ORIGINAL

Very low-frequency rTMS modulates SEPs over the contralateral hemisphere

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Abstract : In order to investigate the transcallosal effects of repetitive transcranial magnetic stimulation (rTMS), we studied median somatosensory evoked potentials (SEPs) before and after applying monophasic very low-frequency (0.2 Hz) subthreshold rTMS over the right motor cortex. For SEPs, median nerve was stimulated on each side. Sham rTMS served as the control. Twelve healthy subjects participated in this study. After rTMS over the right hemisphere, the amplitude of N34 component in right median SEPs recorded from the left parietal scalp (C3') increased significantly. Other components of right or left median SEPs or those after sham stimulation showed no changes. Monophasic 0.2 Hz subthreshold rTMS over the motor cortex predominantly affected the contralateral SEPs, probably through the transcallosal pathway. J. Med. Invest. 57 : 109-113, February, 2010

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1. INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) over the motor cortex modifies cortical excitability that outlasts the period of stimulation (1-5). The effect of rTMS has been explored by examining the changes of motor evoked potentials (MEPs), which reflect activities of the corticospinal tract. However, only a few studies of somatosensory evoked potentials (SEPs) have been reported on the effects caused by rTMS. The right median SEP components N20-P25 and P25-N33 generated in the left hemisphere significantly decreased in amplitude after low-frequency rTMS (1 Hz, biphasic, 200 times) applied over the ipsilateral left motor cortex (6). Their study suggested sensory inhibition occurred by direct cortico-cortical connection between motor and sensory areas because the N20 component reflects an activation of the sensory cortex by thalamocortical fibers. However, our previous study (7) found no changes of these SEP components after very low-frequency rTMS (0.2 Hz, monophasic, 250 times) over the left motor cortex. The discrepancy between these studies may be due to the different stimulation parameters ; frequency (1 Hz *vs.* 0.2 Hz) or phase (biphasic *vs.* monophasic) of rTMS.

Seyal et al. reported significant reduction at base-to-peak amplitude of N20 and peak-to-peak amplitude of N20-P25 after very low-frequency rTMS (0.3 Hz, monophasic, 20 min.) applied over the contralateral hemisphere, but they did not examine ipsilateral effects (8). The present study aimed at examining not only ipsilateral but also contralateral SEP changes after very low-frequency monophasic rTMS

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given over the right motor cortex. In search for stimulus parameters suitable for this use, we used monophasic very low-frequency (0.2 Hz) rTMS, which was efficacious in treating writer's cramp (9).

2. MATERIALS AND METHODS

2. 1. Subjects

Twelve healthy right- handed male volunteers (33.0± 9.5 years) participated in this study. All subjects were free from neurological and phychiatric diseases. They gave their informed consent for the study, which was approved by the Ethics Committee of the University of Tokushima. Handedness was established by a detailed questionnaire, the Edinburgh Handedness Inventory (10).

2. 2. Experimental design

In an electrically and auditory shielded room, the subjects relaxed on a reclining chair with their feet on the foot-rest and were instructed to keep eyes open. SEPs were recorded before and after application of monophasic 0.2 Hz rTMS or sham stimulation over the left cortex hand motor area. Two sessions (rTMS ; real *vs.* sham) were performed on each separate day in a counterbalanced order at 1 week or longer intervals for each session.

2. 3. rTMS

In monophasic rTMS, we used the figure-of-eight stimulation coil (outside diameter of one half-coil, 8.7 cm ; Magstim Col Ltd., OHR Wales, UK) connected to Magstim 200 stimulator (2.2 T at the coil surface). Magnetic stimuli of 250 times were delivered at 0.2 Hz to the right motor cortex, 2 cm anterior and 3.5 cm lateral to Cz (International 10-20 System). We determined the optimal position for activation of the left first dorsal interosseous muscle by moving the coil in 0.5 cm steps around the presumed motor area. Motor response was recorded using electromyography. Threshold was defined as minimum stimulation level necessary to evoke motor response of >50 μV peak-to-peak amplitude in five out of ten trials. The coil was positioned tangentially to the curvature of the head and handle of the coil formed a 45° angle with subject's body midline. Stimulation intensity was 85% of the resting motor threshold for the motor cortex.

Sham stimulation was performed by the same procedure as that of rTMS using a figure-of-eight sham coil (a placebo system ; Magstim Co. Ltd., OHR Wales, UK ; the same shape as that of a true coil) connected to Magstim 200 Stimulator (0.44 tesla at coil surface).

These parameters of rTMS were in accordance with the international safety guidelines (11).

2. 4. SEPs

SEPs were obtained by electric median nerve stimulation at right or left wrist respectively in each session. Each recording took for about 10 minutes. Sides of stimulation were randomly assigned. Electric stimuli (0.2 ms duration) were delivered at 1 Hz through surface electrodes. Positive electrodes were placed on distal and negative ones were on proximal side of wrist. Intensity was adjusted just above the motor threshold of abductor polices muscle. SEPs were recorded with silver chloride disk surface electrodes at F3, F4, 2 cm posterior to C3 (C3') and 2 cm posterior to C4 (C4'), according to the International 10-20 system. The linked earlobe electrodes served as reference. The impedance of these electrodes were kept below 5 kΩ. The electrooculogram (EOG) was also recorded with a pair of silver chloride disk electrodes at 2 cm above the left outer canthus and 2 cm below the right outer canthus. Signals from scalp electrodes and EOG were amplified and acquired at a sampling rate of 10 kHz and filtered at 1-5000 Hz and 0.5-1000 Hz respectively (MEB2200 amplifier ; Nihon Kohden, Tokyo, Japan). All signals were recorded for 100 ms after the onset of median nerve stimulation and stored on a personal computer for off-line analysis. We collected at least 200 artifact-free sweeps and then averaged them on off-line.

2. 5. Data analysis

Components with clear peak were all analyzed. Among SEP components from right median nerve stimulation, P14, P22, N30 and N60 were determined at F3 ; P14, N20, P26, N34 and P45 at C3' ; P14, P22 and N30 at F4 ; P14 was at C4'. Among left median SEP components, P14, P22, N30 and N60 were determined at F4 ; P14, N20, P26, N34, and P45 at C4' ; P14, P22 and N30 at F3 ; P14 was at C3'. We measured the baseline-to-peak amplitudes of these components. The baseline was defined as the segment between 2 and 6 ms after electrical stimulation.

We analyzed the amplitudes of all SEP components by two- way repeated measures analysis of variance (ANOVA) with conditions (real *vs.* sham rTMS stimulation) and time course (before *vs.*

after stimulation). When statistical significance was reached, we used two tail paired t-test to analyze the amplitude change after rTMS compared with that of before.

All data were analyzed with SPSS version 11.01 J for Windows (SPSS Japan Institute Inc., Tokyo). Significant level of all analysis were defined $p < 0.05$.

3. RESULTS

Figure 1 shows the grand-averaged waveforms obtained from twelve subjects. Table 1 shows the peak amplitudes of each component before and after application of rTMS and sham stimulation. In rTMS condition, only N34 amplitude at C3' of right median SEPs reached a significance level by repeated measures ANOVA ($F=4.585$, $p=0.044$). Post hoc analysis disclosed N34 amplitude after rTMS increased significantly ($t=-3.332$, $p=0.007$) (Fig. 2). Figure 3 shows the amplitude change of the N34

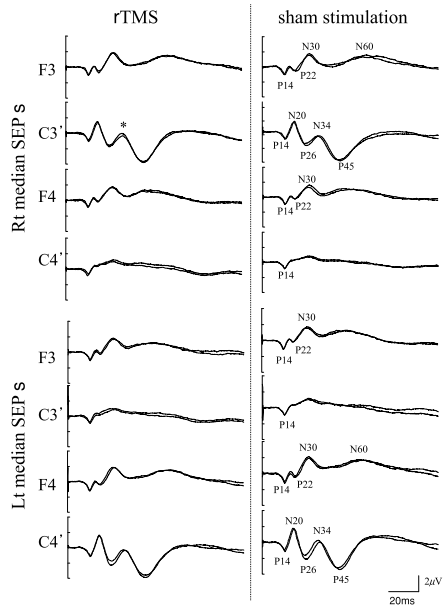

Fig. 1 Grand-averaged waveforms of SEPs by right or left median nerve stimulation before (thin line) and after (thick line) intervention. Left column shows intervention by real rTMS and right shows that of sham stimulation. Asterisk shows significant change detected by paired t-test (* ; $p < 0.05$).

Table 1 Peak amplitudes of each SEP component before and after the application of rTMS and sham stimulation

Rt median nerve stimulation

	F3								F4					
	P14		P22		N30		N60		P14		P22		N30	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
rTMS	-1.34± 0.39	-1.30± 0.33	-1.13± 0.69	-1.01± 0.82	2.44± 1.53	2.52± 1.41	2.28± 1.02	2.27± 1.51	-1.20± 0.39	-1.06± 0.39	-0.48± 0.56	-0.38± 0.55	2.34± 1.12	2.37± 1.08
sham	-1.40± 0.34	-1.33± 0.29	-1.02± 0.92	-1.11± 0.76	2.22± 1.29	2.43± 1.70	2.51± 1.37	2.12± 1.51	-1.24± 0.40	-1.17± 0.39	-0.48± 0.66	-0.54± 0.46	1.97± 0.85	2.29± 1.16

	C3'										C4'		
	P14		N20		P26		N34		P45		P14		
	before	after	before	after	before	after	before	after	before	after	before	after	
rTMS	-1.40± 0.41	-1.23± 0.34	2.01± 1.07	2.18± 0.97	-2.53± 2.06	-2.28± 2.24	-0.02± 1.05	0.39± 0.95	-4.81± 2.51	-5.09± 2.92	-1.14± 0.42	-0.92± 0.31	
sham	-1.30± 0.46	-1.29± 0.32	2.26± 0.89	2.25± 1.23	-2.51± 2.28	-2.98± 3.19	-0.08± 0.95	-0.02± 0.94	-5.51± 4.01	-5.56± 3.94	-1.06± 0.40	-1.05± 0.29	

Lt median nerve stimulation

	F3						F4							
	P14		P22		N30		P14		P22		N30		N60	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after
rTMS	-1.26± 0.24	-1.39± 0.36	-0.30± 0.59	-0.56± 0.42	2.52± 0.97	2.30± 1.18	-1.36± 0.34	-1.56± 0.27	-0.66± 0.71	-0.99± 0.64	2.82± 1.34	2.57± 1.49	2.59± 1.18	2.42± 1.05
sham	-1.43± 0.31	-1.39± 0.38	-0.49± 0.58	-0.41± 0.51	2.21± 0.76	2.38± 0.90	-1.51± 0.41	-1.54± 0.44	-0.84± 0.70	-0.92± 0.72	2.63± 1.01	2.49± 1.30	2.40± 1.25	2.26± 1.50

	C3'		C4'									
	P14		P14		N20		P26		N34		P45	
	before	after	before	after	before	after	before	after	before	after	before	after
rTMS	-1.21± 0.34	-1.16± 0.31	-1.29± 0.42	-1.46± 0.34	2.58± 1.39	2.52± 1.55	-2.44± 2.30	-2.99± 3.04	0.66± 0.65	0.49± 0.79	-4.51± 2.59	-4.68± 2.78
sham	-1.06± 0.39	-1.24± 0.36	-1.45± 0.34	-1.45± 0.33	2.48± 1.39	2.35± 1.19	-3.08± 2.93	-3.21± 2.95	0.37± 1.00	0.15± 0.82	-5.34± 3.67	-5.30± 3.43

Values are expressed mean± SD. Bold figures show the significantly increased value after than before rTMS by paired t-test
rTMS : monophasic 0.2 Hz rTMS, sham : sham stimulation

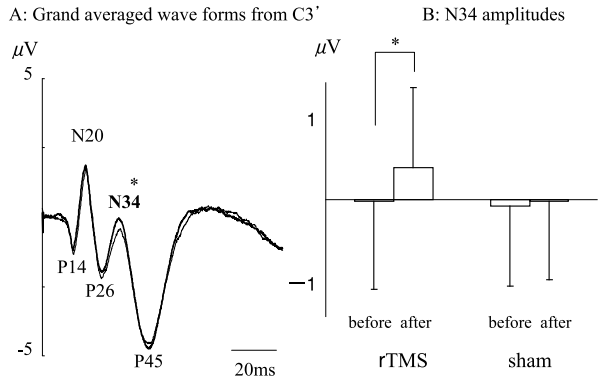

Fig. 2 (A) Grand-averaged waveforms at C3' obtained from SEPs by right median nerve stimulation before (thin line) and after (thick line) rTMS. (B) Amplitudes of N34 component before and after rTMS at C3' (mean± SD). Asterisks shows significant change detected by paired t-test (* ; $p<0.05$). Only real rTMS disclosed significant change of N34 amplitude.

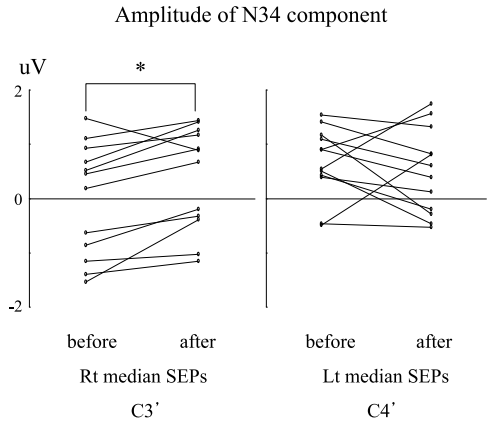

Fig. 3 Amplitude change of the N34 component of SEPs by right (left colum) and left median nerve stimulation (right colum) before and after rTMS in each subject. Asterisk shows significant change detected by paired t-test (* ; $p< 0.05$). Only right median nerve stimulation disclosed significant change of N34 amplitude.

component of SEPs by right and left median nerve stimulation before and after rTMS in each subject. Right median nerve stimulation disclosed significant change of N34 amplitude though left median nerve stimulation showed no significant change. Any components of left median SEPs did not change, or sham stimulation showed no changes of SEPs in each stimulation side.

4. DISCUSSION

Our results showed that monophasic very low-frequency (0.2 Hz) rTMS over the right motor cortex increased the amplitude of N34 component recorded from the left scalp (C3') after right median nerve stimulation. In previous studies, rTMS over the primary motor cortex modified the excitability of the contralateral primary motor cortex (12, 13). This study for the first time showed the rTMS predominantly affects the contralateral SEP component, while leaving the ipsilateral ones unaffected.

Bilateral motor cortices are basically considered to transfer inhibitory effect upon each other (transcallosal inhibition) (14-16). In our study, rTMS applied over the right motor cortex might exert an influence on the contralateral left motor cortex through the mechanism of inter-hemispheric inhibition, which may secondarily affect the contralateral sensory cortex.

Because no significant change was found in P14 or N20 component, we considered that the change of N34 component occurred, not at the sensory pathway up to the primary sensory cortex, but through the interactions between sensory-motor cortices of both hemispheres. It was argued that the increased amplitude of an SEP component reflects inhibition rather than facilitation (7).

Seyal et al. already reported this contralateral effect on N20-P25, but did not investigate the effect of the ipsilateral sensory cortex. Our study is the first to show this contralateral SEP effects with no ipsilateral changes. Although the stimulation condition of rTMS used by Seyal et al. was a monophasic pulse of 0.3 Hz just as we used, their stimulation intensity was 10% above the intensity of visual muscle contraction, being much stronger than ours (85% of resting motor threshold) . The difference of intensity may mainly the reason of different influence on contralateral sensory cortex. We suspect the decreased amplitude by strong rTMS stimulation may relate with a kind of gating through contralateral motor cortex. On the other hand increased amplitude by weak rTMS stimulation in our result may originate mainly from contralateral sensory cortex probably through opposite mechanism of gating.

Left median nerve stimulation did not disclose any significant change of SEP components. The previous studies reported significant decrease of both N20a-P25 amplitude and P25-N33 amplitude (6) or no significant change (7). This difference may be due to the stimulation condition of rTMS. Urushihara's and our study used 0.2 Hz monophasic pulse whereas Enomoto's 1 Hz biphasic pulse. Recent study reported that phase was more important than frequency to induce SEP change (17).

Left median nerve stimulation did not disclose any significant change of SEPs, possibly because N34 amplitude on the left hemisphere was larger in the

right rather than left median nerve stimulation and the finite change of N34 component on the left hemisphere was sensitively detected through right median nerve stimulation. It supports our result that sensory modification predominantly occurs on the contralateral side of rTMS.

In conclusion, monophasic very low-frequency rTMS over the motor cortex significantly increased the amplitude of N34 component generated on the contralateral hemisphere. Our results suggests that rTMS parameters used in this study could modify the cortical sensory processing predominantly on the contralateral hemisphere, possibly through the transcallosal pathways.

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