsaicin-induced secondary hyperalgesia: a functional MRI study. *Neurology* 1999;53:548-557.

Beydoun, A., Morrow, T.J., Shen, J.F. and Casey, K.L., Variability of laser-evoked potentials: attention, arousal and lateralized differences. *Electroenceph clin Neurophysiol* 1993.;88:173–181.

Bjerring, P. and Arendt-Nielsen,L., Argon laser-induced single cortical responses: a new method to quantify pre-pain and pain perceptions. *J Neurol Neurosurg Psychiatry* 1988;51:43–49.

Bentley, D.E., Youell, P.D., Crossman, A.R. and Jones, A.K., Source localisation of 62-electrode human laser pain evoked potential data using a realistic head model. *Int J Psychophysiol* 2001;41:187–193.

Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C. and Buchel, C., Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain* 2002;125:1326-1336.

Bragard, D., Chen, A.C.N. and Plaghki, L., Direct isolation of ultra-late (C-fibre) evoked brain potentials by CO_2 laser stimulation of tiny cutaneous surface areas in man. *Neurosci Lett* 1996;209:81–84.

Bromm, B. and Lorenz, J., Neurophysiological evaluation of pain. *Electroenceph clin Neurophysiol* 1998;107:227–253.

Bromm, B., Lorenz, J. and Scharein, E., Dipole source analysis of brain activity in the assessment of pain. In: Kimura, J. and Shibasaki, H., Editors, 1995. *Recent advances in clinical neurophysiology*, Elsevier,

Amsterdam, 1995;pp. 328–335.

Bromm, B. and Treede, R.D., Nerve fibre discharges, cerebral potentials and sensations induced by CO_2 laser stimulation. *Hum Neurobiol* 1984;3:33–40.

Campbell, J.N. and LaMotte, R.H., Latency to detection of first pain. *Brain Res* 1983;266:203–208.

Chen, A.C.N., Arendt-Nielsen, L. and Plaghki, L., Laser-evoked potentials in human pain. *Pain Forum* 7 1998a; pp. 174–184.

Chen, A.C.N., Arendt-Nielsen, L. and Plaghki, L., Laser-evoked potentials in human pain. *Pain Forum* 7 1998b; pp. 201–211.

Chen, A.C.N., Arendt-Nielsen, L. and Plaghki, L., Understanding of human pain in the brain through topographic mapping and quantification of laser-evoked potentials. *Pain Forum* 1998c;7:196–200.

Chung,K., Sharma, J. and Coggeshall, R.E., Numbers of myelinated and unmyelinated axons in the dorsal, lateral, and ventral funiculi of the white matter of the S2 segment of cat spinal cord. *J Comp Neurol* 1985;234:117–121.

Cruccu, G., Iannetti, G.D., Agostino, R., Romaniello, A., Truini, A. and Manfredi, M., Conduction velocity of the human spinothalamic tract as assessed by laser evoked potentials. *Neuroreport* 2000;11:3029–3032.

Derbyshire, S.W., Jones, A.K., Gyulai, F., Clark, S., Townsend, D. and Firestone, L.L., Pain processing during 3 levels of noxious stimulation produces differential patterns of central activity. *Pain* 1997;73:431–445.

Frot, M., Garcia-Larrea, L., Guenot, M. and Mauguiere, F., Responses of the supra-sylvian (SII) cortex in humans to painful and innocuous stimuli. A study using intra-cerebral recordings. *Pain* 2001;94:65–73.

Garcia-Larrea, L., Multimodal approaches to laser-evoked potential generators. *Pain Forum* 1998;7:216–220.

Garcia-Larrea, L., Peyron, R., Laurent, B. and Mauguiere, F., Association and dissociation between laser-evoked potentials and pain perception. *NeuroReport* 1997;8:385–389.

Hari, R., Kaukoranta, E., Reinikainen, K., Huopaniemie, T. and Mauno, J., Neuromagnetic localization of cortical activity evoked by painful dental stimulation in man. *Neurosci Lett* 1983;42:77–82.

Hari, R., Portin, K., Kettenmann, B., Jousmaki, V. and Kobal, G., Right-hemisphere preponderance of responses to painful CO_2 stimulation

of the human nasal mucosa. *Pain* 1997;72:145–151.

Hoshiyama, M. and Kakigi, R., After-effect of transcutaneous electrical nerve stimulation (TENS) on pain-related evoked potential and magnetic fields in normal subjects. *Clin Neurophysiol* 2000;111:717–724.

Hutchison, W.D., Davis, K.D., Lozano, A.M., Tasker, R.R. and Dostrovsky, J.O., Pain-related neurons in the human cingulate cortex. *Nat Neurosci* 1999;2:403–405.

Huttunen, J., Kobal, G., Kaukoranta, E. and Hari, R., Cortical responses to painful CO_2 stimulation of nasal mucosa; a magnetoencephalographic study in man. *Electroenceph clin Neurophysiol* 1986;64:347–349.

Iadarola, M.J., Berman, K.F., Zeffiro, T.A., Byas-Smith, M.G., Gracely, R.H., Max, M.B. and Bennett, G.J., Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. *Brain* 1998;121:931–947.

Iannetti, G.D., Truini, A., Romaniello, A., Galeotti, F., Rizzo, C., Manfredi, M. and Cruccu, G., Evidence of a specific spinal pathway for the sense of warmth in humans. *J Neurophysiol* 2003;89:562-570.

Inui, K., Tran, T.D., Qiu, Y., Hoshiyama, M. and Kakigi, R., A Comparative magnetoencephalographic study of cortical activations evoked by noxious and innocuous somatosensory stimulations. *Neuroscience* 2003;120:235–248.

Kakigi, R., Endo, C., Neshige, R., Kuroda, Y. and Shibasaki, H., Estimation of conduction velocity of A delta fibers in humans. *Muscle Nerve* 1991a;14:1193–1196.

Kakigi, R., Koyama, S., Hoshiyama, M., Kitamura, Y., Shimojo, M. and Watanabe, S., Pain-related magnetic fields following painful CO_2 laser stimulation in man. *Neurosci Lett* 1995;192:45–48.

Kakigi, R., Koyama, S., Hoshiyama, M., Kitamura, Y., Shimojo, M. and Watanabe, S., Pain-related brain responses following CO_2 laser stimulation: magnetoencephalographic studies. *Electroenceph clin Neurophysiol Suppl* 1996;47:111–120.

Kakigi, R., and Shibasaki, H., Estimation of conduction velocity of the spinothalamic tract in man. *Electroenceph clin Neurophysiol* 1991b;80:39–45.

Kakigi,R., Tran,T.D., Qiu,Y., Wang,X., Nguyen,T.B., Inui,K., Watanabe, S. and Hoshiyama,M., Cerebral responses following stimulation of

unmyelinated C-fibers in humans, electro- and magneto-encephalographic study. *Neurosci Res* 2003;45:255–275.

Kakigi, R., Watanabe, S. and Yamasaki, H., Pain-related somatosensory evoked potentials. *J Clin Neurophysiol* 2000;17;295–308.

Kanda, M., Fujiwara, N., Xu, X., Shindo, K., Nagamine, T., Ikeda, A. and Shibasaki, H., Pain-related and cognitive component of somatosensory evoked potentials following CO_2 laser stimulation in man. *Electroenceph clin Neurophysiol* 1996;100:105–114.

Kanda, M., Nagamine, T., Ikeda, A., Ohara, S., Kunieda, T., Fujiwara, N., Yazawa, S., Sawamoto, N., Matsumoto, R., Taki, W. and Shibasaki, H., Primary somatosensory cortex is actively involved in pain processing in human. *Brain Res* 2000;853:282–289.

Kitamura, Y., Kakigi, R., Hoshiyama, M., Koyama, S., Shimoji, M. and Watanabe, S., Pain-related somatosensory evoked magnetic fields. *Electroenceph clin Neurophysiol* 1995;95:463–474.

Kitamura, Y., Kakigi, R. and Hoshiyama, M., Effects of sleep on somatosensory evoked responses in human: a magnetoencephalographic study. *Brain Res Cogn Brain Res* 1996;4:275–279.

Kitamura, Y., Kakigi, R., Hoshiyama, M., Koyama, S., Watanabe, S. and Shimojo, M., Pain-related somatosensory evoked magnetic fields following lower limb stimulation. *J Neurol Sci* 1997;145:187–194.

Konietzny, F., Perl, E.R., Trevino, D., Light, A. and Hensel, H., Sensory experiences in man evoked by intraneural electrical stimulation of intact cutaneous afferent fibers. *Exp Brain Res* 1981;41:219–222.

Legrain, V., Guerit, J.M., Bruyer, R. and Plaghki, L., Attentional modulation of the nociceptive processing into the human brain: selective spatial attention, probability of stimulus occurrence, and target detection effects on laser evoked potentials. *Pain* 2002;99:21–39.

Lenz, F.A., Rios, M., Chau, D., Krauss, G.L., Zirh, T.A. and Lesser, R.P., Painful stimuli evoke potentials recorded from the parasylvian cortex in humans. *J Neurophysiol* 1998;80:2077–2088.

Malisza, K.L. and Docherty, J.C., Capsaicin as a source for painful stimulation in functional MRI. *J Magn Reson Imaging* 2001;14:341–347.

Mima, T., Nagamine, T., Nakamura, K. and Shibasaki, H., Attention modulates both primary and second somatosensory cortical activities in humans: a magnetoencephalographic study. *J Neurophysiol*

1998;80:2215–2221.

Mor, J., and Carmon, A., Laser emitted radiant heat for pain research. *Pain* 1975;1:233–277.

Naka, D. and Kakigi, R., Simple and novel method for measuring conduction velocity of Aδ fibers in humans. *J Clin Neurophysiol* 1998;15:150–153.

Ochoa, J. and Mair, W.G.P., The normal sural nerve in man. I: ultrastructure and numbers of fibers and cells. *Acta Neuropathol (Berl)* 1969;13:197–216.

Ochoa, J. and Torebjork, E., Sensations evoked by intraneural microstimulation of C nociceptor fibres in human skin nerves. *J Physiol* 1989;415:583–599.

Opsommer, E., Ultra-late (C-fibre) laser evoked potentials: exogenous and endogenous components, dynamic scalp topography and dipolar source modeling. Thesis dissertation, Université catholique de Louvain, Louvain. 1999a;pp.76–92.

Opsommer, E., Masquelier, E. and Plaghki, L., Determination of nerve conduction velocity of C-fibers in humans from thermal thresholds to contact heat (thermode) and from evoked brain potentials to radiant heat (CO_2 laser). *Neurophysiol Clin* 1999b;29:411–422.

Opsommer, E., Weiss, T., Plaghki, L. and Miltner, W.H.R., Dipole analysis of ultralate (C-fibers) evoked potentials after laser stimulation of tiny cutaneous surface areas in humans. *Neurosci Lett* 2001;298:41–44.

Ploner, M., Schmitz, F., Freund, H.J. and Schnitzler, A., Parallel activation of primary and secondary somatosensory cortices in human pain processing. *J Neurophysiol* 1999;81:3100–3104.

Ploner, M., Schmitz, F., Freund, H.J. and Schnitzler, A., Differential organization of touch and pain in human primary somatosensory cortex. *J Neurophysiol* 2000;83:1770–1776.

Price, D.D., Hu, J.W., Dubner, R. and Gracely, R.H., Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses. *Pain* 1997;3:57–68.

Qiu, Y., Fu, Q., Wang, X., Tran, T.D., Inui, K., Iwase, S. and Kakigi, R., Microneurographic study of C fiber discharges induced by CO2 laser stimulation in humans. *Neurosci Lett* 2003;353:25-28.

Qiu, Y., Inui, K ., Wang, X., Nguyen, B.T., Tran, T.D., Kakigi, R., Effects of distraction on magnetoencephalographic responses ascending through C-fibers in humans. *Clin Neurophysiol* 2004;115:636-646.

Qiu, Y., Inui, K., Wang, X., Tran, T.D. and Kakigi, R., Conduction velocity of the spinothalamic tract in humans as assessed by CO_2 laser stimulation of C-fibers. *Neurosci Lett* 2001;311:181–184.

Qiu, Y., Inui, K., Wang, X., Tran, T.D. and Kakigi, R., Effects of attention, distraction and sleep on CO_2 laser evoked potentials related to C-fibers in human. *Clin Neurophysiol* 2002;113:1579–1585.

Rossi, P., Serrao, M., Amabile, G., Parisi, L., Pierelli, F. and Pozzessere, G., A simple method for estimating conduction velocity of the spinothalamic tract in healthy humans. *Clin Neurophysiol* 2000;111:1907–1915.

Rechtschaffen, A.,and Kales, A., A manual for standardized terminology, techniques and scoring for sleep stages in human subjects. Public health service, US govorment printing office, Washington, DC, 1968.

Schmidt, R.F., Schaible, H.G., Messlinger, K., Heppelmann, B., Hanesch, U. and Pawlak, M., 1994. Silent and active nociceptors: structure, functions, and clinical implications. In: Gebhart, G.F., Hammond, D.L. and Jensen, T.S., Editors, *Proceedings of the 7th World Congress on Pain*, IASP Press, Seattle, WA, 1994;pp. 213–250.

Schneider, F., Habel, U., Holthusen, H., Kessler, C., Posse, S., Muller-Gartner, H.W. and Arndt, J.O., Subjective ratings of pain correlate with subcortical-limbic blood flow: an fMRI study. *Neuropsychobiology* 2001;43:175–185.

Siedenberg, R., and Treede, R.D., Laser-evoked potentials: exogenous and endogenous components. *Electroenceph clin Neurophysiol* 1996;100:240–249.

Spence, C., Bentley, D.E., Phillips, N., McGlone, F.P. and Jones, A.K., Selective attention to pain: a psychophysical investigation. *Exp Brain Res* 2002;145:395–402.

Towell, A.D., and Boyd, S.D., Sensory and cognitive components of the CO_2 laser evoked potential. *Electroenceph clin Neurophysiol* 1993;88:173–181.

Tran, T.D., Inui, K., Hoshiyama, M., Lam, K. and Kakigi, R., Conduction velocity of the spinothalamic tract following CO_2 laser stimulation of C-fiber in humans. *Pain* 2002a;95:125–131.

Tran, T.D., Inui, K., Hoshiyama, M., Lam, K., Qiu, Y. and Kakigi, R., Cerebral activation by the signals ascending through unmyelinated C-fibers in humans: a magnetoencephalographic study. *Neuroscience* 2002b;113:375–386.

Tran, T.D., Lam, K., Hoshiyama, M. and Kakigi, R., A new method for measuring the conduction velocities of Aβ-, Aδ- and C-fibers following electric and CO_2 laser stimulation in humans. *Neurosci Lett* 2001;301:187–190.

Treede, R.D., Apkarian, A.V., Bromm, B., Greenspan, J.D. and Lenz, F.A., Cortical representation of pain: functional characterization of nociceptive areas near the lateral sulcus. *Pain* 2000;87:113–119.

Treede, R.D., Kenshalo, D.R., Gracely, R.H. and Jones, A.K., The cortical representation of pain. *Pain* 1999;79:105–111.

Treede, R.D., Meyer, R.A. and Lesser, R.P., 1994. Similarity of threshold temperatures for first pain sensation, laser-evoked potentials, and nociceptor activation. In: Gebhart, G.F., Hammond, D.L. and Jensen, T.S., Editors, *Proceedings of the 7th World Congress on Pain*, IASP Press, Seattle, WA, 1994;pp. 857–865.

Vallbo, A.B., Hagbarth, K.E., Torebjork, H.E. and Wallin, B.G., Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev* 1979;59:919–957.

Valeriani, M., Rambaud, L. and Mauguiere, F., Scalp topography and dipolar source modeling of potentials evoked by CO_2 laser stimulation of the hand. *Electroenceph clin Neurophysiol* 1996;100:343–353.

Valeriani, M., Restuccia, D., Le Pera, D., Fiaschetti, L., Tonali, P. and Arendt-Nielsen, L., Unmasking of an early laser evoked potential by a point localization task. *Clin Neurophysiol* 2000;111:1927–1933.

Valeriani, M., Restuccia, D., Le Pera, D., Armas, L., Maiese, T. and Tonali, P., Attention-related modifications of ultra-late CO_2 laser evoked potentials to human trigeminal nerve stimulation. *Neurosci Lett* 2002;329:329–333.

Wang, X., Inui, K., Qiu, Y., Hoshiyama, M., Tran, T. and Kakigi, R., Effects of sleep on pain-related somatosensory evoked potentials in humans. *Neurosci Res* 2003;45:53–57.

Watanabe, S., Kakigi, R., Koyama, S., Hoshiyama, M. and Kaneoke, Y., Pain processing traced by magnetoencephalography in the human brain. *Brain Topogr* 1998;10:255–264.

Weinder, C., Schmelz, M., Schmidt, R., Hansson, B., Handwerker, H.O. and Torebjork, H.E., Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. *J Neurosci* 1999;19:10184–10190.

William, W.D., From nociception to cortical activity. In: Bromm, B. and Desmedt, J.E., Editors, *Pain and the brain*, Raven, New York, 1995;pp. 1–19.

Yamasaki, H., Kakigi, R., Watanabe, S. and Naka, D., Effects of ditraction on pain perception: magneto- and electro-encephalographic studies. *Brain Res Cogn Brain Res* 1999;8:73–76.

Yamasaki, H., Kakigi, R., Watanabe, S. and Hoshiyama, M., Effects of distraction on pain-related somatosensory evoked magnetic fields and potentials following painful electrical stimulation. *Brain Res Cogn Brain Res* 2000;9:165–175.

Zaslansky, R., Sprecher, E., Katz, Y., Rozenberg, B., Hemli, J.A. and Yarnitsky, D., Pain-evoked potentials: what do they really measure?. *Electroenceph clin Neurophysiol* 1996;100:384–391.

12. Tables

Table 1. Stable single unit recording in each subject

Subject (total of no.)	receptor type	Conduction velocity (m/s)	Latency (P) of LEPs (ms)
S1	C (1)	1.44	1248
S2	C (2)	0.71 and 1.30	1360 and 1327
S3	C (1)	1.25	1346
S4	C (1)	0.72	1356
Mean		1.1±0.3	1327.4±46.2

Table 2. Mean and SD of ultra-late LEPs and late LEPs following stimulation of the skin overlying the vertebral spinous processes at different spinal levels, and the calculated CV in the STT of Aδ- and C-fibers.

Peak latency (ms)	Mean±SD	Range
Late LEP		
P1 (C7)	322.3±27.8	273-363
P1 (T12)	362.6±26.7	317-391
Ultra-late LEP		
P1 (C7)	458.2±40.2	395-539
P1 (T4)	516.7±45.3	455-602
P1 (T8)	579.0±61.2	474-701
P1 (T12)	629.0±56.5	546-725
CV-STT (m/s)		
Aδ-fibers	10.0±4.5	5.8-22.1
C-fibers	2.2±0.6	1.4-4.0

Table 3. Peak latency and amplitude of the P1 component of ultra-late LEP following stimulation of the dorsum of the right hand under different conditions (control, attention, distraction and drowsiness).

	Mean±SD	Range
Control (n=10)		
Latency (ms)	970±106	874 –1136
Amplitude (μV)	8.9±2.4	4.8 – 12
Attention(n=10)		
Latency (ms)	979±101	884 – 1130
Amplitude (μV)	11.3±4.0[b]	5.4 – 18.4
Distraction(n=10)		
Latency (ms)	968±71	860 – 1046
Amplitude (μV)	3.4±3.3[c]	0.1 – 8.2
Drowsiness(n=5)		
Latency (ms)	955±108	858 – 1078
Amplitude (μV)	4.9±2.2 [d]	2.0 – 6.8

a Twenty-five trials were averaged for each recording.

b Amplitude in attention is slightly larger than control.
(P<0.2, Bonferroni-Dunntest)

c Amplitude in distraction is significantly smaller than control.
(P<0.01, Bonferroni-Dunntest)

d Amplitude in drowsiness is significantly smaller than control.
(P<0.01,Paired t test).

Table 4. The coordinate of the each dipole location in each subject

Subject	contralateral hemisphere (X, Y, Z)				ipsilateral hemisphere (X,Y,Z)		
	SI	SII	cingulate	MT	SII	cingulate	MT
S1	41, 16, 105	55, 22, 64.	4, 52, 77	32, 12, 45	-44, 22., 69	4, 52, 77	-30, 18, 34
S2	32, 12, 91	52, 31, 63	-7, 46, 80	30, 3, 30	-51, 27, 65	-7, 46, 80	-20, 15, 32
S3	47, 13, 89	53, 29, 62	7, 37, 90	39, 7, 27	-47, 26, 54	7, 37, 90	-28, 7, 39
S4	38, 22, 86	51, 29, 60	-4, 48, 78	21, 29, 20	-53, 28, 60	-4, 48, 78	-26, 31, 23
S5	30, 3, 96	49, 25, 66	4, 49, 84	34, 23, 34	-38, 14, 71	4, 49, 84	-32, 15, 36
S6	32, 23, 107	58, 18, 80	-0.7, 23, 94	21, 1, 45	-47, 21, 63	-0.7, 23, 94	- 24, 15, 51
S7	35, 12, 102	52, 29, 63	2, 50, 93	38, 18, 43	-53, 28, 62	2, 50, 93	-32, 21, 35
S8	29, 6, 108	55, 13, 65	-6, 57, 79	25, 21, 21	-40, 19, 82	-6, 57, 79	-32, 13, 34
S9	38, 27, 85	55, 2, 71	1, 40, 94	31, 19, 34	-65, 9, 68	1, 40, 94	-38, 2, 29
S10	36, 23, 87	42, 29, 66	-2, 51, 89	39, 36, 12	-53, 16, 79	-2, 51, 89	-43, 25, 30
S11	43, 15, 99	39, 4, 67	1, 52, 63	36, 13, 31	-44, -0.2, 76	1, 52, 63	-30, 21, 35
S12	23, -3, 102	50, -0.3, 70	8, 51, 68	24, 16, 25	-36, -0.6, 79	8, 51, 68	-38, -2, 37
S13	32, -12, 112	55, 9, 77	4, 58, 83	27, 19, 32	-48, -8, 64	4, 58, 83	-36, 31, 36
Mean	35, 12, 98	51, 18, 67	0.9, 47, 82	31, 17, 31	-48, 15, 69	0.9, 47, 82	-31, 16, 35

SI, primary somatosensory cortex; SII, secondary somatosensory cortex; MT, medial temporal area around the amygdala or hippocampal. X indicates the coronal plane with positive values toward the right preauricular point, Y indicates the mid-sagittal plane with a positive value toward the anterior direction, and Z indicates the transverse plane perpendicular to the x-y line with a positive value toward the upper side.

Table 5. The contribution of each dipole to the GOF value

Subject	**SI** (contralateral hemisphere)	**SII** (bilatera hemisphere)	**cingulate**	**MT** (bilateral hemisphere)
S1	30.7	70.5	82.6	94.5
S2	41.4	75.5	86.6	94.5
S3	29.3	52.1	68.8	91.6
S4	22.5	62.8	80.1	91.7
S5	29.8	62.0	81.2	93.5
S6	26.4	55.5	78.5	93.3
S7	37.8	69.2	77.7	93.2
S8	28.7	64.4	80.8	92.6
S9	33.4	65.5	84.8	93.0
S10	30.4	67.1	79.6	93.3
S11	20.1	54.8	83.3	92.1
S12	38.6	72.8	76.7	91.1
S13	46.2	69.5	78.6	92.4
Mean±SD	31.9±7.	64.7±7.2	80.0±4.4	92.9±1.1

These values show the change in GOF value on adding each Source. For example, in Subject 1 (S1) in the contralateral hemisphere, when the one-source model (only SI) was adopted, the GOF value was 30.7%. Then, Source 2 (SII) was added for the two-source model including both hemispheres, and the GOF value increased to 70.5%. next, Source 3 (Cingulate) was added for the three-source model, and the GOF value increased to 82.6%. Finally, Source 4 (MT) was added for the four-source model including both hemispheres, and the GOF value increased to 94.5%. By adopting this four-source model, the GOF value was over 90 % in all subjects and its mean in all subjects was 92.9%.

13. Legends for Figures

Fig. 1: A thin aluminum plate (0.1mm in depth, 40mm in length and 60mm in width) used in the experiment for the recording of ultra-late LEP. In a 25mm x 25mm square on this plate, parallel lines were drawn every 1mm, so that, there were 26 x 26 intersections. A total of 676 (26x26) tiny holes were drilled at these intersections, each with a diameter of 0.4mm, corresponding to an area of 0.125 mm^2 for each hole (A). A tungsten microelectrode was inserted percutaneously into the peroneal nerve in the popliteal area without local anesthesia (B)

Fig. 2: C-fiber action and cerebral potentials in subject 2. (a) Raster display of C-fiber responses to transcutaneous electrical stimulation and laser stimulation through on aluminum plate on the dorsal foot. C-fibers action potentials (in the circle of dots) were recorded from the peroneal nerve in the popliteal area (left side of above). (b) Single C-fiber responses to seven electrical and eight laser stimuli were superimposed, respectively.

Fig. 3: (a) Aδ-fiber action in subject 2. Arrows point to the Aδ-fiber responses to the transcutaneous electrical stimulation on the dorsal foot. Right hand traces are expanded views. (b) There were no Aδ-fiber responses found after laser stimulation through on aluminum plate. Ten single responses were superimposed. These findings indicated that this new stimulation method selectively activated C-fiber nociceptors of the skin.

Fig. 4: A thin aluminum plate was used as a spatial filter and placed on the skin overlying the vertebral spinous processing at T8 in this figure. The array of holes allowed the 2mm laser beam to pass through one to four holes to reach the skin.

Fig. 5: Late LEP and ultra-late LEP following stimulation of various levels of the spinous processes in a subject. Two averaged waveforms are superimposed to indicate the reproducibility of responses. The vertical bars indicate the positive peak and the latency is shown under the peak. The CV in STT related to Aδ-fibers measured from the late LEP and that related to C-fibers in this subject was 9.1m/sec and 2.4m/sec, respectively.

Fig. 6: Scatterplots and regression of individual latencies of ultra-late LEP in 11 subjects. Y-axis: peak latency of the positive component. Each square indicates the peak latency obtained from one site of stimulation. Thin lines were drawn to show intraindividual latency changes, which represent the individual regressions in the eleven subjects who had 44 sites stimulated. The reciprocal of the slope of the regression line indicates the CV related to C-fibers for each subject. The mean and SD was 2.2±0.6 m/s.

Fig. 7: Effects of attention and distraction on the ultra-late LEP in two subjects. The right hand was stimulated during the control, attention and distraction conditions. Latencies and amplitudes represent the average of two overlapping trials.

Fig. 8: Mean (10 subjects) P1 amplitudes in the 3 different conditions. It was only slightly increased under attention (P=0.18) and significantly decreased during distraction (P=0.0068), relative to the control, respectively. There was a significant difference between the amplitude change of attention and distraction (P=0.0003). Results were statistically analyzed by ANOVA followed by Bonferroni–Dunn test.

Fig. 9: Effects of levels of arousal on the ultra-late LEP in two subjects. The right hand was stimulated during control (wakefulness), drowsiness and stage 2 sleep. The P1 is flat during drowsiness and absent during stage 2 sleep. Latencies and amplitudes represent the average of two overlapping trials. Waveforms recorded during drowsiness and sleep appeared smooth, since EMG noise was very low. However, relatively large slow waves during sleep sometimes overlapped with averaged waveforms.

Fig. 10: Mean P1 amplitudes of 5 subjects in the control and drowsiness conditions. The P1 disappeared in the other 5 subjects. The P1 amplitude was decreased during drowsiness (P=0.0044, n=5, paired t test) relative to the control.

Fig. 11: Effects of Distraction on the ultra-late LEF and ultra-late LEP in subject 1. Superimposed waveforms recorded from 37 channnels at positions C4 and C3, corresponding to the hemisphere contralateral and ipsilateral to

the stimulated hand and the simultaneously recorded ultra-late LEP at the Cz electrode are shown. All components, 1M and 2M in LEF and N1 and P1 in LEP, were much reduced in amplitude during Distraction. The isocontour map of 1M at the peak latency in the contralateral hemisphere shows a complicated magnetic field, which implied multiple sources, at least two. The isocontour map of 1M in the ipsilateral hemisphere shows a typical one-dipole pattern.

Fig. 12: Above: mean and standard deviation of the peak latency of N1 and P1 in the ultra-late LEP, and of 1M and 2M in the ultra-late LEF in thirteen subjects during the Control and Distraction condition. We compared peak latencies of the primary component (N1 and 1M) and second component (P1 and 2M) separately. The peak latency of N1 was significantly longer than that of 1M recorded from the contralateral hemisphere in Control, 26 ms on average (P=0.0022,). The latencies of 1M and 2M were significantly longer in the ipsilateral hemisphere than in the contralateral hemisphere, 21 and 32 ms on average, respectively (P=0.01 for 1M and P=0.002 for 2M). Below: amplitudes of both N1 and P1 during Distraction were significantly smaller than those in Control, P=0.037 for N1 and P=0.0029 for P1, respectively. Amplitudes of 1M and 2M recorded from the contralateral and ipsilateral hemispheres were much reduced during Distraction as compared with Control, P=0.01 for 1M and P=0.005 for 2M in the contralateral hemisphere, and P=0.005 for 2M and P=0.002 for 2M in the ipsilateral hemisphere.

Fig. 13: Source generators analyzed by BESA during Control and Distraction in subject 1. SI and SII-insula seem the main generators for 1M,while MT and cingulate cortex seem the main generators for 2M. During Distraction, the activities of SI, SII-insula, MT and cingulate cortex were much reduced. Notice the difference of current strength scale, 10 nAm for SI and SII-insula and 30 nAm for MT and Cingulate. These source locations overlapped on MRI.

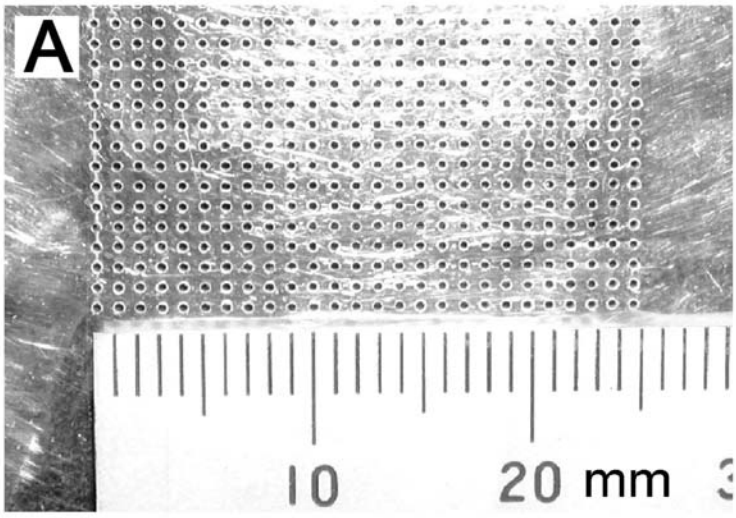
Fig. 14: The mean location of each source. Each source location is overlaid on standard brain MRI.

Fig. 15: Above: the mean latency of SII-insula and MT was significantly

longer in the hemisphere ipsilateral than contralateral to the stimulation. Below: the current strength of all sources was significantly lower in Distraction than Control, and the SII-insula and cingulate were more affected than the SI and MT.

14. Figures

Fig. 1



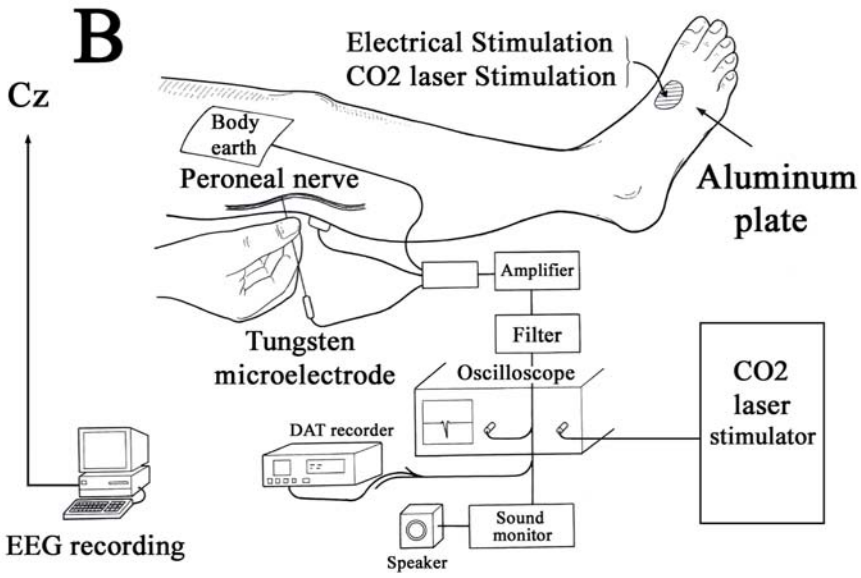


Fig. 2

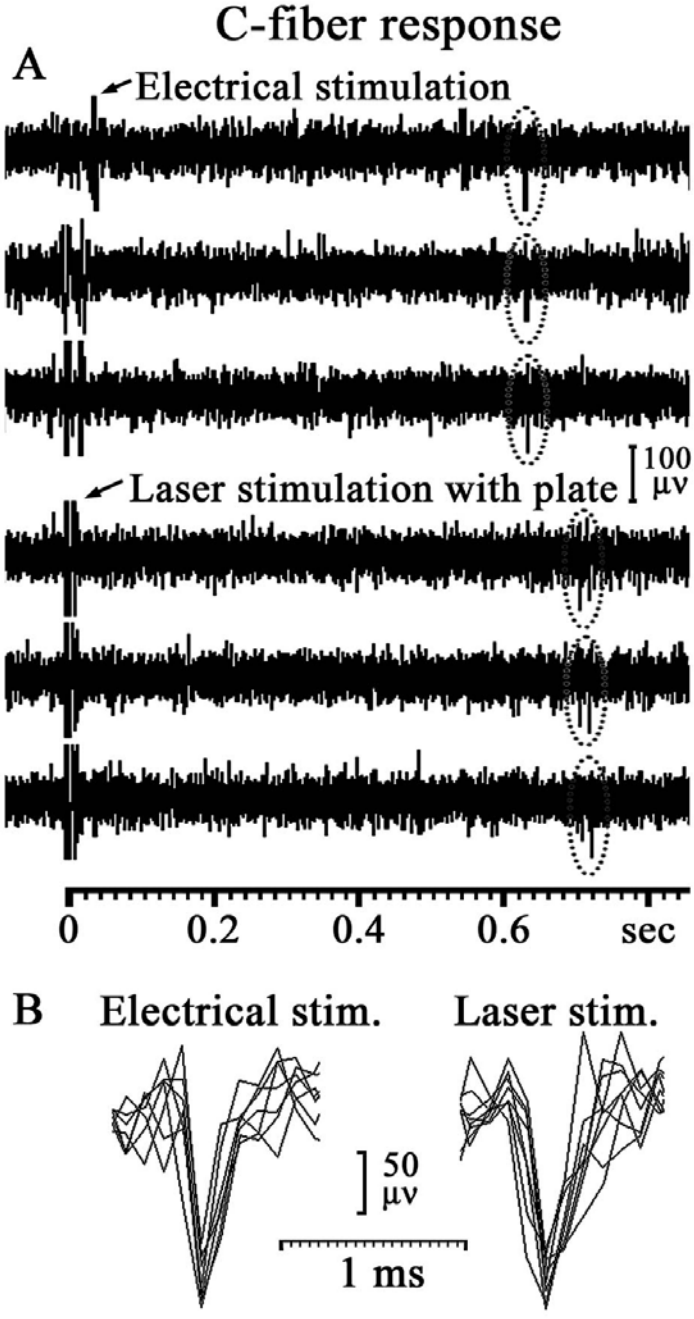

C-fiber response
A
Electrical stimulation
Laser stimulation with plate
100 μv
0
0.2
0.4
0.6
sec
B
Electrical stim.
Laser stim.
50 μv
1 ms

Fig. 3

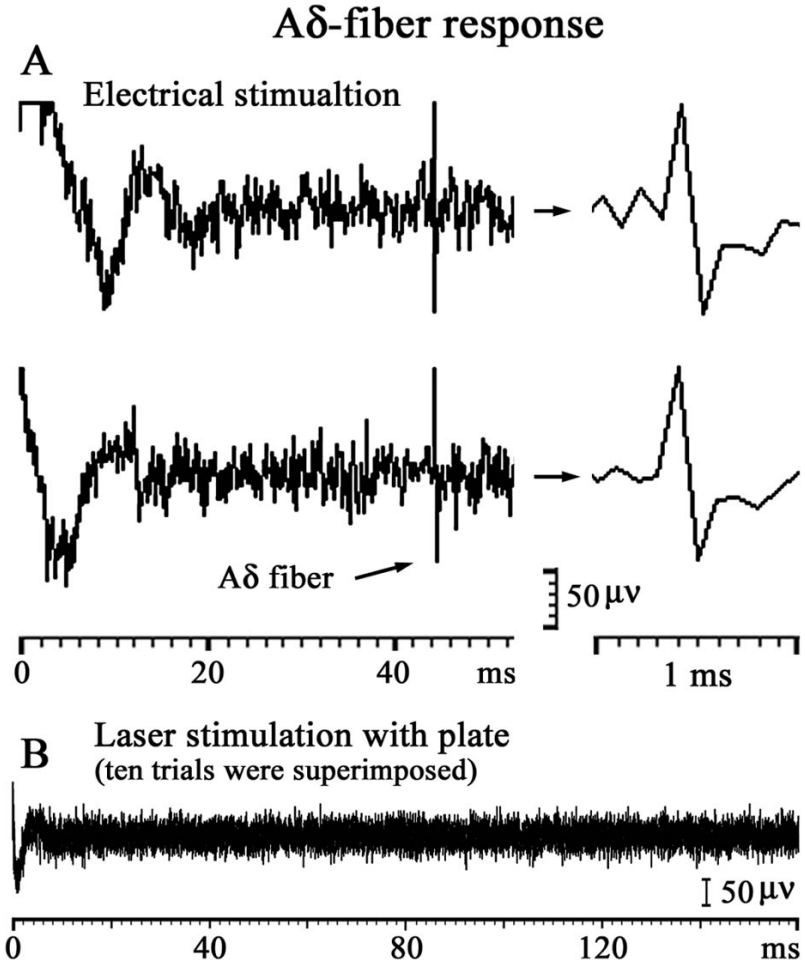

Aδ-fiber response
A
Electrical stimualtion
Aδ fiber
50 μv
0
20
40
ms
1 ms
B
Laser stimulation with plate
(ten trials were superimposed)
50 μv
0
40
80
120
ms

Fig. 4

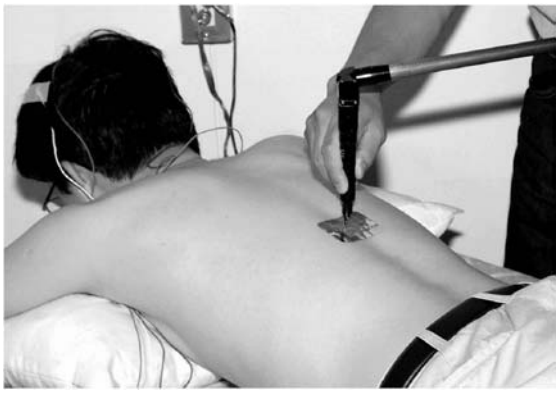


Fig. 5

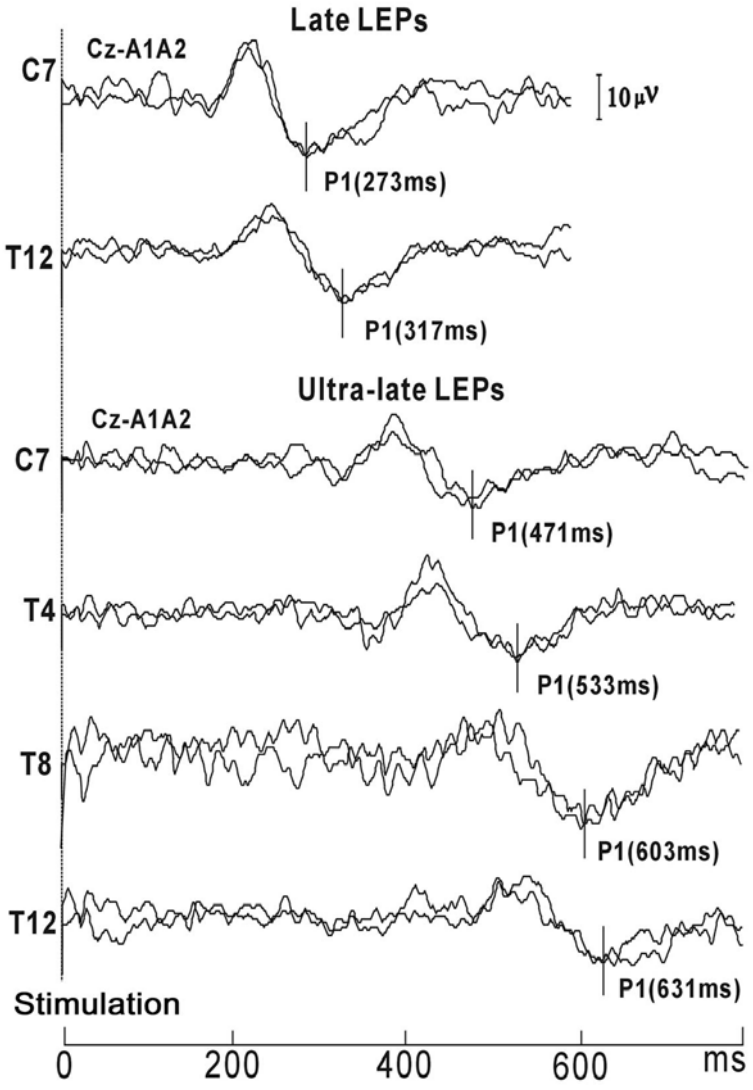

Late LEPs
Cz-A1A2
C7
10μV
P1(273ms)
T12
P1(317ms)
Ultra-late LEPs
Cz-A1A2
C7
P1(471ms)
T4
P1(533ms)
T8
P1(603ms)
T12
P1(631ms)
Stimulation
0
200
400
600
ms

Fig. 6

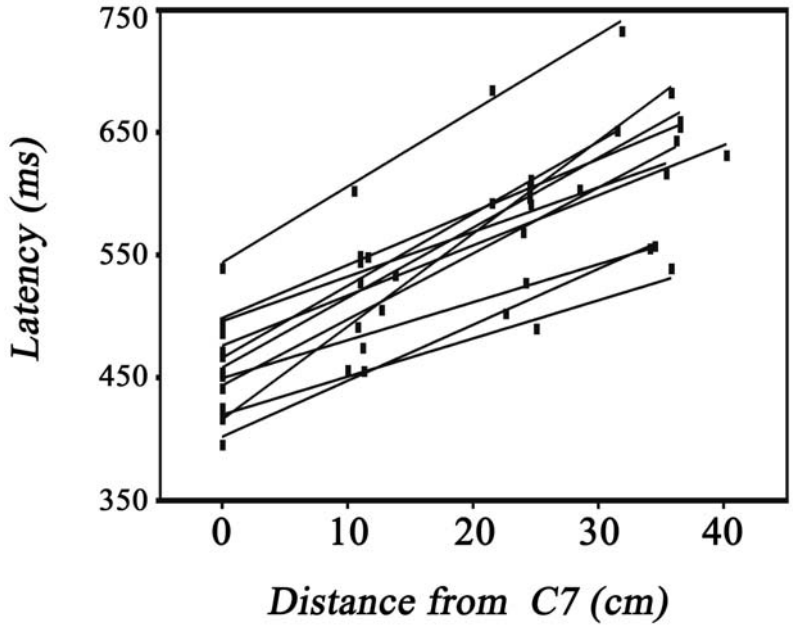

750
650
550
450
350
Latency (ms)
0
10
20
30
40
Distance from C7 (cm)

Fig. 7

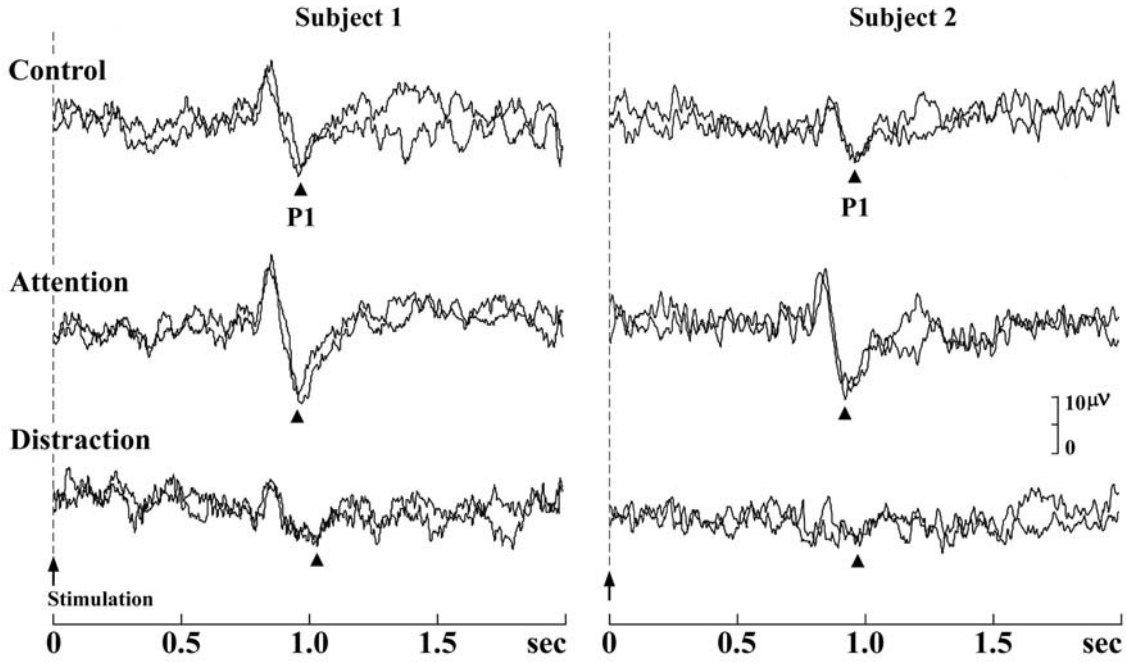

Subject 1
Subject 2
Control
Attention
Distraction
P1
P1
10μV
0
Stimulation
0
0.5
1.0
1.5
sec
0
0.5
1.0
1.5
sec

Fig. 9

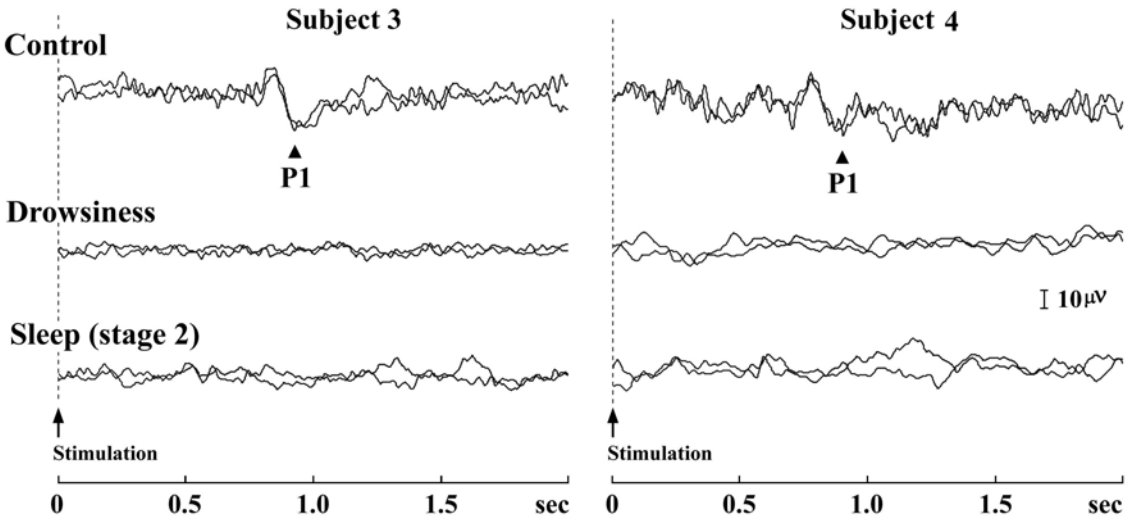


Fig. 8

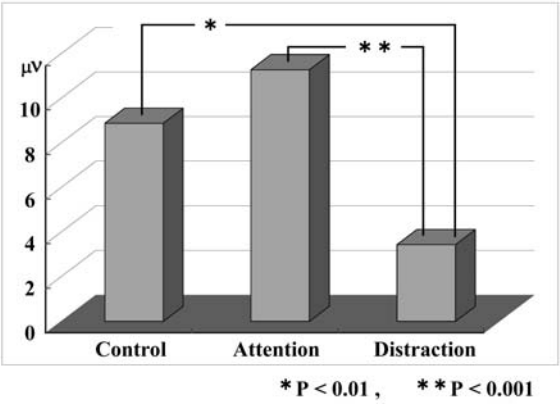
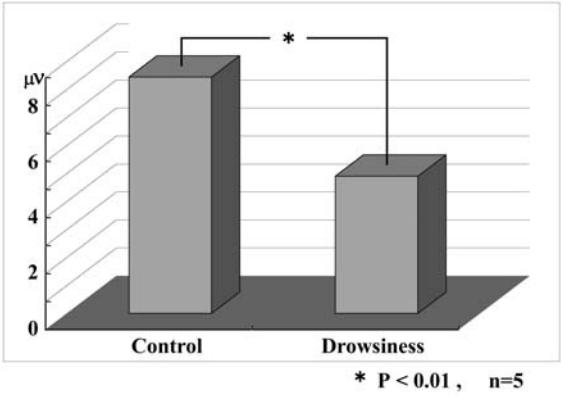


Fig. 10



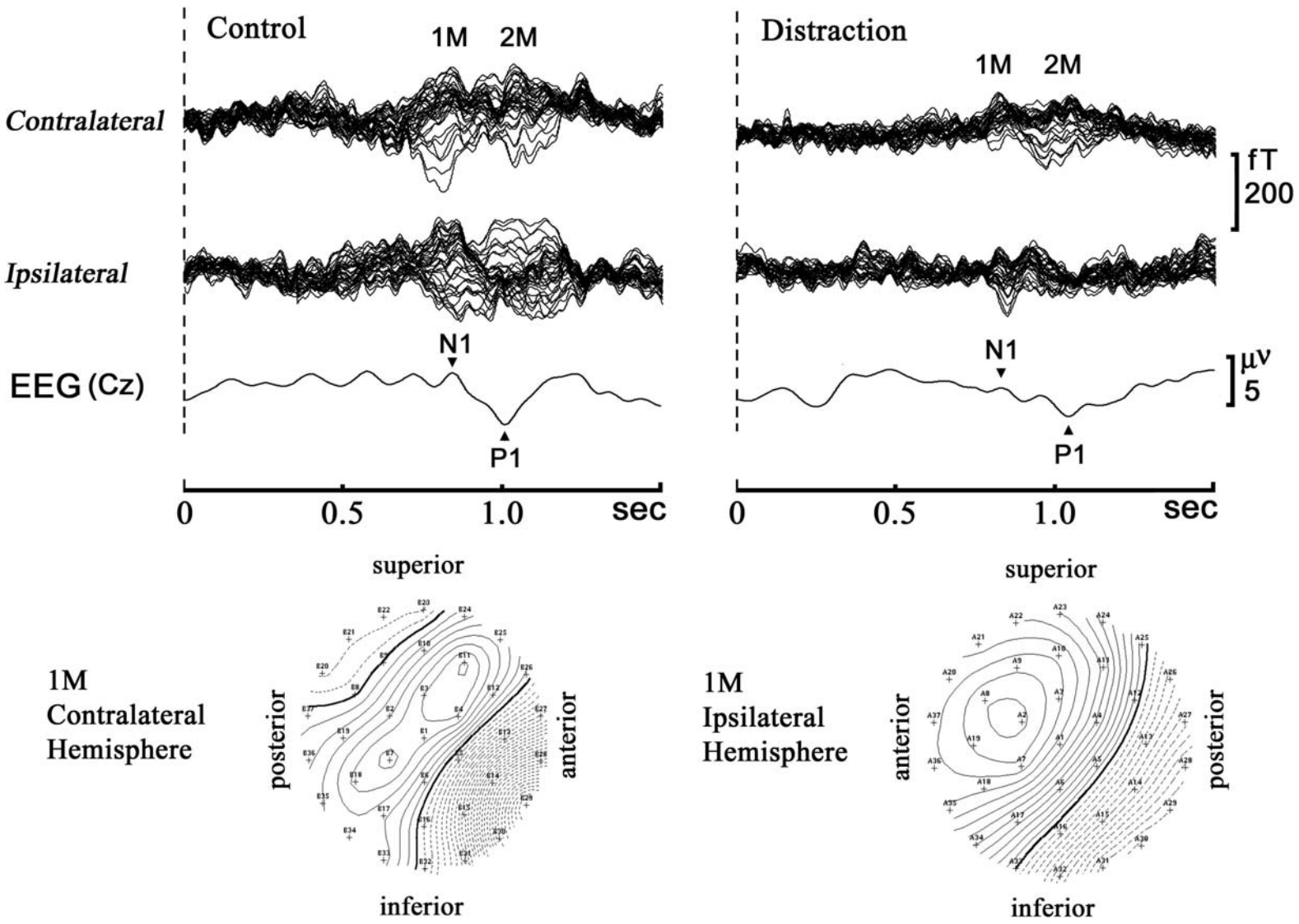
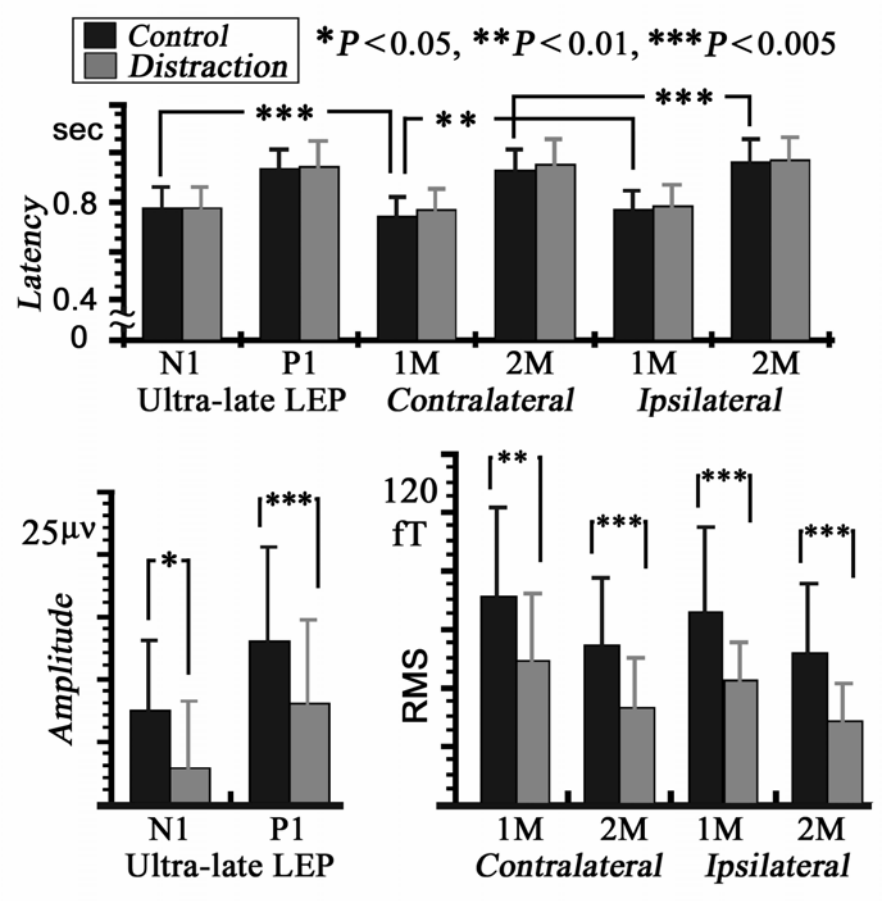

Control
1M 2M
Contralateral
Ipsilateral
EEG (Cz)
N1
P1
0
0.5
1.0
sec
Distraction
1M 2M
fT
200
N1
P1
μν
5
0
0.5
1.0
sec
superior
1M
Contralateral
Hemisphere
posterior
anterior
inferior
superior
1M
Ipsilateral
Hemisphere
anterior
posterior
inferior

Fig. 11

Fig. 12


Control
Distraction
*P<0.05, **P<0.01, ***P<0.005
sec
0.8
0.4
0
Latency
***
**
***
N1
P1
1M
2M
1M
2M
Ultra-late LEP
Contralateral
Ipsilateral
25μv
Amplitude
*
***
N1
P1
Ultra-late LEP
120
fT
RMS
**
***
***
***
1M
2M
1M
2M
Contralateral
Ipsilateral

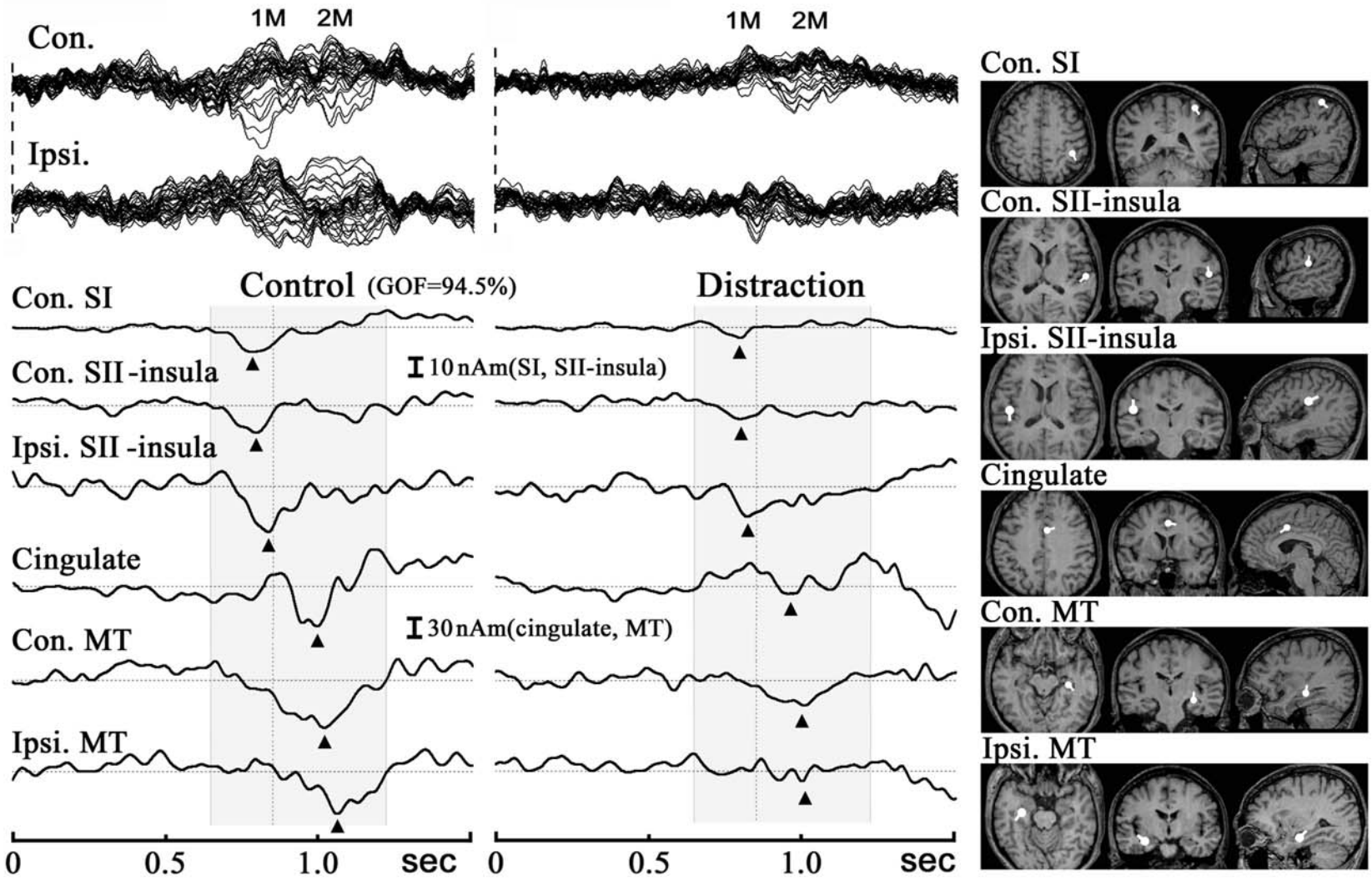

Con.
1M
2M
Ipsi.
Control (GOF=94.5%)
Distraction
Con. SI
Con. SII-insula
Ipsi. SII-insula
Cingulate
Con. MT
Ipsi. MT
10 nAm(SI, SII-insula)
30 nAm(cingulate, MT)
0
0.5
1.0
sec
Con. SI
Con. SII-insula
Ipsi. SII-insula
Cingulate
Con. MT
Ipsi. MT

Fig. 13

Fig. 14

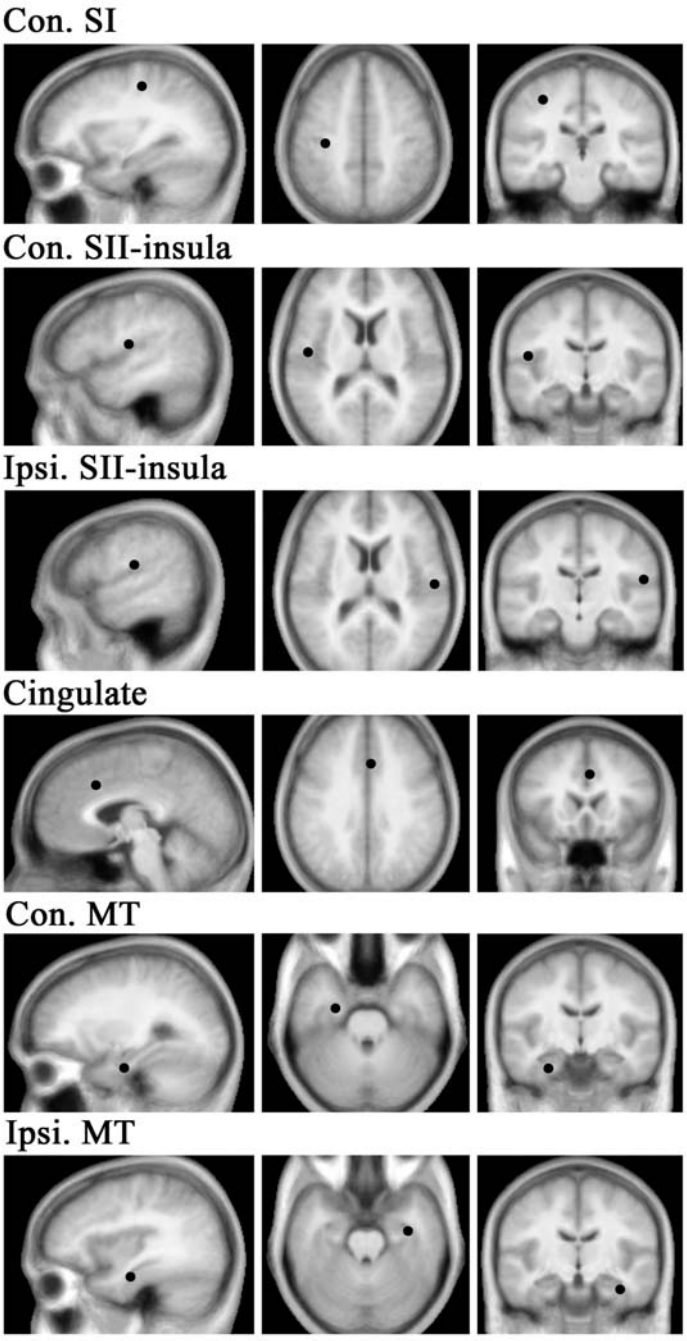
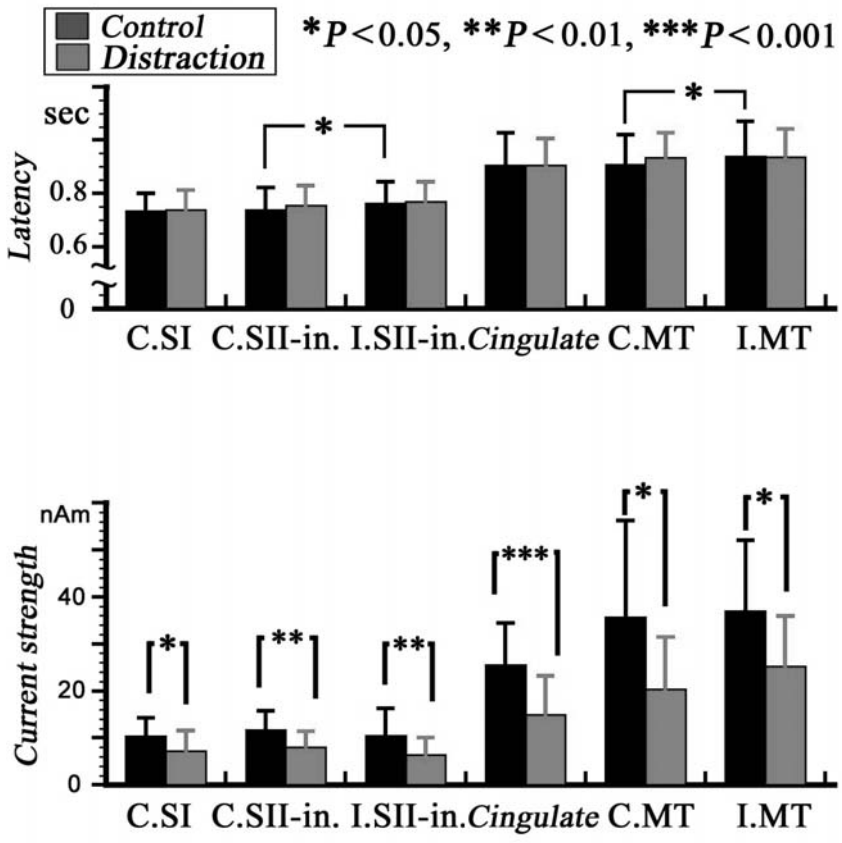

Con. SI
Con. SII-insula
Ipsi. SII-insula
Cingulate
Con. MT
Ipsi. MT

Fig. 15


Control
Distraction
*P<0.05, **P<0.01, ***P<0.001
Latency
sec
0.8
0.6
0
C.SI C.SII-in. I.SII-in. Cingulate C.MT I.MT
Current strength
nAm
40
20
0
C.SI C.SII-in. I.SII-in. Cingulate C.MT I.MT

15. Publications

Original articles as first author

Qiu Y, Inui K, Wang X, Nguyen BT, Tran TD, Kakigi R.
Effects of distraction on magnetoencephalographic responses ascending through C-fibers in humans. *Clin Neurophysiol.* 2004;115(3):636-646.

Qiu Y, Fu Q, Wang X, Tran TD, Inui K, Iwase S, Kakigi R.
Microneurographic study of C fiber discharges induced by CO2 laser stimulation in humans. *Neurosci Lett.* 2003;353(1):25-28.

Qiu Y, Inui K, Wang X, Tran TD, Kakigi R.
Effects of attention, distraction and sleep on CO2 laser evoked potentials related to C-fibers in humans. *Clin Neurophysiol.* 2002;113(10):1579-1585.

Qiu Y, Inui K, Wang X, Tran TD, Kakigi R.
Conduction velocity of the spinothalamic tract in humans as assessed by CO2 laser stimulation of C-fibers. *Neurosci Lett.* 2001;311(3):181-184.

Original articles as co-author

Tamura Y, Hoshiyama M, Inui K, Nakata H, **Qiu Y**, Ugawa Y, Inoue K, Kakigi R., Facilitation of Aδ-fiber-mediated acute pain by repetitive transcranial magnetic stimulation. *Neurology.* 2004; 62(12): 2176 - 2181.

Nakata H, Inui K, Wasaka T, Tamura Y, Tran TD, **Qiu Y**, Wang X, Nguyen TB, Kakigi R., Movements modulate cortical activities evoked by noxious stimulation. *Pain.* 2004;107(1-2):91-8

Wang X, Inui K, **Qiu Y**, Hoshiyama M, Tran TD, Nguyen TB, Kakigi R., Effects of sleep on pain-related somatosensory evoked magnetic fields in humans. *Brain Res Cogn Brain Res.* 2003;17(2):388-99.

Wang X, Inui K, **Qiu Y**, Hoshiyama M, Tran TD, Kakigi R., Effects of sleep on pain-related somatosensory evoked potentials in humans. *Neurosci Res.* 2003;45(1):53-7.

Inui K, Wang X, **Qiu Y**, Nguyen BT, Ojima S, Tamura Y, Nakata H, Wasaka T, Tran TD, Kakigi R., Pain processing within the primary somatosensory cortex in humans. *Eur J Neurosci.* 2003;18(10):2859-66.

Inui K, Tran TD, **Qiu Y**, Wang X, Hoshiyama M, Kakigi R., A comparative magnetoencephalographic study of cortical activations evoked by noxious and innocuous somatosensory stimulations. *Neuroscience.* 2003;120(1):235-48.

Inui K, Tran TD, **Qiu Y**, Wang X, Hoshiyama M, Kakigi R., Pain-related magnetic fields evoked by intra-epidermal electrical stimulation in humans. *Clin Neurophysiol.* 2002;113(2):298-304.

Tran TD, Inui K, Hoshiyama M, Lam K, **Qiu Y**, Kakigi R., Cerebral activation by the signals ascending through unmyelinated C-fibers in humans: a magnetoencephalographic study. *Neuroscience.* 2002;113(2):375-86.

Review articles

Kakigi R, Watanabe S, Tran TD, Inui K, Lam K, **Qiu Y**, Wang X, Yamasaki H, Hoshiyama M. Neurophysiologic assessment of pain. *Advances in Clinical Neurophysiology.* 2002;54:151-55.

Kakigi R, Tran TD, **Qiu Y**, Wang X, Nguyen TB, Inui K, Watanabe S, Hoshiyama M. Cerebral responses following stimulation of unmyelinated C-fibers in humans: electro- and magneto-encephalographic study. *Neurosci Res.* 2003;45:255-75.

Kakigi R, Naka D, Okusa T, Wang X, Inui K, **Qiu Y**, Tran TD, Miki K, Tamura Y, Nguyen TB, Watanabe S, Hoshiyama M. Sensory perception during sleep in humans: a magnetoencephalograhic study. *Sleep Med.* 2003;4:493-507.