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L'EFFETTO DELL'OSSERVAZIONE DI OGGETTI AFFERRATI VS LA LORO PRESENTAZIONE STATICA SULLA MEMORY-RECALL: UNO STUDIO COMPORTAMENTALE E NEUROFISIOLOGICO

THE EFFECT OF THE OBSERVATION OF GRASPING OBJECTS VS THEIR STATIC PRESENTATION ON MEMORY-RECALL: A BEHAVIOURAL AND NEUROPHYSIOLOGICAL STUDY

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Abstract

The objective of this study is to investigate the relationship between the individual motor responsiveness to the presentation of grasped vs static objects, and the ability to recall objects previously observed within a grasping action, or in an isolated, static presentation.

For this purpose, twenty healthy subjects underwent the following procedures. First, the effect of the observation of grasping actions toward objects (AO) and the observation of objects in isolation (OO) on (1) corticospinal excitability and (2) intracortical inhibition was assessed with Transcranial Magnetic Stimulation (TMS). Subsequently, participants underwent a behavioural task were the effect of action (AO) vs object (OO) observation on the recall of correspondent objects was assessed.

At a mere behavioural level, neither AO nor OO induced an advantage on subsequent recall. However, a significant correlation between the behavioural performance and the individual corticospinal facilitation by AO/OO emerged. In particular, the greater was corticospinal relative facilitation by AO/OO, the greater was AO/OO impact on subsequent recall. The same relationship was not found for short-interval intra-cortical inhibition.

In conclusion, this study identified for the first time the motor neurophysiological signatures explaining the effect of the observation of grasping objects vs their static presentation on their subsequent recall. The fact that corticospinal (and not intracortical) excitability modulation by action/object observation is endowed with such a predictive power, suggests an extra-M1 location of the neurophysiological substrates. Besides its theoretical significance, this study could pave the way for the development of implementation of novel treatment approaches, where the traditional training for memory abilities is integrated with the personalized administration of action/objects observation, according to individual motor signatures.

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Introduction

2.1 Action observation and *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., 2005). 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 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).

The most widely accepted hypothesis 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.

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., 1995a). 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. 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., 2004).

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 group 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 performer. 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.

2.2 Object observation and *Affordances*

When we look at objects, we directly perceive not only their physical properties, but also the constellation of potential actions we can perform on and with them. Gibson (Gibson, 2014) called this set of potential actions "affordances" and stated that they are generated without the need or the intention to act on the observed object; at the same time, they are highly constrained by the observer's motor repertoire. This theory suggests that a sensory-motor system, able to transform the physical properties of an object into motor commands at first sight and with minimum effort, is a pre-requisite for successfully interacting within a competitive environment.

A strong support to the affordance idea comes from neurophysiological studies in macaque monkeys, showing a cortico-cortical network devoted to transform object visual information into grasping actions which includes the anterior intraparietal region (AIP) and the ventral premotor cortex (in particular area F5). Premotor cortex, in turn, sends projections to the primary motor cortex (Jeannerod et al., 1995).

Rizzolatti and colleagues (1988), firstly reported a relevant population of neurons within premotor area F5, whose activity was strongly related to specific goal-directed actions such as grasping or manipulating specific objects. The authors argued that motor neurons with different discharge properties code different goal-directed actions and all together they constitute a "motor vocabulary" always accessed by visual information. Among these motor neurons, about 20% showed object-related visual properties. More recently, area F5 visual properties were formally tested and it was described a set of bimodal visuomotor neurons with similar discharge pattern when a monkey grasps an object and when it simply watches a similar object without making any movement (Murata et al., 1997a). The visuomotor neurons belonging to this class have been successively named "canonical neurons" to distinguish them from the other class of visuomotor neurons in area F5, the "mirror" neurons, responding instead to action observation (Gallese et al., 1996). Further studies confirmed the existence of "canonical" neurons within both ventral and dorsal premotor cortex (Raos et al., 2006), and within intraparietal region AIP and posterior parietal cortex (Murata et al., 1997b).

Consistent with monkey evidence, behavioral studies in humans have demonstrated that the mere observation of a graspable object potentiates the observer's motor programs necessary to interact with it, even in absence of an explicit intention to act. This effect has been referred to as "visuomotor priming" or "affordance effect"

(Tucker & Ellis, 1998). In a series of experiments, it was demonstrated that, when subjects were viewing an object with a handle oriented to the left or to the right, they reacted faster when the response hand was congruent with a given handle orientation, even if it was irrelevant for executing the task. The same authors further showed that Reaction Times (RTs) to visually presented large or small objects were significantly affected by the type of response executed by the participants (precision vs whole hand prehension), being faster in presence of congruence between response type and object affordance (Ellis & Tucker, 2000; Tucker & Ellis, 2004). These results seem to confirm that, in the observer, the sight of the object recruits populations of neurons coding the motor program necessary to grasp it. The activity of such population may interact positively or negatively with the one coding the motor program of the selected hand response, both executed with the same muscular effectors.

Similar results have been successively confirmed and extended in the framework of the more general premotor theory of attention (Rizzolatti et al., 1994; Tucker & Ellis, 1998). Other authors described the time-course of the "affordance effect" adopting similar visual stimuli in the context of a Stimulus Onset Asynchrony (SOA) paradigm (Phillips & Ward, 2002). The reported effect was minimum for SOA=0 and increased progressively with SOA, reaching its maximum for SOA=800 and 1200. They thus concluded that the "affordance effect" develops gradually and persists for a relatively long period of time. These results were in agreement with a distributional analysis of reaction times performed in a prior study (Tucker & Ellis, 2001), allowing a further distinction from other Stimulus-Response Compatibility effects.

Human neuroimaging studies have consistently shown that, during the mere observation of graspable objects, a parieto-frontal circuit involved in visually guided

grasping becomes significantly active. A Positron Emission Tomography (PET) experiment with right-handed subjects, reported bilateral activation of premotor cortex during the observation of familiar tools (Rizzolatti et al., 1996). A functional Magnetic Resonance Imaging (fMRI) study, reported a correlation between the size of the "affordance effect" and the activity in the left posterior parietal and premotor cortices and showed increased activity in the left ventral precentral sulcus and in the left intraparietal cortex during the observation of object pictures (Grèzes, Tucker, et al., 2003). Similar increased activations in ventral premotor and posterior parietal cortices (including the intraparietal sulcus and inferior parietal lobule) have been confirmed during the observation of pictures depicting tools (Chao & Martin, 2000). Taken together, all these data seem to suggest that the regions within the dorsal stream automatically activated during object observation may constitute the neural substrate for the "affordance effect" originally hypothesized by Gibson.

Despite behavioral and brain imaging evidence, neurophysiological studies in humans have shown contradictory results. For example, Fadiga et al. (Fadiga et al., 1995a) reported no effects on motor evoked potentials (MEPs) following single pulse transcranial magnetic stimulation (TMS) of motor cortex (M1), when objects were visually presented. However, TMS delivering was not finely time-locked with stimuli presentation and occurred with few seconds of delay with respect to the onset of object observation. More recently, a paired-pulse TMS protocol was applied to subjects preparing to grasp one of two possible objects, requiring different shaping of the hand and thus implying a different recruitment of the two recorded hand muscles, the first dorsal interosseus (FDI) and the abductor digiti minimi (ADM) (Cattaneo et al., 2005). The paired-pulse TMS protocol applied is known to produce higher MEPs, probably because it interacts with repetitive discharges of cortical output neurons (Amassian &

Stewart, 2003; Di Lazzaro et al., 1999) and, ultimately, it could be more effective than single-pulse TMS in revealing excitability differences at cortical level. In this experiment, TMS was delivered during motor preparation, well in advance of any visible electromyographic (EMG) activity, showing a facilitation pattern of MEPs which predicted the subsequent muscle activity during grasping for each object. The MEPs facilitation was absent during preparation to execute simple and complex intransitive movements and also during mere object presentation, not followed by a grasping action. This suggests that object vision does not induce any excitability change in the stimulated areas, unless an object-directed action is prepared. The same group replicated part of those results, failing in finding relevant differences in MEPs during object presentation alone (Prabhu et al., 2007). Oppositely, in another TMS experiment (Buccino et al., 2009), subjects were presented with pictures of familiar objects that could be normally grasped from a handle (tea-caps, tea-pots), oriented to the left or to the right and being thus compatible with a left-hand or right-hand grasp. It was also added a control condition comprising objects with broken handles and single TMS pulses were delivered to the left M1 200 milliseconds after stimuli onset. A significant contralateral MEPs facilitation was present only for objects with the handle oriented to the right side, therefore affording a movement of the right hand.

Recently, another group (Makris et al., 2011) used a combined approach to investigate the "affordance effect": a reaction-time study and a TMS experiment, both comprising as stimuli, the presentation of pictures of familiar objects shown on a computer screen. They first replicated a behavioral study (Tucker & Ellis, 2001) by means of a similar apparatus to collect reaction times but, in addition to "pinchable" and "graspable" objects, they introduced a "neutral" condition with objects that could not be classified in any of the two previous groups (e.g sofa, carpet, door, etc). Behaviorally, a

significant interaction between type of object and type of response was reported only for short SOA (400 milliseconds). In the TMS part of the experiment, they administered TMS at three different timings (300, 600, 900 milliseconds from stimulus onset) to the dominant M1 of subjects watching pictures selected from the behavioral experiment ("pinchable", "graspable" and "neutral") and recorded MEPs from FDI and ADM muscles, while participants were required to perform an attentional task not related to the presented object. The results showed a significant increase in MEPs amplitude congruent with the afforded grasp only for "pinchable" objects as compared only to "graspable" ones, if TMS was delivered 300 milliseconds after stimulus onset.

A recent TMS study (Franca et al., 2012) explored the time-course of the "affordance effect" elicited by the observation of everyday-life graspable objects on motor cortex of resting observers. Motor evoked potentials (MEP) were from three intrinsic hand muscles (two "synergic" for grasping, OP and FDI and one "neutral", ADM). The observation of objects determined an increased excitability at 120 milliseconds after their presentation. Moreover, this modulation was proved to be specific to the cortical representations of synergic muscles. The authors suggested that this timing perfectly fits with a fast recruitment of the motor system aimed at rapidly and accurately choosing the appropriate motor plans in a competitive environment.

2.3 The interplay between motor system and memory processes

The study of the contribution of the motor system on the storage and retrieval of mnemonic traces showed heterogeneous findings. On one hand, some investigations failed to detect an increase in working memory performance after affordances of manipulable objects (Pecher, 2013; Quak et al., 2014), as well as in long-term memory domain (Canits et al., 2018). On the other hand, several empirical evidence showed that

the retrieval of memory traces elicits the activation of sensorimotor brain areas (Ianì, 2019). Moreover, the haptic exploration of familiar objects is able to affect the storing of object's mnemonic traces, even without the intention to memorize them (Hutmacher & Kuhbandner, 2018), and activations in the left medial frontal gyrus and the intraparietal cortex have been described during the recall test of haptically encoded objects (Stock et al., 2009). When action and objects are present, the role sensory-motor system in supporting memory processes is even more evident. Indeed, the adoption of interfering posture (e.g. keeping hands behind the back) during manipulable objects presentation decreased the recall of the same objects (Dutriaux & Gyselinck, 2016), indicating that action simulation processes evoked by objects presentation may support the recall performance. An investigation that adopted fMRI and pupillometry showed that goal-directed action boost episodic memory engaging the medial temporal lobe and locus coeruleus (via noradrenaline transmission) (Yebra et al., 2019). Recently, Calvo-Merino (2020) hypothesised that "brain regions participating in action observation are likely candidates to maintain bodily stimuli in memory". Indeed, several evidence including behavioural performance, fMRI and electrophysiological data, underline a similar activation during observation of stimuli and their memory storage and retrieval. The posterior areas elicited during the observation of visual stimuli were equally engaged during memory storage of those stimuli; at the same way, body stimuli and body-related stimuli activated anterior frontoparietal areas belonging to the action observation network (Galvez-Pol et al., 2020).

In a behavioural-neuroimaging study, Dickenson et al (Dickerson et al., 2007) asked participants to observe common objects and then to perform a free recall test of those same objects. Results showed that greater were activations of hippocampus, fusiform and inferior parietal regions, medial and ventral temporal regions during viewing

and encoding of some objects, greater was their recall comparing to missed items. The authors found additional activations in the right precentral gyrus, in the inferior parietal lobule and intraparietal sulcus.

The fact that memory tasks such as object recall may be associated to the activation of brain substrates typically involved in sensorimotor function, open the way to neurophysiological investigations aimed at forging a theoretical framework linking the modulation of motor system activity with the recall performances.

For this purpose, we conducted a behavioural-neurophysiological study aimed at investigating the relationship between the individual motor responsiveness to the presentation of grasped vs static objects, and the ability to recall objects after their previous visual exposure, either within a grasping action, or in an isolated, static presentation.

The experimental study

3.1 Objectives

The main objective of this thesis is to study the effect of the observation of grasping objects compared to their static presentation on their subsequent recall. The secondary objective is to investigate the neurophysiological substrates of objects' recall facilitation by action observation (AO)/object observation (OO), using Transcranial Magnetic Stimulation (TMS- see Appendix I). For this purpose individual indexes of corticomotor modulation by AO/OO has been correlated with the relative effect of AO/OO on memory recall.

3.2 Participants

Twenty right-hand subjects (12 women, 8 men, mean age 26 ± SD 2,6 years) were enrolled for this study and underwent the behavioural-neurophysiological investigation. None of them had any history of neurological/psychiatric diseases or contraindications to TMS administration. Participants were informed about the experimental procedures and gave their written consent according to the Helsinki Declaration.

3.3 Methods and Material

The experimental design was composed by two separate sessions (administered in different days): (1) the TMS experiment assessing the corticomotor modulation by AO/OO, and (2) the behavioural evaluation of the AO/OO effect on objects' recall.

The behavioral and neurophysiological experiments were performed in the Institute of Neuroscience of National Research Council (CNR, Parma, Italy) between October 2021 and February 2022.

3.3.1 Neurophysiological data recording session

Transcranial Magnetic Stimulation (TMS) was delivered by a figure-of-eight coil (70 mm) connected to a Magstim BiStim stimulator (Magstim, Whitland, UK) and combined with electromyographic (EMG) measurements to assess motor evoked potentials (MEPs). TMS was applied to the scalp with the coil handle rotated 45° from the sagittal plane. EMG signal from the right First Digital Interosseus (R-FDI) muscle was recorded using surface Ag–AgCl electrodes. The optimal stimulation location (hotspot) corresponding to the R-FDI was determined before the experimental session. The hotspot was defined as the scalp location providing the highest peak-to-peak MEP amplitude in the relaxed FDI averaged over five consecutive stimuli. The coil position and orientation were co-registered to a brain template obtained from individual head landmarks (nasion, ears, scalp surface), using an optoelectronic neuronavigation system (VISOR 2, ANT Neuro, the Netherlands).

The EMG signal was amplified ×1000 employing a CED1902 amplifier (Cambridge Electronic Design), sampled at 4 kHz, and acquired using Spike2 software (Cambridge Electronic Design). An additional channel containing digital markers of the TMS trigger was integrated into the same EMG file. The data were stored for subsequent analyses.

The corticomotor excitability was assessed recording the following TMS parameters:

a) The Resting Motor Threshold (RMT) was defined as the lowest stimulator output intensity capable of inducing MEPs of at least 50 μV peak-to-peak amplitude in relaxed FDI in at least 5 out 10 trials.

b) Peak-to-peak MEP amplitudes of MEPs elicited by single-pulse TMS (120% RMT intensity) were measured in the resting FDI.

c) Short-interval intra-Cortical Inhibition (sICI) was obtained from paired-pulse TMS protocol. A sub-threshold conditioning stimulus was delivered at 80% of

RMT, at the inter-stimulus interval (ISI) of 3 ms before a supra-threshold, conditioned, test stimulus (120% RMT). Both stimuli were delivered by the same coil in the same scalp position. ICI was expressed as the percentage decrease of MEPs amplitude from the single-pulse TMS condition, according to the following formula: $sICI = \left(1 - \frac{\text{Conditioned MEP amplitude}}{\text{Single-pulse MEP amplitude}}\right) * 100$.

Participants performed the experiment seated in an armchair in front of a 17'' LCD computer monitor (1024 x 768 pixels), placed at 60 cm from their frontal plane. In each session, participants were asked to keep their upper limbs relaxed.

First, the above mentioned TMS parameters were administered during the continuous observation of a black screen with a white cross in its center. Two separate sessions lasting 2 minutes were administered, one for each specific TMS parameter (standard MEPs, sICI). Within each session, 10 TMS pulses were administered. The items were chosen from a dataset including sixty-four common objects by Snodgrass & Vanderwart (1980, see Appendix II).

Within each TMS protocol, TMS was randomly delivered in ten out of the twenty-four video-clips: five pulses were delivered 120 ms prior to hand-object contact in action observation stimulus and five pulses were delivered 120 ms after the object presentation. These latencies have been selected as time-point were the maximal MEP amplitude is expected, on the basis of previous studies (Franca et al., 2012; Gangitano et al., 2001). Accounting for potential repetition suppression phenomena related to the TMS series, protocol sequences were randomized across subjects.

3.3.2 Behavioural experiment

The following behavioural experiment was implemented with Gorilla Experiment Builder (*www.gorilla.sc*). All the videocalls were recorded and stored.

The behavioural paradigm included three parts: (1) observation and encoding, (2) distractor task and (3) recall test.

1) In the first part, participants were asked to carefully observe and memorize as many objects as possible that were presented in a videosequence so structured: twenty objects were randomly presented (ratio 1:1) either as target of a reach-to-grasp action (i.e. "action observation" - AO), or statically, without any biological effector (i.e. "objects observation"- OO). The order of presentation was randomized. The overall duration of the video was 60 s (3 s for each object). The items were chosen from a dataset including sixty-four common objects by Snodgrass & Vanderwart (1980, see Appendix III).

2) Then, participants were required to perform a distractor task (about 60 s) where they had to solve twenty-two simple mathematical calculations (e.g., 5x3), with a time limit of 5 seconds for each operation.

3) Finally, subjects were asked to perform a recall test of the items observed in (1). During this task, subjects were placed in front a black screen and instructed to "recall as many objects as they can remember", having a maximum time of 2 minutes. Performance was videorecorded and subsequently scored offline.

After the recall experiment, subjects were asked to fill out an online form (https://www.google.it/intl/it/forms/about/) aimed to investigate their memory strategies, as well as their familiarity toward the just observed objects (see appendix IV).

3.4 Neurophysiological and Behavioral indexes

3.4.1 Neurophysiological indexes

Beyond investigating the modulations induced by action observation and object observation at the population level, we analysed at individual level thus computing the ratio between action observation (AO), object observation (OO) and rest condition for each of the TMS parameters:

a) $\frac{\text{MEPs amplitude }_{AO}}{\text{MEPs amplitude }_{REST}}$, obtained from the peak-to-peak MEPs amplitude ratio between action observation and rest condition, i.e., the MEPs *amplitude gain* induced by action observation;

b) $\frac{\text{MEPs amplitude }_{OO}}{\text{MEPs amplitude }_{REST}}$, obtained from the peak-to-peak MEPs amplitude ratio between object observation and rest condition, i.e., the MEPs *amplitude gain* induced by object observation;

c) $\frac{\text{MEPs GAIN}_{AO}-\text{MEPs GAIN}_{OO}}{_{mean}\text{MEPs GAIN}}$, namely the *corticospinal modulation index (Cs-I)*, i.e. the relative gain of corticospinal excitability induced by action observation vs object observation;

d) $\frac{\text{sICI}_{AO}}{\text{sICI}_{REST}}$, i.e. intra-cortical inhibition ratio between action observation and rest condition;

e) $\frac{\text{sICI}_{OO}}{\text{sICI}_{REST}}$, i.e. intra-cortical inhibition ratio between object observation and rest condition;

f) $\frac{\text{GAIN ICI }_{AO}-\text{GAIN ICI }_{OO}}{_{mean}\text{GAIN ICI}}$, namely the *intracortical inhibition modulation index (ICI-I)*, i.e the relative gain of intracortical inhibition induced by action observation vs object observation.

3.4.2 Behavioural indexes

The behavioural outcome of the recall test performance was indexed by the following endpoints:

- The total number of recalled objects belonging to the action observation condition (Recall$_{AO}$)

- The total number of recalled objects belonging to the static object observation condition (Recall$_{OO}$)

- A recall index (R-I), computing according the following formula: (Recall$_{AO}$-Recall$_{OO}$/ Recall$_{AO}$+Recall$_{OO}$); if higher than zero, R-I indicates that recall is more facilitated by action observation; on contrary, if R-I lower than zero, it indicates that recall is more facilitated by static object presentation.

3.5 Statistical analysis

The effect of AO vs OO on the subsequent recall was assessed comparing the performance in each condition using non-parametric comparison (Wilcoxon test).

The modulation of corticomotor excitability by AO/OO was assessed comparing the TMS parameters (corticospinal excitability and intracortical inhibition) collected at rest vs observation conditions, by means of non-parametric contrasts (Wilcoxon test).

In order to explore the link between the behavioral effect on memory recall exerted by AO vs OO and their reciprocal effect on corticomotor modulation, a correlation between the recall index (R-I) and both corticospinal (Cs-I) and intracortical inhibition (ICI-I) modulation indexes were evaluated using Spearman's rank correlation coefficient. In case of significant correlations, the capability of the basal neurophysiological features to predict recall performance in subjects was tested by applying a linear regression model.

Statistical analyses were performed using JASP version 0.16.1. The significance threshold was set at 0.05.

Results

4.1 The effect of action and object observation on corticomotor excitability

Even if slightly higher from a merely descriptive point of view (see figure 1), MEPs elicited during action observation and object observation were not significantly facilitated respect to rest condition (1.40 ± 0.9 mV vs 1.46 ± 0.9 mV vs 1.34 ± 0.8 mV). Looking at the single-subject level, 60% of subjects showed an increase of MEPs amplitude during action observation (MEPs GAIN AO>1) and 55% of subjects showed an increase of MEPs amplitude during object observation (MEPs GAIN OO>1), with an overlap of 40% between AO and OO facilitators.
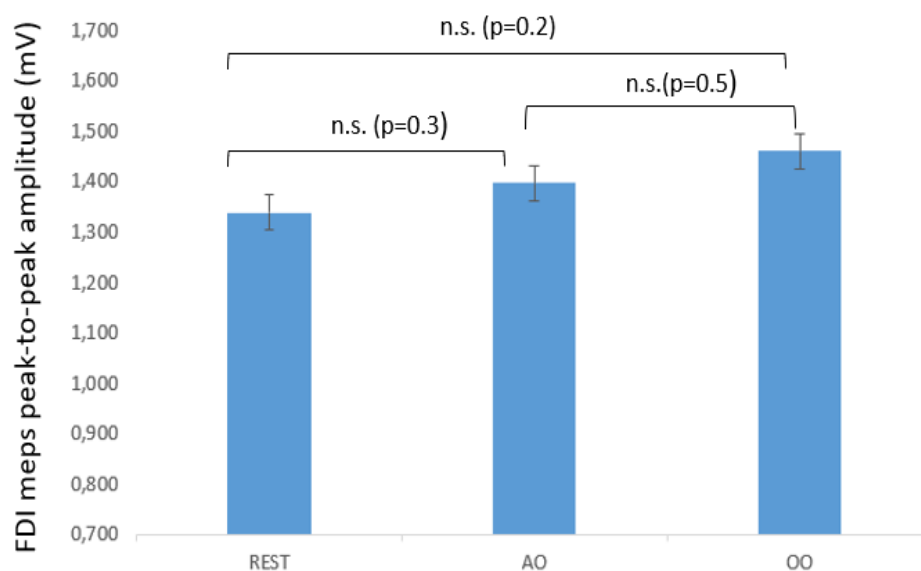

Figure 1 Effect of action and object observation on peak-to-peak MEPs amplitude. Bar charts represent the mean value at rest, during action observation (AO) and object observation (OO).

Similarly, AO and OO did not induce any significant change of sICI (see figure 2) in comparison to rest condition (87% ±10 vs 86% ±12 vs 84%±12 vs).
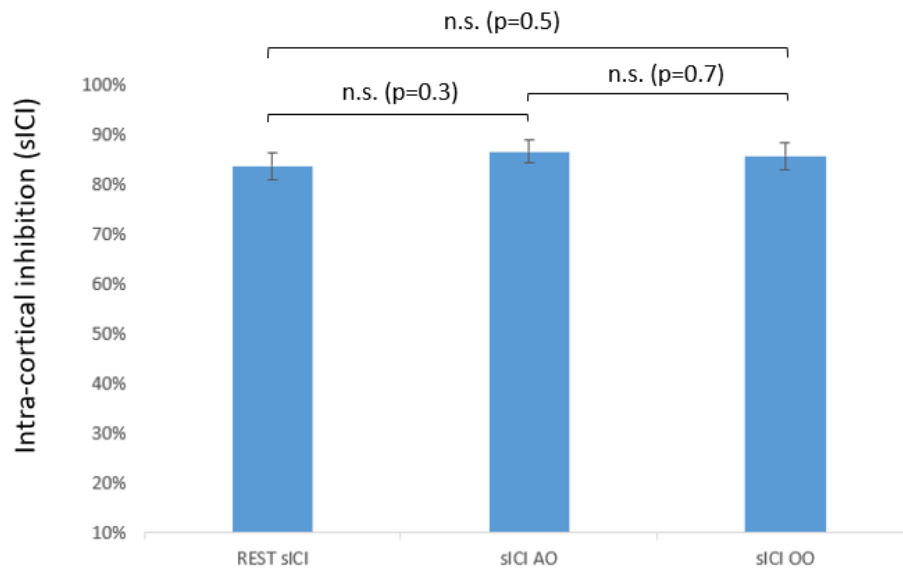

Figure 2 Effect of action and object observation on short-interval intra-cortical inhibition (sICI). Bar charts represent the mean value at rest, during action observation (AO) and object observation (OO)

However, looking at the single-subject level's suppression in the two conditions, 58% of subjects showed a decrease of MEPs amplitude during action observation (ICI GAIN AO>1); 53% of subjects showed a decrease of MEPs amplitude during object observation (ICI GAIN OO>1).

4.2 The effect of action and object observation on recall test

Moving to the behavioral domain, participants recalled on average 10.7±1.7 items. No advantages from previous exposure to AO vs OO on recall performance was observed (Recall$_{AO}$=5.38±0.9 vs Recall$_{OO}$=5.27±1.2).

Four participants had a recall index equal to zero (item recall wasn't affected by previous AO vs OO); nine participants had a recall index higher than zero (recall was more affected by AO); seven participants had a recall index lower than zero (recall was

more affected by OO). Two subjects showed a RI exceeding 2 standard deviations from the mean of the sample (i.e. they were behavioural outliers), so they were excluded from the correlational analyses.

Looking at the form filled online by participants, the results don't show any significant difference about the familiarity toward the just observed objects.

4.3 Correlational analyses

A non-parametric correlation analysis between Recall Index (R-I) and Corticospinal modulation index (Cs-I) aiming at investigating the interplay between the recall performance and the corticomotor modulation by AO/OO, returned a positive, significant, correlation ($\rho s = 0.47$, $p=0.04$). In other words, the higher was corticospinal relative facilitation by AO/OO, the higher was AO/OO impact on subsequent, correspondent, object recall (see figure 3).
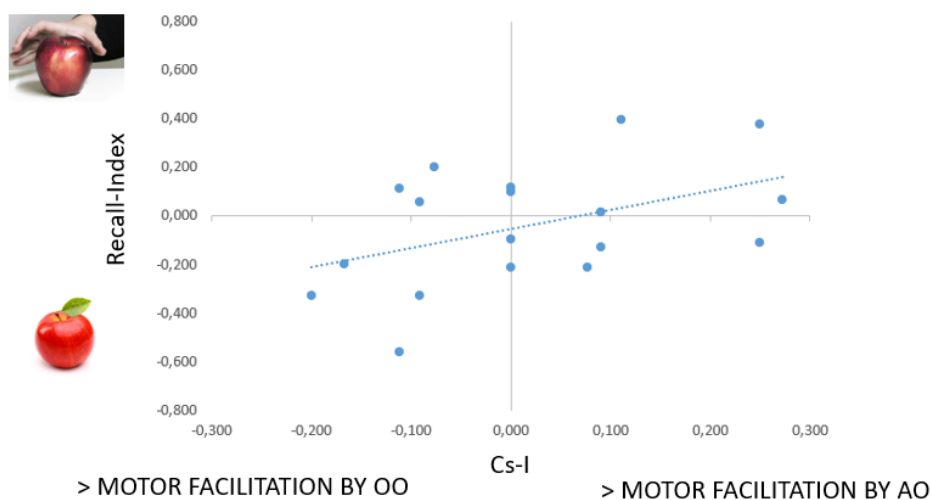

Figure 3 Scatterplot showing the interplay between recall index (R-I) and corticospinal modulation index (Cs-I) induced by action and object observation.

To measure the extent to which the neurophysiological features above could predict the recall performance, a linear regression model was applied. The model showed values close to the statistical significance (R^2=0.20, p=0.06).

On contrary, the correlational analyses linking the recall index (R-I) with intracortical inhibition modulation index (ICI-I), did not show any significant results (ρs =0.03, p=0.9) (see figure 4).
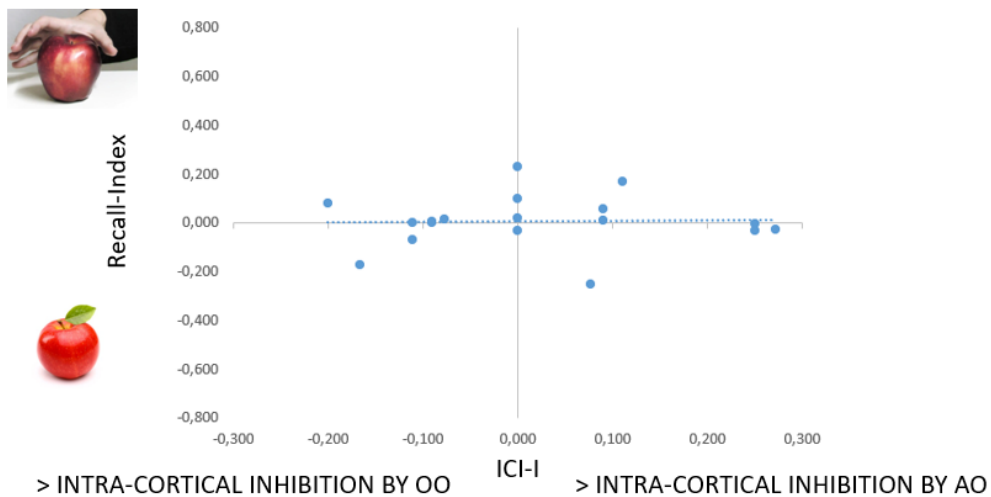

Figure 4 Scatterplot showing the interplay between recall index (R-I) and intra-cortical inhibition modulation index (ICI-I) induced by action and object observation.

Discussion

In this behavioural-neurophysiological study we aimed at investigating the neurophysiological signatures explaining the effect of the observation of grasping objects compared to their static presentation on their subsequent recall. For this purpose, we first collected TMS measures assessing the effect of action observation (AO) vs static object observation (OO) on corticomotor excitability. Then, in a behavioural experiment, we assessed the impact of AO vs OO on the subsequent recall of correspondent objects. Finally, we investigated the relationship between the behavioural performance and the neurophysiological measures of corticomotor excitability.

5.1 Action/object observation effect on corticomotor excitability

A huge body of research have documented that both action observation (Fadiga et al., 1995b; Naish et al., 2014b; Nuara et al., 2021) and object observation (Franca et al., 2012) are able to modulate neurophysiological parameters of corticomotor excitability, even if with a remarkable discrepancy in terms of motor responsiveness across subjects (Naish et al., 2014b; Nuara et al., 2021). Our results confirm such an interindividual heterogeneity: despite corticospinal excitability was slightly increased by AO/OO at population level, a relevant proportion of subjects showed a "motor suppression" while observing actions (40%) or objects (45%). Although surprising, this finding is in line with previous studies, (Hardwick et al., 2012; Nuara et al., 2021; Sartori et al., 2012) and can be explained according to a *"behavioral strategy"* view (Ebbesen & Brecht, 2017; Freud, 1923; Naish et al., 2014a; Nuara et al., 2021) where the excitability of motor pathways is first enhanced by action/object observation but subsequently suppressed to a greater extent by inhibitory projections from the prefrontal cortex and inferior frontal gyrus (Hannah & Aron, 2021; Krams et al., 1998; Tremblay et al., 2004), when subjects

requested to volitionally refrain from movement would "repress the urge to act" (Ebbesen & Brecht, 2017; Freud, 1923).

Another interpretation is that the decrease in MEP amplitude could reflect an action observation–induced inhibitory activity of interneurons hosted in the primary motor cortex (Naish et al., 2014a). However, the notion that MEP "suppressors" do not correspond to sICI "enhancers" and the absence of a correlation between MEP suppression and sICI during action/object observation make this latter perspective less likely.

5.2 Neurophysiological features explaining action/object observation effect on memory recall

Looking at population level, the mere behavioral results did not show a significant advantage of Action/Object observation administration on the subsequent recall of correspondent objects. A remarkable proportion of subjects showed an unbalanced impact of either one action or object observation on recall performance (20% RI=0; 45% RI>1; 35% RI<1).

May this behavioral heterogeneity be explained by individual neurophysiological differences in motor responsiveness to AO/OO?

We found that the recall performance was correlated with, and predicted by, the relative corticospinal excitability modulation exerted by AO/OO. In other words, the greater was corticospinal relative facilitation by AO/OO, the greater was AO/OO impact on subsequent object recall. Interestingly, this correlation was not extended to sICI modulation, suggesting that the putative neuroanatomical substrates of such relationship maybe mostly driven by brain structures outside the primary motor cortex. In this regard, different, not mutually exclusive neurophysiological models can be advanced.

In the realm of subject whose recall is more facilitated by AO (Recall Index>0), higher responsiveness of the parietal hubs endowed with mirror mechanism, may in parallel (1) facilitate the motor output (via parieto-premotor-M1 patways) and, via parieto-temporal projection (2) exert a "gain of function" on temporo-mesial structures primarily involved in memory processes. This view is supported by the acknowledged connection between parahippocampal structures and parietal areas (Dijkerman & de Haan, 2007; Friedman et al., 1986; Kaas, 2004) specifically activated by action observation, but not by object observation (Grèzes, Armony, et al., 2003).

Moving to the subject whose recall is more facilitated by OO (Recall Index<0), a more proficient interplay between temporal structures belonging to the visual ventral stream (involved in object recognition) (Grèzes, Armony, et al., 2003) and prefrontal areas (in turn projecting to motor descending pathways) may have explained the association between a greater recall facilitation by OO and its greater facilitatory effect on corticospinal excitability.

5.3 Limitations of the study

One of the main limitations of this study is intrinsically related to the method used to collect the neurophysiological data. First, single-pulse TMS technique doesn't disentangle the neural substrates of motor output modulations. Future experiments integrating neuroimaging methods and repetitive TMS will contribute to explain the contribution of different neural substrates on corticospinal excitability modulation by action and object observation.

Second, we don't perform a re-test reliability assessment of TMS measures. However, at least two elements may have counterbalanced the potential instability of the neurophysiological measures: first, the adoption of relative, within-subject TMS

measures (e.g. the gain of MEPs amplitude relative to a resting condition) instead of absolute neurophysiological indexes; second, the use of a neuronavigation device ensuring the topographical steadiness of TMS delivery.

Moving to the behavioural performance, a limitation is may related to elements that can affect memory processes such as attention, stress (Schoofs et al., 2013), sleep deprivation (Chee & Chuah, 2008), motivation (Miendlarzewska et al., 2016). Future investigations assessing these factors on the participants will help to fill this gap.

5.4 Future perspectives

Future studies including functional neuroimaging data will help to uncover the neural substrates of the ability of action/object observation to facilitate memory-recall processes. In addition, experiments adopting TMS "virtual lesion" approach will be helpful to weight the functional role of cortical gateways between motor and memory domains (e.g. prefrontal and inferior parietal cortex) in these processes.

For this purpose, also the involvement of people suffering from neurological disorders characterized by mnesic (e.g. Alzheimer disease), motor (e.g. Amyotrophic Lateral Sclerosis) or combined mnesic/motor deficits (e.g. ALS-FDT spectrum) will be able to provide valuable neurophysiological insights. Moreover, the extension of our experimental findings to these neurological frameworks will give the basis for the implementation of novel treatment approaches where the traditional training for memory abilities is integrated with the personalized administration of action/objects observation, according to individual motor features.

Conclusion

In conclusion, our study identified for the first time the motor neurophysiological signatures explaining the effect of the observation of grasping objects vs their static presentation on their subsequent recall. The fact that corticospinal (and not intracortical) excitability modulation by action/object observation is endowed with such a predictive power, suggests an extra-M1 location of the neurophysiological substrates.

Besides its theoretical significance, our study could pave the way for the implementation of novel treatment approaches, where the traditional training for memory abilities is integrated with the personalized administration of action/objects observation, according to individual motor signatures.

Appendix

(I)   The Transcranial magnetic stimulation

The transcranial magnetic stimulation (TMS) is a neurophysiologic technique which allows non-invasive stimulation of human brain. It has been widely used to investigate the central nervous system physiology and the neurobiological basis of motor disorders.

Biophysical mechanism TMS follows the physical principle of electromagnetic induction discovered in 1831 by Faraday. He observed that a pulse of electric current passing through a wire coil generates a changing magnetic field which in turn induces a secondary current, referred to as the eddy current, of opposite direction in a nearby conductor. The magnetic and electric fields lie at right angle to each other. During TMS, electric charge stored in a capacitor is discharged through a stimulation coil held next to the skull. A rapidly changing magnetic field induces a surface parallel electric field in underlying brain tissue. Depolarization of the membranes of the neurons occurs, resulting in excitatory (or inhibitory) postsynaptic potential.
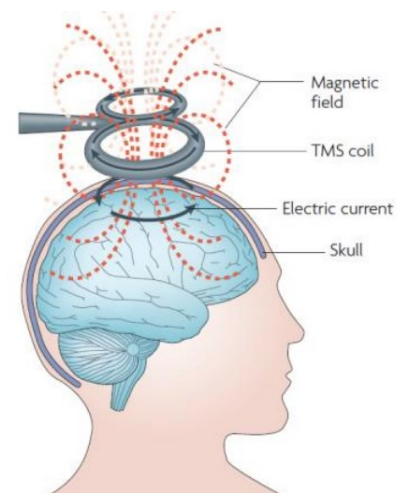
- *Stimulation devices*

The design of transcranial magnetic stimulators may differ in:

- Biophysical characteristics of the produced pulse (monophasic vs biphasic). The current induced in the brain creates itself a magnetic field that tends to cancel the applied one. Therefore, if the current is allowed to freely flow in the circuit, an oscillatory magnetic field would be generated in the nervous tissue (Chen et al., 1998). The circuit of a monophasic stimulator is provided with a diode that

prevents the coil current from flowing in the reverse direction, not allowing subsequent oscillations, whereas in a biphasic stimulator, the inductance and resistance of the circuit is set so that the first rise and fall of the coil current are the major components of the stimulating electric field.

- Geometric shape of the coil, resulting in variations of focality and depth of magnetic field penetration. Available devices include:

  ➢ Round coil. The induced current flows in an annulus underneath the coil. On its central axis the eddy current is quite zero, increasing to maximum towards the coil winding where stimulation occurs. With a typical 14-cm-diameter round coil the electric field strength falls by half at a distance of 4–5 cm from the coil surface (Roth et al., 1990). Considering that cerebral cortex is generally 1–2 cm deep from the surface of the scalp the stimulation is severely attenuated.

  ➢ Figure-eight coil (butterfly coil). It consists of two adjacent circular coils, placed side by side, each traversed by an opposite current. The induced tissue current is at its maximum directly under the central segment, where the two windings meet, giving a more accurately defined area of stimulation. The main advantage of this coil shape is the focal stimulation of superficial cortical regions. These tools have a fundamental limitation: stimulation is severely attenuated with increasing of depth. Therefore, to stimulate deep brain regions, very high intensity would be needed, resulting in facial and cervical muscle contractions over the level that may lead to pain. Only the double-cone coil was reported to have the ability to stimulate the leg motor area, which is 3 to 4 cm in depth (Stokić et al., 1997). It is made of two large adjacent circular wings at an angle of 95°

and induces a stronger but less focal electric field compared to a figure-eight coil (Lontis et al., 2006).

- *Single pulse TMS*

Transcranial magnetic stimulation of the brain induces muscle responses termed motor-evoked potentials (MEPs). MEPs are widely used to study the physiology of corticospinal conduction in healthy subjects and in patients with diseases of the central nervous system. A variety of parameters of MEPs can be studied, including the latency yielding the central motor conduction time (CMCT), the size of the MEP (amplitude, duration and area), and others (such as stimulation thresholds, silent period, facilitation, etc.). If a peripheral nerve is stimulated, the resulting compound motor action potential (CMAP) reflects the number of activated motor units (McComas, 1995), which is roughly proportional to the number of activated motor axons. Theoretically, the size of an MEP should also relate to the number of activated corticospinal motor neurons. If electrical stimuli of increasing intensity are applied to a peripheral nerve, the size of the induced muscle response increases. When the stimulus is strong enough, the CMAP size saturates, i.e. the size will not increase if the stimulus intensity is further increased. These characteristics of peripheral nerve stimulation are explained by increasing recruitment of more and more nerve fibers by increasing stimulus. Also, MEPs increase with increasing stimulus intensity, suggesting that stronger stimuli may also recruit more corticospinal and spinal motor neurons, or both.

However, this relation is obscured by some characteristic of MEPs, making the interpretation of its size measurements difficult. Three basic physiological mechanism may influence the size of MEPs. These are:

1) The number of recruited motor neurons in the spinal cord
2) The number of motor neurons discharging more than once to the stimulus

3) The synchronization of the TMS-induced motor neuron discharges.

In studies of corticomuscular conduction in healthy subjects and patients, it is often assumed that the size of MEP reflects the number of activated motor neurons.

The MEP is influenced by the excitability of the corticospinal pathway, which is variable and can be facilitated by several different mechanisms. For example, voluntary background contraction of the target muscle facilitates the MEP by reducing its threshold, shortening its latency and increasing its size, probably caused by an increasing number of spinal motor neuron brought to fire by the TMS.

- *Paired pulse TMS*

Paired pulse techniques provide measures of both intracortical and cortico–cortical interactions. One of the most common approaches used is the conditioning–test paradigm. This refers to how MEP amplitude changes when it is preconditioned by another stimulus.

Short-interval intracortical inhibition (SICI). (Kujirai et al., 1993a) first observed that a subthreshold conditioning stimulus could suppress a MEP evoked by a later suprathreshold test stimulus if the interval between the stimuli (ISI) was between 1 and 5 ms. In normal individuals, the curve plotting the conditioning stimulus intensity and the percentage of inhibition is U-shaped with maximum SICI (20-30% decrease in MEP amplitude) achieved when the conditioning intensity is 80% of RMT. Because the conditioning stimulus is too low to activate pyramidal cells, the authors suggested that the interaction occurred at the cortical level. Later evidence provided by (Di Lazzaro et al., 1998) confirmed this hypothesis. They showed as the I1-wave was nearly unaffected while the I3-wave and later volleys were the most sensitive to this stimulation paradigm, supporting the idea that SICI on reflects the excitability of distinct, low-threshold, GABAergic interneural circuits within the motor cortex (Di Lazzaro et al., 2006; Kujirai et al., 1993b; Müller-Dahlhaus et al., 2008; Wassermann et al., 2021; Ziemann et al.,

2001). In fact, functional properties of the inhibitory networks make them able to discharge more efficiently than excitatory ones at ISIs as short as those used by Kujirai et al., because of a pronounced tendency to synchronize through axonal interconnections (Hasenstaub et al., 2005). In particular, SICI consist of at least two phases: at an ISI of 1ms this phenomenon is caused by axonal refractoriness rather than by synaptic inhibition, responsible for the second phase starting at ISI of 2,5ms (Fisher et al., 2002). Interestingly, SICI is modulated by muscle contraction, being reduced during contraction of the target muscle and increased during contraction of nearby muscles, a condition where the target muscle must remain relaxed. Finally, proximal arm muscles motor areas have a significantly less SICI amount rather those of intrinsic hand muscles, indicating a fundamental role of inhibitory control in finely tuned finger movements (Stinear & Byblow, 2003).

Previous reports (Fadiga et al., 1995b) show how action observation overall enhances corticospinal excitability, as measured by MEPs amplitude, and how the sICI decreasing is related to action observation (Patuzzo et al., 2003; Strafella & Paus, 2000), joint action (Cardellicchio et al., 2020), as well as action mistake observation (Cardellicchio et al., 2018).

(II)   The twenty-four objects used in the neurophysiological experiment:

Video AO_recall1/2: a grape, a pinecone, a bulb, blue scotch, a stamp, a cotton ball, a cup, a lemon, a chestnut, a tennis ball, a spoon, a candle, a shell, a white scotch, a small yellow clothespin, a lighter, matchbox, bar of soap, a little stone, a felt-tip pen, a white clothespin, a rubber, a nut, a red ball.

(III)   The forty objects used in the behavioral experiment and their assignment to the four videos:

Video 1/3: an apple, a bottle, a shoe, a pencil, a comb, a fork, a flower, a watch, a light bulb, a hammer, a leaf, a toothbrush, a glass, a key, a bag, a hat, a telephone, a carrot, scissors, a ring.

Video 2/4: a pipe, a knife, a button, a ruler, a syringe, a whistle, a screwdriver, a feather, a cigarette, a corkscrew, a book, a strawberry, a brush, a glove, pliers, a pan, a bell, a gun, eyeglasses, a funnel.

(IV)   The online form:

- Qual è la sua occupazione?

- Quali sono i suoi interessi, passioni, hobby?

- Durante il compito di memoria, ha notato di aver adottato delle strategie per facilitare il ricordo degli oggetti presenti nel video?

- Di seguito le verranno presentate le immagini degli oggetti che ha visto nel video precedente. Indichi, su una scala da 1 a 5, quanto l'oggetto mostrato è familiare nella sua vita di tutti i giorni. Non ci sono risposte giuste o sbagliate. Tenga in considerazione tutti i valori della scala, considerando che 1=Per nulla familiare; 5=Molto familiare.

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