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1 **Beyond variability: subjective timing and the neurophysiology of motor cognition**

2

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25 **Abstract**

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

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

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

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

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

45

46

47 **Keywords**

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

50

51 **Introduction**

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

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

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

77 650ms (5), or several stimulus-locked time points (7, 10, 11)]. The onset-locked approach risks
78 neglecting the large inter- and intra-subject variability of RTs for mental rotation of hands, varying
79 from about 950ms (6), to 1500ms (12), or even 2200ms (13) as average. Thus, the traditional onset-
80 locked TMS approach risks probing different phases of mental rotation, in different participants, in
81 different conditions, which in turn could be the cause of inconsistent data.

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

92

93 **Methods**

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

100

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

103 cross, one hand image was presented on the screen (visual angle of about 7.5° at 1m distance). The
104 hand images could vary in terms of laterality (left or right), view (palm or dorsum), and orientation
105 (0°, 90°, 180°, 270°), with a total of 16 different images (15). All images were normalized for
106 luminance and automatically presented using the E-Prime2 software (Psychology Software Tools
107 Inc., Pittsburgh USA) (16). Participants were asked to verbally judge, as quickly and accurately as
108 possible, the laterality (left or right) of the displayed hand image. The hand image remained visible
109 until the verbal response was given. RTs were defined as the time from the image onset to the verbal
110 response and were automatically recorded by a microphone. Accuracy was manually recorded by the
111 experimenter.

112

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

127

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

137
138 *Control for Variability* - A training session preceded the experiment and ensured that participants’
139 performance was constant in terms of RTs. To account for intra- and inter-subject variability in RTs
140 (represented in Figure S1), our approach comprised three steps (Figure 1): 1) “baseline” - we
141 computed the average RTs for each image in each participant, without TMS (*image-for-participant*
142 *baseline*; RTs); 2) “stimulation” - we delivered the TMS pulse at different time points with respect to
143 the image-for-participant baseline; 3) “timing” - in post-processing, we determined when the TMS
144 pulse happened with respect to the beginning of the trial (percentage with respect to the trial duration).
145 First, in the “baseline” step, we recorded two baseline blocks to identify the average RTs profile for
146 each of the 16 images in each participant (the view-by-rotation RTs profile). The computation of these
147 image-for-participant RTs baselines constituted the reference to calculate the timing of the TMS
148 pulses. Second, in the “stimulation” step, we delivered the TMS pulses at specific delays with respect
149 to the image onset. The delays could be delivered at the 50%, 60%, or 70% of the duration of the
150 corresponding image-for-participant RTs baseline. Third, considering intra-subject variability, it is
151 unlikely that a TMS pulse delivered at a given percentage of the corresponding baseline will happen
152 exactly at the same percentage of the current trial’s duration. For example, if in participant “X” the
153 image-for-participant baseline RT is 1000ms, the TMS delivered at 50% of the baseline would be at

154 500ms. However, if in the current trial participant “X” needs 1200ms, the TMS delivered at 500ms
155 will not happen at 50%, but at 41.6% of the current trial. For this reason, in the timing step, we post-
156 hoc calculated when the TMS pulse happened with respect to the percentage of the current trial’s
157 duration (not to the absolute delay between the image onset and the TMS pulse, as in the onset-locked
158 approach).

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164 As the timing of each TMS pulse was expressed in terms of percentage of the current trial’s
165 duration, this approach allowed us to perform direct comparisons between different participants,
166 regardless of the absolute RTs (as in previous studies). For example, if for participant “Y” the image-
167 for-participant baseline RT is 2000ms, the TMS pulse at 50% would be delivered at 1000ms after
168 image onset (in participant “X” it was 500ms). Then, if in the current trial participant “Y” needs
169 2400ms, the TMS at 1000ms from the image onset will happen at 41.6% of the current trial (the same
170 percentage as in participant “X”). In this way, two TMS pulses delivered at two different time points
171 (500ms for participant “X” and 1000ms for participant “Y”), in two trials with different duration
172 (1200ms for participant “X” and 2400ms for participant “Y”), will be classified together as belonging
173 to the same percentage bin (41.6% for both participants) a graphical example of this approach is
174 represented in Figure 1. Thus, the timing of TMS pulse was normalized across participants and across
175 images, resulting in a normal distribution of the timing of the TMS pulses with respect to the current
176 trials’ duration (Figure S2). In sum, using this approach it is likely that the TMS was delivered at the
177 same phase of mental rotation in different trials (intra-subject variability) and in different participants
178 (inter-subject variability).

179

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

189
190 *Cortico-spinal excitability (MEPs analysis)* - The variation of MEPs amplitude was calculated
191 with respect to each participant's baseline MEPs (average of MEPs baseline1 and MEPs baseline2).
192 These MEPs were pooled into percentage-bins of the associated trial's duration. The selection of a
193 specific size (%) for the percentage-bins was made on the basis of a trade-off between statistical
194 power and temporal resolution. Statistical power was based on the inclusion of enough data-points
195 (at least 5) in each percentage-bin for each subject. Temporal resolution was obtained by maximizing
196 the number of bins. In this vein, we compared statistical power and temporal resolution of three
197 different percentage-bins: 10%, 15%, and 20% of the trial duration. With nine bins of 10% of the trial
198 duration, the temporal resolution was high but four bins (0%-10%, 10% to 20%, 30-40%, and 40%-
199 50%) presented less than 2 data points for at least one subject. Thus, using 10% bins would mean
200 losing statistical power for half of trial duration (0% to 50%). With six bins of 15% of the trial
201 duration, the temporal resolution was reasonably high and only in two bins (10%-25% and 25%-40%)
202 the minimum number of data points in at least one subject was less than 5. Thus, the use of 15% bins
203 would result in enough statistical power from 40% to 100% of the trial duration. With five bins of
204 20% of the trial duration, the temporal resolution was low and, as for 15% bins, the minimum number
205 of data points in at least one subject was less than 5 in two bins (0%-20% and 20%-40%). Thus, using

206 20% bins would provide the same statistical power as with 15% bins (from 40% to 100%), but with
207 a lower temporal resolution. These data are represented in Figure S3. As the 15% size offered the best
208 trade-off between statistical power and temporal resolution, we classified the data in bins of 15% of
209 the trial duration, starting from 100% and going backwards. Thus the following analyses took into
210 account four percentage-bins (40%-55%, 55%-70%, 70%-85%, and 85%-100%). A graphical
211 example of this logic is represented in Figure 1. Despite the time points at which the TMS pulse was
212 delivered were different between two participants (700ms for participant A; 1260ms for participant
213 B), considering that both TMS pulses happened at the 61% of the current trial's duration (1150ms for
214 participant A; 2100ms for participant B), both trials will be classified as belonging to the 55%-70%
215 percentage bin, separated for each participant (Figure 1). In this framework, for each muscle, MEPs
216 were analyzed by means of paired t-tests corrected for multiple comparisons (False Discovery Rate,
217 FDR; $p < 0.05$). Separated for each muscle (FDI, AMD, FDS), by mean of these t-tests we directly
218 compared the MEPs amplitude associated with mental rotation of hand images, as a function of three
219 aspects: 1) *awkwardness* (19) - images representing postures assumable via anatomically easy
220 (upright and medial rotations) versus difficult movements (upside-down and lateral rotations); 2) *view*
221 (3) - images shown from the palm versus the dorsum view; 3) *side* (20) - left-lateralized versus right-
222 lateralized images. All statistical analyses were carried out with the R software (21).

223

224 **Results**

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

230

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

232 *awkwardness* is reported in Figure 2. MEPs variations in FDI showed that the difference between
233 easy versus difficult rotations was significant in the 55%-70% percentage bin [$T(11)=3.21$; $p_{FDR}<0.05$],
234 with larger MEPs during the difficult (104% of the MEP baseline; $SD=23\%$) with respect to the easy
235 rotations (96% of the MEP baseline; $SD=24\%$). Such a modulation of MEPs amplitude was specific
236 for the 55%-70% bin, as it was not significant in any of the other percentage bins (all $p_{FDR}>0.05$).
237 Similarly, also MEPs variations in ADM showed the significant difference between easy and difficult
238 rotation in the 55%-70% percentage bin ($T(11)=3.65$; $p_{FDR}<0.05$), with larger MEPs during difficult
239 rotations (118% of the MEP baseline; $SD=25\%$) with respect to easy rotations (105% of the MEP
240 baseline; $SD=21\%$). As for FDI, also in ADM such difference was specific for the 55%-70%
241 percentage bin, as it did not reach statistical significance in any of the other percentage bins (all
242 $p_{FDR}>0.05$). In addition, the influence of awkwardness of mental rotation on MEPs amplitude was
243 specific for FDI and ADM, as the difference between the MEPs for easy and difficult rotations was
244 not significant in any percentage bin of the FDS (all $p_{FDR}>0.05$). In regards to *view*, only for the FDS
245 the 70%-85% percentage bin the MEPs variations were significantly different between dorsum-view
246 and palm-view images ($T(11)=3.53$; $p_{FDR}<0.05$), with a larger MEPs for palm-view (108% of the MEP
247 baseline; $SD=38\%$) with respect to dorsum-view images (102% of the MEP baseline; $SD=39\%$). Such
248 a difference was specific for the 70%-85% percentage bin for the FDS, as it was not significant in
249 any other percentage bin of the FDS, nor in any percentage bin of either FDI or AMD (all $p_{FDR}>0.05$)
250 (Figure S4). Finally, the side of images did not seem to influence MEPs amplitude as the difference
251 between left- and right-lateralized images was not significant in any percentage bin of any muscle
252 (all $p_{FDR}>0.05$).

253

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255

256

Please insert Figure 2 about here

257

258 **Discussion**

259 Everyday we effortlessly predict, or imagine, the consequences of our actions. This ability lets
260 us think outside the borders of our perceptual reality and to weigh alternatives against one another.
261 To study the neural counterpart of this ability, here (i) we introduce a new phase-locked approach to
262 analyze TMS data, then (ii) we validate this approach by neurophysiologically identifying the phase-
263 and effector-specific involvement of M1 in mental rotation of hands. Previous attempts produced
264 inconsistent data including, for instance, increased (6) or unaffected (5, 7) MEPs associated with
265 mental rotation at 400ms after the image onset. Such inconsistencies might derive from the lack of
266 consideration of within- and between-participants variability. To solve this issue, the phase-locked
267 approach computes the TMS timing as a function of the participant-specific phase of mental rotation.
268 Thus, we identified the specific time-windows (expressed in terms of percentage of trial duration)
269 during which corticospinal excitability was modulated by particular features of mental rotation:
270 between 55% and 70% of the duration of mental rotation, the M1-FDI and M1-ADM corticospinal
271 excitability was greater during anatomically difficult than easy mental rotations. We interpret this
272 MEPs modulation as a sign that the involvement of M1 in mental rotation of hands is specific at two
273 levels: (i) *Timing* – M1 is active only at specific time points with respect to the subjective performance
274 (55%-70% of the duration); (ii) *Functional Equivalence* - M1 is muscle-specifically (FDI and ADM,
275 but not FDS) more active for more awkward mental rotations.

276

277 *Timing*

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

283 interpreted before the motor imagery phase takes place (12, 22). On the other hand, the last portion
284 of the total RT is used to translate the response into a physical action (e.g. button press), lasting about
285 80-120ms (23), grossly corresponding to the last percentage bin in the present study (85%-100%).
286 Furthermore, based on the difference of the mean RTs between their fastest and slowest orientations,
287 Sauner et al. (7) suggested that the motor rotation phase of the mental rotation of hands should last a
288 minimum of 150ms. Applying the same principle to our data, the motor rotation phase would be
289 lasting at least 30% of our normalized trial, on average. Therefore our 15% bin-size is expected to
290 allow the identification of any potential effect of interest.

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

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

309

310 *Functional Equivalence*

311 With respect to anatomically-easy hand movements, anatomically-difficult hand movements are
312 more demanding for specific muscles (e.g. FDI and ADM) but not others (e.g. FDS). Similarly, we
313 found that anatomically difficult mental rotations were associated with greater excitability of the M1-
314 FDI and M1-ADM (not M1- FDS) corticospinal excitability. In addition, taking into consideration
315 the image view, our data support a consistency between corticospinal excitability during mental
316 rotation and muscular activity during movement execution. Accordingly, mental rotation of palm-
317 view images resulted in increased MEPs amplitude in FDS compared to dorsum-view, within the
318 70%-85% percentage bin of trial duration. The activation of FDS during physical wrist supination
319 has been previously demonstrated (24). In the present study we extend this evidence, proposing that
320 the larger M1-FDS corticospinal excitability during mental rotation of palm-view images might result
321 from the fact that participants used their hand configuration (palm-down) as a reference frame and
322 imagined the supination of the wrist. This interpretation is in line with previous work showing the
323 correspondence between physical and mental constraints (25). In addition, in the present study we
324 also identified the timing of this activation. In particular, the view-related MEPs modulation between
325 70% and 85% of the trial duration places this phase after the activation we found for processing
326 awkwardness (55%-70% of the trial duration). This suggests a sequential organization of imagined
327 movements, starting with the activation of hand muscles and continuing with the activation of the
328 corticospinal pathway devoted to wrist supination.

329

330 *From onset- to phase-locked and back*

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

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

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

341

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343 S. Ionta developed the study concept. All authors contributed to the study design. Testing, data
344 collection, and data analysis were performed by D. Perruchoud, with input from the other authors. S.
345 Ionta and D. Perruchoud drafted the manuscript. M. Fiorio and P. Cesari provided critical revisions.
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347

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352

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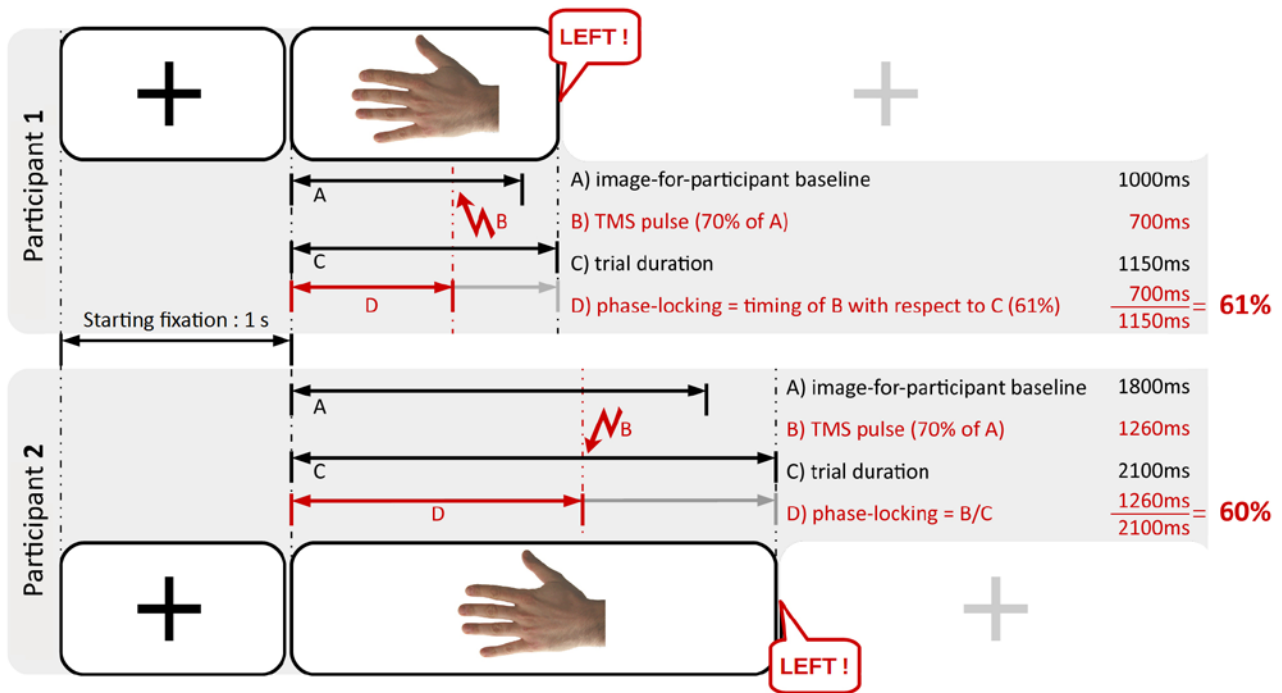
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417 FIGURES

418

419 Figure 1

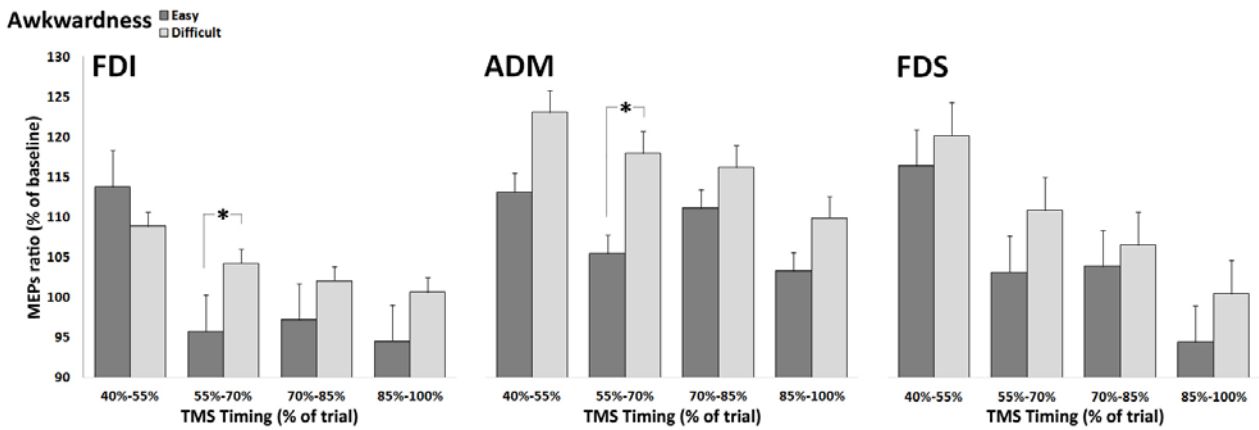


420

421 **Figure 1 : Phase-locked approach to control variability** - In participant 1 (upper panel) the trial
 422 duration is 1150ms and the TMS pulse delivered at 70% of the image-for-participant baseline
 423 (1000ms) occurred 700ms after the onset of the image. According to the phase-locked approach, this
 424 corresponds to the 61% of the trial duration, and this trial will be pooled in the bin of trials in which
 425 the TMS occurred between 55%-70% of the trial duration. Applying the same phase-locked logic to
 426 participant 2, despite different values, the trial will be classified in the same bin (55%-70%), because
 427 the TMS pulse occurred at the same phase (60%) of the trial duration.

428

429 **Figure 2**



430

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

436