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***ACTION OBSERVATION TRAINING FOR THE ACQUISITION OF  
NOVEL COMPLEX MOTOR SKILLS: A BEHAVIORAL AND  
NEUROPHYSIOLOGICAL STUDY***

***ACTION OBSERVATION TRAINING NELL'APPRENDIMENTO DI  
NUOVI COMPITI MOTORI COMPLESSI: UNO STUDIO  
COMPORIMENTALE E NEUROFISIOLOGICO***

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## **ABSTRACT**

Via mirror mechanism, the observation of other's action can evoke in the perceiver the same motor representation concerning the same action, in terms of both action goal and kinematic features. Following this principle, the reiterated observation of an action, followed by its executional attempt, (i.e. action observation training - AOT) is widely adopted for the empowerment of motor skills. The objective of this study was to investigate the relationship between the AOT efficacy and the degree of kinematic similarity between the trainee and the observed model.

For this purpose, twelve subjects underwent an AOT protocol consisting in six consecutive sessions of a reach to-grasp-and-lift trials, using chopstick as effectors. Trial speed and secondary dexterity measures were collected across the training session. Muscular activity of the right hand was recorded across sessions in each subject by surface EMG, and subsequently compared with that previously recorded from the model performing the same task, computing their reciprocal kinematic similarity.

After training, participants showed a significant improvement of trial speed ( $p=0.007$ ) and hand dexterity, as measured by grasp attempts ( $p=0.023$ ) and lifting errors ( $p=0.036$ ). Trial speed improvement was correlated with the

convergence in kinematic similarity of muscle Opponens Pollicis: the greater was the similarity convergence between trainee and model, the greater was the motor improvement. Such a relationship, significant in the full trial ( $\rho=0.594$ ,  $p=0.046$ ), was even stronger considering the reaching phase ( $0.867$ ,  $p<0.001$ ), here predicting the 70% of motor improvement.

In conclusion, our study provided the first, preliminary evidence that the outcome of an Action Observation Training (AOT) is associated with (or even predicted by) the motor similarity between trainee and observed model. This finding would open to the introduction of dynamic manipulations of the kinematic features of the observed stimuli to maximize AOT efficacy.

# 1. INTRODUCTION

## 1.1 Mirror mechanism

Mirror neurons are a distinct class of motor neurons that discharge both when a subject performs a specific motor act and when it observes the same or a similar motor act done by another individual. Originally discovered in a specific sector (area F5c) of the ventral premotor cortex (vPMC) of the macaque monkey (di Pellegrino et al., 1992; Gallese et al., 1996), they have been subsequently described also in the rostral inferior part of its parietal lobe (area PFG and PF, Fogassi et al., 2005a). A fundamental property of mirror neurons is that they are activated by the observation of motor acts having the same goal they encode motorically.

In the framework of mirror neurons, there could be two main levels of congruence between the observed action and the executed one. Strictly congruent mirror neurons discharge when observed and executed effective motor acts are identical in terms of goal and in terms of the way to achieve it. By contrast, broadly congruent mirror neurons are triggered by similarity – but not identity – between the observed and executed motor act (Rizzolatti & Craighero, 2004).

One of the most widely accepted hypotheses on the function of the parieto-frontal mirror circuit is that it mediates the understanding of the observed motor acts. This issue has been addressed by two series of experiments. In the first one (Umiltà et al., 2001), mirror neurons responding exclusively during the observation of the late phase of grasping were tested in

two conditions. In the first one the monkey saw an object-directed action (“full vision” condition), in the other one the same action was presented with its final critical part (the hand-object interaction) deliberately hidden (“hidden condition”). The results showed that most of the neurons responding to the observation of grasping in the “full vision” condition, also discharge in the “hidden condition”, suggesting that it is the meaning of observed action – and not the mere vision of it – that triggers mirror neurons. The second study (Kohler et al., 2002) tested whether mirror neurons were able to represent action from their sound. Mirror neurons were studied while the monkey was observing a motor act characterized by a typical sound and while this sound was presented without the associated vision of the related motor act. The results showed that many mirror neurons that were responding to the observation of motor act accompanied by its sound, also discharged when the sound was presented alone. These neurons were named “audio-visual” mirror neurons.

The role of mirror neurons for the prediction of other’s actions has been demonstrated by neurophysiological studies by showing the relationship between motor intention and mirror activity. The first study providing evidence for this was conducted in monkeys in a single-neuron recording study. The experiment consisted of two parts (Fogassi et al., 2005b): In the first part, monkeys were trained to grasp an object with two different intentions: eating it or placing it in a container (motor paradigm). In the second part, monkeys observed the experimenter grasping the same object with the same intentions (visual paradigm). Neurons were recorded from the inferior parietal lobule (IPL), and their discharge during grasping was studied in the motor and visual paradigm. The results obtained during active movements showed that many IPL grasping-

neurons discharge with markedly different intensity according to the intention of the action (i.e., eating or placing).

The aim of the second experiment (visual paradigm) was to find out whether the visual responses of grasping neurons were modulated by the intention of the actions in which grasping was embedded. The discharge of most of the IPL mirror neurons was modulated by the observed agent's intention, thus showing that the observation of the action and context allows one to understand the agent's intention. Similar results were subsequently found in the premotor areas (F5) of the monkey (Bonini et al., 2010).

Thanks to neuroimaging and neurophysiological studies, the existence of a mirror system has been reported even in humans: when we observe an action, our motor system generates its inner representation, as if the action had been really executed. Such a property to transform sensory representations of others' behaviour into one's own motor or visceromotor representations concerning that behaviour, is named *mirror mechanism* (Rizzolatti & Sinigaglia, 2016).

Two are the main nodes endowed with mirror mechanism in humans: the frontal one includes the ventral premotor cortex (PMv) and the caudal part of the inferior frontal gyrus (IFG); the parietal one is the inferior parietal lobule (IPL) (Hardwick et al., 2018). On the basis of anatomical and functional observations, the frontal and parietal nodes can be globally regarded as homologous areas of F5 and PFG in the monkey. Of note, a huge and growing body of experimental evidences showed that, beyond premotor and inferior-parietal regions, a large number of cortical areas involved in the production of specific motor behaviors, selectively responds even when that behavior is merely perceived, indicating that the *mirror mechanism* is a basic principle of brain functioning (5,6).

Unlikely to monkeys, motor activation in humans can be evoked by the observation of both transitive and intransitive actions. Indeed, one of the first transcranial stimulation (TMS) study investigating mirror mechanism in humans showed that the observation of meaningless movements enhances cortical excitability, with a somatotopical correspondence to the effector performing the observed movements (Fadiga et al., 1995). The finding that mirror neuron system may also encode intransitive movements is very relevant and outlined the fact that mirror mechanism is involved in the process of imitation. In a fMRI study -in which volunteers movement in response to an observed action (imitative behavior) was compared to movement triggered by a cross (non-imitative behavior)- , Iacoboni et al (1999) showed that areas endowed in mirror mechanism (in particular the posterior part of IFG) were wider activated during imitation. Further neuroimaging studies suggested that mirror neuron system could carry out the first steps of learning by imitation. In particular, they are responsible for the discrimination of the individual elements of the action to be imitated and their transformation into the corresponding potential motor act by the observer (Buccino, Vogt, et al., 2004a).

A central feature of brain motor areas endowed with mirror mechanism is that their activation depends by the motor knowledge of the perceiver, being favored by the belonging of the perceived motor act to the observer's motor repertoire. This has been shown by a series of neuroimaging studies that investigated mirror activation in subjects trained in specific motor skills, comparing it to the activation induced by the same stimuli in untrained persons. Buccino et al.(Buccino, Lui, et al., 2004), in a fMRI study, investigated brain activation in response to observation of motor acts performed by different species: human, monkey and dog. Two types of gestures were shown: the act of



biting a piece of food and oral silent communicative gestures (e.g. speech reading, lip smacking and barking). Biting, regardless of the action's agent, produced bilateral activations in the IPL-IFG circuit, virtually identical for three species especially in the left hemisphere. Communicative gestures performed by a human activated the mirror system (in particular IFG); those performed by non-conspecifics only weakly activated it (monkey gesture) or did not activate it at all (silent barking). In a further study, the mirror responses have been investigated in three different groups of participants: classical dancers, teachers of Capoeira and dance-naïve people. Stimuli consisted in videos of either Capoeira or classical dance. The two expert groups showed a strong mirror activation accordingly to their specific expertise: mirror responses to Capoeira steps were stronger in Capoeira experts, while mirror responses to classical dance steps were stronger in ballet performers. In the following experiment, the authors disentangled the “visual” knowledge of dance steps with their motor expertise. Studying classical dancers, the effect of the observation of steps done by different genders on brain activity of male and female dancers was studied: mirror system was activated more strongly by steps executed by individuals of the same gender of the observer. Thus, we can conclude that the activation of mirror system depends on motor expertise and not on visual experience.

To date, most of the neurophysiological research pointed out that motor areas endowed with mirror mechanism mainly encode action goals rather than just mere joint displacements. However, also qualitative aspects of movement, including kinematic features, may be processed in brain areas belonging to the action observation network.

In a fMRI experiment, Casile et al (2010) compared the levels of blood oxygen level–dependent activity elicited by human actions complying with or violating the kinematic laws of human movements. Actions complying with normal kinematic laws of motion differentially activated the left dorsal premotor and dorsolateral prefrontal cortex as well as the medial frontal cortex. These findings suggest that the kinematic laws of human movements specifically modulate the responses of neuronal circuits also involved in action recognition.

To investigate whether decoding others' intentions on the basis of their kinematics depends solely on how much the kinematics varies across different actions, or rather it is also influenced by its similarity with the observer motor repertoire, De Marco et al (2020) performed the following kinematic experiment. The execution of reach-to-grasp and place actions, differing for target size and context, was recorded in terms of upper-limb kinematics in 21 volunteers and in an actor. Volunteers had later to observe the sole reach-to-grasp phase of the actor's actions and predict the underlying intention. Although participants performed above chance in intention recognition, the similarity of motor patterns positively correlated with recognition accuracy. Overall, these findings indicated that kinematic similarity exerts a facilitative role in intention recognition, providing further support to the view of action intention recognition as a visuo-motor process grounded in motor resonance.

However, especially when dealing with human-android interaction or in virtual reality environments, a higher perceived similarity with the observed model is not always predictive of a greater sensorimotor resonance. This is the case of the uncanny valley phenomenon: a high degree of subjective similarity leads to a decrease of perceiver's affinity for the observed model, producing a

sense of eeriness that would disrupt motor resonance process (Mori et al., 2012; Saygin et al., 2012).

## **1.2 Action Observation Training**

The neural bases of *imitation learning* were addressed by Buccino et al. (2004), using fMRI. Naive participants were scanned during four phases: 1. observation of guitar chords played by a guitarist (action observation); 2. a pause following observation during which the participants were instructed to perform motor imagery of the observed actions (motor imagery); 3. active performance of the observed chords (execution); and finally 4. rest. The results indicated that the inferior parietal lobule and the inferior frontal gyrus (i.e., mirror network) keep active during the whole imitation learning process, i.e., throughout action observation, motor imagery, and execution. This fronto-parietal network started to be active since the observation of the guitar chords, persisted during the following motor imagery with the additional recruitment of the middle frontal gyrus (area 46), and remained active also during the chords execution, together with motor and somatosensory areas. In a recent meta-analysis, Hardwick and colleagues investigated the respective networks underlying the three stages (action observation, motor imagery, and action execution) over hundreds of neuroimaging experiments, providing a comprehensive map of the neural substrates of each phase. Further, the intersection among these three renders a robust topography of the fronto-parietal regions endowed with the mirror mechanism.

The schema of the experiment by Buccino (2004) - observation, motor imagery, and execution - represents the scaffold of the Action Observation

Training (AOT). This training approach, used in motor rehabilitation as well as in motor training for healthy subjects, starts with the observation of actions to be performed. Action observation has been proven to be effective, even in isolation, to induce lasting changes in excitability within M1 cortical representations of muscles/movements involved in observed and executed actions both in healthy individuals (Celnik et al., 2006) and in stroke patients (Celnik et al., 2008). Of note, a functional reorganization of the primary motor cortex driven by training is a key index of neuroplasticity, associated with functional improvement and motor skills empowerment (Nudo et al., 1996; Traversa et al., 1997; Chieffo et al., 2016, 2013).

The main effect of AOT onto the cortical motor system is the potentiation of the proper motor program (i.e., the series of cortical and subcortical activations leading to the execution of a given action) across premotor and parietal sites. How does this potentiation impact the motor execution? Different anatomical substrates might underlie the motor improvement driven by AOT. One possibility is that premotor activation increases the excitability of M1; an alternative possibility is that the direct premotor descending projections determine the behavioral improvement (Rizzolatti et al., 2014). There is also evidence in the monkey that action observation directly might activate M1 neurons giving origin to corticospinal pathways (Vigneswaran et al., 2013).

Although action observation *per se* could lead to positive outcomes, there is evidence that linking action observation with action execution sets the premises for an optimal outcome. Indeed, while action observation is already capable of activating the fronto-parietal network underlying a given action, the final motor execution would then be facilitated by the progressive pre-activation of its neural substrates due to the previous stage.

Thanks to its capacity to activate the motor system, the action observation treatment represents a valuable tool to intervene on the motor system following peripheral or central motor damage, as well as to promote motor skills maintenance and acquisition (Rizzolatti et al., 2021).

Traditionally, AOT protocols have been tuned on action goal rather than action kinematic (Buchignani et al., 2019; Nuara et al., 2019; Sgandurra et al., 2013). Consistently, the motor outcomes of trials where AOT was adopted evidenced greater improvement in goal-oriented endpoints rather than in qualitative aspects of movement. (Buchignani et al., 2019; Sgandurra et al., 2013).

To date, no studies have investigated the relationship between the AOT efficacy and the kinematic relationship between the trainee and the observed model. To fill this gap, we conducted a AOT protocol aimed at perfecting a complex motor task, recording the electromyographic activity of the hand muscles of both trainee and observed model, finally comparing their kinematic similarity across the training sessions.

Even if not directly reflecting the spatial displacement of limb's segments during movement, in line with previous literature, we opted to recognize the electromyography as a *kinematic* measure, since its acknowledged role in the fine characterization of voluntary movement patterns.

## **2. OBJECTIVE**

The objective of this study to investigate the relationship between the AOT efficacy and the degree of kinematic similarity between the trainee and the observed model.

For this purpose, twelve subjects underwent an AOT protocol consisting in six consecutive sessions of a reach to-grasp-and-lift of marbles motor task, using chopstick as effectors. In each subject, muscular activity of the right upper limb was recorded by surface EMG and subsequently compared to those previously acquired from the model performing the same task. Trainees-model EMG similarity was then computed and related with the amount of the behavioral improvement.

## **3. METHODS**

### **3.1 Participants**

Twelve volunteers (1 M, 11 F, mean age  $24.25 \pm 3.39$ ) participated to the study. All the participants were selected according to the following inclusion criteria:

- Age 18-40
- Being right-handed according to Edinburgh Handedness Inventory (Oldfield, 1971)
- Having normal or corrected-to-normal vision
- Having no history of neurological/psychiatric disorders

All the participants provided written informed consent.

### **3.2 Hand dexterity baseline assessment**

Before the training procedure, participant's dominant hand dexterity was assessed with the Nine-hole-peg test (9HPT), a brief, standardized, quantitative test of upper extremity function. The participants, seated at a table with a small container holding nine pegs and a wood block containing nine empty holes, had to pick up on a start command the nine pegs one at a time as quickly as possible, put them in the nine holes, and, once they are in the holes, remove them again as quickly as possible one at a time, replacing them into the container. The total time to complete the task was recorded. [44].

### **3.3 Experimental design**

Each participant underwent an Action Observation Training composed by six consecutive sessions (S1-S6) made by fifteen consecutive trials, structured as follows.

First, participants were asked to observe a visual stimulus (duration 64.7 s) showing, from an egocentric perspective, the execution of the following reach-to-grasp and lift motor task: an expert model, using two chopsticks as motor effector, transported fifteen marbles from a plate to fifteen holes of a wooden board (see figure 1). During the observation, subjects were asked to keep their upper limbs relaxed. The visual stimulus was administered twice before the executorial task.

Then, adopting the same kit used by the model, subjects were instructed to execute as quickly and accurately as possible the action represented in the just observed stimulus, with the middle point of the board chosen as the starting

position of the motor task (for a detailed description of the kit adopted for the motor task, see appendix 1).

The time of execution was recorded session-by-session, considering as time onset the first effector displacement from the starting position. The time recording of each session was stopped in these three alternative conditions:

- 1) When the fifteenth marble was collocated on a hole
- 2) In case of falling of the fifteenth marble during the transport
- 3) When the time of the execution exceeded 180''.

Each observation-execution block was preceded by a “rest” condition where subjects were asked to observe a black screen (duration 64.7'').

Study design is summarized in figure 2.

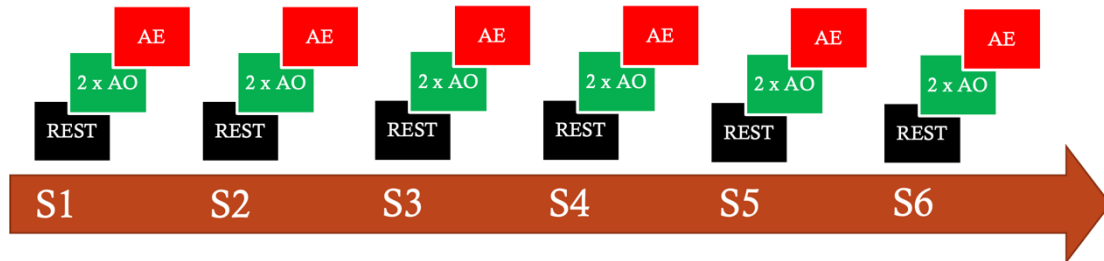
During each session, surface EMG was continuously recorded with wireless clip-electrodes in five muscles of the right upper limb: Opponens Pollicis (OP), First Digital Interosseous (FDI), Abductor Digiti Minimi (ADM), Extensor Digitorum Communis (EDC) and Flexor Digitorum Superficialis (FDS). The EMG signal was amplified ( $\times 1000$ ) using a digital amplifier (Cometa EMGandMotionTools), sampled at 2.5 kHz, filtered with an online band-pass (20–250 Hz) and a notch (50 Hz) filter, and acquired with Cometa device interfaced with a dedicated software. The data were stored for subsequent analyses.

EMG recording was synchronized with a videorecording acquired from two cameras, providing respectively a top- and lateral view of the participant.





**Figure 1:** *Representative series of frames extracted from the visual stimulus, showing an expert model (i.e. a person adopting wood chopstick as a usual feeding effector) transporting fifteen marbles from a plate to fifteen holes of a wooden board.*



**Figure 2.** *Study design. The Action Observation Training was composed by six sessions consisting in the chaining of (1) a rest period, (2) a double exposure to a visual stimulus representing the action to be trained, (3) the execution of the action observed just before.*

### 3.4 Data processing and analysis

The main behavioral endpoint was the Mean trial duration, i.e., the average time required to complete a reach-to-grasp-and-lift trial.

Other, secondary behavioral endpoints were the following:

- Number of grasp attempts

- Number of failed transports (i.e., accidental fallings of the marbles impeding the correct positioning in the board).

In each participant, the video-EMG recordings of the execution trials were segmented off-line in the following phases:

- 1) Reach, starting after the deposition of the previous marble and ending in the timepoint correspondent to the chopstick-marble contact. The only exception here was represented by the first reach, where the onset corresponded to the chopsticks lifting from the midpoint of the wood board.
- 2) Holding, starting at the chopstick-marble contact and ending at the onset of the lifting of the marble from the board surface
- 3) Transport, from the lifting of the marble from the board surface to the deposition of the marble in the board.

Noteworthy, only holding/transport phases without grasping errors/failed transports were included in the subsequent analyses. Then, the time duration of each phase was computed.

### **3.5 Statistical analyses of the behavioral endpoints**

Behavioral changes (mean time duration, grasping attempts, failed liftings) were analyzed using non-parametric tests (Wilcoxon test), comparing the performance of first executional attempt (S1) with those of the following training session (S2-S6).

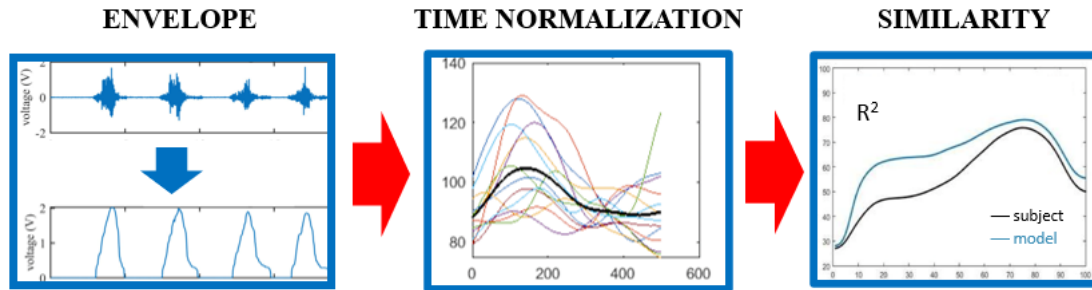
### 3.6 EMG processing and analysis

Participant's muscular activity was analyzed from two points of view: (1) the mean amount of muscular contraction and (2) the EMG pattern similarity between the trainee and the observed model.

The former characterization consisted in computing the mean amount of contraction in each phase (Reach, Holding, Transport) in the recorded muscles (OP, FDI, ADM, EDC, FDS) across the six training sessions (S1-S6). Such an amount of contraction was expressed in terms of percentage of that obtained during the maximal contraction of each muscle.

The latter described the profile of activation of each muscle for each specific phase, regardless of its duration. For this purpose, the EMG curves were processed according to the following steps (see figure 3):

- 1) *EMG envelope*. EMG traces were rectified and filtered in order to increase their smoothness.
- 2) *Time normalization*. EMG traces were time-normalized in all phases in order to be matched in duration.
- 3) *EMG similarity computation*. The extraction of patterns of EMG activity allowed us to compare the participant's EMG patterns with those previously obtained from the expert model, finally computing their kinematic similarity. This comparison was performed adopting the Linear Fit Method (LFM, Iosa et al., 2014). LFM calculates the linear regression between the subject dataset under investigation and model one, returning information and the trueness of the linear relation between them ( $R^2$ ). When the curves under analysis are equal, the values of LFM  $R^2$  tend to its ideal value of 1.



**Figure 3.** *Graphic representation of EMG processing phases (i.e., envelope, time normalization, similarity) adopted for the computation trainee-model EMG similarity.*

### 3.7 Correlational analyses

In order to explore the relationship between the AOT efficacy and the degree of kinematic similarity between the trainee and the observed model, the S1-S6 change in behavioral performance (mean trial duration) was correlated with the S1-S6 change of EMG similarity in the full trial and within each separate phase, by means of non parametric test (Spearman rho).

## 4. RESULTS

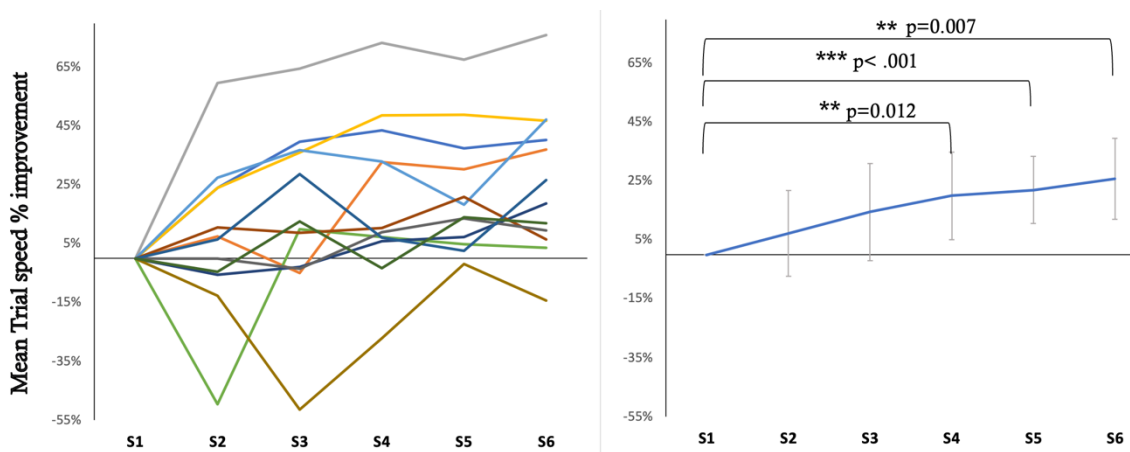
### 4.1 Compliance to experimental procedures

The procedures were well tolerated by all participants. No drop-out events were registered. Mild, subjective fatigue was reported by three participants, starting from the 4<sup>th</sup> or 5<sup>th</sup> session.

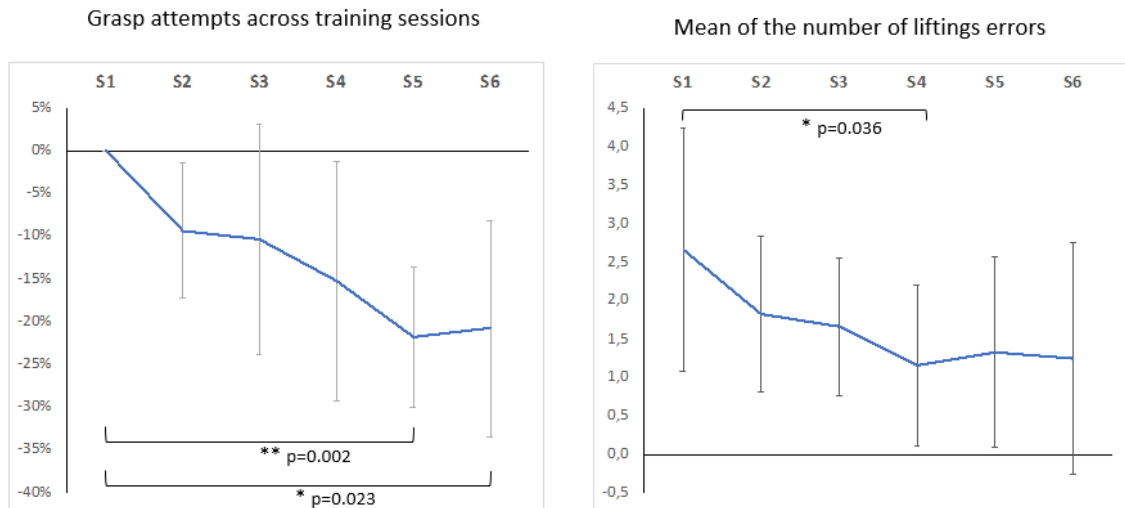
## 4.2 Behavioral endpoints

Participants showed a significant improvement of executional speed (figure 4). Significant changes of time of execution emerged at the following timepoints: S4 ( $z=2.432$ ,  $p=0.012$ ), S5 ( $z=2.981$ ,  $p<.001$ ), S6 ( $z=2.314$ ,  $p=0.023$ ).

Also, the number of grasp attempts (S5:  $z=3.059$ ,  $p=0.002$ ; S6:  $z=2.314$ ,  $p=0.023$ ) and the transport errors ( $z=2.201$ ,  $p=0.036$ ) improved across training sessions (figure 5).



**Figure 4.** Mean trial speed change (percentage from the first session) across training sessions.

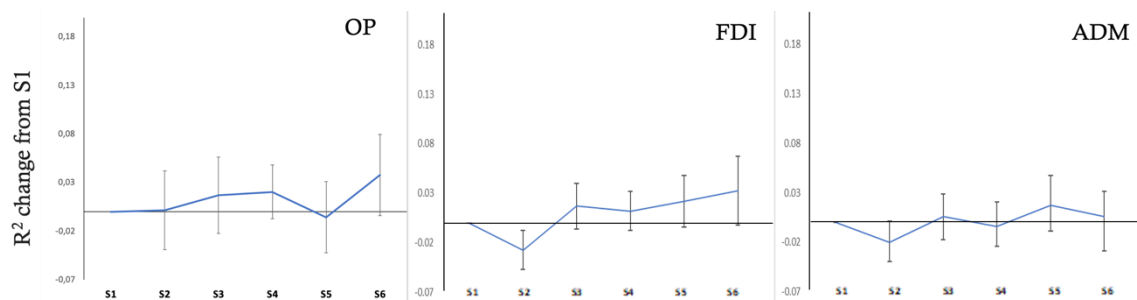


**Figure 5.** Left panel: change (percentage from the first session) of grasping attempts across training sessions. Right panel: mean of the number of lifting errors across training sessions

### 4.3 EMG endpoints

Within the full trials, no significant training-induced changes emerged in term of both (1) EMG amount of contraction and (2) EMG similarity across training sessions (see figure 6).

Looking within each single movement phases (i.e., reaching, holding, transport), a slight convergence of EMG similarity emerged in the reaching phase, limitedly to OP (S4:  $z = 0.042$ ,  $p < 0.001$ ).



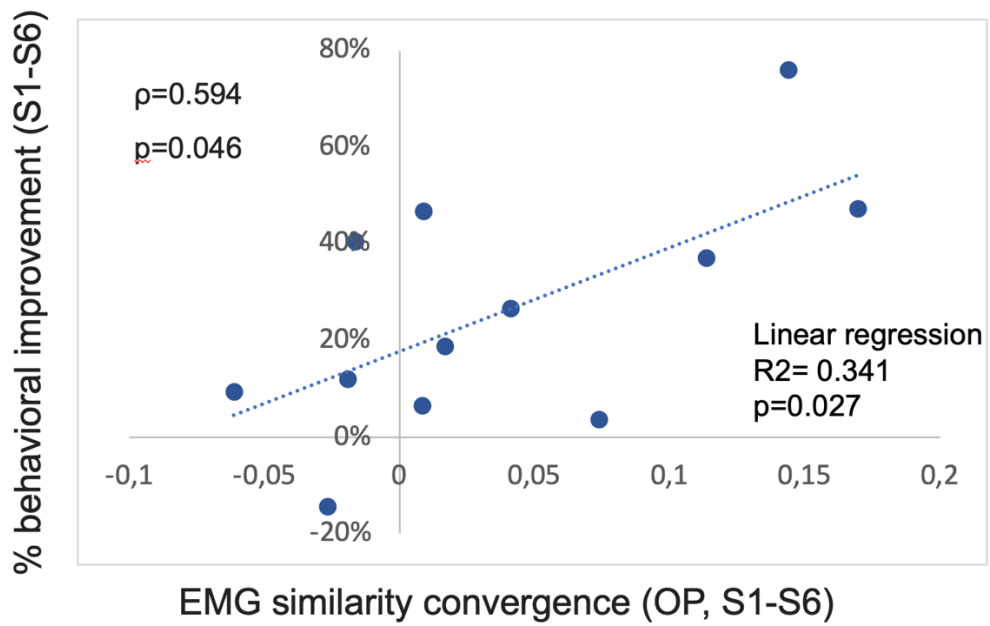
**Figure 6.** *Change in EMG similarity across training sessions. Graphic representation is limited to hand muscles (OP, FDI, ADM).*

#### **4.4 Correlational analyses**

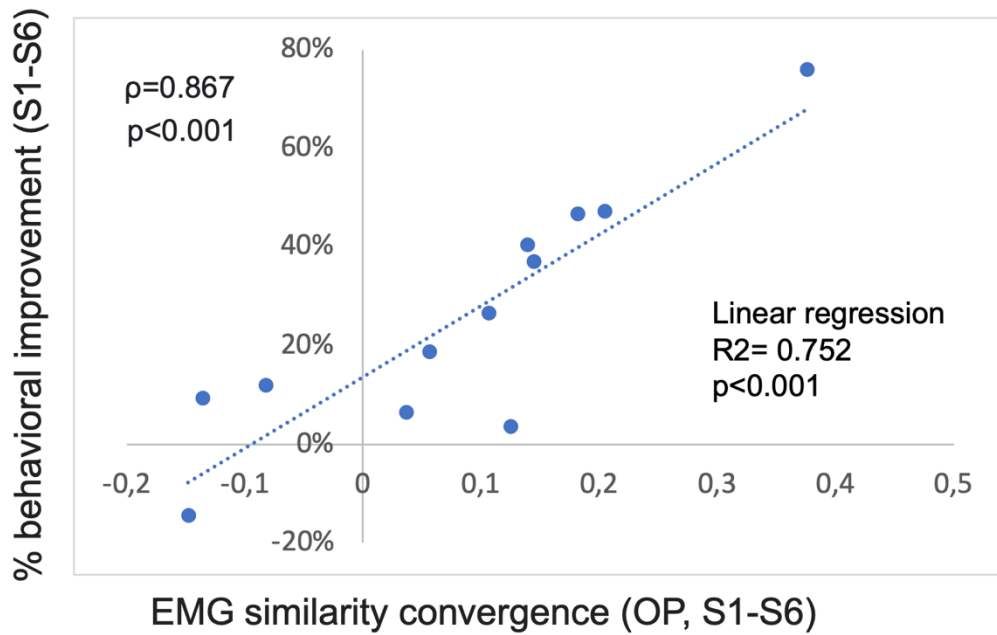
A positive, significant correlation was found between the S1-S6 improvement of EMG similarity in OP between trainee and model and the mean trial duration S1-S6 improvement (Spearman's  $\rho = 0.594$ ,  $p = 0.046$ ). In other word, the greater is the EMG similarity convergence between trainee and model, the greater is the motor improvement induced by the AOT. A linear regression model performed to assess the capability of EMG similarity convergence to predict behavioral outcome, resulted significant ( $p = 0.027$ ), returning a  $R^2 = 0.341$  (see figure 7).

In the field of the specific trial phases, an even stronger correlation emerged within reaching, where the S1-S6 improvement of EMG similarity in OP was positively associated to the mean trial duration S1-S6 improvement (Spearman's  $\rho = 0.867$ ,  $p < 0.001$ ). Here, the linear regression model ( $p < 0.001$ ) returned a  $R^2 = 0.752$  (see figure 8).

Moving to the relationship between the mean amount of EMG contraction and the behavioral improvement, no significant correlations were found.



**Figure 7.** Correlation between behavioral improvement (S1-S6% change in trial execution speed) and EMG similarity convergence in opponens pollicis (S1-S6  $R^2$  change) in the full trial.





**Figure 8.** *Correlation between behavioral improvement (S1-S6% change in trial execution speed) and EMG similarity convergence in opponens pollicis (S1-S6  $R^2$  change) in the reaching phase.*

## 5 DISCUSSION

In the present study we aimed at exploring the relationship linking the motor similarity between the trainee and observed model with the motor improvement promoted by an action observation training. For this purpose, we administered a AOT protocol consisting in six consecutive sessions of a reach-to-grasp-and-lift trials. Muscular activity of the right upper limb was recorded in each subject by surface EMG and subsequently compared with that previously acquired from the model performing the same task. We found that the trainee-model convergence of EMG similarity induced by the training is able to predict the AOT motor improvement.

The finding that action observation training (AOT) can induce the improvement of motor abilities, is in line with recent studies where AOT was applied for the acquisition of complex motor skills, showing an higher efficacy on motor outcome in comparison with action observation or motor practice administered in isolation (Bazzini et al., 2022).

The absence of a control group (i.e., not observing action-related stimuli before action execution) does not allow in our study conclusive remarks on the specific role of action observation in driving the observed motor improvement. However, some theoretical speculation about AOT neural substrates may be advanced, based on recent, controlled, neurophysiological investigations where AOT was applied for the improvement of reach-to-grasp-

and-transport hand dexterity tasks (Nuara et al., 2021). First, cortico-cortical projections from the premotor (Rizzolatti & Luppino, 2001) and parietal (Bruni et al., 2017) areas endowed with mirror mechanism to primary motor cortex, may have promoted short-term plastic changes subserving the performance improvement. Second, direct (Dum & Strick, 1991) descending corticospinal projections from premotor areas endowed with mirror mechanism may have induced at a spinal level neurophysiological readaptation favoring hand motor control improvement. Third, the sustained activation of cortico-striatal projections endowed with a mirror mechanism may have favored the automatization of the motor task (Bonini, 2017; Prather et al., 2008).

Differently from the behavioral realm, neurophysiological results did not show a significant impact of AOT in changing EMG activity over time, neither in terms of amount of muscular contraction, nor in terms of trainee-model similarity. Evidence of similarity convergence has been limitedly found for reaching, in the muscle opponens pollicis, with a remarkable heterogeneity in terms of EMG pattern modifications in the whole population.

Then, we hypothesized that individual propension to converge toward the observed model in terms of EMG similarity along the training sessions could affect the chances to respond to AOT. The finding that the greater is the EMG similarity convergence between trainee and model, the greater is the motor improvement induced by the AOT, supported this view. The power of opponens pollicis similarity convergence to predict AOT outcome, being moderate within the full trial, resulted even more stronger for the specific reaching phase: here, the EMG similarity convergence explained more than 70% of the behavioral improvement. Even if supporting the active role of the observed model in

driving the observer's motor learning, such a correlation needs to be interpreted with caution.

Indeed, other complementary hypotheses – not necessarily grounded on *mirror mechanism* – may be envisioned. For example, the kinematic confluence toward the expert EMG pattern may reflect a “trial-and-error” motor learning process, favoring the convergence toward a more proficient motor program, this latter similar to that traditionally adopted by the expert model.

Future, controlled experiments including a group not exposed to action-related stimuli, will help to define the specific role of action observation in driving motor learning, as well as to assess its role in the relationship between kinematic similarity and motor learning induced by AOT.

Another future perspective regards the inclusion of functional-neuroimaging endpoints. In addition to provide novel insights on the neural substrates of AOT efficacy, such investigations would uncover the role of brain areas encoding kinematic features of the observed model (e.g., dorsal-premotor cortex) in mediating the relationship between changes in kinematic similarity and motor improvement induced by AOT.

Another open question regards the role of model's expertise in tuning the trainee's responsiveness to AOT. What would have happened if, instead of a person who has always been used to handle chopsticks, the model had been unfamiliar with chopsticks use? The answer is far from obvious: on one hand, some clinical AOT studies suggest that is preferable for a trainee to observe a more expert model (Nuara et al., 2019), on the other hand, recent neuroimaging findings evidenced that observation of actions performed by a naïve model produce a stronger activation in parieto-premotor circuits (including the

superior parietal lobule and dorsal premotor cortex), compared to observation of an expert actor (Errante & Fogassi, 2019). Future neuroimaging-behavioral investigation administering AOT by models with different degrees of expertise will help to answer this question.

## **6.CONCLUSION**

In conclusion, our study provided the first, preliminary evidence that the outcome of an Action Observation Training (AOT) is associated with (or even predicted by) the motor similarity between trainee and observed model. If confirmed in controlled studies, these findings would open to the exploitation of novel treatment elements within AOT protocols: currently tuned on action goal, AOT would thus benefit from the addition of a novel ingredient (i.e., the dynamic manipulation of the kinematic features of the observed stimuli), in order to maximize its beneficial effect on motor performance.

## 7. Appendix 1

### Kit adopted for the motor task

The kit adopted for the motor task was composed by a squared plane bowl (size 15.2 x 15.2 x 1.5 cm) and a rectangular wood board (20.4 x 14.5 x 1.5 cm) with fifteen holes, distanced by 7.2 cm. The holes diameter is 1.1 cm (1 mm lower than the marbles diameter). Each hole was distanced from the other by 2.5 cm. The holes were collocated in the middle of the wood board. The length of the wood chopsticks is 24 cm.



**Figure 9:** The kit adopted for the motor task.

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