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Effects of thirty-minute mobile phone exposure on saccades

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Abstract

Objective: To investigate whether exposure to pulsed high-frequency electromagnetic field (pulsed EMF) emitted by a mobile phone has short-term effects on saccade performances.

Methods: A double blind, counterbalanced crossover design was employed. In 10 normal subjects, we studied the performance of visually guided saccade (VGS), gap saccade (GAP), and memory guided saccade (MGS) tasks before and after exposure to EMF emitted by a mobile phone for thirty minutes or sham exposure. We also implemented a hand reaction time (RT) task in response to a visual signal.

Results: With the exception of VGS and MGS latencies, the parameters of VGS, GAP and MGS tasks were unchanged before and after real or sham EMF exposure. In addition, the latencies of VGS and MGS did not change differently after real and sham exposure. The hand RT shortened with the repetition of trials, but again this trend was of similar magnitude for real and sham exposures.

Conclusions: Thirty minutes of mobile phone exposure has no significant short-term effect on saccade performances.

Significance: This is the first study to investigate saccade performance in relation to mobile phone exposure. No significant effect of mobile phone use was demonstrated on the performance of various saccade tasks, suggesting that the cortical processing for saccades and attention is not affected by exposure to EMF emitted by a mobile phone.

Keywords: Mobile phone; Safety; High-frequency electromagnetic field; Saccade; Attention

1. Introduction

Widespread use of mobile phones has aroused a growing concern for its possible adverse effects on human brain function. Due to the relative closeness of the antenna of mobile phones to the head, the brain should absorb part of the electromagnetic field (EMF) energy emitted by them (Schönborn et al., 1998). The antenna of the mobile phone held for daily use is located just below the tip to the inferior part of temporal lobe (Haarala et al., 2003; Aalto et al., 2006). Although SAR distribution may be maximal here, it extends far beyond this region into the frontal and parietal region as well (Huber et al., 2005). Indeed, EMF is documented to have some effects on the EEG power or amplitudes of event-related potentials at the underlying cortex (Reiser et al., 1995; Freude et al., 1998; Huber et al., 2000, 2002; Lee et al., 2001). On the other hand, it is reported to induce regional cerebral blood flow changes (rCBF) in the dorsolateral prefrontal cortex on the exposed side after 30-min use of a digital mobile phone (Huber et al., 2002, 2005).

The possible influences of mobile phones on cognitive function have been studied in many ways, where both inhibitory (Maier et al., 2004) and facilitatory effects are reported (Preece et al., 1999; Koivisto et al., 2000a,b; Sandstrom et al., 2001; Edelstyn and Oldershaw, 2002; Lee et al., 2001; Smythe and Costall, 2003). Whereas most of the studies give negative results (see Sienkiewicz et al.,

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2005 for review), many of the positive results have been unreplicated by the same group who reported positive results (e.g., compare Preece et al., 1999, 2005; Koivisto et al., 2000a; Haarala et al., 2004; Hamblin et al., 2004, 2006). Given the vital importance of attention in cognitive processing, it has also been addressed extensively in association with the effect of EMF, again with controversial results (Freude et al., 1998; Preece et al., 1999; Lee et al., 2001; Huber et al., 2002; Edelstyn and Oldershaw, 2002; Russo et al., 2006).

One way to settle the problem is to study saccade performance, since above all the physiological means to investigate behavior, oculomotor measurements (e.g., latency, velocity and amplitude) are particularly accessible for recording and lend themselves to quantification, allowing the precise analysis of control mechanisms (White et al., 1983). According to the wide SAR distribution, EMF emitted from a mobile phone may well affect the function of cortical regions implicated in saccade generation, although rCBF changes may not necessarily be observed in all the areas where SAR is relatively high (Haarala et al., 2003; Huber et al., 2002, 2005).

Recent brain-imaging studies have demonstrated that control of visual spatial attention and eye movements share most of the underlying cortical structures; similar patterns of activations are obtained in attention and oculomotor tasks in frontal cortical areas such as the lateral and medial premotor and prefrontal areas, including the area of the frontal and supplementary eye fields, the anterior cingulate cortex, and the anterior insula, as well as posterior parietal and temporal areas bilaterally including the intraparietal sulcus and the inferior parietal lobule (e.g., Nobre et al., 1997, 2000; Büchel et al., 1998; Corbetta et al., 1998; Schall and Thompson, 1999; Mort et al., 2003). A potential effect of EMF over these common regions is expected to have effect both on attention and saccades. Furthermore, at the behavioral level, there is also a strong link between attention and gaze shift achieved by saccades; when manipulating an object, we tend to direct our gaze to it (Land et al., 1999; Ballard et al., 1992; Johansson et al., 2001), where peripheral vision as well as central vision combined with gaze shifts may be used to gather visuospatial information (Terao et al., 2002). Since a shift of the visual attention focus is known to precede saccades (Liversedge and Findlay, 2000; also see Findlay and Gilcrhist, 2005 for review), an effect on attention would also be noted along with the delay in saccade latency or dysmetria of saccade amplitude toward the target. In some tasks, saccade may be a more sensitive indicator of EMF effect than for attention (Ditterich et al., 2000).

To our knowledge, there have not been any reports on the possible influence of EMF emitted by mobile phones on saccades. In the present paper, we investigated whether mobile phone use for 30 min has any short-term effects on saccades. In order to monitor the general arousal level during the experiments and also to investigate the possible effect of EMF on spatial attention, we also implemented a reaction time (RT) task during the same session, in which the subjects were required manually respond to the appearance of cue at the same eccentricities.

2. Methods

2.1. Subjects

Ten normal subjects (4 male, 6 female, age 33.1 ± 8.6 years [mean ± standard deviation], range 23–52 years), all right-handed, participated in the present study. The subjects gave their written informed consent to the study, which was approved by the Ethics Committee of the University of Tokyo according to the Declaration of Helsinki. None of the participants reported any psychological or neurological disorders, or serious head injury, and none of them had used handsfree devices prior to or during the experiments. An account of subject characteristics is given in Table 1 in terms of age, occupation, psychosocial workload of each subject, and VDT work, time with mobile phone, calling times, and number of calls per day.

2.2. Experimental setup

The setup for mobile phone was the same as that described by Arai et al. (2003) and Terao et al. (2006).

Table 1
Subject characteristics

Age	
<30 years	5
0–39 years	3
40–49 years	1
>50 years	1
Occupation	
Management	1
Professionals	9
Intermediate	0
Psychosocial workload	
Low	2
Medium	8
High	0
No VDT work	0
VDT work	
<1 h/day	2
1–4 h/day	3
>4 h/day	5
Time with MP	
2–7 years	5
8–14 years	5
15–22 years	0
Calling time (min/day)	
<2 min/day	9
2–15 min/day	1
>15 min/day	0
Number of calls/day	
<2 calls/day	8
3-8 calls/day	2

Pulsed EMF was given with a handset (Matsushita communication P97-7051-0) connected to a cellular phone simulator (Digital cellular phone communication tester, NJR-920). They were set to emit maximum output simulating the ordinary Japanese mobile phone (800 MHz EMF at 0.27 W net antenna input power). The pulse structures of this system were: transmitting frequency; 800 MHz band, modulation scheme; $\pi/4$ shifted quadrature phase-shift keying, multiple access; three channel time division multiple access, time division of multiple access frame period; 20 ms, time slot; 6.7 ms, maximum transmitting power; 270 mW as the averaged value (0.8 W of burst power). The audio circuitry of the handset was disabled so as not to provide the subjects with acoustic cues to indicate the status of the phone. During the exposure for 30 min, the handset was held over the right ear in the same manner as when calling in daily life, and the subjects spoke to one of the nearby examiners through the air but not on the phone. Although some of the previous studies use a holder or a strap to fix the handset to the head, we considered it optimal to have the subjects hold the phones as they usually call because we wanted to know how daily mobile phone use affected cognitive performance in a realistic way. The antenna was located about 2 cm from the head, and the depth from the brain tissue should amount to 30 mm.

Although the subjects could not keep holding the handset exactly at the same position during exposure, we estimated the exposure by the specific absorption rate (SAR) since this approximates what occurs in the brain when using a mobile phone in daily life. To control the exposure dosage, SARs at the brain area (30 mm below the skull under the coil) were measured using a phantom system for SAR measurement according to the instrumentation and procedures recommended by the International Electrotechnical Commission (International Electrotechnical Commission, 2005) while the handset was held at several different positions to the phantom (Fig. 1). Measured values were within 0.054 ± 0.02 W/kg of 10 g averaged value, and did not differ significantly between positions (Terao et al., 2006).

2.3. Recording procedure

For the measurement of saccades, we recorded electro-oculography (EOG) as described previously (Terao et al., 1998; Fig. 2a). A personal computer was used to control the visual stimulus presentation of the task and data acquisition in real-time. The recording setup for target presentation was originally developed by Kato and Hikosaka (1992) and Kato et al. (1995) and modified by Hikosaka et al. (1993) for human use. A microcomputer system built according to this prototype controlled the behavioral paradigms in an interactive manner and stored analog (e.g., eye movement) and digital (e.g., the time of pressing and releasing the switch button) data for later offline analysis.

The subjects were seated in front of a black, concave dome-shaped screen of 90-cm diameter with their heads placed on a chin rest to restrain head movement. They faced the center of the screen with a viewing distance of ~66 cm, where the pinhole used for the central fixation point was located. The subjects held a microswitch button connected to the microcomputer, allowing them to initiate and terminate a task trial by pressing and releasing the button.

Electro-oculographic (EOG) recordings were made by two Ag-AgCl gel electrodes placed at the bilateral outer canthi to record horizontal eye movements, and the signals were fed to a DC-amplifier (EOG amplifier, AN-601G, Nihon-Kohden, Tokyo, Japan), low-pass filtered at 20 Hz, and then digitized (500 Hz). Vertical EOG was also recorded by electrodes placed just above the upper lid and below the lower lid, which, together with the video camera, monitored the occurrence of blinks.

The room for recording was dimmed so that the unlit pinholes were not seen. After placing the electrodes, we waited for a period of at least 30 min to allow the subjects to become adapted to the dark and ready to start. Eye movement calibration was administered before each test session started. A target was presented at 20° to the left and right of the fixation spot which the subjects were required to fixate. While the subjects fixated this spot, we adjusted the gain of the EOG so that the current eye position displayed on the computer monitor would match the target position simultaneously displayed on the screen. Thus calibrated, EOG is known to be roughly linear over a range of 5–30°, and had a resolution of 0.5°. The gain of EOG was continuously monitored and adjusted when necessary throughout the experiments. The adjustment took place during calibration before recording EOG and in between different task performances, since impedance changes of electrodes tended to occur during protracted recordings.

The data were analyzed off-line. For each trial, four parameters were determined: saccade onset latency, the amplitude of the first saccade (before any corrective saccade was made), duration, and peak velocity. The detection of saccades was performed automatically by the computer as previously described (Terao et al., 1998). The onset of an eye movement was defined as the time when velocity and acceleration exceeded predetermined values (28°/s and 90°/s^2, respectively). After the onset, the velocity had to exceed 88°/s, and this suprathreshold velocity had to be maintained for at least 10 ms. The end of the eye movement was determined where the velocity decreased below 40°/s. The above procedure was performed automatically by the computer, although the final judgment of appropriate and inappropriate results was based on visual inspection of the individual eye movement records on the computer monitor. After these parameters for each task trial were determined, statistical analyses and displaying of the parametric data were performed.

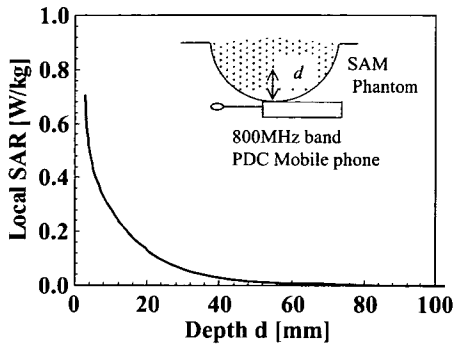

Fig. 1. Simulation used to estimate the specific absorption rate (SAR). A phantom was used to estimate SAR when the personal digital cellular mobile phone as used in the present experiment was placed at different depths according to the procedures recommended by IEC. The abscissa in the plot gives the depth from the mobile phone, and the ordinate gives the local SAR in W/kg.

2.4. Behavioral paradigms

In the present study, three oculomotor paradigms were used: the visually guided saccade (VGS), gap saccade (GAP), and memory guided saccade (MGS) tasks (Fig. 2b–d).

In the VGS task (Fig. 2b), a central spot of light came on shortly after the subject pressed a button either with their left or right finger, and the subject was required to fixate this spot. After a random period of time (1.2–2.0 s), the fixation spot was extinguished, and, simultaneously, a target stimulus appeared randomly at locations of various eccentricities, i.e., 5°, 10°, 20°, 30° either to the left or to the right of the fixation point. The subjects were instructed to foveate the target as quickly as possible. 0.5–1.5 s after the target presentation, the target point dimmed for 0.5 s. The subject had to release the button immediately after they detected this dimming, thereby ensuring foveation of the target with central vision. A sound signaled a successfully performed trial when the button release was made within 0.5–1.0 s after the target dimming. If the response was made later than the allowed period (usually 3 s after the target presentation), there was no sound.

The GAP task (Fig. 2c) was identical to the VGS task, except that the target spot was turned on 200 ms after the fixation point was turned off. We also studied the gap effect, i.e., the difference between latencies of saccades made in the VGS and GAP paradigms, which is an indicator of attentional disengagement from the central fixation point (see Section 4).

In the MGS task (Fig. 2d), the subjects were required to make a saccade to a remembered target location. While the subject fixated the central spot, a peripheral target stimulus appeared briefly for a period of 50 ms as a cue to indicate the future location of the saccade target, typically 1000 ms after the onset of the fixation spot. The subjects were required to maintain fixation until the

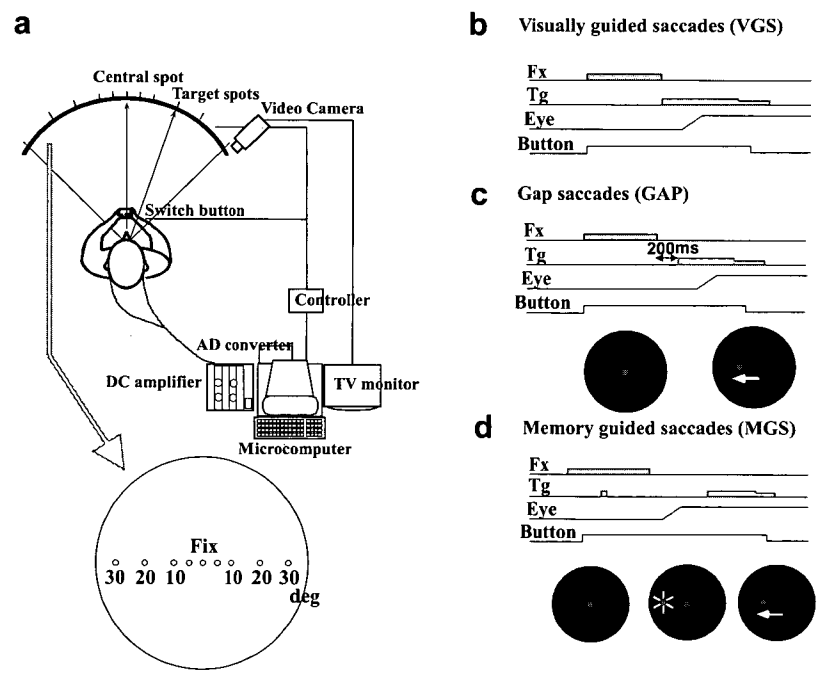

Fig. 2. Experimental setup and task procedure. In each task, after pressing the button, the central fixation spot was lit at the center of the dome, which the subjects were required to fixate. In VGS and GAP (b and c), after a random period of time (1.2–2.0 s), the target appeared at the same time or 200 ms after the central fixation point was extinguished. As soon as the subjects detected the dimming of the target LED which occurred 0.5–1.5 s after the presentation of target, they were asked to release the button. In MGS (d), a cue was presented at the position of the target for a brief period of time (50 ms), typically 1000 ms after the onset of the fixation spot, and the subject was required to memorize its location. 0.5–1.5 s after the cue was presented, the central fixation point was extinguished, at which time the subject was required to make a saccade to the remembered location. The target reappeared 600 ms later, and the subjects were asked to release the button as soon as they detected the dimming of target that occurred 0.5–1.5 s later. The saccade latency was measured from the offset of central fixation point to the onset of saccades, except that in GAP, it was measured from the onset of the target.

fixation point was turned off, when the subjects had to make a saccade based on their memory to the spatial location where the cue had appeared. The target spot turned on again 600 ms after the offset of the fixation point, and thereafter dimmed as in the VGS task. Again, the subjects had to release the button when they had detected the dimming of the target. The saccades unintentionally made to the cue during the delay period (i.e., before the central fixation point was turned off) were termed saccades to cue. The frequency of saccades to cue was considered to be an indicator of distractibility since the subjects were required to suppress its occurrence while the central fixation was presented.

In addition to the three oculomotor paradigms described above, a visual detection task (hereafter termed the reaction time [RT] task) was designed to monitor the general arousal level and to test simple visual perception or spatial attention by means of manual reaction time (Hikosaka et al., 1993). In this task, a central spot of light came on shortly after the subject pressed the button with their thumb, which stayed throughout each trial and the subjects were required to fixate. After a random period of time, another spot came on randomly at locations of 5°, 10°, 20°, 30° either to the left or right of the fixation point. On detecting this target, the subjects were required to release their fingers from the button as soon as possible while keeping fixation on the central cross. The interval between the appearance of the central spot and that of the target was randomized between 2000 and 6500 ms. In the visual detection task as well as in the oculomotor paradigms, the finger to press the button was alternated between the left and right hands for consecutive sessions.

2.5. Experimental design

A double blind, counterbalanced crossover design was employed. One of the experimenters controlled the cellular phone stimulator and the other implemented the saccade and RT tasks to the subjects. Each subject served as his/her own control, i.e., the performance before exposure was compared with his/her own performance after exposure. The subjects underwent two experimental conditions, i.e., real and sham exposures. The order in which sham or real exposure was given was randomized and counterbalanced across subjects, which was known only to the experimenter who controlled the cellular phone simulator; 5 subjects underwent the real exposure first and the other five underwent the sham exposure first. To exclude any lasting effect of having undergone an experimental procedure, the performances of each subject under the real and sham phone exposures were separated by 7 days or more (Besset et al., 2005; Terao et al., 2006).

Prior to the test sessions, the subjects were given one or two practice sessions of 10–20 trials each (without mobile phone) until their performance became stable. After these practice sessions, all the subjects underwent 2 sessions of 30 trials each for each saccade task before and after exposure. Similarly in the RT task, after practicing 10–20 trials, the subjects performed 40 trials (20 trials × 2 sessions) before and after each exposure. Sham exposure was performed by having the subject hold the handset and speak in the same manner as during real exposure but no EMF was given. The subjects could not differentiate real from sham exposure, and were completely unaware which exposure they were in.

2.6. Data analysis and statistical assessment

Only trials with correct responses were included in the analyses. The mean saccade latency, peak velocity and amplitude of the first saccade were calculated for each saccade task. In the MGS task, the frequency with which erroneous saccades to cue occurred was calculated by dividing the number of saccades to cues by the total number of trials in the same block of session. The gap effect was calculated by subtracting the mean GAP latency from the mean VGS latency of saccades at each eccentricity. The frequency of saccades to cue was pooled across all target eccentricities since it was generally small for all the subjects (around 8–9% of the total trial number, see Section 3).

The statistical analysis addressed two aspects of the effect of EMF exposure: first whether the EMF exposure significantly changed the saccade performance relative to baseline (prior to exposure), and second whether the effect, if present, differed from that of sham exposure. Using SPSS 10 software (SPSS Japan, Inc., Tokyo), the saccade measures (saccade latency, peak velocity, amplitude of first saccade) were subjected to repeated measures analysis of variance (ANOVA) with factors time (before and after exposure), mode of exposure (real or sham phone use), and target eccentricity (5°, 10°, 20°, 30° to the left or right of the fixation point). For the RT task, the factors were time, mode of exposure and target eccentricity plus the response hand (left or right hand). For all the analyses, the significance criterion was set at $p < 0.05$. Post hoc analyses (Bonferroni/Dunn's correction for multiple comparisons) were performed in order to assess which differences contributed to the significant effects in the ANOVA ($p < 0.0011$).

3. Results

3.1. Performance in control trials before exposure

For VGS, saccade latency was reliably influenced by target eccentricity, with the former increasing with the latter [$F(7,63) = 10.59$, $p < 0.0001$] (Table 1 and Fig. 5, figures at the top row). Saccade velocity also increased reliably with target eccentricity [$F(7,63) = 1460.61$, $p < 0.0001$]. The amplitude of the first saccade towards targets of larger eccentricities tended to undershoot the target eccentricity. This undershoot became more evident as the target eccentricity increased (10–15% of the target eccentricity at 30° in contrast to 5–10% at 5°).

A similar trend was noted for GAP. Saccade latency and velocity increased reliably with target eccentricity [effect of target eccentricity: saccade latency: $F(7,63) = 6.42$, $p < 0.0001$] (Table 1, Fig. 5, figure at the middle row), as did saccade velocity [$F(7,63) = 1427.50$, $p < 0.0001$]. In a similar manner to the VGS task, the amplitude of first saccade tended to undershoot the target eccentricity. Again, the undershoot was more prominent for larger target eccentricities than for smaller target eccentricities.

For MGS, saccade latency was comparable across different target eccentricities [$F(7,63) = 0.44$, $p = 0.88$]. Meanwhile, saccade velocity increased significantly with target eccentricity [$F(7,63) = 1257.18$, $p < 0.0001$]. Again, there was a trend for saccades to undershoot the targets, especially those of larger eccentricities.

3.2. The effect of EMF and sham exposure on task performance

The superimposed traces of VGS and GAP exhibited no apparent changes when compared before and after real phone exposure or sham. This suggested that, overall, the saccade parameters for each saccade task did not change significantly before and after either real (Fig. 3) or sham exposure (Fig. 4).

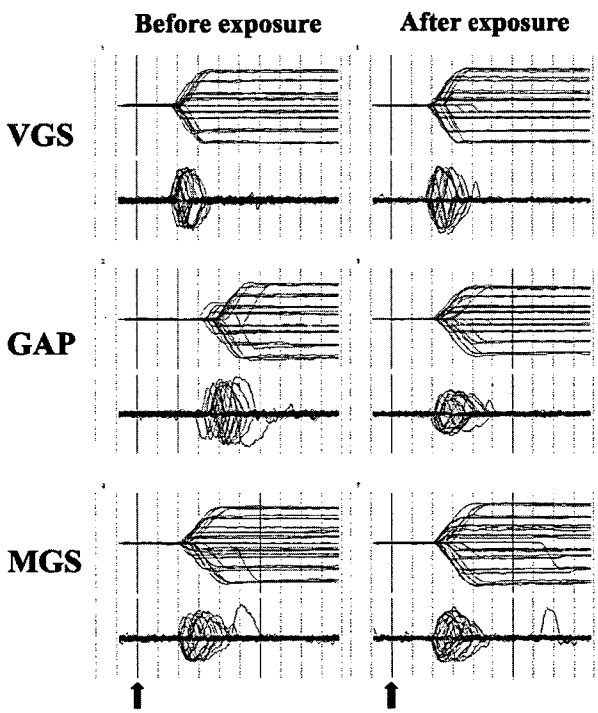

Fig. 3. Traces of saccade performance before and after real EMF exposure. Each of the figures represents an overlay of 30 traces of saccade performance in the task (upper traces: eye position, lower traces: saccade velocity) shown on the left. In the upper traces of each figure, the ordinate shows the position of gaze in degrees (saccade velocity in lower traces) and the abscissa the time axis. Upward deflections denote rightward saccades and downward deflections show leftward saccades. The dashed vertical lines are drawn at a time interval of 100 ms. Left column before exposure, right column after exposure. Arrows indicate the time of offset of the fixation spot.

For VGS (Figs. 5 and 6, top row, Table 2A-1), the saccade latency was slightly but significantly longer post-exposure (244.7 ± 3.1 ms; mean $\pm$ standard error unless otherwise indicated) than before (232.8 ± 3.2 ms) [$F(1,9) = 9.52$, $p = 0.0022$]. There was no interaction between time and eccentricity [$F(7,63) = 0.052$, $p = 0.99$], showing that a similar increase of saccade latency with increasing target eccentricity was noted before and after exposure. Importantly, the interaction between the factors time and mode of exposure (sham or real) failed to reach significance [$F(1,9) = 1.30$, $p = 0.25$], which implied that the prolongation of saccade latency was similarly noted for real or sham EMF exposure. Furthermore, no significant effect of the mode of exposure (sham or real phone use) [$F(1,9) = 0.65$, $p = 0.42$] or target eccentricity [$F(9,63) = 0.24$, $p = 0.98$] was noted. Nor was there any significant three-way interaction among the three factors (eccentricity, time, and mode of exposure) [$F(7,63) = 0.118$, $p = 0.99$]. These findings suggested that the prolongation of VGS latency after exposure occurred across all the target eccentricities, but similarly for real and sham exposures. There was no significant change in peak velocity before and after exposure, as evidenced by the lack of effect for any of the factors or of interactions between any of the factors [effect of time: $F(1,9) = 0.86$, $p = 0.36$, effect of mode: $F(1,9) = 0.04$, $p = 0.84$, time $\times$ eccentricity: $F(7,63) = 1.37$, $p = 0.22$, mode $\times$ time: $F(1,9) = 0.47$, $p = 0.50$, mode $\times$ eccentricity: $F(7,63) = 0.87$, $p = 0.53$, mode $\times$ eccentricity $\times$ time: $F(7,63) = 0.68$, $p = 0.69$]. Similarly, VGS amplitude was slightly but significantly smaller

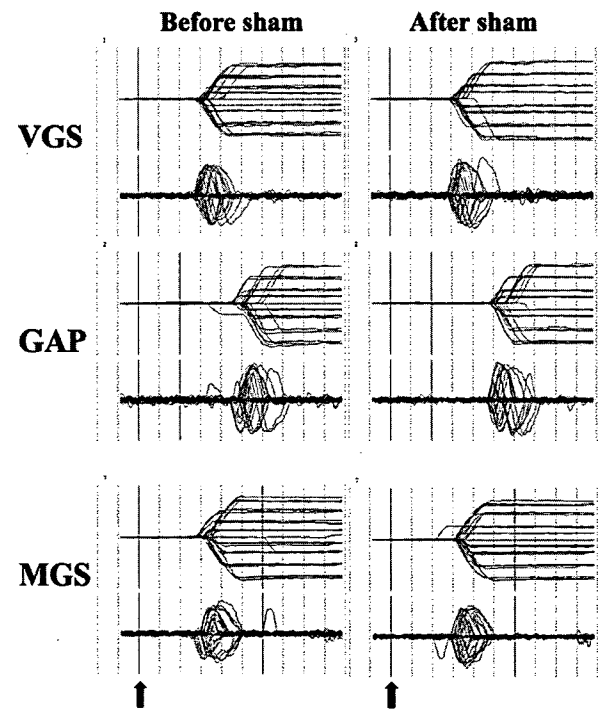

Fig. 4. Traces of saccade performance before and after sham EMF exposure. Conventions as in Fig. 3.

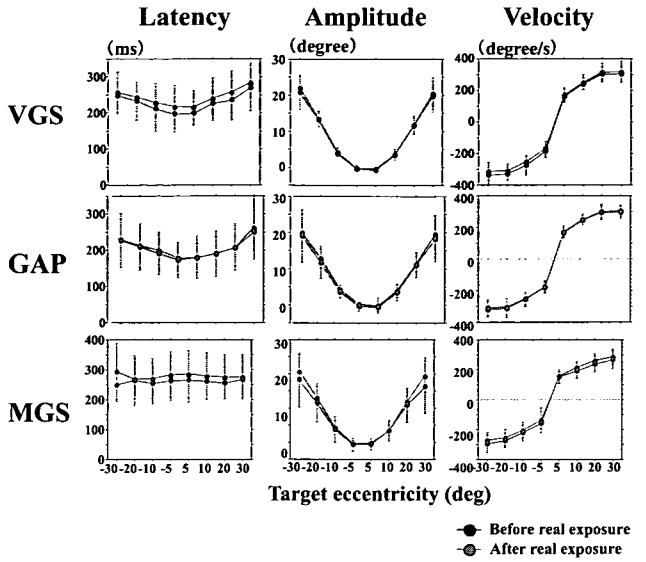

Fig. 5. Changes in saccade parameters before and after real EMF exposure. Changes in saccade parameters before and after real EMF exposure are shown for the three different tasks (top row, VGS; middle row, GAP; bottom row, MGS). Plots are separately given for latency (left column), amplitude (middle column) and velocity (right column). For velocity, positive values correspond to rightward saccades and negative values to leftward saccades. In each figure, the ordinate shows the saccade parameters and the abscissa shows the target eccentricity. Blue curves represent pre-exposure, and the red curves post-exposure. Error bars give the standard error. Positive velocity values indicate saccades towards the right and negative values indicate those to the left.

after exposure than before, but this trend was not affected by the mode of exposure (real or sham); the effect of any of the factors or their interactions failed to reach significance [effect of time: $F(1,9) = 4.74$, $p = 0.03$, effect of mode: $F(1,9) = 1.36$, $p = 0.24$, time × eccentricity: $F(7,63) = 0.46$, $p = 0.86$, mode × time: $F(1,9) = 1.21$, $p = 0.27$, mode × eccentricity: $F(7,63) = 0.49$, $p = 0.84$, mode × eccentricity × time: $F(7,63) = 0.14$, $p = 0.99$]. Altogether, there were

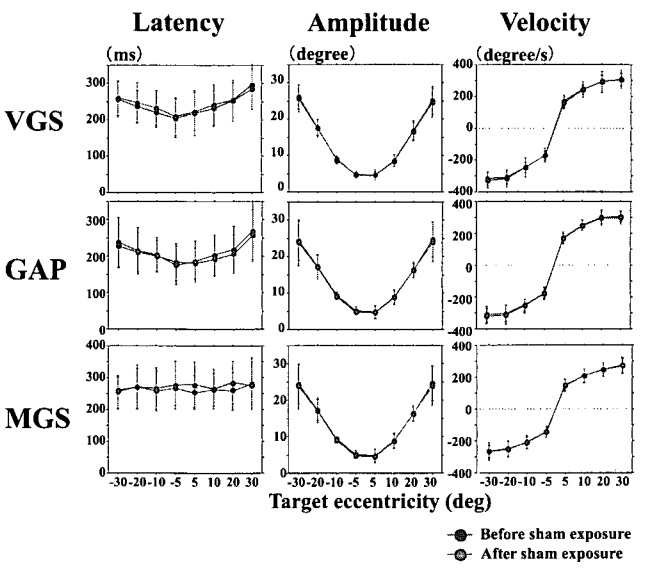

Fig. 6. Changes in saccade parameters before and after sham EMF exposure. Conventions as in Fig. 5.

no significant changes in VGS parameters before and after exposure, except that saccade latency increased slightly after exposure. However, as described above, the amount of prolongation was similar between real and sham exposure.

The saccade latency of GAP (Figs. 5 and 6, middle row, Table 2A-2) was unchanged post-exposure (204.0 ± 3.4 ms) as compared with pre-exposure (204.8 ± 4.3 ms) [effect of time: $F(1,9) = 0.026$, $p = 0.87$]. No interaction was noted between time and eccentricity [$F(7,63) = 0.17$, $p = 0.99$], implying that an increase of saccade latency with increasing target eccentricity was similarly noted before and after exposure. On the other hand, no significant effect of the mode of exposure (sham or real phone use) was noted [$F(1,9) = 2.82$, $p = 0.094$], while the interaction between the factors time and mode of exposure (sham or real) failed to reach significance [$F(1,9) = 0.72$, $p = 0.40$]. This indicated that the latency did not change significantly both after sham and real exposures. Furthermore, there was no significant three-way interaction among the three factors (information, time, and mode of stimulation) [$F(7,63) = 0.32$, $p = 0.94$]. There was no significant change in peak velocity before and after exposure whether it was real or sham, as shown by the insignificant effect of any of the factors or interactions among them [effect of time: $F(1,9) = 0.54$, $p = 0.46$, effect of mode: $F(1,9) = 0.04$, $p = 0.84$, time × eccentricity: $F(7,63) = 0.95$, $p = 0.47$, mode × time: $F(1,9) = 0.40$, $p = 0.53$, mode × eccentricity: $F(7,63) = 0.21$, $p = 0.98$, mode × eccentricity × time: $F(7,63) = 0.26$, $p = 0.97$]. Overall, the amplitude was slightly but significantly smaller after exposure than before [effect of time: $F(1,9) = 4.36$, $p = 0.038$], but this trend was not affected by the mode of exposure (real or sham) across all the target eccentricities [effect of mode: $F(1,9) = 0.04$, $p = 0.84$, time × eccentricity: $F(7,63) = 0.23$, $p = 0.98$, mode × time: $F(1,9) = 0.16$, $p = 0.97$, mode × eccentricity: $F(7,63) = 0.63$, $p = 0.73$, mode × eccentricity × time: $F(7,63) = 0.28$, $p = 0.96$].

Overall, the gap effect (difference between the mean VGS and GAP latencies at each target eccentricity) was 34.4 ± 5.5 ms (Table 3A). This was slightly but significantly larger post-exposure (40.7 ± 2.3 ms) than pre-exposure (28.0 ± 3.1 ms) [$F(1,9) = 10.18$, $p = 0.0016$], but was not

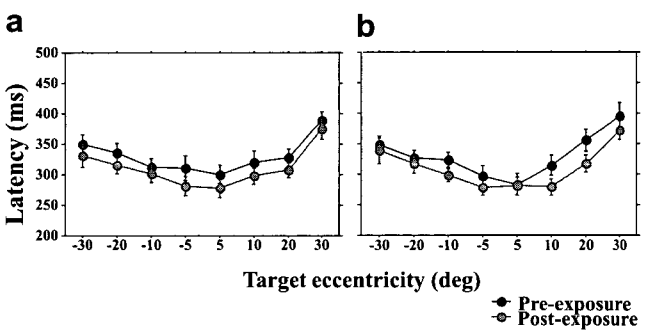

Fig. 7. Changes in RT before and after real EMF exposure. Changes in RT in the visual detection task before and after real EMF exposure are shown for real (a) and sham (b) exposures. Conventions as in Fig. 5. (a) Real exposure. (b) Sham exposure.

Table 2
Performance measures of saccade and reaction time tasks. Mean ± standard error

		−30°	−20°	−10°	−5°	5°	10°	20°	30°	Total
(A) Saccade latency										
(1) VGS										
Real	Before	247.7 ± 9.9	233.9 ± 10.7	211.7 ± 10.8	196.5 ± 12.4	199.2 ± 5.5	225.4 ± 10.5	235.9 ± 10.1	269.0 ± 9.9	227.6 ± 8.3
Exposure	After	257.9 ± 10.2	244.9 ± 7.5	229.7 ± 12.9	218.6 ± 13.6	216.5 ± 9.3	241.4 ± 12.5	258.5 ± 9.8	284.4 ± 10.9	243.6 ± 9.2
Sham	Before	256.2 ± 11.1	236.2 ± 10.2	221.4 ± 10.5	204.2 ± 14.9	218.1 ± 13.0	231.5 ± 10.1	253.2 ± 11.3	285.0 ± 11.2	238.0 ± 10.5
Exposure	After	260.2 ± 9.4	247.8 ± 11.6	233.7 ± 8.0	209.1 ± 12.8	233.2 ± 10.3	241.9 ± 9.4	254.9 ± 10.9	296.6 ± 10.9	245.7 ± 9.0
(2) GAP										
Real	Before	221.0 ± 19.8	208.1 ± 15.2	180.8 ± 18.4	174.2 ± 8.7	182.0 ± 10.2	181.8 ± 15.3	198.9 ± 17.0	254.9 ± 22.0	200.5 ± 15.0
Exposure	After	232.7 ± 13.1	213.8 ± 14.3	196.2 ± 11.0	167.0 ± 8.7	172.7 ± 7.1	189.9 ± 9.7	204.8 ± 9.8	237.8 ± 12.2	201.8 ± 8.9
Sham	Before	238.9 ± 12.9	214.6 ± 15.5	203.3 ± 11.1	174.7 ± 11.5	185.8 ± 15.3	203.3 ± 15.2	218.1 ± 15.6	270.7 ± 19.9	212.6 ± 13.0
Exposure	After	228.0 ± 12.2	211.2 ± 12.1	200.8 ± 11.7	184.2 ± 12.5	191.2 ± 10.5	205.7 ± 10.3	256.4 ± 13.8	207.2 ± 15.5	237.6 ± 11.4
(3) MGS										
Real	Before	293.6 ± 14.8	269.3 ± 8.1	271.3 ± 8.8	283.9 ± 13.3	285.7 ± 14.5	279.6 ± 16.5	275.7 ± 11.4	276.0 ± 14.0	279.3 ± 10.7
Exposure	After	251.7 ± 10.2	269.1 ± 16.2	256.8 ± 13.0	264.2 ± 13.8	267.4 ± 12.4	264.3 ± 7.8	260.8 ± 10.2	274.3 ± 14.2	263.3 ± 10.2
Sham	Before	260.3 ± 6.9	271.4 ± 12.7	266.1 ± 9.7	277.3 ± 11.3	278.2 ± 14.0	265.1 ± 13.3	285.2 ± 9.6	275.0 ± 15.0	272.3 ± 9.1
Exposure	After	255.5 ± 11.9	270.9 ± 11.7	259.0 ± 6.7	266.8 ± 17.8	252.5 ± 11.8	262.3 ± 8.7	259.2 ± 10.7	281.2 ± 20.2	263.1 ± 8.1
(B) Reaction time										
Real	Before	352.1 ± 20.0	338.6 ± 19.3	307.8 ± 16.9	313.2 ± 25.3	298.9 ± 18.5	324.0 ± 27.5	329.4 ± 13.2	382.6 ± 14.1	330.1 ± 17.8
Exposure	After	276.9 ± 22.8	255.1 ± 16.7	269.7 ± 17.5	234.4 ± 17.6	245.4 ± 17.7	268.9 ± 15.5	274.7 ± 13.1	353.5 ± 17.6	269.6 ± 15.5
Sham	Before	343.3 ± 21.4	328.0 ± 16.8	324.6 ± 14.3	294.5 ± 18.3	286.8 ± 23.8	316.3 ± 22.1	358.0 ± 25.6	385.8 ± 26.0	328.8 ± 16.7
Exposure	After	330.7 ± 22.1	314.4 ± 12.4	299.5 ± 10.0	285.7 ± 11.2	275.0 ± 11.7	284.4 ± 11.2	310.2 ± 13.1	369.3 ± 10.3	308.6 ± 9.8

Table 3
Gap effect and frequency of saccades to cue. Mean ± standard error

		−30°	−20°	−10°	−5°	5°	10°	20°	30°	Total
(A) Gap effect										
Real	Before	27.1 ± 17.8	29.2 ± 11.8	31.1 ± 14.0	26.4 ± 6.1	18.5 ± 8.8	46.8 ± 11.9	34.3 ± 11.7	16.4 ± 20.1	28.7 ± 9.7
Exposure	After	23.5 ± 10.3	30.9 ± 8.9	34.3 ± 6.7	49.4 ± 7.5	43.7 ± 9.5	52.1 ± 9.9	53.5 ± 7.8	45.2 ± 10.9	41.6 ± 4.6
Sham	Before	15.6 ± 30.7	28.6 ± 24.2	21.5 ± 26.9	29.9 ± 26.9	35.9 ± 26.9	27.2 ± 26.9	35.6 ± 26.9	24.6 ± 18.0	27.4 ± 7.8
Exposure	After	32.5 ± 11.2	37.5 ± 10.0	34.3 ± 6.0	25.3 ± 11.8	46.2 ± 10.6	51.5 ± 6.1	50.6 ± 10.5	40.4 ± 9.4	39.8 ± 6.4

(B) Frequency of saccades to cue		
Real	Before	8.3 ± 2.0%
Exposure	After	8.2 ± 2.4%
Sham	Before	8.5 ± 2.7%
Exposure	After	8.0 ± 2.8%

reliably influenced by target eccentricity [effect of eccentricity: $F(7,63) = 1.44$, $p = 0.19$]. Effect of mode as well as the interactions involving this factor did not reach significance, suggesting that the mentioned trend was not affected by the mode of exposure (real or sham) across all the target eccentricities [effect of mode: $F(1,9) = 0.16$, $p = 0.69$, mode × time: $F(1,9) = 0.003$, $p = 0.95$, mode × eccentricity: $F(7,63) = 0.37$, $p = 0.92$, mode × eccentricity × time: $F(7,63) = 0.59$, $p = 0.76$].

The saccade latency of MGS (Figs. 5 and 6, bottom row, Table 2A-3) was slightly but significantly shorter post-exposure (265.7 ± 3.1 ms) as compared with pre-exposure (276.1 ± 3.0 ms) [effect of time: $F(1,9) = 5.47$, $p = 0.02$], whereas no interaction was noted between time and eccentricity [$F(7,63) = 0.78$, $p = 0.60$], suggesting that this shortening was common to all target eccentricities. On the other hand, no significant effect of the mode of exposure (sham or real phone use) was noted [$F(1,9) = 0.11$, $p = 0.74$], and the interaction between the factors time and mode of exposure (sham or real) failed to reach significance [$F(1,9) = 0.86$, $p = 0.35$]. Furthermore, there was no significant three-way interaction among the three factors (time, mode of stimulation, and target eccentricity) [$F(7,63) = 0.43$, $p = 0.89$]. These implied that the shortening of latency was noted similarly after sham and real exposures across all target eccentricities. There was no significant change in peak velocity before and after exposure [effect of time: $F(1,9) = 0.54$, $p = 0.46$, effect of mode: $F(1,9) = 0.04$, $p = 0.84$, time × eccentricity: $F(7,63) = 0.95$, $p = 0.47$, mode × time: $F(1,9) = 0.40$, $p = 0.53$, mode × eccentricity: $F(7,63) = 0.21$, $p = 0.98$, mode × eccentricity × time: $F(7, 63) = 0.26$, $p = 0.97$]. Similarly, the amplitude was unchanged after exposure than before, and this trend was not affected by the mode of exposure (real or sham) at all target eccentricities [effect of time: $F(1,9) = 0.45$, $p = 0.51$, effect of mode: $F(1,9) = 0.59$, $p = 0.44$, time × eccentricity: $F(7,63) = 0.57$, $p = 0.78$, mode × time: $F(1,9) = 0.56$, $p = 0.46$, mode × eccentricity: $F(7, 63) = 0.72$, $p = 0.65$, mode × eccentricity × time: $F(7,63) = 0.92$, $p = 0.49$].

The frequency of erroneous saccades to cue was 8.3 ± 2.4% on the average (Table 3B). It was not significantly different after than before exposure [$F(1,9) = 0.45$, $p = 0.51$], nor was it affected by the mode of stimulation (real/sham) [$F(1,9) = 0.35$, $p = 0.56$]. The interaction between these two factors also failed to reach significance [$F(1,9) = 0.41$, $p = 0.53$]. Therefore, the frequency of saccades to cue was not affected by exposure, regardless of whether it was real or sham.

Taken together, there was a slight but significant change in saccade latency after exposure than before for VGS and MGS, whereas the other saccade parameters were totally unchanged before and after exposure. In addition, saccade latency did not show a differential effect of real or sham exposure, suggesting that there was no significant effect of EMF exposure on saccade parameters.

3.3. The effect of EMF on the performance of RT task

Before exposure, RT to the appearance of a visual cue was 340.2 ± 6.2 ms for the left hand and 320.7 ± 6.1 ms for the right hand, i.e., significantly shorter for the latter [Table 2B; effect of hand: $F(1,9) = 5.96$, $p = 0.02$]. Reaction time increased slightly but significantly with increasing target eccentricity [effect of target eccentricity: $F(7,63) = 7.03$, $p < 0.0001$].

Reaction time was significantly shorter post-exposure (310.5 ± 4.1 ms) as compared with pre-exposure (330.5 ± 4.4 ms) [effect of time: $F(1,9) = 12.49$, $p = 0.0004$; Fig. 7]. No interaction was noted between time and eccentricity [$F(7,63) = 0.18$, $p = 0.99$], implying that the increase of RT with increasing target eccentricity was similarly noted before and after exposure. On the other hand, no significant effect of the mode of exposure (sham or real phone use) was noted [$F(1,9) = 0.009$, $p = 0.93$], and the interaction between the factors time and mode of exposure (sham or real) failed to reach significance [$F(1,9) = 0.0002$, $p = 0.99$]. Furthermore, there was no significant three-way interaction among the three factors (time, mode of stimulation, and target eccentricity) [$F(7,63) = 0.21$, $p = 0.98$]. These indicated that the shortening of RT was noted similarly for sham and real exposure conditions at all target eccentricities. Finally, any interaction involving the factor hand did not reach signifi-

cance, suggesting that the EMF exposure did not have any differential effect on the RTs of both hands [mode × hand: $F(1,9) = 0.91$, $p = 0.34$, time × hand: $F(1,9) = 0.29$, $p = 0.51$, eccentricity × hand: $F(7,63) = 0.17$, $p = 0.99$, time × mode × hand: $F(1,9) = 3.26$, $p = 0.07$, mode × eccentricity × hand: $F(7,63) = 0.25$, $p = 0.97$, time × eccentricity × hand: $F(7,63) = 0.34$, $p = 0.56$, time × mode × eccentricity × hand: $F(7,63) = 0.57$, $p = 0.78$]. The lack of statistical significance also persisted when we collapsed the data for all eccentricities [mode: $F(1,9) = 0.003$, $p = 0.95$, mode × time: $F(1,9) = 0.0003$, $p = 0.98$, mode × hand: $F(1,9) = 0.19$, $p = 0.67$, time × mode × hand: $F(1,9) = 66$, $p = 0.42$].

4. Discussion

The present study showed that, with the exception of VGS and MGS latencies, the parameters of saccades were unchanged after real or sham exposure at all target eccentricities. Even with the parameters that did change significantly, the change was of similar magnitude after real and sham exposures. In addition, there was no significant difference in RT shortening after real and sham exposures. Collectively, no significant acute effect of EMF was detected in these measures due to use of a mobile phone for 30 min, consistent with the negative results observed by previous studies employing various electrophysiological methods as well as cognitive and reaction time tasks (Edelstyn and Oldershaw, 2002; Haarala et al., 2003, 2004; Hinrichs and Heinze, 2004; Papageorgiou et al., 2004; Krause et al., 2004; Hamblin et al., 2006; Terao et al., 2006). The results provide an indication that there is no effect of EMF exposure on the cortical regions involved in the control of saccades, especially the frontal and parietal cortical regions.

The reason is unclear why some previous studies have found significant EMF effects on attention, whereas our study did not (Freude et al., 1998; Preece et al., 1999; Lee et al., 2001; Huber et al., 2002; Edelstyn and Oldershaw, 2002; Russo et al., 2006). One possible reason for this may be the lack of statistical power due to a small sample size. Although power analysis is difficult to apply with the present experimental design, at least 50–100 subjects would be required if we were to detect a small change of the order of 1–2%, whereas our sample was 10 subjects. However, detection of a difference of this order (several ms) would be difficult to achieve with a RT task. Another reason may be that we mainly studied spatial attention required for the performance of the oculomotor and RT tasks, whereas other studies have addressed non-spatial aspects of attention. Although it is difficult to generalize across different paradigms for studying attention, the insignificant effect of EMF on the RT task indicated that there was no significant change in general arousal level and/or spatial attention differentially induced by real nor sham EMF exposure. On the other hand, the frequency of saccades to cues in the MGS paradigm, serving as an indicator of distractibility toward the presented cue (Powell and Goldberg, 2000), was affected neither by real or sham exposure, suggesting that the distractibility towards the briefly presented cue was unchanged.

The saccade latency in the GAP paradigm is shorter than that in the VGS paradigm, even for saccades made to targets of the same eccentricity, which is known as the gap effect (Fischer and Ramsperger, 1984). This effect is considered to arise from the fact that in the VGS paradigm, the central fixation point is extinguished at the same time as the peripheral target is presented, necessitating disengagement of attention from the fixation point when initiating a saccade toward the target, whereas in the GAP paradigm, this disengagement is not necessary. The gap effect did not change differently whether the exposure was real or sham, implying that the disengagement of attention from the fixation point was not affected by EMF exposure.

As stated in the introduction, very few studies that made the measurements before and after but not during exposure to EMF have ever demonstrated a significant effect of non-thermal levels of electromagnetic radiation on brain or behavior. In other words, exposure to EMF has not been demonstrated to produce short or long lasting effects so far. Along with our previous demonstrations that mobile phone use for thirty minutes has no short-term adverse effects on the central auditory pathways (Arai et al., 2003), sensory system (Yuasa et al., 2006) or cortical regions involved in motor preparation (Terao et al., 2005, 2006), the results of the present study suggest that there is no significant effect of EMF emitted by mobile phone on the cerebral cortex. Replication of the current study with a larger sample size on the behavioral aspects should focus on the effects of long-term exposure as well as short-term ones. In addition, different oculomotor paradigms that would be more sensitive to the effect of EMF should be considered for future studies.

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RESEARCH ARTICLE

Hemoglobin concentration changes in the contralateral hemisphere during and after theta burst stimulation of the human sensorimotor cortices

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Abstract Using near infrared spectroscopy and repetitive transcranial magnetic stimulation (rTMS), we studied interhemispheric interactions between bilateral motor and sensory cortices in humans. RTMS consisted of a triple-pulse burst (50 Hz) repeated every 200 m for 2 s (10 bursts, 30 pulses); one kind of theta burst TMS (TBS) (Huang et al. in Neuron 45:201–206, 2005). The hemoglobin concentration changes were recorded at the right prefrontal cortex, premotor area (PM), primary hand motor area (M1) and primary sensory area (S1) during and after TBS over the left PM, M1 and S1 or sham stimulation in eight normal volunteers. In addition, motor evoked potentials (MEPs) to TMS over the right M1 were recorded from the left first dorsal interosseous muscle after the conditioning TBS over left S1. TBS over PM induced a significant oxy-Hb decrease at the contralateral PM. TBS over M1 elicited a significant oxy-Hb decrease at the contralateral S1, and TBS over S1 significant oxy-Hb decreases at the contralateral M1 and S1. MEPs to TMS of the right M1 were significantly suppressed by the conditioning TBS over the left S1. These results suggest that there are mainly inhibitory interactions between bilateral PMs and bilateral sensorimotor cortices in humans. Those are partly compatible with the previous findings. In addition to between the primary motor cortices, bilateral connection is requisite for smooth bimanual coordination between the sensory cortices or premotor cortices.

Keywords Interhemispheric interaction · Transcranial magnetic stimulation · Near-infrared spectroscopy · Motor cortex

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Introduction

To investigate the interactions between two distant cortical areas in humans, several non-invasive methods have been used. They are neuroimaging methods, electrophysiological methods including transcranial magnetic stimulation (TMS) over two sites (paired stimulation method) and their combinations. Paired TMS techniques were first applied to see the connections, such as between bilateral motor cortices (Ferbert et al. 1992), cerebello-motor cortical (Ugawa et al. 1995) connections. The inhibitory interaction between bilateral primary motor cortices (M1) was demonstrated by paired TMS over the bilateral M1s (Ferbert et al. 1992). Similar paired TMS also showed interactions between premotor areas (PMs) and bilateral M1s (Civardi et al. 2001; Mochizuki et al. 2004a) and between motor related areas and the contralateral primary sensory cortex (S1) (Mochizuki et al. 2004b). These physiological studies can evaluate interactions between the sensorimotor area and other areas, but can hardly evaluate interactions between other areas (e.g., between bilateral PMs) because we have no good

physiological measures to evaluate the excitability of areas other than the sensorimotor cortex.

Recent neuroimaging studies could show some functional connections between two distant cortical areas other than sensorimotor cortices. Positron emission tomography (PET) revealed regional cerebral blood flow (rCBF) changes at bilateral motor related areas including the supplementary motor area, PM and cerebellum elicited by repetitive TMS over M1 or PM (Siebner et al. 2000; Chouinard et al. 2003). These results suggest some tight connections between the stimulated area and areas showing rCBF changes. However, one major limitation of PET study is due to the irradiation of the subjects. Near-infrared spectroscopy (NIRS) is one of alternative noninvasive methods to see cerebral functional changes. It has four distinct advantages over preexisting techniques: high signal-to-noise ratios for single events, noninterference with magnetic field changes elicited by TMS, higher time resolution than PET and the lack of irradiation. This technique estimates hemoglobin (Hb) concentration changes by measuring reflected light, based on the differences in absorption spectra between oxy- and deoxy-Hb (Jöbsis 1977; Chance et al. 1988; Villringer et al. 1993). In previous studies we successfully recorded Hb concentration changes by a single pulse TMS (Noguchi et al. 2003; Mochizuki et al. 2006). In the present paper, using combination of NIRS and TMS, we studied the interhemispheric interactions between bilateral motor and sensory cortical areas in humans.

Subjects and methods

Subjects

Eight healthy volunteers (two women and six men, 28–53 years old) participated in this study. All subjects were right handed based on the Edinburgh Handedness Inventory (Oldfield 1971) and they all gave written informed consent to participate in the study. The experimental procedures used here were approved by the Ethics Committee of the University of Tokyo and were carried out in accordance with the Declaration of Helsinki.

Electromyographic recordings

Surface electromyograms (EMGs) were recorded from the bilateral first dorsal interosseous muscles (FDIs) with 9 mm diameter, Ag–AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified with an amplifier (Biotop, NEC Medical Systems, Japan) through filters set at 100 Hz and 3 kHz, then recorded with a sampling rate of 10 kHz and stored by a computer (Signal Processor DP-1200, NEC Medical Systems, Japan) for later off-line analysis of the data.

TMS

TMS was delivered with a figure-of-eight-shaped coil (outer diameter of each wing was 7 cm) connected to a Magstim 200 magnetic stimulator or a Magstim Super Rapid Package (The Magstim Co. Ltd, Whitland, UK). The coil was positioned over the left PM, M1 or S1. M1 was defined as the "hot spot" where stimulation evoked the largest MEP in the right FDI. In two of the subjects, that position was confirmed to be over the primary motor cortex by the neuronavigation system (Spetzger et al. 1995; Boroojerdi et al. 1999). PM is proved to be located 2–3 cm anterior site from M1 by PET study (Fink et al. 1997). To minimize stimulation effect spreading by TMS, we kept the distance between two stimulation sites as long as possible, and defined PM at a 3 cm anterior site from M1. In our previous paper (Enomoto et al. 2001), the S1 demonstrated by functional magnetic resonance images was located at 2 cm posterior to C3 of the International 10–20 system, the recording site for SEP. We selected a parietal lobe at a 3 cm posterior, 3 cm lateral site from M1. It was a bit posterior to the recording site for SEP. However, we have confirmed it to be a posterior part of somatosensory cortex, and we defined it as S1 (Fig. 1). At 2 cm away from the coil, the magnetic field elicited by single pulse TMS decreased to the half of that provoked just underneath the coil (Bohning et al. 1997). So, 3 cm apart from the coil may be long enough for excluding the stimulation spread. In fact, repetitive TMS (rTMS) at an intensity of 90% AMT could activate PM (2.5 cm anterior from M1) and M1 separately (Gerschlager et al. 2001).

The coil was oriented to induce medially directed currents in the brain when monophasic TMS was delivered, and medially directed currents at the first phase when delivering biphasic TMS. The intensity was adjusted to be 80 and 100% of the active motor threshold (AMT) at M1 for biphasic conditioning TMS. We defined the AMT as the lowest intensity that evoked five small responses (about 100 μV) in a series of ten stimuli when the subject made a 5% maximal voluntary contraction (about 50 μV). Sham stimulation was performed with two coils. One non-discharging coil was positioned at the midpoint between left M1 and PM,

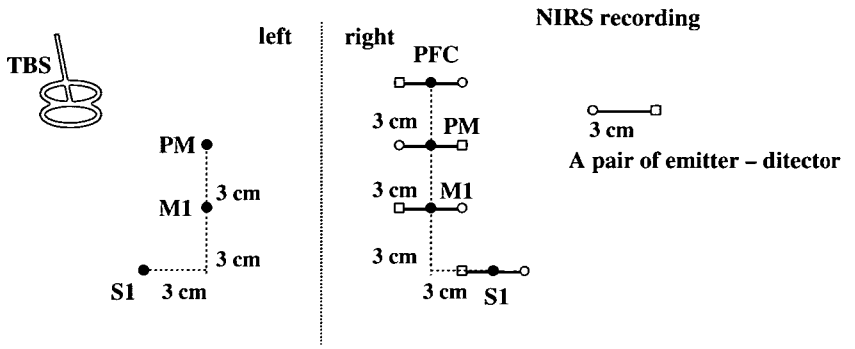

Fig. 1 Locations of TBS and NIRS recording sites. TBS was applied over left PM, M1 or S1. NIRS recordings were made at four measurement points. Each measurement pair of emitter (*open circle*) and detector (*open square*) was located on medio-lateral line at a distance of 3 cm. Measurement points (mid-points) were arranged on right PFC, PM, M1 and S1

and the other was positioned 10 cm above the head and the same currents as real stimulation were induced in it to make sound. The holder of the first coil was touched to the edge of the other one for delivering vibration to the subject when giving a stimulus. As the conditioning stimulus, theta burst TMS (TBS) was used since it has good modulation effects on the stimulated site (Huang et al. 2005).

One train of TBS consisted of triple-pulse bursts (50 Hz) repeated every 200 m for 2 s (10 bursts, 30 pulses). TBS over three sites and sham stimulation were applied at an intensity of 80 or 100% AMT. Eight different stimulation conditions (four sites of stimulation × two intensities) were done in all the subjects. Each stimulation condition consisted of two sessions. Then, in total, 16 sessions (two sessions × eight conditions) were done in one subject. Each session consisted of ten trains of TBS (total 100 bursts, 300 pulses). Each train was given at random inter-train intervals of 34–36 s. We cannot completely exclude the possibility of some accumulative effects even at this interval of 34–36 s. But continuous NIRS recording showed that in most subjects Hb concentration changes by TBS returned to the baseline before the next TBS. In addition, since all experiments were done in the same manner and compared the results between different stimulation conditions, we can safely say that some difference should be present between different stimulation conditions. The interval between the end of a session and the onset of next session was 15 min, which was long enough for the Hb concentration changes to return to the baseline. The order of sessions was counterbalanced within and across subjects. At most, six sessions were done on one experimental day (1,800 pulses at most). In one subject, all the experiments were performed on 3 days separated by a week or more.

To see some adverse effect and cope with it immediately, we recorded electroencephalograms continuously and one doctor observed the subject carefully during whole the experiments. We also sometimes talked with the subject in the experiments to check their condition.

NIRS measurement

We used a NIRS system (ETG-A1; Hitachi Medical Corporation, Tokyo, Japan) having four emitters and four detectors, and the four measurement points (mid-points between emitters and detectors) were placed on the right PM, M1, S1 and a site at 3 cm anterior from PM [which is corresponding to prefrontal cortex (PFC)] (Fig. 1). The homologous positions in the contralateral hemisphere were also marked as the stimulated sites. Near-infrared laser diodes with two wavelengths, 790 and 830 nm, were used as the light sources, and transmittance data of the light beams were obtained every 500 m. We calculated concentrations of oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) from the transmittance data. In this study, each event period ranged from 5 s before the TBS onset to 30 s after the end of TBS. Under each condition, the average Hb concentration changes were obtained from the results of two sessions and they were used in statistical analyses.

Additional physiological experiment

In the main experiments, we observed a large oxy-Hb decrease and small deoxy-Hb increase at the right M1 after TBS over the left S1 (see "Results", third column in Figs. 3, 5). To confirm this finding electrophysiologically, we examined the excitability of the right M1 after TBS over the left S1 using a randomized conditioning-test design reported previously (Hanajima et al. 2001).

In this experiment, the test stimulus was a single pulse, monophasic TMS over the right M1 with a figure-of-eight-shaped coil (outer diameter of each wing was 7 cm). The coil was oriented to induce anteriorly directed currents in the brain. The intensity of the test stimulus was adjusted to evoke an MEP of approximately 0.2–0.5 mV peak to peak in the relaxed left

FDI. The conditioning stimulus was TBS over the left S1. TBS was composed of triple-pulse bursts repeated every 200 ms for 2 s (10 bursts, 30 pulses) at an intensity of 80% AMT. Three conditions [the test stimulus (15 trials) or conditioning TBS given alone (5 trials), and the test stimulus preceded by the conditioning TBS by an interval of 200 m from the onset of the last burst (15 trials)] were intermixed randomly in one block. Data were analyzed off-line after the experiments.

The amplitude of each single MEP from the left FDI in each condition was measured in order to compare amplitudes of the control and conditioned MEPs in the same block. We calculated the ratio of the mean amplitude of the conditioned MEP to that of the control MEP in each subject. The order of two blocks (TBS over S1 and sham stimulation) was counterbalanced across subjects. This additional experiment was performed 1 week or longer interval after the last day of the main experiments.

Statistical analysis

In the main experiments, the 95% confidence interval was calculated for each time point of oxy- and deoxy-Hb changes in each condition. We obtained the mean Hb concentration changes by averaging Hb data from 5 to 20 s after a TBS pulse in each condition, and used these values in statistical comparisons. In each NIRS recording point, two-way analysis of variance (ANOVA) [with factors of stimulation type (three sties and sham stimulation) and INTENSITY (80 and 100%)] was performed on the mean Hb concentration changes. When an ANOVA test showed significant effects, we further performed post-hoc analyses with Scheffe's method for the significant effects. In the additional experiment, we used a paired *t*-test for comparison. The statistical significance was set at $P = 0.05$.

Results

Neither abnormal electroencephalograms nor any adverse effects were noted during or after the experiments in any subjects.

Figures 2, 3, 4 and 5 show averaged relative Hb-concentration changes and the 95% confidence intervals at right PFC (Fig. 2), PM (Fig. 3), M1 (Fig. 4) or S1 (Fig. 5) when TBS was applied over left PM, M1 or S1 or when sham stimulation was given.

No significant changes in oxy-Hb concentration (longer than 15 s) were observed at right PFC (Fig. 2). At right PM, significant oxy-Hb decreases were observed in the left PM stimulation condition (80 and 100% AMT) (Fig. 3). At an intensity of 80% AMT, oxy-Hb began to decrease after the TBS onset and returned to the baseline around 25 s later at the right PM (Fig. 3a, first column). The oxy-Hb significantly decreased as compared to the baseline 4–25 s after the TBS onset, as shown by the 95% confidence lines (the upper thin line was lower than zero), but the deoxy-Hb did not change significantly. No significant changes were seen when TBS was applied over the other sites.

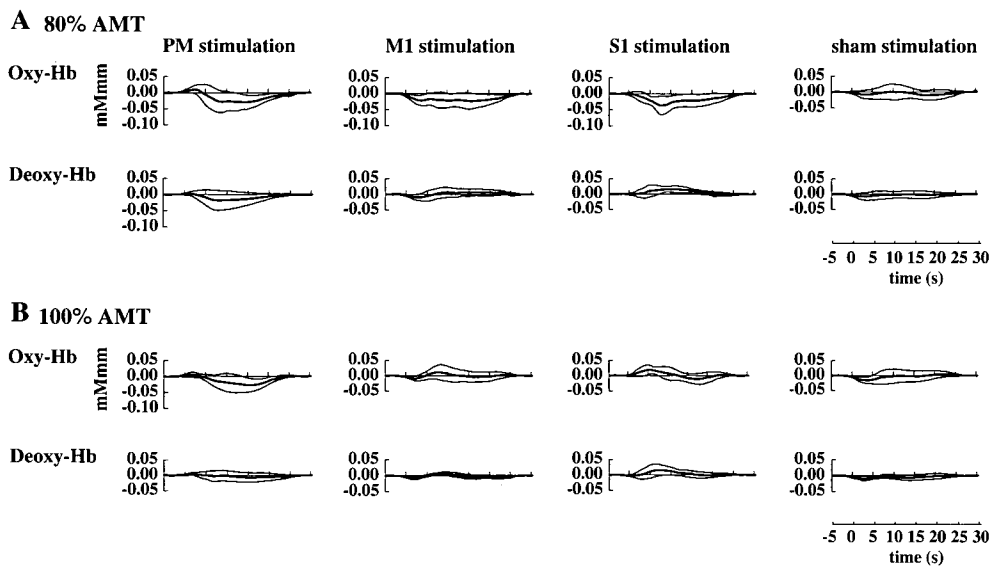

Fig. 2 NIRS recordings at right PFC. TBS was applied over left PM (*first column*), M1 (*second column*), S1 (*third column*) or sham stimulation (*fourth column*) at 80% AMT **a** or 100% AMT **b**. The first and third rows show oxy-Hb concentration changes, and the second and fourth deoxy-Hb. The ground averaged data ($n = 8$) for the four stimulation conditions are separately shown. The averages are depicted by thick lines and the 95% confidence intervals thin lines. No significant changes were evoked by any stimulation

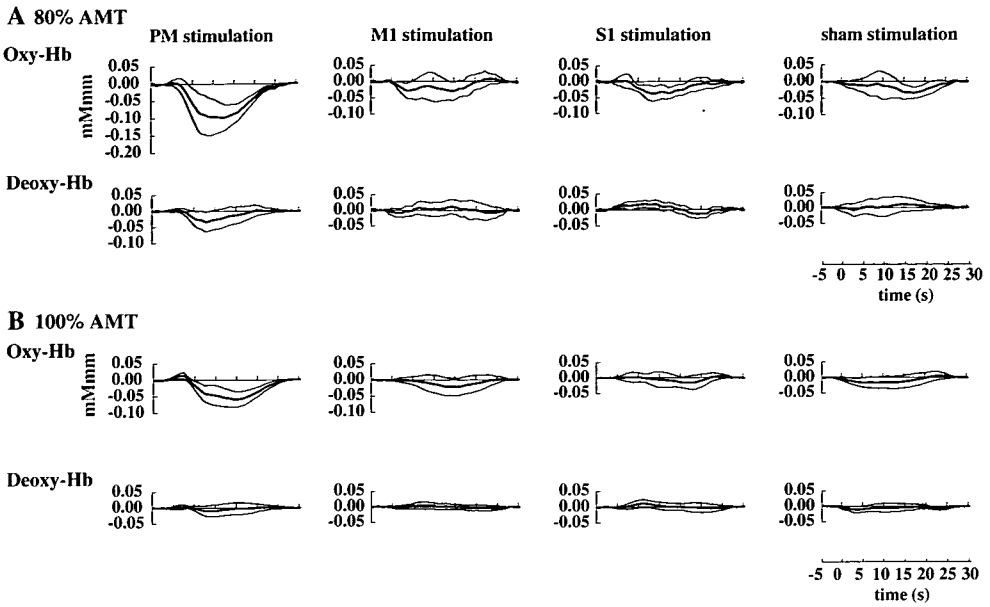

Fig. 3 NIRS recordings at right PM. Figure settings are all the same as Fig. 2. The PM stimulation evoked significant reduction of oxy Hb whereas no other stimulations elicited any changes

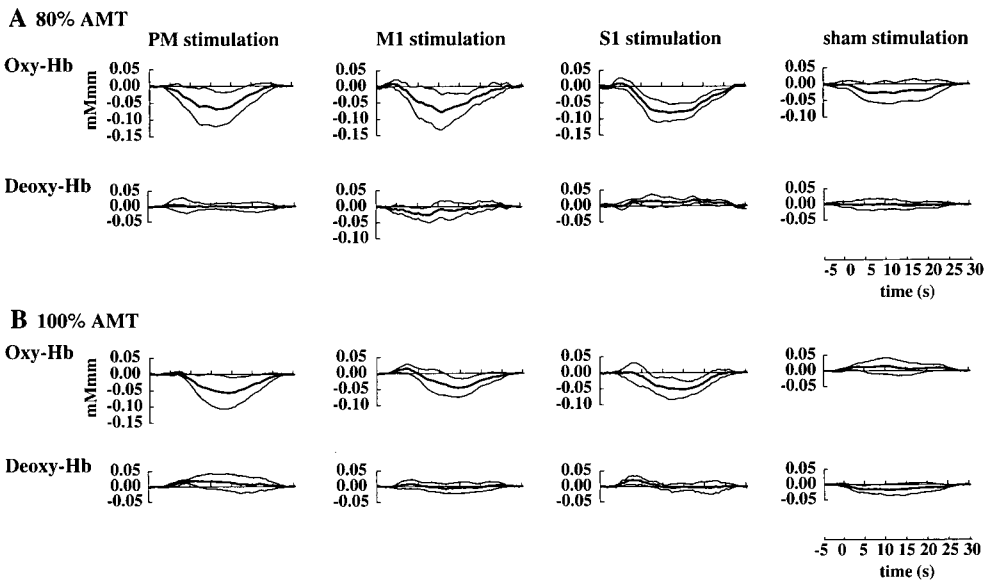

Fig. 4 NIRS recordings at right M1. Figure settings are all the same as Fig. 2. Significant oxy-Hb decrease was observed in the left PM (80% AMT) and S1 (80 and 100% AMT) stimulation conditions

At right M1, significant oxy-Hb decreases were observed in the left PM (80% AMT) and S1 (80 and 100% AMT) stimulation conditions (Fig. 4). At the right S1, significant decreases were observed in the left M1 (80 and 100% AMT) and S1 (80 and 100% AMT) stimulation conditions (Fig. 5). Sham stimulation induced no significant changes in oxy-Hb. No significant changes were observed in deoxy-Hb in any conditions.

Comparisons of mean oxy-Hb concentration changes between four sites of stimulation (stimulation SITE) or TBS intensities (INTENSITY) at each recording site showed some significant effects (SITE effects of 80% AMT TBS were summarized in Fig. 6). At the right PM, two-way ANOVA showed a significant stimulation SITE effect [SITE, F (3, 56) = 7.38, $P < 0.001$; INTENSITY, F (1, 56) = 3.59, $P = 0.06$] without any significant SITE × INTENSITY interaction

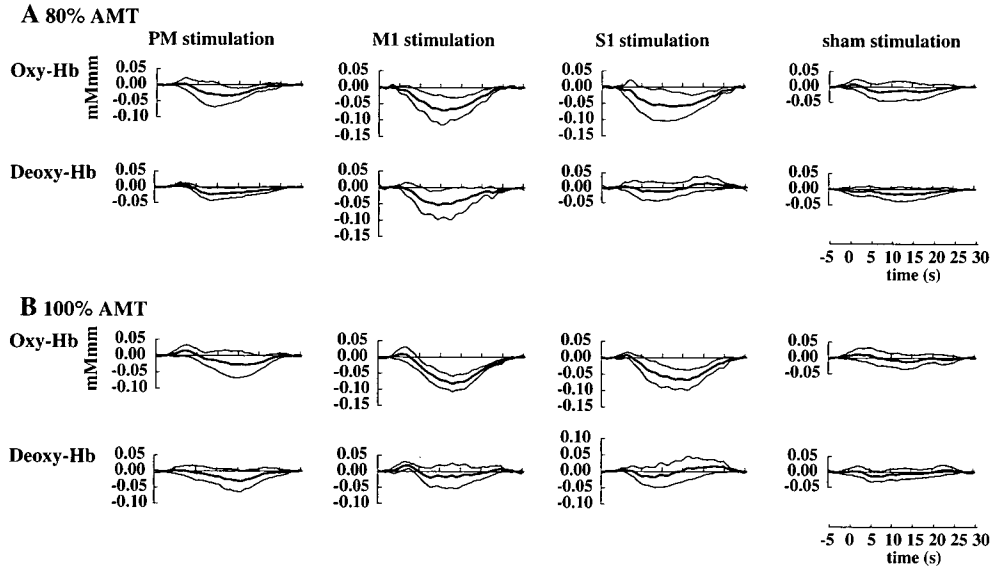

Fig. 5 NIRS recordings at right S1. Figure settings are all the same as Fig. 2. Significant oxy-Hb decreases were evoked by the left M1 (80 and 100% AMT) and S1 (80 and 100% AMT) stimulations

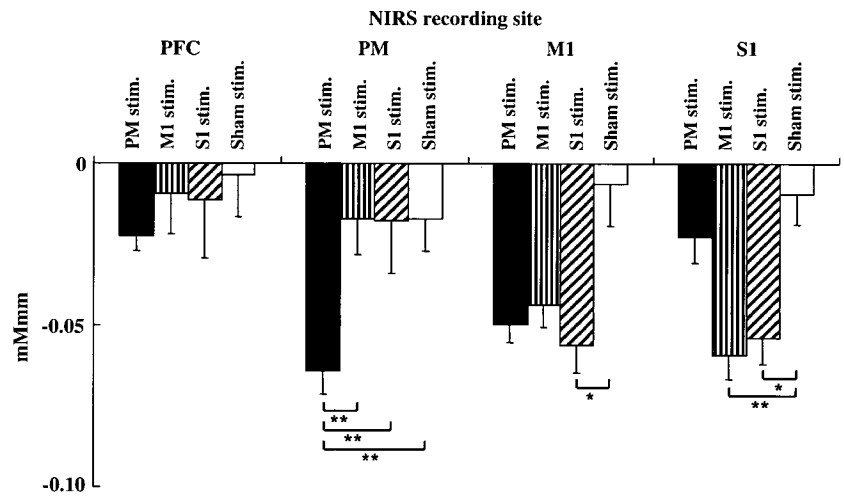

Fig.6 Mean changes of relative oxy-Hb concentrations (averages of Hb concentration values from 5 to 20 s after the onset of TBS at 80% AMT) at right PFC, PM, M1 and S1. Concentration changes elicited by TBS over left PM are denoted by filled columns, M1 by longitudinal stripe columns, S1 by oblique stripe columns, and sham stimulation by non-filled columns. At PM, the contralateral PM stimulation induced a significant oxy-Hb decrease. At M1, the contralateral S1 stimulation induced a significant oxy-Hb decrease; the contralateral M1 or S1 stimulation evoked a significant oxy-Hb decrease at S1. *Error bars* indicate standard errors. *Asterisks* indicate statistically significant changes (*$P < 0.05$, **$P < 0.01$, one-way ANOVA and post-hoc analysis with Scheffe's method)

[F (3, 56) = 0.62, $P = 0.61$] and post-hoc analysis revealed that TBS over left PM induced stronger oxy-Hb decrease than the other three stimulation sites ($P < 0.05$). At the right M1, there were significant SITE and INTENSITY effects [SITE, F (3, 56) = 3.95, $P = 0.01$; INTENSITY, F (1, 56) = 4.79, $P = 0.03$] without any significant SITE × INTENSITY interaction [F (3, 56) = 0.23, $P = 0.88$]. Post-hoc analysis disclosed that TBS over left S1 induced deeper oxy-Hb reduction than sham stimulation ($P = 0.03$) whereas its effect did not significantly differ from those evoked by TBS over left PM ($P = 0.07$) or M1 ($P = 0.15$). The significant INTENSITY effect meant that TBS effect at an intensity of 80% AMT was larger than that at 100% AMT. At the right S1, there was a significant SITE effect [SITE, F (3, 56) = 5.98, $P = 0.001$; INTENSITY, F (1, 56) = 0.03, $P = 0.86$] without any significant SITE × INTENSITY interaction [F (3, 56) = 0.14, $P = 0.94$]. TBS over the left M1 or S1 induced more prominent oxy-Hb decrease than sham stimulation. At the right

PFC, there were no significant effects or interactions [SITE, F (3, 56) = 1.34, P = 0.34; INTENSITY, F (1, 56) = 3.30, P = 0.08; SITE × INTENSITY, F (3, 56) = 1.47, P = 0.23].

The result of the additional physiological experiment was shown in Fig. 7. The MEP inhibition by TBS over S1 (28%) was significantly larger than that by sham stimulation (1%) (paired t-test: P = 0.01). This indicates that the right M1 was functionally suppressed after TBS over the left S1 in parallel with its oxy-Hb concentration reduction.

Discussion

The results of our present study can be summarized as follows. (1) TBS over PM induced a significant oxy-Hb decrease at the contralateral PM. (2) TBS over M1 induced significant oxy-Hb decrease at the contralateral S1 and TBS over S1 significant oxy-Hb decrease at the contralateral M1 and S1. (3) 80% AMT TBS over S1 induced larger oxy-Hb decrease at the contralateral hemisphere than that by 100% AMT TBS. (4) MEPs were also suppressed by conditioning TBS over the contralateral S1.

Oxy-Hb decrease

Three different patterns of NIRS changes have been reported in natural brain activation and TMS stimulation. First, large oxy-Hb increase and small deoxy-Hb decrease were observed in natural brain activation (Chance et al. 1988; Villringer et al. 1993; Kleinschmidt et al. 1996) or after low frequency rTMS (0.25 Hz; 30 stimuli) over the NIRS probes at an intensity of 100% of the maximal stimulator output, which was above the resting motor threshold (Oliviero et al. 1999). This pattern is usually called as activation. Another is large oxy-Hb decrease and small deoxy-Hb increase (Wenzel et al. 2000; Fabbri et al. 2003). This pattern is usually called as deactivation. The other one is small oxy-Hb change and large deoxy-Hb decrease. This pattern is observed only after a single pulse TMS under the center of the coil (Mochizuki et al. 2006). The pattern observed in the present study, large oxy-Hb decrease and small deoxy-Hb increase, is almost the same as that seen in natural deactivation among the above three patterns. Deoxy-Hb concentration, however, did not show any significant changes in the present results. This discrepancy might be due to the lower sensitivity of deoxy-Hb changes than oxy-Hb changes in NIRS measurement (Madsen and Secher 1999). We conclude that some deactivation must occur at the sensori-motor cortices after TBS over the contralateral sensori-motor cortices.

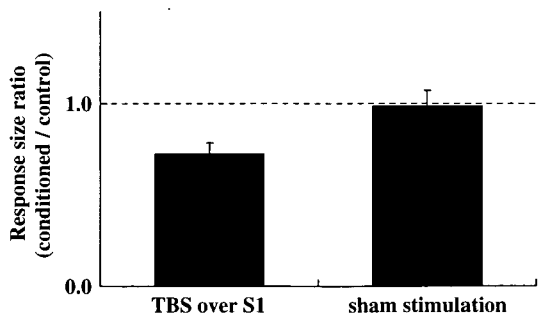

Fig. 7 Comparisons of the effects on MEPs from the left FDI evoked by TMS over right M1 between the conditioning TBS over left S1 and sham stimulation. The ratios were the average values of conditioned MEP/control MEP. TBS over left S1 induced a significant MEP decrease even though sham stimulation did not induce significant MEP changes. *Error bars* indicate standard errors

Some Hb concentration changes seemed to start a bit before the stimulation (e.g., in Fig. 4). Similar results are sometimes seen in some experiments. In the study of NIRS recording before and during gait (Miyai et al. 2001), oxy-Hb increase at supplementary motor areas started before gait. One explanation for this phenomenon is that Hb concentration changes begin when subjects can expect the start of the experiments. In our experiments, even the start of stimulation was randomized in time, randomization was not much enough to escape the expectation. It may partly explain the early start of Hb changes.

Interhemispheric connection

In animal studies, motor related areas and sensory cortex have direct connections with the contralateral homotopic and non-homotopic areas through the corpus callosum (Karol and Pandya 1971; Jenny 1979; Marconi et al. 2003). In humans, direct homotopic or non-homotopic connections have also been suggested to be present between bilateral primary motor and sensory cortices (Ferbert et al. 1992; Hanajima et al. 2001; Mochizuki et al. 2004a, b). The interhemispheric connections between other areas (e.g., PM to the contralateral PM) have not been studied physiologically because of the lack of detection methods for the function of those areas. Using a combination of NIRS and TMS, this study has physiologically proved that PM has a functional connection with the contralateral PM (directly or indirectly) in humans and that motor and sensory cortices interacted densely with the contralateral motor and sensory cortices.

The excitability changes underneath the coil most probably explain the inhibition of the contralateral

hemisphere after a short train of TMS through some interhemispheric interactions. The contralateral changes may be evoked without any changes under the coil. However, it is not plausible because we showed some changes under the coil in a previous paper (Mochizuki et al. 2006).

Intensity of TBS

The effect of 80% AMT TBS over S1 was larger than that of 100% AMT TBS. Münchau et al. (2002) reported that slow repetitive TMS over PM at an intensity of 80% AMT evoked intercortical facilitatory effects on the ipsilateral M1, but repetitive TMS at an intensity of 90% AMT had no effects. They speculated that these two areas have mutual facilitatory and inhibitory connections and stimulation at a higher intensity might evoke a mixture of effects that cancel out each other at 80% AMT. In another paired pulse TMS study (Civardi et al. 2001), single pulse TMS over PM at 90% AMT induced an inhibitory effect on ipsilateral M1, but when TMS was 110% AMT this inhibitory effect was changed to facilitatory one. In our study, 100% AMT TBS might have also evoked a mixture of facilitatory and inhibitory effects on the contralateral side and masked the inhibitory effect observed at 80% AMT TBS.

Physiological meaning

Between bilateral M1s the predominant connection is inhibitory. When one M1 is active, the contralateral M1 must be deactivated (Ferbert et al. 1992; Schambra et al. 2003; Plewnia et al. 2003). This connection plays critical roles in bimanual movements. Our study has shown the physiological inhibitory connection between bilateral PMs in humans for the first time. This connection must much contribute to the interactions between both hemispheres in bimanual and one-hand movements. Our results also demonstrated dense inhibitory connections between bilateral M1s and S1s. S1 was strongly influenced by the ipsilateral M1 (Enomoto et al. 2001). Even though we cannot conclude whether the bilateral sensori-motor cortical connections are direct one or indirect one from the present results, our findings indicate dense interactions between bilateral primary motor and sensory cortices. Smooth, fine and coordinated bimanual movements must be guaranteed by combination of powerful bilateral PM connection and dense bilateral sensori-motor cortical connections.

In conclusion we found the interhemispheric inhibitory connections between bilateral PM, M1 and S1 using TBS and NIRS in humans, and these connections would be requisite for bimanual coordinated movements in humans.

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