that circulating levels of BDNF are negligible. In addition, Karege et al. [26] also reported that an alteration of serum or plasma BDNF is not due to the change in blood BDNF but rather is probably related to the mechanisms of BDNF release, and that depression results from lowered platelet BDNF release. Moreover, Lommatzsch et al. [30] demonstrated that age, weight, gender, and the menstrual cycle have a specific impact on platelet and plasma BDNF levels in healthy adults. In the present study, however, no association was found between age, weight, and the gender and plasma BDNF levels in depressed patients. In addition, we have demonstrated that no significant correlation was observed between serum levels of BDNF and age, weight, and the gender in 103 healthy volunteer (manuscript in preparation). Taking these findings together, although it still remains controversial whether the plasma levels of MHPG and BDNF reflect those of the brain, we speculate that rTMS influences noradrenergic neurons and BDNF, which might be related to the improvement of depressive symptoms, especially agitation/anxiety. Actually, several reports have demonstrated that exposure to stress induced an increase in the MHPG levels, suggesting hyperactivity of noradrenergic systems [43, 47, 49] and decreased BDNF mRNA levels [45]. The results in the present study are in accordance with these findings.

We are aware of the limitations of the present study; i.e., our sample size was very small and heterogeneous, and the duration of treatment was not adequate. The most serious problem in the present study is that there was no control group without rTMS (the sham-controlled group), which makes it difficult to attribute the improvements of Ham-D to rTMS rather to a placebo response. In addition, the patients were taking antidepressants. Moreover, neurochemical changes are also not incompatible with a placebo effect, as imaging findings in depressed patients showed that the clinical improvement following placebo treatment was substantiated by regional metabolic changes in the cortical and subcortical regions [33]. Thus, definitively attributing the behavioral or neurochemical changes to rTMS is not possible until these results are replicated in a controlled fashion. In addition, we used antidepressant drugs combined with rTMS treatment, which could not rule out the effects of ongoing drugs on plasma levels of catecholamine metabolites and BDNF. Therefore, further study will be needed to confirm these preliminary findings.

In conclusion, we have found that rTMS results in some improvement and is well tolerated for treatment-refractory depression, especially in those for whom the symptom of agitation is dominant. In addition, the efficacy of rTMS for treatment-refractory depression might be related to its effect on noradrenergic neurons and BDNF.

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References

[1] Altar CA, Whitehead RE, Chen R, Wortwein G, Madsen TM. Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. Biol Psychiatry 2003; 54: 703 – 709
[2] Angelucci F, Oliviero A, Pilato F, Saturno E, Dileone M, Versace V, Musumeci G, Batocchi AP, Tonali PA, Di Lazzaro V. Transcranial magnetic stimulation and BDNF plasma levels in amyotrophic lateral sclerosis. Neuroreport 2004; 22: 717 – 720
[3] Ballenger JC. Psychopharmacology of the anxiety disorders. Psychiatr Clin North Am 1984; 7: 757 – 771
[4] Barker AT. An introduction to basic principles of magnetic nerve stimulation. J Clin Neurophysiol 1991; 8: 26 – 37
[5] Ben-Shachar D, Belmaker RH, Grisaru N, Klein E. Transcranial magnetic stimulation induces alterations in brain monoamines. J Neural Transm 1997; 104: 191 – 197
[6] Ben-Shachar D, Gazawi H, Riboyad-Levin J, Klein E. Chronic transcranial magnetic stimulation alters beta-adrenergic and 5-HT_2 receptor characteristics in rat brain. Brain Res 1999; 816: 78 – 83
[7] Berman RM, Narasimhan M, Sanacora G, Miano AP, Hoffman RE, Hu XS et al. A randomized clinical trial of repetitive transcranial magnetic stimulation in the treatment of major depression. Biol Psychiatry 2000; 48: 332 – 337
[8] Burt T, Lisanby SH, Sackeim HA. Neuropsychiatric applications of transcranial magnetic stimulation: a metaanalysis. Int J Neuropsychopharmacol 2002; 5: 73 – 103
[9] Couturier JL. Efficacy of rapid-rate repetitive transcranial magnetic stimulation in the treatment of depression: a systematic review and meta-analysis. J Psychiatry Neurosci 2005; 30: 83 – 90
[10] Eschweiler GW, Plewnia C, Bartels M. Which patients with major depression benefit from prefrontal repetitive magnetic stimulation? Fortschr Neurol Psychiatr 2001; 69: 402 – 409
[11] Eschweiler GW, Wegerer C, Schlotter W, Spandl C, Stevens A, Bartels M et al. Left prefrontal activation predicts therapeutic effects of repetitive transcranial magnetic stimulation (rTMS) in major depression. Psychiatry Res 2000; 99: 161 – 172
[12] Figiel GS, Epstein C, McDonald WM, Amazon-Leece J, Figiel L, Saldivia A et al. The use of rapid-rate transcranial magnetic stimulation (rTMS) in refractory depressed patients. J Neuropsychiat Clin Neurosci 1998; 10: 20 – 25
[13] Fitzgerald PB, Brown TL, Daskalakis ZJ. The application of transcranial magnetic stimulation in psychiatry and neurosciences research. Acta Psychiatr Scand 2002; 105: 324 – 340
[14] Fitzgerald PB, Brown TL, Marston NA, Daskalakis ZJ, De Castella A, Kulkarni J. Transcranial magnetic stimulation in the treatment of depression. A double-blind, placebo-controlled trial. Arch Gen Psychiatry 2003; 60: 1002 – 1008
[15] Fleischmann A, Sternheim A, Etgen AM, Li C, Grisaru N, Belmaker RH. Transcranial magnetic stimulation downregulates β-adrenoreceptors in rat cortex. J Neural Transm 1996; 103: 1361 – 1366
[16] Garcia-Toro M, Mayol A, Arnillas H, Capllonch I, Ibarra O, Crespi M et al. Modest adjunctive benefit with transcranial magnetic stimulation in medication-resistant depression. J Affect Disord 2001; 64: 271 – 275
[17] Garcia-Toro M, Pascual-Leone A, Romera M, Gonzalez A, Mico J, Ibarra O et al. Prefrontal repetitive transcranial magnetic stimulation as add on treatment in depression. J Neurol Neurosurg Psychiat 2001; 71: 546 – 548
[18] George MS, Lisanby S, Sackeim HA. Transcranial magnetic stimulation: applications in neuropsychiatry. Arch Gen Psychiatry 1999; 56: 300 – 311
[19] George MS, Wassermann EM, Kimbrell TA, Little JT, Williams WE, Danielson AL et al. Mood improvement following daily left prefrontal repetitive transcranial magnetic stimulation in patients with depression. A placebo-controlled crossover trial. Am J Psychiatry 1997; 154: 1752 – 1756
[20] George MS, Wassermann EM, Williams WA, Callanhan A, Ketter TA, Basser P et al. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. Neuroreport 1995; 6: 1853 – 1856
[21] Gershon AA, Dannon PN, Grunhaus L. Transcranial magnetic stimulation in the treatment of depression. Am J Psychiatry 2003; 160: 835 – 845

[22] Hashimoto K, Shimizu E, Iyo M. Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res Rev 2004; 45: 104 – 114
[23] Jaconsen JP, MØrk A. The effect of escitalopram, desipramine, electroconvulsive seizures and lithium on brain-derived neurotrophic factor mRNA and protein expression in the rat brain and the correlation to 5-HT and 5-HIAA levels. Brain Res 2004; 1024: 183 – 192
[24] Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002; 109: 143 – 148
[25] Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett 2002; 328: 261 – 264
[26] Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry J-M, Bertschy G. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol Psychiatry 2005; 57: 1068 – 1072
[27] Keck ME, Welt T, Müller MB, Uhr M, Ohl F, Wigger A et al. Repetitive transcranial magnetic stimulation increases the release of dopamine in the mesolimbic and mesostriatal system. Neuropharmacology 2002; 43: 101 – 109
[28] Kole MH, Fuchs E, Ziemann U, Paulus W, Ebert U. Changes in 5-HT1A and NMDA binding sites by a single rapid transcranial magnetic stimulation procedure in rats. Brain Res 1999; 826: 309 – 312
[29] Lang UE, Hellweg R, Gallinat J. BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. Neuropsychopharmacology 2004; 29: 795 – 798
[30] Lommatzsch M, Zingeler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, Virchow JC. The impact of age and gender on BDNF levels in human platelets and plasma. Neurobiology Aging 2005; 26: 115 – 123
[31] Loo C, Mitchell P, Sachdev P, Mcdarmant B, Parker G, Gandevia S. Double-blind controlled investigation of transcranial magnetic stimulation for the treatment of resistant major depression. Am J Psychiatry 1999; 156: 946 – 948
[32] Marangell LB, Silver FM, Yudofsky SC. Psychopharmacology and electroconvulsive therapy. In Hales RE, Yudofsky SC, Talbott JA, eds. Textbook of Psychiatry; 3rd edition Washington DC: American Psychiatric Press, 1999: 1025 – 1132
[33] Mayberg HS, Silva JA, Brannan SK, Tekell JL, Mahurin RK, McGinnis S, Jerabek PA. The functional neuroanatomy of the placebo effect. Am J Psychiatry 2002; 159: 728 – 737
[34] Minegishi A, Ishizaki T. Determination of free 3-methoxy-4-hydroxyphenylglycol with several other monoamine metabolites in plasma by high-performance liquid chromatography with amperometric detection. J Chromatogr 1984; 311: 51 – 57
[35] Muller MB, Toschi N, Kresse AE, Post A, Keck ME. Long-term repetitive transcranial magnetic stimulation increases the expression of brain-derived neurotrophic factor and cholecystokinin mRNA, but not neuropeptide tyrosine mRNA in specific areas of rat brain. Neuropsychopharmacol 2000; 23: 205 – 215
[36] Nakamura J, Yoshimura R, Okuno T, Ueda N, Hachida M, Yasumoto K et al. Association of plasma free-3-methoxy-4-hydroxyphenyl(ethylene)glycol, natural killer cell activity and delirium in postoperative patients. Int Clin Psychopharmacol 2001; 16: 339 – 343
[37] Pan W, Banks WA, Fasold MB, Blush J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood-brain barrier. Neuropharmacology 1998; 37: 1553 – 1561
[38] Pascual-Leone A, Rubio B, Pallardo F, Catala MD. Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. Lancet 1996; 348: 233 – 237
[39] Radka SF, Holst PA, Fritsche M, Altar CA. Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected and by a sensitive and specific immunoassay. Brain Res 1996; 709: 122 – 130
[40] Rumi DO, Gattaz WF, Rigonatti SP, Rosa MA, Fregni RF, Rosa MA, Fregni F, Rosa MO, Mansur C, Myczkowski ML, Moreno RA, Marcolin MA. Transcranial magnetic stimulation accelerates the antidepressant effect of amitriptyline in severe depression: a double-blind placebo-controlled study. Biol Psychiatry 2005; 57: 162 – 166
[41] Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP. Physical activity-antidepressant treatment combinations: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav Brain Res 2001; 120: 87 – 95
[42] Shimizu E, Hashimoto K, Okuma N, Koike K, Komatsu N, Kumakiri C et al. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 2003; 54: 70 – 75
[43] Shinkai K, Yoshimura R, Ueda N, Okamoto K, Nakamura J. Associations between baseline plasma MHPG (3-methoxy-4-hydroxyphenylglycol) levels and clinical responses with respect to milnacipran versus paroxetine treatment. J Clin Psychopharmacol 2004; 24: 11 – 17
[44] Siever LJ, Davis KL. Overview: toward a dysregulation hypothesis of depression. Am J Psychiatry 1985; 142: 1017 – 1031
[45] Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 1995; 15: 1768 – 1777
[46] Stahl SM. Essential Psychopharmacology Cambridge University Press. Cambridge: 2000
[47] Ueda N, Yoshimura R, Shinkai K, Nakamura J. Plasma levels of catecholamine metabolites predict the response to sulpiride or fluvoxamine in major depression. Pharmacopsychiatry 2002; 35: 175 – 181
[48] Weissman MM, Bland RC, Canino GJ, Faravelli C, Greenwald S, Hwu HG et al. Cross-national epidemiology of major depression and bipolar disorder. JAMA 1996; 276: 293 – 299
[49] Yamada S, Yajima J, Harano K, Miki K, Nakamura J, Tsuda A et al. Saliva level of free 3-methoxy-4-hydroxyphenylglycol in psychiatric outpatients with anxiety. Int Clin Psychopharmacol 2000; 13: 213 – 217
[50] Yeung PK, Buckley SJ, Pedder SC, Dingemanse J. Determination of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid in human plasma by a simple and rapid high-performance liquid chromatography assay. J Pharm Sci 1996; 85: 451 – 453
[51] Yoshimura R, Ueda N, Shinkai K, Nakamura J. Plasma levels of homovanillic acid and the response to risperidone in first episode untreated acute schizophrenia. Int Clin Psychopharmacol 2003; 18: 107 – 111
[52] Yoshimura R, Nakamura J, Shinkai K, Ueda N. Clinical response to antidepressant treatment and 3-methoxy-4-hydroxyphenylglycol levels: mini review. Prog Neuro-Psychopharmacol Biol Psychiatry 2004; 28: 611 – 616
[53] Yoshimura R, Nakamura J, Shinkai K, Goto M, Yamada Y, Kaji K et al. An open study of risperidone liquid in the acute phase of schizophrenia. Hum Psychopharm 2005; 20: 243 – 248

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RESEARCH ARTICLE

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Cortical hemoglobin-concentration changes under the coil induced by single-pulse TMS in humans: a simultaneous recording with near-infrared spectroscopy

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Abstract We measured cortical hemoglobin-concentration changes under the coil induced by single-pulse transcranial magnetic stimulation (TMS) using a technique of simultaneous recording with near-infrared spectroscopy (NIRS). Single-pulse TMS was delivered over the hand area of the left primary motor cortex at an intensity of 100, 120, or 140% of the active motor threshold (AMT). NIRS recordings were also made during sham stimulation. These four different stimulation sessions (TMS at three intensities and sham stimulation) were performed both when the subject slightly contracted the right first dorsal interosseous muscle and when relaxed it (active and resting conditions). Under the active condition with TMS at 100% AMT, we observed a transient increase in oxy-hemoglobin (oxy-Hb), which was significantly larger than sham stimulation. Under the resting conditions with TMS at 120 and 140% AMT, we observed significant decreases in both deoxy-hemoglobin (deoxyHb) and total-hemoglobin (total-Hb) as compared to sham stimulation. We suggest that the increase of oxy-Hb concentration at 100% AMT under the active condition reflects an add-on effect by TMS to the active baseline and that decrease of deoxy-Hb and total-Hb concentrations at 120 and 140% AMT under the resting condition are due to reduced baseline firings of the corticospinal tract neurons induced by a lasting inhibition provoked by a higher intensity TMS.

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

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Introduction

Transcranial magnetic stimulation (TMS) has been widely used in both clinical neurological (Currà et al. 2002) and neurophysiological examinations (Petersen et al. 2003; Chen 2004). Regional cerebral blood flow (rCBF) and cerebral metabolic changes induced by repetitive transcranial magnetic stimulation (rTMS) over the motor cortex have been studied by several researchers using positron emission computed tomography (PET), single-photon emission computed tomography (SPECT), or functional magnetic resonance imaging (fMRI) (Brandt et al. 1996; Fox et al. 1997; Wassermann et al. 1997; Paus et al. 1998; Bohning et al. 1999; Siebner et al. 2000, 2001; Baudewig et al. 2001; Bestmann et al. 2003; Okabe et al. 2003a). However, the results are inconsistent, presumably because of differences in the stimulation parameters of TMS: e.g., intensity, frequency, duration (total number of stimuli), and direction of currents in the brain. For example, at the site of stimulation (the motor cortex), rCBF or metabolic activity has been reported to increase (Brandt et al. 1996; Fox et al. 1997; Bohning et al. 1999; Paus et al. 1998; Siebner et al. 2000, 2001), decrease (Wassermann et al. 1997; Paus et al. 1998), or show no significant changes (Okabe et al. 2003a) during or after rTMS.

These previous functional imaging investigations have utilized rTMS with more than ten pulses and only a few studies have investigated rCBF changes induced by single-pulse TMS because of the following technical difficulties. First, the hemodynamic changes associated with single-pulse TMS are too small and transient to be

suitable for temporal resolution of SPECT or PET studies. Second, the large magnetic field produced by magnetic stimulation, as well as the mere presence of a TMS coil, interferes with the fMRI measurements due to low signal-to-noise ratio.

Near-infrared spectroscopy (NIRS) is one of appropriate non-invasive methods that allows visualization of the effect of single-pulse TMS. This method has three distinct advantages over the preexisting techniques: high signal-to-noise ratios for single events, non-interference with magnetic field changes, and no use of radioisotopes. This technique estimates hemoglobin (Hb) concentration changes by measuring the reflected light, based on the differences in absorption spectra between oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) (Jöbsis 1977; Chance et al. 1988; Villringer et al. 1993). Our previous study with NIRS has successfully detected Hb concentration changes evoked by single-pulse TMS using a novel technique to record NIRS signals just beneath the coil (Noguchi et al. 2003). Significant oxy-Hb increase was observed after single-pulse TMS when the subjects voluntarily contracted a target hand muscle.

Our previous result of oxy-Hb increase is consistent with a transient activation of the motor cortex above the active baseline by TMS. From a physiological point of view, it is known that transient, monosynaptic facilitation is almost always followed by di- or oligosynaptic inhibition in the central nervous system. In humans, such later inhibition at the motor cortex has also been known as the intracortical inhibition demonstrated by paired-pulse TMS (Kujirai et al. 1993; Ridding et al. 1995; Berardelli et al. 1996; Hanajima et al. 1998; Chen 2004) or as the silent period after motor-evoked potentials (MEPs) elicited by TMS (Inghilleri et al. 1993; Chen et al. 1999). The rCBF changes elicited by TMS may thus reflect the final outcome produced by a combination of all these short-lasting facilitation (facilitatory I-wave interaction), moderately lasting inhibition (mainly synaptic activities), and lasting inhibition of the postsynaptic neurons. According to analyses in vivo, synaptic activity causes an rCBF increase (Mathiesen et al. 1998, 2000; Strafella and Paus 2001), but it remains unknown whether the decrease of baseline activity at the postsynaptic neurons influences the rCBF or not. Such modification after TMS may be masked by voluntary activity of the motor cortex when the subjects contract the target muscle. In the present study, therefore, to study metabolic changes produced by TMS, we measured cortical Hb concentration changes induced by single-pulse TMS under active and resting conditions using a NIRS method and compared them.

Materials and methods

Subjects

Eight healthy volunteers (8 men, 28–51 years old) participated in this study. All subjects were right-handed, scoring 70–100 on the laterality quotient of the Edinburgh Handedness Inventory (Oldfield 1971). Written informed consent was obtained from all subjects after the nature and possible consequences of the studies were explained. The experimental procedures used here were approved by the Ethics Committee of the University of Tokyo, Hongo and were carried out in accordance to the Declaration of Helsinki.

Transcranial magnetic stimulation

Single-pulse TMS was delivered with a figure-of-eight-shaped coil (outer diameter of each wing was 7 cm) connected to a Magstim 200 magnetic stimulator (The Magstim Co., Ltd, Whitland, UK). The coil was positioned over the hand area of the left primary motor cortex (M1). M1 was defined as the "hot spot" where a stimulation evoked the largest MEP from the right first dorsal interosseous (FDI) muscle. In two of them, that position was confirmed to be over the primary motor cortex by the neuronavigation system (Spetzger et al. 1995; Boroojerdi et al. 1999). The coil was oriented to induce medially directed currents in the brain. The intensity was adjusted to be 100, 120, and 140% of the active motor threshold (AMT) at M1. We defined the AMT as the lowest intensity that evoked five small responses (about 100 μV) in a series of ten stimulations when the subject made a 5% maximal voluntary contraction (MVC) (about 50 μV). Sham stimulation was performed as described in our previous report (Okabe et al. 2003b). During sham stimulation, the coil was positioned 10 cm above the head and discharged, while an electric stimulus was given to the skin of the head with electrodes fixed on the head to mimic skin sensation associated with real TMS. For this stimulation, we used a conventional electrical stimulator for peripheral nerves. The anode was placed over the left M1 and the cathode was over 5 cm anterior to the left M1. The duration of the electric stimulus was 0.2 ms, and the intensity was fixed at twice the sensory threshold for skin sensation. This protocol aimed to exclude non-specific effects associated with TMS, such as noise and skin sensation. TMS was tested under the eight different conditions in all the subjects. TMS pulses at three different intensities and sham stimulation were applied when the subject sustained a 10% MVC or when they maintained the relax condition. Each session consisted of 20 single TMS pulses given at random inter-trial intervals of 24–26 s. The same session was repeated two to four times to confirm the reproducibility of the results. The order of sessions was counterbalanced within and across the subjects.

NIRS measurement

We used the same NIRS system as described previously (Noguchi et al. 2003). In brief, the NIRS system (ETG-A1; Hitachi Medical Corporation, Tokyo, Japan)

consisted of two emitters and two detectors, and the four measurement points (midpoints) were placed on the center of the left-hand M1. These measurement points were aligned parallel to the medio-lateral line for minimizing the influence of the Hb-concentration change in the pre-motor and sensory cortices. Near-infrared laser diodes with two wavelengths, 790 and 830 nm, were used as the light sources, and transmittance data of the light beams were obtained every 500 ms. The combination of these wavelengths may not be the best selection because some degree of cross-talks between oxy-Hb and deoxy-Hb may occur in this combination (Uludag et al. 2002; Strangman et al. 2003) and the signal-to-noise ratio is not the highest (Yamashita et al. 2001; Sato et al. 2004). However, even using this combination of wavelengths, other groups (Watanabe et al. 1996, 1998; Isobe et al. 2001; Noguchi et al. 2003) have obtained several typical Hb-concentration changes same as those obtained by using another better combination of wavelengths. These suggest that our method could show

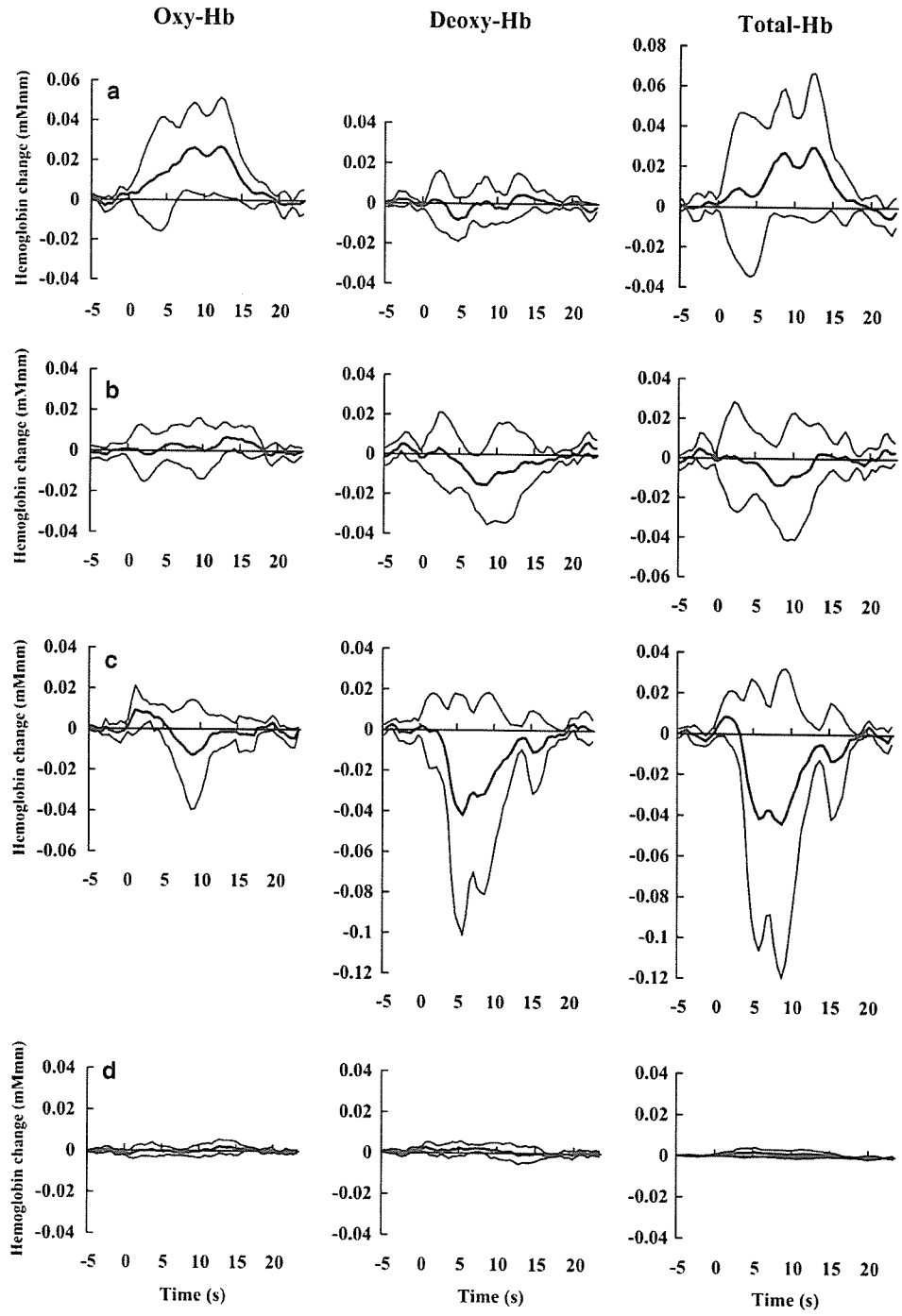

Fig. 1 Oxy-Hb (*left column*), deoxy-Hb (*middle column*), and total-Hb (*right column*) concentration changes after single-pulse TMS when the subject contracted FDI muscle (*active condition*): TMS at 100% AMT (**a**), 120% AMT (**b**), 140% AMT (**c**), and sham stimulation (**d**). Averaged data ($n=8$) obtained at three TMS intensities and sham stimulation are separately shown by *thick lines* and the 95% confidence intervals were indicated by *thin lines*

compatible results to other studies even though the wavelengths are not the best for NIRS recordings. The TMS coil was placed over the fiber probes on the scalp. The minimum distance between the coil and the scalp was 8.5 mm. We calculated concentrations of oxy-Hb, deoxy-Hb, and total hemoglobin (total-Hb) from the transmittance data with the two wavelengths. In this study, each event period ranged from 5 s before the TMS onset to 23 s thereafter. Each Hb change in each session was calculated by averaging the two data at the two measurement points. The Hb change was calculated under each condition by averaging the results of two to four sessions. The 95% confidence interval was also calculated for each time point of oxy-Hb, deoxy-Hb, and total-Hb changes. Two-way analysis of variance (ANOVA) (factors: active/resting condition and four

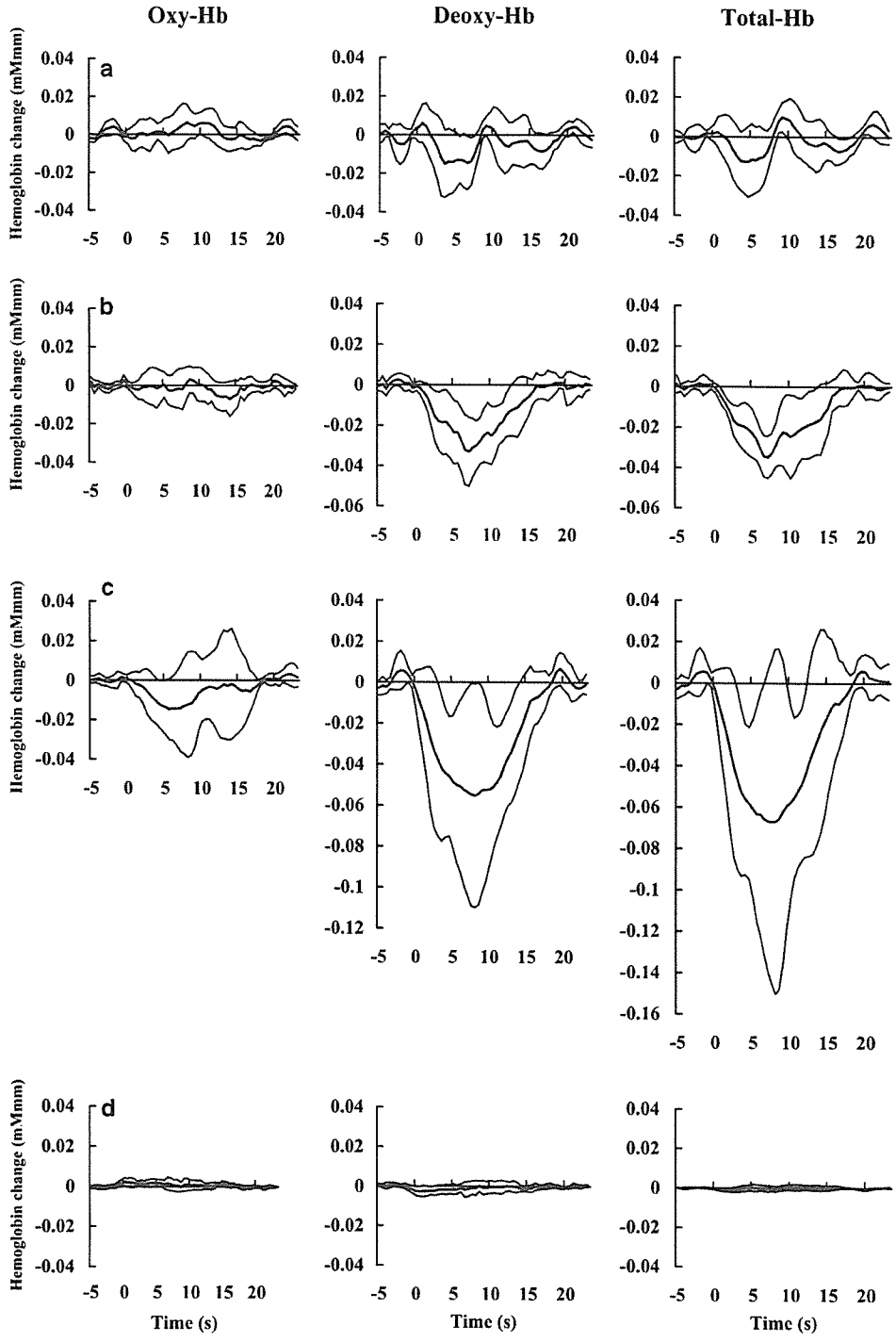

Fig. 2 Oxy-Hb (*left column*), deoxy-Hb (*middle column*), and total-Hb (*right column*) concentration changes after single-pulse TMS when the subject kept the FDI muscle relaxed (resting condition): 100% AMT (**a**), 120% AMT (**b**), 140% AMT (**c**), and sham stimulation (**d**). Averaged data ($n = 8$) obtained at three TMS intensities and sham stimulation are separately shown by *thick lines* and the 95% confidence intervals are indicated by *thin lines*

types of TMS) was performed on the mean Hb changes by averaging the Hb data from 3 to 15 s after each TMS pulse.

Results

TMS under the active condition

Figure 1 shows averaged relative Hb-concentration changes and the 95% confidence intervals when the subjects made a 10% MVC (the active condition). Under the active condition with TMS at 100% AMT, oxy-Hb and total-Hb began to increase after the TMS onset, and returned to the baseline around 15 s later (Fig. 1a). The oxy-Hb significantly increased as compared to the baseline 7–14 s after the TMS onset, as shown by the 95% confidence lines (the lower dotted line was more than zero). In contrast, there were no significant changes in any Hb parameters at 120 and 140% AMT (Fig. 1b, c). We confirmed that the sham stimulation evoked no significant NIRS changes (Fig. 1d).

TMS under the resting condition

Figure 2 shows averaged relative Hb-concentration changes and the 95% confidence intervals, when the subject kept the right FDI relaxed (the resting condition). Under the resting conditions with TMS at 120 and 140% AMT, deoxy-Hb and total-Hb began to decrease 2–3 s after the TMS onset, and returned to the baseline about 15 s later (Fig. 2b, c). In contrast, neither TMS at 100% AMT nor the sham stimulation evoked significant changes in any Hb parameters. The deoxy-Hb at 120% AMT significantly decreased at 2–12 s after the TMS onset, and the total-Hb at 1–13 s. Similarly, at 140% AMT, the deoxy-Hb significantly decreased at 3–14 s after the TMS onset, and the total-Hb also decreased at 3–6 and 10–12 s.

Comparison across conditions

For comparisons between several conditions, we further calculated mean Hb changes in the oxy-Hb, deoxy-Hb, and total-Hb by averaging Hb values from 3 to 15 s after each TMS pulse (Fig. 3). Two-way ANOVA (factors: active/resting conditions and four TMS types) was performed for each parameter. It showed a significant main effect of TMS type on all the parameters (oxy-Hb, $F=5.1$, $P=0.003$; deoxy-Hb, $F=6.6$, $P=0.001$; total-Hb, $F=6.6$, $P=0.001$), as well as a significant main effect of conditions on oxy-Hb and total-Hb (oxy-Hb, $F=4.7$, $P=0.04$; deoxy-Hb, $F=3.7$, $P=0.06$; total-Hb, $F=4.5$, $P=0.04$), but without any significant interactions ($P>0.05$). The oxy-Hb and total-Hb concentrations after the TMS pulse under the active condition were higher than those under the resting condition. Further analyses using paired t-test with corrections for multiple comparisons revealed that the oxy-Hb increase under the active condition with TMS at 100% AMT was significantly larger than the sham stimulation (Fig. 3a). Furthermore, the deoxy-Hb and total-Hb decreases under the resting condition with TMS at 120 and 140% AMT were significantly larger than the sham stimulation (Fig. 3b).

Discussion

The present study with NIRS technique has demonstrated cortical Hb-concentration changes under the coil induced by single-pulse TMS of the motor cortex. The oxy-Hb and total-Hb concentrations after TMS pulse under the active condition were higher than those under the resting condition. From the results of the 95% confidence intervals and comparisons with sham stimu-

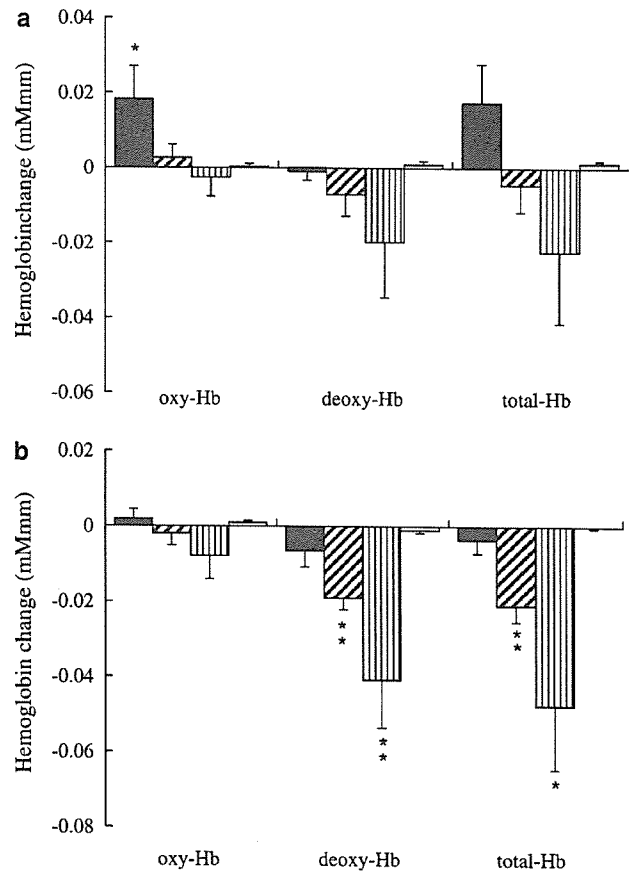

Fig. 3 Mean changes of relative oxy-Hb, deoxy-Hb, and total-Hb concentrations (averages of Hb concentration values from 3 to 15 s after TMS pulse) under the active (**a**) and resting (**b**) conditions. Concentration changes by TMS at 100% AMT are denoted by *filled columns*, 120% by *oblique stripe columns*, 140% by *longitudinal strip columns*, and sham stimulation by *non-filled columns*. *Error bars* indicate standard errors. *Asterisks* indicate the statistical significances ($^{*}P<0.05$; $^{**}P<0.01$, t-test corrected for multiple comparisons)

lation, TMS at 100% AMT produced a significant increase in oxy-Hb under the active condition. Under the resting condition, in contrast, TMS at 120 and 140% AMT produced significant decreases in total-Hb and deoxy-Hb.

The increment of oxy-Hb concentration under the active condition is consistent with the observation seen in other natural brain activations. It has no problems to explain what occurs in this condition. By contrast, our findings under resting condition are not consistent with any typical NIRS patterns previously reported in natural brain activation (oxy-Hb and total-Hb increases and slight deoxy-Hb decrease; Chance et al. 1988; Villringer et al. 1993; Kleinschmidt et al. 1996; Watanabe et al. 1996, 1998; Isobe et al. 2001; Mehagnoul-Schipper et al. 2002) or deactivation (oxy-Hb decrease and deoxy-Hb increase; Wenzel et al. 2000).

Two possibilities may explain our findings which have not been reported yet. One is that our findings are wrong probably because of some flaw of our method. The NIRS recording using the 790- and 830-nm wavelength combination is reported to be affected by some degree of cross-talks between oxy-Hb and deoxy-Hb (Uludag et al. 2002; Strangman et al. 2003). This may cause inconsistency of our results. However, even using this combination, we got several typical patterns of Hb-concentration changes in natural brain activations (Watanabe et al. 1996, 1998; Isobe et al. 2001; Noguchi et al. 2003). Moreover, in weak TMS (100% AMT) experiments during contraction in our present results, we got a typical pattern of Hb-concentration changes same as that already shown in natural brain activation. This may be due to the fact that weak stimulation is nearer to natural brain activation than strong TMS. These facts suggest that our combination of wavelength must give us reasonable results of Hb-concentration changes even with a small contamination of cross-talks. Based on these arguments, we conclude that our result under resting condition is not just a wrong result due to cross-talks. The other possibility is that our present new pattern of Hb-concentration changes must have some physiological meanings (see later discussion). The most important difference of our experiment from other previous studies using NIRS is that we investigated the effect of TMS, an artificial neural activation and not the natural brain activation. Weak TMS pulses at around 100% AMT may mimic a natural activation, but strong TMS pulses at more than 120% AMT must evoke unusual powerful synchronization. It should never occur in natural brain activations. Therefore, it is not surprising that our results are not the same as any other previous patterns.

We can exclude the possibility that the associated scalp movement or Hb-concentration changes in the skin cause the present results. No scalp movement was evoked even by TMS at 140%, and the higher intensity TMS evoked only slight contraction of right-hand muscles without any contraction of neck muscles. Moreover, the 32-mm distance between two NIRS probes is appropriate for detecting the Hb-concentration changes at the cortex, but it is too long to detect Hb-concentration changes at the skin (Germon et al. 1999). Electric stimulation on the skin in sham stimulation, which may elicit skin Hb-concentration changes similar to the real stimulation, evoked no detectable NIRS changes. Therefore, we conclude that the observed NIRS changes were the Hb-concentration changes at the cortex directly elicited by TMS.

Studies on exposed brain tissues of animals have demonstrated that brain activation is associated with an early decrease (initial dip) followed by a subsequent increase of oxy-Hb concentration (Malonek and Grinvald 1996). The initial dip is not always detected with conventional imaging methods because it is small and short lasting. The observable oxy-Hb concentration increase has been confirmed with the NIRS method in previous reports during voluntary muscular contraction (Kleinschmidt et al. 1996; Mehagnoul-Schipper et al. 2002). Using the same NIRS recording probes as those used in the present experiments, we also recorded oxy-Hb-concentration increase associated with a slight decrease of deoxy-Hb over the primary motor cortex during a natural self-paced voluntary contraction of the target muscle (data not shown). In those records, the oxy-Hb concentration began to increase about 4 s after the onset of contraction, gradually decrease toward the baseline and finally returned to it about 15 s after the contraction. No decrease was observed in the oxy-Hb concentration. An increase in oxy-Hb concentration observed under the active condition with TMS at 100% AMT is consistent with our previous results (Noguchi et al. 2003), and compatible with the above-mentioned brain activation during the physiological process (voluntary contraction). Under this condition, the overall contraction makes the cortex more excitable than the resting condition, and TMS further adds facilitation to this active level for a short period. Weak TMS pulses at around 100% AMT may mimic a natural activation and may not produce large inhibitory effects of TMS, because voluntary contraction usually cancels the inhibitory effect in the target muscle (Ridding et al. 1995). This cancellation of inhibition would induce the oxy-Hb and total-Hb increases under the active condition. But the deoxy-Hb concentration did not show such a significant increase. This discrepancy would be due to the lower sensitivity of deoxy-Hb changes than oxy-Hb or total-Hb changes in NIRS measurement (Madsen and Secher 1999). In the case of TMS at 140% AMT, a long-lasting inhibition after short-lasting facilitation has been reported (Inghilleri et al. 1993; Berardelli et al. 1996; Hanajima et al. 1998; Moliadze et al. 2003; Chen 2004), which would not be masked by the voluntary contraction. This may explain why TMS at 140% AMT produced a different pattern of signal changes from that observed during TMS at 100% AMT (see later discussion on mechanisms for this pattern).

Under the resting condition, decreases of total-Hb and deoxy-Hb occurred without any changes of oxy-Hb

concentration. No reports have shown this pattern of changes in Hb concentration. Reafferentation due to movements does not explain these changes because 100% RMT is about 130–140% AMT (Tergau et al. 1999; Khedr et al. 2004) and so TMS at 120% AMT usually elicited no movements in the resting condition, and because reafferentation causes an increase (not a decrease) of rCBF in the PET study (Mima et al. 1999). In this study, there was an obvious total-Hb (rCBF) decrease after TMS. Even though animal experiments have had no good explanation for rCBF decrease, many PET or SPECT studies have shown reduction of rCBF or metabolism evoked by rTMS (Wassermann et al. 1997; Paus et al. 1998; Okabe et al. 2003a; Hayashi et al. 2004). A possible explanation is vasoconstriction elicited by TMS, but this contribution may be small, because vasoconstriction should produce large decreases of total-Hb and oxy-Hb (Fantini 2002; Fabbri et al. 2003), while oxy-Hb showed no change in our results.

In animal experiments on cerebellar cortex, synaptic activity produces a rCBF increase whether it is facilitatory or inhibitory, and firings of Purkinje cells partly contribute to the rCBF changes (Mathiesen et al. 1998). Since the resting firing frequency of the corticospinal tract (CST) neurons is moderately high (Evarts 1964), the postsynaptic firing reduction must cause a CBF decrease. Furthermore, because a TMS pulse evokes synchronous suppression of many CST neurons after activation, such as known as the silent period after activation, the resting firings of CST neurons decrease dramatically after single-pulse TMS. A practical recording showed dramatic firing decrease after TMS pulse in cat visual cortex (Moliadze et al. 2003). So, the total firing number of CST neurons must be reduced after single-pulse TMS. These suppressions physiologically seen at 100–200 ms after stimulation must cause CBF changes seen at several seconds after stimulation. This lag time between the physiological event and the CBF change is always observed (Villringer et al. 1993; Malonek and Grinvald 1996; Mathiesen et al. 1998; Noguchi et al. 2003). At the site under the coil, during stimulation, both facilitatory and inhibitory synaptic activities evoke a rCBF increase and postsynaptic long-lasting reduction of firings must cause a rCBF decrease. The combination of these two opposite effects may provoke a rCBF decrease and postsynaptic neuronal suppression. These rCBF changes must be detected as Hb-concentration changes in our study. Consequently, all of oxy-Hb, deoxy-Hb, and total-Hb supplies to these regions will decrease. The oxygen consumption must also be reduced in the inhibited areas, and then the oxy-Hb is not used. The reduction of oxy-Hb supply and decrease of its consumption finally result in the unchanged oxy-Hb concentration. Therefore, these changes will finally lead to decreases in total-Hb and deoxy-Hb and absence of oxy-Hb changes.

As mentioned above, under the active condition with weak stimulation, voluntary motor command would surpass the inhibition elicited by TMS and thus only a short-latency facilitation was observed. However, under the active condition with strong intensity, powerful inhibition may become greater than the facilitatory effect produced by voluntary contraction. The NIRS pattern of TMS at 140% AMT under the active condition thus becomes very similar to that under the resting condition.

In conclusion, the present study showed cortical Hb-concentration changes induced by single-pulse TMS of the motor cortex, suggesting that the oxy-Hb increase at 100% AMT under the active condition reflects a facilitatory effect by TMS, and that the decreases of deoxy-Hb and total-Hb at 120 and 140% AMT under the resting condition are due to postsynaptic firing-frequency reduction induced by a higher intensity TMS.

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References

Baudewig J, Siebner HR, Bestmann S, Tergau F, Tings T, Paulus W, Frahm J (2001) Functional MRI of cortical activations induced by transcranial magnetic stimulation (TMS). Neuroreport 12:3543–3548

Berardelli A, Rona S, Inghilleri M, Manfredi M (1996) Cortical inhibition in Parkinson's disease. A study with paired magnetic stimulation. Brain 119:71–77

Bestmann S, Baudewig J, Siebner HR, Rothwell JC, Frahm J (2003) Subthreshold high-frequency TMS of human primary motor cortex modulates interconnected frontal motor areas as detected by interleaved fMRI-TMS. Neuroimage 20:1685–1696

Bohning DE, Shastri A, McConnell KA, Nahas Z, Lorberbaum JP, Roberts DR, Teneback C, Vincent DJ, George MS (1999) A combined TMS/fMRI study of intensity-dependent TMS over motor cortex. Biol Psychiatry 45:385–394

Boroojerdi B, Foltys H, Krings T, Spetzger U, Thron A, Töpper R (1999) Localization of the motor hand area using transcranial magnetic stimulation and functional magnetic resonance imaging. Clin Neurophysiol 110:699–704

Brandt SA, Davis TL, Obrig H, Meyer BU, Belliveau JW, Rosen BR, Villringer A (1996) Functional magnetic resonance imaging shows localized brain activation during serial transcranial stimulation in man. Neuroreport 7:734–736

Chance B, Leigh JS, Miyake H, Smith DS, Nioka S, Greenfeld R, Finander M, Kaufmann K, Levy W, Young M, Cohen P, Yoshioka H, Boretsky R (1988) Comparison of time-resolved and -unresolved measurements of deoxyhemoglobin in brain. Proc Natl Acad Sci USA 85:4971–4975

Chen R, Lozano AM, Ashby P (1999) Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res 128:539–542

Chen R (2004) Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res 154:1–10

Currà A, Modugno N, Inghilleri M, Manfredi M, Hallett M, Berardelli A (2002) Transcranial magnetic stimulation techniques in clinical investigation. Neurology 59:1851–1859

Evarts EV (1964) Temporal patterns of discharge of pyramidal tract neurons during sleep and waking in the monkey. J Neurophysiol 27:152–171

Fabbri F, Henry ME, Renshaw PF, Nadgir S, Ehrenberg BL, Franceschini MA, Fantini S (2003) Bilateral near-infrared monitoring of the cerebral concentration and oxygen-saturation of hemoglobin during right unilateral electro-convulsive therapy. Brain Res 992:193–204

Fantini S (2002) A haemodynamic model for the physiological interpretation of in vivo measurements of the concentration and oxygen saturation of haemoglobin. Phys Med Biol 47:N249–N57

Fox P, Ingham R, George MS, Mayberg H, Ingham J, Roby J, Martin C, Jerabek P (1997) Imaging human intra-cerebral connectivity by PET during TMS. Neuroreport 8:2787–2791

Germon TJ, Evans PD, Barnett NJ, Wall P, Manara AR, Nelson RJ (1999) Cerebral near infrared spectroscopy: emitter-detector separation must be increased. Br J Anaesth 82:831–837

Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I (1998) Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. J Physiol 509:607–618

Hayashi T, Ohnishi T, Okabe S, Teramoto N, Nonaka Y, Watabe H, Imabayashi E, Ohta Y, Jino H, Ejima N, Sawada T, Iida H, Matsuda H, Ugawa Y (2004) Long-term effect of motor cortical repetitive transcranial magnetic stimulation induces. Ann Neurol 56:77–85

Inghilleri M, Berardelli A, Cruccu G, Manfredi M (1993) Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. J Physiol 446:521–534

Isobe K, Kusaka T, Nagano K, Okubo K, Yasuda S, Kondo M, Itoh S, Onishi S (2001) Functional imaging of the brain in sedated newborn infants using near infrared topography during passive knee movement. Neurosci Lett 299:221–224

Jöbsis FF (1997) Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 198:1264–1267

Khedr EM, Gilio F, Rothwell JC (2004) Effects of low frequency and low intensity repetitive paired pulse stimulation of the primary motor cortex. Clin Neurophysiol 115:1259–1263

Kleinschmidt A, Obrig H, Requardt M, Merboldt KD, Dirnagl U, Villringer A, Frahm J (1996) Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy. J Cereb Blood Flow Metab 16:817–826

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Assleman P, Marsden CD (1993) Cortical inhibition in human motor cortex. J Physiol 471:501–519

Madsen PL, Secher NH (1999) Near-infrared oximetry of the brain. Prog Neurobiol 58:541–560

Malonek D, Grinvald A (1996) Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. Science 26:551–554

Mathiesen C, Caesar K, Akgören N, Lauritzen M (1998) Modification of activity-dependent increases of cerebral blood flow by excitatory synaptic activity and spikes in rat cerebellar cortex. J Physiol 512:555–566

Mathiesen C, Caesar K, Lauritzen M (2000) Temporal coupling between neuronal activity and blood flow in rat cerebellar cortex as indicated by field potential analysis. J Physiol 523:235–246

Mehagnoul-Schipper DJ, van der Kallen BFW, Colier WNJM, van der Sluijs MC, van Erning LJTO, Thijssen HOM, Oeseburg B, Hoefnagels WHL, Jansen RWMM (2002) Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. Hum Brain Mapp 16:14–23

Mima T, Sadato N, Yazawa S, Hanakawa T, Fukuyama H, Yonekura Y, Shibasaki H (1999) Brain structures related to active and passive finger movements in man. Brain 122:1989–1997

Moliadze V, Zhao Y, Eysel U, Funke K (2003) Effect of transcranial magnetic stimulation on single-unit activity in the cat primary visual cortex. J Physiol 553:665–679

Noguchi Y, Watanabe E, Sakai KL (2003) An event-related optical topography study of cortical activation induced by single-pulse transcranial magnetic stimulation. Neuroimage 19:156–162

Okabe S, Hanajima R, Ohnishi T, Nishikawa M, Imabayashi E, Takano H, Kawachi T, Matsuda H, Shiio Y, Iwata NK, Furubayashi T, Terao Y, Ugawa Y (2003a) Functional connectivity revealed by single-photon emission computed tomography (SPECT) during repetitive transcranial magnetic stimulation (rTMS) of the motor cortex. Clin Neurophysiol 114:450–457

Okabe S, Ugawa Y, Kanazawa I (2003b) The effectiveness of rTMS on Parkinson's disease study group 0.2-Hz repetitive transcranial magnetic stimulation has no add-on effects as compared to a realistic sham stimulation in Parkinson's disease. Mov Disord 18:382–388

Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9:97–113

Paus T, Jech R, Thompson CJ, Comeau R, Peters T, Evans AC (1998) Dose-dependent reduction of cerebral blood flow during rapid-rate transcranial magnetic stimulation of the human sensorimotor cortex. J Neurophysiol 79:1102–1107

Petersen NT, Pyndt HS, Nielsen JB (2003) Investigating human motor control by transcranial magnetic stimulation. Exp Brain Res 152:1–16

Ridding MC, Tavlor JL, Rothwell JC (1995) The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 487:541–548

Sato H, Kiguchi M, Kawaguchi F, Maki A (2004) Practicality of wavelength selection to improve signal-to-noise ratio in near-infrared spectroscopy. Neuroimage 21:1554–1562

Siebner HR, Peller M, Willoch F, Minoshima S, Boecker H, Auer C, Drzrezga A, Conrad B, Bartenstein P (2000) Lasting cortical activation after repetitive TMS of the motor cortex: a glucose metabolic study. Neurology 54:956–963

Siebner HR, Takano B, Peinemann A, Schwaiger M, Conrad B, Drzezga A (2001) Continuous transcranial magnetic stimulation during position emission tomography: a suitable tool for imaging regional excitability of the human cortex. Neuroimage 14:883–890

Spetzger U, Laborde G, Gilsbach JM (1995) Frameless neuronavigation in modern neurosurgery. Minim Invasive Neurosurg 38:163–166

Strangman G, Franceschini MA, Boas DA (2003) Factors affecting the accuracy of near-infrared spectroscopy concentration calculations for focal changes in oxygenation parameters. Neuroimage 18:865–879

Strafella AP, Paus T (2001) Cerebral blood-flow changes induced by paired-pulse transcranial magnetic stimulation of the primary motor cortex. J Neurophysiol 85:2624–2629

Tergau F, Wanschura V, Canelo M, Wischer S, Wassermann EM, Ziemann U, Paulus W (1999) Complete suppression of voluntary motor drive during the silent period after transcranial magnetic stimulation. Exp Brain Res 124:447–454

Uludag K, Kohl M, Steinbrink J, Obrig H, Villringer A (2002) Cross talk in the Lambert–Beer calculation for near-infrared wavelengths estimated by Monte Carlo simulations. J Biomed Opt 7:51–59

Villringer A, Planck J, Hock C, Schleinkofer L, Dirnagl U (1993) Near infrared spectroscopy (NIRS): a new tool to study hemodynamic changes during activation of brain function in human adults. Neurosci Lett 154:101–104

Wassermann EM, Kimbrell TA, George MS, Danielson AL, Herscovitch P, Hallett M, Post RM (1997) Local and distinct changes in cerebral glucose metabolism during repetitive transcranial magnetic stimulation (rTMS). Neurology 48:A107–A108

Watanabe E, Yamashita Y, Maki A, Ito Y, Koizumi H (1996) Non-invasive functional mapping with multi-channel near infrared spectroscopic topography in humans. Neurosci Lett 205:41–44

Watanabe E, Maki A, Kawaguchi F, Takashiro K, Yamashita Y, Koizumi H, Mayanagi Y (1998) Non-invasive assessment of language dominance with near-infrared spectroscopic mapping. Neurosci Lett 256:49–52

Wenzel R, Wobst P, Heekeren HH, Kwong KK, Brandt SA, Kohl M, Obrig H, Dirnagl U, Villringer A (2000) Saccadic suppression induces focal hypooxygenation in the occipital cortex. J Cereb Blood Flow Metab 20:1103–1110

Yamashita Y, Maki A, Koizumi H (2001) Wavelength dependence of the precision of noninvasive optical measurement of oxy-, deoxy-, and total-hemoglobin concentration. Med Phys 28:1108–1114

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Short communication

Pathology of the sympathetic nervous system corresponding to the decreased cardiac uptake in ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy in a patient with Parkinson disease

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Abstract

Decreased cardiac uptake in ^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy has been adopted as one of the most reliable diagnostic tests for Parkinson disease (PD) in Japan. To investigate the morphological basis for this finding, we performed a detailed neuropathological study of the cardiac sympathetic nervous system of a 71-year-old autopsy-proven PD patient, who presented with a marked decrease in cardiac uptake of MIBG, just 1 year prior to death. We carefully examined the intermediolateral column at several levels of the thoracic spinal cord, the sympathetic trunk and ganglia, and the nerve plexus of the anterior wall of the left ventricle and compared the findings with those of five age-matched controls. We found that the cardiac plexus was more heavily involved than the sympathetic ganglia in this patient with PD. Our study may provide further evidence that the markedly decreased cardiac uptake of MIBG observed in PD cases represents preferential involvement of the cardiac sympathetic nerve plexus in this disorder.

Keywords: Lewy body; α-synuclein; Distal axonopathy

1. Introduction

^{123}I-metaiodobenzylguanidine (MIBG) is an analogue of noradrenaline and is metabolized by noradrenergic neurons. It is therefore used as a tracer in myocardial scintigraphy for the evaluation of cardiac sympathetic innervation. Markedly decreased cardiac uptake of MIBG shown by myocardial scintigraphy is a specific finding in Parkinson disease (PD) or dementia with Lewy bodies (DLB) and is useful for the differential diagnosis of other Parkinsonian syndromes [1–4] or Alzheimer's disease [5]. This decrement has been seen even in PD patients without autonomic symptoms [2–4].

A follow-up MIBG scintigraphy study recently revealed the occurrence of a progressive decrement of MIBG uptake in cases of Yahr Stage I PD (Dr. S. Orimo, abstract of the 45th Annual Meeting of the Japanese Association of Neurology, May 2004, Tokyo) while another report showed that PD patients with normal MIBG scintigraphy have a higher incidence of mutations of the *parkin* gene (Dr. M. Yamamoto, abstract of the 45th Annual Meeting of the Japanese Association of Neurology, May 2004, Tokyo). These observations suggest that the decreased uptake of MIBG is not necessarily a finding invariably observed in patients with levodopa-responsive-Parkinsonism.

Orimo et al. reported markedly decreased tyrosine hydroxylase (TH)-immunoreactive nerve fibers in the heart of a patient with pathologically proven PD, whose

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cardiac uptake of MIBG had been found to be severely decreased 1 year before death [6]. Amino et al. reported that not only TH-immunoreactive but also neurofilament (NF)-immunoreactive nerve fibers were markedly decreased in heart tissues from patients with pathologically proven PD [7]. Recently, Orimo et al. examined heart tissues together with sympathetic ganglia from patients with pathologically proven PD, and concluded that although sympathetic ganglia were relatively preserved, TH-immunoreactive nerve fibers were markedly decreased in heart tissues [8].

Orimo's report is the only report describing an autopsy of a PD patient who had undergone MIBG scintigraphy in situ, because the examination is usually done in the very early clinical stage of the disorder. The purpose of this study was to examine in detail the neuropathological findings of the cardiac sympathetic nervous system in a patient with PD who was examined by MIBG scintigraphy 1 year prior to death.

2. Case report and methods

2.1. Case report

A 73-year-old right-handed man visited our outpatient clinic with chief complaints of progressive gait disturbance and bradykinesia. He had been well until 9 months before this visit, at which point he noticed slowness in walking and a tendency to fall backward. His gait disturbance and bradykinesia gradually deteriorated until he required help to rise from his bed. He had a past history of exposure to the atomic bomb in Hiroshima at age 19, at which time temporarily lost his hair. He also had an 11-year history of diabetes mellitus (DM) with excellent control using glibenclamide. On neurological examination, he showed mild rigidity in his neck and four extremities, severe bradykinesia and gait of short stride with loss of arm swing. His postural reflex was also impaired but resting tremor was absent. His deep tendon reflexes were preserved and no sensory disturbances were present and he did not have any symptoms of constipation, urinary disturbances or orthostatic hypotension.

The patient's fasting blood sugar was 106 mg/dl and his hemoglobin $A1_c$ was 6.0% (normal range: 4.3–5.8%). Magnetic resonance images of the brain were unremarkable except for mild cortical atrophy, and the electrocardiogram showed unremarkable results. The coefficient of variation of the R–R interval for the electrocardiogram was 1.03% (normal range: 1.27–3.69) but the head-up tilt test showed no evidence of orthostatic hypotension. Positron emission tomography (PET) studies showed reduced ^{18}F-fluorodopa uptake with mild laterality (right>left) and increased ^{11}C-*N*-methylspiperone uptake in the striatum with mild laterality (right<left), findings which were consistent with PD.

The patient received levodopa and experienced transient amelioration, but subsequently deteriorated into a wheelchair-bound state. At age 74, he had repeated hemorrhagic episodes from diverticulitis of the colon, subsequently followed by subacutely progressive dementia with a score by Mini-Mental Stage Examination of 3, one year and six months from the onset of Parkinsonism. He unexpectedly died of massive hemorrhage 5 months later. His clinical diagnosis was PD with dementia, following the "one year rule" of the Consensus Guidelines [9]. The total clinical course was 2 years.

2.2. MIBG myocardial scintigraphy

After the patient was in the supine position for 20 min, 111 MBq of ^{123}I-MIBG (Daiichi Radioisotope Laboratories Co, Tokyo, Japan) was intravenously injected. Planar imaging and single photon emission computed tomography were performed using a triple headed gamma camera (GCA9300A, Toshiba Co, Tokyo, Japan) after 15 min (early phase) and 3 h (late phase). Photopeak energy was centered at 159 keV with a 20% window and relative organ uptake of ^{123}I-MIBG was determined by setting the region of interest on the anterior planar image. Using average counts per pixel for the heart and mediastinum, the ratio of the uptake by the heart to that by the mediastinum was calculated.

2.3. Neuropathology

A postmortem examination was performed 18 h after death. The brain and spinal cord were fixed in 20% buffered formalin for two weeks and the appropriate areas were embedded in paraffin for routine morphological examinations. To study the cardiac sympathetic innervation in detail, the intermediolateral column at several levels of the thoracic spinal cord, the sympathetic trunk and ganglia, and the nerve plexus of the anterior wall of the left ventricle were carefully examined and compared with those of five age-matched controls.

Six micron-thick sections were stained with hematoxylin and eosin by the Klüver–Barrera method. Antibodies raised against Aβ (12B2, monoclonal, aa. 11–28, IBL, Maebashi, Japan); phosphorylated τ (ptau) (AT8, Innogenetics, Temse, Belgium); phosphorylated α-synuclein (psyn) (psyn#64, monoclonal, and Pser129, polyclonal, kind gifts from Dr T. Iwatsubo), phosphorylated neurofilament (SMI31, Sternberger Immunochemicals, Bethesda, MD); HLA-DR (CD68, Dako, Glostrup, Denmark); tyrosine hydroxylase (TH, polyclonal, Calbiochem, Parmstadt, Germany); and glial fibrillary acidic protein (GFAP, polyclonal, Dako, Glostrup, Denmark) were employed. The sections were visualized with a Ventana NX20 system as previously reported [10].

The control cases died of systemic disorders that did not affect the heart.

3. Results

3.1. MIBG myocardial scintigraphy

MIBG myocardial scintigraphy revealed that the uptake ratio of the heart to that of the mediastinum was 1.58 (normal mean of 2.76) during the early phase and 1.35 (normal mean of 3.45) during the late phase.

3.2. Neuropathology

The brain weighed 1250 g and the temporal lobe was slightly atrophic. Serial coronal slices of the brain showed mild dilatation of the lateral and third ventricles and serial axial sections revealed the loss of pigmentation in the substantia nigra and locus ceruleus. Histologically, neuronal loss and gliosis were present in the substantia nigra, locus ceruleus, and basal nucleus of Meynert. Lewy bodies (LBs) were present in the substantia nigra, locus ceruleus, dorsal vagal motor nucleus, raphe nucleus, hypothalamus, basal nucleus of Meynert, amygdala, anterior cingulate gyrus, transentorhinal region and second temporal gyrus, but not present in the frontal or parietal cortex. The LB score of this case was 4, following the Consensus Guidelines for DLB [9]. Senile plaques were absent and neurofibrillary tangles were only scattered in the transentorhinal cortex (Braak Stage I).

In the sympathetic nerves innervating the heart, LBs were present in the intermediolateral column of the thoracic spinal cord and sympathetic ganglia. In contrast, LBs were completely absent in the control subjects. Multiple levels of the intermediolateral column of the thoracic spinal cord were examined with anti-phosphorylated α-synuclein antibody (psyn). Scattered psyn-immunoreactive neuronal intracytoplasmic inclusions, threads and dots were present there. Immunohistochemistry with anti-psyn antibodies showed positive axons in the thoracic ventral roots, sympathetic trunk and cardiac plexus (Fig. 1D–F) and Nageotte's residual nodules were scattered among relatively preserved sympathetic ganglia (Fig. 1A). In the cardiac plexus, total loss of TH-immunoreactivity (Fig. 1H) compared with the normal control (Fig. 1G) and a marked decrease of axons (Fig. 1C) compared with the normal control (Fig. 1B) were evident. In contrast, the dorsal root ganglia and the sural nerve, including unmyelinated fibers, were well preserved, as shown by ultrastructural studies (data not shown). The heart itself did not show any valvular, coronary or myocardial change.

4. Discussion

This study found cardiac sympathetic denervation in a patient with PD, which was well correlated with severely decreased uptake in MIBG scintigraphy.

Previous studies demonstrated that neuronal degeneration with LBs occurs in broad areas of the sympathetic nervous system, including the sympathetic ganglia and the cardiac plexus, in patients with PD [11]. In the cardiac plexus, LBs and α-synuclein positive axons [12] or

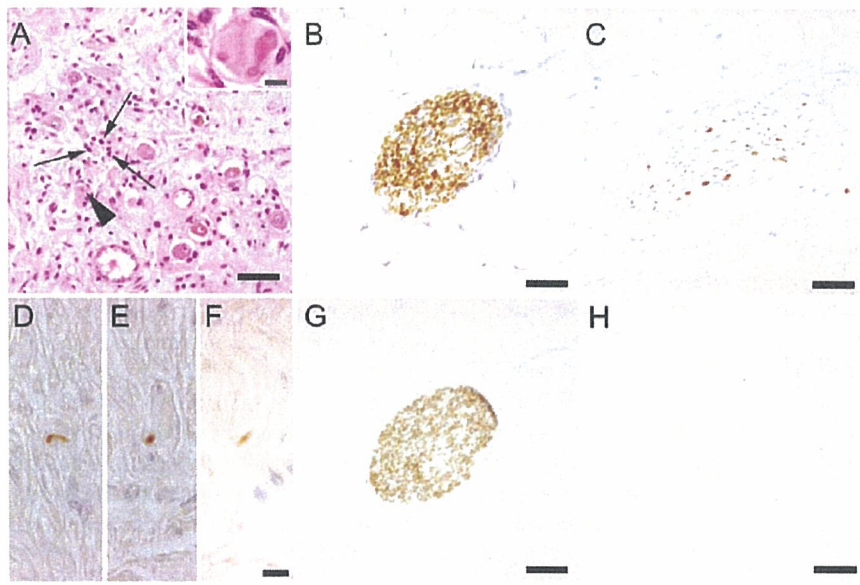

Fig. 1. Pathology of the sympathetic nervous system of a case of Parkinson disease A: a sympathetic ganglion showing a Nageotte's residual nodule (arrows) with Lewy bodies (LBs) (arrowhead) (hematoxylin and eosin staining, bar=50 μm). Inset: a typical LB in the sympathetic ganglion (bar=10 μm). B and C: unmyelinated fibers in the epicardial fatty tissue immunostained with anti-phosphorylated neurofilament antibody (SMI 31). Abundant axons from a control (B) and marked loss of axons from the case (C) (bar=50 μm). D–F: Lewy axons visualized by immunohistochemistry with anti-phosphorylated α-synuclein antibody (psyn#64) in the same fascicle as in section C (bar=10 μm). G and H: serial sections from section B (G) and C (H) immunoassayed with anti-tyrosine hydroxyls (TH) antibody. Abundant TH-immunoreactive fibers from the control (G) and total loss of immunoreactivity from the patient (H) (bar=50 μm).

markedly decreased TH-positive nerve fibers [7,8] were reported, which is consistent with our findings.

The present study found that the pathology of the sympathetic ganglia consisted of prominent α-synucleinopathy with a relatively preserved neuronal population. This was in sharp contrast with the severe axonal loss of sympathetic nerves in the cardiac muscle. Thus, LB-related α-synucleinopathy may cause distal axonopathy of the postganglionic sympathetic nerves.

It is difficult to exclude the possibility that the clinical history of DM may have made some contribution to the findings of MIBG scintigraphy and the pathology of the peripheral autonomic nervous system in this case, although the extremely low MIBG uptake and intact unmyelinated fibers in the sural nerve and dorsal root ganglia as well as pathologically unremarkable heart itself suggest that this possibility is not likely.

This study suggested that MIBG scintigraphy could be used to detect the presence of LB-related α-synucleinopathy in the cardiac sympathetic nervous system. Further prospective pathological studies on cardiac sympathetic innervation in PD or DLB patient who underwent MIBG scintigraphy should be carried out.

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References

[1] Orimo S, Ozawa E, Nakade S, Sugimoto T, Mizusawa H. ^{123}I-metaiodobenzylguanidine myocardial scintigraphy in Parkinson's disease. J Neurol Neurosurg Psychiatry 1999;67:189–94.

[2] Taki J, Nakajima K, Kwang EH, Matsunari I, Komai K, Yoshita M, et al. Peripheral sympathetic dysfunction in patients with Parkinson's disease without autonomic failure is heart selective and disease specific. Eur J Nucl Med 2000;27:566–73.

[3] Braune S, Reinhardt M, Schnitzer R, Riedel A, Lücking CH. Cardiac uptake of [^{123}I] MIBG separates Parkinson's disease from multiple system atrophy. Neurology 1999;53:1020–5.

[4] Yoshita M. Differentiation of idiopathic Parkinson's disease from striatonigral degeneration and progressive supranuclear palsy using iodine-123meta-iodobenzylguanidine myocardial scintigraphy. J Neurol Sci 1998;155:60–7.

[5] Yoshita M, Taki J, Yamada M. A clinical role for [^{123}I] MIBG myocardial scintigraphy in the distinction between dementia of Alzheimer's-type and dementia with Lewy bodies. J Neurol Neurosurg Psychiatry 2001;71:583–8.

[6] Orimo S, Ozawa E, Oka T, Nakade S, Tsuchiya K, Yoshimoto M, et al. Different histopathology accounting for a decrease in myocardial MIBG uptake in PD and MSA. Neurology 2001;57:1140–1.

[7] Amino T, Orimo S, Itoh Y, Takahashi A, Uchichara T, Mizusawa H. Profound cardiac sympathetic denervation occurs in Parkinson disease. Brain Pathol 2005;15:29–34.

[8] Orimo S, Amino T, Itoh Y, Takahashi A, Kojo T, Uchichara T, et al. Cardiac sympathetic denervation precedes neuronal loss in the sympathetic ganglia in Lewy body disease. Acta Neuropathol 2005; 109:583–8.

[9] McKeith I.G., Galasko D, Kosaka K, Perry EK, Dickson DW, Hansen LA, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996;47: 1113–24.

[10] Saito Y, Kawashima A, Ruberu NN, Fujiwara H, Koyama S, Sawabe M, et al. Accumulation of phosphorylated α-synuclein in aging human brain. J Neuropathol Exp Neurol 2003;62:644–54.

[11] Wakabayashi K, Takahashi H. Neuropathology of autonomic nervous system in Parkinson's disease. Eur Neurol 1997;38(Suppl 2):2–7.

[12] Iwanaga K, Wakabayashi K, Yoshimoto M, Tomita I, Satoh H, Takashima H, et al. Lewy body-type degeneration in cardiac plexus in Parkinson's and incidental Lewy body diseases. Neurology 1999;52: 1269–71.

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Task-Guided Selection of the Dual Neural Pathways for Reading

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Summary

The visual perception of words is known to activate the auditory representation of their spoken forms automatically. We examined the neural mechanism for this phonological activation using transcranial magnetic stimulation (TMS) with a masked priming paradigm. The stimulation sites (left superior temporal gyrus [L-STG] and inferior parietal lobe [L-IPL]), modality of targets (visual and auditory), and task (pronunciation and lexical decision) were manipulated independently. For both within- and cross-modal conditions, the repetition priming during pronunciation was eliminated when TMS was applied to the L-IPL, but not when applied to the L-STG, whereas the priming during lexical decision was eliminated when the L-STG, but not the L-IPL, was stimulated. The observed double dissociation suggests that the conscious task instruction modulates the stimulus-driven activation of the lateral temporal cortex for lexico-phonological activation and the inferior parietal cortex for spoken word production, and thereby engages a different neural network for generating the appropriate behavioral response.

Introduction

Behavioral studies have shown that printed words rapidly activate the representation of their spoken forms, even when the stimuli are perceived unconsciously (Frost, 2003). This phonological activation constitutes a mandatory and crucial component of visual word recognition, the dysfunction of which is known to correlate with difficulties in literacy acquisition (Paulesu et al., 2000; Shaywitz et al., 1998). The automatic nature of such print-to-sound translation during reading is mirrored by brain imaging data showing that the mere exposure to visual words induces widespread activation of the left perisylvian area, associated with phonology, beyond the functional demands of the task (Price et al., 1996).

Typically, the stimulus-driven response to pronounceable letter-strings extends beyond the left occipitotemporal cortex (associated with visual word perception), which responds approximately 150–200 ms after the stimulus onset (Cohen et al., 2000; Nobre et al., 1994; Tarkiainen et al., 1999), down to a middle part of the left superior temporal gyrus/sulcus (L-STG/STS) that is associated with the auditory representation of spoken words (Demonet et al., 1992; Howard et al., 1992; Price, 1998). More recent work, however, has shown that the distributed activation during reading is drastically reduced and localized to the left occipitotemporal region under the visual masking procedure where participants are unable to perceive written words consciously (Dehaene et al., 2001). This observation indicates that it is an open question what neural mechanisms mediate the connection between the occipitotemporal region and the auditory association cortex to achieve the feedforward activation of phonological code from letter-strings, because such automatic phonological computation is in itself an obligatory component of reading, which operates even under the subliminal perception of words.

Several other recent studies have further suggested that the interregional connectivity changes in the left-hemisphere language network as a function of task requirements (Bokde et al., 2001; Horwitz et al., 1998; Mechelli et al., 2005). Thus, the neural connections mediating the cross-modal translation from print to sound may be modulated by task instructions, presumably under the executive control of the prefrontal cortex (Fuster, 1997; Miller and Cohen, 2001). Critically, such top-down, strategic control may further exert a modulatory influence over the stimulus-driven neural activation triggered by unconsciously perceived words. In fact, past behavioral studies have shown that the voluntary allocation of attentional resources produces a measurable impact on the unconscious or autonomous processing of visual stimuli (Naccache et al., 2002; Ramachandran and Cobb, 1995).

Specifically, there are at least two candidate large-scale neural networks involved in automatic phonological computation during reading. First, brain imaging and neuropsychological studies both point to a central role of the left inferior parietal lobe (L-IPL) in orthography-to-phonology conversion during reading (Price, 1998). Here, the spreading activation from the occipitotemporal cortex is assumed to reach the L-IPL before activating the phonological representation associated with the L-STG region. Indeed, behavioral studies have suggested that the serial computation of phonology occurs in an earlier stage of reading prior to the lexical activation (Frost, 2003). Second, recent brain imaging studies have suggested the existence of polysensory neurons in a posterior part of the STS which respond to both visual and auditory stimuli (Beauchamp et al., 2004; Wright et al., 2003). As an anatomical substrate for multisensory

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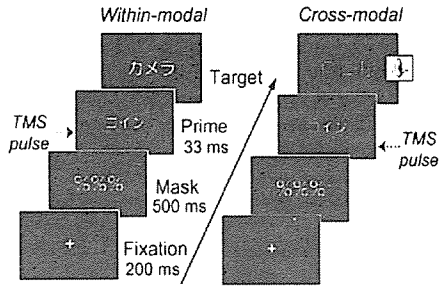

Figure 1. Sequence of Events Used for the Behavioral Paradigm

Each trial consisted of a masked prime followed either by a visual target ("within-modal trials") or by an auditory target synchronized with a string of three of four letter-like symbols (∈,∩,∋,∪) as a backward mask ("cross-modal trials"). The two types of trials were randomly intermixed in each TMS session, which lasted 480 s. For the LD task, participants determined whether or not the target stimuli represented a real Japanese word irrespective of their modality, while for the PRN task, they simply read aloud the visual targets or repeated the auditory targets. Experiment 1 was a behavioral experiment using the same event sequence without TMS. A post-hoc prime-visibility test confirmed that participants were unable to see the primes consciously (see Experimental Procedures). In Experiment 2, a single-pulse TMS synchronized to the fixation cross was applied to the target brain site in each trial.

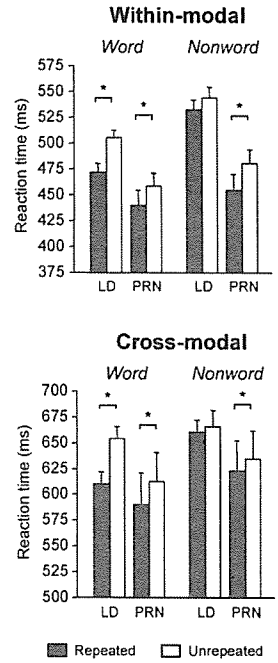

Figure 2. Mean Reaction Times, ±SEM, During the LD and PRN Tasks in Experiment 1

For the within-modality condition, the priming effect in LD was significant only for words, whereas the onset priming effect in PRN was significant both for words and nonwords (upper panel). This same pattern of priming was also obtained for the cross-modal condition (lower panel). (*) indicates a priming effect significant in the planned pairwise comparison (see Results).

integration (Beauchamp, 2005), this posterior lateral temporal sector might constitute an alternative neural pathway that can relay an input signal from the occipito-temporal area for cross-modal word processing.

Using event-related transcranial magnetic stimulation (TMS) with a masked repetition priming paradigm (Figure 1), we explored the degree of relative contribution of the L-IPL and L-STG/STS in unconscious phonological activation during reading under two different task instructions, lexical decision (LD) and pronunciation (PRN). The behavioral response to a written word is known to be facilitated after the brief, subliminal presentation of the same word, both for LD (Forster et al., 2003) and PRN (Kinoshita, 2003). We examined the TMS-induced interference in the two types of repetition priming while manipulating the stimulation site (L-IPL and L-STG), target modality (visual and auditory), and lexicality (word and nonword). The cross-modal manipulation of the priming condition has previously been used as a behavioral index of phonological activation during reading (Grainger et al., 2003; Kouider and Dupoux, 2001). Crucially, our experimental paradigm with visual masking enables minimization of potential strategic or attentional effects induced by the conscious perception of words and thereby targets bottom-up activations of the neural code. Combined with a real-time 3D navigation system, the present TMS experiment thus allowed inferences about the neural processes operating during the later stage of reading (>200 ms after the stimulus onset) at the cerebral loci targeted with high-resolution structural MRI.

Results

Experiment 1

We first conducted a behavioral experiment without TMS to confirm a reliable masked repetition priming effect under within- and cross-modal conditions under the two task instructions (see Figure 1). Participants made few errors during the two tasks (mean error rate = 3.48% for LD and 0.89% for PRN). Mean reaction times (RT) for each of the four priming conditions are presented in Figure 2. Planned pairwise comparison was made separately for words and nonwords using analysis of variance (ANOVA). For words, a significant priming effect was obtained under the LD task in the within-modal ($F[1,13] = 20.56$, $p < 0.001$) and cross-modal ($F[1,13] = 18.98$, $p < 0.001$) conditions. There was also a significant priming effect under the PRN task in the within-modal ($F[1,13] = 15.76$, $p < 0.005$) and cross-modal ($F[1,13] = 34.51$, $p < 0.001$) conditions. For nonwords, the priming effect in LD did not reach significance for the within-modal condition ($F[1,13] = 3.78$, $p = 0.07$) or for the cross-modal condition ($F[1,13] < 1$). For PRN, there was a robust "onset priming" effect (Kinoshita, 2003) for the within-modal condition ($F[1,13] = 31.13$, $p < 0.001$), while the same effect did not reach significance in the cross-modal condition ($F[1,13] = 3.01$, $p = 0.11$).

The same participants also performed a forced-choice test designed to evaluate the visibility of primes (80 trials). Each trial comprised the same sequence of masks and words as in the main experiment described above, except that the prime stimuli appeared as a left-right inverted mirror image of the original words with a probability of 50%. Participants were asked to determine whether or not prime words were flipped as accurately as possible without time pressure. The mean

accuracy of this forced-choice test was 50.59% for the within- and 48.27% for the cross-modal conditions, respectively. This accuracy level did not depart significantly from the chance-level trials, neither for the within-modal (t = 0.33, p > 0.5) nor for the cross-modal (t = −0.72, p = 0.48) ones, suggesting that participants were unable to perceive the masked primes consciously in both conditions.

Experiment 2

In this experiment, we examined the impact of TMS on the repetition priming effects observed in Experiment 1. Guided by the real-time MRI-based monitoring, we delivered a precisely targeted single-pulse TMS either to the L-IPL or L-STG during the execution of the LD and PRN tasks (Figures 3A and 3B). The overall spatial displacement of the stimulation points during the TMS session was ~8 mm for the L-IPL and ~11 mm for the L-STG across participants, respectively. The potential effect of coil motion on the stimulation intensity would be negligible for this range of displacement given the estimated spatial extent of the electric field generated by the TMS (see Experimental Procedures) and the approximate volume of the anatomical target sites (>10 x 10 x 10 mm^3 for both the IPL and STG, according to the stereotaxic atlas of Talairach and Tournoux [1988]).

Participants achieved a high level of accuracy for both tasks during the TMS session (mean error rate = 7.04% for LD and 3.39% for PRN). Mean RTs with respect to the lexicality and modality are presented in Figures 3C and 3D. An omnibus ANOVA for the RTs revealed significant main effects of lexicality and modality (p < 0.001 for both), suggesting that participants responded more quickly to (1) words than nonwords and to (2) visual targets over auditory targets, respectively. In the following analysis, the effects of repetition priming, task type, and stimulation site were examined separately for words and nonwords with respect to their modality.

For words, there was a significant priming effect under the within-modal condition (F[1,15] = 18.14, p < 0.005), whereas the effects of task and site did not reach significance (both Fs < 1). Crucially, there was a significant three-way interaction between these factors (F[1,15] = 9.82, p < 0.01). None of the other interactions were significant (p > 0.1). Under the cross-modal condition, there was also a significant priming effect (F[1,15] = 8.64, p < 0.05), while the effects of task and site were both nonsignificant (p > 0.1). The three-way interaction between these factors was marginally significant (F[1,15] = 4.31, p = 0.06). No other interactions reached significance (p > 0.1).

For nonwords, there was a significant effect of the within-modal priming (F[1,15] = 12.61, p < 0.005). The effect of task type was marginally significant (F[1,15] = 4.42, p = 0.05), suggesting that the response latency was longer for LD than for PRN when targets were presented in the visual modality. All of the other main effects and interactions were nonsignificant (p > 0.1). For the cross-modal condition, none of the main effects and interactions reached significance (all Fs < 1).

Planned Comparisons for the Priming Effect

Planned comparisons for words revealed a significant priming effect in the LD task when the stimulation was applied to the L-IPL, both for the within-modal (F[1,15] = 33.98, p < 0.001) and cross-modal (F[1,15] = 4.49, p = 0.05) conditions. However, the priming effect in the PRN task did not reach significance when the same region was stimulated; this was also the case for the within-modal (F[1,15]< 1) and cross-modal conditions (F[1,15] < 1). In contrast, the priming for LD was eliminated when the L-STG was stimulated, both for the within-modal (F[1,15] = 1.01, p = 0.33) and

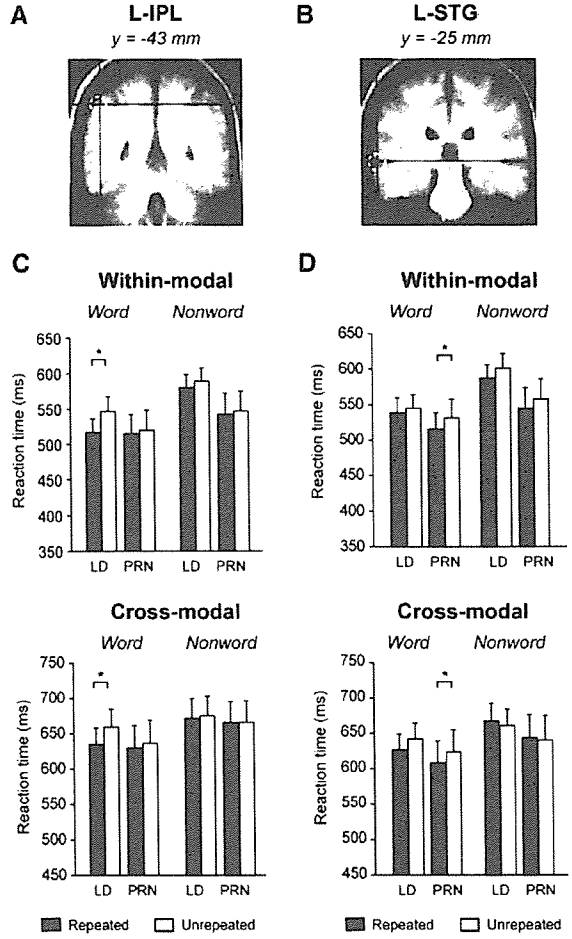

Figure 3. Locations of the Anatomical Targets and Behavioral Effects of the TMS

Each target site is rendered on a structural MRI averaged across participants. The dotted circles represent the approximate spatial extent of the induced electric field around the maximum stimulation point. (A) The L-IPL target. The mean coordinates (± SD) of the stimulation points were x = −48 ± 6 mm, y = −43 ± 7 mm, z = 48 ± 9 mm according to the stereotaxic atlas of Talairach and Tournoux (1988). (B) The L-STG target. The mean coordinates (± SD) of the stimulation points were x = −62 ± 4 mm, y = −25 ± 7 mm, z = 2 ± 8 mm. (C) Mean reaction times (±SEM) for the LD and PRN tasks during the stimulation of L-IPL. For words, the priming effect in PRN was eliminated irrespective of the target modality when the L-IPL was stimulated. In contrast, the priming for LD remained significant for both the within- and cross-modal conditions. For nonwords, the onset priming effect in PRN was also disrupted by the TMS. (D) Mean reaction times (±SEM) for the LD and PRN tasks during the stimulation of L-STG. For words, the priming effect in LD was eliminated regardless of the target modality when the L-STG was stimulated. In contrast, the onset priming for PRN remained significant across the within- and cross-modal conditions. For nonwords, the priming effect in PRN was also affected by the TMS.

cross-modal ($F[1,15] = 2.74$, $p = 0.12$) conditions. The priming effect in PRN remained significant when the same region was stimulated in the within-modal condition ($F[1,15] = 10.47$, $p < 0.01$) and in the cross-modal condition ($F[1,15] = 9.14$, $p < 0.01$).

On the other hand, nonwords produced no significant priming effect when the L-STG was stimulated during the LD task in either within- ($F[1,15] = 1.99$, $p = 0.18$) or cross-modal ($F[1,15] < 1$) conditions. There was a significant effect of priming for PRN in the within-modal condition ($F[1,15] = 8.13$, $p < 0.05$). No significant priming was obtained for the PRN task in the cross-modal condition ($F[1,15] < 1$). When the L-IPL was stimulated, no priming effect was found for either of the two tasks irrespective of the modality ($p > 0.08$ for all Fs).

Combined Analysis of the Behavioral and TMS Experiments

For each of the planned comparisons reported above, we further examined the effect of TMS on the magnitude of the priming effect in a joint analysis of the two datasets, one from Experiment 1 without TMS and the other from Experiment 2. For each stimulation site, the mean RTs were analyzed as a 2 × 2 factorial arrangement with the repetition priming as a within-participant factor (identical versus unrelated) and the stimulation as a between-group factor (with TMS versus without TMS).

For words, the stimulation of the STG interfered with the priming in LD for both the within-modal and cross-modal conditions (interaction, $F[1,28] = 9.42$, $p < 0.01$ and $F[1,28] = 4.92$, $p < 0.05$, respectively). The stimulation of the same site did not produce a significant impact on the magnitude of priming in PRN ($F < 1$ for both within- and cross-modal trials). For IPL, the priming × stimulation interaction was significant for PRN in the cross-modal condition ($F[1,28] = 4.22$, $p < 0.05$) and marginally significant in the within-modal condition ($F[1,28] = 3.28$, $p = 0.08$). However, stimulation of the same site produced no significant effect on the priming for LD ($F[1,28] < 1$ and $F[1,28] = 2.32$, $p = 0.14$ for within- and cross-modal conditions, respectively). For nonwords, stimulation of the IPL interacted with the priming in PRN only for the within-modal condition ($F[1,28] = 10.55$, $p < 0.005$). This same priming effect was also reduced by the stimulation of the STG ($F[1,28] = 4.44$, $p < 0.05$). None of the other interactions reached statistical significance.

Discussion

By the combined use of masked priming and TMS, the present study focused on the neural substrate of the bottom-up phonological activation during reading, while eliminating the influence of postperceptual processes, i.e., the distant global broadcasting that may occur when words cross the threshold of consciousness (Dehaene et al., 2004; Lamme, 2003; Lamme et al., 2002). Behavioral results from Experiment 1 show a significant masked priming effect across the two tasks, not only when primes and targets were presented in the same modality, but also when they were presented in differing modalities. It is important to note that each of these priming effects has a distinct cognitive locus involved in visual word processing. For the LD task, the within-modal priming is thought to reflect mainly a lexical-level activation, while the earlier, orthographic contribution is relatively smaller and no greater than 10 ms (Forster et al., 2003). For PRN, the within-modal priming has been known to occur both for words and nonwords and has been attributed to either the orthography-to-phonology computation (Forster and Davis, 1991) or further downstream toward the speech planning process (Kinoshita, 2003). This latter process could be also involved in the cross-modal priming in PRN as a final common pathway for spoken output. On the other hand, the cross-modal priming in LD has been attributed to the activation of phonological codes through visual input (Kouider and Dupoux, 2001).

Previous behavioral studies with alphabetic readers have reported conflicting results about whether or not subliminal primes indeed facilitate the participants' responses during LD. For instance, Grainger et al. (2003) demonstrated significant masked cross-modal priming with the prime duration of 57 ms, while another study failed to obtain a significant priming effect at the same or even longer prime duration (Kouider and Dupoux, 2001). The inconsistency across studies has been attributed to several different factors in the experimental setting, such as the differences in prime duration, interstimulus interval between primes and targets, and backward masking (Grainger et al., 2003). Although the present design does not allow us to determine the relative contribution of these factors in the mechanics of masked cross-modal priming, our results provide additional evidence showing that the response facilitation across different modalities does occur even at a very brief prime duration (33 ms). The robust effect obtained here could be partially attributed to a possible advantage of the syllabic script in phonological conversion, i.e., a higher degree of transparency between orthography and phonology which would allow a more rapid computation of phonology from print.

Our TMS results revealed that the within- and cross-modal priming conditions for words both produced a significant triple interaction between repetition, task type, and stimulation site. Coupled with the joint analysis of the two datasets, this finding suggests a net component of regional-specific interference by TMS which cannot be attributed to a nonspecific, global cortical inhibition associated with the stimulation. That is, the stimulation of the L-STG and the L-IPL each produced a differential impact on the within- and cross-modal priming according to the task instruction and lexical property of the stimuli.

Specifically, the stimulation of the L-IPL eliminated the masked onset priming effect for words and nonwords, whereas these same effects were not affected by the stimulation of the L-STG. This finding is consistent with past neuropsychological and brain imaging studies suggesting that the L-IPL plays a leading role in phonological computation from orthography (Friedmann et al., 1993; Price, 1998). Importantly, however, our results suggest that when top-down effects by attentional or strategic control are eliminated by visual masking, this neural pathway for letter-to-sound mapping opens only when participants are engaged in the cognitive set for word production. Moreover, the serial phonological conversion procedure subserved by the IPL runs nonspecifically to the pronounceable word-like stimuli

irrespective of whether they represent a real word or not, since the effect size for the onset priming in PRN did not differ between words (=20 ms) and nonwords (=24 ms) in the within-modal condition (Experiment 1). In fact, several past studies have consistently suggested that it is the left inferior prefrontal cortex, rather than the L-IPL, that is sensitive to the lexicality and regularity of orthography of written words (Fiebach et al., 2002; Fiez et al., 1999; Herbster et al., 1997; Rumsey et al., 1997).

On the other hand, we also found that the stimulation of the L-STG, but not that of the L-IPL, reduced the masked priming effect of words in the LD task, for both the within- and cross-modal conditions. This finding suggests that the lexical activation and phonological processing subsequent to it are mediated by the ventral neural pathway extending from higher-order visual cortex to the L-STG, of which the L-IPL does not constitute a pivotal component. Since participants were unaware of the identity of primes as confirmed by the prime visibility test, this interpretation in turn implies that the unconscious perception of visual words leads to the activation of their spoken forms without the implicit mobilization of the neural mechanism for speech production.

Functional brain imaging studies converge to suggest that the ventro-lateral temporal cortex comprises distinct subregions distributed between the occipitotemporal cortex for visual object recognition and the middle part of the L-STG associated with the phonological processing of spoken words. That is, a posterior sector of the STS responding to both visual and auditory stimuli is currently thought to represent a human homolog of the polysensory neurons in the macaque STS (Beauchamp et al., 2004; Wright et al., 2003). A functional MRI study by Cohen et al. (2004) has also shown that a middle portion of the left middle temporal gyrus adjacent to the polysensory STS is associated with the abstract, lexical-level representation of words and termed it a "lateral inferotemporal multimodal area."

More generally, a current neural model of reading proposes an anterior-to-posterior processing stream of the left occipitotemporal cortex for a progressive abstraction process in visual word recognition (Dehaene et al., 2004), which conforms to a global framework of the cortical object recognition mechanism whereby raw visual features of stimuli are transformed increasingly from perceptual to conceptual (Henson et al., 2000; Lerner et al., 2001; van Turennout et al., 2000). Indeed, a recent study using diffusion tensor tractography revealed white matter fiber bundles connecting the extrastriate cortex and lateral temporal cortex (Catani et al., 2003). Taken together with the present finding, this suggests that the ventral pathway linking the occipitotemporal cortex and L-STG via the intermediate polysensory area is "turned on" and receptive of unconsciously perceived words only when observers are in the cognitive set for word recognition. This latter neural mechanism is sensitive to the lexicality of stimuli and works for the lexical- and subsequent phonological-level activation independently of the L-IPL, which operates in the phonological computation of both words and nonwords.

Importantly, our results provide direct evidence for the proposal that even the unconscious processing of incoming stimuli operates under the strong influence

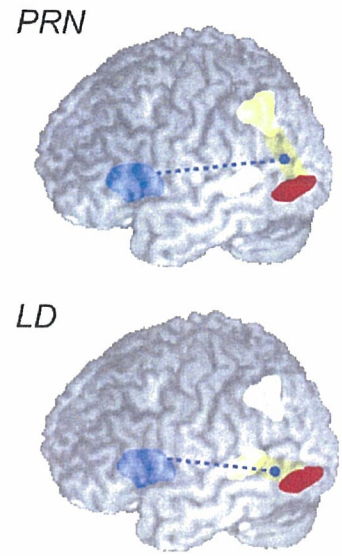

Figure 4. A Possible Schematic of the Neural Connections for Processing Subliminal Words in the Two Different Task Contexts

(Top) The lateral prefrontal cortex (blue) sends a biasing influence over the neural pathway from the left fusiform cortex (red) via the IPL (yellow) to prepare the spoken response when participants are in the cognitive set for pronunciation. This unconscious word processing proceeds without the contribution of the L-STG (gray). (Bottom) In lexical decision, the lateral prefrontal cortex (blue) selects the ventral processing stream to produce lexical- and phonological-level activation by directing the neural signal from the fusiform area to the L-STG (yellow). The L-IPL (gray) is not involved in the unconscious word processing during this task operation.

of the conscious task instructions (Dehaene et al., 1998). The finding that TMS produced a differentiated impact on the subliminal priming effect as a function of the stimulation site and task requirement is likely to reflect the task-dependent dynamic change of connection strength within the brain network involved in reading. Indeed, a recent fMRI study has shown that the effective connectivity between the left inferior frontal gyrus and task-specific temporo-parietal regions is modulated by the nature of behavioral tasks during conscious language operations (Bitan et al., 2005). The selection of these task-relevant neural pathways in posterior brain regions is thought to be achieved by the biasing influence exerted by the prefrontal cortex (Miller and Cohen, 2001). As illustrated in Figure 4, our results further suggest that such top-down, strategic control modulates the bottom-up neural activation produced by unconsciously perceived words to set up a different neural circuit for generating the intended behavioral response.

In addition, it is noteworthy that the well-known dual-route model of reading posits at least two different processing components, i.e., the lexical or whole-word system available for real words and the phonological assembly which plays an important role during reading of nonwords and low-frequency words (Coltheart et al., 2001). Under this framework, it might be said that these two components each are subserved by the ventral temporal pathway linking the occipitotemporal cortex and the L-STG and the more dorsal, parieto-temporal circuitry involving the L-IPL. This proposal seems partially