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Beyond variability: subjective timing and the neurophysiology of motor cognition

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Abstract

Background: In everyday life we frequently use (consciously or unconsciously) mental simulation of movements to predict the outcome of our actions and anticipate possible corrections. In experimental setups, the neuro-behavioral aspects of movement simulation can be studied via a cognitive task called mental rotation: people mentally re-orient rotated pictures of body parts. Despite mental rotation is supposed to activate the motor brain network, the involvement of the primary motor cortex (M1) in mental rotation is largely controversial.

Hypothesis: Such inconsistency could arise from potential methodological flaws in experimental procedures and data analysis. In particular, until now, the timing of M1 activity has been computed in *absolute* terms: from the onset of mental rotation (onset-locked), neglecting intra- and inter-subject variability.

Methods: A novel phase-locked approach is introduced to synchronize the same phases of cognitive processing among different subjects and sessions. This approach was validated in the particular case of corticospinal excitability of the motor cortex during mental rotation.

Results: We identified the *relative* time-windows during which the excitability of M1 is effector-specifically modulated by different features of mental rotation. These time windows correspond to the 55% to 85% of the subjective timing.

Conclusions: In sum, (i) we introduce a new method to study the neurophysiology of motor cognition, and (ii) validating this method, we shed new light on the involvement of M1 in movement simulation.

Keywords

Transcranial magnetic stimulation; phase-locked data analysis; motor-evoked potentials; mental rotation of hands; primary motor cortex; functional equivalence; temporal dynamics.

Introduction

How do you enter and sit down in a car? How do you program the exact movements to make your body fit the size of the car's door and then the shape of the seat? This apparently simple action is, instead, the result of the combination of highly complex processes. First, we compute the spatial difference between the seat and ourselves. Second, we gather information about the current state of our body. Finally, we anticipate the movements required to let our body enter the car and fit in the seat. Such ability to simulate and predict multiple stages of our actions entails motor imagery, i.e. the activation of motor representations in absence of real movements (1).

In experimental setups, motor imagery can be investigated through the so-called mental rotation of hands, in which participants are asked to determine the laterality of rotated images of hands (2). To this aim people imagine moving their own hand towards the image's orientation (3), starting from the current hand's position (4). Thus, mental rotation of hands would recruit sensorimotor simulation mechanisms, which could trigger the activation of the primary motor cortex (M1). One way to probe such involvement of M1 is offered by transcranial magnetic stimulation (TMS). As TMS can perturb the neural activity of a specific brain region (including M1), it is used to assess the consequences of this neural perturbation at the behavioral level, expressed in variations of response times (RTs) for specific tasks. However, the available data are controversial and TMS over M1 either affected (5, 6) or did not influence mental rotation of hands (7, 8). Another way to use TMS is to measure the cortico-spinal excitability of M1 [expressed in variations of motor evoked potentials (MEPs)] at different stages of specific cognitive tasks, i.e. motor imagery (9). However, even with this approach the involvement of M1 in mental rotation of hands is uncertain and MEPs modulations due to TMS over M1 have been associated with mental rotation of hands (e.g. 10) as well as any other object (e.g. 11).

Where do these inconsistencies arise from? One possibility is that previous studies might be biased by a potential methodological flaw which might undermine the reliability of the obtained results: the *onset-locked* approach. This approach means attempting to target M1 with TMS at a fixed time, the same for all participants, calculated from the onset of the target image [e.g. 400ms (6),

650ms (5), or several stimulus-locked time points (7, 10, 11)]. The onset-locked approach risks neglecting the large inter- and intra-subject variability of RTs for mental rotation of hands, varying from about 950ms (6), to 1500ms (12), or even 2200ms (13) as average. Thus, the traditional onset-locked TMS approach risks probing different phases of mental rotation, in different participants, in different conditions, which in turn could be the cause of inconsistent data.

To overcome this issue, here we propose and validate a novel *phase-locked* TMS normalization approach, in which the TMS timing is based on the participant-and-condition specific timing. First, we calculated the baseline RTs for each participant in each condition of mental rotation. Second, we delivered the TMS in different participant-specific phases of mental rotation with respect to the participant's baseline. Third, offline, we estimated the phase of mental rotation in which the TMS pulse occurred expressed as a percentage of the total trial duration. With this approach the TMS pulses are distributed along the whole duration of the task. Thus, at the behavioral level we hypothesized that TMS should not affect participants' performance (RTs). Conversely, as TMS pulses are classified as belonging to normalized phases of mental rotation, at the neurophysiological level we hypothesized the involvement of M1 only in some specific phases of mental rotation (MEPs).

Methods

Participants - Twelve participants, right-handed (14), male (age 23.2 ± 4.3 years) with normal or corrected-to-normal vision, signed a written informed consent prior to the experiment. The procedure was approved by the local Ethics Committee of the University of Verona (Italy), financially supported by the Swiss National Science Foundation, and conducted at the Department of Neurosciences, Biomedicine, and Movement Sciences of University of Verona in accordance with the Declaration of Helsinki 1964.

Setup and Procedure - Participants sat comfortably on a chair, in front of a computer screen, with their hands palm-down on the laps, hidden from view. At the beginning of each trial, after a fixation

cross, one hand image was presented on the screen (visual angle of about 7.5° at 1m distance). The hand images could vary in terms of laterality (left or right), view (palm or dorsum), and orientation (0°, 90°, 180°, 270°), with a total of 16 different images (15). All images were normalized for luminance and automatically presented using the E-Prime2 software (Psychology Software Tools Inc., Pittsburgh USA) (16). Participants were asked to verbally judge, as quickly and accurately as possible, the laterality (left or right) of the displayed hand image. The hand image remained visible until the verbal response was given. RTs were defined as the time from the image onset to the verbal response and were automatically recorded by a microphone. Accuracy was manually recorded by the experimenter.

Neurophysiological measurements - We measured cortico-spinal excitability throughout the experiment by means of MEPs recorded at the level of the hand. To record MEPs, three pairs of disposable bipolar electromyographic electrodes were positioned on the participant's right hand and forearm, in a belly-tendon montage. In particular, these electrodes were positioned on three muscles: (i) First Dorsal Interosseus (FDI) of the index finger; (ii) Abductor Digiti Minimi (ADM) of the little finger; and (iii) Flexor Digitorum Superficialis (FDS) of the forearm. For each participant, we individuated a cortical motor *hotspot*, defined as the position of the TMS coil which elicited the maximal FDI excitation. In addition we identified the minimal motor *threshold* at rest, defined as the minimal TMS output necessary to trigger five MEPs of at least 50μV (in FDI), out of ten trials. TMS was carried out by a STM9000 Magnetic stimulator (ATES-EB Neuro, Italy) using a figure-of-eight coil (diameter: 70mm), producing a maximum output of 2Tesla at the coil surface. MEPs were collected by a Digitimer D360 8-channel amplifier (Digitimer Ltd, Welwyn Garden City, UK) coupled with a CED Power 1401 and a Spike2 acquisition system (Cambridge Electronic Design Ltd, Cambridge, UK) to record and pre-process the data.

TMS protocol – After the identification of the motor threshold, we recorded ten MEPs at rest in each participant (MEPs baseline1). The experiment consisted of six blocks of mental rotation, each composed by 64 trials, with a delay of 6s between two consecutive trials and 5min between two consecutive blocks. In each block the TMS pulse was delivered between 50% and 70% of the participant-and-condition baseline RTs ("image-for-participant" baseline, see Control of Variability). As a control condition, some mental rotation trials did not imply TMS. To exclude any potential influence of time on the baseline rest activity, at the end of the experiment, ten more MEPs at rest were recorded (MEPs baseline2). Then, MEPs baseline1 and MEPs baseline2 were averaged and used in post-processing as a reference for the MEPs recorded during the experimental blocks.

Control for Variability - A training session preceded the experiment and ensured that participants' performance was constant in terms of RTs. To account for intra- and inter-subject variability in RTs (represented in Figure S1), our approach comprised three steps (Figure 1): 1) "baseline" - we computed the average RTs for each image in each participant, without TMS (*image-for-participant* baseline; RTs); 2) "stimulation" - we delivered the TMS pulse at different time points with respect to the image-for-participant baseline; 3) "timing" - in post-processing, we determined when the TMS pulse happened with respect to the beginning of the trial (percentage with respect to the trial duration). First, in the "baseline" step, we recorded two baseline blocks to identify the average RTs profile for each of the 16 images in each participant (the view-by-rotation RTs profile). The computation of these image-for-participant RTs baselines constituted the reference to calculate the timing of the TMS pulses. Second, in the "stimulation" step, we delivered the TMS pulses at specific delays with respect to the image onset. The delays could be delivered at the 50%, 60%, or 70% of the duration of the corresponding image-for-participant RTs baseline. Third, considering intra-subject variability, it is unlikely that a TMS pulse delivered at a given percentage of the corresponding baseline will happen exactly at the same percentage of the current trial's duration. For example, if in participant "X" the image-for-participant baseline RT is 1000ms, the TMS delivered at 50% of the baseline would be at

500ms. However, if in the current trial participant “X” needs 1200ms, the TMS delivered at 500ms will not happen at 50%, but at 41.6% of the current trial. For this reason, in the timing step, we post-hoc calculated when the TMS pulse happened with respect to the percentage of the current trial’s duration (not to the absolute delay between the image onset and the TMS pulse, as in the onset-locked approach).

Please insert Figure 1 about here

As the timing of each TMS pulse was expressed in terms of percentage of the current trial’s duration, this approach allowed us to perform direct comparisons between different participants, regardless of the absolute RTs (as in previous studies). For example, if for participant “Y” the image-for-participant baseline RT is 2000ms, the TMS pulse at 50% would be delivered at 1000ms after image onset (in participant “X” it was 500ms). Then, if in the current trial participant “Y” needs 2400ms, the TMS at 1000ms from the image onset will happen at 41.6% of the current trial (the same percentage as in participant “X”). In this way, two TMS pulses delivered at two different time points (500ms for participant “X” and 1000ms for participant “Y”), in two trials with different duration (1200ms for participant “X” and 2400ms for participant “Y”), will be classified together as belonging to the same percentage bin (41.6% for both participants) a graphical example of this approach is represented in Figure 1. Thus, the timing of TMS pulse was normalized across participants and across images, resulting in a normal distribution of the timing of the TMS pulses with respect to the current trials’ duration (Figure S2). In sum, using this approach it is likely that the TMS was delivered at the same phase of mental rotation in different trials (intra-subject variability) and in different participants (inter-subject variability).

Behavior (RTs analysis) - Trials with RTs shorter than 500ms or longer 3500ms and incorrect trials were excluded from the following analyses (17), with a total loss of 8% of the trials. Typically, mental rotation of hands is faster for rotations towards (medial rotations) than away from (lateral rotations) the midsagittal plane (18). This effect has been taken into account by including the factor direction in the following analyses. In addition, we introduced the factor TMS timing to ensure that the TMS pulse per se did not affect mental rotation. Thus, RTs were analyzed according to a 4-way repeated-measures ANOVA with *TMS timing* (no-TMS, 50%, 60%, 70%), *laterality* (left, right), *view* (palm, dorsum), and *direction* (upright, medial, upside-down, and lateral) as main factors. Post-hoc comparisons were performed with the Tukey test ($p<0.05$).

Cortico-spinal excitability (MEPs analysis) - The variation of MEPs amplitude was calculated with respect to each participant's baseline MEPs (average of MEPs baseline1 and MEPs baseline2). These MEPs were pooled into percentage-bins of the associated trial's duration. The selection of a specific size (%) for the percentage-bins was made on the basis of a trade-off between statistical power and temporal resolution. Statistical power was based on the inclusion of enough data-points (at least 5) in each percentage-bin for each subject. Temporal resolution was obtained by maximizing the number of bins. In this vein, we compared statistical power and temporal resolution of three different percentage-bins: 10%, 15%, and 20% of the trial duration. With nine bins of 10% of the trial duration, the temporal resolution was high but four bins (0%-10%, 10% to 20%, 30-40%, and 40%-50%) presented less than 2 data points for at least one subject. Thus, using 10% bins would mean losing statistical power for half of trial duration (0% to 50%). With six bins of 15% of the trial duration, the temporal resolution was reasonably high and only in two bins (10%-25% and 25%-40%) the minimum number of data points in at least one subject was less than 5. Thus, the use of 15% bins would result in enough statistical power from 40% to 100% of the trial duration. With five bins of 20% of the trial duration, the temporal resolution was low and, as for 15% bins, the minimum number of data points in at least one subject was less than 5 in two bins (0%-20% and 20%-40%). Thus, using

20% bins would provide the same statistical power as with 15% bins (from 40% to 100%), but with a lower temporal resolution. These data are represented in Figure S3. As the 15% size offered the best trade-off between statistical power and temporal resolution, we classified the data in bins of 15% of the trial duration, starting from 100% and going backwards. Thus the following analyses took into account four percentage-bins (40%-55%, 55%-70%, 70%-85%, and 85%-100%). A graphical example of this logic is represented in Figure 1. Despite the time points at which the TMS pulse was delivered were different between two participants (700ms for participant A; 1260ms for participant B), considering that both TMS pulses happened at the 61% of the current trial's duration (1150ms for participant A; 2100ms for participant B), both trials will be classified as belonging to the 55%-70% percentage bin, separated for each participant (Figure 1). In this framework, for each muscle, MEPs were analyzed by means of paired t-tests corrected for multiple comparisons (False Discovery Rate, FDR; $p<0.05$). Separated for each muscle (FDI, AMD, FDS), by mean of these t-tests we directly compared the MEPs amplitude associated with mental rotation of hand images, as a function of three aspects: 1) *awkwardness* (19) - images representing postures assumable via anatomically easy (upright and medial rotations) versus difficult movements (upside-down and lateral rotations); 2) *view* (3) - images shown from the palm versus the dorsum view; 3) *side* (20) - left-lateralized versus right-lateralized images. All statistical analyses were carried out with the R software (21).

Results

Behavior (RTs) - The 4-way ANOVA on RTs showed that TMS did not affect mental rotation, as the factor TMS was not significant either as a main effect ($p=0.68$) or in interaction with any other factor (all $p>0.8$). The other significant main effects and interactions of laterality, view, and rotation generally confirmed previous findings about mental rotation of hands, and are reported as Supplemental Material.

Cortico-spinal excitability (MEPs) – Statistical analysis of MEPs amplitude as a function of

awkwardness is reported in Figure 2. MEPs variations in FDI showed that the difference between easy versus difficult rotations was significant in the 55%-70% percentage bin [$T(11)=3.21$; $p_{FDR}<0.05$], with larger MEPs during the difficult (104% of the MEP baseline; SD=23%) with respect to the easy rotations (96% of the MEP baseline; SD=24%). Such a modulation of MEPs amplitude was specific for the 55%-70% bin, as it was not significant in any of the other percentage bins (all $p_{FDR}>0.05$). Similarly, also MEPs variations in ADM showed the significant difference between easy and difficult rotation in the 55%-70% percentage bin ($T(11)=3.65$; $p_{FDR}<0.05$), with larger MEPs during difficult rotations (118% of the MEP baseline; SD=25%) with respect to easy rotations (105% of the MEP baseline; SD=21%). As for FDI, also in ADM such difference was specific for the 55%-70% percentage bin, as it did not reach statistical significance in any of the other percentage bins (all $p_{FDR}>0.05$). In addition, the influence of awkwardness of mental rotation on MEPs amplitude was specific for FDI and ADM, as the difference between the MEPs for easy and difficult rotations was not significant in any percentage bin of the FDS (all $p_{FDR}>0.05$). In regards to *view*, only for the FDS the 70%-85% percentage bin the MEPs variations were significantly different between dorsum-view and palm-view images ($T(11)=3.53$; $p_{FDR}<0.05$), with a larger MEPs for palm-view (108% of the MEP baseline; SD=38%) with respect to dorsum-view images (102% of the MEP baseline; SD=39%). Such a difference was specific for the 70%-85% percentage bin for the FDS, as it was not significant in any other percentage bin of the FDS, nor in any percentage bin of either FDI or AMD (all $p_{FDR}>0.05$) (Figure S4). Finally, the side of images did not seem to influence MEPs amplitude as the difference between left- and right-lateralized images was not significant in any percentage bin of any muscle (all $p_{FDR}>0.05$).

Please insert Figure 2 about here

Discussion

Everyday we effortlessly predict, or imagine, the consequences of our actions. This ability lets us think outside the borders of our perceptual reality and to weigh alternatives against one another. To study the neural counterpart of this ability, here (i) we introduce a new phase-locked approach to analyze TMS data, then (ii) we validate this approach by neurophysiologically identifying the phase- and effector-specific involvement of M1 in mental rotation of hands. Previous attempts produced inconsistent data including, for instance, increased (6) or unaffected (5, 7) MEPs associated with mental rotation at 400ms after the image onset. Such inconsistencies might derive from the lack of consideration of within- and between-participants variability. To solve this issue, the phase-locked approach computes the TMS timing as a function of the participant-specific phase of mental rotation. Thus, we identified the specific time-windows (expressed in terms of percentage of trial duration) during which corticospinal excitability was modulated by particular features of mental rotation: between 55% and 70% of the duration of mental rotation, the M1-FDI and M1-ADM corticospinal excitability was greater during anatomically difficult than easy mental rotations. We interpret this MEPs modulation as a sign that the involvement of M1 in mental rotation of hands is specific at two levels: (i) *Timing* – M1 is active only at specific time points with respect to the subjective performance (55%-70% of the duration); (ii) *Functional Equivalence* - M1 is muscle-specifically (FDI and ADM, but not FDS) more active for more awkward mental rotations.

Timing

Using a phase-locked approach and a within-subject design, in the present study we excluded the risks of uncontrolled biases related to intra- and inter-subject variability. In this way we demonstrated that M1 is differentially involved in different phases of mental rotation of hands, i.e. the timing of the activation reported here is consistent with the current models of the sequential phases of mental rotation of hands. On the one hand, during the first portion of the total RT the image has to be visually

interpreted before the motor imagery phase takes place (12, 22). On the other hand, the last portion of the total RT is used to translate the response into a physical action (e.g. button press), lasting about 80-120ms (23), grossly corresponding to the last percentage bin in the present study (85%-100%). Furthermore, based on the difference of the mean RTs between their fastest and slowest orientations, Sauner et al. (7) suggested that the motor rotation phase of the mental rotation of hands should last a minimum of 150ms. Applying the same principle to our data, the motor rotation phase would be lasting at least 30% of our normalized trial, on average. Therefore our 15% bin-size is expected to allow the identification of any potential effect of interest.

Altogether, the MEPs modulations we found between 55% and 85% of the baseline suggest that the timing of the activation of M1 during mental rotation varies across different subjects but corresponds to the (subjective) intermediate phase of mental rotation.

Two previous studies (7, 10) aimed at unraveling whether and when M1 is involved in mental rotation of hands and reported inconsistent findings, potentially due to methodological flows (beyond the intrinsic limitations of the onset-locked approach). In particular, while recording MEPs, Sauner et al. (7) delivered the TMS pulses over M1 at several onset-locked time-points, but did not find any variation of either RTs or MEPs. This null result could be due to the relatively small sample size with respect to the complex multi-factorial analysis. Indeed, RTs for left hand rotations around the upside-down direction showed a clear increase for TMS at 400ms after the image onset, with respect to 0ms. However, this effect went unnoticed and unreported. Conversely, Hyde et al. (10) delivered the TMS pulse at similar onset-locked time points of mental rotation (50ms, 400ms, and 650ms) and reported that MEPs were modulated by (i) the cognitive strategy (recruiting or not on motor simulation) for mental rotation and (ii) the anatomical awkwardness of mental rotation. Again, it is worth noting that the study was performed according to a between-subject design (different people were classified post-hoc as good or bad performers in mental rotation), and the two groups were not balanced (good: N=16; bad: N=8). These methodological issues undermine the reliability of data-driven conclusions on the involvement of M1 in mental rotation.

Functional Equivalence

With respect to anatomically-easy hand movements, anatomically-difficult hand movements are more demanding for specific muscles (e.g. FDI and ADM) but not others (e.g. FDS). Similarly, we found that anatomically difficult mental rotations were associated with greater excitability of the M1-FDI and M1-ADM (not M1- FDS) corticospinal excitability. In addition, taking into consideration the image view, our data support a consistency between corticospinal excitability during mental rotation and muscular activity during movement execution. Accordingly, mental rotation of palm-view images resulted in increased MEPs amplitude in FDS compared to dorsum-view, within the 70%-85% percentage bin of trial duration. The activation of FDS during physical wrist supination has been previously demonstrated (24). In the present study we extend this evidence, proposing that the larger M1-FDS corticospinal excitability during mental rotation of palm-view images might result from the fact that participants used their hand configuration (palm-down) as a reference frame and imagined the supination of the wrist. This interpretation is in line with previous work showing the correspondence between physical and mental constraints (25). In addition, in the present study we also identified the timing of this activation. In particular, the view-related MEPs modulation between 70% and 85% of the trial duration places this phase after the activation we found for processing awkwardness (55%-70% of the trial duration). This suggests a sequential organization of imagined movements, starting with the activation of hand muscles and continuing with the activation of the corticospinal pathway devoted to wrist supination.

From onset- to phase-locked and back

The phase-locked approach solves any potential bias associated with variability which might dramatically affect past and future scientific research on the timing of cortical activity associated with cognitive tasks. We identified a characteristic increase of M1 excitability within a specific time-window during mental rotation of hands. This time-window occurred relatively late, as the main

MEPs modulations happened between 55% and 85% of the trial duration.

In sum, here we propose a new method for TMS studies and validate it by positioning the involvement of M1 in the late phases of mental rotation. This is in line with previous studies which exploited different neuroinvestigation techniques [TMS (5, 26), magnetoencephalography (27), and electroencephalography (22)] and consistently reported that motor simulation starts only after visual inspection and a “guess" implicit perceptual analysis (12).

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S. Ionta developed the study concept. All authors contributed to the study design. Testing, data collection, and data analysis were performed by D. Perruchoud, with input from the other authors. S. Ionta and D. Perruchoud drafted the manuscript. M. Fiorio and P. Cesari provided critical revisions. All authors approved the final version of the manuscript for submission.

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FIGURES

Figure 1

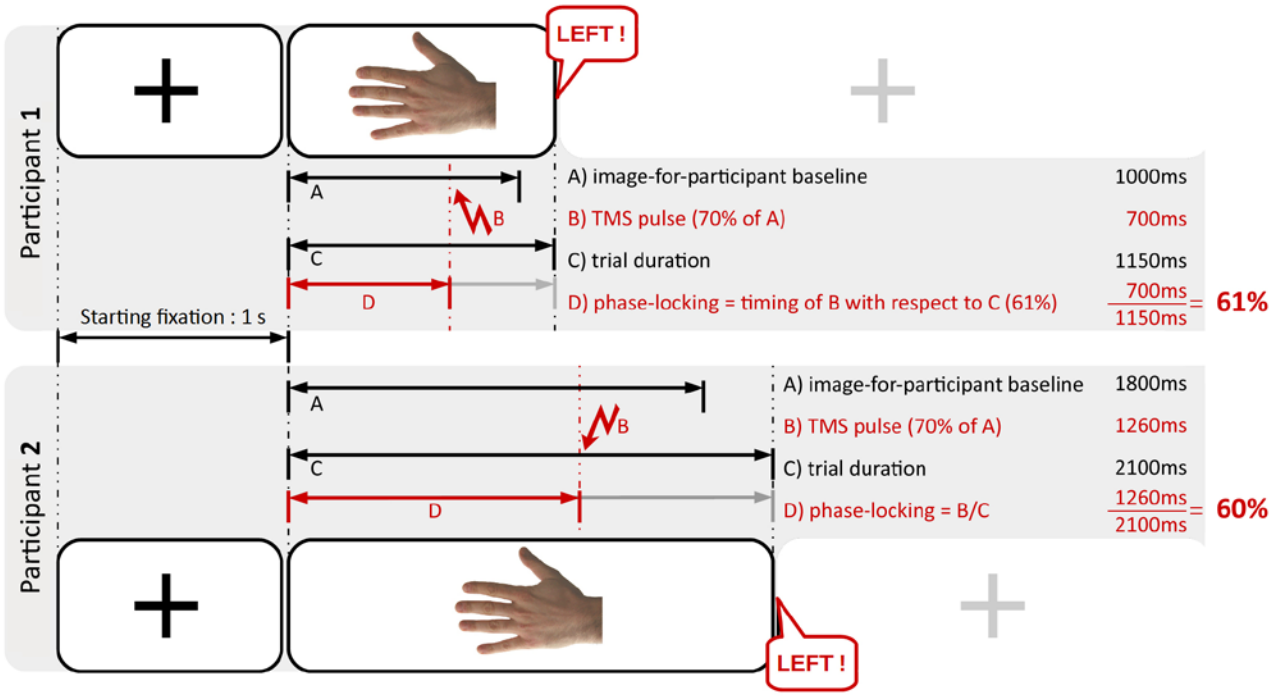

Figure 1 : Phase-locked approach to control variability - In participant 1 (upper panel) the trial duration is 1150ms and the TMS pulse delivered at 70% of the image-for-participant baseline (1000ms) occurred 700ms after the onset of the image. According to the phase-locked approach, this corresponds to the 61% of the trial duration, and this trial will be pooled in the bin of trials in which the TMS occurred between 55%-70% of the trial duration. Applying the same phase-locked logic to participant 2, despite different values, the trial will be classified in the same bin (55%-70%), because the TMS pulse occurred at the same phase (60%) of the trial duration.

Figure 2

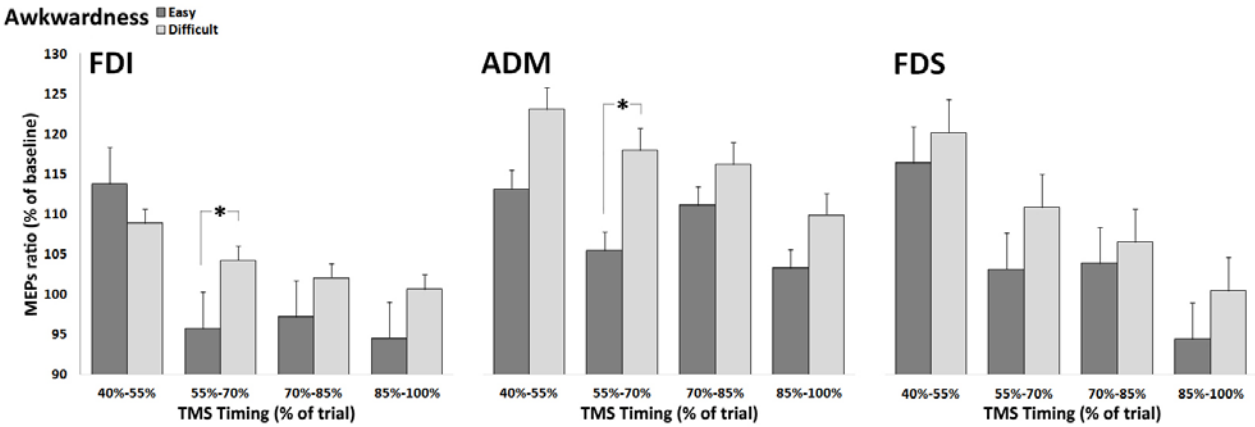

Figure 2 : Cortico-spinal excitability and Awkwardness - Variations of MEP amplitudes (with respect to baseline) for each percentage bin at which the TMS pulse was delivered. When the TMS pulse was delivered between 55% and 70% of the trial duration, MEPs from FDI and ADM (not FDS) were larger during anatomically difficult than easy mental rotations (* = pFDR<0.05). Error bars represent standard error.