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PIÙ MI MUOVO COME TE, PIÙ IMPARO DA TE.
STUDIO COMPORTAMENTALE ED ELETTROFISIOLOGICO SULL'APPRENDIMENTO MOTORIO TRAMITE UN TRAINING DI OSSERVAZIONE DELL'AZIONE

THE MORE I MOVE LIKE YOU, THE MORE I LEARN FROM YOU.
A BEHAVIOURAL AND ELECTROPHYSIOLOGICAL STUDY ON THE MOTOR LEARNING *VIA* ACTION OBSERVATION TRAINING

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ABSTRACT

Action Observation Training (AOT), exploiting the properties of the mirror mechanism, represents an effective tool to promote the acquisition of new motor abilities (Rizzolatti et al., 2021; Bazzini et al., 2022). However, whether the motor improvement depends on the convergence of the observer's motor pattern onto the observed model remains unsettled. Thus, we designed an EMG experiment to investigate whether responsiveness to AOT depends upon a gain of similarity in terms of temporal patterns of muscular activity between the trainee and the model.

Seventy-two healthy subjects were enrolled in the study and randomly subdivided into two groups (AOT & CTRL). All participants had to learn to position 15 marbles on a board with holes using chopsticks. The training consisted of six sessions of observation and execution phases. During the observation phase, the AOT group observed an expert performing the task, while the CTRL group observed landscape videos. For each participant, the number of grasping attempts, the number of failed liftings, and the mean duration of the reach-to-place action were measured. Furthermore, during the six executions, the EMG pattern of 3 hand muscles was collected and compared with that of the expert. The gains in similarity were correlated with behavioural improvement for both groups.

While both AOT and CTRL groups improved upon the training, participants receiving AOT presented a larger improvement than CTRL, especially concerning decreased grasping attempts. A significant correlation was found between behavioural improvements and the degree of convergence toward the muscular pattern of the model. Interestingly, this applies to first digital interosseus - FDI, and abductor digiti minimi -

ADM in the reaching and holding phases of the action. Of note, these correlations were specific for AOT participants.

These results confirm the previous findings on the key role played by AOT in driving motor learning, linking it to the convergence of neuromuscular pattern of the trainee towards the observed model, thus extending the assessment of the neurophysiological correlates of AOT beyond the boundaries of the cortical motor system.

1. INTRODUCTION

1.1. Mirror System in Monkeys and Humans

Originally found in the region F5 of the monkey premotor cortex, mirror neurons are a specific subclass of visuomotor neurons that fire both when the monkey performs a specific action and when it witnesses another person (or monkey) performing the same action (Di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a). Specifically, they were discovered in the sector F5c of the ventral premotor cortex, which controls hand and mouth movements (Rizzolatti et al., 1981; Rizzolatti et al., 1988). In the F5 area of the monkey, there is another class of visuomotor neurons, canonical neurons active when a 3D object is presented, and action on the object is not needed (Luppino et al., 2005).

Beside the promotor cortex (PM), mirror neurons have also been recorded in the inferior parietal lobule (IPL) (Fogassi et al., 2005; Rozzi et al., 2008). The network composed by IPL and PMv is the most studied among the networks having a mirror mechanism (mirror systems). This network transforms sensory representations of observed (or heard, Kohler, 2002) motor actions into motor representations of that actions. The congruence between the action motorically coded by the neuron and that observed triggering the same neuron can be strict or broad. More precisely, we have the class of strictly congruent mirror neurons discharging when the observed and executed effective motor acts are identical both in terms of goal (e.g., grasping) and in terms of the way in which that goal is achieved (e.g., precision grip), and the class of broadly congruent mirror neurons activated by similarity – not identity – between the observed action and the executed one (Gallese et al., 1996).

Research on mirror neurons has a long tradition. Many studies suggest that neurons with mirror properties typically encode the goal of the action. The expression "action goal" indicates the overall aim of an action (Rizzolatti & Sinigaglia, 2016). It has been discovered that F5 mirror neurons respond to the observation of an action presented with the final part hidden, suggesting that the meaning of the action triggers the neuron and not just the mere vision of it (Umiltà et al., 2001). Furthermore, mirror neurons discharge in conditions where the monkey has enough clues to represent a mental description of what the agent does (Rizzolatti & Craighero, 2004).

A large number of studies provide indirect evidence that a mirror system also pertains to humans. Neurophysiological and brain imaging studies provided proof of this. Despite the lack of overt (i.e., action imitation) motor activity, neurophysiological studies showed that when people witness another person doing an action, their motor cortex becomes active (Rizzolatti & Craighero, 2004). The reactivity of brain rhythms during the observation of movements was validated by EEG research carried out around the 1950s (Gastaut & Bert, 1954), supporting the existence of what is now known as the mirror mechanism in humans (Rizzolatti & Sinigaglia, 2006).

Relevant insights into the human mirror mechanism were also provided by transcranial magnetic stimulation (TMS) studies. TMS is a non-invasive technique allowing exogenously evoke the activity of a given cortical region. When applied to the motor cortex, it is possible to record the motor-evoked potentials (MEPs) from the muscles of the contralateral body part. By using TMS on the left motor cortex, Fadiga and co-workers (1995) evoked motor potentials in different muscles of the right hand and arm in subjects who were asked to observe an experimenter grasping objects with his hand (transitive hand actions, i.e., direct toward an object) or making apparently insignificant gesture (intransitive arm movements, i.e., not direct to an object). In both conditions, a selective increase in MEPs of those muscles that are activated by the production of the observed movement was found (Rizzolatti & Craighero, 2004).

An important property of human mirror system is that the cortical facilitation time course during action observation follows the temporal course of the movement execution as demonstrated by Gangitano and co-workers (2001). This suggested that the mirror system in humans codes the action goal plus the temporal aspects of the movements that compose the action.

While EEG and TMS suffer from low spatial resolution, brain imaging techniques can provide a three-dimensional and spatially resolved view of the metabolic changes caused by the execution and observation of specific motor actions, thus estimating the relative activation level. In particular, Positron Emission Tomography (PET) data confirmed results from studies on monkeys' mirror neuron system, indicating that frontal areas are active during the observation of hand actions (Rizzolatti et al., 1996b). Similar results were found in later Functional Magnetic Resonance Imaging (fMRI) studies (see Caspers et al., 2010; Hardwick et al., 2018).

Understanding the goal of other people's acts and their intentions is one of the functions that the human parieto-frontal mirror system supports. The inferior parietal lobule (IPL) and the ventral premotor cortex (PMv) are the two primary nodes of the human mirror system (see Figure 1.1), according to numerous brain imaging studies (see Fabbri-Destro & Rizzolatti, 2008). According to Buccino and colleagues (2001), the mirror responses of the premotor node are somatotopically organized. The observation of motor actions performed with three effectors (hand, foot, mouth) activates the corresponding fields in the motor system.

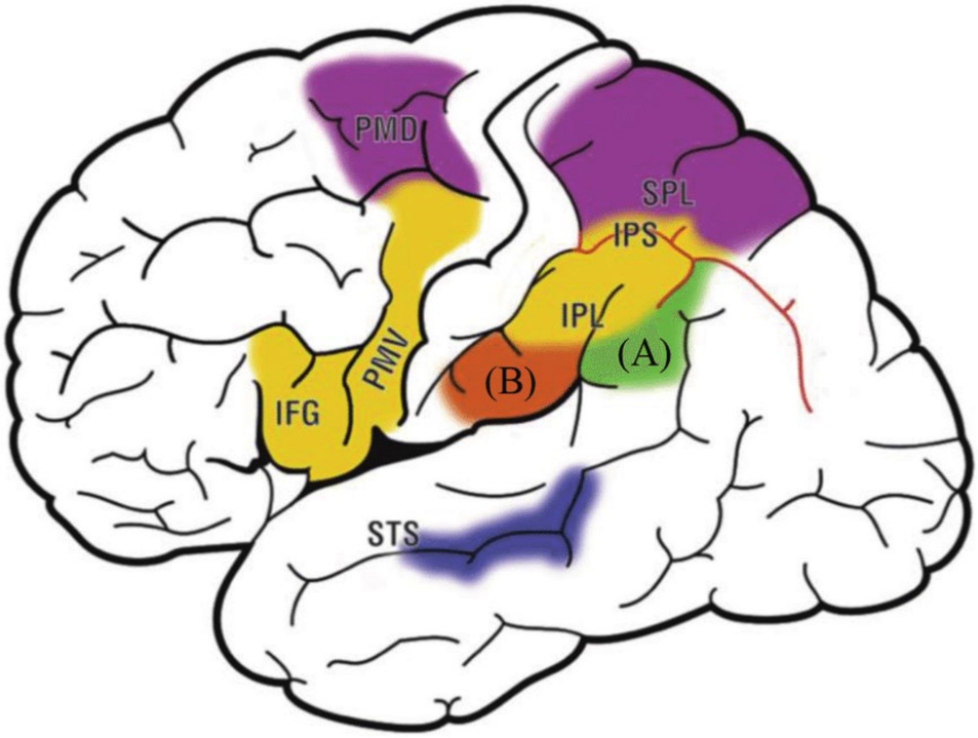

Figure 1.1 Mirror neuron system in humans. *Purple areas (PMD and SPL) are involved in reaching movements. Yellow areas (IFG, PMV, IPL, and IPS) are involved in transitive distal movements. Blue areas (STS) are involved in the observation of upper-limb movements. Green areas (A) are involved in intransitive movements. Orange areas (B) are involved in tool use. PMD indicates dorsal premotor cortex; SPL, superior parietal lobule; IFG, inferior frontal gyrus; PMV, ventral premotor cortex; IPL, inferior parietal lobule; IPS, intraparietal sulcus; STS, superior temporal sulcus*. *From Nakano & Kodama, 2017*.

A larger activation of the mirror mechanism is determined by motor actions that are well-represented in the observer's motor repertoire. This was demonstrated by brain imaging experiments (Calvo-Merino et al., 2005, 2006) that compared mirror activations of experts in specific motor abilities with activations dictated by the same stimuli in individuals with diverse motor expertise. The findings revealed that the observed activities are mapped onto the correspondent motor programs of the observer and that activation is stronger in people skilled at performing them (Calvo-Merino et al., 2005).

In conclusion, observing acts performed by others generates an immediate involvement of the motor areas involved in the organization and execution of those acts in the observer. This engagement enables decoding the meaning of observed motor events.

1.2. AOT: Action Observation Training

In an fMRI study, Buccino and co-workers (2004) addressed the neural bases of imitation learning by administering subjects four different 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 4. rest. The study's results showed that the mirror network, comprising the inferior parietal lobule and inferior frontal gyrus, was active during the entire imitation learning (action observation, motor imagery, and execution) process. Using hundreds of neuroimaging studies, Hardwick and co-workers (2018) examined the corresponding networks supporting the three stages (action observation, motor imagery, and action execution), producing a detailed map of the brain substrates of each phase (see Figure 1.2). The conjunction between action observation and execution provides a reliable image of the fronto-parietal regions endowed with the mirror mechanism, interestingly largely overlapping also with the cortical territories mediating motor imagery. The structure of the study by Buccino et al. (2004) – observation, motor imagery, and execution – stands as the scaffold for the Action Observation Treatment (AOT).

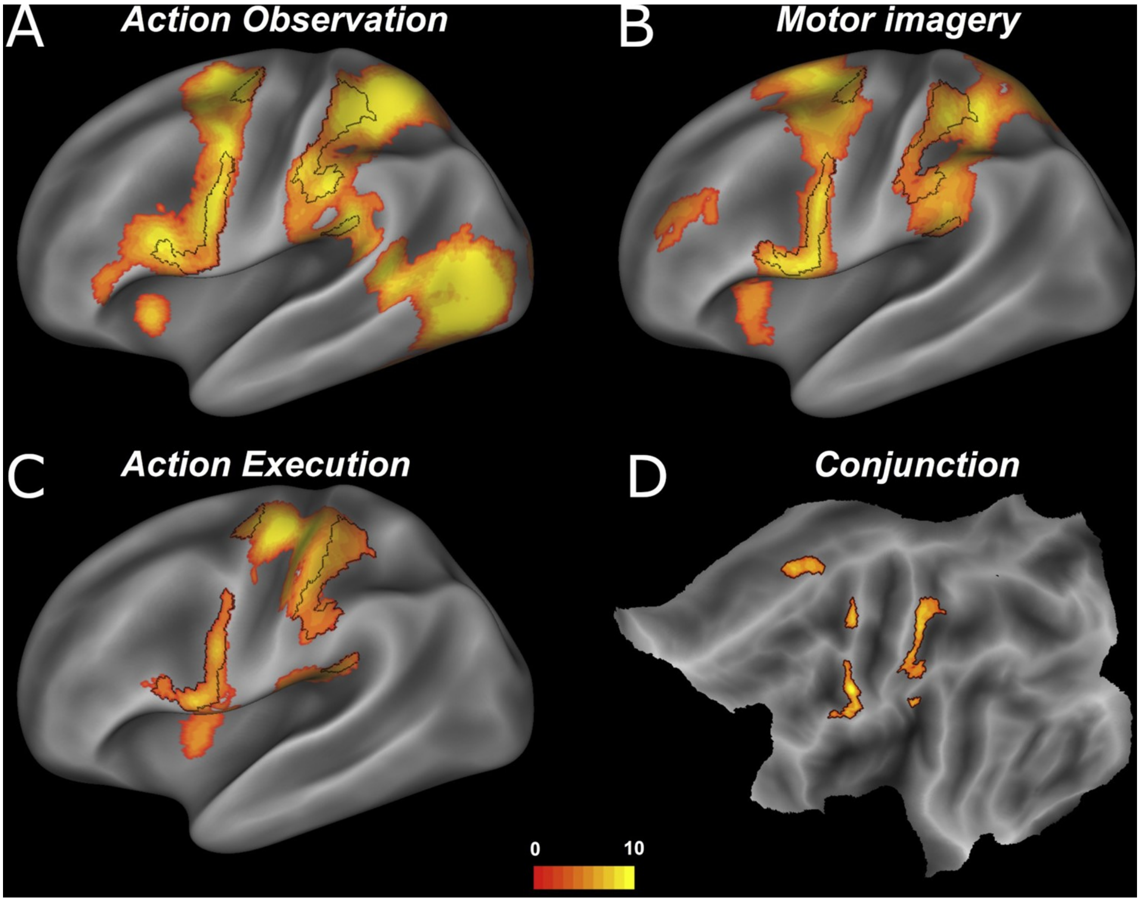

Figure 1.2 Brain substrates of AO, MI, and AE. *Neural substrates of action observation (AO, panel A), motor imagery (MI, panel B), action execution (AE, panel C), and conjunction across AO, MI, and AE (panel D). From Rizzolatti et al., 2021.*

In healthy individuals (Celnik et al., 2006) and stroke patients (Celnik et al., 2008), action observation has been shown to cause, even in isolation, long-term alterations in the excitability within M1 cortical representations of muscles/movements involved in the action. Noteworthy, functional rearrangement of the primary motor cortex is an important indicator of neuroplasticity, as it is connected to functional improvement and motor skill empowerment.

Although action observation itself might point the way to positive results, connecting action observation with action execution via motor imagery, i.e., asking participants to repeat the previously observed action, establishes the prerequisites for a successful outcome. Combining action observation, motor imagery, and action execution in a sequential procedure would integrate reciprocal benefits. While action observation can immediately activate the fronto-parietal network supporting a given action, subsequent motor imagery prolongs this activity in time, extending recruitment to a broader cortical network. Therefore, the final motor execution would be supported by the pre-activation of its neural substrates driven by the previous stages. AOT is a powerful tool for interventions on the motor system, positively biasing the trajectory of motor skills during motor recovery or motor training (Rizzolatti et al., 2021).

A study by Mattar & Gribble (2005) has provided evidence that combining action execution and observation promotes motor learning. The study aimed to investigate whether watching another person going through the motor learning process could influence the performance of naïve observers. In the first experiment, subjects were sub-divided into three groups: 1. observation of a person learning a clockwise force field; 2. observation of a person learning a counter-clockwise force field; 3. no observation (control condition). Subjects who observed a video of another person learning in a novel environment outperformed subjects who saw similar movements but did not learn in the same environment. The observer could extract the information required at the motor execution level based on observation.

A great number of animal and human research have shown that traumatic injuries do not affect only the musculoskeletal system but also the entire motor system, including the cortical neural level. Thus, AOT and traditional rehabilitative techniques could be combined in the rehabilitation pathway. Noteworthy, training a certain part of the body expands its specific representation in the cortex (Rizzolatti et al., 2021). Notwithstanding, active exercise is not always possible, particularly in the case of post-injury situations. Especially in these cases, the mirror mechanism is a plausible approach to preserve the neural motor program, that is, the sequence of neuronal events occurring during action execution (Rizzolatti et al., 2014).

In a recent study, Bazzini and co-workers (2022) extended the application of AOT beyond therapeutic and rehabilitative settings to everyday activities demanding the acquisition and refinement of new motor abilities. Participants were subdivided into three groups: Action Observation Training (AOT), Observational Learning (OL), and Motor Practice (MP). The three experimental procedures compared the efficacy of training primarily based on action observation and execution and the alternation of both methodologies. AOT subjects were asked to alternate observation and execution of nautical knots; during the observation, they had to observe an expert performing the task. Similar to the AOT group, the OL group started the experiment with an observation-execution block in which, for one time, the participants were asked to observe the expert performing the knot and then replicate it. Subsequently, the training consisted of 9 trials in which the participants observed the expert performing the knot. At the end of the observation trials, subjects were asked to execute the observed knot. Finally, the same starting procedure (observation-execution block) was proposed to MP group. However, the training for this group consisted in 10 execution trials of a mere motor practice. The results showed that the AOT group improved significantly more than the OL and MP groups, suggesting that the constant alternation between observation and execution is the most effective strategy to enhance motor learning. Therefore, only AOT integrates all aspects of the motor experience. This result depends on the ability of action observation to engage the cortical motor system, hence modulating the creation of new motor programs. These findings suggest several opportunities for extending the application of AOT from the therapeutic, rehabilitative context to daily routines involving learning and perfecting new motor abilities (Bazzini et al., 2022).

1.3. Action Observation and Electromyographic Changes

To have an AOT-induced behavioural effect, a modification must be generated in the cortical areas of the motor system. Action observation has been shown to cause long-term alterations in excitability in M1 representations of muscles/movements involved in witnessed and executed actions. Furthermore, AOT induces behavioural improvements, and action observation has a specific role in motor training (Bazzini et al., 2022; Rizzolatti et al., 2021). Whether this behavioural improvement originates from general activations of the motor system or specific task-related activations, then reinforced by motor practice, remains to be addressed. Several findings have shown that training (i.e., alternating action observation and action execution) triggers a peripheral modification of muscle activity. In particular, electromyographic (EMG) measures stand as an electrophysiological parameter of behavioural modification considering muscular activity.

A study by Obhi & Hogeveen (2010) presented how EMG measures could be used to investigate the specificity of the motor response to observed actions. The EMG activity from the first dorsal interosseous and the abductor pollicis brevis was measured. At the same time, participants held a rubber ball between their forefinger and thumb and reacted to colour cues instructing a strong or soft pressure. The cues were presented on videos with the two types of squeezing, thus generating two congruent and two incongruent situations. The muscles involved in the two types of squeezing were the same, the only difference being the degree of the contraction. The findings revealed that the congruence between the video and the cue had a significant effect on squeezing response EMG activity. Thus, the observer's motor system was influenced by the video in terms of muscular activity amount.

Di Rienzo et al. (2019) broaden the understanding of the effects of action observation (AO) and action observation combined with motor imagery (AO + MI) on

EMG activations. Participants were administered a training of three sessions, each one lasting thirty minutes and separated from the others by two days; each trial included ten maximal elbow flexions against a platform. There were three conditions: AO, observation of a bodybuilder executing the task; AO + MI, observation of a bodybuilder executing the task and MI of himself/herself performing the task; control, watching a basketball documentary. EMG was recorded from the biceps brachii. Findings of this study show that AO and AO+MI conditions lead to greater EMG activation than controls.

In another study, Losana-Ferrer and co-workers (2018) aimed to determine how motor imagery and action observation paired with a hand grip strength program influenced the electromyographic (EMG) activity of forearm muscles. Participants were administered a training protocol of ten days. The main task consisted of 10 maximal isometric hand grip contractions. There were three conditions. The first was motor imagery (MI), with participants instructed to complete a daily program consisting of two parts: 1. the subject had to visualize himself completing the task; 2. the subject had to physically perform the task while imagining himself executing the task. The second condition was action observation (AO), in which subjects had to complete a two parts daily program: 1. the subject had to watch a video depicting a forearm doing the task; 2. the subject had to execute the task while observing the video. The third condition was the control condition, in which participants were instructed to perform the task daily. EMG of the forearm muscles (extensor carpi radialis longus and extensor carpi radialis brevis) was collected before, during, and after the training. Results revealed that both motor imagery and action observation combined with a hand grip strength program effectively presented a significant change in forearm muscles EMG activity and strength gain.

In recent research, Romano Smith and colleagues (2019) conducted a study consisting of five conditions: 1. action observation (AO); 2. motor imagery (MI); 3.

simultaneous imagery and observations (S-AOMI); 4. alternate imagery and observation (A-AOMI); 5. control. Participants were divided into five groups; all participants had to perform a dart-throwing task before and after the test. AO subjects were asked to watch a video of an intermediate player performing 30 dart throws. MI subjects had to depict a mental image of themselves holding a dart. S-AOMI subjects received a pre-recorded video and imagery instructions; they had to observe the video while imagining the experience. A-AOMI subjects had to alternate imagery and action observation. The tests had to be repeated three times per week for six weeks. The results revealed that S-AOMI and A-AOMI training allow a higher performance improvement than AO and MI alone. The findings suggest that combining MI and AO significantly impacts on motor control by requiring less EMG activation to perform the throwing task efficiently, regardless of how the combination is organized. The EMG activity reduction could indicate more expert like motor control and could be supported by the recruitment of fewer motor units (Duchateau et al., 2006).

Whether the efficacy of AOT originates from a general unspecific activation of cortical motor areas induced by repeated action observation (action observation as "amplifier" of motor system activation) or from absorption of the observed model that forges the motor representation of the action reaching a peripheral level and thus modifying the muscular pattern, remains to be addressed. A way to tackle this issue is to evaluate the similarity between the observed and performed motor actions and how it develops during the training time. De Marco and colleagues (2020) have provided a positive correlation between the similarity of motor patterns and accuracy in action recognition. Two experiments were conducted. In the first one, subjects were asked to reach and grasp an object and then transport it into a container; there were four conditions: container big or small, human agent making a container by hands that were big or small.

An optoelectronic system recorded the reaching and grasping kinematics to analyze the movement features. In the second experiment, an observation task was presented. The stimuli consisted of a video depicting an actor performing the same task in the first experimental conditions; participants were asked to recognize the intention behind the reach-to-grasp acts by seeing only the reaching part of the action. Results showed that accuracy in motor intention identification was enhanced by the similarity between the actor's kinematic parameters and the parameters of the observer. These findings suggest that a major similarity level between the model and the observer leads to better recognition of the observed action.

A still unsolved question is whether, during an action observation training, the convergence toward the model motor pattern explains the behavioural improvement. In other words, whether the observed model intrudes into the observer's motor system enough to reach and bias his EMG activity. To address this issue, we administered participants an AOT training aimed at acquiring a complex motor task while recording the electromyographic activity of the hand muscles of both the model and subjects. The trainee-model similarity was computed by comparing each subject's muscular activity with that recorded from the model executing the same task.

Suppose the AOT participants display a greater behavioural improvement, but this improvement does not reflect an increase in similarity with the model. In that case, action observation activates the cortical motor system but likely for encoding more goal related features than the way the action was executed. In turn, if the participants involved in AOT during the training converge toward the model's motor pattern and that relates to a better behavioural improvement, this would favour the idea that the observed model (composed of both motor action goal and kinematics) intrudes *via* the mirror mechanism into the motor system of the observer, not limiting to the cortical motor system but propagating up to the neuromuscular control.

2. OBJECTIVE

The objective of this study was: 1) to investigate the relationship between Action Observation Training (AOT) and performance improvement in a motor task by evaluating AOT efficacy in promoting the acquisition of a motor task with chopsticks; 2) to explore the relationship between the AOT efficacy and the degree of similarity between the trainee and the observed model.

For these purposes, seventy-two participants underwent motor training consisting of six consecutive sessions of reach-to-grasp and lift marbles using chopsticks. The participants were equally distributed into two groups: Action Observation Training (AOT) and control (CTRL). For each subject, the muscular activity of the right hand 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 to the amount of behavioural improvement.

3. MATERIALS AND METHODS

3.1. Participants

Power analysis for within/between-subjects ANOVA with G-Power 3.1 was conducted to define the sample size suitable for our study. The analysis output showed a minimum sample of 70 subjects (35 for each group) to obtain a significant effect on the dependent variable with an $\alpha = 0.05$, a power $\beta = 0.90$, and a Cohen's $F = 0.2$.

Seventy-two healthy naïve volunteers (age $M = 26.03$, $SD = 4.52$, 55 females) were enrolled for the experiment. Participants were right-handed ($M = 0.82$, $SD = 0.17$) according to Edinburgh Handedness Inventory (Oldfield, 1971), had normal or corrected-to-normal vision, and presented no history of neurological or psychiatric disorders.

Participants were further randomly subdivided into two groups: Action Observation Training (AOT, $n = 36$; age $M = 26.08$, $SD = 4.25$) and control group (CTRL, $n = 36$; age $M = 25.97$, $SD = 4.84$). The local ethics committee approved the study (Comitato Etico dell'Area Vasta Emilia Nord, n. 10084, 12.03.2018), which was conducted according to the principles expressed in the Declaration of Helsinki. The participants provided written informed consent.

3.2. Baseline Assessment

Before the experimental procedures, participants were administered a subjective questionnaire to evaluate their expertise with chopsticks. They were asked to rank on a Likert scale their chopsticks' frequency use (*scale* = 1 - less than once a year; 2 - once or twice a year; 3 - once or twice a month; 4 - once a week; 5 - more than once a week) and their ability in using this tool (*scale* = 1 – 6; 6 indicating the highest proficiency with chopstick use).

Furthermore, the hand dexterity of both dominant and non-dominant hands was assessed by the Nine Hole Peg Test (Mathiowetz & Weber, 1985). Participants were instructed to accomplish the test as quickly as possible, consisting of a small container for the nine pegs and a square wooden board with nine holes. They had to pick up the nine pegs once at a time as quickly as possible, place them in the nine holes, and then put them back into the container. We recorded the total time to complete the task for both the right and left hands.

In addition, as the experimental procedures would have encompassed the recording of EMG from three hand muscles, subjects were required to produce the maximal contraction of the three muscles in separate blocks of about 10 seconds. These indices were later used for EMG amplitude normalization across participants.

3.3. Stimuli and Experimental Design

Before the participants' recruitment, an expert in chopsticks use was invited to perform the chopstick task, consisting in grasping with the chopsticks 15 marbles positioned on a plate and placing them into fifteen holes in a wooden board (see Figure 3.1). From an egocentric perspective, the expert's performance was video-recorded, using a high-definition camera to maximize a potential motor resonance effect (see Angelini et al., 2018).

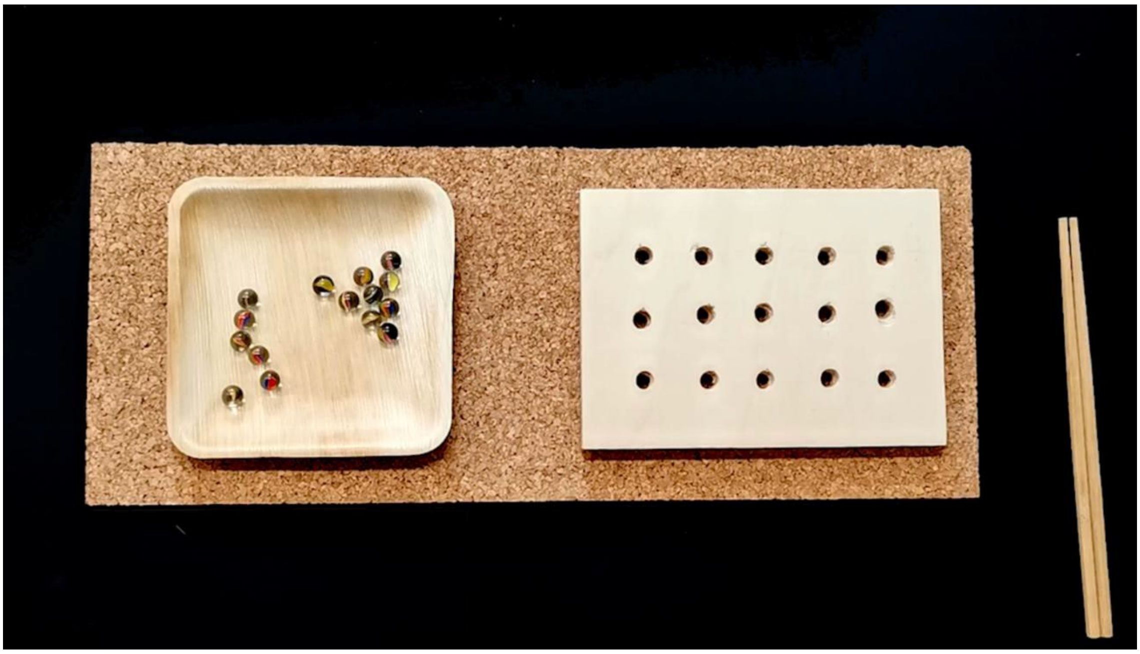

Figure 3.1 Kit adopted for the motor task. *The kit adopted for the motor task was composed of a squared plane bowl (size 15.2 x 15.2 x 1.5 cm) and a rectangular wood board (20.4 x 14.5 x 1.5 cm) with fifteen holes distantiated by 7.2 cm. The holes' diameter is 1.1 cm (1 mm lower than the diameter of the marbles). Each hole was distantiated from the closest one by 2.5 cm. The length of the wood chopsticks is 24 cm.*

The experiment consisted of six training sessions (S1 – S6) to learn the chopsticks task previously performed by the expert. Each training session was structured as follows: observation and execution phases. The AOT participants observed a video showing the expert performing the task and then executed the same task. At the same time, the CTRL subjects watched a landscape video (without any biological movement to minimize motor system activation) and then verbally instructed to execute the task.

During the expert's execution, surface EMG was recorded via wireless clip-electrodes positioned on three muscles of the right hand: Opponens Pollicis (OP), First Digital Interosseous (FDI), and Abductor Digiti Minimi (ADM) using a wireless EMG system (Cometa Wave Plus, Cometa System Inc., Italy). The EMG signals were amplified

(×1000), sampled at 2000 Hz, and filtered with an online first-order band-pass filter (10-500). The same procedure was applied to each participant. The six executions of each subject were video-recorded from two cameras (lateral and top-frontal views), synchronized with the simultaneous surface EMG recording.

The stimuli were presented using PsychToolbox-3 (Brainard, 1997; Pelli, 1997; Kleiner et al., 2007) on a monitor (22-inch LCD) placed 60 cm from the participant's forehead. The duration of the observation phases was 64.7 seconds. The chopsticks displacement from the wooden board was considered the starting position. The maximum duration of the execution was 180". See figure 3.2.

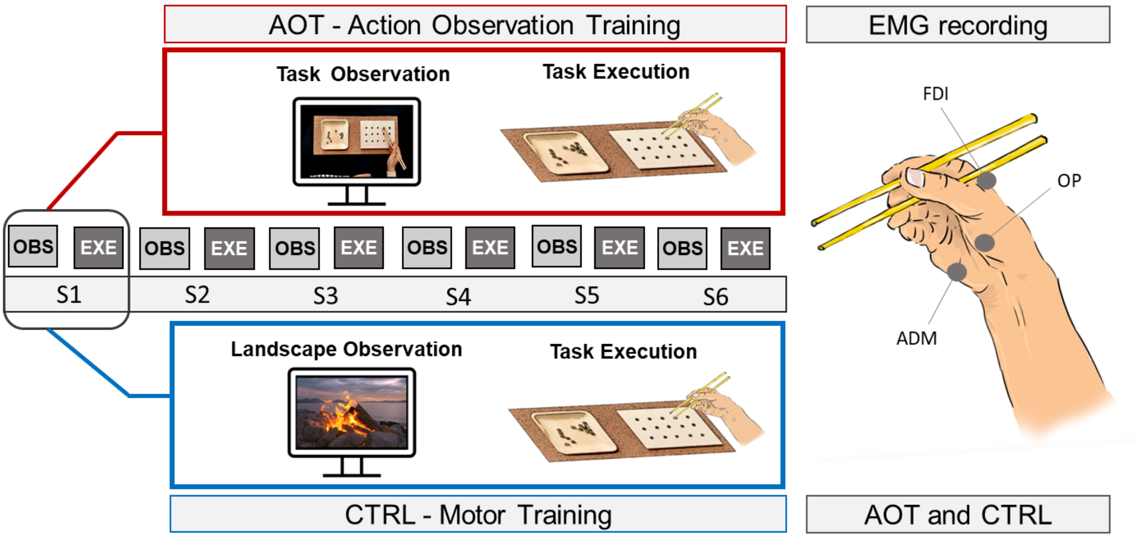

Figure 3.2 Experimental design. *The two groups underwent a training characterized by six sessions (S1-S6), each composed of an observation (OBS) period and an execution (EXE) one. The AOT participants observed a video showing the expert performing the task and then executed the same task (red panel), while the CTRL subjects observed a landscape video and then executed the task (blue panel). EMG was recorded for both AOT and CTRL participants from three hand muscles:* Opponens Pollicis *(OP),* First Digital Interosseous *(FDI), and* Abductor Digiti Minimi *(ADM).*

3.4. Data Analysis

3.4.1. Behavioural Outcomes

For each participant, the video-EMG recordings of the execution trials (reach-to-place) were off-line segmented into three phases:

(1) reaching: starting after the placement of marble or when the chopsticks leave the wooden board (first marble) and ending in the timepoint correspondent to the chopstick-marble contact;

(2) holding: starting at the chopstick-marble contact and ending at the onset of the marble lifting from the plate surface;

(3) transport: starting from the marble lifting and ending at the marble placement.

The duration of the three phases was calculated for each marble and then averaged for each session. See figure 3.3.

The behavioural endpoints were:

1. the number of grasping attempts (GA), i.e., the number of contacts between the chopsticks and the marble during the attempt to grab it. In principle, the ideal execution would comprise a number of GA equal to the number of marbles. Conversely, the higher is GA, the more inaccurate the motor performance;
2. the number of failed liftings (FL), i.e., the number of accidental fallings of the marble during the transport phase, thus impeding its correct positioning on the board;
3. the mean duration of the reach-to-place action (MD), obtained by summing the mean duration of the three phases. In this way, we excluded from the mean duration the time spent failing to grasp, thus obtaining a temporal index completely independent from GA.

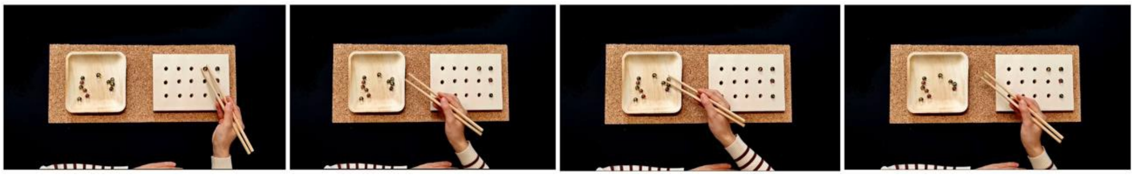

Figure 3.3 Trial segmentation. *Representative series of frames extracted from the visual stimulus showing an expert model (i.e., a person adopting a wood chopstick as a usual feeding effector) transporting fifteen marbles from a plate to fifteen holes of a wooden board.*

3.4.2 EMG Outcomes

Participants' muscular activity was analyzed in terms of 1) the mean amount of muscular contraction and 2) the EMG pattern similarity (R^2) between the trainee and the observed model.

The EMG signals were segmented using the same time points extracted from the videos and analyzed using a homemade code developed in MATLAB (R2021a). For each execution, the amount of muscular contraction in each phase (Reaching, Holding, Transport) and muscles (OP, FDI, ADM) was normalized according to the individual (participant and muscle) maximal contraction. The contraction was expressed in terms of muscle maximal contraction percentage. The same parameters were also calculated on the whole trial, averaging the 3 phases.

The EMG pattern of each phase and muscle was processed according to three steps (see figure 3.4): (1) *EMG envelope*, EMG traces were rectified and filtered to increase their smoothness, using a band-pass filter 3-1000Hz and an envelope lowpass 2Hz filter, and segmented using the same time points extracted from the videos; (2) *Time normalization*, EMG traces were time-normalized (temporal axis 1-100) to be matched in duration; (3) *EMG similarity computation*, the participant's EMG patterns were compared with the model's EMG pattern. The comparison was performed adopting the Linear Fit

Method (LFM, Iosa et al., 2014) already used to assess the kinematics similarity in upper limb reach-to-grasp actions (De Marco et al., 2020). LFM calculates the linear regression between the subject and the model, returning the coefficient R^2 as a measure of the temporal similarity between the two curves. When the curves follow the same pattern, the value of R^2 tends to be the ideal value of 1.

We extrapolated two EMG outcomes for each muscle: the full trial average contraction and the full trial similarity (R^2). Noteworthy, the full trial similarity was also considered as a full trial Δ similarity calculated by subtracting the R^2 similarity at S1 from the R^2 similarity at S6. This index displays the convergence toward the model induced by the training.

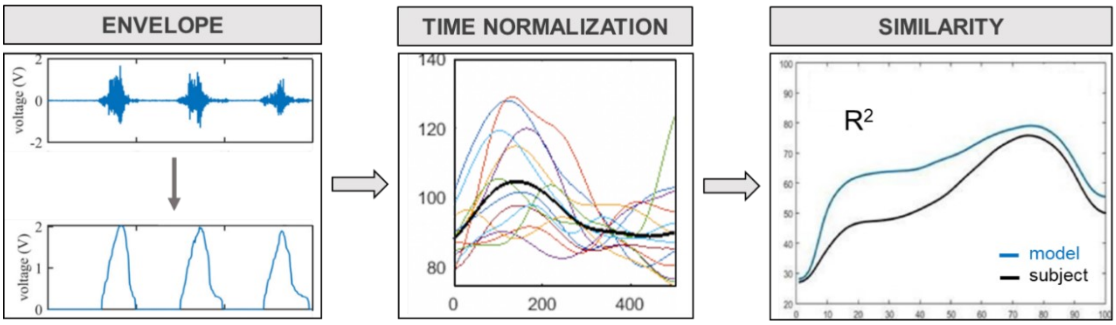

Figure 3.4 EMG processing. *Graphic representation of EMG processing phases (i.e., envelope, time normalization, similarity) adopted for the computation of trainee-model EMG similarity.*

3.5. Statistical Analyses

3.5.1. Baseline Evaluation

To ensure that the two groups were homogeneous, a two-sample t-test was applied to age, chopsticks' frequency use and ability, and hand dexterity (right and left).

3.5.2. Behavioural Analyses

When considering the behavioural performance, the three behavioural outcomes were baseline corrected by subtracting the initial performance at S1 from all other sessions' performance scores. The group baseline performance, considering the behavioural outcomes of GA, FL, and MD, was verified via a two-sample t-test to ascertain the balance between the two groups (AOT and CTRL) at S1. A one-sample t-test (against zero) was applied for each behavioural variable, baseline corrected, to evaluate the performance improvement between S1 and S6 (S1 & S6) within the group. Finally, a two-sample t-test between groups was performed on each behavioural outcome at S6, baseline corrected, to evaluate whether the extent of the learning rate was different across the two groups.

3.5.3. EMG Analyses

Considering the full trial average amplitude of muscular contraction, three repeated measures ANOVAs (*between factor* = group, *within factor* = time) were conducted to evaluate whether the level of contraction changed over time and between groups. The similarity with the model (R^2) was also submitted to a similar analysis to evaluate whether our participants approached the muscular pattern of the model during the training in any of the three investigated muscles. In case of significant effects, planned contrasts were performed between the initial and final scores within each group. As a note, three subjects had instability of one of the EMG electrodes (1 OP in AOT, 1 OP in CTRL, 1 ADM in CTRL) during the recording. Thus, they have not been included in the analyses.

3.5.4. Correlational Analyses

Preliminarily, we converted the behavioural improvement in standardized scores based on the initial and final performance to weight the absolute increase over the average performance [Δ = (S6-S1)/(S6+S1)]. The correlation analysis took into account the behavioural variables highlighting a significant difference between groups and the EMG outcomes showing a significant modulation over time to test the link between the improved behavioural variables and the EMG parameters relative to both amplitude and similarity.

To investigate whether the initial full trial similarity (at S1) is associated with the initial behavioural performance (GA), Pearson correlation was performed between the behavioural performance and the initial full trial similarity for each muscle (OP, FDI, ADM) and for each group, separately. The same analysis was conducted between the full trial similarity with the model at S1 and the behavioural performance improvement (ΔGA), aiming to evaluate whether the initial similarity with the model sets better promises for improvement.

To explore the relationship between the AOT efficacy and the degree of similarity between the trainee and the observed model, the percentage of the behavioural performance improvement (ΔGA) was correlated with the full trial Δsimilarity. Furthermore, the same analysis was performed within each separate phase of the reach-to-place action (reaching, holding, transport) for each muscle and each group to evaluate whether some muscles in some phases play a major role in sustaining the behavioural outcome and, ultimately, the motor learning.

4. RESULTS

4.1. Baseline Results

The two-sample t-tests applied to the baseline variables showed no significant differences (all $p > 0.31$) between the two groups (AOT vs. CTRL); all groups were homogeneous in terms of age, chopsticks' frequency use and ability, and hand dexterity. See Table 4.1.

Table 4.1 *The table reports mean and standard deviation of AOT and CTRL group, t and p values of the two-sample t-tests conducted on each baseline variable*

BASELINE VARIABLES	M_{AOT}	M_{CTRL}	SD_{AOT}	SD_{CTRL}	*t*	*p*
AGE	26.08	25.97	4.25	4.84	0.10	0.92
FREQUENCY USE	2.39	2.47	0.90	4.84	-0.40	0.69
USING ABILITY	2.80	2.92	1.26	1.25	-0.37	0.71
R HAND DEXTERITY	18.34	19.09	3.08	2.58	-1.03	0.31
L HAND DEXTERITY	20.06	20.10	3.14	3.12	-0.06	0.95

Notes: M = mean; SD = standard deviation; t = Student's t; p = significance level of the t-test; R = right, L = left, AOT = action observation training; CTRL = control group.

4.2. Behavioural Results

Figure 4.1 illustrates the time course of the behavioural indices for both groups over the six training sessions. As one can see, all of them show a marked performance improvement over the training, with larger values occurring in the initial session (S1) and the lowest generally found in the final session (S6).

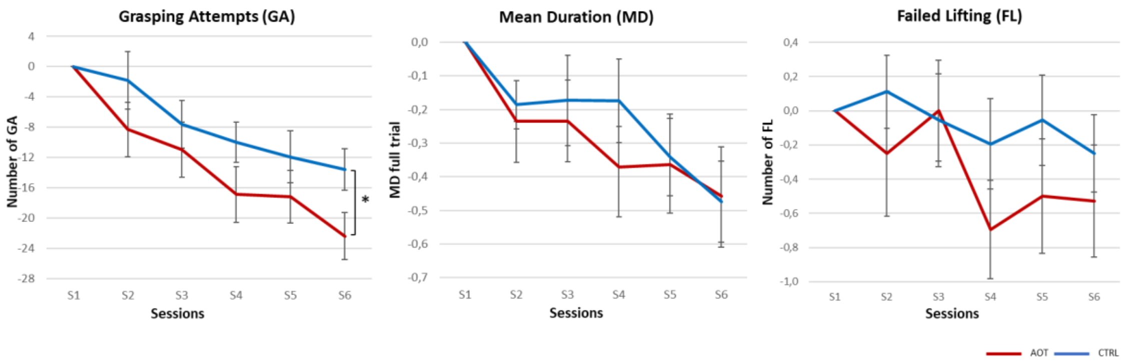

Figure 4.1 Performance scores. *Behavioural performance scores, baseline corrected, in terms of Grasping Attempts (GA), Mean Duration (MD), and Failed Lifting (FL) in the six sessions, showing the development of performance scores in AOT (red line) and CTRL (blue line). In particular, a significant difference emerged between AOT and CTRL group at S6 in GA, indicated by the asterisks (p < 0.05). Graphic representation of MD highlights a rapid decrease in AOT, although S6 performance is similar between the two groups. The FL performance displays a decrement in AOT, while the CTRL performance has a stable pattern with a slight decrease.*

Two-sample t-tests performed on the baseline behavioural performance between groups showed no significant differences (all $p > 0.26$) between the $S1_{AOT}$ mean and the $S1_{CTRL}$ mean in terms of GA ($M_{AOT} = 51.42$ vs $M_{CTRL} = 50.17$), FL ($M_{AOT} = 1.42$ vs $M_{CTRL} = 0.97$), and MD ($M_{AOT} = 3.91$ vs $M_{CTRL} = 3.93$), confirming that the initial abilities were very well balanced. See Table 4.2.

Table 4.2 *The table reports mean and standard deviation of AOT and CTRL group, t and p values of the two-sample t-tests conducted on the initial behavioural performance between groups*

PERFORMANCE	M_{AOT}	M_{CTRL}	SD_{AOT}	SD_{CTRL}	*t*	*p*
GA_{S1}	51.42	50.17	22.22	15.11	0.28	0.78
FL_{S1}	1.42	0.97	1.84	1.46	1.13	0.26
MD_{S1}	3.91	3.93	0.96	0.95	-0,08	0.94

Notes: M = mean; SD = standard deviation; t = Student's t; p = significance level of the t-test; GA = grasping attempts; FL = failed lifting; MD = mean duration; S1 = initial performance, AOT = action observation training; CTRL = control group.

The three one-sample t-tests conducted on the baseline corrected outcomes were the following. Considering the GA, a significant effect was found within each group [AOT: $t_{(35)} = -7.20$, $p < 0.001$; CTRL: $t_{(35)} = -4.96$, $p < 0.001$], taking into account the initial and final performance baseline corrected. All groups exhibited a significant learning effect along the training regarding the MD, as demonstrated by the significantly better performance at the end of the training compared to baseline [AOT: $t_{(35)} = -3.00$, $p = 0.005$; CTRL: $t_{(35)} = -3.91$, $p < 0.001$]. Regarding FL, a trend toward significance emerged for the AOT group, decreasing the number of failed liftings over time [AOT: $t_{(35)} = -1.61$, $p = 0.12$; CTRL: $t_{(35)} = -1.10$, $p = 0.28$]. This result could be due to the very low values (range between -7 and 0) and their variability. No significant results emerged for the CTRL group.

Finally, a significant difference between AOT and CTRL emerged considering GA at S6 [$t_{(35)} = -2.12$, $p = 0.04$]. On the contrary, the two-sample t-tests on FL and MD did not produce significant differences between the two groups [FL: $t_{(35)} = -0.70$, $p = 0.49$; MD: $t_{(35)} = 0.09$, $p = 0.93$]. These results show a higher performance improvement in the

AOT condition in terms of GA, proving that AOT has slightly higher efficacy than motor practice.

In summary, the two groups exhibit a significant learning effect over time considering GA and MD and a trend toward the significance considering FL. In addition, both AOT and CTRL showed a similar performance at S1 for all the behavioural endpoints. Still, the AOT group outperformed the CTRL group, especially concerning a decrease in grasping attempts (see Figure 4.1).

4.3. EMG Results

The repeated measure ANOVAs conducted on the full trial average contraction returned no significant effect of Group or Time for any muscle except for FDI with a significant main effect of time [$F_{(5, 350)} = 4.17$, $p = 0.001$]. However, such an effect appeared due to an exceedingly low level of contraction at S1, with all other sessions having balanced values (see Figure 4.2). Thus, no relevant differences emerged regarding the modulation of muscular contraction along the training. Therefore, muscular contraction seems not related to behavioural improvement.

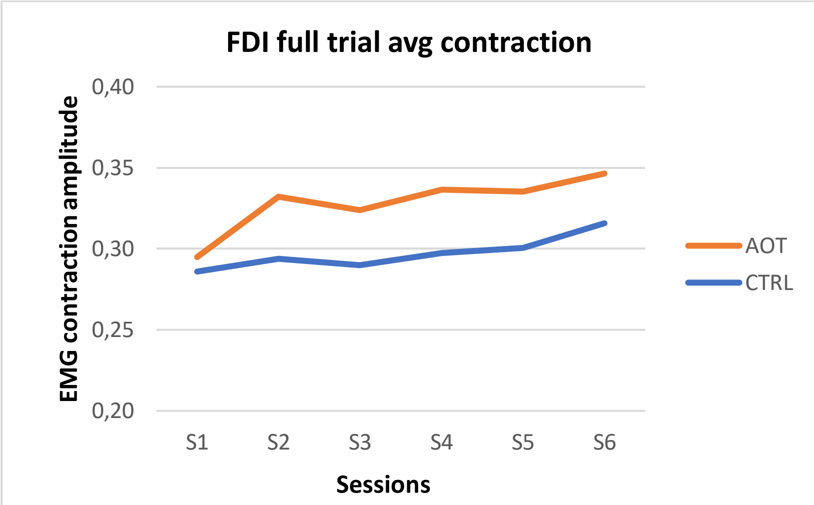

Figure 4.2 Muscular contraction. *Full trial average contraction of the FDI muscle during the six execution sessions. The red line represents the AOT values, and the blue line represents the CTRL values. Post hoc comparisons for the time effect revealed a significant difference between S1 and S6 (p < 0.001). However, the significant main effect is primarily due to the average contraction at S1, which showed a lower value than the other sessions. For this reason, the effect does not represent a gradual muscle contraction increase along the training but rather a sharp increase from S1 to S2 that remains constant from S2 to S6.*

Considering the temporal dynamics of the muscular contraction and its similarity with the model, the rmANOVAs subsequently performed on the full trial similarity (R^2) for the OP showed a trend toward the significance for Time [$F_{(5, 350)} = 2.05$, $p = 0.07$], a significant effect of the interaction Time*Group [$F_{(5, 350)} = 2.33$, $p = 0.04$] and no main effect of Group. Considering FDI muscle, we found a significant main effect of Time [$F_{(5, 350)} = 3.16$, $p = 0.008$], a trend toward the significance for the interaction Time*Group [$F_{(5, 350)} = 1.84$, $p = 0.10$] and no main effect of Group. The repeated measures ANOVA revealed no main effect of Group or Time regarding the ADM muscle. See table 4.3 for a summary of the significant effect and trends.

Table 4.3 *Table reports the results of the rmANOVA considering similarity in OP and FDI muscles, showing the significant main effect and the trends toward the significance*

rmANOVA OP

	SS	*dF*	*MS*	*F*	*p*
Time	0.08	5	0.02	2.04	0.07
Time*Group	0.1	5	0.02	2.33	0.04*

rmANOVA FDI

	SS	dF	MS	F	p
Time	0.15	5	0.03	3.16	0.008**
Time*Group	0.09	5	0.02	1.84	0.1

Notes: rm = repeated measures; SS = Sum of Squares; dF = degree of freedom; MS = Mean Square
*$p < 0.05$
**$p < 0.01$

Taking into consideration the main effects previously exposed, the full trial similarity improvement was investigated inside each group (AOT and CTRL), comparing the initial similarity at S1 and the final similarity at S6, referring to the OP full trial similarity and the FDI full trial similarity. The paired t-test on S1 and S6 full trial similarity showed a significant difference between the R^2 values for OP and FDI in the AOT group [OP_{AOT}: $M_{S1} = 0.36$, $M_{S6} = 0.41$, $t_{(35)} = -2.55$, $p = 0.01$; FDI_{AOT}: $M_{S1} = 0.36$, $M_{S6} = 0.42$, $t_{(35)} = -2.57$, $p = 0.01$], while no significant differences emerged in the CTRL group [OP_{CTRL}: $M_{S1} = 0.36$, $M_{S6} = 0.36$, $t_{(35)} = 0.09$, $p = 0.93$; FDI_{CTRL}: $M_{S1} = 0.39$, $M_{S6} = 0.41$, $t_{(35)} = -1.08$, $p = 0.29$]. See table 4.3. The significant effect was specific for the AOT condition, evidencing that the AOT condition could affect the convergence of the trainee toward the model while the CTRL group could not.

Table 4.3 *The table reports mean and standard deviation of S1 and S6, t and p values of the paired t-tests conducted on OP and FDI muscles in both groups*

MUSCLES	M_{S1}	M_{S6}	SD_{S1}	SD_{S6}	t	p
OP_{AOT}	0,36	0,41	0,26	0,26	-2,55	0,01*
OP_{CTRL}	0,36	0,36	0,28	0,29	0,09	0,93
FDI_{AOT}	0,36	0,42	0,23	0,25	-2,57	0,01*
FDI_{CTRL}	0,39	0,41	0,25	0,27	-1,08	0,29

Notes: M = mean; SD = standard deviation; t = Student's t; p = significance level; S1 = first session; S6 = sixth session; AOT = Action observation Training; CTRL = control group
*$p < 0.05$

In summary, from these analyses emerged no relevant differences in terms of modulation of the muscular contraction in the AOT and CTRL groups along the training. This indicates that the behavioural improvement due to the training could not be explained by the increase or decrease in muscular contraction. On the other hand, considering the temporal dynamics of the muscular contraction, AOT participants showed a significant increase of similarity with the model during the training, especially concerning OP and FDI muscles. At the same time, the same does not apply to the CTRL participants (not exposed to the model observation) who showed no similarity modulation along the training. Starting from these results, a correlational analysis was performed to investigate the relationship between the convergence toward the model and the behavioural performance improvement.

4.4. Behavioural and EMG Correlations

The parametric correlational analysis (Pearson) reveals that the initial performance at S1 expressed as GA did not correlate with the initial similarity (all $p > 0.29$) of the considered muscles in both groups. The same analysis was conducted having ΔGA as behavioural outcome; the results showed no correlations in each muscle between the behavioural improvement and the capacity to learn during the training (all $p > 0.19$). Therefore, the initial similarity level cannot determine a better initial behavioural performance and cannot lead to a larger amount of behavioural improvement along the training.

To explore whether the AOT causes the different behavioural outcomes, we performed a correlational analysis between the behavioural performance improvement (ΔGA) and the full trial Δsimilarity of each muscle (OP, FDI, ADM). The results did not show significant correlations in both groups for each muscle (AOT: all $p > 0.64$, CTRL: all $p > 0.16$).

Since the three investigated muscles could play a different role in the specific phases that compose the reach-to-place action, a further correlational analysis was carried out considering the gain of similarity in the Reaching, Holding, and Transport phases separately and the ΔGA. The correlational analysis showed no significant effect for the three phases in both groups considering the OP muscle (all $p > 0.16$). As reported in figure 4.3, the correlational analysis revealed a significant correlation ($r = -0.34$, $p = 0.045$) between FDI Δsimilarity in the holding phase and the ΔGA in the AOT group, no correlations were found considering the same muscle similarity in the reaching and transport phase (all $p > 0.21$). The correlational analysis between ΔGA and FDI Δsimilarity showed no significant differences for the CTRL group in all three phases (all $p > 0.54$). Significant correlations emerged between the ΔGA and ADM Δsimilarity in the reaching phase ($r = -0.39$, $p = 0.018$) and in the holding phase ($r = -0.39$, $p = 0.019$) for the AOT group (see figure 4.3). No significant difference was found in the transport phase ($r = 0.08$, $p = 0.63$) in this group. The correlational analysis between ΔGA and ADM Δsimilarity showed no significant differences for the CTRL group in all three phases (all $p > 0.39$).

The analyses highlighted significant correlations between the behavioural improvement in GA and the degree of convergence toward the model, specifically for the AOT group in FDI and ADM muscles for the reaching and holding phases of the reach-to-place action. A remarkable aspect is that the muscles are involved in the grasping movement, and the phases surrounding the grasping event (reaching precedes the grasp and holding follows the grasp). The correlations between the behavioural improvement and the Δsimilarity of the phases indicate a link between the performance improving ability and the aptitude in embodying the motor schema of the observed model specific for AOT.

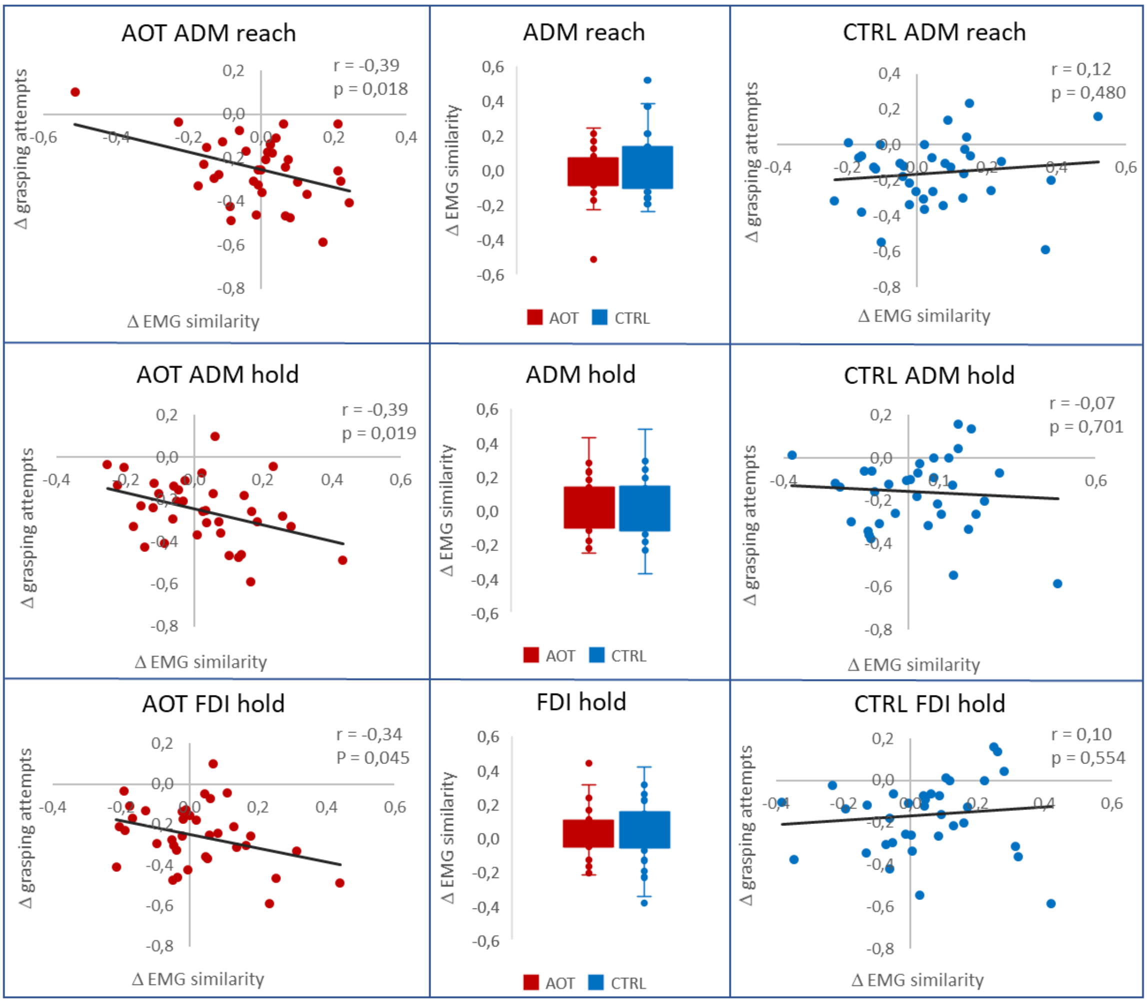

Figure 4.3 Distribution of the similarity changes due to the training and correlations between performance improvement and similarity. *The scatterplot shows the relationship between the convergence toward the model during the training and the improvement of grasping attempts in the AOT group (left side) and the CTRL group (right side). In the central section, the box and whiskers show the distribution of the Δ similarity scores, red represents the AOT group, and blue represents the CTRL group.*

5. DISCUSSION

The current study aimed to (1) examine the relationship between Action Observation Training (AOT) and motor performance improvement, evaluating the efficacy of AOT in promoting the acquisition of a new motor task and, (2) investigate the relationship between the AOT efficacy and the degree of trainee-model's similarity. For these purposes, seventy-two healthy subjects were enrolled and randomly subdivided into two groups (AOT & CTRL). The AOT group was administered a training consisting of action observation and execution. The selected action implied grasping fifteen marbles on a plate with chopsticks and placing them into fifteen holes in a wooden board. The structure of the CTRL group training was the same except for the visual stimuli, as CTRL participants were exposed to landscape videos without any motor content. All training was characterized by six consecutive sessions, each composed of a regular alternation between observation and execution trials.

In humans (Hardwick et al., 2018; Rizzolatti et al., 2014) and non-human primates (Bonini, 2017; Nelissen et al., 2011), action execution and action observation share motor representations in the fronto-parietal networks. It has been demonstrated that the regular alternation of action observation and action execution assists the acquisition of new motor skills in a motor learning task (Bazzini et al., 2022).

At the behavioural level, the result of the present study highlights a significant decrease in grasping attempts (GA), indicating a larger motor improvement for AOT with respect to the CTRL group. At the same time, a smaller but significant improvement was found in the CTRL group, indicating that the sole practice leads to a meaningful yet suboptimal motor learning rate. The previous study by Bosch and co-workers (2018) used a similar paradigm based on motor practice with chopsticks, demonstrating that the performance improvement follows a logarithmic trend. In such a scenario, the improvement is reasonably due to the capacity of subjects to adjust their performance according to internal feedback (e.g., proprioception or goal achievement) without external inputs. This latter information could explain the advantage of AOT over control training, as alternating external inputs intrinsic to action observation and internal feedback could result in a better outcome.

While the behavioural results confirmed the efficacy of AOT in motor improvement, the EMG results indicated that the similarity between the subject and the observed model is a key element during motor learning. Indeed, a significant correlation between the behavioural improvement and EMG similarity increase was found for both FDI and ADM muscles, but only for the AOT group.

In general, the idea of investigating similarity is not novel, as previous studies have already tackled its relevance not only in documenting motor abilities but also in supporting motor-related cognitive functions. De Marco et al. (2020) reported a significant correlation between the accuracy in intention prediction and the similarity between the observed action's kinematics and the observer's motor repertoire. Starting from these results, we wondered whether, beyond facilitating the *cognitive* comprehension of the observed action, motor similarity could also set better premises for the outcome of motor learning. This is even more relevant in the case of AOT, in which the gain of similarity during the training could explain the motor learning rate regardless of the initial similarity. Interestingly, we relied on the EMG signal for the similarity computation instead of movement kinematics, and two are the reasons underlying this choice. First, the selected task involves much finer motor control than praxic organization, with performance better indexed by EMG signals rather than the overall movement kinematics. Second, such a signal reflects processes nearer to the central nervous system relative to kinematics.

Comparing the EMG pattern of each participant with the model, we revealed a gain in EMG similarity for the AOT (5%) and not for the CTRL group (0%). This notion is even more relevant if one considers that both groups presented a significant behavioural improvement. Indeed, this demonstrates that subjects can improve their performance through various learning trajectories, which remain unbiased in the absence of action observation. In other words, CTRL participants are free to identify their ameliorative trajectory, which results in no increase of similarity with the given expert at the population level. Different is the case of AOT, since the exposure of subjects to expert observation and rehearsing their motor system accordingly, polarizes the learning trajectory towards a similarity increase, ultimately explaining why AOT participants significantly increase their similarity over the training.

What is the basis of the convergence towards the model? In a general motor system model, a visual input reaches the cortical areas and the mirror networks. Through multiple possible corticospinal pathways, action observation may forge the motor representation of the movements involved in the complex task and the temporal dynamics of the muscular contraction. Facilitated by action observation, this motor representation is recruited more faithfully during the execution. AOT, exploiting these neural substrates, could therefore promote (1) the behavioural performance improvement and (2) the convergence of the temporal dynamics of participants' muscular contraction toward the given model (Figure 5.1). Based on recent, controlled neurophysiological studies in which AOT was employed to improve reach-to-grasp and transport tasks, some theoretical speculations about AOT neural mechanisms may be discussed (Nuara et al., 2021). Cortico-cortical projections from the premotor (Rizzolatti & Luppino, 2001) and parietal

(Bruni et al., 2017) areas, which are endowed with mirror mechanisms, to the primary motor cortex may have stimulated short-term plastic alterations that contributed to achieve high-performance improvement.

In parallel, also descending corticospinal projections from mirror premotor regions (Dum & Strick, 1991) may have contributed to neurophysiological re-adaptation, promoting hand motor control improvement at the spinal level. Finally, the persistent activation of cortico-striatal projections endowed with a mirror mechanism may have encouraged motor task automatization (Bonini, 2017; Prather et al., 2008). Cortico-spinal projection affects the degree of muscular contraction at a peripheral level.

In summary, the findings of this study outlined that AOT can promote the acquisition of complex motor skills. Additionally, the behavioural improvement due to AOT reflects an increase in the similarity of EMG patterns between the observer and the model. To better investigate the effect of AOT during the acquisition of novel motor skills, starting from preliminary evidence that TMS modulation induced by action observation predicts the amount of motor improvement induced by AOT (Nuara et al., 2021), future studies combining behavioural results with neurophysiological technique (i.e., TMS), should aim at evaluating the temporal dynamics of motor learning during action observation. Moreover, future research might apply to comprehend which pathway is mainly involved in the muscular EMG similarity, a direct or mediated one (Figure 5.1).

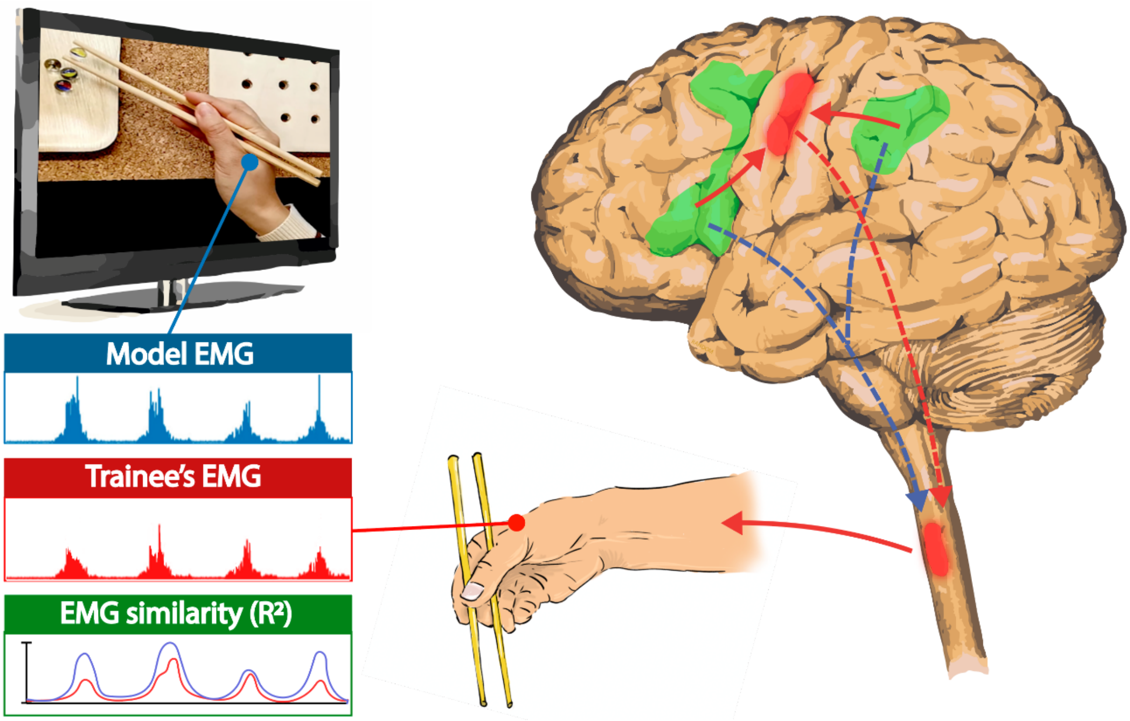

Figure 5.1 Model explaining the AOT effect on behavioural improvement and the similarity convergence toward the model. *Green areas represent frontal and parietal areas endowed with mirror mechanisms. Their projections to the primary motor cortex (red area) are highlighted with continuous red arrows; corticospinal projections (from premotor, parietal and primary motor cortex) are represented with dashed arrows. Multiple corticospinal pathways contribute in making action observation forge the motor representation of the movements involved in the complex task and the temporal dynamics of the muscular contraction. This motor representation could be subsequently straightened during the execution. AOT, exploiting these neural substrates, could therefore mediate (1) the behavioural performance improvement and (2) the convergence of the participants' muscular contraction temporal dynamics toward model one.*

6. CONCLUSION

In conclusion, Action Observation Training (AOT) improves behavioural performance and is linked with a gain in the motor similarity between the trainee and the observed model. Noteworthy, these findings provide additional information about the role of peripheral muscular patterns in the learning process. The similarity of EMG patterns seems to mediate motor learning *via* AOT. Moreover, the behavioural changes seem rooted in the motor system reactivity to action observation.

The possibility of a causal relationship between the muscular similarity and the behavioural outcomes warrants further investigation. In addition, these results could pave the way for developing individualized models to monitor the training *via* AOT over time, identifying the timing for shifting the task complexity during AOT in real-life scenarios. Future studies could focus on determining which mechanism is primarily involved in inducing muscular EMG similarity.

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