Motor training strengthens corticospinal suppression during movement preparation

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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; a phenomenon called preparatory suppression. Here, we investigated the effect of motor training on preparatory suppression of CSE in humans, as compared to modulatory changes at rest. 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 baseline CSE at rest. Most interestingly, we found that motor activity during action preparation did not mirror the training-related rise in resting CSE. Rather, the degree of preparatory suppression from the rising baseline strengthened with practice. This training-related change in preparatory suppression predicted RT gains (but not the changes in baseline CSE): subjects showing a stronger expansion 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: RTs were generally faster in trials where preparatory suppression was deeper. These findings suggest that training induces changes in motor preparatory processes that are linked to an enhanced ability to initiate fast movements.

New and Noteworthy

Any movement is preceded by a period of preparation, which involves a broad suppression 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 an acceleration of movement initiation. Our findings yield an extension of former work, highlighting a key role of preparatory suppression in training-driven behavioral improvements.

57   **Keywords:** motor training; action preparation; transcranial magnetic stimulation; motor-evoked

58   potentials; primary motor cortex; corticospinal excitability; corticospinal suppression, reaction

59   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; Quoilin et al. 2018). Indeed, the amount of preparatory suppression was shown to scale with the complexity of selection processes (Duque et al. 2016; Klein et al. 2014). Another hypothesis is that preparatory suppression eases action 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, these hypotheses could be both valid

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91   as they focus on different levels of control, which are both known to shape motor activity: while

92   the choice hypothesis suggests that suppression originates from processes that help select accurate

93   actions (*i.e.,* therefore reducing the error rate), the motor hypothesis entails that preparatory

94   suppression is also generated by processes speeding up action initiation (*i.e.,* therefore reducing

95   RTs).

96   Here, we investigated the impact of motor training on preparatory suppression, while

97   subjects practiced an instructed-delay RT task. The choice aspects were clear-cut, as evident from

98   the low error rates, even before training. Hence, in such task, the selection requirements are so low

99   that there is no room for improvement; subjects can only become more skilled at the motor level,

100   by initiating their action faster. Based on this, we predicted that RTs would decrease over the course

101   of practice but that error rates would remain marginal. In addition, we expected resting CSE to

102   increase with training, in accordance with previous work (Butefisch et al. 2000; Christiansen et al.

103   2018; Duque et al. 2008; Galea and Celnik 2009; Pascual-Leone et al. 1995). Based on the motor

104   hypothesis (*i.e.,* that preparatory suppression fastens RTs), we expected that training would deepen

105   the drop in excitability during action preparation with respect to rest, reflecting an increased

106   preparatory suppression. Hence, we predicted that preparatory activity would not follow the

107   training-related rise in resting CSE.

108   **2. Materials and Methods**

109

110   **2.1. Participants**

111   Fifteen right-handed healthy subjects participated in the present study (n=15; 10 women;

112   22.4±1.63 years old). Handedness was assessed via Edinburgh Handedness inventory (Oldfield

113   1971). Participants filled out a TMS safety questionnaire to look for any contra-indication and gave

114   written informed consent in accordance with the Ethics Committee of the Université Catholique de

115   Louvain and the principles of the Declaration of Helsinki. We had to exclude one subject because

116   we encountered a technical problem during the experiment; hence, analyses were run on the

117   fourteen remaining subjects. Part of the data reported here has been exploited in a separate study

118   (Vassiliadis et al. 2018). All of the data are expressed as mean±SE.

119

120   **2.2. Task**

121   Subjects were sited in front of a computer screen with the hands on response devices

122   **(Fig.1A,** (Quoilin et al., 2016, 2018, 2019; Grandjean et al., 2019). They performed an instructed-

123   delay RT task, which required them to choose between abduction movements of the left or right

124   index finger according to the position of a preparatory cue (*i.e.,* a left- or right-sided ball separated

125   from a goal by a gap). Participants had to prepare their movement once the ball appeared but to

126   withhold responding until the onset of a "Go" signal (*i.e.,* a bridge). When the bridge appeared on

127   the screen, subjects had to respond as fast as possible, allowing the ball to roll on the bridge and to

128   reach the goal. To reduce anticipation of the "Go" signal, the bridge did not appear in some of the

129   trials (5%). Trials always ended with a feedback score reflecting performance (see **Fig.1B**).

130

131   **2.3 TMS Protocol**

132   Monophasic pulses were delivered through one or two figure-of-eight shaped coils, each

133   connected either to a Magstim 200² magnetic stimulator (Magstim, Whitland, Dyfed, UK) or a

134   Magstim Bistim² magnetic stimulator. The TMS machine used to stimulate each hemisphere was

135   counterbalanced between subjects. Pulses could be triggered in one (*i.e.,* single-coil TMS) or in the

136   two coils (*i.e.,* double-coil TMS, **Fig.1C**) because the dataset was initially acquired for a separate

137   study to establish the reliability of double-coil TMS to probe CSE bilaterally (Grandjean et al.

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138   2018; Vassiliadis et al. 2018). Here, MEPs are considered regardless of the protocol used to elicit
139   them.

140   Each TMS coil was placed tangentially over one M1 with the handle pointing backward
141   and laterally at a 45° angle away from the midline **(Fig.1C)**. TMS was applied over the hotspot of
142   the first dorsal interosseous muscle (FDI), which was the prime-mover in our task (Duque et al.,
143   2014; Derosiere et al., 2017a, 2017b). The resting Motor Threshold (rMT) was determined for each
144   M1. It was defined as the minimal intensity required to evoke MEPs of 50µV at rest in at least 5
145   out of 10 stimulations. The rMTs equalled 41.7±5.05% and 40.8±6.39% of the maximum
146   stimulator output for the left and the right FDI, respectively. For each hemisphere, the intensity
147   used throughout the experiment was set at 115% of the individual rMT (Derosiere et al. 2019).

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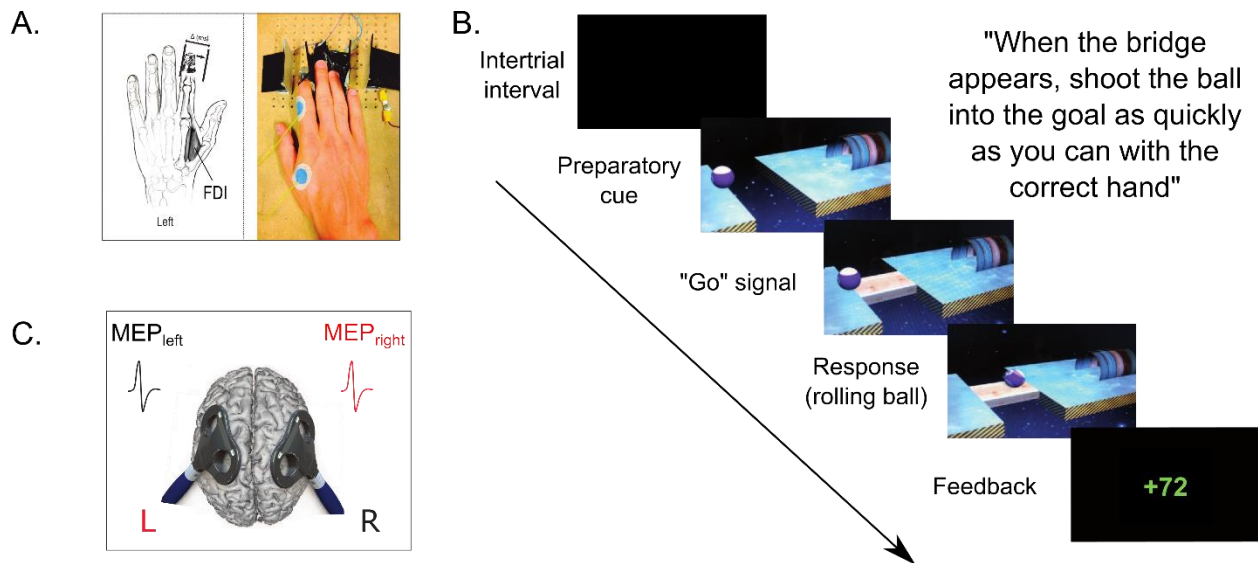
150   **Figure 1. A, The response device.** Index finger responses were recorded using a home-made
151   device composed of two pairs of metal edges fixed on a wooden platform and positioned under the
152   left (graphic representation) and right (photographic representation) hands. **B, "Rolling Ball" task.**
153   Subjects were asked to choose between responding with the left or right index finger according to
154   the position of a ball (Preparatory cue) appearing on the left or right part of the screen (left in the
155   current example). They had to wait until the onset of a bridge (Go signal) to release their response
156   as quickly as possible. The ball then rolled on the bridge (when the subjects answered correctly) to
157   reach a goal located on the other side of the gap. A feedback reflecting how fast and accurate the
158   subjects were concluded each trial. On correct trials, scores ranged from 1 to 100 points and were
159   displayed in green. Participants were informed that the score was inversely proportional to the RT:

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160   the faster the response, the higher the score. In order to homogenize the score across subjects,

161   scores on correct trials were individualized according to RTs measured during a familiarization

162   session just before the main experiment (Vassiliadis et al., 2018; Grandjean et al., 2019). Incorrect

163   responses were penalized with negative scores displayed in red. They involved responses occurring

164   too early (RT<100 ms), referred to as "anticipation errors" (-75 points), responses occurring too

165   late (RT>500 ms), referred to as "time-out errors" (-50 points), responses provided with the

166   incorrect hand (-20 points), referred to as "choice errors" and responses provided on catch trials (-

167   12 points), referred as "catch errors". When subjects succeeded not to respond on a catch trial, they

168   were rewarded by +12 points. The total score was displayed at the end of each block. **C, TMS**

169   **protocol.** Two figure-eight-shaped coils were placed over the subject's M1, eliciting MEPs in the

170   left and/or right FDI. In double-coil trials, a 1-ms interval separated the onset of the two pulses,

171   eliciting MEPs in both hands at a near simultaneous time (Algoet et al. 2018; Grandjean et al. 2018;

172   Quoilin et al. 2019; Vassiliadis et al. 2018). This interval was used to avoid direct electromagnetic

173   interference between the two coils (Cincotta et al. 2005), while preventing transcallosal interactions

174   that would occur between motor areas with longer delays (Ferbert et al. 1992; Hanajima et al.

175   2001). Notably, in double-coil trials, half of the trials involved a pulse over left M1 first whereas

176   the other half involved a pulse over right M1 first (1ms delay). These data were assembled because

177   the a prior analysis reported elsewhere showed that the order of stimulation does not influence the

178   double-coil MEP amplitudes, which were identical to single-coil MEPs (Vassiliadis et al. 2018).

179

2.4 Experimental procedure

181   The experiment started with two familiarization blocks. The first block allowed subjects to

182   become acquainted with the task. The second block involved TMS and served to compute the

183   median RT for each subject. The latter was used to individualize the feedback scores on correct

184   trials according to the initial performance (see **Fig.1B**).

185   Then, subjects performed 400 trials of the task, divided in 10 blocks. Each block involved

186   an equal combination of single- and double-coil stimulations, occurring in a random order (*i.e.,*

187   subjects could not anticipate the type of stimulation they would face). Given that both techniques

188   produce equivalent MEPs (Grandjean et al. 2018; Vassiliadis et al. 2018), these data were

189   considered regardless of the protocol used to elicit them.

190   TMS could occur in three different settings. First, some TMS pulses were delivered outside

191   the blocks (TMS$_{baseline-out}$), providing MEPs reflecting baseline CSE at complete rest. TMS$_{baseline-}$

192    $_{out}$ pulses occurred every other block, starting before block 1 and ending after block 8 (5 time points;

193   30 MEPs per time point; **Fig.2A**). Second, TMS occurred during the intertrial interval, 300 ms

194   before the beginning of the trial (**Fig.2B**). MEPs recorded at this time (12 per block) provided

195   another baseline measure of CSE, with subjects at rest but engaged in the task (TMS$_{baseline-in}$

196   Labruna et al., 2011). Finally, TMS occurred at 900 or 950 ms after the onset of the preparatory

197   cue (TMS$_{preparation}$). Since no difference was found between MEPs recorded at these two timings in

198   our previous analysis (Vassiliadis et al. 2018), these data were pooled together (48 MEPs per

199   block). Half of these MEPs fell in left response trials, while the other half occurred in right response

200   trials. Hence, MEPs could either fall in a hand that was selected for the forthcoming response

201   (MEP$_{selected}$; *e.g.,* left MEPs preceding a left index finger response) or in a hand that was non-

202   selected (MEP$_{non-selected}$).

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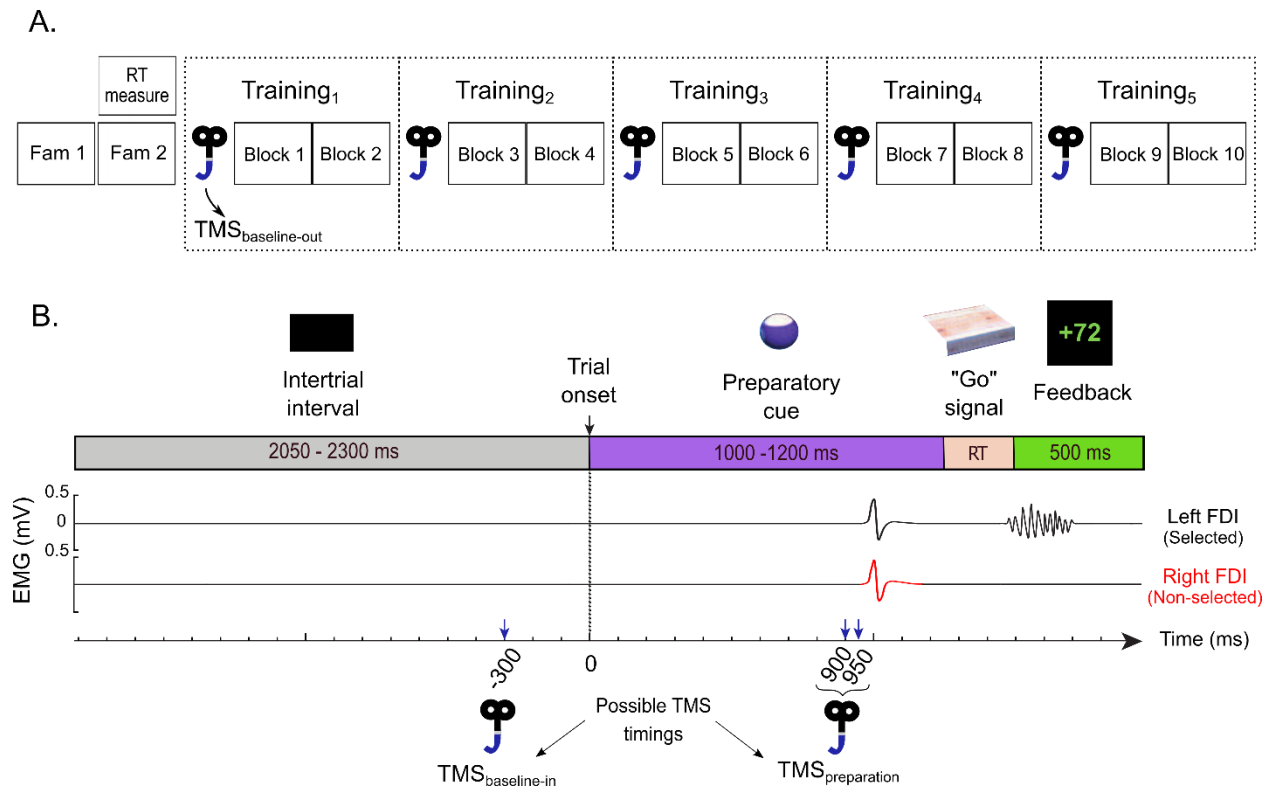
205   **Figure 2. A, Time-course of the experiment.** After two familiarization blocks (Fam1: 20 trials

206   without TMS and Fam 2: 40 trials with TMS), subjects executed ten blocks of forty trials during

207   which MEPs were elicited at $TMS_{baseline-in}$ or $TMS_{preparation}$ (see 2A). The effect of training was

208   assessed by comparing five sets of data ($Training_1$ to $Training_5$), each involving MEPs pooled over

209   two consecutive blocks. MEPs were also elicited outside the blocks ($TMS_{baseline-out}$) at five points

210   in time, before block 1 and after blocks 2, 4, 6 and 8, categorized as $Training_1$ to $Training_5$, similar

211   to the MEPs elicited during the blocks. We obtained 15 $TMS_{baseline-out}$ MEPs per hand for each

212    $Training_{STAGE.}$ Comparing these data sets allowed us to consider potential training-related changes

213   in resting CSE outside the context of the task. **B, Time course of a trial.** Trials were separated by

214   a blank screen (intertrial interval; 2050 to 2300 ms) and always started with a preparatory cue

215   appearing for a variable delay period (1000 to 1200 ms). Then, a Go signal was presented and

216   remained on the screen until a response was detected, hence for the duration of the reaction time

217   (RT). The feedback was presented at the end of each trial for 500 ms and depended on the RT on

218   correct trials. Importantly, the variable delays in the task were sampled from uniform distributions

219   to induce temporal uncertainty and therefore reduce anticipation that could emerge with the

220   repetition of trials. TMS pulses occurred either during the intertrial interval (300 ms before the

221   beginning of the trial; $TMS_{baseline-in}$), or during the delay period (900 or 950 ms after the preparatory

222   cue onset, timings pooled; $TMS_{preparation}$). In double-coil trials, motor-evoked potentials (MEPs)

223   were elicited in the first dorsal interosseous (FDI) of both hands at a near simultaneous time (1 ms

224   delay); in single-coil trials, MEPs were elicited in the left or right hand. The figure displays a left

225   hand trial with double-coil TMS at $TMS_{preparation}$.

226

227   **2.5 Data processing and statistical analyses**

228   The purpose of the study was twofold: (1) to characterize changes in CSE at rest and during

229   action preparation occurring along with training in a basic instructed-delay RT task, (2) to assess

230   whether modulations in CSE were correlated to training-related improvements in RTs. To do so,

231   the behavioral and MEP data were evaluated according to the block within which they were elicited

232   and data from two consecutive blocks were pooled together. Given the 10 blocks, we obtained five

233   data sets reflecting five training stages ($Training_{STAGE}$: $Training_1$ to $Training_5$; **Fig.2A**).

234   Statistical analyses were carried out with Matlab 2018a (the Mathworks, Natick,

235   Massachusetts, USA) and Statistica 10 (StatSoft Inc., Tulsa, Oklahoma, USA). All data were

236   systematically tested for the sphericity assumption using Maunchley's tests. The Greenhouse–

237 Geisser (GG) correction was used for sphericity when necessary. Post-hocs comparisons were

238 always conducted using the Fisher's LSD procedure. The significance level was set at $p \leq 0.05$.

239

2.5.1 RTs and errors

241 Left and right hand RTs were computed as the difference between the onset of the Go signal

242 and movement onset (when the finger quitted the outer metal edge of the device). Trials where

243 subjects made an error were removed from the data set for the RT analysis. An average of 35 left

244 and 34 right response trials remained for each subject at each Training$_{STAGE}$. We computed the

245 mean RT for left and right responses separately and then averaged these data together. Besides, we

246 also assessed response accuracy over training, by computing, for each Training$_{STAGE}$, the amount

247 of anticipation, time-out and catch errors as well as the total error rate. For each of these variables,

248 we expressed the number of incorrect trials in percentage of the total amount of trials, regardless

249 of the responding hand. Choice errors were not analysed because they were rare (4 choice errors

250 across all subjects). For the statistical analysis of RTs and errors (*i.e.,* anticipation, time-out, catch

251 and global errors), we used one-way analyses of variance for repeated measures (ANOVA$_{RM}$) with

252 the factor Training$_{STAGE}$ (Training$_1$ to Training$_5$).

253

2.5.2 MEP amplitudes

255 MEPs were obtained by recording electromyography (EMG) bilaterally from surface

256 electrodes (Neuroline, Medicotest, Oelstykke, Denmark) placed over the FDI. The signals were

257 amplified (x1000), bandpass filtered (10-500Hz; NeuroLog; Digitimer), digitalized at 2000 Hz

258 and collected with Signal (Signal 3.0, Cambridge, UK) for offline analysis. Trials with background

259 EMG activity preceding the pulse exceeding 3 SDs above the mean were discarded ($1.68\pm0.30\%$

260 removal; Vassiliadis et al., 2018; Grandjean et al., 2018, 2019). This was done to prevent

261 contamination of the MEP measurements by significant fluctuations in background EMG.

262 To assess training-related changes in resting CSE based on MEPs elicited at TMS$_{baseline-out}$

263 and TMS$_{baseline-in}$, we averaged separately left and right hand MEPs for each Training$_{STAGE}$ before

264 computing the mean of these averages. These data were analysed using a two-way ANOVA$_{RM}$ with

265 TMS$_{TIMING}$ (TMS$_{baseline-out}$ or TMS$_{baseline-in}$), and Training$_{STAGE}$ (Training$_1$ to Training$_5$) as within-

266 subject factors. To assess training-related changes in preparatory suppression based on MEPs at

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267 $TMS_{preparation}$ (expressed in percentage of MEPs at $TMS_{baseline-in}$), we first removed the trials in

268 which subjects made a mistake (10.78±1.81% removal) and then grouped left and right hand MEPs

269 according to whether they corresponded to a $MEP_{selected}$ or $MEP_{non-selected}$. Within these categories,

270 we averaged the separate means of left and right hand MEPs for each $Training_{STAGE}$. To analyse

271 these data, we first focused on percentage MEPs at $Training_1$, assessing with t-tests (against a

272 constant value of 100%) the significance of preparatory suppression at the beginning of training.

273 Then, we analyzed all training stages using a two-way $ANOVA_{RM}$ with the factors $MEP_{SELECTION}$

274 ($MEP_{selected}$ or $MEP_{non-selected}$) and $Training_{STAGE}$ ($Training_1$, to $Training_5$). This ANOVA was also

275 run on absolute MEP amplitudes (in mV).

276

2.5.3 Relationship between training-related changes in RTs and CSE

278 As described in the Result section, training influenced RTs and CSE. We studied the

279 relationship between changes at these two levels, with CSE considered separately at rest and during

280 action preparation. We computed ratios reflecting training-related changes. Based on the RT data,

281 we realized that the subjects' behavior improved substantially during the first practice stage

282 ($Training_1$ to $Training_3$) but then, RTs remained quite stable (from $Training_3$ to $Training_5$; Result

283 section). For this reason, we considered ratios for these two phases of training separately, providing

284 us with an indication of early ($Training_{ratio-early}$: $Training_3/Training_1$) and late ($Training_{ratio-late}$:

285 $Training_5/Training_3$) training-related changes in RTs and CSE. For the latter, we computed separate

286 ratios for MEPs at $TMS_{baseline-out}$, $TMS_{baseline-in}$ and $TMS_{preparation}$ (expressed in percentage of MEPs

287 at $TMS_{baseline-in}$). We then examined the correlation between the RT and MEP $Training_{ratios}$ by using

288 least squares linear regressions.

289 Finally, we compared the strength of the RT relationship to training-related changes in MEP

290 amplitudes at $TMS_{baseline-in}$ (reflecting resting CSE) and changes in percentage MEPs at

291 $TMS_{preparation}$ (reflecting preparatory suppression of CSE). To do so, in order to obtain a robust

292 estimate of the absolute Pearson's R, we ran a bootstrap analysis with 10000 resamples and

293 calculated a median R for each correlation (Efron 1979). These R-values were then compared to

294 each other using Pearson and Fillon's z test (Pearson and Filon 1898).

295

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 $TMS_{baseline-in}$. 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 ($MEP_{non-selected}$) 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 $MEP_{non-selected}$. Within each arrangement, trials were grouped into 6 consecutive percentile bins (MEP_{BIN}: MEP_{BIN-1} = 0 to 16.7%, MEP_{BIN-2} = 16.7 to 33.3% … MEP_{BIN-6} = 83.3 to 100% of the data). MEP_{BIN-1} contained the trials with the stronger preparatory suppression whereas the MEP_{BIN-6} included the trials with the weaker preparatory suppression. We then computed the mean RT of trials within each MEP_{BIN} (23 trials per condition on average and never less than 19 trials), and then averaged the corresponding RTs. 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-1} to MEP_{BIN-6}).

13

319 ## 3. Results

320

321 ### 3.1. RTs and errors

322 **Fig.3A** shows the evolution of RTs with training. The ANOVA$_{RM}$ revealed a significant

323 influence of Training$_{STAGE}$ on RT ($F_{(4,52)}$=4.31, p=0.0043). Post-hoc tests showed that RTs

324 measured from Training$_3$ to Training$_5$ were shorter than at Training$_1$ (all p<0.004). In contrast, the

325 total error rate remained stable over the blocks ($F_{(4,52)}$=0.82, p=0.52, **Fig.3B**). We did not observe

326 any modification of the percentage of anticipation ($F_{(4,52)}$=1.12, p=0.36), time-out (GG-corrected

327 $F_{(2.50,32.50)}$=0.90, p=0.44) or catch errors ($F_{(4,52)}$=1.73, p=0.16). Hence, training enabled subjects to

328 respond more quickly while maintaining the same accuracy level.

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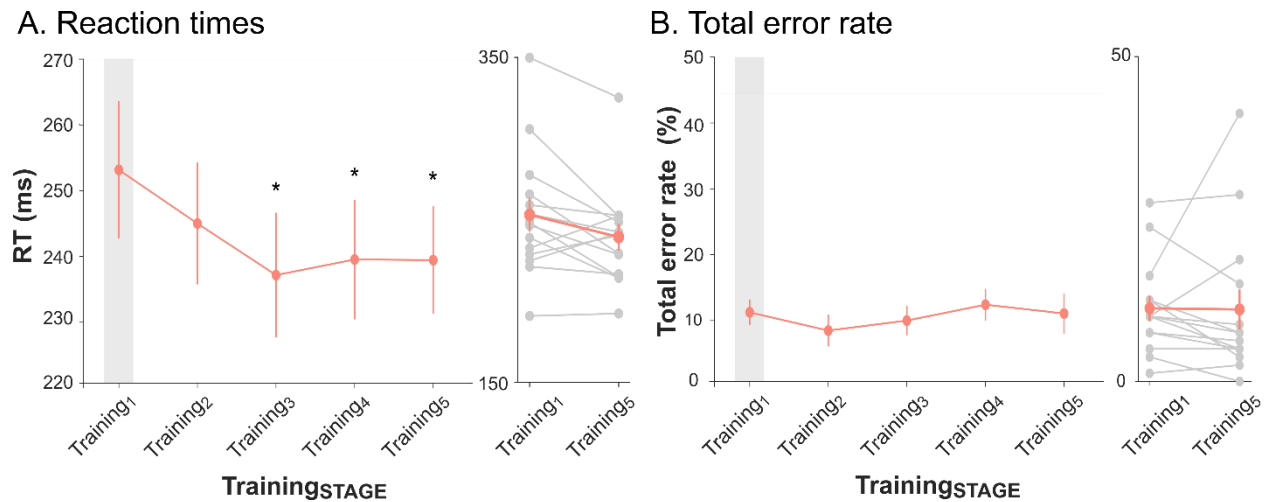
330

331 **Figure 3. Evolution of reaction times (RTs) and total error rate throughout training.** The

332 mean RTs (**A**, in ms) and total error rate (**B,** in % of all trials) are represented for each

333 Training$_{STAGE}$, regardless of the responding hand. Stars denote a significant difference between a

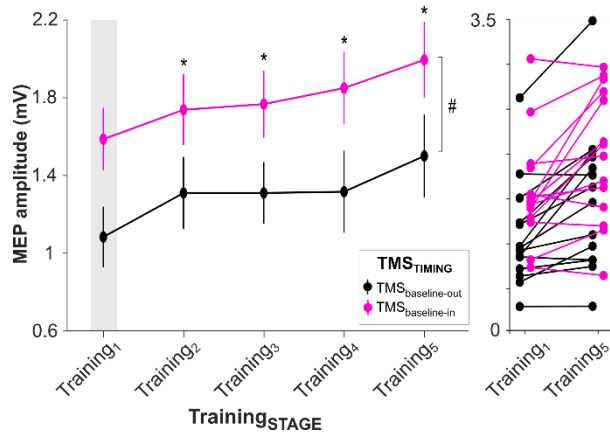334 given Training$_{STAGE}$ and Training$_1$ (p<0.05). Individual data for Training$_1$ and Training$_5$.

335

336 ### 3.2. MEP amplitude

337 First, we evaluated the effect of training on MEPs acquired at rest. As evident on **Fig.4,**

338 MEPs were larger when assessed in the context of the task (TMS$_{baseline-in}$: 1.79±0.17mV) compared

339 to when subjects were fully at rest (TMS$_{baseline-out}$: 1.34±0.17mV), as supported by the significant

340 factor TMS$_{TIMING}$ ($F_{(1,13)}$=28.43, p<0.001) and consistent with previous studies (Derosière et al.

14

341   2015; Labruna et al. 2011). The ANOVA$_{RM}$ also revealed an effect of Training$_{STAGE}$ on baseline

342   MEPs ($F_{(4,52)}$=6.34, p<0.001). MEPs recorded at Training$_2$ to Training$_5$ were larger than at

343   Training$_1$ (all p<0.03). This training effect on MEPs occurred independently of the TMS$_{TIMING}$:

344   there was a parallel increase in the amplitude of MEPs elicited at TMS$_{baseline-out}$ and TMS$_{baseline-in}$

345   (Training$_{STAGE}$xTMS$_{TIMING}$: $F_{(4,52)}$=0.18, p=0.95).

346

347

348   **Figure 4. Evolution of baseline MEPs throughout training.** MEP amplitudes (in mV) elicited at

349   TMS$_{baseline-out}$ (black) and TMS$_{baseline-in}$ (pink) at the different Training$_{STAGES}$. Hash signs indicate a

350   TMS$_{TIMING}$ effect. Stars denote a significant difference between a given Training$_{STAGE}$ and

351   Training$_1$ (p<0.05). Individual data for Training$_1$ and Training$_5$ are also displayed.

352

353   Second, we analyzed the effect of training on preparatory suppression by considering MEPs

354   elicited at TMS$_{preparation}$ (expressed in percentage of TMS$_{baseline-in}$). As evident on **Fig.5A**,

355   percentage FDI MEPs were initially suppressed at Training$_1$ (MEPs smaller than 100%), consistent

356   with the presence of preparatory suppression in the prime-mover, whether selected for the

357   forthcoming response (MEP$_{selected}$: 73.98±4.00%; $t_{(13)}$=-6.50, p<0.0001) or not (MEP$_{non-selected}$:

358   76.26±4.36%; $t_{(13)}$=-5.44, p<0.001). Interestingly, preparatory suppression became more

359   prominent with training: the ANOVA$_{RM}$ revealed a significant decrease in percentage MEP

360   amplitudes over the Training$_{STAGES}$ ($F_{(4,52)}$=2.79, p=0.036). This change was marginal at Training$_4$

361   (*i.e.,* Training$_4$: p=0.058 when compared to Training$_1$) and became significant at Training$_5$

362   (p=0.006). It concerned MEPs obtained from the selected and non-selected hands

363   (Training$_{STAGE}$xMEP$_{SELECTION}$: $F_{(4,52)}$=0.56, p=0.70). To further our understanding of training-

15

364   related changes of preparatory activity, we ran another set of $ANOVA_{RM}$ on absolute MEP

365   amplitudes (rather than percentages) at $TMS_{preparation}$ (**Fig.5B**). These MEPs did not show any

366   fluctuation over the $Training_{STAGES}$ ($F_{(4,52)}=1.30$, $p=0.28$). Moreover, we did not find any

367    $MEP_{SELECTION}$ effect ($F_{(1,13)}=1.69$, $p=0.22$) or $Training_{STAGE}xMEP_{SELECTION}$ interaction

368   ($F_{(4,52)}=0.85$, $p=0.50$).

369   In conclusion, our results indicate that training did not produce even modulatory changes

370   in motor activity at rest and during action preparation: while resting CSE increased, preparatory

371   activity remained flat over the blocks, thus revealing an augmenting drop (*i.e.,* preparatory

372   suppression) with respect to the rising baseline excitability state. These changes in CSE occurred

373   in parallel with an acceleration of RTs.

374

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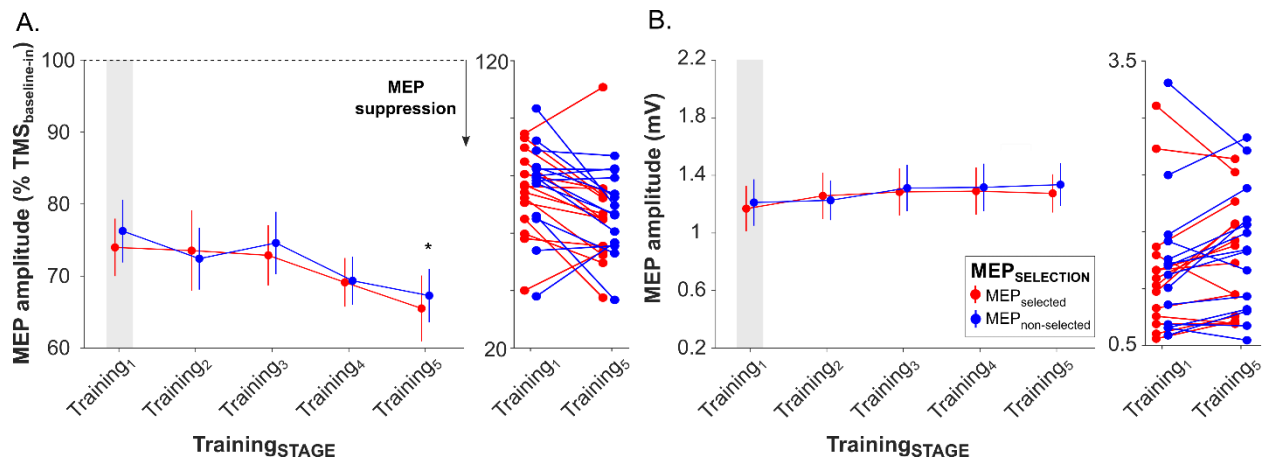
376   **Figure 5. Evolution of preparatory MEPs throughout training.** Normalized MEP amplitudes

377   recorded at $TMS_{preparation}$ (in percentage of MEPs elicited at $TMS_{baseline-in}$) muscles at the different

378    $Training_{STAGES}$ (**A**). Absolute MEP data (in mV) are also represented muscles (**B**). The star denotes

379   a significant difference between a $Training_5$ and $Training_1$ ($p<0.05$). Note that the change in

380   preparatory suppression was close to significance at $Training_4$ (*i.e.,* $p=0.058$ when compared to

381    $Training_1$). Individual data for $Training_1$ and $Training_5$ are also displayed.

382

383   Because RTs became shorter over the blocks, one may argue that MEPs at $TMS_{preparation}$

384   were not recorded in a comparable preparatory state throughout training; that is, the delay between

385   TMS and movement onset ($Delay_{TMS-TO-MOVE}$) may have decreased over the blocks. Importantly,

386   we shuffled the delay between the pulse and the Go signal in the present study (see Methods), in

16

387   order to prevent changes in RT to convert into equivalent changes in the $\text{Delay}_{\text{TMS-TO-MOVE}}$.

388   However, because TMS fell on average closer to movement onset at Training_5 (399.70 ± 8.48ms)

389   than Training_1 (419.99 ± 9.99ms, $t_{(13)}=-3.10$, $p=0.008$), we performed an additional analysis to

390   control for a potential bias of the $\text{Delay}_{\text{TMS-TO-MOVE}}$. We conducted a response-locked analysis

391   whereby we classified MEP data at $\text{TMS}_{\text{preparation}}$ (regardless of the $\text{Training}_{\text{STAGE}}$) according to the

392     $\text{Delay}_{\text{TMS-TO-MOVE}}$ in 5 consecutive bins of trials ($\text{Delay}_{\text{BIN}} = \text{Delay}_{\text{BIN-1}} = 0$ to 20%, $\text{Delay}_{\text{BIN-2}} = 20$

393   to 40%, ..., $\text{Delay}_{\text{BIN-5}} = 80$ to 100% of the $\text{Delay}_{\text{TMS-TO-MOVE}}$ data). An ANOVA_{RM} ran on these

394   data did not reveal any effect of $\text{Delay}_{\text{BIN}}$ ($F_{(4,52)}=1.45$; $p=0.23$), nor was there any significant

395     $\text{MEP}_{\text{SELECTION}}\text{x}\text{Delay}_{\text{BIN}}$ interaction ($F_{(4,52)}=0.40$; $p=0.81$; **Fig.6**). These results indicate that MEPs

396   elicited preceding a Go signal remain quite unaffected by the delay separating the $\text{TMS}_{\text{preparation}}$

397   pulse and movement onset.
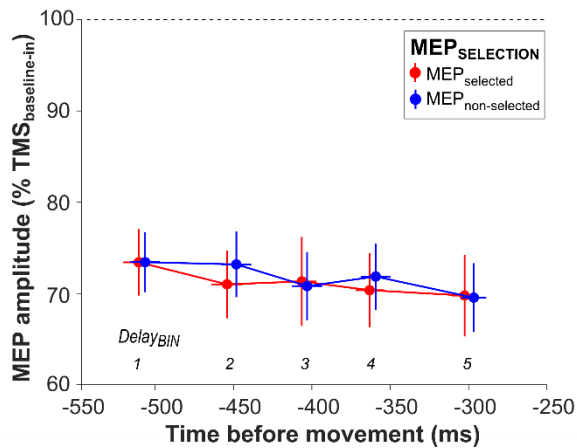
398

399   **Figure 6. Preparatory suppression according to time before movement onset.** MEP amplitudes

400   recorded at $\text{TMS}_{\text{preparation}}$ (in percentage of MEPs elicited at $\text{TMS}_{\text{baseline-in}}$) are represented for each

401     $\text{Delay}_{\text{BIN}}$ in a selected (red) or non-selected (blue) muscle.

402

3.3 Relationship between training-related changes in RTs and CSE

404   Given that training influenced RTs and CSE, we studied the relationship between changes

405   at these two levels, with CSE considered separately at rest and during action preparation. To assess

406   the relationship between RTs and resting CSE, we ran correlations between training-related

407   changes in RTs and changes in MEPs at $\text{TMS}_{\text{baseline-in}}$ and $\text{TMS}_{\text{baseline-out}}$. These analyses did not

408   reveal any link between variations in resting measures of CSE and changes observed in RTs, neither

409   at $\text{Training}_{\text{ratio-early}}$ **(Fig.7A,** R=-0.27, p=0.36 and R=0.079, p=0.79 for $\text{TMS}_{\text{baseline-in}}$ and $\text{TMS}_{\text{baseline-}}$

410    $_{\text{out}}$, respectively) nor at $\text{Training}_{\text{ratio-late}}$ (R=-0.28, p=0.33 and R=-0.16, p=0.59).

411   In contrast, changes in RTs at $\text{Training}_{\text{ratio-early}}$ were linked to variations in preparatory

412   suppression observed in the selected **(Fig.7B;** R=0.55, p=0.043**)** and non-selected FDI **(Fig.7C;**

413   R=0.74, p=0.0027): subjects showing a greater training-related strengthening of preparatory

414   suppression also showed larger improvements in RTs. This correlation was not significant at

415    $\text{Training}_{\text{ratio-late}}$, neither for the selected (R=0.12, p=0.67) nor for the non-selected effectors

416   (R=0.48, p=0.084). Our results suggest that RT improvements were related to early changes in

417   preparatory suppression.

418   This conclusion is further supported by an additional analysis showing that the strength of

419   the correlation between RTs and CSE at $\text{Training}_{\text{ratio-early}}$ was significantly higher when considering

420   percentage MEPs at $\text{TMS}_{\text{preparation}}$ (*i.e.,* preparatory suppression) in the non-selected FDI (bootstrap

421   estimate of absolute R=0.76), than when MEPs were considered at $\text{TMS}_{\text{baseline-in}}$ (R=0.29; z=1.75;

422   p=0.040, **Fig.7D**). This difference was not significant when taking preparatory suppression in the

423   selected FDI (R=0.56; z-score=0.85, p=0.20). Hence, training-related changes in preparatory

424   suppression of the non-selected effector turned out to be the best predictor of RT improvements.

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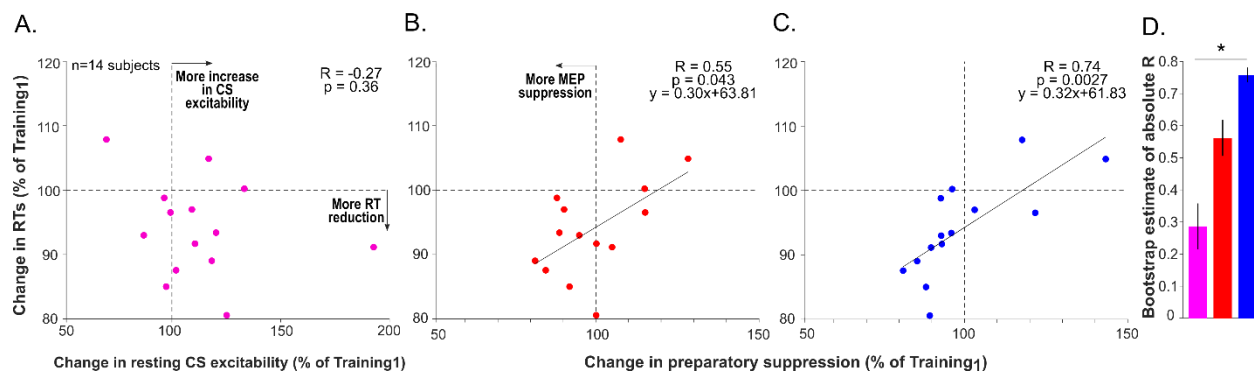
427   **Figure 7. Correlation between early training-related changes in RTs and CSE.** Changes in

428   RTs as a function of changes in MEP amplitudes at $\text{TMS}_{\text{baseline-in}}$ (reflecting resting CSE, **A**) and

429   changes in percentage MEPs at $\text{TMS}_{\text{preparation}}$ (reflecting preparatory suppression of CSE) in the

430   selected **(B)** and non-selected FDI muscle **(C)** during the early $\text{Training}_{\text{stage}}$. For this analysis,

431   changes in RTs and MEPs were assessed by computing percentage ratios between the values

432   obtained at Training_3 and Training_1. **(D)** Bootstrap estimates of absolute R values are also displayed

433   (± standard deviation of the samples) for each condition. These R values were compared by means

434   of a Pearson and Fillon's z test. One tail p-values were used given our a priori hypothesis

435   concerning the directionality of the effect (p<0.05).

436

3.4 Single-trial relationship between RTs and preparatory suppression

438   Finally, we asked whether the dependency of RTs to preparatory suppression is also evident

439   on a single-trial basis. This was the case for MEPs recorded from the non-selected hand: the greater

440   the preparatory suppression in that hand, the shorter the following RT (**Fig.8**, right panel), as

441   supported by the ANOVA$_{RM}$ revealing an effect of the factor MEP$_{BIN}$ on RTs ($F_{(5,65)}=2.57$,

442   p=0.035). Post-hoc tests revealed that RTs in MEP$_{BIN-1}$ and MEP$_{BIN-2}$ (*i.e.,* strongest preparatory

443   suppression) were systematically shorter than those in MEP$_{BIN-6}$ (p=0.0021 and p=0.0090). We did

444   not observe any relationship between RTs and MEPs obtained in the selected hand (MEP$_{BIN}$: GG-

445   corrected $F_{(2.22,28.88)}=0.85$, p=0.45; **Fig.8**, left panel**).** Hence, the training-related effects and the

446   single-trial relationship indicates that preparatory suppression in the non-selected (non-responding)

447   hand is a predictor of the following RT. The lower this activity, the faster the response.

448
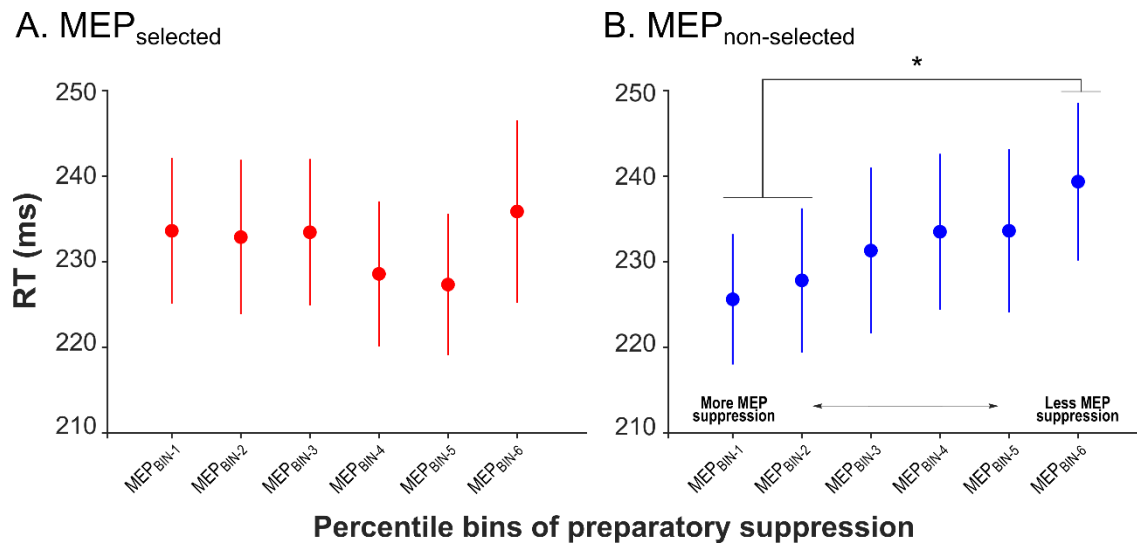
Figure 8. Single-trial MEP-RT relationship. Averaged RTs as a function of the preceding preparatory suppression in a selected (left panel) or non-selected muscle (right panel). For this analysis, the MEP data were divided in 6 MEP$_{BIN}$ of increasing amplitude and the RTs corresponding to each MEP$_{BIN}$ 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

19

455   there was also a trend for RTs at MEP_{BIN-1} to be shorter than those in MEP_{BIN-4} (p=0.070) and

456   MEP_{BIN-5} (p=0.066).

457 **4. Discussion**

458

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

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

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

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

21

487   in the selected and non-selected hands from the beginning of the training. Contrary to rest, the

488   amplitude of MEPs at TMS$_{preparation}$ did not increase with practice (they remained unchanged),

489   reflecting a strengthening drop in CSE from the rising baseline state. Notably, although at the group

490   level this reinforcement of preparatory suppression appeared late (**Fig.5A**), at the individual level,

491   a majority of subjects already exhibited a strengthening of preparatory suppression at early training

492   stages (**Fig.6**).

493   Based on these findings, one could propose that changes in resting excitability are key to

494   RT improvements, as suggested by the inverse relationship between baseline CSE and RTs

495   described recently (Greenhouse et al. 2017). Yet, we did not find a relationship between training-

496   related changes in baseline excitability and improvements in performance. This is in line with the

497   idea that increased resting CSE is not crucial for immediate performance (Bologna et al. 2015), but

498   may be involved in the long-term retention of the motor behavior (Cantarero et al. 2013). Rather,

499   what was predictive of RT gains in the present study was the change in relative CSE, as measured

500   during action preparation: subjects showing the strongest reinforcement of preparatory suppression

501   at the early Training$_{STAGES}$ were those who became fastest. These results are consistent with animal

502   studies showing that behavioral improvements in motor learning tasks are associated with changes

503   in relative preparatory activity (Mandelblat-Cerf et al. 2009; Paz et al. 2003; Vyas et al. 2018).

504   Similarly, a recent study using paired-pulse TMS showed that changes in preparatory activity of

505   M1 intra-cortical circuits are correlated to training-related behavioral gains, contrary to changes

506   observed at rest (Dupont-Hadwen et al. 2018). More generally, our findings agree with the idea

507   that efficient action preparation relies on dynamical shifts of neural activity from a baseline state

508   to a preparatory state (Churchland et al. 2012). From this point of view, training may allow tuning

509   the dynamics of preparatory activity, bringing it closer to an optimal state for action initiation (Vyas

510   et al. 2018). In this line, strengthening of preparatory suppression would facilitate action initiation

511   by allowing excitatory inputs targeting the selected motor representation to better stand out against

512   a quiescent background (mostly reflected in the excitability of non-selected effector), ultimately

513   speeding up RTs (Greenhouse et al. 2015; Hasbroucq et al. 1997; Hasegawa et al. 2017).

514   This interpretation was reinforced by our single-trial analysis showing that RTs depended

515   on the foregoing amount of preparatory suppression. That is, stronger levels of suppression were

516   related to faster initiation times in the very same trials, in agreement with previous results (Hannah

517   et al. 2018; Hasegawa et al. 2017). Interestingly, we found such relationship when considering the

22

518   non-selected prime-mover but not the selected one. This was also the case for training-related

519   effects, with preparatory suppression in the non-selected effector appearing as the best predictor of

520   RT changes, possibly because the selected effector is targeted by too many overlapping inputs to

521   supply as meaningful MEP amplitudes (Duque and Ivry 2009). Overall, our data support the view

522   that preparatory suppression facilitates rapid motor initiation.

523   **Conclusion**

524   This study shows that a simple training paradigm can lead to improvements in action

525   initiation that are accompanied by an increase in resting CSE and a strengthening of corticospinal

526   suppression from the rising baseline state. Moreover, contrary to changes in resting CSE, such

527   strengthening of preparatory suppression was linked to RTs improvements. These findings could

528   have implications for the rehabilitation of patients suffering from impaired action initiation such as

529   in cerebellar ataxia (Battaglia et al. 2006) or Parkinson's disease (Mure et al. 2012).

530 **Additional information**

531 Data availability:

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

533 2020).

534

535 Conflict of interest:

536 The authors declare no conflict of interest.

537

538 Author contributions:

539 PV, GD, JG, JD: conception and design of the work; PV, JG: acquisition of data; PV: analysis of

540 data; PV, GD, JD interpretation of data; PV: drafting; PV, GD, JG, JD revising the manuscript.

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