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Using Simultaneous Repetitive Transcranial Magnetic Stimulation/Functional Near Infrared Spectroscopy (rTMS/fNIRS) to Measure Brain Activation and Connectivity

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Abstract

Introduction—Simultaneously acquiring functional Near Infrared Spectroscopy (fNIRS) during Transcranial Magnetic Stimulation (rTMS) offers the possibility of directly investigating superficial cortical brain activation and connectivity. In addition, the effects of rTMS in distinct brain regions without quantifiable behavioral changes can be objectively measured.

Methods—Healthy, non-medicated participants age 18–50 years were recruited from the local community. After written informed consent was obtained, the participants were screened to ensure that they met inclusion criteria. They underwent two visits of simultaneous rTMS/fNIRS separated by 2 to 3 days. In each visit, the motor cortex and subsequently the prefrontal cortex (5 cm anterior to the motor cortex) were stimulated (1 Hz, max 120% MT, 10 secs on with 80 secs off, for 15 trains) while simultaneous fNIRS data were acquired from the ipsilateral and contralateral brain regions.

Results—Twelve healthy volunteers were enrolled with one excluded prior to stimulation. The 11 participants studied (nine male) had a mean age of 31.8 (s.d. 10.2, range 20–49) years. There was no significant difference in fNIRS between Visit 1 and Visit 2. Stimulation of both the motor and prefrontal cortices resulted in a significant decrease in oxygenated hemoglobin (HbO₂) concentration in both the ipsilateral and contralateral cortices. The ipsilateral and contralateral changes showed high temporal consistency.

Discussion—Simultaneous rTMS/fNIRS provides a reliable measure of regional cortical brain activation and connectivity that could be very useful in studying brain disorders as well as cortical changes induced by rTMS.

Keywords

Brain stimulation; functional neuroimaging; optical imaging; healthy subjects; oxygenated hemoglobin; deoxygenated hemoglobin; rTMS; TMS; fNIRS

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Introduction

Transcranial magnetic stimulation (TMS) is a technology that is being used to investigate normal brain function and various neuropsychiatric disorders. TMS involves using rapidly alternating current in a coil to induce magnetic fields. These magnetic fields pass from the coil relatively unimpeded to the brain and induce electrical fields that can depolarize neurons (e.g., stimulating the thumb area of the motor strip cortex can make the thumb twitch) (George and Belmaker, 2007). When TMS is provided repeatedly at a specific frequency, it is referred to as repetitive TMS (rTMS).

In addition to investigating brain function, rTMS is being investigated as a possible treatment for various brain disorders. As an example, daily prefrontal rTMS has been found to have antidepressant properties when treating patients with depression (Burt et al., 2002; Couturier, 2005; Holtzheimer et al., 2001; Kozel and George, 2002; Martin et al., 2003; Martin et al., 2002; McNamara et al., 2001). Whether or not the statistically significant differences were clinically meaningful has been debated. The largest (n=301) trial ever conducted was a multisite, industry supported clinical trial that found left high frequency rTMS relative to sham rTMS improved depressive symptoms (O'Reardon et al., 2007). Although the clinical response was comparable to results seen with antidepressants (O'Reardon et al., 2007), the response rate at 6 weeks (50% improvement in the Montgomery Åsberg Depression Rating Scale (MADRS)) of 23.9% indicates that the potential exists to improve clinical outcome. One mechanism to improve clinical outcome is to better target treatment to patients. A greater understanding of brain activation and connectivity associated with a disease (e.g., major depressive disorder) and the impact that rTMS can have on that brain could enable the tailoring of treatments to better fit patients.

The majority of rTMS (and TMS) studies of brain function and treatment effects have occurred with stimulation of the motor cortex (Hallett, 2007). Only the motor cortex produces a rapidly observable and quantifiable behavioral change when that cortical region is stimulated. When the area of the motor cortex corresponding to a particular muscle group (e.g., Abductor Pollicis Brevis) is stimulated, then the respective muscle can be made to contract and move a body part (e.g., thumb moves). The degree of muscle contraction can be measured with electromyography (EMG). Although TMS can also produce phosphenes and speech arrest, these behavioral changes are difficult to quantify.

To better understand brain changes associated with rTMS in areas other than the motor cortex, researchers have used various neuroimaging techniques. Neuroimaging has been used to assess the impact of rTMS on the brain over different time courses: (1) the immediate effect of rTMS on the brain (Bestmann et al., 2004; Bohning et al., 2003; Daskalakis et al., 2008; Nahas et al., 2001); (2) the cumulative effect of one session of rTMS (Fitzgerald et al., 2002; Li et al., 2003; Loo et al., 2003; Mottaghy et al., 2003; Nahas et al., 1999; Paus et al., 1998); and (3) the cumulative effect of multiple days of rTMS (Kozel et al., 2009 (in press); Nahas et al., 2000; Speer et al., 1999). These studies have addressed various questions ranging from brain changes associated with rTMS to parameter selection to assessing safety of rTMS. The focus of this paper is on the immediate brain effects of rTMS, which provides the ability to assess the functioning of the brain (e.g., activation and connectivity).

Because of the temporal resolution required to assess the immediate brain changes associated with rTMS, there are only several modalities that have been able to investigate this aspect of brain changes associated with rTMS. These modalities include interleaved TMS/functional MRI (TMS/fMRI) (Bestmann et al., 2008; Bohning et al., 1998), Electroencephalography (EEG) (Amassian et al., 1992; Bohning et al., 1998; Cracco et al., 1989; Daskalakis et al.,

2008; Farzan et al., 2008), Magnetoencephalography (MEG) (Shibasaki, 2008), and functional Near Infrared Spectroscopy (fNIRS) (Akiyama et al., 2006; Hanaoka et al., 2007; Mochizuki et al., 2006). Each modality has strengths and weaknesses. Functional MRI, EEG, and MEG, however, all share the technical difficulty of measuring brain changes associated with rTMS using a signal that involves the electromagnetic spectrum. Since rTMS produces magnetic and electrical fields, measuring brain changes using the electromagnetic spectrum is limited by the co-production of significant measurement artifacts. Conversely, fNIRS measures an optical signal that does not interact with the magnetic or electric fields produced by the TMS coil.

Functional NIRS (fNIRS) is a developing optical imaging technology that offers a method to investigate brain changes associated with rTMS that has some unique beneficial properties (Boas et al., 2004; Kim and Liu, 2007; Villringer and Chance, 1997). It measures the light attenuation of the biological tissues in near infrared spectrum (670–900 nm). The near infrared light penetrates tissues to a depth of several centimeters and is mainly absorbed by two principal chromophores in blood flow: the oxygenated hemoglobin (HbO₂) and the deoxygenated hemoglobin (Hb). Therefore, by measuring the change of light attenuation from a baseline stage at two or more wavelengths, the changes of HbO₂ and Hb concentrations in the tissues can be quantified (Kim and Liu, 2007). Because fNIRS is measuring functional brain changes without using electrical or magnetic signals, there are no electromagnetic interactions between the rTMS and fNIRS.

Functional NIRS offers the potential of measuring immediate brain effects of rTMS in areas of the brain that do not have an immediate and quantifiable behavioral change. In support of this, Allen et al. (2007) have demonstrated in an animal model that optical imaging of hemodynamic changes provides an effective means to monitor neuronal changes from TMS. Several investigators have employed optical imaging to study brain changes associated with single pulse TMS (Mochizuki et al., 2006; Noguchi et al., 2003). In addition, Hanaoka et al. (2007) have investigated brain changes in the left prefrontal cortex that correspond to stimulating the right prefrontal cortex for 60 seconds with 1 Hz rTMS at a calculated dose of 50% motor threshold. They found that the contralateral side displayed significantly decreased HbO₂ during stimulation that tended to increase during the latter portion of stimulation compared to the sham condition. Thus, fNIRS has the potential to study brain changes associated with stimulation with rTMS.

The two technologies have also been used together in a number of different ways. Eschweiler et al. (2000) found that lack of activation as measured by NIRS to a mental task at the site of rTMS stimulation predicted a clinical response to rTMS for depression. Another way the technologies have been used together is to use TMS to provide localization of a region of the motor cortex and NIRS to measure the brain changes associated with the respective movement with a high temporal resolution (Akiyama et al., 2006). These two studies illustrate the tremendous potential that integrating TMS with fNIRS has for neuroscience research and clinical applications.

We hypothesized that TMS would produce bilateral changes in cortical activation that would be measurable by fNIRS. Further, we hypothesized that functional brain changes induced by TMS over the motor cortex and the prefrontal cortex would be similar and consistent for an individual participant across two time points.

Materials and methods

Study Sample

Healthy participants age 18–50 years were recruited from the local community. Participants could not have a past or current psychiatric disorder, history of a significant medical disorder,

a presently unstable medical condition, caffeinism (i.e., withdrawal symptoms with 3 days of abstinence), nicotine use, be pregnant or breast feeding, or currently taking any medication.

The study protocol was approved by the University of Texas Southwestern Medical Center at Dallas Institutional Review Board. Written informed consent was obtained from all participants. A physician screened participants with a Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al., 1995), a Transcranial Magnetic Stimulation Adult Safety Screen (TASS) form (Keel et al., 2001), a medical history review, and a physical exam. A urine sample was obtained for a drug screen and a urine pregnancy test (if the participant was a woman with childbearing potential). After the screening was completed, eligible participants were taken to the Neurostimulation Lab to undergo fNIRS measurement of brain changes associated with rTMS.

TMS and NIRS Procedures

The study involved two visits that were 2–3 days apart to reduce the chance of carryover effects from Visit 1 to Visit 2.

Visit 1—Participants were positioned in the TMS device chair and adjusted to ensure comfort throughout the experimental procedure. All external light was blocked from entering the Neurostimulation Lab, and the ambient lighting was tested to ensure that it did not contaminate the fNIRS signal. The fNIRS probe consisting of 8 sources and 16 detectors was then positioned bilaterally over the region of the primary motor cortex with the optode (i.e., source and detector) grid aligned parallel to the mediolateral line. The probe was fastened using Velcro straps and retained in place using a tubular elastic bandage. The optodes were attached against the scalp with their tips directly touching the skin for maximum efficiency of light transmission. The hair strands under the optodes were manually moved away using combs to facilitate optimal optode-scalp contact. This helped avoid contamination by light absorption in hair. On a line from the nasion to the vertex, the distance from the nasion to the probes was measured and recorded for use in Visit 2 (see Figure 1).

Once the fNIRS probes were in place, the location of maximal stimulation of the right abductor pollicis brevis (left motor cortex) was determined using the visual method (Pridmore et al., 1998). Using the T.M.S. Motor Threshold Assessment Tool (Borckardt et al., 2006), the motor threshold (MT) was determined three times and averaged. Stimulation during the experiment was 120% MT or 100% of TMS machine output if the MT was greater than 83% (i.e., 120% MT of greater than 83% machine output would be greater than 100% machine output). As the fNIRS probes required the TMS coil to be about 15 mm away from the scalp, the resulting MT were greater than they otherwise would have been without this additional distance from coil to cortex (Cukic et al., 2009; Kozel et al., 2000; McConnell et al., 2001; Mosimann et al., 2002; Stokes et al., 2005). Thus, the location and dose of TMS over the motor cortex were determined over the NIRS probes.

With the participants instructed to sit quietly, eyes open, stay awake, and not move, fNIRS data was acquired one minute prior to stimulation, during the 15 stimulation/rest epochs (90 seconds per epoch), and during a one-minute poststimulation period resulting in a total recording time of 24 minutes and 30 seconds. The fNIRS system was a continuous-wave, MRI compatible system (CW5, TechEn Inc, MA) (Franceschini et al., 2006). The rTMS was performed using a Neuronetics Model 2100 CRS Repetitive Transcranial Magnetic Stimulation (rTMS) System (Malvern, PA) (O'Reardon et al., 2007). The stimulation epochs consisted of 10 seconds of 1 Hz stimulation and 80 seconds of rest that were repeated 15 times. After the fNIRS recording was complete, the location of the TMS stimulation relative to the fNIRS system was determined.

Subjects were given a short rest (~10 minute break) and then placed back in the TMS device chair using the coordinates previously determined. The fNIRS probes were positioned over the prefrontal cortex (see Figure 1) – defined as 5 cm anterior to the motor cortex position in a para-sagittal line. The repositioning of the fNIRS probes required an additional 20–30 minutes resulting in a minimum of 30–40 minutes between stimulations. Once the fNIRS probes were in position, the coil was moved 5 cm anteriorly in a para-sagittal line. The fNIRS recording and TMS stimulation were exactly the same as for the motor cortex stimulation. After the fNIRS recording was complete, the location of the TMS stimulation relative to the fNIRS system was determined.

Visit 2—Two to three days after Visit 1, participants returned for a second visit of fNIRS measurement of brain changes associated with rTMS stimulation. They were interviewed by a physician to ensure there were no changes to their medical condition and to assess for any adverse effects related to the prior rTMS. Using the coordinates of the TMS device chair, the subjects were placed back in the same position as the first visit. The fNIRS probes were positioned over the motor cortex with the same position of Visit 1 by referring to the distance from the nasion to the fNIRS probes on a line with the vertex. The location of maximal stimulation of the abductor pollicis brevis was confirmed and the MT was determined again using the T.M.S. Motor Threshold Assessment Tool. This was to confirm that no significant change in MT (greater than 10% change) occurred since Visit 1. In order to make the results from the two visits more comparable, the MT from Visit 1 was used to determine the stimulation dose. The simultaneous TMS/fNIRS over the motor cortex and the prefrontal cortex were performed as in Visit 1.

fNIRS Statistical Analyses—The fNIRS data were processed and analyzed channel-wise for each session (two sessions per visit) and for each individual, where a channel primarily represents one source-detector pair. The setup comprised of 28 channels in total with 14 channels on each hemisphere. In addition, the raw data for each channel was inspected during the entire time course to exclude the epochs with significant discontinuity – which usually resulted from the motion artifacts during measurement. Some channels had no good epochs throughout the measurement due to the loose contact of its source or detector on the scalp. Subsequently, these channels were excluded. Once the quality of the data was ensured, then a low-pass filter (cutoff frequency at 0.2 Hz) was applied to remove the instrumental noise. The changes of HbO_2 and Hb concentrations during the session were calculated based on equation (2) where $DPF = 1.0$ for both wavelengths. To get the TMS effect from each session, all of the quality checked epochs (90 seconds) in the session were averaged by channel. Since the probe area of fNIRS on the left hemisphere was larger than the rTMS stimulating area, only the channels directly under the site of stimulation (ranged from 2–4 channels based on position of the rTMS with respect to the fNIRS grid) were selected. The site of direct stimulation was slightly variable for each subject. The recorded location of rTMS with reference to the fNIRS determined which channels were used based on the following: four channels were selected if the center of the rTMS coil was positioned directly above a source; two channels were selected if the center of the rTMS coil was directly over the detectors; and three channels were selected if the center of the rTMS coil was located between the sources and detectors. The reason to utilize a variable number of channels was to maximize the uniformity of the optical signals by only averaging those signals from brain regions directly stimulated. The hemodynamic changes within the selected channels were averaged to get the eventual ipsilateral response under the rTMS coil. Similarly, the mirror channels on the right hemisphere were selected and averaged to get the eventual contralateral response due to rTMS. These were the time courses on which the subsequent analyses were performed that included determining if: (1) there were significant differences between Visit 1 and Visit 2; (2) there were significant brain changes associated with motor cortex stimulation; (3) there were significant brain changes associated with

prefrontal cortex stimulation; (4) there was concordance between changes in the ipsilateral and contralateral hemispheres; and (5) there were significant differences between the motor and prefrontal cortices.

For each series of measurements, a repeated measures analysis of variance using a general linear models technique was performed on the data. The information from this analysis was used to perform a comparison to the baseline value using Dunnett's procedure at the 0.05 significance level. Dunnett minimum significant differences were used to create and plot 95% confidence limits for each value in the series. Using this technique, confidence limits that do not cover the baseline value are considered significantly different at the $p < 0.05$ level. Calculations were performed using SAS 9.2 (Cary, N.C.).

In addition to looking at the temporal changes induced focally for the ipsilateral and contralateral cortices, spatial differences were investigated to determine whether the changes seen were just global brain changes or more specific to the ipsilateral and contralateral regions stimulated. All of a participant's individual channels (ipsilateral and contralateral) were inspected to determine that they were of sufficient quality to be included in the analysis. Only those participants that had quality data in all channels were included to reconstruct the hemodynamic images of the cortex frame by frame (Arridge, 1999). This was felt to be necessary to accurately represent the spatial distribution of the brain changes induced by rTMS. Concentration changes in reconstructed HbO₂ and Hb images were measured from the 6th to the 10th seconds of stimulation since this was felt to be the time of greatest change in the hemodynamic response. Data was averaged for all participants with adequate quality data.

Results

Participants

Twelve healthy volunteers were enrolled. One subject had periodic spontaneous hand movements prior to the study and was excluded. For the 11 participants studied with a mean age of 31.8 (s.d. 10.2, range 20–49) years, nine were male (mean age 31.4 years) and two were female (mean age 33.5 years). Four participants were Caucasians, two were African-Americans, one was Asian, and four were Hispanic. Eight participants were right-handed and three were left-handed.

rTMS

A resting motor threshold (RMT) was obtained for nine of the eleven subjects. The mean RMT for these participants was 84% (s.d. 8, range 69–96) machine output. The other two subjects had active motor thresholds (AMT) of approximately 91% machine output and 100% machine output. The motor thresholds (MT) were higher than generally seen as a result of the increased distance from the coil to the cortex due to the fNIRS probes. There were no significant changes from Visit 1 to Visit 2 in MT ($t(10) = 0.10$, two-tailed $p = 0.92$). Stimulation used was the same for Visit 1 and Visit 2 for all participants. The range of stimulation intensities was 83 to 100% machine output (mean 98 s.d. 5). For those with RMT, this resulted in stimulating at a mean of 114% (s.d. 8) motor threshold. For those with RMT higher than 100% of the machine output, obviously their stimulation would be below 100% motor threshold.

Simultaneous rTMS/fNIRS of Visit 1 and Visit 2

There was no significant difference between Visit 1 and Visit 2 in the motor or prefrontal cortex supporting the reliability of the measure. Thus, the time courses of Visit 1 and Visit 2 were combined.

Simultaneous rTMS/fNIRS of Motor Cortex

The fNIRS measurements of 1 Hz rTMS stimulation demonstrated a significant reduction in HbO₂ during stimulation in both the ipsilateral and contralateral motor cortex. Interestingly, the degree of decrease appeared to be less for the ipsilateral versus the contralateral motor cortex. There was a trend for an increase but no significant change in Hb signal was found across the time course. The results for the Hb signal ipsilateral and contralateral sides were similar (see Figure 2).

Simultaneous rTMS/fNIRS of Prefrontal Cortex

Similar to the motor cortex, the fNIRS measurements of 1 Hz rTMS stimulation demonstrated a significant reduction in HbO₂ during stimulation in both the ipsilateral and contralateral prefrontal cortex. For the prefrontal cortex, however, the decrease for the contralateral versus the ipsilateral cortices was similar. The Hb signal was similarly increased for both the ipsilateral and contralateral prefrontal cortex, but only the ipsilateral cortex was marginally significant (see Figure 2).

Comparing Prefrontal and Motor Cortices of Simultaneous rTMS/fNIRS

One question often asked is how applicable are neurophysiologic changes associated with rTMS of the motor cortex to other brain regions. At least under the conditions studied (healthy participants stimulated for 10 secs with 1 Hz rTMS), the brain changes associated with motor cortex stimulation and prefrontal cortex stimulation were very comparable (See Figure 2).

Spatial Distribution of Brain Changes Associated with Focal rTMS

Due to technical artifacts in various channels, the averaged prefrontal cortex spatial response included four subjects and the averaged motor cortex spatial response included three. There was indeed a spatial distribution of brain changes for the focal rTMS. The area directly under the coil and its mirror contralateral brain region demonstrated the greatest brain changes for the HbO₂ (see Figure 3).

Discussion

For the first time to our knowledge, brain changes both under the rTMS coil and contralaterally have been measured with fNIRS. A significant decrease in HbO₂ was found at the site of rTMS stimulation and the contralateral brain region for both the motor and prefrontal cortices. The robustness of these findings was evident in the consistency of results across subjects and across two visits. This opens an exciting new area of investigation using simultaneous rTMS/fNIRS to probe the degree of reactivity and connectivity of superficial cortical structures as well as to investigate brain mechanisms of rTMS.

Intervening in the course of neurological and psychiatric disorders with rTMS is a growing area of therapeutic development. Gaining a better understanding of how different treatment parameters interact to produce clinical outcome is critical to optimizing the effectiveness of these new treatments. Unlike medications that involve the “parameter space” of only needing to choose whether to use the medication, at which dosage, and for how long, neuromodulation devices like rTMS require the additional choices of where to stimulate, what frequency, what on-off cycle, and for how long. The additional parameters not only increase the number of options to consider, but they also dramatically increase the number of potential interactions between these parameters. Testing all of these various permutations with clinical trials would require an almost infinite number of patients. Also, this assumes that there is one optimal parameter set for all patients. Conversely, if brain changes associated with response can be identified (e.g., need to increase prefrontal cortex activation), simultaneous rTMS/fNIRS could

be used to determine more efficiently which parameters most effectively brought about the needed change. In addition, what is learned from evaluating the reactivity and connectivity of superficial cortical structures with simultaneous rTMS/fNIRS could inform which treatment parameters are most likely to work. As a potential example, suppose patients with major depressive disorder are found to have a variable degree of prefrontal activation and connectivity with rTMS. This degree of activation and connectivity may predict which parameters are most likely to produce remission of depressive symptoms. The rTMS/fNIRS could measure that degree of activation and connectivity to help determine which parameters were most likely to relieve the depressive symptoms. Obviously, these optimized parameter methods would need to be formally tested against a standard treatment method in a masked, randomized, controlled clinical trial.

There are a number of factors that should be considered when interpreting these results. Although our sample was from a broad spectrum of racial groups, all of our subjects were healthy men and women who had no history of psychiatric or neurologic disorders and were not taking any medications. The results from this sample can in no way be applied to patients with neurologic or psychiatric disorders, or patients who are taking medications. Also, the sample size did not enable us to investigate the relationship between handedness and brain effects of rTMS. Future studies should address how different neuropsychiatric diseases and handedness impact on these results.

In addition to only studying healthy participants, only the brain effects of 1 Hz stimulation were evaluated. There is mounting evidence that different frequencies of rTMS differentially impact the brain (Edwards et al., 2008; George and Belmaker, 2007; Speer et al., 2000). This technology advantageously lends itself to studying a broad range of frequencies since its method of measuring brain activity does not involve electromagnetic signals. Understanding how cortical regions other than the motor cortex immediately respond to different stimulation frequencies is an important area of future research.

Because the focus of this initial study was to determine if a reliable signal could be obtained with the simultaneous rTMS/fNIRS, no sham stimulation group was included. One concern would be that the brain changes observed were nonspecific variations related to the experience and not specific to the rTMS brain stimulation. The fact that spatial differentiation was observed with the greatest changes occurring with respect to the location of the TMS coil, however, argue against this possibility. In addition, two studies have demonstrated that at least in the contralateral prefrontal cortex, there were clear differences between sham and active rTMS that are consistent with our results (Aoyama et al., 2009; Hanaoka et al., 2007). Future work should include a sham condition to better understand how factors other than the rTMS impact on the fNIRS signal.

Another concern related to factors that could potentially confound brain changes related to rTMS stimulation is if the fNIRS was simply measuring scalp and not brain changes to the stimulation. There are several factors that argue against this possibility. First, the clear and consistent change seen in the contralateral cortices in which there was no scalp stimulation demonstrated a measurable change – similar to what was observed under the coil. Another argument against the measured fNIRS changes being simply from the scalp is the reduced decrease in HbO₂ in the ipsilateral motor cortex compared to the contralateral motor cortex (and ipsilateral and contralateral prefrontal cortex). Previous experiments have shown that participant initiated simple movements of the fingers reliably produce increases in the HbO₂ over the motor cortex (Huppert et al., 2006). The *relatively lessened decrease* in the HbO₂ in the ipsilateral motor cortex may be the result of integration of the *decrease* in HbO₂ from the 1 Hz rTMS and *increase* in HbO₂ from the movement induced by the rTMS. If this hypothesis

is correct, then clearly the signal being measured by the fNIRS is from the brain and not the scalp. Further work is required to address this issue.

Because of the increased distance from coil to cortex as a result of the fNIRS system, the rTMS machine was unable to provide 120% MT stimulation to all participants. In fact, due to lack of power, the TMS was unable to determine a resting MT in two subjects. The resulting difference in stimulation dose as reflected in the percent MT may have been problematic. From interleaved TMS/fMRI studies (Bestmann et al., 2005; Nahas et al., 2001), one would expect that the differences may result in increased variability in activation. This variability may have reduced our ability to detect changes, but would not be expected to change the direction of the finding.

Due to the design of our experiment, the effect of order of brain region being stimulated could not be tested. All subjects were first stimulated over the motor cortex and then the prefrontal cortex. Because we wanted to confirm that our technique provided reliable and consistent results, we assessed the motor threshold prior to each rTMS session (Visit 1 and Visit 2) to ensure that we were comparing trials with similar levels of stimulation. Since determining the motor threshold required stimulating over the motor cortex, there would be no way to stimulate the prefrontal cortex without having first stimulated the motor cortex. This study supported the reliability of this technique; future studies can test for order effects by counterbalancing the order in which brain regions are stimulated.

The location of prefrontal stimulation was determined using the standard “5-cm rule.” Although it has been shown to not always result in stimulation of the prefrontal cortex (Herwig et al., 2001), it is the method most often used in clinical studies of rTMS to treat depression. Future work investigating rTMS/fNIRS could use structural MRIs to better localize site of prefrontal cortex stimulation.

One interesting aspect of this study is the potentially conflicting results compared to what has been observed with interleaved TMS/fMRI. This study found a very consistent decrease in HbO₂ under the coil and in the mirror contralateral cortical region. This decrease in HbO₂ has been found by other investigators for contralateral brain regions (Aoyama et al., 2009; Hanaoka et al., 2007). Conversely, a number of interleaved TMS/fMRI studies have found activation under the coil and contralaterally (Bestmann et al., 2003, 2005; Bohning et al., 1999; Bohning et al., 1998; Nahas et al., 2001). This apparent discrepancy may be explained by differences in paradigm design, data analysis methods, or differences in fNIRS’ HbO₂/Hb signal and functional MRI’s blood oxygen level dependent (BOLD) signal.

In summary, this is the first report to our knowledge of using fNIRS to simultaneously measure brain changes induced by rTMS in the ipsilateral and contralateral cortices. Low frequency rTMS produced significant decreases in HbO₂ under the coil and in the mirror contralateral brain region. These brain changes were consistent across treatment days and specific to stimulation location. This technology offers a unique opportunity to study brain function in health and disease, and to gain a better understanding of brain changes associated with different rTMS treatment parameters.

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Figure 1. Location of the rTMS Coil and fNIRS Sources and Detectors

Location of the Transcranial Magnetic Stimulation coil (light grey figure 8) with respect to functional Near Infrared Spectroscopy (sources are circles and detectors are squares) on the participant's head. The fNIRS probe comprised of 28 source-detector pairs (marked with solid lines between sources and detectors) in total with 14 source-detector pairs on each hemisphere.

Figure 2. Functional brain changes under the coil and in the contralateral region

Averaged time course of deoxygenated hemoglobin (Hb) and oxygenated hemoglobin (HbO₂) signals for the motor cortex and the prefrontal cortex. Measurements were made under the coil and in the corresponding contralateral cortex. One hertz stimulation was performed for 10 seconds followed by 80 seconds of rest for 15 epochs. The x-axis represents the time from 0 to 90 seconds in the epoch, and the y-axis represents the mean and 95% confidence interval for hemoglobin (Hb or HbO₂) concentration in $\mu\text{M/L}$. (A) is the ipsilateral and contralateral Motor Cortex and (B) is the ipsilateral and contralateral Prefrontal Cortex.

Averaged PFC Response (N=4)

Averaged MC Response (N=3)

Figure 3. Spatial distribution of functional brain changes associated with stimulation from rTMS
 Spatial distribution related to Transcranial Magnetic Stimulation of Motor and Prefrontal Cortex measured with functional Near Infrared Spectroscopy. Concentration changes in oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (Hb) were measured from the 6th to the 10th seconds of stimulation. The concentration changes of HbO₂ and Hb were in μM/L with the color-coded scale to the right of the image. The x-axis and y-axis were in cm (the probe size was 20×6 cm²). PFC – prefrontal cortex, MC – motor cortex.