Brain Stimulation 8 (2015) 603–612

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: www.brainstimjrnl.com

Reversal of Practice-related Effects on Corticospinal Excitability has no Immediate Effect on Behavioral Outcome

Matteo Bologna [a], Lorenzo Rocchi [b], Giulia Paparella [b], Andrea Nardella [a], Pietro Li Voti [a], Antonella Conte [a], Maja Kojovic [c,d], John C. Rothwell [c], Alfredo Berardelli [a,b,*]

[a] Neuromed Institute (IRCCS), Pozzilli, Isernia, Italy
[b] Department of Neurology and Psychiatry, Sapienza University of Rome, Italy
[c] Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, UK
[d] Department of Neurology, University of Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form
12 January 2015
Accepted 14 January 2015
Available online 17 February 2015

Keywords:
Transcranial magnetic stimulation
Depotentiation
Primary motor cortex

ABSTRACT

Background: Motor training usually increases the excitability of corticospinal outputs to the trained muscles. However, it is uncertain to what extent the change in excitability is a critical component of behavioral learning or whether it is a non-specific side effect.
Objective/Hypothesis: We used a depotentiation protocol to abolish the training-induced increase of corticospinal excitability and tested whether this had any immediate effect on the improved motor performance.
Methods: We used an index finger abduction task in which behavioral improvement is known to be associated with M1 excitability changes as monitored by the amplitude of motor-evoked potentials produced by single-pulse transcranial magnetic stimulation (TMS). These effects could be reversed by a depotentiation protocol using a short form of continuous theta-burst stimulation (cTBS150). Participants underwent three experimental interventions: 'motor training', 'motor training plus cTBS150' and 'cTBS150'. M1 excitability and TMS-evoked finger movements were assessed before the experimental interventions and 5 min, 15 min, and 30 min thereafter. Motor retention was tested 45 min after the experimental interventions.
Results: During training, acceleration of the practiced movement improved. At the end of training, M1 excitability and the acceleration of TMS-evoked index finger movements in the direction of training had increased and the enhanced performance was retained when tested 45 min later. The depotentiation protocol, delivered immediately after the end of training, reversed the excitability changes in M1 but did not affect the acceleration of the TMS-evoked finger movement nor the retention of performance. The depotentiation protocol alone did not modify M1 excitability.
Conclusions: The present study indicates that in the short term, increases in corticospinal excitability are not related to immediate changes in behavioral motor outcome.

Abbreviations: ADM, abductor digiti minimi; APB, abductor pollicis brevis; AMT, active motor threshold; ANOVA, analysis of variance; cTBS, continuous theta burst stimulation; DePO, de-potentiation; EMG, electromyographic; FDI, first dorsal interosseus muscle; I/O, input–output; LTD, Long-term depression; LTP, Long-term potentiation; M1, primary motor cortex; MEP, motor-evoked potentials; MSO, maximal stimulator output; RMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation.

Competing interests: None.

Financial disclosures: Dr. Matteo Bologna receives support from the Dystonia Coalition (NS065701). Drs. Lorenzo Rocchi, Giulia Paparella, Andrea Nardella, Pietro Li_Voti, Antonella Conte, and Maja Kojovic have no relevant disclosures. Professor John C Rothwell receives research support from the Medical Research Council UK (MR/K01384X/1), The Stroke Association UK (2014-04) and European Union FP7 (316639). Professor Alfredo Berardelli has received financial support to speak/attend meetings from Allergan and Boehringer Ingellheim.

* Corresponding author. Department of Neurology and Psychiatry, "Sapienza" University of Rome, Viale dell'Università 30, 00185 Rome, Italy. Tel./fax: +390649914700.
E-mail address: alfredo.berardelli@uniroma1.it (A. Berardelli).

http://dx.doi.org/10.1016/j.brs.2015.01.405
1935-861X/

Introduction

Studies on animals demonstrate that repeated stimulation of a pathway can produce long lasting changes in the efficiency of synaptic transmission known as long-term potentiation (LTP) and long-term depression (LTD) and it is postulated that they are the basis of learning and memory [1]. In humans, it is possible to investigate mechanisms related to synaptic plasticity in the motor system by applying repetitive transcranial magnetic stimulation (rTMS) protocols to the motor cortex [2–6]. The result (which depends on the rTMS protocol used) is an increase or decrease in corticospinal excitability, that is N-methyl-D-aspartic acid receptor-dependent and therefore thought to involve LTP and LTD-like processes [7,8]. We can refer to these rTMS methods as 'exogenous' probes of probes of LTP- and LTD-like plasticity.

'Endogenous' plasticity in contrast is produced by natural behaviors. In humans, repeated performance of a voluntary movement leads to effects that can be termed motor memories [9–19] which are thought to involve LTP-like changes at synaptic connections [20,21]. Motor practice-related plasticity changes at the level of primary motor cortex (M1) can be quantified by various methods such as (i) increases in corticospinal excitability, (ii) changes in TMS-evoked movements and (iii) retention of the improved performance achieved after practice [9–19]. The question we ask here is whether all three methods measure the same thing.

In order to test this we made use of a phenomenon known as depotentiation (DePo) [22,23]. A short rTMS protocol that is alone insufficient to produce any lasting change in corticospinal excitability can abolish the lasting increase evoked by an excitatory rTMS protocol [3,4]. We ask whether DePo applied immediately after a period of repeated voluntary movement will abolish all three measures of endogenous plasticity. Previous work by Canterero et al. [24], has shown that DePo abolishes/reduces the immediate increase in corticospinal excitability that usually accompanies motor training. They also showed that DePo interfered with task consolidation since it reduced performance when tested 24hr after training. The conclusion was that the motor-training related effects on corticospinal excitability assessed shortly after performance were strongly related, and perhaps causal to the long-term retention of improvements in performance when tested the next day. However, they did not examine whether DePo had an immediate effect on performance tested on the day of training.

Previous work has shown that the mechanisms of short term improvement differ from those tested 24 h later: Hotermans et al. [25], found that 1Hz rTMS given immediately after training abolished the immediate improvement in performance but did not affect performance tested 24 h later. The present experiment was therefore designed to examine the effect of DePo on the behavioral consequences of a training protocol shortly after training was complete and compare this with its effect on corticospinal excitability as assessed with single-pulse TMS. The results are relevant to studies investigating plasticity mechanisms and measure of immediate changes in corticospinal excitability as a proxy for expected behavioral gains.

Materials and methods

Participants

Fifteen healthy right-handed subjects (8 females; mean age ± 1 standard deviation: 27.6 ± 4.4 years, age range: 24–39 years) participated in the study. None of the subjects had a history of neurological or psychiatric disorders and were not taking any medications at the time of experiments. None of the subjects had ever been engaged in professional training in music. All gave their informed consent to the experimental procedures, which were approved by the local Institutional Review Board and conducted in accordance with international safety recommendations [26]. All experiments adhered to the tenets of the Declaration of Helsinki. None of the participants reported fatigue or adverse effects during or after the experiments.

TMS and electromyographic recordings

Single-pulse TMS was delivered to the dominant M1 using a Magstim 200^2 magnetic stimulator with a monophasic current waveform. Repetitive TMS was delivered using a Magstim Rapid with a biphasic current waveform (Magstim, Carmarthenshire, Wales, UK). The magnetic stimulators were connected to a focal figure-of-eight-shaped coil (outer diameter of each wing, 90 mm). The intersection of the coil was placed tangentially to the scalp with the handle pointing backward and laterally at ~45° angle away from the midline. During the experiments, the hot spot of the dominant first dorsal interosseous (FDI) muscle, i.e. the optimal scalp position for eliciting motor-evoked potentials (MEP) of maximal amplitudes, was defined in the contralateral M1. To ensure consistency of coil positioning over the FDI hot spot we used a neuronavigation system (SofTaxic Neuronavigation System for TMS, EMS Corporation, Italy).

Corticospinal excitability was tested with single-pulse TMS on the dominant FDI hot spot with the hand at complete rest as confirmed by visual inspection of the EMG records. Traces with background electromyographic (EMG) activity exceeding 50 μV in the 200 ms time window preceding stimulation were rejected online (~1% of trials). Visual feedback of EMG level was provided to help subjects maintain the hand at complete rest. We first determined the resting motor threshold (RMT) to the nearest 1% of the maximal stimulator output (MSO). This was defined as the minimal stimulus intensity required to produce MEPs larger than 50 μV peak-to-peak amplitude, in the contralateral FDI, in at least five out of 10 consecutive trials. Evaluation of RMT was followed by measurement of the MEP input–output (I/O) curve using single-pulse TMS stimuli at 3 intensity levels: 100%, 120% and 140% of RMT. Fifteen trials were recorded at each intensity level. Recordings at each intensity level were randomly collected in order to avoid the hysteresis effects on I/O curves [27].

DePo protocol was a short form of continuous theta burst stimulation, i.e. cTBS150 [3,4,24]. In brief, cTBS150 consists of a 10 s train of stimuli, with bursts of three pulses delivered at 50 Hz over the FDI hot-spot repeated at 5 Hz (i.e. 200 ms intervals). The stimulation intensity was set to 80% active motor threshold (AMT) [3,4,24] which was defined as the minimum stimulation intensity required to produce an MEP of greater than 200 μV on more than 5 out of 10 trials while the subject maintained a ~20% maximal voluntary contraction of the FDI. Visual feedback of EMG level was provided to help subjects maintain a constant level of contraction.

MEPs were recorded from the dominant FDI, abductor pollicis brevis (APB) and abductor digiti minimi (ADM) using silver-chloride surface cup electrodes (9 mm in diameter), taped in a belly tendon-montage with the active electrode centered over the muscle belly and the reference electrode over the metacarpophalangeal joint of the respective fingers (inter-electrode distance ~2 cm). The raw EMG signals were amplified (1000 X) and band-pass filtered (20 Hz to 2 kHz) by a Digitimer D360 amplifier (Digitimer, Welwyn Garden City, Hertfordshire, UK), digitized at a sampling rate of 4 kHz by an analog-to-digital interface (Micro 1401; Cambridge Electronic Design, UK) and stored on a laboratory PC for offline analyses. The EMG recordings were analyzed using Signal® version 4.00 (Cambridge Electronic Design, UK). The MEP peak-to-peak amplitude was measured within a time window of 20–40 ms after the TMS artefact.

Motor training and kinematic recordings

The motor training task was adopted from previous studies [8–11,28]. In brief, participants were comfortably seated in a chair with the arms slightly abducted from the trunk (~45–50°) and the elbow flexed (~90°). The dominant forearm and wrist rested pronated on a desk. Participants extended their index finger in line with the forearm and made rapid abductions of their finger, so as to achieve the highest acceleration possible following a 'go' signal, given every 5 s with a random jitter of ±0.5 s. Participants were also instructed to return to the starting position after the movement by allowing the finger to relax back to original position. In each motor training session 225 movements were collected; 15 consecutive movements were considered as a trial block and averaged (15 acquisition trials). A rest interval of 15–20 s was left between acquisition blocks to avoid fatigue. The motor training task lasted approximately 20 min. One retention trial block (15 movements) was collected 45 min after the end of motor training (Fig. 1). Before starting the motor training, one practice trial was allowed for the participants to become familiar with the experimental setting.

Index finger abductions were recorded using a 3D optoelectronic system (SMART motion system, BTS Engineering, Milan, Italy). This system comprises three infrared cameras (sampling rate, 120 Hz) that follow the 3D displacement of reflective markers taped on the hand of the subjects. We used four reflective markers (5 mm in diameter) of negligible weight. One marker was placed on the tip of the index finger. Three additional reflective markers were placed on the hand (one marker on the head of the 2nd metacarpal bone, one marker on the base of the 2nd metacarpal bone and one marker on the base of the 5th metacarpal bone) to define a reference plane that was used to mathematically exclude from index finger recordings possible contamination due to hand movements. A dedicated software (SMART Analyzer, BTS Engineering, Milan, Italy) reconstructed the 3D spatial displacement of the reflective markers offline and determined the acceleration of index finger movements over the abduction axis (horizontal axis). The first acceleration peak of each index finger abduction was expressed in m/s^2.

To quantify motor performance, we measured changes in the peak acceleration of the index finger abduction throughout the motor training task (Fig. 2) and quantified online and offline effects, as defined by Cantarero et al. [24,29], and by Reis et al. [30]. The online effect was quantified as the acceleration peak in the 15th trial block minus (Δ) the acceleration peak in the 1st trial block. The offline effect was measured as the acceleration peak in the retention trial block minus (Δ) the acceleration peak in the 15th trial block (Fig. 3). We also quantified the effects of training or DePo in terms of increased acceleration of the movement evoked by a single TMS pulse in the same direction as the practiced movement. For this purpose we delivered single-pulse TMS over the FDI hand area at an intensity of 150% RMT while the subjects kept their index finger actively extended and in line with the forearm and measured the first acceleration peak of the TMS-evoked index finger movement in the direction of training. At the same time of the TMS-evoked index finger movement we recorded the MEPs from the FDI, APB and ADM. We asked the subjects to keep their index finger actively extended because in our preliminary observations we found that it was not always possible to record reliable TMS-evoked index finger movements by stimulating the M1FDI area with the hand at complete rest (unless using higher stimulation intensities). In addition, without keeping the finger extended the TMS-evoked movements could have been affected by friction against the plane of the desk; whereas by keeping the arm slightly supinated TMS-evoked finger movements could have been affected by gravity. Finally, since the FDI is not the prime mover for keeping the index finger slightly extended and the fact that the duration of the muscle activation during TMS-evoked movements was so short (~1 min for each measurement time point), we do not believe that these methodological issues would affect the results.

Experimental design

Participants underwent three main sessions consisting of different experimental interventions: (i) 'motor training', (ii) 'motor training plus cTBS150' (in this session the cTBS150 was delivered 1 min after the end of motor training) and (iii) 'cTBS150'. The order of the three main experimental sessions was randomized between subjects. Recordings of MEP I/O curves were performed in all the three main experimental sessions at four time points: before the interventions and 5 min, 15 min and 30 min thereafter (Fig. 1). At each measurement time point we also collected ten TMS-induced index finger movements.

In a first control experiment, performed after the three main experimental session (on the 15 subjects who participated in the main experiments), we tested the time specificity of the DePo protocol on M1 excitability changes. For this purpose the 'cTBS150' was delivered ~5 min after the end of motor training. Recordings of I/O curves were made at four time points: before the intervention and 5 min, 15 min and 30 min thereafter. In this control experiments we did not collect TMS-evoked movements since in the main experimental session we observed that the DePo protocol had no effects on these measures (Fig. 1).

In a second control experiment, performed after the previous experiments (on 9 of the original 15 subjects), we tested the possible confounding effect of over-training. Participants underwent two experimental sessions: (i) 'short-motor training' consisting of 3 trial blocks of 15 movements each and (ii) 'short-motor training plus cTBS150', in this session the cTBS150 was delivered 1 min after the end of motor training (Figs. 1 and 2). One retention trial block (15 movements) was collected 45 min after the end of the 'short motor training' in both sessions. The order of the two sessions of the second control experiment was randomized between subjects. In both sessions MEP and TMS-evoked movements were collected following the same methods of the main experiment (Fig. 1). In control experiments we did not collect MEPs from the APB and ADM since they had shown no effect of motor training in the main experimental session.

Statistical analysis

To evaluate the time course of peak acceleration during motor training, absolute values (m/s^2) were entered into a repeated measures analysis of variance (ANOVA) using SESSION (two levels: 'motor training' and 'motor training plus cTBS150') and TRIAL NUMBER (sixteen levels: 1st to 15th acquisition trial blocks, plus the retention trial block) as within-subject factors. Online and offline effects of motor performance were also compared across the two training sessions using two-tailed paired samples *t*-tests (Fig. 3). Peak acceleration (m/s^2) from both the first and second control experiments was analyzed in distinct repeated measures ANOVAs because these control experiments were performed separately, i.e. after the three main experimental sessions; moreover the second control experiment testing the possible confounding effect of over-training was performed on 9 of the original 15 subjects and it consisted of a different number of acquisition blocks (Figs. 1 and 2). In the first control experiment (evaluating the time specificity of the DePo protocol) we used the within-subject TRIAL NUMBER (fifteen levels: 1st to 15th acquisition trial blocks) and in the second control experiment (evaluating the short period of training) we used the within-subject factors SESSION (two levels:

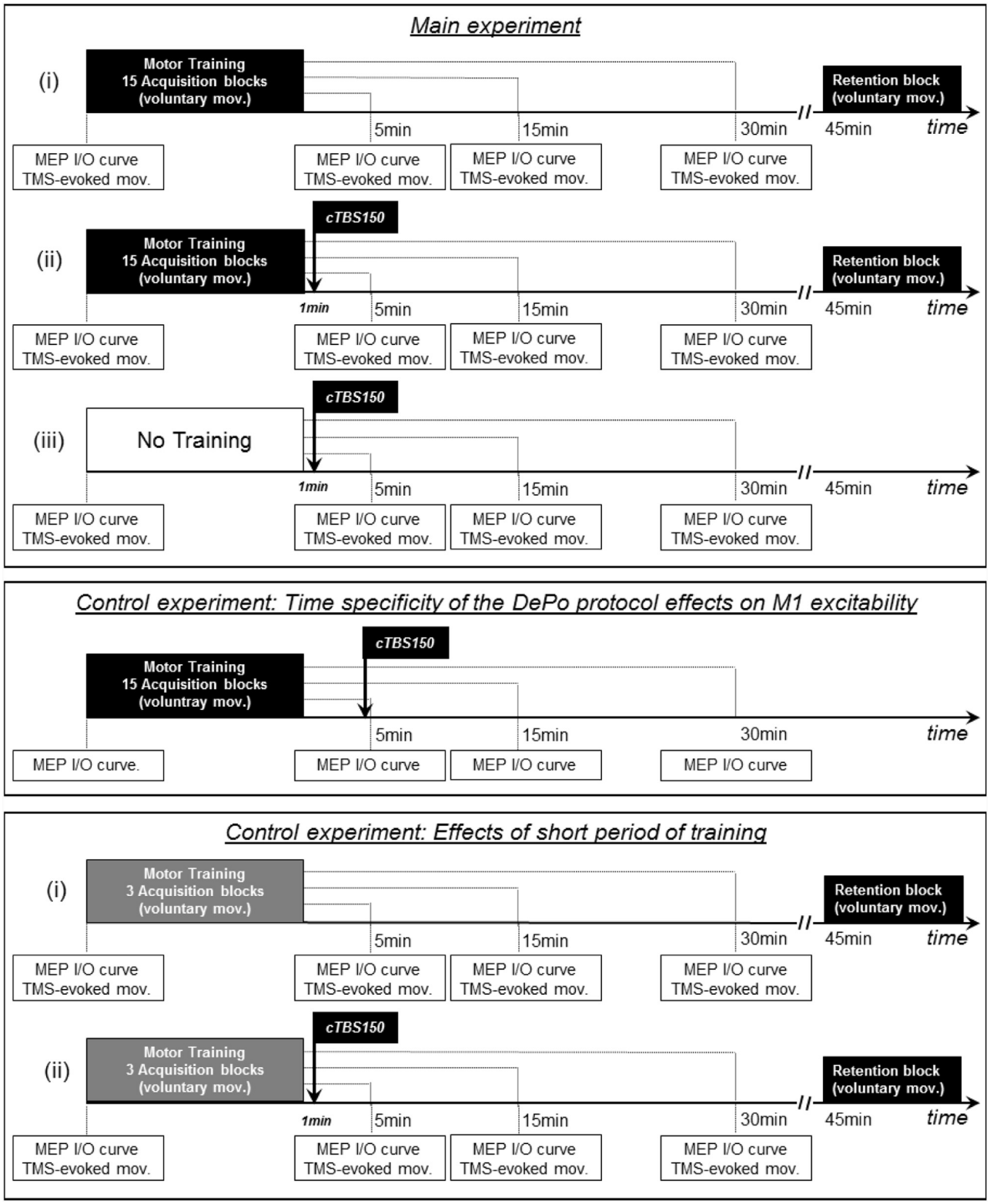

Figure 1. Experimental design. Participants underwent three main experimental sessions (upper panel) consisting of different interventions: (i) 'motor training', (ii) 'motor training plus cTBS150' and (iii) 'cTBS150'. In a control experimental session (middle panel), we tested the time specificity of the of DePo protocol effects on M1 excitability by delivering the 'cTBS150' ~5 min after the end of motor training. In a second control experiment (lower panel), consisting of two sessions: (i) 'short-motor training' and (ii) 'short-motor training plus cTBS150' we tested the possible confounding effects of over-training.

'short-motor training' and 'short-motor training plus cTBS150') and TRIAL NUMBER (four levels: 1st to 3rd acquisition trial blocks, 4th retention trial block).

Comparisons of baseline RMT/AMT values across the three main experimental sessions and the three control experiments were performed using one-way ANOVA with the between-group factor

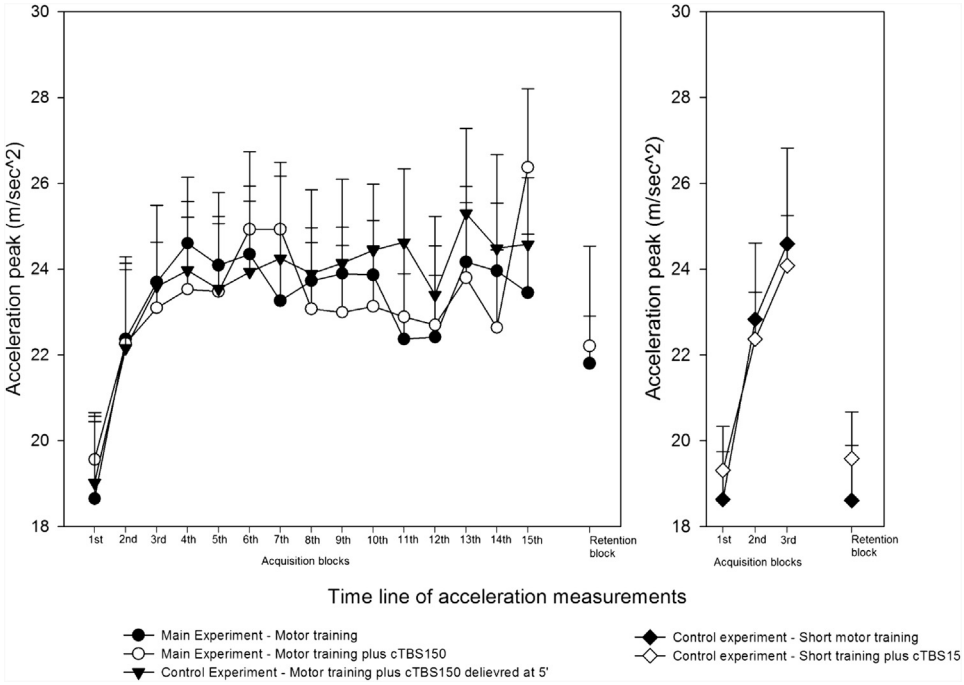

Figure 2. Course of the acceleration peak expressed in m/s^2 (*y* axis) throughout the motor task. The *x* axis refers to the timeline of acceleration measurement in the two training sessions of the main experiment and in the control experiments examining the time specificity of the DePo protocol (left panel), consisting of 15 acquisition trials and in 1 retention trial collected 45 min after the end of training. The right panel refers to the control experiment evaluating the effects of a short period of training, consisting of 3 acquisition trials and in 1 retention trial collected 45 min after the end of training. Vertical bars indicate 1 standard error of the mean.

SESSION. Motor training-related changes of M1excitability (MEP I/O curves) in the three sessions of the main experiment were evaluated using a repeated measures ANOVA with within-subject factors MUSCLE (three levels: FDI, APB, ADM), SESSION (three levels: 'motor training', 'motor training plus cTBS150' and 'cTBS150'), TIME POINT (four levels: before the intervention and 5 min, 15 min and 30 min after the intervention) and STIMULUS INTENSITY (three levels: 100%, 120% and 140% RMT), as within-subject factors. The first control experiment employed the within-subjects factors TIME POINT (four levels: before the intervention and 5 min, 15 min and 30 min after the intervention) and STIMULUS INTENSITY (three levels: 100%, 120% and 140% RMT). The second control experiment employed SESSION (two levels: 'short-motor training' and 'short-motor training plus cTBS150'), TIME POINT (four levels: before the intervention and 5 min, 15 min and 30 min after the intervention) and STIMULUS INTENSITY (three levels: 100%, 120% and 140% RMT), as within-subject factors.

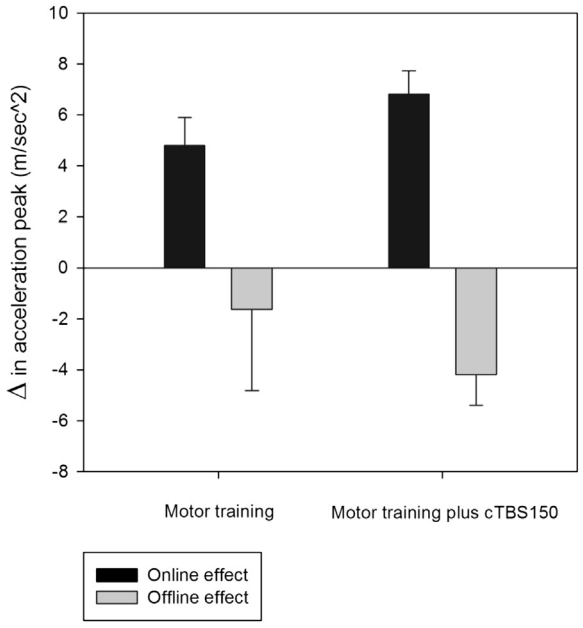

Figure 3. Motor performance in the two training sessions of the main experiment. The online effect (black histograms) was quantified as the acceleration peak in the 15th trial block minus (Δ) the acceleration peak in the 1st trial block (*y* axis). The offline effect (gray histograms) was measured as the acceleration peak in the retention trial block minus (Δ) the acceleration peak in the 15th trial block (*y* axis). Vertical bars indicate 1 standard error of the mean. Two-tailed paired *t*-tests showed no significant difference in online and offline effects in both the 'motor training' and 'motor training plus cTBS150' sessions.

Analysis of the acceleration peak (m/s^2) of the TMS-evoked finger movements in the three sessions of the main experiment used a repeated measures ANOVA with SESSION (three levels: 'motor training', 'motor training plus cTBS150' and 'cTBS150') and TIME POINT (four levels: before the intervention and 5 min, 15 min and 30 min after the intervention) as within-subject factors. The within-subject factor MUSCLE (three levels: FDI, APB and ADM) was added when evaluating the MEP collected during TMS-evoked finger movements recordings. Analysis of the TMS-evoked finger movements in the second control experiments employed SESSION (two levels: 'short-motor training' and 'short-motor training plus cTBS150') and TIME POINT (four levels: before the intervention and 5 min, 15 min and 30 min after the intervention) as within-subject factors. If needed, Greenhouse–Geisser correction was applied to ANOVAs. Tukey's honest significant difference test was used for post-hoc analysis following the ANOVAs and results were corrected for multiple comparisons using Bonferroni's method.

Pearson's product–moment correlation coefficient was calculated to evaluate possible associations between M1 excitability changes in 'motor training' and 'motor training plus cTBS150' sessions of the main experiments. For this purpose we used the MEP ratio: average MEP at 5 min post intervention/average MEP at

baseline in both sessions. We also evaluated possible associations between the reversal of M1 excitability in the 'motor training plus cTBS150' session (with respect to the M1 excitability changes in the 'motor training' session) and motor retention. To do so we calculated a 'reversal index': [MEP ratio in the "motor training" session] − [MEP ratio in the 'motor training plus cTBS150' session], larger values for the 'reversal index' indicates a more significant effect of the DePo protocol. Then, we tested a possible correlation between the 'reversal index' of the MEP and offline changes of motor performance in the 'motor training plus cTBS150' session [29,30]. Unless otherwise stated, all results are indicated as mean values ± 1 standard error of the mean (SEM). In all tests the level of significance was set at $P < 0.05$. Data were analyzed using STATISTICA® (StatSoft, Inc).

Results

Main experiment

Motor training task

The time course of the acceleration peak throughout the motor task in the two training sessions of the main experiments is shown in Fig. 2. Two-way repeated-measures ANOVA showed a significant effect of TRIAL NUMBER [$F_{(15,210)} = 5.56$, $P < 0.001$]. Post-hoc analysis revealed higher acceleration values in the 3rd to 15th acquisition trial blocks and for the retention trial block, in comparison to the 1st acquisition block, indicating that participants improved performance of the practiced movement and retained the improvement at 45 min after training (P values all <0.001). However, there was no effect of SESSION [$F_{(1,14)} < 0.01$, $P = 0.94$] and no interaction TRIAL NUMBER × SESSION [$F_{(15,210)} = 0.87$, $P = 0.59$]. These results were further confirmed by two-tailed paired t-tests showing no significant difference in online ('motor training' session:4.80 ± 1.10 m/s^2, 'motor training plus cTBS150' session: 6.81 ± 0.92 m/s^2; $P = 0.38$) and offline effects ('motor training' session:−1.64 ± 3.17 m/s^2, 'motor training plus cTBS150' session: −4.19 ± 1.20 m/s^2; $P = 0.20$) of motor performance (Fig. 3). We conclude that participants' performance was the same in the two training sessions of the main experiment and that motor performance was retained to the same extent in both sessions when tested 45 min after training. Thus the DePo protocol did not interfere with practice-related motor retention.

M1 excitability

Baseline motor thresholds (expressed as percentage of MSO) are summarized in the Table 1. There was no difference in motor thresholds between experimental sessions [RMT: $F_{(5,72)} = 0.04$, $P = 0.99$; AMT: $F_{(5,72)} = 0.08$, $P = 0.99$]. Results on MEP I/O curves for FDI are shown in Fig. 4 (data for the APB and ADM muscles are reported in the Supplementary Table 1). The overall excitability of the FDI output (but not of the APB or ADM) increased in the 'motor training' session (but not in the 'motor training plus cTBS150' or in the 'cTBS150' sessions), indicating a focal effect of training on cortical representations of the involved muscle without spread to surround muscles. This was supported by a repeated-measures ANOVA, showing a significant MUSCLE × TIME POINT interaction [$F_{(6,84)} = 4.32$, $P = 0.01$], and most importantly, a significant MUSCLE × SESSION × TIME POINT interaction [$F_{(12,168)} = 3.09$, $P = 0.02$]. Post-hoc analysis revealed that the excitability of the FDI output was enhanced 5 min after motor training with respect to baseline measures ($P < 0.001$) and then gradually returned to the baseline (baseline vs. post 15′: $P = 0.10$; baseline vs. post 30′: $P = 1.00$, Fig. 3). As expected, repeated measures ANOVA also revealed a significant effect of the factors MUSCLE [$F_{(2,28)} = 55.3$, $P < 0.001$], with higher MEPs values being recorded in the FDI muscle than in the APB ($P < 0.001$) and ADM ($P < 0.001$) and of STIMULUS INTENSITY [$F_{(2,28)} = 80.0$, $P < 0.001$] confirming that the mean MEPs amplitude increased with increasing stimulus intensity. The interaction term MUSCLE × STIMULUS INTENSITY was also significant [$F_{(4,56)} = 41.4$, $P < 0.001$] due to a steeper MEP I/O curve in the FDI than in the APB and ADM muscles. There were no other significant main or interaction effects: SESSION [$F_{(2,28)} = 1.39$, $P = 0.26$]; TIME POINT [$F_{(3,42)} = 2.07$, $P = 0.14$]; MUSCLE × SESSION [$F_{(4,56)} = 2.13$, $P = 0.13$]; SESSION × TIME POINT [$F_{(6,84)} = 2.43$, $P = 0.07$]; SESSION × INTENSITY [$F_{(4,56)} = 1.14$, $P = 0.32$]; TIME POINT × INTENSITY [$F_{(6,84)} = 0.92$, $P = 0.43$]; MUSCLE × SESSION × INTENSITY [$F_{(8,112)} = 1.72$, $P = 0.18$]; MUSCLE × TIME POINT × INTENSITY [$F_{(12,168)} = 1.23$, $P = 0.30$]; SESSION × TIME POINT × INTENSITY [$F_{(12,168)} = 1.36$, $P = 0.25$]; MUSCLE × SESSION × TIME POINT × INTENSITY [$F_{(24,336)} = 1.40$, $P = 0.16$]. We conclude that training fast index finger abductions selectively increased the excitability of the FDI output and that this was abolished if motor training was followed by the cTBS150 protocol; finally cTBS150 alone had no effect on corticospinal excitability (Fig. 4).

TMS-evoked movements

As shown in Fig. 5, after motor training, the acceleration peak of the TMS-evoked index finger movement increased in the direction of training (finger abduction axis). Analysis of the acceleration peak revealed no significant effect of SESSION [$F_{(2,28)} = 2.64$, $P = 0.09$] although there was a significant effect of TIME POINT [$F_{(3,42)} = 4.67$, $P = 0.02$] and SESSION × TIME POINT interaction [$F_{(6,84)} = 3.0$, $P = 0.03$]. Post hoc analysis revealed that the acceleration peak of the TMS-evoked index finger movement increased at 5 min after training in both the 'motor training' (baseline vs. post 5 min: $P = 0.002$) and 'motor training plus cTBS150' session (baseline vs. post 5 min: $P = 0.001$).

Results on MEP recordings during TMS-evoked movements for FDI, APB and ADM muscles are reported in the Supplementary Table 2. Repeated measures ANOVA revealed no significant effect of SESSION [$F_{(2,28)} = 0.96$, $P = 0.39$], TIME POINT [$F_{(3,42)} = 0.69$, $P = 0.55$] and interactions MUSCLE × SESSION [$F_{(4,56)} = 1.09$, $P = 0.36$], MUSCLE × TIME POINT [$F_{(6,84)} = 0.57$, $P = 0.74$], SESSION × TIME POINT [$F_{(6,84)} = 0.99$, $P = 0.43$] and MUSCLE × SESSION × TIME POINT [$F_{(12,168)} = 0.97$, $P = 0.47$]. As expected, the only significant factor was MUSCLE [$F_{(2,28)} = 2.64$, $P = 0.09$], with higher values being observed in the FDI, than in the APB and ADM (both $P < 0.001$) and in the ADM than in APB ($P < 0.001$). These results indicate that training fast index finger abductions increased the acceleration of TMS-evoked movements in the direction of training. However these kinematic changes were unaffected by the DePo protocol. Finally changes in TMS-evoked movement were not associated with any clear changes in the amplitude of evoked MEPs measured in the same task.

Table 1
Baseline resting motor threshold (RMT) and active motor threshold (AMT) values in the three sessions of the main experiment and in the control experiments. Plus and minus values are means ± 1 standard error of the mean (SEM). Resting motor threshold (RMT) measured in experiments using a monophasic transcranial magnetic stimulator. Active motor threshold (AMT) measured in experiments using a biphasic repetitive transcranial magnetic stimulator.

	Main experiment			Control experiments		
	Motor training	Motor training plus cTBS150	cTBS150	Motor training plus cTBS150 (5′)	Short motor training	Short motor training plus cTBS150
RMT	44.6 ± 1.4	44.1 ± 1.7	44.2 ± 1.8	44.9 ± 1.6	44.4 ± 1.7	44.6 ± 1.7
AMT	37.1 ± 1.8	38.1 ± 1.9	37.7 ± 2.1	36.4 ± 1.9	37.4 ± 1.5	37.1 ± 1.2

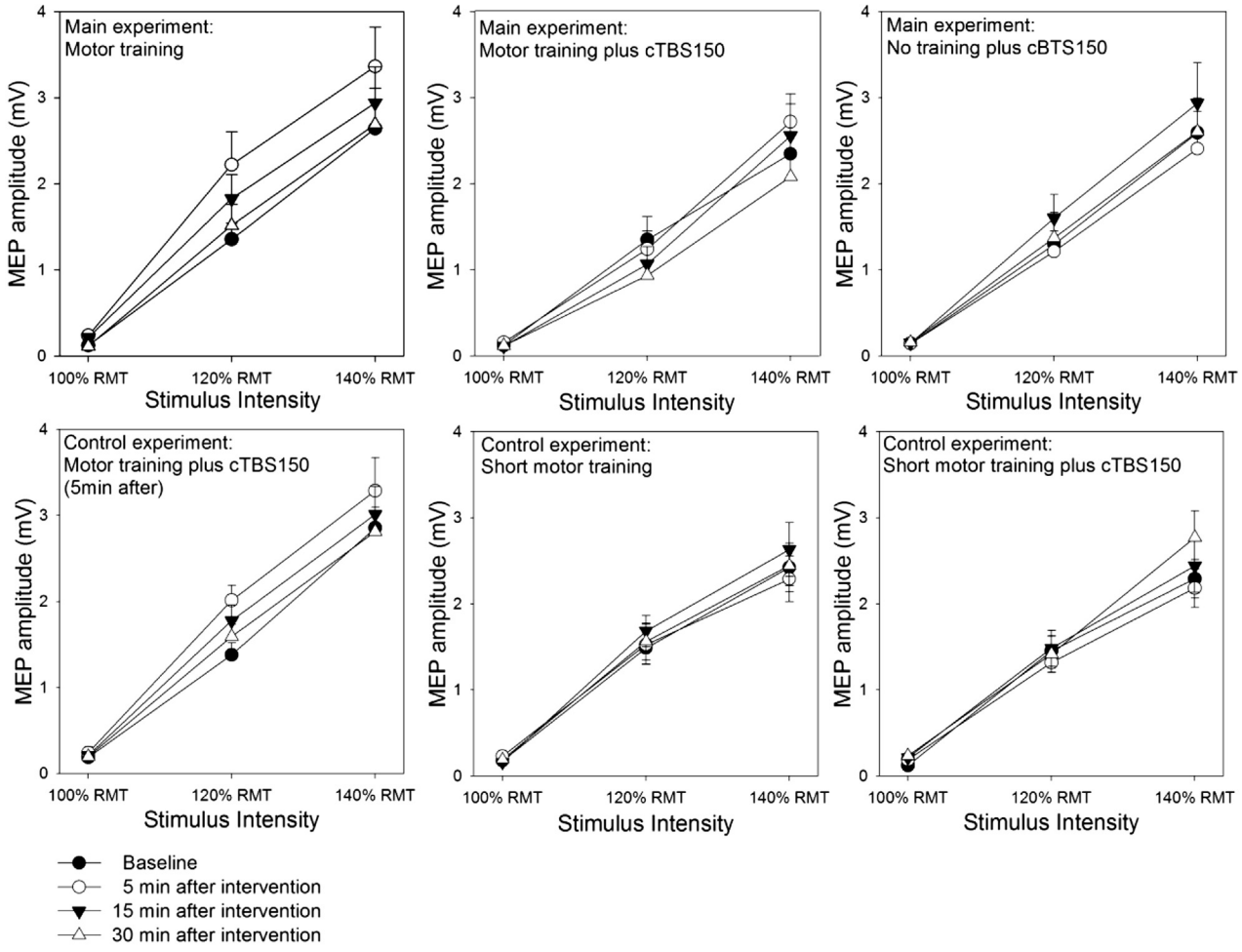

Figure 4. Cortical excitability changes (motor evoked potentials – MEP amplitude in mV – *y* axis) in the FDI area in the three main experimental sessions and in controls experiments. Vertical bars indicate 1 standard error of the mean. In the main experiment, motor training increased the excitability of corticospinal output to FDI (but not to muscles unrelated to the task: APB and ADM); the cTBS150, however, was able to erase the increased corticospinal excitability produced by motor training; finally, cTBS150 alone had no effect on corticospinal excitability (significant MUSCLE × SESSION × TIME POINT interaction [$F_{(12,168)} = 3.09$, $P = 0.02$] by repeated measure ANOVA). In the control experiment testing the time specificity of the of DePo protocol effects on M1 excitability higher MEPs values occurred 5 min after motor training in comparison to baseline thus indicating that the cTBS150 did not reverse the increase in M1 excitability if it was delivered ~5 min after motor training (significant effect of the main factors TIME POINT [$F_{(3,42)} = 8.7$, $P = 0.02$] and TIME POINT × STIMULUS INTENSITY [$F_{(6,84)} = 3.86$, $P = 0.02$ by repeated measure ANOVA). Finally the excitability of the FDI output did not change after 'short-motor training.'

Correlation analysis

There was no evidence of a correlation between M1 excitability changes in the 'motor training' and 'motor training plus cTBS150' sessions ($r = -0.21$, $P = 0.44$). Similarly, no relationship emerged between the 'reversal index' of the MEP and offline changes of motor performance in the 'motor training plus cTBS150' sessions ($r = -0.29$, $P = 0.28$). Thus at this stage in learning, changes in M1 excitability were unrelated to motor performance.

Control experiments

Time specificity of the of DePo protocol effects on M1 excitability

The time course of the acceleration peak in this control experiment is shown in Fig. 2. Repeated-measures ANOVA disclosed a significant effect of TRIAL NUMBER [$F_{(14,196)} = 4.53$, $P = 0.001$]. Post-hoc analysis revealed higher acceleration values of the index finger abductions for the 3rd to 15th acquisition trial blocks in comparison to the 1st acquisition block, confirming that participants increased their acceleration during training (*P* values all <0.001). As the original 15 subjects trained on the same behavioral task consisting of 15 acquisition trials blocks on three different occasions, we tested for any possible carry-over effects across sessions on baseline measurements using a repeated measures ANOVA using SESSION ORDER as within-subject factor (three levels: 1st, 2nd and 3rd training session), the analysis revealed no significant effect, thus indicating no effects of practice order [$F_{(2,28)} = 0.27$, $P = 0.76$].

Repeated-measures ANOVA on I/O MEP values also revealed a significant effect of the main factors TIME POINT [$F_{(3,42)} = 8.7$, $P = 0.02$] and STIMULUS INTENSITY [$F_{(2,28)} = 64.81$, $P < 0.001$] together with a significant interaction TIME POINT × STIMULUS INTENSITY [$F_{(6,84)} = 3.86$, $P = 0.02$]. Post hoc analysis showed that higher MEPs values occurred 5 min after motor training in comparison to baseline ($P < 0.001$), indicating that the DePo protocol did not reverse the increase in M1 excitability if it was delivered ~5 min after motor training, in contrast to its effect when applied 1 min after training (Fig. 4). We also tested for any possible difference across sessions (Motor training alone vs. DePo delivered 5 min after training) on percentage changes in FDI MEPs (average MEP at 5 min post intervention/average MEP at baseline in both sessions) using a two-tailed paired samples *t*-test. The analysis revealed that the percentage MEP change at 5 min in the motor training session (main experiment) and in the DePo delivered 5 min after training session did not significantly differ (141.63 ± 8.36 vs.130.96 ± 5.90;

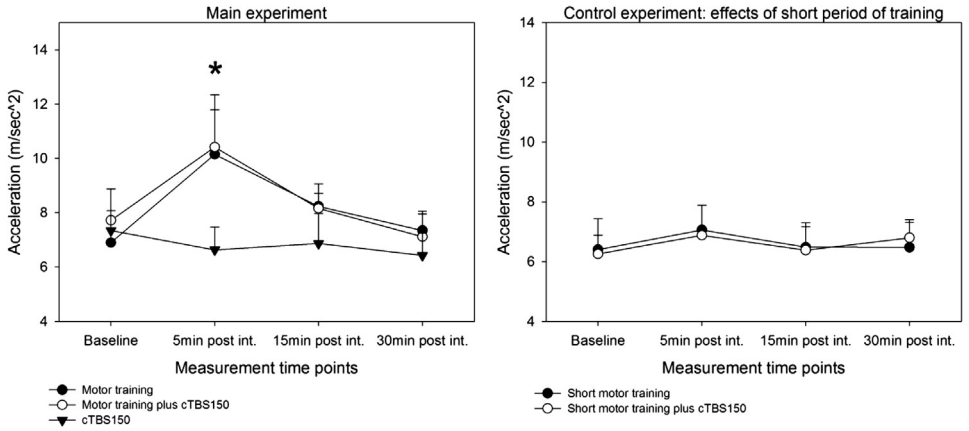

Figure 5. Course of the acceleration peak, expressed in m/s^2 of the TMS evoked index finger movements toward the direction of training in the four measurements time points: baseline, 5 min, 15 min and 30 min in the three main experimental sessions (left panel) and in control experiment evaluating the effects of a short period of training (right panel). Vertical bars indicate 1 standard error of the mean. Asterisk indicates significant SESSION × TIME POINT interaction [$F_{(6,84)} = 3.0$, $P = 0.03$] by repeated measure ANOVA (post-hoc analysis: 'motor training' session − baseline vs. post 5 min: $P = 0.002$; 'motor training plus cTBS150' session − baseline vs. post 5 min: $P = 0.001$).

$P = 0.33$). It is worth noting that in the present control experiment MEPs were tested immediately after cTBS150 whereas in the main experimental session cTBS150 was applied at 1 min after training and MEPs were tested at 5 min after training, i.e. there was a delay of 4 min between cTBS150 and MEP testing. Thus, there is no time-matched testing of MEPs with respect to cTBS150. A more detailed evaluation of the timing-dependency of the DePo protocol is beyond the aim of the study.

Effects of short period of training

Figure 2 depicts the time course of the acceleration peak in the two sessions of this control experiment. Repeated-measures ANOVA showed a significant effect of TRIAL NUMBER [$F_{(3,24)} = 19.19$, $P < 0.001$]. Post-hoc analysis revealed higher acceleration values in the 3rd acquisition trial blocks ($P < 0.01$), but not for the retention trial block ($P = 0.99$), compared with the 1st acquisition block, indicating that participants improved performance of the practiced movement during a short period of training, but they did not retain it when tested 45 min after training. In addition, the participants' performance was the same in both of the short-training sessions in this control experiment as revealed by no significant effects of SESSION [$F_{(1,8)} = 0.02$, $P = 0.87$] or TRIAL NUMBER × SESSION interaction [$F_{(3,24)} = 0.43$, $P = 0.73$] (Fig. 2).

The excitability of the FDI output did not change after 'short-motor training': there were no significant effects of SESSION [$F_{(1,8)} = 0.15$, $P = 0.70$] or TIMEPOINT [$F_{(3,24)} = 1.02$, $P = 0.39$]. As expected, the only significant factor was STIMULUS INTENSITY [$F_{(2,16)} = 54.9$, $P < 0.001$]. Finally there was no significant interaction between the main factors of analysis [SESSION × TIME POINT: $F_{(3,24)} = 0.25$, $P = 0.85$; SESSION × STIMULUS INTENSITY: $F_{(2,16)} = 0.49$, $P = 0.61$; SESSION × TIME POINT × STIMULUS INTENSITY: $F_{(2,16)} = 0.39$, $P = 0.87$].

There was also no significant effect of the factors SESSION [$F_{(1,8)} < 0.01$, $P = 0.97$] or TIME POINT [$F_{(3,24)} = 0.58$, $P = 0.62$] and no significant interaction SESSION × TIMEPOINT [$F_{(3,24)} = 0.06$, $P = 0.97$] in the analysis of TMS-evoked finger movements (Fig. 5).

We conclude that a short training session did not increase the excitability of the FDI output and did not induce significant behavioral effects.

Discussion

As expected we found that subjects improved performance during training. Second, motor training transiently and focally increased the excitability of corticospinal output to FDI but not to muscles unrelated to the task (APB and ADM). Third, motor training encoded the kinematics of the practiced movement in M1 as revealed by an increased peak acceleration of the TMS-evoked index finger movements in the direction of training. Finally, subjects retained the enhanced performance when tested 45 min after training. However, the effects of training on M1 excitability and TMS-evoked peak acceleration lasted only 5 min whereas retention of the motor skill lasted at least 45 min following training. The differences in time course suggest that the effects are mechanistically distinct. This hypothesis is further supported by the effects of the DePo protocol. The DePo protocol abolished the practice-related potentiation of MEPs if it was given 1 min after the end of training but it had no effect when delivered alone or ~5 min after training. Thus, the DePo protocol could interact with the motor practice-related changes of corticospinal excitability. Despite this, the DePo protocol had no effect on the increased acceleration of TMS-evoked movements or on the retained performance gain at 45 min after training.

Depotentiation abolishes motor-training related short term increases of corticospinal excitability

The DePo protocol, which by itself has no effect on excitability of corticospinal output, was able to erase the increased corticospinal excitability produced by motor training. The effect is not explained by different levels of motor performance during training, since participants improved their index finger acceleration at similar rates in the two training sessions of the main experiment. Since baseline corticospinal excitability was constant in the different experimental sessions we also can exclude this as a possible confound. Nor is it likely that the reversal of motor-training related increases of corticospinal excitability reflects any carry-over effect between experimental sessions since these were randomized and performed at least one week apart. We can also exclude the possibility that non-specific factors related to cortical stimulation (e.g. scalp sensation or noise of stimulation) were responsible since there was no effect if cTBS150 was delivered 5 min rather than 1 min after the end of training.

Our results are in line with previous studies showing that the effect of DePo on corticospinal excitability is time-dependent, i.e. that DePo is less effective if there is a longer interval between the corticospinal excitability changes induced by applying rTMS protocols to M1 and delivery of DePo [3]. We conclude that

reversal of practice-related excitability changes in M1 is a process that results from the interaction between 'endogenous' mechanisms involved in repetition of a simple movement and the DePo intervention. The results confirm and extend previous observations by Canterero et al. [24] who showed that increases in M1 excitability produced after a different training protocol were also abolished by applying the DePo protocol immediately after training.

The nature of the reversibility of the corticospinal excitability changes in M1 is still matter of debate. It is still not entirely clear whether the mechanisms underlying the reversal of exogenously or endogenously induced M1 plasticity reflect a partial 'homeostatic' interaction [3,24,30–32] due to the relative weakness of the DePo protocol [3]. In our experiments there was no correlation between the increase in corticospinal excitability elicited by motor training and its reversal in the 'motor training plus cTBS150' session. This is consistent with the idea that the effects of DePo on endogenously induced plasticity in healthy humans reflects phenomena similar to those observed in animal studies [22,23] and in humans [3,4,24].

Depotentiation has no effect on the practice-related acceleration changes of TMS-evoked movements

In the present study we observed that repeated abduction of the index finger increased the acceleration of TMS-evoked index finger movements in the direction of training. Classen et al. [12], proposed that training 'established a change in the cortical network representing the thumb, which encoded kinematic details of the practiced movement'. They suggested that this could be 'regarded as a short-term memory for movement and be the first step of skill acquisition'. The process has also been termed 'use-dependent learning', to emphasize that it is acquired automatically after repetition of any, usually attention demanding, movement [33]. It may be that when TMS is applied to this changed network, then the movement evoked is changed in the same way as the practiced movement.

Changes in TMS-evoked movement, however, were not associated with any clear changes in the amplitude of MEPs recorded simultaneously in FDI. One possibility is that the lack of changes of the MEPs is due to a ceiling effect. An alternative explanation is that the change in the cortical network produced during learning involves not only outputs to FDI, but also to other agonist and antagonist muscles. Thus the change in TMS-evoked movement could reflect different patterns of activation of these muscles rather than FDI. Indeed, Butefisch et al. [34], found that a period of practice increased MEPs in the relaxed agonist muscle whereas those in the antagonist decreased. Applied to the present results, this could mean that after practice, TMS evokes a smaller MEP in the antagonist and that this was responsible for the larger movement. However as we did not examine excitability in the antagonist, this needs to be confirmed in future experiments with careful measurement of all agonist and antagonist muscle MEPs involved in the evoked movement.

An original finding of the study was that changes in TMS-evoked movement were unaffected by the DePo protocol. Similarly, the DePo protocol had no effect on MEPs recorded during TMS-evoked movements despite the clear effects on the practice-induced increase in M1 excitability measured at rest. The probable reason is that we measured movement produced by TMS pulses while subjects actively held their index finger extended. This movement activates the FDI muscle to a small extent, and it is possible that effects of practice or DePo differ when cortical motor areas are stimulated at rest vs. activation. This is true, for example for 1Hz rTMS: the usual MEP suppression is no longer present when tested during active muscle contraction [35].

Depotentiation has no effect on short-term retention of the practiced movements

Reversal of motor-practice related changes of corticospinal excitability did not affect early retention of the practiced movements. Ghilardi et al. [36] demonstrated that motor retention was prone to disruption by a subsequent period of training but that this depended on the duration of the first training period. If subjects performed only few trials during the first session, retention was easily disrupted by subsequent training of a second motor task. However, if subjects were over-trained on the first task, then retention of motor performance was no longer disrupted by subsequent training of a second behavioral task. In the current study performance plateaued quite quickly after the initial sessions of training, potentially leading to over-training effects. We tested for this by examining the effects of a short period of training. In this case, training produced an immediate increase in finger acceleration but this was not retained when tested 45 min later. The short training session also failed to produce significant changes in M1 excitability. A possible explanation is that changes of M1 excitability and motor retention processes started later on during the training. However, if this task requires more than three sessions to produce any retention (and MEP changes), then it seems unlikely that the total of 15 training sessions used in the main experiment would have resulted in significant over-training, although without testing additional training durations we cannot exclude this as a potential explanation of our results.

As shown by Muellbacher et al. [14], and Baraduc et al. [37], 1Hz rTMS applied immediately after practicing a ballistic movement task completely abolished all the improvement in performance when participants were tested a few minutes later. However, in both cases, the TMS pulses were supra-threshold, causing muscle twitches in the targeted muscle. A later investigation by Lundbye-Jensen et al. [38], showed that the motor memory could be abolished purely by stimulation of the peripheral muscle nerve, suggesting that suppression of performance gains was due to re-afference from the movement evoked by the TMS pulse rather than interference with a memory encoded in cortex.

More recently Cantarero et al. [24], used the same sub-threshold DePo technique as employed in the present experiments, and found that it suppressed both MEPs and performance gain. However, they tested MEPs immediately after practice whereas retention of performance was tested 24 h later. Thus it appears from the present results that DePo has no effect on immediate performance gains even though the data from Canterero et al. [24], show that it depresses long-term retention. A similar, but inverse dissociation was described by Hotermans et al. [25], who found that 1Hz rTMS at 90% resting threshold could abolish the immediate improvement in performance of a finger tapping task, but had no effect on retention of the task 24 h later. These studies suggest that different mechanisms are involved in immediate improvements in performance vs. longer-term retention, 1Hz rTMS appears to interfere with early improvement whereas DePo interferes with late retention. We conclude that in the short term, increases in corticospinal excitability are not related to immediate changes in performance. An alternative explanation for the different effects of DePo on motor retention in the study by Cantarero et al. [24], could relate to differences in the behavioral tasks. The task used in this study likely relies on use-dependent learning mechanisms whereas Cantarero et al. [24], used a skill task, which might involve forms of reinforcement learning that may have a different neurophysiology.

Limitations

In the present study we specifically examined the effects of DePo protocol on electrophysiological and behavioral effects of a relatively short duration motor training task and therefore whether results will generalize to other stages of motor learning, such as consolidation is unknown [18,39]. For example, Walker et al. [40], showed that it is possible to interfere with motor performance the following day by training 2 skills in close succession. However, if performance was probed immediately after motor training (rather than the next day), there was no difference in performance between subjects who trained one task in isolation vs. 2 tasks in an interference paradigm. Also, since we examined the movement retention 45′ after the end of training we cannot exclude the possibility that performance gains earlier in the time course could be modulated by the DePo protocol. Finally, it is important to note that in the present study we adopted a simple motor training task; further experiments are needed to clarify the effects of DePo on more complex forms of motor learning.

Conclusions

The present study further explores the mechanisms of DePo in healthy humans. The present experiments suggest that in the short term, increases in corticospinal excitability are not related to immediate changes in motor performance and therefore immediate changes in corticospinal excitability are not necessarily a good indicator of expected behavioral gains. They might also be helpful to prompt further research on mechanisms that reverse plasticity in other forms of motor learning in healthy humans and in pathological conditions.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.brs.2015.01.405.

References

[1] Cooke SF, Bliss TV. Plasticity in the human central nervous system. Brain 2006;129:1659–73.
[2] Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201–6.
[3] Huang YZ, Rothwell JC, Lu CS, Chuang WL, Lin WY, Chen RS. Reversal of plasticity-like effects in the human motor cortex. J Physiol 2010;588:3683–93.
[4] Huang YZ, Rothwell JC, Lu CS, Chuang WL, Chen RS. Abnormal bidirectional plasticity-like effects in Parkinson's disease. Brain 2011;134:2312–20.
[5] Ziemann U, Paulus W, Nitsche MA, et al. Consensus: motor cortex plasticity protocols. Brain Stimul 2008;1:164–82.
[6] Chen R, Udupa K. Measurement and modulation of plasticity of the motor system in humans using transcranial magnetic stimulation. Motor Control 2009;13:442–53.
[7] Huang YZ, Rothwell JC, Edwards MJ, Chen RS. Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. Cereb Cortex 2008;18:563–70.
[8] Teo JT, Swayne OB, Rothwell JC. Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol 2007;118:1649–51.
[9] Agostino R, Iezzi E, Dinapoli L, et al. Effects of 5 Hz subthreshold magnetic stimulation of primary motor cortex on fast finger movements in normal subjects. Exp Brain Res 2007;180:105–11.
[10] Agostino R, Iezzi E, Dinapoli L, Suppa A, Conte A, Berardelli A. Effects of intermittent theta-burst stimulation on practice-related changes in fast finger movements in healthy subjects. Eur J Neurosci 2008;28:822–8.
[11] Li Voti P, Conte A, Suppa A, et al. Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. Exp Brain Res 2011;212:91–9.
[12] Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol 1998;79:1117–23.
[13] 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.
[14] Muellbacher W, Ziemann U, Wissel J, et al. Early consolidation in human primary motor cortex. Nature 2002;415:640–4.
[15] Ziemann U, Ilić 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.
[16] Stefan K, Classen J, Celnik P, Cohen LG. Concurrent action observation modulates practice-induced motor memory formation. Eur J Neurosci 2008;27:730–8.
[17] Jung P, Ziemann U. Homeostatic and nonhomeostatic modulation of learning in human motor cortex. J Neurosci 2009;29:5597–604.
[18] Censor N, Cohen LG. Using repetitive transcranial magnetic stimulation to study the underlying neural mechanisms of human motor learning and memory. J Physiol 2011;589:21–8.
[19] Rosenkranz K, Rothwell JC. Modulation of proprioceptive integration in the motor cortex shapes human motor learning. J Neurosci 2012;32:9000–6.
[20] Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP. Strengthening of horizontal cortical connections following skill learning. Nat Neurosci 1998;1:230–4.
[21] Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. Science 2000;290:533–6.
[22] Huang CC, Liang YC, Hsu KS. A role for extracellular adenosine in time dependent reversal of long-term potentiation by low-frequency stimulation at hippocampal CA1 synapses. J Neurosci 1999;19:9728–38.
[23] Huang CC, Hsu KS. Progress in understanding the factors regulating reversibility of long-term potentiation. Rev Neurosci 2001;12:51–68.
[24] 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.
[25] Hotermans C, Peigneux P, de Noordhout AM, Moonen G, Maquet P. Repetitive transcranial magnetic stimulation over the primary motor cortex disrupts early boost but not delayed gains in performance in motor sequence learning. Eur J Neurosci 2008;28:1216–21.
[26] Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008–39.
[27] Möller C, Arai N, Lücke J, Ziemann U. Hysteresis effects on the input-output curve of motor evoked potentials. Clin Neurophysiol 2009;120:1003–8.
[28] Bologna M, Caronni A, Berardelli A, Rothwell JC. Practice-related reduction of electromyographic mirroring activity depends on basal levels of interhemispheric inhibition. Eur J Neurosci 2012;36:3749–57.
[29] Cantarero G, Tang B, O'Malley R, Salas R, Celnik P. Motor learning interference is proportional to occlusion of LTP-like plasticity. J Neurosci 2013;33:4634–41.
[30] 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.
[31] Bienenstock EL, Cooper LN, Munro PW. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 1982;2:32–48.
[32] Abraham WC. Metaplasticity: tuning synapses and networks for plasticity. Nat Rev Neurosci 2008;9:387.
[33] Diedrichsen J, White O, Newman D, Lally N. Use-dependent and error-based learning of motor behaviors. J Neurosci 2010;30:5159–66.
[34] Bütefisch CM, Khurana V, Kopylev L, Cohen LG. Enhancing encoding of a motor memory in the primary motor cortex by cortical stimulation. J Neurophysiol 2004;91:2110–6.
[35] Touge T, Gerschlager W, Brown P, Rothwell JC. Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? Clin Neurophysiol 2001;112:2138–45.
[36] Ghilardi MF, Moisello C, Silvestri G, Ghez C, Krakauer JW. Learning of a sequential motor skill comprises explicit and implicit components that consolidate differently. J Neurophysiol 2009;101:2218–29.
[37] Baraduc P, Lang N, Rothwell JC, Wolpert DM. Consolidation of dynamic motor learning is not disrupted by rTMS of primary motor cortex. Curr Biol 2004;14:252–6.
[38] Lundbye-Jensen J, Petersen TH, Rothwell JC, Nielsen JB. Interference in ballistic motor learning: specificity and role of sensory error signals. PLoS One 2011;6:e17451.
[39] Walker MP, Brakefield T, Hobson JA, Stickgold R. Dissociable stages of human memory consolidation and reconsolidation. Nature 2003;425:616–20.
[40] Walker MP, Brakefield T, Seidman J, Morgan A, Hobson JA, Stickgold R. Sleep and the time course of motor skill learning. Learn Mem 2003;10:275–84.