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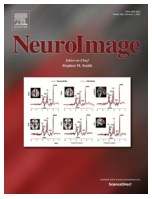

The value of corticospinal excitability and intracortical inhibition in predicting motor skill improvement driven by action observation

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ABSTRACT

The observation of other's actions represents an essential element for the acquisition of motor skills. While action observation is known to induce changes in the excitability of the motor cortices, whether such modulations may explain the amount of motor improvement driven by action observation training (AOT) remains to be addressed. Using transcranial magnetic stimulation (TMS), we first assessed in 41 volunteers the effect of action observation on corticospinal excitability, intracortical inhibition, and transcallosal inhibition. Subsequently, half of the participants (AOT-group) were asked to observe and then execute a right-hand dexterity task, while the controls had to observe a no-action video before practicing the same task. AOT participants showed greater performance improvement relative to controls. More importantly, the amount of improvement in the AOT group was predicted by the amplitude of corticospinal modulation during action observation and, even more, by the amount of intracortical inhibition induced by action observation. These relations were specific for the AOT group, while the same patterns were not found in controls. Taken together, our findings demonstrate that the efficacy of AOT in promoting motor learning is rooted in the capacity of action observation to modulate the trainee's motor system excitability, especially its intracortical inhibition. Our study not only enriches the picture of the neurophysiological effects induced by action observation onto the observer's motor excitability, but linking them to the efficacy of AOT, it also paves the way for the development of models predicting the outcome of training procedures based on the observation of other's actions.

1. Introduction

Throughout the human lifespan, the observation of others' actions represents an essential element for attaining and empowering motor abilities (Rizzolatti et al., 2021; Rizzolatti and Sinigaglia, 2016; Whiten et al., 2009). Seminal examples are the predisposition to learn by imitation during childhood (Vygotskij and Cole, 1981; Whiten et al., 2009) as well as the acquisition of complex motor skills by expert model observation in adolescence and adulthood (Bazzini et al., 2022; Rizzolatti et al., 2021).

Motor skills improvements are associated with neuroplastic changes at multiple levels of the central nervous system (Caroni et al., 2012), first among all, the functional reorganization of the primary motor cortex (M1) (Chieffo et al., 2016; Nudo and Milliken, 1996). This applies also to the case of action observation, which has been proven able to induce lasting changes within primary-motor representations in both healthy individuals (Celnik et al., 2006; Stefan et al., 2008) and people with brain damage (Celnik et al., 2008), as well as to prevent the corticomotor depression following peripheral limb immobilization (Bassolino et al., 2014). Such long-term modulations likely explain why motor training approaches based on the alternation of action observation and execution (i.e., Action Observation Training, AOT) are capable of promoting the acquisition (Bazzini et al., 2022), recovery (Ertelt et al., 2007), and maintenance (De Marco et al., 2021) of motor abilities (see Rizzolatti et al., 2021 for a recent review.

A still unsettled piece of knowledge, however, is whether the efficacy of AOT can be explained, or even predicted, by the reactivity of the motor system to action observation. In other words, whether the mirror mechanism indexes the potential of learning via action observation. Among the recording techniques allowing to probe the reactivity of

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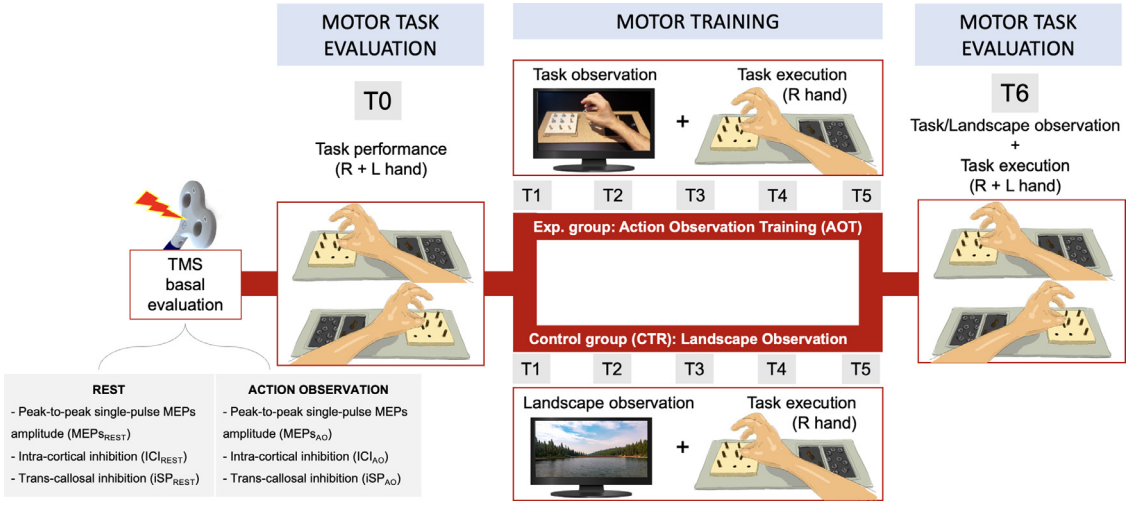

Fig. 1. Study design.
In the first phase (i.e., baseline evaluation), the modulatory effect by action observation on TMS parameters and bilateral hand motor performance were assessed (T0). In the second phase, participants were randomized into two groups. Action observation training (AOT) subjects were asked to observe a video clip showing a correct execution of the modified version of the Nine Hole Peg Test (mNHPT) and then execute it as quickly and accurately as possible. (Of note, the video used for AOT was different from that adopted in the TMS basal evaluation). This observation-execution combination was repeated six consecutive times (T1–T6). The last trial (T6) also included left-hand mNHPT execution. Participants in the control group (CTR) followed the same procedure, except the video clip preceding mNHPT execution depicted an animated lake landscape. mNHPT performance was recorded across T0–T6 timepoints. Notes: TMS, transcranial magnetic stimulation; R, right; L, left.

motor cortices to action observation, transcranial magnetic stimulation (TMS) combines a relative easiness of administration with a high richness of information. Indeed, by delivering a single pulse of suprathreshold TMS on a cortical motor map, one can measure the motor-evoked potentials (MEPs, reflecting the corticospinal excitability), previously shown to increase during action observation (Fadiga et al., 1995; Naish et al., 2014; Patuzzo et al., 2003; see, however Hardwick et al., 2012; Sartori et al., 2012). At the same time, paired-pulse TMS measures of intracortical inhibition (sICI) reflect the excitability of distinct, low-threshold, GABAergic interneural circuits within M1 (Di Lazzaro et al., 2006; Ferland et al., 2021; Kujirai et al., 1993; Müller-Dahlhaus et al., 2008; Wassermann et al., 2021), whose activity appears downregulated by action observation (Cardellicchio et al., 2020, 2018; Patuzzo et al., 2003; Strafella and Paus, 2000). Finally, the single-pulse TMS administration on M1 may lead to a short-lasting disruption of the ipsilateral voluntary motor output (ipsilateral Silent Period - iSP) due to the interhemispheric inhibitory transfer mediated by callosal fibers (Meyer et al., 1998). Also iSP has been reported to be enhanced during action observation, with a modulation tuned to the kinematics of the observed movements (Gueugneau et al., 2016).

Taken together, these data indicate that action observation evokes transitory but reliable changes in corticospinal pathways (Fadiga et al., 1995; Gangitano et al., 2001; Patuzzo et al., 2003), cortical inhibitory circuits (Cardellicchio et al., 2020, 2018; Patuzzo et al., 2003), and transcallosal motor connections (Gueugneau et al., 2016), and that such a multifaceted picture can be documented via TMS. However, whether *transient* responses to action observation have the potential to predict the *efficacy* of AOT on motor learning remains to be addressed.

To address this issue, we probed the effect of action observation on (1) corticospinal excitability, (2) intracortical inhibition (sICI), and (3) transcallosal inhibition (iSP) in 41 healthy participants. Subsequently, we administered either an AOT or, as a control, motor training with the observation of non-action videos. Finally, we assessed the capacity of each neurophysiological marker in predicting the motor learning outcome

Such procedures allow not only to test whether the rate of motor learning achieved *via* action observation is explained by the observer's initial motor reactivity *to* action observation but also indicate which neural mechanisms within motor cortices mostly promote motor learning via AOT. Consequently, our data could inform the procedures for optimizing and individualizing treatments based on action observation.

2. Methods

2.1. Participants

Forty-one subjects (11 males and 30 females, mean age 36 ± SD 9 years [range 22–61 years]) were recruited for the study. All subjects were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). None of them had any history of neurological/psychiatric diseases or contraindications to TMS administration (Rossi et al., 2021). Participants were informed about the experimental procedures and gave their written consent according to the Helsinki Declaration. Subjects were randomized into two groups, namely Action Observation Training (AOT, n = 20) and controls (CTR, n = 21). Two subjects (belonging to controls) performed only the baseline neurophysiological evaluation, as they decided not to continue with the following training procedures. Concerning sICI, one subject (AOT group) was excluded from the neurophysiological evaluation due to the triggering system malfunctioning.

The experiment was approved by the local ethical committee "Area Vasta Emilia Nord" (n. 10084, 13.03.2018).

In the following paragraphs, we will detail the experimental design, which is graphically summarized in Fig. 1.

2.2. Baseline neurophysiological evaluation

TMS was delivered by a figure-of-eight coil (70 mm) connected to a Magstim BiStim stimulator (Magstim, Whitland, UK) and combined with electromyographic (EMG) measurements to assess MEPs. TMS, delivering monophasic pulses inducing current in a posterior-anterior direction, was applied to the scalp, with the coil handle rotated 45° from the sagittal plane. Before the experimental session, the optimal stimulation location (hotspot) corresponding to the right first digital interosseous (R-FDI) was determined with a procedure similar to that described by

Bonzano et al. (2022), as follows. First, the TMS coil was positioned over the hand knob area in the precentral gyrus (as marked on the brain template provided by the neuronavigation system) and a few TMS pulses were delivered at 35% of the maximal stimulator output, within a distance below 2 cm from the initial location. If no MEPs were induced, the TMS intensity was increased by 5%, and the procedure was repeated until MEPs (> 50 μV) were recorded. At this point, the position of the coil was recorded in the neuronavigation system. Then, a few TMS pulses were delivered moving the coil around the recorded position within a distance of maximum 1 cm. The location inducing the highest and most reliable MEPs was finally set as hotspot. The coil position and orientation were coregistered to a brain template obtained from individual head landmarks (nasion, ears, scalp surface) using an optoelectronic neuronavigation system (visor 2, ANT Neuro, Netherlands). A dedicated monitor (out of the visual field of the participants) provided the experimenter with online visual feedback informing the reciprocal coil-target placing. The maximum tolerated spatial errors for stimulus delivery were the following: (a) 10° tilt angle from the target; (b)10° rotation angle from the target; (c) 5 mm linear distance from the target. All the TMS stimuli were properly delivered, below the error threshold.

EMG signals from the R-FDI muscle and the left opponens pollicis (L-OP) were continuously recorded using surface Ag–AgCl electrodes. The EMG signal was amplified (× 1000) using a CED1902 amplifier (Cambridge Electronic Design), sampled at 2.5 kHz, filtered with an analogical online band-pass (20–500 Hz) and a notch (50 Hz) filter, and acquired with CED Micro 1401 interfaced with Spike2 software (Cambridge Electronic Design). An additional channel containing digital markers of the TMS trigger was integrated into the same EMG file. The data were stored for subsequent analyses.

The following TMS parameters have been collected:

(a) The resting motor threshold (RMT), defined as the lowest stimulator output intensity capable of inducing MEPs greater than 50 μV peak-to-peak amplitude in relaxed R-FDI in at least 5 of 10 trials (Rossini et al., 1999).
(b) The corticospinal excitability (CSE), measured as the peak-to-peak amplitude of MEPs elicited in the resting R-FDI by single-pulse TMS (120% RMT intensity).
(c) The short-interval intracortical inhibition (sICI) was obtained from a paired-pulse TMS protocol (Kujirai et al., 1993). A subthreshold conditioning stimulus was delivered at 80% of the RMT and at an interstimulus interval of 3 ms before a suprathreshold, conditioned, test stimulus (120% RMT). The same coil delivered both stimuli in the same scalp position. sICI was expressed as the percentage decrease of MEP amplitude relative to the single-pulse TMS condition, according to the following formula:

$$sICI = \left(1 - \frac{Conditioned\ MEP\ amplitude}{Single\ pulse\ MEP\ amplitude}\right) * 100.$$

(d) The ipsilateral silent period (iSP) was acquired by delivering single-pulse TMS to the right opponens pollicis hotspot (obtained with a procedure like that described for the R-FDI muscle) while the participant maintained a maximal contraction of the L-OP.

The iSP parameters were computed from the rectified traces of the L-OP EMG. The iSP onset was defined as the point at which EMG activity decreased (minimum duration 10 ms) of at least 2 standard deviations relative to the baseline (60-10 ms prestimulus). The iSP offset was defined as the first point after iSP onset at which the EMG activity regained the baseline value. The iSP_{AREA} was defined as the EMG area between iSP offset and iSP onset, while $Baseline_{AREA}$ as the EMG area between 60 and 10 ms before the TMS stimulus (Spagnolo et al., 2013). We then calculated the iSP amount according to the formula: (Kuo et al., 2017)

$$iSP_{AMOUNT} = \left(1 - \frac{iSP_{AREA}}{Baseline_{AREA}}\right) * 100.$$

Subjects performed the experiment seated in a comfortable armchair and in front of a 17-inch LCD computer monitor (1024 × 768 pixels) placed 60 cm from their frontal plane. First, the abovementioned TMS parameters were measured during the continuous observation of a black screen with a white cross in its center (REST). Three separate sessions lasting two minutes were administered, one for each specific TMS protocol (standard MEPs, sICI, and iSP). While subjects were asked to keep their upper limbs relaxed during standard MEPs and sICI assessments, during the iSP assessment, they were requested to start the voluntary contraction upon the verbal request of the experimenter, who controlled and jittered the delivery of TMS pulses. For each TMS parameter, 15 TMS pulses were administered.

After the REST protocol, the same parameters listed above were estimated during action observation. In this protocol, subjects were asked to carefully observe 24 video clips depicting reach-to-grasp actions toward different objects. Each video, showing a pinch- or tri-digital grasp, represented the action from a first-person perspective and lasted 3.5 s. An intertrial (2 s, black background) was interposed between the videos. The overall action observation trial duration was about 2 min, in line with the resting condition. During the iSP assessment, subjects were requested to start the voluntary contraction at each action onset and relax during the intertrial. Within each session, in 15 of the 24 videos, TMS was randomly delivered 200 ms prior to hand-object contact. Such a latency has been previously shown as the timepoint providing the maximal MEP amplitude (De Stefani et al., 2013; Gangitano et al., 2001). Considering potential repetition suppression phenomena related to the TMS series (Pitkänen et al., 2017), the protocol sequence was randomized across participants. MEPs preceded by a muscular contraction (identified by an EMG amplitude > 50 μV within the 2" preceding the TMS delivery) and iSP preceded by an EMG activity < 90% of the maximal contraction, were excluded from the analyses.

2.3. Motor training

A modified version of the Nine Hole Peg Test (mNHPT) — a quantitative test of upper extremity function (Oxford Grice et al., 2003) — was adopted to assess motor performance. Previous studies have shown that the performance of the standard NHPT strongly depends on frontoparietal network functioning (Uggetti et al., 2016, Fiori et al., 2018). Moreover, NHPT performance improves with repetition over time (Solari et al., 2005), denoting the test's suitability as a motor learning endpoint. At baseline (T0), both the right and left hands were tested (see Fig. 1, task performance). Participants were seated at a table hosting a woodblock with nine empty holes on one side and a small container on the other. The latter was further split into two parts holding nine pegs and nine nuts, respectively.

On a start cue, subjects had to pick up the nine pegs one at a time as quickly as possible and put them in the nine holes according to a preestablished order (left to right, top to bottom). After placing the pegs in the holes, they had to apply the nuts in correspondence with each peg, following the same insertion order. Finally, they had to remove the nuts and pegs as quickly as possible—one at a time, placing them back into the proper container. Noteworthy, subjects were asked to adopt a first–fifth pinch grasp (thumb–little finger) throughout the task. This constraint, as well as the adding of the nuts, was introduced in the modified version of the test to increase task difficulty, thus delaying the performance "ceiling effect." The task was video-recorded and scored offline. The time required to perform the mNHPT was selected as the primary endpoint. In addition, errors, defined as placing, sequence, or hand-posture inaccuracies, were registered.

Participants belonging to the experimental (AOT) group were asked to observe a video clip showing a correct right-hand execution of the mNHPT (duration 1:16 min) and then to execute it as quickly and accurately as possible. This observation-execution combination was repeated six consecutive times (namely, T1–T6). The last trial (T6) also included left-hand mNHPT execution, thus allowing a direct before and

after training comparison of both hands' performance. Participants in the control group followed the same procedure, except the content of the video clip preceding the mNHPT execution depicted a landscape.

The time required to perform the mNHPT was recorded at each timepoint. The percentage decrease of total time relative to T0 (in other words, the increased speed) was computed. The T0–T6 percentage difference in right-hand mNHPT time of execution was set as the main behavioral endpoint. As secondary endpoint, also T0–T6 left-hand improvement in mNHPT execution speed was assessed.

2.4. Data analysis

Statistical analyses were performed using IBM SPSS version 25.0 for MacOs.

Since previous evidences showed that age can modulate motor excitability (Cuypers et al., 2013; Levin et al., 2011), intracortical inhibition (Hermans et al., 2018), and motor outcomes such as reaction times (Cuypers et al., 2013; Levin et al., 2011), a Spearman's rank correlation between participant's age and basal behavioral/neurophysiological features was performed as control analysis. Furthermore, the absence of significant differences between groups in basal behavioral/neurophysiological features was assessed with an independent-sample t-test .

The effect of action observation on motor cortex excitability was assessed by comparing the TMS parameters (MEPs, sICI, iSP) between the *rest* and *action observation* protocols by means of direct, nonparametric contrasts (Wilcoxon test). The choice of nonparametric tests was due to the absence of normality assumption.

Beyond investigating the modulations induced by action observation at the population level, we also moved to the individual level, thus computing the ratio between action observation and REST protocols for each of the TMS parameters:

(a) $MEPs_{(AO)}/MEPs_{(REST)}$
(b) $sICI_{(AO)}/sICI_{(REST)}$
(c) $iSP_{(AO)}/iSP_{(REST)}$.

Mixed ANCOVA was applied to the right-hand mNHPT speed increase, considering TIME as a within-subject factor, GROUP as a between-subjects factor and "age" as covariate.

As T0 served as a baseline for individual data, six levels were included in TIME (T1–T6). Planned multiple comparisons (Bonferroni correction) were made using independent sample, two-tailed t-tests, limited to the comparison between groups at each timepoint. A preliminary analysis was conducted to evaluate the correlation between basal TMS parameters estimated at rest (MEP amplitude, sICI) and motor improvement in both groups, using Spearman's rank correlation.

Subsequently, the capability of basal neurophysiological features assessing left-hemisphere excitability modulation by action observation ($MEPs_{(AO)}/MEPs_{(REST)}$, $sICI_{AO}/sICI_{REST}$) to predict motor outcomes (right- and left-hand T0–T6 mNHPT improvement) was separately evaluated in each group using a linear regression model; also, iSP_{AO}/iSP_{REST} ability to predict left-hand T0–T6 mNHPT improvement was assessed with a linear regression model .

In case of multiple significances, multiple linear regression models were also applied to evaluate the cross-talk between individual regressors. For each significant regression, a Bayesian factor ($BF_{1|0}$) was computed to quantify the evidence in favor of the alternative hypothesis (i.e., the neurophysiological feature *predicts* motor outcome) relative to the null hypothesis (i.e., the neurophysiological feature *does not predict* motor outcome).

Despite being widely adopted and easy to interpret as a motor learning endpoint, the mere difference between T0 and T6 does not account for the temporal dynamics of the learning process. Indeed, regardless of the T6 performance, the learning curve at T6 could exhibit higher/lower slopes. Thus, for each subject, we applied a regression model to fit the timewise performances into a logarithmic curve defined by the following equation: $y = A * \log(bx)$, where x indicates the trial number and the A coefficient indexes the slope of the curve. In the case of significant regression, the A coefficient can be regarded as a time-independent index of *motor learning drive*. Significant results would extend the validity of timepoint-specific observations to a global, time-independent dynamic. Then A values were compared between groups by direct contrast (independent samples, two-tailed t-test), and following the same statistical procedures described above, a linear regression was performed against baseline neurophysiological variables. The statistical significance threshold was set at p=0.05

3. Results

3.1. Participant's baseline features

No significant differences of the baseline behavioral/neurophysiological features were found between groups. Moreover, participant's age did not show any significant correlation with basal behavioral/neurophysiological features (see supplementary material 1).

3.2. Effect of action observation on neurophysiological parameters

In line with previous literature, CSE was higher during action observation than rest (1.46 ± 1.06 vs 1.65 ± 1.09 mV, Z [41] = -2.611, p = 0.007, *effect size* r = 0.408), indicating an average facilitation effect of 13%. Although the overall effect was significant, a consistent variability emerged at the single-subject level, as 14 out of 41 subjects (34%) showed a decrease in MEPs amplitude during action observation.

Action observation induced a significant reduction of sICI (78.25% ± 20.95% vs. 73.26% ± 25.00%, Z [40] = 1.747, p = 0.04, *effect size* r = 0.276). Even in this case, despite the overall significant decrease, 13 out of the 40 participants (32%) displayed an increase in sICI (i.e., an increase in the degree of inhibition) during action observation. Little overlap (n = 1) was observed between the 14 MEP suppressors and the 13 sICI enhancers. No significant change was observed comparing iSP amount at rest vs. action observation (90.47% ± 27.35 vs 90.88% ± 28.63 p = 0.35).

3.3. Effect of action observation on motor learning

Mixed ANCOVA showed that both TIME (T0-T6, F [5, 185], p<0.001) and GROUP (AOT vs controls, F [1, 37], p<0.001) factors had a significant effect on motor improvement. No significant time*group, nor time*age interactions were found. An exploratory analysis based on planned contrasts indicated that subjects undergoing AOT outperformed controls already after the first session of the training and throughout its entire duration (Contrast at T6: 27.67% ± 6.4 vs. 19.01% ± 3.1; t[39] = −5.362; p<0.001; Cohen's d = 1.718, see Fig. 2, Panel A)."

To evaluate the effect of training on the hand contralateral to that actively practiced, we assessed hand motor performance even in the left hand (LH) before and after training (i.e. at T0 and T6). Also LH performance improved more in subjects undergoing AOT (20.01% ± 7.16 vs 14.55% ± 7.95; t[39] = −2.288; p = 0.028; Cohen's d = 0.733; see Fig. 3, Panel A).

3.4. Neurophysiological signatures predicting motor learning

Neither MEPs nor sICI collected at baseline in rest condition were correlated with the motor improvement of right- or left-hand performance (see supplementary material 1).

To investigate whether the CSE gain driven by action observation ($MEPs_{(AO)}/MEPs_{(REST)}$) and the modulation of intracortical inhibition by action observation ($sICI_{(AO)}/sICI_{(REST)}$) can predict the improvement

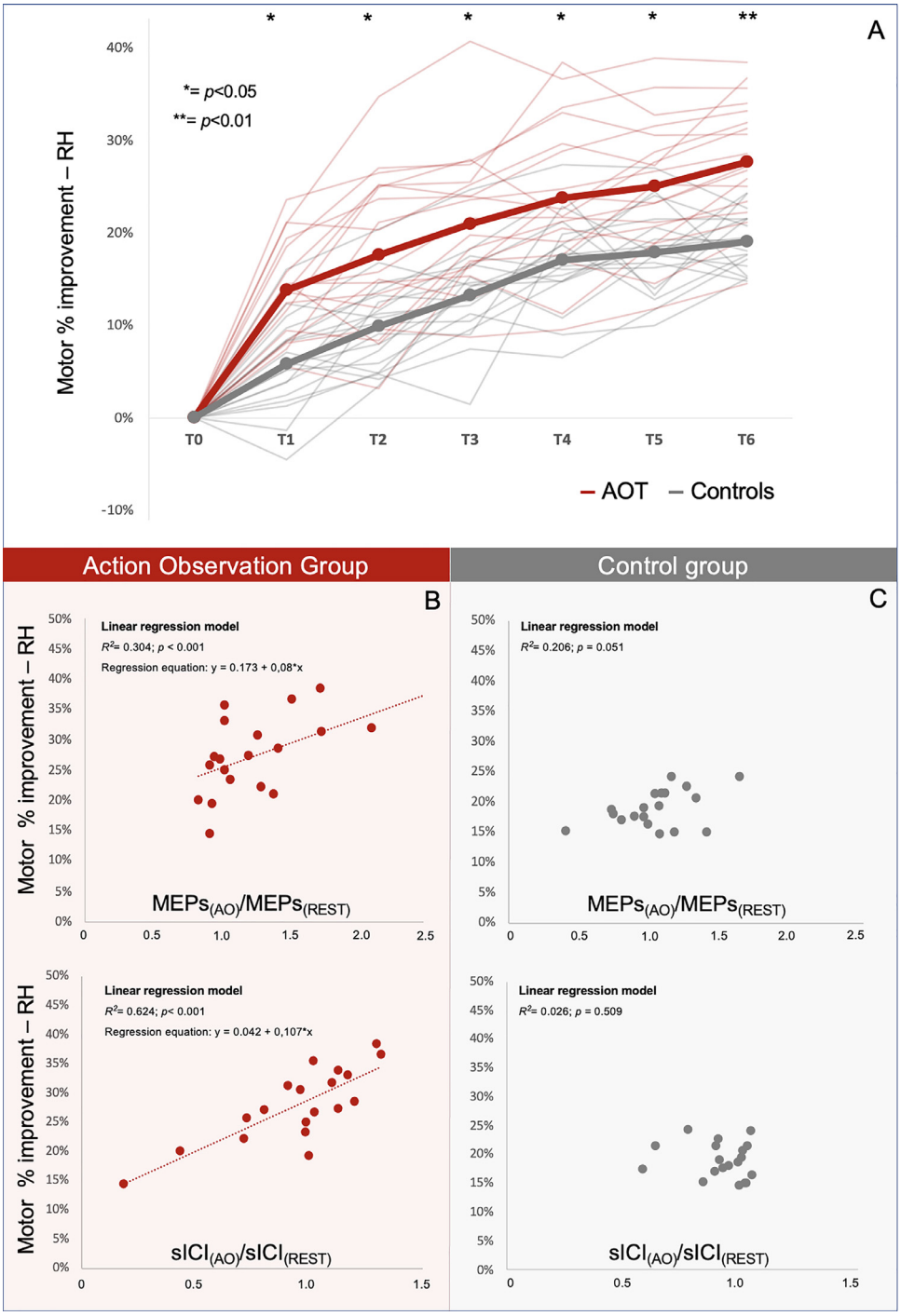

Fig. 2. Right-hand motor improvement induced by action observation training and neurophysiological predictors of efficacy.
Panel A. Right-hand modified version of the Nine Hole Peg Test variations across evaluation timepoints in action observation training (red lines) and control group (gray lines). Single-subject learning trajectories and mean values are represented in thin and thick lines, respectively. Asterisks indicate the level of significance in between-groups multiple comparisons (Bonferroni correction) across timepoints. **Panel B.** Scatterplot showing the interplay between right-hand modified version of the Nine Hole Peg Test T0–T6 improvement in the action observation training group and (1) motor evoked potential amplitude gain induced by action observation (top) and (2) intracortical inhibition relative increase during action observation (bottom). **Panel C.** Scatterplot representing the same variables of Panel B in the control group.

in right- and left- hand motor performance, linear regression models were separately applied in the two groups.

Linear regression showed that both $MEPs_{(AO)}/MEPs_{(REST)}$ ($R^2 = 0.304$, $p<0.001$) and $sICI_{(AO)}/sICI_{(REST)}$ ($R^2 = 0.624$, $p<0.001$) are significant predictors of the right-hand motor improvement following AOT (see Fig. 2, Panel B). A multiple linear regression model confirmed the stronger predictive value of $sICI_{(AO)}/sICI_{(REST)}$ in comparison to $MEPs_{(AO)}/MEPs_{(REST)}$ ($R^2 = 0.624$ vs. $R^2 = 0.339$, $p<0.001$) but also indicated their combination as the best predictor of right-hand motor improvement ($R^2 = 0.680$, $p<0.001$). Bayesian factors confirmed a lower level of evidence for $MEPs_{(AO)}/MEPs_{(REST)}$ ($BF_{1|0} = 5.64$) relative to $sICI_{(AO)}/sICI_{(REST)}$ ($BF_{1|0} = 312.01$) and their combination ($BF_{1|0} = 234.33$), both indicating a decisive level of evidence (Kass and Raftery, 1995).

Moving to the left hand, the linear regression model identified $MEPs_{(AO)}/MEPs_{(REST)}$ as a predictor of motor improvement ($R^2 = 0.366$, $p<0.01$, see Fig. 3, Panel B). Here, the correspondent Bayesian model returned a $BF_{1|0}$ of 5.193, indicating a substantial level of evidence (Kass and Raftery, 1995) in favor of the alternative hypothesis.

It is worth noting that the linear regression models applied to the controls did not return any significant results (see Panel C of Figs. 2 and 3), supporting that the AOT motor outcome is specifically predicted by the effect of action observation on corticospinal excitability and intracortical inhibition.

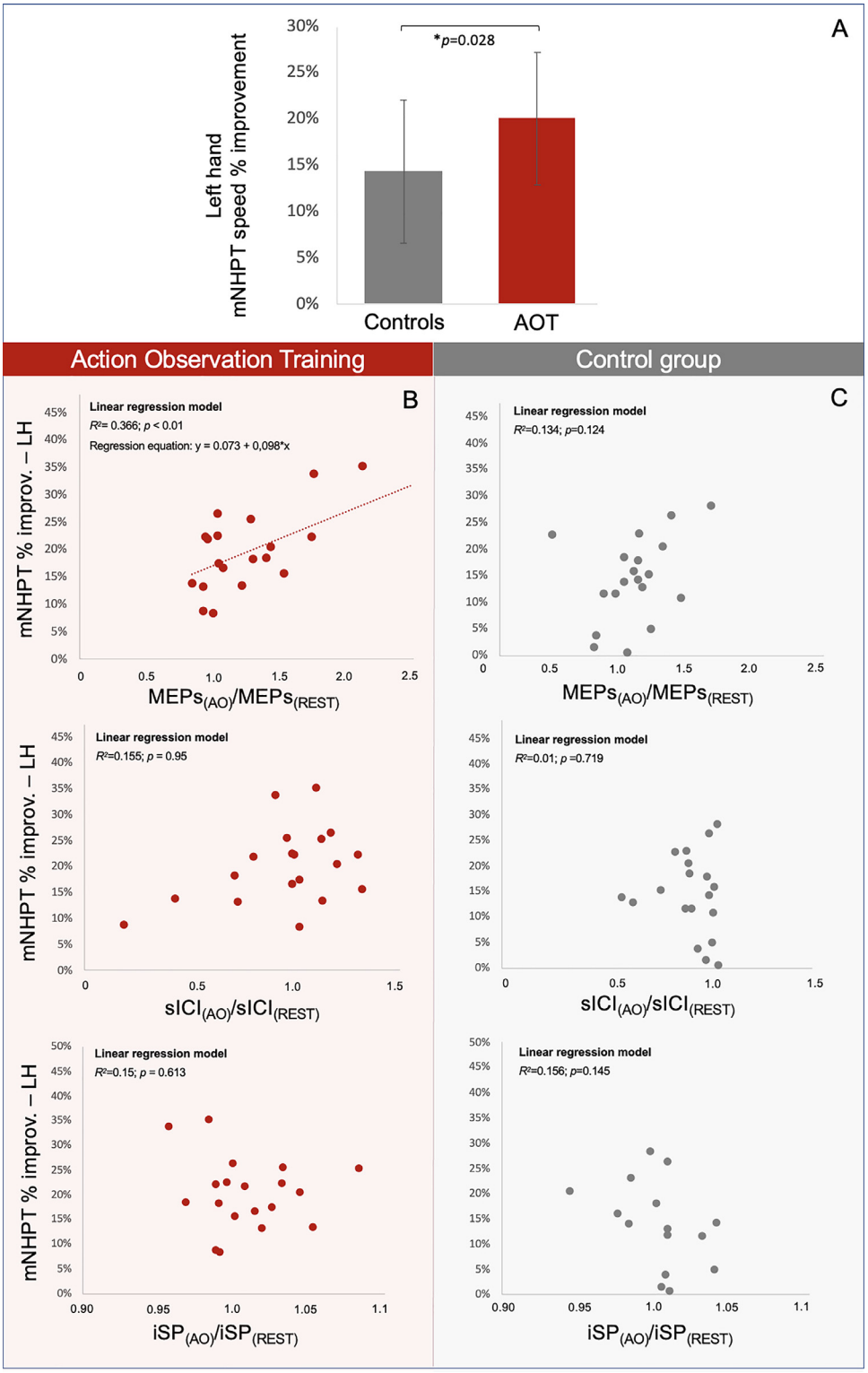

Fig. 3. Left-hand motor improvement induced by action observation training and neurophysiological predictors of efficacy.
Panel A. Left-hand modified version of the Nine Hole Peg Test T0–T6 changes between two groups. **Panel B.** Scatterplot showing the interplay between left-hand modified version of the Nine Hole Peg Test T0–T6 improvement in the action observation training group and (1) motor evoked potential amplitude gain induced by action observation, (2) intracortical inhibition relative increase during action observation, and (3) interhemispheric inhibition relative increase during action observation. Motor evoked potential amplitude gain induced by action observation and resultant left-hand motor improvement significantly correlated. **Panel C.** Scatterplot representing the same variables of Panel B in the control group.

3.5. Regression fitting model

Individual data of right-hand performance were fitted with a logarithmic model ($y = A * \log(bx)$, where x indicates the trial number [see Methods]). Subjects' curves showed excellent fitting values (all $p<0.05$), with adjusted R^2 ranging from 0.605 to 0.960 (mean $R^2 = 0.833$). The comparison of A coefficients between groups showed higher values in AOT subjects than in controls ($t[39] = -3.785$; $p<0.001$; $\eta^2 = 0.279$), supporting that AOT biases the whole motor learning trajectory beyond the single time points.

In line with the previous analysis, a linear regression was tested between the estimates of the A coefficient and neurophysiological features. Both $MEPs_{(AO)}/MEPs_{(REST)}$ ($R^2 = 0.329$, $p<0.001$) and $sICI_{(AO)}/sICI_{(REST)}$ ($R^2 = 0.575$, $p<0.001$) were significant predictors of A, thus extending the predictive power of such neurophysiological signatures on time-independent AOT outcome.

4. Discussion

In the present study, we first assessed the modulatory effect of action observation on motor system excitability using TMS, confirming that – despite a remarkable interindividual variability – action observation overall induces a corticospinal facilitation (Fadiga et al., 1995; Naish et al., 2014; Patuzzo et al., 2003) and a decrease of intracortical inhibition (Cardellicchio et al., 2020, 2018; Patuzzo et al., 2003; Strafella and Paus, 2000). Subsequently, the same participants were randomized to receive either (1) an Action Observation Training (AOT), consisting of the repeated alternation of observation and execution of the action to be trained, or (2) a control training, where participants had to observe a no-action video before performing the same task. AOT participants outperformed controls and –more interestingly – the behavioral improvement induced by AOT was predicted by the modulation of corticospinal excitability and intracortical inhibition driven by action observation assessed before the training.

4.1. *Insights on motor circuitry underlying motor learning* via *action observation*

A large body of research indicates that action observation can promote the acquisition of new motor skills and especially the perfectioning of abilities requiring fine motor control (Rizzolatti et al., 2021). Our results confirmed that integrating the observation of unimanual actions within motor learning procedures amplifies the learning magnitude (Bazzini et al., 2022), and that improvement extends also to the limb contralateral to the one observed and actively practiced. In addition, the extent of motor improvement driven by AOT is predicted by the modulations of corticospinal excitability induced by action observation, indicating that a greater MEPs facilitation evoked by action observation is predictive of a more favorable AOT outcome. From a neurophysiological perspective, we could speculate that the neural mechanism transiently ignited by action observation could lead to neuroplasticity changes, supporting the better AOT outcomes.

Looking at the organization of the motor system, three (non-mutually exclusive) scenarios might explain why action observation enhances corticospinal excitability, in turn favoring neuroplasticity changes (see Fig. 4). First, sustained inputs from excitatory cortico-cortical projections from premotor (Rizzolatti and Luppino, 2001) and parietal (Bruni et al., 2017) areas hosting mirror mechanism may increase M1 excitability, forging the motor representations of the muscles and movements involved in the trained task. In support of this view, it has been recently demonstrated that the synaptic potentiation of premotor-to-M1 connections—a key neuroanatomical pathway underlying motor facilitation *via* mirror mechanism (Bruni et al., 2017; Kilner et al., 2009; Rizzolatti and Sinigaglia, 2016; Shimazu, 2004)—improves the performance of a dexterity task highly similar to that adopted in our study (Fiori et al., 2018). A second contribution could derive from corticospinal *mirror* neurons outside M1 (e.g. ventral- and dorsal- premotor cortex, supplementary motor area and inferior parietal lobule, Hardwick et al., 2018a), which affect the spinal excitability *via* disynaptic outputs (Borra et al., 2010; Dum and Strick, 1991, 1996; Kraskov et al., 2014, 2009; Morecraft et al., 2019; Rathelot et al., 2017; Strick et al., 2021), thus potentially supporting spinal plastic changes underlying hand's motor control improvement (Weiler et al., 2019; Wolpaw and Tennissen, 2001). Third, cortico-striatal neurons endowed with a *mirror* mechanism (Bonini, 2017; Prather et al., 2008) may in parallel modulate the corticospinal gain and favor the automatization of the motor acts composing the observed action. While we cannot be definite about the individual weights of these three branches on the increase of CSE recorded in the present study, this topic is worth being deepened for two essential reasons. On one side, experimental procedures on animal and human models could illuminate the motor pathways traveled by mirror-triggered neuronal information. Alongside, non-invasive neuromodulatory techniques (TMS, tDCS) and neuropharmacological interventions (e.g., dopaminergic modulators) could be designed specifically to enhance these components, thus increasing the chances of action observation to induce neuroplastic changes and ultimately increase the motor learning outcome.

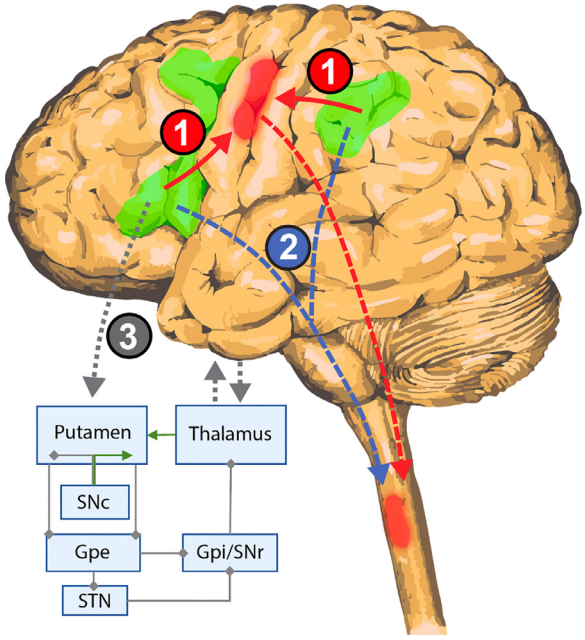

Fig. 4. Anatomical models explaining the link between corticospinal facilitation induced by action observation and AOT outcome. Corticospinal projections represented in dashed lines. (1) sustained inputs from excitatory cortico-cortical projections (red continue arrows) from premotor and parietal areas hosting mirror mechanism (in green) may increase M1 excitability, forging the motor representations of muscles/movement involved in the trained task. (2) Corticospinal projections from premotor and parietal cortices hosting mirror mechanism (blue dashed arrows) may induce spinal plastic changes underlying hand's motor control improvement. (3) Cortico-striatal neurons endowed with a mirror mechanism may modulate the corticospinal gain and favor the cortico-basal loops (grey arrows) subserving the automatization of basic movements required in the motor task.
Abbreviations. SNc: Substantia Nigra, pars compacta; SNr: Substantia Nigra, pars reticolata; Gpe: Globus pallidus externum; STN: Sub-thalamic nucleus; Gpi: Gpe: Globus pallidus internum. Mesial structure of motor cortex endowed with mirror mechanism, e.g. Supplementary Motor Area, are not displayed.

Interestingly, the right-hand muscle facilitation evoked by action observation predicts both right- and left-hand improvement. While the transcallosal transfer between contralateral and ipsilateral M1 could in principle explain this finding, the lack of correlation between the left-hand motor improvement and the iSP modulation does not support this view. A more likely explanation has then to be found in the bilateral *mirror* activation typically evoked by the observation of unimanual actions (Hardwick et al., 2018b).

Despite offering a comprehensive and network-scale perspective on corticospinal excitability, single-pulse TMS does not disambiguate the modulations induced by different neuronal populations and neural structures. Conversely, paired-pulse TMS allows measuring the intracortical inhibition (i.e., sICI) driven by the modulation of local GABAa interneurons (Cardellicchio et al., 2021; Kujirai et al., 1993; Leodori et al., 2019), moving from a *global* view of the whole motor chain to a *local* view of the inhibitory circuitry within M1. Such a local perspective is even more relevant when investigating the neural substrates of dexterous tasks performance (Strick et al., 2021), in which speed and accuracy depend on the proper selection of which muscles *to move* or *not to move* in each action instant. This ability strongly relies on cortico-cortical inhibitory projections, which sustain the precise recruitment of neural representations of appropriate movements and suppress the

undesired motor activity (Beck and Hallett, 2011; Greenhouse, 2022). This tuning of excitatory and inhibitory mechanisms throughout cortical circuits (Ebbesen and Brecht, 2017) plays a major role in the context of visuomotor tasks (Giboin et al., 2021), precise hand movements (Maier et al., 1993), and tool use (Quallo et al., 2012).

We found that the modulation of sICI by action observation largely predicts AOT efficacy, explaining more than 60% of its variance. Specifically, larger increases in intracortical inhibition during action observation corresponded to better AOT learning curves. The evidence in favor of this relationship is very strong, more than three hundred times more likely than the *no link* hypothesis. Interestingly, the pattern of sICI findings revealed two major distinctions from the MEPs ones. First, the predictive power of sICI almost doubled the MEPs one. Second, sICI modulations predicted specifically the contralateral hand improvement, contrary to the bilateral predictive capacity of MEPs. Taken together, these aspects corroborate the view of MEPs as a system-level indicator lacking specificity, and sICI as a local indicator highly specific for the sampled motor chain. Findings intriguingly complementary to our study have been recently reported by Ferroni et al (2021), showing that within the action observation network mirror responses are found also for inhibitory interneurons. Integrating these multi-scale perspectives, we propose that the individual propensity to upregulate such inhibitory circuits in response to action observation may drive the functional shaping of M1 cortico-motoneuronal populations subserving highly fractionated patterns of muscle activity (Lemon, 2008; Rathelot and Strick, 2009; Shimazu, 2004; Strick et al., 2021), ultimately improving hand dexterity (Strick et al., 2021).

Finally, it is worth discussing whether and how the processes underlying MEPs and sICI modulations by action observation crosstalk. Despite reflecting opposite phenomena in terms of neural excitability, corticospinal facilitation and cortical inhibition are not antithetical actors within motor learning *via* action observation, but rather can be regarded as complementary. Indeed, when using both MEPs and sICI gain in a multiple regression framework, both remained significant and with almost stable predictive performance. Tentatively, it could be envisioned a *"spotlight"* model (see Greenhouse, 2022), where the gain of intracortical inhibition induced by action observation increases the signal-to-noise ratio within motor output pathways, favoring at the same time the "fine sculpting" of the observed action representation at the cortical level.

4.2. Corticospinal excitability and intracortical inhibition modulation by action observation: revisiting the dichotomic view toward a continuum

Most of previous TMS investigations on the *mirror mechanism* indicate that action observation exerts a facilitatory effect on corticospinal excitability (Fadiga et al., 1995; Naish et al., 2014; Patuzzo et al., 2003). Nevertheless, a significant interindividual variability in terms of both *direction* (i.e. facilitation, Fadiga et al., 1995; Naish et al., 2014; Patuzzo et al., 2003 vs. suppression, Hardwick et al., 2012; Sartori et al., 2012) and *amount* of modulation has been reported in most studies (see Naish et al., 2014 for a review). Accordingly, we found that action observation exerts an overall facilitatory effect at the population level, but with a relevant proportion (35%) of participants showing suppression of MEP amplitude during action observation.

These findings are not incompatible with the neurophysiological models described in the previous paragraph. Indeed, a *"behavioral strategy"* view (Ebbesen and Brecht, 2017; Freud, 1923; Naish et al., 2014) would account that motor pathways excitability is first enhanced by action observation, and subsequently suppressed by inhibitory projections from frontal cortices (Hannah and Aron, 2021; Krams et al., 1998; Tremblay et al., 2004), when subjects volitionally refrain from movement. In this view, suppressors could result when the latter, inhibitory drive outweighs the former, facilitatory component. Another interpretation could be that the suppression of MEPs amplitude during action observation reflects a larger inhibitory response evoked for the interneurons hosted in the primary motor cortex (Naish et al., 2014). However, the notion that MEPs suppressors do not correspond to sICI enhancers and the absence of correlation between MEPs suppression and sICI during action observation make this latter hypothesis unlikely. Finally, recent evidence proved that MEPs amplitude depends on the phase of the prominent motor oscillations at the time of the TMS pulse delivery (Zrenner et al., 2022). It is thus tempting to hypothesize that a larger variability of MEPs amplitude is found at baseline, i.e., when motor rhythms are synchronized alternating up- and down-states, compared to the action observation condition, during which motor rhythms are known to be desynchronized (Avanzini et al., 2012). In this perspective, a fairer comparison would require the administration of a TMS-EEG protocol controlling for the phase-locking of the TMS pulses with the underlying motor rhythms, ultimately disentangling the phase-locking contribution from the quantification of MEPs enhancers/suppressors ratio.

Moving to the intracortical inhibition, we found that – even with a remarkable interindividual variability – action observation provokes a transient downregulation of intracortical inhibitory circuits. Our results are in line with previous research, where sICI decrease has been related to action observation (Patuzzo et al., 2003; Strafella and Paus, 2000), joint actions (Cardellicchio et al., 2020) and action mistake observation (Cardellicchio et al., 2018). Previous literature addressing sICI modulation induced by action observation is sparser than its MEP counterpart, thus it is hard to establish whether variability is a fingerprint also of this TMS parameter. However, it seems relevant to highlight that in our study also sICI distributed its values quite openly, with about 20% of the participants showing marked sICI upregulation during action observation and another 20% presenting the opposite pattern.

Taken together, MEP and sICI findings at the population level indicate that motor susceptibility to action observation does not follow a dichotomic canon, instead positioning along a *continuum* in which the individual placement predicts, or even determines, the ability to learn *via* action observation.

Conversely, we did not find modulation of interhemispheric inhibition during action observation, in partial contrast with a previous report (Gueugneau et al., 2016). Methodological differences, such as the use of video clips instead of a live actor, as well as the absence of EMG-based dynamical TMS triggering, could have determined such divergences in results.

5. Conclusions

In the present study, we documented how action observation modulates different neuroanatomical substrates of the human motor system, providing an insight on the mechanisms making action observation training effective in promoting motor learning. We further demonstrated the predictive value of these electrophysiological signatures in explaining the amount of motor improvement driven by action observation, with a major role of intracortical inhibition upregulation in the fine-tuning of motor programs. Besides its theoretical significance, our study could pave the way for the development of neurophysiological models predicting the outcome of widespread training approaches grounded on the observation of other's actions. Extending such knowledge to clinical and rehabilitative contexts would help clinicians to improve the accuracy of prognoses and tune treatment plans, ultimately optimizing patients' rehabilitation pathways.

Declaration of Competing Interest

The authors confirm that they had no interests which might be perceived as posing a conflict or bias.

Credit authorship contribution statement

Arturo Nuara: Methodology, Conceptualization, Writing – original draft. **Maria Chiara Bazzini:** Data curation, Writing – review & edit-

ing. **Pasquale Cardellicchio:** Methodology. **Emilia Scalona:** Methodology, Writing – review & editing. **Doriana De Marco:** Methodology, Writing – review & editing. **Giacomo Rizzolatti:** Writing – review & editing. **Maddalena Fabbri-Destro:** Methodology, Writing – review & editing. **Pietro Avanzini:** Methodology, Formal analysis, Writing – review & editing.

Data Availability

Data will be available on an apposite data repository online

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.119825.

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