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

Running title: Using directional TMS during motor imagery

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Florent Lebon: Conceptualization, Data curation, Supervision, Funding acquisition, Writing - Review & Editing.

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Abstract

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

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

Introduction

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

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

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

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

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

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

Material and Methods

Participants

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

Experimental setup

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

Electromyographic recordings

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

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

Transcranial Magnetic Stimulation

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

Please Insert Fig. 1 here

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

MEPs latency

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

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

Adaptive threshold-hunting technique

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

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

MEP_{target}

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

Single-pulse TMS

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

Short-interval intracortical inhibition (SICI)

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

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

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

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

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

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

General procedure

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

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

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

Statistical analysis

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

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

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

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.

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.

Results

Motor thresholds

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).

Please Insert Table 1 here

MEP latency

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.

Unconditioned TS Intensity

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

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

Please Insert Fig. 2 here

Conditioned TS Intensity (SICI)

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

Please Insert Fig. 3 here

RMS

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

SICI increase during MI is restricted to PA orientation

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

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

is mediated by the recruitment of inhibitory interneurons generating late I-waves.

By comparing the extent of SICI modulation obtained with a PA-induced current, we found that there was significantly more inhibition during MI when compared to rest. This finding also corroborates a previous study showing that when tested with low CS intensities, as in the current study ($< 70\%$ rMT), SICI is greater during MI than at rest (Neige *et al.*, 2020). This increase in SICI could reflect the crucial role played by cortical interneurons within M1 in the fine-tuning neural processes required during MI. This may prevent the production of an overt movement when the mental representation of that movement is activated.

Conversely, by comparing the extent of SICI modulation obtained with an AP-induced current, we found a SICI decrease during MI compared to rest. Moreover, contrary to what was found during the resting state, the level of SICI assessed during MI using AP- compared with PA orientation was not significantly greater. These results, combined with the unconditioned TS intensity findings, indicate that the specific early- and late I-waves evoked by PA and AP orientation are differentially modulated by MI.

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

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

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

Limitations and perspectives

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

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

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

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

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

Competing interests

The authors declare no competing interests.

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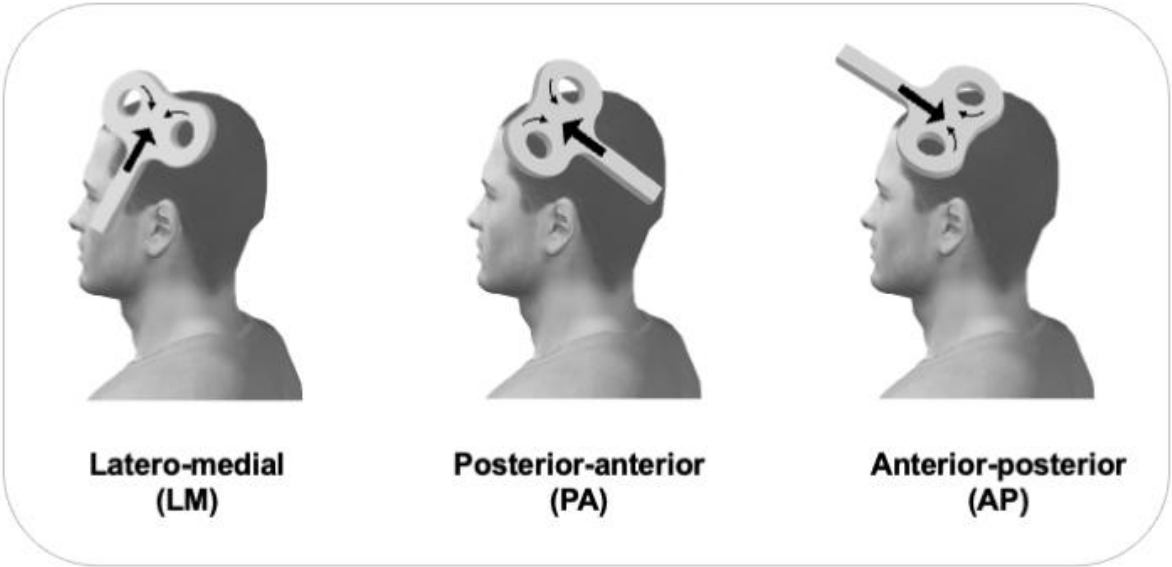
758 **Table**

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



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.

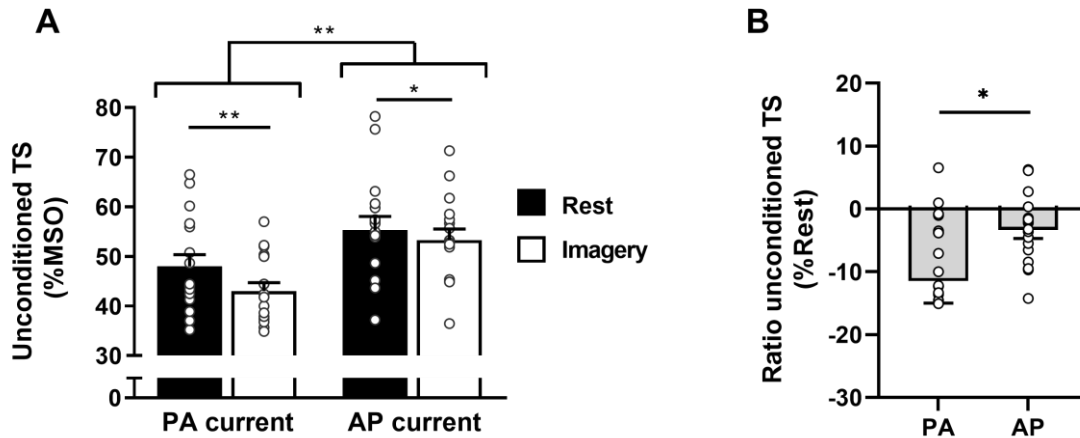


Figure 2: **A)** Mean \pm SE for the unconditioned TS Intensity (%MSO) obtained with the hunting-threshold technique at rest and during motor imagery for the two current orientations. Lower values of %MSO indicate lower TS intensities to reach the MEP_{target} amplitude. **B)** Ratio for the unconditioned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

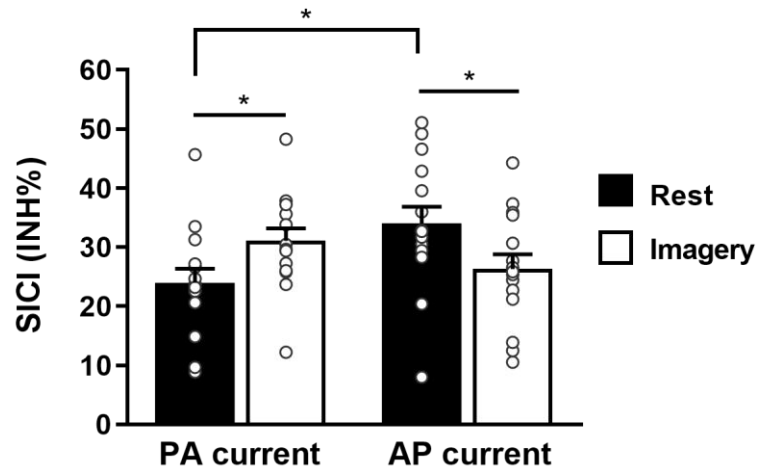


Figure 3: Mean \pm SE for the SICI (%INH) obtained with the hunting-threshold technique at rest and during motor imagery for the two current orientations. Data points represent individual participants. PA: posterior-anterior; AP: anterior-posterior. * $p < .05$

787 **Data Accessibility**

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

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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