Motor imagery practice during arm-immobilization benefits sensorimotor cortical functions and plasticity-related sleep features

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Abstract

Limb disuse has substantial maladaptive behavioral consequences, suggesting that a sustained stream of sensory inputs and motor outputs is necessary for the maintenance of optimal motor functions. Besides the actual execution of movements, the activation of mental representation of movements alone, or Motor Imagery (MI), is known to significantly engage motor networks. Based in such observation, MI should, at least in part, compensate for maladaptive neuroplasticity elicited by immobilization. Although highly relevant for clinical conditions associated with transient (e.g. after orthopedic injury) or chronic (e.g. after stroke) limb immobilization, this hypothesis and associated underlying neural mechanisms remain underexplored. Moreover, durable changes due to immobilization may also implicate sleep processes. To address these questions, here we investigated the potential benefits of MI practice during 11 h of unilateral arm-immobilization on the sensorimotor and cortical representations of the arms, as well as on subsequent sleep characteristics. Using a within-subjects design, fourteen participants were first tested after a normal day of motor activity (without immobilization, Baseline), followed by two randomized 11-h conditions of immobilization of the right (dominant) arm, either with concomitant MI practice (15 min every two hours; MI) or without MI training but a neutral task during an equivalent time (NoMI), one week apart. At the end of each condition (Baseline, MI, NoMI), participants were tested on a hand laterality judgment task to assess changes in the sensorimotor representation of the hands, followed by a measure of cortical excitability of the primary motor cortices (M1). Polysomnography recordings were acquired during the subsequent night to assess condition-dependent modulations of sleep features. Data revealed that MI practice prevented the following consequences of arm immobilization: (i) alteration of the sensorimotor representation of hands, (ii) decrease of

cortical excitability over M1 contralateral to arm-immobilization, (iii) reduction of sleep spindles over both M1s, and (iv) decrease in total time spent in REM sleep. Altogether, these results provide strong evidence that implementing MI within the context of limb-immobilization can substantially limit the deleterious effects of limb disuse, at several levels of sensorimotor functioning.

Key words: motor imagery, sensorimotor representation, cortical excitability, sleep, arm-immobilization

Highlights

- MI prevents the impairment of sensorimotor representation caused by immobilization
- M1 excitability contralateral to immobilization is preserved with MI practice
- REM sleep duration is prolonged following MI practice
- Reduced sleep spindles over both M1s after immobilization is counteracted by MI practice

A period of immobilization impairs not only the musculoskeletal motor functions, but elicits substantial modifications at the brain level [1]. Specifically, short-term immobilization of the upper-limb (10 h to 24 h) may modulate the excitability of the primary motor cortex (M1) [2], reorganize sensorimotor functions [3], alter the sensorimotor representation of the nonused hand [4, 5], and affect sleep characteristics [6]. Previous research established that ensuring a stream of sensory inputs during immobilization can prevent motor degradation [7, 8]. For instance, recent data showed that proprioceptive vibrations (80 Hz) delivered onto the immobilized arm contributed to prevent the depression of excitability over contralateral M1 [8]. Based on this finding, here we postulated that it might be possible to induce comparable benefits during arm-immobilization using motor imagery practice (MI). MI typically involves the mental rehearsal of an action through visual and proprioceptive inputs without any overt body movement [9]. In the clinical context, accumulated evidence suggests that MI is a reliable, cost-effective and easily implementable substitute for executed movements: MI activates motor networks, which in turn can prompt and/or promote functional recovery [10, 11]. In case of upper limb immobilization, however, contrasting results on MI efficiency have been reported, hence precluding general conclusions. Interestingly, MI practice during arm-immobilization was found to attenuate strength loss [12], prevent the impairment of movement preparation time [13] and of the sensorimotor representation of the immobilized limb [14]. Crew et al. [15] and Bassolino et al. [16] however failed to show that MI was effective in compensating the maladaptive plasticity induced by limb disuse. Further investigations are therefore required to clarify whether and how MI practice may alleviate some of the deleterious effects caused by short-term immobilization.

The effects of short-term immobilization have also been studied and revealed in the sleeping brain. Notably, Huber and collaborators [6] observed a reduction of slow wave activity (1-4 Hz) and spindles activity (13-14 Hz) over M1 contralateral to the immobilized arm, during the first NREM sleep period (< 1 hr) following a day with 12 h of immobilization. The authors reported that brain plasticity after immobilization may be linked to local sleep regulation, mirroring those induced after motor learning [6, 17, 18]. While it has been established that motor consolidation also occurred after MI, much like for actual motor practice, and yielded subsequent motor improvement [19, 20], changes in plasticity-related sleep features following MI are still underexplored. While Debarnot et al. [21] emphasized the importance of NREM sleep for effective MI consolidation, Speth and Speth [22] underlined the key role of REM sleep in mediating MI-related phenomenon. In addition to being a common clinical concern, limb immobilization might be a suitable experimental context to clarify the effects of daytime MI practice during immobilization on subsequent sleep features.

The present study used a randomized crossover within-subjects design to investigate whether MI training during arm-immobilization might counteract the adverse behavioral and neural effects observed after the immobilization period, as well as during subsequent sleep. We expected that MI practice during arm-immobilization would (i) prevent the decrement in the sensorimotor representation, (ii) preserve cortical excitability over M1, and (iii) normalize sleep features.

RESULTS

Fourteen healthy participants (mean age ± SD = 25.43 ± 2.56, nine women) were tested after three different daytime conditions of 11 h, which were separated by 1 week (Figure 1). All participants first spent 11 h of normal daily routine (i.e. Baseline condition), then they

underwent two conditions of 11-h immobilization of the right, dominant arm (in pseudo-random order), one condition with MI treatment (15 min each two hours; MI condition) and one control condition without MI but a neutral task during an equivalent time (NoMI condition). At the end of the 11-h periods (Baseline, MI, NoIM), all participants were tested for sensorimotor representations of both hands using a mental rotation task (i.e. hand laterality judgment task), cortical excitability of both M1s by means of transcranial magnetic stimulation (TMS), and sleep features using polysomnography (PSG) recordings. More details about the procedure can be found in the STAR Methods section.

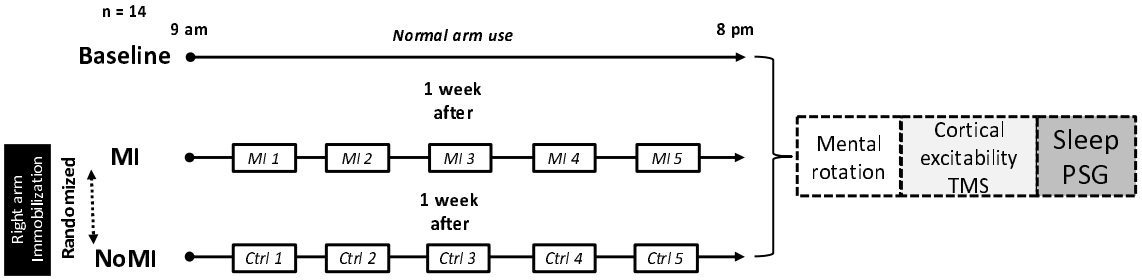

Figure 1: Experimental design. Participants performed three experimental conditions, separated by a week, to compare the effects of a normal 11 h awake period without immobilization (Baseline), 11 h of arm immobilization with MI treatment (MI condition) including 5 MI sessions, or a similar immobilization period without MI (NoMI condition). After each experimental condition, we assessed changes in the sensorimotor representation (mental rotation task) and cortical excitability (TMS) over both primary motor cortices. Then, PSG data were recorded during the night following each daytime condition.

Preliminary note

The mean score of the Stanford Sleepiness Scale assessed at 8 pm (i.e. before starting the multimodal assessment) did not differ as a function of conditions (mean +/- SD; Baseline: 1.92 ± .64, MI: 1.83 ± .55, NoMI: 2.23 ± .89; $F(2, 24) = 1.74$). Actigraphy data confirmed that all participants complied with the instructions regarding the right-arm immobilization procedure. A repeated-measures ANOVA on the MET (i.e. metabolic equivalent of

task)scores revealed main effects of CONDITION (Baseline, MI, NoMI); $F(2, 52) = 37.99$, $p < .001$, $\eta_p^2 = .59$) and HAND (Right, Left; $F(1, 26) = 135.29$, $p < .001$, $\eta_p^2 = .83$), as well as a significant CONDITION X HAND interaction ($F(2, 52) = 70.21$, $p < .001$, $\eta_p^2 = .73$). Bonferroni post-hoc tests showed that during the Baseline condition, physical activity with the right arm was higher relative to the left ($p < .01$), in line with the subjects' right hand dominance. Importantly, activity of the right immobilized arm significantly decreased in both MI and NoMI conditions compared to Baseline (all $p < .001$), and was significantly lower compared to the left arm activity ($p < .001$ for both MI and NoMI conditions). During both MI and NoMI conditions, activity of the left arm did not differ from that in Baseline ($p \geq .32$ for both comparisons), suggesting that the non-immobilized arm was not more used than usual during right-arm disuse. Regarding MI performance across the 5 MI sessions, participants reported achieving easily mental exercises (4.15 over a maximum score of 5) and reaching vivid imagery (3.87 over a maximum of 5; $p > .1$ for all comparisons between MI sessions).

Mental rotation tasks

Hand laterality judgment

An ANOVA performed on the percentage of correct responses did not yield a main effect of CONDITION ($F(2,104) = .85$, $p = .43$, $\eta_p^2 = .01$), but showed a main effect of CONFIGURATION (two or three dimensional hand stimuli; $F(1,52) = 10.21$, $p < .01$, $\eta_p^2 = .16$), a main effect of LATERALITY (right or left; $F(1,52) = 11.87$, $p < .01$, $\eta_p^2 = .18$), and a significant CONFIGURATION × LATERALITY interaction ($F(1,52) = 4.32$, $p < .05$, $\eta_p^2 = .07$). Post-hoc comparisons revealed that the percent of correct responses was higher for the 3D (95.31 ± 1.81) compared to the 2D (92.11 ± 1.99) configurations, and that left hand stimuli (96.14 ± 1.71) were more correctly identified compared to the right hand ones (91.96 ± 2.09).

For response times, an ANOVA with the same factors did not reveal an effect of LATERALITY ($F(1,52) = .45$, $p = .50$, $\eta_p^2 = .01$), but yielded a main effect CONFIGURATION ($F(1,52) = 4.54$, $p < .05$, $\eta_p^2 = .08$) and CONDITION ($F(2,104) = 24.05$, $p < .001$, $\eta_p^2 = .32$), but no interaction between those factors. Bonferroni post-hoc tests revealed that participants were faster at recognizing 3D (vs. 2D; $p < .05$) configurations, and also both left and right hand stimuli after the MI condition compared to Baseline and NoMI conditions ($p < .001$ for each condition comparison with MI; Figure 2) whereas there was no difference between Baseline and NoMI ($p = .69$). Thus, these results showed that immobilization impacted hand representations after the NoMI condition (i.e. no improvement relative to the Baseline), while MI exercises during arm-immobilization had a beneficial impact on hand representations.

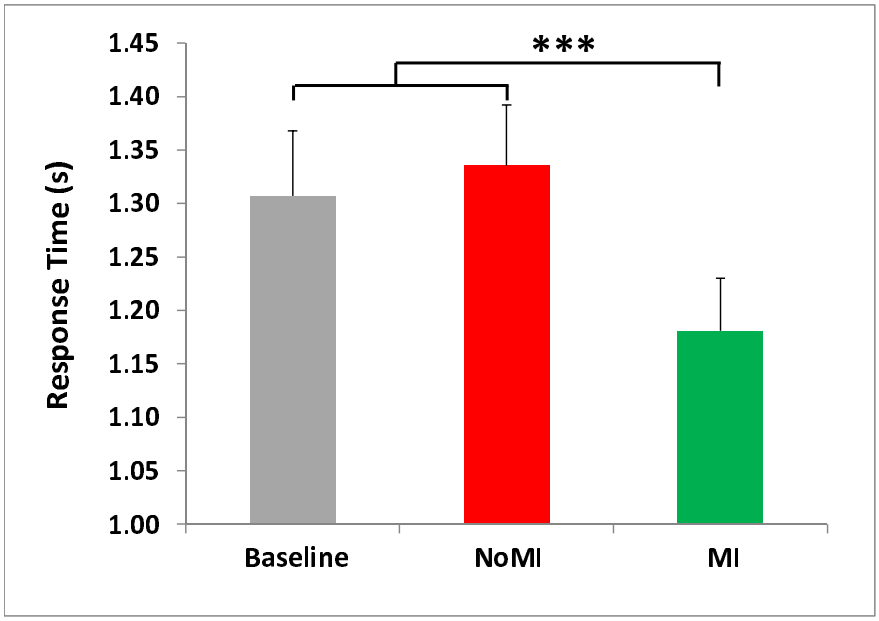

Figure 2: Response times during the mental rotation task. Independently of hand laterality, response times after the MI condition was significantly faster compared to Baseline and NoMI conditions. Error bars indicate the standard error of the mean. *** $p < .001$.

Alphanumeric normal-mirror judgment task (control task)

The ANOVA on the percentage of correct responses for the control mental rotation task revealed a significant effect of CONFIGURATION ($F(1,26) = 48.71$, $p < .0001$, $\eta_p^2 = .65$) which showed that participants were more accurate to identify the normal (94%) rather than the mirror (72%) configuration of stimuli. The ANOVA yielded CONDITION X CONFIGURATION interaction ($F(2,52) = 3.88.71$, $p < .0001$, $\eta_p^2 = .65$), and post hoc analyses only showed that normal stimuli were better identified after the MI compared to the Baseline ($p < .05$) but not compared to the NoMI condition ($p = .20$).

The ANOVA on response times showed a main effect of CONFIGURATION ($F(1,26) = 4.55$, $p < .05$, $\eta_p^2 = .15$), as well as a main effect of CONDITION ($F(2,52) = 4.36$, $p < .01$, $\eta_p^2 = .14$), but no CONDITION X CONFIGURATION interaction ($F(2,52) = .28$, $p = .75$, $\eta_p^2 = .01$). Again, post-hoc tests indicated faster responses for normal (vs. mirror) configurations in both MI and NoMI conditions compared to the Baseline ($p < .01$ and $p < .05$, respectively).

Together, the results from the mental rotation task suggest that while immobilization affected the sensorimotor representation of hand (hand laterality judgment) it did not in the control task, and MI training may somehow have led to better performance on these tasks possibly because they also rely on mental imagery skills.

Transcranial Magnetic stimulation

One participant was excluded from TMS analysis due to very high resting motor threshold (RMT > 80% machine output).

Corticomotor excitability

Left M1 (Contralateral to the immobilized arm)

Analysis of the resting motor threshold (RMT) in left M1 showed a main CONDITION effect ($F(2,24) = 3.57$, $p < .05$, $\eta_p^2 = .23$), and post-hoc analyses revealed a higher RMT in the MI condition compared to the Baseline ($p < .01$). Comparing the recruitment curve (RC) data

between conditions (Figure 3A) revealed a main effect of INTENSITY ($F(4,46) = 10.44$, $p < .001$, $\eta p2 = .47$), a main effect of CONDITION ($F(2,98) = 19.86$, $p < .001$, $\eta p2 = .30$), as well as a significant INTENSITY X CONDITION interaction ($F(8,92) = 2.27$, $p < .05$, $\eta p2 = .16$). Post-hoc analyses showed that the motor evoked potential (MEP) amplitude recorded after the NoMI condition was significantly lower than after Baseline at 15% ($p < .01$), 20% ($p < .001$) and 25% ($p < .001$) of maximum stimulator output (MSO). For the MI condition, MEP amplitudes were less than during the Baseline conditions only at 25% MSO ($p < .05$). Furthermore, lower MEP at 20% ($p < .01$) and 25% ($p < .001$) were observed between the NoMI and the MI conditions, hence providing further evidence of the positive impact of MI training during arm immobilization. Most importantly, ANOVA performed on slope of left M1 showed an effect of CONDITION ($F(2,24) = 7.73$, $p < .01$, $\eta_p^2 = .39$), and further post-hoc confirmed that MI treatment was able to cope against the reduction caused by immobilization on corticospinal excitability. Indeed, while immobilization reduced the slope of left M1 after the NoMI condition relative to Baseline ($p <.001$), there was only a non-significant trend when comparing the MI and the Baseline conditions ($p = .06$, Figure 3B). No significant correlation between the RC (at 15%, 20% and 25%) and the slopes of NoMI and MI conditions (relative to Baseline values) were found with response time data on the mental rotation task (i.e. independently of the hand stimuli, as no laterality effect was previously found).

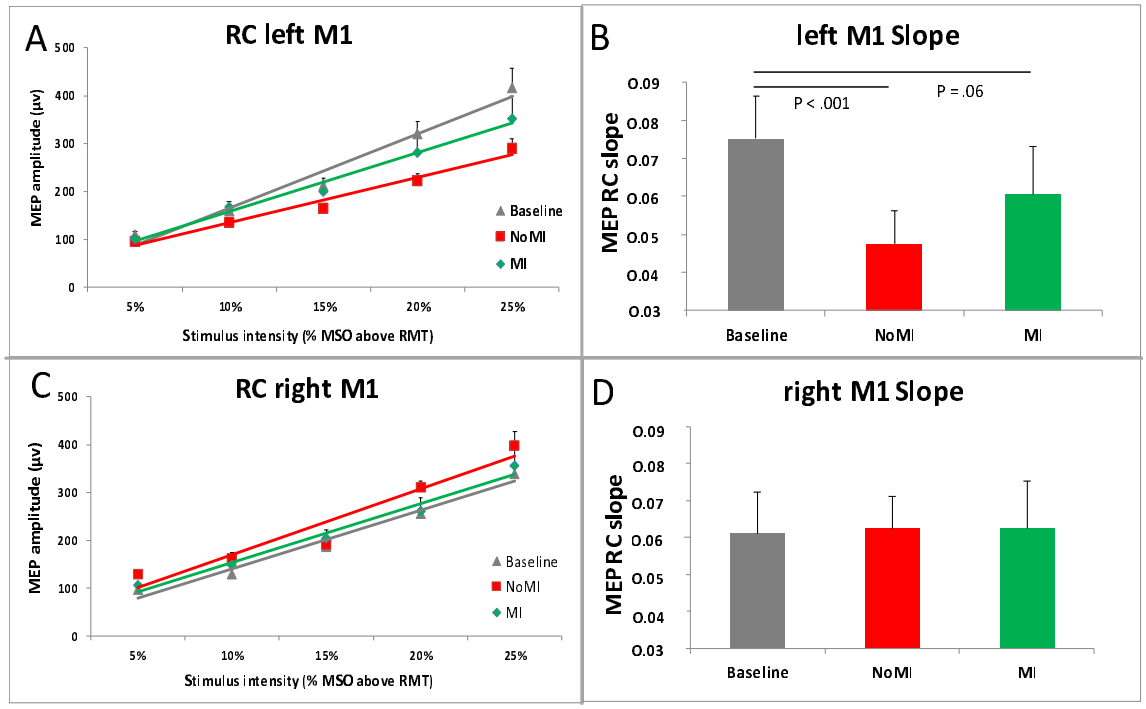

Figure 3: Decrease of cortical excitability over left and right M1 after MI. (A) MEP amplitude from the left M1 at increasing strength of MSO intensity after Baseline, NoMI, and MI conditions. (B) Slope data showed a significant reduction in the cortical excitability over left M1 after immobilization alone (NoMI) relative to the Baseline condition, while MI practice limited the deleterious effect of impairment. (C) MEP amplitudes over right M1. (D) Slope data showed no significant differences over the right M1 as a function of conditions. Key: RC, recruitment curve; MEP, motor evoked potential; MSO, maximum stimulator output; RMT, resting motor threshold; M1, primary motor cortex.

Right M1 (ipsilateral to the immobilized arm)

First, the RMT of right M1 were equivalent in the three experimental conditions ($F(2,24) = 1.06$, $p = .36$, $\eta_p^2 = .08$). RC data revealed a significant main effect of INTENSITY ($F(4, 38) = 33.02$, $p < .001$, $\eta_p^2 = .77$), and a main effect of CONDITION ($F(2, 76) = 3.36$, $p < .05$, $\eta_p^2 = .08$), due to globally higher MEP after NoMI compared to both Baseline and MI (all P = .06), but no INTENSITY X CONDITION interaction ($F(8, 76) = .51$, $p = .85$, $\eta_p^2 = .05$; Figure 3C). Moreover, the ANOVA on the slope of right M1 did not show any difference between conditions (Figure 3D) (Baseline vs. NoMI: $p = .89$; Baseline vs. MI: $p = .83$; NoMI vs MI: $p = .62$).

POLYSOMOGRAPHIC DATA

Three subject were excluded from spindles and spectral power analysis due to poor EEG signal quality, while sleep scoring was still possible for these participants for the three experimental nights.

SLEEP ARCHITECTURE

Participants showed normal sleep patterns across the three recorded nights (Supp. Table 2). Analysis of sleep architecture during the 1st sleep cycle revealed a significant interaction between conditions and sleep stage duration ($F(2, 26) = 7.37$; $p = .003$), due to an increase in the time spent in REM after MI treatment (10.73% ± 2.02%), as compared to NoMI (12.92 ± 1.29, $p = .02$) and Baseline (10.78% ± 1.43%, $p = .003$; Figure 4A) conditions. A similar analysis of the sleep architecture on the whole night data showed a significant interaction ($F(2, 26) = 5.33$; $p < .01$) due to an increase in REM duration after MI condition (27.17% ± 1.69%) relative to Baseline condition (22.57% ± 1.22%, $p < .01$), but not to NoMI (26.42% ± 1.14%, $p > .05$; Figure 5A). No other changes (duration of wakefulness or total sleep duration measured by time in bed, total sleep period [TSP], and total sleep time [TST]; all $p > .05$) were found between conditions during the 1st cycle of sleep, or during the whole night. No correlation emerged between the duration (min) and TSP (%) in REM sleep (1st cycle or whole night) and response time data on the mental rotation task nor on both [MI – Baseline] and [NoMI – Baseline] MEP slopes.

Spindles during the 1st NREM sleep period

We first look at the first cycle of sleep as previous data by Huber et al. [6] indicated that a decrease of spindle activity mostly occurred at the beginning of the night following 12h of arm-immobilization. Spindles data acquired during the 1st NREM cycle revealed significant a main effect of CONDITION for both the NUMBER of sleep spindles (N2 +N3) over left M1 ($F(2, 24) = 6.74$, $p < .01$) and right M1 ($F(2, 24) = 8.32$, $p < .01$), and DENSITY over the left M1 ($F(2,24) = 7.66$, $p < .01$), and right M1 ($F(2,24) = 9.32$, $p < .01$) (Supp. Table 3). Bonferroni post-hoc tests revealed a decreased number of spindles after the NoMI condition relative to the Baseline and MI conditions over motor areas ($p < .01$ over the left and right M1), as well as compared to the MI condition ($p < .05$ for both M1s). Spindle density was reduced in the NoMI condition over both M1s as compared to Baseline ($p < .01$ over the left and right M1) and MI condition ($p < .05$ over the left and right M1Left, Figure 4 B and C). Importantly, there was no differences between Baseline and MI condition over both M1s ($p = 1.0$ for each M1). No correlation between the number of spindles at each derivation and response time data on the mental rotation task, nor on both [MI – Baseline] and [NoMI – Baseline] MEP slopes were observed.

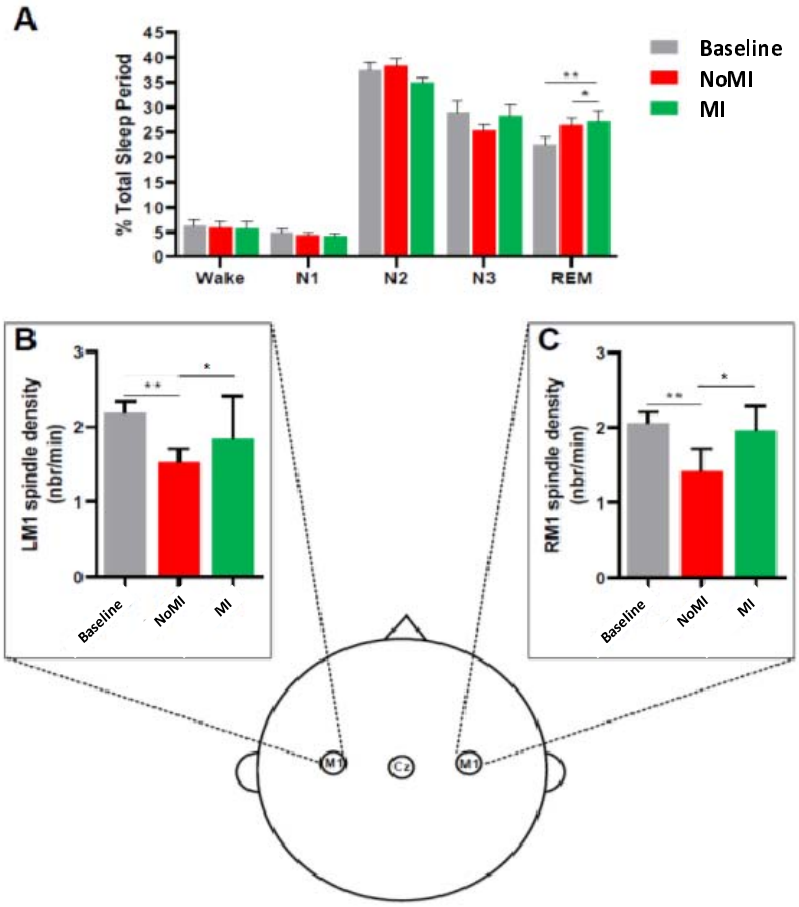

Figure 4: Sleep architecture and spindles density over both M1 during the 1st cycle of sleep after each condition (Baseline, MI, NoMI). (A) Percentage of total sleep period for each sleep phase and condition. REM sleep was longer in the MI condition relative to both Baseline and NoMI. (B) Lower spindle density over the Left M_1 and (C) Right M_1 in NoMI compared to Baseline and MI conditions.

Spindles during the NREM of the whole night

A similar pattern of results was found on the NUMBER of spindles during the whole NREM sleep (N2 + N3) with main effects of CONDITION over Cz ($F(2, 24) = 3.63$, $p < .05$), left M1 ($F(2, 24) = 12.69$, $p < .001$), and right M1 ($F(2, 24) = 14.51$, $p < .001$), with similar effect for DENSITY measures over Cz ($F(2,24) = 5.82$, $p < .01$) left M1 ($F(2,24) = 10.95$, $p < .001$), and right M1 ($F(2,24) = 12.29$, $p < .001$; Supp. Table 4). Bonferroni post-hoc showed that the number of spindles remained lower during the NoMI condition compared to Baseline over both motor areas (all p values < .001), and only over the right M1 relative to MI condition ($p < .05$) but

not over the left M1 (p = .24). Number of spindles decreased in the MI condition compared to the Baseline over the Left M1 (p < .05) but not over the right M1 (p = .11). Spindle density was reduced in the NoMI condition over both M1s as compared to Baseline (p < .001 over the left and right M1) and MI condition (p < .05 over the left and p < .01 over the right M1, Figure 5 B and C). Importantly, there was no differences between Baseline and MI condition over both M1s over both M1s (p > .10 for each M1). No correlation was observed between the number of spindles at each derivation and response time data on the mental rotation task, nor on both [MI – Baseline] and [NoMI – Baseline] MEP slopes were observed.

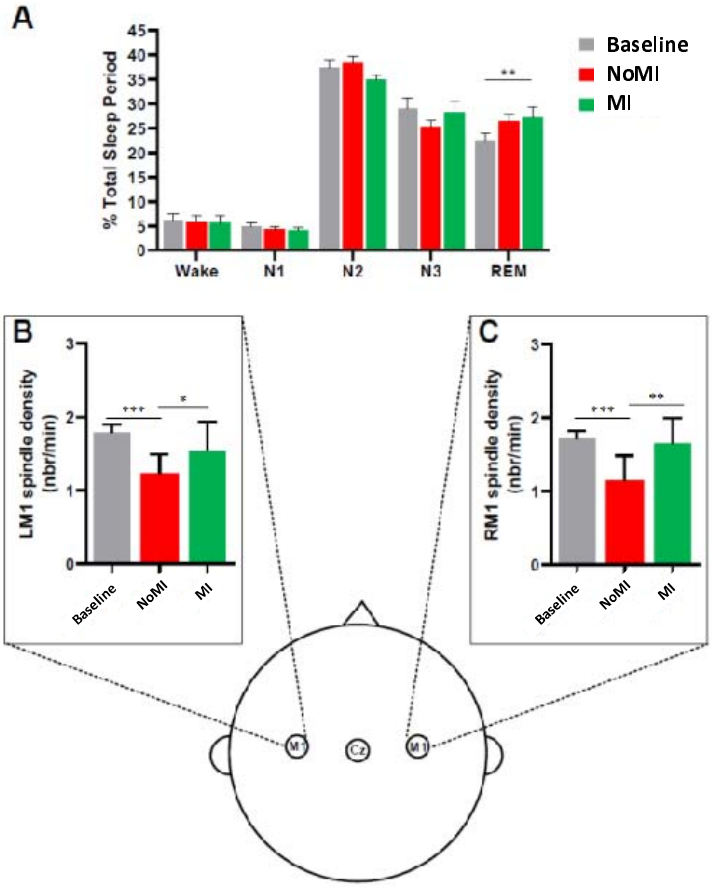

Figure 5: Sleep architecture and spindles density over both M1 during whole night of sleep after each condition (Baseline, MI, NoMI). (A) Percentage of total sleep period for each sleep phase and condition. REM sleep remained longer in the MI condition relative to the Baseline.

(B) Lower spindle density over the Left M1 and (C) Right M1 in NoMI compared to Baseline and MI conditions.

EEG Spectral analysis

ANOVARM performed separately on Cz and both M1s channels during NREM (N2, N3) and REM of the 1st cycle of sleep, with CONDITION and FREQUENCY BANDS, only yielded a main effect of FREQUENCY BANDS ($p < .001$ SO, delta, theta, slow spindle, fast spindle, beta, and alpha), but no effect of CONDITIONS ($p \geq .11$ for each channel and phase). Similarly, analysis performed on the EEG spectrum of the whole night showed a main effect of FREQUENCY BANDS ($p < .001$ on each channel).

DISCUSSION

The present study was designed to investigate the effects of MI practice administered during 11 h of arm-immobilization on the sensorimotor and cortical representations of both hands, as well as on sleep features. MI practice during unilateral immobilization was found to substantially prevent the degradation of sensorimotor representation. Likewise, MI practice allowed for the maintenance of the cortical excitability over left M1 contralateral to the arm-immobilization. Furthermore, the time spent in REM sleep was significantly longer during the 1st cycle of sleep following the MI condition compared to that after Baseline and NoMI conditions. Finally, data revealed that, while immobilization decreased the number of sleep spindles compared to the condition without immobilization, this decrease was no longer significant after the MI condition over both M1s.

Effect of MI practice on the sensorimotor representation of hands

The first important finding of the present study is that mental rotation performance improved after the MI but not the NoMI condition, for both right and left hand stimuli. The lack of task-repetition benefit after the NoMI condition for both right and left hand stimuli

confirms and extends previous findings by Toussaint et al. [23] obtained after 48h of non-dominant hand immobilization. Performance gains observed following the MI condition further support previous data showing that 15 min of kinesthetic MI performed right before splint removal following 24 hr of left hand immobilization, contributed to improve the response time to identify left hand stimuli [14]. Based on this result, kinesthetic MI was thought to reactivate proprioceptive and motor processing, counteracting the effects of immobilization. In the present study, participants were involved in extensive MI practice (75 min vs. 15 min), with the last MI session performed 2 h before the splint removal. Present results therefore support that practicing MI several times during the 12 h of arm-immobilization may have not only transiently activated sensorimotor processing, but also promoted its maintenance at a normal functioning level. Interestingly, a recent neuroimaging study conducted by Burianova et al. [3] showed that 24 h of hand immobilization decrease the neural activation underpinning MI practice, specifically over the sensorimotor areas contralateral to the limb disused. Taken together, these observations suggest that reactivation of the sensorimotor system by means of MI practice during immobilization contributed to prevent such maladaptive brain plasticity. This issue is of particular importance in the clinical context where MI is increasingly used to reactivate injured sensorimotor networks to assist the recovery of lost motor functions [24-26].

Effect of MI practice on corticomotor excitability

The second remarkable result of the present experiment is that MI prevented the decrease of cortical excitability in the left M1 caused by the contralateral arm-immobilization. As expected, we found a significant reduction in the slope of RC data after the NoMI condition, hence reflecting the typical local synaptic depression induced by unilateral short-term immobilization [2, 6, 8, 27]. Such downregulation of left M1 excitability was not observed

after the MI condition. This finding challenges data by Bassolino et al. [16] and Crew et al. [15] who showed that MI training did not prevent corticomotor depression after upper-limb immobilization. Such discrepancy may be due to differences in the nature and dosage of the MI intervention. In the present study, the experimenter guided participants to mentally rehearse movements using different upper-limb joints, i.e., wrist, elbow and shoulder, and the MI program was structured with a regular increment in task complexity up to bilateral upper-limb movements. This thorough and progressive MI training design differs from previous studies where participants performed MI of simple arm/hand movements without any emphasis on the voluntary/effortful engagement of complex motor representations. Moreover, instead of an interactive and tailored guidance, previous studies used audiotaped guidelines or visual stimuli displayed on a computer screen. Crucially, the overall time spent to practice MI was significantly longer in the present study (i.e., 75 min vs. 40 min [16] and 30 min across 3 successive days [15]). We therefore postulate that a relatively heavy MI intervention might be needed to promote significant M1s (re)activation, hence protecting against loss of sensorimotor functions during limb immobilization. This assumption is also supported by reports in the motor learning domain showing that MI increases corticospinal excitability in M1 and that such increase is specific to the representation of the limb involved during imagery [28, 29] .

Effect of MI practice on NREM and REM sleep

A third original finding from the present study is that MI practice during immobilization elicited an increase in the time spend in REM sleep. Furthermore, and relative to Baseline, the density of sleep spindles over both M1s after the MI condition remained stable, while it decreased in the NoMI condition. Motor circuits are known to be activated during REM sleep [30-32]; this activation may support MI in dreams and the consolidation of motor skills

learning [22, 33-39], although a causal role of REM sleep for motor learning is still debated [40]. Our result is further consistent with clinical data providing evidence that patients with REM sleep behavior disorders exhibit a large range of motor behaviors while enacting their dreams [41, 42]. Although in the current experimental design we did not test whether increased REM sleep after MI induced better consolidation of MI skills, it is likely that practicing MI exercises during arm-immobilization might have potentiated the demand for REM sleep related motor rehearsal and consolidation, yielding longer REM duration. As expected and consistent with the findings from Huber and collaborators [6], we found that 11 h of immobilization decreased the density of spindles over both M1s after the NoMI condition. Importantly, spindle density over motor regions after the MI condition was unchanged as compared to Baseline. Moreover, sleep spindles may play a critical role in memory reactivation and consolidation during NREM sleep, including for procedural learning [43, 44]. In line with this hypothesis, Debarnot et al. [21] reported similar MI performance gains after both a short (10 min NREM) and long (NREM and REM) intervening naps (compared to wake period), suggesting that NREM sleep, and particularly spindles activity may have contributed to the consolidation process.

To conclude, present results provide strong evidence that MI practice can alleviate the deleterious impact of arm-immobilization on sensorimotor representations, and motor cortex excitability. Our findings also reveal lasting effects of MI practice on subsequent NREM sleep (limitation of the reduction in spindle number) and REM sleep (longer REM duration). Overall, MI contributed to reactivate sensorimotor networks suboptimally activated during immobilization, hence protecting for the maladaptive neuroplasticity occurring during and after immobilization. Based on these observations, we would like to suggest that an early integration of MI in the context of limb disuse might help to promote

recovery of lost motor functions (e.g. in the classical course of physical therapy after orthopaedic intervention). Moreover, MI is increasingly used to control external device such as in electroencephalographic-based brain computer interfaces [45]. Early MI practice after immobilization or in post-stroke rehabilitation programs might help to preserve the cortical excitability over the contralateral M1 to the limb disused, and thereby increase the opportunity for the patient to benefit from brain computer interface-based interventions.

STAR METHODS

Participants

To be eligible for the study, the fourteen healthy participants had to comply with the following criteria: (1) be right-handed according to the Edinburgh inventory [score >70, 46]; (2) have a Pittsburg Sleep Quality Index < 5 [47] ; (3) have an intermediate circadian typology score from 42 to 58 [48]; (4) have Epworth Sleepiness Scale score < 11; (5) have a Beck Depression inventory score < 9; (6) have a total MI ability score on the Movement Imagery Questionnaire ≥ 63 [corresponding to 75% of the maximum score; MIQ-3, 49], and (7) have no previous history of orthopedic problems for the right hand and arm. Exclusion criteria included (1) the presence of ferromagnetic metallic implants or a pacemaker; (2) previous neurosurgery, or (3) history of seizures, major head trauma, alcoholism, drug addiction, or any psychiatric or neurological disorder. All participants were instructed to be alcohol and caffeine free for 24hr hours prior to and during the experimental days. The protocol was carried out in accordance with ethical standards and was approved by the ethical committee of the Geneva University Hospital. After the nature of the procedure had been fully explained, participants gave written informed consent before inclusion. All participants were naive to the purposes of the study and received an attendance fee.

Immobilization procedure

During the immobilization conditions, participants were requested to not move the right arm (from the fingers to the shoulder) for 11 h from the morning (9 am) until the beginning of the mental rotation task (8 pm). The duration of the sensorimotor deprivation was chosen based on recent reports demonstrating modifications of M1s excitability [27], with additional local changes during the subsequent night of sleep [6]. Here, we used a silk glove to reduce the contact between each finger. We further put on a splint, typically used in clinical practice (FERULA, Malleable aluminum thumb-hand right, model "OM6101D/2") to ensure a complete immobilization of the wrist, as well as the carpometacarpal and metacarpophalangeal joints of all fingers. In addition, a soft shoulder and elbow splint was used to support the forearm in a comfortable way during the 11-h immobilization (DONJOY model IMMO, "DGO GLOBAL").

To ensure that immobilization was effective, we quantitatively monitored physical activity of both arms during the three experimental conditions, using actimetry (Actigraph GT3X-BT, Pensacola, Florida, USA) on their left and right forearms. Data were sampled at 30 Hz. Energy cost of physical activity was calculated in metabolic equivalent of task (MET), i.e. one MET equals the resting metabolic rate obtained during quiet sitting [50]. We also verified that all subjects maintained a regular sleep-wake schedule with a minimum of 7 h of sleep per night, at least three days before each experimental day. Compliance to the schedule was assessed using both sleep diaries and wrist actigraphy measures. Finally, every hour during the 3 experimental days (i.e. 12 times for each experimental day), all participants assessed their current state of alertness by filling out the Stanford Sleepiness Scale [51].

Motor imagery sessions

During the MI condition, participants performed 5 sessions (one session every two hours, 15 min each) of mental training with the immobilized arm, in a quiet room, without any

distracting stimuli in order to help them to focus on MI exercises. The content of MI exercises was varied and included monoarticular and polyarticular movements. Specifically, participants were requested to mentally perform 15 different "monoarticular" movements (e.g. flexion/extension movements of the wrist) with the right-immobilized arm during 1 min each during the two first MI sessions. Then, during the three next MI sessions, they performed five different and complex "polyarticular" movements (e.g. throwing a ball) during 3 min each, i.e. each movement was performed 2 min first and then repeated again during 1 min in order to minimize mental fatigue resulting from maintaining focused attention. For each exercise, the experimenter gave precise instructions about the movement to be performed and asked participants to either sit or stand during MI in order to facilitate the postural congruency related to the movement (e.g. finger tapping was performed in a seated position, performing arm circle was executed while standing up). If needed, participants could first practice the movement with the non-immobilized arm prior to MI rehearsal with the immobilized arm [52]. They were asked to imagine themselves performing movements using a combination of visual and kinaesthetic imagery, i.e. imagining movement within one's own body and perceiving the induced proprioceptive sensations. They were instructed to say "go" when they started the mental simulation of movement with the actual immobilized arm. The experimenter watched the participant continuously in order to check that no physical movement with the immobilized arm was produced during MI, and used a stopwatch to indicate the end of an exercise to the participant. After each exercise, participants were asked to rate the difficulty and the quality of their mental images using two Liker-type scales (from 1 = very difficult/poor mental representation to 5 = very easy/vivid mental representation).

Post-immobilization/baseline assessment of sensorimotor functions

Mental rotation tasks

After the 11-h immobilization or baseline period, participants were administered two mental rotation tasks, i.e. a hand laterality judgment task and an alphanumeric normal-mirror judgment task (control task). They sat on a chair at a distance of 50 cm from a 17-inch computer screen with their hands on their knees. They were in a quiet room, without any distracting stimuli, in order to help them to focus on the task. In the hand laterality judgment task, participants were asked to identify whether a hand stimulus presented visually on the screen was depicting a left or right hand. Hand stimuli were either in two dimensional (dorsum or palm view with four possible orientations: 0°, 90°, 180°, or 270°) or three dimensional (palm from finger views, dorsum from wrist views, palm from wrist views, or dorsum from finger views). In the alphanumeric normal-mirror judgment task, participants had to determine whether the letter "R" or number "2" were presented in a normal or mirror view. Alphanumeric stimuli could be rotated by 0°, 90°, 180°, or 270°. Upright hand and alphanumeric stimuli had 13 cm in height and 7 cm in width.

At the beginning of each trial, a fixation cross was displayed (0.5 s) in the center of the screen. Then, the stimulus was presented and remained visible during 2.5 s. Participants gave a verbal response (left/right in the hand mental rotation task; yes/no in the alphanumeric mental rotation task for normal or mirror image, respectively). Verbal responses were recorded using a microphone connected to the computer. Response time was defined by the time from stimulus onset to the beginning of the audio signal corresponding to the participants' response. For each trial, the experimenter manually collected response accuracy.

During the Baseline condition, all participants familiarized with the hand laterality judgment task which consisted in practicing 6 trials of hand stimuli. Right after, the mental

rotation test included four randomized blocks of hand laterality judgment task (two blocks of two dimensional, and two blocks of three dimensional items). Each block contained 16 trials with 8 left and 8 right hand photographs, presented in a random order. Then, participants familiarized with the alphanumeric normal-mirror judgment task which consisted in practicing 6 trials of alphanumeric stimuli (three letters and three numbers). Right after, they performed two blocks of 16 trials with 8 normal and 8 mirror alphanumeric items.

The hand mental rotation task was always performed before the alphanumeric mental rotation task, as Toussaint and Meugnot [23] demonstrated that effects induced by arm immobilization may be reduced when participants first perform a non-body mental rotation task. During each condition (Baseline, MI and NoMI), participants thus completed a total of 64 trials of hand laterality judgment task and 32 trials of alphanumeric normal-mirror judgment task. During the MI and NoMI conditions, participants were no longer familiarized with the tests.

Stimulus presentation, data recording and analysis were handled by a homemade MATLAB program (The MathWorks, Natick, MA, USA), incorporating the Psychtoolbox (Brainard, 1997).

Transcranial Magnetic Stimulation

We used TMS to assess cortical excitability of left M1 (contralateral to the immobilized-arm) and then right M1 (ipsilateral to the immobilized-arm) by means of resting motor threshold (RMT) and recruitment curve (RC) after each condition. A figure-of-eight coil with wing diameters of 70 mm, connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK) was placed tangentially on the scalp over M1; the handle pointed backward and laterally at a 45° angle to the sagittal plane inducing a posteroanterior current in the brain. First, we identified the optimal spot for eliciting motor-evoked potentials (MEPs) in the right

first dorsal interosseous (FDI) muscle and the location was marked on an EEG cap fitted on the participant's head to provide a reference landmark throughout each TMS session. Additionally, motor cortices marks over the cap was also visible on the scalp of participants to attach EEG electrodes for sleep polysomnographic recording. The RMT was defined as the minimal TMS intensity required to evoke MEPs of about 50 µV peak-to-peak in the targeted muscle in 5 out of 10 consecutive trials and was expressed as a percentage of maximum stimulator output (MSO). Once we found the RMT over left M1, RC was assessed by measuring peak-to-peak amplitude (expressed in µV) of MEPs elicited at stimulus intensities of 5%, 10%, 15%, 20%, and 25% of MSO above individual RMT (obtained each experimental day). Five trials were recorded at each intensity, and MEP amplitude was then averaged. The same RMT and RC procedure was then performed over right M1.

Electromyography recording

Electromyography (EMG) was recorded and monitored continuously on-line during each TMS sessions. Similar EMG montage was carry out bilaterally using active electrodes attached to the skin overlying first dorsal interosseous muscle, reference electrodes placed over the first metacarpophalangeal joints and ground electrodes placed over the wrist bone. AcqKnowledge 4 software along with MP36 unit (Biopac systems, Goleta, CA, USA) was used to acquire EMG signal. The EMG signals were amplified and filtered online (20 Hz to 1 kHz), sampled at a rate of 5000 Hz and stored on a personal computer for display and later offline data analysis. Trials with background EMG activity were excluded from analysis.

Polysomnographic recording

Data acquisition

PSG data were acquired in all participants during three nights of sleep following Baseline, MI and NoMI conditions, with a V-Amp 16 system (Brain Products, Gilching, Germany), from 11

EEG electrodes (international 10–20 system: LM1, CZ, RM1, F3, FZ, F4, P3, PZ, P4, A1, A2), 3 electrooculogram electrodes, and 2 chin electromyogram electrodes (sampling rate: 250 Hz).

Sleep data analysis

For polysomographic analyses, we used the PRANA software (version 10.1. Strasbourg, France: Phitools) and custom written MATLAB scripts and function (MATLAB R2009b, Natick, Massachusetts: The MathWorks Inc., 2009). Two scorers blind to the experimental conditions determined the different sleep stages (NREM 1, 2, 3, REM sleep and wake) for each recorded night of sleep on 30-s epochs. From the scoring of the sleep architecture, we computed the duration (min) of each sleep stage, as well as the percentage of each sleep stage relative to the total sleep period (TSP; from sleep onset to wake up time) and relative to the total sleep time (TST; TSP minus intra-wake epochs). Sleep efficiency (TST/time in bed*100), and number of intra-awakenings were also calculated. Epochs affected by artefacts were excluded from further quantitative EEG analysis. The EEG spectrum power average was calculated with a 0.2 Hz resolution, by applying a Fast Fourier Transformation (FFT; 50% overlapping, 5 s windows, Hanning filter) using the each channel derivations. Mean power was calculated for each 0.2 bins between 0.4 and 30 Hz and for the following frequency bands: slow oscillations (0.4-1 Hz), delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), spindle power range (12-15 Hz), and beta (15-30 Hz). Following the procedure of Ray and colleagues [53], spindles were detected automatically using the PRANA software. The detection was performed on each channel following the global standards used in sleep research [43, 54]: spindles were required to have a duration between 0.5 s and 3 s, an inter-spindle interval no less than 1 s, and fusiform (waxing and waning amplitude) with typical spindle morphology. An experienced scorer visually supervised all detected spindle for each night of each participant.

Data analysis

For the mental rotation task, we analyzed the percentage of correct responses and mean response time. Only data from correct responses were used to analyze response times. Results obtained on the hand laterality judgment for the three conditions were evaluated by means of ANOVA with CONDITIONS (Baseline, MI and NoMI), LATERALITY (left, right) and CONFIGURATION (two or three dimensional) as within-subjects factors. Likewise, an ANOVA was performed for the alphanumeric normal-mirror judgment task, with CONDITIONS (Baseline, MI and NoMI), CONFIGURATION (normal, mirror) and ORIENTATION (0°, 90°, 180°, or 270°) as within-subjects factors.

To evaluate the effects of immobilization on cortical excitability, we compared the resting motor threshold (RMT) of the left and right M1 separately for each condition (Baseline, MI and NoMI) by means of ANOVA. Recruitment curve (RC) values were analyzed separately for each hemisphere by means of an ANOVA with CONDITIONS (Baseline, MI and NoMI) and INTENSITY (5%, 10%, 15%, 20%, and 25% of MSO above RMT) as within-subjects factors. Using an ANOVA (and post-hoc t tests), we then evaluated the differences between the slopes of the 3 regression lines obtained from the data in the different experimental CONDITIONS (Baseline, MI and NoMI), separately for the left and right M1. We further examined the relationship between cortical excitability (RC and slope) modulations, mental rotation performance (sensorimotor representation of both arm), and sleep features by performing Pearson's correlations.

To examine whether daytime CONDITIONS (Baseline, MI and NoMI) influenced sleep architecture, several ANOVAs were performed on sleep stage variables. We also analysed spindle features (number, density, duration, peak amplitude and mean frequency) by means of ANOVAs with CONDITIONS as within-subjects factor. Similar analyses were performed on

each power SPECTRUM BANDS from channels Cz, LM1, RM1 with CONDITIONS as within-subjects factor. All sleep analyses (sleep architecture, spindles features and spectral power data) were conducted separately during whole night of sleep and the 1st sleep cycle.

Both the Stanford Sleepiness Scale and MET scores for the Baseline, MI, NoMI conditions were evaluated by means of ANOVA.

Whenever interactions were significant, the origin of the interaction was investigated using planned comparisons with Bonferroni corrected post-hoc tests. The alpha-level of all analyses was set at $p < .05$.

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