UNIVERSITA’ DEGLI STUDI DI PARMA

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DOTTORATO DI RICERCA IN NEUROSCIENZE

CICLO XXV

# Objects Representations in Motor Cortex: Neurophysiological and Behavioral Evidence

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# OVERVIEW

The present series of experimental studies had the main aim of investigating if, and in such case how, graspable objects are represented within the Motor System of human observers.

The first study had four different goals: to verify if object sight induces an automatic excitability increase within the Cortico-Spinal pathway of human participants, as it is known to occur in macaque monkeys; to verify to what extent this excitability modulation, if present, is effector-specific (i.e. occurring exclusively in those Cortico-Spinal neurons targeting muscles involved in reach-to-grasp action toward the observed object); to verify to what extent this excitability modulation, if present, is time-specific (i.e. occurring within a discrete time-window locked to object presentation); finally, differently from previous studies published on this issue, as the objects used as visual stimuli were always in the same central position relative to the observer, any spatial compatibility arising between participant's hand and presented objects was ruled out as major determinant (as well as confounding factor) of any excitability modulation.

To this aim, Transcranial Magnetic Stimulation was adopted as the most suitable technique device to address these topics. Indeed, by using this approach it is possible to verify in vivo the occurrence of subtle changes in excitability in the Primary Motor cortex, with an extremely high time-resolution (in the millisecond order).

Furthermore, differently from animal studies, in the present one participants were not trained, nor required to grasp any of the objects, ruling out any priming effect due to the training period that necessarily precedes any experimental work performed in monkeys.

The second study aimed at shedding further light on the same topic. Specifically, it primarily aimed at verifying if "graspability" was the sole and sufficient object-feature required to induce excitability changes within the observer's Motor System. For this purpose, the object adopted was a small-sized cylinder, always presented in the same central position, and never grasped throughout the study. By so doing, differently from previous studies, any possible confounding factors related to familiarity with the object and its usage were contextually excluded.

Participants were asked to perform a choice-reaction time moving the right or left index finger by contracting the right or left first dorsal interosseous muscles as fast and as accurately as possible, as soon as they recognized the cylinder or its absence (the control condition), respectively. The first two experiments were performed in two different groups of right-handed participants, but in the second one a transparent barrier interposed between them and visual stimuli prevented any hand-object interaction. Finally, to verify the influence of the observer's motor repertoire on the recorded effects, the last experiment was performed in  a group of left-handed participants.

# PART 1

# Corticospinal Facilitation During Observation of Graspable Objects: a Transcranial Magnetic Stimulation Study

## 1.1 Introduction

In his ecological approach to vision [1], Gibson argued that, 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. He 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 very 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 both to the primary motor cortex and to the cervical enlargement [2-6].

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 [7]. 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 the same object without making any movement [8]. The visuomotor neurons belonging to this class have been successively named "canonical neurons" [9] to distinguish them from the other class of visuomotor neurons in area F5, the “mirror” neurons, responding instead to action observation [10-11]. Further studies confirmed the existence of “canonical” neurons within both ventral and dorsal premotor cortex [12-13], and within intraparietal region AIP and posterior parietal cortex [14-15].

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” [16-17]. 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 [18-20]. 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 [17, 21]. Other authors described the time-course of the "affordance effect" adopting similar visual stimuli in the context of a Stimulus Onset Asynchrony (SOA) paradigm [22]. The reported effect was minimum for SOA=0 and increased progressively with SOA, reaching its maximum for SOA = 800 ms and 1200 ms. 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 [19], 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 Tomoraphy (PET) experiment with right-handed subjects, reported bilateral activation of premotor cortex during the observation of familiar tools [23]. A functional Magnetic Resonance Imging (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 [24]. 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 [25]. 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. [26] 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) [27]. The paired-pulse TMS protocol is known to produce higher MEPs, probably because it interacts with repetitive discharges of cortical output neurons [28-29] 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 [30]. Oppositely, in another TMS experiment [31], 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 [32] used a combined approach to investigate the “affordance effect”: a reaction-time study and a TMS experiment, both using as stimuli the presentation of pictures of familiar objects shown on a computer screen. They first replicated a behavioral study [19] 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. No other significant differences were observed.

In the present study, we decided to disentangle these ambiguous results by verifying if a significant modulation of M1 excitability dependent on objects observation is detectable in healthy human subjects by using TMS in a very controlled situation. To this aim, we used real objects as stimuli and required subjects to sit still and to relax while looking at them. In so doing, no explicit movement preparation was affecting our results. Objects were always kept in front of each participant, within their peripersonal space. Objects were all small-sized, normally graspable with a grip involving the opposition of thumb and index finger (precision grip), while in the control condition everything was visually identical, apart from the  absence of the object. TMS was applied to the left M1 of each subject and MEPs were recorded from three different intrinsic right hand muscles. Differently from previous studies, we decided here to investigate the time-course of corticospinal excitability in the time interval immediately following stimulus onset, at 120, 150 and 180 ms, prompted by the evidence that in monkey ventral premotor cortex, the majority of visuomotor neurons with object-related responses (“canonical” neurons) show a phasic peak of firing very soon after object presentation [8].

## 1.2 Materials and Methods

### *1.2.1 Subjects*

Twenty-one healthy volunteers (8 males, 13 females; age 23-40) gave their written informed consent and participated in the study. The experimental procedures were approved by Ferrara University Ethics Committee and were performed according to the Declaration of Helsinki. All subjects had normal or corrected-to-normal vision and were naïve as to the aims of the experiment. According to the Edinburgh Handedness Inventory [33], 19 participants were right-handed while 2 were left-handed. Prior to entering the study, the subjects were screened for possible adverse reaction to TMS [34] and were monetarily compensated at the end of the experiment.

### *1.2.2 EMG recordings and TMS*

Surface EMG traces were recorded from three different intrinsic hand muscles of the right hand by using 9-mm-diameter adhesive Ag/AgCl surface electrodes (Kendall, GbmH, DE), in a belly-tendon montage. The muscles were the opponens pollicis (OP), the first dorsal interosseus (FDI) and the abductor digiti minimi (ADM), chosen because of their different involvement in grasping movements [27], [30]. Signals were amplified and band-pass filtered (50-1000Hz) by means of a wireless EMG system (ZeroWire, Aurion s.r.l., IT), then digitized at 2000 Hz and stored on a PC for off-line analysis with Signal Software (2.02 Version, Cambridge Electronic Design, UK). EMG recordings started 200 milliseconds before visual stimuli presentation (which lasted 300 milliseconds) and ended 2 seconds after its conclusion (total duration 2.5 seconds).

Focal cortical stimulation was performed by means of a figure-of-eight coil (90 mm outer diameter) connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK). Magnetic pulses had a nearly monophasic configuration, with a rise time of ~100μs, decaying back to zero over ~0.8 milliseconds. The coil was positioned over the left motor cortex (M1), tangentially to the scalp, with the handle oriented 45° laterally and backwards with respect to the sagittal inter-hemispheric plane, inducing a medially and anteriorly directed current flow in the underlying cortex, approximately perpendicular to the central sulcus. First the hand motor area was localized and the "hot spot" for the right FDI defined as the scalp site, at which stimulation evoked the largest MEPs from the homonymous muscle. Then, the resting motor threshold for the FDI muscle (RMT) was identified, according to [35]. The stimulation intensity used in the experimental procedure was set to 120% of RMT of each subject to consistently evoke stable MEPs from all muscles in resting conditions.

### *1.2.3 Experimental Procedure*

During the whole study, participants sat on a comfortable chair, with their hands resting pronated on a table in front of them. A 20x20x20 cm black box was placed on the table, its opening facing the subject at 40-centimeter distance and its upper edge being at the same height of the subject's eyes. The centre of the floor of the box - where objects were placed in the "object" condition – coincided with subject's mid-sagittal plane. There were two experimental condition: no-object (the box was left empty) and object (one object at a time was placed inside the box chosen from a pool of ten, see Fig. 1a). All objects were of similar, small size, to evoke similar type of grasping, i.e., thumb-to-index opposition (precision grip). Each object was presented 3 times for a total of 30 trials for object condition and 30 trials for the non-object one. We decided to test only small objects because of some evidence favoring the representation of precision grip over power grasp [36], [37]. With this setup (Fig 1b), objects were within subject's peripersonal space (i.e. reaching distance), and their 3D features (i.e. depth), were fully perceived even at the short viewing interval adopted. All objects were presented centrally and with the longest dimension facing the subjects, so that they were all subtended by a visual angle raging between 1° and 6° in height and width.

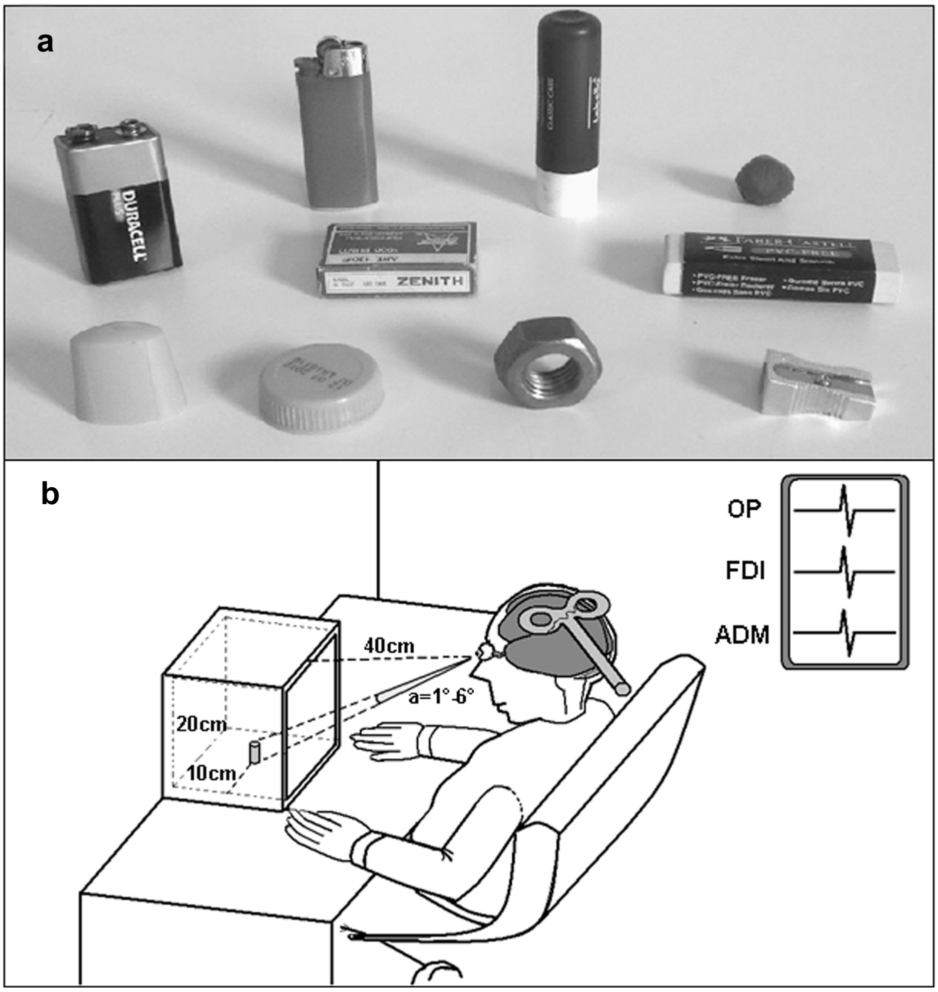

**Fig 1 a.** The set of objects that, one at time, were presented to subjects inside the box. **b.** Schematic drawing depicting the adopted setup: 20-centimeter-sided square box (with an object inside) lying 40 cm in front of our subject. All the objects subtended a visual angle varying between 1° to 6° in width and height.

Participants were required to keep their head on a chinrest, while TMS coil was kept in place by a mechanical arm (Manfrotto, IT). This guaranteed constant head and coil positions throughout the study. The room was completely darkened before the session started. Every trial was structured as follows (Fig 2): a warning sound advised the subject to open his/her eyes. After an interval of 1 to 2 seconds, the box with its content was illuminated for 300 milliseconds (Stimulus Presentation). A LED lamp inside the box, but hidden to the subject, lit up the box. Then, after one of three possible delays from stimulus onset (120, 150 or 180 milliseconds), a TMS pulse was delivered to the subject's left. At the end of the trial, the subject had to close his/her eyes waiting for the next warning sound, while the stimulus was manually changed, according to a precompiled randomized list. Inter-trial interval was always longer than 10 seconds, avoiding any possible unwanted interference of one magnetic pulse on the next. Participants were requested to just watch the stimulus while keeping their hands relaxed in order to then answer a question asked after the end of the stimulus presentation (end of trial). The questions were about the presence or the absence of an object, or about its salient features (mainly color, shape, name or normal use). Questions related to manipulation or interaction with objects were explicitly avoided. By this way, corticospinal excitability as revealed by MEPs area, could not be influenced by any explicit ongoing motor program. Ten practice trials preceded the experimental procedure recordings. Randomization of trials was performed by E-Prime 2.0 Software, which also triggered EMG acquisition, magnetic stimulator and visual stimulus presentation (i.e. turning on and off the LED) via the adoption of the Usb to Parallel FIFO (UPF), [38].

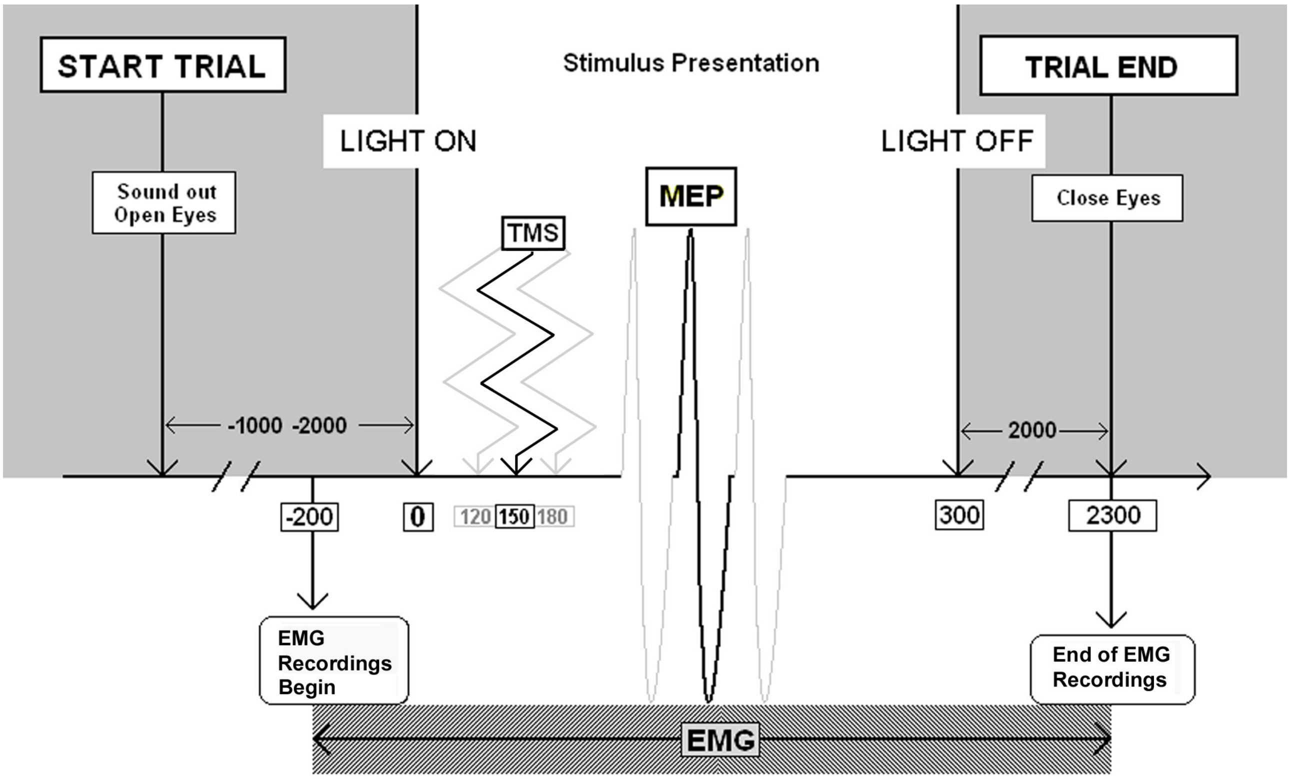

**Fig 2.** Schematic time-line of events in each trial. All numbers indicate milliseconds relative to the turning on of the LED inside the box (time 0). In black, magnetic pulse delivered 150 milliseconds

after stimulus presentation onset, while alternative delays of magnetic stimulation are depicted in light grey.

### *1.2.4 Data Analysis*

Trials showing EMG activity prior to TMS were discarded from further analysis (less than 5% of all trials, equally distributed between the two conditions). After calculation on a trial by trial basis of MEPs area from rectified EMG, MEPs area values were normalized (z-scores) separately for every subject and for each muscle to reduce inter-subject variability. Offline analysis of MEPs area was carried out with MATLAB (version 9.a, The Mathworks Inc., Natick, USA) and STATISTICA (www.statsoft.com) to perform the statistical analysis. For each dependent variable, data regarding each muscle were entered in a 2x3 within-subject ANOVA. Main effects of Object Presence (two levels, object and no-object), TMS Delay (three levels, 120, 150 and 180 ms) and the interaction between these 2 factors were computed. Subsequently, paired t-test comparisons were performed between conditions of interest.

## 1.3 Results

None of the subjects ever made any mistake answering questions related to the presented stimuli. Superimposed MEP traces from the three muscles of a representative subject are shown in Fig. 3 Mean MEP areas (+/-standard deviation) from OP, FDI and ADM across all subjects were equal to 10.9 (+/-11.8), 11.9 (+/-7.5), 7.1 (+/-4.8) mV*s respectively.

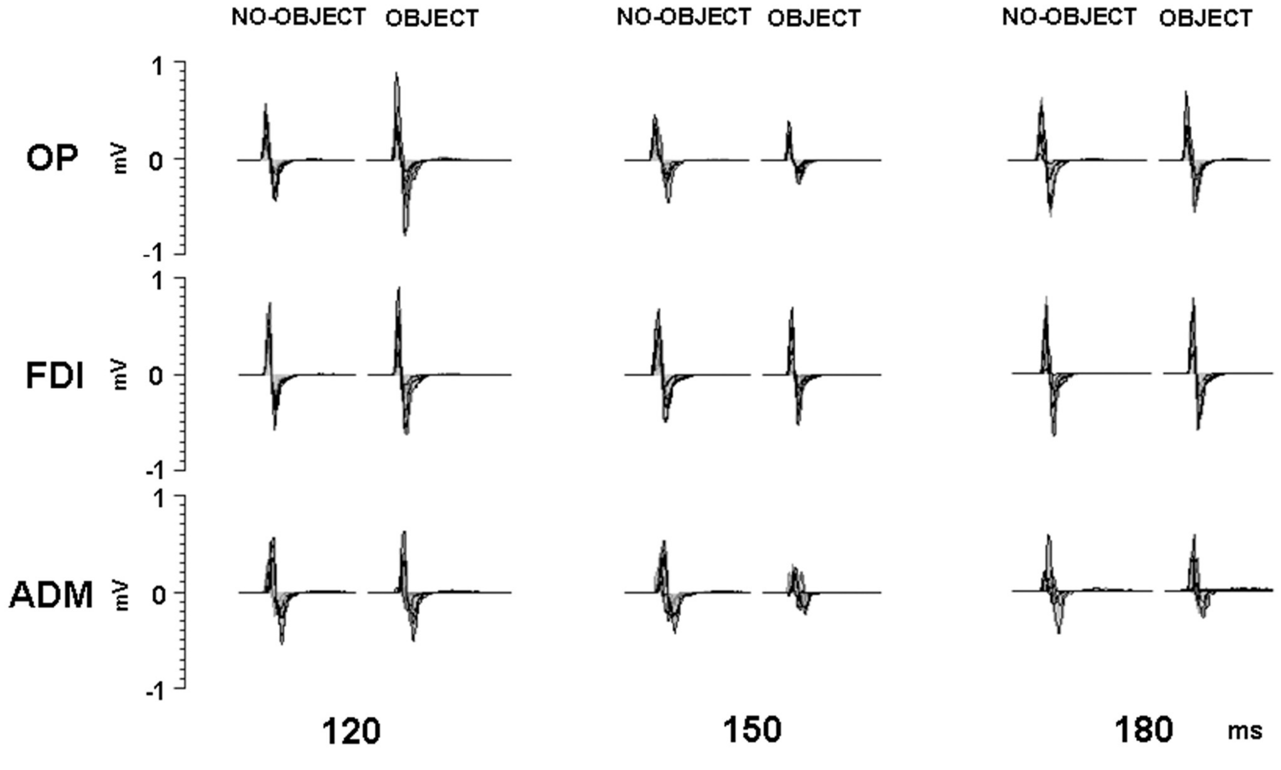

**Figure 3**. Effects of different conditions on MEPs, recorded in a representative subject. Each panel shows superimposed the traces (n=10) evoked from the indicated intrinsic hand muscle.

### *1.3.1 Within-subject ANOVA for MEP area and post hoc comparisons*

The within-subject ANOVA performed for the OP showed no significant result for the main effect of Object Presence ($F_{1,20} = 2.520$, p =0.128) and for the main effect of TMS Delay ($F_{2,40} = 1.011$, p =0.373), but the interaction was significant ($F_{2,40} = 4.375$, p = 0.019). Comparisons between the two conditions of interest ("object" and "no-object") for each time interval revealed a significant difference for 120 ms delay ($p<0.05$). For the FDI muscle, the main effect of Object Presence was significant ($F_{1,20} = 5.086$, p = 0.035), while TMS Delay and the interaction were not ($F_{2,40} = 2.058$, p = 0.141 and $F_{2,40} = 0.469$, p = 0.629, respectively). Post-hoc comparisons between object and no-object at the three delays revealed a significant difference for the 120 ms only ($p<0.05$). For the ADM muscle, while the main effect of Object Presence and the interaction were not significant ($F_{1,20} = 0.177$, p =0.678 and $F_{2,40} = 0.253$, p =0.778, respectively) the main effect of TMS Delay was significant ($F_{2,40} = 3.719$, p =0.033). Post-hoc comparisons revealed a significant difference between 120 and 180 ms ($p<0.05$) independent from the presence of the object. Figure 4 summarizes the obtained results.

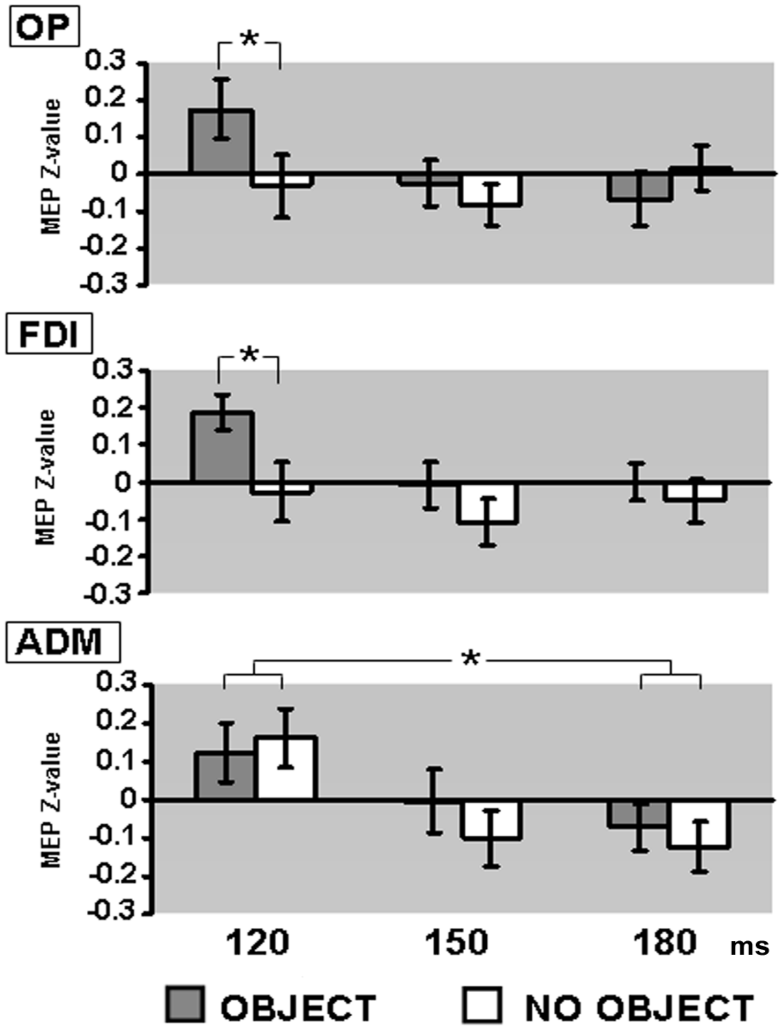

**Figure 4.** Graph representing normalized MEP data for three intrinsic hand muscles at the three timings studied (milliseconds from stimulus onset). From top to bottom of figure: opponens pollicis (OP),

first dorsal interosseus (FDI), abductor digiti minimi (ADM). Dark grey histograms correspond to “object” condition, white histograms to “no-object” condition. Error bars indicate standard error of the mean. Asterisk indicates significant difference of post-hoc comparison ($p<0.05$).

## 1.4 Discussion

The main finding of the present study is that the motor system is automatically facilitated by the observation of graspable objects within a small time interval after their visual presentation. This facilitation was muscle-specific, i.e. limited to the muscle involved in the actual manipulation of the object, and occurred very early, i.e. 120 milliseconds after stimulus presentation. These results support the notion of a sensory-motor system automatically converting visual description of objects into pragmatic coordinates to act on them [2].

### *1.4.1 Selectivity in modulation of corticospinal excitability*

Corticospinal excitability modifications reported in our study seem to reflect the sensory-motor activations that neuroimaging studies have already shown during object observation, along the parieto-frontal circuit for grasping [23-25, 39, 40]. It has to be noticed that, differently from previous studies [17-20, 22, 31-32], the stimuli adopted here were all symmetrical, centrally presented and without differences in orientation. This means that although they possess fewer local spatial and functional differences with respect to objects with handles, they cannot evoke strong attentional spatial shifts. Nevertheless, we were able to report a significant increase in MEP areas recorded from the right FDI in the "object" respect to the "no- object" (control) condition, with a transient modulation occurring in its cortical representation, while noticeably, no differences could be found for ADM relatively to presented stimuli. Moreover, MEP recorded from OP revealed a significant interaction between stimuli and TMS-timing only 120 milliseconds after stimulus presentation. This pattern of muscle involvement agrees with an effector-specific behavior (i.e. an action-specific behavior) and, at the same time, the modulation was time-specific, being recorded only after 120 milliseconds from stimulus onset.

Another interesting result concerns the right ADM muscle, whose MEPs seem to reveal an increase excitability of its cortical representation, 120 milliseconds from stimulus onset. The excitability pattern, observed across the representations of the three different effectors studied (OP, FDI, ADM) known to be widely intermingled in M1, could reflect a transient cortical activation occurring simultaneously (i.e. 120 milliseconds from the instructing stimulus onset), but with a different level of sensitivity to the stimulus instructed movement. In fact, 120 milliseconds after "object" stimulus onset, both right OP and FDI muscles excitabilities increase, since both muscles are involved in the most probable associated movement (right hand precision grip in "object" conditions); while right ADM excitability increases without any stimulus specificity. These results rule out any unspecific attentional factor (e.g. arousal) as cause of the observed effect, avoiding as well the possibility that the effect of object observation on motor cortices relies exclusively on spatial compatibility between visual stimulus and motor response, as previously hypothesized [41].

The results from MEPs data analysis are congruent with the grip the objects would have "afforded", and it is tempting to say that a modulation dependent on the affordance is the most likely explanation. We could have expected that the effect of object observation in M1, if already present after 120 milliseconds, were still present at later timings and maybe until the object were in view. Since this was not the case, the explanation might be that, as our task involved a simple detection of the object, the effect lasted until stimulus detection was achieved. Had the task required a more complex cognitive process or the actual grasp of the object, the effect would have shown a longer persistence. Following this simple idea, it may be interesting to understand similarities and differences between the present findings and previously reported ones in terms of the adopted task and of the time-window in which the effect was present.

### *1.4.2 Comparison of the present study with previous investigations*

Previous behavioral studies reported that the effect of affordance on reaction times increases slowly and lasts longer than what we observed in our data [22, 32, 42]. It should be noted, however, that the conclusions on activation timing that can be drawn from reaction time studies are not easily transferable to TMS experiments. In fact, in RTs studies, once the instruction-cue was recognized and the instructed response selected, the preparation-state was maintained until the go-signal. In this case, interactions between the motor representations related to the affordance and those coding the instructed motor response (at the basis of the effect) are more likely to occur for longer SOAs. Since in our study movements were to be suppressed, the time-window during which object-related modulation of M1 excitability occurred vanished soon after object recognition, instead of lasting several hundreds of milliseconds after stimulus onset, as when a reaction is to be implemented. Furthermore, of relevance to the present discussion, an interference effect due to TMS pulses delivered on the dominant M1 was reported on EMG recordings from FDI and ADM on the subsequent self-paced reach-to-grasp movement only at 150 milliseconds from stimulus onset (TMS timings were 50, 100, 150 and 800 milliseconds after stimulus onset) [30]. Although different methodological issues suggest that a general conclusion could not be drawn from those results (as for example, about its validity in a reaction time paradigm), still they suggest that visual information processing of objects occurs in motor cortices from the very beginning of stimulus presentation. TMS interference lasted 50 milliseconds at most, and was already present for the 50 milliseconds-timing, vanishing apparently 150 milliseconds after stimulus onset (as proved by the absence of any interference in the two later timings). This could mean that the magnetic pulse prevented the stimulated area from receiving and processing object-related visual information necessary to select the following action, but only if delivered at the very beginning of object presentation, specifically before 150 milliseconds.

As already mentioned, relevant differences in cortico-spinal excitability were absent at 50 and 100 milliseconds, but this is not surprising as such time intervals are probably too early for visual input to influence M1 excitability. TMS was shown to interfere with occipital cortex processing if delivered 100 milliseconds after visual stimuli onset [43, 44], and it seems conceivable to assume that if magnetic pulses on M1 interfere with its activity (in relation to object vision), they might do so only few milliseconds later. If a similar process had occurred in our subjects, then we should have expected a significant difference between "object" and "no-object" conditions also at 150 milliseconds and not only at 120 milliseconds. Nevertheless, as the subjects did not move, it is possible that M1 modulation due to object vision came up soon after the presentation started, lasting only for a very brief period, as intracortical inhibitory processes soon prevailed, dramatically shortening the time-window of the "affordance effect" reported by others. In this scenario, the sight of the object might have behaved as a "distractor", pushing the excitability of M1 away from the resting/inhibited state required by the task, and closer to the action-related state. As a consequence, it is not too surprising to obtain an effect of affordance on MEPs around 120 milliseconds, dissipating soon afterwards. This implies a neural structure receiving object-related visual information and able to transform it into motor representations, in a very short time, and specifically by 120 milliseconds. In agreement with currently accepted models [45-46], this structure is likely to be represented by the parieto-frontal "dorsal" stream and within it, by the cortical areas with the most direct access to the primary motor cortex and to the spinal cord, specifically by premotor cortices. Single-cell recordings from monkey ventral premotor cortex have demonstrated the existence of neurons discharging during object presentation, as well as during the execution of the action needed to grasp a particular object [14]. Their discharge frequency pattern showed a phasic increase immediately after stimulus onset, peaking very soon. Furthermore, it was demonstrated that neurons from premotor cortex can influence the excitability of neurons in the ipsilateral primary motor cortex already after 1 millisecond (peaking in 6 ms), through abundant and fast conducting cortico-cortical projections [47-48]. The same premotor-to-motor cortical circuit has been tested also in human subjects with the aid of TMS, and the short latency of these influences was demonstrated on MEPs, and most interestingly excitability changes showed a state-dependent behavior [49-51]. Although, we do not think that a system analogue to the "canonical neuron system" is the only explanation for the present results, we believe it could be one of the most likely candidates, because of its efferent connections to neurons in the primary motor cortex and because of its stimulus response properties (relevant stimulus features, phasic firing pattern, short latency increase in firing compatible with the early observed effect). Our results seem to be further supported by the recent observation of a significant activation of the sensorimotor cortex, contralateral to the forthcoming movement, 120 milliseconds after the instructing stimulus appearence [52]. Although different in many aspects, the implied methodologies (EEG and TMS) share similar time-resolution allowing a direct comparison [53]. The authors interpreted their results as dependent on the subject's intention to act, as no activation could be detected during motor imagery tasks. The present work extend their results even further, strongly arguing against the occurrence of any effect due to the subject's intention to act at the base of their and our results, as in our work no action had to be executed. On the contrary, the differences observed in the present as well as in their work, can be explained more easily assuming that stimulus detection, in the motor system is "translated" into the motor program required by the task 120 milliseconds after its onset. Finally, from an evolutionary perspective, an "affordance" effect at around 120 milliseconds seems to better fit the need for a rapid, accurate, on-line, visual information processing. As a consequence, this may be considered the most basic demonstration of the "affordance" effect, as it is evident in a simple detection task, whereas the effects reported in other studies may be caused by the same process (most likely canonical activation) persisting for the duration of more complex tasks, or by the combination of this process together with others more closely related to attention or motor preparation.

### 1.5 Conclusions

In the present study, we reported evidence of an automatic recruitment of the dorsal stream during simple perception of graspable objects. This "affordance" effect was extremely selective both in terms of muscle activity and in terms of timing, suggesting that a "potential" action, specific for the observed object, was directly accessed following stimulus presentation.

It is tempting to interpret such effect within the theoretical framework of the "affordance competition hypothesis" recently proposed [46], where the processes of action selection and specification evolve simultaneously and continuously, and where different motor plans offered by the environment are progressively selected, through inhibitory processes, leading to the "winning" motor program to be implemented. The increased activity documented in motor areas by the present results due to graspable object observation, could be interpreted as the initial stage of preparation of motor programs needed to grasp the object, as a "potential" action to be executed. As the subjects know that no subsequent movement directed towards the object has to be initiated, the initial recruitment of the system may rapidly decay or may be actively inhibited in favor of other motor plans. Nevertheless, other possibilities cannot be excluded, as for example our evidence could describe the contribution of motor cortices to a more complete perception of graspable objects, especially when no action has to be implemented.

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# PART 2

# Affordance, Handedness and Stimulus-Response Compatibility: a Choice-Reaction Time study

## 2.1 Introduction

In the following series of behavioural experiments, we verified if "graspability" of a real object is a sufficient and necessary feature as to determine the recruitment of observers' motor system, in accordance with the Theory of Affordances [1]. Indeed, even if a great wealth of behavioural, neurophysiological and neuroimaging studies already support this view [2-14], other authors have proposed that spatial features of objects, rather than their affordance, might be at the basis of all previous results as they could induce attentional shifts in the observer [15-17]. Hence, the effect described as visuomotor or affordance-related, could be simply due to spatial stimulus-response compatibility. To further disentangle these two different hypotheses, in the first two experiments object graspability was varied while keeping constant its visual features and by eliminating any factor related to spatial compatibility between visual stimuli and motor responses.

The third experiment was designed to compare left-handed with right-handed participants that took part in the first study, while object graspability remained constant. So, this last control studied verified if the so called "affordance effect" is modulated by the observer's motor repertoire as predicted by Gibson's Theory of Affordances [1].

To this purpose, we asked participants to judge the presence or absence of a real object of small size (exclusively graspable with a precision grip), responding as soon as possible by moving either the right or left index finger by contracting the right or left first dorsal interosseous (FDI), respectively. The object always appeared in the same central position and consisted of a small-size cylinder (1 cm diameter, 2 cm height). RTs were calculated from electromyographic (EMG) traces recorded from the right and left FDI, normally involved in grasping the presented object.

## 2.2 Materials and Methods

### *2.2.1 Subjects*

Forty volunteers (17 males, 23 females; age 22-40) participated in the first experiment, while another group of thirty-nine participants (14 males, 25 female; age 23-45) took part in the second study. All of them were right-handed. The third study was carried out in twenty left-handed volunteers (7 males, 13 female; age 23-36). Hand dominance was assessed according to the Edinburgh Handedness Inventory [19]. All participants had normal or corrected-to-normal vision and were naïve as to the aim of the research. Written informed consent was obtained from each volunteer. The experimental procedures were approved by Ferrara University Ethics Committee and were performed according to the Declaration of Helsinki. The same EMG-recording technique, visual stimuli, experimental set-up and data analysis were used in all three experiments.

### *2.2.2 Visual Stimuli and experimental conditions*

Participants were presented with two experimental conditions: an empty 20x20x20 cm black box (“no-object” condition) or the same box containing a small metallic cylinder (diameter, 1 cm; height, 2 cm; “object” condition, see Figure 1) kept at the center of the box by an invisible black rod. Cylinder major axis was oriented antero-posteriorly and it was located 5 cm from box open side. The box was placed on a table, facing the subject at a 45 cm distance from its open side (within subjects’ reaching distance), with its upper edge maintained at the same height of the subject’s eyes. The type of grasping afforded by the cylinder was a precision grip (i.e. thumb-to-index opposition). Throughout the study, the background luminance level in the two conditions was 0.3 Lux, as measured at the open face of the box with a photometer (Reed ST-1301 Light Meter).

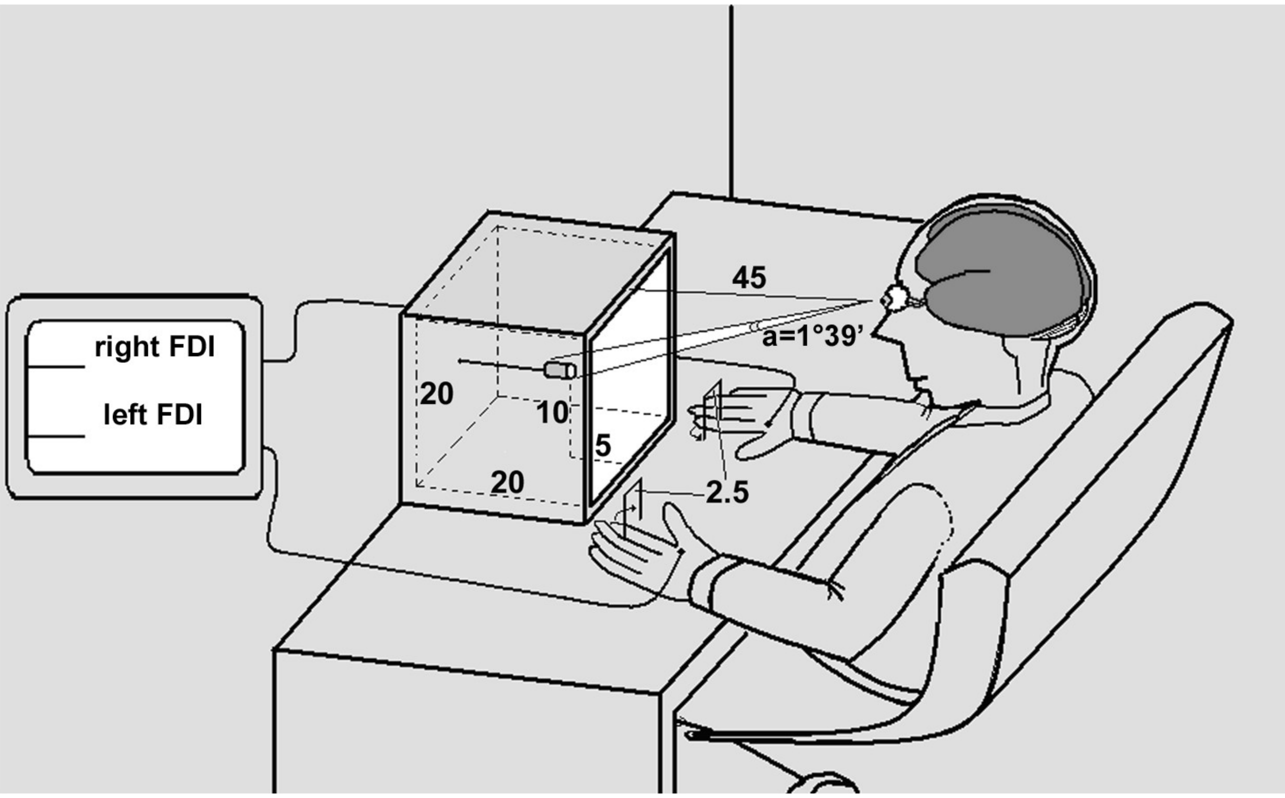

**Figure 1.** Schematic drawing of the experimental set-up (all measurements are expressed in centimeters).

### *2.2.3 Experimental Procedure*

During the whole study, subjects sat on a comfortable chair, with their hands resting pronated on the table, in front of their shoulders. Each index finger was positioned between two vertical bars (about 2.5 cm apart) with both fingers touching the lateral bars with their lateral side. The bars were symmetrically positioned relative to the black box and to the subjects' head (Fig. 1). Participants were required to keep the head on a headrest with the eyes closed. Before starting the experiment, the room was completely darkened. The study consisted of 96 trials, half belonging to the "no-object" condition and half to the "object" condition. Each trial was structured as follows (Fig. 2): an acoustic warning cue (750 Hz, 100 ms duration) advised the subject to open her/his eyes and to fixate a white LED, embedded in the mid-point of the box upper edge, so that it was at the same height as subjects' eyes (fixation light). After a delay randomly varying between 800 and 1000 ms, the fixation light switched off and a multiple LED lamp (not in subject's view) lit up the inner part of the box with its content (visual stimuli presentation). The box could be illuminated for 25, 50, 100 or 150 ms. The fixation light prevented participants' recognition of stimuli before their presentation, avoiding any effect secondary to adaptation to dark. Participants were instructed to remain as relaxed as possible with both hands, before stimulus presentation and to abduct their right or left index finger in order to touch one of the two medial bars (according to the assigned stimulus-response mapping), as soon as they had recognized it. They were required to be as fast and as accurate as possible. After each response, subjects had to reposition their fingers in the starting position and close their eyes, waiting for the next warning sound, while an experimenter manually changed the stimuli. Inter-trial interval was about 10 seconds. The order of box-illumination time, as the order of stimuli presentation ("no-object" vs "object" conditions) were pseudo-randomized within each subject and across them, according to precompiled lists. In each study, half participants were required to touch the right bar, abducting the right index finger, if they had seen the cylinder, and to touch the left bar, abducting the left index, if they had recognized the empty box. Opposite instruction was given to the other half of volunteers (right index-empty box, left index-cylinder). Participants were randomly assigned to one of the two groups. To ensure that all subjects understood the task and could achieve complete muscle relaxation (that was continuously monitored by EMG, see below) before movement, each experiment was preceded by few practice trials. Acoustic cue, fixation light and visual stimuli presentation were precisely timed by using Signal Software (4.02 Version) and CED micro 1401 (Cambridge Electronic Design, Cambridge, UK).

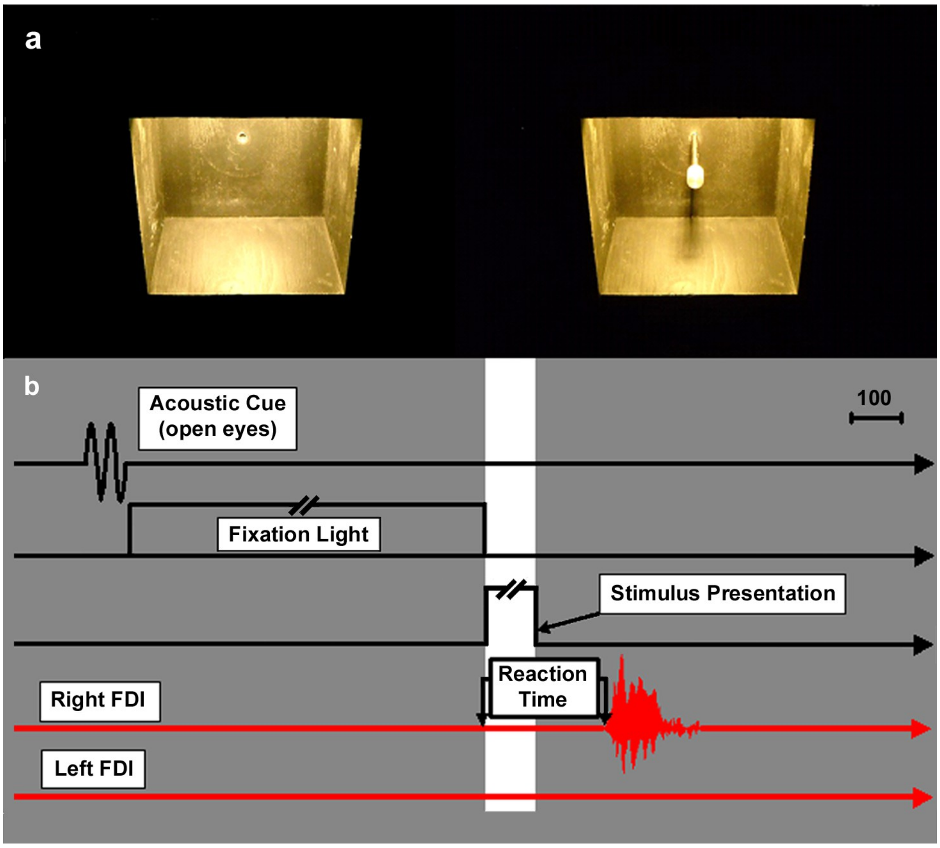

**Figure 2.** Stimuli and Trial Structure. **a)** Illustrates the two visual stimuli adopted in the study: on the left, the "no-object" condition, on the right, the "object" condition. The object colour was metallic grey and its shadow is visible only in the picture, due to camera flash artefact (not visible in any experiments thanks to the multiple LED lamps). **b)** Time-line of events in each trial with an example of a motor response from the right FDI. Time-scale shown in milliseconds on the top right.

### *2.2.4 EMG recordings*

Surface EMG traces were recorded from the right and left First Dorsal Interosseus (FDI) muscles by using 9-mm-diameter adhesive Ag/AgCl surface electrodes (Kendall, GbmH, DE), in a belly-tendon montage. Signals were amplified (1000x), band-pass filtered (2Hz-1kHz) and sampled (2kHz) by means of a wireless EMG system (ZeroWire, Aurion s.r.l., IT), digitized with a CED Micro 1401 analog-to-digital converter and stored on a PC with Signal Software (4.08 Version, Cambridge Electronic Design, Cambridge, UK) for off-line analysis. Data were analyzed with MATLAB Software (version 9.a, The Mathworks Inc., Natick, USA). EMG recordings started, in each trial, 200 ms before an acoustic cue, signaling the beginning of the trial, and ended 3.8 sec after it (the total recording lasted 4 seconds). RTs were collected directly from FDI-EMG, during index finger abduction for three reasons: it is an agonist muscle during precision grip, which was the type of grasp afforded by that small-sized cylinder; it requires a cortico-spinal tract activation, involving intrinsic hand muscles to perform such a peculiar digit movement [20]; it facilitates RT calculation from EMG traces, as FDI contraction determines index finger abduction. By so doing, not differently from previous RTs studies on this topic, the present experimental task required to associate a visual stimulus with an action having a different goal, but performed with effectors partially in common with those involved in the action that most likely would be performed with that stimulus on sight (i.e. affordance). Hence, the task exploits the overlap between the cortical representations of the action afforded by the sight of the cylinder (reach to grasp with right or left upper limb) and of the action for the required response (right or left index finger abduction).

### *2.2.5 EMG data processing and analysis*

In order to obtain objective measurements for RTs, we calculated the mobile arithmetic mean of the rectified EMG signal. To this purpose, each trace was divided in 20 ms bins, spaced by one sample (0.5 ms). Then, each RT was identified as the time interval between stimulus onset and the first bin at which the mobile mean reached the 15% of its maximum (Fig. 3). We further verified that at this time-bin the average EMG activity was significantly higher than the recorded activity in the first 120 ms baseline EMG activity period (one-tailed t-test, p=0.01). The present procedure for RT latency detection was chosen because it had turned out to be the most effective in comparison with others in a previous pilot study. Finally, by visual inspection it was verified that the timing of first increase in EMG activity was always considered as RT latency. Trials with no motor response, with response obtained by the “wrong” muscle or with activity in both FDI muscles were all considered “error trials” and discarded from analysis. When an error occurred, after the end of the trial participants were asked about its reason and specifically whether it was due to visual discrimination or motor selection error. Subjects with a number of “error trials” exceeding 10% were not analyzed.

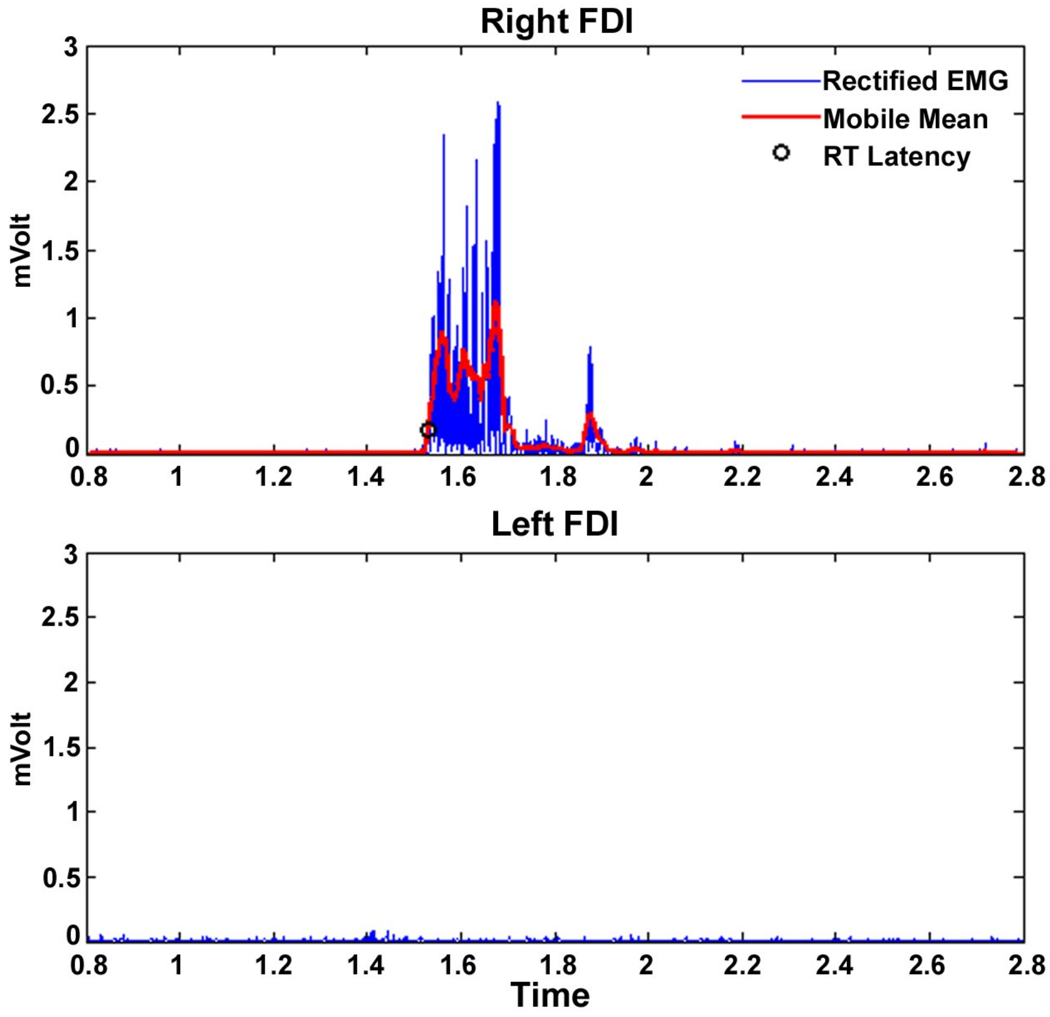

**Figure 3.** Example of a representative trial with right FDI contraction on EMG. Time units are seconds relative to start of trial (beginning of acoustic cue). On the ordinate, mVolt indicates EMG amplitude in millivolts. In blue, the rectified EMG trace; in red, the Mobile Mean of rectified EMG activity; the black circle illustrates the determined RT latency

Mean RTs per condition were calculated in each participant using only correct trials after removing outliers, i.e. RTs falling outside two standard deviations from the mean. STATISTICA software (www.statsoft.com) was adopted to perform a 2x4x2 analysis of variance (ANOVA), with "Object Presence" (two levels: "no-object" and "object") and "Presentation Time" (25, 50, 100 and 150 ms) as within subject factors, and "S-R mapping" ("right-object/left-no-object" and "left-object/right-no-object") as between-subjects factor. Main effects of "Object Presence", "Presentation Time", "S-

R mapping" and the interactions among these 3 factors were computed. Subsequently, post-hoc t-test comparisons were performed between the conditions of interest.

## 2.3 Results

### *2.3.1 Experiment 1*

One participant was discarded by data analysis because he showed outlying mean RT (longer than 1 sec), exceeding three standard deviations from the group RT mean, and three were discarded because of their error rate exceeded 10% of trials. According to subjects' reports, mistakes were always consequence of an incorrect response selection and never due to wrong visual stimuli discrimination. Statistical analysis was thus performed on the RTs of 36 subjects, 18 per mapping group. The ANOVA showed a significant main effect of "S-R mapping" ($F_{1,34} = 5.563$, $p < 0.05$), with shorter RTs when subjects had to move their right index finger in response to the cylinder presence and their left index finger in response to the empty box (355 ± 11 ms) than when the opposite mapping was required (420 ± 25 ms). A significant effect was also found for "Object Presence" ($F_{1,34} = 4.832$, $p < 0.05$), with shorter RTs when subjects responded to the cylinder (383 ± 14 ms) than when they responded to the empty box (392 ± 14 ms). Finally, a significant interaction between "S-R mapping" and "Presentation Time" ($F_{3,102} = 3.665$, $p < 0.05$) was observed, and post-hoc tests showed that RTs differed significantly as a function of the assigned stimulus-response mapping in all presentation intervals but 50 ms, for which a statistical trend was anyway detected ($p = 0.072$). Results are summarized in Figure 4.

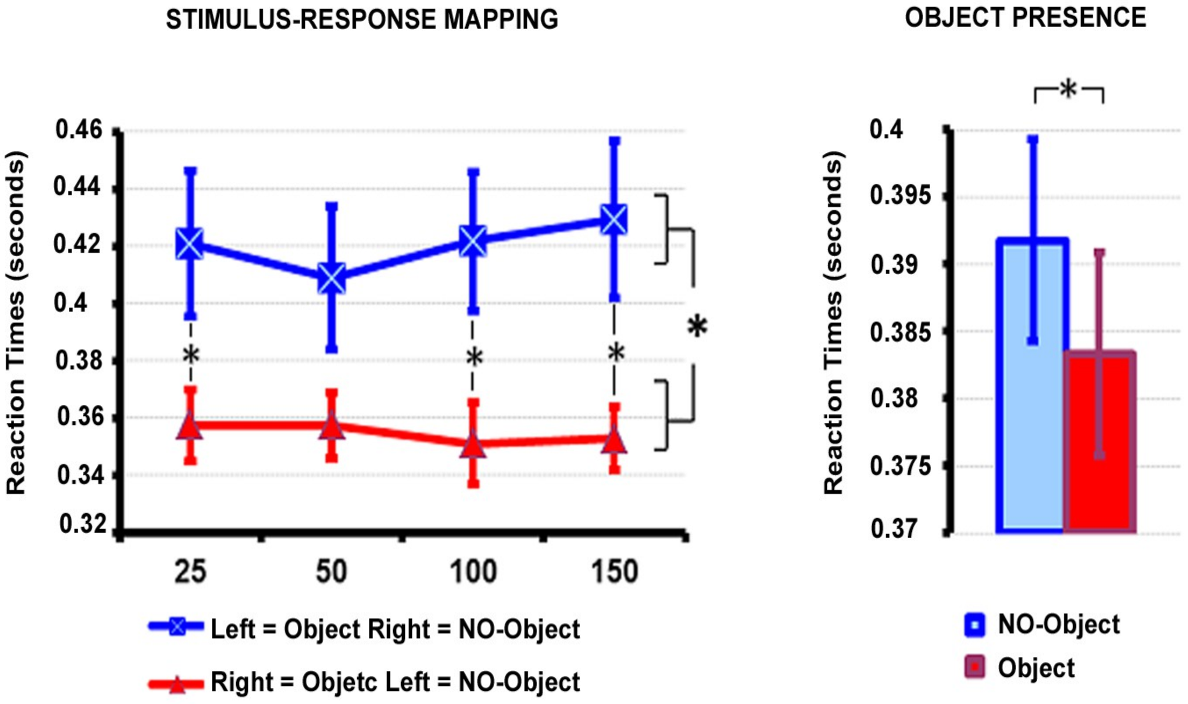

**Figure 4.** Results of Experiment 1. **a)** Average RTs at different Presentation timings (abscissa, units in milliseconds) of participants belonging to the two mapping groups (Blue = Left-Object/Right-NO-Object; Red = Right-Object/Left-NO-Object). Reaction Times (ordinate) are expressed in seconds from stimulus onset. **b)** Average RTs from participants in the "Object" (red) and "No-Object" (blue) conditions. Error bars indicate the standard error of the mean. Asterisks indicate significant differences.

### *2.3.2 Discussion (Experiment 1)*

The main result of the first study is that the recognition of an object inside a box determines significantly shorter RTs than the recognition of the same empty box (Experiment 1). This difference perfectly agrees with the "Theory of Affordances" [1]. Moreover, in light of the adopted stimuli, the present study supports and extends conclusions already drawn about the so called "affordance effect" [2-12, 14-18], showing that such effect does not require any previous experience, familiarity with the object, or knowledge about its usage (that might have behaved to some extent as confounding factors in earlier studies adopting pictures of familiar objects as stimuli). Furthermore, as the presented cylinder was symmetrical and always presented in the same central position, no spatial compatibility code between visual stimulus and motor response could account for the observed RT difference. Hence, we argue that this difference was strictly related to the presence/absence of the object per se, thus favouring the hypothesis of an "affordance effect". In particular, we believe that the present findings offer a clear demonstration of Gibson's definition of "Affordances" [1], and rule out as well other alternative explanations, and specifically the compatibility between the spatial codes activated by the object sight and the required motor response, as other studies might suggest [15-17].

Another interesting result is that right-handed people responded faster when the presence of a graspable object was associated with a movement executed with the right-hand and its absence with a movement executed with the left hand. They were slower when the opposite association was required. Of notice, in all experiments participants never grasped the cylinder. At first glance, the difference between the two S-R mappings could be linked to participants' hand dominance. As in every-day life right-handed people grasp objects more often with their dominant hand, they might be more prone to react with the right hand to the "object" and with the left hand to the "no-object" condition than to the opposite association. If this was the case, the feature of the stimuli determining the observed difference would have been their "graspability". The effect of S-R mapping observed in our study parallels that found by Borghi and Scorolli [21] in which right-handers were required to indicate whether word pairs composed of a verb and a noun (both action-related) made sense or not. Participants showed faster RTs with the right hand than with the left hand when they had to respond to sensible combinations and faster RTs with the left hand than with the right hand when they had to respond to non-sensible combinations. Hence, the interpretation of those results (i.e. reading verb-noun pairs describing manual actions, activates the dominant hand) is consistent with our own. Moreover, our findings are consistent with previous studies showing that right-handers' attention is biased to their dominant hand, so that their right hand is more readily primed to move compared to their left hand [22-23]. Nevertheless, even if less intuitive, other explanations might be advanced. For example, it might be argued that, independently of any graspable feature of the stimuli and of the observer's hand dominance, a "Positive/Affirmation" as opposed to a "Negative/Denial" response could be preferentially associated with the right and left hand, respectively. Indeed, such explanation of the present results would be in agreement with the "polarity correspondence principle", according to which the association of "yes" and "no" responses respectively with right and left key-presses elicits faster reaction times compared to the opposite mapping [24]. Similarly, the mere "Presence" and "Absence" of any stimuli might be linked respectively to the right and left hand movement. In turn, this could represent an instance of the spatial-numerical association of response codes (SNARC) effect [25], according to which faster reaction times are observed when the responses to large and small numbers are associated with right and left key-presses respectively, compared to the opposite mapping. Although in our research both the presence and the absence of the stimulus might be considered – at first blush – as small numbers (i.e., 1 and 0 respectively), it should be noted that the categorization of a number as large or small is contingent on the specific stimulus set [25-26]. The second and third experiments were designed in order to evaluate the plausibility of these alternative explanations and whether results could be replicated in left-handers.

### *2.3.3 Experiment 2*

The analysis was performed on RTs of 38 subjects (19 per mapping group), as one participant was discarded because his error rate exceeded 10% of all trials. The ANOVA showed no significant effect or interaction and demonstrated that interposing a plexi-glass screen between the observing subject and the cylinder inside the box cancelled all the RT differences observed in the first experiment.

A 2x4x2x2 ANOVA taking into account the factors "S-R mapping", "Presentation Time", "Object Presence" and "Screen Presence" ("no-screen" and "screen") was performed on RTs from all subjects participating in Experiment 1 and Experiment 2. The analysis confirmed a significant main effect of “S-R mapping” ($F_{1,70} = 7.566$, $p < 0.01$) with shorter RTs for subjects that had to match the object presence with their right index finger and its absence with their left one (374±11 ms vs 426±16 ms in the opposite mapping group), and a significant interaction among the factors “S-R mapping”, “Presentation Time” and “Screen Presence” ($F_{3,210} = 3.804$, $p <0.05$). Post-hoc t tests showed that, while in the first experiment RTs of the two S-R mapping groups were significantly different at all presentation intervals ($p < 0.05$ in all cases) apart from 50 ms, in the second study the two S-R mappings did not differ at any interval.

### *2.3.4 Discussion (Experiment 2)*

The results of the second study indicate that both the polarity correspondence principle and the SNARC effect are implausible explanations for our findings, demonstrating that the screen presence prevented the possibility to observe any significant difference in RTs due to factors “Object Presence” and “S-R mapping”. Hence, based on results from the first two experiments, the most parsimonious conclusion is that the major determinant of both reported effects relied on the affordance meaning of the stimuli, that is, on the potentiality to grasp the object, while all other factors proved to be inconsistent.

The ANOVA performed on data from the first two experiments indicated that contextual information, represented by the screen interposition, strongly reduced the effect of object sight (the main effect of “Object Presence” was not significant), whereas only mildly affected the “S-R mapping” effect. Its persistence might suggest that it reflects a functional property of the motor system of both hemispheres, likely represented as a pre-set excitability default state, that made it less dependent on the actual contextual information than the effect of object sight. The fact that the interaction between factors “Screen” and “S-R Mapping” was not significant should not be surprising but it rather suggests that interposing a transparent barrier only reduced to a variable degree the affordance content of the presented object (i.e. its actual “graspability”) and its effectiveness in recruiting the observer's motor system. So, the final outcome of this intervention proved to be only a reduction in the intensity of the effect of "S-R mapping", without deleting or inverting its direction.

### *2.3.5 Experiment 3*

Data from all twenty left-handed volunteers were analyzed and the ANOVA showed no significant effect.

A further 2x4x2x2 ANOVA was conducted pooling together data from the first and from the third experiment, considering the factors "S-R mapping", "Presentation Time", "Object Presence" and "Handedness" ("right-handed" and "left-handed"). A significant interaction between "S-R mapping" and "Handedness" was found ($F_{1,52} = 5.547$, $p < 0.05$). Post-hoc comparisons showed that RTs differed between the two mappings only in right-handed participants (as reported above in Experiment 1), whereas no significant differences could be observed among left-handed subjects (Fig. 5).

**Figure 5.** Graph depicting interaction between Response Mapping and Handedness, obtained pooling together data from Experiment 1 and 3. In ordinate: RTs expressed in seconds from visual stimulus onset. Error bars indicate standard error of the mean. Asterisk indicates significant difference.

### *2.3.6 Discussion (Experiment3)*

The third experiment aimed at testing the influence of observer's handedness in the same choice-reaction time task, as from affordance definition we might expect a consequent modulation of RTs. Even if the limited number of left-handed volunteers greatly reduced the possibility of observing significant differences among left-handed participants, the ANOVA conducted on data from the first and third experiments showed that the interaction between the two between-subjects factors type of "S-R mapping" and "Handedness" was significant, demonstrating their close association, as hypothesized initially. Also, these findings make the SNARC effect account of our results extremely unlikely, given that such phenomenon is present in both right-handers and left-handers [25-26], supporting the conclusions already drawn on the basis of the first two experiments.

## 2.4 General discussion

The “S-R mapping” effect found in the present studies can be explained within the framework of the premotor theory of action selection [27-28]. In this perspective, a rapid and efficient behaviour is insured by afferent information (e.g. visual input from the environment, task instructions, proprioceptive information, past experiences) which is constantly mapped onto excitability differences among parallel neural networks, serving competing motor programs. In right handers, the RT advantage of one “S-R mapping” over the other might rely on the excitability unbalance between the motor cortices of the two hemispheres favouring the left over the right primary motor cortex (M1). The cortical network responsible for this difference in excitability is likely to be found within the dorsal stream for vision. A plausible candidate is the cortico-cortical network devoted to transform object visual information into grasping actions, which includes the anterior intraparietal (AIP) region and the ventral premotor cortex [29-36]. In particular, a crucial role is likely played by a class of visuo-motor neurons sited in the ventral premotor cortex (F5) that discharge both during object observation and during a reach to grasp action on the same object. These neurons, named “Canonical Neurons” [37], through their connections with M1 could modify the excitability pattern across different motor representations in M1, according to the type of grip afforded by the object. Moreover, the stimulus adopted here (a small-sized cylinder) is analogous (shape, material, afforded grip, color) to those that proved so effective in triggering the discharge of such premotor neurons in monkeys [38].

If subjects are forced to choose the hand to act with, in presence of a graspable object, their motor cortices are in a sort of “default-excitability state” with slight activation in the left hemisphere and de-activation/inhibition in the non-dominant right one. When the association no-object-right hand and object-left hand is required, the plasticity of the motor system allows subjects to successfully and rapidly carry over the task, but at a cost of few tens of milliseconds (on average about 70 msec), needed to counteract the excitability states of their motor pathways. The fact that errors were never related to difficulties in visual stimuli recognition confirmed that the recorded behaviour was primarily due to a phenomenon occurring at the response selection stage. Intuitively, this difference seems related to subjects' handedness, which was stabilized in the early stages of development as the result of the combination of two other factors: the known tendency of vertebrate brain towards a hemispheric specialization on the one hand [39-40], and the use-dependent reinforcement occurring after each successful grasping on the other [41]. In this regards, the finding that "S-R mapping" did not turn out to be a significant factor in left-handers (study 3) could be accounted for by left-handers' motor representations being less lateralized than those of right-handers, as shown by both behavioural [23, 42-47] and neuroimaging studies [48-49]. On the other hand, the finding that "Object Presence" affected RTs in right-handed participants but not in left-handed participants could be due to the fact that the automatic action simulation evoked by the sight of the cylinder could rely more on sensorimotor processes in right-handers than in left-handers, and more on visual processes in left-handers than in right-handers, as already proposed for hand laterality judgments [50-53] and mental simulation of others' actions [45-47].

In summary, the present study demonstrated the independence of the so called "affordance effect" from any spatial compatibility code arising between visual stimulus and motor response. Indeed, these as well as other behavioural effects are likely the final outcome of a series of neural events weighted according to: stimuli features (i.e. size, shape, distance, degree of spatial asymmetry), task demands, and the characteristics of the sensory-motor system of the observer. In this view, results obtained previously by different groups [2, 15-17] appear perfectly reconcilable. Of notice, stimuli used in studies that reported a spatial-compatibility effect [15-17] were extremely poor concerning their affordance content, but their asymmetry might have prompted a difference in RTs mainly due to spatial stimulus-response compatibility. Instead, in paradigm such that adopted in other studies [2-12, 14, 18], the stimuli affordance content might have become more relevant and effective enough in modulating M1 excitability, through cortico-cortical connections originating in the ventral premotor cortex. Finally, the present work shows a new approach to study affordances, specifically analyzing participants' hand dominance (as reflected by RT differences between hands) in task requiring the detection of stimuli with different content in affordance. The extendibility of these results to different tasks might shed further light on the relation between hand dominance and the "Affordance effect".

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# FINAL REMARKS

The present studies represent an attempt to ground an “affordance theory” of perception in neurophysiology of the cortico-spinal tract. The first one clearly illustrates that visual information constantly exerts its influence on muscles representations within M1, highly likely through the dense, short-latency connections arising within the vPM. Of notice, as would be predicted if a system analogous to the one described in macaque monkeys really exists also in humans, the increase in excitability was largely limited to the very same effectors normally involved in manipulating the observed object. Moreover, this modulation was extremely early relative to visual stimulus onset, although short lasting. The fast modulation observed constitutes a pre-requisite for a Motor System constantly “translating” visual inputs into different action opportunities (i.e. excitability modulation), which in turn is a pre-requisite for a successful interaction in a competitive environment.

The second study, supports previous works showing shorter reaction times in presence of a graspable object (the so called “affordance effect”). Contextually, it demonstrates that this effect relies mainly on objects graspable features. Nevertheless, obviously these conclusions hold true in those conditions. Indeed, in other cases (e.g. in presence of clearly asymmetric objects) other factors or their interactions with object graspability might become relevant. Furthermore, the adopted paradigm - presence vs absence and choice-reaction times - offers an original way to study affordances, and at the same time it might give new insight in Motor System organization, specifically on the origin of hemispheric dominance for hand action and on bimanual coordination, which is strictly linked to handedness. Future neurophysiological (TMS/EEG) studies might exploit this S-R mapping effect in order to clarify the relevance of different cortical networks as well as the precise timings of their involvement.