# Motor training strengthens corticospinal suppression during movement preparation

Pierre Vassiliadis[1,2]*, Gerard Derosiere[1], Julien Grandjean[1], Julie Duque[1]

[1] Institute of Neuroscience, Université Catholique de Louvain, 1200, Brussels, Belgium.

[2] Center for Neuroprosthetics (CNP) and Brain Mind Institute (BMI), Swiss Federal Institute of Technology (EPFL), 1202, Geneva, Switzerland

Abbreviated title: Training strengthens preparatory suppression

*** CORRESPONDENCE TO:**
**Pierre Vassiliadis**
Institute of Neuroscience
Université catholique de Louvain
53, Avenue Mounier
COSY- B1.53.04
B-1200 BRUSSELS
Belgium
Email: pierre.vassiliadis@uclouvain.be
Web: http://www.coactionslab.com

## Abstract

Training can improve motor skills and modify neural activity at rest and during movement execution. Learning-related modulations may also concern motor preparation but the neural correlates and the potential behavioral relevance of such adjustments remain unclear. In humans, preparatory processes have been largely investigated using transcranial magnetic stimulation (TMS) with several studies reporting decreased corticospinal excitability (CSE) relative to a baseline measure at rest; a phenomenon called preparatory suppression. Here, we investigated the effect of motor training on such preparatory suppression, in relation to resting CSE, in humans. We trained participants to initiate quick movements in an instructed-delay reaction time (RT) task and used TMS to investigate changes in CSE over the practice blocks. Training on the task speeded up RTs, with no repercussion on error rates. Training also increased resting CSE. Most interestingly, we found that CSE during action preparation did not mirror the training-related increase observed at rest. Rather, compared to the rising baseline, the degree of preparatory suppression strengthened with practice. This training-related change in preparatory suppression (but not the changes in baseline CSE) predicted RT gains: the subjects showing a greater strengthening of preparatory suppression were also those exhibiting larger gains in RTs. Finally, such relationship between RTs and preparatory suppression was also evident at the single-trial level, though only in the non-selected effector: RTs were generally faster in trials where preparatory suppression was deeper. These findings suggest training induces changes in motor preparatory processes that are linked to an enhanced ability to initiate fast movements.


## New and Noteworthy

Movement preparation involves a broad suppression in the excitability of the corticospinal pathway, a phenomenon called preparatory suppression. Here, we show that motor training strengthens preparatory suppression and that this strengthening is associated with faster reaction times. Our findings highlight a key role of preparatory suppression in training-driven behavioral improvements.

**Keywords:** motor training; action preparation; transcranial magnetic stimulation; motor-evoked potentials; primary motor cortex; corticospinal excitability; corticospinal suppression, reaction time.

## 1. Introduction

Motor training improves the speed and/or accuracy at which movements are selected, initiated and executed. Significant research has been devoted to unveiling the functional changes at the basis of such improvements (Krakauer et al. 2019). At the neural level, neuroimaging (*e.g.,* Wiestler & Diedrichsen, 2013; Wenger et al., 2017; Yokoi & Diedrichsen, 2019) and transcranial magnetic stimulation (TMS) studies (*e.g.,* Rosenkranz et al., 2007; Reis et al., 2008; Mawase et al., 2017) have shown that training is accompanied by a plastic reorganization of the motor system, supporting the formation of new motor memories. Specifically, training amplifies resting motor activity (*e.g.,* Pascual-Leone et al., 1995; Butefisch et al., 2000; Duque et al., 2008; Galea & Celnik, 2009; Christiansen et al., 2018) and induces learning-specific changes of motor activity during movement execution (Krakauer et al. 2004; Shmuelof et al. 2014; Steele and Penhune 2010). Animal studies also show learning-related modulations of motor activity during action preparation (Makino et al. 2017; Paz et al. 2003; Vyas et al. 2018, 2020) that could reflect an optimization of preparatory processes with training (Mawase et al. 2018). Yet, the behavioral relevance of the effects of training on action preparation remain unclear.

In humans, the excitability of the motor system can be assessed by applying TMS over primary motor cortex (M1), eliciting motor-evoked potentials (MEPs), whose amplitude reflects the excitability of the corticospinal pathway (Derosiere et al. 2020; Derosiere and Duque 2020). When applied during reaction time (RT) tasks, TMS elicits MEPs that are used to assess corticospinal excitability (CSE) changes associated with action preparation and initiation. CSE is often suppressed during action preparation when compared to a baseline, measured at rest. The function of this preparatory suppression (or inhibition) remains unclear (*e.g.,* Greenhouse et al., 2015; Duque et al., 2017; Derosiere, 2018; Hannah et al., 2018). A prominent view is that it assists action selection processes, by preventing the release of premature or incorrect responses (Duque et al. 2010; Grandjean and Duque 2020; Quoilin et al. 2018, 2020). Indeed, the amount of preparatory suppression seems to scale with the complexity of selection processes (Duque et al. 2016; Klein et al. 2014). Another hypothesis is that preparatory suppression eases movement initiation (Greenhouse et al. 2015; Hasegawa et al. 2017). In this line, a study showed a dependence of RTs on the amount of preparatory suppression on a single-trial basis: the stronger the suppression, the faster the initiation of the ensuing movement (Hannah et al. 2018).

Importantly, both hypotheses could be valid as they focus on different levels of control: while the "selection hypothesis" suggests that suppression originates from processes that help select accurate actions (*i.e.,* therefore reducing the error rate), the "motor hypothesis" entails that preparatory suppression is also generated by processes speeding up movement initiation (*i.e.,* therefore reducing RTs).

Here, we investigated the impact of motor training on preparatory suppression, while subjects practiced an instructed-delay RT task. The selection aspects were clear-cut, as evident from the low error rates, even before training. Hence, in such task, there is no room for improvement at the selection level and subjects can only become more skilled at the motor level, by initiating their response faster. Based on this, we predicted that RTs would shorten over the course of practice but that error rates would remain marginal. In addition, we expected resting CSE to increase with training, in accordance with previous work (Butefisch et al. 2000; Christiansen et al. 2018; Duque et al. 2008; Galea and Celnik 2009; Pascual-Leone et al. 1995). Based on the motor hypothesis (*i.e.,* that preparatory suppression fastens RTs), we expected that the suppression of CSE during action preparation would strengthen along with the RT decrease over the practice blocks.

## 2. Materials and Methods

### 2.1. Participants

Fifteen right-handed healthy subjects participated in the present study (n=15; 10 women; 22.4±1.63 years old). Handedness was assessed via Edinburgh Handedness inventory (Oldfield 1971). Participants filled out a TMS safety questionnaire to look for any contra-indication and gave written informed consent in accordance with the Ethics Committee of the Université Catholique de Louvain (approval number: 2012/22MAR/119) and the principles of the Declaration of Helsinki. We had to exclude one subject because we encountered a technical problem during the experiment; hence, analyses were run on the fourteen remaining subjects. Part of the data reported here has been exploited in a separate study (Vassiliadis et al. 2018). All of the data are expressed as mean±SE.

### 2.2. Task

Subjects were seated in front of a computer screen with the hands on response devices **(Fig.1A,** Grandjean et al. 2019; Grandjean and Duque 2020; Quoilin et al. 2016, 2018, 2019, 2020). They performed an instructed-delay RT task, which required them to choose between abduction movements of the left or right index finger according to the position of a preparatory cue (*i.e.,* a left- or right-sided ball separated from a goal by a gap). Participants had to prepare their movement once the ball appeared but to withhold responding until the onset of a "Go" signal (*i.e.,* a bridge). When the bridge appeared on the screen, subjects had to respond as fast as possible, allowing the ball to roll on the bridge and to reach the goal (**Fig.1B**). To reduce anticipation of the "Go" signal, the bridge did not appear in some of the trials (5%). Subjects were required not to respond on these trials and were penalized if they did so.

Trials always ended with a feedback score reflecting performance. On correct trials, scores ranged from 1 to 100 points and were displayed in green. Participants were informed that the score was inversely proportional to the RT: the faster the response, the higher the score. In order to homogenize the score across subjects, scores on correct trials were individualized according to RTs measured during a familiarization block just before the main experiment (Vassiliadis et al., 2018; Grandjean et al., 2019). Incorrect responses were penalized with

negative scores displayed in red. They involved responses occurring too early (RT<100 ms), referred to as "anticipation errors" (-75 points), responses occurring too late (RT>500 ms), referred to as "time-out errors" (-50 points), responses provided with the incorrect hand (-20 points), referred to as "selection errors" and responses provided on catch trials (-12 points), referred as "catch errors". When subjects succeeded not to respond on a catch trial, they were rewarded by +12 points. The total score was displayed at the end of each block.

### 2.3 TMS Protocol

Monophasic pulses were delivered through one or two small figure-of-eight shaped coils (internal diameter: 3.5 cm), each connected either to a Magstim 200² magnetic stimulator (Magstim, Whitland, Dyfed, UK). The TMS machine used to stimulate each hemisphere was counterbalanced between subjects. Pulses could be triggered in one (*i.e.,* single-coil TMS) or in the two coils (*i.e.,* double-coil TMS, **Fig.1C**) because the dataset was initially acquired for a separate study to establish the reliability of double-coil TMS to probe CSE bilaterally (Grandjean et al. 2018; Vassiliadis et al. 2018). In double-coil trials, a 1-ms interval separated the onset of the two pulses, eliciting MEPs in both hands at a near simultaneous time (Algoet et al. 2018; Grandjean et al. 2018; Quoilin et al. 2019; Vassiliadis et al. 2018). This interval was used to avoid direct electromagnetic interference between the two coils (Cincotta et al. 2005), while preventing transcallosal interactions that would occur between motor areas with longer delays (Ferbert et al. 1992; Hanajima et al. 2001). Notably, in double-coil trials, half of the trials involved a pulse over left M1 first whereas the other half involved a pulse over right M1 first (1ms delay). These data were assembled because a prior analysis reported elsewhere showed that the order of stimulation does not influence double-coil MEP amplitudes, which are equivalent to single-coil MEPs (Vassiliadis et al. 2018).

Each TMS coil was placed tangentially over one M1 with the handle pointing backward, laterally at a 45° angle away from the midline to induce a posterior-anterior current in the underlying cortical tissue **(Fig.1C)**. TMS was applied over the hotspot of the first dorsal interosseous muscle (FDI), which was the prime-mover in our task (Duque et al., 2014; Derosiere et al., 2017a, 2017b). The resting Motor Threshold (rMT) was determined for each M1. It was defined as the minimal intensity required to evoke MEPs of 50μV at rest in at least 5 out of 10 stimulations (Groppa et al. 2012; Wessel et al. 2020). The rMTs equalled 41.7±5.05% and

40.8±6.39% of the maximum stimulator output for the left and the right FDI, respectively. For each hemisphere, the intensity used throughout the experiment was set at 115% of the individual rMT (Derosiere et al. 2019).

### 2.4 Experimental procedure

The experiment started with two familiarization blocks. The first block included 20 trials and allowed subjects to become acquainted with the task. The second block involved 40 trials with TMS (as in the experimental blocks) and served to compute the median RT for each subject. The latter was used to individualize the feedback scores on correct trials according to the initial performance.

Then, subjects performed 400 trials of the task, divided in 10 experimental blocks. Each block involved an equal combination of single- and double-coil stimulations, occurring in a random order (*i.e.,* subjects could not anticipate the type of stimulation they would face). Given that both techniques produce equivalent MEPs (Grandjean et al. 2018; Vassiliadis et al. 2018), these data were considered regardless of the protocol used to elicit them.

TMS could occur in three different settings. First, some TMS pulses were delivered outside the blocks ($TMS_{baseline\text{-}out}$), providing MEPs reflecting baseline CSE at complete rest. $TMS_{baseline\text{-}out}$ pulses occurred every other block, starting before block 1 and ending after block 8 (5 time points; **Fig.2A**). Each time point involved 10 double-coil pulses (i.e. 10 pulses on both M1) and 10 single-coil pulses (5 pulses on each M1), allowing us to collect 15 MEPs in each hand at each of the 5 different stages of the experiment. Second, TMS occurred during the intertrial interval, 300 ms before the beginning of the trial (**Fig.2B**). MEPs recorded at this time provided another baseline measure of CSE, with subjects at rest but engaged in the task ($TMS_{baseline\text{-}in}$ Labruna et al., 2011). In each block, $TMS_{baseline\text{-}in}$ occurred in 4 double-coil trials (i.e. 4 pulses on both M1) and 4 single-coil trials (2 pulses on each M1), allowing us to collect a total of 6 baseline MEPs per block in each hand (i.e., 12 for each $Training_{STAGE}$). Finally, TMS occurred at 900 or 950 ms after the onset of the preparatory cue ($TMS_{preparation}$). Since no difference was found between MEPs recorded at these two timings in our previous analysis of the same data set (Vassiliadis et al. 2018), these MEPs were pooled together. $TMS_{preparation}$ occurred in 16 double-coil trials (i.e., 16 pulses on both M1) and 16 single-coil trials (8 pulses on each M1), allowing us to collect a total of 24 preparatory MEPs per block in each hand (48 MEPs for

each $Training_{STAGE}$). Half of these MEPs fell in left response trials, while the other half occurred in right response trials. Hence, MEPs could either fall in a hand that was selected for the forthcoming response ($MEP_{selected}$; *e.g.,* left MEPs preceding a left index finger response) or in a hand that was non-selected ($MEP_{non\text{-}selected}$).

### 2.5 Data processing and statistical analyses

The purpose of the study was twofold: (1) to characterize changes in CSE at rest and during action preparation occurring along with training in a basic instructed-delay RT task, (2) to assess whether modulations in CSE were correlated to training-related improvements in RTs. To do so, the behavioral and MEP data were evaluated according to the block within which they were elicited and data from two consecutive blocks were pooled together. Given the 10 blocks, we obtained five data sets reflecting five training stages ($Training_{STAGE}$: $Training_1$ to $Training_5$; **Fig.2A)**.

Statistical analyses were carried out with Matlab 2018a (the Mathworks, Natick, Massachusetts, USA) and Statistica 10 (StatSoft Inc., Tulsa, Oklahoma, USA). All data were systematically tested for the sphericity assumption using Maunchley's tests. The Greenhouse–Geisser (GG) correction was used for sphericity when necessary. Post-hocs comparisons were always conducted using the Fisher's LSD procedure. The significance level was set at $p \leq 0.05$.

#### 2.5.1 RTs and errors

Left and right hand RTs were computed as the difference between the onset of the "Go" signal and movement onset (when the finger quitted the outer metal edge of the device). Trials where subjects made an error were removed from the data set for the RT analysis. An average of 34.9 left and 33.6 right response trials remained for each subject at each $Training_{STAGE}$ (Table 1). We computed the mean RT for left and right responses separately and then averaged these data together. We choose this two-step method to make sure that left and right hand RTs would have the same weight in the averaged data for each $Training_{STAGE}$, regardless of the number of errors in each hand. Besides, we also assessed response accuracy over training, by computing, for each $Training_{STAGE}$, the amount of anticipation, time-out and catch errors as well as the total error rate. For each of these variables, we expressed the number of incorrect trials in percentage of the total

amount of trials, regardless of the responding hand. Selection errors were not analysed because they were rare (4 selection errors across all subjects). For the statistical analysis of RTs and errors (*i.e.,* anticipation, time-out, catch and global errors), we used one-way analyses of variance for repeated measures ($ANOVA_{RM}$) with the factor $Training_{STAGE}$ ($Training_1$ to $Training_5$).

### 2.5.2 MEP amplitudes

MEPs were obtained by recording electromyography (EMG) bilaterally from surface electrodes (Neuroline, Medicotest, Oelstykke, Denmark) placed over the FDI. The signals were amplified (x1000), bandpass filtered (10-500Hz; NeuroloLog; Digitimer), digitalized at 2000 Hz and collected with Signal (Signal 3.0, Cambridge, UK) for offline analysis. Trials with background EMG activity in the 200 ms window preceding the pulse exceeding 3 SDs above the mean were discarded (1.68±0.30% removal; Vassiliadis et al., 2018; Grandjean et al., 2018, 2019). This was done to prevent contamination of the MEP measurements by significant fluctuations in background EMG.

To assess training-related changes in resting CSE based on MEPs elicited at $TMS_{baseline\text{-}out}$ and $TMS_{baseline\text{-}in}$, we averaged separately left and right hand MEPs for each $Training_{STAGE}$ before computing the mean of these averages. These data were analysed using a two-way $ANOVA_{RM}$ with $TMS_{TIMING}$ ($TMS_{baseline\text{-}out}$ or $TMS_{baseline\text{-}in}$), and $Training_{STAGE}$ ($Training_1$ to $Training_5$) as within-subject factors. To assess training-related changes in preparatory suppression based on MEPs at $TMS_{preparation}$ (expressed in percentage of MEPs at $TMS_{baseline\text{-}in}$), we first removed the trials in which subjects made a mistake (10.78±1.81% removal) and then grouped left and right hand MEPs according to whether they corresponded to a $MEP_{selected}$ or $MEP_{non\text{-}selected}$. Within these categories, we averaged the separate means of left and right hand MEPs for each $Training_{STAGE}$. The number of trials that remained in each condition after data cleaning is provided in Table 1. To analyse these data, we first focused on percentage MEPs at $Training_1$, assessing with t-tests (against a constant value of 100%) the significance of preparatory suppression at the beginning of training. Then, we analyzed all training stages using a two-way $ANOVA_{RM}$ with the factors $MEP_{SELECTION}$ ($MEP_{selected}$ or $MEP_{non\text{-}selected}$) and $Training_{STAGE}$ ($Training_{1,}$ to $Training_5$). This ANOVA was also run on absolute MEP amplitudes (in mV).

| | Left hand | Right hand |
|---|---|---|
| RTs | 34,9 [28-40] | 33,6 [14-39] |
| MEPs at $TMS_{baseline\text{-}out}$ | 14,6 [11-15] | 14,7 [13-15] |
| MEPs at $TMS_{baseline\text{-}in}$ | 11,2 [9-12] | 11,2 [9-12] |
| MEPs at $TMS_{preparation}$ - selected | 20,7 [12-24] | 20,0 [9-24] |
| MEPs at $TMS_{preparation}$ - non-selected | 20,1 [8-24] | 20,8 [15-24] |

Table 1: Number of trials included per condition in the main analysis (mean [range]).

### 2.5.3 Relationship between training-related changes in RTs and CSE

As described in the Result section, training influenced RTs and CSE. We studied the relationship between changes at these two levels, with CSE considered separately at rest and during action preparation. We computed ratios reflecting training-related changes. Based on the RT data, we realized that the subjects' behavior improved substantially during the first practice stage ($Training_1$ to $Training_3$) but then, RTs remained quite stable (from $Training_3$ to $Training_5$; Result section). For this reason, we considered ratios for these two phases of training separately, providing us with an indication of early ($Training_{ratio\text{-}early}$: $Training_3/Training_1$) and late ($Training_{ratio\text{-}late}$: $Training_5/Training_3$) training-related changes in RTs and CSE. For the latter, we computed separate ratios for MEPs at $TMS_{baseline\text{-}out}$, $TMS_{baseline\text{-}in}$ and $TMS_{preparation}$ (expressed in percentage of MEPs at $TMS_{baseline\text{-}in}$). We then examined the correlation between the RT and MEP $Training_{ratios}$ by using least squares linear regressions.

Finally, we compared the strength of the RT relationship to training-related changes in MEP amplitudes at $TMS_{baseline\text{-}in}$ (reflecting resting CSE) and changes in percentage MEPs at $TMS_{preparation}$ (reflecting preparatory suppression of CSE). To do so, in order to obtain a robust estimate of the absolute Pearson's R, we ran a bootstrap analysis with 10000 resamples and calculated a median R for each correlation (Efron 1979). These R-values were then compared to each other using Pearson and Fillon's z test (Pearson and Filon 1898).

### 2.5.4 Single-trial relationship between RTs and preparatory suppression

The correlation analyses revealed a relationship between RTs and preparatory suppression: the subjects who showed the greatest training-related reduction in RTs were also those who displayed the strongest deepening in preparatory suppression (see Result section). To better understand the dependency of RTs to the strength of preparatory suppression, we investigated whether this relationship was evident on a single-trial basis, as suggested previously (Hannah et al. 2018). We selected the MEPs elicited at $TMS_{preparation}$ and again, expressed them as a percentage of $TMS_{baseline\text{-}in}$. We only used the double-coil trials, to consider a homogeneous set of data, with preparatory MEPs falling in both hands systematically. For each trial, we extracted the RT, as well as the MEPs recorded at $TMS_{preparation}$ in both selected ($MEP_{selected}$) and non-selected ($MEP_{non\text{-}selected}$) hands. Hence, for each trial, we obtained one RT measure linked to two different MEPs.

To examine the relationship between RTs and preparatory suppression, we pooled the trials from all 10 blocks together and sorted them according to the amplitude of MEPs within each trial. Given that there were two MEPs in each trial, we repeated this procedure twice, providing us with two different orderings of the trials according to the $MEP_{selected}$ or $MEP_{non\text{-}selected}$. Within each arrangement, trials were grouped into 6 consecutive percentile bins ($MEP_{BIN}$: $MEP_{BIN\text{-}1}$ = 0 to 16.7%, $MEP_{BIN\text{-}2}$ = 16.7 to 33.3% … $MEP_{BIN\text{-}6}$ = 83.3 to 100% of the data). $MEP_{BIN\text{-}1}$ contained the trials with the stronger preparatory suppression whereas the $MEP_{BIN\text{-}6}$ included the trials with the weaker preparatory suppression. We then computed the mean RT of trials within each $MEP_{BIN}$ (23.5 trials per condition on average and never less than 19 trials). Notably, because each $MEP_{BIN}$ involved a limited number of trials in this analysis, RTs were pooled together regardless of whether they were obtained in a left or right hand trial. Hence, we obtained six average RT values (*i.e.,* one for each $MEP_{BIN}$) for each of the trial arrangements based on the two MEP types. These two sets of RT data were analysed using two separate $ANOVA_{RM}$ with the factor $MEP_{BIN}$ ($MEP_{BIN\text{-}1}$ to $MEP_{BIN\text{-}6}$).

## 3. Results

### 3.1. RTs and errors

**Fig.3A** shows the evolution of RTs with training. The $ANOVA_{RM}$ revealed a significant influence of $Training_{STAGE}$ on RT ($F_{(4,52)}$=4.31, p=0.0043). Post-hoc tests showed that RTs measured from $Training_3$ to $Training_5$ were shorter than at $Training_1$ (all p<0.004). In contrast, the total error rate remained stable over the blocks ($F_{(4,52)}$=0.82, p=0.52, **Fig.3B**). We did not observe any modification of the percentage of anticipation ($F_{(4,52)}$=1.12, p=0.36), time-out (GG-corrected $F_{(2.50,32.50)}$=0.90, p=0.44) or catch errors ($F_{(4,52)}$=1.73, p=0.16). Hence, training enabled subjects to respond more quickly while maintaining the same accuracy level.

### 3.2. MEP amplitude

First, we evaluated the effect of training on MEPs acquired at rest. As evident on **Fig.4,** MEPs were larger when assessed in the context of the task ($TMS_{baseline\text{-}in}$: 1.79±0.17mV) compared to when subjects were fully at rest ($TMS_{baseline\text{-}out}$: 1.34±0.17mV), as supported by the significant factor $TMS_{TIMING}$ ($F_{(1,13)}$=28.43, p<0.001) and consistent with previous studies (Derosière et al. 2015; Labruna et al. 2011). The $ANOVA_{RM}$ also revealed an effect of $Training_{STAGE}$ on baseline MEPs ($F_{(4,52)}$=6.34, p<0.001). MEPs recorded at $Training_2$ to $Training_5$ were larger than at $Training_1$ (all p<0.03). This training effect on MEPs occurred independently of the $TMS_{TIMING}$: there was a parallel increase in the amplitude of MEPs elicited at $TMS_{baseline\text{-}out}$ and $TMS_{baseline\text{-}in}$ ($Training_{STAGE}$x$TMS_{TIMING}$: $F_{(4,52)}$=0.18, p=0.95).

Second, we analyzed the effect of training on preparatory suppression by considering MEPs elicited at $TMS_{preparation}$ (expressed in percentage of $TMS_{baseline\text{-}in}$). As evident on **Fig.5A**, percentage FDI MEPs were initially suppressed at $Training_1$ (MEPs smaller than 100%), consistent with the presence of preparatory suppression in the prime-mover, whether selected for the forthcoming response ($MEP_{selected}$: 73.98±4.00%; $t_{(13)}$=-6.50, p<0.0001) or not ($MEP_{non\text{-}selected}$: 76.26±4.36%; $t_{(13)}$=-5.44, p<0.001). Interestingly, preparatory suppression became more prominent with training: the $ANOVA_{RM}$ revealed a significant decrease in percentage MEP amplitudes over the $Training_{STAGES}$ ($F_{(4,52)}$=2.79, p=0.036). This change was marginal at $Training_4$ (*i.e.,* $Training_4$: p=0.058 when compared to $Training_1$) and became significant at

336 $Training_5$ (p=0.006). It concerned MEPs obtained from the selected and non-selected hands
337 ($Training_{STAGE}xMEP_{SELECTION}$: $F_{(4,52)}$=0.56, p=0.70). To further our understanding of training-
338 related changes of preparatory activity, we ran another set of $ANOVA_{RM}$ on absolute MEP
339 amplitudes (rather than percentages) at $TMS_{preparation}$ (**Fig.5B**). These MEPs did not show any
340 fluctuation over the $Training_{STAGES}$ ($F_{(4,52)}$=1.30, p=0.28). Moreover, we did not find any
341 $MEP_{SELECTION}$ effect ($F_{(1,13)}$=1.69, p=0.22) or $Training_{STAGE}xMEP_{SELECTION}$ interaction
342 ($F_{(4,52)}$=0.85, p=0.50).

343 In conclusion, our results indicate that training did not produce even modulatory changes
344 in motor activity at rest and during action preparation: while resting CSE increased, preparatory
345 activity remained flat over the blocks, thus revealing an augmenting drop in preparatory activity
346 (i.e. a strengthening of preparatory suppression) with respect to the rising baseline excitability
347 state. These changes in CSE occurred in parallel with an acceleration of RTs.

348 Because RTs became shorter over the blocks, one may argue that MEPs at $TMS_{preparation}$
349 were not recorded in a comparable preparatory state throughout training; that is, the delay
350 between TMS and movement onset ($Delay_{TMS\text{-}TO\text{-}MOVE}$) may have decreased over the blocks.
351 Importantly, we shuffled the delay between the pulse and the "Go" signal in the present study
352 (see Methods), in order to prevent changes in RT to convert into equivalent changes in the
353 $Delay_{TMS\text{-}TO\text{-}MOVE}$. However, because TMS fell on average closer to movement onset at $Training_5$
354 (399.70±8.48ms) than $Training_1$ (419.99±9.99ms, $t_{(13)}$=-3.10, p=0.008), we performed an
355 additional analysis to control for a potential bias of the $Delay_{TMS\text{-}TO\text{-}MOVE}$. We conducted a
356 response-locked analysis whereby we classified MEP data at $TMS_{preparation}$ (regardless of the
357 $Training_{STAGE}$) according to the $Delay_{TMS\text{-}TO\text{-}MOVE}$ in 5 consecutive bins of trials ($Delay_{BIN}$ =
358 $Delay_{BIN\text{-}1}$= 0 to 20%, $Delay_{BIN\text{-}2}$= 20 to 40%, …, $Delay_{BIN\text{-}5}$= 80 to 100% of the $Delay_{TMS\text{-}TO\text{-}}$
359 $_{MOVE}$ data). An $ANOVA_{RM}$ ran on these data did not reveal any effect of $Delay_{BIN}$ ($F_{(4,52)}$=1.45;
360 p=0.23), nor was there any significant $MEP_{SELECTION}xDelay_{BIN}$ interaction ($F_{(4,52)}$=0.40; p=0.81;
361 **Fig.6**). These results indicate that MEPs elicited preceding a "Go" signal remain quite unaffected
362 by the delay separating the $TMS_{preparation}$ pulse and movement onset.

363 Moreover, as an additional safety check, we also considered whether potential changes in
364 background EMG activity could be responsible for the training-related changes in CSE reported
365 here. To do so, we ran two additional analyses on pre-TMS EMG RMS amplitudes. First, we
366 analysed RMS amplitude before the $TMS_{baseline\text{-}in}$ pulses by means of a one-way ANOVA with the

factor $Training_{STAGE.}$ This analysis revealed a stable EMG background at $TMS_{baseline\text{-}in}$ over the $Training_{STAGE}$ (GG-corrected $F_{(1.65,\ 21.45)}$=0.89; p=0.41). In a second step, in order to make sure that a change in the relationship between EMG background activity at $TMS_{preparation}$ with respect to $TMS_{baseline\text{-}in}$ was not responsible for the observed training-related change in preparatory suppression, we expressed the RMS computed at $TMS_{preparation}$ in percentage of the values obtained at $TMS_{baseline\text{-}in}$ for each $Training_{STAGE}$. As in the main analysis, we did so separately for the selected and non-selected FDIs ($FDI_{SELECTION}$: $FDI_{selected}$ and $FDI_{non\text{-}selected}$). We found that RMS amplitudes at $TMS_{preparation}$ were very similar to the ones obtained at $TMS_{baseline\text{-}in}$ for all $Training_{STAGES,}$ with the RMS recorded at $TMS_{preparation}$ ranging from 99.79+/-0.76 to 101.85+/-1.77 % of values obtained at $TMS_{baseline\text{-}in}$. Consistently, the two-way ANOVA run on these data did not show any effect of $Training_{STAGE}$ (GG-corrected $F_{(1.88,\ 24.49)}$=0.91; p=0.41), $FDI_{SELECTION}$ ($F_{(1,13)}$=1.40; p=0.26) nor any $Training_{STAGE}$x$FDI_{SELECTION}$ interaction (GG-corrected $F_{(1.02,\ 13.20)}$=1.12; p=0.31). Hence, changes in EMG background activity are unlikely to be responsible for the training-related changes in preparatory suppression observed in the present study.

### 3.3 Relationship between training-related changes in RTs and CSE

Given that training influenced RTs and CSE, we studied the relationship between changes at these two levels, with CSE considered separately at rest and during action preparation. To assess the relationship between RTs and resting CSE, we ran correlations between training-related changes in RTs and changes in MEPs at $TMS_{baseline\text{-}in}$ and $TMS_{baseline\text{-}out}$. These analyses did not reveal any link between variations in resting measures of CSE and changes observed in RTs, neither at $Training_{ratio\text{-}early}$ **(Fig.7A,** R=-0.27, p=0.36 and R=0.079, p=0.79 for $TMS_{baseline\text{-}in}$ and $TMS_{baseline\text{-}out}$, respectively) nor at $Training_{ratio\text{-}late}$ **(**R=-0.28, p=0.33 and R=-0.16, p=0.59).

In contrast, changes in RTs at $Training_{ratio\text{-}early}$ were linked to variations in preparatory suppression observed in the selected **(Fig.7B;** R=0.55, p=0.043**)** and non-selected FDI **(Fig.7C;** R=0.74, p=0.0027): subjects showing a greater training-related strengthening of preparatory suppression also showed larger improvements in RTs. This correlation was not significant at $Training_{ratio\text{-}late}$, neither for the selected (R=0.12, p=0.67) nor for the non-selected effectors (R=0.48, p=0.084). Our results suggest that RT improvements were related to early changes in preparatory suppression.

This conclusion is further supported by an additional analysis showing that the strength of the correlation between RTs and CSE at $Training_{ratio\text{-}early}$ was significantly higher when considering percentage MEPs at $TMS_{preparation}$ (*i.e.,* preparatory suppression) in the non-selected FDI (bootstrap estimate of absolute R=0.76), than when MEPs were considered at $TMS_{baseline\text{-}in}$ (R=0.29; z=1.75; p=0.040, **Fig.7D**). This difference was not significant when taking preparatory suppression in the selected FDI (R=0.56; z-score=0.85, p=0.20). Hence, training-related changes in preparatory suppression of the non-selected effector turned out to be the best predictor of RT improvements.

### 3.4 Single-trial relationship between RTs and preparatory suppression

Finally, we asked whether the dependency of RTs to preparatory suppression is also evident on a single-trial basis. This was the case for MEPs recorded from the non-selected hand: the greater the preparatory suppression in that hand, the shorter the following RT (**Fig.8**, right panel), as supported by the $ANOVA_{RM}$ revealing an effect of the factor $MEP_{BIN}$ on RTs ($F_{(5,65)}$=2.57, p=0.035). Post-hoc tests revealed that RTs in $MEP_{BIN\text{-}1}$ and $MEP_{BIN\text{-}2}$ (*i.e.,* strongest preparatory suppression) were systematically shorter than those in $MEP_{BIN\text{-}6}$ (p=0.0021 and p=0.0090). We did not observe any relationship between RTs and MEPs obtained in the selected hand ($MEP_{BIN}$: GG-corrected $F_{(2.22,28.88)}$=0.85, p=0.45; **Fig.8**, left panel). Hence, the training-related effects and the single-trial relationship indicates that preparatory suppression in the non-selected (non-responding) hand is a predictor of the following RT. The lower this activity, the faster the response.

## 4. Discussion

Training accelerated RTs while errors remained low. CSE became larger at rest and preparatory suppression of CSE was stronger after training. Interestingly, subjects who showed the strongest RT improvements at the early $Training_{STAGES}$ were also those displaying the largest initial strengthening in preparatory suppression, especially when probed in the non-selected hand. Such a relationship between RTs and preparatory suppression was also evident at a single-trial level: RTs were generally faster in trials where preparatory suppression was deeper.

Subjects responded faster with training. RTs reflect the sum of the time required for processing the imperative cue, preparing the motor command and initiating the action (Derosiere et al. 2019; Haith et al. 2016) and, theoretically, training may impact any of these sensory-motor components. Previous studies have shown that RT improvements can result from both faster sensory processing (Clark et al. 2015) and more efficient motor preparation (Mawase et al. 2018). Yet, in an instructed-delay task, the time required for sensory processing and motor preparation is strongly constrained and most of the RT is assumed to reflect the time needed for action initiation (Haith et al. 2016). Hence, the RT gains reported here are likely to reflect a reduction in initiation time. Our findings thus yield an extension of former work, suggesting that, in addition to accelerating sensory processing and motor preparation, training can also boost action initiation.

Resting CSE was higher when assessed in the context of the task (*i.e.,* at $TMS_{baseline\text{-}in}$) than between the blocks (*i.e.,* at $TMS_{baseline\text{-}out}$), consistent with previous data (Labruna et al. 2011; Vassiliadis et al. 2018) and with the observation that task-driven increases in attention amplifies cortical excitability (Kastner et al. 1998, 1999). As expected based on prior observations (*e.g.,* (Butefisch et al. 2000; Christiansen et al. 2018; Duque et al. 2008; Galea and Celnik 2009; Pascual-Leone et al. 1995), practicing the task led to an increase in resting CSE. Interestingly, this increase was not exclusive to the task and was in fact strongly similar at $TMS_{baseline\text{-}in}$ and $TMS_{baseline\text{-}out}$, ruling out the possibility that it resulted from a change in task-related attention over practice (Derosière et al. 2015). Rather, our findings support the idea of a plastic reorganization of the motor system, measurable when engaged in the task as well as at rest.

CSE was reduced during action preparation when compared to baseline (during the task), reflecting the well-known preparatory suppression effect (Duque et al. 2017), which was evident

448 in the selected and non-selected hands from the beginning of the training. Contrary to rest, the
449 amplitude of MEPs at $TMS_{preparation}$ did not increase with practice (they remained unchanged),
450 reflecting a strengthening of preparatory suppression with respect to the rising baseline. Notably,
451 although at the group level this strengthening of preparatory suppression appeared late (**Fig.5A**),
452 at the individual level, a majority of subjects already exhibited this effect at early training stages
453 (**Fig.6**).

454 Based on these findings, one could propose that changes in resting excitability are key to
455 RT improvements, as suggested by the inverse relationship between baseline CSE and RTs
456 described recently (Greenhouse et al. 2017). Yet, we did not find a relationship between training-
457 related changes in baseline excitability and improvements in performance. This is in line with the
458 idea that increased resting CSE is not crucial for immediate performance (Bologna et al. 2015),
459 but may be involved in the long-term retention of the motor behavior (Cantarero et al. 2013).
460 Rather, what was predictive of RT gains in the present study was the change in relative CSE, as
461 measured during action preparation: subjects showing the greater strengthening of preparatory
462 suppression at the early $Training_{STAGES}$ were those who became fastest. These results are
463 consistent with animal studies showing that behavioral improvements in motor learning tasks are
464 associated with changes in relative preparatory activity (Mandelblat-Cerf et al. 2009; Paz et al.
465 2003; Vyas et al. 2018). Similarly, a recent study using paired-pulse TMS showed that changes in
466 preparatory activity of M1 intra-cortical circuits are correlated to training-related behavioral
467 gains, contrary to changes observed at rest (Dupont-Hadwen et al. 2018). More generally, our
468 findings agree with the idea that efficient action preparation relies on dynamical shifts of neural
469 activity from a baseline state to a preparatory state (Churchland et al. 2012). From this point of
470 view, training may allow tuning the dynamics of preparatory activity, bringing it closer to an
471 optimal state for action initiation (Vyas et al. 2018). In this line, strengthening of preparatory
472 suppression would facilitate action initiation by allowing excitatory inputs targeting the selected
473 motor representation to better stand out against a quiescent background (mostly reflected in the
474 excitability of non-selected effector), ultimately speeding up RTs (Greenhouse et al. 2015;
475 Hasbroucq et al. 1997; Hasegawa et al. 2017).

476 This interpretation was reinforced by our single-trial analysis showing that RTs depended
477 on the foregoing amount of preparatory suppression. That is, stronger levels of suppression were
478 related to faster initiation times in the very same trials, in agreement with previous results

(Hannah et al. 2018; Hasegawa et al. 2017). Interestingly, we found such relationship when considering the non-selected prime-mover but not the selected one. This was also the case for training-related effects, with preparatory suppression in the non-selected effector appearing as the best predictor of RT changes. Hence, despite the fact that preparatory suppression was broad, affecting selected and non-selected muscles, suppression in the non-selected effector turned out to be a better predictor of RTs than suppression in the selected muscle. This difference may be due to the fact that MEP amplitudes in the selected effector are influenced by many overlapping inputs, potentially reflecting the operation of response preparation processes (Duque and Ivry 2009), while MEP amplitudes in the non-selected effector reflect a purer form of suppression. For instance, Duque and Ivry (2009) found that action preparation entails a release of M1-intracortical inhibition in parallel of the suppression of CSE, specifically in the selected effector. The multiplicity of inputs targeting the selected effector during action preparation may therefore have weakened the link between our measure of preparatory suppression and RTs. Overall, we propose that motor initiation is eased by a form of preparatory suppression encompassing selected and non-selected effectors (Greenhouse et al. 2015) but that the link between suppression and initiation is best evidenced when considering the excitability of non-selected effectors.

## Conclusion

This study shows that a simple training paradigm can lead to improvements in action initiation that are accompanied by an increase in resting CSE and a strengthening of corticospinal suppression from the rising baseline state. Moreover, contrary to changes in resting CSE, such strengthening of preparatory suppression was linked to RT improvements, especially in the non-selected effector. These findings could have implications for the rehabilitation of patients suffering from impaired action initiation such as in cerebellar ataxia (Battaglia et al. 2006) or Parkinson's disease (Mure et al. 2012).

## Additional information

Data availability:

The data that support the findings of this study are available at: https://osf.io/8p5wm/ (Vassiliadis 2020).

Conflict of interest:

The authors declare no conflict of interest.

Author contributions:

PV, GD, JG, JD: conception and design of the work; PV, JG: acquisition of data; PV: analysis of data; PV, GD, JD interpretation of data; PV: drafting; PV, GD, JG, JD revising the manuscript.

Funding:

P.V. and J.G. were PhD students supported by the Fund for Research training in Industry and Agriculture (FRIA/FNRS; FC29690 and FC09115). G.D. was a post-doctoral fellow supported by the Belgian National Funds for Scientific Research (FNRS, 1B134.18). J.D. was supported by grants from the Belgian FNRS (F.4512.14) and the Fondation Médicale Reine Elisabeth (FMRE).

## References


**Algoet M**, **Duque J**, **Iannetti GD**, **Mouraux A**. Temporal Profile and Limb-specificity of Phasic Pain-Evoked Changes in Motor Excitability. *Neuroscience* 386: 240–255, 2018.

**Battaglia F**, **Quartarone A**, **Ghilardi MF**, **Dattola R**, **Bagnato S**, **Rizzo V**, **Morgante L**, **Girlanda P**. Unilateral cerebellar stroke disrupts movement preparation and motor imagery. *Clin Neurophysiol* 117: 1009–1016, 2006.

**Bologna M**, **Rocchi L**, **Paparella G**, **Nardella A**, **Li P**, **Conte A**, **Kojovic M**, **Rothwell JC**, **Berardelli A**. Brain Stimulation Reversal of Practice-related Effects on Corticospinal Excitability has no Immediate Effect on Behavioral Outcome. *Brain Stimul* 8: 603–612, 2015.

**Butefisch CM**, **Davis BC**, **Wise SP**, **Sawaki L**, **Kopylev L**, **Classen J**, **Cohen LG**. Mechanisms of use-dependent plasticity in the human motor cortex. *Proc Natl Acad Sci* 97: 3661–3665, 2000.

**Cantarero G**, **Lloyd A**, **Celnik P**. Reversal of Long-Term Potentiation-Like Plasticity Processes after Motor Learning Disrupts Skill Retention. *J Neurosci* 33: 12862–12869, 2013.

**Christiansen L**, **Madsen MJ**, **Bojsen-møller E**, **Thomas R**, **Nielsen JB**. Brain Stimulation Progressive practice promotes motor learning and repeated transient increases in corticospinal excitability across multiple days. *Brain Stimul* 11: 346–357, 2018.

**Churchland MM**, **Cunningham JP**, **Kaufman MT**, **Foster JD**, **Nuyujukian P**, **Ryu SI**, **Shenoy K V.**, **Shenoy K V.** Neural population dynamics during reaching. *Nature* 487: 51–56, 2012.

**Cincotta M**, **Borgheresi A**, **Jung P**, **Balestrieri F**, **Giovannelli F**, **Zaccara G**, **Ziemann U**. Physical interactions between induced electrical fields can have substantial effects on neuronal excitation during simultaneous TMS of two brain areas. *Clin Neurophysiol* 116: 1733–1742, 2005.

**Clark K**, **Gregory Appelbaum L**, **van den Berg B**, **Mitroff SR**, **Woldorff MG**. Improvement in visual search with practice: Mapping learning-related changes in neurocognitive stages of processing. *J Neurosci* 35: 5351–5359, 2015.

**Derosiere G**. A Dynamical System Framework for Theorizing Preparatory Inhibition. *J Neurosci* 38: 3391–3393, 2018.

**Derosière G**, **Billot M**, **Ward ET**, **Perrey S**. Adaptations of motor neural structures' activity to

lapses in attention. *Cereb Cortex* 25: 66–74, 2015.

**Derosiere G**, **Duque J**. Tuning the Corticospinal System: How Distributed Brain Circuits Shape Human Actions. *Neurosci* 107385841989675, 2020.

**Derosiere G**, **Thura D**, **Cisek P**, **Duque J**. Motor cortex disruption delays motor processes but not deliberation about action choices. *J Neurophysiol* 122: 1566–1577, 2019.

**Derosiere G**, **Vassiliadis P**, **Demaret S**, **Zénon A**, **Duque J**. Learning stage-dependent effect of M1 disruption on value-based motor decisions. *Neuroimage* , 2017a. doi:10.1016/j.neuroimage.2017.08.075.

**Derosiere G**, **Vassiliadis P**, **Duque J**. Advanced TMS approaches to probe corticospinal excitability during action preparation. *Neuroimage* 213: 116746, 2020.

**Derosiere G**, **Zénon A**, **Alamia A**, **Duque J**. Primary motor cortex contributes to the implementation of implicit value-based rules during motor decisions. *Neuroimage* 146: 1115–1127, 2017b.

**Dupont-Hadwen J**, **Bestmann S**, **Stagg CJ**. Motor training modulates intracortical inhibitory dynamics in motor cortex during movement preparation. *Brain Stimul* , 2018. doi:10.1016/j.brs.2018.11.002.

**Duque J**, **Greenhouse I**, **Labruna L**, **Ivry RB**. Physiological Markers of Motor Inhibition during Human Behavior. *Trends Neurosci* 40: 219–236, 2017.

**Duque J**, **Ivry RB**. Role of corticospinal suppression during motor preparation. *Cereb Cortex* 19: 2013–2024, 2009.

**Duque J**, **Labruna L**, **Cazares C**, **Ivry RB**. Dissociating the influence of response selection and task anticipation on corticospinal suppression during response preparation. *Neuropsychologia* 65: 287–296, 2014.

**Duque J**, **Lew D**, **Mazzocchio R**, **Olivier E**, **Richard B**. Evidence for two concurrent inhibitory mechanisms during response preparation. *J Neurosci* 30: 3793–3802, 2010.

**Duque J**, **Mazzocchio R**, **Stefan K**, **Hummel F**, **Olivier E**, **Cohen LG**. Memory formation in the motor cortex ipsilateral to a training hand. *Cereb Cortex* 18: 1395–1406, 2008.

**Duque J**, **Petitjean C**, **Swinnen SP**. Effect of aging on motor inhibition during action preparation under sensory conflict. *Front Aging Neurosci* 8: 1–14, 2016.

**Efron B**. Bootstrap Methods: Another Look at the Jackknife. *Ann Stat* 7: 1–26, 1979.

**Ferbert A**, **Priori A**, **Rothwell JC**, **Day BL**, **Colebatch JG**, **Marsden CD**. Interhemispheric inhibition of the human motor cortex. *J Physiol* 453: 525–546, 1992.

**Galea JM**, **Celnik P**. Brain Polarization Enhances the Formation and Retention of Motor Memories. *J Neurophysiol* 102: 294–301, 2009.

**Grandjean J**, **Derosiere G**, **Vassiliadis P**, **Quemener L**, **Wilde Y de**, **Duque J**. Towards assessing corticospinal excitability bilaterally: Validation of a double-coil TMS method. *J Neurosci Methods* 293: 162–168, 2018.

**Grandjean J**, **Duque J**. A TMS Study of Preparatory Suppression in Binge Drinkers. *NeuroImage Clin* 28: 102383, 2020.

**Grandjean J**, **Quoilin C**, **Duque J**. Investigating the effect of anticipating a startling acoustic stimulus on preparatory inhibition. *Neurophysiol Clin* 49: 137–147, 2019.

**Greenhouse I**, **Sias a.**, **Labruna L**, **Ivry RB**. Nonspecific Inhibition of the Motor System during Response Preparation. *J Neurosci* 35: 10675–10684, 2015.

**Greenhouse XI**, **King XM**, **Noah XS**, **Maddock XRJ**, **Ivry RB**. Individual Differences in Resting Corticospinal Excitability Are Correlated with Reaction Time and GABA Content in Motor Cortex. *J Neurosci* 37: 2686–2696, 2017.

**Groppa S**, **Oliviero A**, **Eisen A**, **Quartarone A**, **Cohen LG**, **Mall V**, **Kaelin-Lang A**, **Mima T**, **Rossi S**, **Thickbroom GW**, **Rossini PM**, **Ziemann U**, **Valls-Solé J**, **Siebner HR**. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clin Neurophysiol* 123: 858–882, 2012.

**Haith AM**, **Pakpoor J**, **Krakauer JW**. Independence of Movement Preparation and Movement Initiation. *J Neurosci* 36: 3007–3015, 2016.

**Hanajima R**, **Ugawa Y**, **Machii K**, **Mochizuki H**, **Terao Y**, **Enomoto H**, **Furubayashi T**, **Shiio Y**, **Uesugi H**, **Kanazawa I**. Interhemispheric facilitation of the hand motor area in humans. *J Physiol* 531: 849–859, 2001.

**Hannah R**, **Cavanagh SE**, **Tremblay S**, **Simeoni S**, **Rothwell JC**. Selective suppression of local interneuron circuits in human motor cortex contributes to movement preparation. *J Neurosci* 38: 2869–17, 2018.

**Hasbroucq T**, **Kaneko H**, **Akamatsu M**, **Possamaï CA**. Preparatory inhibition of cortico-spinal excitability: A transcranial magnetic stimulation study in man. *Cogn Brain Res* 5: 185–192, 1997.

**Hasegawa M**, **Majima K**, **Itokazu T**, **Maki T**, **Albrecht UR**, **Castner N**, **Izumo M**, **Sohya K**, **Sato TK**, **Kamitani Y**, **Sato TR**. Selective Suppression of Local Circuits during Movement Preparation in the Mouse Motor Cortex. *Cell Rep* 18: 2676–2686, 2017.

**Kastner S**, **Pinsk MA**, **De Weerd P**, **Desimone R**, **Ungerleider LG**. Increased Activity in Human Visual Cortex during Directed Attention in the Absence of Visual Stimulation. *Neuron* 22: 751–761, 1999.

**Kastner S**, **De Weerd P**, **Desimone R**, **Ungerleider LG**. Mechanisms of Directed Attention in the Human Extrastriate Cortex as Revealed by Functional MRI. *Science (80- )* 282: 108–111, 1998.

**Klein PA**, **Petitjean C**, **Olivier E**, **Duque J**. Top-down suppression of incompatible motor activations during response selection under conflict. *Neuroimage* 86: 138–149, 2014.

**Krakauer JW**, **Ghilardi MF**, **Mentis M**, **Barnes A**, **Veytsman M**, **Eidelberg D**, **Ghez C**. Differential Cortical and Subcortical Activations in Learning Rotations and Gains for Reaching: A PET Study. *J Neurophysiol* 91: 924–933, 2004.

**Krakauer JW**, **Hadjiosif AM**, **Xu J**, **Wong AL**, **Haith AM**. Motor Learning. 9: 613–663, 2019.

**Labruna L**, **Fernández-Del-Olmo M**, **Ivry RB**. Comparison of different baseline conditions in evaluating factors that influence motor cortex excitability. *Brain Stimul* 4: 152–155, 2011.

**Makino H**, **Ren C**, **Liu H**, **Kim AN**, **Kondapaneni N**, **Liu X**, **Kuzum D**, **Komiyama T**. Transformation of Cortex-wide Emergent Properties during Motor Learning. *Neuron* 94: 880-890.e8, 2017.

**Mandelblat-cerf Y**, **Paz R**, **Vaadia E**. Trial-to-Trial Variability of Single Cells in Motor Cortices Is Dynamically Modified during Visuomotor Adaptation. *J Neurosci* 29: 15053–15062, 2009.

**Mawase F**, **Lopez D**, **Celnik PA**, **Haith AM**. Movement Repetition Facilitates Response Preparation. *Cell Rep* 24: 801–808, 2018.

**Mawase F**, **Uehara S**, **Bastian AJ**, **Celnik P**. Motor Learning Enhances Use-Dependent Plasticity. *J Neurosci* 37: 2673–2685, 2017.

637 **Mure H**, **Tang CC**, **Argyelan M**, **Ghilardi MF**, **Kaplitt MG**, **Dhawan V**, **Eidelberg D**.
638 Improved sequence learning with subthalamic nucleus deep brain stimulation: Evidence for
639 treatment-specific network modulation. *J Neurosci* 32: 2804–2813, 2012.

640 **Oldfield RC**. The assessment and analysis of handedness: The Edinburgh inventory.
641 *Neuropsychologia* 9: 97–113, 1971.

642 **Pascual-Leone A**, **Nguyet D**, **Cohen LG**, **Brasil-Neto JP**, **Cammarota A**, **Hallett M**, **Nguyen**
643 **KT**, **Cohen a D**, **Brasil-Neto JP**, **Cammarota A**, **Hallett M**, **Nguyet D**, **Cohen LG**, **Brasil-**
644 **Neto JP**, **Cammarota A**, **Hallett M**, **Cohen G**, **Cammarota A**, **Pascual-Leone A**, **Nguyet D**,
645 **Cohen LG**, **Brasil-Neto JP**, **Hallett M**, **Cammarota A**, **Hallett M**, **Cohen G**, **Cammarota A**,
646 **Pascual-Leone A**, **Nguyet D**, **Cohen LG**, **Brasil-Neto JP**, **Hallett M**. Modulation of muscle
647 responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor
648 skills. [Online]. *J Neurophysiol* 74: 1037–1045,
649 1995http://www.ncbi.nlm.nih.gov/pubmed/7500130.

650 **Paz R**, **Boraud T**, **Natan C**, **Bergman H**, **Vaadia E**. Preparatory activity in motor cortex
651 reflects learning of local visuomotor skills. 6: 882–890, 2003.

652 **Pearson K**, **Filon L**. VII. Mathematical contributions to the theory of evolution.— IV. On the
653 probable errors of frequency constants and on the influence of random selection on variation and
654 correlation. *Philos Trans R Soc London* 191: 229–311, 1898.

655 **Quoilin C**, **Fievez F**, **Duque J**. Preparatory inhibition: Impact of choice in reaction time tasks.
656 *Neuropsychologia* 129: 212–222, 2019.

657 **Quoilin C**, **Grandjean J**, **Duque J**. Considering Motor Excitability During Action Preparation
658 in Gambling Disorder: A Transcranial Magnetic Stimulation Study. *Front Psychiatry* 11: 1–13,
659 2020.

660 **Quoilin C**, **Lambert J**, **Jacob B**, **Klein PA**, **Duque J**. Comparison of motor inhibition in
661 variants of the instructed-delay choice reaction time task. *PLoS One* 11: 1–16, 2016.

662 **Quoilin C**, **Wilhelm E**, **Maurage P**, **Timary P De**, **Duque J**. Deficient inhibition in alcohol-
663 dependence : let's consider the role of the motor system ! *Neuropsychopharmacology* 0–8, 2018.

664 **Reis J**, **Swayne OB**, **Vandermeeren Y**, **Camus M**, **Dimyan MA**, **Harris-Love M**, **Perez MA**,
665 **Ragert P**, **Rothwell JC**, **Cohen LG**. Contribution of transcranial magnetic stimulation to the

understanding of cortical mechanisms involved in motor control. *J Physiol* 586: 325–351, 2008.

**Rosenkranz K**, **Kacar A**, **Rothwell JC**. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. *J Neurosci* 27: 12058–12066, 2007.

**Shmuelof L**, **Yang J**, **Caffo B**, **Mazzoni P**, **Krakauer JW**. The neural correlates of learned motor acuity. 971–980, 2014.

**Steele CJ**, **Penhune VB**. Specific increases within global decreases: A functional magnetic resonance imaging investigation of five days of motor sequence learning. *J Neurosci* 30: 8332–8341, 2010.

**Vassiliadis P**. Training_Motor_Preparation_TMS [Online]. 2020.https://osf.io/8p5wm/.

**Vassiliadis P**, **Grandjean J**, **Derosiere G**, **Wilde Y De**. Using a Double-Coil TMS Protocol to Assess Preparatory Inhibition Bilaterally. *Front Neurosci* 12: 1–14, 2018.

**Vyas S**, **Even-Chen N**, **Stavisky SD**, **Ryu SI**, **Nuyujukian P**, **Shenoy K V.** Neural Population Dynamics Underlying Motor Learning Transfer. *Neuron* 97: 1177-1186.e3, 2018.

**Vyas S**, **O'Shea DJ**, **Ryu SI**, **Shenoy K**. Causal role of motor preparation during error-driven learning. *Neuron* 1–11, 2020.

**Wenger E**, **Kühn S**, **Verrel J**, **Mårtensson J**, **Bodammer NC**, **Lindenberger U**, **Lövdén M**. Repeated Structural Imaging Reveals Nonlinear Progression of Experience-Dependent Volume Changes in Human Motor Cortex. *Cereb Cortex* 27: 2911–2925, 2017.

**Wessel MJ**, **Draaisma LR**, **de Boer AFW**, **Park C hyun**, **Maceira-Elvira P**, **Durand-Ruel M**, **Koch PJ**, **Morishita T**, **Hummel FC**. Cerebellar transcranial alternating current stimulation in the gamma range applied during the acquisition of a novel motor skill. *Sci Rep* 10: 1–12, 2020.

**Wiestler T**, **Diedrichsen J**. Skill learning strengthens cortical representations of motor sequences. *Elife* 2013: 1–20, 2013.

**Yokoi A**, **Diedrichsen J**. Neural Organization of Hierarchical Motor Sequence Representations in the Human Neocortex. *Neuron* 103: 1178-1190.e7, 2019.

| | Left hand | Right hand |
|---|---|---|
| RTs | 34,9 [28-40] | 33,6 [14-39] |
| MEPs at $TMS_{baseline\text{-}out}$ | 14,6 [11-15] | 14,7 [13-15] |
| MEPs at $TMS_{baseline\text{-}in}$ | 11,2 [9-12] | 11,2 [9-12] |
| MEPs at $TMS_{preparation}$ - selected | 20,7 [12-24] | 20,0 [9-24] |
| MEPs at $TMS_{preparation}$ - non-selected | 20,1 [8-24] | 20,8 [15-24] |

Table 1: Number of trials included per condition in the main analysis (mean [range]).

**Figure legends**

**Figure 1. A, The response device.** Index finger responses were recorded using a home-made device composed of two pairs of metal edges fixed on a wooden platform and positioned under the left (graphic representation) and right (photographic representation) hands. **B, "Rolling Ball" task.** Subjects were asked to choose between responding with the left or right index finger according to the position of a ball (Preparatory cue) appearing on the left or right part of the screen (left in the current example). They had to wait until the onset of a bridge ("Go" signal) to release their response as quickly as possible. The ball then rolled on the bridge (when the subjects answered correctly) to reach a goal located on the other side of the gap. A feedback reflecting how fast and accurate the subjects were concluded each trial. **C, TMS protocol.** Two small figure-eight-shaped coils were placed over the subject's M1, eliciting MEPs in the left and/or right FDI.

**Figure 2. A, Time-course of the experiment.** After two familiarization blocks, subjects executed ten blocks of forty trials during which MEPs were elicited at $TMS_{baseline\text{-}in}$ or $TMS_{preparation}$. The effect of training was assessed by comparing five sets of data ($Training_1$ to $Training_5$), each involving MEPs pooled over two consecutive blocks. MEPs were also elicited outside the blocks ($TMS_{baseline\text{-}out}$) at five points in time, before block 1 and after blocks 2, 4, 6 and 8, categorized as $Training_1$ to $Training_5$, similar to the MEPs elicited during the blocks. Comparing these data sets allowed us to consider potential training-related changes in resting CSE outside the context of the task. **B, Time course of a trial.** Trials were separated by a blank screen (intertrial interval; 2050 to 2300 ms) and always started with a preparatory cue appearing for a variable delay period (1000 to 1200 ms). Variable delays were sampled from uniform distributions to induce temporal uncertainty and therefore reduce anticipation of the pulses that could emerge with the repetition of trials. Then, a "Go" signal was presented and remained on the screen until a response was detected, hence for the duration of the reaction time (RT). The feedback was presented at the end of each trial for 500 ms and depended on the RT on correct trials. TMS pulses occurred either during the intertrial interval (300 ms before the beginning of the trial; $TMS_{baseline\text{-}in}$), or during the delay period (900 or 950 ms after the preparatory cue onset, timings pooled; $TMS_{preparation}$). In double-coil trials, motor-evoked potentials (MEPs) were elicited in the first dorsal interosseous (FDI) of both hands at a near simultaneous time (1 ms delay); in single-coil trials, MEPs were elicited

in the left or right hand. The figure displays a left hand trial with double-coil TMS at $TMS_{preparation}$.

**Figure 3. Evolution of reaction times (RTs) and total error rate throughout training.** The mean RTs (**A**, in ms) and total error rate (**B,** in % of all trials) are represented for each $Training_{STAGE}$, regardless of the responding hand. Stars denote a significant difference between a given $Training_{STAGE}$ and $Training_1$ ($p<0.05$). Individual data for $Training_1$ and $Training_5$.

**Figure 4. Evolution of baseline MEPs throughout training.** MEP amplitudes (in mV) elicited at $TMS_{baseline\text{-}out}$ (black) and $TMS_{baseline\text{-}in}$ (pink) at the different $Training_{STAGES}$. Hash signs indicate a $TMS_{TIMING}$ effect. Stars denote a significant difference between a given $Training_{STAGE}$ and $Training_1$ ($p<0.05$). Individual data for $Training_1$ and $Training_5$ are also displayed.

**Figure 5. Evolution of preparatory MEPs throughout training.** Normalized MEP amplitudes recorded at $TMS_{preparation}$ (in percentage of MEPs elicited at $TMS_{baseline\text{-}in}$) muscles at the different $Training_{STAGES}$ **(A)**. Absolute MEP data (in mV) are also represented muscles **(B)**. The star denotes a significant difference between a $Training_5$ and $Training_1$ ($p<0.05$). Note that the change in preparatory suppression was close to significance at $Training_4$ (*i.e.,* $p=0.058$ when compared to $Training_1$). Individual data for $Training_1$ and $Training_5$ are also displayed.

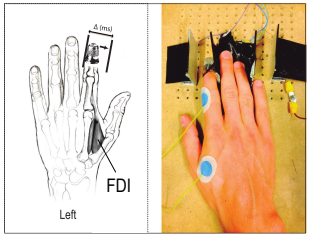
**Figure 6. Preparatory suppression according to time before movement onset.** MEP amplitudes recorded at $TMS_{preparation}$ (in percentage of MEPs elicited at $TMS_{baseline\text{-}in}$) are represented for each $Delay_{BIN}$ in a selected (red) or non-selected (blue) muscle.

**Figure 7. Correlation between early training-related changes in RTs and CSE.** Changes in RTs as a function of changes in MEP amplitudes at $TMS_{baseline\text{-}in}$ (reflecting resting CSE, **A**) and changes in percentage MEPs at $TMS_{preparation}$ (reflecting preparatory suppression of CSE) in the selected **(B)** and non-selected FDI muscle **(C)** during the early $Training_{stage}$. For this analysis, changes in RTs and MEPs were assessed by computing percentage ratios between

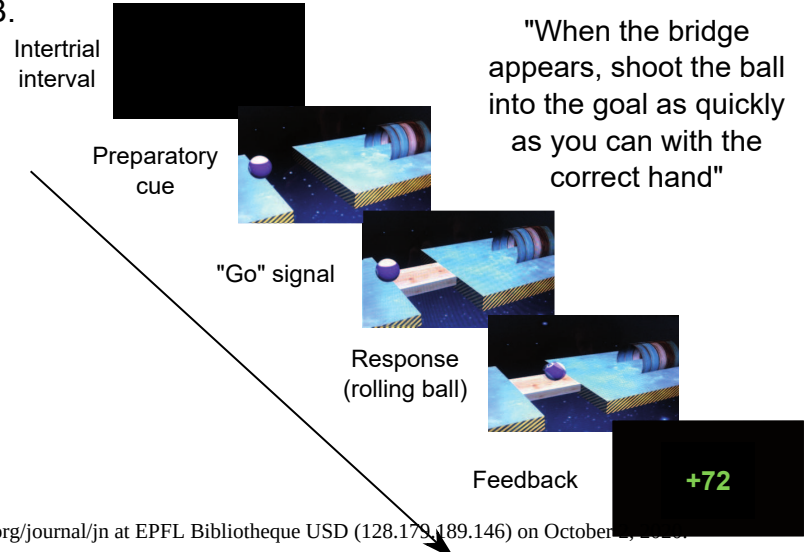
the values obtained at $Training_3$ and $Training_1$. **(D)** Bootstrap estimates of absolute R values are also displayed (± standard deviation of the samples) for each condition. These R values were compared by means of a Pearson and Fillon's z test. One tail p-values were used given our a priori hypothesis concerning the directionality of the effect ($p<0.05$).

**Figure 8. Single-trial MEP-RT relationship.** Averaged RTs as a function of the preceding preparatory suppression in a selected (left panel) or non-selected muscle (right panel). For this analysis, the MEP data were divided in 6 $MEP_{BIN}$ of increasing amplitude and the RTs corresponding to each $MEP_{BIN}$ were averaged. The star denotes a significant difference between RTs at $MEP_{BIN-1}$ and $MEP_{BIN-2}$ and RTs at $MEP_{BIN-6}$ in the non-selected muscle ($p<0.05$). Note that there was also a trend for RTs at $MEP_{BIN-1}$ to be shorter than those in $MEP_{BIN-4}$ ($p=0.070$) and $MEP_{BIN-5}$ ($p=0.066$).

A.




B.




C.