

Changes in Cerebello-motor Connectivity during Procedural Learning by Actual Execution and Observation

Sara Torriero^{1,2}, Massimiliano Oliveri^{1,2}, Giacomo Koch^{1,3},
Emanuele Lo Gerfo¹, Silvia Salerno¹, Fabio Ferlazzo⁴,
Carlo Caltagirone^{1,3}, and Laura Petrosini^{1,4}

Abstract

■ The cerebellum is involved in motor learning of new procedures both during actual execution of a motor task and during observational training. These processes are thought to depend on the activity of a neural network that involves the lateral cerebellum and primary motor cortex (M1). In this study, we used a twin-coil TMS technique to investigate whether execution and observation of a visuomotor procedural learning task is related to modulation of cerebello-motor connectivity. We observed that, at rest, a magnetic conditioning pulse applied over the lateral cerebellum reduced the motor-evoked potentials obtained by stimulating the contralateral M1, indicat-

ing activation of a cerebello-motor connection. Furthermore, during procedural learning, cerebellar stimulation resulted in selective facilitation, not inhibition, of contralateral M1 excitability. The effects were evident when motor learning was obtained by actual execution of the task or by observation, but they disappeared if procedural learning had already been acquired by previous observational training. These results indicate that changes in cerebello-motor connectivity occur in relation to specific phases of procedural learning, demonstrating a complex pattern of excitatory and inhibitory drives modulated across time. ■

INTRODUCTION

In motor learning, plastic changes occur in primary motor cortex (Monfils, Plautz, & Kleim, 2005; Pascual-Leone, Grafman, & Hallett, 1994) and in interconnected areas, such as the cerebellum, leading to consolidation of new motor engrams. Experimental findings show that the cerebellum is involved in learning new procedures during the actual execution of a task and during observational training (Ito, 2008; Petrosini, 2007; Torriero, Oliveri, Koch, Caltagirone, & Petrosini, 2004, 2007; Leggio et al., 1999, 2000) and, in particular, that it plays a key role in sequential procedural learning, as measured by a serial reaction time task (SRTT) (Gómez-Beldarrain, García Moncò, Rubio, & Pascual-Leone, 1998; Molinari et al., 1997; Pascual-Leone, Grafman, Clark, Stewart, & Massaquoi, 1993). Furthermore, it has been shown that cerebellar rTMS decreases procedural learning during performance of an SRTT (Torriero et al., 2004) and interferes with acquisition of procedural competencies through observation (Torriero et al., 2007).

The pathway connecting the cerebellum and primary motor cortex has been identified. Specifically, cerebellar cortical efferents exert inhibitory control over deep cere-

bellar nuclei, and thus, modulate the excitatory projections toward contralateral brain areas (Ito, 1984). In healthy subjects at rest, when a TMS conditioning pulse (CS) was applied over the lateral cerebellum, it reduced the amplitudes of motor-evoked potentials (MEPs) from contralateral motor cortex when the ISI was 5 to 6 msec (Koch, Mori, et al., 2008; Iwata & Ugawa, 2005; Luft, Manto, & Taib, 2005; Oliveri, Koch, Torriero, & Caltagirone, 2005; Daskalakis et al., 2004; Iwata et al., 2004; Werhahn, Taylor, Ridding, Meyer, & Rothwell, 1996; Ugawa, Uesaka, Terao, Hanajima, & Kanazawa, 1995). Interestingly, this inhibitory effect was absent in patients with cerebellar lesions (Di Lazzaro et al., 1994).

Although these previous studies were conducted with subjects at rest, recent evidence suggests that with paired-pulse TMS protocols, it is possible to investigate task-related changes in the excitability of a given cortical connection. The advantage of probing these pathways with a TMS method is that the response to a TMS conditioning pulse depends on the state of excitability of the pathways at the time the stimulus is applied. Thus, changes in the effectiveness of the conditioning pulse indicate how the excitability of the connection changes over time when the cortical networks become active during a specific motor task (Davare, Lemon, & Olivier, 2008; Koch, Del Olmo, et al., 2008; O'Shea, Sebastian, Boorman, Johansen-Berg, & Rushworth, 2007; Koch et al., 2006).

¹IRCCS Fondazione Santa Lucia, Rome, Italy, ²Università di Palermo, Palermo, Italy, ³Università di Roma Tor Vergata, Rome, Italy, ⁴Università "Sapienza" di Roma, Rome, Italy

Although this approach has revealed selective activation of connections originating in posterior parietal cortex and premotor cortex (Koch & Rothwell, 2009), this method has not yet been adopted to investigate cerebellar connections.

Therefore, the present study was designed to investigate the changes that occur in the connection between the cerebellum and primary motor cortex while subjects are performing a procedural learning task.

We speculated that if these connections were involved in different aspects of procedural motor learning, we would find task-related changes in the excitability of the cerebello-thalamo-motor pathways. We hypothesized that the strength of these connections would increase during acquisition of novel motor procedures, both if the subjects were actually executing the motor task and if they were observing other subjects learning the same procedure. Therefore, we studied the changes in MEP amplitudes in the right M1 provoked by CS over the left cerebellar hemisphere at different ISIs when this protocol was applied during a modified version of an SRTT. First, we investigated the role of the cerebellum in modulating contralateral motor cortex activity during the actual execution of ordered and random sequences. Then, we analyzed cerebello-motor connectivity during observational learning of the same sequences.

METHODS

We studied 22 right-handed healthy subjects (5 men, 17 women; mean age = 26.2 ± 4.3 years). All participants gave

their written informed consent to take part in the research. The study was conducted according to the Declaration of Helsinki and the experiments were approved by the IRCCS “S. Lucia” Ethical Committee.

Procedural Learning Task

Subjects were seated in front of a computer touch screen and MEPs were recorded from the right first dorsal interosseus (FDI) muscle during execution or observation of a modified version of the SRTT (Figure 1). Subjects were instructed to use their right index finger to touch the black squares (1 cm × 1 cm) that appeared on a white background. The black squares could appear in four different positions (1, 2, 3, 4, from left to right) along a virtual horizontal line; the distance between the squares’ positions was such that the subjects were able to reach them by adduction or abduction of the finger without moving the wrist. Each black square disappeared as soon as the subject touched it and a new black square appeared in another position after 250 msec. The task consisted of performing different sequences, each composed of 240 squares. The sequences differed in that square positions were: (1) randomly selected (*random*) or (2) in an ordered sequence of eight items, repeated 30 times (*ordered*). In Nissen and Bullemer’s (1987) SRTT, the same sequence was composed of succeeding blocks: In the first and last blocks, trials were random, and in the central

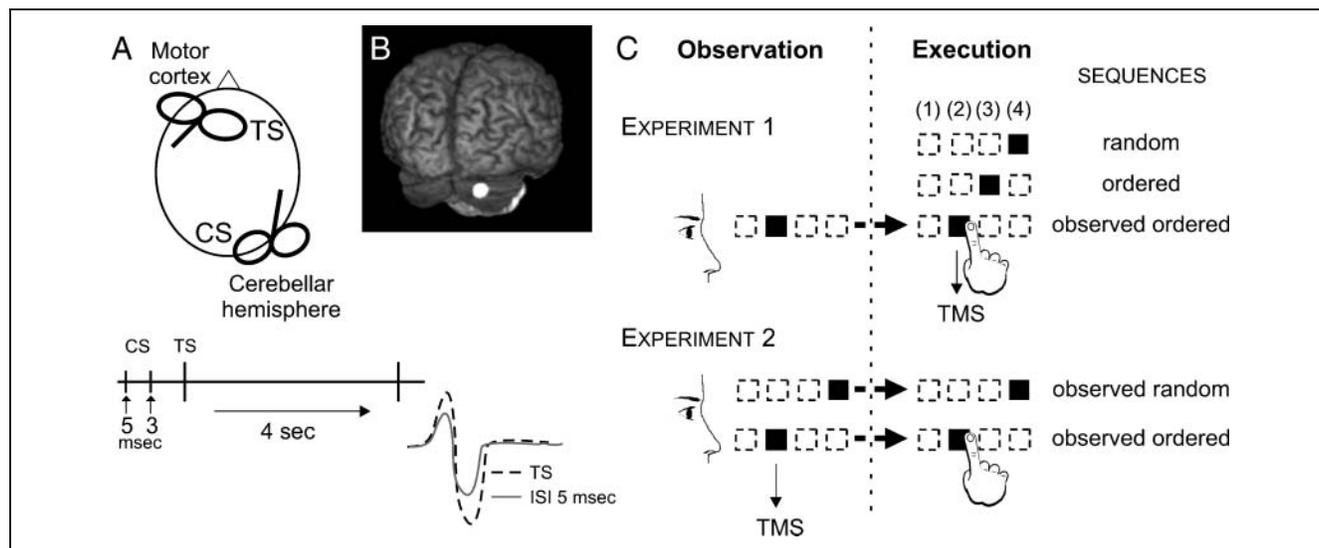


Figure 1. Experimental procedure. (A) Using a twin-coil technique, the test stimulus (TS) was applied over left motor cortex and the conditioning stimulus (CS) was applied over the right cerebellar hemisphere. The TS was delivered alone or preceded by a CS, with an ISI of 3 or 5 msec; TMS pulses were applied every 4 sec and MEPs were recorded from the right FDI muscle. At rest, the 5-msec ISI induced a decrease in MEP amplitudes. (B) The coil position for the right cerebellar hemisphere was determined using the neuronavigation system, in individual anatomic magnetic resonance images (a white circle indicates the Talairach and Tournoux coordinates in a representative subject: 24, -95, -22). (C) The procedural learning task consisted of performing sequences that differed for square positions, which were randomly selected (*random*) or in an ordered repeated sequence of eight items (*ordered*). In one condition in Experiment 1 and throughout Experiment 2, subjects observed an actor’s performance and then executed the observed sequences (*observed ordered* and *observed random*). TMS pulses were applied approximately every six visual cues of the sequences during actual execution of the task (Experiment 1) and during observational learning of the same sequences (Experiment 2).

blocks, trials were ordered. In this study, we separated random and ordered blocks into two different sequences. This adjustment of the classic SRTT allowed us to differentiate changes in connectivity occurring in random and ordered conditions. The sequences were performed either without training (*random sequence* and *ordered sequence*) or after observational training (*observed random sequence* and *observed ordered sequence*). In the observed random and observed ordered sequence conditions, subjects were told to observe carefully as an actor performed the task and were informed that at the end of the observation phase they would have to perform the same task. Subjects observed the actor's performance (which lasted approximately 3 min) and were requested to execute it immediately.

Subjects were not informed about the presence of random or ordered sequences in any of the conditions and were only instructed to touch the squares that appeared on the screen. The order of the conditions was counter-balanced among subjects.

As the SRTT is a blend of procedural and declarative components, the reciprocal interaction between declarative and procedural memory systems cannot be excluded (Robertson, 2007). To minimize the contribution of declarative aspects, at the end of the experiments we asked the subjects whether they had become aware of the presence of a sequence during observation/execution of the task. The subjects were asked how many items there were in the sequence. Then they were shown the squares in the four positions and were asked to indicate the order of the items. Subjects who were able to describe the proposed sequence totally or partially were excluded.

Transcranial Magnetic Stimulation

Paired TMS was applied to explore the connectivity between the cerebellar hemisphere and the contralateral motor cortex during the procedural learning task. EMG traces were recorded from the FDI muscles of the right hand using 9-mm diameter Ag–AgCl surface cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified with a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK) through filters set at 20 Hz and 2 kHz, with a sampling rate of 5 kHz; then, they were recorded by a computer using SIGNAL software (Cambridge Electronic Devices, Cambridge, UK). We used a paired-pulse stimulation technique with two high-power Magstim 200 machines (Magstim Co., Whitland, Dyfed, UK). The magnetic stimulus had a nearly monophasic pulse configuration with a rise time of about 100 μ sec, decaying back to zero over about 0.8 msec.

Magstim 200 Mono Pulse magnetic stimulators were connected to two separate figure-of-eight coils (70 mm in diameter). The paired TMS technique consists of a con-

ditioning stimulus (CS) followed by a suprathreshold test stimulus (TS) at different ISIs. As all subjects were right-handed, TS was applied over left motor cortex in the optimal scalp position for induction of the largest MEPs in the right FDI muscle. The coil was positioned tangentially to the scalp, with the handle pointing backward and laterally at a 45° angle away from the midline (Figure 1A). TS was set at an intensity that, when given alone, would evoke an EMG response of about 1 mV peak-to-peak whether the experiments were conducted with muscle contraction (see Experiment 1 below) or at rest (Experiment 2).

We determined the CS application site by means of the neuronavigation system (Softaxic, E.M.S., Bologna, Italy), using individual anatomical magnetic resonance images. The coil was positioned over the superior posterior lobule of the right cerebellar hemisphere (Koch et al., 2007); the individual coordinates of each stimulation site were normalized a posteriori into the Talairach coordinate system and averaged (21, -94, -25) (Figure 1B). The coil was positioned tangentially to the scalp, with the handle pointing upward. CS intensity was set at 90% of the resting motor threshold measured over contralateral motor cortex (Koch, Mori, et al., 2008). We tested the 5-msec ISI to study modulation of the inhibitory drive from the cerebellum to contralateral motor cortex (Figure 1A). We tested the 3-msec ISI as a control condition. Previous studies have reported contrasting effects of cerebellar stimulation over contralateral motor cortex at the 3-msec ISI; these range from facilitatory effects using electrical stimulation (Iwata et al., 2004) to the absence of any effect using magnetic stimulation with a figure-of-eight coil (Koch, Mori, et al., 2008). Independently of its effects, we considered the 3-msec ISI as a noninhibitory condition in contrast with the expected inhibitory effect of the 5-msec ISI.

MEPs were recorded in separate conditions: first, in a resting condition, that is, with the hand relaxed and without performing any actual or observational sequential task, and then during actual execution or observation of the task. Each recording session consisted of 36 trials: 12 single pulses (TS), 12 paired pulses (CS + TS) at the 3-msec ISI, and 12 paired pulses (CS + TS) at the 5-msec ISI, applied randomly. Single or paired magnetic pulses were delivered randomly during actual execution of the task, regardless of the actual finger position (Experiment 1), or during observational training (Experiment 2), with an intertrial interval of 4 sec \pm 10%. Therefore, magnetic stimuli were applied in approximately every six visual cues of the sequence and were not triggered by the presentation of the visual stimuli. Thus, application of TMS pulses could be associated with different finger positions and, consequently, with different levels of FDI muscle contraction. Because MEPs are highly dependent on the state of activation of the hand, in Experiment 1, MEPs varied during the same sequence in response to TS and CS. Nevertheless, this variability was present in all sequences and its effects were balanced among subjects.

Experiments

In the first experiment, we studied cerebello-motor connectivity by analyzing the influence of paired-pulse TMS applied during actual execution of the procedural learning task. Twelve subjects (3 men, 9 women; mean age = 28.1 ± 4.9 years) were recruited for this experiment. They were instructed to touch the squares that appeared on the screen. They were required to perform the task three times, for the three different sequences. Subjects were not informed about the differences among the three sequences. They had to perform: a *random sequence*, an ordered sequence never seen before (*ordered sequence* — 41324231) and an ordered sequence previously observed (*observed ordered sequence* — 14232413). The sequences were performed without any training. Before executing the observed ordered sequence, subjects were submitted to observational training, that is, they had to carefully observe the actor's performance. The presentation order of the three sequences was counterbalanced among subjects. TMS was always applied during actual execution of the sequences (Figure 1C).

In the second experiment, we studied cerebello-motor connectivity by analyzing the influence of paired-pulse TMS applied during observational training prior to actual execution of the task. Ten subjects were recruited for this experiment (2 men, 8 women; mean age = 24.8 ± 2.6 years). As in Experiment 1, they were instructed to touch the squares that appeared on the screen. Unlike Experiment 1, in this experiment, two sequences were proposed: an *observed random sequence* and an *observed ordered sequence* (14232413). Subjects were not informed about the difference between the two sequences. They observed the actor perform the ordered and the random sequence. Immediately after they had observed each sequence, the subjects were requested to execute it. The presentation order of the sequences was counterbalanced among subjects. TMS was always applied during the observational training preceding actual execution of the sequences (Figure 1C).

Data Analysis

RTs, defined as the latencies between the appearance of the visual cue and the correct touch on the screen, were recorded during execution of the procedural learning task, both in random and ordered sequences. For all conditions, we calculated mean RTs for five blocks; each block was equivalent to six repetitions of the eight-item ordered sequence. Procedural learning was defined as a significant difference in RTs between random and ordered sequences along the five blocks of the task. A two-way ANOVA was performed with sequence (random vs. ordered vs. observed ordered in Experiment 1; random vs. ordered in Experiment 2) and block (I–V) as within-subjects factors.

MEP amplitudes and background EMG activity were recorded for each task condition. Mean peak-to-peak am-

plitude of the unconditioned MEPs evoked by TS alone was compared among the different conditions by means of a one-way ANOVA, with sequence (random vs. ordered vs. observed ordered) as within-subjects factor. Mean peak-to-peak amplitude of the conditioned MEP at each ISI was then expressed as the percentage of the mean peak-to-peak amplitude of the unconditioned test MEP in that condition. Two-way ANOVAs were performed, with sequence (random vs. ordered vs. observed ordered in Experiment 1; baseline vs. random vs. ordered in Experiment 2) and ISI (3 msec vs. 5 msec) as within-subjects factors.

In Experiment 1, a two-way ANOVA was also performed on rectified EMG activity of the 80-msec period preceding the first TMS pulse, with sequence (random vs. ordered vs. observed ordered) and ISI (test pulse vs. 3 msec vs. 5 msec) as within-subjects factors.

Post hoc Duncan's tests were performed when required.

RESULTS

Experiment 1

Procedural Learning Task

At the end of the experiment, no subject was able to detect the presence of the ordered sequences and to reproduce them; therefore, the data of all subjects who performed the task were included in the analysis. Although TMS pulses were delivered during the execution of the task, the evoked twitches of the index finger used for the task did not disturb the correctness of the response. Thus, all RTs were included in the analyses. A two-way ANOVA (3 Sequences \times 5 Blocks) on RTs displayed during execution of the random, ordered and observed ordered sequences along the five blocks of the task showed significant sequence [$F(2, 22) = 11.60, p = .0004$] and block [$F(4, 44) = 17.06, p < .0001$] effects, as well as a significant Sequence \times Block interaction [$F(8, 88) = 2.53, p = .016$]. Post hoc comparisons indicated that, in the first block, RTs of the random and ordered sequences were similar ($p = .614$); on the contrary, the subjects performed the first block of the observed ordered sequence significantly faster than the random ($p < .0001$) and ordered ($p < .0001$) sequences. In the last block, the subjects became significantly faster in the ordered than the random sequence ($p < .0001$) and they were as fast as in the observed ordered sequence ($p = .384$) (Figure 2). These results indicate that the subjects started at the same speed when they performed the random and the ordered sequences, but started faster when they performed the previously observed ordered sequence. This demonstrates the beneficial influence of the previous observational phase. As subjects were not aware of the presence of an ordered sequence, the advantage when they performed the observed ordered sequence should be considered an indication that procedural learning had taken place. When they performed the ordered sequence, the subjects acquired procedural competence only after

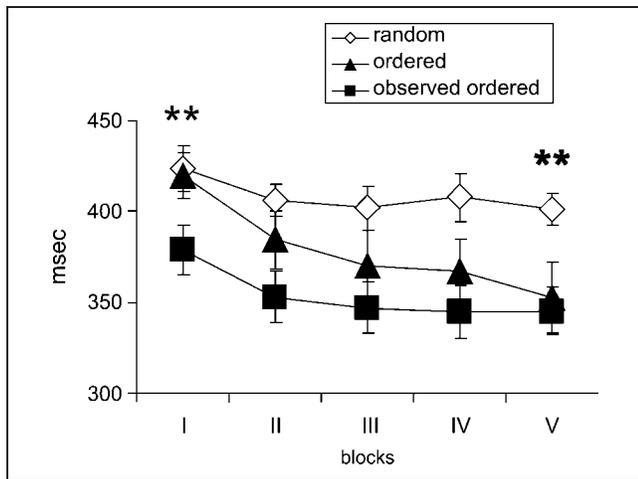


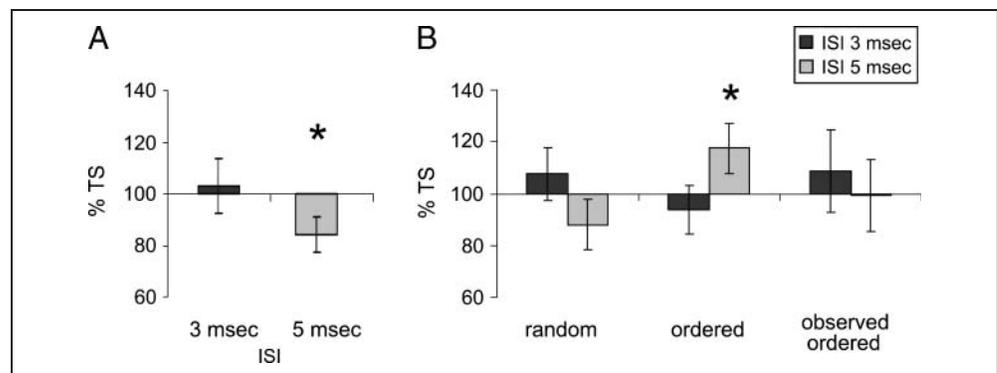
Figure 2. Mean RT during execution of the task (modified SRTT) in the three sequences. When performing the observed ordered sequence, but not the other sequences, the subjects started with an advantage. At the end of the task, the RTs in the ordered sequence decreased significantly and reached the values recorded during performance of the observed ordered sequence, indicating the acquisition of learning. White diamonds: random sequence; black triangles: ordered sequence; black squares: observed ordered sequence. In this and in the following figures, vertical bars indicate mean standard errors. Asterisks indicate the significance level of the comparison between observed ordered versus random and ordered sequences in the first block and between ordered versus random sequences in the last block. $**p < .0001$.

repeated practice of the task and they reached the same speed as in the observed ordered sequence only in the late phase of the task (Figure 2).

Cerebellar Motor Connectivity

Once we had verified that the task allowed measuring procedural learning, we analyzed the effects of different ISIs on MEP amplitude during execution of the task.

Figure 3. Cerebello-motor connectivity during execution of the task. (A) In the resting condition, the 5-msec ISI induced a significant inhibition of MEP amplitude (expressed as percentage of TS), whereas the 3-msec ISI induced no significant changes. (B) The cerebellar CS applied during execution of the task provoked an increase in MEP amplitudes only during performance of the ordered sequence and at the 5-msec ISI. Dark gray columns: 3-msec ISI; light gray columns: 5-msec ISI. Asterisks indicate the significance level of the comparison between TS and CS in (A), and between ordered and random sequences in (B). $*p < .05$.



The effect of 3-msec and 5-msec ISIs on test MEP amplitude at rest was first investigated by separately comparing test and conditioned MEPs mean amplitude for each ISI. Although there was no significant effect for the 3-msec ISI [$F(1, 11) = 0.454, p = .514$], the 5-msec ISI induced a significant inhibitory effect on MEP amplitudes [$F(1, 11) = 6.987, p = .023$] (Figure 3A).

A two-way ANOVA (2 ISIs \times 3 Sequences) on MEP amplitudes during execution of the different sequences revealed a significant ISI \times Sequence interaction [$F(2, 22) = 3.788, p = .039$], but the main factors were not significant. Post hoc comparisons indicated that the 5-msec ISI induced a significant increase in MEP amplitudes during execution of the ordered sequence with respect to the random sequence ($p = .033$), but failed to reveal any significant difference between the random and the observed ordered sequences ($p = .375$). The difference between ordered and observed ordered sequences was not significant ($p = .162$). The 3-msec ISI did not induce any significant change in MEP amplitudes either during the ordered ($p = .278$) or the observed ordered ($p = .917$) sequences compared to the random sequence (Figure 3B).

It is noteworthy that the significant increase of MEP amplitudes induced by the 5-msec ISI was shown during execution of the ordered sequence. On the contrary, the lack of significant facilitation during execution of the observed ordered sequence can be explained by the fact that the procedural learning shown during execution of this task had been acquired previously, that is, during the preceding observation phase.

A one-way ANOVA comparing MEP amplitudes evoked by TS alone during execution of the three sequences revealed no significant effect [$F(2, 22) = 0.270, p = .766$] (Figure 4A).

A two-way ANOVA (3 Sequences \times 3 ISIs) on background EMG activity revealed no significant effect [main effect of sequence: $F(2, 22) = 1.711, p = .204$; main effect

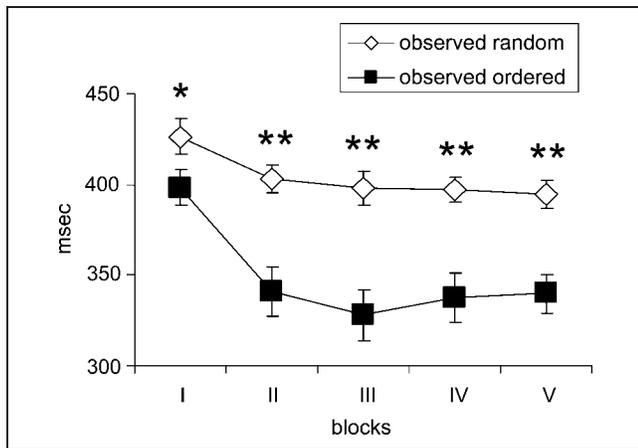


Figure 6. Mean RTs during execution of random and ordered sequences after observational training. Observational training of the ordered sequence induced a significant advantage during its actual execution compared to the observed random sequence. White diamonds: observed random sequence; black squares: observed ordered sequence. Asterisks indicate the significance level of the comparison between observed ordered and observed random sequences. * $p < .05$; ** $p < .0001$.

was found from Block I to Block II (random sequence, $p = .012$; ordered sequence, $p < .0001$), but RTs did not change across the other blocks (Figure 6). As in Experiment 1, although both sequences had been observed previously, the results indicate that the subjects started with an advantage when they performed the ordered sequence rather than the random sequence, which suggests that observation of a specific sequence, not mere observation of the task, improved the subsequent actual performance of that sequence. In fact, when we compared performance of the random (Experiment 1) versus observed random (Experiment 2) sequences, we found no significant differences in RTs during execution of the two sequences. A two-way ANOVA (2 Groups \times 5 Blocks) showed a significant effect of block [$F(4, 72) = 6.841, p = .0001$], but the effect of the group and Group \times Block interactions was not significant.

Cerebello-motor Connectivity

The effect of CS at the 3-msec and 5-msec ISIs on test MEP amplitude in the baseline condition was investigated. Separate t tests comparing test and conditioned MEP mean amplitudes for each ISI showed that the 5-msec ISI induced a significant reduction of MEP amplitudes [$F(1, 9) = 5.284, p = .047$], whereas the 3-msec ISI failed to induce any significant effect (Figure 7A).

Unlike Experiment 1, in this experiment, all MEPs were recorded at rest. Thus, we were able to include the resting condition in the analysis of the effects of 3-msec and 5-msec ISIs.

A one-way ANOVA comparing MEP amplitudes evoked by TS during execution of the three sequences revealed no significant effect (Figure 8).

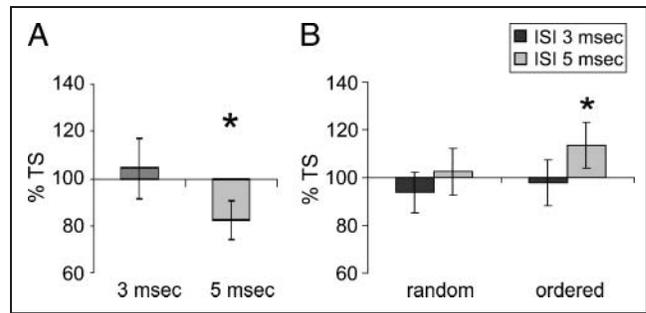


Figure 7. Cerebello-motor connectivity during observation of the task performed by an actor. (A) In the resting condition, the 5-msec ISI induced a significant suppression of MEP amplitude (expressed as percentage of TS), whereas the 3-msec ISI induced no significant changes. (B) The cerebellar CS applied during observational training provoked an increase in MEP amplitudes only during observation of the ordered sequence with the 5-msec ISI. Dark gray columns: 3-msec ISI; light gray columns: 5-msec ISI. Asterisks indicate the significance level of the comparison between TS and CS in (A), and between observed ordered and observed random sequences in (B). * $p < .05$.

A two-way ANOVA (2 ISIs \times 3 Conditions) on MEP amplitudes in the baseline condition and during observation of an actor performing the random and ordered sequences showed a significant ISI \times Condition interaction [$F(2, 18) = 4.466, p = .027$]. In fact, the 5-msec ISI induced a significant increase in MEP amplitudes during observation of the ordered sequence compared to the baseline condition ($p = .005$) and compared to observation of the random sequence ($p = .050$). On the contrary, observation of the random sequence induced no significant change in MEP amplitudes compared to the baseline condition. The 3-msec ISI failed to induce any significant change in MEP amplitudes during observation of both random and ordered sequences (Figure 7B).

Interestingly, although execution of the previously observed ordered sequence was not associated with increased cortical excitability (Experiment 1), a significant facilitation was found during observation of the ordered sequence (Experiment 2) (Figure 7B). Moreover, facilitation was not related to mere observation but was specific for a kind of observation that induced procedural learning.

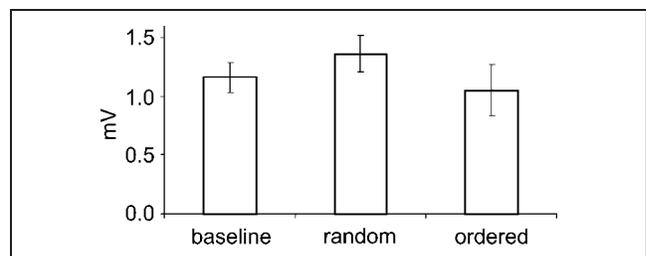


Figure 8. Mean peak-to-peak amplitude of the MEPs evoked by TS alone was comparable in the baseline condition and during observation of the random and ordered sequences. White columns: test stimulus.

DISCUSSION

The present study provides evidence of functional changes in cerebello-motor connectivity when procedural learning is ongoing. In fact, while procedural competencies were being acquired by actually executing the task, motor cortex was not more inhibited but was actually facilitated by the cerebellar projection. Interestingly, similar cerebello-motor facilitation has been found during acquisition of procedural knowledge through observational learning. It is noteworthy that the cerebello-motor facilitation was lacking once procedural learning had already been acquired through previous observational training.

Previous studies have shown that acquisition of new motor skills is associated with modulation of cortical motor output to the muscles involved in the task (Pascual-Leone et al., 1995). Although most studies have shown that visuomotor procedural learning is related to changes in the synaptic efficacy of motor cortex, the hypothesis of a contribution of other cortical and subcortical structures to the learning-associated modulation of cortical excitability has not yet been investigated. In the last decade, the role of the cerebellum in the acquisition of procedural competencies has been widely demonstrated in studies on rats, healthy human subjects, and cerebellar patients (Torriero et al., 2004, 2007; Leggio et al., 1999; Gómez-Beldarrain et al., 1998; Petrosini, Molinari, & Dell'Anna, 1996). A recent magnetoencephalography study demonstrated that after a perceptual-motor task had been learned a decrease in motor cortical activity was accompanied by an increase in cerebellar activity (Houweling, Daffertshofer, van Dijk, & Beek, 2008). Furthermore, recent studies have demonstrated that when procedural learning has already been acquired, motor cortex excitability increases and a modulation of short intracortical inhibition occurs (Perez, Wise, Willingham, & Cohen, 2007; Nordstrom & Butler, 2002). These findings show the effects of visuomotor learning on cortical excitability but they do not allow drawing any conclusions about which modifications take place during learning acquisition. The present study shows that procedural learning is related to a modulation of cortical excitability via cerebello-thalamo-cortical projections.

Neurophysiological studies using electrical or magnetic stimulation in healthy subjects and in patients have provided evidence that the cerebellum decreases motor cortex excitability (Daskalakis et al., 2004; Ugawa et al., 1995; Di Lazzaro et al., 1994). It has been suggested that applying TMS to healthy subjects might activate inhibitory Purkinje cells, which interfere with the tonic facilitatory effect of cerebellar nuclei on motor cortex (Pinto & Chen, 2001). Consistent with previous studies (Iwata & Ugawa, 2005), we found that in the resting condition cerebellar conditioning pulses delivered at 5-msec ISI induced a decrease in motor cortex excitability. This effect was smaller than the inhibition reported by Ugawa et al. (1995) using a double cone-coil for cerebellar stimulation. Further, we

found that, unlike the 5-msec ISI, the 3-msec ISI did not induce any effect. This result is at variance with the facilitatory effect reported over contralateral motor cortex following electrical cerebellar stimulation at a 3-msec ISI (Iwata et al., 2004). Similarly, a recent study reported the absence of any 3-msec ISI effect when magnetic cerebellar stimulation was used with a figure-of-eight coil (Koch, Mori, et al., 2008). The different results obtained in the reported experiments suggest that methodological variations might have had different effects on cerebellar excitability.

In fact, it can be hypothesized that the facilitatory or inhibitory effect of the cerebellar stimulation depends on which cerebellar structure is activated by the stimulus. The activation of the dentate nucleus or the superior cerebellar peduncle could be responsible for the facilitatory effect found at the 3-msec ISI by Iwata et al. (2004). On the other hand, the longer interval needed to induce an inhibitory effect (5-msec ISI) over contralateral motor cortex is consistent with the idea that another synaptic delay occurs. In this view, the stimulus would activate Purkinje cells, leading to inhibition of the dentate nucleus and a reduction of the tonic facilitation of motor cortex through the thalamus (Ugawa & Iwata, 2005).

Our finding of an inhibitory effect at the 5-msec ISI and the absence of effects at the 3-msec ISI might be explained by assuming that the TMS pulses over the cerebellum activated Purkinje cells, perhaps through simple spikes from the parallel fibers, but did not reach deeper cerebellar structures, such as the dentate nucleus (Schmolesky, Weber, De Zeeuw, & Hansel, 2002; Medina & Mauk, 2000). In fact, the stimuli were delivered through a figure-of-eight coil at an intensity that was equal to 90% of the resting motor threshold measured over contralateral motor cortex (Koch, Mori, et al., 2008). If you correct for the difference in distances according to the method of Stokes et al. (2005), the intensity of cerebellar stimulation is equivalent to 68% of the resting motor threshold over motor cortex (Fernandez Del Olmo, Cheeran, Koch, & Rothwell, 2007). In motor cortex, stimulation at this intensity is below the threshold for activating cortico-spinal output but is within the range of intensities conventionally used to activate cortico-cortical circuits when testing short-interval intracortical inhibition and facilitation (SICI/ICF) (Chen et al., 1998; Kujirai et al., 1993). Therefore, we believe that the TMS pulses were able to activate at least some elements in cerebellar cortex, showing the inhibitory effect at 5-msec ISI, but were not able to activate the dentate nucleus, which might have shown a facilitatory effect at 3-msec ISI.

The most intriguing result of the present study is that inhibition of motor cortex, induced by conditioning cerebellar pulses at 5-msec ISIs, was replaced by a facilitation of motor output related to the phase of procedural learning. This task-specific change in the direction of the effects induced by cerebellar TMS is not surprising. Indeed, similar phenomena have also been reported for premotor-motor connections (Davare, Montague, Olivier,

Rothwell, & Lemon, 2009; Davare et al., 2008). It is well known that cerebellar output involves both facilitatory and inhibitory projections (Daskalakis et al., 2004). Although inhibition is more readily evoked at rest, facilitation of the contralateral M1 is a subtle but reliable phenomenon (Iwata & Ugawa, 2005; Daskalakis et al., 2004). Our data seem to suggest that the latter facilitatory effect, which is almost negligible at rest, is specifically enhanced during the acquisition of novel motor procedures. This might indicate that these connections are open when new motor engrams have to be acquired and that they might be involved in the plastic changes that occur in cerebellar and motor cortex during these motor learning tasks (for a review see Ito, 2008).

Another interpretation of the lack of inhibition in motor cortex during motor learning is offered by the theory of cerebellar learning. In animal models, when two inputs, one from a climbing fiber and the other from a set of granule cell axons, are repeatedly associated in Purkinje cells, the input efficacy of the granule cell axons in exciting the Purkinje cells is persistently depressed (see Ito, 2001, 2002). Thus, the cellular phenomenon that underlies motor learning consists of activating the climbing fiber input to cerebellar cortex, which suppresses the excitability of the Purkinje cells by activating a complex spike (cerebellar LTD) (Hansel, Linden, & D'Angelo, 2001; Ito, 2001). This mechanism of plasticity leads to an increase in cerebellar excitatory output toward contralateral motor cortex, which is associated with motor learning.

This physiological mechanism could explain why cerebellar-induced motor cortical inhibition turns into a motor cortical facilitation when the learning process reaches its maximum. In fact, during motor learning, the Purkinje cells might be already inhibited and the 5-msec ISI TMS pulses might enhance the effect of cerebellar LTD, resulting in an increase in the excitatory output from the dentate nucleus to contralateral motor cortex. However, the mechanisms underlying the effects of cerebellar paired-pulse TMS during learning have still not been clarified and further studies are needed to completely resolve this issue.

Further evidence of the role of the cerebellum in modulating cortical excitability comes from a recent study by Battaglia et al. (2006). In patients with cerebellar stroke, these authors demonstrated decreasing excitability of motor cortex contralateral to the cerebellar lesion during preparation and imaging of sequential movements. In the past few years, a large number of studies have focused on the neural circuitry subserving the perception–action matching system in humans and have demonstrated that the same brain areas (frontal, parietal, temporal, and cerebellar regions) are involved in preparing actions, in mentally representing one's own action, and in observing another's action (Grèzes & Decety, 2001). It is now recognized that observational learning plays an important role in the acquisition of new skills. Recent findings demonstrate that observing another's actions is sufficient to induce changes in motor cortex activity. In particular, repeated observation of repetitive movements of the thumb modifies the direction

of thumb movements subsequent to TMS over M1 (Stefan et al., 2005). Brown, Wilson, and Gribble (2009) also demonstrated that rTMS of motor cortex, applied after observation of another person learning to reach in a novel force environment, reduced the beneficial effect of observing congruent forces and eliminated the detrimental effect of observing incongruent forces. Another TMS study demonstrated that actually performing, imaging, and, to a lesser extent, observing finger movements enhanced excitability of the cortico-spinal system, targeting the FDI muscle (Porro, Facchin, Fusi, Dri, & Fadiga, 2007). An increasing number of experimental and neuroimaging findings point to cerebellar involvement in “motor thought” (Schmahmann, 2004). In rats, cerebellar lesions markedly impaired the learning of new procedures not only during the actual execution of a task (Mandolesi, Leggio, Spirito, Federico, & Petrosini, 2007; Mandolesi, Leggio, Spirito, & Petrosini, 2003; Mandolesi, Leggio, Graziano, Neri, & Petrosini, 2001; Petrosini et al., 1996) but also during observation (Leggio et al., 2000). Likewise, cerebellar activation has been reported during the observation of actions that have to be subsequently imitated (Frey & Gerry, 2006; Jackson, Meltzoff, & Decety, 2006; Leslie, Johnson-Frey, & Grafton, 2004; Chaminade, Meltzoff, & Decety, 2002; Decety et al., 1994).

In the present study, we found that previous observational training prevented facilitation of motor cortex excitability during the actual execution of the task. We also found a facilitatory effect of cerebellar conditioning stimulation during observational training. These findings demonstrate that observational training elicits the development of procedural learning, as indicated by the advantage obtained when the subjects actually performed the previously observed ordered sequence. In fact, given that this advantage was present from the beginning of the task procedural learning could have been gained through the previous observational training. This is in accordance with a recent study demonstrating that observation supports implicit sequence learning even in the absence of any explicit knowledge (Song, Howard, & Howard, 2008). Nevertheless, the different performances shown after observational learning and practice-based learning might also have been due to differences in the mechanism responsible for supporting skill acquisition. For example, oculomanual transfer may have been responsible for the skill shown in performing the observed ordered sequence.

However, the present results indicate that the cerebellum modulates cortical excitability when learning is being acquired (such as when a new sequence is actually performed or observed) and that this modulation is weaker when learning has already been acquired (as occurs when a previously observed sequence is actually performed). This is coherent with neuroimaging data showing marked cerebellar activation during the acquisition phases of learning and decreased cerebellar activation after prolonged practice (Petersen, van Mier, Fiez, & Raichle, 1998; Vaina, Belliveau, des Roziers, & Zeffiro, 1998). Experimental findings have also revealed the cerebellar role in the acquisi-

tion of procedural competencies, even when learning is acquired by observation. Cerebellar lesions in rats induced deficits in spatial learning by observation; nevertheless, once acquired, spatial procedures were not impaired even in the presence of cerebellar damage (Petrosini et al., 2003; Leggio et al., 2000). It was also found that although rTMS applied over the cerebellum interferes with observational learning of new procedures, cerebellar rTMS applied after observational training does not influence task execution (Torriero et al., 2007).

In conclusion, although future studies are needed to identify the specific neurophysiological mechanisms underlying anatomo-functional relations between cerebellar and cortical regions, the present study contributes to our understanding of cerebello-motor connectivity during action, apart from motor execution (Frey & Gerry, 2006).

Reprint requests should be sent to Dr. Sara Torriero, Laboratorio di Neurologia Clinica e Comportamentale, Fondazione Santa Lucia IRCCS, Via Ardeatina, 306, 00179 Rome, Italy, or via e-mail: s.torriero@libero.it.

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