

Effects of volitional walking control on postexercise changes in motor cortical excitability

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To explore the effects of qualitative or quantitative changes in walking on motor cortical excitability, a transcranial magnetic stimulation procedure was used to examine the alterations of motor-evoked potential (MEP) amplitude following walking. Eight healthy participants completed a series of two walking tasks on a treadmill at 2 km/h. The ratio of the left stance duration to the right stance duration was 1 : 2 in the asymmetrical walking task and 1 : 1 in the symmetrical walking task. In each task, walking for 10 min followed by MEP measurement for ~ 4 min was repeated three times. MEP measurements were also performed before a walking task as a baseline and continued every 10 min for a further 30 min after the completion of the walking task. During slight voluntary contraction of the left tibialis anterior muscle, MEP measurements were conducted four times. Although a significant MEP depression was found after the asymmetrical walking task with increasing amount of walking, no significant decrease in MEP below baseline was observed after the symmetrical walking task throughout all measurement sessions. This MEP depression was the prominent response to the

asymmetrical walking task compared with the symmetrical walking task. These findings indicate that the intentional control of walking pattern has both temporal and task-specific influences on excitability changes in the cerebral cortex, and suggest that motor cortical excitability may be altered by controlling the amount of central commands to the legs even during gait exercise.

NeuroReport 25:44–48 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

NeuroReport 2014, 25:44–48

Keywords: motor-evoked potentials, primary motor cortex, transcranial magnetic stimulation, walking control

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Received 22 August 2013 accepted 9 September 2013

Introduction

Recent studies using functional MRI [1–3] and transcranial magnetic stimulation (TMS) [3–5] have demonstrated that motor skill training can cause plastic changes in the cerebral cortex. These findings indicate the possibility of anatomical or physiological changes in the primary motor cortex caused by repetitive training and suggest that the human primary motor cortex is involved in motor learning. Although several studies have utilized brief motor skill training related to the hands [3,5], there are few reports regarding motor training of the lower extremities [6]. Unlike the hands, where fine movements of individual fingers are required for function, the main role of the lower extremities is gait, which requires dynamic and coordinated movements of the bilateral limbs. Therefore, in the clinical setting, therapists generally place more emphasis on gait training and less on motor skill training as a crucial treatment for affected lower extremities in most patients.

Human locomotion is generated predominantly at the spinal level [7], and stepping-like electromyographic (EMG) activity was elicited using epidural spinal cord

stimulation even when isolated from brain control [8]. Previous studies using TMS techniques showed that normal walking induced no significant changes in motor-evoked potential (MEP) after a walking task compared with before walking [9–11]. Although these results may be consistent with the theory that locomotion is an automated movement performed with a minimum of conscious control [7], recent studies have suggested evidence of plastic changes of the brain in stroke patients caused by more than several weeks of gait training [12–14]. Thus, from the point of motor learning, it is assumed that consecutive gait exercise is also able to produce excitability changes in the cerebral cortex, particularly during the training session to reacquire walking ability.

Some patients with brain damage due to stroke exhibit gait disturbance with an asymmetrical walking (AW) pattern. In these cases, the period spent on the supporting leg on the unaffected side is extended and the affected leg is consciously advanced. Thus, we hypothesized that the volitional control of walking pattern likely mediates the leg area in the primary motor cortex, eventually causing excitability changes of the corticospinal tract. Our aim

was to assess the process of excitability changes in the cerebral cortex with increasing amount of walking, and confirm the contribution of intentional walking to motor cortical excitability by comparing the excitability changes caused by unconscious symmetrical walking (SW) with those caused by conscious AW.

Methods

Participants

Eight healthy male volunteers without neurological or orthopedic disabilities in their lower extremities or trunks participated in this study. The mean \pm SD values for age, height, and weight were 20.6 ± 0.7 years, 169.9 ± 4.1 cm, and 60.9 ± 8.3 kg, respectively. Before the experiments, we clearly explained the purpose and methods of the study to all participants, and each participant provided written informed consent. The study was approved by our Institutional Research Ethics Committee for Human Subjects.

Condition of walking tasks

Each individual cadence was measured using a metronome while the participant walked on a treadmill (Sakai Medical Co. Ltd, Tokyo, Japan) at 2 km/h. The following two walking tasks were developed on the basis of the measured cadence.

- (1) The ratio of the left stance duration to the right stance duration was set up at 1:2 to create an AW task according to the individual cadence.
- (2) The ratio of the left stance duration to the right stance duration was 1:1 in SW for the control task.

Two walking tasks were performed on the treadmill with a walking speed at 2 km/h. Walking paces were guided by the metronome rate set to the individual's routine cadence. The participants practiced the two different walking tasks before MEP measurements. Each walking task consisted of three 10-min walking trials.

General procedures

While the participants were seated, MEPs were initially measured from the left tibialis anterior (TA) before walking tasks to determine the baseline values. Subsequently, 10-min walking trials on the treadmill were repeated three times (first, second, and third trials), and MEPs were measured in the sitting position immediately after completion of each walking trial. It took ~ 4 min for each measurement process after the participants stopped walking. In addition, MEP measurements were continued every 10 min for a further 30 min after the three walking trials (10, 20, and 30 min later). Thus, a total of seven measurements were collected for each walking task. Two individual walking tasks were implemented on each separate day with a randomized experimental sequence.

Transcranial magnetic stimulation

Weak voluntary contraction is known to exhibit facilitatory effects on MEP and reduce the threshold inducing MEP. To maintain the muscle contraction force constant during the measurements, an isokinetic dynamometer (BIODEX SYSTEM 3; Biodex Medical Systems Inc., Shirley, New York, USA) was used as a visual feedback tool of ankle dorsiflexion torque values. The participants were told to sit on the chair of the BIODEX SYSTEM 3 with their left feet fixed to the attachment. The axis of the dynamometer then coincided with the ankle joint axis. Before the walking tasks, measurements of maximum isometric dorsiflexion torque (MIDFT) were performed three times, and the three MIDFTs acquired were then averaged for each participant. On the basis of these values, $\sim 15\%$ of MIDFT in the left TA was determined for each participant. Before electrophysiological measurements the participants practiced to maintain the fixed volitional contraction of the TA using the BIODEX SYSTEM 3 monitor.

TMS was caused using a magnetic stimulator (Magstim 200; Magstim Co., Dyfed, UK). A double-cone coil (110 mm in outer diameter) was used to stimulate the leg area of the primary motor cortex. The coil was moved over the scalp to find an optimal location where the left TA was elicited by the lowest stimulus intensity at baseline measurements, and was oriented so that the induced current flowed in the posterior-anterior direction in the brain. To stimulate the same location of the motor cortex during each measurement, each participant put on a swimming cap tightly fixed to the skin with surgical tape. The most suitable coil placement to activate the TA was marked on the cap.

TMS intensity was increased in steps of 10% until MEP reached maximum amplitude or its plateau and four stimuli were delivered at each intensity. Stimulus intensities were expressed as percentage of the maximum output of the stimulator. From the result of the initial MEP measurement, the following two stimulus intensities were determined for each participant and used to compare changes in MEP amplitude off-line [8].

- (1) The stimulus intensity at which the maximum MEP amplitude was obtained at the baseline measurements (TMS_{max}).
- (2) The stimulus intensity at which a half of the maximum MEP amplitude was obtained at the baseline measurements (TMS_{half}).

Electromyographic recording

Disposable silver chloride surface electrodes of 10 mm in diameter (Blue Sensor P-00-S; Ambu Co., Ballerup, Denmark) were used for recording EMG activity and MEP from the left TA. Bipolar electrode pairs were placed longitudinally on the muscle belly at an interelectrode

distance of 20 mm. A ground electrode was placed on the left lateral malleolus. The amplified EMG signals were sampled at 5 kHz, and filtered by a frequency band of 20 Hz to 1 kHz using an electromyography apparatus (Neuropack-S1; Nihon Kohden Co., Tokyo, Japan) for later off-line analysis. EMG signals in response to each test stimulus were recorded for 600 ms, including a pre-stimulus period of 60 ms, so that the background EMG activities could be calculated from the rectified signals just before TMS.

Statistical analyses

Three similar values of four recorded MEP amplitudes were included to determine the average of MEP amplitude for reduction of measurement error. IBM SPSS Statistics 20 (IBM SPSS Statistics Inc., Tokyo, Japan) was used for statistical analyses. A one-way repeated measures analysis of variance was used to assess the background EMG level over each measurement period and to investigate the effect of the amount of walking task on MEP amplitude. When a significant effect was detected, Dunnett's post-hoc analysis was used to compare the value following the three walking trials with the baseline value. Furthermore, the MEP amplitude in each walking task was divided by the baseline values (relative MEP value: R-MEP) for comparison of AW and SW tasks in each measurement session. A paired *t*-test was used for this statistical examination. Values of *P* less than 0.05 were considered statistically significant. Data are shown as mean ± SE.

Results

Relationship between amount of walking and cortical excitability

The background EMG activities at all TMS intensities were not significantly different over the experimental period. The mean values and SEs of the MEP amplitude are shown in Table 1. One-way repeated measures analysis of variance revealed a significant effect of the amount of walking on the MEP amplitude under the TMS_{max} condition during the AW task [$F(3, 21) = 5.711, P = 0.005$]. Post-hoc analyses showed a statistically significant reduction below the baseline after the second ($P = 0.033$) and third ($P = 0.002$) trials. However, no significant differences of MEP amplitude were observed during the SW task or under the TMS_{half} condition.

Relationship between different walking patterns and cortical excitability

The changes of the average R-MEP during the two different walking tasks are plotted in Fig. 1. Under the stimulus condition of TMS_{max}, significant reductions in R-MEPs following the AW task were found after the second [$t(7) = 2.400, P = 0.047$] and third [$t(7) = 2.718, P = 0.030$] trials compared with the SW task (0.93 ± 0.04 vs. $0.88 \pm 0.04, 0.91 \pm 0.03$ vs. 0.82 ± 0.04). Under the TMS_{half} condition, no significant differences in R-MEP

were observed between the SW and AW task in all measurement periods.

Discussion

Effect of the amount of walking on motor-evoked potential amplitude

In the present study, significant decreases in MEP amplitude following the AW task were detected under the TMS_{max} condition compared with the baseline amplitude. However, there were no significant changes under the TMS_{half} condition. The different outcomes observed between the stimulus conditions of TMS_{half} and TMS_{max} may depend on to what extent cortical interneurons and pyramidal neurons were elicited by TMS. When the lower limb area of the motor cortex was stimulated by TMS at high intensity, the induced current was reported to evoke not only an indirect wave but also a direct wave [15]. Thus, the induced current under the TMS_{max} spread to the extensive brain regions, suggesting that the MEP under TMS_{max} may reflect the excitability changes in cortical interneurons and pyramidal neurons more strongly than that under TMS_{half}.

Previous studies considering the change of MEP amplitude following some tasks reported that increased MEP was observed in the TA cortical area after a brief motor skill training [6] or complicated walking tasks using a split-belt treadmill [11]. These responses are different from the cortical alteration induced by normal walking in the present study.

Our data are consistent with previous studies examining the influence of normal walking on MEP, where MEP depression [9,16] or no significant change in MEP [9–11] was observed after various measurement conditions of normal walking (i.e. walking velocity, walking duration, stimulus intensity to measure). Thus, the changes of MEP after walking itself are similar to those following passive training or repetition of nonskilled tasks, which do not require complex motor performance [3,4,6,17].

Relative to MEP depression found after the AW task, Brasil-Neto *et al.* [18] described a marked transient depression of MEP amplitude as a result of fatiguing wrist exercise, and suggested that the decreased MEP correlated with central fatigue caused by a depletion of neurotransmitters. In addition, postexercise depression of MEP is related to the period of exercise [19]. Thus, we suggest that the MEP depression in our study was attributed to central fatigue as the amount of walking appeared to mediate the MEP across the time points of MEP measurement.

Effect of different walking patterns on motor-evoked potential amplitude

We hypothesized that the control of walking pattern produced the different changes of MEP amplitude compared with those elicited by normal walking. In the

Table 1 Effects of the amount of walking on motor-evoked potential amplitude during different walking tasks

Stimulus intensity	Walking task	Experimental time course				Multiple comparison
		Baseline	First trial	Second trial	Third trial	
TMS _{max}	SW	2.43±0.24	2.35±0.30	2.28±0.24	2.18±0.19	NS Rest period >2nd trial, rest period >3rd trial
	AW	2.65±0.33	2.49±0.37	2.31±0.27	2.16±0.28	
TMS _{half}	SW	1.38±0.16	1.45±0.18	1.41±0.19	1.34±0.12	NS NS
	AW	1.66±0.20	1.61±0.27	1.55±0.23	1.56±0.26	

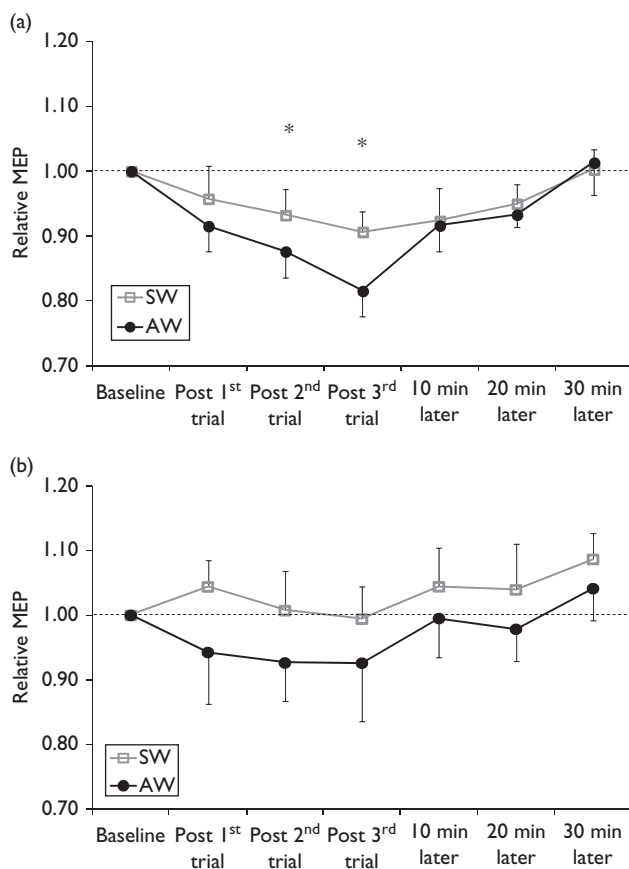
The following two stimulus intensities were determined from the result of baseline MEP measurement.

TMS_{max}: the stimulus intensity at which maximum MEP amplitude was obtained.

TMS_{half}: the stimulus intensity at which approximately a half of maximum MEP amplitude was obtained. The ratio of the left stance duration to the right stance duration was set at 1:2 in an asymmetrical walking task and 1:1 in a symmetrical walking task in accordance with individual cadence.

The data shown are the mean±SE, expressed in millivolts. The level of statistical significance is defined as $P < 0.05$.

TMS, transcranial magnetic stimulation; MEP, motor-evoked potential; SW, symmetrical walking; AW, asymmetrical walking; NS, not significant.

Fig. 1

The serial changes in motor-evoked potential (MEP) during two different walking tasks. MEPs were divided by the initial values at baseline, and are shown as relative MEPs (R-MEPs). Circles represent changes of mean values in cases of asymmetrical walking (AW) task. Squares represent changes of mean values in cases of symmetrical walking (SW) task. Error bars indicate \pm SE. (a) Serial changes of R-MEP under the stimulus intensity at which maximum MEP amplitude was obtained (TMS_{max}). (b) Serial changes of R-MEP under the stimulus intensity at which approximately a half of maximum MEP amplitude was obtained (TMS_{half}). Under the TMS_{max} condition, significant decreases in R-MEPs following the AW task were detected after the second and third trials compared with the SW task. Asterisks indicate significant differences of R-MEP value between different walking tasks ($P < 0.05$). TMS, transcranial magnetic stimulation.

present study, we constructed an AW task that required intentional control to maintain the determined walking pattern to probe whether the cerebral cortex contributed to walking control or motor learning process to obtain a new walking pattern. We detected significant MEP depressions after the second and third trials during the AW task compared with the SW task under the TMS_{max} condition.

A potential mechanism underlying the significant MEP depressions after the AW task may involve central fatigue as described above. The rate of oxygen consumption in patients with hemiplegia was reported to be higher than that in healthy individuals when walking was adjusted to the same speed [20]. AW characterized as hemiplegic gait [21,22] also requires higher energy consumption than for normal gait. As such, the AW task in the present study might represent a more fatigable walking pattern than the SW task, so that R-MEP following the AW task was significantly reduced from baseline compared with the SW task.

MEP depression was also related to the amount of central commands or activation to control the TA during walking tasks. In a recent study of post-task MEP depression, a significant decrease in MEP amplitude was observed only with finger tapping and imagined grip task compared with fatiguing hand-grip task, suggesting the possibility that the increase in repeated initiation of central commands or sustained central activation was the main element underlying MEP depression [23]. The walking task used in this study involved a change in the duration of the stance phase rather than the swing phase on the left. With respect to the timing of muscle activity, the onset of the TA activity starts in preswing, and cessation is toward the end of the loading response [24]. Consequently, muscle activity in the TA was required to augment during a gait cycle in the AW condition. Thus, the significant reduction of MEP after the AW task likely resulted from the increase in central drive compared with the SW task.

With regard to the association between MEP depression and MEP increment, Jayaram *et al.* [11] implemented complex walking tasks using a split-belt treadmill, which

consisted of an independent speed of two separate belts or a changing unpredictable speed of tied belts, and demonstrated that significant augmentations in MEP amplitude were caused by these two tasks. By contrast, we observed a significant decrease of MEP generated by volitional AW in the present study. Teo *et al.* [25] indicated that simple-to-execute motor tasks were followed by a period of reduced excitability, and suggested that more demanding tasks are likely required for the increment of MEP amplitude. Therefore, as MEP changes were dependent on performance difficulty, the AW task was enough to induce excitability changes, but not enough to generate MEP increment, so that a more general period of MEP depression was detected as a postexercise excitability change.

Conclusion

We demonstrated that the intentional control of walking pattern could induce the alteration of cerebral excitability, which likely depends on the learning difficulty of walking control. Although this novel finding suggested that motor cortical excitability might be changed by controlling volitional drive to the legs even during walking, we need further studies to clarify this mechanism in the future.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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