Activation of fast sleep spindles at the premotor cortex and parietal areas contributes to motor learning: A study using sLORETA

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Abstract

Objective: The present study examined whether slow and/or fast sleep spindles are related to visuomotor learning, by examining the densities of current sleep-spindle activities.

Methods: Participants completed a visuomotor task before and after sleep on the learning night. The task was not performed on the non-learning night. Standard polysomnographic recordings were made. After the amplitudes of slow and fast spindles were calculated, sLORETA was used to localize the source of slow and fast spindles and identify their relationship to motor learning.

Results: Fast spindle amplitude was significantly larger on the learning than on the non-learning nights, especially at the left frontal area. sLORETA analysis revealed that fast spindle activities in the left frontal and left parietal areas were enhanced when a new visuomotor skill was learned. There were no significant learning-dependent changes in slow spindle activity.

Conclusions: Fast spindle activity increases in cortical areas that are involved in learning a new visuomotor skill. The thalamocortical network that underlies the generation of fast spindles may contribute to the synaptic plasticity that occurs during sleep.

Significance: Activity of fast sleep spindles is a possible biomarker of memory deficit.

1. Introduction

Our daily lives constitute a series of highly sophisticated motor actions, the performance of which requires numerous skills. Whenever we start acquiring a new motor skill, we must carefully train ourselves. This continuous and effective training ultimately enables automation of the movement. Presumably, this automation occurs as a result of consolidation and stabilization of the skill in procedural memory (Hikosaka et al., 2000). Recently, numerous reports have indicated that sleep plays an essential role in this memory-consolidation process (Karni et al., 1998; Gais et al., 2000; Stickgold et al., 2001; Fischer et al., 2002; Walker et al., 2002, 2003; Tamaki et al., 2007, 2008). These studies demonstrate that one intervening night of sleep can increase motor skills to an astoundingly high level. Therefore, it is possible that neuroplastic changes occur in the brain, not only during wakefulness, but also during sleep. However, the actual mechanisms underlying this plasticity during sleep have yet to be fully investigated.

Recently, it has been suggested that sleep spindles contribute to the synaptic plasticity that facilitates both learning and memory. Sleep spindles are a major characteristic patterns of electroencephalograms (EEG) observed during non-rapid eye movement (NREM) sleep. Their frequency ranges from 10 to 16 Hz, and continues for at least 0.5 s (Sleep Computing Committee of the Japanese Society of Sleep Research Society, 2001). They mainly appear during Stage 2 of NREM sleep. Some studies have suggested that sleep spindles are related to memory consolidation during sleep (Gais et al., 2002; Clemens et al., 2005; Fogel and Smith, 2006; Schmidt et al., 2006; Tamaki et al., 2008). For example, temporal correlations between hippocampal ripples (140–200 Hz) and cortical

spindles (originating in the prefrontal and somatosensory cortices) have been demonstrated in both mice and rats (Siapas and Wilson, 1998; Sirota et al., 2003). In a study of humans, sleep spindle density was observed to be significantly higher following a paired association task, as compared to a non-memory control task (Gais et al., 2002). Clemens et al. (2005) demonstrated that overnight verbal memory retention after a face-name association test was highly correlated with the number of sleep spindles. Finally, Fogel and Smith (2006) demonstrated that spindle (12–16 Hz) density (the number of spindles that occurred in 1 min.) increased after participants learned four types of motor tasks.

Sleep spindles have been classified into two types. The first are the slow spindles, which have a frequency of approximately 12 Hz, and predominantly distributed in the frontocentral area of the scalp. The second are the fast spindles, which have a frequency of approximately 14 Hz and predominantly distributed in the centroparietal area of the scalp (Jobert et al., 1992; Werth et al., 1997; De Gennaro et al., 2000). Although the topological distribution differs between fast and slow spindles, they can both be observed extensively, throughout the surface of the head. Also, the incidence of these two types of spindles differs during nightly sleep. Slow spindles appear predominantly in the early part of sleep, and fast spindles generally appear later.

The two types of sleep spindles may play different roles in the memory consolidation process. Walker et al. (2002) used a finger-taping task and showed a significant relationship between task improvement and the amount of NREM Stage 2 (r=.66). A particularly significant relationship was found in the fourth cycle of NREM Stage 2 (r=.72), when data were divided into each sleep cycle. Fast spindles were predominant in comparison to slow spindles during this period.

Tamaki et al. (2008) used a visuomotor task to demonstrate that fast sleep spindle activity is correlated with improvements in motor skills. Fast spindles had a greater amplitude and a longer duration on the learning night than during the non-training control night. Furthermore, a significant relationship was found between skill improvement and fast spindle activity with regard to density, amplitude, and duration. In contrast to these findings, no significant relationships were observed between the improvement of motor skills and slow spindle activity. Therefore, between the two types of sleep spindles, fast spindles may be closely involved in motor-memory consolidation. However, the brain location where neural plasticity related to the activation of fast spindles occurs, remains to be clarified.

It is possible to demonstrate the contribution of spindle activity in neural plasticity by analyzing the current density and source localization of the spindle generators. Standardized low-resolution brain electromagnetic tomography (sLORETA) could provide one approach to such a demonstration. sLORETA is a functional source imaging method based on certain electrophysiological and neuroanatomical constraints (Pascual-Marqui, 2002) and has been identified as an efficient functional mapping tool, as it is both consistent with physiology and capable of correct localization (Anderer et al., 2001). The sLORETA approach was developed to reduce the speculation error that is observed in low-resolution brain electromagnetic tomography (LORETA) (Pascual-Marqui et al., 1994; Pascual-Marqui, 2002) to zero, and this has been mathematically confirmed (Greenblatt et al., 2005; Sekihara et al., 2005). Using these efficient tools, functional sources have been demonstrated in many basic and clinical EEG and ERP studies (see Anderer et al., 1998; Brandeis et al., 1998; Strik et al., 1998;

Babiloni et al., 2006; Zumsteg et al., 2006; Gianotti et al., 2007; Rodriguez et al., 2007). With regard to EEG activity during sleep, Anderer et al. (2001) analyzed scalp-recorded sleep spindles via LORETA, showing that different activated cortical areas are associated with the two types of spindles.

We generated the following hypotheses: (1) fast spindle activity is enhanced after learning a skill, as opposed to when no skill is learned, (2) The activation of fast spindle activity is observed most strongly at the scalp sites that overlie brain regions involved in consolidating visuomotor skills, (3) localization of the source of enhanced spindle generators are estimated as being in the neocortical areas, which are involved in consolidating visuomotor skills, and (4) no significant learning-associated activation will be observed for slow spindle activity. Spindle activities were measured and compared between learning and non-learning nights. A visuomotor task was performed before and after nocturnal sleep on the learning night. The amplitudes of fast and slow spindles were measured and the amplitudes during the non-learning night were subtracted from those during the learning night. In order to discriminate learning effects from the sleep-initiation process, subtracted amplitudes were used in sLORETA analysis for detecting learning-related spindle activation.

2. Methods

2.1. Participants

Ten healthy student volunteers (7 females and 3 males; mean 22.0 years) participated in the study. None of the volunteers had physical or psychiatric diseases that required concurrent medical treatment, and none were suspected of suffering from sleep disorders. After the purpose and the procedure of the study were explained to them, all the participants provided written informed consent for

participating in the study. All the participants were also informed that they were free to discontinue the experiment at any time.

Prior to the experiment, participants completed a questionnaire that assessed their sleep-wake habits, including usual sleeping and waking times, phases of circadian activity cycle, regularity of sleep habits, napping, sleep complaints, and regularity of lifestyle (e.g., mealtimes). They also completed a Morningness-Eveningness questionnaire (Horne and Östberg, 1976), as well as questionnaires designed to measure physical and psychiatric health and sleep conditions.

All participants had regular sleep-wake cycles and slept for 6–9 hours daily. None of them took regular naps, drank alcoholic beverages before going to sleep, smoked cigarettes, or were taking any prescription medications from one month prior to the experiment. Finally, all participants were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), and possessed normal or corrected-to-normal vision.

2.2. Stimuli and Apparatus

We used a five-pointed star and 6 irregular figures as the stimuli. The star was drawn using 2 lines that were 3 mm apart, forming a path. The perimeter of the star was 400 mm in total, with each of the 10 angles being 50-mm apart. Similarly, each of the 6 irregular figures was drawn using 2 lines that were 3 mm apart. Figure perimeters were 600 mm, and each possessed 11 angles spaced at 50-mm intervals. These figures were made from basal plates. A test lead was used as a stylus to trace the paths, which made it possible for an electric current to pass when they were contacted by the stylus. Each participant's right hand was placed out of view by covering it with an open-ended box (width, 300 mm; height,

280 mm; depth, 210 mm) with an 85-mm closed-circuit camera (Akizuki Denshi Tsusho, Saitama, Japan) mounted on top. Stimuli were presented under the box, and images were projected on a 14-inch display (KV-14AF1; Sony, Tokyo, Japan) placed in front of the participants. Turning the camera could rotate the view.

2.3. Task

A modified version of the classic mirror-tracing task was used. Stimuli were presented in front of the participant's hands that were covered with a box. The participants kept their eyes closed during the preparation of the stimuli, so that they could not directly see the stimuli. They were asked to trace the figures projected on the display, from start to the finish, as quickly as possible by using the stylus. They were advised to stay within the path without looking at their hands. During the pre- and post-sleep sessions, the experimenter gave the starting signal ("Ready, set, go") through a microphone in the console room. The star (Fig. 1 A) and the 6 irregular figures (Fig. 1 B) were alternately presented 6 times each, for a total of 12 presentations. The 6 irregular figures were presented pseudorandomly, with each figure visually rotated 90° clockwise (rotated image), while the visual image of the star was not manipulated (non-rotated image). The order of the images was counterbalanced across both participants and sessions (pre-sleep/post-sleep). The duration of each session was approximately 30 min.

2.4. Procedure

Starting 1 week prior to the experiment (the preparation week), participants were instructed to maintain their sleep-wake habits; such as their usual schedule of waking and sleeping, as well as their normal sleep duration. During this time, they were also instructed to refrain from excessive alcohol consumption, physical exercise (apart from their usual activities), and napping.

Their sleep-wake habits were monitored with a self-reported sleep log in order to ensure that the above-described preparatory procedures were closely followed throughout this week.

On the day before the experiment, participants were instructed to refrain from consuming alcohol and caffeine, from performing unusual physical or psychological exercises, and from napping. Standard polysomnographic (PSG) recordings were conducted once during three nights for each of the following three conditions: Adaptation, control non-learning, and learning. Adaptation night was established in consideration of the *first night effect* (Agnew et al., 1966; Tamaki et al., 2005). This refers to the distortion in sleep patterns that is seen during the first night of sleep in a laboratory. On the learning night, participants completed the motor task one hour before retiring (pre-sleep session) and the next morning one hour after they woke up (post-sleep session). We measured tiredness and sleepiness before each session by using 100-mm Visual Analogue Scales (VAS; range 0 to 100, 0 corresponds to no tiredness or sleepiness, 100 corresponds to the strongest tiredness or sleepiness). These sessions were not performed on the adaptation and non-learning nights. The order of the learning and non-learning nights was counterbalanced across the participants, intervening one week or more. Considering variations in biological rhythms, retiring and waking times were individualized according to each participant's routine.

2.5. Polysomnograph Recordings

By using Ag/AgCl electrodes (EEG 1000/9000 ver.03-11, Nihon Koden Inc.), EEG was recorded at 28 scalp sites (Fp1, Fp2, F3, F4, FC3, FC4, C3, C4, P3, P4, O1, O2, F7, F8, FT7, FT8, T7, T8, P7, P8, Fpz, AFz, Fz, FCz, Cz, Pz, POz, and Oz) according to the 10-20 system (American Electroencephalographic Society, 1991).

The scalp electrodes were fixed with collodion. The system reference (mean amplitude between C3 and C4) was used for recording. The data were re-referenced offline to the linked earlobes. Electrooculograms (EOGs) were recorded at 4 electrodes: 2 were placed at the outer canthi of both eyes and 2 were placed 1.5 cm superior and inferior to the left eye. A bipolar submental electromyogram (EMG) was also recorded. Electrode impedance was maintained below 5 kΩ, and the sampling rate was 500 Hz. The time constants for each recording were as follows: 0.3 s for the EEG, 5.0 s for the EOG, and 0.003 s for the EMG. The low-pass filter was set at 120 Hz.

2.6. Data Analysis

Perceptual motor task. Motor skill performance was quantified as the time taken to trace each rotated image from the start to the goal (tracing time) and the number of times that the stylus deviated from the path of the figures (error rate). The first of the 6 trials in each session was considered to be an adaptation trial and was therefore excluded from the analyses. Performance in the last trial of the pre-sleep session (5th trial) and that of the trial after the adaptation trial (1st trial) were compared. Improvement in motor-task performance was assessed by dividing the performance score of the 1st trial of the post-sleep session by that of the 5th trial of the pre-sleep session for the rotated image [skill improvement: (100 – 1st trial in the post-sleep session/5th trial in the pre-sleep session × 100)]. Performance (tracing time and error rate) was automatically measured by detecting the electrical signal (BASIC; see *2.2. Stimuli and Apparatus*).

Sleep-stage scoring and variables. Sleep-stage scoring was completed according to Rechtschaffen and Kales' (1968) criteria and the supplements and amendments of the Sleep Computing Committee of the Japanese Society of Sleep

Research (JSSR, 2001). The sleep stages indicated in the EEGs were manually scored for every 20 s epoch. EEG recordings from the C3 electrode were used for this scoring; if they were contaminated by artifacts, C4 recordings were used instead. The same scalp area was used for scoring across individuals and across nights. Sleep efficiency (total sleep time (TST) / time in bed × 100), latency to sleep onset, and the percentage of time spent in each sleep stage (% TST) were calculated for each participant. Sleep onset was defined as the time between lights-off and NREM Stage 2 and in all cases sleep onset continued for at least 2 min.

Sleep Spindles. The three sites at the frontal pole (Fpz, Fp1, and Fp2) were excluded from further analysis, due to the artifacts that were derived from background EEGs. For example, K-complexes ($\geq$ 200μV, $\geq$ 0.5sec) and slow waves ($\leq$ 2Hz, $\geq$ 75μV) are partially recognized during NREM Stage 2 (Rechtschaffen and Kales, 1968; JSSR, 2001). Measures of sleep spindle activity at the frontal pole could be contaminated by these extraneous activities. Therefore, the remaining 25 sites were used (F3, F4, FC3, FC4, C3, C4, P3, P4, O1, O2, F7, F8, FT7, FT8, T7, T8, P7, P8, AFz, Fz, FCz, Cz, Pz, POz, and Oz). On the learning and non-learning nights, the fast and slow spindles that appeared during NREM Stage 2 were detected, measured, and analyzed automatically, and confirmed visually. A Butterworth-type zero-phase digital filter (10–16 Hz: Matlab version 6.5, The MathWorks) was applied to the EEG recordings during NREM Stage 2 (Fig. 2 A, B). The spindles were then classified into fast and slow types as described in previous studies (Werth et al., 1997; De Gennaro et al., 2000; Anderer et al., 2001; Tamaki et al., 2008). Slow spindles were classified as those with frequencies greater than 10 Hz and less than 13 Hz, with a predominant scalp distribution in

the frontal regions (Fz > Pz). Fast spindles were classified as those with a frequency greater than or equal to 13 Hz, with a predominant scalp distribution in the parietal regions (Pz > Fz). Spindle epochs with no artifacts or extraneous EEG activities (such as K-complexes and slow waves) were extracted, for further analysis of each spindle. This extraction procedure was concluded when first 20 artifact-free epochs were derived. Amplitudes of positive five waves (including the peak) were calculated for each epoch (see Fig. 2 C). The 100 samples (20 epochs × 5 waves) for amplitude were averaged for each site. This figure was log-transformed for each condition to control the variance. To detect the changes in sleep-spindle activity associated with learning, the difference in amplitudes between the learning and non-learning nights (learning − non-learning) was calculated for both slow and fast spindles (subtracted amplitude). Bandpass filtering and spindle detection was performed by Matlab version 6.5 (The MathWorks). Analysis of amplitude was completed using Bimutas® 2 (KISSEI COMTEC, Nagano, Japan).

sLORETA. sLORETA (The KEY Institute for Brain-Mind Research, Zurich; Pascual-Marqui, 2002) was used on the subtracted amplitudes to estimate the three-dimensional distribution of electrical activity (the current density) of sleep spindles. The cortex has been modeled as a collection of volume elements (voxels) in the digitized atlas provided by the Brain Imaging Center, Montreal Neurological Institute (MNI; Talairach and Tournoux, 1988). The sLORETA algorithm solves the inverse problem by assuming related orientations and strengths of neighboring neuronal sources (represented by adjacent voxels). Regarding the technical details for the sLORETA procedure, the MNI brain volume was scanned at 5-mm resolution. Then, MNI coordinates were converted

to “corrected” Talailach coordinates, and were finally given to the Talailach Daemon. Voxels that were unambiguously labeled as cortical gray matter, and that were unambiguously within the brain compartment, were retained. Therefore, sLORETA’ s solution space is restricted to cortical and hippocampal gray matter whose images represent the power in 6239 voxels, with a spatial resolution of 5 mm. The digitized structural MRI template selected for brain imaging in the present study was Colin 27 (Holmes et al., 1998). The magnitude of computed sLORETA activity (current density) was calculated from subtracted amplitudes of the fast spindles at 25 scalp sites for each participant. More detailed information on sLORETA can be found in the paper of Pascual-Marqui (2002).

2.7. Statistical Analysis

To examine the effect of sleep on the improvement of motor skills, two-tailed t-tests were performed on performance data (tracing time and error rate) across the sessions (pre-sleep/post-sleep). To examine the differences in subjective states before the sessions, two-tailed t-tests were conducted on the scores of VAS (both tiredness and sleepiness). To examine the differences between the scores obtained from the questionnaire and the sleep log, two-tailed t-tests were conducted on sleep duration. To examine the effects of learning on the activity of sleep spindles, a one-way ANOVA was performed on the amplitudes of each spindle across the conditions (learning/non-learning) for each site. Post-hoc tests were performed as multiple comparisons using Shaffer’s modified sequentially rejective multiple test procedure (Shaffer, 1986). This is an improved version of Holm’s sequentially rejective Bonferroni procedure (Holm, 1979), and was used to obtain an increase in power. To examine the increment in spindle

activity related to motor learning, the sLORETA image for subtracted amplitude (learning − non-learning) was compared with the baseline (i.e., 0 μV) using a paired *t*-test. A randomization test of statistical non-parametric mapping (SnPM) was used to correct for use of multiple tests (Nichols and Holmes, 2002).

3. Results

One of the participants did not improve following an intervening night of sleep, and the spindles of another participant were too few to be analyzed, so these data were excluded from the analyses reported below. The final remaining participants were 8 healthy students (7 females and 1 males, mean 21.8 years old).

The sleep-wake habits obtained from the questionnaire are shown in Table 1. According to the sleep log, the average retiring time was 0:46, the average time of final awakening was 8:02, and the average TST was 7.3±0.29 hours. There was no significant difference between the scores of the questionnaire and the sleep log (paired t-test for TST: $t = 1.74$, $p = 0.12$).

3.1. Sleep Variables

During the learning and non-learning nights, the latency to sleep onset was less than 15 min, the sleep efficiency was greater than 98%, and the wake time after sleep onset was under 5 min. Percentages of slow wave sleep, including NREM sleep Stages 3 and 4, were greater than 15%, and those of REM sleep were over 20%. For both the learning and the non-learning nights, no distortion in sleep structure was detected by observation of sleep hypnograms. One-way ANOVAs conducted on each of the variables did not show any significant differences: learning (%; mean ± S.E.): NREM1, 7.7 ± 1.22; NREM2, 48.6 ± 0.86; NREM3, 12.0

± 0.82; NREM4, 5.8 ± 0.64; REM, 23.0 ± 0.99; non-learning (%; mean ± S.E.): NREM1, 6.5 ± 0.41; NREM2, 51.0 ± 1.33, NREM3, 12.6 ± 0.48; NREM4, 4.4 ± 0.77; REM, 22.7 ± 1.00; all non-significant (*n.s.*).

3.2. Improvement in Motor Skill Performance

The drawing time for the rotated images was 31.2 ± 2.99 *s* at the last trial of the pre-sleep session; this value decreased to 24.0 ± 2.32 *s* at the first trial of the post-sleep session. Compared to the last trial of the pre-sleep session, the drawing time was 7.2 s (23.3 ± 2.00%) shorter for the first trial of the post-sleep session (Fig. 3). Error rate was reduced from 11.6 ± 1.12 in the last trial of the pre-sleep session to 8.6 ± 0.73 in the first trial of the post-sleep session (22.0 ± 1.21%). Two-tailed t-tests demonstrated significant differences in performance between the pre-sleep and post-sleep sessions (tracing time: $t = 7.49$, $p < .001$; error rate: $t = 2.85$, $p = .025$).

The tiredness score of VAS conducted before each session was 30.3 ± 6.10 for the pre-sleep session and 36.9 ± 7.49 for the post-sleep session. Sleepiness score was 49.9 ± 6.94 for the pre-sleep and 38.5 ± 5.40 for the post-sleep session. There were no significant differences between the sessions for the two scores (Tiredness: $t = -1.172$; Sleepiness: $t = 1.28$; both *n.s.*).

3.3. Topographical analysis of spindle activity

In both conditions, scalp distribution was predominant at the parietal area for fast spindles (Fig. 4 A, B). One-way ANOVAs conducted across conditions (learning/non-learning) for each site revealed significant differences at 18 sites, but not at sites O1, F8, T3, T4, T6, FC3, and FT8. Subsequent post-hoc tests revealed that the fast spindle amplitudes were greater on the learning than on the non-learning nights (all *ps* < 0.01). For both conditions, the scalp distribution was

predominant at the frontal area for slow spindles (Fig. 4 C, D), and one-way ANOVAs conducted across the conditions (learning/non-learning) for each site did not reveal any significant difference (all *n.s.*). To examine learning-dependent enhancements in fast spindle activity, subtracted amplitudes (learning − non-learning) were calculated for each site. Subtracted amplitudes revealed that fast spindle activity was greater at the left-frontal area on the learning night (see Fig. 5 A). On the other hand, there were no learning-dependent differences observed in slow spindles (Fig. 5 B).

3.4. sLORETA for fast spindle

To examine possible increments in fast spindle activity related to motor learning, the source of subtracted amplitude was compared with the baseline (0 μV) using sLORETA. There were two main regions that were significantly enhanced—the left frontal and left parietal areas (Fig. 6, Table 2). In the left frontal area, fast spindle activity at the middle frontal (BA6, 32 voxels) and pre-central gyri (BA4, 22 voxels; BA6, 23 voxels) were significantly increased. On the parietal area, fast spindle activity at the precuneus (BA7, 114 voxels), superior parietal lobule (BA7, 69 voxels), and postcentral gyrus (BA2, 18 voxels; BA3, 24 voxels) were significantly increased (all ps < 0.01).

4. Discussion

The present study demonstrated enhanced fast spindle activity in the cortical areas involved in learning and consolidating new motor skills. No distortion in sleep structure occurred during the learning or non-learning nights. For both conditions, the scalp distribution for fast spindles was predominant at the parietal area (Fig. 4 A) and for slow spindles in the frontal area (Fig. 4 B). When spindle amplitudes were compared across conditions, the fast spindle

amplitude was significantly larger on the learning nights than on the non-learning nights. When the amplitude on the non-learning night was subtracted from that on the learning night, the subtracted amplitude value revealed that left frontal activity was enhanced on the learning night (Fig. 5 A). The sLORETA analysis of the subtracted fast spindle amplitude revealed that activity in the left frontal and left parietal areas was enhanced as the new skill was learned (Fig. 6). There were no significant learning-dependent changes for slow spindles (Fig. 5 B).

The newly acquired motor skill (tracing a rotated image) improved by 23.3% for tracing time and 22% for accuracy, in the post-sleep session in comparison to the pre-sleep session. Therefore, our hypothesis 1 was confirmed. This result is consistent with previous studies that demonstrated the facilitatory effects of sleep on motor-skill learning (Karni et al., 1998; Gais et al., 2000; Stickgold et al., 2001; Walker et al., 2002, 2003; Tamaki et al., 2007). Sleep after training appears to be critical for the improvement of visuomotor performance.

For fast spindles, scalp distribution in the parietal area was predominant for each condition (Fig. 4 A), and for slow spindles, the frontal area was predominant (Fig. 4 B). These results are consistent with numerous previously published works that have analyzed sleep spindles both qualitatively and quantitatively (Gibbs and Gibbs, 1950; Broughton and Hasan, 1995; Dijk, 1995; Werth et al., 1997; Anderer et al., 2001). This consistency demonstrates the adequacy of the selection decisions and analysis methods used in the present study. Moreover, subtracted amplitude measure showed activation in the left frontal area for the fast spindles (Fig. 5 A). There were no obvious changes (activation or deactivation) for slow spindles (Fig. 5 B). In the present study, sleep

spindles were carefully selected by excluding those data points that contained artifacts or other extraneous EEG activities such as slow waves and K-complexes. Our use of such rigorous methods suggests that the enhancement observed on the learning night (as compared to the non-learning night) is not a mere artifact that occurred independently of the learning that took place. Rather, it is more likely that our results reflect the increment of fast spindle activity that occurs in the brain during the consolidation of procedural memory during sleep.

The sLORETA analysis revealed that learning a new skill enhanced activity in both the left frontal and left parietal areas. In the left frontal area, the middle frontal (BA6) and pre-central gyri (BA4, BA6) were significantly activated. These areas correspond to the premotor cortex, which receives neuronal projections from the thalamus where the sleep spindle generator is thought to exist (Steriade et al., 2001). The premotor cortex is an important structure that integrates sensory and motor information (Halsband and Lange, 2006), and it is activated when a new visuomotor skill is learned (Astafiev et al., 2003; Iacoboni and Zaidel, 2004; Graydon et al., 2005; Lee and van Donkelaar, 2006). The premotor cortex receives several sensory stimuli to consolidate and transfer information to the motor-output systems (Kurata, 1994). Though fast spindles are distributed dominantly in the parietal area, they also appear in other areas, such as the frontal area. The finding that motor learning causes changes in the distribution of spindle activity indicates that sleep spindles contribute to neural plasticity during sleep.

In the parietal area, the precuneus (BA7), superior parietal lobule (BA7), and postcentral gyrus (BA2, BA3) were significantly activated. These areas correspond to the parietal associative area, which also receives neuronal

projection from the thalamus and, like the premotor cortex, appears to play an important role in visuomotor learning. Because these areas are activated at the middle-to-late phases of learning, they could be involved in automation of movement (i.e., the transition of the skill from the declarative stage to the procedural stage (Sakai et al., 1998). Moreover, these areas are activated when a new motor skill is learned, but not when an old pre-learned skill is repeated (see Jenkins et al., 1994). The premotor and parietal association areas appear to exchange perceptual and motor information, in order to integrate them into an applicable state (Kurata, 1994). During the learning of a new motor skill, fast spindles occur during subsequent sleep periods and activate the perceptual-motor brain areas. Therefore, visual and motor information acquired during the waking period could be consolidated during the subsequent sleep, via the activity of fast sleep spindles. It is possible that the fast sleep spindles are themselves a mechanism in memory consolidation. These results taken together lend support to hypotheses 2, 3, and 4.

We found that fast spindle enhancement seemed to involve a disproportionate emphasis on the left hemisphere. Indeed, there is a hemispheric asymmetry in the function of the premotor cortex. The left premotor cortex is activated in response to tasks that request cross-interaction with the outer environment, such as the description or a pursuit task (Seitz et al., 1997; van Mier et al., 1998). The left premotor cortex does not appear to activate in response to simple finger movement tasks (Seitz et al., 1990). The left premotor cortex is not recruited during simple motor commands; rather, it is involved in integrating perceptual and motor information (Halsband and Lange, 2006). However, there are fewer reports regarding hemispheric asymmetry in the parietal associative

area. There is also the possibility that our finding is the product of participants using their right hand during the task, which could produce neural plasticity in the hemisphere contralateral to the hand used, i.e. left motor areas. Walker et al. (2005) examined brain activation during motor tasks, 12 hours after the first motor-task session, using functional magnetic resonance imaging. In this study, participants used their left hands. Eventually, activation was greater in the *right* primary motor cortex when participants were performing the task after sleeping, compared to after being awake for the same amount of time. It is suggested that future studies should employ multiple conditions that involve the use of both right and left hand, to ascertain the mechanisms underlying the hemispheric asymmetry shown in the present study.

On the other hand, several studies have proposed that generation of sleep spindles is related to synaptic plasticity in the neocortex and therefore, for the enhancement of memory (Chapman et al., 1998; Trepel and Rasine, 1998; Fogel and Smith, 2006). Long-term potentiation (LTP) and long-term depression respectively refer to the sustained increase or decrease in synaptic efficacy induced by stimulation of presynaptic terminals. These phenomena are proposed to play a critical role in learning and memory (Rioult-Pedotti et al., 2000). Rosanova and Ulrich (2005) clearly demonstrated that spindle-like stimulation can produce LTP in neocortical pyramidal cells *in vitro*, and that LTP is not consistently induced by a regular firing pattern (such as a mirrored spindle stimulation pattern). The augmentation of fast spindles at the premotor and parietal areas, found in the present study, could be a reflection of the neural plasticity induced by learning a new skill.

In clinical research, Ferrarelli et al. (2007) reported that sleep spindle

power (13.75 - 15 Hz) was reduced in patients with schizophrenia compared to non-patient participants. It was suggested that the decreased spindle activity in schizophrenia patients might be a marker of an attentional gating deficit of sensory information. Also, it has been reported that sleep in Parkinson's disease patients showed a significant deactivation of sleep spindles and that patients with dementia have less spindle activity (Ktonas et al., 2007). Spindle activity could be both a biomarker of memory deficits and contribute to finding clues to the treatment of memory deficits in these patients.

There are however, several limitations to the present study. As Stickgold (2005) pointed out, the occurrence of sleep dependent consolidation, both in terms of sleep stages and the time of the night, remains poorly understood. Also, other oscillatory activities, such as slow-wave activity can contribute to motor learning during sleep. Tononi and Cirelli (2006) have proposed a synaptic homeostasis hypothesis. According to this theory, slow waves promote a generalized downscaling of synaptic strength that results in regulating the synaptic weight to a suitable state. There are several studies that support this hypothesis. Huber et al. (2004) for example, used an adaptation-rotation task and demonstrated a local enhancement of slow wave activity (1-4 Hz) in the parietal area. In addition, they found a significant relationship between performance improvements using a rotation-adaptation task (r=.86). They also demonstrated that arm immobilization resulted in the suppression of activity in the same motor-related cortical area. It is also possible that not only NREM sleep, but also REM sleep play a certain role in motor-memory consolidation (Huber et al., 2006). Fisher et al. (2002) have shown that sleep during the first day or night following training was critical for delayed performance improvements and described a correlation between overnight

improvement and the amount of REM sleep, rather than NREM Stage 2. Walker and Stickgold (2006) suggested that a new finger-to-thumb task requires more REM sleep, whereas a keyboard-typing task, which is a simple variant of a well-learned skill (typing), is consolidated during NREM Stage 2.

In conclusion, the present study is the first to demonstrate that fast spindle activity increases at the cortical areas involved in visuomotor learning and consolidating a new motor skill. Fast spindle amplitude was larger on the learning than on the non-learning nights, and subtracted amplitude values that we computed demonstrated that left frontal activity was enhanced on the learning night. Furthermore, sLORETA analysis of the subtracted fast spindle amplitude demonstrated that activation of the left frontal and left parietal areas was enhanced during learning. The thalamocortical network underlying the generation of fast spindles may contribute to synaptic plasticity that occurs during sleep.

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Table 1
Sleep-wake habits obtained from sleep questionnaire

Sleep Variables	M	SE
Retiring time	0:26	0.01
Time of final awakening	8:07	0.02
TST (hrs)	7.6	0.32
Sleep latency (min)	13.4	4.32
WASO	0.4	0.18
MEQ score	49.9	2.18

TST: Total sleep time; WASO: number of time awakening after sleep onset; MEQ score: Morningness-Eveningness questionnaire score (Horne and Östberg, 1976).

Table 2
Brain areas that show a stronger activation during fast spindle activity on the night after motor learning (compared with the control night)

Anatomical locations		Brodmann area (BA)	MNI coordinates			t value
			x	y	z	
Frontal Lobe	Precentral Gyrus	6	-55	5	50	5.29
		4	-50	-10	50	5.24
	Middle Frontal Gyrus	6	-45	5	55	5.18
Parietal Lobe	Precuneus	7	-25	-60	55	5.15
	Superior Parietal Lobule	7	-35	-60	60	5.35
	Postcentral Gyrus	2	-55	-25	50	5.14
		3	-55	-15	50	5.40
		5	-35	-50	60	4.91

Brain areas with statistical differences (t >4.064, p <.01) are shown. MNI coordinates and t values show the maximum value for each location. Coordinates are given in millimeters, and the origin is at the anterior commissure. For x, negative values represent left, positive values represent right. For y, negative values represent posterior, positive values represent anterior. For z, negative values represent inferior, positive values represent superior.

Fig1

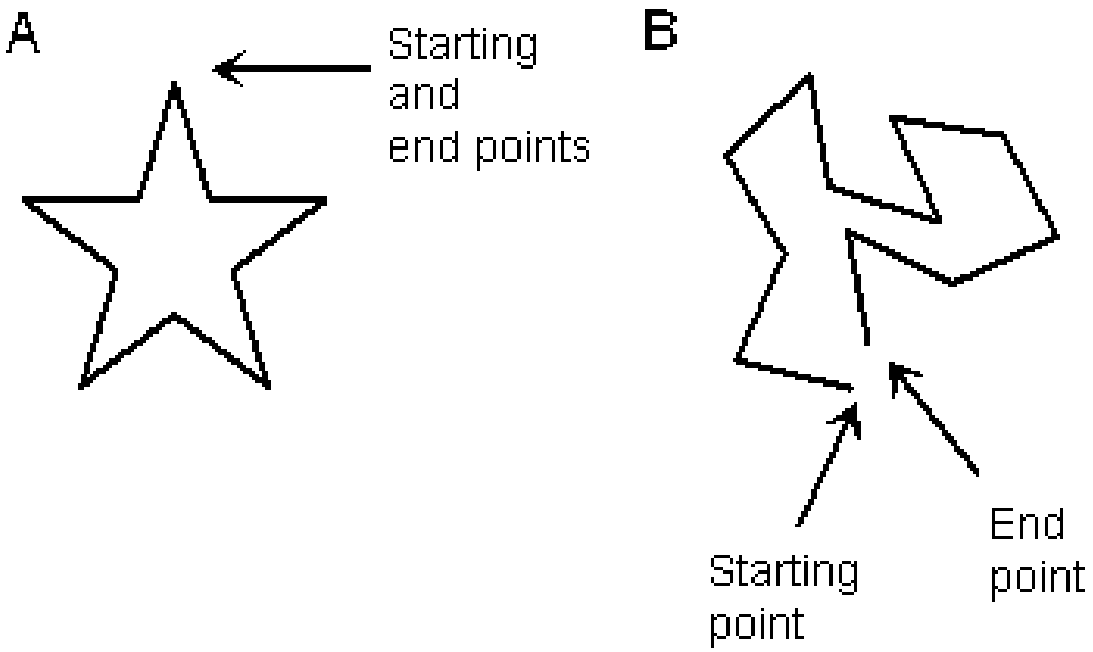


Fig2

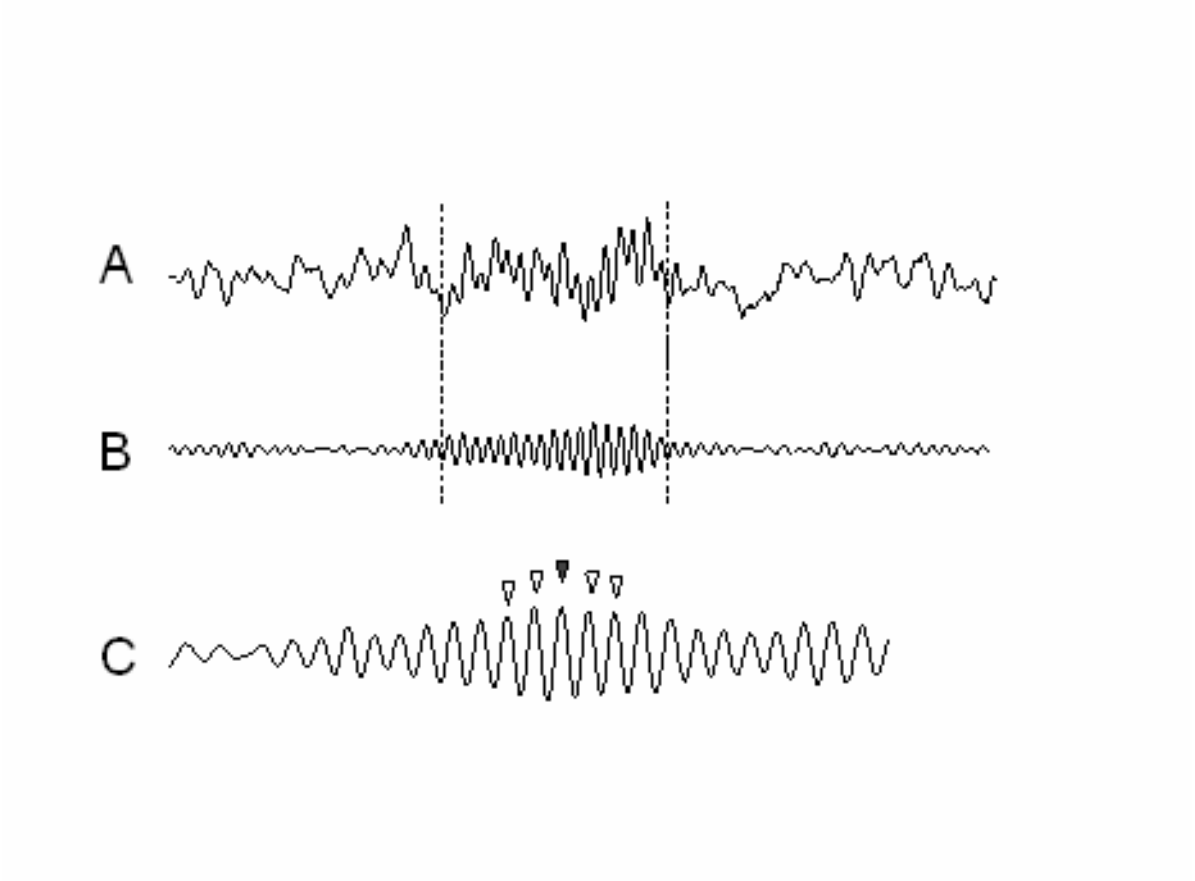


Fig3

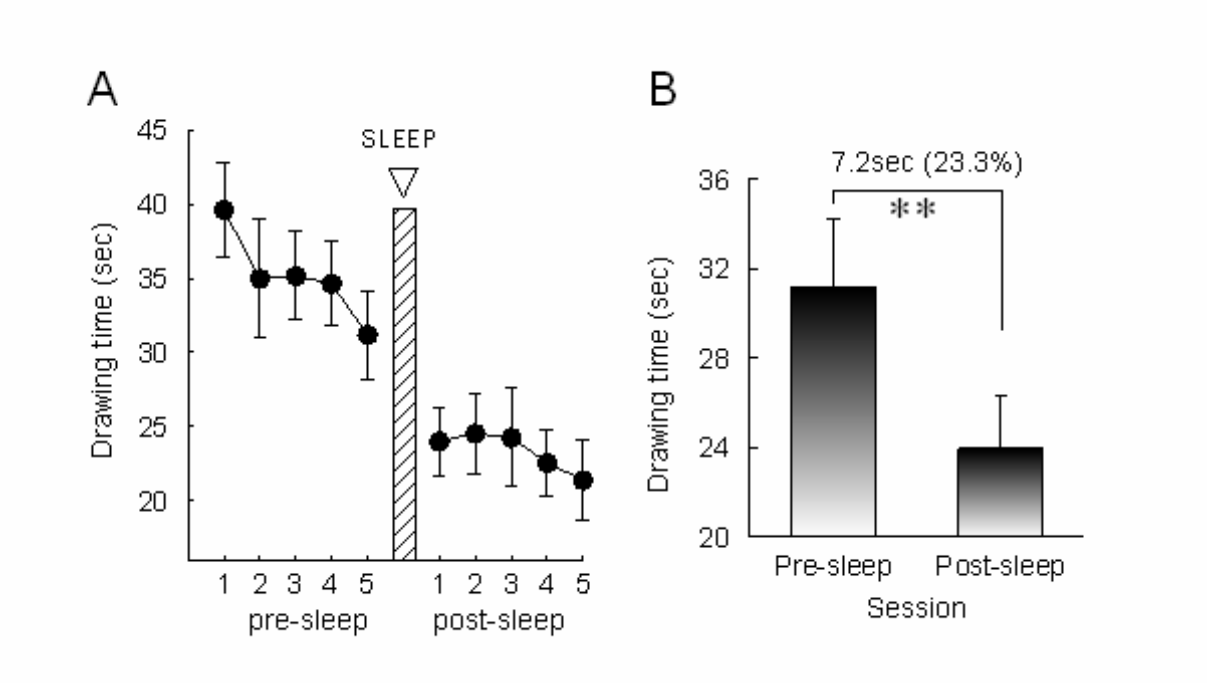

A
45
40
35
30
25
20
Drawing time (sec)
SLEEP
1 2 3 4 5
pre-sleep
1 2 3 4 5
post-sleep
B
36
32
28
24
20
Drawing time (sec)
7.2sec (23.3%)
**
Pre-sleep
Post-sleep
Session

Fig4

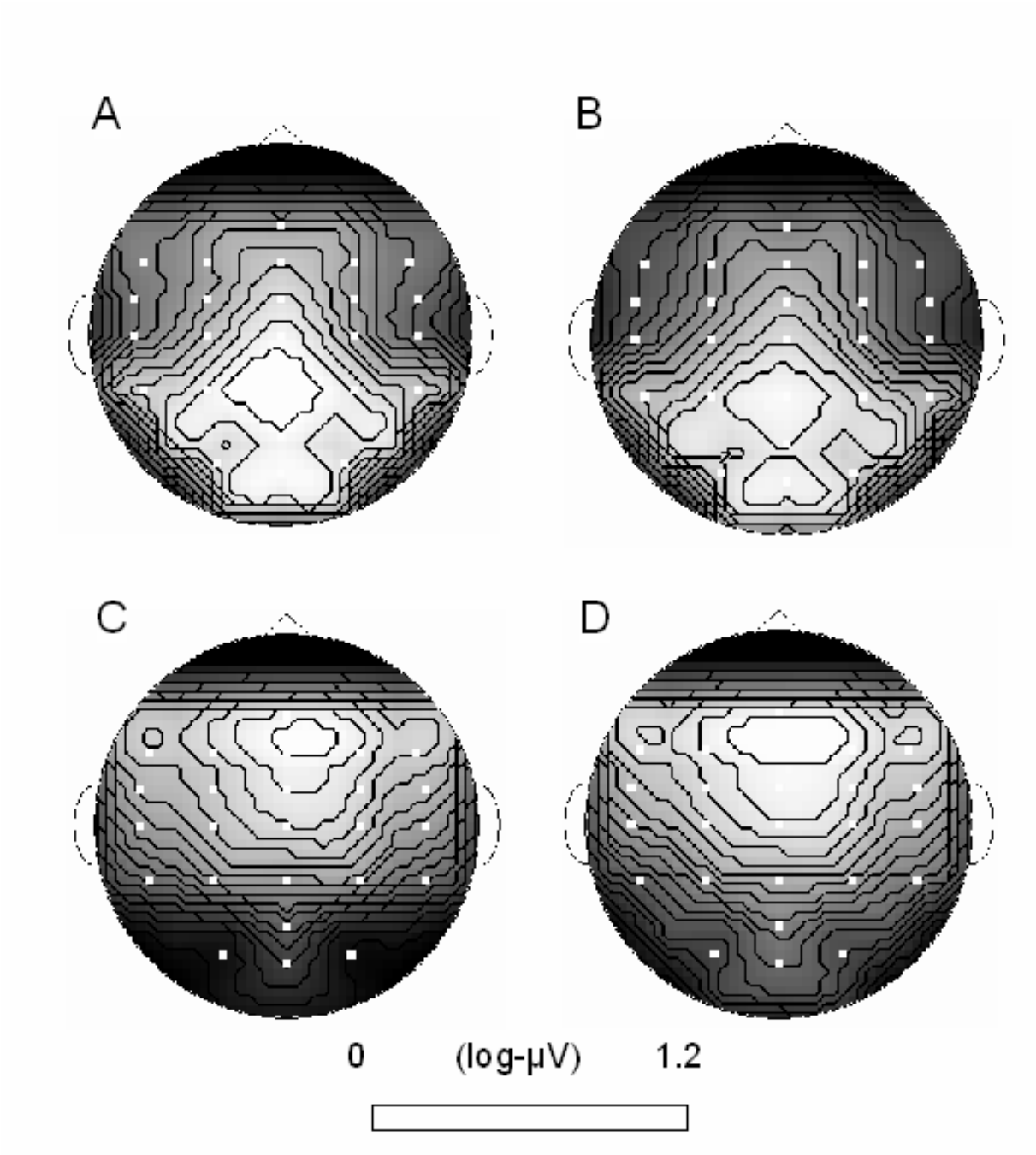


Fig5

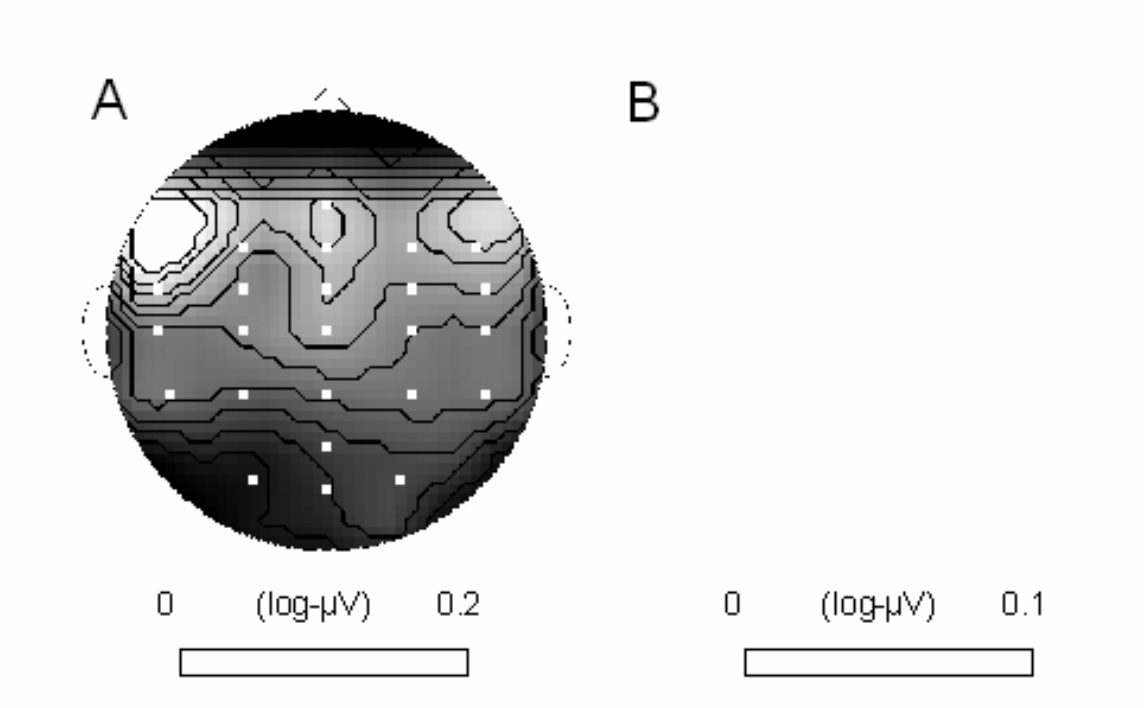
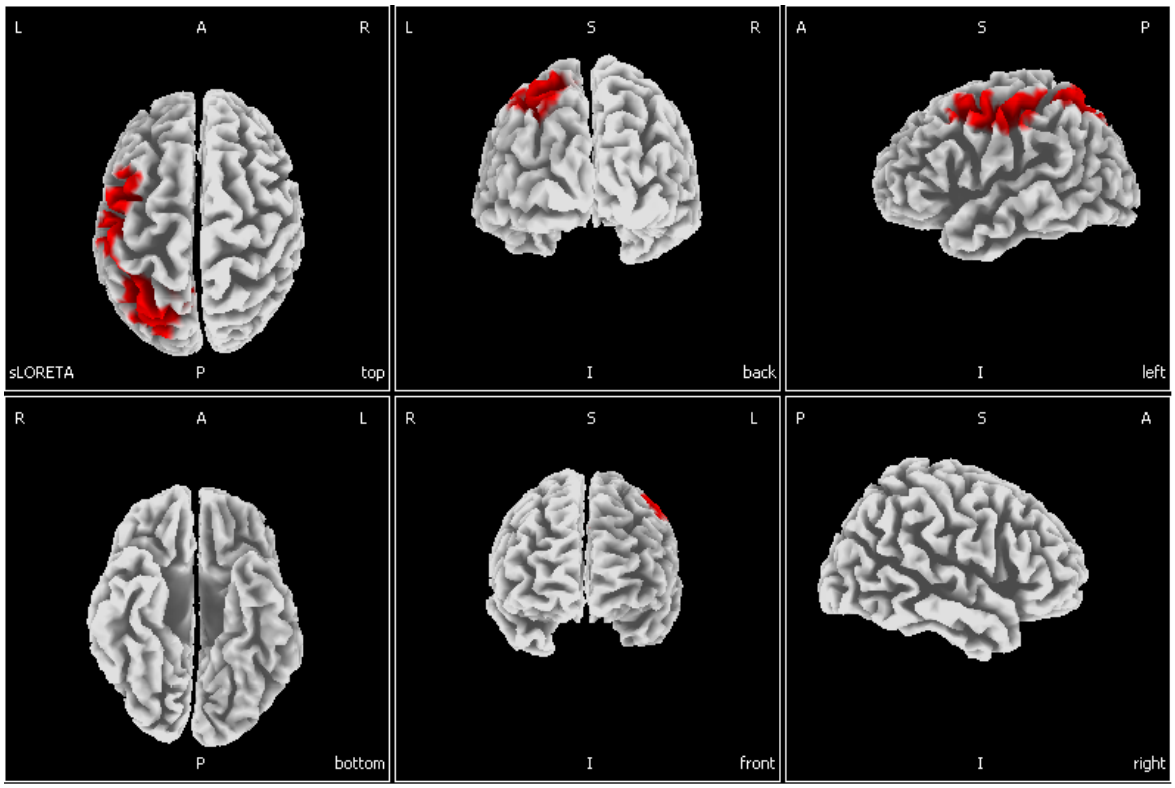


Fig6


L A R
sLORETA P top
L S R
I back
A S P
I left
R A L
P bottom
R S L
I front
P S A
I right