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1 **The recruitment of indirect-waves within primary motor cortex during**
2 **motor imagery: A directional Transcranial Magnetic Stimulation study**

3
4 **Running title:** Using directional TMS during motor imagery

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32 **Abstract**

33 Motor imagery (MI) refers to the mental simulation of an action without overt movement.
34 While numerous transcranial magnetic stimulation (TMS) studies provided evidence for a
35 modulation of corticospinal excitability and intracortical inhibition during MI, the neural
36 signature within the primary motor cortex is not clearly established. In the current study, we
37 used directional TMS to probe the modulation of the excitability of early and late indirect-
38 waves (I-waves) generating pathways during MI. Corticospinal responses evoked by TMS
39 with posterior-anterior (PA) and anterior-posterior (AP) current flow within the primary
40 motor cortex evoke preferentially early and late I-waves, respectively. Seventeen participants
41 were instructed to stay at rest or to imagine maximal isometric contractions of the right flexor
42 carpi radialis. We demonstrated that the increase of corticospinal excitability during MI is
43 greater with PA than AP orientation. By using paired-pulse stimulations, we confirmed that
44 short-interval intracortical inhibition (SICI) increased during MI in comparison to rest with
45 PA orientation, whereas we found that it decreased with AP orientation. Overall, these results
46 indicate that the pathways recruited by PA and AP orientations that generate early- and late I-
47 waves are differentially modulated by MI.

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51 **Key words:** Primary motor cortex, adaptive threshold-hunting, motor-evoked potentials, short
52 interval intracortical inhibition, corticospinal excitability.

53

54 **Introduction**

55 Motor imagery (MI) is a cognitive process that refers to the mental simulation of an action
56 without overt movement (Jeannerod & Decety, 1995). MI is known to activate brain regions
57 also involved during motor execution but is accompanied by a voluntary inhibition of the
58 actual movement (Decety, 1996). Using vascular space occupancy method combined with
59 high-resolution (7T) functional magnetic resonance imaging, Persichetti et al. (2020)
60 supported the idea that MI activated only the superficial layers II/III of the primary motor
61 cortex (M1) with cortico-cortical connections from somatosensory and premotor areas (Huber
62 *et al.*, 2017). In contrast, actual finger movements activated both superficial layers and the
63 deeper layers Vb/VI in M1 with descending excitatory corticospinal projections. These results
64 would nicely explain the absence of muscle activity during MI. However, this exclusive
65 activation of superficial layers within M1 during MI is at odds with numerous observations in
66 the current literature. Indeed, past studies using different methodological approaches found
67 neural modulations downstream of the pyramidal cells while imagining (Li *et al.*, 2004;
68 Grosprêtre *et al.*, 2015). For example, by using a combination of different techniques during
69 MI, Grosprêtre et al. (2015) provided evidence for a subliminal motor output that traveled
70 along the corticospinal tract and reached the spinal level but did not activate alpha-
71 motoneurons. These modulations following motor imagery practice, besides the changes
72 within M1, would also explain the improvement in motor learning (Ruffino *et al.*, 2017).

73 Transcranial magnetic stimulation studies (TMS) provided evidence of the activation of the
74 corticospinal pathway during MI, compared to rest (Yahagi & Kasai, 1999; Grosprêtre *et al.*,
75 2016). This activation is classically marked by an increase in the amplitude of the motor
76 evoked potentials (MEPs) evoked by single-pulse TMS and recorded in the specific muscle
77 involved in the imagined movement (Yahagi & Kasai, 1999; Lebon *et al.*, 2012; Grosprêtre *et*
78 *al.*, 2016; Neige *et al.*, 2020, 2021). According to Di Lazzaro and Ziemann (2013), the axons
79 of the more superficial pyramidal neurons (P2/P3) are conceivably the most excitable neural
80 elements to low-threshold TMS, due to their location close to the stimulating coil. Moreover,
81 these axons also represent the primary source of excitatory descending input to pyramidal
82 tract neurons of layer V (Anderson *et al.*, 2010). Therefore, if TMS activates axons of
83 superficial layer cells preferentially and MI induces an increase in TMS-evoked responses, it
84 is most likely that superficial pyramidal neurons activated during MI directly excite deeper
85 layers, contradicting the findings by Persichetti et al. (2020).

86 Previous studies that used TMS during MI rely on interpreting the MEPs amplitude evoked by
87 the posterior-anterior (PA) current direction. However, MEPs amplitude is a complex and
88 global readout that is thought to reflect the summation of several monosynaptic and
89 polysynaptic descending inputs, termed D- (direct) and I-(indirect) waves, as evidenced from
90 spinal epidural recordings (Di Lazzaro *et al.*, 2012). The first descending volley is thought to
91 originate from direct activation (D-wave) of corticospinal tract axons, whereas the latter I-
92 waves are thought to derive from indirect, trans-synaptic activation of the corticospinal
93 neurons (Di Lazzaro & Rothwell, 2014; Ziemann, 2020). These I-waves usually appear at ~
94 1.2–1.5 ms intervals are numbered in order of their appearance, and are referred to as either
95 early (I1) or late (I2, I3, I4) I-waves (Di Lazzaro *et al.*, 2012; Ziemann, 2020). The directional
96 TMS technique is a non-invasively and valuable approach used to activate distinct sets of
97 synaptic inputs to corticospinal neurons responsible for the early and late I-waves pathway. It
98 has been proposed that TMS-induced electric currents flowing from LM (latero-medial), PA
99 and AP (anterior to posterior) directions activate different sets of excitatory synaptic inputs
100 that arrive at the pyramidal tract neurons several milliseconds apart (Di Lazzaro & Rothwell,
101 2014; Di Lazzaro *et al.*, 2017). LM stimulation at high intensity can directly activate the
102 corticospinal axons of pyramidal tract neurons, evoking a short-latency D-wave (Di Lazzaro
103 & Rothwell, 2014). PA stimulation preferentially elicits primarily early I-wave, which is
104 thought to originate from excitatory inputs to the basal dendrites of the corticospinal neurons
105 in layer V of M1 (Di Lazzaro & Ziemann, 2013; Hannah, 2020). AP stimulation preferentially
106 elicits later and more dispersed I-waves which are thought to result from mono- and poly-
107 synaptic inputs from layers II/III of M1 (Ziemann, 2020), as well as the activation of
108 horizontal cortico-cortical connections from surrounding brain regions to M1 (Di Lazzaro *et*
109 *al.*, 2017; Hannah, Cavanagh, *et al.*, 2018). Therefore, comparing MEPs amplitude recorded
110 in hand muscles induced by PA and AP current directions allows us to infer the different I-
111 wave contributions evoked by separate subpopulations of interneurons. To our knowledge,
112 whether MI differentially modulates specific circuits with each current direction remains
113 unexplored. As MI is an important field of research in cognitive neuroscience and motor
114 rehabilitation, it is important to decipher the neural circuits underlying imagined movements.

115 In the current study, we investigated the modulation of early and late I-waves generating
116 pathways specifically activated by PA and AP-directed currents during MI and rest
117 conditions. We used single-pulse and paired-pulse TMS to probe corticospinal excitability
118 and short-interval intracortical inhibition (SICI). Interestingly, SICI affects mainly later I-

119 waves that are mainly targeted by AP orientation (Nakamura *et al.*, 1997; Hanajima *et al.*,
120 1998; Cirillo & Byblow, 2016; Wessel *et al.*, 2019), and SICI increases during MI but is only
121 observed with PA orientation (Neige *et al.*, 2020).

122 If MI preferentially recruits superficial layers within M1 (Persichetti *et al.*, 2020), we would
123 observe a greater increase in corticospinal excitability and SICI with AP than with PA
124 orientation. On the contrary, if MI activates neural circuits downstream of the pyramidal cells
125 (Grosprêtre *et al.*, 2015), we expect that MI would induce a specific modulation of the
126 pathway recruited by PA orientation that preferentially generates early I-wave. This pathway
127 would be critical in modulating both corticospinal excitability and SICI.

128 **Material and Methods**

129 **Participants**

130 Seventeen healthy volunteers were recruited in the current study after providing written
131 informed consent (3 females; age = 24.3 years, range 21-31 years; height = 177 ± 8 cm;
132 weight = 69 ± 10 kg; right-handed as assessed by the Edinburgh Handedness Inventory
133 (Oldfield, 1971)). All volunteers were screened by a medical doctor for contraindications to
134 TMS (Rossi *et al.*, 2009). The protocol was approved by the CPP SOOM III ethics committee
135 (number 2017-A00064-49) and complied with the Declaration of Helsinki.

136 **Experimental setup**

137 Participants were seated in an isokinetic dynamometer chair (Biodex System 3, Biodex
138 Medical Systems Inc., Shirley, NY, USA). Participants' right hand was firmly strapped in a
139 neutral position to a custom-built accessory adapted for wrist isometric contraction. The
140 rotation axis of the dynamometer was aligned with the styloid process of the ulna. The upper
141 arm was vertical along the trunk (shoulder abduction and elevation angles at 0°), and the
142 forearm was semipronated and flexed at 90° . First, participants familiarized themselves with
143 the voluntary force production feedback procedure during an approximately 5-min warm-up
144 of wrist flexions. Next, they received online visual feedback of the real-time exerted force
145 contraction on a computer screen located 1 m in front of them. Then, participants performed
146 three maximal voluntary isometric contractions lasting 3 seconds with verbal encouragement,
147 separated by at least 30 seconds of rest in between. The maximum of the three trials was
148 defined as the participant's maximal voluntary isometric contractions.

149 **Electromyographic recordings**

150 Surface electromyographic (EMG) activity was recorded from the right flexor carpi radialis
151 (FCR) using two silver-chloride (Ag/AgCl) electrodes placed over the muscle belly at 1/3 of
152 the distance from the medial epicondyle of the humerus to the radial styloid process. In
153 addition, a ground electrode was placed over the medial epicondyle to the radial styloid.
154 Signals were amplified (gain of 1000), band-pass filtered (10–1000 Hz), digitized at a
155 sampling rate of 2000 Hz and stored for off-line analysis (Biopac Systems Inc. Goleta, CA,
156 USA).

157 Background root mean square (RMS) of the surface EMG was calculated during the 100 ms
158 epoch before TMS to ensure the absence of muscle contraction in each condition.

159 **Transcranial Magnetic Stimulation**

160 Transcranial magnetic stimuli were applied using a 70-mm figure-of-eight coil through a
161 Magstim BiStim² stimulator (The Magstim Co., Whitland, UK) with a monophasic current
162 waveform. The optimal stimulation site on the scalp (hotspot) was defined as the location
163 eliciting the largest MEP amplitude in the FCR muscle with PA-induced currents for a given
164 intensity. This location was marked by a color marker on a tight-fitting cap worn by the
165 participant. For other coil orientations, the same hotspot was used since previous experiments
166 have shown that the direction of the induced current does not significantly influence the
167 position of the hotspot (Sakai *et al.*, 1997; Hamada *et al.*, 2013) (see Figure 1).

168

169

Please Insert Fig. 1 here

170

171 The resting motor threshold (rMT) was determined for PA and AP directions as the lowest
172 stimulus intensity required to evoke at least 5 MEPs of 50 μ V peak-to-peak amplitude out of
173 10 consecutive trials in the relaxed muscle (Rossini *et al.*, 1994). The active motor threshold
174 (aMT) was determined for PA, LM and AP directions as the lowest stimulus intensity
175 required to evoke at least 5 MEPs of 200 μ V peak-to-peak amplitude out of 10 consecutive
176 trials during 10% of their Maximal Voluntary Contraction (MVC) (Rossini *et al.*, 1994).

177 **MEPs latency**

178 The onset latency of MEPs obtained between PA-LM and AP-LM was used as an individual
179 index of early (I1) and late (I2, I3) I-waves recruitment (Hamada *et al.*, 2013; Neige &
180 Beynel, 2020).

181 MEPs onset latency was determined for the FCR while participants maintained approximately
182 10% of their MVC, with online feedback visualizing the generated force recorded by the
183 isokinetic dynamometer (Hamada *et al.*, 2013). This was done to ensure that low stimulus
184 intensities could be used, thereby maximizing the selectively recruiting early or late I-waves
185 with PA or AP currents. Higher stimulus intensity was used for LM to ensure that
186 corticospinal neurons were directly stimulated (D-wave) at this coil orientation (Werhahn *et*
187 *al.*, 1994). Stimulation intensities were set at 110% of aMT_{PA} , 110% of aMT_{AP} and 150% of
188 aMT_{LM} (or 50% of maximum stimulator output (%MSO) in participants whose 150% aMT_{LM}
189 did not reach 50 %MSO) (Hamada *et al.*, 2013). Fifteen MEPs were recorded for each current
190 direction, with the order of currents pseudo-randomized. The participants were instructed to
191 maintain their contraction, and the interval between stimulations was fixed at 4s. At the end of
192 each condition (i.e., 15 trials) participants were asked to relax their wrists to avoid fatigue.
193 The onset latency of MEPs assessed during muscle contraction was measured from the
194 superimposed raw EMG waves-forms by visual inspection (Hamada *et al.*, 2013; Hannah,
195 Rocchi, *et al.*, 2018).

196 **Adaptive threshold-hunting technique**

197 To probe the distinct cortical elements recruited within M1 during MI, we used the adaptive
198 threshold-hunting technique, which consists in maintaining a constant MEP amplitude (called
199 the MEP_{target} , see below) by adjusting the TS (Test Stimulus) stimulation intensity. The
200 adaptive threshold-hunting paradigm offers several advantages when compared to the
201 conventional protocol used to assess corticospinal excitability and SICI modulations. First, it
202 allows for overcoming the intrinsic MEPs amplitude variability, thus providing more reliable
203 results with a shorter acquisition time (Samusyte *et al.*, 2018). Then, it minimizes the
204 potential “floor/ceiling effect” when complete inhibition is observed with the conventional
205 SICI paradigm (Cirillo & Byblow, 2016). Finally, the adaptive threshold-hunting technique
206 relies on a weaker TS intensity (see below) than conventional paradigms (usually MEP_{test}
207 1mV or 120-130% rMT), which is thought to recruit more selectively early and later I-waves
208 generating pathways (Di Lazzaro *et al.*, 2001; Cirillo *et al.*, 2020).

209 In the current study, unconditioned MEP_{test} and SICI modulation obtained during MI and
210 compared to the rest will be assessed by using the adaptive threshold-hunting technique with
211 PA and AP current orientations known to elicit early- preferentially and late I-waves,
212 respectively.

213 **MEP_{target}**

214 The hunting threshold was defined as the TS intensity (expressed in %MSO) required to elicit
215 a MEP_{target} in the relaxed FCR muscle corresponding to the mean of 15 MEPs elicited at
216 115% rMT_{PA} in peak-to-peak amplitude. This led to a MEP_{target} of 0.251 ± 0.141 mV
217 amplitude (see Table 1 for individual values). Generally, a non-personalized fixed 0.2 mV
218 MEP_{target} amplitude is selected in studies using the adaptive threshold-hunting technique,
219 corresponding approximately to 109% rMT (Fisher *et al.*, 2002; Awiszus, 2003; Vucic *et al.*,
220 2006; Menon *et al.*, 2015; Cirillo & Byblow, 2016; Cirillo *et al.*, 2018; Samusyte *et al.*, 2018;
221 Van den Bos *et al.*, 2018; Neige *et al.*, 2020). However, in the current study, a subject-
222 specific MEP_{target} was chosen since 1) a huge between-subject variability in the intrinsic
223 excitability of the corticospinal pathway exists, 2) a TS delivered at a lower intensity (i.e.,
224 below 110% rMT) could fail to evoke late I-waves, and limits SICI magnitude (Garry &
225 Thomson, 2009) and 3) a TS delivered at a higher intensity can also elicit early I-waves when
226 using an AP current direction, therefore limiting the interpretation differences obtained
227 between PA and AP findings.

228 **Single-pulse TMS**

229 The adaptive threshold-tracking single-pulse TMS technique was used to assess the
230 unconditioned TS stimulation intensity required to reach the MEP_{target} amplitude (see General
231 procedure) at rest vs. during MI, with PA and AP currents direction. The unconditioned TS
232 intensity (expressed in %MSO) was quantified and compared across all experimental
233 conditions.

234 **Short-interval intracortical inhibition (SICI)**

235 The adaptive threshold-hunting technique was then used to investigate SICI modulation at rest
236 vs. during MI, with PA and AP currents direction. A sub-threshold conditioning pulse (CS)
237 was applied before the TS. The conditioned TS stimulation intensity required to reach the
238 MEP_{target} amplitude was quantified. The CS intensity was fixed at 60% rMT_{PA} for $SICI_{PA}$ and
239 60% rMT_{AP} for $SICI_{AP}$, based on a previous study showing that higher CS intensities could

240 lead to the unwanted recruitment of excitatory interneurons during MI, biasing the result
241 interpretation (Neige *et al.*, 2020). The inter-stimulus interval (ISI) between CS and TS was
242 set at 3 ms to induce the greatest inhibition when using the AP current direction (Kujirai *et*
243 *al.*, 1993; Cirillo *et al.*, 2018).

244 To probe the influence of the Task and Orientation on intracortical inhibition, the amount of
245 SICI (expressed in INH%) was quantified for each condition using the following equation
246 (Fisher *et al.*, 2002):

$$INH(\%) = \frac{(\text{conditioned TS Intensity}) - (\text{unconditioned TS Intensity})}{(\text{unconditioned TS Intensity})} \times 100$$

247 The higher values characterize the higher TS Intensity required to overcome the inhibitory
248 influence of the CS and reach the MEP_{target} amplitude (Cirillo *et al.*, 2020).

249 It has to be noted that only SICI data from 15 participants were used in the subsequent
250 analysis because it was not possible to reach the MEP_{target} amplitude even at high stimulation
251 intensity (>90% MSO) in 2 participants.

252 **General procedure**

253 Experimental conditions (single- vs. paired-pulse; rest vs. MI; PA vs. AP current direction)
254 were performed in different recording blocks and were randomized and counterbalanced
255 across participants. An available online freeware (TMS Motor Threshold Assessment Tool,
256 MTAT 2.0), based on a maximum-likelihood Parameter Estimation by Sequential Testing
257 (PEST) strategy (Awiszus, 2003) was used with “assessment without a priori information” in
258 line with previous studies (Cirillo & Byblow, 2016; Cirillo *et al.*, 2018). The stimulation
259 sequence always began with the TS at 37 %MSO. One experimenter held the coil over M1,
260 while the other indicated whether (or not) the MEP amplitude was \geq MEP_{target}. The predictive
261 algorithm then determined the next TS intensity to be delivered and was stopped after twenty
262 stimulations, which provides sufficient accuracy for the threshold estimate according to
263 previous studies (Awiszus, 2003, 2014; Ah Sen *et al.*, 2017).

264 For MI trials, participants were instructed to perform explicit and kinesthetic (somatosensory)
265 MI of right wrist maximal isometric contractions with the first-person perspective for a
266 duration of 3 s following an auditory cue (Hanakawa, 2016). Participants were reminded that
267 they had already performed this movement during maximal contractions at the beginning of
268 the experiment. The following instructions (in French) were carefully given to the

269 participants: “When you hear the cue, try to imagine yourself performing the movement,
270 feeling the movement, i.e., the muscle contraction and the tension that you would experience
271 when performing the actual action. Be sure not to contract any muscles during the task and
272 keep your eyes open” (Lebon *et al.*, 2019; Neige *et al.*, 2021). The kinesthetic MI strategy is
273 thought to produce the greater muscle-specific and temporally modulated facilitation of the
274 corticospinal pathway compared to the visual MI strategy (Stinear *et al.*, 2006). The TMS
275 pulses were triggered 1250 ± 250 ms after the onset of the auditory cue during the execution
276 phase of MI trials (Neige *et al.*, 2021) and the inter-trial interval was 8 s. Finally, the RMS
277 preceding the TS for each trial was inspected during the experiment. Trials contaminated by
278 pre-stimulus EMG activity (RMS $>10 \mu\text{V}$; 100 ms before stimulation) were rejected online
279 and repeated immediately (Mooney *et al.*, 2018).

280 **Statistical analysis**

281 Statistical analyses were performed using Statistical Program for the Social Sciences (SPSS)
282 version 24 software (SPSS Inc., Chicago, IL, USA). Data distribution was assessed using the
283 Shapiro-Wilk test. Homogeneity of variances was assessed by Mauchly’s test. If the
284 sphericity assumption was violated, a Greenhouse-Geiser correction was applied. Pre-planned
285 posthoc analyses were performed on significant interactions after applying a Bonferroni
286 adjustment for multiple comparisons. Corrected p values for multiple comparisons are
287 reported in the results section. The α level for all analyses was fixed at .05. Partial eta squared
288 (η_p^2) values are reported to express the portion of the total variance attributable to the tested
289 factor or interaction. For t-test analyses, effect sizes (Cohen’s d) are reported to indicate small
290 ($d = 0.2$), moderate ($d = 0.5$) and large ($d = 0.8$) comparative effects. Values in parentheses in
291 the text represent mean \pm SD.

292 The first set of analyses was performed to control for potential methodological biases. A
293 Student’s two-tailed paired sample *t*-tests were used to compare the rMT and aMT (%MSO)
294 obtained for PA and AP current direction and to analyze the MEPs latency difference between
295 PA-LM and AP-LM.

296 Then, a repeated-measure ANOVA was performed on the unconditioned TS Intensity
297 (%MSO) with two within-subject factors: Task₂ (Rest vs. MI) and Orientation₂ (PA vs. AP).
298 Moreover, to complement this analysis and test specifically how changes observed between
299 rest and MI differ according to the Orientation, MI-rest ratios were calculated and compared
300 (PA vs. AP) by using a two-tailed Student *t*-test for paired samples.

301 The same repeated-measure ANOVA was also performed on SICI measurements (INH %) with Task₂ (Rest vs. MI) and Orientation₂ (PA vs. AP) within-subject factors.

303 The RMS values were compared across conditions using a repeated-measures analysis of variance (ANOVA) with two within-subject factors: Task₂ (Rest vs. MI), and Orientation₂ (PA vs. AP). This ANOVA was performed separately for the unconditioned TS and the SICI measures. We predict no significant difference for these comparisons since an absence of any volitional muscle activity is expected for all the experimental conditions.

308 **Results**

309 **Motor thresholds**

310 Overall, both rMT ($t(16) = -3.55$, $p = .003$; Cohen's $d = -0.862$) and aMT ($t(16) = -8.147$, $p < .001$; Cohen's $d = -1.976$) were significantly lower for PA compared to AP orientation (Table 1) as observed in previous studies using the adaptive-hunting threshold technique (Cirillo & Byblow, 2016; Cirillo *et al.*, 2018).

314

315 Please Insert Table 1 here

316

317 **MEP latency**

318 The analysis of MEPs latency difference revealed that PA-LM latency was significantly shorter compared with AP-LM latency (0.90 ± 0.7 ms vs. 2.56 ± 1.1 ms; $t(16) = -10.042$, $p < .001$; Cohen's $d = -2.436$). This result was consistent across participants and suggested that the early wave recruited with PA orientation (mean latency = 17.06 ± 1.2 ms) and late I-waves recruited with AP orientation (mean latency = 18.72 ± 1.4 ms) could be differentially recruited within individuals.

324 **Unconditioned TS Intensity**

325 Figure 3A illustrates the unconditioned TS Intensity obtained at rest and during MI for PA and AP current directions. We found a significant main effect of Orientation ($F_{(1,16)} = 39.338$, $p < .001$; $\eta_p^2 = .711$) indicating that the unconditioned TS Intensity required to reach the MEP_{target} was significantly higher for the AP orientation than the PA orientation. A main effect of Task was also observed ($F_{(1,16)} = 11.004$, $p = .004$; $\eta_p^2 = .409$) but more importantly

330 the Orientation by Task interaction was significant ($F_{(1,16)} = 5.130$, $p = .038$; $\eta_p^2 = .243$). Post
331 hoc analyses revealed that for both PA and AP orientations, the unconditioned TS Intensity
332 required to reach the MEP_{target} was significantly lower during MI than at rest ($p = .005$ for PA
333 and $p = .025$ for AP) indicating that MI increased corticospinal excitability. Importantly, when
334 comparing the rest vs. MI ratios according to the Orientation, the reduction of the
335 unconditioned TS Intensity for MI when compared to rest was significantly more important
336 for PA than AP direction ($t(16) = -2.601$, $p = .019$; Cohen's $d = -0.631$) (see Figure 3B).
337
338 Taken together, these results suggest that the classical corticospinal excitability increase
339 during MI is mainly driven by early I-waves recruitment.

340

341

Please Insert Fig. 2 here

342

343 **Conditioned TS Intensity (SICI)**

344 Figure 4 illustrates the percentage of inhibition (SICI) obtained at rest and during MI for the
345 PA and AP current direction. We did not find any main effects of Orientation ($F_{(1,14)} = 1.107$,
346 $p = .311$) or Task ($F_{(1,14)} < 1$, $p = .895$), but the Orientation by Task interaction was significant
347 ($F_{(1,14)} = 11.995$, $p = 0.004$, $\eta_p^2 = .461$). Post hoc comparisons showed that at rest, the amount
348 of SICI was higher for AP orientation than the PA orientation ($p = .031$) whereas it was not
349 significant when comparing orientations during MI ($p = .106$). Moreover, SICI was greater
350 during MI compared to rest with the PA orientation ($p = .028$), whereas SICI was lower
351 during MI compared to rest with the AP orientation ($p = .033$).

352

353

Please Insert Fig. 3 here

354

355 **RMS**

356 The analysis of RMS of EMG background for the unconditioned TS showed no significant
357 difference between PA and AP orientation ($F_{(1,16)} < 1$, $p = .548$) and more importantly
358 between rest and MI ($F_{(1,16)} < 1$, $p = .542$). Nor was the Orientation by task interaction ($F_{(1,16)}$
359 $= 3.594$, $p = .076$).

360 Similarly, the analysis of the RMS values for the conditioned TS yielded no significant main
361 effect of Orientation ($F_{(1,14)} < 1$, $p = .997$), Task ($F_{(1,14)} = 1.77$, $p = .204$) or Orientation by
362 Task Interaction ($F_{(1,13)} < 1$, $p = .563$).

363 Together, these results indicate that any changes in corticospinal excitability cannot be
364 attributed to differences in the EMG levels prior to the TMS pulse.

365 **Discussion**

366 In the current study, we demonstrated for the first time that MI activates different subsets of
367 neurons within M1 by means of directional TMS and the adaptive threshold-hunting
368 technique. The increase of corticospinal excitability during MI may originate from an increase
369 in the excitability of the pathway known to generate early I-waves rather than the pathway
370 that preferentially generates late I-waves, as evidenced by a greater increase observed with
371 PA orientation when compared to AP orientation. By using paired-pulse stimulation, the
372 results confirmed that the amount of SICI measured at rest is higher for AP orientation than
373 the PA orientation (Neige *et al.*, 2020). Interestingly, the SICI increase observed during MI
374 (vs. at rest) was also restricted to PA orientation. On the contrary, when using AP orientation
375 SICI was lower during MI compared to rest. Taken together, it suggests that pathways
376 recruited by PA and AP orientations generating early- and late I-waves respectively are
377 differentially modulated by MI. This result also confirms the hypothesis that MI activates
378 neural circuits downstream of the pyramidal cells and produces a subliminal motor output that
379 reaches the spinal level (Grosprêtre *et al.*, 2015) rather than induces a specific superficial
380 activation restricted to the superficial layers within M1 that could explain the absence of
381 muscle activity during motor imagery (Persichetti *et al.*, 2020). The excitability of the
382 pathway activated by PA orientation that generates early I-wave may be critical in modulating
383 both corticospinal excitability and intracortical inhibition.

384 **Corticospinal excitability increase observed during MI is greater for PA than AP** 385 **orientation**

386 It is well known that MEPs amplitude increases during MI compared to rest reflect an
387 increase in neuron responsiveness to TMS (Grosprêtre *et al.*, 2016). However, MI is a
388 complex state that also involves mechanisms that actively suppress the transmission of the
389 motor command into the efferent pathway, supporting the action of inhibitory pathways
390 during MI (Jeannerod & Decety, 1995). Actually, it is still unclear which inhibitory

391 mechanisms counteract the corticospinal excitability increase in order to prevent the
392 production of an overt movement.

393 Based on previous reports demonstrated that single-pulse TMS with a PA orientation
394 preferentially recruits early I-waves (I1), whereas AP orientation preferentially recruits later I-
395 waves (I3) (Zoghi *et al.*, 2003; Di Lazzaro *et al.*, 2017), we used directional TMS to activate
396 different sets of excitatory synaptic inputs within M1.

397 First, our results demonstrate that our experimental setting (coil orientation) was correct, with
398 a significant latency difference between PA-LM and AP-LM, supporting differential
399 recruitment of cortical neurons in M1 relative to the current orientation (Werhahn *et al.*, 1994;
400 Hamada *et al.*, 2013; Di Lazzaro & Rothwell, 2014). If it has already been shown in a forearm
401 extensor muscle (McCambridge *et al.*, 2015), our study is the first to demonstrate latency
402 difference in the FCR muscle.

403 To further investigate the involvement of different subsets of cortical neurons during MI
404 compared to the resting state, we used the adaptive threshold-hunting technique and compared
405 the unconditioned TS intensity (%MSO) required to reach the MEP_{target} at rest and during MI
406 with PA and AP orientation. We found that the unconditioned TS Intensity required to reach
407 the MEP_{target} was significantly lower during MI than at rest for both the PA and the AP
408 orientations. However, when comparing the ratios MI/rest according to the orientation, the
409 reduction of the unconditioned TS Intensity for MI was significantly more important for PA
410 than AP direction. Taken together, these results indicate that the early I-wave generating
411 pathway within M1 possibly mediated the MEPs amplitude increases observed during MI.
412 The exact underpinning neurophysiology of I-waves generation remains largely
413 misunderstood (Ziemann, 2020). However, it has been suggested that the early I-wave evoked
414 by TMS with PA orientation is the result of the activation of monosynaptic cortico-cortical
415 connections projecting onto the large corticospinal neurons of layer V (Di Lazzaro &
416 Ziemann, 2013; Di Lazzaro *et al.*, 2017; Hannah, 2020). This result seems coherent with
417 previous literature assuming that the early I-wave is produced by a different anatomical
418 substrate and mechanism than the late I-waves (Ziemann, 2020). Crucially, specific early I-
419 wave recruitment evoked by TMS with PA orientation is enhanced when corticospinal
420 excitability increases (Di Lazzaro *et al.*, 1998a, 2017), which was the case during MI. For
421 example, voluntary muscle contraction increased both corticospinal excitability and the
422 relative contribution of the early I-wave (Di Lazzaro *et al.*, 1998a, 2017).

423 The findings of the current study also extend and consolidate our knowledge regarding the

424 distinct I-wave circuits recruitment during behavioral states that share analogous control
425 mechanisms and neural circuits with overt movements but without any muscle activity (i.e.,
426 covert actions) (Hannah, 2020). Indeed, recent studies exploited the directional TMS
427 technique to probe the different subsets of cortical neurons recruited during motor preparation
428 (Hannah, Cavanagh, *et al.*, 2018) and action observation (Hannah, Rocchi, *et al.*, 2018). The
429 results showed that during motor preparation, the decrease of the corticospinal excitability in
430 the selected and non-selected muscles was accompanied by selective suppression of the subset
431 of excitatory inputs to corticospinal neurons responsible for late I-waves. In contrast, the
432 subsets of neurons responsible for early-I wave generation remain unaffected (Derosiere,
433 2018; Hannah, Cavanagh, *et al.*, 2018). However, when using the directional TMS technique
434 during action observation, other authors failed to observe selective recruitment of the early or
435 late I-waves pathway, probably due to large intersubject variability in corticospinal
436 modulations (Hannah, Rocchi, *et al.*, 2018). Overall, the recent use of the directional TMS
437 technique applied during motor preparation, action observation and MI allows further insight
438 into the distinct circuitry recruited with TMS that contributes to the corticospinal excitability
439 modulation.

440 **SICI increase during MI is restricted to PA orientation**

441 The adaptive threshold-hunting paired-pulse TMS technique was also used in the current
442 study to examine modulations of SICI during MI and at rest and how they are influenced by
443 TMS coil orientation.

444 SICI involves a subthreshold CS, which is thought to activate low-threshold inhibitory
445 interneurons that employ gamma-aminobutyric acid type A receptor (GABA_A). The effect of
446 the activation of these GABAergic inhibitory interneurons is the reduction of the excitatory
447 inputs activated by the TS (Kujirai *et al.*, 1993; Di Lazzaro *et al.*, 1998b, 2017). Importantly,
448 it has been described previously that SICI affects predominantly later I-waves (I₃), mainly
449 targeted by AP orientations (Nakamura *et al.*, 1997; Hanajima *et al.*, 1998; Di Lazzaro *et al.*,
450 2012; Higashihara *et al.*, 2020). Moreover, it is known that the use of the adaptive threshold-
451 tracking technique with an AP-induced current with a 3-ms ISI provides a more robust and
452 sensitive measure of SICI than with a PA-induced current (Cirillo & Byblow, 2016).
453 Therefore, a greater level of SICI assessed at rest using AP- compared with PA-orientation
454 demonstrated in the present study corroborates and replicates earlier findings (Cirillo &
455 Byblow, 2016; Cirillo *et al.*, 2018, 2020). This result also indicates further evidence that SICI

456 is mediated by the recruitment of inhibitory interneurons generating late I-waves.
457 By comparing the extent of SICI modulation obtained with a PA-induced current, we found
458 that there was significantly more inhibition during MI when compared to rest. This finding
459 also corroborates a previous study showing that when tested with low CS intensities, as in the
460 current study ($< 70\%$ rMT), SICI is greater during MI than at rest (Neige *et al.*, 2020). This
461 increase in SICI could reflect the crucial role played by cortical interneurons within M1 in the
462 fine-tuning neural processes required during MI. This may prevent the production of an overt
463 movement when the mental representation of that movement is activated.
464 Conversely, by comparing the extent of SICI modulation obtained with an AP-induced
465 current, we found a SICI decrease during MI compared to rest. Moreover, contrary to what
466 was found during the resting state, the level of SICI assessed during MI using AP- compared
467 with PA orientation was not significantly greater. These results, combined with the
468 unconditioned TS intensity findings, indicate that the specific early- and late I-waves evoked
469 by PA and AP orientation are differentially modulated by MI.

470 **MI influences a specific distributed circuit that can differentially contribute to early and**
471 **late I-waves**

472 Neuroimaging studies provided evidence that MI activates a premotor-parietal network,
473 including cortical and subcortical brain regions such as the dorsolateral prefrontal cortex,
474 supplementary motor area, premotor cortex, posterior parietal regions, putamen and
475 cerebellum (Hardwick *et al.* 2018). Crucially, M1 is known to integrate inputs from some of
476 these structures, and the latter are differentially recruited according to the current orientation.
477 For example, late I-waves evoked by AP orientation could activate axons of neurons of the
478 premotor cortex projecting to the corticospinal cells (Groppa *et al.*, 2012; Volz *et al.*, 2015;
479 Aberra *et al.*, 2020; Siebner, 2020; Desmons *et al.*, 2021). Recently, Oldrati *et al.* (2021)
480 reported that following offline 1Hz inhibitory repetitive TMS over the dorsal premotor cortex
481 (PMd), corticospinal excitability assessed during kinesthetic MI was not significantly higher
482 than rest condition (Oldrati *et al.*, 2021). These findings suggest facilitatory connectivity from
483 PMd to M1 during MI. Although this remains speculative, the facilitatory input from PMd to
484 M1 during MI has decreased the SICI level within M1. Moreover, the opposite higher level of
485 SICI during MI (vs. rest) observed with PA current reflect the activation of inhibitory inputs
486 received from the somatosensory cortex and the supplementary motor area, both areas known
487 to functionally inhibit M1 when imagining (Kasess *et al.*, 2008; Oldrati *et al.*, 2021). Finally,

488 it is also possible that the cerebellum, which facilitates M1 excitability during MI (Tanaka *et*
489 *al.*, 2018; Rannaud Monany *et al.*, 2022), also contributes to the result of the current study
490 since the influence of the cerebellum on M1 might occur via interactions with specific I-
491 waves generating circuits (Spampinato *et al.*, 2020).

492 **Limitations and perspectives**

493 Several limitations need to be taken into consideration when interpreting the results of this
494 study. First, SICI modulations tested with the adaptive-threshold hunting technique also
495 depend on CS intensity (particularly during MI) (Vucic *et al.*, 2009; Ibáñez *et al.*, 2020; Neige
496 *et al.*, 2020) and interstimulus intervals (Fisher *et al.*, 2002). These two parameters were not
497 manipulated in the current study, and the careful consideration of stimulation parameters
498 selected for SICI assessment deserves further investigations. Moreover, the activation of
499 distinct subsets of neurons within M1 according to the PA or AP orientation is known to be
500 sensitive to specific stimulation parameters such as pulse duration, pulse shape, and phase-
501 amplitude (D'Ostilio *et al.*, 2016; Hannah & Rothwell, 2017; Hannah *et al.*, 2020;
502 Spampinato, 2020).

503 Future studies should use a controllable pulse parameter TMS device with 1) monophasic
504 pulses and 2) short duration pulses (i.e., 30 μ s) for AP current and long duration pulses (i.e.
505 120 μ s) for PA current to determine whether the different activation of subsets of neurons in
506 AP and PA current change during MI.

507 To gain further insight into the different subsets of cortical neurons and interneuronal circuits
508 recruited during MI, it would be worthwhile to exploit recent techniques also developed to
509 probe the separate subsets of inputs within M1. For example, Kurz *et al.* (2019) developed a
510 novel non-invasive method that combines single-pulse TMS with peripheral nerve
511 stimulations of the median nerve generating an H-reflex. This technique makes it possible to
512 estimate excitability changes of different micro-circuits of M1, which reflect layer-specific
513 activity (Dukkipati & Trevarrow, 2019; Kurz *et al.*, 2019). Since layer-specific cortical
514 circuits activity has been recently evidenced during MI (Persichetti *et al.*, 2020) and
515 corticospinal neurons responsible for the early and late I-waves pathways are thought to
516 originate from layer-specific cortical circuits, the technique of Kurz *et al.* could be a
517 promising tool to delineate further the different subsets of neurons in M1 activated during MI.
518 Finally, the exact contribution of the early and late I-waves can be captured by delivering
519 paired-pulse TMS at precise intervals approximating the different I-wave latency (Tokimura

520 *et al.*, 1996; Hanajima *et al.*, 2002). This technique has been recently applied during grasping
521 observation to isolate the contribution to early and late excitatory inputs to M1 (Cretu *et al.*,
522 2020) and could be tested during MI.

523 In conclusion, this study is the first to present evidence that the increase of corticospinal
524 excitability and intracortical inhibition during MI may originate from a specific modulation of
525 the excitability of the pathway activated by PA orientation that generates early I-wave (rather
526 than later I-waves generated by AP orientation). This finding is reflected by a greater
527 corticospinal excitability increase observed during MI (compared to rest) with PA than AP
528 orientation. Moreover, the SICI increase during MI was only restricted to PA orientation. We
529 found decreased SICI when using AP orientation, which is more sensitive to later I-waves
530 generating pathways. Taken together, the results confirm that MI modulates the excitability of
531 the pathway that generates early I-wave preferentially.

532 **Competing interests**

533 The authors declare no competing interests.

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757

758 **Table**

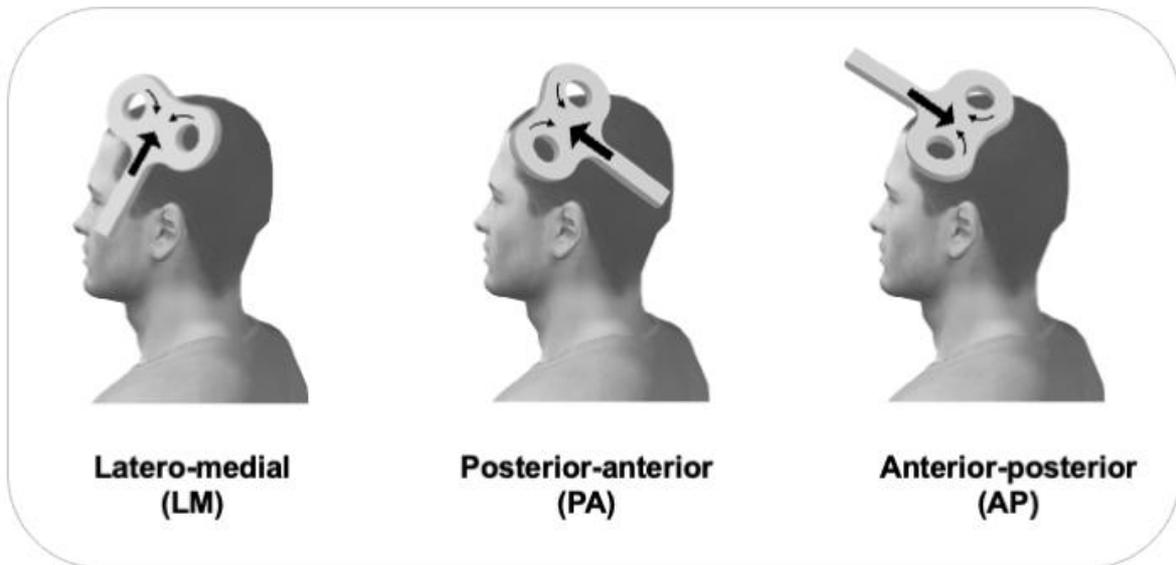
Subject	rMT _{PA}	rMT _{AP}	aMT _{PA}	aMT _{AP}	aMT _{LM}	MEP _{target}
1	40	46	26	35	30	0.213
2	32	41	28	35	29	0.384
3	50	35	28	39	35	0.109
4	46	55	39	48	40	0.095
5	39	51	37	44	44	0.079
6	32	32	25	28	29	0.337
7	45	49	39	41	44	0.291
8	45	56	35	50	35	0.647
9	43	55	33	47	34	0.255
10	30	35	26	30	31	0.234
11	50	52	43	46	43	0.170
12	36	40	27	34	30	0.162
13	35	48	29	42	33	0.258
14	38	42	31	36	34	0.368
15	43	49	34	42	37	0.263
16	40	45	36	41	39	0.313
17	41	49	32	42	35	0.119
Mean	40.3	45.9	32.2	40	35	0.252
SD	6.2	7.6	5.5	6.4	5.2	0.14

759

760 **Table 1:** Individual rMT and aMT expressed in %MSO (Rossini et al., 1994) according
761 to PA and AP orientations. The individual MEP_{target} amplitude (mV) has been calculated
762 from the mean of 15 MEPs elicited at 115% rMT_{PA}.

763

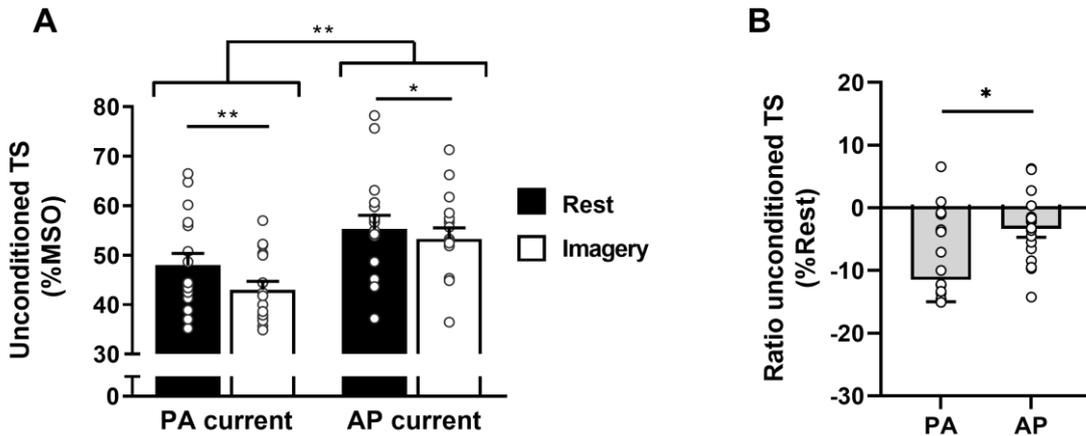
764 **Figures**



765

766 **Figure 1:** Illustration of the coil orientations and their direction of currents induced in
767 the brain (large arrows) by single- and paired-pulse TMS.

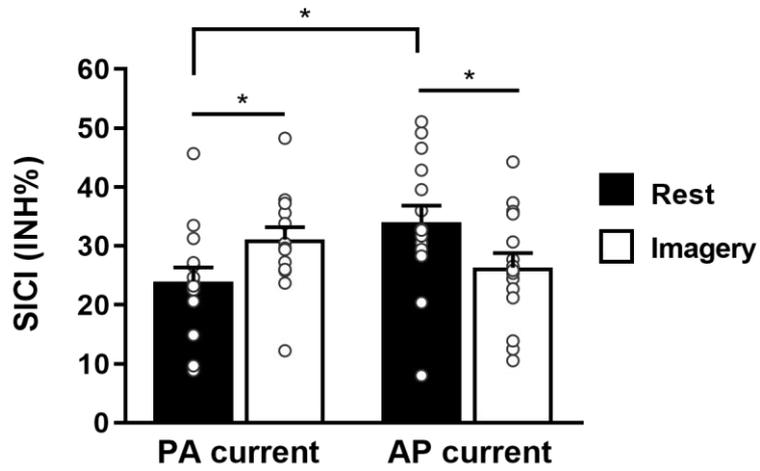
768



769

770 **Figure 2:** A) Mean \pm SE for the unconditioned TS Intensity (%MSO) obtained with the
 771 hunting-threshold technique at rest and during motor imagery for the two current
 772 orientations. Lower values of %MSO indicate lower TS intensities to reach the MEP_{target}
 773 amplitude. B) Ratio for the unconditioned TS Intensity obtained during motor imagery
 774 and expressed as a percentage of rest condition for the two current orientations.
 775 Negative values indicate lower TS intensities during Imagery compared to rest, and
 776 therefore, an increase in corticospinal excitability which is greater with PA orientation
 777 than with AP orientation.
 778 Data points represent individual participants. PA: posterior-anterior; AP: anterior-
 779 posterior. * $p < .05$; ** $p < .01$.

780



781

782 **Figure 3:** Mean \pm SE for the SICI (%INH) obtained with the hunting-threshold
 783 technique at rest and during motor imagery for the two current orientations.
 784 Data points represent individual participants. PA: posterior-anterior; AP: anterior-
 785 posterior. * $p < .05$

786

787 **Data Accessibility**

788 All datasets will be freely available on the Open Science Framework repository upon
789 publication at <https://osf.io/ks92r/>

790

791

792 **List of abbreviations**

- 793 AP –Anterior-Posterior
- 794 aMT –active Motor Threshold
- 795 CS –Conditioned Stimulus
- 796 EMG –Electromyographic
- 797 FCR –Flexor Carpi Radialis
- 798 GABA_A –Gamma-aminobutyric acid type A receptor
- 799 INH –Inhibition
- 800 M1 –Primary Motor Cortex
- 801 MEPs –Motor Evoked Potentials
- 802 MI –Motor imagery
- 803 MSO –Maximum Stimulator Output
- 804 MVC –Maximal Voluntary Contraction
- 805 PA –Posterior-Anterior
- 806 PMd –dorsal Premotor cortex
- 807 RMS –Root Mean Square
- 808 rMT –resting Motor Threshold
- 809 SICI –Short-Interval Intracortical Inhibition
- 810 TS –Test Stimulus
- 811 TMS –Transcranial magnetic stimulation