Title

Action observation effects reflect the modular organization of the human motor system

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Abstract: Action observation, similarly to action execution, facilitates the observer's motor system and Transcranial Magnetic Stimulation (TMS) has been instrumental in exploring the nature of these motor activities. However, contradictory findings question some of the fundamental assumptions regarding the neural computations run by the Action Observation Network (AON). To better understand this issue, we delivered TMS over the observers' motor cortex at two timings of two reaching-grasping actions (precision vs power grip) and we recorded Motor-Evoked Potentials (4 hand/arm muscles; MEPs). At the same time, we also recorded whole-hand TMS Evoked Kinematics (8 hand elevation angles; MEKs) that capture the global functional motor output, as opposed to the limited view offered by recording few muscles. By repeating the same protocol twice, and a third time after continuous theta burst stimulation (cTBS) over the motor cortex, we observe significant time-dependent grip-specific MEPs and MEKs modulations, that disappeared after cTBS. MEKs, differently from MEPs, exhibit a consistent significant modulation across pre-cTBS sessions. Beside clear methodological implications, the multidimensionality of MEKs opens a window on muscle synergies needed to overcome system redundancy. By providing better access to the AON computations, our results strengthen the idea that action observation shares key organizational similarities with action execution.

Keywords: Action Observation; Kinematic; Motor Cortex; Modularity; Transcranial Magnetic Stimulation

1. Introduction

Action execution and action observation evoke similar activities in the human brain (Rizzolatti & Sinigaglia, 2016). However, there is a considerable debate around the specificity and purposes of action observation-evoked motor facilitation (D'Ausilio, Bartoli, & Maffongelli, 2015).

Dozens of studies have been published using Transcranial Magnetic Stimulation (TMS) and Motor Evoked Potentials (MEPs) to investigate how modulations of corticospinal excitability (CSE), during action observation, reflect action execution features (Fadiga, Craighero, & Olivier, 2005; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Naish, Houston-Price, Bremner, & Holmes, 2014). Some studies show that MEPs are modulated by observation of low-level motor features, such as kinematic features (e.g. fingers aperture during grasping action, Gangitano et al., 2001), EMG temporal coupling (Borroni, Montagna, Cerri, & Baldissera, 2005; Cavallo, Becchio, Sartori, Bucchioni, & Castiello, 2012) or forces (observation of lifting of objects of different weight, Alaerts et al., 2010; Senot et al., 2011). Others works report higher level modulations, such as action goals (Cattaneo et al., 2009, 2013; high-level features). For instance, MEPs modulations do not seem to depend on the effector used in the observation of the same object grasping goal (Borroni & Baldissera, 2008; Finisguerra et al., 2015; Senna, Bolognini, & Maravita, 2014), suggesting their independence from low-level movement features. Lastly, studies trying to separately analyse these aspects, highlight the multi-dimensionality of Action Observation Effects (AOEs), which may depend on several details of the experimental protocol such as instructions (Mc Cabe, Villalta, Saunier, Grafton, & Della-Maggiore, 2014; Sartori, Betti, Chinellato, & Castiello, 2015), TMS trigger timing (Cavallo, Bucchioni, Castiello, & Becchio, 2013) and number of recorded muscles (Betti, Castiello, & Sartori, 2015). External influences such as learning (Catmur et al., 2008; Catmur, Walsh, & Heyes, 2007) or context (Brass, Schmitt, Spengler, & Gergely, 2007) may modulate AOEs as well.

However, apart from identifying key features of the AOEs, these studies rarely tested the reproducibility of their effects. In fact, MEPs are highly variable across time (Schmidt et al., 2009) and hugely dependent on cortical states (Klein-Flügge, Nobbs, Pitcher, & Bestmann, 2013) and on spontaneous cortical oscillatory dynamics (Elswijk et al., 2010; Keil et al., 2014). More importantly, in many cases MEPs might not be the most accurate measure to explore AOEs. In fact, one basic tenet of action observation studies is that the visual appearance of actions is directly mapped onto one unique muscle activity pattern. Based on this assumption, CSE is usually recorded from few muscles at a time, during the observation of often complex kinematic configurations. CSE modulations are then used to build inferences about the functional meaning of motor activities during action observation (Naish et al., 2014). However, it is known that the same kinematic configuration can be achieved via largely different underlying muscle activation patterns (Grasso, Bianchi, & Lacquaniti, 1998; Levin, Wenderoth, Steyvers, & Swinnen, 2003).

Here we suggest that the TMS-evoked kinematic pattern (Motor Evoked Kinematics, MEK) provides a more reliable measure of motor activities induced by action observation. This assumption is based on principles of redundancy and invariance during motor execution (Flash & Hochner, 2005; Guigon, Baraduc, & Desmurget, 2007; Sporns & Edelman, 1993) and it takes into account the fact that the control of grasping actions relies upon the composition of intracortical, corticospinal, spinal and peripheral influences (Fetz, Perlmutter, Prut, Seki, & Votaw, 2002) which in turn regulate the temporal-spatial coordination of multiple agonist and antagonist muscles.

The functional output of the motor system can be extrapolated from TMS-induced MEK (Bartoli, Maffongelli, Jacono, & D'Ausilio, 2014; Finisguerra et al., 2015; Gentner & Classen, 2006). Single finger MEKs are modified by physical practice (Classen, Liepert, Wise, Hallett, & Cohen, 1998) and by action observation training (Celnik et al., 2006; Stefan et al., 2005; Stefan, Classen, Celnik, & Cohen, 2008) thus reflecting short-term cortical plasticity. Whole-hand MEKs replicate the modular organization of hand functions, which are dissociable in discrete postures (Gentner & Classen, 2006), requiring years of practice to be significantly changed (Gentner et al., 2010).

Importantly, MEKs offer a direct measure of the functional motor output, without losing its inherent multidimensionality. This fact may have a significant impact on how we investigate the nature of AOEs (D'Ausilio et al., 2015) and could clarify to what extent action observation and action execution share similar synergistic organization principles.

To this end, we compared side-by-side MEPs and MEKs in a classical action observation protocol. Subjects observed a goal directed grasping action towards one of two simultaneously presented objects, requiring either a precision or a power grip. We recorded MEPs from 4 hand muscles as well as whole-hand MEKs at one of two possible time points during the observed reaching phase. The first time-point corresponds to maximal wrist acceleration, when limited cues are available to predict which object is going to be grasped. The second one was temporally aligned to maximal wrist velocity, occurring during the fingers opening phase, a moment at which the action goal becomes predictable (Gangitano et al., 2001). The experimental design replicates the same paradigm to evaluate the reproducibility of the AOEs. **On day one, the action observation protocol was measured alone, on the second day the action observation protocol was repeated before administering continuous Theta Burst Stimulation (cTBS) over the primary motor cortex**. **The action observation was then repeated a third time after cTBS administration to evaluate a potential causal contribution of M1 excitability to both measures, MEPs and MEKs.** Beside important considerations about the replicability of MEKs and MEPs, results will nourish theoretical considerations about the way by which action observation-induced motor facilitation reflects the functional, synergistic organization of the motor output.

2. Material and methods

2.1. Participants

Fifteen volunteers (5 males, 10 females, mean age 25.4 ± 3.41 years (m±sd)) participated in the study. All participants were right handed (Edinburgh handedness inventory ; Oldfield, 1971), with

normal or corrected to normal vision and no contraindication to TMS according to their personal clinical history. None of them reported after-TMS undesired effects. The whole experimental procedure was approved by the local ethics committee, and was in compliance with National legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki). Participants gave their informed consent before performing the experiment and were remunerated for their participation.

2.2. Stimuli

During the whole experiment, subjects sat on a TMS chair (Rogue Research Inc., Montreal, Quebec), with their elbow flexed at 90° and their hand prone in a relaxed position. **Their head was kept stable via a chin and a head rest**. **The stimuli, two video-clips of reach-to-grasp actions, were displayed through Psychtoolbox-3 software (PTB-3, The MathWorks Inc., Natick, MA, USA), on a computer screen placed in front of the subject (distance of 60 cm). Clips were recorded via a Sony 3D camera (Sony Corporation, Tokyo, Japan) at the format of 800x600 pixel and length of 2500ms**. Each clip showed an actor reaching either one of two different objects, simultaneously present on a table. The distance between the hand resting position and the objects was about 50 cm. The two objects were a small sphere (diameter 2 cm; graspable by precision grip) and a large sphere (diameter 10 cm; graspable by power grip). The two objects were placed on a table at a small distance from each other (10 cm) to create an ambiguity regarding the final target of the grasping action. Actions were shown from a lateral perspective to maximize the visibility of hand trajectory and finger opening but making it difficult to predict the action goal. The two video-clips (one for each object) were selected from a set of 40 video-clips of the same actor reaching for the small sphere (half of the trials) or the large one. During these video recordings, we also captured movement kinematics and electromyography (EMG) of the actor. This information was used to select two movies with similar duration and similar kinematic features (e.g. wrist velocity and grip aperture, Figure 1B). A more detailed description of kinematic and EMG recording of the stimuli are available in supplementary material A.

2.3. Procedure

All subjects completed three experimental sessions over two different days (Figure 1A). During the first day, they performed one experimental run of the action observation protocol (session 1 – day 1). In the second day, the participants completed two experimental runs of the same action observation protocol: one session before (session 2 – day $2_{pre\text{-}cTBS}$) and one after (session 3 – day $2_{post\text{-}cTBS}$) the application of continuous theta burst stimulation (cTBS) over the left primary motor cortex (see the TMS section for more details).

Each day started with the TMS mapping procedure (see the TMS section for more details). Each action observation run started with 15 baseline trials with the subject at rest ($baseline_{pre}$). After the baseline, subjects completed 60 action observation trials (30 trials for each object type, precision and power), followed again by 15 baseline trials ($baseline_{post}$). Each action observation trial began with a fixation cross on the computer screen. After an inter-trial interval (varying from 8 to 12 seconds) the fixation cross disappeared and the movie started. In one third of the trials, subjects were asked if the action just presented was the same as the previous one (to monitor attention). The two first sessions lasted about 30 minutes, and the third session lasted about 50 minutes, including subject preparation, debriefing, and cTBS application (only for the third session). On average the time elapsed between session 1 and 2 was **6 days (+/- 1.2 days (STD)).** The time of the day was kept as constant as possible: it was the same for 10 subjects, while for the remaining participants largest difference was 3 hours.

2.4. TMS, EMG and motion capture

EMG signals were recorded with a standard tendon-belly montage (Ag/AgCl electrodes), on four right intrinsic and extrinsic hand muscles: First Dorsal Interosseus (FDI), Abductor Digiti Minimi (ADM), arm Flexor Digitorum Superficialis (FLX), Extensor Digitorum (EDC). Data was amplified via a wireless electromyography system (ZeroWire EMG, Aurion, Italy), with a band pass between 10-1000Hz. Analog to digital conversion was done via a dedicated board (Power1401 CED,

Cambridge Electronic Design Limited, Cambridge, England) at a sampling rate of 2kHz. Right arm TMS-evoked movements were measured via a passive motion capture system (VICON, Oxford, UK) with 9 near infrared cameras with an acquisition frequency of 100Hz. Nine reflective markers were attached on the right hand. Markers were respectively on the nail of the thumb, nail of the index, nail of the middle finger, nail of the ring finger, nail of the 5th finger, ulnar styloid, radial styloid (thumb knuckle), index knuckle, 5th finger knuckle (Figure 1C). TMS was applied using a Magstim 200 stimulator (Magstim Company, Whitland, UK) and a 70 mm figure of eight coil. Coil position was determined at the beginning of session 1 (day 1) and 2 (day $2_{pre\text{-}cTBS}$) based on standard procedures (Rossini et al., 1994, 2015) to define the optimal coil location for the muscles of interest. In this case, coil position and orientation was optimized to achieve reliable MEPs on all recorded muscles, at the lowest possible intensity. Resting Motor Threshold (rMT) was determined as the intensity evoking at least 50μV MEPs in all the four recorded muscles, at least 5 times out of 10. At the beginning of session 2, the active motor threshold (aMT) was also determined. The aMT was defined as the minimal TMS intensity evoking, in all muscles, 5 out of 10 MEPs of at least 200μV, during voluntary sub-maximal contraction. Once we determined the optimal coil position, we used a mechanical support to fix the coil position with respect to the head. The head was also constrained by a chin-rest and an ark-shaped two-points support on the forehead and on the right lateral side of the head. We additionally marked the coil outline on the head of the participant (five small marks where drawn directly on the skin with an ink marker to match coil position and orientation). An experimenter was standing behind the participant for the whole duration of the experiment to control that the coil was not displaced at any time with respect to the optimal location identified. TMS was delivered during an approximately equal amount of muscles contraction (all four muscles; 30% maximal) lasting 2s and followed by 8-12s of rest. Muscle contraction onset was prompted by a tone sound and was monitored on a screen by the experimenter and the subject, via continuous visual feedback. Between session 2 (day $2_{pre\text{-}cTBS}$) and 3 (day $2_{post\text{-}cTBS}$) we applied a cTBS protocol over the left primary motor cortex. The cTBS protocol consists of a series of TMS

trains (three pulses at 50 Hz) repeated every 200ms for 40s (total of 600 pulses) and it was applied at an intensity of 80% of the aMT (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005). During the baseline and the action observation protocol, the intensity of stimulation was set at 120% of the rMT. During baseline trials, TMS was delivered at random intervals (ranging between 8-12s) while subjects were asked to rest and relax. During action observation trials a single TMS pulse was delivered on each trial at one of the two possible time points (60 total trials, with 15 pulses for each combination of the two object types with the two stimulation time points; Figure 1B). The first stimulation time point (t_1) corresponded to maximal arm transport acceleration, 250ms from the start. This time point was chosen to offer very little visual information to disambiguate which object was going to be grasped. As shown in supplementary material A (Fig. A.2), at timing t_1 (peak acceleration) few differences were visible in the main parameters of the kinematics of the actor (grip aperture, velocity, acceleration, fingers kinematics). The video-clips used as stimuli were chosen specifically to be as similar as possible in the early phase of reaching. The second stimulation timing (t_2) was delivered at maximal transport velocity, 500ms from the start. At this time point a significant amount of visual information about the observed movement is available and this also corresponds to maximal CSE modulation (Gangitano et al., 2001). In total, 30 trials for each of the two timings were recorded (15 per grip type).

2.5. Data analysis

2.5.1. Preprocessing

The data collected (EMG, motion capture and behavioral responses) were processed with custom software written in Matlab (Mathworks, Natick, MA). From EMG recordings, we computed peak-to-peak maximal amplitude of each MEP for all four muscles, on a variable-length window, after the TMS pulse. The exact window length was set separately for each subject and muscles by averaging all trials in all conditions. This procedure ensures that the window of peak-to-peak computation is tailored to the specific MEPs morphology (Figure 1C). Motion capture data were

first low-pass filtered using a digital fifth-order Butterworth filter at a cutoff frequency of 20Hz. We then computed 8 elevation angles (Figure 1C): (1) from radial styloid to nail of thumb, (2) index knuckle to nail of index, (3) index knuckle to nail of middle, (4) 5th finger knuckle to nail of ring, (5) 5th finger knuckle to nail of 5th finger, (6) ulnar styloid to radial styloid, (7) ulnar styloid to index knuckle, (8) ulnar styloid to 5th finger knuckle. Elevation angles are defined by the angle of each segment with the vertical axis. This measure represents not just the displacement of a unique finger, but rather its movement with respect to the movement of the hand and is comparable to previous investigations using inductive sensors (Gentner & Classen, 2006). Elevation angles were then low-pass filtered (Butterworth filter at a cutoff frequency of 20Hz). To account for slight changes in the initial hand position, we normalized elevation angles, at each trial level, by the pre-stimulation mean amplitude (500 ms period before TMS). After this pre-processing, the peak-to-peak amplitude of each angular displacement was used to define MEKs. Outliers' values, exceeding 2 standard deviations (SD) from the average of each subject, were discarded (around 5% of trials). In addition, MEPs and MEKs data exhibiting excessive muscle activity prior to the TMS pulse within each experimental session were removed from further analysis (>3 SD; MEPs: 1% of trials, MEKs: 3% of trials). Finally, MEPs and MEKs individual trials values were normalized on the basis of the average of the baseline$_{pre}$ for each session and each subject separately.

2.5.2. Permutation tests

Permutation test is a class of randomization test, based on the computation of the values of the statistical test after all possible randomization of the labels between the compared datasets. Contrary to parametric statistics, these tests do not depend on priors or on the form of the populations sampled, and showed more reliability in case of violations of these foundational assumptions (Byrne, 1993; Hunter & May, 2003). Randomization techniques, such as permutations test, are particularly relevant for cognitive/experimental psychology relying on small samples (Byrne, 1993; Hunter & May, 2003; Killeen, 2005), situation in which they outperform the classical parametric approaches (Ludbrook & Dudley, 1998; Nichols & Holmes, 2001). Thus, permutation test, as a

conservative strategy, are becoming the method of reference in EEG, MEG and fMRI studies (Eklund, Nichols, & Knutsson, 2016; Maris & Oostenveld, 2007; Nichols & Holmes, 2001; Pantazis, Nichols, Baillet, & Leahy, 2005; Singh, Barnes, & Hillebrand, 2003). For these reasons, permutation tests are a well-suited tool for the investigation of AOEs via TMS and we present only the statistic values reported by this technique (results from parametric tests can be found in supplementary material C).

Comparing two datasets A and B with permutation tests, an absence of significant differences suggests that, the labelling of the data under investigation could be considered as arbitrary and that the same data would have arisen whatever the experimental condition is. The method generates shuffled data sets by randomly permuting the labels associated to the conditions and estimating the sampling distribution of the test statistic under this strong null hypothesis. Repeating the process many times, a distribution of test statistics is obtained representing the distribution under the null hypothesis. Then, the null hypothesis is rejected at a significance level if the tested statistic is greater than the $1-\alpha$ percentile of the empirical permutation distribution (where α is the significance level). At the end, the final p-value gives the proportion of occasions on which the data would have segregated into such disparate groups by chance. We performed multiple permutation tests using the matlab function 'mult_comp_perm' using 5000 repetitions. When applying permutation tests with multiple comparisons a correction must be performed. The "tmax" method was used for adjusting the p-values of each variable in the same way as Bonferroni correction does for a t-test (Blair & Karniski, 1993; Westfall & Young, 1993).

2.5.3. Statistical analysis

We performed three different groups of multiple comparisons using two-tailed corrected permutation test **on all variables (4 MEPs and 8 MEKs).**

Generic attentional effects: the first analysis was aimed at evaluating non-specific action observation or attentional effects. Specifically, we analysed the temporal evolution of our dependent variables where no AOEs are expected ($baseline_{pre}$ trials vs. $baseline_{post}$ vs. AO trials with

stimulation at t_1 in both grasp-type conditions ($t_{1power\&precision}$)). All possible comparisons between these 3 conditions were run for session 1 and 2 separately (day 1 and day $2_{pre\text{-}cTBS}$).

Action observation effects: the second analysis was directed to the investigation of AOEs. For this purpose, we ran multiple permutation tests to compare the grasp-type conditions (precision and power) and the two timings (t_1 vs. t_2). For each pre-cTBS session separately (day 1 and day $2_{pre\text{-}cTBS}$), all possible comparisons between these 4 conditions were performed.

Effects of cTBS on M1: the third analysis was performed to evaluate the effect of cTBS on baseline trials (pre-post cTBS effects on **all** MEPs and MEKs). Since the effect of cTBS has been reported to be highly variable across participants (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2013; Huang et al., 2005; Palmer, Bunday, Davare, & Kilner, 2016; Ridding & Ziemann, 2010; Vallence et al., 2015; Vernet et al., 2014), we also show the effect of cTBS, on corticospinal excitability, at the single subject level as a separate piece of information (see Figure A.1 in supplementary material A).

At the group level, we ran a simple two-tailed permutation test on each variable. At the subject level, we ran a series of paired two-tailed t-tests, between the measures recorded at rest before the cTBS protocol ($baseline_{post}$ – day $2_{pre\text{-}cTBS}$) and the ones recorded at rest 5 minutes after ($baseline_{pre}$ - day $2_{post\text{-}cTBS}$).

Effect of cTBS on AOEs: the last analysis was performed to evaluate the change in the AOEs following cTBS application. We ran multiple permutation tests to compare the grasp-type conditions (precision and power) on timing t_2, between the two sessions ($day_{2\ pre\text{-}cTBS}$, $day_{2\ post\text{-}cTBS}$). All possible comparisons between these 4 conditions were performed. This analysis was repeating two times: (1) in normalizing by the $baseline_{pre}$ of each session, (2) in normalizing by the $baseline_{pre}$ of the session 2 ($day_{2\ pre\text{-}cTBS}$).

2.5.4. Principal Component Analysis

A Principal Component Analysis was used to investigate the modulation in the whole hand pattern of movement elicited by TMS. This method is classically used as an index of movement

coordination evaluation (Berret, Bonnetblanc, Papaxanthis, & Pozzo, 2009; Daffertshofer, Lamoth, Meijer, & Beek, 2004; Hicheur, Terekhov, & Berthoz, 2007; Paizis, Papaxanthis, Berret, & Pozzo, 2008) and has already been employed in previous investigations on TMS-evoked movements (Gentner & Classen, 2006). This procedure uses an orthogonal transformation to convert selected variables into a set of new variables, less numerous, linearly uncorrelated and named principal components. These new variables are the results of linear combination of the initial variables explaining the maximal variance of the dataset. This operation can be thought as an efficient manner to reveal the hidden internal structure of a multivariate dataset in a way that best explains the variance in the data.

As done by (Gentner & Classen, 2006), we defined for each trial a posture vector formed by the value of the eight elevation angles at a precise time-point. This time-point was computed as the time where the absolute sum of joint angles (relative to baseline) reached a maximum in the temporal window from 0 to 150ms after the TMS pulse. Separate PCAs were performed for each participant and for each condition on a matrix M, composed of m=30 rows (number of trials for each grasp type) and n=8 columns (number of angles). Each column $M_{i\ (1\leq i\leq n)}$ of M was centered and normalized. Based on this transformation, the covariance matrix of M was computed and orthonormally diagonalized to obtain the matrix of the eigenvectors. Eigenvectors were then reordered in a decreasing order based on the value of the associated eigenvalue. This new matrix, denoted W (formed by the columns $(w_{ij})_{i\leq 1,j\leq 1}$) contained the weighting coefficients or loadings associated to the principal components. Then, the principal components (denoted by PC), are defined by the following linear combination: PC = MW. Deduced from this, the first PC is obtained by the following equation:

$$PC_1 = \sum_{i=1}^{8} w_{i,1} M_i \qquad \text{(Eq. 3)}$$

The first eigenvector (associated to the first principal component) represents the direction of the maximum variance. The ratio between the first eigenvalue and all the eigenvalues gives a number between 0 and 1 (converted in percentage and reported as PC%). Expressed at each subject level, variance explained by the first PC captures the amount of "invariance" between movements across trials. Functionally speaking, a high PC% value means that markers movement are dependent and suggest a grouped control of the variables instead of an individual control of each joint.

From this computation, we analysed across subjects the number of components necessary to obtain a PC%≥90, and the PC% value for a number of 3 and 4 components (average number of components found across subjects). We first ran multiple permutation tests defined similarly to the three analyses performed on MEPs and MEKs (see *2.5.3 Statistical Analysis*: Generic attentional effects, Action Observation Effects and Effects of cTBS over M1). These computations showed no significant effect and interaction for any variable. However, since PCA analysis requires a large amount of data (Gentner & Classen, 2006), this absence of significance was expected. We then performed a second analysis by grouping together trials belonging to the two grasp types (precision and power) in order to increase the number of observations. Multiple one-tailed permutation tests were then run to compare baseline vs AO trials (baseline, t_1, t_2) within and between all sessions (day1, day2$_{pre\text{-}cTBS}$, day2$_{post\text{-}cTBS}$).

3. Results

In the following section, we present the modulations observed on the MEPs for the 4 recorded muscles (FDI, ADM, FLX, EDC) and on the MEKs for the 8 elevation angles (thumb, index, middle, ring, 5th finger, thumb knuckle, index knuckle, and 5th finger knuckle), in function of the different experimental conditions: timing of TMS pulse (t_1, t_2), observed grip type (power, precision) and sessions (day 1, day 2$_{pre\text{-}cTBS}$, day 2$_{post\text{-}cTBS}$).

We will first present the generic modulation induced by the observation of an action. In a second part, we will investigate the specific modulation of MEPs and MEKs related to grip type (power vs precision) before cTBS application (day 1 and day $2_{pre\text{-}cTBS}$). Then we will describe the effect of cTBS (day $2_{post\text{-}cTBS}$) on the previously observed modulations. To finish we will analyse modularity of TMS-evoked movements, by applying PCA data reduction to the MEKs data, to explore how these coordination patterns are affected by action observation and cTBS application. For graphical reasons, we present in this section only the principal actors of the movement. The additional variables modulations are shown in supplementary material B.

3.1. Generic attentional effects

These analyses focused on changes of MEPs and MEKs measures that cannot be attributed to specific AOEs (i.e. differences in the observed grasping movements), but rather to a generic modulation related to action observation or attentional effects. The permutation test highlighted a generic action observation effect in the first and second session (day 1 and day $2_{pre\text{-}cTBS}$) on the MEPs from all 4 muscles, by showing an increase of the MEPs recorded at timing $t_{1\ precision\&power}$ with respect to $baseline_{pre}$ ($p<0.05$; Figure 2). On the MEKs, this effect appeared on the index in the day $2_{pre\text{-}cTBS}$ only ($p=0.013$; Figure 2). In addition, an increase from $baseline_{pre}$ to $baseline_{post}$ appeared for FDI ($p=0.007$) and FLX ($p=0.001$) on day $2_{pre\text{-}cTBS}$. Altogether, MEPs measures displayed stronger generic attentional-related effects with respect to MEKs measures.

3.2. Action observation effects

These analyses focused on contrasting the specific modulations induced by the observation of the two grasping actions (i.e., the classical AOEs). The permutation test highlighted a grasp-type related modulation at timing t2 (i.e. mirror-like effect; Figure 3) on EDC MEPs (day $2_{pre\text{-}cTBS}$: $p=0.028$) and thumb MEKs (day 1: $p=0.043$; day $2_{pre\text{-}cTBS}$: $p=0.014$). Therefore, the thumb MEKs tracked the expected AOEs reliably across sessions, whereas the MEPs result was not present in the first session. In addition, the contrast between the two timings revealed an increase of the FDI

MEPs (day $2_{pre\text{-}cTBS,}$ power: p=0.028; day $2_{pre\text{-}cTBS}$, precision: p=0.048), and of the index MEKs (precision, day 1: p=0.001) at timing t_1 with respect to timing t_2 (Figure 3).

3.3. Effect of cTBS over M1

This analysis aimed at verifying the general efficacy of the cTBS protocol in inhibiting TMS-evoked responses at rest ($baseline_{pre}$ and $baseline_{post}$). On average, cTBS reduced the baseline MEPs amplitude of 19% for FDI, 32% for ADM and FLX, and 28% for EDC (Figure 4). Permutation tests showed a significant effect on FLX (p=0.008) and EDC (p=0.035) (Figure 4). On MEKs, an increase of amplitude following cTBS was observed for the thumb (37%), index (10%), and thumb knuckle (15%) while a decrease was found for the middle (19%) and 5th finger (20%). No change (<5%) was noticed for ring, index knuckle and 5th finger knuckle (Figure 4). None of these MEKs modulation are significant after permutation tests. In addition, the effect of cTBS at the subject level, on EDC MEPs and thumb MEKs can be found in supplementary material 1, Figure A.1.

3.4. Effect of cTBS on AOEs

This analysis focused on the inhibitory effect that a cTBS stimulation over the primary motor cortex has on both MEPs and MEKs AOEs, by examining the AOEs after the cTBS protocol (day $2_{post\text{-}cTBS}$). On day $2_{post\text{-}cTBS}$, no significant AOEs modulations (precision vs power and t_1 vs. t_2) were found for MEPs and MEKs (p>0.05; Figure 5). **As shown in 3.2, a significant AOEs modulation was found only for thumb MEK (p=0.007) and EDC MEP (p=0.023). The exact same significant modulations were found for the two types of normalization.** These results show that the cTBS protocol affected the previously reported AOEs on both the MEPs and MEKs.

3.5. Movement modularity evaluation

The PCA analyses were employed to investigate if the whole hand pattern of movement coordination elicited by TMS was altered by action observation and by cTBS over the primary motor cortex. On average, the first four PCs accounted for 93%, 93.1%, 92.5% of the variance in

day1, day2$_{pre\text{-}cTBS}$ and day2$_{post\text{-}cTBS}$ respectively, with the first two accounting for 74.1%, 74.7%, 73.9% of the variance. This result is in agreement with previous reports showing, with a larger amount of data, that the first four PCs accounted for 89.3% of the variance, with the first two accounting for 72.6% (Gentner & Classen, 2006). Furthermore, we intended to measure if cTBS altered the AOEs and baselines. We ran the permutation tests to contrast baseline vs AO trials (baseline$_{pre\&post}$, t1$_{power\&precision}$, t2$_{power\&precision}$) within and between sessions (day 1, day 2$_{pre\text{-}cTBS}$, day 2$_{post\text{-}cTBS}$). A significant PC% reduction of baseline day 2$_{post\text{-}cTBS}$ compared to baseline in day 1 ($p=0.015$) and day2$_{pre\text{-}cTBS}$ ($p=0.049$) was revealed (Figure 6). Moreover, a significant PC% increase at t$_{2power\&precision}$ compared to baseline was found on day 2$_{post\text{-}cTBS}$ ($p=0.029$). These results suggest that the cTBS affected the organization of coordinated hand movements at baseline, while the action observation partially restored it.

4. Discussion

The present study evaluated, side-by-side, motor evoked potential (MEPs) and TMS-evoked kinematics parameters (MEKs) to characterize action observation effects in humans. The experimental protocol consisted in a classical action observation task (i.e. Gangitano et al., 2001), involving reaching-grasping towards either one of two objects with different sizes (thus affording power or precision grip). MEPs amplitude during all action observation conditions increased with respect to baseline. This increase is associated to generic action observation because it is not action-specific (i.e. precision vs. power grasping; Fadiga et al., 1995; Strafella and Paus, 2000; Aziz-Zadeh et al., 2002; Clark et al., 2004). Therefore, it cannot be excluded that in our experiments MEPs modulation is driven by a more general attentional grab due to the increased saliency of moving visual stimuli. On the contrary, this effect did not appear on MEKs, suggesting that these measurements are less prone to attentional modulations.

The critical modulation that we were expecting was related to the grip type (precision vs. power grip) particularly at the later stimulation timing (t_2, as opposed to the earlier timing t_1, when far less action-specific visual cues are available). As we found in our data, larger responses for precision grip were more likely to occur at t_2. Precision grip requires indeed more accuracy in finger positioning and consequently greater control on muscle activity during execution (Gribble, 2003; Marzke, 1997). Moreover, as shown by cortical stimulation and recording experiments performed on monkeys (Fluet, Baumann, & Scherberger, 2010; Rizzolatti et al., 1988) and humans (Pistohl, Schulze-Bonhage, Aertsen, Mehring, & Ball, 2012), precision grip has a larger cortical representation than power grip.

Our results showed a clear difference between the two kind of measures. While MEPs at t_2 increased only for finger extensors and only in one session, a significant MEKs modulation at t_2 was found for the thumb elevation angle in both recording sessions. This major involvement of the thumb could be related to its fundamental role in grasping tasks (Cotugno, Althoefer, & Nanayakkara, 2016) and to the larger probability in evoking thumb movements via TMS stimulation (Gentner & Classen, 2006).

For both measures (extensor muscle MEPs, thumb MEKs), after the application of cTBS over M1 significant AOEs modulation was not observed anymore. This result do not match with previous reports showing no change in CSE-based AOEs (Avenanti, Bolognini, Maravita, & Aglioti, 2007) or in behavioral execution-adaptation effects (Cattaneo & Barchiesi, 2011), after the application of cTBS over M1. While contrasting with previous findings, our results are in line with the recent demonstration that M1 cTBS alters behavioral performance in an action observation task (Palmer et al., 2016). Further studies will be necessary to fully understand the role played by M1 in AOEs, especially in light of the discovery that in non-human primates, neurons with mirror-like properties have also been found in the primary motor cortex (Dushanova & Donoghue, 2010; Kraskov et al., 2014; Tkach, Reimer, & Hatsopoulos, 2007).

Although the use of MEKs requires a greater amount of data processing and the selection of the kinematic parameters of interest (i.e., elevation angles in the present work; Gentner and Classen, 2006), we demonstrated that the use of TMS-evoked thumb kinematics provides a greater reproducibility of AOEs. Importantly, we evaluate AOEs via statistical methods that, by incorporating biophysically motivated constraints in the test statistic, drastically increase sensitivity of the statistical test (Maris & Oostenveld, 2007). Strikingly, the recording of MEPs alone did not show the emergence of consistent AOEs (Fadiga et al., 2005, 1995; Naish et al., 2014). Although reproducibility issues are becoming more and more important (Kobayashi & Pascual-Leone, 2003; Mills, 1999), this is rarely verified. Our findings, together with the known difficulty in publishing negative results (Matosin, Frank, Engel, Lum, & Newell, 2014; Mervis, 2014), suggest that a quite significant number of unpublished studies did not find AOEs using classical CSE measures (i.e. MEPs). Although a larger number of subjects or trials might have shown effects on MEPs in both sessions, the critical point here is that another measure recorded in parallel (MEKs) can show the same AOEs twice, with the same number of trials and subjects. As a consequence, it is here more interesting to discuss why MEKs should be more consistent than MEPs.

To understand why MEPs could be more affected by confounds it is important to consider some key experimental constraints. In action observation studies, the classical procedure consists in focusing on very few muscles (up to two or three) and stimulation is applied just above threshold to maximize response sensitivity to AOEs modulations. Recording MEPs on several muscles would require higher TMS intensities, to accommodate for the different thresholds and partially non-overlapping representations. Increasing stimulation intensities though, would sample from different regions of the recruitment curve in each individual muscle (Devanne, Lavoie, & Capaday, 1997), and this is known to affect MEPs sensitivity to AOEs (Loporto, Holmes, Wright, & McAllister, 2013). Therefore, recording from very few muscles is primarily driven by technical limitations in measuring reliable CSE. This is a potential reason for which we do not find a clear replicable modulation on the MEPs, while we do on the MEKs.

Although the solution may seem to record less muscles, this is a sub-optimal choice to explore AOEs for goal-directed actions. In fact, in a realistic scenario (e.g. movement execution to reach an object), small postural changes (such as those caused by a change in height of the table) have a dramatic influence on the temporal evolution and recruitment of the same muscle in the same action towards the same goal. The same amount of EMG activity in one muscle is present in many different actions and is not necessarily predictive of the action goal. For example, finger extensors activation while lifting an object is in principle against the goal of applying forces onto an object, but it is necessary, via co-contraction with the flexors, to stabilize fingers and wrist joints. Therefore, recording from finger extensor only, would not allow us to discriminate the act of opening or closing fingers. In general, during action execution, little discriminative information can be extracted from the activity of one (or few) muscle(s)).

Many AOE studies instead, used intransitive (non goal-directed) simpler movements, involving few muscles, such as the abduction-adduction of the index or the 5th finger (Catmur et al., 2007; Maeda, Kleiner-Fisman, & Pascual-Leone, 2002; Urgesi, Candidi, Fabbro, Romani, & Aglioti, 2006). This situation offers a direct one-to-one mapping between cortical recruitment, muscle activities and observed movement kinematics. At the same time, these experimental settings may offer a limited insight about the neural mechanisms at play during naturalistic action observation. Nevertheless, these simplified action observation protocols were used to debate about the origin of mirror-like activities in general (Cook, Bird, Catmur, Press, & Heyes, 2014). Specifically, if AOEs are the by-product of sensorimotor associative learning or do they represent a genetic adaptation to fulfil a specific socio-cognitive function? (Barchiesi & Cattaneo, 2012; Catmur et al., 2007; Cavallo, Heyes, Becchio, Bird, & Catmur, 2014).

We concur that understanding the relationship between AOEs and the plastic modulations induced by action observation learning is important. In fact, typical AOEs studies propose long sessions of repetitive action observations, which is the exact same protocol used to induce observational

learning effects (Celnik et al., 2006; Stefan et al., 2005, 2008; Williams & Gribble, 2012), thus creating a fundamental confound between these two components. Here we show a baseline increase from pre- to post-action observation on the MEPs (see "3.1 Generic attentional effects"). Crucially, this effect never appeared on MEKs, indicating greater independence from these learning-induced changes. The reason could be that MEKs convey a richer description of the multidimensional nature of the descending volley. In fact, whole-hand TMS-evoked motor synergies more than muscle-level modulations, have been shown to be relatively robust to long term motor learning (Gentner et al., 2010). It remains to be seen whether MEKs during goal-directed action observation are affected by short-term counter-mirror observational training, as it was the case for CSE in simple intransitive movement observation (Catmur et al., 2007).

More importantly from a theoretical point of view, similar kinematic patterns (and thus visual appearance) may very well be associated to quite different muscle recruitment over time and space. Redundancy and invariance principles in action execution (Flash & Hochner, 2005; Guigon et al., 2007; Sporns & Edelman, 1993), suggest that the functional kinematic output, more than the activities of (few) muscles, provides the best action goal description. These considerations are based on behavioral observations of how kinematics relates to (multi-) muscle activity. At the same time, if we look at the anatomical targets of the descending corticospinal tract, its role and function becomes clearer. In fact, direct corticospinal projections largely target the dorsal horns at the spinal level, meaning that muscle activity is mediated by divergent interneuronal connectivity (Jankowska, 1992; Nielsen, 2016). Projections to the ventral horn, which are a relatively new product of evolution, instead target different spinal motor nuclei, innervating different muscles at the same time (Fetz & Cheney, 1980; Porter & Lemon, 1993). It is for these reasons why MEKs may be better suited to measure goal-directed AOEs. MEKs measure the effect of the synergistic activity of multiple muscles producing coordinated movements, which are driven by intracortical, corticospinal, spinal and peripheral influences.

When we move to the level of whole-hand coordination, we know it is neither based on muscle by muscle nor on single finger movement control. In fact, hand control relies on the temporo-spatial grouping of muscle activities that is further constrained by joint movement biomechanics. Thus, to consider the organization of the motor system, AOEs should be evaluated even beyond separated joint movements. To do so we performed a PCA on the TMS-evoked posture vectors composed by all joints movements. As previously found, a small set of three to four PCs accounted for much of the data variance of TMS-evoked movements (Gentner & Classen, 2006). Whole-hand coordination, however, did not show any modulation for grip type observation. This can be explained by the relatively small amount of data-points we could use to extract uncorrelated whole-hand synergies (i.e. PCs). Previous investigations have indeed shown that at rest, single pulse TMS evoked a quite large number of different postures (Gentner and Classen, 2006). Despite this, we found a significant modulation of whole-hand coordination following cTBS application. Our data revealed a global disturbance of whole-hand coordination due to cTBS-driven injection of noise in the organization of hand movements (Miniussi, Harris, & Ruzzoli, 2013). The same analyses revealed also a significant increase in coordination between action observation (at timing t_2) and baseline recordings after the application of cTBS. This finding suggests that action observation partly countered the interfering effect of cTBS over primary motor cortex.

5. Conclusions

In conclusion, we showed in this study that MEKs act as a more effective measure than MEPs in describing the motor activities triggered by action observation. Specifically, MEKs seem to be more robust to the two critical confounds that can occur when investigating AOEs: observational learning and attentional modulations. These differences are in agreement with other studies showing that while MEKs discriminate between observed actions with different effectors, while MEPs did not (Finisguerra et al., 2015). This lack of sensitivity could ultimately derive from the small amount of information we can extract from MEPs recorded from one muscle. Neural control of arm and hand

movements is the consequence of many adjustments at the muscular level (Bernstein, 1967; Bizzi, Accornero, Chapple, & Hogan, 1984; Gribble, 2003), following possibly a synergistic organization (D'Avella, Portone, Fernandez, & Lacquaniti, 2006; Gentner & Classen, 2006; Leo et al., 2016; Santello, Baud-Bovy, & Jörntell, 2013). In the present study, we demonstrate that recording the net motor output is substantially less ambiguous and more robust in describing the nature of AOEs. The shift from a single muscle to a functional output perspective frames the investigation of AOEs within current models of action control.

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Figure 1: Illustration of the experimental protocol, stimuli and dependent variables. A. Time course of the experiment across the two days, showing the 3 sessions (session 1 – day 1, session 2 – day $2_{pre\text{-}cTBS}$ and session 3 – day $2_{post\text{-}cTBS}$) each starting with a baseline ($baseline_{pre}$), followed by an action observation run (AO) and a second baseline recording ($baseline_{post}$). The cTBS protocol was applied on day 2 (between session 2 and session 3). B. Four representative frames of the two displayed movies (upper panel: power grip, lower panel: precision grip) and associated kinematic (grip aperture and index velocity). Timing t_1 and t_2 are represented by black dotted vertical lines. C. Typical recording for MEPs (four muscles: FDI, ADM, EDC and FLX) and MEKs (8 elevation angles: thumb, index, middle finger, ring finger, 5th finger, index knuckle, 5th finger knuckle, and wrist). In the present study, we used the peak to peak amplitude for both measures.

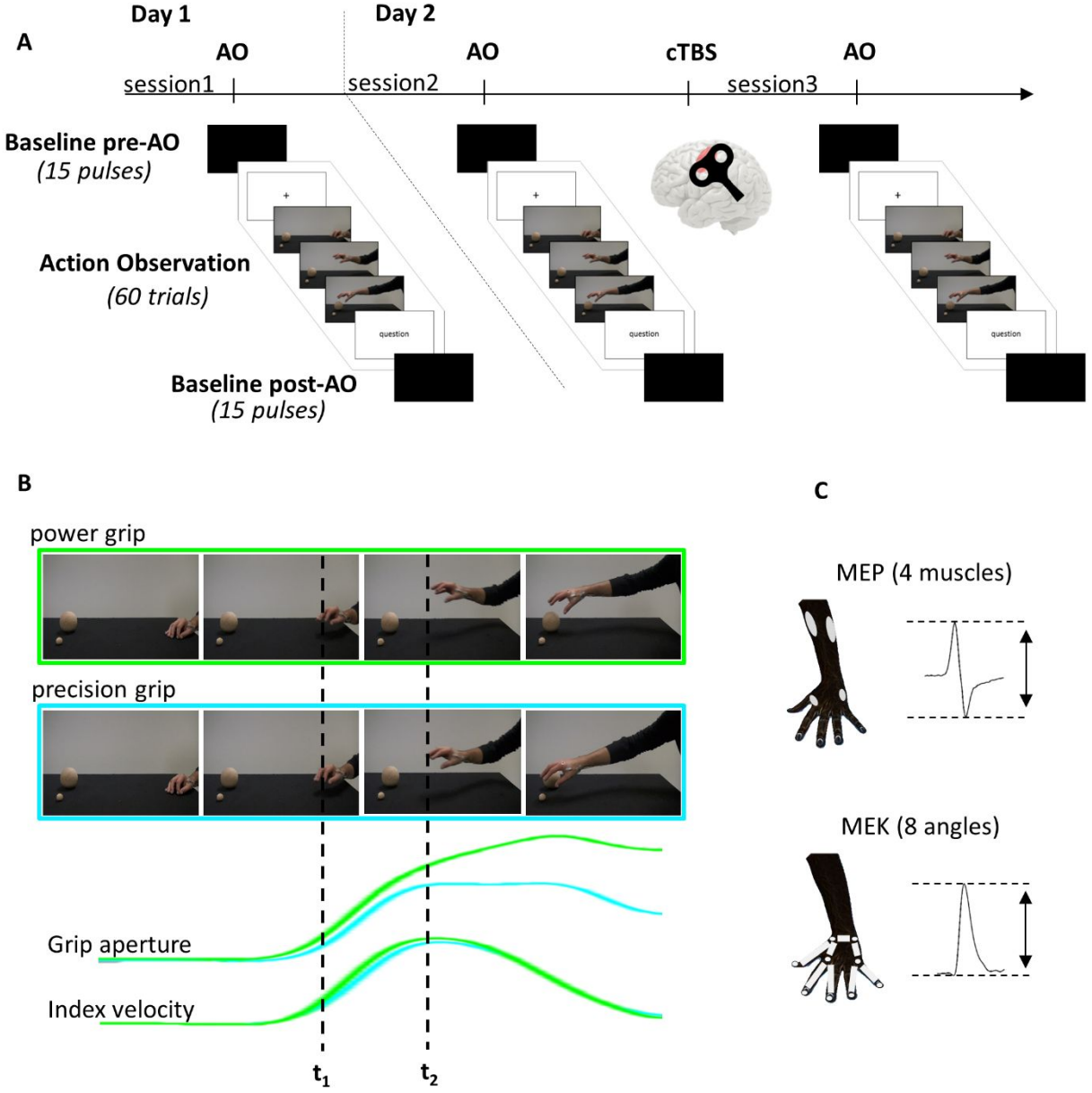


Figure 2: Generic attentional effects on MEPs and MEKs amplitude. Mean and standard error for the four muscles (FDI, ADM, FLX, and EDC) MEPs and three elevation angles MEKs (thumb, index and 5th) are shown as a percentage (%) of the average of $baseline_{pre}$ on the y-axis. The two sessions are stacked vertically for each measure (day 1 on top, day $2_{pre\text{-}cTBS}$ on bottom). The $baseline_{pre}$ level is represented by the low horizontal bar (100%). The 2 phases contrasted (timing $1_{power\&precision}$, $baseline_{post}$) are shown on the x-axis. Significant differences ($p<0.05$) with $baseline_{pre}$ are represented by an asterisk in the top of the value, between the two phases by a horizontal segment surmounted by an asterisk. **The Y-axis scale is the same within variables (MEPs [90 to 210%], MEKs [60 to 180%]). X-axis labels are constant across variables and are reported on for the first variable (FDI).**

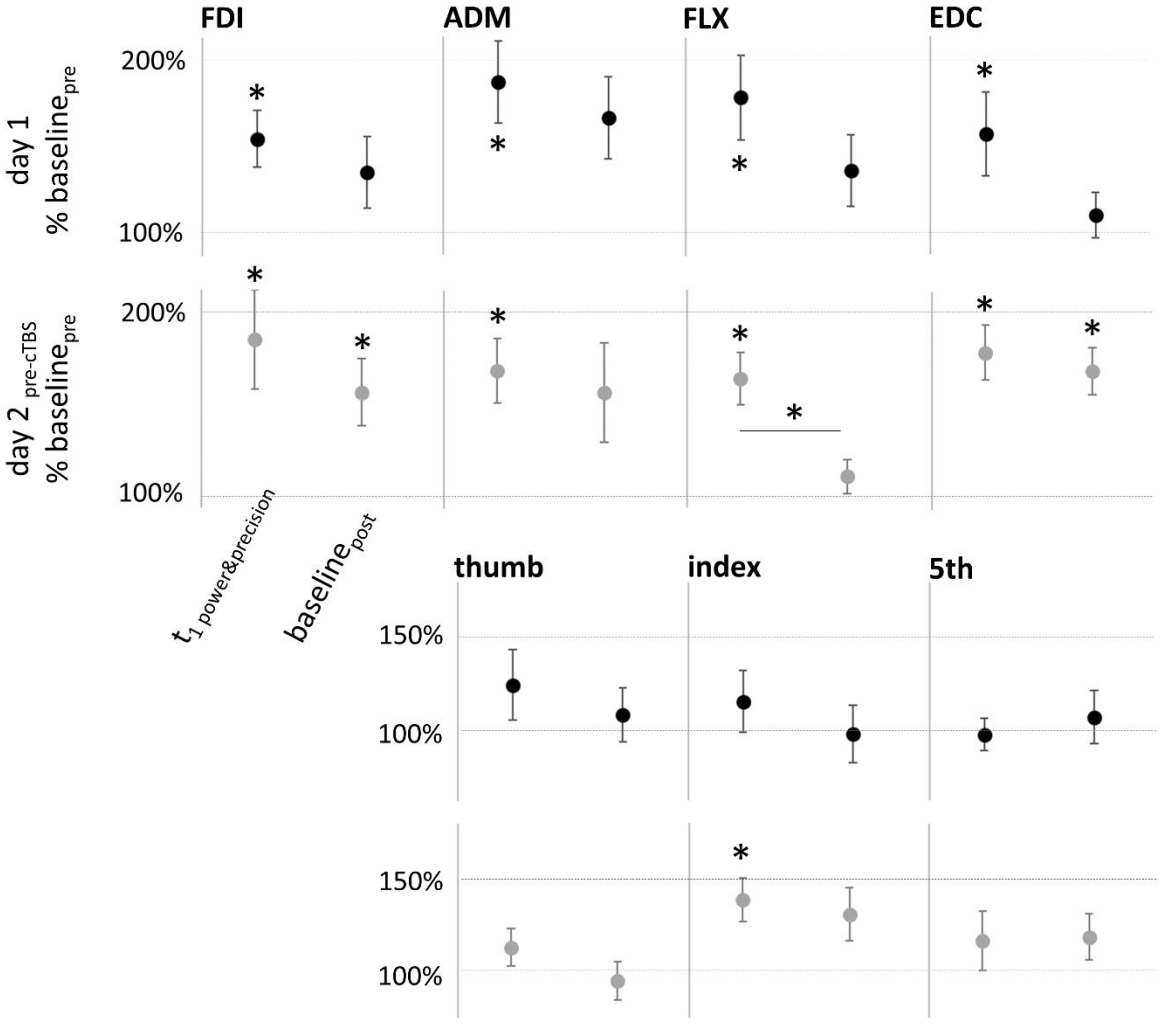


Figure 3: Action observation effects on MEPs and MEKs amplitude. Mean and standard error of the four muscles (FDI, ADM, FLX, and EDC; panel A) and MEKs (thumb, index and 5th; panel B) expressed as a % of the average of baseline$_{pre}$, separately for session (day 1, day 2 $_{pre\text{-}cTBS}$), timing (t_1, t_2), and grasp type (precision (prec), power (pow)). Significant differences are represented by an asterisk ($p<0.05$). **X-axis labels are constant across variables and are reported on for the first variable (FDI).**

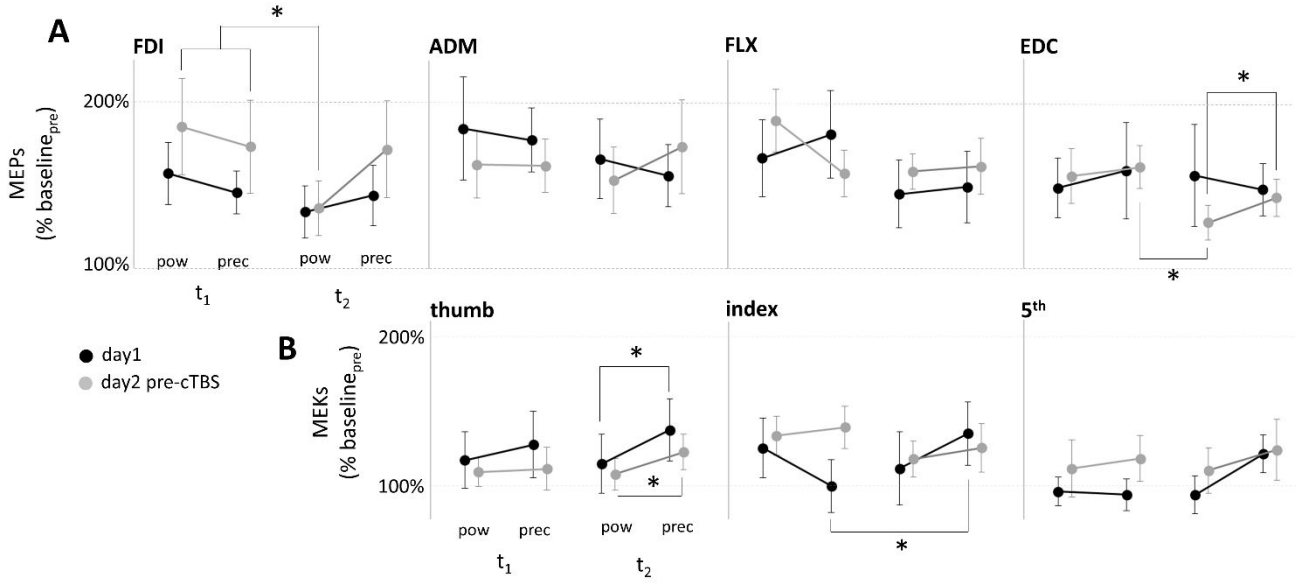


Figure 4: Effects of cTBS on baseline MEPs and MEKs. For each graph, the first point (to the left) represents the mean and standard error of the 15 baseline trials recorded before cTBS (pre-cTBS). The second point (to the right) represents the mean and standard error of the 15 baseline trials recorded after cTBS (post-cTBS). Asterisks denote significant differences ($p<0.05$). **X axis labels are constant across variables (referred to the first panel 'FDI').**

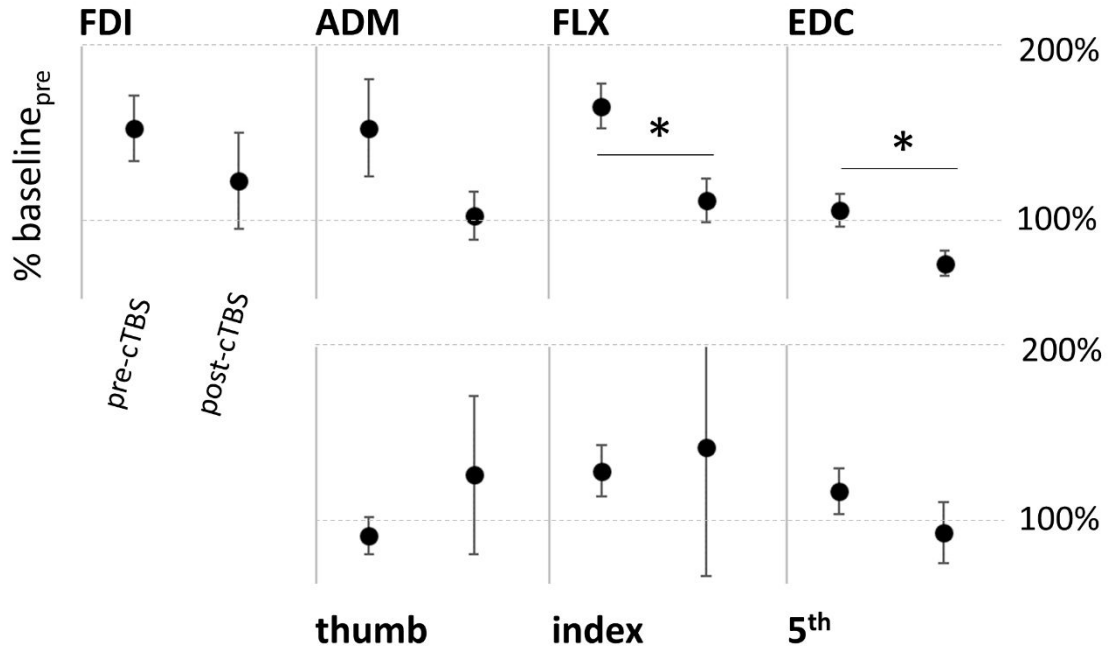


Figure 5: Effects of cTBS on AOEs. Mean and standard error of EDC MEP (A) and thumb MEK (B), as a function of grasp type (precision (prec), power (pow)) at timing t2, before (left side) and after (right side) cTBS protocol over M1. All values are expressed as a % of the average of $baseline_{pre}$ for each session. Asterisks denote significant differences ($p<0.05$). **X axis labels are constant across variables (referred to the first panel 'FDI').**

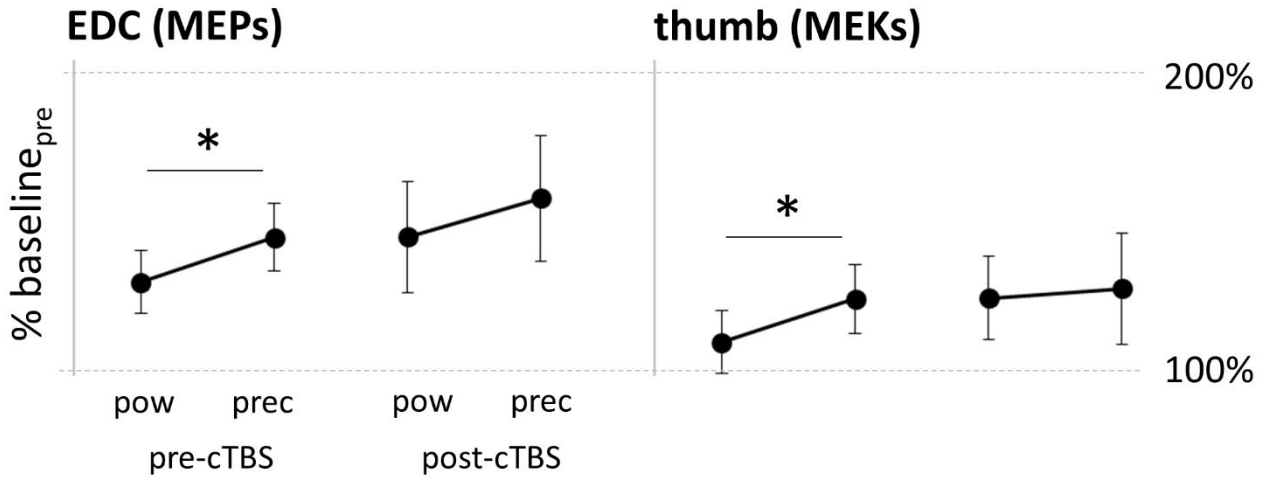
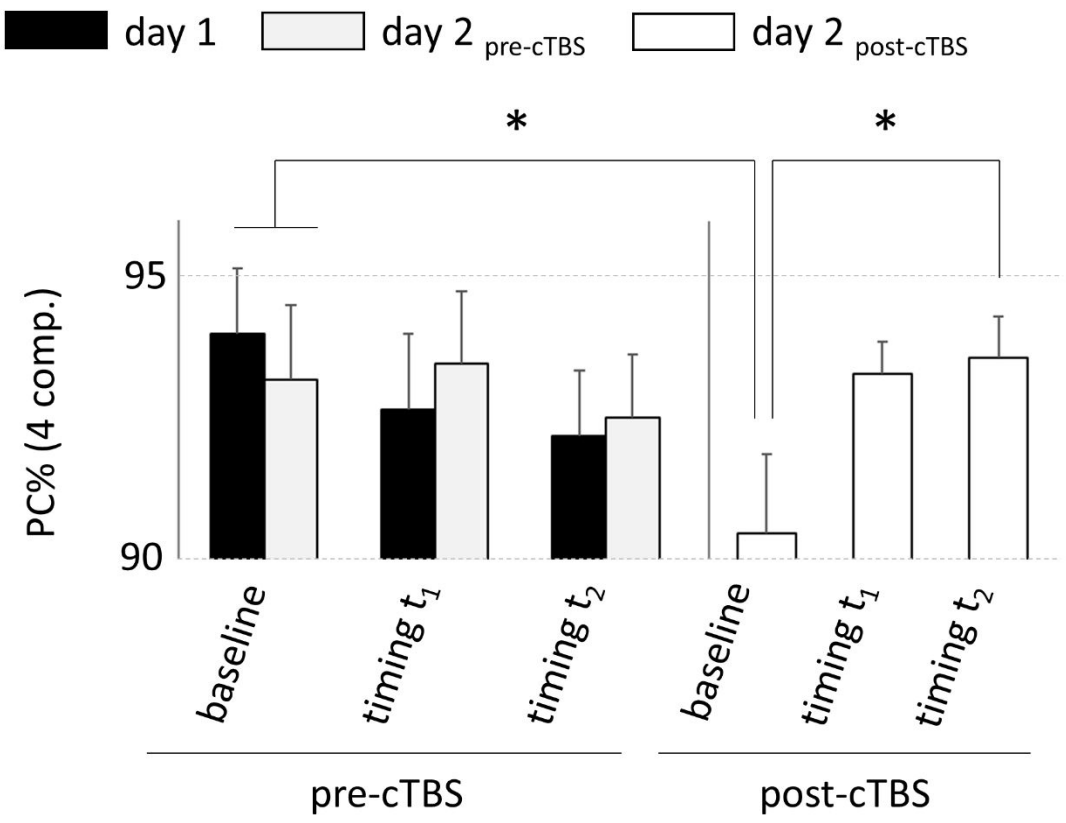


Figure 6: Whole hand configuration changes across sessions and conditions. PC% values of the fourth first components (y-axis), computed on the 8 elevation angles, are shown for baseline and AO trials (baseline, timing t_1 and timing t_2) for the three sessions (day 1, day $2_{pre\text{-}cTBS}$, day $2_{post\text{-}cTBS}$). Asterisks denote significant differences ($p<0.05$).



Supplementary Figures

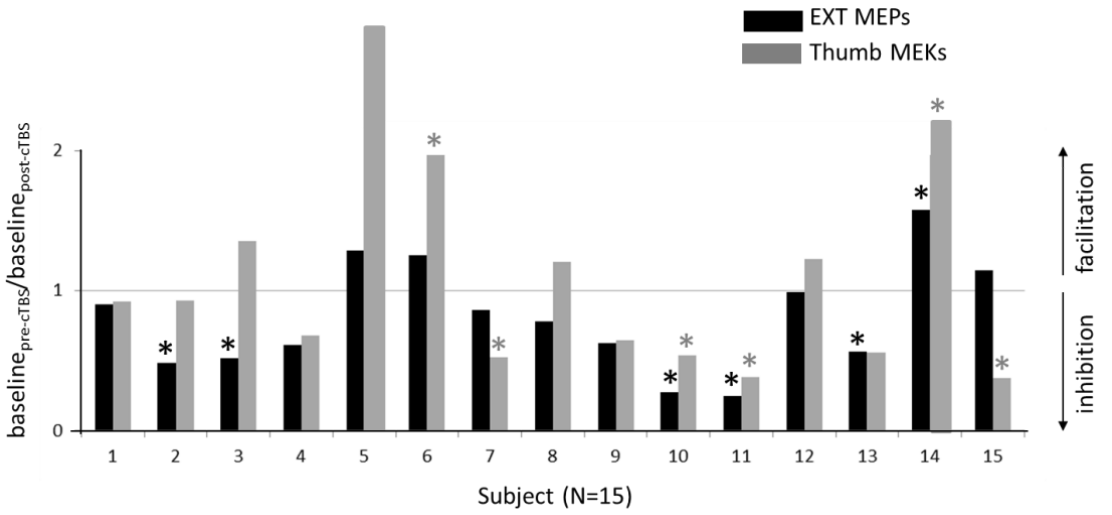


Figure S1.1: Evaluation of cTBS effects at the single subject level. Bars represent, for each subject, the ratio between the average of the 15 $baseline_{post}$ for FDI MEPS (light grey) or thumb MEKs (dark grey) in session 2 ($baseline_{pre\text{-}cTBS}$) with the 15 $baseline_{pre}$ recorded in session 3 ($baseline_{post\text{-}cTBS}$). Values smaller than 1 indicate a reduction of MEPs amplitude in post-cTBS baseline recordings, indexing the expected inhibitory effect of cTBS. Asterisks denote a subject-wise significant cTBS effect (t-test; $p<0.05$). Large Inter-subject variability of cTBS effects is also supported by studies and reviews (Ridding and Ziemann 2010; Vallence et al. 2015; Vernet et al. 2014)

Supplementary Method

(A) Stimuli kinematic and muscular description

In order to choose the most relevant stimuli, we recorded 40 repetitions of an actor performing reach-to-grasp movements toward the two objects (small and large sphere, 20 movements each, **Figure 1B**). We selected one movie per grip type (pow and prec) based on duration, wrist velocity, wrist acceleration and grip aperture. The following section shows a detailed description (EMG and kinematic) of the 40 repetitions of the movements and of the two stimuli selected.

Kinematic: By analyzing the trajectories for the two movements selected as stimuli in the present work, it is possible to notice that the thumb elevation angle increased more in precision grip than power grip, and that this change appeared late relatively to timing 2. The precision grip movement was associated to a smaller displacement of the index at both timing 1 and 2. The index knuckle, reflecting wrist movement, was unchanged in function of grip type (**Figure S2**).

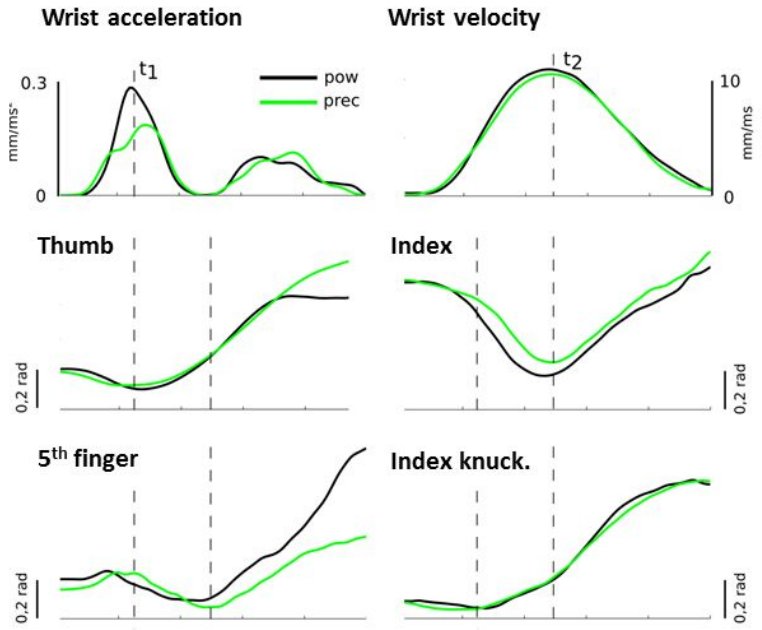


Figure S1.2: Stimuli kinematic features. Wrist velocity and acceleration and angular trajectories of thumb, index, 5th finger and index knuckle recorded during the execution of the two movements selected as stimuli, showing the power grasp in black and precision in green. The two vertical dashed lines denote the two time-points (t_1 and t_2) selected to deliver the single-pulse TMS during the action observation part of the experiment.

By analyzing the average across the 20 repetitions of the two movements (pow and prec), the kinematic parameters did not show any clear modulation related to grip type around timing t_1 and t_2 (**Figure S1.3**). It is important to note that one marker (thumb knuckle) was missing in the actor kinematic as compared to the MEKs recording. To compute the thumb elevation angle we then used the segment from the thumb apex to index knuckle (**Figure S1.2 and S1.3**). This change in computation could influence the trajectory showed here.

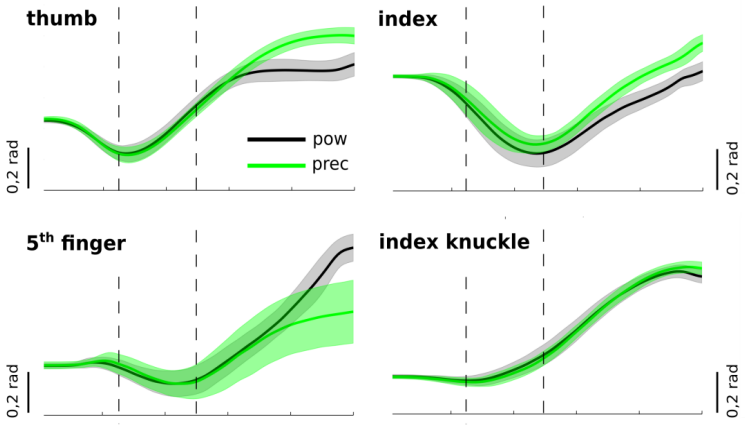


Figure S1.3: Movement repetitions kinematic features. Mean and standard deviation of four elevation angles trajectories (thumb, index, 5th finger, index knuckle), for the 40 repetitions of the reaching movements recorded (20 precision grip, 20 power grip). The power grasp is plotted in

black and precision in green. The two vertical dashed lines denote the two timings (t_1 and t_2) selected to deliver the single-pulse TMS during the action observation part of the experiment.

EMG: By analyzing the EMG data from the 20 repetitions of the two movements, EXT and FLX muscles revealed no clear grip-type-modulation. FDI and ADM muscles showed a difference around timing t2: a greater and earlier increase in amplitude when performing a reach-grasp movement aiming at a precision grip compare to power grip (**Figure S1.4**).

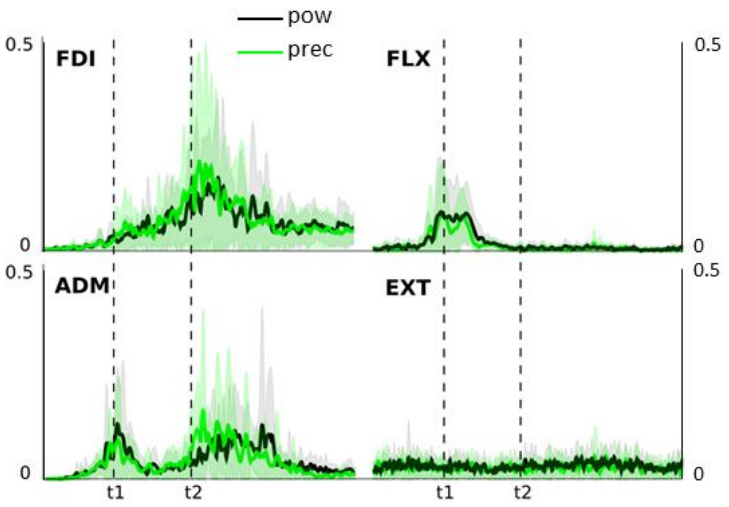


Figure S1.4: Movement repetitions EMG recordings. Mean and standard deviation of activation of the four muscles (FDI, ADM, FLX, EXT) calculated across the 40 repetitions of the reaching movements recorded on the actor (20 precision grip, 20 power grip). The power grasp is plotted in black and precision in green. The two vertical dashed lines denote the two timings (t_1 and t_2) selected to deliver the single-pulse TMS during the action observation part of the experiment

By analyzing the EMG activity recorded during the execution of the two movements selected as stimuli in the present work, we showed that FDI activation amplitude changes depending on the grip type. This difference was in the opposite direction as compared to the data from the 40 repetitions, being increased for power grip with respect to precision grip around timing 2 (**Figure S1.5**). The data recorded from FLX and ADM also showed a modulation, with greater activity for the movement aimed at the power grip around timing 1.

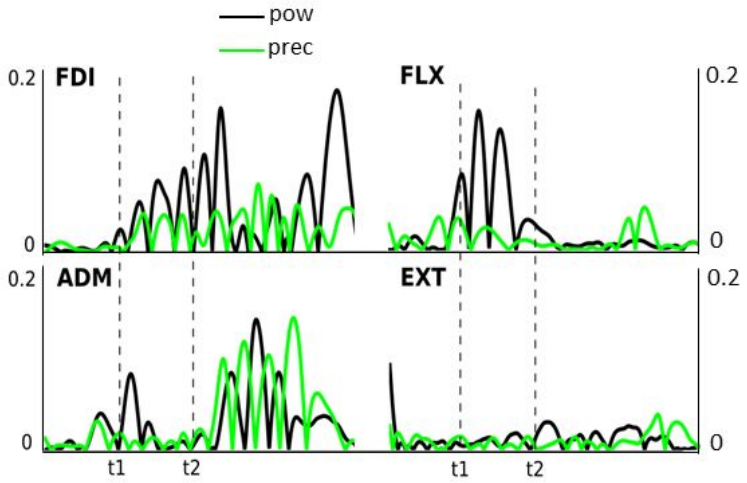


Figure S1.5: Stimuli EMG recordings. Muscular activation for the four muscles (FDI, ADM, FLX, EXT) in the two movements selected as stimuli. The power grasp is plotted in black and precision in green. The two vertical dashed lines denote the two timings (t_1 and t_2) selected to deliver the single-pulse TMS during the action observation part of the experiment.

References

Ridding, Michael C. and Ulf Ziemann. 2010. "Determinants of the Induction of Cortical Plasticity by Non-Invasive Brain Stimulation Inn Healthy Subjects." *The Journal of physiology* 13(Pt 13):2291–2304. Retrieved (http://www.ncbi.nlm.nih.gov/pubmed/20478978).

Vallence, A. M. et al. 2015. "Inter- and Intra-Subject Variability of Motor Cortex Plasticity Following Continuous Theta-Burst Stimulation." *Neuroscience* 304:266–78. Retrieved (http://dx.doi.org/10.1016/j.neuroscience.2015.07.043).

Vernet, Marine et al. 2014. "Reproducibility of the Effects of Theta Burst Stimulation on Motor Cortical Plasticity in Healthy Participants." *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* 125(2):320–26. Retrieved (http://www.sciencedirect.com/science/article/pii/S1388245713009334).

Supplementary results 2 (presentation of the additional MEKs data)

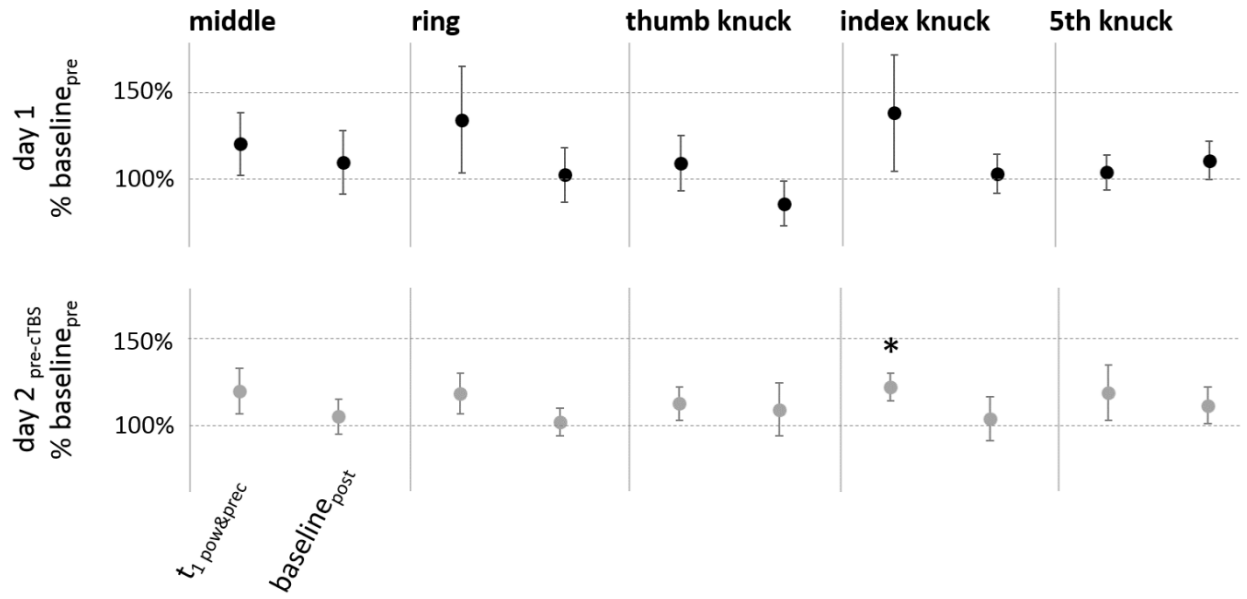


Figure S2.1 : Generic attentional effects on MEPs and MEKs amplitude. Mean and standard error for the five elevation angles MEKs (middle, ring, thumb knuckle, index knuckle and 5th knuckle) are shown as a percentage (%) of the average of baseline$_{pre}$ on the y-axis. The two sessions are stacked vertically for each measure (day1 on top, day2$_{pre\text{-}cTBS}$ on bottom). The baseline$_{pre}$ level is represented by the low horizontal bar (100%). The 2 phases contrasted (timing $_{1pow\&prec}$, baseline$_{post}$) are shown on the x-axis. Significant differences (p<0.05) with baseline$_{pre}$ are represented by an asterisk in the top of the value, between the two phases by a horizontal segment surmounted by an asterisk.

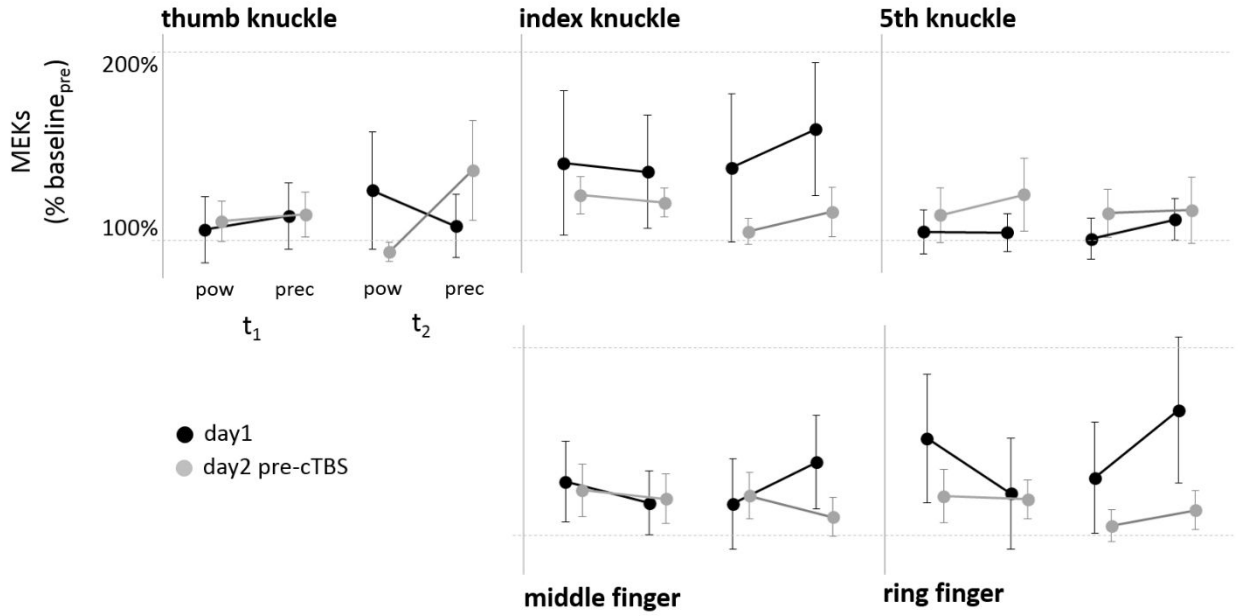


Figure S2.2 : Action observation effects on MEKs amplitude. Mean and standard error of the five MEKs (middle, ring, thumb knuckle, index knuckle and 5th knuckle) expressed as a % of the average of baseline$_{pre}$, separately for session (day1, day2pre-cTBS), timing (t1, t2), and grasp type (prec, pow). Significant differences are represented by an asterisk (p<0.05).

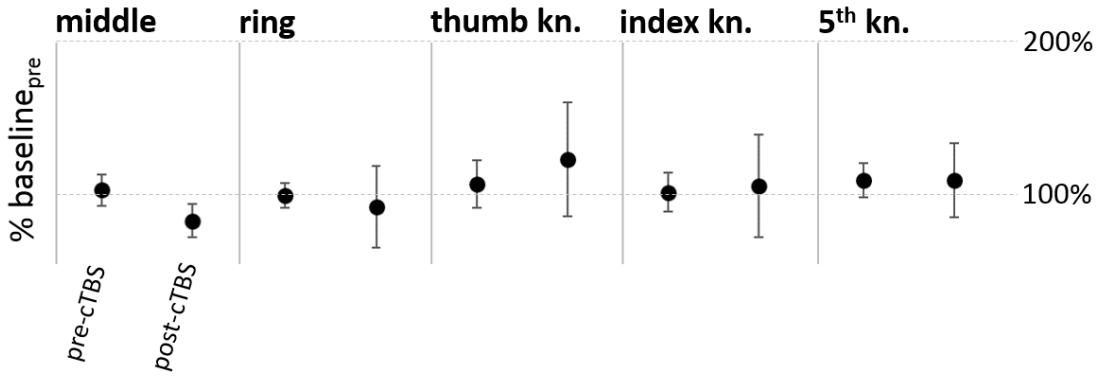


Figure S2.3 : Effects of cTBS on baseline MEKs. For each graph, the first point (to the left) represents the mean and standard error of the 15 baseline trials recorded before cTBS (pre-cTBS). The second point (to the right) represents the mean and standard error of the 15 baseline trials recorded after cTBS (post-cTBS). Asterisks denote significant differences (p<0.05).

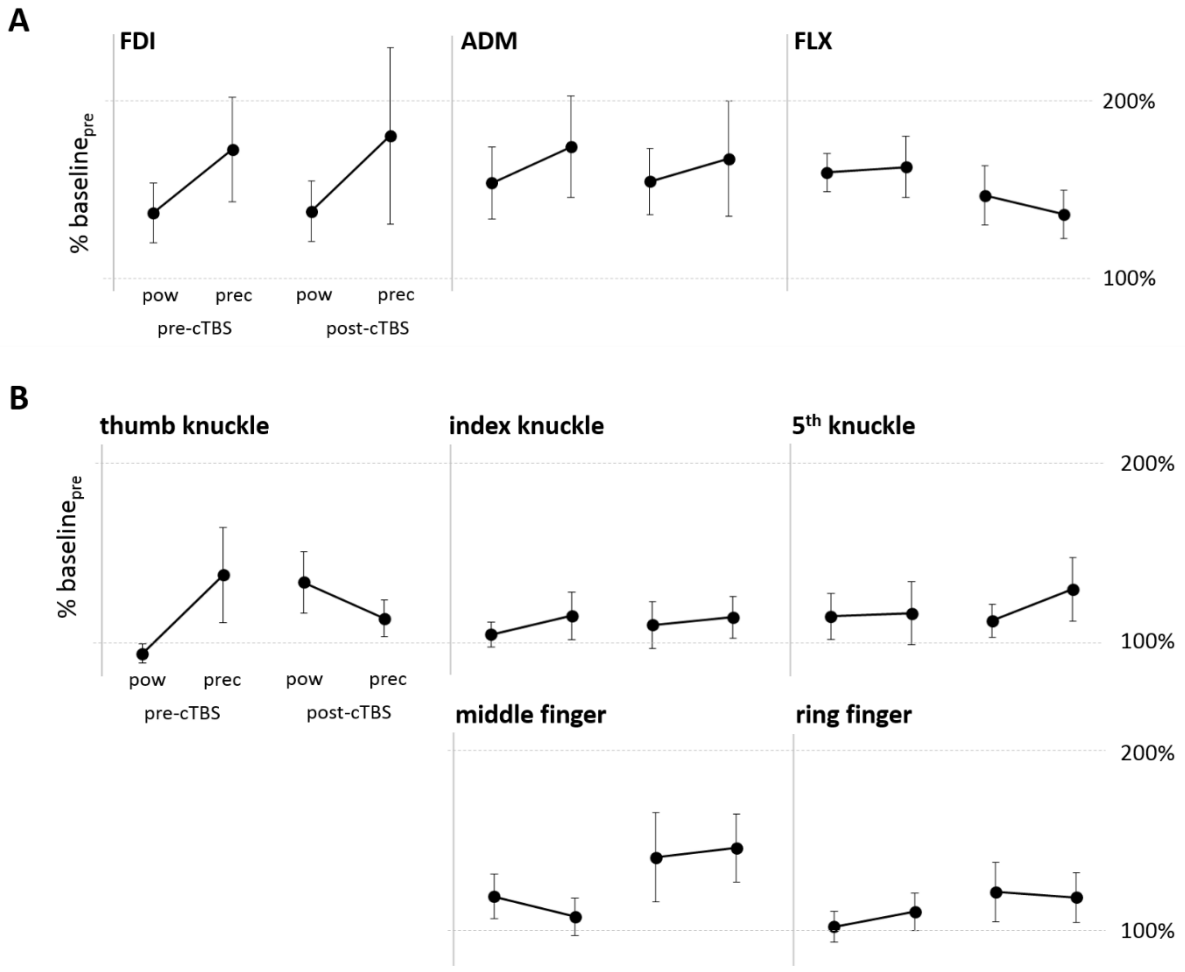


Figure S2.4 : Effects of cTBS on AOEs. Mean and standard error of FDI, ADM and FLX MEPs (A) and middle, ring, thumb knuckle, index knuckle and 5th knuckle MEKs (B), as a function of grasp type (prec, pow) at timing t_2, before (left side) and after (right side) cTBS protocol over M1. All values are expressed as a % of the average of baseline$_{pre}$ for each session. Asterisks denote significant differences (p<0.05).

Supplementary results 3 (compare permutation test and parametric method (corrected multiple t-test))

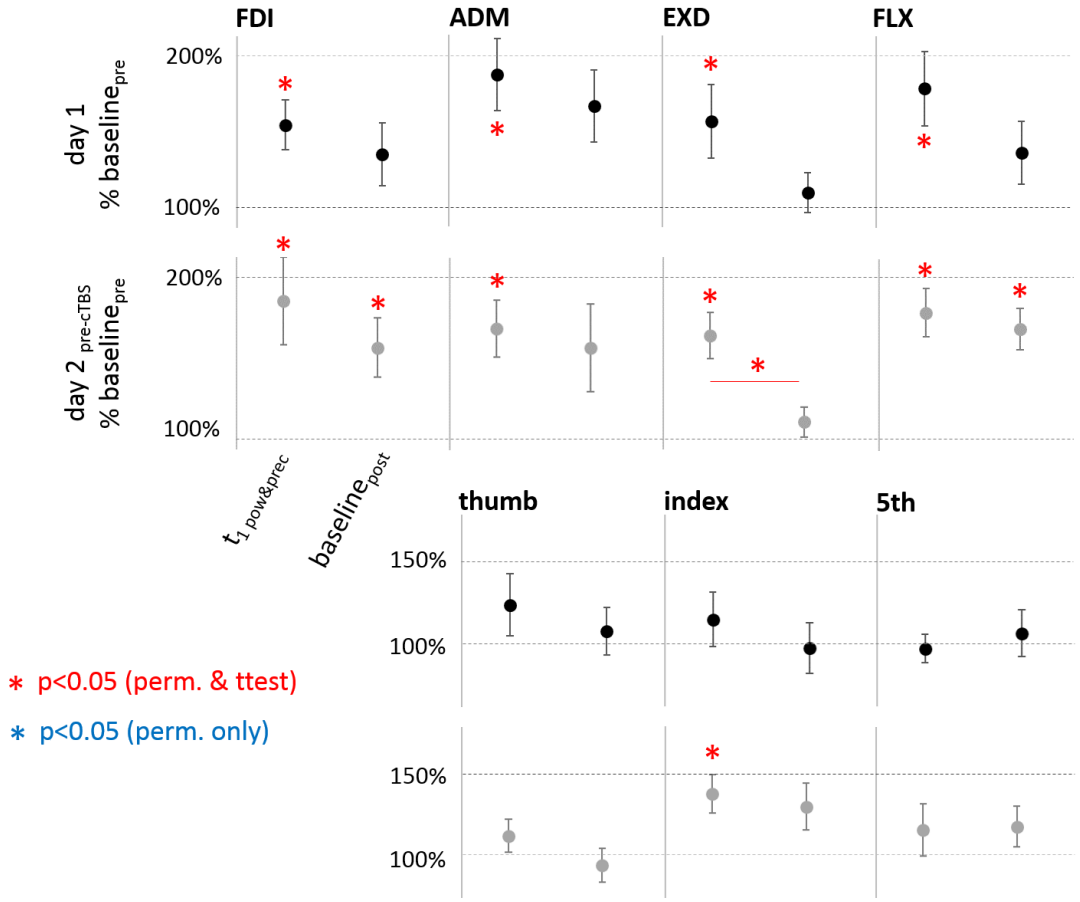


Figure S3.1 : Generic attentional effects on MEPs and MEKs amplitude. Mean and standard error for the four muscles (FDI, ADM, FLX, and EXD) MEPs and three elevation angles MEKs (thumb, index and 5th) are shown as a percentage (%) of the average of $baseline_{pre}$ on the y-axis. The two sessions are stacked vertically for each measure (day1 on top, $day2_{pre\text{-}cTBS}$ on bottom). The $baseline_{pre}$ level is represented by the low horizontal bar (100%). The 2 phases contrasted (timing $_{1pow\&prec}$, $baseline_{post}$) are shown on the x-axis. Significant differences with $baseline_{pre}$ are represented by an asterisk in the top of the value, between the two phases by a horizontal segment surmounted by an asterisk (red if both statistic methods give p<0.05 ; blue if only permutation test gives p<0.05).

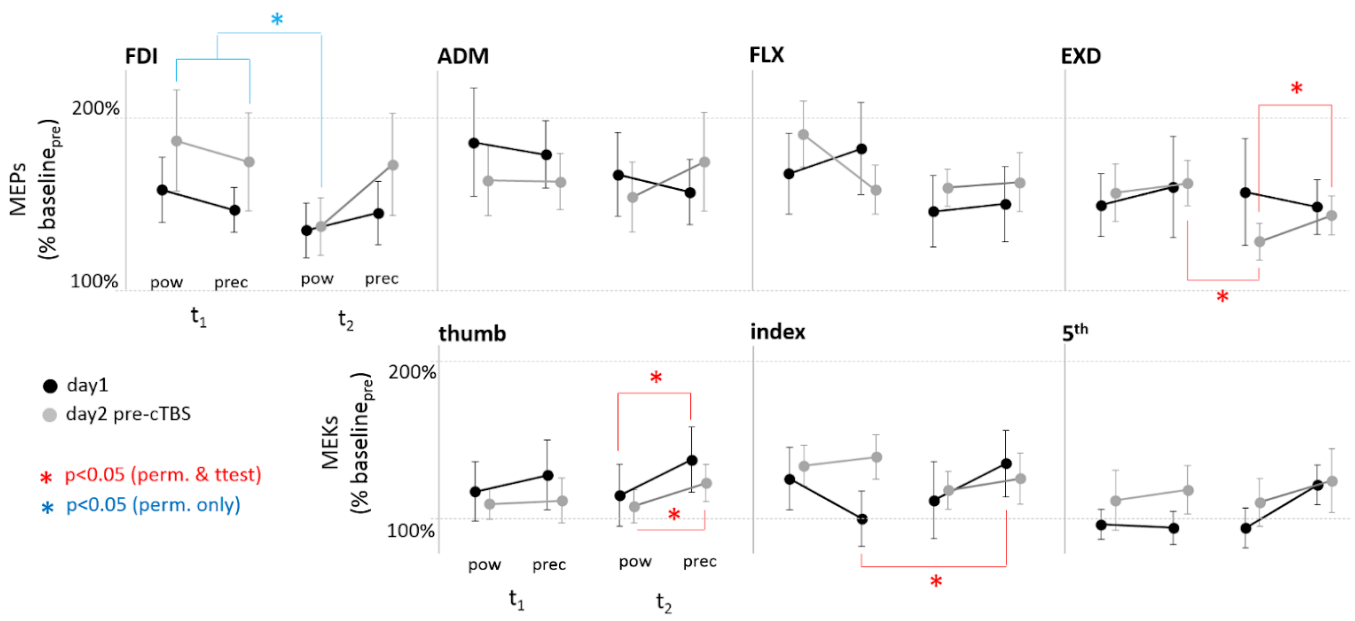


Figure S3.2 : Action observation effects on MEPs and MEKs amplitude. Mean and standard error of the four muscles (FDI, ADM, FLX, and EXD; panel A) and MEKs (thumb, index and 5th; panel B) expressed as a % of the average of baselinepre, separately for session (day1, day2pre-cTBS), timing (t1, t2), and grasp type (prec, pow). Significant differences are represented by an asterisk (red if both statistic methods give p<0.05 ; blue if only permutation test gives p<0.05).

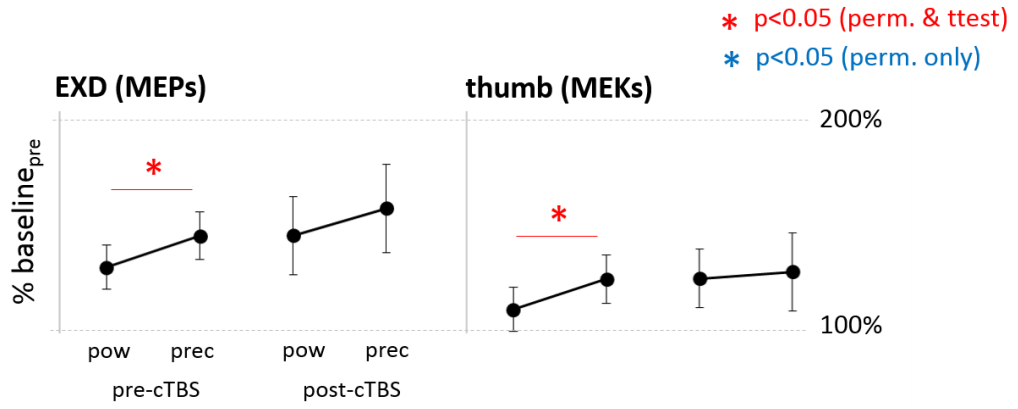


Figure S3.3 : Effects of cTBS on AOEs. Mean and standard error of EXD MEP and thumb MEK, as a function of grasp type (prec, pow) at timing t_2, before (left side) and after (right side) cTBS protocol over M1. All values are expressed as a % of the average of baseline$_{pre}$ for each session. Significant differences are represented by an asterisk (red if both statistic methods give p<0.05 ; blue if only permutation test gives p<0.05).

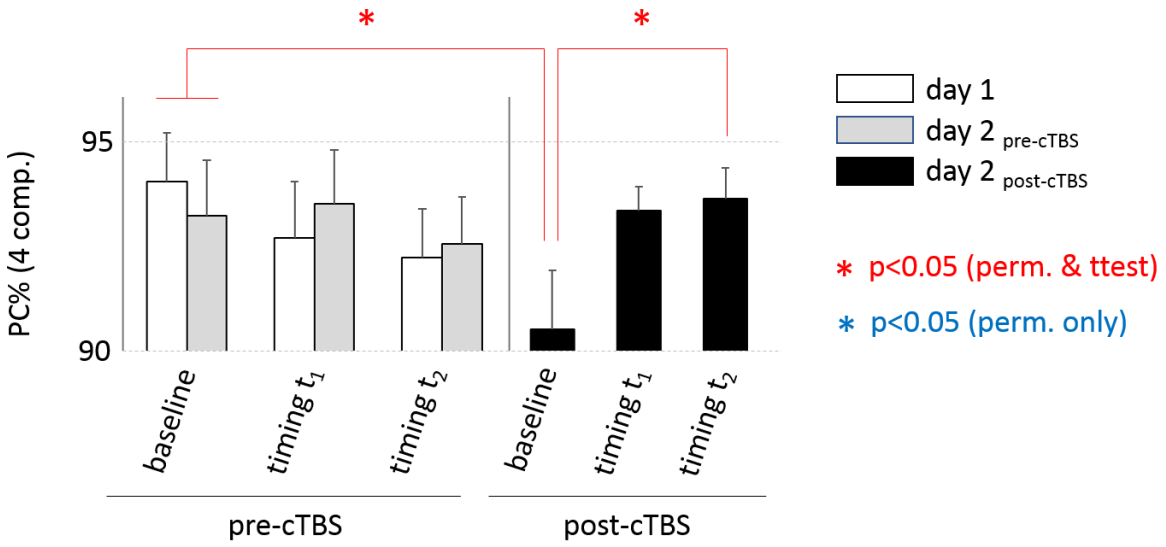


Figure S3.4 : Whole hand configuration changes across sessions and conditions. PC% values of the fourth first components (y-axis), computed on the 8 elevation angles, are shown for baseline and AO trials (baseline, timing 1 and timing 2) for the three sessions (day 1, day 2 pre-cTBS, day 2 post-cTBS). Significant differences are represented by an asterisk (red if both statistic methods give p<0.05 ; blue if only permutation test gives p<0.05).