Weight dependent modulation of motor resonance induced by weight estimation during observation of partially occluded lifting actions

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ABSTRACT

Seeing others performing an action induces the observers' motor cortex to "resonate" with the observed action. Transcranial magnetic stimulation (TMS) studies suggest that such motor resonance reflects the encoding of various motor features of the observed action, including the apparent motor effort. However, it is unclear whether such encoding requires direct observation or whether force requirements can be inferred when the moving body part is partially occluded. To address this issue, we presented participants with videos of a right hand lifting a box of three different weights and asked them to estimate its weight. During each trial we delivered one transcranial magnetic stimulation (TMS) pulse over the left primary motor cortex of the observer and recorded the motor evoked potentials (MEPs) from three muscles of the right hand (first dorsal interosseous, FDI, abductor digiti minimi, ADM, and brachioradialis, BR). Importantly, because the hand shown in the videos was hidden behind a screen, only the contractions in the actor's BR muscle under the bare skin were observable during the entire videos, while the contractions in the actor's FDI and ADM muscles were hidden during the grasp and actual lift. The amplitudes of the MEPs recorded from the BR (observable) and FDI (hidden) muscle increased with the weight of the box. These findings indicate that the modulation of motor excitability induced by action observation extends to the cortical representation of muscles with contractions that could not be observed. Thus, motor resonance appears to reflect force requirements of observed lifting actions even when the moving body part is occluded from view.

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1. Introduction

When observing a box being lifted by somebody, most of the time, people can easily estimate its weight. The mere observation of another person acting has been shown to activate several brain areas that are also engaged during action execution (Caspers et al., 2010). Additionally it has been shown that observing actions modulates the excitability of the primary motor cortex (M1) of the observer, i.e. that it induces "motor resonance in M1" (Fadiga et al., 2005).

Fadiga et al. (1995) were the first to use single pulse transcranial magnetic stimulation (TMS) to assess the excitability of

M1 during action perception. They found that seeing others' actions increased the amplitude of TMS-induced motor-evoked potentials (MEPs) and that this increase in corticospinal excitability was specific to the muscles used to perform the observed actions. Since the work of Fadiga et al. (1995), the facilitation of M1 during action observation has been replicated numerous times (Aziz-Zadeh et al., 2002; Candidi et al., 2010; Fadiga et al., 2005; Roosink and Zijdewind, 2010; Sartori et al., 2012; Schütz-Bosbach et al., 2009; Strafella and Paus, 2000). This motor facilitation appears to (1) be present for transitive actions (Fadiga et al., 1995; Sartori et al., 2012) and intransitive movements (Borroni et al., 2005; Burgess et al., 2013; Fadiga et al., 1995; Romani et al., 2005); (2) be temporally coupled with the phases of the observed actions (Alaerts et al., 2012; Borroni et al., 2005; Gangitano et al., 2001, 2002; Urgesi et al., 2010); (3) depend on muscular involvement rather than direction features of observed movements (Alaerts et al., 2009; Urgesi et al., 2006a); (4) be causally linked to signals

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from the same premotor and parietal regions that are involved in action performance (Avenanti et al., 2007, 2013; Catmur et al., 2011; Koch et al., 2010). Remarkably, such fronto-parietal regions correspond to the regions where mirror neurons were first discovered in the monkey brain (di Pellegrino et al., 1992; Fogassi et al., 2005; Gallese et al., 1996) and where blood-oxygen-level-dependent (BOLD) signal is increased during action observation (Caspers et al., 2010).

These findings have supported the assumption that motor resonance is dependent on activity in the mirror neurons (Fadiga et al., 1995) and similar somatotopical organization of motor resonance has also been found in premotor and parietal brain regions using functional magnetic resonance imaging (fMRI). Buccino et al. (2001) showed that the activations in the premotor and parietal regions corresponded to the effector used in the observed actions. Their results show that when participants observed actions performed with the mouth, hand or foot, different parts of frontal and parietal cortex were activated. Brain stimulation studies indicate that activity in parietal and premotor regions is necessary for action perception (Avenanti et al., 2013). In particular, Pobric and Hamilton (2006) first used repetitive transcranial magnetic stimulation (rTMS) to investigate whether the motor system plays a crucial role in weight estimation. They found that when participants watched a hand lifting a box and were instructed to estimate its weight, repetitive rTMS, delivered to the inferior frontal gyrus (IFG) disrupted their performance. In contrast, rTMS to the occipital cortex did not affect performance, and rTMS over the frontal or the occipital cortex did not affect performance when people had to judge the weight of a bouncing ball. This observation implies that motor system activation is crucial for weight estimation when a human hand is lifting the object and not for weight estimation of objects as such.

Alaerts et al. (2010) have shown that when watching somebody lift an object M1 excitability is proportionally modulated by the weight of the object being lifted. They recorded MEPs from the first dorsal interosseous (FDI) muscle while participants passively observed an actor lifting two different objects of different weights using a precision grip. Results showed that the amplitude of the MEPs was modulated in accordance with the weight of the objects being lifted. In a complementary experiment, Alaerts et al. (2010) compared the amplitudes of the MEPs measured from the opponens pollicis (OP, thumb), flexor carpi radialis (FCR, wrist) and extensor carpi radialis (ECR, wrist) muscles while participants passively observed videos of a hand lifting an empty, half-full or full bottle. The amplitude of the MEPs measured from the OP and ECR muscles was higher when the observed videos showed a hand lifting a transparent half-full or full (heavier) bottle as compared with an empty one. Additional studies demonstrated that the weight-dependent modulation of motor resonance in M1 persisted when the agent lifted objects that were visually identical but had different weights (Alaerts et al., 2010; Senot et al., 2012; Tidoni et al., 2013). Weight dependent motor resonance in the observers' M1 was observed even when only kinematic information (e.g. trajectories, speed, acceleration) associated to lifting light and heavy objects was available. In some experiments, weight related information conveyed by muscle contraction and local skin tone changes associated to grasping and moving objects with different weights were minimized either using digital movie editing (Tidoni et al., 2013) or by asking the moving agent to wear a glove (Alaerts et al., 2010, 2012). However, in these studies, the moving hand and arm were entirely visible for the observers.

In the monkey brain, a significant proportion of mirror neurons in the premotor cortex has been shown to fire also when the object is occluded from view (Umiltá et al., 2001). Umiltá et al. (2001) have shown that "grasping" mirror neurons in the ventral premotor cortex (vPM) of the monkey fire both during the observation of a hand reaching for and grasping an object in full sight, and of a hand reaching behind an occluding screen to grasp a hidden object. Recently, Villiger et al. (2011) have used TMS to investigate motor resonance in M1 using a similar occluding paradigm. The authors presented participants with videos of actual or mimed grasping movements of visible or hidden objects and measured the MEPs from the grasping muscles of the observers. Results showed that MEPs are modulated by object presence. Thus, even when the object was hidden behind a screen, but participants were aware of its existence, MEPs were larger compared to when the object was visible. This effect was pronounced during the grasping phase, but not when seeing the hand at rest. We therefore hypothesize that, motor resonance in M1 will be present when the final phase of the action is occluded from view and that the amount of motor resonance in M1 measured from muscles not directly observed by the participant will be proportional to the inferred force requirements of the action. In the present study we therefore asked participants to estimate the relative weight of a box being lifted. In the videos, only the hand and arm could be observed during the reaching phase, while the grasp and lift of the object were hidden behind a screen and only the forearm proximal to the wrist was visible. This approach allowed us to determine whether modulation of the MEPs according to the weight of the box being lifted occurs only in the muscles in which contractions can be observed by the participants (arm muscles) or also in the muscles involved in the action but that are hidden behind a screen during the actual lift. In the latter (hand) muscles contractions are not directly observable. The distinction between observable and not-observable (occluded from view) muscles allows to explore whether motor resonance in M1 is triggered only by the observation (detectable only for the observable forearm muscle) or whether it can be detected for the hand muscles for which involvement in the action can only be inferred based on the motion and muscle contraction in the visible part of the arm.

2. Materials and methods

2.1. Participants

A total of 28 participants took part in the study. Of these seven were excluded during the experiment because their resting motor threshold (rMT) was too high (above 80% of the maximal stimulator output),[1] six because they failed to learn to discriminate between the videos during the practice run of the experiment (their performance was lower than or approximately at chance level), and three because the total fraction of valid MEPs (absent EMG background activity and correct response) was less than 65%. The data of 12 participants was analyzed (5 males, 7 females, M age=25 years, SD=7.27). All participants were right-handed (Edinburgh handedness inventory M=79.85, SD=27.38) with normal or corrected to normal vision. None of them had neurological, psychiatric, or other medical problems, or had any contraindication to TMS (Rossi et al., 2009). The protocol was approved by the local ethics committee of the University Medical Center Groningen and was carried out in accordance with the ethical standards of the 2008 Declaration of Helsinki. No discomfort or adverse effects during TMS were reported or noticed.

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[1] MEPs are recorded after a single TMS pulse is delivered to the motor cortex at an intensity of 120% of the individual rMT. Participants who have a rMT above 80% of the maximal stimulator output would need to receive single pulse TMS of intensity close to the maximum output of the machine.
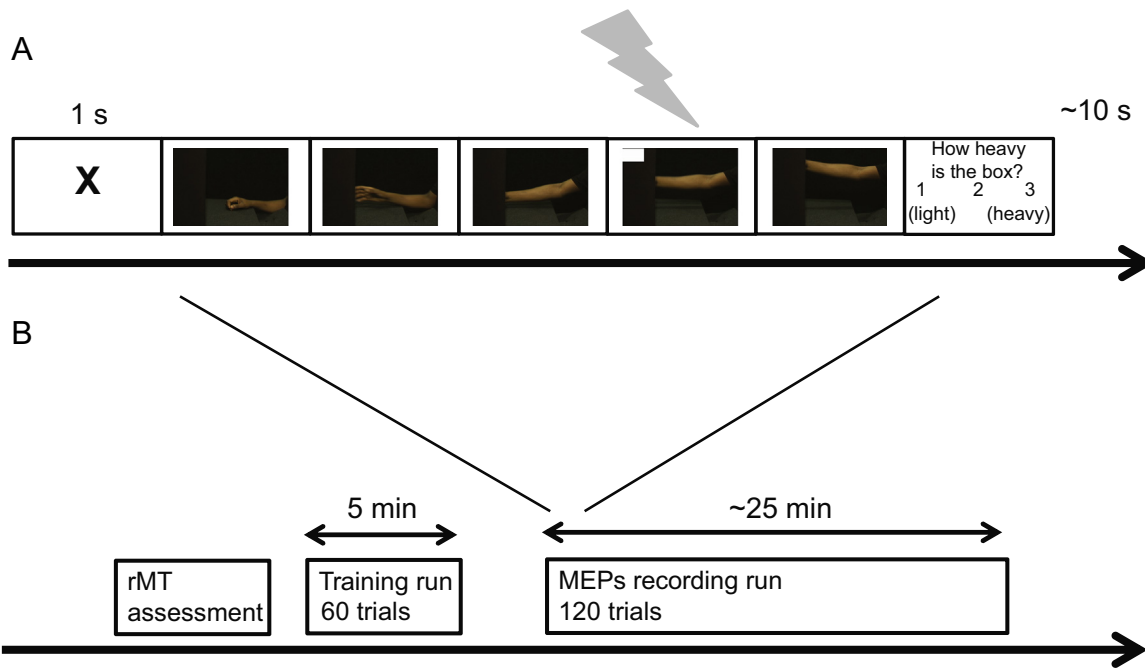
Fig. 1. (A) Single trial procedure (videos had an average duration of 3 s) and (B) experimental design.

2.2. Experimental stimuli

Video stimuli consisted of short movies (3–5 s) of a right hand entering from the right side of the visual scene and lifting an object hidden behind a screen (see Fig. 1A). Only one actor was used for all the videos. The actor was seated in front of the table with the object. The screen hiding the object was placed perpendicular to the viewing direction of the camera. In this way, in the recorded videos, participants saw the medial side of the arm and could observe contractions of the brachioradialis (BR) muscle of the right forearm (bulging of the muscle during the contraction) but not the FDI or abductor digiti minimi (ADM) muscles, during actual weight lifting. In order to create the videos as similarly as possible, the movement speed of the actor's hand was paced with a metronome. The highest point of the lift was determined with a visual mark visible only to the actor. Three different weights were used: 185 g, 900 g and 3500 g.

2.3. Experimental design, task and procedures

Each participant participated in one experimental session composed of three parts: preparation, practice run and MEP recording run (see Fig. 1A), in that order. For each of the three weights 20 different videos were presented once (practice run) or twice (MEP recording run) for a total of 60 (practice run) or 120 trials (MEP recording run). Movies were presented in a semi-randomized order, such that no more than two videos of the same weight were presented consecutively Table 1.

During the preparation part of the experiment the optimal scalp position (OSP) and the rMT were determined by recording MEPs (see Section 2.4 for details). Once the OSP was identified, it was marked on an electroencephalography cap placed on the participant's head which was secured with a chin strip so that the mark did not move.

During the practice run participants watched and evaluated 60 trials of the experimental task to become familiar with it. Each trial was composed of a 1-s fixation cross, followed by the presentation of the video and the question "How heavy is the box?" (Fig. 1B). Participants were required to answer as accurately and

Table 1
Average Pearson correlations between the P–P amplitudes of the valid MEPs recorded from the FDI, BR and ADM muscles for each weight.

		BR			ADM		
		185	900	3500	185	900	3500
FDI	185	0.50*			0.47*		
	900		0.50*			0.54*	
	3500			0.44*			0.53*
BR	185				0.51*		
	900					0.41**	
	3500						0.53*

* *t*-Test against zero after Fischer z-transformation, $p < 0.001$.
** Idem but $p < 0.02$.

quickly as possible and to pay attention to the effort of the lifting action. Responses were given after the end of each video. To respond, participants used their left hand to press one of three keys on the computer keyboard. The interval between trials was set to 2 s. If the performance on the practice run was close to chance level the participant was asked to perform the training trials again and if the score did not improve after the additional block, the subject was excused from further participation in the experiment. One of the 12 participants had to perform the practice run twice (120 trials) in order to learn the task.

MEPs were recorded after the practice run while participants were executing the same task. Participants responded in 120 trials and one TMS pulse was delivered to the OSP during every trial in the interval between the grasp and the highest point of the lifting movement. Trials were composed in an identical way to the practice run, but the interval between the trials was randomly varied between 8 and 12 s to avoid any influence of one TMS pulse on the next one (Chen et al., 1997). The TMS pulse was triggered by a voltage change in a photo cell placed on the screen on which the movies were displayed. A white square in the top left corner of the video (not visible to the subject) activated the photo cell. The
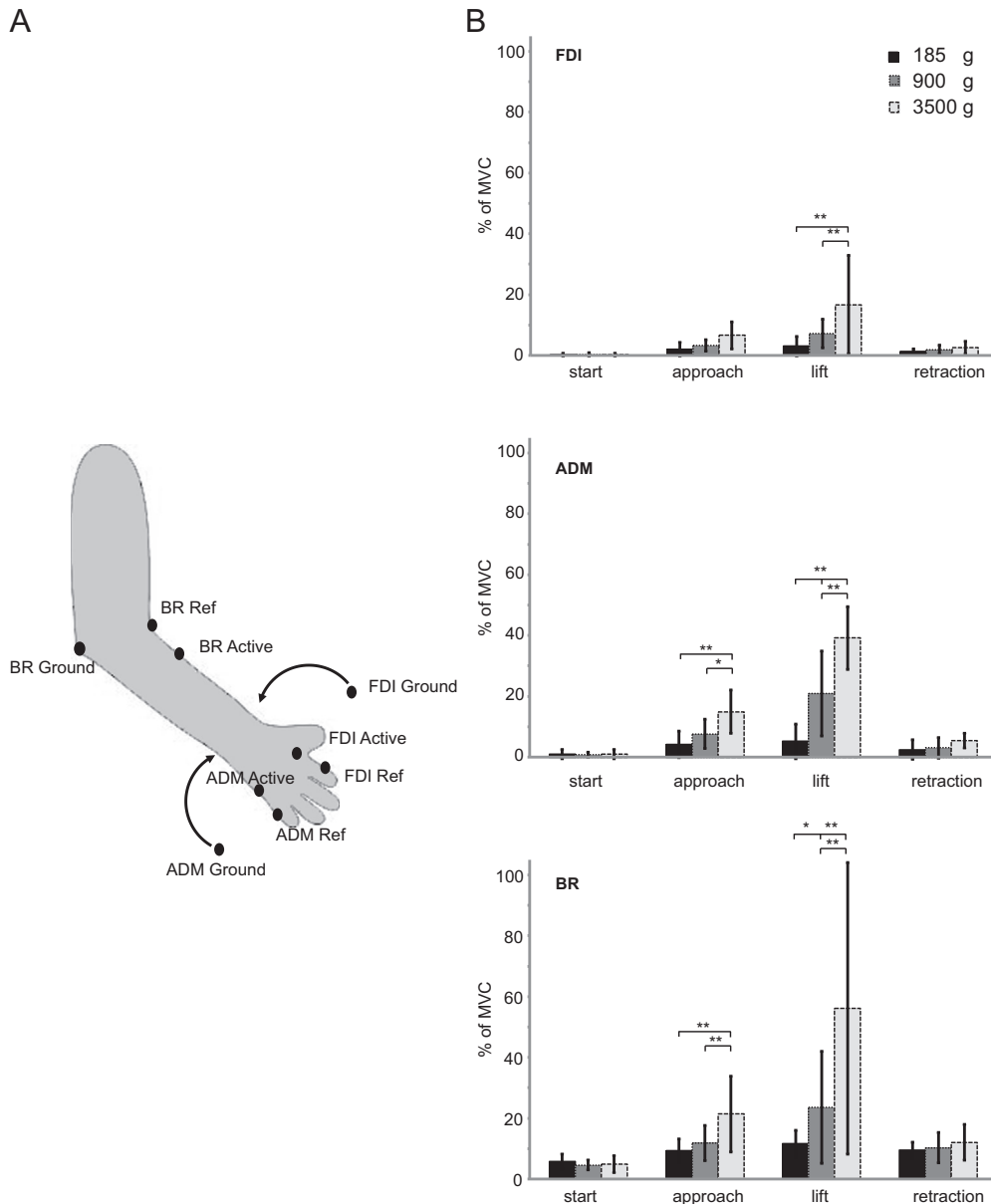
Fig. 2. (A) Electrodes setup and (B) median and interquartile range of the activation of the FDI, ADM and BR muscles expressed in %MVC of the corresponding muscle during lifting of three weights for each of the four phases of the movement (start; approach; lift; retraction). Stars indicate a significant difference between the levels of activation corresponding to the indicated weights ($**=p < 0.01$; $*=p < 0.05$).

frame in which the white square was embedded was chosen at random for each video and varied between the second frame after the grasp (in which there was visible movement in all videos) and two frames before the end of the video.

2.4. TMS

TMS during the experiment was delivered with a 70 mm figure-eight stimulation coil connected to a Magstim Rapid2 (The Magstim Company, Carmarthenshire, Wales, UK).

The rMT was determined by recording MEPs induced by single-pulse TMS over the left M1. MEPs were recorded from the right FDI, right ADM and right BR muscles by means of a Biopac MP-150 electromyograph (Biopac Corp, Goletta, CA.). Pairs of silver/silver chloride surface electrodes (active and reference) were placed over the muscle belly and over the associated joint of the muscle (on the first metacarpophalangeal joint for the FDI, on the fourth

metacarpophalangeal joint for the ADM and at the end of the proximal tendon for the BR muscle). Since each differential channel of the EMG amplifier used in this experiment requires a separate ground electrode, two were placed on the ventral surface of the right wrist (for the FDI and ADM muscles) and another one at the olecranon (for the BR muscle) (see Fig. 2A for electrode placement). EMG signals were band-pass filtered (20 Hz to 1.0 kHz, sampled at 5 kHz), digitized and displayed on a computer screen. The OSP was determined such that reliable MEPs were produced in all three muscles. The rMT was defined as the lowest level of stimulation able to induce MEPs of at least 50 μV in at least 5 out of 10 TMS pulses in all three muscles simultaneously (Rossini et al., 1994). The coil was positioned in such a way that the intersection of the figure of eight was tangential to the scalp with the handle towards the back of the head at an angle of 45° relative to the mid sagittal line. In this way, induced current was approximately perpendicular to the line of the central sulcus and

could trans-synaptically activate the corticospinal pathway (Brasil-Neto et al., 1992; Mills et al., 1992).

During the experimental task MEPs were recorded while stimulating the OSP for each individual subject. The intensity was set at 120% of the individual rMT.

2.5. EMG activity validation

To determine the extent to which the muscles we are measuring MEPs from, were activated during the lifting of a box of 185, 900 or 3500 g, we recorded EMG activity from the FDI, ADM and BR muscles in four participants, who did not participate in the main experiment (one female: age 27, Edinburgh handedness score 75 and three male: ages 45, 40 and 33, Edinburgh handedness scores respectively: 100, 70 and 100). Each subject lifted each of the three boxes five times and the average absolute EMG signal was calculated during four phases of the movement: 500 ms before the start of the action (start), approach phase (approach), lifting period (lift) and retraction of the hand (retraction). Two mechanical sensors sending a stimulus to the EMG amplifier detected the moment when the hand was lifted from the table (start of action) or returned to the table (end of retraction) and the moment when the object was lifted from the table (end of approach, start of lifting) and was placed back on the table (end of lifting, start of retraction). The EMG during a maximum voluntary contraction (MVC) of each muscle separately (i.e., index finger abduction, little finger abduction and elbow flexion) was determined for each individual muscle by calculating the average absolute signal during 500 ms of maximal contraction. We computed the activation of each muscle as %MVC during the four phases of the action.

These results were compared with a repeated measures ANOVA with phase (4 levels: start, approach, lift and retraction), weight (3 levels: 185, 900 and 3500 g) and muscle (3 levels: FDI, BR and ADM) as repeated factors. Note that, since the number of participants is small, the results of this test should be interpreted with caution. We detected significant main effects of the factors phase ($F_{(3,9)}=19.22$, $p < 0.001$), weight ($F_{(2,6)}=22.67$, $p=0.002$) and muscle ($F_{(2,6)}=8.55$, $p=.018$). We found that %MVC was higher for the BR than ADM ($p=0.04$) and higher for the BR than FDI ($p=0.05$; main effect of muscle). However, it should be noted that EMG activity during action execution was expressed as %MVC, where for each muscle the maximal contraction was assessed using a specific movement (i.e., index finger abduction for FDI, little finger abduction for ADM and elbow flexion for BR). In contrast, during reach and grasp both FDI and ADM are not active in their 'preferred' direction. FDI is an index finger abductor and is a (important) synergist during flexion. The ADM is the little finger abductor and is a synergist during flexion. This implies that EMG activity expressed as % of the MVC would be lower during reaching and grasping in these two muscles as compared to the BR. In this latter muscle MVC is measured during flexion, which is the "preferred" direction for this muscle. The comparison of the EMGs across muscles therefore does not tell us more than we may expect from the role of these muscles in the movement under consideration. The data also showed that %MVC was higher during the lifting phase as compared to the start phase ($p=0.02$), approach phase ($p=0.03$), and retraction phase ($p=0.02$; main effect of phase). Finally, %MVC was higher for 3500 g than 900 g ($p=0.014$) and for 3500 g than 185 g ($p=0.018$; main effect of weight). The ANOVA showed that main effects were qualified by the two-way interactions muscle × phase ($F_{(6,18)}=3.91$, $p=.011$) and phase × weight ($F_{(6,18)}=18.12$, $p < 0.001$). The interaction muscle × weight was marginally significant ($F_{(4,12)}=3.17$, $p=.054$). By contrasting the values from the lifting phase against all other phases for each pair of weights (i.e. 3500 vs 900 g and 3500 vs 185 g, and 185 vs

900 g), we found that the phase × weight interaction was due to the difference in %MVC between weights being larger for the lifting phase than for any other phase (all $p < 0.03$). By contrasting the values from the lifting phase against all other phases for each muscle (BR vs FDI, BR vs ADM, and FDI vs ADM) we found no significant contrasts. Notably, the two-way interactions were qualified by the significance of the three way interaction muscle × weight × phase ($F_{(12,36)}=4.85$, $p < 0.001$). Evaluating the three-way interaction by means of Fisher post-hoc tests we established that the phase and weight which showed the highest % MVC for all three muscles is the lifting phase and the 3500 g weight (all significant $p < 0.001$). Comparing the %MVC from each phase and each weight across muscles showed that %MVC for BR is higher than for FDI for all weights during the approach, lifting and return phases (all significant $p < 0.02$). In addition, %MVC for BR is higher than for ADM for the 3500 g weight only, during the approach and lifting phases, while for the return phase %MVC for BR is higher than for ADM for all weights (all significant $p < 0.02$). This was expected due to the contraction requirements of the executed movement. Finally, the %MVC for the ADM was higher than for FDI during the lifting phase for the 900 and 3500 g weights (all significant $p < 0.01$). Overall these findings confirm that during action execution the three muscles were recruited and finely modulated as a function of weight and phase.

2.6. Data analysis

Performance for each participant was calculated during both the practice run and MEP recording run. Subject performance was summarized by the IntraClassCorrelation (ICC) (McGraw and Wong, 1996). We chose to use the ICC index as opposed to a Pearson correlation index, since the latter only evaluates the degree to which there is a linear relationship between two variables. In our case we aim at evaluating how well one variable (y vector of responses given by the subject) can be equated to another variable (x vector of correct responses). We used the ICC(A,1) of McGraw and Wong (1996), where A stands for absolute agreement, and 1 denotes that the number of observations per subject are fixed.

EMG data were analyzed offline. For all 120 MEPs per participant we determined the peak to peak (P–P) amplitude in mV. We analyzed only the MEPs for which there was no background muscle activity in the 100 ms before the trigger, as assessed visually. MEPs with a P–P amplitude smaller than 50 μV were also excluded. Additionally all MEPs associated with an incorrect estimation of the observed weight were excluded from the analysis. A total of 692 MEPs were included in the analysis (on average 58 per subject from which 24 for the 185 g weight, 15 for the 900 g and 19 for the 3500 g).

P–P amplitudes recorded from the three muscles and associated with the observation of the three weights were compared by means of a multilevel analysis as implemented in SPSS 17.0 (mixed models). We chose this analysis method because it allows to use all MEPs without averaging them per subject and per weight. Additionally multilevel analysis is applicable on less homogeneous data and allows the variation of both the slope and the intercept of the estimated models. We evaluated the fit of three models by means of comparing the resulting −2 Restricted Log Likelihood (−2LL). The first model did not allow for a random variation of the slopes and intercepts and resulted in the highest −2LL. The second which allowed for a random variation of the intercept resulted in a lower −2LL. The third model which allowed for a random variation of the slope and intercept did not decrease the −2LL. Consequently the data were analyzed using a linear mixed model which allows for a random variation of the intercept.

3. Results

3.1. Performance

We calculated the performance score of all participants, as measured by the ICC, to assure that participants were able to discriminate between the videos of different weights. (for analyzed participants: M ICC(A,1)=0.68, SD=0.09). On average analyzed participants had 74 correct responses out of 120 (SD=8). A one sample t-test showed that the number of correct responses

was significantly different from chance level ($t_{(11)}=13.52$, $p<0.01$).

A repeated measure ANOVA was used to compare the number of correct responses per weight and showed a significant main effect for weight ($F_{(2,22)}=4.87$, $p=0.02$). In addition, a Fisher post-hoc test indicated that the number of correct responses was higher for the 185 g box than the 900 ($p=0.01$) and 3500 g ($p=0.04$) boxes while the number of correct responses did not differ between the 900 and 3500 g boxes ($p=0.38$). The number of correct responses was above chance level for each of the conditions (for
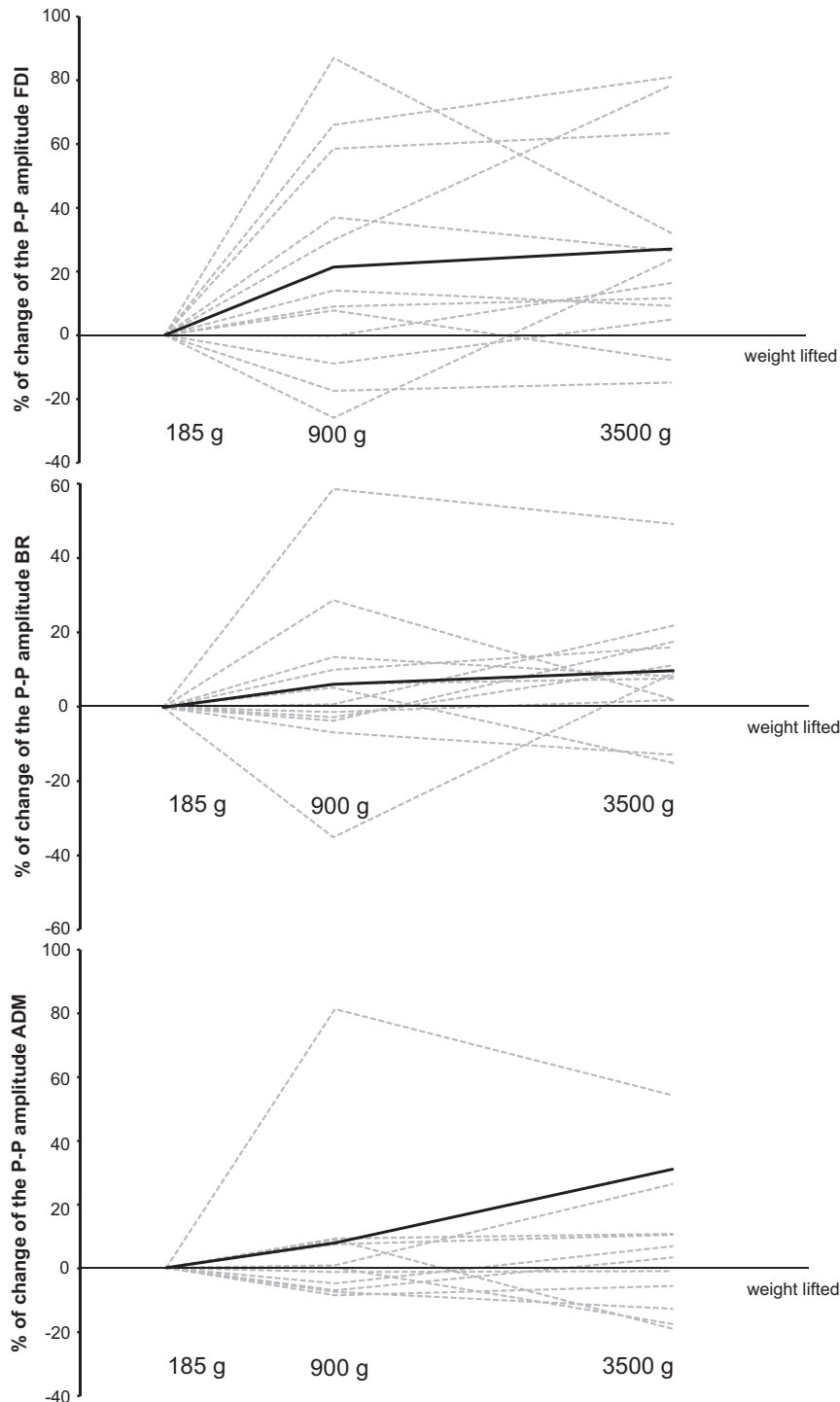

Fig. 3. Change of the P–P. amplitude of the MEPs (in %) relative to the lightest weight (185 g) for each recorded muscle. Broken lines represent the % change of the P–P. amplitude for each subject, solid lines represent the mean % of change of the P–P amplitudes.

185 g $M=72.5\%$, SD$=15\%$; for 900 g $M=52.5\%$, SD$=12.5\%$; for 3500 g $M=60\%$, SD$=15\%$).

3.2. Effect of weight on MEP amplitude

As a first step of the analysis we evaluated the covariation of the MEPs recorded from the FDI, BR and ADM muscles while participants watched the movies. For each weight and participant separately, we correlated the P–P amplitudes of the MEPs from the three muscles. Only the valid MEPs were considered for this analysis. We then used a Fisher z-transform and *t*-tests to compare the average correlations across muscles of the 12 participants against zero and against each other. Results showed that for each weight there is a strong significant correlation between the MEPs across all pairs of muscles (two-tailed *t*-test against zero, all $p < 0.01$ (FDR-corrected)). Pairwise comparisons revealed that there is no significant difference in correlation across muscles (two-tailed, paired *t*-tests comparing normalized correlations across pairs of muscles for any given weight, all $p > 0.12$).

The valid MEPs that were obtained when a correct response was given suggest an increase in MEP amplitude when observing larger weights being lifted compared to lighter weights. The data were analyzed by means of a multilevel linear model. Data from each muscle was analyzed separately, including the P–P amplitude of the MEPs as a dependent variable and the weight of the box being lifted as a covariate (185, 900 and 3500 g). We left the intercept to vary across participants. For illustration of the results see Fig. 3.

Results show that the P–P amplitude of the MEPs measured from the FDI muscle were modulated by the weight of the observed box ($F_{(1,716)}=7.28$, $p=0.02$). The same effect was observed in the BR muscle ($F_{(1,716)}=4.26$; $p=0.04$). No significant effect was observed for the ADM muscle ($F_{(1,716)}=0.99$, $p=0.23$). After applying an FDR correction for multiple comparisons results were still marginally significant for the FDI ($p_{(FDR)}=0.06$) and BR ($p_{(FDR)}=0.06$) muscles and non-significant for the ADM muscle ($p_{(FDR)}=0.23$).

4. Discussion

In this study we investigated whether motor resonance in M1, during weight estimation through observation, is only induced by visual cues derived from visibly active muscles, or whether it can be detected in muscles that are involved in the action but in which contractions are unobservable at the time of the grasp and lift. We compared MEP amplitudes measured from three muscles of the right hand (FDI and ADM) and forearm (BR) while participants were watching videos of a hand lifting objects of different weights and estimating their weight. Two of these muscles (ADM and FDI) play their main role in the grasping of the object and one (BR) in the lifting. Our results showed a significant increase with weight in the amplitudes of the MEPs recorded from the FDI and the BR muscles and no significant effect for the amplitudes of the MEPs recorded from the ADM muscle. The findings support the notion that M1 receives information regarding both observable and unobservable muscles during action observation. The increase in M1 excitability for the muscles for which contractions were unobservable may be caused by recognition of the weight and related prior expectations about force requirements or may be based on prior expectations about the co-activation of the observable and unobservable muscles in the context of the observed action. Our results can therefore be interpreted in light of the theories that link motor resonance with action understanding (see Friston et al., 2011; Rizzolatti and Craighero, 2004).

Recently, Alaerts et al. (2010) found that when observing another person lift objects of different weights the motor system of the observer is activated proportionally to the weight of the object being lifted. We extended on those results by showing that motor resonance in M1 is not only triggered and modulated by the immediately available visual information. Although the effect observed in the BR muscle could be induced by immediately visible cues, the increased excitability of the FDI cannot be explained by visual information regarding this muscle at the time of grasping, as this muscle could not be observed while it contracted (during the lifting phase). In this context we suggest that there could be a functional top-down influence on the processing of the visible cues, i.e, the prior knowledge about lifting objects of different weights influences activity in the M1 as measured by the increase of MEPs amplitudes in both observable and unobservable muscles. In their work, Hamilton and Grafton (2007); see also: Thioux et al., 2008, define several levels of "action understanding": (1) long term intention; (2) short term goals necessary to achieve the long term intention; (3) the kinematics that describe the movement; (4) the pattern of muscle activations required by the action. Taking this distinction as a starting point we can place motor resonance in M1 in the third and fourth levels of action understanding. The effect of somatotopically distributed activation in M1 during action observation (Fadiga et al., 1995) demonstrates that the motor system of the observer is primed by the kinematics and pattern of muscle activations in the observable environment. This effect has been explored further and replicated in a number of studies which demonstrate its robustness (Aziz-Zadeh et al., 2002; Candidi et al., 2010; Fadiga et al., 2005; Roosink and Zijdewind, 2010; Sartori et al., 2012; Schütz-Bosbach et al., 2009; Strafella and Paus, 2000; Urgesi et al., 2006b). Rizzolatti and Craighero (2004) have suggested that automatically matching the observed action into the motor representation in the observer's brain would be sufficient to infer the intentions and goals, i.e. the actions are "recognized" by the motor system of the observer (Beudel et al., 2011). However, in the monkey brain Umiltá et al. (2001) have shown that mirror neurons in the premotor cortex respond to partially occluded actions, as well. Grasping mirror neurons responded when the monkey was observing a hand reaching for an object hidden behind a screen. Additionally, researchers found that these mirror neurons were not reacting when the hand was mimicking grasping on an empty table or reaching behind a screen where the monkey knew there was no peanut. Here we go one step further in showing that motor resonance measured in muscles which were not observed by the participant is proportional to their supposed involvement in the action.

Theories of the development of the mirror neurons could explain our results. A Hebbian learning account of mirror neurons proposes that due to the temporal delay of approximately 250 ms that separates premotor commands from visual re-afference, synapses connecting visual neurons responding to the sight of reaching and premotor neurons causing grasping would be systematically enhanced during self-observation (Keysers and Gazzola, 2014). Within this framework of Hebbian learning, it is likely that the sight of a strong BR contraction would have been associated via Hebbian learning mechanisms with the sight and motor programs of lifting involving heavier objects than those involving weaker BR contractions, explaining how the lacking visual information for the FDI could be 'filled in' by the synaptic connections selected via Hebbian learning mechanisms in both the visual to premotor and the premotor to visual direction. Heyes and colleagues (Heyes, 2010, 2001) make a similar argument based on associative learning rules. In this view associative learning (prediction-error) is sufficient for the development of mirror neurons (Cooper et al., 2012) and to provide the development of the mapping mechanisms which link the observed and executed actions in the brain. In the present experiment we cannot distinguish

between the associative sequence learning and Hebbian learning theories but, we can conclude that motor resonance in M1, as measured by the increase of the amplitude of the MEPs, is influenced by predictive processes that add information to the immediately visible information about the BR.

It should be noted that behavioral performance indicates that light weights were easier to recognize. One possible concern regards the relationship between changes in MEPs and task difficulty. In particular, one may ask whether the increase in motor excitability for larger weights may reflect the greater difficulty in the recognition of heavier weights in the weight estimation task. Two aspects of the data speak against this possibility. First, MEPs data indicate a linear increase in motor excitability from light to mid and heavy objects, whereas no similar trend was found in behavioral performance. Second, and most importantly, significant motor modulations were specific for the FDI and BR muscles and were not found in the ADM muscle. Task difficulty would hardly account for any muscle-specific change in motor excitability. Rather, our data indicate that weight estimation task induced weight-dependent and muscle-specific motor modulations likely reflecting motor resonance processes in M1.

The fact that there was a significant modulation of MEPs amplitude from the FDI and BR but not from the ADM muscle could be due to the experimental setup. When grasping and lifting an object, the FDI and ADM muscles control the index and little fingers involved in grasping, whereas the BR is implicated in the lifting phase. However, the ADM is typically activated for grasping big objects with a whole hand grip, but not for grasping little objects with a precision grip which does not involve the little finger. In contrast, the FDI muscle is active both during whole hand and precision grips and the BR is constantly recruited during the lifting phase (Lemon et al., 1995; Long II et al., 1970). In fact in our EMG activity validation we found that both the ADM and FDI are activated during the lift of a box using a full grasp. It is important to note that the size and the shape of the lifted boxes were never shown to the participants and they did not know the real weight of the objects. Thus, it may be that in the absence of such information (and in the absence of direct sight of the ADM involvement), the observation of a partially occluded action specifically triggers motor resonance in the cortical representation of the muscles that are most reliably associated with any grasping-and-lifting action.

Based on our results we can only speculate on the automaticity of the effects. Some studies have already shown muscle specific and weight dependent motor resonance modulation during passive observation (Alaerts et al., 2012) and tasks that do not explicitly require weight estimation (Tidoni et al., 2013). It remains for future experiments to evaluate whether resonance in muscles which cannot be directly observed is modulated by weight automatically.

In conclusion, we have shown that the excitability of M1 induced during the observation of a hand lifting an object influences the amplitude of the MEPs measured from the FDI muscle, which is involved in the action but the contractions of which cannot be directly observed. Moreover, the amplitude of the MEPs measured from this muscle in the observer – which is hidden in the actor during the lifting phase – is proportional to the estimated weight of the observed box. The same effect is present in the BR muscle, which is also involved in the action and the contractions of which are observable during the whole action. We therefore propose that our findings support the notion that visually triggered motor resonance in M1 can go beyond the direct visual evidence, and include graded responses in muscles the contractions of which cannot be directly observed.

Author contributions

Acknowledgements

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