DIFFERENTIAL EFFECTS OF MOTOR SKILL ACQUISITION ON THE PRIMARY MOTOR AND SENSORY CORTICES IN HEALTHY HUMANS

Giulia Paparella,[1] Lorenzo Rocchi,[2] Matteo Bologna,[1,3] Alfredo Berardelli,[1,3] John Rothwell[2]

[1]*Neuromed Institute IRCCS, Pozzilli (IS), Italy;*

[2]*Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology,*

University College London, London, United Kingdom

[3]*Department of Human Neurosciences, Sapienza University of Rome, Italy*

Corresponding author:

Dr. Giulia Paparella

IRCCS Neuromed, Via Atinense 18, 86077 Pozzilli, (IS), Italy.

Tel: +39 0865 929600

giulia.paparella@uniroma1.it

https://orcid.org/0000-0002-7760-9442

somatosensory evoked potentials (SEPs), short-interval intracortical inhibition (SICI), transcranial magnetic stimulation (TMS).

Key points summary:

- We explored the large variability in motor skill acquisition-related effects on the primary and sensory cortices. Namely, we tested whether this variability depends on interindividual variance or the type of motor task investigated.

- We compared different motor learning tasks, i.e. model-free vs. model-based learning tasks, and their possible differential effects on the primary motor and sensory cortices by using transcranial magnetic stimulation techniques.

- The model-free learning task induced an increase in corticospinal excitability and a reduction in the amplitude of somatosensory evoked potentials. Conversely, the model-based learning tasks induced a decrease in intracortical inhibition.

- No correlations were found between neurophysiological changes and motor performance, indicating that this differential modulation may be secondary to the motor skill acquisition.

- The study results suggest differential motor skill acquisition-related effects on cortical parameters, possibly due to the engagement of specific neurophysiological substrates.

ABSTRACT

A large variability in learning-related neurophysiological changes in the primary motor and sensory cortices has been observed. It is unclear whether these differential effects are due to the different tasks investigated or to interindividual variance. Only a few studies have assessed different motor learning tasks and their effects on neurophysiological features within the same group of participants, and several issues are unclear. Here, we compared the effects

of different tasks within each individual. We investigated the effects on motor and sensory cortex parameters after a model-free learning task, i.e. a ballistic motor task, compared to model-based learning tasks, i.e. visuomotor learning tasks. Motor and sensory evoked potentials, intracortical excitability as assessed by short-interval intracortical inhibition, and sensorimotor interaction, i.e. short-latency afferent inhibition, were recorded from 15 healthy subjects before and after the tasks. The ballistic motor task induced an increase in corticospinal excitability but did not change motor cortex intracortical inhibition or sensorimotor integration. In addition, it decreased the amplitude of cortical components of the somatosensory evoked potentials. The visuomotor learning tasks induced a reduction in motor cortex intracortical inhibition but did not modulate corticospinal and sensory cortex excitability or sensorimotor integration. This differential modulation is likely secondary to the motor skill acquisition, since no correlation was observed between neurophysiological changes and motor performance. Our results demonstrate differential motor skill acquisition-related effects on cortical parameters, possibly reflecting the engagement of specific neurophysiological substrates, and contribute in-depth knowledge of the mechanisms involved in different types of motor skill acquisition in humans.

INTRODUCTION

Experimental studies have consistently pointed to a prominent role of the primary motor (M1) and somatosensory cortices (S1) in motor learning (Pascual-Leone *et al.*, 1995; Classen *et al.*, 1998; Muellbacher *et al.*, 2001; Rosenkranz *et al.*, 2007; Bologna *et al.*, 2015, 2019; Andrew *et al.*, 2015; McGregor *et al.*, 2016; Berghuis *et al.*, 2017; Hannah *et al.*, 2019). Learning-related M1 changes include increased corticospinal excitability as reflected by increased motor evoked potential (MEP) amplitude, as well as cortical map enlargement of

the trained muscles. More recent neurophysiological studies have also demonstrated a learning-related decrease in M1 intracortical excitability as assessed by short-interval intracortical inhibition (SICI) (Pascual-Leone *et al.*, 1995; Muellbacher *et al.*, 2001; Rosenkranz *et al.*, 2007; Cirillo *et al.*, 2011; Smith *et al.*, 2014; Coxon *et al.*, 2014; Berghuis *et al.*, 2017; Hannah *et al.*, 2019). However, the results of these studies are not always consistent, and a number of reports have shown no significant learning-related changes in MEPs or SICI (Rogasch *et al.*, 2009; Smith *et al.*, 2014; Berghuis *et al.*, 2017; Koizume *et al.*, 2017). Similarly, learning-related effects on S1, including changes in somatosensory evoked potentials (SEPs) (Murphy *et al.*, 2003; Haavik & Murphy, 2013; Andrew *et al.*, 2015; Dancey *et al.*, 2016; McGregor *et al.*, 2016) and sensorimotor interactions (Rosenkranz & Rothwell, 2012; Andrew *et al.*, 2015; Pelosin *et al.*, 2016; Matur & Öge, 2017) are variable.

One explanation for these discrepancies is the interindividual variability in cortical plasticity induced by motor learning, similar to what has been observed with exogenous plasticity-inducing protocols (Hamada *et al.*, 2013; López-Alonso *et al.*, 2014; Rocchi *et al.*, 2018). An alternative explanation is that this variability is due to the motor task investigated. Motor tasks ranged from simple ballistic movements (Classen *et al.*, 1998; Muellbacher *et al.*, 2001; Agostino *et al.*, 2008; Bologna *et al.*, 2015, 2019; Hussain *et al.*, 2016), reflective of model-free learning (Hamada *et al.*, 2014), to more complex tasks (Hirano *et al.*, 2015; Lopez-Alonso *et al.*, 2018), e.g. visuomotor tracking tasks, which are representative of model-based learning (Hamada *et al.*, 2014). These tasks may differently affect various physiological circuits, which could explain the variability of previous results.

Notably, only a few studies have compared different motor learning tasks and their relative effects on neurophysiological features in the same group of subjects (Muellbacher *et al.*,

2001; Hamada *et al.*, 2014; Andrew *et al.*, 2015; Lopez-Alonso *et al.*, 2018). Hamada and colleagues, for example, showed that performance improvement in ballistic movements and visuomotor gain adaptation tasks depended on different interneuronal circuits within M1 (Hamada *et al.*, 2014).

An additional issue to be clarified concerns the relationship between learning-related excitability changes in M1 and S1 and motor behaviour (Bologna *et al.*, 2015; Hirano *et al.*, 2015; Veldman *et al.*, 2016; Hussain *et al.*, 2016; Lopez-Alonso *et al.*, 2018; Hannah *et al.*, 2019). While Hamada and colleagues showed a relation between interneuronal circuit modulation within M1 and motor learning (Hamada *et al.*, 2014), most studies found no clear relationship between cortical neurophysiological changes and motor performance (Bologna *et al.*, 2015; Veldman *et al.*, 2016; Hussain *et al.*, 2016; Lopez-Alonso *et al.*, 2018; Hannah *et al.*, 2019). Hence, it is still unclear whether learning-related changes in corticospinal excitability or other cortical parameters reflect changes in behavioural motor outcome. Clarifying these issues may provide further insight into the physiological mechanisms of motor learning.

In the present study, we first aimed to investigate the possible differential effects on M1 and S1 excitability of different types of motor skill acquisition tasks within the same group of participants, with one representing a model-free learning task and the others representing model-based learning tasks. We used transcranial magnetic stimulation (TMS) to record MEPs, SEPs, SICI, and sensorimotor interaction as assessed by short-latency afferent inhibition (SAI) before and after the ballistic motor task (BMT) and two visuomotor tasks (VMTs). We also investigated possible correlations between cortical baseline neurophysiological measurements, skill acquisition related cortical effects, and motor performance.

MATERIALS AND METHODS

Ethical Approval

All participants gave their informed consent to the experimental procedures, which were approved by the local institutional review board (ID Number 03 N108) and conducted in accordance with international safety recommendations (Rossi *et al.*, 2009). The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

Participants

We enrolled 15 young healthy volunteers (6 females; mean age ± 1 standard deviation (SD): 27.0 ± 5.4 years). All participants were right-handed, as evaluated by the Handedness Questionnaire (Oldfield, 1971). None had a history of neurological or psychiatric disorders or medication intake. None had previously been exposed to professional training in specific hand movements. No individuals had contraindications to non-invasive brain stimulation involving TMS or reported any adverse effects during or after the experiments (Rossi *et al.*, 2009).

Motor skill acquisition tasks

Ballistic motor task

Tasks were adopted from previous studies (Classen *et al.*, 1998; Muellbacher *et al.*, 2001; Agostino *et al.*, 2008; Hamada *et al.*, 2014; Bologna *et al.*, 2015, 2019; Lopez-Alonso *et al.*, 2018; Hannah *et al.*, 2019). During the BMT, participants were seated on a chair with their right arm abducted, the elbow flexed to 90º, and the forearm pronated (Classen *et al.*, 1998;

Muellbacher *et al.*, 2001; Agostino *et al.*, 2008; Hamada *et al.*, 2014; Bologna *et al.*, 2015, 2019; Hannah *et al.*, 2019). The volar surface of the hand was placed on the edge of the chair handle. The index finger was extended and aligned with the forearm, and the remaining fingers and thumb were flexed, forming a firm grip on the chair handle. Subjects were instructed to perform a rapid abduction with the index finger and return to the initial neutral position in response to a brief auditory tone (given at 0.2 Hz, with a jitter of 10% to avoid prediction) in order to achieve the highest acceleration possible along the horizontal axis. The electromyographic (EMG) activity during the BMT was recorded through a pair of adhesive electrodes placed over the right first dorsal interosseous (FDI) in a belly-tendon montage. The ground electrode was placed over the styloid process of the right ulna. The acceleration of the index finger was measured with a triaxial accelerometer (ACL300; Biometrics Ltd., UK 1 mV/g; DataLOG; Biometrics Ltd., UK) and stored on a personal computer for offline analysis. Subjects were verbally encouraged to optimise their performance, taking into consideration the acceleration values recorded online. Each subject performed 15 consecutive movements in one block, and repeated the same procedure for 10 blocks, for a total of 150 movements. The total task duration was approximately 20 min. Previous studies demonstrated that this duration was sufficient to induce a plateau in corticospinal learning-related changes (Bologna *et al.*, 2015, 2019). Before starting, subjects performed one practice block of five movements in order to familiarise themselves with the task. To prevent subjects from fatiguing, a rest interval of 30 s was administered between each block (Lopez-Alonso *et al.*, 2018). We measured the root mean square (RMS) of the EMG activity recorded from the FDI (Farina *et al.*, 2014), using Signal software (Cambridge Electronic Design). Acceleration values (m/s^2) obtained using the triaxial accelerometer during the BMT were analysed using MATLAB R2018b (Version 9.5). Motor performance during the BMT was expressed as the

mean acceleration on the x-axis for each block (m/s^2) (Bologna *et al.*, 2015, 2019; Hannah *et al.*, 2019).

Visuomotor tasks

The VMTs were adaptations of the sequential visual isometric pinch task (Lopez-Alonso *et al.*, 2018). Participants were seated with their dominant arm lying on a pillow, as detailed for the BMT. The index finger was extended while the other fingers were relaxed in a neutral position. A force transducer (BT/P 200; Biometrics Ltd., UK) was attached to the edge of a fixed metal block, placed medially to the lateral margin of the distal phalanx of the index finger. Subjects were instructed to push against the surface of the transducer by abducting their index finger; the force applied resulted in the vertical displacement of a cursor, displayed on a PC screen. In each trial, subjects were asked to modulate their force to track the outline of a pattern containing five square waveforms. The total duration of a single trial was 11 s, and the duration of each waveform was 1 s. Waveforms were separated by 1 s, during which subjects returned to the neutral index finger position. The height of each of the five consecutive waveforms in the VMT was set at 15%, 5%, 20%, 10%, and 25% of each subject's respective MVC. Patients completed six blocks and performed 15 such trials per block, resulting in a total of 90 trials per subject. Subjects had one practice trial to familiarise themselves with the task. In order to prevent fatigue, a rest interval of 30 s was administered between each block. The total duration of the task was approximately 20 min (Lopez-Alonso *et al.*, 2018). The RMS of the EMG activity recorded from FDI was measured Signal software. Our primary measure was the absolute error, i.e. the distance between the red cursor tracing and the target square waveforms at every sampled time point (11000 total points, 1000 points/s) (Hamada *et al.*, 2014; Lopez-Alonso *et al.*, 2018). The average error represented motor performance for each block during the VMT (we excluded from the

average the middle 800 points of the intervals between the waveforms, during which the subject's finger returned to the neutral position, since they corresponded to 0, and we only considered the first and last 100 points of these intervals).

To exclude that possible differential skill acquisition-related effects of the two tasks were exclusively due to differences in the muscle force needed to perform the tasks, which was possibly lower during the VMT, we also performed a modified version of the VMT. In this control experiment session, the VMT was performed applying higher muscle forces (HF-VMT). Participants followed the same procedure as they did for the VMT, but 37.5%, 12.5%, 50%, 25%, and 62.5% of each subject's MVC values (2.5 times higher than respective percentages in the VMT) were used to set the peaks of each successive square wave. As in the VMT, motor performance for each block during the HF-VMT was represented by the average error (Lopez-Alonso *et al.*, 2018).

Neurophysiological M1 assessment

The EMG activity was recorded from the right FDI and from abductor digiti minimi (ADM) muscle, which was used as a control muscle. Raw signals were sampled at 5 kHz with a CED 1401 analog-to-digital laboratory interface (Cambridge Electronic Design) and amplified and filtered (bandwidth 5 Hz to 2 kHz) with a Digitimer D360 (Digitimer, Ltd.). Data were stored on a laboratory computer for online visual display and additional offline analysis using Signal software (Cambridge Electronic Design). Single and paired-pulse TMS was delivered on the left M1 using a Magstim 200 stimulator (Magstim Company) connected to a figure-of-eight coil held tangentially to the scalp with the handle pointing backwards at a 45º angle laterally to the midline. We first defined the hot spot of the FDI muscle as the optimal scalp position to obtain MEPs of maximal amplitude in the target muscle. In each session, the resting motor threshold (RMT) required to generate an MEP of 0.05 mV in the FDI was identified to the

nearest 1% of the maximal stimulator output (MSO) (Bologna *et al.*, 2015, 2019). The intensity able to elicit MEPs with an amplitude of approximately 1 mV from the FDI was then found. Subsequently, neurophysiological measures were recorded in one recording block (See experimental design). Fifteen MEPs using the test stimulus intensity (TS-MEPs) were recorded at complete rest, as confirmed by visual inspection of the EMG recording. We used paired-pulse TMS to assess SICI at an interstimulus interval (ISI) of 2 ms (Berardelli *et al.*, 2008). Three different conditioning stimulus intensities (CS-int) were used (50%, 70%, and 90% of the RMT), with 15 trials for each CS-int collected randomly with the other neurophysiological measures in a single recording block. The rationale for including 3 different CS intensities in the SICI assessment was twofold. First, by measuring the SICI at 3 different CS-intensities, it was more likely to detect possible changes related to motor skill acquisition which we could not predict a priori. Secondly, our approach followed the guidelines regarding the intracortical excitability assessment (Rossini *et al.*, 2015). MEP size was expressed as peak-to-peak amplitude (mV). SICI was expressed as the ratio between the conditioned and unconditioned MEPs (Berardelli *et al.*, 2008).

Sensorimotor interaction was assessed by recording SAI (Tokimura *et al.*, 2000; Hwang *et al.*, 2018). Conditioning stimuli consisted of single electrical pulses (0.2 ms) applied through bipolar electrodes to the ulnar nerve at the wrist, since it innervates the target muscle. Stimulus intensity was set at just over the motor threshold for evoking a compound muscle action potential higher than 100 microvolts. Two different ISIs between the conditioning stimulus and the TMS pulse, delivered on the contralateral M1, were explored (N20 + 2 ms and + 4 ms, based on the individual latency of N20 components of the SEP) (Tokimura et al., 2000). Fifteen trials for each ISI were collected and randomized with the other neurophysiological measures in a single recording block (see also Experimental design). SAI was expressed as the ratio between conditioned and unconditioned MEPs.

Neurophysiological S1 assessment

The ulnar nerve was stimulated using a constant current stimulator (Digitimer DS7AH; Digitimer Ltd, Hertfordshire, UK) and through surface adhesive electrodes placed on the wrist. The cathode was proximal in order to minimize the risk of anodal block. Digital nerves of the right index finger were stimulated using the same device via ring electrodes. The cathode was placed at the base of the proximal phalanx with the anode placed 2 cm distally (Kwast-Rabben *et al.*, 2002; Rocchi *et al.*, 2017). Stimuli consisted of square wave pulses with a duration of 0.2 ms given at a 3-Hz frequency with a 10% jitter. For ulnar nerve stimulation, 500 single pulses were delivered. To test S1 intracortical excitability, we also performed a paired-pulse stimulation, delivering 500 paired-pulses with a time interval of 5 ms and another 500 trials with an ISI of 30 ms in two separate blocks (Erro *et al.*, 2018). For digital nerve stimulation, 500 single pulses were given at rates of 3 Hz (with a 10% jitter). To obtain a clearer response, the stimulus intensity was set at 4 times the subject's somatosensory threshold, defined as the lowest stimulus intensity at which the subject reported sensation of wrist or finger stimulation in half of the trials. The N20-P25 component of SEPs was recorded by an active electrode placed at CP3 and a reference electrode placed at Fz. Signal was recorded from -20 to 100 ms around the pulse, digitized with a 5 kHz sampling frequency, and band-pass filtered (3 Hz–2 kHz) (Cruccu *et al.*, 2008). N20 latency and N20-P25 peak-to-peak amplitude were measured. Responses following the second stimulus in the PP-SEPs were obtained by subtracting the SEP waveform of the first stimulus from the waveform following each double stimulus (Rocchi *et al.*, 2016, 2017).

Experimental Design

BMT and VMTs were randomized in two sessions, performed at least one week apart. Neurophysiological data were collected from the FDI and ADM before and after the tasks,

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including RMT, TS-MEPs, and SICI. Single SEPs from ulnar and digital stimulation, PP-SEPs from ulnar stimulation, and SAI were also measured (Tokimura *et al.*, 2000; Kwast-Rabben *et al.*, 2002; Cruccu *et al.*, 2008; Rocchi *et al.*, 2017). In order to ensure the feasibility of the experiment, we collected neurophysiological parameter measurements immediately after the skill acquisition tasks investigating the earliest phases of motor skill acquisition. TS-MEPs, SICI, and SAI measures were all randomized in a single recording block, overall including 90 randomized pulses, i.e. 15 TS-MEPs, 45 paired pulses (15 for each CS-int) for the SICI assessment, and 30 trials for the SAI assessment (15 paired pulses with a ISI of N20 + 2 ms and 15 paired pulses with a ISI of N20 + 4 ms). The interval between stimuli was 4 seconds (with a 10% jitter). The HF-VMT control experiment was performed in all participants of the original sample, at least two weeks after the main sessions. This time interval between the main and the control experiment was relatively long and thus likely excluded a carry-over effect (Kim et al., 2014). Moreover, we also performed an additional analysis comparing the baseline error measurements recorded during the block 1 of the VMT and those recorded during the block 1 of the HF-VMT. In the HF-VMT session we recorded MT, TS-MEP, SICI, and SAI.

Statistical analysis

Motor skill acquisition during BMT was represented by changes in the acceleration across the 10 blocks (Bologna *et al.*, 2015, 2019; Hannah *et al.*, 2019). As a synthetic index of acquisition of practiced movement, we calculated the BMT ratio between the mean acceleration of the block 10 and the mean acceleration of the block 1. Similarly, motor skill acquisition during the VMT and HF-VMT was calculated as the ratios between the mean error of the block 6 and the mean error of the block 1 (Lopez-Alonso *et al.*, 2018).

To analyse the course of the acceleration reached during the BMT, as well as the course of the error recorded in the VMTs, raw values were entered into different repeated measures analyses of variance (rmANOVAs), one for each task, using 'block' (ten levels: block 1 to block 10 for the BMT; six levels: block 1 to block 6 for the VMT and HF-VMT) as a factor of analysis. To quantify possible differences between motor skill acquisition in the two different VMTs, error values were entered in an additional rmANOVA with the factors 'task' (two levels: VMT and HF-VMT) and 'block' (six levels: block 1 to block 6). Moreover, we performed a paired t-test between the baseline error values recorded during the block 1 of the two VMTs in order to exclude any carry-over effects. Finally, we compared by a paired t-test the VMT and HF-VMT ratios, i.e. mean error of the block 6 divided by mean error of the block 1 for each VMT.

To analyse differences in baseline measurements and stimulation intensities between sessions, we performed one-way rmANOVAs. To analyse possible changes in RMT intensities induced by the tasks, values were entered in an rmANOVA with 'time' (pre, post) and 'task' (BMT, VMT, HF-VMT) as factors of analysis. To analyse changes in N20-P25 peak-to-peak amplitude, a similar rmANOVA was performed, with two levels for the factor 'task' (BMT, VMT). To analyse changes in TS-MEP amplitude before and after the tasks, we performed two separate rmANOVAs, one for each muscle, using 'time' (pre, post) and 'task' (BMT, VMT, HF-VMT) as factors of analysis. Similarly, for SICI, the conditioned/unconditioned MEP ratios were inserted in two separate rmANOVAs (one for each muscle), with factors 'task' (BMT, VMT, HF-VMT), 'CS-int' (50%, 70%, and 90% RMT), and 'time' (pre, post). For SAI analysis, we considered the factors 'task' (BMT, VMT, HF-VMT), 'ISI' (N20+2 ms and N20+4 ms), and 'time' (pre, post) from each muscle. Bonferroni's correction was used for post-hoc comparisons. Pearson's correlation coefficient was adopted to test possible correlations between the motor ratios of the BMT (block 10 /

block 1 acceleration) and the motor ratio of the VMT (block 6 / block 1 error). Pearson's

correlation coefficient was also used to test possible correlations between baseline

neurophysiological measures and behavioural outcome, as well as between changes in

neurophysiological parameters induced by skill acquisition and motor performance. The

Shapiro-Wilk statistic was computed to test whether the distribution of neurophysiological

values was normal. All results are expressed as mean values ± standard deviation (SD) and

the level of significance for all tests was set at $p<0.05$. All data were statistically analysed

using IBM SPSS Statistics (Version 25.0).

RESULTS

Motor skill acquisition tasks

The mean RMS of the EMG activity recorded from the FDI during the BMT was 0.60 ± 0.19

mV in the block 1 and 0.69 ± 0.26 mV in block 10. The course of the mean index finger

accelerations in the x-axis throughout the 10 blocks of the BMT are depicted in Fig. 1. The

rmANOVA showed a significant effect of the factor 'block' ($F_{9,126} = 21.43$, $p<0.0001$),

indicating that participant acceleration values changed during training. Post-hoc analysis

showed that accelerations reached from block 3 to block 10 differed from those recorded in

block 1 (all p values<0.004).

The mean RMS of the EMG activity recorded from the FDI during the VMT was 0.23 ± 0.02

mV in the block 1 and 0.18 ± 0.03 mV in block 6. The mean RMS of the EMG activity

recorded from the FDI during the HF-VMT was 0.37 ± 0.01 mV in the block 1 and $0.27 \pm$

0.02 mV in block 6. The course of the mean error throughout the six blocks of the VMTs is

depicted in Fig. 1. The rmANOVAs showed a significant effect of the factor 'block' for the

VMT ($F_{5,70} = 57.78$, $p<0.0001$) and HF-VMT ($F_{5,70} = 12.97$, $p<0.0001$). Post-hoc comparisons for the VMT showed that participants decreased their error during training, with values of each block differing from those obtained in block 1 (all p values <0.0001). Similarly, the error decrease in the HF-VMT and post-hoc comparison showed that values of each block differed from those obtained in block 1 (all $p<0.002$), except for block 3. The rmANOVA comparing the two VMTs showed a significant effect of the factor 'block' ($F_{5, 70}=30.923$, $p<0.0001$), as well as a significant effect of the factor 'task' ($F_{1, 14}=374.47$, $p<0.0001$), but no significant 'block x task' interaction ($F_{5, 70}=1.9043$, $p=0.1046$). Notably, post-hoc comparison showed that the error values were higher in the HF-VMT, meaning that, even if the performance curve was similar between the two sessions, the HF-VMT was more difficult to perform than the VMT. This result was also confirmed by the analysis on the baseline error measures recorded in the block 1 of the two VMTs, which showed higher error values in the HF-VMT ($p=0.003$ with paired t-test).

Neurophysiological M1 assessment

No differences between sessions were found in baseline RMT or TS-MEPs (Table 1). No changes in RMT were found after the tasks (all $p>0.05$). The results on TS-MEP amplitude are shown in Fig. 2. The overall excitability of the FDI increased after the BMT but not after the VMT and HF-VMT, indicating a specific effect of BMT on the corticospinal excitability. This was supported by rmANOVA, which showed a significant 'task x time' interaction ($F_{2,28} = 7.69$, $p=0.0022$). Post-hoc comparisons showed an increase in TS-MEP amplitude after the BMT but not after the VMTs. There was a trend toward a reduction in the TS-MEP amplitude recorded from the FDI after the HF-VMT, but it did not reach statistical significance. The rmANOVA for the ADM showed no significant effects or interaction, thus demonstrating the focal effect of the ballistic task on the involved muscle.

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SICI was also specifically modified by the tasks (Fig. 3A and 3B). As expected, the rmANOVA for the FDI showed a significant effect of the factor 'CS-int' ($F_{2,28} = 32.1$, p<0.0001), whereby larger inhibitions were obtained at higher CS-int. The factor 'time' ($F_{1,14} = 11.65$, p=0.004) and the 'task x time' interaction ($F_{2,28} = 5.04$, p=0.01) were also significant. The non-significant interaction of 'CS-int x task' ($F_{4,56} = 0.59$, p=0.67) reflected that the SICI curve did not globally differ between the sessions. The factor 'task' and the 'time x CS-int' and 'time x CS-int x task' interactions were not significant. Post-hoc comparisons showed that SICI values decreased after the VMT and reached a significant reduction when the CS-int was 50% RMT (p=0.03). SICI values were significantly reduced for every CS-int in the HF-VMT (p=0.023 for CS-int of 50% RMT, p=0.005 for CS-int of 70% RMT, and p=0.016 for CS-int of 90% RMT). Conversely, SICI did not change after the BMT. In the control muscle, SICI values were not modified (all p>0.05 except for the factor 'CS-int').

No differences in baseline SAI values were found between sessions. No changes in SAI were found after the BMT or VMTs (Fig. 4). However, from the inspection of raw data, a higher SD in SAI values was observed after the BMT, particularly for SAI 4 ms (Fig. 4). To investigate this, we performed an additional rmANOVA, using each subject's SD values of SAI 4 ms and the factors 'task' (three levels: BMT, VMT, HF-VMT) and 'time' (two levels: pre vs. post). No significant effects of the factors 'task' or 'time' were found, while the interaction 'task x time' was significant ($F_{2,28}=5.1004$, p=0.0129). Post-hoc comparison showed that SAI 4 ms SD values increased after the BMT but not after the VMT or HF-VMT. Thus, the ballistic acquisition task caused an increase in SAI 4 ms variability.

Neurophysiological S1 assessment

No differences were found in baseline sensory thresholds (mean ± SD: 1.9 ± 0.5 in the BMT, 1.9 ± 0.8 in the VMT, 2.0 ± 0.5 in the HF-VMT) or N20 latency between sessions (Table 1). No changes in amplitude of the N20-P25 component of single SEPs from ulnar stimulation after the tasks were found. Similarly, paired ulnar nerve SEP amplitude did not change after the BMT or VMT, indicating that no tasks modified S1 excitability as assessed by paired stimulation (all p>0.05). In contrast to what was observed for SEPs from ulnar stimulation, the analysis on SEPs from digital stimulation showed a significant effect of the factor 'time' ($F_{1,14}$ = 7.34, p=0.017) indicating that the amplitude of the SEP from digital stimulation changed after the motor performance. There was no effect of 'task' ($F_{1, 14}$=2.0684, p=0.1723) nor interaction 'task x time' ($F_{1, 14}$=1.8678, p=0.1933). Post-hoc comparison showed that digital SEP amplitude was reduced after the BMT (p=0.028) but not after the VMT (Fig. 5). Thus, when electrical stimulation was applied on the specific area involved in the motor task, differential effects of the two tasks on S1 excitability were found.

Correlation analysis

Correlation analysis showed a correlation between motor performance expresses as motor ratios in the BMT and VMT (i.e. mean acceleration/error of the last block divided by the mean acceleration/error of the first block 1), meaning that subjects who acquired the most during the BMT also learned the most during the VMT (r= -0.74, p=0.01) (Fig. 6). We did not find any significant correlation between neurophysiological measures at baseline and behavioural outcome, nor between changes in neurophysiological parameters induced by the tasks and motor performance (all p>0.05)

Median split procedure

In order to further address the relationship between neurophysiological changes and measures of motor skill acquisition, we applied a median split procedure (Nowak *et al.*, 2017; Guerra *et al.*, 2018). We divided participants into two subgroups for each motor task session, according to the level of the motor improvement, i.e. the mean acceleration of the block 10 divided by the mean acceleration of the block 1 for the BMT and the mean error of the block 6 divided the mean error of the block 1 for the VMTs. We identified a high-learner (HL) subgroup (8 subjects for the BMT, mean motor ratio ± SD: 3.04±0.67; 8 for the VMT, mean motor ratio ± SD: 0.72±0.15, 8 for the HF-VMT, mean motor ratio ± SD: 0.90±0.07) and a low-learner (LL) subgroup (7 subjects for the BMT, 1.89±0.21; 8 for the VMT, 1.62±0.28, and 8 subjects for the HF-VMT, 0.68±0.02). We then considered the neurophysiological measures that had changed in relation to the motor tasks in the main analysis (TS-MEPs, SICI at 3 CS-int and digital SEPs). We normalised these measures to their corresponding baseline values and compared the ratios between the HL and LL subgroups using the Mann-Whitney test (notably we used non-parametric test given the number of subjects in HL and LL subgroups). No differences between the HL and LL emerged from the analysis (Table 2). Finally, there was no correlation between neurophysiological measures at baseline and behavioural outcome, nor between changes in neurophysiological parameters induced by the tasks and motor performance in the two subgroups.

DISCUSSION

The novel aspect of the present study is the demonstration that, within the same group of subjects, different motor skill acquisition tasks, one representing a model-free and the other a model-based learning task (Hamada *et al.*, 2014), have distinct effects on M1 and S1

excitability, thus indicating a task-related engagement of specific neurophysiological substrates. Since participants were naïve to the tasks and improved their performance at similar rates in all training sessions, the differential effects of the motor tasks cannot be explained by different levels of motor performance. Furthermore, since baseline corticospinal excitability was constant in all experimental sessions, it can be excluded as a possible confounder. It is also unlikely that the modification of cortical parameters reflected any carry-over effect between the main experimental sessions since these were randomised and performed at least one week apart. The lack of correlation between neurophysiological changes and motor performance may suggest that this differential motor skill acquisition-induced modulation of motor and sensory circuits is more likely secondary to the task rather than part of the physiological mechanisms underlying learning.

Motor skill acquisition-related effects on M1 and sensorimotor interaction

The first result of the study was that the BMT increased corticospinal excitability, as indexed by MEP amplitude changes, whereas the VMT decreased intracortical inhibition, as assessed by SICI. When performed with a higher force, the HF-VMT induced a stronger reduction in SICI. Although the HF-VMT was performed as a control experiment and not randomized within the main sessions, the time interval between the main and control experiment was relatively long and thus likely excluded a carry-over effect (Kim et al., 2014). Moreover, the analysis on the baseline errors recorded during the two VMTs showed that the error measures in the block 1 of the HF-VMT were higher than those recorded in the block 1 of the VMT. This result reflects the higher difficulty in performing the HF-VMT (compared to VMT) and supports the lack of any carry-over effect. Finally, although the effect on SICI in the VMT was observed only with the lowest conditioning stimulus intensity (50% RMT), effects were

seen at all CS intensities in the HF-VMT reinforcing the conclusion that a visual tracking task can modulate intracortical excitability.

Our observations are consistent with previous reports that have shown an increase in corticospinal excitability after ballistic movement. Several studies have shown an increase in MEP amplitude after a ballistic learning (Classen *et al.*, 1998; Rosenkranz *et al.*, 2007; Agostino *et al.*, 2008; Bologna *et al.*, 2015; Veldman *et al.*, 2016; Hannah *et al.*, 2019). However, others reported opposite results, i.e. a null effect after a finger abduction training task in old adults (Rogasch *et al.*, 2009). A recent meta-analysis highlighted the broad heterogeneity of these neurophysiological results (Berghuis *et al.*, 2017). Moreover, the hypothesis that SICI modulation contributes to motor learning is supported by a number of studies that have demonstrated reduced intracortical inhibition after different types of visuomotor tasks (Cirillo *et al.*, 2011; Smith *et al.*, 2014; Coxon *et al.*, 2014; Berghuis *et al.*, 2017). Although the removal of intracortical inhibition is an important substrate for optimal motor learning (Ziemann *et al.*, 2001), most studies did not show any correlation between the degree of SICI changes and learning (Berghuis *et al.*, 2017), therefore supporting the hypothesis that SICI modulation is more likely secondary, rather than a contributing mechanism, to motor learning.

No changes in the global values of SAI were found after the motor skill acquisition tasks, confirming previous reports (Koizume *et al.*, 2017). It is well known that the cholinergic system modulates arousal state in relation to concentration or attention during task performance (Koizume *et al.*, 2017) and that SAI may be considered an indirect electrophysiological parameter of cortical acetylcholine activity in humans (Tokimura *et al.*, 2000). Notably, recent evidence suggests that abnormal cholinergic transmission may play a role in motor control in conditions characterised by altered voluntary movements (Schirinzi *et*

al., 2018; Bologna *et al.*, 2020). The lack of SAI changes we observed in our study indicates that the acquisition of motor skills does not induce any significant effects on cortical cholinergic activity. Ballistic movements, however, induced an increase in SAI variability in our sample, as shown by the analysis on the SD.

Motor skill acquisition-related effects on S1

The BMT and VMT induced differential effects on S1, with a decrease in the amplitude of the cortical components of digital SEPs observed only after the BMT. Evidence on the role of S1 in motor learning comes from studies showing that disruption of somatosensory function leads to an impairment in motor skill acquisition in animals and humans (Pavlides *et al.*, 1993; Vidoni & Boyd, 2009). More recent studies have demonstrated that human learning induces changes in SEP cortical components (Andrew *et al.*, 2015; Dancey *et al.*, 2016; McGregor *et al.*, 2016). However, results are characterised by a marked heterogeneity, and both no changes (Haavik & Murphy, 2013) or an increase (Dancey *et al.*, 2016) in amplitude of the N20-P25 complex have been reported after a typing task. To the best of our knowledge, no studies have compared the effects induced by different learning tasks on SEPs, nor have learning-related effects on digital SEPs been demonstrated. Our results on the effects on the SEPs evoked from digital stimulation may suggest that digital input is specific to the task itself.

Comparison between tasks

By comparing the two motor tasks within the same sample of participants, we demonstrated the selectivity of cortical, corticospinal, and intracortical changes induced by the BMT and VMTs, thus providing an important insight into the physiological effects of the acquisition of different motor skills. We adopted these tasks from previous study tasks so as to include examples of model-free and model-based learning (Hamada *et al.*, 2014). The BMT

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represents a model-free learning task (Classen *et al.*, 1998; Hamada *et al.*, 2014). In model-free learning, the repetition of an action causes future movements to be biased toward that action. Conversely, the VMTs represent model-based learning, in which subjects learn an internal model predicting the consequences of motor commands (Hamada *et al.*, 2014). Previous studies have demonstrated that model-free learning depends on local M1 networks (Classen *et al.*, 1998; Bütefisch *et al.*, 2000), while model-based learning involves both M1 (Bagce *et al.*, 2013) and the cerebellum (Butler *et al.*, 2000; Krakauer *et al.*, 2005, 2019). In the latter case, M1 is likely to store an updated internal model built by comparing the prediction of the sensory consequences of a movement with the actual sensory consequences (Shadmehr & Krakauer, 2008). Previous studies have suggested that distinct cortical interneuron circuits link the two types of learning (Hamada *et al.*, 2014). In the Hamada's study, however, the main aim was not to compare the effect of these two learning models on cortical excitability, but instead to investigate whether specific TMS interventions on M1 had independent effects on two forms of motor learning. Our results, showing selective motor skill acquisition-related effects on M1, corroborate the hypothesis that different interneuronal circuits are involved in different learning tasks.

Although we tried to match the forces exerted in the BMT and VMT by performing the HF-VMT session, we only have a surrogate parameter of the muscle contraction levels during the motor skill acquisition tasks, i.e. the RMS of the EMG activity recorded from the FDI. Moreover, we did not match the rate of force development. The ballistic task requires a very rapid recruitment of motor neurons and this demands a large synchronised descending corticospinal excitation. This difference in the rate of force development should be considered an alternative hypothesis to explain the differences in M1 effects between the two tasks. A second difference between the tasks that should be mentioned is the total duration of the muscle contraction, which was longer for the VMTs. We can hypothesise that

intracortical inhibition modulation is not heavily involved in the BMT, but may be required in longer lasting movements, such as in the VMT.

Our study also demonstrated a selectivity in S1 motor skill acquisition-related responses, as suggested by the differential effects on the SEP cortical component as a result of digital stimulation. It is possible that sensory gating, expressed by the attenuation of SEP signals (Macerollo *et al.*, 2018), is mainly involved in the BMT and is less common in the VMT.

Of note, since we showed different effects related to differential motor skill acquisition within the same group of subjects, our results may help explain previous contradicting motor-learning results in literature, since these contradictions may be related to the specific task adopted rather than interindividual variance. We do note that recording of a retention block, which was not included in the present study in order to maintain the experimental sessions of reasonable duration, would provide important additional information to our results and would clarify whether they can be linked to learning through off-line consolidation (Tunovic *et al.*, 2014).

Correlation between neurophysiological measurements and motor performance

The lack of correlation between M1 and S1 neurophysiological changes after the skill acquisition tasks and motor performance in both the BMT and VMTs confirms previous observations (Bologna *et al.*, 2015, 2019; Hussain *et al.*, 2016; Berghuis *et al.*, 2017; Hannah *et al.*, 2019). However, the specificity of the effects we observed after the BMT and VMTs suggests that cortical changes are not solely due to motor practice or they would have been similar after both tasks. One hypothesis to explain the lack of correlation is that the skill acquisition tasks may directly involve the corticospinal and intracortical circuits in M1 and S1, but complex signals such as MEPs and SEPs, which contain cortico-cortical as well as intracortical and spinal contributions, may not clearly reflect these effects (Gedankien *et al.*,

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2017). Another hypothesis is that a wider neuronal network involving other cortical and subcortical areas is implicated in skills acquisition processes, and this makes it difficult to find a direct correlation between M1 and S1 parameters and motor behaviour. Furthermore, many additional factors may influence plasticity response but not motor acquisition, such as the attentional demand required for the task, the history of prior synaptic activity, and genetic effects (Ridding & Ziemann, 2010; Cirillo *et al.*, 2011).

Alternatively, the lack of correlation may suggest that none of the M1 or S1 neurophysiological changes should be considered underlying mechanisms, but rather effects of the motor skill acquisition. Thus, model-free learning tasks such as the BMT induce an increase in MEP amplitude because the control of corticospinal excitability is not fundamental in improving motor performance and if this increases it may be beneficial. Accordingly, animal data have previously demonstrated that movement strength and speed are encoded in M1 (Pasquereau *et al.*, 2016). We may hypothesise that the repeated firing of neurons encoding aspects strictly related to the BMT (i.e. strength and speed) lead to an increase in their excitability due to activity-dependent plasticity mechanisms. Thus, the increase in excitability may be explained by the repeated action and still not be correlated with performance.

In contrast, the need for accuracy in model-based learning tasks may imply a strict control of corticospinal excitability. Indeed, although no MEP changes were induced by the VMTs, a decrease in SICI was observed, reflecting intracortical excitability regulation. Previous observations have demonstrated an early decrease in intracortical inhibition within the corticomotor system in the observation of a motor execution error (Cardellicchio *et al.*, 2018). The reduction in SICI after the VMTs may thus be interpreted as reflective of a mismatch between the observed erroneous action and the expectation (i.e. the difference between the

observed trajectory generated by the force applied and the track to follow during the VMTs). The results obtained in the HF-VMT, in which the error was even higher than the VMT, strongly support this hypothesis. The higher the error, the larger the SICI decrease. Conversely, the BMT, in which there was no possible error, did not induce any changes in SICI values.

The same did not hold true for the SEP decrease after the BMT, implying that during this task, in which there was no sensory endpoint, afferent input was not fundamental in improving motor performance, thus resulting in SEP decrease. This did not occur after the VMT, in which the role of sensory prediction and afferent input may be important.

Conclusion

Our study represents the first direct comparison of the effect of two different motor skill acquisition tasks within the same group of subjects, thus providing novel information on the role of M1 and S1 in learning processes. The differential effects of model-free and model-based learning tasks on neurophysiological parameters demonstrate how different neuronal circuits are related to different forms of motor leaning. These differential effects likely reflect neurophysiological products of different motor learning tasks, rather than direct involvement in motor performance improvement. Our results emphasise the importance of selecting the most appropriate motor-learning task for the neurophysiological parameter to be studied in future investigations. Further studies on wider samples of subjects and including a retention recording block are needed to provide a deeper understanding of the neurophysiological mechanisms of motor learning in healthy humans.ù

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	BMT	VMT	HF-VMT	P values
RMT	49.3 ± 9.0	48.6 ± 11.0	50.0 ± 9.8	0.77
TS-MEPs intensity	61.3 ± 12.1	60.4 ± 12.7	63.6 ±	0.06
Sensory threshold	1.9 ± 0.5	1.9 ± 0.8	2.0 ± 0.5	0.90
N20 latency	20.2 ± 1.2	20.2 ± 1.0	20.1 ± 1.2	0.6

Table 1: Resting motor threshold (RMT), test stimulus-motor evoked potentials (TS-MEPs) intensity expressed as a percentage of the maximal stimulator output (MSO), sensory thresholds expressed in mA, N20 latency expressed in s for the 3 experimental sessions from the 15 subjects sample. BMT: ballistic motor task, VMT: visuomotor task, HF-VMT: high-force visuomotor task. Results are shown as mean values ± 1 standard deviation (SD). P values by one-way rmANOVAs. Significant p values are in bold.

	BMT		VMT		HF-VMT	
	HL	LL	HL	LL	HL	LL
TS-MEPs ratio	1.64 ± 0.59 / 1.59 ± 0.79		1.10 ± 0.24 / 0.98 ± 0.28		0.76 ± 0.26 / 0.81 ± 0.39	
SICI ratio 50%RMT	0.84 ± 0.29 / 1.12 ± 0.51		1.53 ± 0.82 / 1.32 ± 0.2		1.69 ± 0.59 / 1.54 ± 1.35	
SICI ratio 70%RMT	1.27 ± 0.56 / 1.18 ± 0.45		1.26 ± 0.47 / 1.08 ± 0.43		1.59 ± 0.54 / 1.44 ± 0.62	
SICI ratio 90%RMT	1.03 ± 0.51 / 1.43 ± 0.96		1.36 ± 1.02 / 1.25 ± 0.64		1.61 ± 0.96 / 1.93 ± 1.62	
Digital SEPs ratio	0.90 ± 0.24 / 0.77 ± 0.36		0.89 ± 0.24 / 0.97 ± 0.22		-	

Table 2: Median split procedure results. Test stimulus-motor evoked potentials (TS-MEPs) ratio was calculated dividing the TS-MEPs recorded after the motor tasks in the three motor sessions by the TS-MEPs recorded before the motor tasks in the three motor sessions. The short-intracortical inhibition (SICI) ratios were calculated dividing the unconditioned/conditioned MEPs recorded after the motor tasks in the three motor sessions by the unconditioned/conditioned MEPs recorded before the motor tasks at the 3 different conditioning stimulus intensities, i.e. 50, 70 and 90% of the resting motor threshold (RMT). The digital sensory evoked potentials (SEPs) ratios were calculated diving the digital SEPs amplitude obtained after the motor tasks in the three motor sessions by the post by digital SEP amplitude obtained before the motor tasks. BMT: ballistic motor task, VMT: visuomotor task, HF-VMT: high-force visuomotor task. HL: high-learners subgroup, LL: low-learners subgroup. Results are shown as mean values ± 1 standard deviation (SD). P values by the Mann-Whitney test were all >0.05.

Figure legends:
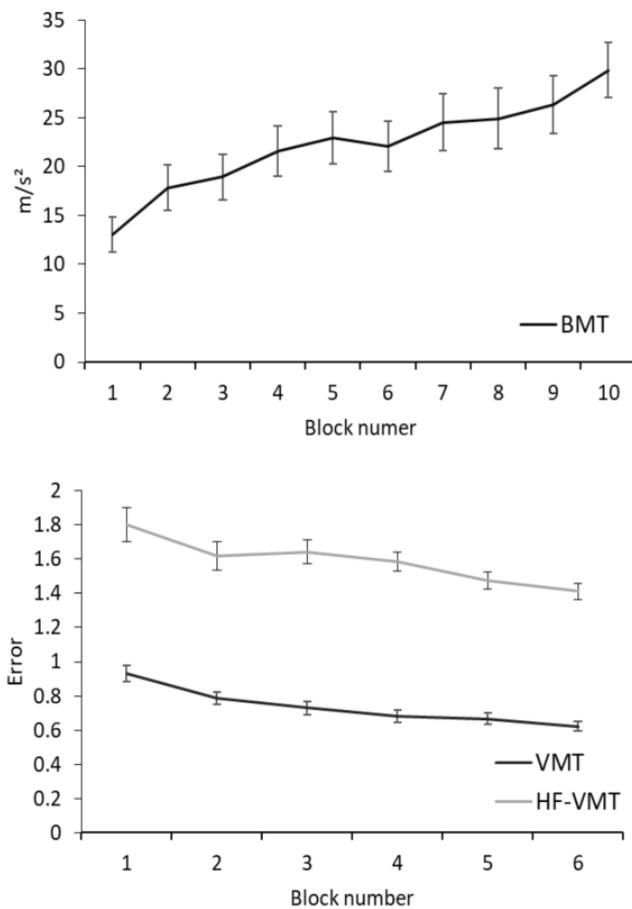
Fig. 1 Motor skill acquisition across the motor task sessions

Upper part: Mean accelerations of the index finger abductions throughout the 10 blocks of the ballistic motor task (BMT). The x-axis shows the block number and the y-axis shows the mean accelerations (m/s^2) across the 15 subjects. Lower part: Trajectory error recordings throughout the 6 blocks of the visuomotor task (VMT) (dark grey), and throughout the 6 blocks of the high-force visuomotor task (HF-VMT) (light grey). The x-axis shows the block number and the y-axis shows the mean error across the 15 subjects. Error bars denote ± 1 SEM.
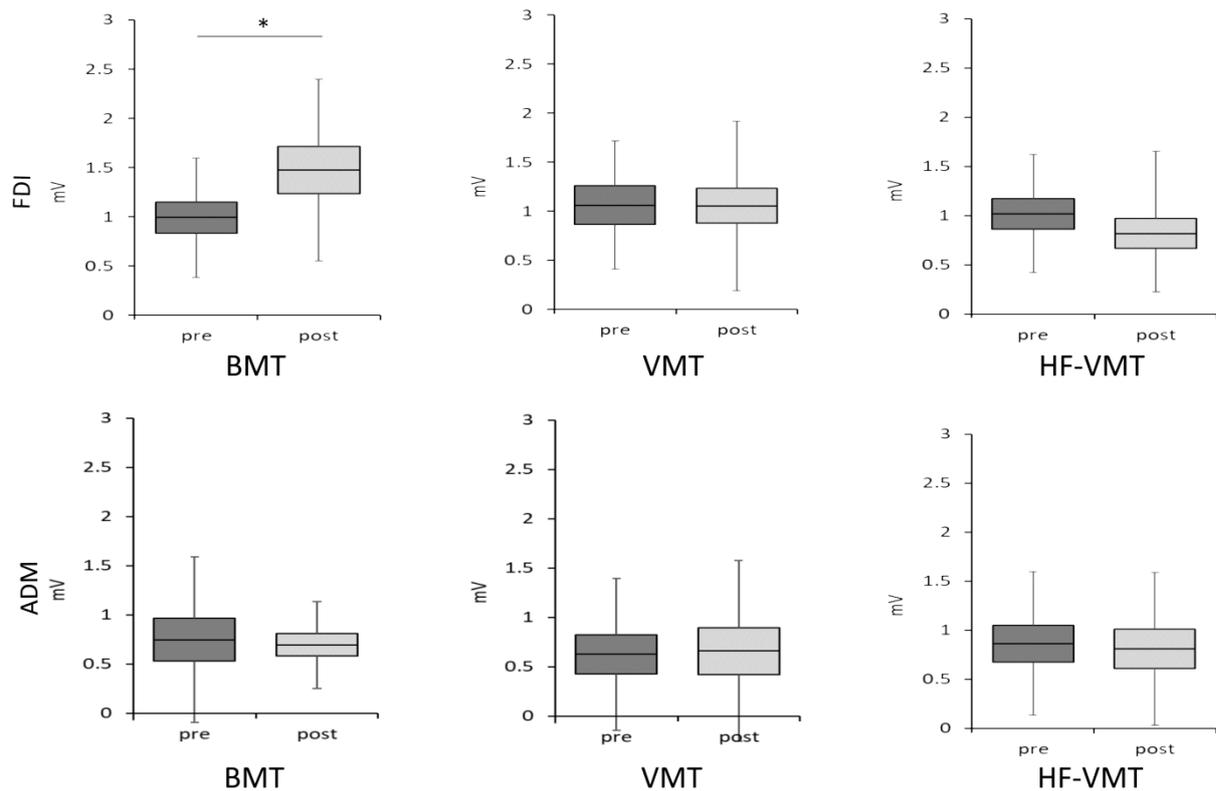
Fig. 2 Motor skill acquisition-related effects on MEPs amplitude

Test stimulus motor-evoked potential (TS-MEP) amplitude (mV) recorded before (dark grey) and after (light grey) the ballistic motor task (BMT), visuomotor task (VMT), and high-force visuomotor task (HF-VMT) from the first dorsal interosseous (FDI) and the adductor digiti minimi (ADM) were compared by a rmANOVA in 15 subjects. Horizontal lines denote the average values. The boxes contain the mean value ± 1 SE of the mean. Whiskers contain the mean value ± 1 SD of the mean. Asterisks indicate P < 0.05 in the post-hoc comparisons.
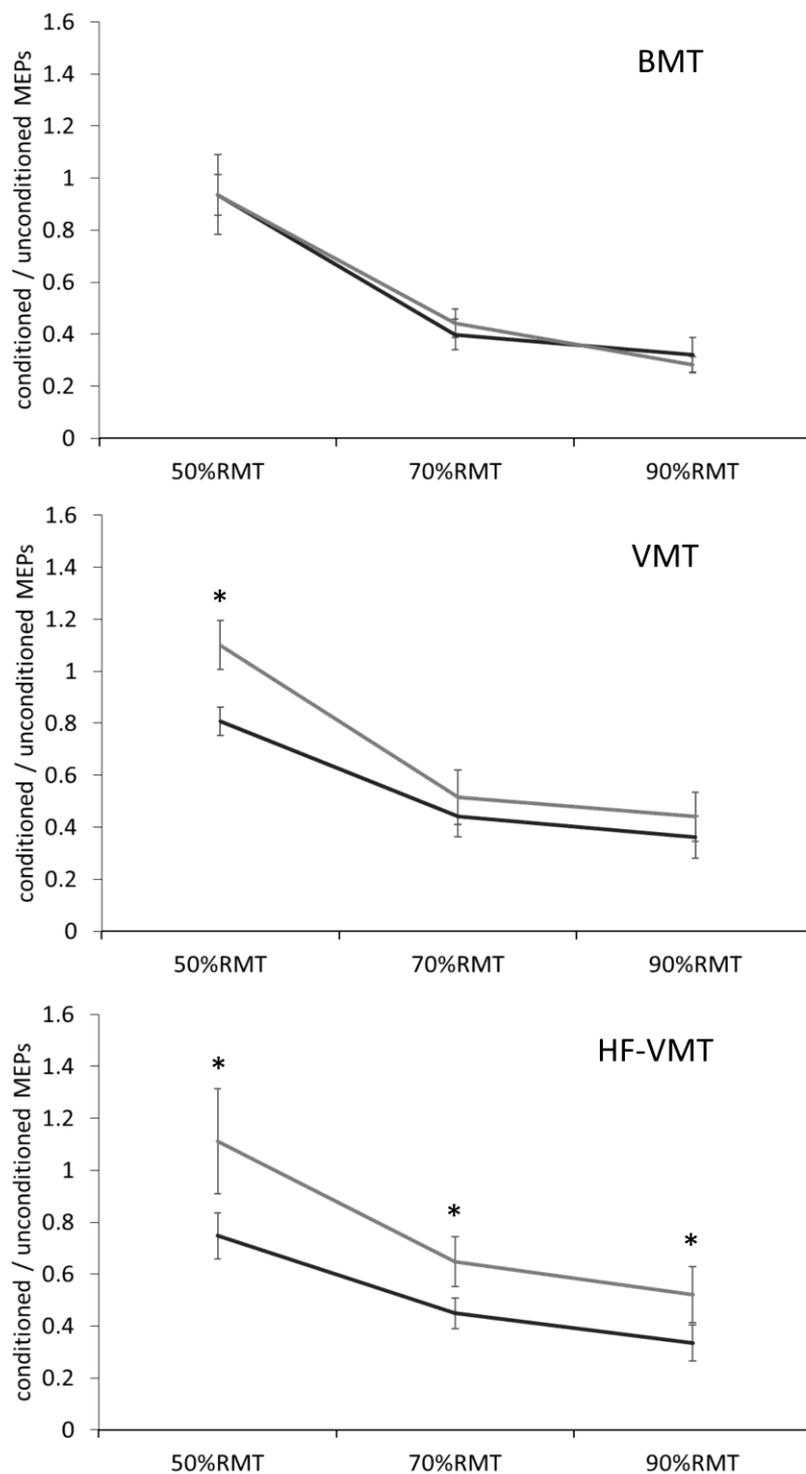
Fig. 3 Short-interval intracortical inhibition (SICI) curve recorded from the first dorsal interosseous (FDI) before and after the ballistic motor task (BMT), the visuomotor task (VMT), and the high-force visuomotor task (HF-VMT). SICI values at 3 conditioning stimulus intensities (50%, 70%, and 90% of the resting motor threshold, RMT) are expressed

as the ratio of conditioned MEPs/unconditioned MEPs. Dark grey curve: data collected before the tasks (pre). Light grey curve: values collected after the tasks (post). Data were compared by a rmANOVA in 15 subjects. Error bars denote ± 1 SEM. Asterisks indicate P < 0.05 in the post-hoc comparisons.
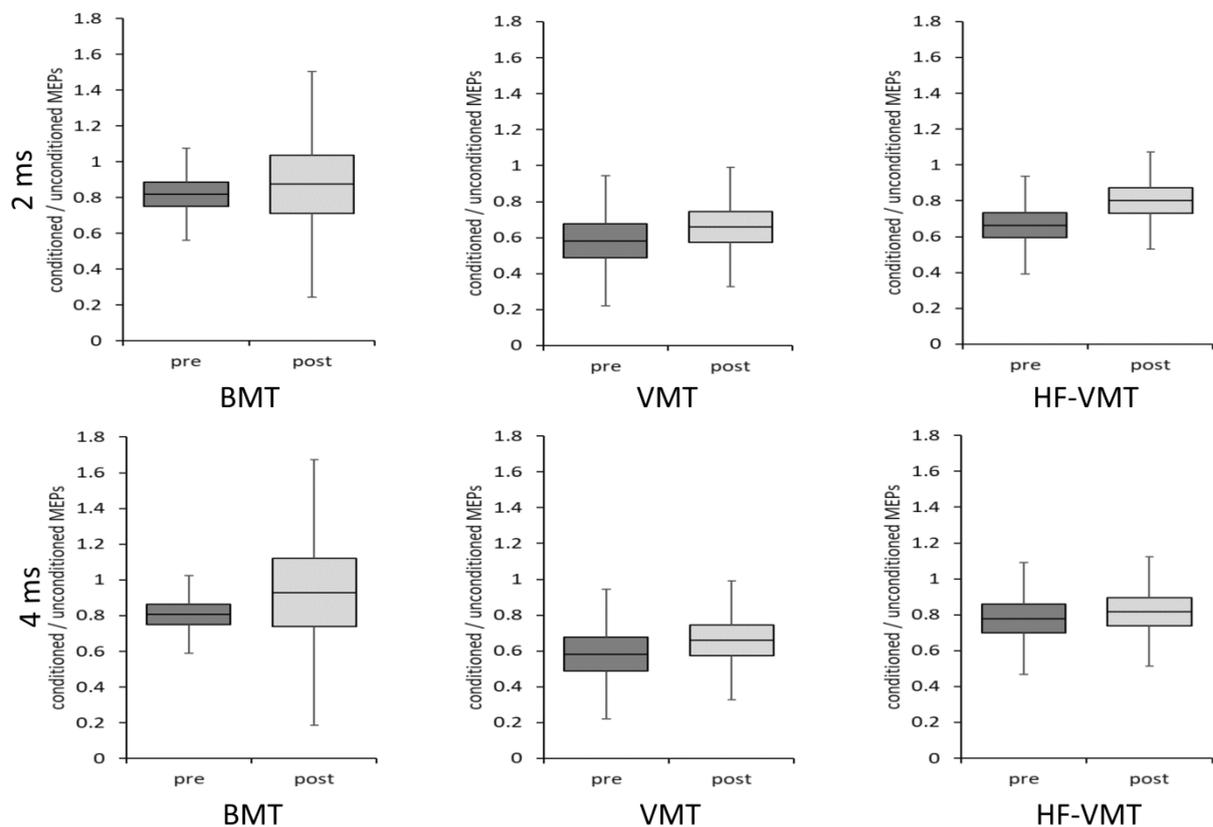
Fig. 4 Short-latency afferent inhibition (SAI) values recorded from the first dorsal interosseous (FDI) before and after the ballistic motor task (BMT), the visuomotor task (VMT) and the high-force visuomotor tasks (HF-VMT). SAI values at the 2-ms interstimulus interval (N20 latency + 2 ms on the left in each task condition) and SAI values at the 4-ms interstimulus interval (N20 latency + 4 ms on the right in each task condition) are expressed as the ratio of conditioned MEPs/unconditioned MEPs. Dark grey: data collected before the tasks. Light grey: values collected after the tasks. Data were compared by a

rmANOVA in 15 subjects. Horizontal lines denote the average values. The boxes contain the

mean value ± 1 SE of the mean. Whiskers contain the mean value ± 1 SD of the mean.

Asterisks indicate $P < 0.05$ in the post-hoc comparisons.
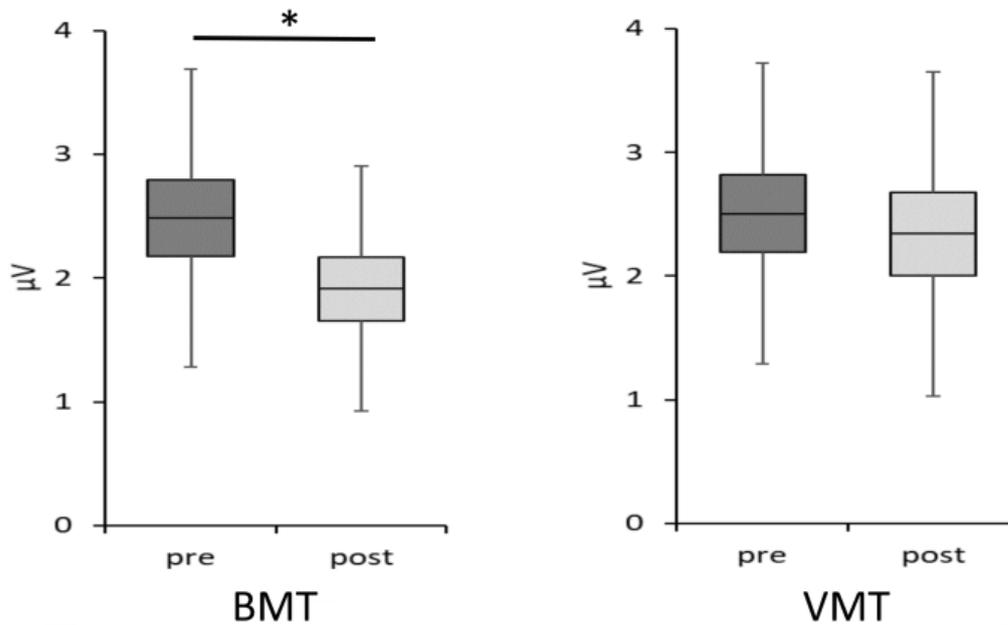
Fig. 5 Amplitude of the N20-P25 component from digital stimulation of the index finger in the two main sessions.

Dark grey boxes represent values collected before the ballistic motor task (BMT) and the visuomotor task (VMT). Light grey boxes represent values collected after the tasks. Data were compared by a rmANOVA in 15 subjects. Horizontal lines denote the average values. The boxes contain the mean value ± 1 SE of the mean. Whiskers contain the mean value ± 1 SD of the mean. Asterisks indicate $P < 0.05$ in the post-hoc comparisons.
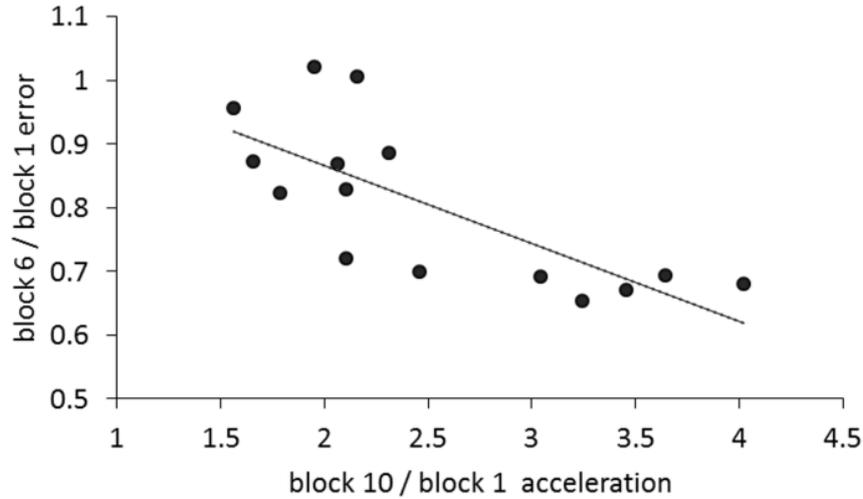
Fig. 6 Correlation between the learning ratios recorded from 15 subjects during the ballistic motor task (BMT) and the visuomotor task (VMT).

The BMT ratio was calculated dividing the acceleration value recorded in the block 10 of the experimental session by the acceleration value recorded in the block 1 of the experimental session. The VMT ratio was calculated dividing the error value recorded in the block 6 of the experimental session by the error value recorded in the block 1 of the experimental session. The Pearson's correlation coefficient was calculated to test correlation.

Additional information session:

The experiments were performed in the laboratories of the Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom.

Author contributions

G.P.: conception and design of the work, acquisition, analysis and interpretation of data for the work, drafting the work

L.R.: conception and design of the work, analysis and interpretation of data for the work, revising the work critically for important intellectual content

M.B.: conception and design of the work, interpretation of data for the work, revising the work critically for important intellectual content

A.B.: conception and design of the work, revising the work critically for important intellectual content

J.R.: conception and design of the work, interpretation of data for the work, revising the work critically for important intellectual content

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Dr. Giulia Paparella is a PhD student at the Department of Human Neurosciences, Sapienza University of Rome, under the supervision of Professor Alfredo Berardelli. She obtained her medical degree with honours in 2013 and specialized in Neurology in 2018. She is trained in general neurology and neurophysiology, with a special interest in movement disorders. Between 2018 and 2019, she spent a year at the Department of Clinical and Movement Neurosciences, University College of London, thanks to a grant from the International Federation for Clinical Neurosciences (IFCN Research Scholarship 2019). During this period, she worked with Professor John Rothwell and his research team. Her research activity mainly focuses on neurophysiological investigations on motor control in healthy humans and in patients with movement disorders through non-invasive brain stimulations techniques.