



Contents lists available at ScienceDirect

## Brain Stimulation

journal homepage: [www.brainstimjrn.com](http://www.brainstimjrn.com)

# Interactions Among Learning Stage, Retention, and Primary Motor Cortex Excitability in Motor Skill Learning



Masato Hirano<sup>a</sup>, Shinji Kubota<sup>a,1</sup>, Shigeo Tanabe<sup>b</sup>, Yoshiki Koizume<sup>a</sup>, Kozo Funase<sup>a,\*</sup>

<sup>a</sup> Human Motor Control Laboratory, Graduate School of Integrated Arts and Sciences, Hiroshima University, Hiroshima, Japan

<sup>b</sup> Faculty of Rehabilitation, School of Health Sciences, Fujita Health University, Aichi, Japan

## ARTICLE INFO

### Article history:

Received 16 January 2015

Received in revised form

9 July 2015

Accepted 9 July 2015

Available online 07 August 2015

### Keywords:

Motor skill learning

Primary motor cortex excitability

Learning stage

Performance retention

## ABSTRACT

**Background:** Previous studies have shown that primary motor cortex (M1) excitability is modulated by motor skill learning and that the M1 plays a crucial role in motor memory. However, the following questions remain: 1) At what stage do changes in M1 excitability occur? 2) Are learning-induced changes in leg M1 excitability associated with motor memory? Here, we did two experiments to answer these questions.

**Methods and results:** In experiment 1, subjects learned a visuomotor tracking task over two consecutive days. Before and after the task in Day 1, we recorded input–output curves of the motor evoked potentials (I–O curve) produced in the tibialis anterior muscle by transcranial magnetic stimulation. We found that the changes in M1 excitability were affected by learning stage. In addition, the changes in M1 excitability in Day 1 were correlated with the retention. In experiment 2, we recorded I–O curves before learning, after the fast-learning stage, and after learning. We found no changes in M1 excitability immediately after the fast-learning stage. Furthermore, a significant relationship between the length of slow-learning stage and the changes in M1 excitability was detected.

**Conclusions:** Previous studies have suggested that optimal motor commands are repeatedly used during the slow-learning stage. Therefore, present results indicate that changes in M1 excitability occur during the slow-learning stage and that such changes are proportional to motor skill retention because use-dependent plasticity occur by repetitive use of same motor commands during the slow-learning stage.

© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

The learning of motor skills is a basic requirement of daily life. Many transcranial magnetic stimulation (TMS) studies have demonstrated that motor skill learning induces changes in primary motor cortex (M1) excitability [1–4]. However, the modulation of M1 excitability is highly variable, although all participants improve their performances [5]. This indicates that various factors impact

on changes in M1 excitability induced by motor skill learning. The process of motor skill learning consists of fast- and slow-learning stages [6–8]. Previous studies have found that different neural networks are involved in these stages [9,10]. In addition, Adi-Japha et al. (2008) suggested that a shift in the processing mode occurs between these stages [11]. Thus, we considered that changes in M1 excitability induced by motor skill learning might be learning stage dependent. A recent study suggested that same motor commands acquired during the fast-learning stage are repeatedly used to perform the task during the slow-learning stage [12]. Therefore, we hypothesized that changes in M1 excitability occur during the slow-learning stage because the M1 exhibits use-dependent plasticity [13].

One of the important factors in the motor skill learning is performance retention [14]. Some previous studies have demonstrated that the M1 plays a crucial role in the retention of the newly acquired motor skills [15–18]. However, only one study has examined the relationship between the changes in M1 excitability induced by

This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant no. 25350815). S.K. was supported as a Research Fellow of the Japan Society for the Promotion of Science. The authors declare no competing financial interests.

\* Corresponding author. Graduate School of Integrated Arts and Sciences, Hiroshima University, 1-7-1 Kagamiyama, Higashi-Hiroshima 739-8521, Japan. Tel./fax: +81 82 424 6590.

E-mail address: [funase@hiroshima-u.ac.jp](mailto:funase@hiroshima-u.ac.jp) (K. Funase).

<sup>1</sup> Research Fellow of the Japan Society for the Promotion of Science.

<http://dx.doi.org/10.1016/j.brs.2015.07.025>

1935-861X/© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

motor skill learning and the retention of the new skills without using intervention techniques [19]. Therefore, further investigation is required to corroborate the relationship between them.

To the best of our knowledge, there are few previous studies that have investigated the relationship between learning-induced changes in M1 excitability and learning stages. Furthermore, the role of the leg M1 in motor skill retention remains unknown. The leg M1 possesses similar plasticity to the hand M1 [20–24]. Therefore, we focused on the leg M1 and leg motor skill learning. Here, we investigated the following two questions: 1) is there a relationship between changes in M1 excitability induced by motor skill learning and stages of motor skill learning? 2) Do changes in M1 excitability affect retention processes? In order to answer these questions, we did two experiments.

## Materials and methods

### Subjects

Thirty one healthy right-footed subjects (6 females; mean age:  $22.5 \pm 2.3$  years) participated in this study after giving their written informed consent. We checked the footedness of each subject using a custom-made questionnaire based on that described in a previous study [25]. All experimental procedures were carried out in accordance with the Declaration of Helsinki and were approved by the ethics committee of Hiroshima University. During the experiments, the subjects were seated in an armchair with their right lower leg secured to a semi-flexed ankle foot brace at the hip ( $120^\circ$ ) and knee ( $120^\circ$ ) with their ankle plantar-flexed ( $110^\circ$ ).

### EMG recording

Surface electromyogram (EMG) recordings were taken from the right tibialis anterior (TA) with Ag/AgCl surface electrodes (diameter: 9 mm). In one subject, we recorded surface EMG activity from TA and soleus muscle (SOL) during visuomotor tracking task, control task, and maximum voluntary isometric contraction of ankle dorsi-/plantar-flexion (see below; Fig. 1B and C). All EMG recordings

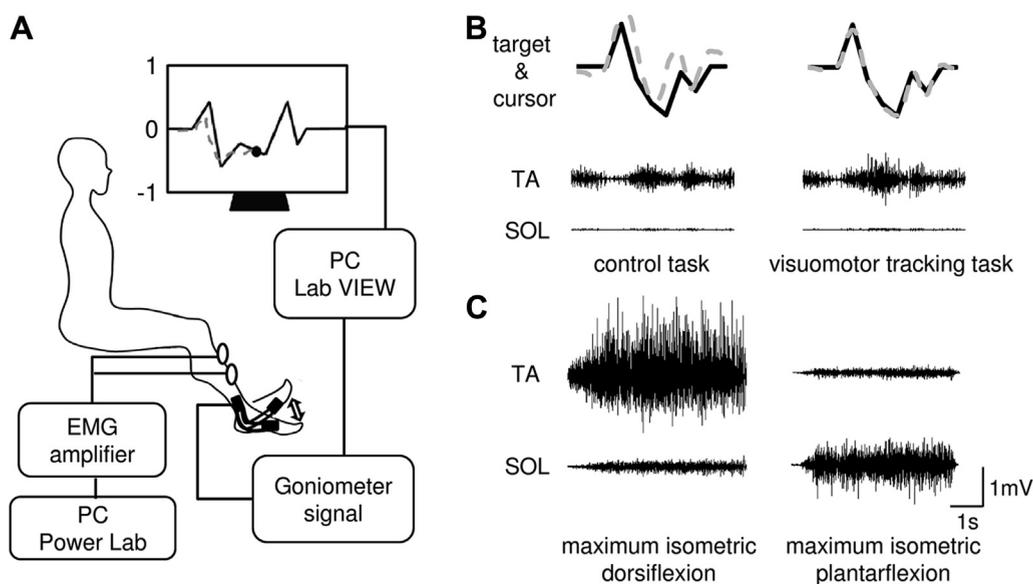
were amplified at a bandwidth ranging from 5 Hz to 3 kHz, and all amplification procedures were controlled using a signal processor (7S12, NEC San-ei Co. Ltd., Japan). The analog outputs from the signal processor were digitized at a sampling rate of 4 kHz and then fed into a computer for off-line analysis (PowerLab system, AD Instruments Pty. Ltd., Australia).

### TMS application and MEP recording

TMS was delivered using a Magstim 200 stimulator connected to a double cone coil with a diameter of 110 mm. We determined the optimal position for evoking MEP in the TA and marked it on a swimming cap worn by the subjects with a soft-tip pen to ensure reliable coil placement between trials. To reduce the threshold required to evoke MEP in the TA, the subjects performed an isometric dorsiflexion of the ankle. The intensity of isometric dorsiflexion was set to the 10% EMG level of the maximum voluntary contraction (MVC) throughout the MEP recordings. We monitored the strength of the isometric dorsiflexion by measuring background EMG activity (bEMG) for the 200 ms before the TMS trigger with the Integral Abs, Scope (version 3.7.6.), PowerLab system. We delivered visual feedback to the subjects to help them to maintain isometric dorsiflexion at a constant level. The active motor threshold (aMT) was defined as the lowest intensity required to evoke MEP of  $>200 \mu\text{V}$  in 5 out of 10 trials during dorsiflexion. We obtained the Input–Output curves of MEP before and after the learning session. The intensity of the TMS was set at 1.0, 1.2, 1.4, or  $1.6 \times \text{aMT}$ . Seven to 10 MEP were evoked at intervals of 5–7 s during each measurement. The peak-to-peak MEP amplitudes and the slope of the I–O curve (I–O slope) were measured off-line.

### H-reflex recording

The ratio of the maximum H-reflex amplitude ( $H_{\text{max}}$ ) to the maximum M-response amplitude ( $M_{\text{max}}$ ) for the TA was calculated to investigate spinal motoneuron pool excitability before and after the motor task as described below. These recordings were carried out during isometric dorsiflexion at 10% MVC EMG level. The



**Figure 1.** A: A block diagram of the experimental equipment. The subjects were asked to track the target black line by controlling the red cursor line (illustrated as a dashed gray line in Fig. 1). B: Recording examples during control tracking task (left side) and visuomotor task (right side). Upper panel represents target (solid black line) and cursor (dashed gray line) trajectories. Lower panel represents raw EMG activities of TA and SOL at each task. C: Raw EMG activities of TA and SOL at maximum isometric dorsiflexion (left side) and plantar flexion (right side).

H-reflex was evoked by applying an electrical stimulus (a rectangular pulse of 1 ms duration) to the common peroneal nerve at the caput fibulae using paired bar-type electrodes. We measured the H-reflex at different stimulus intensities around the threshold of the M-response in order to determine  $H_{max}$ .  $H_{max}$  was calculated by averaging 20 waves. Then, the stimulus intensity was increased to obtain  $M_{max}$ , which was calculated by averaging 5 waves. The electrical stimulus was applied every 3 s during the H-reflex recording and every second during the  $M_{max}$  recording. The ratio of  $H_{max}$  to  $M_{max}$  was measured off-line.

### Motor task

We used a custom-built PC program (LabVIEW, National Instruments Japan, Co.) for the visuomotor tracking task (Fig. 1A). During the learning session, a goniometer was attached to the subject's right ankle. The goniometer signal was acquired at a sampling rate of 200 Hz with an analog/digital converter (USB6212, National Instruments Japan, Co.) and stored on a PC. The position of the ankle joint was displayed as a red circular cursor on a 26-inch display connected to the PC. The subjects were able to control the cursor by performing ankle dorsiflexion and plantar flexion. During maximal dorsiflexion, the cursor moved to the bottom of the display and vice versa. To maintain task complexity, we inversely set the direction of the cursor with respect to the actual ankle movement. The cursor moved automatically from left to right at 5 s. A random target line (black line) was presented on the display, and we instructed the subjects to trace it as accurately as possible by controlling the cursor. Target line during initial 1 s and last 0.5 s were always horizontal. The subjects performed 10 training blocks, each of which consisted of 10 trials. The training blocks were separated by 1-min rest periods. In experiment 1, we set 3-min rest periods between block 3 and 4, and between block 6 and 7 in order to avoid the muscle fatigue. The value of the vertical axis of the bottom and top of the display is arbitrarily defined  $-1$  and  $1$ , respectively. We calculated the performance score for each trial as the mean difference between the target line and the actual position of the ankle joint (red cursor) at 200 points. In this task, the subjects were required to adjust the red cursor in the middle of the display without any feedback at the beginning of the first trial in each block because the initial position of the target line was set to the middle of the display. Therefore, the performance of the first trial in each block was always wrong. For that reason, we excluded the data in the first trial in each block from the analysis.

### Experiment 1

Twenty subjects (4 females; mean age:  $22.5 \pm 2.5$  years) participated in this experiment over two consecutive days. Before the learning session on Day 1, we recorded the aMT, the I–O curve,  $H_{max}$ , and  $M_{max}$ . After the learning session, we recorded the I–O curve,  $H_{max}$ , and  $M_{max}$ . On the next day, the subjects performed the visuomotor tracking task without measurement of any physiological data (Day 2).

### Control experiment

Seven subjects participated in control experiment (2 females; mean age:  $22.3 \pm 1.3$  years). All subjects participated in experiment 1 and they are in long slow-learning group (see below). The interval between the main experiment and control experiment were at least 3 months. We asked subjects to perform the tracking task without visual feedback. The target line was displayed in the same way as main experiment, but red cursor (subject's cursor) was only visible during initial 1 s and last 0.5 s in each trial in order to move the

cursor to the initial position. During the cursor was disappeared, subjects performed the ankle movement with the imagination that the cursor matches to the target. The protocol of the control experiment was same as the experiment 1. The performance score was calculated during the cursor was not visible (150 points). In this control task, EMG activity during the task was almost as much as the task with visual feedback (Fig. 1B). However, the learning did not occur (see Fig. 4).

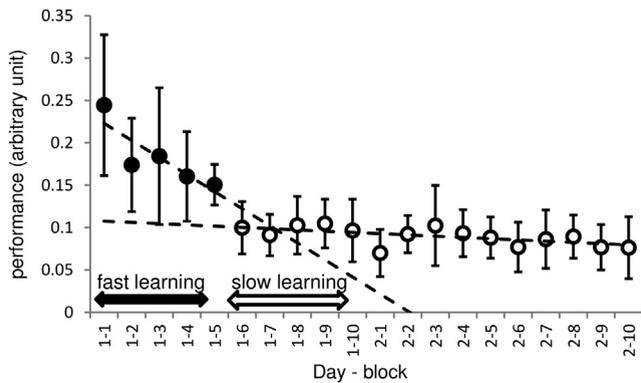
### Experiment 2

Eleven subjects (2 females; mean age:  $22.1 \pm 1.8$  years) participated in this experiment. As with the experiment 1, we recorded I–O curves and  $M_{max}$  before and after the task. In addition, we obtained I–O curve and  $M_{max}$  immediately after the fast-learning stage (post fast-learning) in order to clarify whether the fast-learning stage contributed to the changes in the I–O curve. The subjects performed 10 training blocks. The training blocks were separated by 1-min rest periods. Because the endpoint of the fast-learning stage is different between subjects, the time point of the post fast-learning was different between subjects. We defined the endpoint of the fast-learning stage based on the data of experiment 1. To define the endpoint of the fast-learning stage, we set two requirements: 1) relative performance values against the performance of block 1 must be less than 0.65 consecutively twice. 2) The difference between the two consecutive relative values that are below 0.65 must be less than 0.15 (i.e. no improvement within these two consecutive blocks). The 0.65 is mean (0.55) plus 1SD (0.10) at endpoint of fast-learning stage in experiment 1. When we used this definition to data of experiment 1, we can detect the endpoint of the fast-learning stage within  $\pm 3$  blocks compared with estimating it by two-piece linear regression method (see below) in 19 out of 20 subjects. Furthermore the coefficient of determination between the endpoints of fast-learning stage estimated by two-piece linear regression method and estimated by this definition is 0.73 ( $P < 0.01$ ). Therefore, we use this definition in order to detect the endpoint of the fast-learning stage in the experiment 2. Three subjects did not satisfy these requirements within 10 blocks. We excluded the data of the three subjects from analysis.

### Data analysis

In order to define the stage of motor learning in experiment 1, we approximated the learning curve using two-piece linear regression by minimizing the residual sum of the squares of the two types of regression line (Fig. 2 and Supplementary Fig. 1). We set the following requirement for the approximate: the slope of the left regression line must be negative. Because the blocks approximated by left regression line are defined as the fast-learning stage that is represented by great performance improvement. We separated the fast- and slow-learning stages based on the inflection point. Furthermore, we divided all of the subjects into two groups. We classified the subjects who exhibited a longer slow-learning stage into the long slow-learning group (LSL:  $n = 10$ , 2 females), and the other subjects were termed the short slow-learning group (SSL:  $n = 10$ , 2 females).

In experiment 1, between-group differences in the performance score for block 1, performance improvement, age,  $M_{max}$ , inflection point, and aMT were assessed using a non-paired *t*-test. The differences in the pre and post bEMG activity at each stimulus intensity were assessed using paired *t*-test. In control experiment, the differences between the pre and post I–O slope, the pre and post bEMG activity at each stimulus intensity, and the performance score in the block 1 and block 10 were assessed using a



**Figure 2.** A specimen learning curve (mean  $\pm$  SD). The dashed lines represent the regression equations derived from the two-line regression analysis. We separated the fast (white circles) and slow (black circles) learning stages using the inflection point. The number of blocks in the slow-learning stage was calculated in order to perform correlation analysis and to classify the subjects into the LSL and SLS groups.

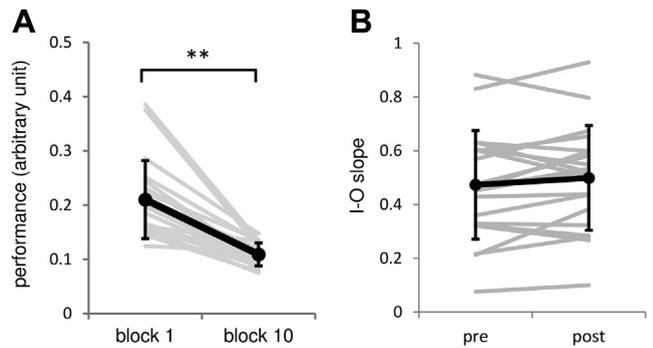
paired *t*-test. Three-way repeated measures ANOVA was performed to test for differences in the I–O curve (group  $\times$  intensity  $\times$  time). Two-way repeated measures ANOVA was also used to test for differences in the time course of performance score (group  $\times$  block), retention (day  $\times$  group), and  $H_{max}/M_{max}$  (group  $\times$  time). Bonferroni's post-hoc test for multiple comparisons was used for further analyses. To analyze of the correlations between the changes in the I–O slope and 1) the number of blocks in the slow-learning stage or 2) the degree of motor skill retention, we performed analyses using Spearman's rank correlation coefficient. To further analysis, we performed partial correlation and semi-partial correlation analysis using Spearman's partial rank correlation coefficient. We calculated performance improvement as the difference between the performance scores for blocks 1 and 10. We normalized the mean performance data for each block to the mean performance data for block 1 when we calculated the performance improvement. In experiment 2, between-experiment differences in the block 1 performance, performance improvement, endpoint of the fast-learning stage, age, baseline I–O slope,  $M_{max}$ , and aMT were assessed using a non-paired *t*-test. The differences in the pre, post fast-learning, and post bEMG activity were assessed using paired *t*-test. One-way repeated measures ANOVA was used to test for difference in the changes in the I–O slope (time). Shaffer's post-hoc test for multiple comparisons was used for further analyses. We test the relationships between the changes in the I–O slope at post fast-learning and the number of blocks in the fast-learning stage, the changes in the I–O slope at post and the number of blocks in the slow-learning stage using Spearman's rank correlation coefficient. All data are shown as the mean  $\pm$  SD values.

## Results

### Experiment 1

#### Changes in performance and I–O curves in all subjects

All subjects in the experiment 1 improved their task performance during the 10 blocks (Fig. 3A; block 1:  $0.21 \pm 0.08$ , block 10:  $0.11 \pm 0.02$ ;  $t = 6.64$ ,  $P < 0.01$ ). However, there was no significant difference between the pre and the post I–O slopes in the average of all subjects (Fig. 3B; pre:  $0.47 \pm 0.20$ , post:  $0.50 \pm 0.19$ ;  $t = 1.39$ ,  $P = 0.18$ ). In addition, there was no significant difference in bEMG activity between the pre and the post sessions at any TMS intensity (Table 1).



**Figure 3.** A: Performance scores for blocks 1 and 10. All subjects improved their performance. B: The pre and post I–O slope for all subjects. The changes in the slope of the curve were highly variable. \*\* $P < 0.01$ .

#### Relationship between changes in I–O slope and motor learning stage

First, to examine the effects of motor learning stage, we divided the subjects into the LSL and the SSL groups. There were no significant differences in any fundamental parameters, such as block 1 performance (LSL:  $0.21 \pm 0.07$ , SSL:  $0.22 \pm 0.08$ ;  $t = 0.30$ ,  $P = 0.76$ ), performance improvement (LSL:  $0.48 \pm 0.17$ , SSL:  $0.41 \pm 0.14$ ;  $t = 0.95$ ,  $P = 0.36$ ), age (LSL:  $21.8 \pm 1.32$ , SSL:  $23.2 \pm 3.26$  years;  $t = 1.26$ ,  $P = 0.23$ ),  $M_{max}$  (LSL:  $5.83 \pm 1.66$  mV, SSL:  $6.95 \pm 1.86$  mV;  $t = 1.43$ ,  $P = 0.17$ ), or aMT (LSL:  $41.30 \pm 6.89\%$ , SSL:  $43.40 \pm 6.13\%$ ;  $t = 0.72$ ,  $P = 0.48$ ) between the two groups. Figure 4 shows the time course of the changes in the mean performance score of each group. We performed two-way repeated measures ANOVA between the LSL and the SSL group. A significant effect of block on the performance was detected (main effect of block:  $F_{19,342} = 42.07$ ,  $P < 0.01$ ). A significant difference in the performance between the two groups was also detected (main effect of group:  $F_{1,18} = 4.61$ ,  $P < 0.05$ ). In addition, the positions of the inflection points also differed significantly between the two groups (LSL:  $4.0 \pm 0.67$ , SSL:  $8.3 \pm 1.49$ ,  $t = 8.31$ ,  $P < 0.01$ ). These results suggest that the subjects in the LSL group learned faster than those in the SSL group.

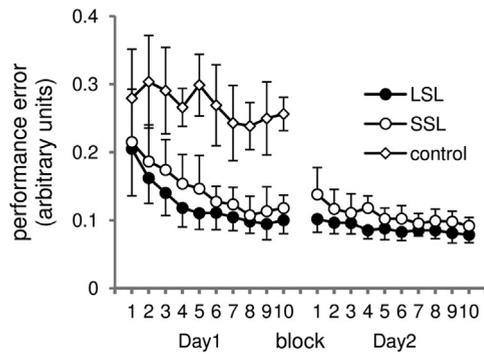
Figure 5A–C shows the pre and the post I–O curves of the each group, respectively. The result of three-way repeated measures ANOVA was shown in Table 2. There was a significant effect of intensity and time. In addition, there was a significant group  $\times$  time interaction. Then, we detected a simple main effect of time at the LSL group ( $F_{1,36} = 21.35$ ,  $P < 0.01$ ), but not in the SSL group ( $F_{1,36} = 0.01$ ,  $P = 0.93$ ). Post-hoc test revealed significant differences between the pre and the post values at 1.4 and 1.6  $\times$  aMT in the LSL group (Fig. 5A).

To investigate the notion that just performing the ankle movement simply causes the changes in the I–O slope, we asked the subjects in the control group to perform the tracking task without visual feedback. We found no improvement of the task performance (Fig. 4; block 1:  $0.27 \pm 0.07$ , block 10:  $0.25 \pm 0.02$ ,  $t = 0.95$ ,  $P = 0.38$ ) and no changes in the I–O slope in control experiment (Fig. 5C; pre:  $0.47 \pm 0.17$ , post:  $0.46 \pm 0.14$ , paired *t* test;  $t = 0.11$ ,  $P = 0.91$ ). In

**Table 1**  
bEMG (% of MVC) activity observed in each condition.

	bEMG activity (% of MVC)		
	Pre	Post	Paired <i>t</i> -test
1.0 $\times$ aMT	10.84 $\pm$ 1.42	10.97 $\pm$ 1.26	$P = 0.72$ , NS
1.2 $\times$ aMT	10.49 $\pm$ 1.28	10.95 $\pm$ 1.10	$P = 0.07$ , NS
1.4 $\times$ aMT	10.93 $\pm$ 1.48	11.04 $\pm$ 1.19	$P = 0.66$ , NS
1.6 $\times$ aMT	11.25 $\pm$ 1.40	11.06 $\pm$ 1.60	$P = 0.48$ , NS

NS: not significant, bEMG: background EMG, MVC: maximum voluntary contraction, aMT: active motor threshold.



**Figure 4.** The time courses of the performance scores for the LSL group (black circles), SSL group (white circles) and control group (white rhombus). The SSL group exhibited worse performance than the LSL group in most blocks. The learning did not occur in the control group.

addition, there was no significant difference in bEMG activity between the pre and the post sessions at any TMS intensity ( $P > 0.05$ ). These results suggest that the I–O slope did not change when learning did not occur.

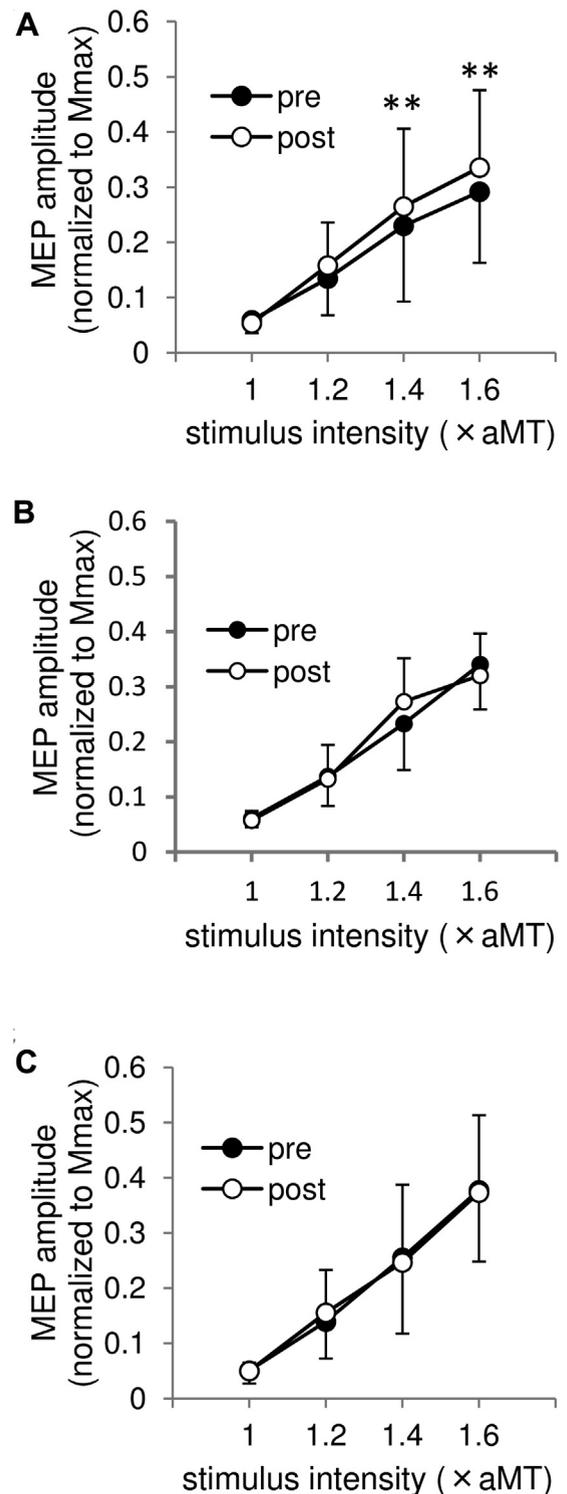
We also performed an analysis of the correlation between the degree of variability in the I–O slope and the number of blocks in the slow-learning stage. Surprisingly, the changes in the I–O slope were strongly correlated with the number of blocks in the slow-learning stage (Fig. 6A; Spearman's  $r = 0.70$ ,  $P < 0.01$ ). However, we also detected a significant negative relationship between the baseline I–O slope and the change in the I–O slope (Fig. 6B; Spearman's  $r = -0.47$ ,  $P < 0.05$ ). Furthermore, the group means of the baseline I–O slope tend to be larger in the SSL than the LSL group (paired  $t$  test;  $t = 1.76$ ,  $P = 0.10$ ). It is possible that the changes in the I–O slope induced by motor skill learning depend on the baseline excitability. Accordingly, we performed semi-partial correlation analysis. This method could be used to analyze a correlation between two variables except for the effects of one related variable. We analyze the correlation between the changes in the I–O slope and the number of blocks in the slow-learning stage after removing the influence of the baseline I–O slope only from the changes in the I–O slope. Then, we detected a significant relationship between the changes in the I–O slope and the number of blocks in the slow-learning stage (Spearman's semi-partial  $r = 0.62$ ,  $P < 0.01$ ). Similarly, we also performed semi-partial correlation analysis between the changes in the I–O slope and the baseline I–O slope after removing the influence of the number of blocks in the slow-learning stage. We found the no significant correlation between the changes in the I–O slope and the baseline I–O slope (Spearman's semi-partial  $r = -0.38$ ,  $P = 0.09$ ). Although the trend of correlation ( $P = 0.09$ ) was observed between the baseline I–O slope and the changes in the slope, these findings indicate that the subjects that displayed longer slow-learning stages also exhibited the larger increases in the I–O slope.

#### $H_{max}/M_{max}$

In order to investigate the spinal motoneuron pool excitability before and after the motor task, we calculated the ratio of the  $H_{max}$  to the  $M_{max}$  for the TA (LSL: pre;  $5.40 \pm 2.65\%$ , post;  $6.01 \pm 2.64\%$ , SSL: pre;  $4.93 \pm 3.84\%$ , post;  $5.34 \pm 3.88\%$  of  $M_{max}$ ). Two-way repeated measures ANOVA detected no significant main effect of group ( $F_{1,18} = 0.16$ ,  $P > 0.05$ ) and time ( $F_{1,18} = 0.97$ ,  $P > 0.05$ ) and group  $\times$  time interaction ( $F_{1,18} = 17.47$ ,  $P > 0.05$ ).

#### Relationship between changes in I–O slope and retention

All of the subjects performed the same task on two consecutive days. We calculated the degree of variability between the mean



**Figure 5.** Pre and post I–O curves for the LSL group (A), SSL group (B), control (C). \*\* $P < 0.01$ .

performance score for blocks 9–10 on Day 1 and the mean performance score for blocks 1–2 on Day 2 to evaluate the extent of skill retention ((Day 2 – Day 1)/Day 1). Figure 7A shows the mean performance score for blocks 9–10 on Day 1 and that for blocks 1–2 on Day 2 in each group. Two-way repeated measures ANOVA detected significant main effects of day ( $F_{1,18} = 5.32$ ,  $P < 0.05$ ) and group ( $F_{1,18} = 5.53$ ,  $P < 0.05$ ). In the SSL group, the mean

**Table 2**

Summary of three-way repeated measures ANOVA with factors of “group,” “intensity,” and “time” on I–O curve.

	df	F value	P value
Group	1	0.692	0.408, NS
Intensity	3	48.827	<b>&lt;0.001</b>
Time	1	6.874	<b>0.011</b>
Group × intensity	3	0.754	0.524, NS
Intensity × time	3	1.294	0.283, NS
Group × time	1	6.140	<b>0.016</b>
Group × intensity × time	3	1.894	0.138, NS

NS: not significant. Bold represents statistically significant results,  $P < 0.05$ .

performance score for the first 2 blocks on Day 2 was significantly worse than that for the last 2 blocks on Day 1 ( $t = 2.96$ ,  $P < 0.05$ ). The correlation between the degree of variability in the I–O slope and the retention was also analyzed. Interestingly, we detected a strong negative correlation between these variables (Fig. 7B; Spearman's  $r = -0.59$ ,  $P < 0.01$ ). Furthermore, we performed partial correlation analysis between the retention and the changes in the I–O slope and the baseline slope because these three factors were correlated with each other, respectively (Fig. 7B; retention and changes in the I–O slope:  $r = -0.59$ ,  $P < 0.01$ , Fig. 6B; changes in the I–O slope and baseline slope:  $r = -0.47$ ,  $P < 0.05$ , Fig. 7C; baseline slope and retention:  $r = 0.60$ ,  $P < 0.01$ ). The significant relationships between the retention and the changes in the I–O slope (Spearman's partial  $r = -0.43$ ,  $P = 0.05$ ) and between the retention and the baseline slope (Spearman's partial  $r = 0.46$ ,  $P < 0.05$ ) were also detected. These results suggest that both baseline I–O slope and changes in the I–O slope are good predictors of retention of newly acquired motor skills.

#### Sensitivity analysis about length of the slow-learning stage

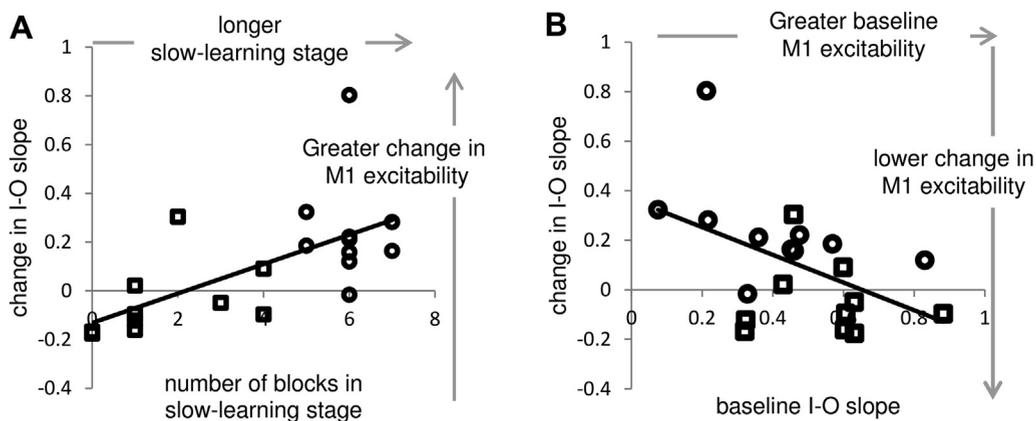
As described above, we found the significant relationships between the number of blocks in the slow-learning stage and the changes in the I–O slope, between the changes in the I–O slope and the retention. In this study, we measured the number of blocks in the slow-learning stage of Day 1 by the two-piece linear regression method using the data of Day 1 and Day 2. There is a question that the data of Day 2 affected the estimation of the number of blocks in the slow-learning stage of Day 1. Therefore, we should investigate whether our estimation method that includes the data of Day 1 and Day 2 could reflect the length of the slow-learning stage of Day 1 well. In order to solve this question, we performed a sensitivity

analysis about the length of the slow-learning stage. We fit the two piece-linear regression function on the data of Day 1 with the length of slow-learning stage fixed to the number of blocks that was determined by the two-piece linear regression function on the data of Day 1 and Day 2. Then we approximated the data of Day 1 with the length of slow-learning stage increased and decreased. We computed the  $R$  square value for each of three different fits. Based on the  $R$  square values, we redetermined the number of blocks in the slow-learning stage for each subjects (Table 3). And then, we performed a correlation analysis between the number of blocks in the slow-learning stage that was redetermined by above sensitivity analysis and the changes in the I–O slope. We found that the relationship between the number of blocks in the slow-learning stage and the changes in the I–O slope was still significant (Fig. 8, Spearman's  $r = 0.67$ ,  $P < 0.01$ ). The result suggests that our estimation could detect the slow-learning stage of Day 1 well.

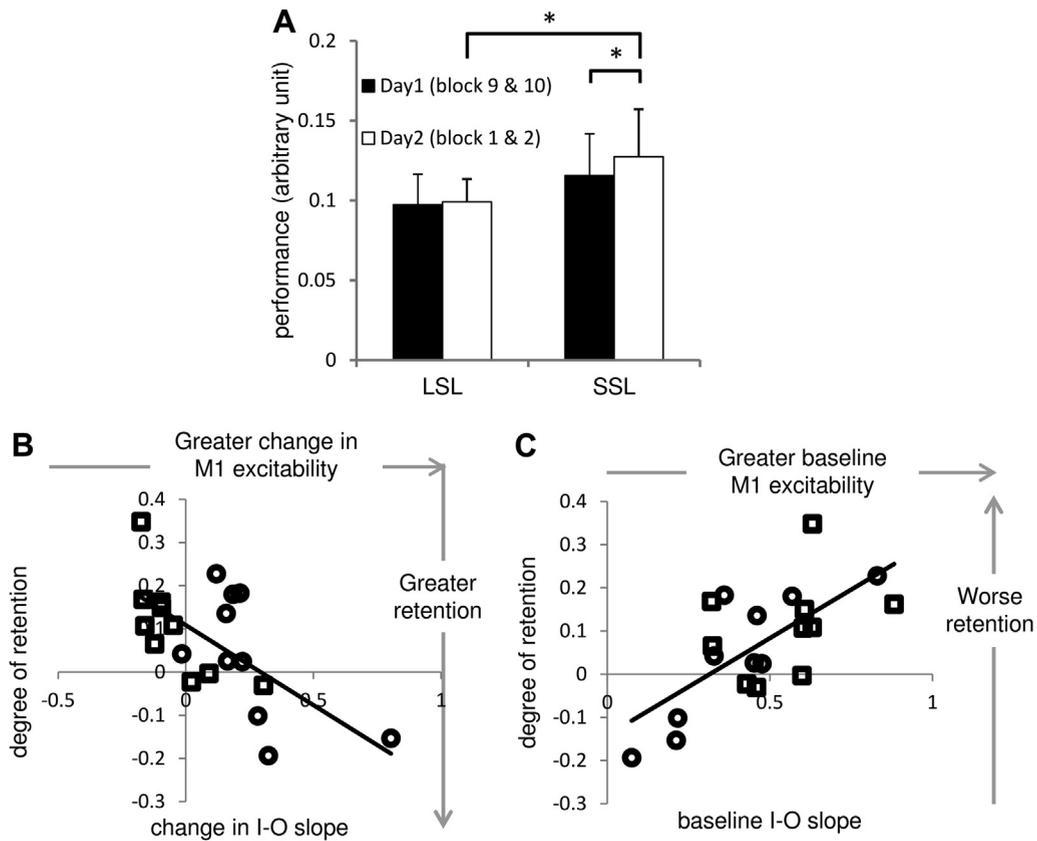
#### Experiment 2

##### Changes in I–O slope after fast-learning stage

All subjects in experiment 2 improved their performance during 10 blocks (1block:  $0.21 \pm 0.07$ , 10block:  $0.11 \pm 0.03$ ,  $t = 5.46$ ,  $P < 0.01$ ). There were no significant differences in any fundamental parameters, such as block 1 performance (experiment 1:  $0.21 \pm 0.07$ , experiment 2:  $0.21 \pm 0.07$ ;  $t = 0.027$ ,  $P = 0.98$ ), performance improvement (experiment 1:  $0.45 \pm 0.15$ , experiment 2:  $0.44 \pm 0.09$ ;  $t = 0.06$ ,  $P = 0.95$ ), endpoint of the fast-learning stage (experiment 1:  $6.10 \pm 2.55$ , experiment 2:  $4.75 \pm 1.83$ ;  $t = 1.36$ ,  $P = 0.19$ ), age (experiment 1:  $22.50 \pm 2.52$ years, experiment 2:  $22.50 \pm 1.77$ years;  $t = 0.00$ ,  $P = 1.00$ ), aMT (experiment 1:  $42.35 \pm 6.43\%$ , experiment 2:  $40.25 \pm 5.68\%$ ;  $t = 0.80$ ,  $P = 0.43$ ), and baseline I–O slope (experiment 1:  $0.47 \pm 0.20$ , experiment 2:  $0.65 \pm 0.25$ ,  $t = 1.95$ ,  $P = 0.06$ ) between the experiment 1 and 2. However, there was a significant difference in  $M_{max}$  (experiment 1:  $6.38 \pm 1.76$  mV, experiment 2:  $4.70 \pm 1.76$  mV,  $t = 2.42$ ,  $P < 0.05$ ) between the two experiments. Figure 9A shows the changes in the I–O slope at pre, post fast-learning, and post. One-way repeated ANOVA detected significant main effect of time ( $F_{2,14} = 8.69$ ,  $P < 0.01$ ). Post-hoc Shaffer's test revealed significant increases in the I–O slope at post compared with pre (pre:  $0.64 \pm 0.25$ , post:  $0.72 \pm 0.24$ ,  $P < 0.05$ ) and compared with post fast-learning (post fast-learning:  $0.67 \pm 0.26$ , post:  $0.72 \pm 0.24$ ,  $P < 0.05$ ). There are no significant changes in the I–O slope at post fast-learning (pre:  $0.64 \pm 0.25$ , post fast-learning:  $0.67 \pm 0.26$ ,  $P > 0.05$ ). Furthermore, there were no significant differences in bEMG activity between the



**Figure 6.** A: The relationship between the change in I–O slope and the number of blocks in the slow-learning stage. Circles represent LSL group and squares represent SSL group. B: The relationship between the baseline I–O slope and the change in I–O slope. Circles represent LSL group and squares represent SSL group. Spearman's  $r = -0.47$ ,  $P < 0.05$ .



**Figure 7.** A: The mean performance scores for blocks 9–10 on Day 1 and blocks 1–2 on Day 2 in each group. \* $P < 0.05$ . B: Correlation between the change in I–O slope and motor skill retention. Circles represent LSL group and squares represent SSL group. C: Correlation between the baseline I–O slope and motor skill retention. Spearman’s  $r = 0.60$ ,  $P < 0.01$ . Circles represent LSL group and squares represent SSL group.

pre ( $10.28 \pm 0.82\%$  of MVC), post fast-learning ( $9.93 \pm 0.72\%$ ), and post sessions ( $9.92 \pm 1.10\%$ ). As with experiment 1, we performed correlation analysis. We found significant relationship between the number of blocks in the slow-learning stage and the changes in the I–O slope at post (Fig. 9B; Spearman’s  $r = 0.75$ ,  $P < 0.05$ ), but not between the number of blocks in the fast-learning stage and the

changes in the I–O slope at post fast-learning (Fig. 9C; Spearman’s  $r = -0.05$ ,  $P = 0.91$ ). These results indicate that the changes in the I–O slope induced by motor skill learning occur during the slow-learning stage.

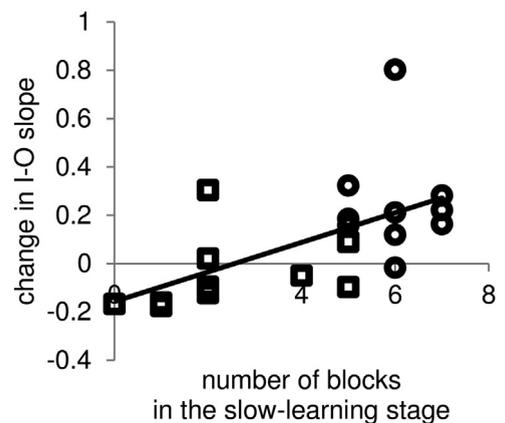
**Discussion**

The main findings of this study were as follows: 1) significant changes in the I–O slope were only observed in the LSL group, and

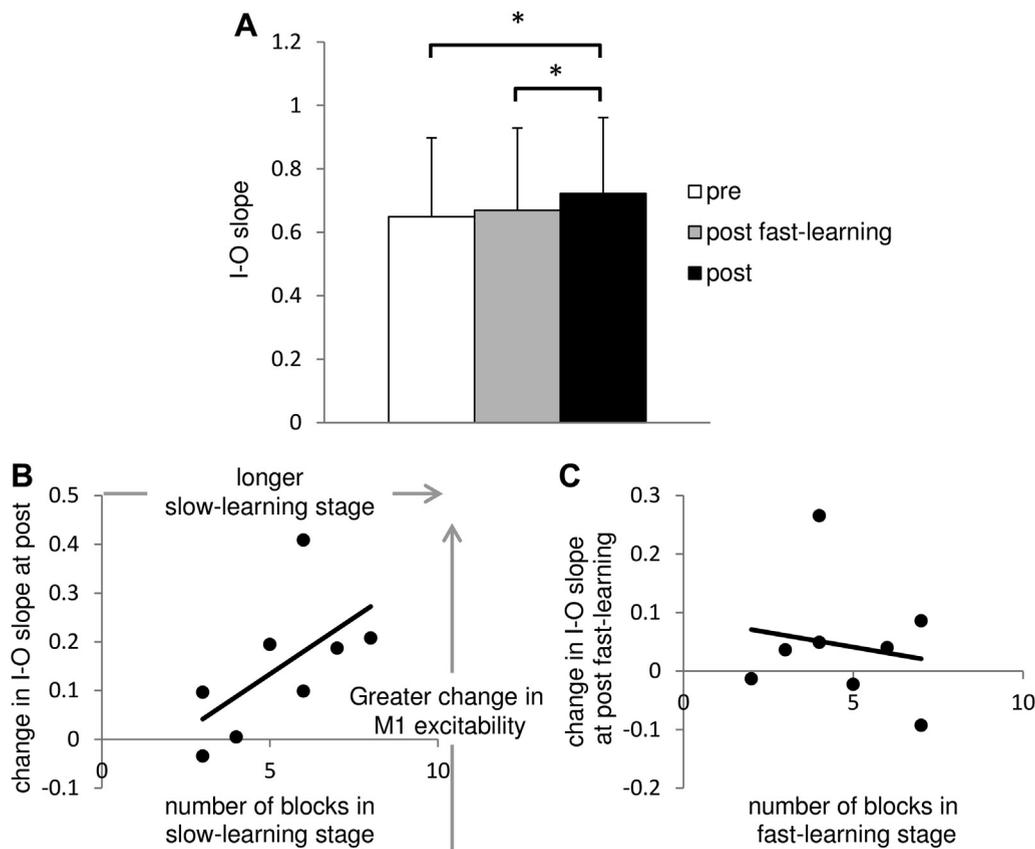
**Table 3**  
R square values for each of three different fits in all subjects.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
+1	<b>0.84539</b>	<b>0.55275</b>	0.841119	0.873338	<b>0.93013</b>
0	0.832081	0.270067	<b>0.87847</b>	<b>0.90855</b>	0.917469
-1	0.743708	–	0.777598	0.904241	0.881984
	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10
+1	0.966494	0.879416	0.96237	0.939964	0.572007
0	<b>0.97274</b>	<b>0.88957</b>	<b>0.9705</b>	<b>0.97325</b>	<b>0.80005</b>
-1	0.958644	0.790895	0.913931	0.867648	0.580349
	Subject 11	Subject 12	Subject 13	Subject 14	Subject 15
+1	0.532587	<b>0.72139</b>	0.887563	<b>0.92677</b>	0.844023
0	<b>0.77206</b>	0.72069	<b>0.93518</b>	0.923776	<b>0.88642</b>
-1	0.441165	0.711349	0.925175	0.915804	0.720391
	Subject 16	Subject 17	Subject 18	Subject 19	Subject 20
+1	<b>0.95555</b>	<b>0.74978</b>	0.921798	0.747663	<b>0.97598</b>
0	0.939831	0.744434	0.921323	<b>0.77583</b>	0.970946
-1	0.904503	0.735205	<b>0.92393</b>	–	0.967851

0 means the inflection point of the learning stages that was determined using the data of day1 and day2. +1 means plus 1 block to the 0 and -1 means minus 1 block from the 0. The R square values were determined using the data of day 1 only. The boldface represents the best R square value in each subjects. Because Subject 2 and Subject 19 did not have slow-learning stage in day 1, there are blank columns in -1.



**Figure 8.** The relationship between the I–O slope and the number of blocks in the slow-learning stage that redetermined by the sensitivity analysis. Circles represent LSL group and squares represent SSL group.



**Figure 9.** A: The changes in the I–O slope at pre, post-fast-learning, post in experiment 2. \* $P < 0.05$ . B: Correlation between the change in I–O slope at post and the number of blocks in the slow-learning stage. C: Correlation between the change in I–O slope at post fast-learning and the number of blocks in the fast-learning stage.

the changes were significantly correlated with the number of blocks in the slow-learning stage; 2) A strong relationship was detected between the learning-induced changes in the I–O slope and the retention of the skills required for the visuomotor tracking task. 3) The significant changes in the I–O slope occur after the slow-learning stage, but not after the fast-learning stage.

#### Relationship between changes in M1 excitability and learning stage

In experiment 1, the significant changes in the I–O slope were only observed in the LSL group, and the changes were correlated with the number of blocks in the slow-learning stage. In experiment 2, we found the significant changes in the I–O slope occur during the slow-learning stage, but not during the fast-learning stage. Furthermore, no changes in the I–O slope were found in the control task. The spinal motoneuron pool excitability ( $H_{max}/M_{max}$ ) was not changed by motor skill learning. These results indicate that M1 excitability is modulated during slow-learning stage of motor skill learning. Based on our results, we propose a possible mechanism for changes in M1 excitability induced by motor skill learning.

Two types of processing modes are known to operate during motor skill learning: an initial mode, which requires a great degree of effort to control, and an automatic mode [26]. Previous studies have suggested that a shift in processing mode from the initial to the automatic mode occurs during motor skill learning [6,11,27,28]. The initial mode is in operation during the fast-learning stage, and the automatic mode is employed during the slow-learning stage. Karni et al. (1998) suggested that the fast-learning stage involves processes aimed at searching for the optimal way of performing the

task [29]. In the present task, the subjects search the optimal way of performing the task with trial and error during the fast-learning stage. Therefore, it is possible that various motor commands are used to perform the task during the fast-learning stage. Conversely, optimal motor commands that are acquired during the fast-learning stage are repeatedly used to perform the task during the slow-learning stage. M1 plasticity is use-dependent [30,31]; in other words, changes in M1 excitability occur when same neural networks are repeatedly used. In an animal study, Kleim et al. (2004) demonstrated that motor map reorganization and synaptogenesis occur during late phase of motor skill learning [32]. Rioult-Pedotti et al. (2000) reported that motor learning occurs through long-term potentiation (LTP) involving horizontal connections in the M1 [33]. Masamizu et al. (2014) found that accuracy of lever movement (forelimb movement learned through training) predicted from neuronal ensemble activity in layer 5a of the forelimb M1 improves during the late stage of learning [34]. Previous study demonstrated that the disruption of M1 function by TMS in the slow-learning stage of adaptive learning could reduce adaptation [12]. Taken together, these findings suggest that plastic changes in the M1 such as LTP or rewiring of the neuronal circuits might occur during the slow-learning stage. A more plausible hypothesis is that changes in M1 excitability are induced by repeated use of same neural networks (i.e. optimal motor commands) during the slow-learning stage.

In this study, we found a significant negative relationship between the baseline M1 excitability and changes in M1 excitability induced by motor skill learning in experiment 1. It is possible that further increasing M1 excitability is difficult when the baseline excitability was already high. Therefore, no changes in M1

excitability in the SSL group might be caused by the high baseline excitability. However, we detected a significant correlation between the changes in I–O slope and the number of blocks in the slow-learning stage although we performed semi-partial correlation analysis which removes the influence of the baseline slope only from the changes in I–O slope. Furthermore, the relationships between the baseline I–O slope and the changes in the I–O slope diminished when we performed semi-partial correlation analysis. Therefore, the relationship between the changes in M1 excitability and slow-learning stage certainly exists.

#### *No changes in M1 excitability on average of all subjects in experiment 1*

We observed a high degree of inter-individual variability in the learning-induced changes in M1 excitability in experiment 1, although all of the subjects improved their performance during the study period. For this reason, significant changes in M1 excitability were not detected by the average of all subjects in experiment 1. Recently, Schlerf et al. (2012) demonstrated that visuomotor adaptation does not cause changes in cortico-spinal excitability [35]. Furthermore, it was demonstrated that changes in cortico-spinal excitability after walking adaptation are linked to task difficulty but not to adaptation [36]. However, many studies have detected significant changes in M1 excitability induced by motor skill learning [37–40]. There are some possibilities that the different results are observed among studies including our study. First, it is possible that different processes involve in motor adaptation and motor skill learning. Second, the differences of the tasks may affect the results. Target line was changed every trial in our visuomotor tracking task. However, in most of the studies using visuomotor tracking task, several type of target lines were presented repetitively during the task [20,37]. This reason might lead slow performance improvement to our subjects than other previous studies and that's why there were no changes in M1 excitability on average. Third, we recorded the MEP from TA by double-cone coil. It has been demonstrated that the component of I-wave is different between recording MEP from hand muscle by figure-of-eight coil and from leg muscle by double cone coil [41]. Furthermore, we asked the subjects to maintain isometric ankle dorsiflexion during MEP recording in order to reduce the threshold required to evoke MEP in the TA. Voluntary muscle contraction induces some physiological phenomena in M1 (e.g. reduction of short-interval intracortical inhibition) [42]. Most of previous studies that demonstrated changes in M1 excitability after motor skill learning measured the MEP in a resting condition [20,37]. These possibilities might affect the results. However, we detected significant increases in M1 excitability after learning in experiment 2. It is because that we excluded the subjects who did not finish the fast-learning stage within 10 training blocks. When we exclude the data of subjects that the number of blocks in the slow-learning stage is 0 or 1, we could detect a significant increase in the I–O slope in experiment 1. Therefore we emphasize that inter-individual variability of length of the slow-learning stage affects strongly to the results.

#### *Relationship between changes in M1 excitability and motor skill retention*

To best of our knowledge, our study is the first to demonstrate the relationship between the leg M1 excitability and the performance retention. Recently, several studies have demonstrated that the M1 plays a crucial role in the retention of learned motor skills [43,44]. Most of these studies investigated the role of the hand M1 in the modulation of cortical excitability before or after task

learning using rTMS or tDCS [45,46]. However, motor skill learning itself is able to induce changes in M1 excitability. In addition, the homeostatic meta-plastic principle, which is the alteration of the efficacy of the intervention depend on “cortical history,” should be taken into consideration when such interventions are employed before or after motor learning [47]. However, previous studies that investigated the relationship between motor skill retention and M1 excitability using interventions such as rTMS or tDCS ignored learning-induced changes in M1 excitability and the homeostatic meta-plastic principle. If the M1 stores motor memory, changes in M1 excitability induced by motor skill learning will be associated with retention of learned motor skills. Previous study demonstrated that LTP-like plasticity is essential for performance retention [48,49]. In the present study, a reduction in the performance score during the first two blocks performed on Day 2 only occurred in the SSL group. Furthermore, we detected a significant correlation between the changes in leg M1 excitability and the motor skill retention. Our results agree with those of a previous study [19]. Previous study demonstrated that the disruption of the M1 function by TMS in the slow-learning stage of adaptive learning could impede the adaptation [12]. As mentioned above, the repeated use of optimal motor commands during the slow-learning stage induces changes in M1 excitability. We speculate that increasing M1 excitability during the slow-learning stage reflects the strong formation of the optimal neural networks. Therefore, the strong correlation between the changes in M1 excitability and the retention was found.

Strangely, we observed a significant negative relationship between the baseline M1 excitability and the performance retention. Many previous studies indicated the M1 plays critical role in performance retention. However, we are not able to explain well why the baseline M1 excitability had relationship with the retention (the lower the baseline excitability, the retention is good). This result suggests that baseline M1 excitability might have relationship with retention.

## Conclusions

The results of the present study provided two insights about motor skill learning. 1) M1 excitability is modulated during the slow-learning stage of motor skill learning. 2) The degree of motor skill retention observed on the next day was strongly correlated with the learning-induced changes in leg M1 excitability. We suggest that changes in M1 excitability reflects the strong formation of the neural networks which represents the optimal motor commands, and such phenomenon occurs during the slow-learning stage of motor skill learning because optimal motor commands were repeatedly used during the slow-learning stage. We argue that the present results improve our knowledge regarding motor skill learning.

## Appendix. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.brs.2015.07.025>.

## References

- [1] Jensen JL, Marstrand PCD, Nielsen JB. Motor skill training and strength training are associated with different plastic changes in the central nervous system. *J Appl Phys* 2005;99:1558–68. <http://dx.doi.org/10.1152/jappphysiol.01408.2004>.
- [2] Ljubisavljevic M. Transcranial magnetic stimulation and the motor learning-associated cortical plasticity. *Exp Brain Res* 2006;173:215–22. <http://dx.doi.org/10.1007/s00221-006-0538-z>.
- [3] Bagece HF, Saleh S, Adamovich SV, Krakauer JW, Tunik E. Corticospinal excitability is enhanced after visuomotor adaptation and depends on learning

- rather than performance or error. *J Neurophysiol* 2013;109:1097–106. <http://dx.doi.org/10.1152/jn.00304.2012>.
- [4] Sanes JN, Donoghue JP. Plasticity and primary motor cortex. *Annu Rev Neurosci* 2000;23:393–415. <http://dx.doi.org/10.1146/annurev.neuro.23.1.393>.
- [5] Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. *Exp Brain Res* 2001;136:431–8. <http://dx.doi.org/10.1007/s00221000614>.
- [6] Park J-W, Kim Y-H, Jang SH, Chang WH, Park C, Kim ST. Dynamic changes in the cortico-subcortical network during early motor learning. *Neuro-Rehabilitation* 2010;26:95–103. <http://dx.doi.org/10.3233/NRE-2010-0540>.
- [7] Dayan E, Cohen LG. Neuroplasticity subserving motor skill learning. *Neuron* 2011;72:443–54. <http://dx.doi.org/10.1016/j.neuron.2011.10.008>.
- [8] Doyon J, Benali H. Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol* 2005;15:161–7. <http://dx.doi.org/10.1016/j.conb.2005.03.004>.
- [9] Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 1995;377:155–8. <http://dx.doi.org/10.1038/377155a0>.
- [10] Müller R-A, Kleinhans N, Pierce K, Kemmotsu N, Courchesne E. Functional MRI of motor sequence acquisition: effects of learning stage and performance. *Cogn Brain Res* 2002;14:277–93. [http://dx.doi.org/10.1016/S0926-6410\(02\)00131-3](http://dx.doi.org/10.1016/S0926-6410(02)00131-3).
- [11] Adi-Japha E, Karni A, Parnes A, Loewenschuss I, Vakil E. A shift in task routines during the learning of a motor skill: group-averaged data may mask critical phases in the individuals' acquisition of skilled performance. *J Exp Psychol Learn Mem Cogn* 2008;34:1544–51. <http://dx.doi.org/10.1037/a0013217>.
- [12] Orban de Xivry J-J, Criscimagna-Hemminger SE, Shadmehr R. Contributions of the motor cortex to adaptive control of reaching depend on the perturbation schedule. *Cereb Cortex* 2011;21:1475–84. <http://dx.doi.org/10.1093/cercor/bhq192>.
- [13] Bütefisch CM, Davis BC, Wise SP, et al. Mechanisms of use-dependent plasticity in the human motor cortex. *Proc Natl Acad Sci U S A* 2000;97:3661–5. <http://dx.doi.org/10.1073/pnas.050350297>.
- [14] Kantak SS, Winstein CJ. Learning-performance distinction and memory processes for motor skills: a focused review and perspective. *Behav Brain Res* 2012;228:219–31. <http://dx.doi.org/10.1016/j.bbr.2011.11.028>.
- [15] Muellbacher W, Ziemann U, Wissel J, et al. Early consolidation in human primary motor cortex. *Nature* 2002;415:640–4. <http://dx.doi.org/10.1038/nature712>.
- [16] Hunter T, Sacco P, Nitsche MA, Turner DL. Modulation of internal model formation during force field-induced motor learning by anodal transcranial direct current stimulation of primary motor cortex. *J Physiol* 2009;587:2949–61. <http://dx.doi.org/10.1113/jphysiol.2009.169284>.
- [17] Smyth C, Summers JJ, Garry MI. Differences in motor learning success are associated with differences in M1 excitability. *Hum Mov Sci* 2010;29:618–30. <http://dx.doi.org/10.1016/j.humov.2010.02.006>.
- [18] Hadipour-Niktarash A, Lee CK, Desmond JE, Shadmehr R. Impairment of retention but not acquisition of a visuomotor skill through time-dependent disruption of primary motor cortex. *J Neurosci* 2007;27:13413–9. <http://dx.doi.org/10.1523/JNEUROSCI.2570-07.2007>.
- [19] Tunovic S, Press DZ, Robertson EM. A physiological signal that prevents motor skill improvements during consolidation. *J Neurosci* 2014;34:5302–10. <http://dx.doi.org/10.1523/JNEUROSCI.3497-13.2014>.
- [20] Perez Ma, Lungholt BKS, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Exp Brain Res* 2004;159:197–205. <http://dx.doi.org/10.1007/s00221-004-1947-5>.
- [21] Roberts DR, Ricci R, Funke FW, et al. Lower limb immobilization is associated with increased corticospinal excitability. *Exp Brain Res* 2007;181:213–20. <http://dx.doi.org/10.1007/s00221-007-0920-5>.
- [22] Roy FD, Norton JA, Gorassini MA. Role of sustained excitability of the leg motor cortex after transcranial magnetic stimulation in associative plasticity. *J Neurophysiol* 2007;98:657–67. <http://dx.doi.org/10.1152/jn.00197.2007>.
- [23] Weier AT, Pearce AJ, Kidgell DJ. Strength training reduces intracortical inhibition. *Acta Physiol* 2012;206:109–19. <http://dx.doi.org/10.1111/j.1748-1716.2012.02454.x>.
- [24] Hirano M, Kubota S, Morishita T, Uehara K, Fujimoto S, Funase K. Long-term practice induced plasticity in the primary motor cortex innervating the ankle flexor in football juggling experts. *Motor Control* 2014;18:310–21. <http://dx.doi.org/10.1123/mc.2013-0059>.
- [25] Chapman JP, Chapman LJ, Allen JJ. The measurement of foot preference. *Neuropsychologia* 1987;25:579–84. [http://dx.doi.org/10.1016/0028-3932\(87\)90082-0](http://dx.doi.org/10.1016/0028-3932(87)90082-0).
- [26] Chein JM, Schneider W. Neuroimaging studies of practice-related change: fMRI and meta-analytic evidence of a domain-general control network for learning. *Brain Res Cogn Brain Res* 2005;25:607–23. <http://dx.doi.org/10.1016/j.cogbrainres.2005.08.013>.
- [27] Korman M, Raz N, Flash T, Karni A. Multiple shifts in the representation of a motor sequence during the acquisition of skilled performance. *Proc Natl Acad Sci U S A* 2003;100:12492–7. <http://dx.doi.org/10.1073/pnas.2035019100>.
- [28] Floyer-Lea A, Matthews PM. Changing brain networks for visuomotor control with increased movement automaticity. *J Neurophysiol* 2004;92:2405–12. <http://dx.doi.org/10.1152/jn.01092.2003>.
- [29] Karni A, Meyer G, Rey-Hipolito C, et al. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. *Proc Natl Acad Sci U S A* 1998;95:861–8.
- [30] Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J Neurosci* 1996;16:785–807.
- [31] Plautz EJ, Milliken GW, Nudo RJ. Effects of repetitive motor training on movement representations in adult squirrel monkeys: role of use versus learning. *Neurobiol Learn Mem* 2000;74:27–55. <http://dx.doi.org/10.1006/nlme.1999.3934>.
- [32] Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, Rempie M. Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *J Neurosci* 2004;24:628–33. <http://dx.doi.org/10.1523/JNEUROSCI.3440-03.2004>.
- [33] Rioult-Pedotti M-S, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. *Science* 2000;290:533–6. <http://dx.doi.org/10.1126/science.290.5491.533>.
- [34] Masamizu Y, Tanaka YR, Tanaka YH, et al. Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task. *Nat Neurosci* 2014;17:987–94. <http://dx.doi.org/10.1038/nn.3739>.
- [35] Schlerf JE, Galea JM, Bastian AJ, Celnik PA. Dynamic modulation of cerebellar excitability for abrupt, but not gradual, visuomotor adaptation. *J Neurosci* 2012;32:11610–7. <http://dx.doi.org/10.1523/JNEUROSCI.1609-12.2012>.
- [36] Jayaram G, Galea JM, Bastian AJ, Celnik P. Human locomotor adaptive learning is proportional to depression of cerebellar excitability. *Cereb Cortex* 2011;21:1901–9. <http://dx.doi.org/10.1093/cercor/bhq263>.
- [37] Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. *Eur J Neurosci* 2011;21:1901–9. <http://dx.doi.org/10.1111/j.1460-9568.2011.07870.x>.
- [38] Lotze M, Braun C, Birbaumer N, Anders S, Cohen LG. Motor learning elicited by voluntary drive. *Brain* 2003;126:866–72. <http://dx.doi.org/10.1093/brain/awg079>.
- [39] Rosenkranz K, Kacar A, Rothwell JC. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. *J Neurosci* 2007;27:12058–66. <http://dx.doi.org/10.1523/JNEUROSCI.2663-07.2007>.
- [40] Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol* 1995;74:1037–45.
- [41] Terao Y, Ugawa Y, Hanajima R, et al. Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. *Brain Res* 2000;859:137–46. [http://dx.doi.org/10.1016/S0006-8993\(00\)01975-2](http://dx.doi.org/10.1016/S0006-8993(00)01975-2).
- [42] Ridding M, Taylor J, Rothwell J. The effect of voluntary contraction on corticocortical inhibition in human motor cortex. *J Physiol* 1995;487:541–8.
- [43] Reis J, Schambra HM, Cohen LG, et al. Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci U S A* 2009;106:1590–5. <http://dx.doi.org/10.1073/pnas.0805413106>.
- [44] Cothros N, Köhler S, Dickie EW, Mirsattari SM, Gribble PL. Proactive interference as a result of persisting neural representations of previously learned motor skills in primary motor cortex. *J Cogn Neurosci* 2006;18:2167–76. <http://dx.doi.org/10.1162/jocn.2006.18.12.2167>.
- [45] Galea JM, Vazquez A, Pasricha N, de Xivry J-JO, Celnik P. Dissociating the roles of the cerebellum and motor cortex during adaptive learning: the motor cortex retains what the cerebellum learns. *Cereb Cortex* 2011;21:1761–70. <http://dx.doi.org/10.1093/cercor/bhq246>.
- [46] Richardson AG, Overduin SA, Valero-Cabré A, et al. Disruption of primary motor cortex before learning impairs memory of movement dynamics. *J Neurosci* 2006;26:12466–70. <http://dx.doi.org/10.1523/JNEUROSCI.1139-06.2006>.
- [47] Ziemann U, Iliac TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci* 2004;24:1666–72. <http://dx.doi.org/10.1523/JNEUROSCI.5016-03.2004>.
- [48] Cantarero G, Lloyd A, Celnik P. Reversal of long-term potentiation-like plasticity processes after motor learning disrupts skill retention. *J Neurosci* 2013;33:12862–9. <http://dx.doi.org/10.1523/JNEUROSCI.1399-13.2013>.
- [49] Orban de Xivry J-J, Ahmadi-Pajouh MA, Harran MD, Salimpour Y, Shadmehr R. Changes in corticospinal excitability during reach adaptation in force fields. *J Neurophysiol* 2013;109:124–36. <http://dx.doi.org/10.1152/jn.00785.2012>.