i.e., the blood oxygen level-dependent (BOLD) signal (Chance et al. 1998; Gratton et al. 1997; Huppert et al. 2006; Ito et al. 2000; Jöbsis 1977; Kameyama et al. 2004; Villringer et al. 1993). When used in combination with TMS, NIRS has three main advantages over PET, SPECT, or fMRI when investigating brain responses to TMS: It is immune to the effect of electromagnetic fields; it is used in a natural measurement setting, with subjects in a sitting position and without gantry requirements; and it allows simultaneous measurements during rTMS (Hanaoka et al. 2007; Mochizuki et al. 2006; Noguchi et al. 2003).

In combination with electrophysiological studies, Hb concentration changes measured by NIRS are expected to provide insights into the mechanisms of stimulation when TMS is applied to the motor cortex, which is not possible with other neuroimaging techniques such as PET and fMRI. Because of its high signal-to-noise ratios for single events and noninterference with magnetic field changes, NIRS has been successfully used to detect Hb concentration changes evoked by single-pulse TMS just beneath the coil (Mochizuki et al. 2006; Noguchi et al. 2003). This very feature allowed us to study CBF changes after TMS on a trial-by-trial basis with a relatively good temporal resolution, and also to investigate the effect of voluntary contraction of varying levels on the changes in hemodynamic responses. In addition, the simultaneous measurement of oxy-Hb and deoxy-Hb concentrations allowed us to evaluate aspects of the hemodynamic responses different from those of PET or fMRI.

Noguchi et al. (2003) and Mochizuki et al. (2006) reported that TMS increased oxy-Hb levels at the motor cortex when it was applied over the motor cortex at 90–100% active motor threshold (AMT) while subjects made weak voluntary contractions. The findings of both Noguchi et al. and Mochizuki et al. were important in demonstrating the neurovascular coupling after a single-pulse TMS. Moreover, Mochizuki et al. showed that TMS at 120% and 140% AMT, i.e., intensities comparable to a relaxed motor threshold, rather decreased deoxy-Hb and total hemoglobin (total Hb) without causing any changes in oxy-Hb levels at the motor cortex while the first dorsal interosseous muscle (FDI) was relaxed. This result is not consistent with any typical NIRS patterns previously reported in natural brain activation or deactivation. For this reason, the authors considered that weak TMS pulses at ~100% AMT may mimic natural activation, but strong TMS may not; this decrease was considered to be due to reduced baseline firings of the corticospinal tract neurons induced by a lasting inhibition provoked by a higher-intensity TMS. However, both of these studies focused only on the motor cortex just underneath the stimulating coil because the measurement systems employed had a single channel, and possible changes in Hb concentration in cortical areas surrounding the motor cortex were not investigated.

In the present study, we addressed two main questions on the effect of TMS of the motor cortex on CBF using NIRS, namely, *1*) how does muscle contraction influence the effect of TMS targeting primary motor cortex (M1) on cortical Hb levels? and *2*) how do cortical Hb dynamics in non-motor cortical regions compare to dynamics in the stimulated motor cortex? To this end, we investigated the change in CBF at or around the motor cortex when the target muscle (the FDI) was maintained at a weak contraction level or was relaxed, using a specially devised double square coil for multichannel NIRS. We used the anteromedial current direction because this direction is most effective for eliciting motor evoked potentials (MEPs).

METHODS

Subjects. Fifteen healthy volunteers (12 men and 3 women), aged 28–54 yr, participated in the study. All the subjects were right-handed, scoring 75–100 on the laterality quotient of the Edinburgh handedness inventory (Oldfield 1971). They had no previous history of any neurological or psychiatric disorders. Written informed consent was obtained from all the subjects after the nature and possible consequences of the studies were explained. The experimental procedures used here were approved by the Ethics Committee of Fukushima Medical University and The University of Tokyo and were carried out in accordance with the Declaration of Helsinki.

NIRS measurements. The NIRS system used (ETG-100; Hitachi Medical, Chiba, Japan) consisted of 6 pairs of optical fibers functioning as emitters and detectors, so that the 17 measurement probes (17 channels) were placed over the left hemisphere centered on the left M1. The ninth channel (ch 9) corresponded to the left M1, illustrated in Fig. 1*C* with a green circle. The measurement points were aligned parallel to the anteromedial line according to the direction of the TMS coil. Neighboring emitters and detectors were arranged 3 cm apart, an optimal spacing for measuring changes in Hb concentration in neural structures at a depth of 15–20 mm from the scalp, i.e., in the cerebral cortex (Germon et al. 1999; Kato et al. 1993) (see Fig. 1). The NIRS probes were provided with plastic insulation. Because NIRS uses infrared light to measure blood flow, it is immune to electromagnetic artifacts that might be caused by the TMS. This is in sharp contrast with fMRI, which measures the electromagnetic field directly and is much more subject to electrical artifacts arising from the TMS. However, the NIRS measurement system can be affected by artifacts due to oscillatory movements of the coil during stimulation procedures. To overcome this problem, a nonmagnetic spring was used to mitigate such movement artifacts. Near-infrared laser diodes operating at two wavelengths, 790 and 830 nm, were used as the light sources, and transmittance data of the light beams were obtained every 500 ms, as detailed in our previous study (Mochizuki et al. 2006). The combination of these wavelengths is cause for concern, with problems such as cross talk (Strangman et al. 2003; Uludag et al. 2002) and the signal-to-noise ratio (Sato et al. 2004; Thomson et al. 2011; Yamashita et al. 2001) discussed. Nevertheless, even when using this combination of wavelengths and sampling rate, other groups (Isobe et al. 2001; Watanabe et al. 1998) obtained results that show typical Hb concentration changes similar to those obtained when using other combinations of wavelengths. In addition, Thomson et al. (2011) obtained similar results using wavelengths of 687 nm and 830 nm and a sampling frequency of 50 Hz. Noguchi et al. (2003) also used a similar experimental setting, and their results were similar to those obtained by fMRI after single-pulse TMS over the motor cortex. Therefore, although the wavelengths and sampling frequency we used might not have been ideal, we believe they were suitable enough to provide reliable results.

We calculated the concentrations of oxy-Hb, deoxy-Hb, and total Hb based on the data for the two wavelengths. We used the same basic procedure as in our previous studies (Mochizuki et al. 2006, 2007), with each event period ranging from 5 s prior to TMS onset (baseline measurement) to 23 s after application. One session consisted of five trials, and each session was repeated twice for all the subjects to confirm reproducibility of the results for each condition. The session order (sham stimulation, 100%, 120%, 140% AMT, see below) was counterbalanced within and across the subjects.

To explore the responses in a broader area centered over M1 in the left hemisphere, we focused on five measurement points anterior and

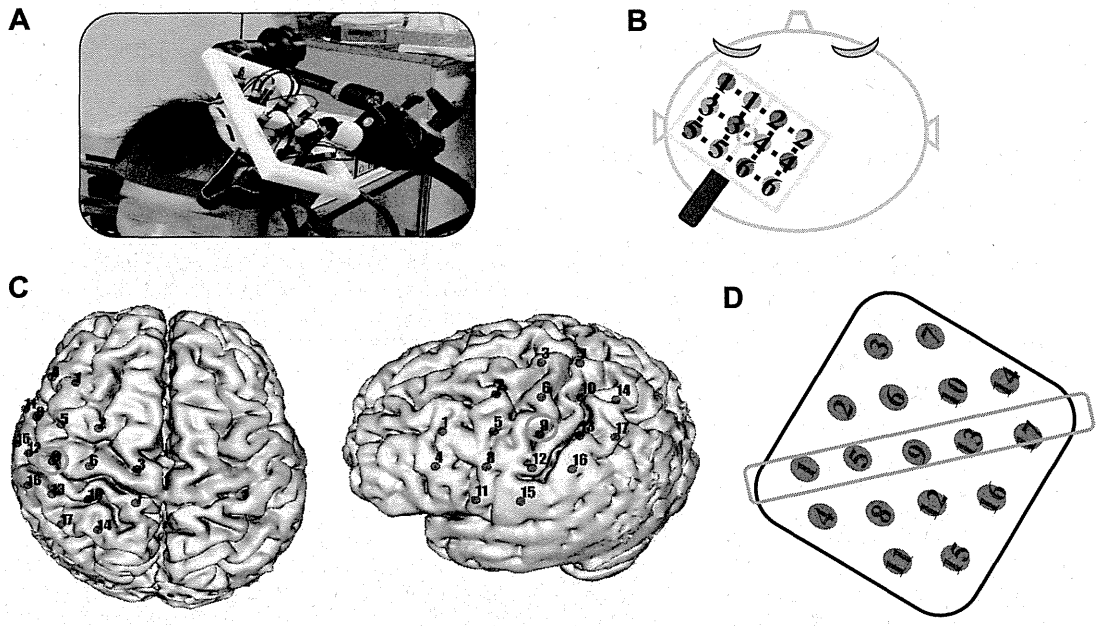

Fig. 1. Experimental setup for near-infrared spectroscopy (NIRS) measurement. *A*: the double square coil was held just above the probe holder, allowing the probes to project beyond the plane of the coil windings. *B*: arrangement of the NIRS probes. The distance between the probe pairs serving as detector and emitter was fixed at 3 cm, so that emitted infrared beams could measure the hemoglobin concentration changes in the cerebral cortex most efficiently (dots). Channel 9 (Ch 9) was placed over the left M1 (green circle in *C*). *C*: the NIRS channels are shown projected over a surface rendering of the brain MRI. *D*: 5 channels in the middle were used for recording: Ch 1, frontal area (FC); Ch 5, premotor area (PM); Ch 9, primary motor cortex (M1); Ch 13, anterior parietal cortex (APC); Ch 17, posterior parietal cortex (PPC).

posterior relative to M1. Although all 12 (6 pairs) probes were fixed to the holder, some of them were displaced from the scalp because of curvature differences between the probe holder and subject's crania, which led to a decrease in light captured by certain probes and a resulting disturbance of proper NIRS recording. Therefore, unfortunately, we had to select the five channels over the midline, nearest the central hole and thus less subject to displacement. Specifically, the ch 9 probe was placed over M1 as defined by the TMS hot spot, and four other probes with dedicated channels were placed as follows: frontal area (FC, ch 1), premotor area (PM, ch 5), anterior parietal cortex (APC, ch 13), and posterior parietal cortex (PPC, ch 17) (Civardi et al. 2001; Terao et al. 2005), based on a surface-rendered brain MR image projected onto the measurement positions (see Fig. 1).

Transcranial magnetic stimulation. Single-pulse TMS was delivered via a double square coil connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, UK). The double square coil (95 × 100 mm, 0.96 T) was specially devised for multichannel NIRS measurements (Fig. 1). The center of the coil was placed just over M1 to induce anteromedially directed currents in the brain. With this coil orientation, the current induced in the brain flows perpendicular to the central sulcus, which leads to a predominantly transsynaptic activation of the corticospinal system (Sakai et al. 1997; Terao et al. 2001). With the coil placed just above the probe holder, the minimum distance between the coil and the scalp was 8.5 mm. MEPs were recorded from the right FDI by a pair of surface cup electrodes with a belly-tendon montage. Signals were amplified, with filters set at 100 Hz and 3 kHz, and recorded by a computer (Viking IV, Nicolet Biomedical) for later off-line analysis. The TMS intensity was adjusted to 100%, 120%, and 140% of the AMT over M1. We defined AMT as the lowest intensity that evoked 5 small responses (of ~100 μV) during a series of 10 stimulations when the subject maintained 10% of maximal voluntary contraction (MVC). Sham stimulation was performed to exclude nonspecific effects associated with TMS, such as the loud pulse noise. During sham stimulation, another coil connected to the same magnetic stimulator was positioned 10 cm above the head and discharged while the normally used double square coil remained in contact with the probe holder. TMS was tested under eight different conditions in all subjects: TMS at three different intensities (100%, 120%, and 140% AMT) and sham stimulation, under both relaxed and active conditions.

Procedures. Subjects were seated on a reclining chair with an armrest and wore earplugs in both ears during the experiment to minimize the suppressive effects of the transient loud sounds associated with TMS over the cerebral cortex (Furubayashi et al. 2000). The optimal stimulus site over the motor cortex for the FDI (the hot spot) was determined by using a figure eight-shaped coil and a double square coil in each subject to make sure that the localization of M1 was the same for the two types of coils. To locate the hot spots, we moved the stimulation site in 1-cm steps starting at a point 5 cm lateral to the vertex and determined the location at which the largest responses were elicited for the same stimulus intensity, and the hot spot was marked with a pen. Then, at this location, we compared the differences between AMTs for the double square coil and the figure eight-shaped coil. After that, the probe holder was placed over the scalp. The probe holder was provided with a number of holes between the locations of the nine NIRS channels. When placing the holder, we carefully checked that the locations of the ch 9 probe and the hot spot were coincident. The probe holder is shaped so that when the double square coil is placed over the holder, its center will automatically be fixed just above the ch 9 probe of the NIRS system. The probe holder has four markers, and the location of the coil was checked for possible displacement throughout the experiment, by checking that hole positions coincided with markers on the scalp.

We also compared AMT values when the double square coil was placed on the probe holder (8.5 mm from the scalp) with those when the same coil was placed in close contact with the scalp (for additional details, see *Transcranial magnetic stimulation*).

Before the main experiment, each subject was instructed to perform self-paced finger tapping in order to confirm the optimal probe location for recording M1 Hb concentration changes. The tapping

task, which consisted of sequential oppositional movements between the thumb and the other four fingers at a pace of ~1 Hz, lasted for 20 s while NIRS measurements were recorded.

During the main experiment, the experimenter spoke the words "start" and "end" at the beginning and the end of the measurement, respectively. Subjects being tested in the active condition were to immediately start and maintain contraction of the FDI at 10% MVC on hearing the word "start," while they were to remain at rest when undergoing relaxed condition tests. Muscle activities were monitored throughout the experiment with an oscilloscope screen. In the active condition, the subjects could maintain constant contraction by monitoring the EMG monitor. In the relaxed condition, subjects could also monitor the state of muscle contraction in order to avoid inadvertent EMG activity. We therefore considered that the attention level was practically the same between these two conditions. Actual measurements commenced when the NIRS measurements reached a stable level, a few trials after the subjects initiated their task.

To minimize factors that might confound the measurement of Hb concentrations, such as counting or irrelevant thoughts, subjects were instructed to strictly focus on maintaining muscle condition and to refrain from predicting the start of the next trial.

Data analyses and statistical assessment. We discarded trials in which inadvertent muscle contraction was observed in the monitored EMG trace. As a result, 0.8% of the trials were discarded from the analysis.

We recorded changes in cortical Hb concentration over the time period starting 3 s before TMS and ending 13.5 s after. On the basis of data obtained during this interval, the 95% confidence interval was calculated at each time point for changes in oxy-Hb, deoxy-Hb, and total Hb for all five probe channels. The statistical analysis sought to evaluate the changes in Hb concentrations over time, and how these values differed according to different stimulus intensities and conditions (relaxed or active) as monitored from the five probe locations. To accomplish this, the value for each Hb concentration at 3, 6, 9, and 12 s after application of TMS was compared with the corresponding baseline (BL) value. Averaged Hb concentrations for the following four periods, with a duration of 3 s each, were calculated: BL (−3 to 0 s before TMS), 3 s (1.5 to 4.5 s after TMS), 6 s (4.5 to 7.5 s), 9 s (7.5 to 10.5 s), and 12 s (10.5 to 13.5 s).

With SPSS 11.5 software (SPSS Japan, Tokyo, Japan), the subscores for Hb concentrations were subjected to repeated-measures analysis of variance (ANOVA). Each of the four following factors was selected according to the issue of interest: TMS intensity (100%, 120%, and 140% AMT and sham); condition (relaxed and active conditions in the FDI); time (BL and 3, 6, 9 and 12 s after TMS); and area (FC, PM, M1, APC, and PPC). For all analyses, the significance criterion was set at $P < 0.05$. Contingent on the significance of analyzed effects and interactions, post hoc analyses (Bonferroni correction for multiple comparisons) were performed to assess which differences contributed to the significance revealed by ANOVA.

RESULTS

Finger tapping. Using NIRS, we confirmed that TMS hot spots corresponded to the M1. The averaged time course of NIRS measurement during the tap task is shown in Fig. 2. The increase

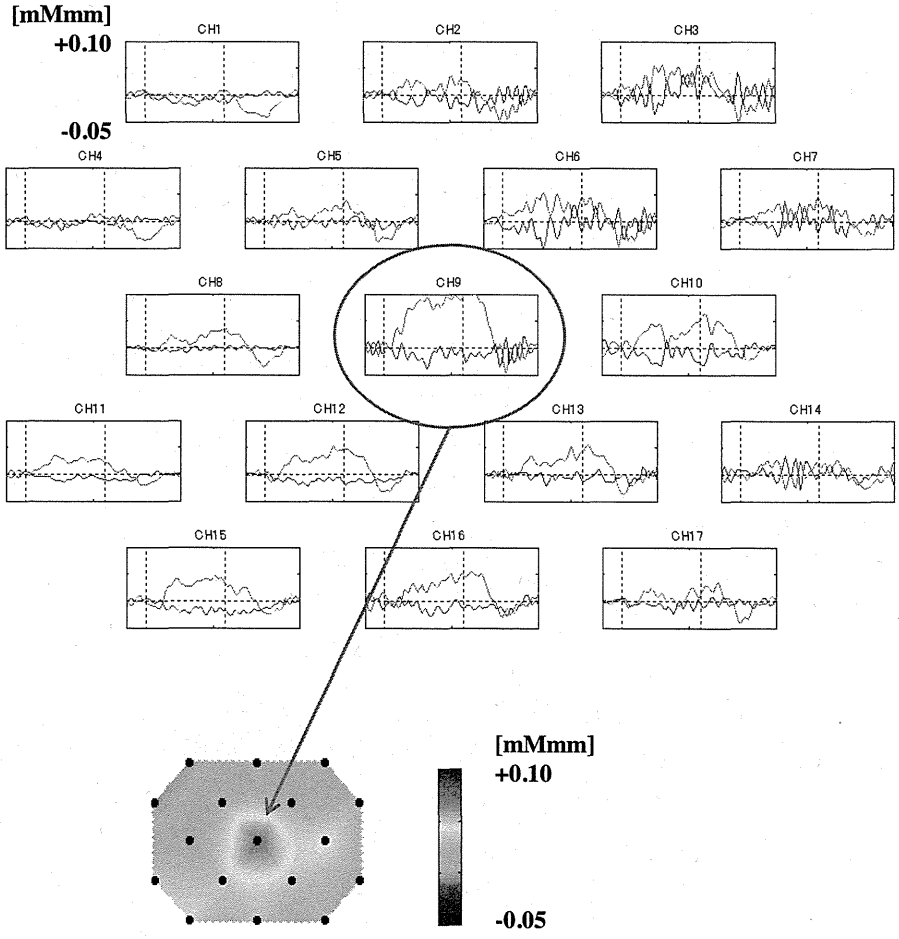

Fig. 2. Hemoglobin concentration changes during the self-paced 1-Hz finger tapping task. In each image, the vertical axis shows the oxy-Hb or deoxy-Hb concentrations (mMmm) and the horizontal axis the time (s). Subjects tapped their fingers during the period indicated by the 2 vertical dashed lines. The interval from the *left* dashed line to the *right* dashed line denotes the duration of the tapping, i.e., from 0 s to 20 s. Red curves represent oxy-Hb concentration and blue curves deoxy-Hb concentration. Ch 9 corresponds to the left M1, where a prominent increase followed by a small decrease is noted in the oxy-Hb concentration. Channels posterior to M1 also show small increases.

in oxy-Hb concentration was predominant at ch 9 when finger tapping was initiated and returned to BL after a plateau period, while deoxy-Hb concentration decreased slightly during tapping. The increase in oxy-Hb concentration observed at 8 s after the initiation of the tapping was more prominent at ch 9 than at any other channel. These results indicate that ch 9 indeed corresponded to M1 as determined by the TMS hot spot location.

Time course of Hb concentration changes after single-pulse TMS. Figure 3 shows the grand averaged relative oxy-Hb concentration changes and the 95% confidence intervals for all subjects when the subject kept the right FDI relaxed (relaxed condition, Fig. 3, *left*) and maintained 10% MVC (active condition, Fig. 3, *right*). Overall, in the relaxed condition, oxy-Hb increased rapidly after TMS and peaked 5–6 s later. Thereafter, the concentration returned swiftly to BL and Hb concentration did not change in response to sham stimulation during the entire recording time. In contrast, in the active condition, oxy-Hb concentration gradually increased after TMS but decreased relative to BL between 9–12 s after TMS. The change in oxy-Hb concentration tended to increase with increasing TMS intensity. Statistical analyses were conducted to confirm these trends by means of repeated-measures ANOVA with four factors [area (FC, PM, M1, APC, and PPC), condition (relaxed, active), intensity (100%, 120%, 140% AMT and sham), and time (BL and 3, 6, 9, and 12 s after TMS)].

There was a significant main effect both of time and condition and the interaction between them (main effect of time: $F = 57.5$, $P < 0.001$ and condition: $F = 85.9$, $P < 0.001$; interaction of condition × time: $F = 4.2$, $P < 0.01$). The main effect of time suggested that oxy-Hb concentration varied significantly after TMS relative to BL (3 s: $P < 0.001$, 6 s: $P < 0.05$, 9 s: $P < 0.001$, 12 s: $P < 0.001$). The main effect of condition reflected the fact that changes in oxy-Hb concentration were significantly smaller when the subjects were in the active condition compared with those in subjects in the relaxed condition throughout the entire time course, i.e., at 3, 6, 9, and 12 s after TMS (3 s after TMS: $P < 0.001$, 6 s: $P < 0.001$, 9 s: $P < 0.001$, 12 s: $P < 0.001$). The significant interaction implied that the time course of oxy-Hb concentration after TMS was different depending on whether the subject was in the active or relaxed condition. When the subjects were in the relaxed condition, oxy-Hb increased rapidly after TMS and peaked 5–6 s later, and the concentration rapidly returned to BL thereafter. However, with subjects in the active condition, oxy-Hb concentration gradually increased after TMS but decreased between 9 and 12 s after TMS. Indeed, in the relaxed condition, significantly increased oxy-Hb concentrations were observed relative to BL at 3 s and 6 s after TMS, but evidence of this significant change disappeared 9 s after TMS (3 s and 6 s: $P < 0.001$). In contrast, when subjects were in the active condition, oxy-Hb increased significantly relative to BL at

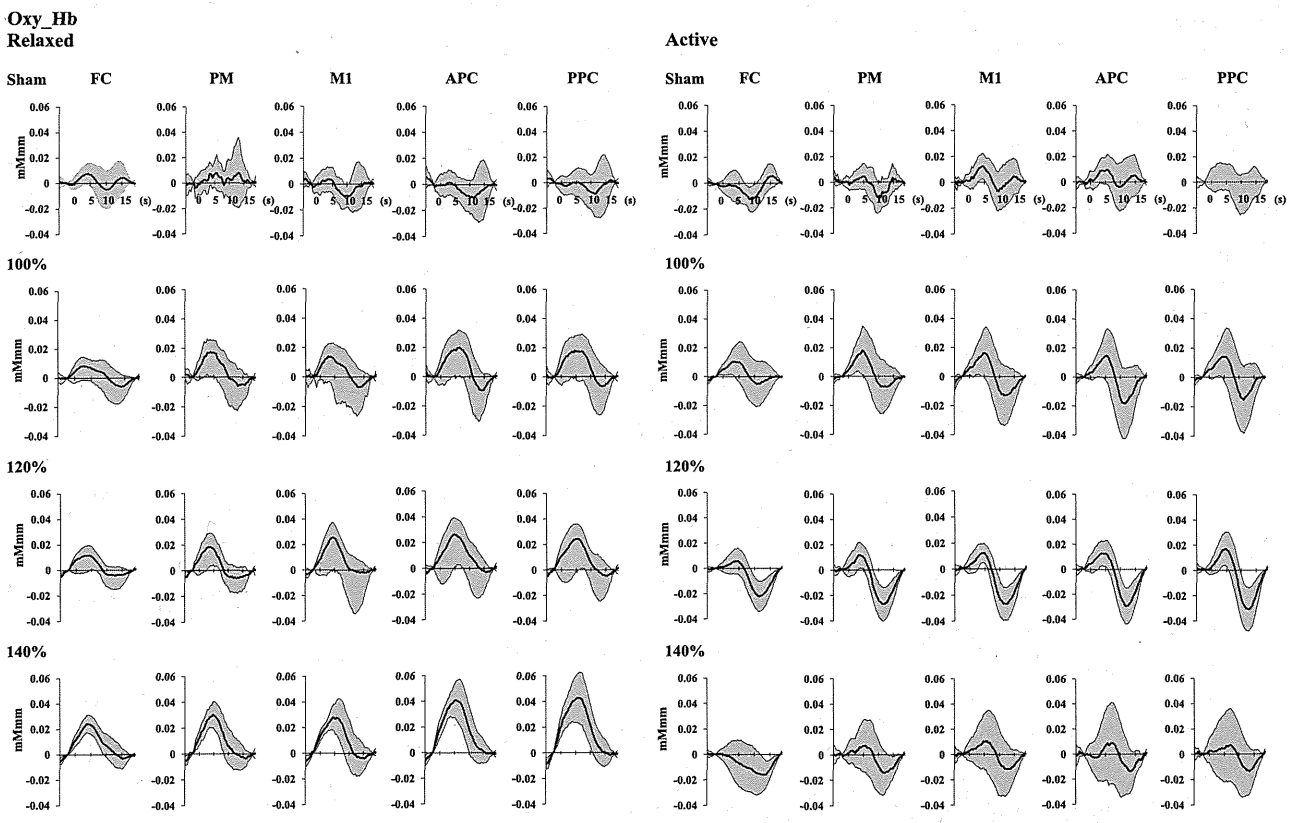

Fig. 3. Averaged oxy-Hb concentration changes (thick line) and 95% confidence intervals (thin lines) while the subject kept the right first dorsal interosseous muscle (FDI) relaxed (relaxed condition, *left*) or maintained 10% maximal voluntary contraction (MVC) (active condition, *right*). The 5 channels from *left* to *right* represent the frontal area (FC), premotor area (PM), motor cortex (M1), anterior parietal cortex (APC), and posterior parietal cortex (PPC). Traces for sham stimulation and 100%, 120%, and 140% active motor threshold (AMT), respectively, are shown from *top* to *bottom*. In each image, the vertical axis shows the Oxy-Hb concentrations and the horizontal axis the time. Transcranial magnetic stimulation (TMS) was applied at 0 ms (*left* end of each plot).

3 s but then decreased significantly at 9 and 12 s (3 s: $P < 0.01$, 9 s and 12 s: both $P < 0.001$).

The main effect of TMS intensity as well as the interaction between factors condition and intensity also reached significance (main effect of intensity: $F = 10.3$, $P < 0.001$; condition × intensity: $F = 18.7$, $P < 0.001$). This suggested that the change in oxy-Hb concentration increased with increasing TMS intensity, but differently for subjects in the active and relaxed conditions. Post hoc analyses showed that in the relaxed condition the change in oxy-Hb concentration increased with increasing intensity relative to the sham stimulation condition (sham vs. 100%: $P < 0.05$, sham vs. 120%: $P < 0.001$, sham vs. 140%: $P < 0.001$). However, in the active condition, increase in oxy-Hb concentration relative to sham stimulation was only noted at 120% AMT ($P < 0.01$). Oxy-Hb increased relative to the sham condition at 3 s (sham vs. 100% and 120%: $P < 0.01$; sham vs.140%: $P < 0.001$), 6 s (sham vs. 100%: $P < 0.01$, sham vs. 140%: $P < 0.001$, 120% vs. 140%: $P < 0.05$), and 9 s (120% vs. 140%: $P < 0.001$). At 12 s, however, a decrease in oxy-Hb concentration was noted at 120% AMT relative to sham stimulation ($P < 0.001$).

The main effect of factor area showed a trend ($F = 2.3$, $P = 0.056$) suggesting that the Oxy-Hb level was different across areas; we noted a tendency for Hb concentration changes to be more pronounced in M1 and cortical regions posterior to it than anterior to it. To confirm this possible difference in anterior (FC) and posterior (APC and PPC) cortical regions, we performed planned comparison between anterior and posterior cortical regions, which showed a significant difference between FC and APC, as well as between FC and PPC (FC vs. APC: $P < 0.001$, FC vs. PPC: $P < 0.05$). No significant interactions were observed between area and condition, area and intensity, or area and time. The differences in Hb concentration changes observed at the different channels are discussed below (see Fig. 6).

Figure 4 shows the averaged relative deoxy-Hb concentration changes and the 95% confidence intervals for the relaxed (Fig. 4, *left*) and active (Fig. 4, *right*) conditions. Here there was neither a significant main effect of time nor a significant interaction between factors time and condition, meaning that deoxy-Hb did not change significantly compared with BL after TMS under either the relaxed or active condition. Indeed, this was true when separate analysis was conducted for each channel under either condition.

Figure 5 shows the averaged relative total Hb concentration changes and the 95% confidence intervals in the relaxed (Fig. 5, *left*) and active (Fig. 5, *right*) conditions. Essentially the same trend was noted as for oxy-Hb (Fig. 3), but some differences were observed. There was a significant main effect of both time and condition, and the interaction between condition and time was also significant (main effect of time: $F = 53.7$, $P < 0.001$ and condition: $F = 60.6$, $P < 0.001$; interaction condition × time: $F = 3.1$, $P < 0.05$). Total Hb concentration varied significantly relative to BL after TMS and was significantly smaller in the active condition than the relaxed condition throughout the time course, i.e., at 3, 6, 9, and 12 s after TMS (3 s: $P < 0.001$, 6 s: $P < 0.001$, 9 s: $P < 0.001$, 12 s: $P < 0.001$). The significant interaction implied that

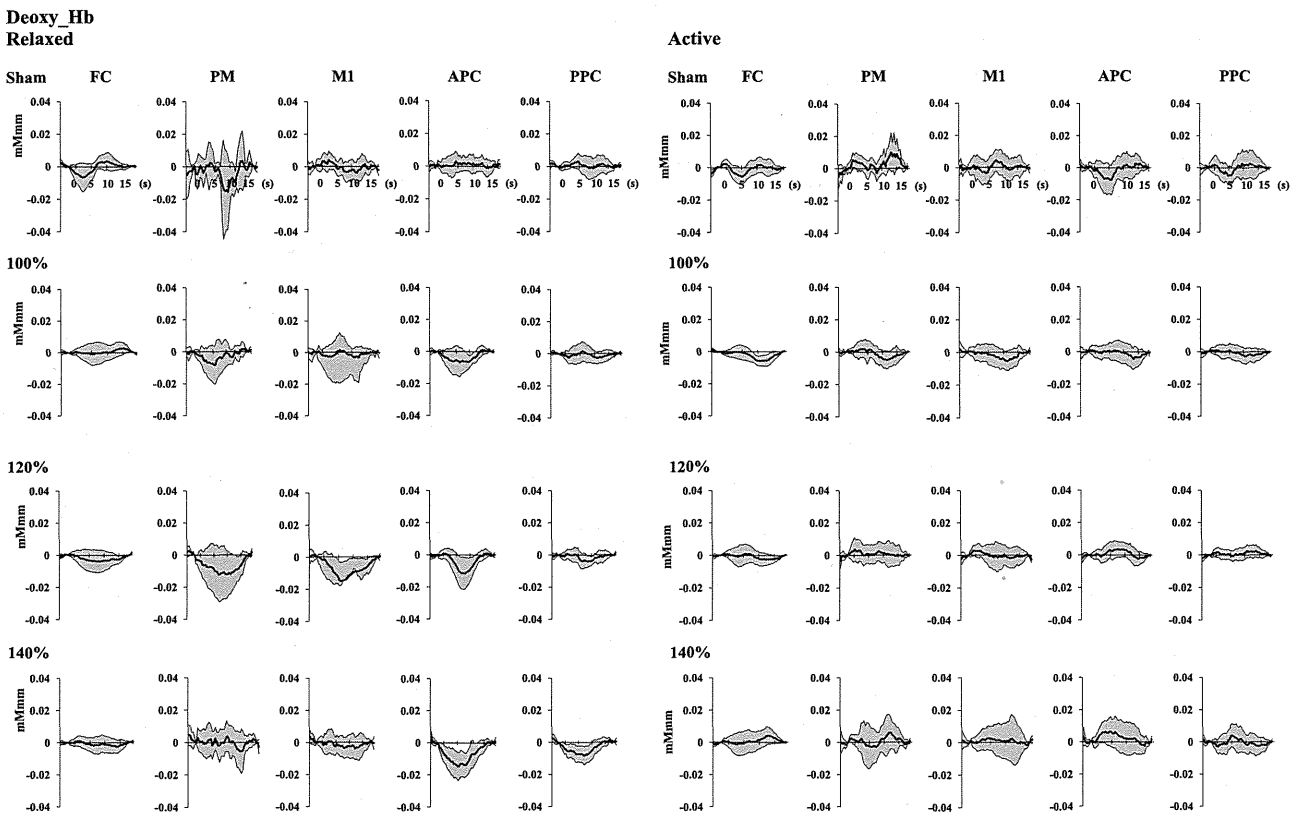

Fig. 4. Averaged deoxy-Hb concentration changes (thick line) and 95% confidence intervals (thin lines) in the relaxed (*left*) and active (*right*) conditions. Conventions are the same as in Fig. 4.

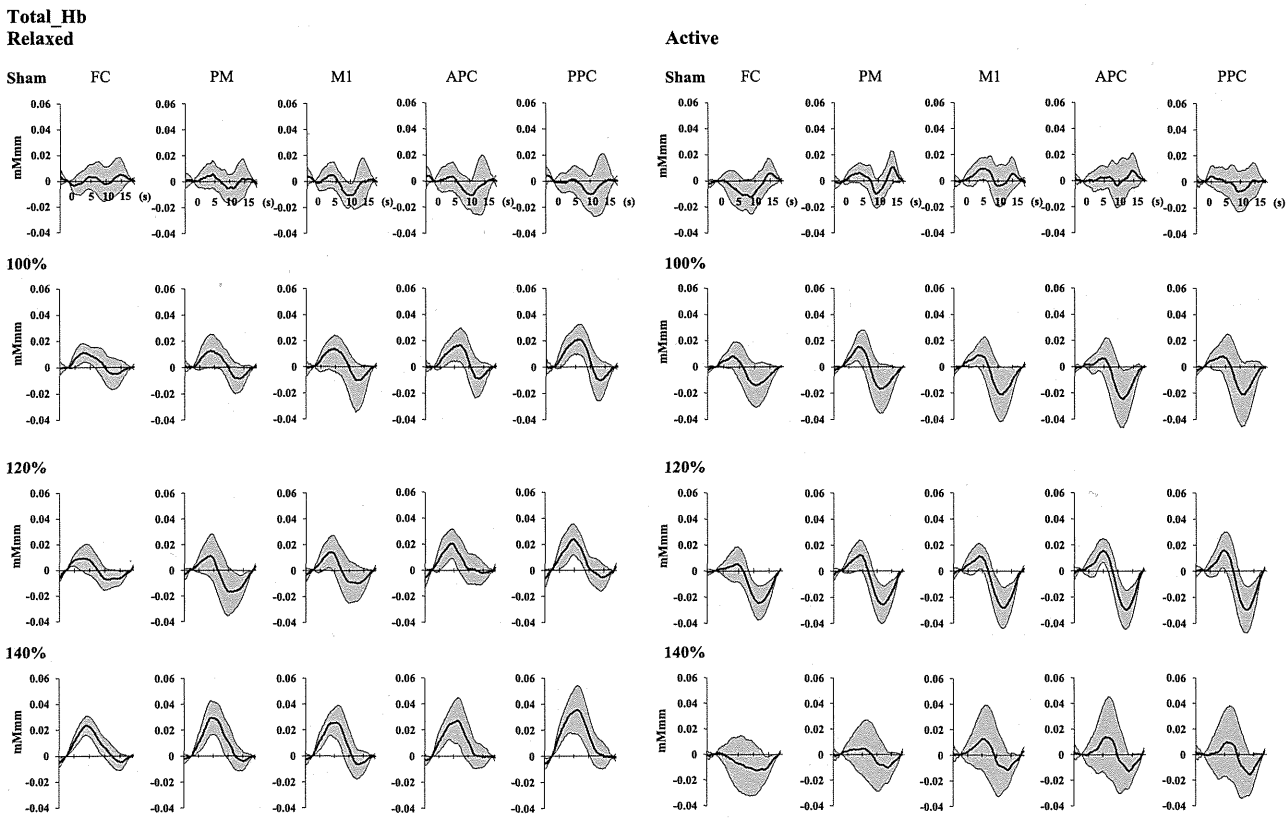

Fig. 5. Averaged total Hb concentration changes (thick line) and 95% confidence intervals (thin lines) in the relaxed (*left*) and active (*right*) conditions. Conventions are the same as in Fig. 4.

the time course of total Hb concentration after TMS was different depending on whether the subjects were in the active or relaxed condition. As with the observed changes in oxy-Hb concentration, total Hb increased rapidly after TMS but decreased slightly relative to BL thereafter and then returned to BL. This later decrease was more prominent for the active condition than for the relaxed condition. Indeed, in the relaxed condition, total Hb increased significantly relative to BL at 3 s after TMS ($P < 0.001$) and decreased significantly at 12 s ($P < 0.05$). In contrast, in the active condition, total Hb decreased significantly at 9 and 12 s (both 9 and 12 s: $P < 0.001$). Again, the main effect of TMS intensity as well as the interaction between factors condition and intensity were significant (intensity: $F = 13.1$, $P < 0.001$; condition × intensity: $F = 9.0$, $P < 0.001$). This suggested that the change in total Hb concentration increased with increasing TMS intensity for both the relaxed and active conditions, but this trend was noted more prominently for the relaxed than the active condition (100% AMT: $P < 0.001$; 120% AMT: $P < 0.001$; 140% AMT: $P < 0.001$).

Because the main effect of factor area showed a trend for oxy-Hb (see above), we later compared areal differences in Hb concentration changes. We focused our analysis on TMS applications with an intensity of 120% AMT because this level provided the most robust Hb concentration changes among both sub- and suprathreshold TMS intensities.

Figure 6 compares the time courses of Hb concentration changes (oxy-Hb, deoxy-Hb, and total Hb) for the different channels for the relaxed and active conditions. As noted above, the time courses for oxy-Hb (Fig. 6, *left*) differed between the two conditions as suggested by the significant main effect of time and condition as well as their interaction. The main effect of area approached a trend ($F = 2.2$, $P = 0.067$) due to the larger Hb concentration changes observed at channels over or posterior to M1 than at frontal channels. This trend was also noted for total Hb (Fig. 6, *right*; $F = 4.2$, $P < 0.01$). As shown in Fig. 4, deoxy-Hb did not change significantly relative to BL after the application of TMS (Fig. 6, *center*).

DISCUSSION

This study gave us some answers to our two main questions mentioned in the introduction: differences between recording sites and those between muscle conditions. A single-pulse TMS induced changes in the oxy-Hb and total Hb concentrations not only in the motor cortex just underneath the coil but also in the surrounding cortical areas. These Hb concentration changes were more prominent at M1 and areas posterior to it. Importantly, we noted drastic differences in Hb concentration changes depending on whether subjects were in an active or relaxed condition. In the relaxed condition, Hb concentration continued increasing up to 3–6 s after single-pulse TMS, peaking at ~6 s (Figs. 3 and 6). In contrast, in the active condition, a smaller increase in oxy-Hb and total Hb concentrations continued up to 3–6 s after single-pulse TMS (early activation) but was followed by a decrease in Hb concentration from 9 to 12 s after the delivery of the TMS pulse (delayed deactivation). The delayed deactivation was consistent

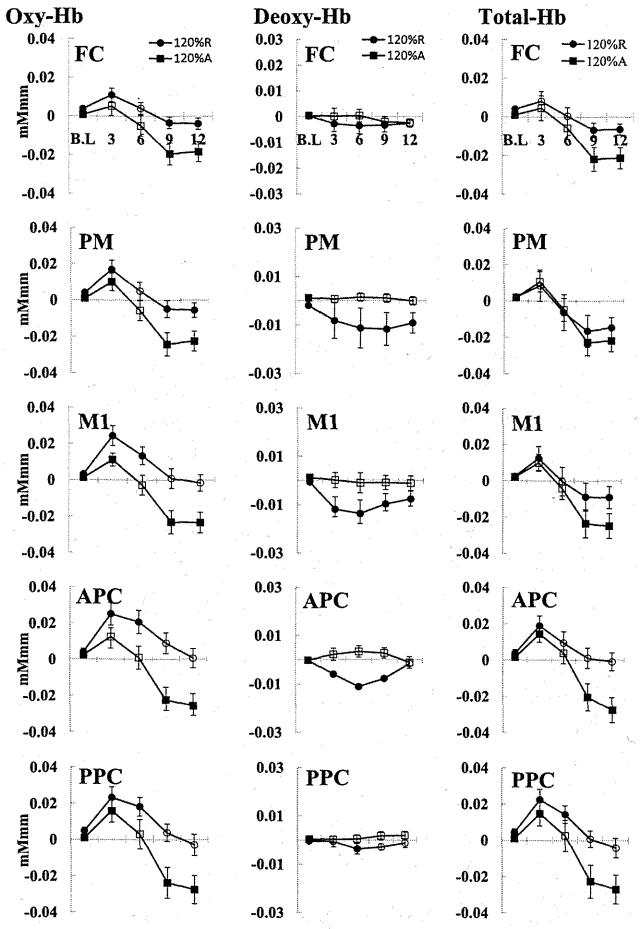

Fig. 6. Time courses of averaged relative oxy-Hb (*left*), deoxy-Hb (*center*), and total Hb (*right*) concentration changes in the relaxed (R, circles) and active (A, squares) conditions induced by TMS at 120% AMT. Filled symbols show significant differences compared with baseline (BL) ($P < 0.05$). In the active condition, prominent early activation was followed by delayed deactivation after single-pulse TMS at the channel over M1 and posterior areas. A decrease was also seen in the relaxed condition in the anterior channels (FC, PM).

with the results of Mochizuki et al. (2006), who also conducted a similar study with subjects in the active condition. These results suggest that the Hb concentration changes observed under the active and relaxed conditions were produced by different mechanisms, which we discuss below.

Hb concentration changes did not exhibit a simple dose dependence. Although the increase in oxy-Hb and total Hb in the relaxed condition increased with increasing stimulus intensity, the deactivation in the active condition was most prominent at 120% AMT.

Finally, the double square coil used in this study allowed close contact with the probe holder and thus good proximity to the scalp even when used in combination with a multichannel NIRS system. This combination of devices may provide an especially effective method for recording Hb concentration changes under or near the TMS coil.

Early activation after TMS over motor cortex. NIRS is assumed to reflect changes in CBF associated with brain activity (Akiyama et al. 2006; Hada et al. 2006; Hirth et al. 1996; Kato et al. 1993; Noguchi et al. 2003; Watanabe et al. 1998). The typical pattern of cerebral activation observed via NIRS measurements consists of an increase in oxy-Hb and total Hb and a small decrease in deoxy-Hb (Abdelnour and Huppert 2009; Hanaoka et al. 2007; Kleinschmidt et al. 1996; Villringer et al. 1993; Watanabe et al. 1998). During physiological brain activation, an increase in blood flow and the resultant increase in oxygen supply are larger than that in the oxygen consumption induced by neuronal activation, resulting in a net increase in oxy-Hb level and decrease in deoxy-Hb level in the cerebral blood vessels (Fantini 2002; Fox and Raichle 1986; Villringer and Chance 1997). Conversely, cortical deactivation is accompanied by oxy-Hb decrease with a small decrease or unaltered deoxy-Hb concentration (Hada et al. 2006; Hanaoka et al. 2007; Kozel et al. 2009; Sakatani et al. 1998). Thus the increase of oxy-Hb and total Hb concentrations we observed in the subjects in the relaxed condition after TMS is compatible with the pattern of typical physiological cerebral activation. We also noted this typical cortical activation pattern during the self-paced finger tapping task that each subject conducted as a preliminary study.

A TMS pulse transsynaptically depolarizes the population of pyramidal tract neurons (PTNs) underlying the coil, which then induces action potentials in the PTNs followed by a subsequent prolonged inhibition (Barker et al. 1998; Seyal et al. 1993). The local field potential caused by postsynaptic processing is considered to correlate with the increase in CBF (Allen et al. 2007; Logothetis et al. 2001; Mathiesen et al. 1998; Moliadze et al. 2005). Therefore, cortical activation after TMS is likely due to spatial summation of the activity of interneurons within the motor cortex induced by TMS. As the TMS intensity is increased, the overall activity induced in the cortical interneurons also increases, resulting in further increase in oxy-Hb and total Hb and a small decrease in deoxy-Hb in the cerebral blood vessels. Devor et al. (2005) stimulated the whiskers of rats and measured the local hemodynamic response, using optical imaging and electrical responses from the somatosensory cortex. Both multiunit activity and local field potentials increased with increasing stimulus intensity and reached a plateau at a certain level.

However, with subjects in the active condition, the prominent increase in oxy-Hb concentration change typically observed after TMS was much less prominent than when subjects were in the rest condition. The reason for this may be that, because of weak muscle contraction in the FDI, the Hb concentration had already become considerably increased. Therefore, the early effect of TMS on Hb concentration changes may have been largely masked in the active condition (Baudewig et al. 2001).

Later deactivation after TMS. Both our study and the study of Mochizuki et al. (2006) demonstrated later deactivation after TMS, which emerged 9–12 s after stimulation and was prominent in the active condition. This deactivation may have been caused by prolonged inhibition due to activity of the inhibitory interneurons in the cortex as noted above (Barker et al. 1998; Seyal et al. 1993). This may correspond to the fact that MEP induced by TMS during target muscle contraction is followed by a transient suppression of ongoing motor activity, called the "silent period" (SP) (Fuhr et al. 1991; Nakamura et al. 1997; Ni et al. 2007; Sanger et al. 2001; Uncini et al. 1993). This later suppression was similar to that observed by Mochizuki et al. (2006). In this study, Hb concentration changes were investigated at M1 just underneath the coil, which showed deactiva-

tion in the active condition at intensities of 100%, 120%, and 140% AMT.

Although the precise mechanism of deactivation is unclear, we also postulate a mechanism similar to that for the SP to explain the observed phenomenon. The later period of the SP is considered to arise because of the decreased excitability of the motor cortex, inducing cessation of the muscle activity, possibly mediated by $GABA_B$ inhibitory interneurons. MEP amplitude induced by TMS shows a correlation with the duration of the following SP, which reflects the increased amount of inhibitory inputs to the PTN (Taylor et al. 1997).

Although largely in agreement, the results of our study exhibited a small difference from those of Mochizuki et al. (2006) in the early phase of activation under the active condition. In the present study, we noted an early activation followed by a later deactivation. Mochizuki et al. (2006) noted an increase of oxy-Hb in the active condition, whereas in the relaxed condition they noted a significant decrease in deoxy-Hb and total Hb concentrations.

The most probable explanation for the difference in outcomes may be the difference in the orientation of the coil. Lateromedial directed induced current in the brain tends to activate the PTNs directly at their axons, whereas an anteromedial current direction tends to activate the PTNs transsynaptically (Sakai et al. 1997; Terao et al. 2001; Werhahn et al. 1994). Transsynaptic stimulation would result in a more natural activation involving the cortical interneurons and cause early activation of the pyramidal cells, followed by a prolonged inhibition. This would not occur with direct stimulation of the pyramidal tract axons in the white matter, because the latter would not contribute to the increase in the metabolism of the cerebral cortex (probably by interneuronal activation) as measured by NIRS. The lack of early activation in the study of Mochizuki et al. would thus be explained by the lack of transsynaptic activation of interneurons due to the lateromedial current direction. Taken together, the results of Mochizuki et al. (2006) and the present study can essentially be explained by a common mechanism, after taking into account the different methods of activating the motor cortex. Another possible explanation could be that the observed differences in Hb concentration change in the cerebral vessels were due to an effect such as vasoconstriction. However, TMS has not been reliably associated with an occurrence of vasoconstriction, and there is no reason why such should occur in the study of Mochizuki et al. but not in ours.

The amount of later deactivation was correlated with the TMS intensity, and this deactivation only emerged when the TMS intensity was above AMT. Mochizuki et al. (2006) reported a transient increase in oxy-Hb concentration at 100% AMT under slight FDI contraction but only decreases in deoxy-Hb and total Hb concentrations at 120% and 140% AMT in the relaxed condition. They suggested that a weak TMS pulse at ~100% AMT may mimic natural brain activation and may not produce the larger inhibitory effects of more intense TMS because voluntary contraction usually cancels out the inhibitory effect in the target muscle (Ridding et al. 1995). In contrast, 140% AMT should induce a long-lasting inhibition after the short-lasting facilitation (Berardelli et al. 1996; Chen 2004; Hanajima et al. 2001; Inghilleri et al. 1993). The same interpretation also applies to our results.

Spatial distribution of hemoglobin concentration changes induced by TMS over motor cortex. By using a multichannel NIRS, we also were able to investigate the spatial spread of Hb concentration changes outside M1. Possible mechanisms that may be responsible for the spatial spread of Hb concentration changes include contributions from neighboring regions, such as lateral cortico-cortical connections, diffuse nonlemniscal input, contributions of subthreshold synaptic activity, and diffusion of vasodilator substances, all of which might widen the distribution of the hemodynamic response compared with neuronal activity changes (Devor et al. 2005).

Although the spatial resolution of NIRS is somewhat limited (Akiyama et al. 2006; Fabbri et al. 2003; Kato et al. 2004), we noted a clear indication that Hb concentration changes were pronounced in M1 and cortical regions posterior to it, possibly including the sensory cortex, rather than in anterior regions. These differences according to location were more pronounced for higher stimulus intensities, and posteriorly dominant activation was more pronounced when subjects were active than when they were relaxed. Oxy-Hb concentrations in the relaxed condition revealed significant increases in APC and PPC only when TMS was given at 140% AMT. One hundred percent resting motor threshold (RMT) roughly corresponds to 130–140% AMT (Khedr et al. 2004; Tergau et al. 1999), and this indicates that in the relaxed condition muscle contraction was only induced in the 140% AMT trials. Therefore, the observed posterior dominant activation is likely to be due to activation of the sensory cortex associated with sensory feedback from muscle contraction induced by the TMS. Concurrent TMS-fMRI/PET studies applying TMS to M1 (at sub- and suprathreshold intensities) have demonstrated modulation of the neural activity in remote regions that have anatomical connections with M1, including the sensory cortex (Bestmann et al. 2004, 2008; Fox et al. 1997; Hanakawa et al. 2009; Paus et al. 1997; Shitara et al. 2011). Shitara et al. suggested that much of the activation seen in response to suprathreshold single-pulse TMS may be ascribed to activity related to the processing of muscle afferents associated with muscle contraction.

We found a tendency for Hb concentration changes to be more pronounced in M1 and cortical regions posterior to it than anterior to it, which could be due to afferent inputs induced by contraction in response to TMS. Hanakawa et al. (2009) showed that subthreshold-intensity TMS over M1 induced S1 activation but not M1 activation. M1 activation became apparent only when the TMS intensity was above motor threshold. BOLD changes in the primary somatosensory cortex could reflect the changes in sensory cortical activity induced by TMS, since there are some reports in which TMS over M1 can affect the amplitudes of sensory evoked potentials (SEPs) in humans by way of the anatomical connections between the primary motor and sensory cortices (Enomoto et al. 2001; Kujirai et al. 1993; Ohki et al. 1994; Seyal et al. 1993). In fact, some studies suggest that there is significant linear neurovascular coupling between SEP amplitude and BOLD changes in S1 (Arthurs and Boniface 2002, 2003; Arthurs et al. 2007). Alternatively, it is also possible that the predominant blood flow changes in posterior cortical regions are due to activation of posterior cortical regions through anatomical connections, but it remains unknown whether such changes can indeed be detected by NIRS, apart from the sensory afferent information induced by muscle contraction. Strangman et al. (2003) re-

ported that NIRS signal levels drop substantially when the probes are placed off target by >1 cm in either the longitudinal or transverse direction. Therefore the wide distribution of hemodynamic changes in the present study may be caused by the cortico-cortical and subcortico-cortical connections between the sensorimotor cortices rather than sensory afferent information per se.

In conclusion, we achieved the two goals raised in the introduction of this paper. First, we could show that Hb concentration changes in the motor cortex after application of single-pulse TMS over the cortex were different depending on whether the target muscle was in an active or relaxed condition. The observed consequent differences consisted of early activation and later deactivation. Early activation is more evident when the current direction is anteromedial than lateromedial. This difference can be understood if we consider that early activation occurs when the motor cortex is stimulated naturally, i.e., transsynaptically. Lateromedial directed current rather tends to activate the PTNs directly at their axons and resulted in smaller early activation. On the other hand, the later deactivation is considered to occur through the subsequent prolonged inhibition of the cerebral cortex, which is presumably due to a mechanism similar to that for the SP. Second, we could show that Hb dynamics in nonmotor cortical regions, i.e., in the sensory cortex, was larger than that in the stimulated motor cortex; higher intensities of stimulation resulted in an additional Hb activation of the sensory cortex due to afferent inputs induced by muscle contraction in response to TMS or to anatomical connections of the primary sensorimotor cortices.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.F. conception and design of research; T.F., H. Mochizuki, N.A., R.H., M.H., H. Matsumoto, S.N.-E., S.O., A.Y., and S.I.-T. performed experiments; T.F. analyzed data; T.F., Y.T., R.H., M.H., and Y.U. interpreted results of experiments; T.F. prepared figures; T.F. drafted manuscript; T.F., H. Mochizuki, Y.T., N.A., R.H., M.H., H. Matsumoto, S.N.-E., S.O., A.Y., S.I.-T., and Y.U. approved final version of manuscript; Y.T. and Y.U. edited and revised manuscript.

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