Cortical hemoglobin concentration changes underneath the coil after single-pulse transcranial magnetic stimulation: A near-infrared spectroscopy (NIRS) study

Toshiaki FURUBAYASHI¹,², Hitoshi MOCHIZUKI², Yasuo TERAO³, Noritoshi ARAI⁴, Ritsuko HANAJIMA³, Masashi HAMADA³,⁵, Hideyuki MATSUMOTO³, Setsu NAKATANI-ENOMOTO², Shingo OKABE³, Akihiro YUGETA³, Satomi INOMATA-TERADA³, Yoshikazu UGAWA²,⁶

1) Faculty of Medical Science and Welfare, Department of Rehabilitation, Tohoku Bunka Gakuen University, Sendai, Japan
2) Department of Neurology, School of Medicine, Fukushima Medical University, Fukushima, Japan
3) Department of Neurology, Division of Neuroscience, The University of Tokyo, Tokyo, Japan
4) Department of Neurology, National Center for Global Health and Medicine, Tokyo, Japan
5) Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, UCL, London, United Kingdom
6) JST, Research Seeds Program, Fukushima, Japan

Correspondence to T. Furabayashi at the following address:
Faculty of Medical Science and Welfare, Department of Rehabilitation, Tohoku Bunka Gakuen University, 6-45-1 Kunimi, Aoba-ku, Sendai 981-8551, Japan
Phone: +81-22-233-6113
Fax: +81-22-233-6299
E-mail: furubayashi-tky@umin.ac.jp

Running title: Hemoglobin concentration changes after single-pulse TMS

Key words: Near-infrared spectroscopy, Magnetic stimulation, Motor cortex,
Hemoglobin concentration changes
ABSTRACT

Using NIRS (near-infrared spectroscopy) and multi-channel probes, we studied hemoglobin (Hb) concentration changes when single-pulse transcranial magnetic stimulation (TMS) was applied over the left hemisphere primary motor cortex (M1). Seventeen measurement probes were centered over left M1. Subjects were studied both in the active and relaxed conditions, with the TMS intensity set at 100%, 120%, and 140% of the active motor threshold. The magnetic coils were placed so as to induce anteromedially directed currents in the brain. Hb concentration changes were more prominent at channels over M1 and posterior to it. Importantly, Hb concentration changes at M1 after TMS differed depending on whether the target muscle was in an active or relaxed condition. In the relaxed condition, Hb concentration increased up to 3-6 s after TMS, peaking at around 6 s, and returned to the baseline. In the active condition, a smaller increase in Hb concentrations continued up to 3-6 s after TMS (early activation), followed by a decrease in Hb concentration from 9-12 s after TMS (delayed deactivation). Hb concentration changes in the active condition at higher stimulus intensities were more pronounced at locations posterior to M1 than at M1. We conclude that early activation occurs when M1 is activated trans-synaptically. The relatively late deactivation may result from the prolonged inhibition of the cerebral cortex after activation. The posterior dominant activation at higher intensities under the active condition may result from an additional activation of the sensory cortex due to afferent inputs from muscle contraction evoked by the TMS.
INTRODUCTION

Transcranial magnetic stimulation (TMS) has been widely used to explore the activity of the human cortex in neurophysiological and psychophysiological research, as well as in clinical practice. By applying single-pulse, paired pulses, or multiple repetitive pulses (rTMS), various methods have been applied to study the excitability of the motor cortex and the activity of the intrinsic circuits in the motor cortex (Kujirai et al. 1993; Ridding et al. 1995; Ziemann et al. 1996, 1998; Hanajima et al. 1998, 2002; Okabe et al. 2003; Chen et al. 2004; Mochizuki et al. 2007; Hamada et al. 2008; Kozel et al. 2009; Shirota et al. 2010; Nakamura et al. 2011), and various techniques have been devised to address the function of excitatory and inhibitory neurons in the motor cortex. For example, the paired-pulse TMS technique using a sub-motor threshold conditioning stimulus followed by a supra-motor threshold test stimulus is now widely used to evaluate the function of GABAergic inhibitory interneurons in the motor cortex (Kujirai et al. 1993; Hanajima et al. 1998).

By combining motor cortex stimulation with stimulation of other brain areas, TMS can also be used to study functional connectivity between the motor cortex and various other cortical areas such as the medial frontal cortex or the cerebellum (Civardi et al. 2001; Ugawa et al. 1997; Iwata et al. 2004). Functional connectivity has also been addressed by combining TMS with neuroimaging techniques such as single-photon emission computed tomography (SPECT), positron emission computed tomography (PET), and functional magnetic resonance imaging (fMRI), as well as electroencephalographic (EEG) recordings (Amassian et al. 1992; Seyal et al. 1993; Bohning et al. 1998; Ilmoniemi et al. 1999; Okabe et al. 2003; Daskalakis et al. 2008; Farzan et al. 2008). The regional cerebral blood flow changes or EEG changes in functionally connected cortical and subcortical areas can also be observed after stimulating one cortical area with a single pulse, or repetitive pulses (Fox et al. 1997; Bestmann et al. 2008; Bohning et al. 1998; Mochizuki et al. 2007; Hanakawa et al. 2009; Shitara et al. 2011). However, the main limitations of these studies are the technical challenges of coping with the artifacts induced by the powerful magnetic and electrical fields resulting from TMS or the stimulating coil itself (Kozel et al. 2009).

Near-infrared spectroscopy (NIRS) is a novel non-invasive neuroimaging
technique which can assess changes in cerebral hemoglobin concentrations with a
temporal resolution that is higher than that of PET or fMRI (i.e., between 0.1 and 0.5 s),
but lower than that of EEG recordings. However, the spatial resolution that NIRS
provides is inferior to that of PET, SPECT or fMRI. NIRS can measure both Oxy-Hb
and Deoxy-Hb concentrations; Oxy-Hb is considered to reflect capillary blood flow
volume whereas Deoxy-Hb reflects the blood flow velocity. In contrast, fMRI is based
mainly on Deoxy-Hb, i.e., the bold oxygen level-dependent (BOLD) signal (Jöbsis
Kameyama et al. 2004; Huppert et al. 2006). 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 (Noguchi et al.
2003; Mochizuki et al. 2006; Hanaoka et al. 2007).

In combination with electrophysiological studies, hemoglobin 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. Due to its high signal-to-noise ratios
for single events and non-interference with magnetic field changes, NIRS has been
successfully used to detect Hb concentration changes evoked by single-pulse TMS just
beneath the coil (Noguchi et al. 2003; Mochizuki et al. 2006). This very feature allowed
us to study the cerebral blood flow 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 level 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 that 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% 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 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 around 100% AMT may mimic natural activation, but strong TMS may not; this decrease was considered 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 employed measurement systems had a single channel, and possible changes in hemoglobin 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 the cerebral blood flow using NIRS, namely 1) how does muscle contraction influence the effect of TMS targeting 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 cerebral blood flow 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 MEPs.

METHODS

Subjects

Fifteen healthy volunteers (12 men and 3 women), aged 28 to 54, 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.
The NIRS system used (ETG-100; Hitachi Medical Corporation, Chiba, Japan) consisted of 6 pairs of optical fibers functioning as emitters and detectors, so that the seventeen measurement probes (17 channels) were placed over the left hemisphere centered on the left primary motor cortex. The ninth channel (ch 9) corresponded to the left primary motor cortex, illustrated in Figure 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 hemoglobin concentration in neural structures at a depth of 15 to 20 mm from the scalp, i.e., in the cerebral cortex (Kato et al. 1993; Germon et al. 1999) (see Fig. 1). The NIRS probes were provided with plastic insulation. Because NIRS uses infra-red 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. In order 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 (Uludag et al. 2002; Strangman et al. 2003) and the signal-to-noise ratio (Yamashita et al. 2001; Sato et al. 2004; Thomson et al. 2011) discussed. Nevertheless, even when using this combination of wave lengths and sampling rate, other groups (Watanabe et al 1998; Isobe et al. 2001) 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 23s after application. One session consisted of 5 trials, and each session was repeated twice for all the subjects to confirm reproducibility of the results for each condition. The session order (sham, 100%, 120%, 140% AMT, see below) was counterbalanced within and across the subjects.

To explore the responses in a broader area centered over the primary motor cortex in the left hemisphere, we focused on 5 measurement points anterior and posterior relative to the primary motor cortex. Although all 12 (6 pairs of) probes were fixed to the holder, some of them were displaced from the scalp due to 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 5 channels over the midline, nearest the central hole and thus less subject to displacement. Specifically, the channel 9 probe was placed over the primary motor cortex (M1) as defined by the TMS hot spot, and four other probes with dedicated channels were placed as follows: frontal area (FC, ch1); premotor area (PM, ch5); anterior parietal cortex (APC, ch13); and posterior parietal cortex (PPC, ch17) (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 (TMS)**

Single-pulse TMS was delivered via a double square coil connected to a Magstim 200 magnetic stimulator (The Magstim Co., Ltd, Whitland, UK). The double square coil (95 x 100 mm, 0.96 T) was specially devised for multi-channel 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 first dorsal interosseous muscle (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 Inc.) for later off-line analysis. The TMS intensity was adjusted to 100%, 120%, and 140% of the active motor threshold (AMT) over M1. We defined AMT as the lowest intensity that evoked five small responses (of about 100 μV) during a series of ten stimulations when the subject maintained 10% of maximal voluntary contraction. Sham stimulation was performed to exclude non-specific 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 the 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 the primary motor cortex 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 hotspot 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 in between the locations of the 9 NIRS channels. When placing the holder, we carefully checked that the locations of the channel 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 channel 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 the section on 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 hemoglobin concentration changes. The tapping task, which consisted of sequential oppositional movements between the thumb and the other four fingers at a pace of about 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 upon hearing the word “start,” while they were to remain at rest when undergoing relaxed condition tests. Muscle activities were monitored throughout the experiment using an oscilloscope screen. In the active condition, the subjects could maintain consistent 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 starting from 3 s before TMS and ending 13.5 s after. Based on 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 BL value. Averaged Hb concentrations for the following four periods,
with a duration of 3s each, were calculated: baseline (-3 to 0 s before TMS); 3 s (1.5 to
4.5 s after the 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).
Using SPSS 11.5 software (SPSS Japan, Tokyo, Japan), the sub-scores 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, 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 hotspots were corresponded to the M1.
The averaged time course of NIRS measurement during the tap task is shown in Figure
2. The increase in the Oxy-Hb concentration (red line) was predominant at channel 9
when finger tapping was initiated, and returned to the baseline (BL) following a plateau
period, while Deoxy-Hb concentration (blue line) decreased slightly during tapping. The
increase in Oxy-Hb concentration observed at 8 s after the initiation of the tapping was
more prominent at channel 9 than at any other channel. These results indicate that
channel 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
(thick line) and the 95% confidence intervals (thin lines) for all subjects when the
subject kept the right FDI relaxed (relaxed condition, 5 traces on the left) and
maintained 10% MVC (active condition, 5 traces on the right). The five channels
represent the frontal area (FA), premotor area (PM), motor cortex (M1), anterior parietal
cortex (APC), and posterior parietal cortex (PPC), from left to right in this order. The
rows from top to bottom show traces for sham, 100%, 120%, and 140% AMT,
respectively. 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
centration 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, 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; condition: F= 85.9,
p<0.001; and interaction of condition x 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; and 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 to 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; and 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
In contrast, when subjects were in the active condition, Oxy-Hb increased significantly relative to BL at 3 s but then decreased significantly at 9 and 12 s (3 s: p<0.01; 9 and 12 s: p<0.001, respectively).

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; and condition x 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 condition (sham vs.100%: p<0.05; sham vs. 120%: p<0.001; sham vs. 140%: p<0.001). However, in the active condition, the 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; and 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 (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 that hemoglobin concentration changes were pronounced in the primary motor cortex and cortical regions posterior to it than anterior to it. To confirm this possible difference in anterior (FC) and posterior cortical regions (APC and PPC), we performed planned comparison between anterior and posterior cortical regions, which showed a significant difference between FC and APC areas, 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 hemoglobin concentration changes observed at the different channels will be discussed later (see Figure 6).

Figure 4 shows the averaged relative Deoxy-Hb concentration changes and the 95% confidence intervals for the relaxed (left 5 traces) and active conditions (right 5 traces). 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 (5 traces on the left) and active (5 traces on the right) conditions. Essentially the same trend was noted as for Oxy-Hb (Figure 3), but some differences were observed. There was a significant main effect both of time and condition, and the interaction between condition and time was also significant (main effect of time: F=53.7, p<0.001; condition: F=60.6, p<0.001; and interaction: condition x 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; and 12 s: p<0.001). The significant interaction implied that 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 x intensity: F=9.0, p<0.001). This suggested that the change in Total-Hb concentration increased with increasing TMS intensity both for 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; and 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 hemoglobin concentration changes. We focused our analysis on TMS applications with an intensity of 120% AMT because this level provided the most robust hemoglobin concentration changes among both sub- and suprathreshold TMS intensities.

Figure 6 compares the time courses of hemoglobin 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 (leftmost figures) 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 hemoglobin concentration changes observed at channels over or posterior to M1 than at frontal channels. This trend was also noted for Total-Hb (rightmost figures, F=4.2, p<0.01). As shown in Figure 4, Deoxy-Hb did not change significantly relative to BL after the application of TMS (middle figures).

DISCUSSION

This study gave us some answers to our two main questions mentioned in 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 hemoglobin concentration changes were more prominent at the primary motor cortex and areas posterior to it. Importantly, we noted drastic differences in hemoglobin concentration changes, depending on whether subjects were in an active or relaxed condition. When in the relaxed condition, hemoglobin concentration continued increasing up to 3-6 s after single-pulse TMS, peaking at around 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 hemoglobin concentration from 9-12 s after the delivery of the TMS pulse (delayed deactivation). The delayed deactivation was consistent 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 hemoglobin concentration changes observed under the active and relaxed conditions were produced by different mechanisms, which we will discuss in the following.

Hemoglobin concentration changes did not exhibit a simple dose dependency. Although the increase in Oxy-Hb and Total-Hb in the relaxed condition increased with the 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 multi-channel NIRS system. This combination of devices may provide an especially effective method for recording hemoglobin concentration changes under or near the TMS coil.

Early activation after TMS over the motor cortex

NIRS is assumed to reflect changes in cerebral blood flow (CBF) associated with brain activity (Kato et al. 1993; Hirthe et al. 1996; Watanabe et al. 1998; Noguchi et al. 2003; Akiyama et al. 2006; Hada et al. 2006). The typical pattern of cerebral activation observed via NIRS measurements consists of an increase in Oxy- and Total-Hb and a small decrease in Deoxy-Hb (Villringer et al. 1993; Kleinschmidt et al. 1996; Watanabe et al. 1998; Hanaoka et al. 2007; Abdelnour and Huppert 2009). 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 (Fox and Raichle 1986; Villringer and Chance 1997; Fantini 2002). Conversely, cortical deactivation is accompanied by Oxy-Hb decrease with a small decrease or unaltered Deoxy-Hb concentration (Sakatani et al. 1998; Hada et al. 2006; Hanaoka et al. 2007; Kozel et al. 2009). 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 (Seyal et al. 1993; Barker et al. 1998). The local field potential caused by postsynaptic processing is considered to correlate with the increase in CBF (Mathiesen et al. 1998; Logothetis et al. 2001; Moliadze et al. 2005; Allen et al. 2007). 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

15
also increases, resulting in further increase in Oxy- 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 multi-unit 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, due to weak muscle contraction in the FDI, the level of hemoglobin concentration had already become considerably increased. Therefore, the early effect of TMS on hemoglobin 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 (Seyal et al. 1993; Barker et al. 1998). 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; Uncini et al. 1993; Nakamura et al. 1997; Sangaer et al. 2001; Ni et al. 2007). This later suppression was similar to that observed by Mochizuki et al. (2006). In this study, hemoglobin concentration changes were investigated at the primary motor cortex just underneath the coil, which showed deactivation 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 due to 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 pyramidal tract neurons directly at their axons, whereas an anteromedial current direction tends to activate the pyramidal neurons transsynaptically (Werhahn et al. 1994; Sakai et al. 1997; Terao et al. 2001). 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 Mochizuki’s study 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 hemoglobin 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 Mochizuki et al.’s study 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 under the relaxed condition. They suggested that a weak TMS pulse at
around 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 (Inghilleri et al. 1993; Berardelli et al. 1996; Hanajima et al. 2001; Chen 2004). The same interpretation also applies to our results.

**Spatial distribution of hemoglobin concentration changes induced by TMS over the motor cortex**

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

Although the spatial resolution of NIRS is somewhat limited (Kato et al. 2004; Akiyama et al. 2006; Fabbri et al. 2003), we noted a clear indication that hemoglobin concentration changes were pronounced in the primary motor cortex 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. 100% RMT roughly corresponds to 130-140% AMT (Tergau et al. 1999; Khedr et al. 2004) 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 the primary motor cortex (at sub- and suprathreshold intensities) has demonstrated modulation of the neural activity in remote regions that
have anatomical connections with the primary motor cortex, including the sensory
cortex (Paus et al. 1997; Fox et al. 1997; Bestmann et al. 2004, 2008; Hanakawa et
al. 2009; 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 that hemoglobin concentration changes were more
pronounced in the primary motor cortex 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 the primary motor
cortex induced S1 activation but not primary motor cortex activation. Primary motor
cortex 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 the primary motor cortex can affect the amplitudes of sensory evoked
potentials in humans by way of the anatomical connections between the primary motor
and sensory cortices (Seyal et al. 1993; Kujirai et al. 1993; Ohki et al. 1994; Enomoto et
al. 2001). 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) reported that NIRS signal levels drop
substantially when the probes are placed off target by more than 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 sensori-motor 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 hemoglobin concentration changes in the motor cortex after
applying 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 when 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
induced rather tends to activate the pyramidal tract neurons 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. Secondly, we could
show that Hb dynamics in non-motor cortical regions, i.e. in the sensory cortex, was
larger from that in the stimulated motor cortex; higher intensities of stimulation resulted
in an additional hemoglobin activation of the sensory cortex due to afferent inputs
induced by muscle contraction in response to TMS or to anatomical connections of the
primary sensori-motor cortices.

ACKNOWLEDGEMENTS

We are grateful to Hitachi Medical Corporation (Kashiwa, Chiba, Japan) for
technical advice.

Part of this work was supported by the following grants:
Research Project Grants-in-aid for Scientific Research from the Ministry of Education,
Culture, Sports, Science and Technology of Japan (No. 22390181, No. 22590954, No.
20591019); the Research Committee on rTMS Treatment of Parkinson disease from the
Ministry of Health, Labour and Welfare of Japan (H20-023); the Research Committee
on Dystonia, the Ministry of Health, Labour and Welfare of Japan; the Research
Committee on Intractable Pain from the Ministry of Health, Labour and Welfare of
Japan; the Research Committee on Degenerative Ataxia from the Ministry of Health and
Welfare of Japan; the Magnetic Health Science Foundation; the Uehara Memorial
Foundation and The Novartis Foundation (Japan) for the Promotion of Science.

REFERENCES

Abdelnour A and Huppert T. Real-time imaging of human brain function by
near-infrared spectroscopy using an adaptive general linear model. Neuroimage


Hanakawa T, Mima T, Matsumoto R, Abe M, Inouchi M, Urayama S, Anami K,


Kameyama M, Fukuda M, Uehara T, Mikuni M. Sex and age dependencies of


FIGURE LEGENDS

Figure 1. Experimental setup for 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). Ch9 was placed over the left M1 (green circle). (C) The NIRS channels are shown projected over a surface rendering of the brain MRI. (D) Five channels in the middle were used for recording: Ch 1: FC; Ch 5: PM; Ch 9: M1; Ch 13: APC; and Ch 17: PPC.

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

Figure 3. Averaged Oxy-Hb concentration changes (thick line) and the 95% confidence
intervals (thin lines) while the subject kept the right FDI muscle relaxed (relaxed
condition, 5 rows on the left) or maintained 10% MVC (active condition, 5 rows on the
right).
The five channels from left to right represent the frontal area (FA), premotor area (PM),
motor cortex (M1), anterior parietal cortex (APC), and posterior parietal cortex (PPC),
in this order. The top to bottom rows show the traces for sham, 100%, 120% and 140%
AMT, respectively. In each figure, the vertical axis shows the Oxy-Hb concentrations
(mMmm) and the horizontal axis the time (s). TMS was applied at 0 ms (the left end of
each plot).

Figure 4. Averaged Deoxy-Hb concentration changes (thick line) and the 95%
confidence intervals (thin lines) in the relaxed (left) and active conditions (right).
Conventions are the same as for the previous figure.

Figure 5. Averaged Total-Hb concentration changes (thick line) and the 95% confidence
intervals (thin) in the relaxed (left) and active conditions (right). Conventions are the
same as for the previous figures.

Figure 6. The time courses of averaged relative Oxy-Hb (left column), Deoxy-Hb
(middle column), and Total-Hb (right column) concentration changes in the relaxed (○)
and active (□) conditions induced by TMS at 120% AMT. Filled symbols such as ● and ■
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).
<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>PM</th>
<th>M1</th>
<th>APC</th>
<th>PPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total_Hb Relaxed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Active</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td>Active</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Active</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
<tr>
<td>Active</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.04</td>
</tr>
</tbody>
</table>