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Cerebral blood flow and metabolic changes produced by repetitive magnetic brain stimulation

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Abstract We evaluated cerebral variation in oxyhemoglobin, deoxyhemoglobin, and cytochrome oxidase before and after transcranial magnetic and electrical stimulation in ten healthy volunteers using near-infrared spectroscopy. Immediately after magnetic but not after electric stimulation a significant increase in oxyhemoglobin and a decrease in cytochrome oxidase were observed ($P < 0.05$). Our data suggest that repetitive transcranial magnetic stimulation induces metabolic activation of the cerebral cortex together with an increase in cerebral blood flow.

Key words Transcranial stimulation · Near-infrared spectroscopy · Brain metabolism

Introduction

Since its first descriptions in 1980s, transcranial electrical stimulation and transcranial magnetic stimulation over the motor cortex have become widely used tools for evaluating the physiology of cortical areas [20]. It is generally believed that electrical stimulation activates the corticospinal system directly by depolarizing the axons, and that magnetic stimulation activates the corticospinal system by stimulating the axons of cortico-cortical interneurons synapsing on the corticospinal neuron [5, 6]. No effects of transcranial magnetic stimulation on the electroencephalogram, cognitive function, or serum hormone levels have been reported [12, 13]. The hemodynamic effects of repetitive transcranial magnetic stimulation (rTMS) of the human brain have been investigated by several authors who report an increase in cerebral blood flow (CBF) [7, 19, 11, 23].

The idea that regional CBF could reflect neuronal activity began with the experiment of Roy et al. [21]. Investigations of the metabolism of the human brain were hampered by the fact that the brain within the skull is not easily accessible for performing experimental procedures, that the nervous tissue is composed of many different cell populations, and that the more recent techniques for studying the metabolic state of brain, such as single photon emission computed tomography, functional magnetic resonance, and positron-emission tomography, are not suitable in many situations.

Near-infrared spectroscopy (NIRS) is a noninvasive, repeatable method that allows regional assessment of the oxygenation state of hemoglobin in tissue and the redox state of cytochrome oxidase (cyt aa3), which reflects the

overall activity of oxidative metabolism in the cells. NIRS measures cerebral concentrations of oxyhemoglobin (HbO_2), deoxyhemoglobin (HHb), and cytochrome aa3 by observing the absorption of near-infrared light. These measures are expressed as micromoles per liter variation from baseline. HbO_2 and HHb have different absorption spectra in the visible and near infrared regions, allowing spectroscopy techniques to be used to provide an indicator of blood oxygenation and hence oxygen delivery, and thus they can be considered a good index of CBF variations.

Cytochrome aa3 is an enzyme located in the mitochondrial membrane and plays a vital role in energy generation. Cytochrome aa3 exists in two states: oxidized, when it loses an electron, and reduced, when it gains an electron. The two different states exhibit different optical absorption spectra, allowing it to be examined spectroscopically. Monitoring the redox state of cyt aa3 provides direct and immediate information on the intracellular utilization of oxygen, and this reflects the metabolic activation of the cell. NIRS is able to detect only the oxidized form of cyt aa3, and for this reason the change in the redox state of cyt aa3 from oxidized to reduced form is evaluated by NIRS as a decrease in the oxidized cyt aa3 concentration (for a review see [2]).

NIRS can provide metabolic information without invasive sampling and detects even small changes in cerebral hemodynamic response to functional stimulation [15]. Several reports describe the ability of NIRS to measure the hemodynamic changes related to the human brain activities such as motor, visual, auditory, cognitive, and language functions [3, 11, 14–16, 24]. Moreover, NIRS has been used to monitor CBF modification in physiological and pathological conditions, with good results both in adults and in children [8, 9, 26].

The purpose of the present study was to evaluate CBF changes induced by rTMS and by repetitive transcranial electric stimulation (rTES) and their relationships with metabolic activation of the tissue. We measured the changes in brain oxygen availability and consumption before and after repetitive transcranial stimulation using NIRS.

Methods

Ten healthy volunteers (five men, five women) aged 24–38 years (mean 28) participated in the study. All subjects were right-handed, with normal and constant arterial pressure and heart rate. The subjects lay on a couch in a warm and semidarkened room, awake, and were instructed to try to think of nothing. Subjects had their eyes closed during the whole experiment. The project was approved by the local ethics committee, and all the subjects gave informed consent. Magnetic stimulation was performed using a Magstim 200 (Novametrix, UK). Thirty stimuli (100% of the maximal stimulator output; 0.25 Hz) were delivered through a figure-of-eight coil over the NIRS probe. Electrical stimulation was performed using a Digitimer D 180A stimulator with a 50-μs time constant; the cathode was located 10 cm in front of the vertex and the anode 7 cm laterally (anodal stimulation). Thirty stimuli (30% of the maximal stimulator output; 0.25 Hz) were used. Both magnetic and electrical stimulation were above motor threshold for evoking muscle twitch when delivered over the motor cortex. A four-wavelength NIRS unit (Critikon Cerebral Research Monitor 2001) was used to quantify the differences in light assorbance before and after rTMS/rTES. The Critikon 2001 research monitor uses a two-channel system to delete the skull and scalp contribution, so that it monitors the concentration of chromophores from a volume of tissue below the superficial extracerebral tissue surface.

The probe of the NIRS unit was placed on the anterior right frontal region of the scalp; the center of the probe corresponded approximately to the Fp2 position of the international electroencephalographic 10–20 system. In four subjects the probe of the NIRS unit was also placed over the right motor area. In two subjects both rTMS and rTES were performed over the anterior right frontal region.

The NIRS examination was performed for at least 10 min before the stimulation and for 5 min following the stimulation. The experimental procedure was performed once for each subject. During the stimulation the NIRS was interrupted to avoid artifacts. To ensure that no modification in evaluated parameters could be due to the effects of rTMS/rTES on the NIRS probe, the latter was placed over an inert material, and the effects of both types of repetitive stimulation were evaluated.

For the analysis of data we used the average of 10 min recording before stimulation and the first 5 min following it. The sample rate was one every second. Difference in mean values before and after the stimulation were compared by the Wilcoxon test.

Results

Our findings are summarized in Table 1. A statistically significant increase in HbO_2 and a decrease in cyt aa3

Table 1 Mean HbO_2, HHB and Cyt aa3 (μM) of difference from the starting baseline considered as zero before and after transcranial stimulation

	HbO_2		HHb		Cyt aa3	
	Magnetic	Electric anodal	Magnetic	Electric anodal	Magnetic	Electric anodal
Right frontal region[a]						
Control	–0.2 ± 2.2	–0.02 ± 1.2	–1.07 ± 2.2	–0.50 ± 1.1	–0.63 ± 1.3	–0.2 ± 0.7
After stimulation	4.14 ± 3.5	–0.02 ± 2.0	–0.12 ± 2.2	–0.55 ± 1.0	–2.24 ± 1.7	–0.15 ± 1.0
P	< 0.05	> 0.05	> 0.05	> 0.05	< 0.05	> 0.05
Right motor area[b]						
Control	–0.82 ± 0.9	np	–0.27 ± 1.4	np	–0.01 ± 0.4	np
After stimulation	6.77 ± 4.1	np	–3.8 ± 3.9	np	–1.52 ± 0.3	np
P	< 0.05		> 0.05		< 0.05	

[a] Magnetic stimulation, ten subjects; anodal stimulation, two subjects
[b] Magnetic stimulation, four subjects

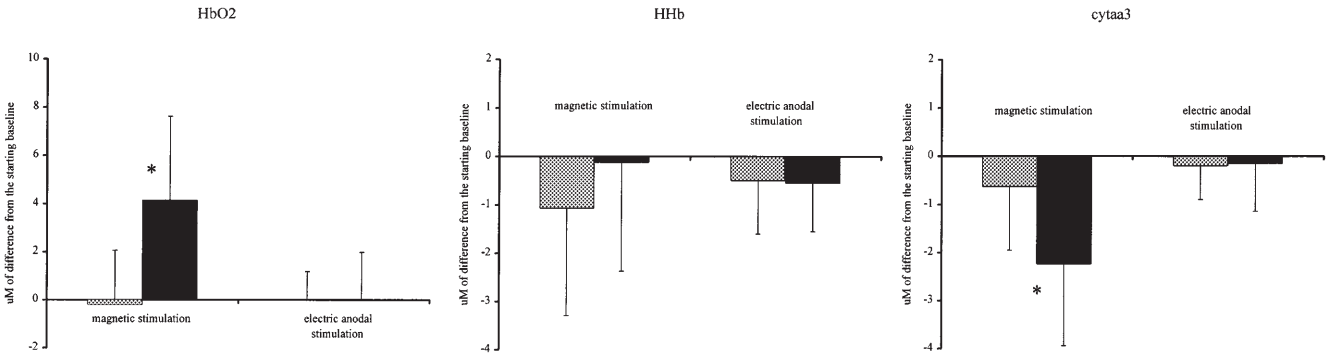

Fig. 1 Mean HbO_2, HHb, and cyt aa3 (μM) of difference from the starting baseline considered as zero before and after transcranial stimulation. A statistically significant increase in HbO_2 and a decrease in cyt aa3 concentrations were observed after rTMS of both the anterior right frontal region and the right motor area (*$P < 0.05$). There was no statistically significant modification in HHb concentration ($P > 0.05$)

concentrations were observed after rTMS of both the anterior right frontal region and the right motor area ($P < 0.05$). There was no statistically significant modification in HHb concentration ($P > 0.05$; Figs. 1, 2). On the other hand, neither transcranial electrical stimulation, acoustic stimuli (acoustic artifact of the coil during discharge), nor voluntary contraction of the left small hand muscles led to a significant increase in HbO_2 or a significant decrease in cyt aa3 concentrations ($P > 0.05$). NIRS measures evaluated after rTMS/rTES over an inert material showed no change.

Discussion

These results provide direct evidence that induced metabolic activation of the brain differs substantially depending on whether electrical or magnetic stimulation is used.

At present there is no full understanding of the site at which transcranial (electric or magnetic) stimulation of the motor cortex activates the corticospinal system in man. The most popular view at present is the "D- and I-wave" hypothesis first proposed by Day et al. [4] on the basis of single motor unit studies in the hand. This hypothesis has recently been supported by our group on the basis of direct recording of descending volleys at cervical level [6]. This view states that transcranial electrical stimulation activates corticospinal fibers directly near the cell body of the corticospinal neuron at a point, such as the bend, where the axons leave the gray matter in their course towards the internal capsule [1]. In contrast, transcranial magnetic stimulation tends to activate corticospinal neurons indirectly via excitatory synaptic inputs. Following Patton and Amassian [17], these are said to produce D- and I-wave volleys in the pyramidal tract.

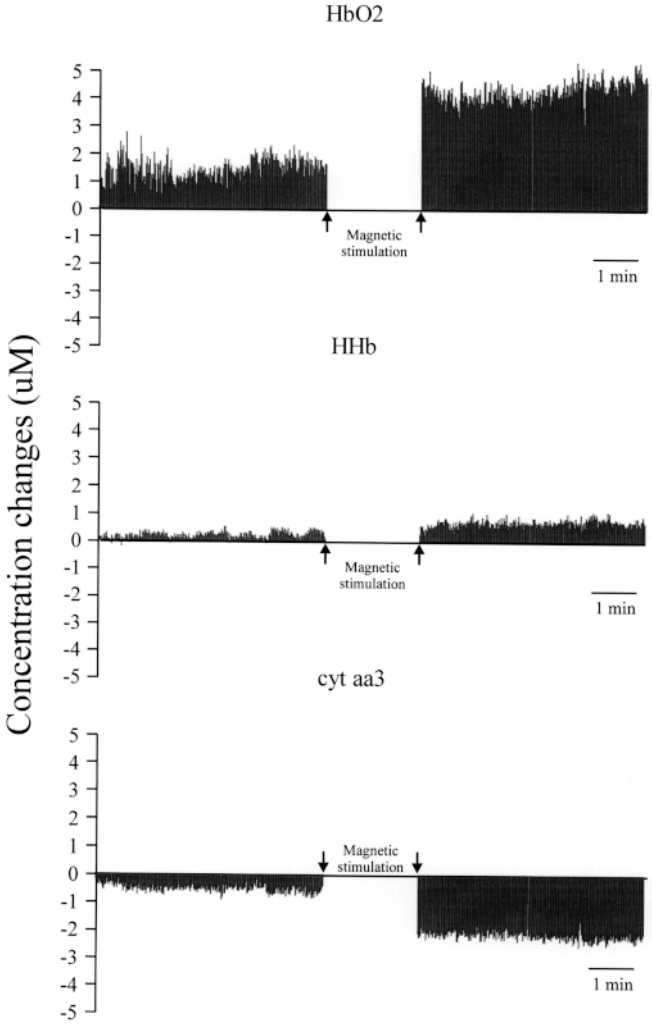

Fig. 2 Effect of magnetic stimulation on NIRS parameters in one of the subjects. HbO_2, HHb, and cyt aa3 (μM) concentration changes. NIRS probe was placed over the right anterior frontal region, and the center of the probe corresponded approximately to the Fp2 position of the international electroencephalographic 10–20 system, and magnetic stimuli were delivered over the NIRS probe. *Arrows* start and the end of magnetic stimulation. An increase in HbO_2, a decrease in cyt aa3, and a slight increase HHb were observed after rTMS

Immediately after brain rTMS a local increase in HbO_2 and a decrease in cyt aa3 concentrations are the typical findings. HbO_2 could be considered as a nondiffusible tracer and, according to the Fick principle, the increase in HbO_2 concentration could be considered an index of increased CBF. This finding has indeed been reported by other authors [7, 19, 22, 23]. A decrease in cyt aa3 means a transformation from the oxidized state of cytochrome to the reduced state, and this provides a direct and immediate information on the increased intracellular utilization of oxygen. Increased consumption of oxygen indicates metabolic activation of the brain. The method used does not provide information about a temporal correlation between CBF changes and the metabolic activation because of the long sample rate of the NIRS (1 s) or about the duration of the activating phenomena because after the rTMS and rTES the NIRS is resettled to a new baseline. The human brain depends virtually completely on glucose for energy metabolism, and most of this energy comes from mitochondria. Glucose utilization reflects neuronal activity, and most nerve cells are not only exclusively dependent on glucose but also lack the ability to store glycogen. This is why nervous tissue when activated requires increased oxygen and glucose availability. The increased use is supported by an increased CBF. Positron-emission tomography and single photon emission computed tomography allow the monitoring of the increased consumption of glucose while transcranial Doppler monitors CBF variation. NIRS allows a contemporary evaluation of both parameters. Our data suggest that only synaptic and cellular activation which is produced mainly by transcranial magnetic stimulation can significantly increase the metabolic requirements that are supplied by the increased CBF. This is in accordance with the presynaptic origin of the I waves produced by magnetic stimulation [5, 6, 20]. On the other hand, rTES, acting at axon level in the white matter [6, 17, 20], fails to produce a metabolic activation of the underlying tissue and consequently any changes in CBF.

Magnetic transcranial stimulation over the motor cortex produced a greater increase in HbO_2 concentration. Previous studies have reported that cerebral activation and regional CBF increase induced by dynamic hand movement can be blocked by regional anesthesia of the working arm, suggesting that such increases in CBF depend at least in part on afferent input from the working muscles [10, 18, 25]. Stimulation of the motor cortex produces large twitches of the contralateral body muscles, and the afferent input from the moving muscles could contribute to the observed increase in CBF. However, neither magnetic nor electric stimulation of the anterior region of the frontal cortex produces any twitch of the contralateral body muscles. Therefore the observed changes after rTMS cannot be due to sensory input but must originate from the transynaptic activation of the cortical cells. Further studies are needed to better define the relationship between the metabolism of activated cells and the increased CBF supplying it.

References

1. Amassian VE, Eberle LP, Maccabee PJ, Cracco RQ (1992) Modelling magnetic coil excitation of human cerebral cortex with a peripheral nerve immersed in a brain shaped volume conductor: the significance of fiber bending in excitation. Electroencephalogr Clin Neurophysiol 85:291–301
2. Chance B (1991) Optical method. Annu Rev Biophys Chem 20:1–28
3. Chance B, Zhuang Z, Unah C, Alter C, Lipton L (1993) Cognition-activated low-frequency modulation of light absorption in human brain. Proc Natl Acad Sci USA 90:3770–3774
4. Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989) Electric magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol (Lond) 412:449–473
5. Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC (1998) Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol (Lond) 508:625–633
6. Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, Rothwell JC (1998) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol 109: 397–401
7. Dressler D, Voth E, Feldmann M, Benecke R (1990) Safety aspects of transcranial brain stimulation in man tested by single photon emission-computed tomography. Neurosci Lett 119: 153–155
8. Edwards AD, Richardson C, Van der Zee P, Elwell JS, Wyatt JS, Cope M, Delpy DT, Reynolds EOR (1993) Measurement of hemoglobin flow and blood flow by near-infrared spectroscopy. J Appl Physiol 75:1884–1889
9. Elwell CE, Owen-Reece, Cope M, Wyatt JS, Edwards AD, Delpy DT, Reynolds EOR (1993) Measurement of adult cerebral haemodynamics using near infrared spectrosopy. Acta Neurochir (Wien) 59 [Suppl]:74–80
10. Friedman DB, Friberg L, Payne G, Mitchell JH, Secher NH (1992) Effects of axillary blockade on regional cerebral blood flow during dynamic hand movement. J Appl Physiol 73:2120–2125
11. Hoshi Y, Hirotaka O, Watanabe Y, Andersonn J, Bergstrom M, Lilja A, Langstom B, Tamura M (1994) Nonsynchronous behavior of neuronal activity, oxidative metabolism and blood supply during mental tasks in man. Neurosci Lett 172:129–133

12. Krain L, Kimura J, Yamada T, Cadwell J, Sakamaki S (1989) Consequences of cortical magnetoelectric stimulation. In: Chokroverty S (ed) Magnetic stimulation in clinical neurophysiology. Butterworths, Boston
13. Levy WJ, Oro J, Tucker D, Haghighi S (1989) Safety studies of electrical and magnetic stimulation for the production of motor evoked potentials. In: Chokroverty S (ed) Magnetic stimulation in clinical neurophysiology. Butterworths, Boston
14. Nakajima T, Fujita M, Watanabe H, Kuwabara T, Takeo K, Iwamoto S, Shimizu S, Kohono K, Naruse S, Yuasa T (1994) Functional mapping of human visual system with near-infrared spectroscopy and BOLD functional MRI. In: Proceedings of the Society of Magnetic Resonance, San Francisco, p 687
15. Obrig H, Hirth C, Junge-Hulsing JG, Doge C, Wolf T, Dirnagl U, Villringer A (1996) Cerebral oxygenation changes in response to motor stimulation. J Appl Physiol 81 : 1174–1183
16. Okada F, Tokumitsu Y, Hoshi Y, Tamura M (1993) Gender and handedness-related differences of forebrain oxygenation and hemodynamics. Brain Res 601 : 337–342
17. Patton HD, Amassian VE (1954) Single- and multiple-unit analysis of cortical stage of pyramidal tract activation. J Neurophysiol 17 : 345–363
18. Roland PE, Larsen B (1976) Focal increase of cerebral blood flow during stereognostic testing in man. Arch Neurol 33 : 53–56
19. Rossini PM, Silvestrini M, Zarola F, Caramia MD, Mariorenzi R, Traversa R, Martino G, Baggio G, Bolcino G (1990) Effects of non-invasive magnetic brain stimulation on transcranial Doppler and computerized EEG. Electroencephalogr Clin Neurophysiol 75 : S 130
20. Rothwell JC (1997) Techniques and mechanism of action of transcranial stimulation of the human motor cortex. J Neurosci Methods 74 : 113–122
21. Roy CS, Sherrington CS (1890) On the regulation of blood supply of the brain. J Physiol 11 : 85–108
22. Sander D, Meyer BU, Roricht S, Klingelhofer J (1995) Effect of hemisphere-selective repetitive magnetic brain stimulation on middle cerebral artery blood flow velocity. Electroencephalogr Clin Neurophysiol 97 : 43–48
23. Shafran B, Maccabee PJ, Amassian VE, Cracco RQ, Strashun A, Vaquer R, Singman R (1989) Spectamine imaging of cerebral blood flow during voluntary movement and focal stimulation of motor cortex with magnetic coil. Soc Neurosci Abstr 15 : 789
24. Villringer A, Planck J, Hock C, Schleinkofer L, Dirnagl U (1993) Near infrared spectroscopy (NIRS) a new tool to study hemodinamic changes during activation of brain function in human adults. Neurosci Lett 154 : 101–104
25. Williamson JW, Friedman DB, Mitchell JH, Secher NH, Friberg L (1996) Mechanisms regulating regional cerebral activation during dynamic handgrip in humans. J Appl Physiol 81 : 1884–1890
26. Wyatt JS (1994) Noninvasive assessment of cerebral oxidative metabolism in the human newborn. J R Coll Physicians (Lond) 28 : 126–132