EFFECTS OF SLEEP ON CEREBRAL ACTIVITIES FOLLOWING PAINFUL STIMULATION

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1. Abbreviations

BESA: Brain electric source analysis

ECD: Equivalent current dipole

EEG: Electroencephalography

ES: Noxious intra-epidermal electrical stimulation

fMRI: Functional magnetic resonance imaging

GOF: Goodness of fit

MEG: Magnetoencephalography

MT: Medial temporal area

PET: Positron emission tomography

RMS: Root mean square

SEF: Somatosensory evoked magnetic field

SEP: Somatosensory evoked potential

SI: Primary somatosensory cortex

SII: Secondary somatosensory cortex

2. Abstract

Cerebral activities in humans, particularly responses to various kinds of sensory stimuli, should be much changed during sleep. We used magnetoencephalography (MEG) to study the effect of sleep on cerebral activities following painful stimulation.

In the first study, while the subjects were awake, non-painful and painful electrical stimulations were applied, and while asleep, painful stimulation was applied to the left index finger. During awake, five components (1M-5M) were identified following both non-painful and painful stimulation, but the 4M and 5M at around 70-100 ms and 140-180 ms, respectively, were significantly enhanced following painful stimulation. During sleep, magnetic fields recorded in stage 1 sleep and stage 2 sleep were analyzed. 1M and 2M generated in the primary somatosensory cortex (SI) did not show a significant change, 3M in SI showed a slight but significant amplitude reduction, and 4M and 5M generated in both SI and the secondary somatosensory cortex (SII) were significantly decreased in amplitude or disappeared during sleep. The 4M and 5M are complicated components generated in SI and SII ascending through both A-beta fibers and A-delta fibers. They are specifically enhanced by painful stimulation due to an increase of signals ascending through A-delta fibers, and are markedly decreased during sleep, because they much involve cognitive function.

In the second study, painful intra-epidermal electrical stimulation (ES), which selectively activates A-delta fibers, was applied to the dorsum of the left hand. While awake, subjects were asked to count the number of stimuli silently (Attention) or ignore the stimuli (Control). During sleep, magnetic fields recorded in stage 1 sleep and stage 2 sleep were analyzed. The contralateral SI, bilateral SII, insular cortex, medial

temporal area (MT) and cingulate cortex were activated by ES. Cortical responses in the contralateral SI, ipsilateral SII and MT, bilateral insula and cingulated cortex were significantly enhanced in Attention as compared with Control. All of these activities were significantly reduced during sleep. The present results suggested that SI, SII, insula, cingulated cortex and MT are involved in pain cognition.

3. Introduction

Mechanisms underlying pain processing have been examined in many studies but are still not well understood. Various investigators have studied cortical activities involved in nociception using different kinds of methods, including positron emission tomography (PET) (Casey et al., 1994; Coghill et al., 1994), functional magnetic resonance imaging fMRI (Davis et al., 1998; Gelnar et al., 1999), magnetoencephalography (MEG) (Kakigi et al., 1995; Watanabe et al., 1998; Ploner et al., 1999b; Inui et al., 2003) and intra-cranial SEP recordings (Lenz et al., 1998; Frot et al., 2001). MEG is the noninvasive measurement of magnetic fields generated by electrical activity in the brain. MEG has excellent spatial and temporal resolution, in the order of millimeter and millisecond. The spatial resolution of MEG is almost the same as that of fMRI and PET, but the temporal resolution is much better. Therefore, we can analyze MEG responses to noxious stimulation for not only detecting cortical sources but also measuring the time taken for activities to transfer in the brain in the order of milliseconds.

Cerebral responses to noxious stimuli are affected by the subject's attentional and arousal levels. To clarify the mechanism of pain processing, several studies have investigated the effects of distraction on pain-related somatosensory evoked potential (SEP) (Beydoun et al., 1993; Garcia-Larrea et al., 1997; Yamasaki et al., 1999, 2000; Qiu et al., 2002). These studies found a decrease of pain-related SEP amplitude during distraction tasks. Examining the effects of sleep on cerebral responses to noxious stimuli is another useful way of understanding nociceptive processing. However, up to now, only a few studies have attempted to investigate the cortical responses to noxious

stimuli during sleep (see Kakigi et al., 2003). Beydoun et al. (1993) reported that pain-related SEPs were markedly decreased during sleep stages, which was confirmed later by Naka and Kakigi (1998), and Qiu et al., (2002).

By applying painful stimuli such as high-intensity electrical stimulation (Becker et al., 1993; Dowman, 1991 Milltner et al., 1989), intra-epidermal electrical stimulation (ES) (Inui et al., 2002, 2003), and CO_2 laser stimulation, pain-related SEF and SEP can be recorded (see reviews by Kakigi et al., 2000a, b). Each method has its own advantages and disadvantages.

In the first study, we used painful electrical stimulation to investigate the effects of sleep on pain-related SEFs. The biggest advantage of using painful electrical stimulation is that this method activates both fast A-beta fibers relating to touch and slow A-delta fibers relating to pain simultaneously and thus enables a comparison of the effects of sleep on tactile- and pain-related cortical responses. Our objective is to compare pain-related SEF between awake and sleep stages to investigate how somatosensory and pain perception are changed during sleep.

In the second study, we used noxious intra-epidermal electrical stimulation (ES), a method we recently developed (Inui et al., 2002a, b, 2003). ES selectively activates cutaneous A-delta fibers and produces weak but well-defined sharp sensations of pricking. As compared with strong painful laser stimulation, which frequently wakes subjects up, ES produces less uncomfortable sensations, and therefore is suitable for the purpose of this study, since the subjects remained asleep even during ES stimulation. It is well known that multiple areas are activated by noxious stimulation such as the

primary somatosensory cortex (SI), secondary somatosensory cortex (SII), medial temporal regions (MT) and cingulate cortex (for review, see Treede et al., 1999; Schnitzler and Ploner, 2000). Therefore, we recorded magnetic fields following noxious stimuli and separated each cortical activity by use of a multiple source analysis method to know the effects of sleep on each region.

4. Painful high-intensity electrical stimulation

4.1 Methods

4.1.1 Subjects

Ten healthy male volunteers participated in the study. Their ages ranged from 27 to 38 years (mean ± SD: 31.0 ± 3.2). None of the subjects had any history of medical abnormalities or sleep disorders. No medication was given to them before the experiment for inducing sleep, and the study was done in the afternoon after taking lunch. Informed consent was obtained from all participants prior to the study which was first approved by the Ethical Committee at our Institute.

4.1.2 Painful electrical stimulation

The electrical stimulus was a constant current square wave pulse delivered transcutaneously to the left index finger using ring electrodes. The stimulus duration was 1.0 ms. Two levels of intensity were adopted, “painful” and “non-painful”. The degree of pain was about 80% of intolerable pain, depending on the subjective feeling of each subject, and it’s intensity ranged from 14 to 16 mA. The level of “non-painful” stimulation was three times the sensory threshold, approximately 3-4 mA. The inter-stimulus interval was random, between 1500 and 5000 ms.

4.1.3 MEG recordings

The evoked magnetic fields were measured with dual 37-channel biomagnetometers (Magnes, Biomagnetic Technologies Inc., San Diego, CA). The detection coils of the biomagnetometer (axial-type gradiometer) were arranged in a uniformly distributed array in concentric circles over a spherically concave surface. Thus, all of the sensor coils were equally sensitive to the brain's weak magnetic signals. The device was 144 mm in diameter, with a radius of curvature of 122 mm. The outer coils were 72.5° apart. Each coil was 20 mm in diameter and had a 50 mm baseline. The distance between the centers of each coil was 22 mm. Each coil was connected to a superconducting quantum interference device (SQUID). The intrinsic noise level of each channel was less than 10 fT/$\sqrt{}$Hz. Two measurement matrices were centered around the C3 and C4 of the international 10-20 system in each subject. The SI and SII in the bilateral hemispheres were mostly covered by these positions. SEF responses were filtered with a 0.1-100 Hz bandpass filter and digitized at a sampling rate of 1042 Hz. The analysis time was 100 ms before and 500 ms after the application of each stimulus. The DC offset procedure was achieved using the pre-stimulus period as the baseline. Trials in which the MEG deflection exceeded 3 pT were excluded from the averaging. Painful and non-painful stimuli were delivered randomly while awake. Each session was made up of an average of 100 trials in each subject. During sleep, only painful stimuli were delivered because we had to save the experiment time to complete recording during the limited period of stable sleep. Because sleep stages tended to fluctuate during the recording in some subjects, we watched an EEG monitor carefully and recorded evoked magnetic fields at different sleep stages separately. For example, when the subject was in stage 1 sleep, sweeps were recorded as the stage 1 section, and

if the sleep stage changed from stage 1 to stage 2, then the data were collected as the stage 2 section. When the subject was awake, the recording was stopped.

4.1.4 Sleep recordings

An EEG was recorded to monitor sleep stage by placing gold disk electrodes at Cz, Pz and Oz of the International 10-20 system referenced with linked earlobes. The Pz and Oz were on the mid-sagittal line in the parietal and occipital region. The sleep stage was determined according to the guidelines of Rechtschaffen and Kales (Rechtschaffen and Kales, 1968). The subject's head was fixed to the lower device by the adhesion tape to minimize undesired head movement. We checked the head position before and after the experiment, and confirmed that they did not differ significantly. In fact, the head position did not differ significantly between before and after the experiment in any subjects. Since most subjects did not reach stage 3 or 4 or rapid eye movement (REM) sleep, and body movements during stage 3 and 4 were sometimes very large, we analyzed SEF only during the awake state, stage 1 sleep and stage 2 sleep in the present study.

4.1.5 Source localization

A spherical model was fitted to the digitized head shape of each subject, and the location (x, y, z positions) (Fig. 1), orientation and amplitude of a best-fitted single equivalent current dipole (ECD) were estimated for each point in time (Sarvas, 1987). The origin of the head-based coordinate system was the midpoint between the

preauricular points. This analysis demonstrated consistent localization of the sources with standard deviations of a few millimeters (Pantev et al., 1991). The correlation coefficient between the theoretical field generated by the model and the measured field was calculated.

Since the measured field in several subjects was considered to contain two or more temporally overlapping sources as will be described in the results, the single ECD model was inappropriate in such cases. Therefore, for the spatio-temporal multi-dipole model, we used brain electric source analysis (BESA 99, NeuroScan, Inc., McLean, VA) for the computation of theoretical source generators in a 3-layer spherical head model. BESA was modified for our 37-channel biomagnetometer (Watanabe et al., 1998; Hoshiyama et al., 2000; Nihashi et al., 2001). This method allows the spatio-temporal modeling of multiple simultaneous sources over defined time intervals. The location and orientation of the dipoles were calculated by an iterative least-squares fit. The residual variance (%RV) indicated the percentage of data that could not be explained by the model. The goodness of fit (GOF) was expressed as a percentage (100 - %RV). The GOF indicated the percentage of the data that can be explained by the model. We used the GOF value to determine whether or not the model was an appropriate one. We considered that the adaptation of the dipoles would be significant, when the GOF was larger than 90%. An increase in the dipole number mathematically increases the GOF, since a greater number of dipoles will account for more variance (Watanabe et al., 1998; Hoshiyama et al., 2000; Nihashi et al., 2001), hence physiologically plausible solutions were taken into account when using this method.

Since the correlation coefficient calculated for a single dipole model and GOF for BESA were different mathematical values, we cannot simply compare them. In addition,

the GOF value is always smaller than the correlation, because the correlation was calculated at one point (latency), whereas, the GOF was calculated for some period, for example almost 150 msec in our study. In general, the GOF value calculated for a long period is much decreased as compared with that for a short period or one point. Therefore, a GOF larger than 90% is considered to indicate a good model for a middle- or long-latency SEF study.

Magnetic resonance imaging (MRI) was performed using a GE signa 1.5T system. T1-weighted coronal, axial and sagittal images 1.5 mm in thickness were adopted for overlays with ECD sources detected by MEG. The nasion and bilateral preauricular points were identified on MRI images with the aid of high contrast cod liver oil capsules (3 mm in diameter).

4.1.6 Statistical analysis

To determine the peak latency and peak amplitude of each MEG component, we used the time course of the amplitude of recorded magnetic fields as measured by the root mean square (RMS). The peak latency of a component meant the latency at which the RMS reached its maximum. The peak amplitude of a component was measured as the RMS at the peak latency. In the analysis using BESA, like a SEF component, the peak latency of a source meant the latency at which the source strength reached its maximum. The paired t-test was used to compare the values of SEF between painful and non-painful sessions. The one-way factorial analysis of variance (ANOVA) followed by Fisher’s protected least significant difference (PLSD) test for multiple comparison was used for the statistical evaluation of the values of SEF while awake, in stage 1 sleep and

in stage 2 sleep. A p value less than 0.05 was considered significant.

4.2 Results

The SEF were recorded successfully in all three conditions, that is, the awake state, stage 1 sleep and stage 2 sleep in eight out of ten subjects. The recording in stage 2 sleep was not successful in one subject, and another subject did not fall asleep.

4.2.1 SEF following non-painful and painful stimulation while awake

In the SEF recording, five consistent components were identified during the awake state from the hemisphere contralateral to the stimulated finger in both non-painful and painful sessions. We termed them 1M, 2M, 3M, 4M, and 5M (Figs. 2 and 3); M means magnetic field. The peak latency and amplitude of each component are shown in Table 1. The peak latency of each component did not differ between the two stimulus conditions. Although 1M, 2M and 3M, which peaked around 20, 35 and 45 ms, respectively, did not differ in amplitude between painful and non-painful stimulation, the amplitudes of 4M and 5M, which peaked around 80 and 160 ms, respectively, were significantly larger in the painful sessions ($P<0.02$).

Correlation values for 1M, 2M and 3M at peak latency were reliably high (>97%), and ECDs for them were located in the posterior bank of the central sulcus, probably the SI, following both non-painful and painful stimulation. The isocontour map of 4M was much different from the maps of 1M-3M, indicating that different mechanisms are involved. The correlation value for 4M was also reliably high (>97%), and the ECD for

4M was localized in the upper bank of the Sylvian fissure, probably in the SII, following both non-painful and painful stimulation (Figs. 2 and 3). Isocontour maps of 5M showed a complicated dipole pattern in both non-painful and painful sessions and the correlation values were lower than 93%. Since the ECD could not be reliably estimated by a single dipole model, 5M was considered to contain two or more sources.

In the hemisphere ipsilateral to the stimulation, two components, 4M(I) and 5M(I) whose latencies were longer than those of 4M and 5M by approximately 10-15 ms, were identified in both stimulus conditions (Fig. 3). Amplitudes of these components were significantly larger for painful stimulation ($P<0.02$) (Table 1). The correlation value for 4M(I) was over 97% following both painful and non-painful stimulation. The ECD for 4M(I) was localized to SII. The correlation value for 5M(I) was relatively low, less than 92%, following both painful and non-painful stimulation, but the ECD of 5M(I) was estimated in SII in all subjects.

4.2.2 Effects of sleep on pain-related SEF using the single ECD model

The amplitude of 1M and 2M was slightly increased and decreased, respectively, during sleep, but these changes did not reach the significant level ($P>0.05$). By contrast, the amplitude of 3M was significantly decreased during sleep ($P<0.05$) (Table 1). For the analysis of 4M and 5M during sleep, it was often very difficult to decide the peak, since they were much decreased in amplitude during sleep. Therefore, we did not measure the peak latency for 4M and 5M, but measured the RMS of 4M and 5M with the same latencies of 4M and 5M during the awake state in such cases. 4M and 5M were significantly smaller in amplitude during sleep (Table 1). The two components

detected in the ipsilateral hemisphere, 4M(I) and 5M(I), were significantly decreased in amplitude ($P<0.005$) during sleep stages in a similar manner to 4M and 5M (Table 1).

4.2.3 Effects of sleep on painful SEF analyzed by BESA

Since both SI and SII were activated following painful electrical stimulation as shown using a single dipole model, at first we made a two-dipole model placing source 1 and 2 at SI and SII, respectively. When the GOF was low in this two-dipole model, we tried to add more sources to improve the model, but, in principle, the smaller the better for the number of sources. In the time range up to 50 msec, it was considered that only S1 was activated from the results of the single dipole model, and the period from the onset of the 4M component to the offset of the 5M component was used as the analysis period for BESA. The GOF value was not reliably high (65.5 ± 25.6%) with the SII source alone, but it markedly and reliably increased when the SI source was added (91.4 ± 1.0%). We could not make a better model than this one. We located the SI source and SII source during sleep in the same region as those while awake, since very good models with high GOF values were obtained by doing so in all subjects. In addition, it was probably the best way to investigate the change of amplitude and latency accurately among each condition. Table 2 shows the strengths and peak latencies of the SII source. The mean x, y, z coordinates for SII source location were 20±9, -43±4 and 70±6, respectively (mean ± SD, mm). Two sequential activities in opposite directions were identified in the SII source. Therefore, we analyzed them separately, and used terms “early component” and “late component” to distinguish the two activities.

The strength of both the early and late components of the SII source was

significantly decreased during sleep ($p<0.001$) (Table 2) (Fig. 4). The source strength of both the early and late components was reduced in stage 2 sleep compared to stage 1 sleep, but the difference was not significant. Peak latencies of early and late components were prolonged along with the stage of sleep, but the prolongation was not significant ($P>0.05$).

The peak latencies of the SI source during the period of analysis differed greatly among subjects, and SI activity did not create consistent components, unlike those in the SII source. However, unexpectedly, SI activity was still strong during the period of the 5M peak, stronger than that at the time of the 4M peak in most subjects (Fig. 4). The inter-individual difference of the changes in the SI source during sleep was large. That is, the activities of the SI source during the analysis period were decreased in five subjects (for example in subject 1, in Fig. 4), whereas they showed no change or were slightly increased in four subjects during sleep (subject 3, in Fig. 6).

4.2.4 Exceptional but interesting cases

Two subjects showed unique but interesting findings. The isocontour map of 4M showed a two-dipole pattern while they were awake, but a typical one-dipole pattern when they were asleep (Fig. 5). BESA revealed that both the SI and SII were activated while awake, but only SI was activated during sleep (Fig. 6). This finding clearly indicated that the SI activity was still present during sleep, but the SII activity disappeared after falling asleep.

4.3 Discussion

Various methods are used to record pain-related SEF such as dental pulp stimulation (Hari et al., 1983), CO_2 gas applied to the nasal mucosa (Huttunen et al., 1986; Hari et al., 1997;), painful electrical stimulation that activates both A-beta and A-delta fibers (Howland et al., 1995; Kitamura et al., 1995; Hoshiyama et al., 1997; Kitamura et al., 1997; Yamasaki et al., 2000; Ninomiya et al., 2001), CO_2 or YAG laser stimulation (Kakigi et al., 1995; Bromm et a., 1996; Kakigi et al., 1996; Watanabe et al., 1998; Ploner et al., 1999; Yamasaki et al., 1999; Kanda et al., 2000; Ploner et al., 2000; Tran et al., 2002), painful impact stimulation (Arendt-Nielsen et al., 1999) and epidermal electrical stimulation (Inui et al., 2002). Each method has its own advantages and disadvantages, but the biggest advantage of painful electrical stimulation used in the present study is that the method is very simple and requires no special equipment. Another advantage is that signals ascending through both fast A-beta fibers relating to touch and slow A-delta fibers relating to pain can be recorded simultaneously. Although it would be difficult to differentiate between the activities, we believe to have been able to clarify this difficult but interesting problem in the present study.

Since the conduction velocity of A-beta fibers and A-delta fibers following the electrical stimulation of skin is approximately 50-60 m/sec and 10-15 m/sec (Naka and Kakigi., 1998), signals ascending through A-beta and A-delta fibers are considered to reach the cerebral cortex approximately 20 msec and 70-100 msec following the stimulation, respectively. Therefore, 1M-3M, whose peak latencies were less than 50 msec and whose ECD were estimated in the SI, should be generated by the signals ascending through A-beta fibers. Since the 1M and 2M were unchanged during sleep, they were considered to be the primary responses. In contrast, the 3M was reduced in

amplitude during sleep. Desmedt et al. (1983) reported that the middle-latency SEP components longer than 40 msec in latency were enhanced in amplitude when using a cognitive task (oddball paradigm). Our finding was compatible with theirs and we believe that the 3M relates to cognitive function to some degree.

The amplitudes of 4M and 5M were significantly larger in the painful than non-painful session. The enhancement of 4M and 5M in amplitude following painful stimulation was largely due to an increase of signals ascending through A-delta fibers. Yamasaki et al. (2000) found no effect of distraction on the magnitude of 4M and 5M following painful electrical stimulation. Mima et al. (1998) used an oddball paradigm asking the subject to pay much closer attention to the stimulation as compared with the paradigm used in our study, and found an enhancement of SII activity. The degree of the enhancement of SII activity in their study was, however, far less than the enhancement in our study. Therefore, attention to the stimulation is less likely to play a major role in the enhancement of 4M and 5M activity in our study.

The mechanisms generating 4M and 5M are considered to be much more complicated than for the early 1M-3M, since signals ascending through A-delta fibers reach the cerebral cortex in this period. Therefore, late activities relating to A-beta fibers and primary activities relating to A-delta fibers should overlap. Therefore, we used a multiple source model, BESA, for analyzing the mechanisms for 4M and 5M. We summarize the findings as follows; (1) The 4M and 5M were recorded for non-painful stimulation, though their amplitudes were not so large. (2) The 4M and 5M should be related to pain to some degree, since they were much enhanced after painful stimulation. (3) Both 4M and 5M following painful stimulation were much reduced in amplitude or disappeared during sleep. (4) Using a single dipole model, the ECD for 4M following

both non-painful and painful stimulation was estimated in SII with a relatively high correlation value, but the ECD for 5M following both non-painful and painful stimulation could not be reliably estimated. (5) Using a multiple dipole model following painful stimulation, both SI and SII were activated at the time of 4M and 5M, but the SI activity was relatively larger in the 5M period. Based on these findings, we made the following hypotheses about the mechanisms generating each component as shown in Table 3.

In principle, we think that both 4M and 5M were generated by a mixture of the following 4 activities;

(A) SII activities ascending through A-beta fibers,

(B) SI activities ascending through A-beta fibers,

(C) SII activities ascending through A-delta fibers,

(D) SI activities ascending through A-delta fibers.

Although there was inter-individual difference, the common mechanisms should be as follows: Following non-painful stimulation, the main contributor for both 4M and 5M was (A). Following painful stimulation while awake, (A), (B), (C) and (D) all contributed to generating both 4M and 5M. The main contributor to the 4M was (C) and the secondary contributor was (A). Therefore, as both (A) and (C) were located in SII, a single dipole model could be adopted for the 4M. However, (B) was still large at the time of 4M and a single dipole model could not be adopted in some subjects (see Figs 4 and 5). The contribution by (D) was small for the 4M. The main and secondary contributor to the 5M was also (C) and (A), respectively, but (D) was relatively large, so that a single dipole model could not be adopted.

SII activities ascending through either A-beta fibers (A) and A-delta fibers (C) and

SI activity ascending through A-delta fibers (D) were much reduced in amplitude or disappeared during sleep. In contrast, SI activity ascending through A-beta fibers (B) was not so reduced in amplitude. Therefore, the ECD of the 4M during sleep in some subjects was estimated in the SI (Fig. 4).

4M(I) and 5M(I) recorded from the hemisphere ipsilateral to the stimulation are generated in SII, and the peak latency was longer than 4M and 5M by approximately 10-15 msec. Therefore, 4M(I) and 5M(I) were considered to be generated by signals transferred from the contralateral SII through the corpus callosum, though it was also possible that they were generated by signals directly from the thalamus.

A problem which must be discussed is whether the location of the activated area in SII following A-beta stimulation is different from that following A-delta stimulation. Frot et al. (2001) reported that non-nociceptive and nociceptive stimulation by stimulating A-beta and A-delta fibers, respectively, activated the same region in SII. Also in our study, one source in SII was enough to explain the SII activities for the period of 4M and 5M. Therefore, we cannot definitely solve this problem in the present study, but we think the same or very close regions in SII are activated by signals ascending through both A-beta and A delta fibers.

All previous studies on pain-related SEF reported activation in SII in the bilateral hemispheres (see reviews by Kakigi et al. 2000a, b), but several recent studies reported activation of SI in the hemisphere contralateral to the stimulation, and that its latency was almost the same as SII activity (Ploner et al., 1999; Kanda et al., 2000; Inui et al., 2002; Tran et al., 2002). Tarkka and Treede (1993) first reported such a finding on analyzing SEP recordings by BESA. Our findings were compatible with theirs. We now speculate that signals ascending through A-delta fibers reach both SI and SII directly

from the thalamus. This is very different from the processing of signals ascending through A-beta fibers in which SI was activated first and then SII. The role of SI in pain perception is yet to be elucidated. We now speculate that SI is mainly responsible for the localization of stimulus or painful sites and the bilateral SII is responsible for the primary processing of pain perception, that is, how painful it is, what kind of painful feeling it is, and so on, but this should be left for a future study.

A pain-related later component, whose peak latency was approximately 250 msec, was reported when the probe was centered at the vertex (Cz position) (Kitamura et al., 1997). However, we could not place the probe at the Cz position when subjects lay on the bed asleep. Therefore, we did not discuss this component in the present report. However, in our previous study on the effects of sleep on pain-related SEP (Wang et al., 2003a), the pain-specific later component, P240, whose peak latency was compatible with the SEF component generated in the cingulate cortex, was much reduced in amplitude or disappeared during sleep. Therefore, pain-specific activities generated in the cingulate cortex were probably much reduced or disappeared during sleep.

5. Painful pure noxious stimulation using a special made needle electrode

5.1 Methods

5.1.1 Subjects

The experiment was performed on ten healthy male volunteers, aged 28-39 years (32.0 ± 3.2). No medication to induce sleep was given to the subjects before the experiment. Informed consent was obtained from all participants prior to the study which was first approved by the Ethical Committee at our Institute.

5.1.2 Noxious stimulation

Intra-epidermal electrical stimulation (ES), a method we recently developed (Inui et al., 2002a, b, 2003) (Fig 7), was used in the present study. In brief, a pushpin-type needle electrode with a needle tip 0.2 mm in length was used. By pressing the electrode plate against the skin gently, the needle tip was inserted adjacent to the nerve endings of the thin myelinated and unmyelinated fibers in the epidermis and superficial part of the dermis. A surface electrode 1.0 cm in diameter was placed on the skin at a distance of 4 cm from the needle electrode as the anode. The electric stimulus was a current constant square wave pulse delivered to the left hand between the first and second metacarpal bones. The intensity ranged 0.1-0.3 mA. The inter-stimulus interval was varied at random between 0.1-0.3 Hz. The stimulus duration was 0.5 ms.

5.1.3 Experimental protocol

First, we obtained data in the awake state. Subjects were instructed to mentally count the number of (Attention) or ignore (Control) the stimuli. Recordings for the two conditions were performed alternately, that is, ten trials of Attention and ten trials of Control were recorded separately as a group. After the collection of data in the awake state, subjects were left to fall asleep. Sleep stage was monitored by the same methods as the first study. Since most subjects did not enter a deep sleep such as stage 3, stage 4 or rapid eye movement (REM) sleep, due to the same reasons shown in the first study, we analyzed SEFs only during stage 1 sleep and stage 2 sleep also in the present study. MEG was recorded in the same methods as the first study. SEF responses were filtered with a 0.1-100 Hz bandpass filter and digitized at a sampling rate of 2083 Hz. The analysis time was 100 ms before and 400 ms after the application of each stimulus. Each session was made up of an average of 60 trials.

5.1.4 Data analysis

First, the root mean square (RMS) of the evoked magnetic fields was calculated at each sampling point in order to compare the amplitude of the response among the four conditions. Second, since several cortical activities following noxious stimulation overlapped temporally, we used the brain electric source analysis (BESA) software package (NeuroScan, Inc, Mclean, VA) for the analysis of theoretical multiple source generators as described elsewhere (Inui et al., 2003). Since the best dipole to explain the

major magnetic component at its peak latency was estimated to be around the sylvian fissure in all cases, we started the analysis with this one-source model. The second source was determined by the distribution of the residual magnetic fields, that is, the magnetic fields that were not explained by the one-source model. We combined the best second source with the first source, and then tried to find the third source, and so on. We continued this procedure until we obtained a GOF value larger than 90%. Magnetic resonance imaging (MRI) was performed using a GE signa 1.5T system. Each source was overlaid on the MRI scans.

5.1.5 Statistical analysis

Data were expressed as the mean ± standard deviation (SD). A paired t-test was used to compare RMS between the control and each task condition every 0.48 ms. A P value less than 0.05 was considered significant. The source strength of each cortical activity was compared among the four conditions using a two-way ANOVA (condition and source as the two factors) followed by Fisher's protected least significant difference (PLSD). A one-way ANOVA was used to examine whether the degree of amplitude change during Attention and Sleep relative to Control was significantly different among each cortical source. P values less than 0.05 were considered significant.

5.2 Results

5.2.1 Waveforms

SEFs were recorded successfully in all four conditions in all the ten subjects. In the recorded magnetic fields, one consistent component was identified in both Attention and Control from each hemisphere. We termed it 1M and 1M (i), recorded from the hemisphere contralateral and ipsilateral to the stimulation (Fig. 8). As shown in Fig. 8, evoked magnetic fields were enhanced in Attention and attenuated in Sleep as compared with Control. Figure 9 depicts the time course of the group-mean RMS. The RMS in Attention was significantly larger than that in Control in both hemispheres at a latency of 130~180 ms (paired t-test, $P<0.05$). During sleep, the RMS around 110~270 ms in stage 1 and stage 2 sleep was significantly smaller than that in Control (paired t-test $p<0.05$).

5.2.2 Results using BESA

In all subjects, sources in the contralateral SI, bilateral SII and insula were identified in Control (Figs. 10 and 11). The peak latencies of the contralateral SI, SII, and insular activities were 152, 149 and 145 ms, respectively, in Control (Table 4), which corresponded approximately to the peak latency of 1M (148 ms). The peak latencies of the ipsilateral SII and insular activities were 159 and 155 ms, respectively, which were longer than the respective latency in the contralateral hemisphere by approximately 10 ms, and also corresponded to the peak latency of 1M (i) (157ms). For magnetic fields later than 1M and 1M (i) components, additional sources in the medial temporal area (MT) and cingulate cortex were necessary. Activation in the ipsilateral MT was identified in all subjects, whereas that in the contralateral MT was found in only two of the subjects. The peak latency of the ipsilateral MT was 193 ms in Control.

Among all ten subjects, activation in the cingulate cortex was identified in five subjects. The peak latency of each source activity is shown in Table 4.

The two-way ANOVA showed a main effect of condition on the source strengths of cortical activities and a significant interaction between the two factors in both hemispheres ($F=12.2$, $P<0.001$ for the contralateral and $F=21.1$, $p<0.001$ for the ipsilateral hemisphere). Fisher's PLSD posthoc test showed that the activities in the contralateral SI, bilateral insula, ipsilateral SII and MT and cingulate cortex were significantly enhanced in Attention as compared with Control ($p<0.05$), whereas the change of the contralateral SII did not reach the significant level (Table 4). As for the cortical responses during sleep, we could not estimate any dipoles for the records during sleep, since magnetic fields during sleep were almost abolished or markedly attenuated in all cases. Therefore, we evaluated the changes during sleep using the model which was determined in Control, assuming that the location and orientation of each dipole did not differ between the Control and Sleep conditions. We considered that such an analysis might reveal the distinct effect of sleep on each cortical activity. All the activities were significantly decreased in sleep stages as compared with Control ($p<0.05$) except for the activity of SI in stage 1 sleep ($p=0.15$) (Table4). The degree of reduction of source strength during sleep did not differ among these cortical sources (one-way ANOVA, $p=0.48$ for Stage 1 and $p=0.96$ for Stage 2). Figure 12 shows the group-averaged waveforms of the time course of each cortical activity in the four conditions and the mean location of each source.

5.3 Discussion

In the first study (Wang et al., 2003b), we reported the effects of sleep on cortical activations following noxious transcutaneous electrical stimulation, and found that pain-related components were significantly reduced in amplitude. However, since that method stimulated not only A-delta fibers but also A-beta fibers, we could not evaluate the pure effects of sleep on pain-specific SEP/SEF components ascending through A-delta fibers. Therefore, this is the first report to investigate changes of cortical activity following pure noxious stimulation during sleep. In this study, we found activations in the contralateral SI, and bilateral SII, insula, MT and cingulate cortex following noxious stimulation confirming our previous results (Inui et al., 2003). Although we could not precisely rate the subjects' attentional levels in the Attention and Control conditions, our results indicated that attentional levels apparently modulated most of the cortical response. All these activities were clearly modulated during sleep, suggesting that they are involved in pain recognition.

The present results showed that the activities generated in insula, cingulate cortex and MT are greater than those in SI or SII. The processing of noxious events has been separated into sensory-discriminative and affective-motivational components in general. SI and SII are involved in the discriminative aspect of pain perception, in which restricted numbers of specific neurons respond to stimuli. For example, SI neurons exclusively respond to stimuli applied to their receptive fields (Chudler et al., 1990; Kenshalo et al., 1988; Lamour et al., 1983). Such a property enables one to discriminate the nature of the stimuli. By contrast, insula, cingulate cortex and MT are involved in the emotional and behavioral aspects of pain perception, in which larger numbers of neurons specific to 'warning' information may be activated by the same stimuli. For example, neurons in the anterior cingulate cortex of humans have been shown to

respond to noxious stimuli applied to various parts of the body (Hutchison et al., 1999). We considered that the difference in source strength between SI and SII on the one hand and insula, MT and cingulate cortex on the other in the present study could be explained by differences in such a response property among these cortical areas.

The involvement of SI in pain processing has been proved by anatomical and physiological studies in animals, as well as functional imaging studies in humans (for review, see Bushnell et al., 1999, and Schnitzler and Ploner, 2000). Since SI nociceptive neurons can encode the location, intensity and duration of a stimulus (Lamour et al., 1983; Kenshalo et al., 1988; Chudler et al., 1990), SI is considered to be primarily involved in the discriminative aspect of pain sensation. However, noxious stimuli-related SI activation is also regulated by cognitive factors (Bushnell et al., 1999). Qiu et al (2004) found that the SI response relating to signals ascending through C-fibers was significantly attenuated during a mental calculation task.

SII responses to noxious stimuli have been identified in a large number of PET and fMRI studies (Casey et al., 1994; Coghill et al., 1994; Davis et al., 1998; Gelnar et al., 1999), as well as in MEG studies (Hari et al., 1983; Kakigi et al., 1995; Watanabe et al., 1998; Ploner et al., 1999b; Kanda et al., 2000). Relative to nociceptive neurons in SI, neurons in SII have larger, bilateral receptive fields and encode the stimulus intensity poorly (Rabinson and Burton, 1980; Dong et al., 1989). In addition, anatomic studies demonstrated that SII projects to the limbic structures (Friedman and Murray, 1986), suggesting that SII is involved in the recognition of nociception. Our first (Wang et al., 2003b) and this study that SII activity was markedly reduced during sleep is consistent with this idea. A study in a patient with cerebral infarction in the postcentral gyrus and the parietal operculum (Ploner et al., 1999a) also supported that SII is involved in pain

recognition.

Activations in the insula to noxious stimuli have been reported in SEF (Inui et al. 2003), SEP (Garcia-Larrea, 1998), intra-cranial SEP (Frot and Mauguière, 2003) and functional imaging (Coghill et al., 1994; Davis et al., 1998) studies. In clinical studies, patients with insular lesions showed reduced feelings of pain and reactions to painful stimuli without changes of pain threshold (Berthier et al., 1988; Greenspan et al., 1999), suggesting that the insula may be involved in the affective and motivational aspects of pain. Our results do not conflict with this notion.

By analyzing the magnetic responses later than 1M and 1M(i), activations in the MT or anterior cingulate cortex were identified. In the present study, activity in the cingulate cortex was found in only five out of ten subjects. This was probably due to the following two reasons: (1) the cingulate cortex is located in a deep region, and (2) the cingulate cortex is usually activated in bilateral hemispheres when a noxious stimulus is applied to part of the body, and these two activities may cancel each other out. Functional imaging studies (Talbot et al., 1991; Rainville et al., 1997) have constantly demonstrated activation in the anterior cingulate cortex during noxious stimulation. In addition, single cell recordings revealed nociceptive neurons in the anterior cingulate cortex in humans (Hutchison et al., 1999). Vaccarino et al (1989) showed that the cingulate cortex was part of the affective component of pain by injecting lidocaine into this region in rats. Unitary recording studies in animals found neurons in the anterior cingulate cortex that were selectively active during pain-avoidance behavior (Koyama et al., 2000), which was consistent with a clinical study (Hurt and Ballantine, 1974) that reported lesions of the cingulate cortex relieved the feeling of persistent pain. In an fMRI study, Sawamoto et al. (2000) reported that activation in the anterior cingulate

cortex was significantly increased even when subjects expected painful laser stimuli. These findings suggest affective or attentional roles of this region in pain perception, which is consistent with the present findings.

Activation in the MT following noxious stimulation in the present study confirmed previous findings in SEF (Watanabe et al., 1998; Inui et al., 2003) and SEP (Valeriani et al., 1996, 2000) studies. In the present study, activation in the ipsilateral MT was identified in all subjects and activation in the contralateral MT in only two subjects, which is consistent with our previous report (Inui et al., 2003). We could not determine whether the discrepancy between the hemispheres came from the dominance of the ipsilateral hemisphere or the dominance of the left hemisphere, since we stimulated the left hand exclusively. However, activities in the contralateral MT (n=2) were increased during Attention and decreased during sleep in a similar manner to activities in the ipsilateral MT, implying a similar function of MT in both hemispheres. Inui et al (2003) found that the time course of activity in MT well corresponded to that of the SEP recorded at the vertex. On the other hand, pain-related vertex potentials were modulated by arousal (Beydoun et al., 1993) and attentional levels (Yamasaki et al., 1999, 2000). Taken together, these findings implied that MT was involved in the attentional and emotional aspect of nociception, which is consistent with the traditional concept that MT is part of the limbic system and involved in the emotional aspect of nociception.

In this study, each cortical activation was equally attenuated during sleep. Although each activation might be decreased equally by different mechanisms, a simple explanation for the the present results is that the similar degree of reduction among all cortical activities was due to inhibition at the stem of the processing flow. Bushnell et al. (1989) reported that nociceptive neurons in the medial thalamus were modulated by

changes in attentional state, supporting this possibility.

6. General discussion and conclusion

In the first study it was established that painful electrical stimulation evoked early activations ascending through A-beta fibers relating to touch and late activations ascending through both A-beta and A-delta fibers. The early activations generated in SI showed no change during sleep. Whereas, the pain-related late activations generated in SI and SII were significantly reduced during sleep. In the second study, ES selectively activated A-delta fibers and evoked activations generated in SI, SII, insula, MT, and cingulate cortex. All of these activities were significantly reduced during sleep. Our studies indicated the different effects of sleep on SI activity and similar effects of sleep on SII activity between noxious and innocuous somatosensory processing. The main role of nociceptive SI neurons may be localizing the stimulated site for quick withdrawal of the body from harm, rather than execution of the very complicated function required in the tactile processing. Therefore, the different effects of sleep on SI activities following noxious and innocuous stimuli might reflect such functional differences of the SI activity. Whereas, SII probably has higher integrative functions for somatosensory perception, and such a role is not necessary during sleep.

Noxious stimuli applied to the skin activate cutaneous-nociceptors. The signals are conveyed through peripheral nociceptive afferents and the spinothalamic tract to reach the thalamus and then cerebral cortices. During sleep, a subject does not feel any pain after receiving the noxious stimuli, indicating the nociceptive pulses may be blocked at certain points along the neural pathway. Our results showed that all cortical activations were significantly reduced during sleep. Bushnell et al (1989) reported that nociceptive neurons in the medial thalamus were modulated by changes in attentional state,

suggesting that changes to cortical activities during sleep or attentional tasks are due to changes in thalamic activities. This hypothesis can explain our results that all activities of the SI, SII, insula, cingulate cortex and MT were decreased during sleep at least in part.

In conclusion, we used MEG to study the effects of sleep on cerebral activities following painful stimulation. Cerebral activities evoked by noxious stimuli were significantly reduced during sleep, suggesting they are involved in pain recognition.

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9. Tables

Table 1. Amplitudes and latencies of SEF components, while awake and in stage 1 and 2 Sleep

		Non-painful	Painful	Painful	Painful
		Awake	Awake	Stage 1	Stage 2
1M	(fT)	31.1 ± 12.2	36.4 ± 15.9	41.7 ± 18.5	39.6 ± 6.1
	(ms)	19.6 ± 0.5	19.3 ± 1.3	19.6 ± 0.9	20.3 ± 1.8
2M	(fT)	42.2 ± 20.4	55.6 ± 24.2	47.5 ± 19.2	52.3 ± 20.0
	(ms)	33.7 ± 5.4	34.0 ± 5.0	34.2 ± 5.2	35.4 ± 5.6
3M	(fT)	44.1 ± 21.2	61.9 ± 24.3	36.1 ± 15.3*	32.2 ± 14.7*
	(ms)	43.3 ± 2.3	43.7 ± 5.6	44.0 ± 2.8	44.3 ± 3.1
4M	(fT)	100.3±47.5¶¶	153.3 ± 62.8	76.4 ± 32.4**	69.7 ± 30.0**
	(ms)	82.0 ± 9.9	86.3 ± 7.6	-	-
5M	(fT)	78.2 ± 33.8¶	119.1 ± 32.8	69.7 ± 28.5**	51.9 ± 15.4**
	(ms)	154.9 ± 22.3	156.2 ± 22.0	-	-
4M(I)	(fT)	65.1 ± 30.6¶¶	103.3 ± 41.6	49.8 ± 20.7**	36.4± 12.0**
	(ms)	92.5 ± 6.4	95.8 ± 5.7	-	-
5M(I)	(fT)	57.0 ± 19.1¶	92.0 ± 28.8	46.4 ± 14.2**	41.1 ± 21.6**
	(ms)	170.2 ± 14.2	172.6 ± 13.3	-	-

¶$p<0.02$, ¶¶$p<0.01$ compared with the results following painful stimulation while awake (Paired t-test). *$p<0.05$, **$p<0.005$ compared with the results following painful stimulation in the awake state (ANOVA followed by post-hoc paired comparisons using Fisher's PLSD procedure). Peak latencies of 4M, 5M, 4M(I) and 5M(I) during sleep are not shown in this table, since they could not be accurately determined in most subjects.

Table 2. Results using BESA

The strengths and peak latencies of components of the SII source while awake, in stage 1 sleep and in stage 2 sleep following painful electrical stimulation.

	Awake	Stage 1	Stage 2
Early-component (nAm)	38.3±16.2	17.0±11.0*	15.4±11.3*
(ms)	84.0±9.6	97.3±13.4	102.4±22.7
Late-component (nAm)	24.1±11.0	6.1±6.8*	3.5±5.5*
(ms)	156.8±14.9	167.0±22.6	165.0±22.0

*$p < 0.001$ compared with the awake state (ANOVA followed by post-hoc paired comparisons using Fisher's PLSD procedure).

Table 3. Cortical activities caused by signals ascending through A-beta and A-delta fibers following painful electrical stimulation, and the changes during sleep stages.

	A-beta				A-delta			
	SI		SII		SI		SII	
	Awake	sleep	awake	sleep	awake	sleep	awake	sleep
1M	+++	+++	–	–	–	–	–	–
2M	+++	+++	–	–	–	–	–	–
3M	+++	++	–	–	–	–	–	–
4M	+	+	+++	–	+	+(?)	++	–
5M	–	–	++	–	++	+(?)	+++	–

+++: very strong; ++: strong; +: weak; –: absent; ?: questionable

Table 4. The source latencies and strengths of each component in the awake state, stage 1 sleep and stage 2 sleep.

		Control	Attention	Stage 1	Stage 2
SI (c)	(ms)	151.6±18.2	146.7±13.3	-	-
	(nAm)	6.6±3.5	11.5±6.0*	3.8±1.6	2.6±1.5*
SII (c)	(ms)	148.7±17.3	142.0±12.1	-	-
	(nAm)	7.0±2.7	10.2±3.0	2.6±1.8*	3.3±2.5*
SII (i)	(ms)	158.6±12.8	156.6±12.8	-	-
	(nAm)	6.2±2.8	13.0±3.2**	2.8±0.7*	3.1±1.5*
Insula (c)	(ms)	144.9±16.8	138.9±15.3	-	-
	(nAm)	17.2±6.5	29.7±12.0**	7.0±2.3*	6.2±5.7*
Insula (i)	(ms)	154.9±14.3	152.3±17.3	-	-
	(nAm)	20.5±8.6	43.9±19.5**	9.0±4.8*	7.8±4.9*
MT (c)	(ms)	186.7±15.4	186.9±13.9	-	-
	(nAm)	96.1±30.5	139.7±53.0	46.1±24.5	47.0±21.2
MT (i)	(ms)	192.6±15.1	190.2±10.2	-	-
	(nAm)	78.2±44.6	138.4±47.3**	20.2±14.3**	19.9±18.6**
Cingulate	(ms)	192.7±16.1	198.1±14.2	-	-
	(nAm)	34.5±15.3	63.2±17.2**	16.8±7.3*	15.3±6.9*

*P<0.05, **P<0.01 compared with Control (Fisher's PLSD procedure). SI and SII, Primary and secondary somatosensory cortex, respectively; MT, medial temporal area; (c) and (i): hemisphere contralateral and ipsilateral to the stimulation, respectively. Since the peak latency of each component was not made entirely clear during sleep in some subjects by reducing the amplitude, we did not calculate the mean and S.D.

10. Figure Legends

Fig. 1. The head-based coordinate system. The origin was the point exactly halfway between the pre-auricular points (PAs). The *x*-axis indicates a line extending through the origin and the nasion, with positive *x* coming out of the head at the nasion. The *z*-axis was a line extending through the origin and the top of the head, with positive values toward the upper side. This axis is perpendicular to the plane formed by the left and right PAs and nasion. The *y*-axis is a line perpendicular to the *x*-axis and *z*-axis extending through the origin and the sides of the head, with positive values toward the left PA. Adopted from Nihashi et al., 2001.

Fig. 2. SEF following painful and non-painful electrical stimulation of the index finger during the awake state, and stage 1 and 2 sleep. Waveforms recorded from 37 channels in the hemisphere contralateral to the stimulation are superimposed. During sleep, 1M and 2M showed no significant change, 3M was reduced in amplitude, and 4M and 5M were much reduced. Isocontour maps of 2M and 4M at the peak latency following painful stimulation while awake. Thin lines show fields directed out of the head, dotted lines into the head, and thick lines the zero fields. Isocontour lines are separated by 20 fT. The ECD of 2M and 4M was estimated in the SI and SII, respectively.

Fig. 3. SEF following painful electrical stimulation of the index finger recorded from the hemisphere contralateral and ipsilateral to the stimulation while awake and in stage 2 sleep. 4M(I) and 5M(I) recorded from the hemisphere ipsilateral to the stimulation were much reduced in amplitude during sleep, like 4M and 5M in the contralateral

hemisphere. Isocontour maps of 4M and 4M(I) at the peak latency following painful stimulation while awake. The ECD of 4M and 4M(I) was estimated in the SII of each hemisphere.

Fig. 4. Multiple source analysis using BESA was applied to the SEF waveforms following painful stimulation in the same subject as shown in Fig. 2. A two-source model (SI and SII) was the most appropriate. The location and orientation of sources were the same while awake, in stage 1 sleep and in stage 2 sleep. Both SI and SII activities were much reduced in amplitude or disappeared during sleep.

Fig. 5. SEF following painful electrical stimulation recorded from the hemisphere contralateral to the stimulation during the awake state, and stage 1 and 2 sleep. This subject showed exceptional but interesting findings. The 4M was not reduced in amplitude much during sleep. The isocontour map of 4M showed a two-dipole pattern in the awake state, but a single dipole pattern while asleep. The dipole of the 4M during stage 1 sleep was estimated in the SI with a high correlation value.

Fig. 6. Multiple source analysis using BESA was applied to the SEF waveforms following painful stimulation in the same subject as shown in Fig. 5. A two-source model (SI and SII) was the most appropriate. During sleep, the activity of the SI source remained, but the activity of the SII source was much reduced or disappeared. The location and orientation of sources were the same while awake, in stage 1 sleep and in stage 2 sleep.

Fig. 7. Picture of a needle electrode (left) and a schematic drawing of its insertion in the epidermis (right). In the most superficial layers, there are only free nerve endings which emerge from subepidermal nerve plexus of the A-delta and C fibers. Encapsulated endings and myelinated A-beta afferents are situated in the deepest papillae of the epidermis or in the deeper structures. Note that the intracutaneous area is enlarged ten times. Adopted from Inui et al., 2002a.

Fig. 8. Magnetic fields following noxious epidermal stimulation of the dorsum of the left hand in the awake state, stage 1 sleep and stage 2 sleep in subject 1. Superimposed waveforms were recorded from 37 channels at positions C4 and C3, corresponding to the hemisphere contralateral and ipsilateral to the stimulated hand. 1M and 1M (i) indicate the first components of the magnetic field in the hemisphere contaralateral and ipsilateral to the stimulation, respectively.

Fig. 9. The group-averaged RMS of all subjects in the four conditions and the paired t-test values at each sampling point between control and each task condition (Attention, Stage 1 and Stage 2). The scale for paired t-test is common logarithm. $P<0.05$ was considered to be significant.

Fig. 10. Time courses of each cortical response in the four conditions in subject 1. Analyzed by BESA. Waveforms obtained in the four conditions are superimposed to clarify the differences in each condition. Note the different vertical scale, 20 nAm for the SI, SII, insula and cingulated, and 50 nAm for the MT.

Fig. 11: Source locations of all components in control are superimposed on MRI. Data from the same subject of figure 10.

Fig. 12. The group-averaged waveforms of each cortical activity in the four conditions and the mean source location. Waveforms obtained in the four conditions are superimposed to clarify the differences in each condition. Since this is the grand-averaged waveform in ten subjects except for cingulated cortex (5 subjects), source locations are shown not on the MRI but in schematic head drawings.

11. Figures

Figure 1

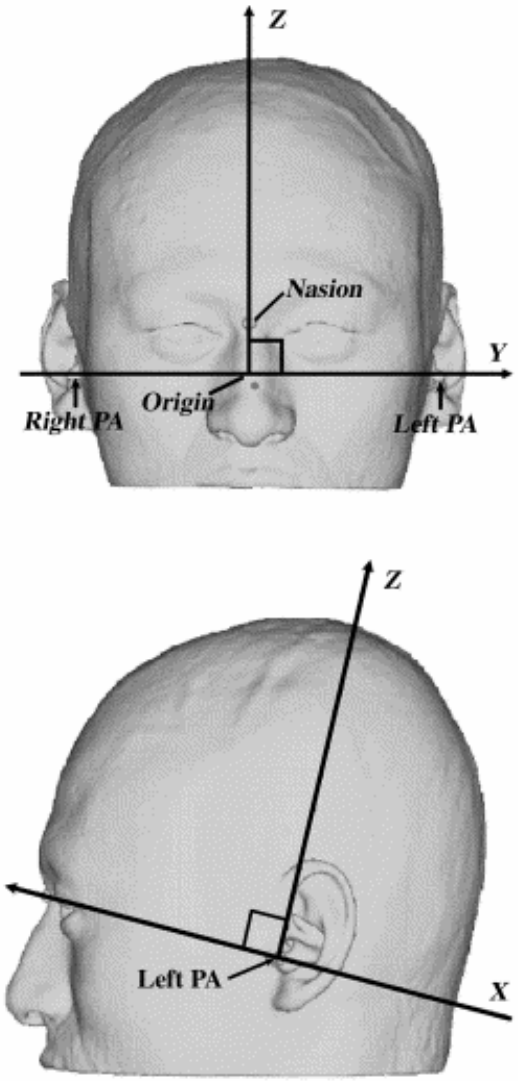


Figure 2

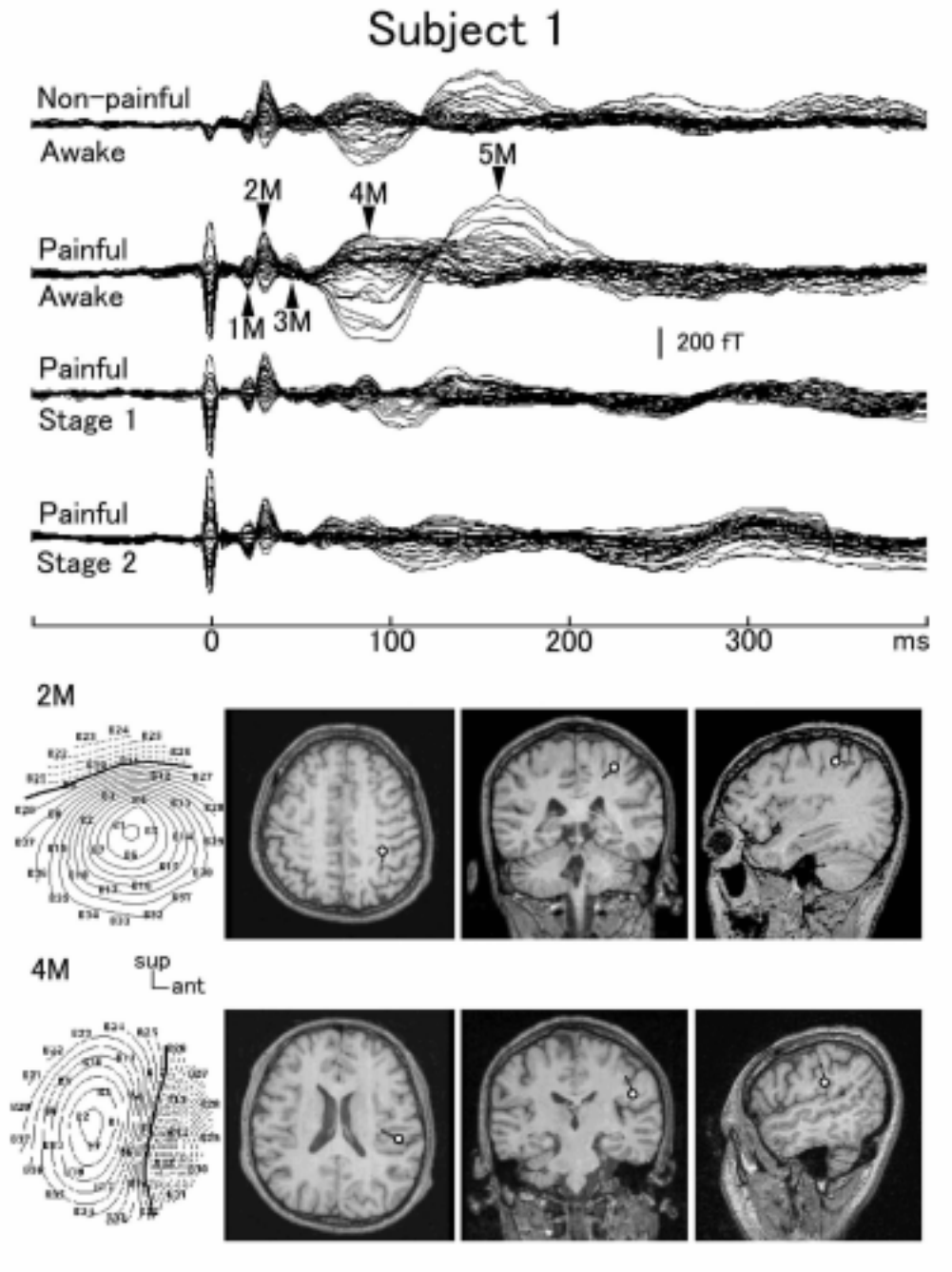

Subject 1
Non-painful
Awake
5M
2M
4M
Painful
Awake
1M
3M
200 fT
Painful
Stage 1
Painful
Stage 2
0
100
200
300
ms
2M
4M
sup
ant

Figure 3

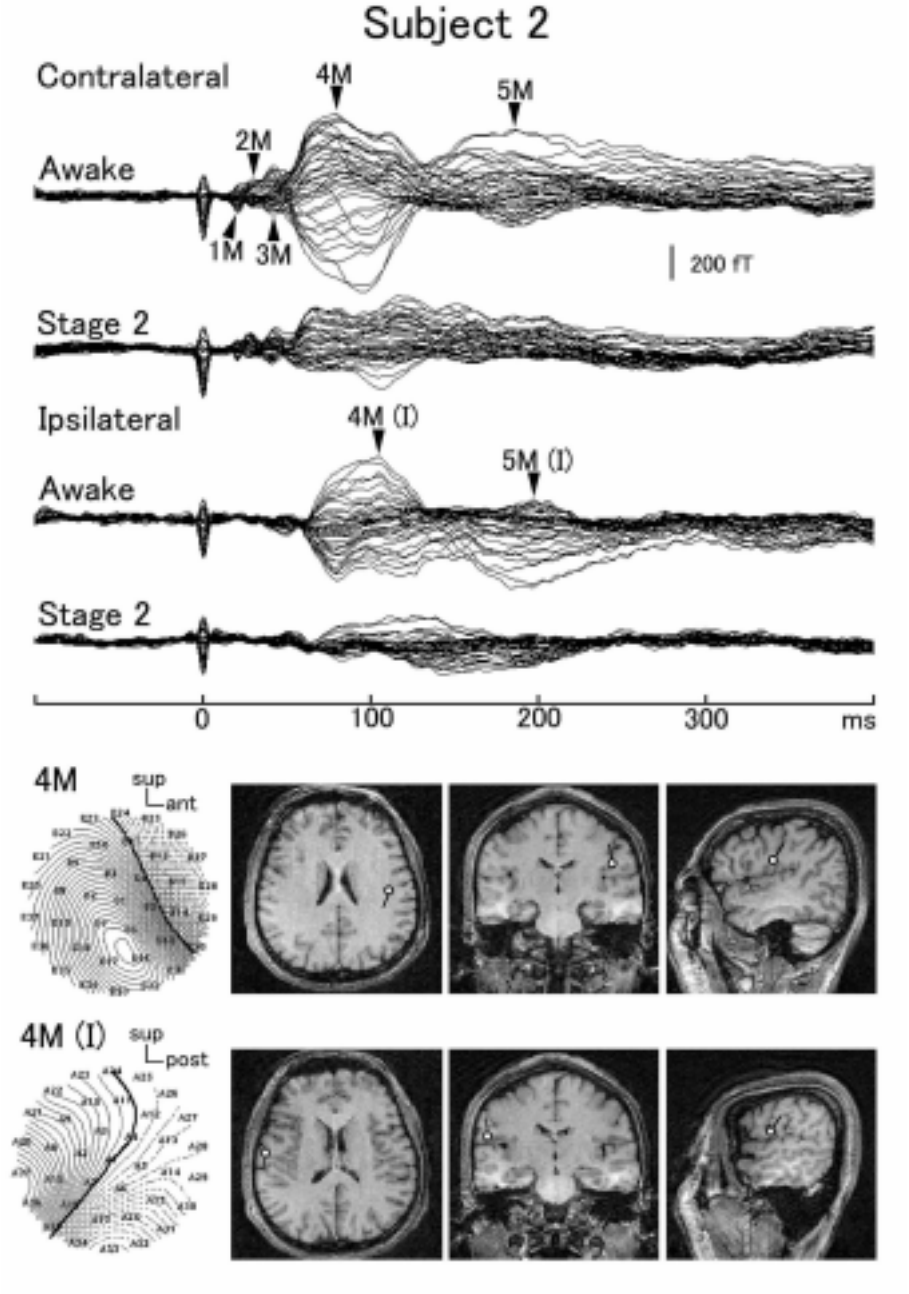

Subject 2
Contralateral
4M
5M
2M
Awake
1M
3M
200 fT
Stage 2
Ipsilateral
4M (I)
5M (I)
Awake
Stage 2
0
100
200
300
ms
4M
sup
ant
4M (I)
sup
post

Figure 4

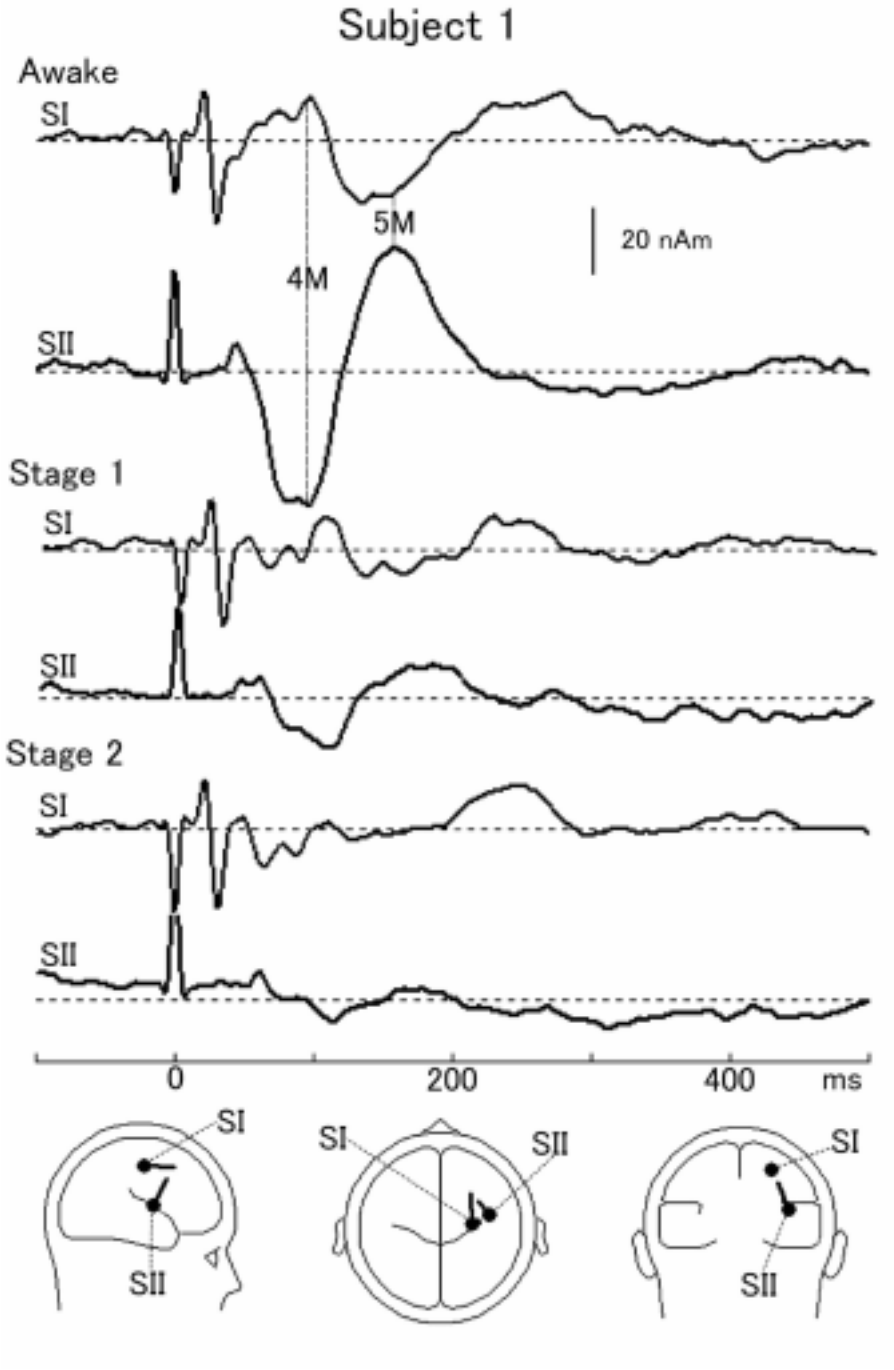

Subject 1
Awake
SI
5M
20 nAm
4M
SII
Stage 1
SI
SII
Stage 2
SI
SII
0
200
400
ms
SI
SII
SI
SII
SI
SII

Figure 5

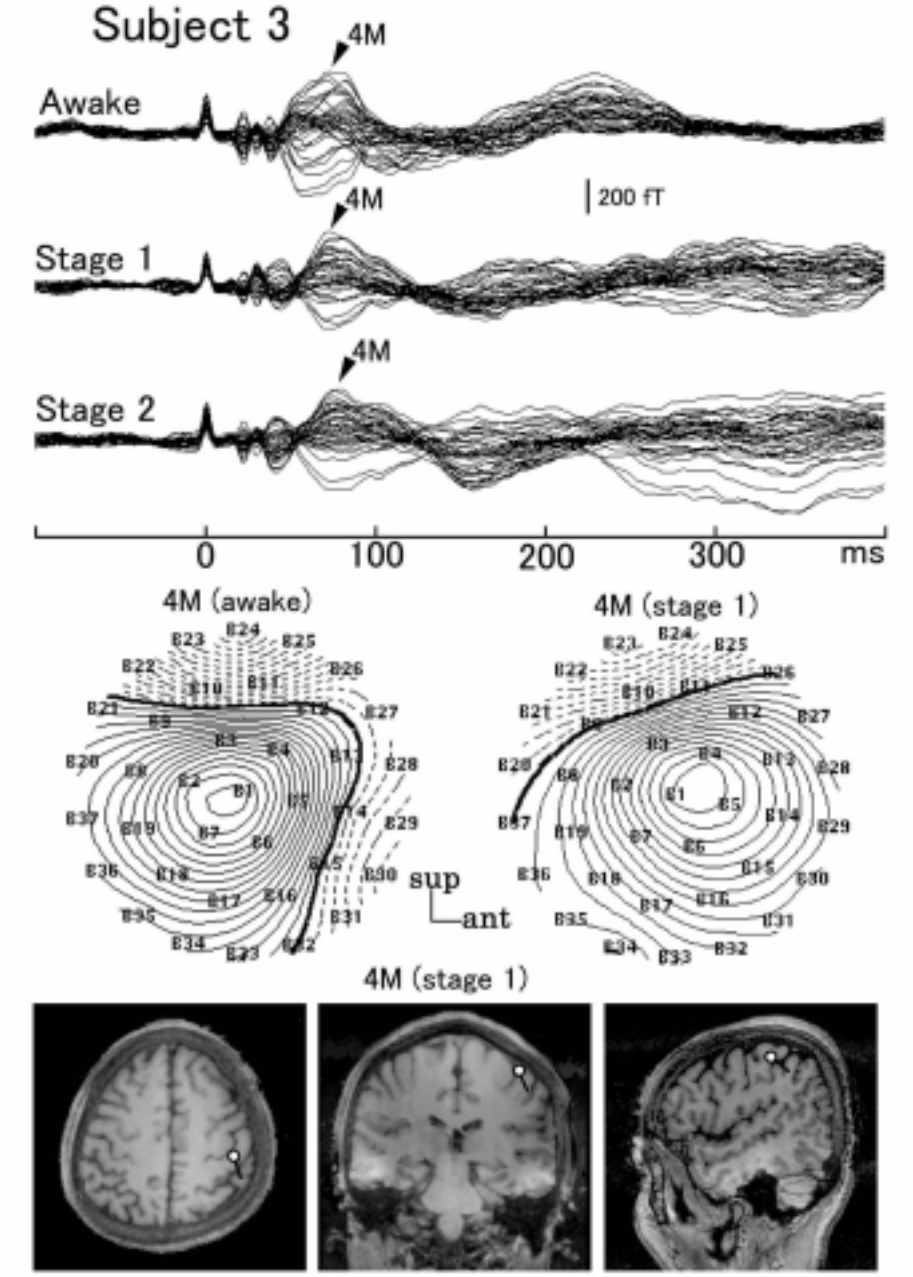

Subject 3
4M
Awake
200 fT
Stage 1
Stage 2
0
100
200
300
ms
4M (awake)
4M (stage 1)
sup
ant
4M (stage 1)

Figure 6

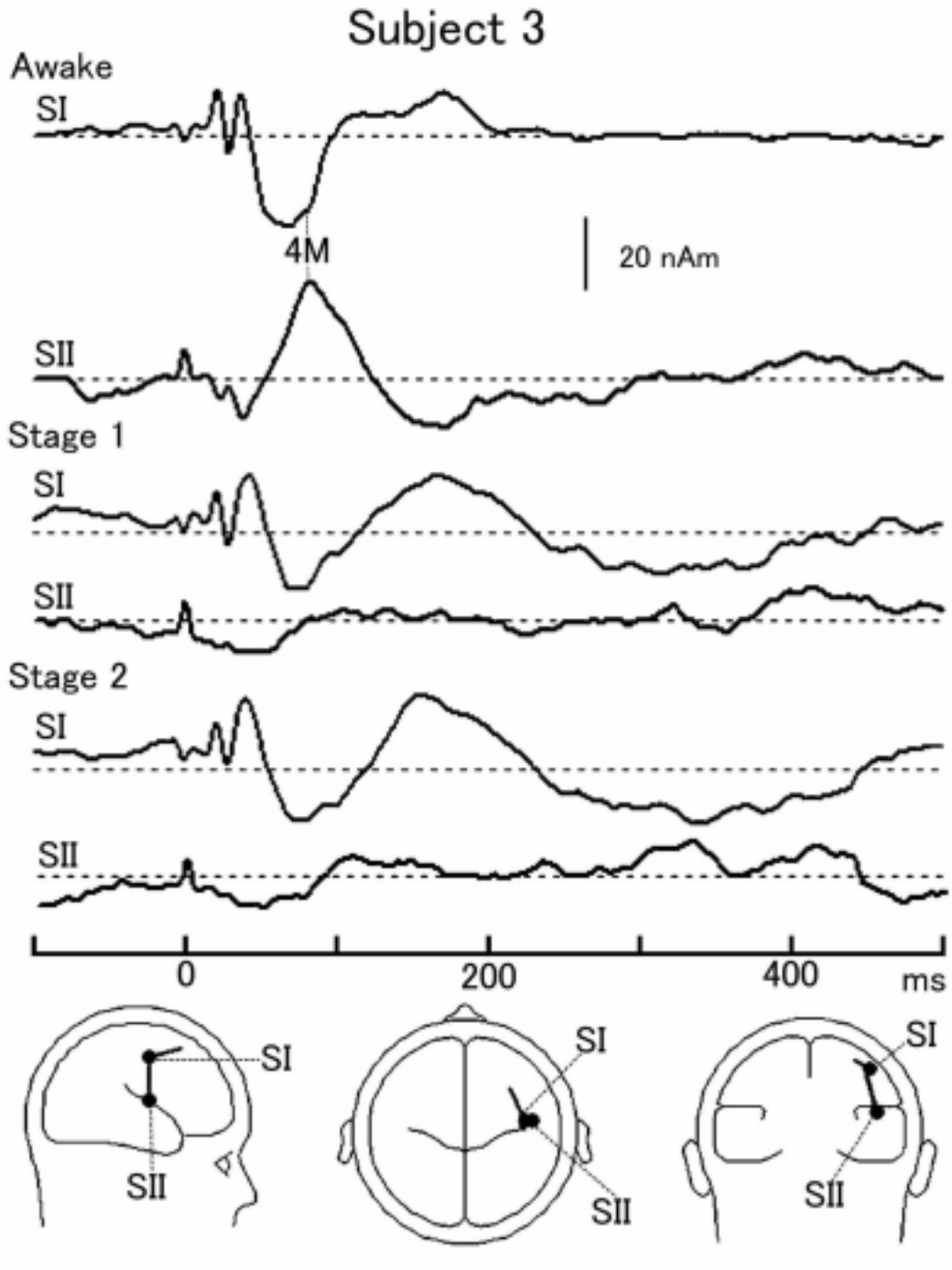

Subject 3
Awake
SI
4M
20 nAm
SII
Stage 1
SI
SII
Stage 2
SI
SII
0
200
400
ms
SI
SII
SI
SII
SI
SII

Figure 7

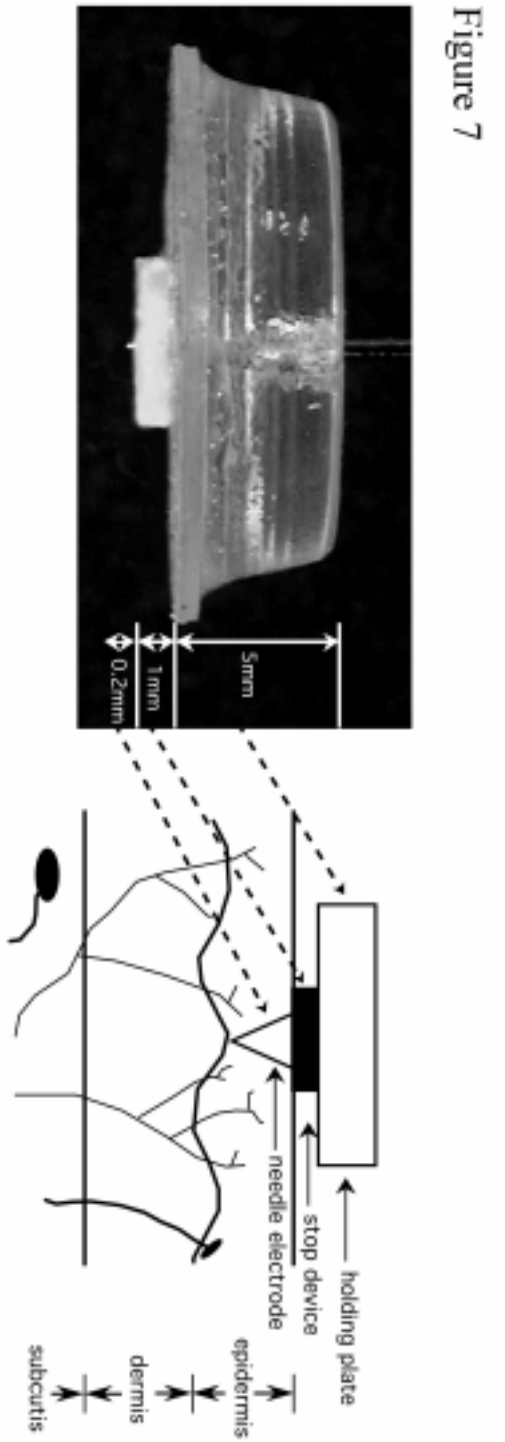

5mm
1mm
0.2mm
holding plate
stop device
needle electrode
epidermis
dermis
subcutis

Figure 8

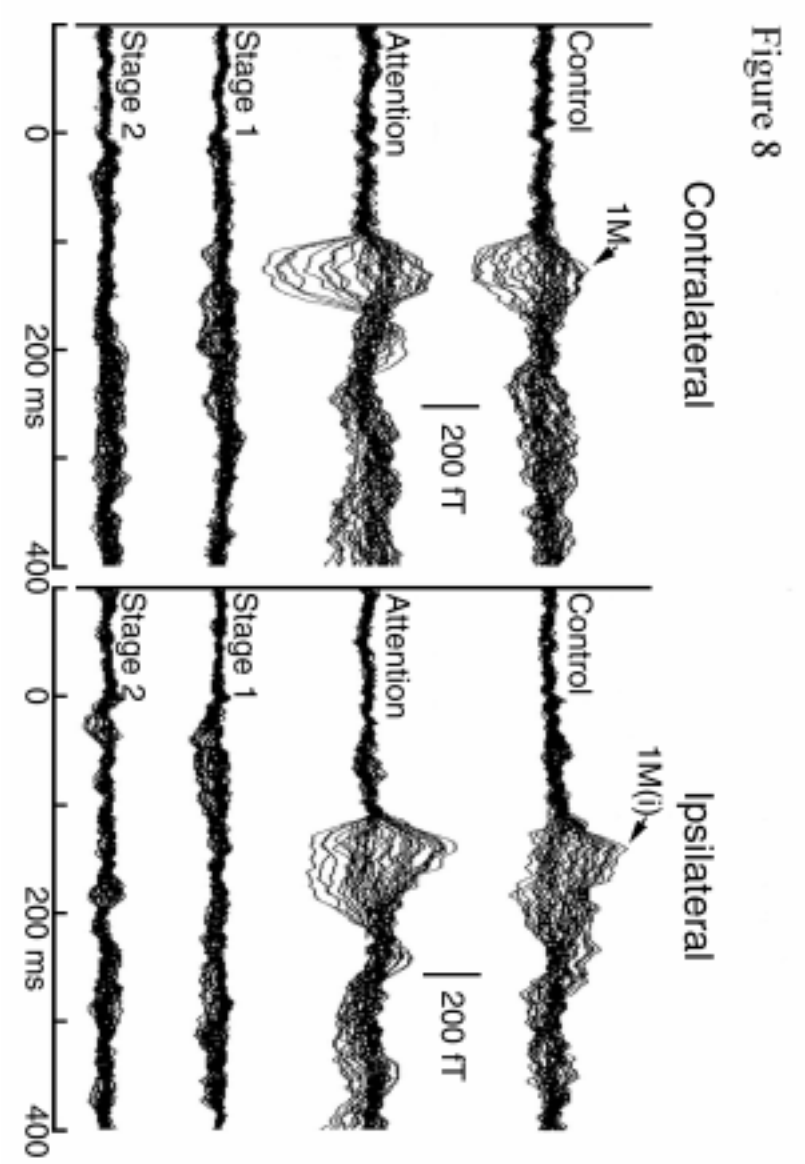

Contralateral
Ipsilateral
Control
Attention
Stage 1
Stage 2
1M
1M(i)
200 fT
0
200 ms
400

Figure 9

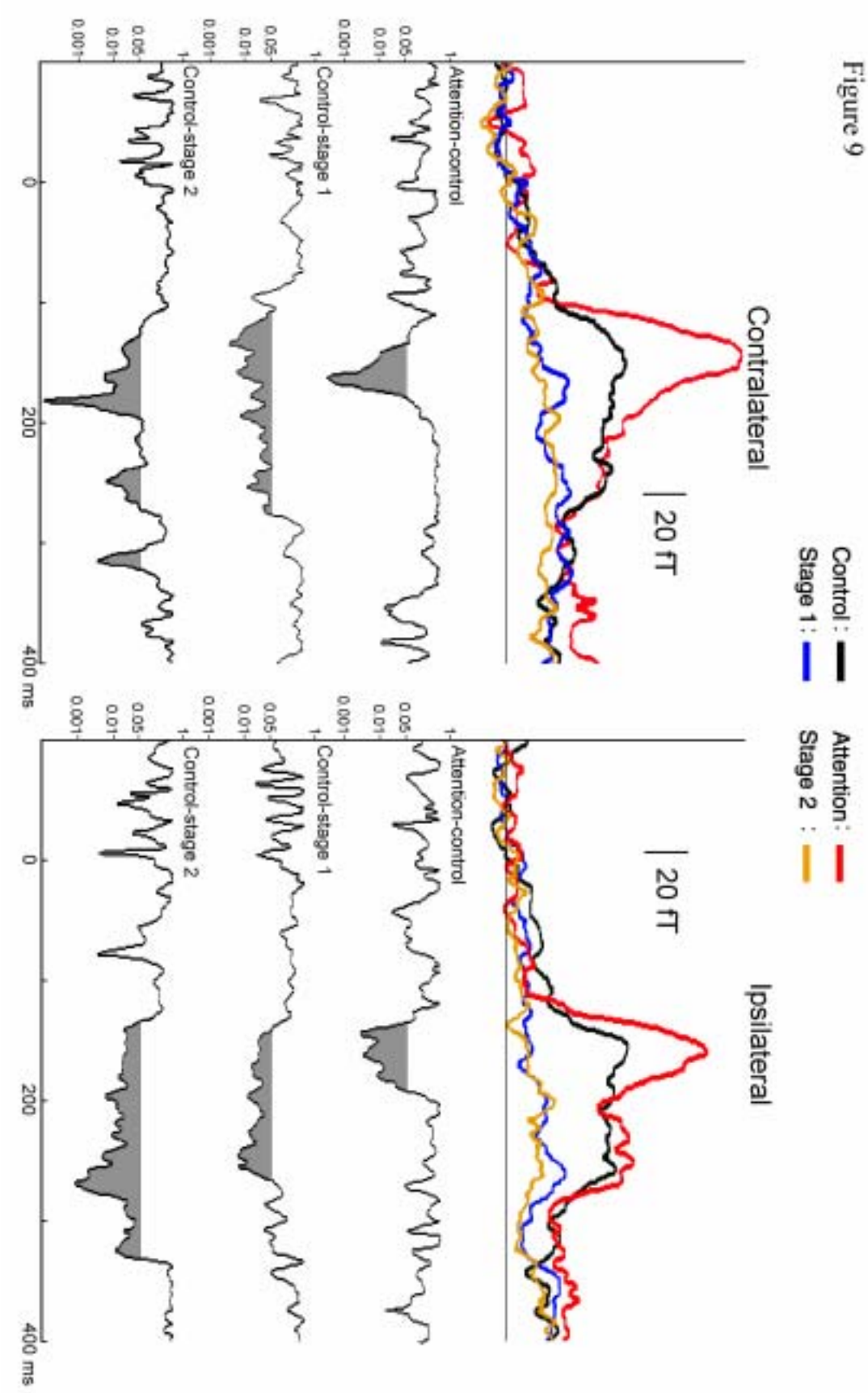

Control :
Stage 1 :
Attention :
Stage 2 :
Contralateral
Ipsilateral
20 fT
Attention-control
Control-stage 1
Control-stage 2
0.05
0.01
0.001
0
200
400 ms

Figure 10

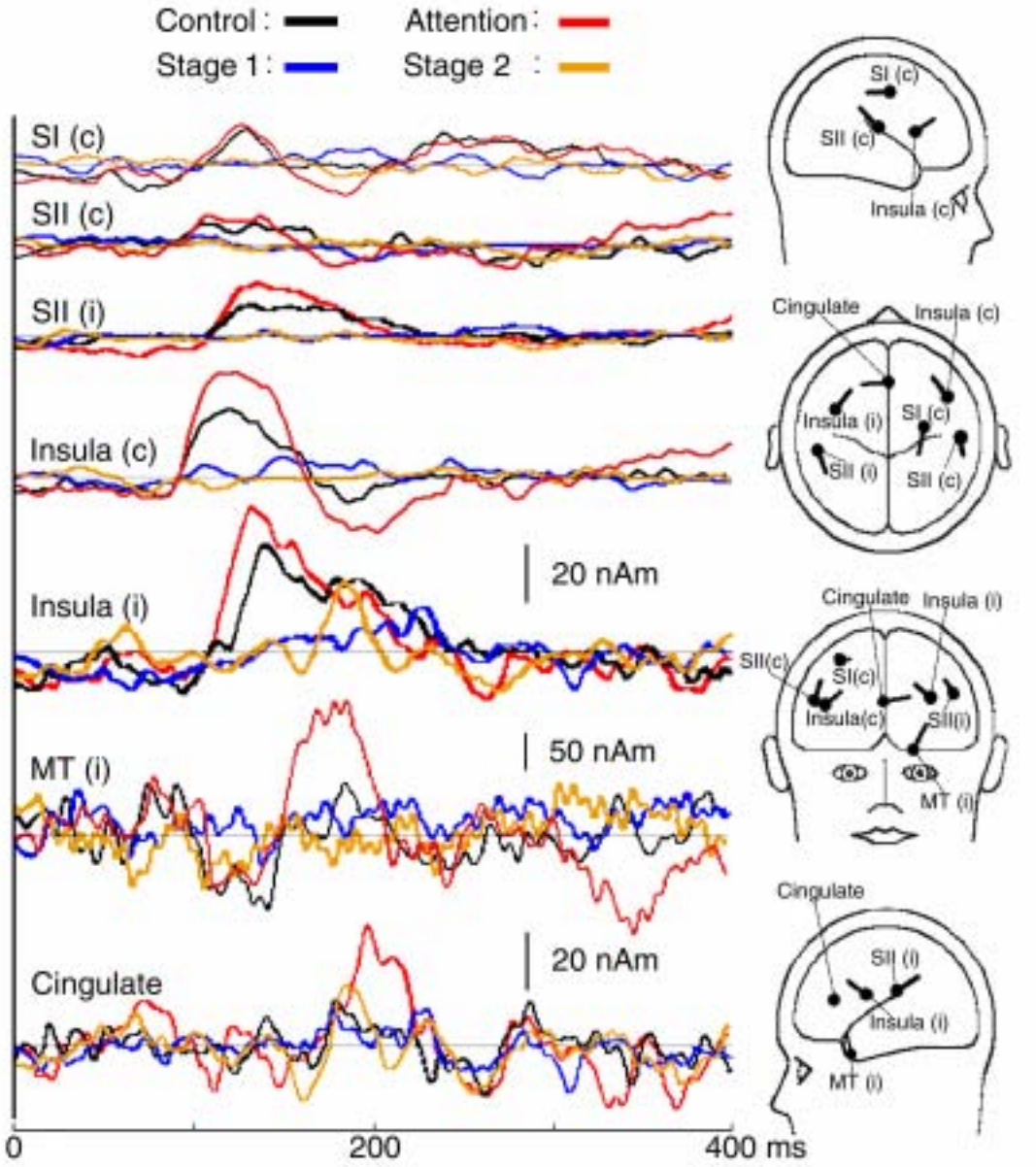

Control :
Attention :
Stage 1 :
Stage 2 :
SI (c)
SII (c)
SII (i)
Insula (c)
Insula (i)
MT (i)
Cingulate
20 nAm
50 nAm
20 nAm
0
200
400 ms
SI (c)
SII (c)
Insula (c)
Cingulate
Insula (c)
Insula (i)
SI (c)
SII (i)
SII (c)
Cingulate
Insula (i)
SII(c)
SI(c)
Insula(c)
SII(i)
MT (i)
Cingulate
SII (i)
Insula (i)
MT (i)

Figure 11

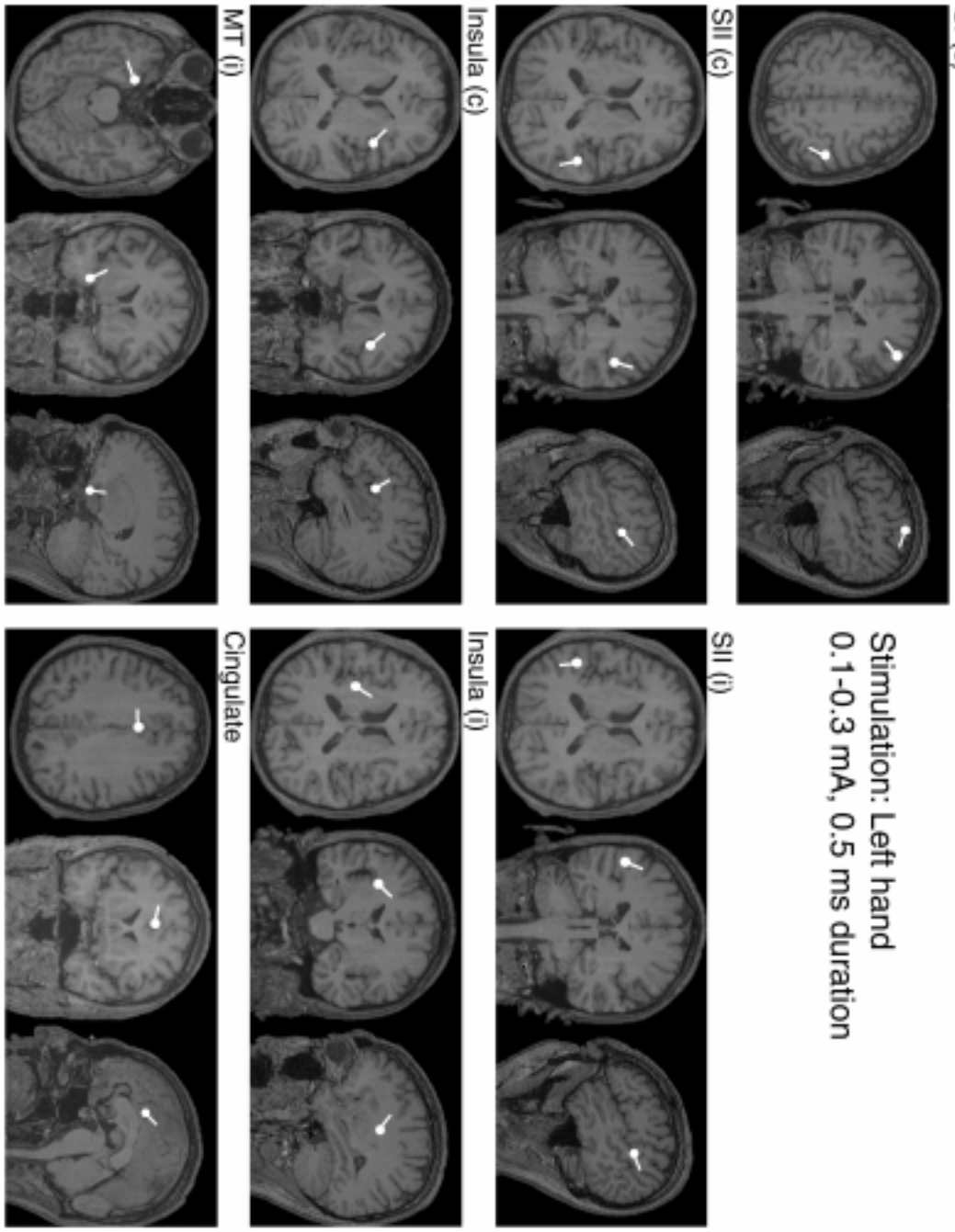
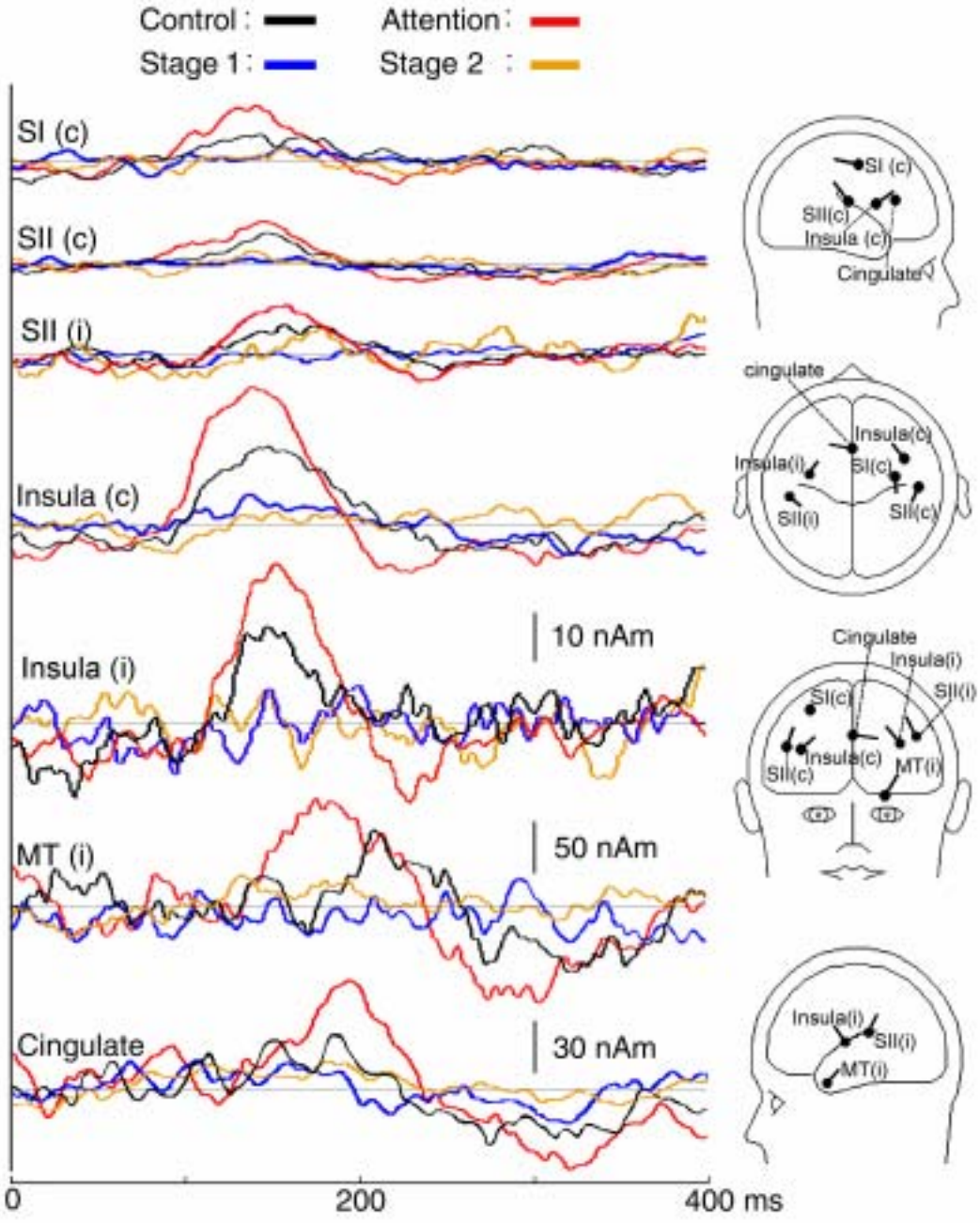

SI (c)
SII (c)
Insula (c)
MT (i)
Stimulation: Left hand
0.1-0.3 mA, 0.5 ms duration
SII (i)
Insula (i)
Cingulate

Figure 12


Control :
Attention :
Stage 1 :
Stage 2 :
SI (c)
SII (c)
SII (i)
Insula (c)
Insula (i)
MT (i)
Cingulate
10 nAm
50 nAm
30 nAm
0
200
400 ms
SI (c)
SII(c)
Insula (c)
Cingulate
cingulate
Insula(c)
Insula(i)
SI(c)
SII(i)
SII(c)
Cingulate
Insula(i)
SI(c)
SII(i)
SII(c)
Insula(c)
MT(i)
Insula(i)
SII(i)
MT(i)

12. Publications

Original articles as first author

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;17:388-399.

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;45:53-57.

Wang X, Inui K, Qiu Y, Kakigi R. Cortical responses to noxious stimuli during sleep. Neuroscience. In revision.

Original articles as co-author

Inui K, **Wang X**, Tamura Y, Kaneoke Y, Kakigi R. Serial processing in the human somatosnesory system. Cerebral Cortex. 2004. In press.

Inui K, **Wang X**, Qiu Y, Nguyen T, Ojima S, Tamura Y, Nakata H, Wasaka T, Tran TD, Kakigi R. Pain processing within the primary somastosensory cortex in humans. Eur J Neurosci. 2003;18:2859-2866.

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

Qiu Y, Fu Q, **Wang X**, Tran TD, Inui, Iwase S, Kakigi R. Microneurographic study of C fiber discharges induced by CO_2 laser stimulation in humans. Neurosci Lett.2003;353:25-28.

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;120:235-248.

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

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

Qiu Y, Inui K, **Wang X**, Tran TD, Kakigi R. Conduction velocity of the spinothalamic tract in humans as assessed by CO_2 laser stimulation of C-fibers. Neuroscience Letters, 2001;311:181-184.

Review articles

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.

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;45:255-275.

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, 54:151-155, 2002.

Proceedings in International Conferences

Wang X, Inui K, Qiu Y, Kakigi R,: Cortical responses to noxious stimuli during sleep. Proceedings of the 4th International Conference on Noninvasive Functional Source Imaging, (Eds:Romani GL, & Pizzella V), (in press).

Wang X, Inui K, Qiu Y, Hoshiyama M, Tran T, Kakigi R. Multiple-source analysis of pan-related somatosensory evoked magnetic fields in humans. In: Haueisen J and Nowak H (Eds.), Proceedings of the 13th International Conference on Biomagnetism, Berlin, pp. 418-420.

Inui K, Tran TD, Qiu Y, **Wang X,** Hoshiyama M, Tran T, Kakigi R. Cortical responses to intra-epidermal electrical stimulation in humans. In: Haueisen J and Nowak H (Eds.), Proceedings of the 13th International Conference on Biomagnetism, Berlin, pp. 415-417.