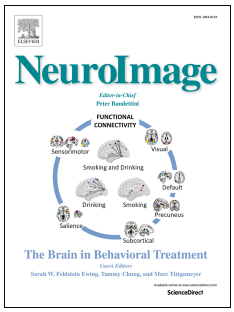

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Authors Contribution

P.C., E.D., P.H., L.F. and A.D. had the idea and design the experiments; P.C., E.D. and A.D. prepared the experimental setup and collected the data. P.C., E.D. and P.H. analyzed the data. All authors participated in interpretation of data and helped draft the manuscript. All authors gave final approval for publication.

Motor cortical inhibition during concurrent action execution and action observation

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Abstract

Action Execution (AE) and Action Observation (AO) share an extended cortical network of activated areas. During coordinative action these processes also overlap in time, potentially giving rise to behavioral interference effects. The neurophysiological mechanisms subtending the interaction between concurrent AE and AO are substantially unknown. To assess the effect of AO on observer's corticomotor drive, we run one electromyography (EMG) and three Transcranial Magnetic Stimulation (TMS) studies. Participants were requested to maintain a steady hand opening or closing posture while observing the same or a different action (hand opening and closing in the main TMS study). By measuring Cortical Silent Periods (CSP), an index of $GABA_B$-mediated corticospinal inhibitory strength, we show a selective reduction of inhibitory motor drive for mismatching AE-AO pairs. The last two TMS experiments, show that this mismatch is computed according to a muscle-level agonist-antagonist representation. Combined, our results suggest that corticospinal inhibition may be the central neurophysiological mechanism by which one's own motor execution is adapted to the contextual visual cues provided by other's actions.

Keywords: Motor cortical inhibition, action execution, action observation, cortical silent period

1. Introduction

Observing others' actions activates an extended parieto-premotor brain network, often referred as the Action Observation Network (AON), which is partially overlapping with the cortical network recruited for action preparation and execution (Giese and Rizzolatti, 2015; Hardwick et al., 2018). Sensorimotor activity during AO may support action-related perceptual processes (Avenanti et al., 2013). According to the predictive coding hypothesis, other's action sensory outcomes are compared to sensory predictions generated by the same hierarchical neural machinery for movement preparation and execution (Donnarumma et al., 2017; Friston, 2011; Friston et al., 2011). Perceptual discrimination and prediction of other's actions, may have a key role in supporting temporal and spatial interpersonal coordination (Pezzulo et al., 2018). We may indeed observe other's actions, to produce complementary responses in a turn-taking fashion (e.g., playing tennis) or to simultaneously coordinate our own movements with those of others (e.g., when moving a heavy object together). However, the cortical response to new stimuli is influenced by ongoing activity in the same neural substrate (Silvanto et al., 2008). We can thus expect that temporal and spatial overlap of the neural processes subtending AE and AO produces functionally relevant interaction.

Nevertheless, little is known about the neurophysiological mechanisms subtending the interaction of concurrent AO and AE. Corticospinal excitability (CSE) modulation has provided direct neurophysiological evidence that passive AO activates the corresponding motor representations in the observer's sensorimotor system (Fadiga et al., 1995). These sensorimotor modulations are characterized by a fine temporal and muscle specificity (Fadiga et al., 2005; Naish et al., 2014; D'Ausilio et al., 2015) and are influenced by proprioceptive feedback (Varlet et al., 2017). However, we yet don't know whether and how a voluntary descending motor drive interacts with the concurrent observation of others' action.

Here we designed four experiments, to elucidate the neurophysiological mechanisms subtending the integration of AO and AE (a schematic illustration of the four experiments in Figure 1). **In the main transcranial magnetic stimulation (TMS) study, participants were asked to keep the same isometric opened or closed hand posture, while observing an intransitive hand opening or closing action. The dependent measure was the length of the Cortical Silent Period (CSP) elicited from the Flexor Digitorum Superficialis (FDS) muscle. Beside the main TMS experiment, an electromyographic (EMG) study, first checked whether the FDS muscle is similarly recruited in both hand opening and closing posture, the former in a postural while the latter in an instrumental role. The other two TMS studies strengthen and specify the results of the main TMS study. The first one tested whether the AE-AO integration is computed at the level of action goals or muscle recruitment by presenting also a wrist flexion action for which the FDS is instrumental but to achieve a different goal. In the second control study we verify if AE-AO integration effects are generalized also to other muscles by testing the same experimental protocol on the Extensor Digitorum Communis (EDC).**

CSP is a corticospinal index of inhibition visible only during a tonic muscular contraction and following a TMS pulse. This $GABA_b$-mediated neurophysiological index has been associated with the voluntary motor drive (Tergau et al., 1999) and, in AE, is regarded as a marker of response selection (Davranche et al., 2007; Tandonnet et al., 2012). During the natural deployment of coordinative behaviors, it is necessary to continuously select and adapt our own motor output to other's action. Consequently, we predict that CSP would be modulated by the mismatch between AO and AE only when FDS plays an instrumental role in the action performed (hand closing posture). All in all, these studies are aimed at verifying whether corticospinal inhibition is sensible to AE-AO mismatch and according to a muscle-level agonist-antagonist mapping of shared action goals.

FIGURE 1

2. Material and methods

2.1. Subjects

A total of 64 healthy naive volunteers took part in the study (31 males; mean age 24.3, SD 2.1). 10 subjects (mean age 29.3, SD: 5.1) participated in the Electromyography (EMG) study and the remaining 54 (mean age 25, SD: 1.7 participated in the Transcranial Magnetic Stimulation (TMS) studies. 21 (mean age 22.8, SD: 2.0) subjects took part in the main TMS experiment, 21 (mean age 24.8, SD: 1.7) in the first TMS control and 12 (mean age 23.5, SD: 2.6) in second TMS control experiment. None of the subjects participated in more than one experiment. All subjects were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). Participants were informed about the experimental procedure and gave their written consent according to the 1964 Helsinki Declaration, as revised in 1983. None of the participants reported neurological, psychiatric or other contraindications to TMS (Rossi et al., 2009). The experiment was approved by the ethical committee "Comitato Etico Unico della Provincia di Ferrara" (approval N. 170592), and participants were compensated for their participation with 12,50 €.

2.2. EMG study

2.2.1. Procedures

Subjects were seated in a comfortable armchair with their right hand in a pronated posture and resting on a pillow. First, subjects learned to perform the two actions (i.e. a whole-hand movement in the direction of closing or opening the hand) and keep the final posture for at least 3s. Once the participant successfully managed to do the task, the recording session started. Each trial began with the presentation of a fixation cross (size: 4° of visual angle) at the center of the screen. After 3 s, the fixation cross was replaced by a color-filled circle (diameter: 8° of visual angle) at the center of the screen. The color (green/red, counterbalanced across subjects) indicated the type of task to perform (hand opening or closing) and prompted the start of the action. Participants were asked to keep a steady posture for 5 s, until the appearance of the fixation cross which duration was 3 s to avoid muscle fatigue (Figure 2B). Participants completed 20 trials for each of the two actions. The duration of the experiment was about 15 min. The task was implemented in E-Prime Software (E-Prime 2.0, Psychology Software Tools, Inc.).

EMG signal was recorded through a wireless EMG system (Zerowire EMG, Aurion, Italy) with a tendon-belly montage (Figure 2A). Electrode locations for both muscles were based on previous literature (Bickerton et al., 1997). EMG traces were digitized (2 kHz) and acquired by a CED Micro 1401 board and data were stored for offline analysis using the Signal 3.09 software (Cambridge Electronic Design, Cambridge, UK).

2.2.2. Analysis

The EMG analysis aimed at determining the level of FDS and EDC recruitment in each action (opening vs closing). For each trial, the muscle activation onset was defined as the time point exceeding an individually set threshold. The threshold was defined as the root mean squared (RMS) muscular activity +3 SD, recorded during a 200-ms baseline preceding the instruction to move. A trial was considered as valid if the muscle activity was kept above this threshold for at

least 500 ms. This criterion was met for all subjects and no trial was discarded from statistical analysis.

Muscle contraction was then quantified in time bins of 50 ms by computing the RMS of the rectified signal over a 1 s time-window (from 250 before to 750 ms after muscle activation onset). The Shapiro-Wilk test was applied to test the normality of the variables. Given the non-normal distribution we performed non-parametric statistics. To evaluate statistically whether muscle activation differs between the two actions (hand opening and closing) we run a two-tailed group-level permutation test (Blair and Karniski, 1993; Groppe et al., 2011; Manly, 1997), separately for the two muscles (FDS and EDC) and for each time bin. Permutation tests do not depend on any statistical assumption on the data (Byrne, 1993; Hunter and May, 2003) and have been shown to outperform classical parametric approaches when the normality assumption is violated (Ludbrook and Dudley, 1998; Nichols and Holmes, 2002; Routledge, 1997). Thus, permutation tests are becoming the method of reference in EEG, MEG and fMRI studies (Eklund et al., 2016; Maris and Oostenveld, 2007; Pantazis et al., 2005; Singh et al., 2003) as well as TMS research (Hilt et al., 2017; Palmer et al., 2016).

Permutations consists in randomly assigning, for each subject, the labels corresponding to the two actions (hand opening/closing) to calculate the (group-level) difference between the obtained RMS. This procedure is repeated 5000 times generating a distribution of the difference in muscle activation under the null hypothesis that the probability distributions for the data belonging to the two actions are mutually exchangeable. The p-value of the statistical test is yielded by the proportion of random permutations that results in a difference that is larger than to the one observed in the original data. This p-value is then corrected for multiple comparisons across time bins by controlling the False Discovery Rate (FDR; Benjamini and Hochberg, 1995). Analyses were run by using MATLAB (MATLAB R2015a,The MathWorks Inc., Natick, MA, 2015).

2.2.3. Results

The level of FDS muscle activation was similar between the two actions. The permutation test yielded no significant difference between the conditions in each time bin (in Supplementary materials 1). This result demonstrated that the FDS muscle was equally recruited in both tasks. The level of EDC muscle activation was significantly different between the two conditions (FDR-corrected for multiple comparisons across time points, Figure 2C). Following these results, we confirmed the selection of the FDS muscle to investigate the modulation of the CSP in the main TMS study.

FIGURE 2

2.3. TMS studies

2.3.1. Main TMS experiment

2.3.1.1. Stimuli

The visual stimuli consisted of short video clips of 3 s, previously used in another study (Finisguerra et al., 2015). Each movie showed the lateral view (thumb-index finger side) of a right-hand opening or closing of all fingers. Video clips had a resolution of 720x576 pixels and were displayed in the center of a 17″ computer screen (1024×768 pixels; refresh rate, 60 Hz) at distance of 57 cm from participants' frontal plane. All videos had a uniform gray background (figure 3A).

2.3.1.2. Procedures

The aim of the TMS study was to investigate CSP modulations while participants maintained a static hand closing/opening posture, with the concurrent observation of a hand closing/opening action. Importantly, in the EMG study, the FDS muscle was shown to be equally recruited while attaining the two different postures of interest (opened and closed hand). **The muscle choice was driven by the need to prevent any modulation of CSP duration due by pre-TMS muscle activity. Although still matter of debate, the level of tonic muscle pre activation could affect CSP-duration (Cantello et al., 1992; Haug et al., 1992; Inghilleri et al., 1993; Kojima et al., 2013; Roick et al., 1993; Säisänen et al., 2008; Stetkarova et al., 1994; Taylor et al., 1997; Triggs et al., 1993; Uncini et al., 1993; Van Kuijk et al., 2005; Wilson et al., 1993; Wu et al., 2002).**

Subjects sat on the same armchair of the EMG study and were asked to maintain the same arm posture. During the study participants were asked to do the same task as in the EMG study (i.e. keeping a static hand opening and closing posture). Here we additionally asked to maintain a constant level of FDS muscle activity (30% of maximal contraction) throughout the static hand posture part of the action. The muscular activation level was constantly monitoring, by the experimenter, via online data visualization. Before the experimental session, they underwent an initial training phase to familiarize with the task and learn how to execute the task and maintain the correct level of FDS contraction (using EMG visual feedback). Once the participant successfully achieved the desired level of EMG activity, we moved to the TMS mapping procedure and motor threshold assessment (see TMS and EMG section).

During the experimental protocol, trials began by the presentation of a fixation cross (4° of visual angle) at the center of the screen. After 3 s, the fixation cross was replaced by a colored circle (green/red, counter-balanced across subjects; diameter, 8° of visual angle), indicating the action to perform (hand opening/closing) and acting as a GO-signal. The video-clip appeared 2 s after the appearance of the circle. Participants were asked to keep the static hand posture, in a state of tonic

FDS muscle contraction, from the presentation of the circle until the end of the movie (Figure 3A). In other words, AE started before AO and persisted until the end of AO. Inter-trial interval was set to 3s. Four experimental conditions were tested (2 video-clips stimuli x 2 hand actions), each containing 20 trials, for a total of 80 trials. For each condition, TMS was delivered in 75% of the trials to reduce predictability. In TMS trials, a single-pulse was released at 90% of the observed action in the video-clip, corresponding to the time preceding maximal (hand opening) or minimal (hand closing) aperture (as in Finisguerra et al., 2015; Figure 3B). To ensure subjects' attention to video-clips, a question was displayed in 8 randomly trials. The question prompted them to verbally report if the last observed action was the same as the previously observed one. Participants had no time limit to give their answer.

In addition, 30 baseline trials consisted in the presentation of a static and uniform grey screen, for the same duration of the video-clip stimuli. In this case, the trial timeline was the same as previously described, with TMS pulses released at the same point in time. Participants were requested to perform the same action execution tasks. Experimental and baseline trials were presented in a fully randomized order. The total duration of the experiment, including training and TMS mapping procedure never exceeded 45 min. The task was implemented in E-Prime Software (E-Prime 2.0, Psychology Software Tools, Inc.).

FIGURE 3

2.3.1.3. TMS

TMS was delivered through a figure-of-eight coil (70 mm) and a Magstim monophasic stimulator (Magstim, Whitland, UK). The FDS Optimal Scalp Position (OSP) was found by moving the coil in

0.5 cm steps around the left primary motor cortex hand area and using a slightly suprathreshold stimulus. The TMS coil was held tangentially to the scalp with the handle pointing backward and laterally to form a 45° angle with the midline. The OSP was marked on a cap, coil position was fixed by a mechanical support and was continuously monitored by the experimenter. Head movements were constrained by a 4-point head blocking system (External occipital protuberance, fontal bone, right parietal bone, as well as the coil on the left lateral surface). The resting motor threshold (rMT) was established as the lowest stimulus intensity eliciting Motor Evoked Potentials (MEPs) on the right FDS muscle, greater than 50 μV amplitude, in at least 5 trials out of 10 (Rossini et al., 1994). EMG signal was recorded with the same wireless system (Zerowire EMG, Aurion, Italy) and analogous tendon-belly montage as in the EMG study. EMG data, collected from 300 ms before to 3 s after the TMS pulse, was, digitized (2 kHz) by a CED micro1401 board and stored on a PC for offline analysis (Signal 3.09 software; Cambridge Electronic Design, Cambridge, UK). The TMS stimulus intensity was set at 120% of the rMT and ranged from 50% to 65% (mean = 57%; SD = 5.45%) of the maximum stimulator output. This intensity is considered appropriate to investigate CSP (Farzan et al., 2013; Giovannelli et al., 2009; Säisänen et al., 2008).

2.3.1.4. Analyses

We first verified that the activation of FDS was comparable for the two actions. We rectified the EMG signal and computed the RMS in time bins of 50 ms over the 0.3 s preceding the TMS pulses. Since the data was not normally distributed (Shapiro-Wilk Test $p < 0.01$), we performed non-parametric statistics. A two-tailed permutation test (corrected for multiple comparisons across time bins by controlling the FDR) was employed, to verify if a difference emerged in the phases leading to the magnetic stimulation.

Then, we explored CSP values. We discarded from the analysis trials with either no visible CSP or trials with outlier (2 SD) pre-TMS EMG activity (total mean 4%, SD = 1.4). CSPs were measured for each trial as the time between the offset of the MEPs and the return of EMG activity, according to standard procedures (Farzan et al., 2013, 2010; Säisänen et al., 2008). The end of the CSP was determined on each individual trial as the resumption of EMG-activity to the level of pre-stimulus EMG-activity (<2SD of the 50 ms pre-stimulus signal). Baseline and action observation raw CSPs lengths were normalized (z-scores) within each subject and then averaged within each condition **(Burle et al., 2002; Davranche et al., 2007; Hoshiyama and Kakigi, 1999; Rothkegel et al., 2010).** Offline extraction of CSPs duration was carried out with Signal 3.09 software (Cambridge Electronic Design, Cambridge, UK). CSP data were normally distributed (Shapiro-Wilk Test $p>0.05$), we thus performed parametric statistics.

The first analysis on CSP was run on baseline trials (i.e. containing action execution without action observation). We compared opening and closing actions trials via paired-samples two-tailed t-tests comparisons. This analysis was implemented to measure any potential effect of execution in absence of actions observation. The second analyses evaluated the modulation of action execution effects by the concurrent action observation. We run a 2×2 within-subjects repeated measures ANOVAs, with factor Action Execution (two levels, hand opening and closing) and Action Observation (two levels, hand opening and closing), with CSP as dependent variable. Finally, a 2×2 within-subjects repeated measures ANOVAs was run on the ratio between the un-transformed CSP length during AO and baseline trials. This latter analysis was run to further investigate the direction of modulation with respect to AE-only. Partial eta-squared was used as a measure of effect size and, in case of a significant interaction, we run Bonferroni post-hoc comparisons. All parametric analyses were run with STATISTICA 9 (StatSoft, Inc.) while non-parametric analyses were run by using MATLAB (MATLAB R2013a, The MathWorks Inc., Natick, MA, 2000).

273

274 *2.3.1.5. Results*

275 The amount of pre-TMS EMG activity of the FDS muscle was comparable during the execution of
276 hand opening and closing (Figure 3C). The permutation test showed that there was no significant
277 difference between the two actions in any time bin preceding the TMS pulse ($p>0.05$). This result
278 confirmed what was observed in the EMG study and allowed us to compare CSP during the
279 execution of the two actions without any confound due to unequal muscle activation. No
280 significant difference was found in baseline CSP during closing (-0.07 ± 0.35 SD) and opening
281 actions (0.09 ± 0.35 SD; $t(20) = 1.112$; $p = 0.27$; Figure 4B), showing that the CSP is not modulated
282 by the type of AE. **Raw measures of CSPs are shown in Table 1**, while Supplementary materials 2
283 shows one subject's data.
284 The 2x2 repeated-measures ANOVA on z-transformed CSP durations showed no main effect of
285 Executed Action ($F(1,20) = 2.70$, $p = 0.11$, $\eta^2p = 0.12$) and a significant main effect of the Observed
286 Action ($F(1,20) = 6.30$, $p = 0.02$; $\eta^2p = 0.23$). CSPs were longer when observing the hand closing
287 action compared to the opening one (closing observation: 0.06 ± 0.40 SD; opening observation: -0.04
288 ± 0.45 SD). The interaction between the Executed Action and the Observed Action ($F(1, 20) = 6.19$,
289 $p = 0.02$; $\eta^2p = 0.22$) was significant. Post-hoc analyses revealed a modulation of CSP during the
290 execution of the closing action ($p = 0.04$). Specifically, CSP recorded during hand closing execution
291 was shorter when observing the hand opening action (opening observation: -0.27 ± 0.35 SD; closing
292 observation: -0.009 ± 0.42 SD). Differently, action observation did not modulate CSP when
293 executing a hand opening action (opening AO: 0.14 ± 0.46 SD; closing AO: 0.10 ± 0.39 SD; $p > 0.05$;
294 Figure 4A).
295 The 2x2 repeated-measures ANOVA on the ratio between mean raw CSP duration during AO+AE
296 and Baseline trials (only AE), showed no main effect of Executed Action ($p = 0.42$) and a main

297 effect of the Observed Action (F (1,20) = 7.78, p = 0.01; η^2p = 0.28). Results reveal a reduction of
298 inhibition when observing the hand opening action (0.98 ± 0.10 SD) compared to the observation of
299 closing action (1.01 ± 0.10 SD). The interaction between the Executed Action and the Observed
300 Action (F (1, 20) = 6.07, p = 0.02; η^2p = 0.23) was significant. Post-hoc analyses revealed a significant
301 (p = 0.04) reduction of inhibition during the execution of a closing action and observation of an
302 opening action (opening observation: 0.95 ± 0.12 SD; closing observation: 1.03 ± 0.09 SD;
303 Supplementary materials 3).
304
305 _____
306 FIGURE 4 – TABLE 1
307
308 ***2.3.2. First TMS control experiment***
309 ***2.3.2.1. Stimuli***
310 The visual stimuli consisted of four short video clips of 3 s. Two were the same used in the main
311 TMS study, while two new ones were added. The new video clips showed the lateral view of a
312 right hand, starting open or close and flexing the wrist (Figure 5A). The two wrist flexion stimuli,
313 with different starting posture, were employed to match the early frames of the other two stimuli.
314 Video clips had the same resolution (720x576 pixels), were displayed on the same screen as the
315 main TMS experiment (17"; 1024×768 pixels; refresh rate: 60 Hz) and at distance of 57 cm from
316 participants' frontal plane. All videos had a uniform gray background.
317
318 ***2.3.2.2. Procedures***
319 In this study, we investigated the modulations of the CSP while participants observed
320 closing/opening hand actions or wrist flexion during the execution of hand opening or closing. The

aim of this first control experiment is to demonstrate that a fundamental driver, into mismatch detection, is the observation of actions recruiting the antagonist muscle. For this reason, we compare motor inhibition in FDS during the observation of two different action goals that require the same involvement of the muscle itself. Participants were asked to do the same task as in the first TMS study (i.e. keeping a static hand opening or closing posture) meanwhile we recorded FDS muscular activation. The procedure of the initial training phase was the same of the main TMS study. Conditions were the same of the main TMS experiment, plus two with wrist flexion video. Each one contained 22 trials, for a total of 132 trials, plus 32 baseline trials were added as described in the first TMS experiment procedure. For each condition, TMS was delivered in 73% of the trials to reduce predictability (6 trials for conditions without TMS). In TMS trials, a single-pulse was released at 90% of the observed action in the video-clip, as explained in the main TMS experiment procedure. Experimental and baseline trials were presented in a fully randomized order. The total duration of the experiment, including training and TMS mapping procedure never exceeded 60 min. The task was implemented in E-Prime Software (E-Prime 2.0, Psychology Software Tools, Inc.).

2.3.2.3. TMS

TMS mapping procedure, motor threshold assessment and EMG recording were implemented as in the main TMS experiment. EMG data were collected from 5 s before to 1.5 s after the TMS pulse.

2.3.2.4. Analyses

Trials with either no visible CSP and MEPs below 50 μV or with outlier (2 SD) pre-TMS EMG activity (mean 1.6%, SD = 2.1) were discarded from the analysis. As in the main TMS experiment, CSPs were measured for each trial as the time between the offset of the MEPs and the return of

EMG activity. Baseline and action observation of CSPs lengths were normalized (z-scores) separately within each subject and then averaged within each condition. Offline extraction of CSPs duration was carried out with Signal 3.09 software (Cambridge Electronic Design, Cambridge, UK). CSP data were normally distributed (Shapiro-Wilk Test $p > 0.05$).

Here we repeated several analyses run the main TMS study. First, we analyzed the amount of pre-TMS EMG activity of the FDS muscle during the execution of hand opening and closing, with a permutation test **(in Supplementary materials 5 we reported analyses of the whole pre-TMS EMG recording)**. Then, we assessed CSP modulation during baseline trials (AE only), with paired-samples two-tailed t-tests. Finally, we repeated the same ANOVA design of the main TMS experiment, on the new data. Thus, we report here a 2×2 within-subjects repeated measures ANOVAs run on CSP, with factor Action Execution (two levels, hand opening and closing) and Action Observation (two levels, hand opening and closing), and Bonferroni post-hoc tests.

Finally, we run a series of planned comparisons on the new conditions, to evaluate generalization of the previous effects. First, we tested whether the observation of wrist flexion with the two starting postures did not differ, with a paired-samples two-tailed t-tests. We then tested, with paired-samples two-tailed Bonferroni-corrected t-tests, whether during the execution of hand closing action, the observation of a wrist flexion (data collapsed from both video clips) differed with respect to the observation of closing or opening hand action.

2.3.2.5. Results

As in the main TMS study, the amount of pre-TMS EMG activity of the FDS muscle during the execution of hand opening and closing, did not differ ($p = 0.15$). Also baseline trials (AE only) did not differ ($t(20) = 0.37$; $p = 0.71$; closing action: 0.06 ± 0.24 SD; opening action: 0.02 ± 0.23 SD). **Raw measures of CSPs are shown in Table 2.**

The 2x2 repeated-measures ANOVA on Z-transformed CSP duration showed no main effect of Executed Action ($F(1,20) = 1.23$, $p = 0.27$, $\eta^2p = 0.05$) and a significant main effect of the Observed Action ($F(1,20) = 19$, $p < 0.01$; $\eta^2p = 0.48$). Post-Hoc Bonferroni corrected reveled that CSPs were longer when observing the hand closing action compared to the opening one: $p < 0.01$ (closing observation: 0.14 ± 0.33 SD; opening observation: -0.05 ± 0.36 SD). The interaction between the Executed Action and the Observed Action ($F(1, 20) = 11.1$, $p < 0.01$; $\eta^2p = 0.35$) was also significant. Post-hoc analyses, on the interaction, revealed the same modulation. Hand closing execution elicited shorter CSPs when observing the hand opening action (opening observation: -0.22 ± 0.07 SD; closing observation: 0.18 ± 0.07 SD; $p < 0.01$). Differently, action observation did not modulate CSP when executing a hand opening action (opening AO: 0.10 ± 0.07 SD; closing AO: 0.10 ± 0.07 SD; $p > 0.05$; Supplementary materials 4). These results critically replicate the same effects of the main TMS study, on a different group of participants.

The paired-samples t-tests on wrist flexion with the two starting postures did not show any difference ($p = 0.12$). Paired-samples Bonferroni-corrected t-tests, during closing action execution, while observing wrist flexion did not differ from observing the hand closing action ($t(20) = 1.19$, $p = 0.24$), while it differed from the opening action observation ($t(20) = 4.97$, $p < 0.01$; hand opening: -0.22 s ± 0.32; wrist flexion: 0.11 s ± 0.26; Figure 5B).

FIGURE 5 – TABLE 2

2.3.3. Second TMS control experiment

2.3.3.1. Stimuli

In this second control experiment we used the same stimuli of the main TMS experiment. Video clips were displayed on the same screen as the main TMS experiment (17"; 1024×768 pixels; refresh rate: 60 Hz) and at a distance of 57 cm from participants' frontal plane.

2.3.3.2. Procedures

The aim of this second control experiment was to validate, on extensor muscles, the modulation of CSP for the mismatch between ongoing executed and observed action. As in the main TMS study, participants executed both hand opening and closing actions, while observing the two video clips either showing a hand opening or closing action (Figure 6A). Otherwise, here we recorded CSP from the EDC muscle. We kept the same design to avoid any bias towards one action goal (opening or closing) but we analyze the data only pertaining to the opening AE. In fact, as demonstrated in the EMG experiment, the EDC muscle would not provide a fair CSP comparison across the two AE tasks.

2.3.3.3. TMS

TMS mapping procedure, motor threshold assessment, EMG recording were implemented as those used in the main TMS experiment. Timing of the TMS pulse was the same as the main TMS experiment. EMG data were collected from 5 s before to 1.5 s after the TMS pulse.

2.3.3.4. Analyses

We analyzed only trials with the execution of hand opening posture. Trials with either no visible CSP and with outlier (2 SD) pre-TMS EMG activity (mean 2.2%, SD = 4.2) were discarded from the analysis. CSPs were measured for each trial as the time between the offset of the MEPs and the return of EMG activity, as in previous experiments. Baseline and action observation CSPs were

normalized (z-scores) separately within each subject and then averaged within each condition. Offline extraction of CSPs duration was carried out with Signal 3.09 software (Cambridge Electronic Design, Cambridge, UK). Data were normally distributed (Shapiro-Wilk Test $p>0.05$), we thus performed parametric statistics. We analyzed CSPs of the two experimental conditions and baseline via Bonferroni-corrected paired samples two tailed t-test analyses. All parametric analyses were run with STATISTICA 9 (StatSoft, Inc.)

2.3.3.5. Results

The paired-samples t-tests analysis showed that during observation of the closing action (-0.22 s ± 0.25) the CSP was significant shorter than the observation of the opening action (Opening: 0.02 s ± 0.18; $t(11) = 2.83$, $p = 0.01$) or baseline (baseline: 0.20 s ± 0.31; $t(11) = 2.77$, $p = 0.01$; Figure 6B). No significant difference was found between observing opening action and baseline ($p=0.2$). **Raw measures of CSPs are shown in Table 3.**

FIGURE 6 – TABLE 3

3. Discussion

Behavioral interaction in natural settings occurs at fast pace and humans coordinate their actions by quickly adapting to other's behavior. This means that neural processes subtending AE and AO can unfold smoothly, notwithstanding their important temporo-spatial overlap (Novembre et al., 2014). However, at the behavioral level AE interferes with the process of visual action recognition (De La Rosa et al., 2016). Proactive eye movements, which are present during visually guided

actions and during AO (Elsner et al., 2013; Flanagan and Johansson, 2003), are reduced when an AO-AE mismatch is present (Costantini et al., 2012). Similarly, the observation of objects affording a specific grasp, biases concurrent grasping performances (Costantini et al., 2010; Rounis et al., 2018). In general, AE is facilitated by compatible and impeded by incompatible AO (Cracco et al., 2018; Kilner et al., 2003). These results suggest that the neural processes subtending AO and AE modulate each other.

Nevertheless, most research has investigated the neurophysiological mechanisms of AO and AE by using a strict temporal separation between observer's and actor's role (Hadley et al., 2015). Conversely, here we considered participants as actors and observers at the same time, in fact they produced a tonic motor descending drive, while observing others' actions. Corticospinal inhibition decreased during mismatching executed and observed actions. In our main experiment, we show reduction of corticospinal inhibition only for the execution of hand closing actions while observing opening ones. The lack of symmetry (e.g. no effects for opening AE during closing AO) can be explained if we consider the function of the muscle recorded here. Although equally recruited in both actions (see first EMG study), the FDS muscle is instrumental in achieving hand closing but has only a postural role in opening, which is instead realized by recruiting forearm extensors (e.g. EDC). Corticospinal inhibition measured on EDC was reduced for opening AE during closing AO (see second TMS control study), suggesting that these effects are not limited to flexor muscles.

More importantly, executing a closing action while observing a wrist flexion did not produce any modulation of FDS corticospinal inhibition (see first TMS control study). Hand closing and wrist flexion mismatch at the level of goals but share a central role for FDS recruitment. All these results together demonstrate that AE-AO mismatch is computed at the level of muscle recruitment and according to an agonist-antagonist mapping of actions. Critically, the functional contribution of

463 muscles to a specific action seems to be the guiding principle in allowing modulation of
464 corticospinal inhibitory circuits for AE-AO mismatching conditions.
465
466 **3.1. The role of corticospinal inhibition in AE**
467 The CSP is measures supraspinal inhibitory activity in the motor system, at least in its late
468 component (Fuhr et al., 1991; Inghilleri et al., 1993; Ziemann et al., 1993) and it is relatively not
469 affected by pre-TMS EMG amplitude (Cantello et al., 1992; Triggs et al., 1993; Taylor et al., 1997;
470 Säisänen et al., 2008). **Despite several studies have demonstrated this, other studies have**
471 **reported shortened duration of CSP with increasing muscle activity (Cantello et al., 1992;**
472 **Stetkarova et al., 1994; Wilson et al., 1993). More recently, it has been shown that CSP might be**
473 **prolonged as a consequence of fatigue (Goodall et al., 2018) or decreased with an increase in**
474 **force output (Matsugi, 2019).** CSP duration reflects motor cortical postsynaptic inhibition and is
475 potentially mediated by $GABA_b$ receptors, thus indexing the involvement of slow metabotropic-
476 mediated inhibitory neural circuits (Ziemann et al., 2015). A likely source of this corticospinal
477 inhibitory mechanism could be the dorsal premotor cortex (PMd; Duque et al., 2013, 2012;
478 Sawaguchi et al., 1996). In fact, changes in reciprocal inhibition between forearm extensor and
479 flexor muscles would be caused by long loop inhibitory connections to supra-spinal centers that
480 receive input from PMd cortex (Huang et al., 2009). Interestingly, TMS-induced interference on
481 PMd activity results in shortened CSP durations (Münchau et al., 2002; Rizzo et al., 2004).
482 The PMd is engaged in response preparation (Terao et al., 2007; Wise et al., 1992), exhibits robust
483 delay-related activity (Cisek and Kalaska, 2005) and, in cooperation with the left supramarginal
484 gyrus (SMG), is a key region for non-routine responses that require the integration of conflicting
485 information during action reprogramming (Hartwigsen et al., 2012; Hartwigsen and Siebner, 2015).
486 It has been hypothesized that the PMd suppresses movements that have been prepared but are not

used (Koch et al., 2006; Kroeger et al., 2010). Greenhouse et al., 2015 recently suggested that motor inhibition is instrumental in "competition resolution" by reducing noise to enhance signal processing and, in turn, modulate the gain of a selected response. According to this view, a response will fail to elicit movement until motor noise has been sufficiently suppressed (Churchland, 2006) across different sub-populations within M1 (Derosiere, 2018).

The PMd could also modulate spinal circuits via direct projections (Dum and Strick, 1991; Bizzi et al., 2000) targeting spinal interneurons (Dum, 2005; Galea and Darian-Smith, 1994) or via sub-cortical structures (Duque et al., 2012) originating indirect descending pathways (primarily the reticulospinal tract) partly involved in the control of distal hand muscles (Cohen et al., 2010; Riddle et al., 2009). In general, direct corticospinal projections as well as indirect pathways via somatosensory cortex, basal ganglia, motor thalamus, brainstem and cerebellum provide essential spinal inhibitory motor control (Ebbesen and Brecht, 2017).

3.2. Corticospinal inhibition during concurrent AO and AE

The monosynaptic spinal reflex (H-reflex), which provide a measure of spinal excitability (Bestmann and Duque, 2015), is facilitated before movement onset (Gottlieb et al., 1970) while it is reduced during passive AO (Baldissera et al., 2001). This latter study shows that spinal centers are suppressed during action observation, possibly to avoid unnecessary automatic action imitation. Conversely, AO induces a reduction of intracortical inhibition thus shifting the balance towards greater local excitation (Cardellicchio et al., 2018; Patuzzo et al., 2003; Strafella and Paus, 2000).

As a consequence, AO might constitute a source of neural noise interfering with the correct execution of actions, both at the cortical and spinal levels. Motor inhibition, with its tightly link to cognitive processes (Hilt and Cardellicchio, 2018; Wessel and Aron, 2017), could have a central role

in enhancing signal processing, facilitating action execution and preventing early change detection signals from translating into behavioral distraction (Greenhouse et al., 2015; Wessel et al., 2019). For instance, when we execute an action (e.g. hand closing) every other action produced by the same effector should be suppressed (e.g. opening is suppressed to effectively execute a closing action). However, in a mismatching AE-AO condition, the observed action (opening), by activating the corresponding cortical representation in the observer (Fadiga et al., 1995), contrasts with its required attenuation. This mechanism of corticospinal disinhibition might explain the numerous evidences showing AO-AE behavioral interference (for a review see Cracco et al., 2018). Conversely, matching AO-AE may facilitate action selection and preparation thus explaining the automatic imitation tendencies for similar actions (Bisio et al., 2010; Heyes, 2011). More importantly, disinhibition does not emerge from mismatching action goals. Rather, attenuation of corticospinal inhibition is selective for the muscle that is functionally involved in the executed Vs. the observed action. Based on our results, mismatch seems to be computed in a muscle space whereby actions are mapped according to an agonist-antagonist representation.

Although here a bidirectional haptic and/or informational exchange between interacting subjects is missing, our results open a window upon the neurophysiological mechanism by which AE is modulated by the concurrent visual cues provided by other's action. Future research will need to clarify whether inputs from premotor and parietal areas or different intracortical populations (e.g. by using paired pulse TMS protocols) contribute to the current phenomenon. Still, our results offer a first demonstration that corticospinal inhibitory mechanisms promoting accurate motor execution are deeply affected by the co-participant's muscle-level state, estimated from action observation.

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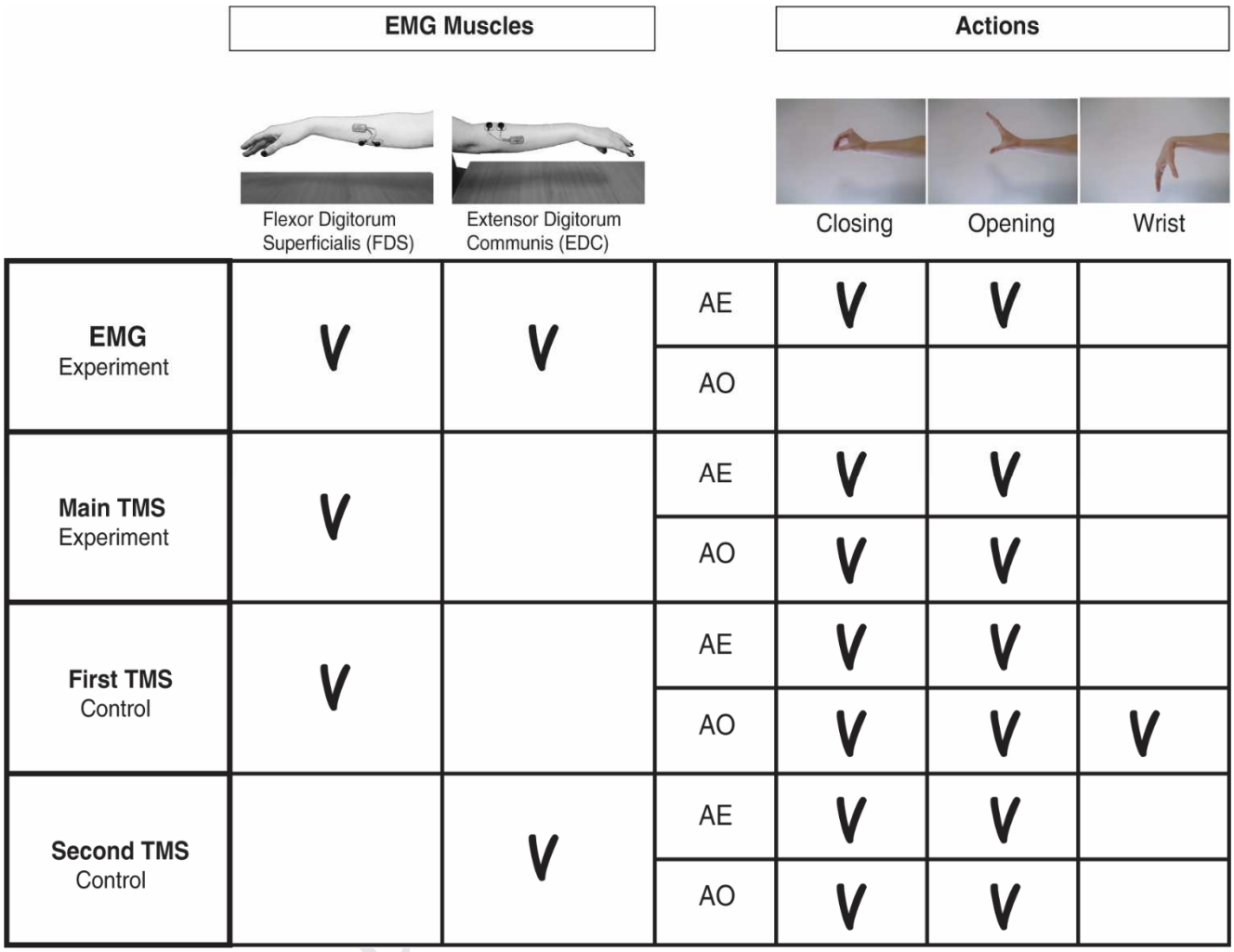
821 **Figures with Captions**



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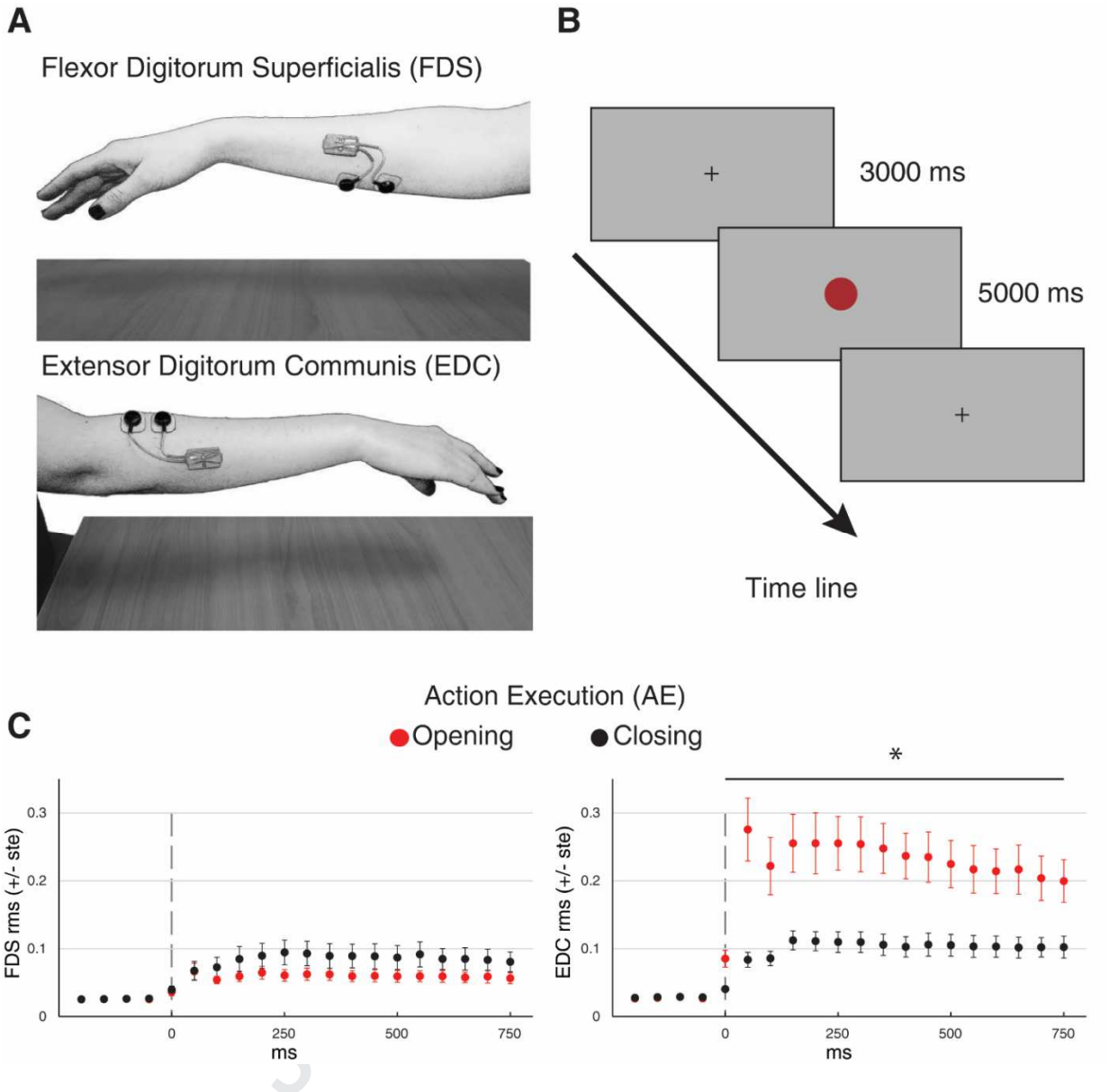
823 **Figure 1. Schematic illustration of all experiments.** Schematic description of the different

824 experimental conditions and measurements across the four experiments (AE: Action Execution;

825 AO: Action Observation).



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827 **Figure 2. EMG experiment.** Panel A: The representation of the EMG montage for Flexor Digitorum
828 Superficialis (FDS) and Extensor Digitorum Communis (EDC). Panel B: The timeline of the
829 experimental trial. Each trial starts with a fixation cross and a colored dot appears indicating the
830 start and the type of action to perform. Panel C: The EMG signal recorded during the two isometric
831 hand postures (hand opening and hand closing) for the FDS (left side) and the EDC (right side)
832 muscle. The RMS signal was averaged in time bins of 50 ms, between -250 ms to 750 ms with
833 respect to EMG onset (vertical dashed line). Whiskers plots on data points represent the standard
834 error. The asterisk with the horizontal line shows the time bins in which the two actions are
835 significantly different.

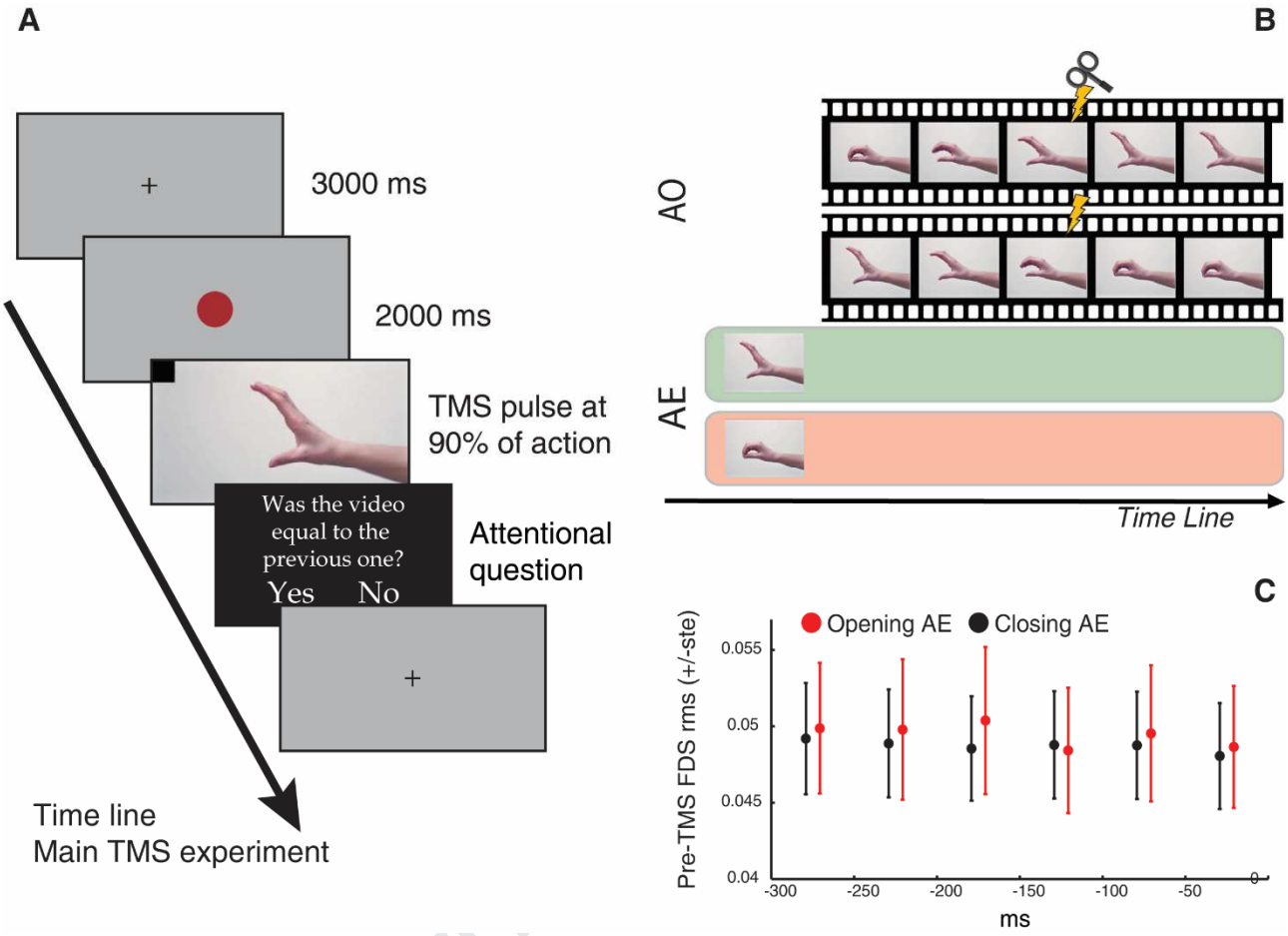

Figure 3. Methods of the main TMS experiment. Panel A: Timeline of the experimental trial. Each trial starts with a central fixation cross. After 3 seconds, a colored dot indicates the type of action to perform and acted as a GO-signal. Two seconds later, a video clip showing a closing or opening hand action was displayed to participants. Participants had to maintain an isometric hand posture (hand opened or closed) until the end of the video clip. At 90% of the observed movement, a single TMS pulse was delivered. In 8 random trials, participants had to answer an attentional question. Panel B: The action video clips and execute action are shown. Panel C: EMG signal preceding the TMS pulse, for the two actions (hand opening and hand closing) recorded from the FDS muscle. The signal was averaged (RMS) in time bins of 50 ms across the 300 ms before the TMS pulse. Whiskers plots indicate the standard error of mean. No significant difference in pre-TMS EMG activity was present between the two actions.

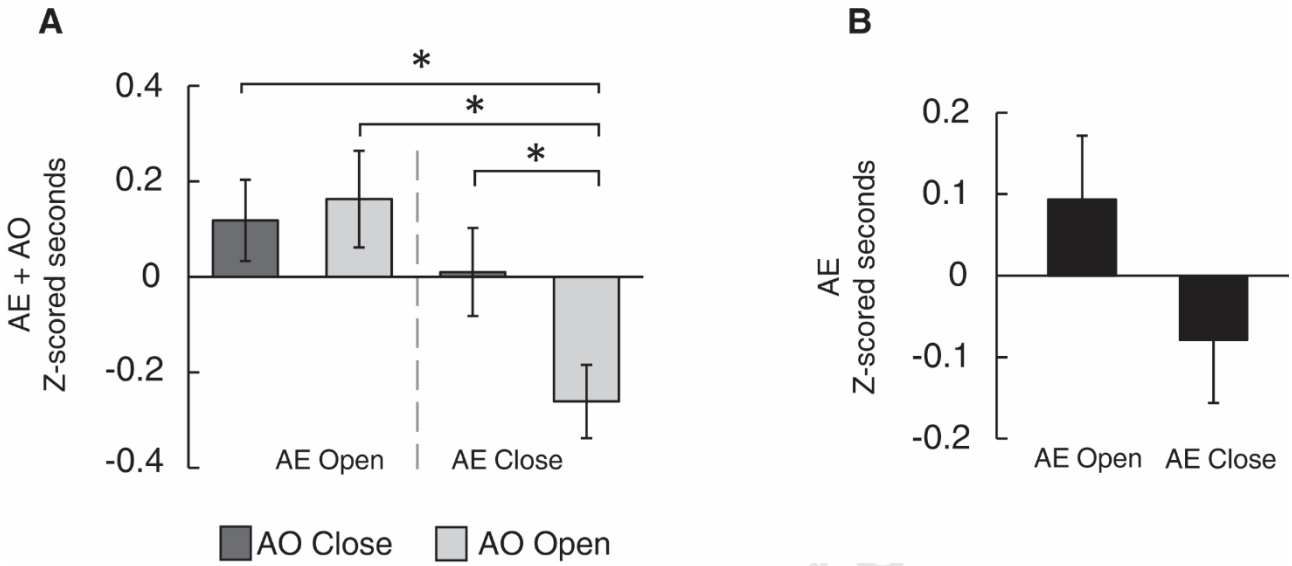

Figure 4. Results of the main TMS experiment. Panel A: Z-scored CSP duration during concurrent AE and AO. A reduction of CSP duration is shown during the execution of a closing action while observing an opening action. Panel B: CSPs during AE alone does not show any differences. Bars indicate the standard error of mean. Asterisks indicate significant comparisons.

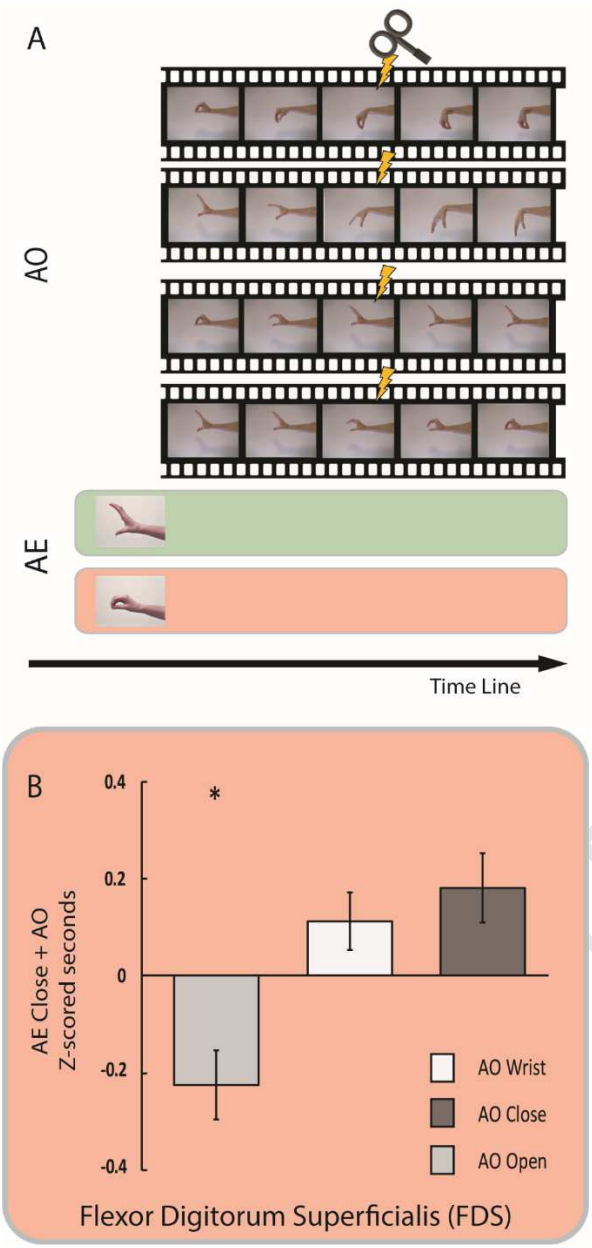

Figure 5. Methods and results of the first TMS control experiment. Panel A: CSPs are recorded in the FDS muscle. The procedure is the same of the main TMS experiment. Two additional video clips are included, describing a wrist flexion with either the finger flexed or extended. Panel B: During the execution of the hand closing action, the planned comparison between wrist flexion observation (both video clips collapsed) and hand opening observation was significantly different. No significant difference is present between wrist flexion and hand closing observation. Bars indicate the standard error of mean. Asterisks indicate significant comparisons.

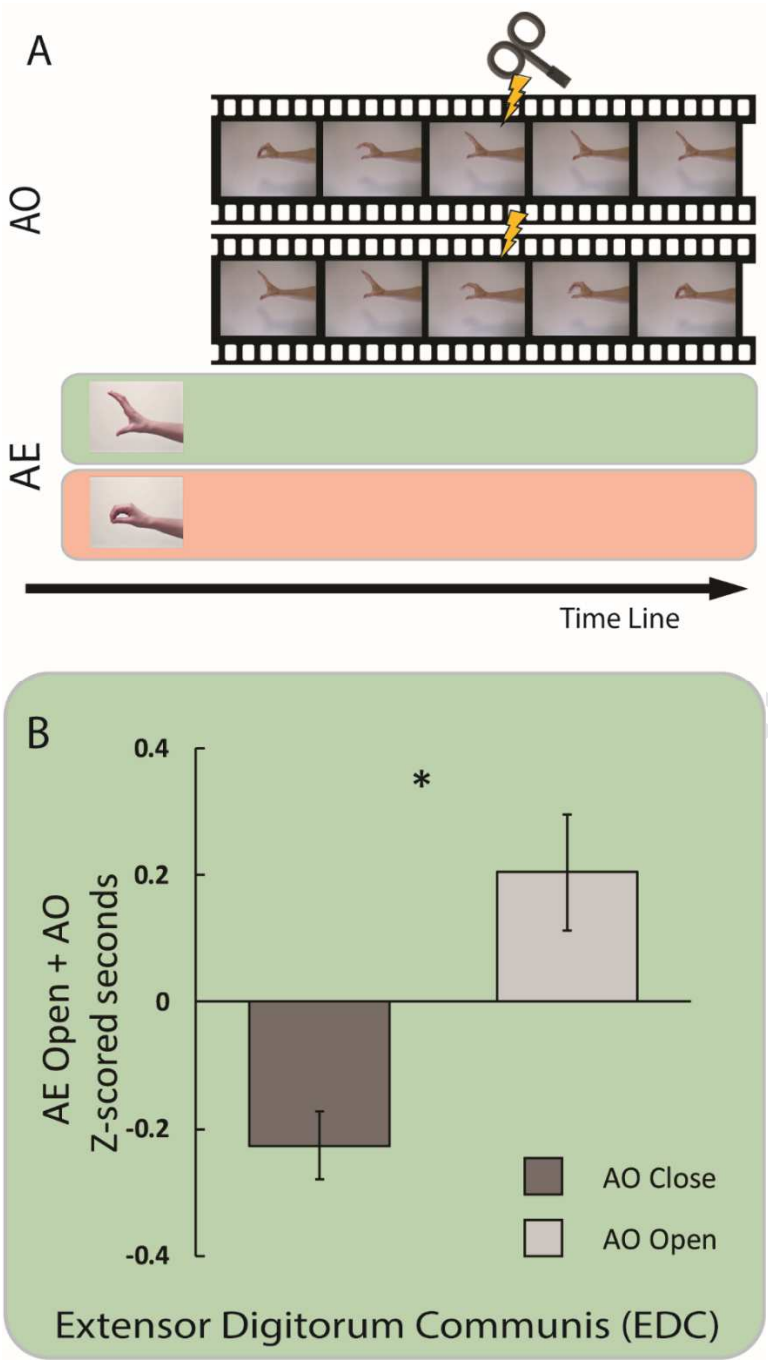

Figure 6. Methods and results of the second TMS control experiment. Panel A: CSPs are recorded from the EDC muscle. The procedure is the same of the main experiment. Panel B: During the execution of the hand opening action, the planned comparison between the observation of closing action and opening action was significantly different. Bars indicate the standard error of mean. Asterisks indicate significant comparisons.

Main TMS experiment			
AO	**AE**	**Mean (ms)**	**St. dev**
open	**open**	109.21	±26,7
	close	99.89	±29,3
close	**open**	108.70	±29,6
	close	106.34	±28,6
baseline	**open**	107.57	±26,6
	close	102.76	±25,2

871

872 **Table 1: Raw measures of CSPs in the Main TMS experiment.** The table shows mean and
873 standard deviation of CSP duration in ms, for each experimental condition.

First TMS control experiment			
AO	**AE**	**Mean (ms)**	**St. dev**
open	**open**	95.81	±28,03
	close	87.39	±27.8
close	**open**	95.14	±28,4
	close	94.7	±28.9
wrist	**open**	94.12	±28.6
	close	95.02	±29.4
baseline	**open**	92.79	±28.07
	close	92.51	±28.81

874

875

876 **Table 2: Raw measures of CSPs in the first control TMS experiments.** The table shows mean and
877 standard deviation of CSP duration in ms, for each experimental condition.

Second TMS control experiment			
AO	**AE**	**Mean (ms)**	**St. dev**
open	**open**	104.55	±22,2
close		100.37	±21,1
baseline		108.48	±19,4

878

879 **Table 3: Raw measures of CSPs in the second control TMS experiments.** The table shows mean

880 and standard deviation of CSP duration in ms, for each experimental condition.

881

882

Conflict of interest

The authors declare no competing financial interests.