Benefits of motor imagery practice during arm-immobilization on the sensorimotor cortical networks and sleep features

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Abstract

Limb disuse has overt substantial measurable maladaptive consequences, which are reinforced during the sleep consolidation process, hence highlighting that a continuous stream of sensory inputs and motor outputs are necessary for the maintenance of intact brain organization. Motor Imagery (MI), which corresponds to the mental representation of movement without its actual execution, is known to significantly activate motor networks, even when overt movement are no longer possible. Whether MI might rapidly compensate the maladaptive neuroplasticity elicited by immobilization remains however a working hypothesis, which has received far less attention. Here, we investigated the potential benefits of MI practice during 12 hr of unilateral arm-immobilization on the sensorimotor and cortical representations of the arms, as well as during subsequent sleep. Using a within-subject design, fourteen participants were first tested after a normal day of motor activity (without immobilization, NoImmo), followed by two randomized 12 hr conditions separated by 1 week, whereby their right (dominant) arm was immobilized either with MI practice 15 min every two hours (ImMI), or without MI (ImCtrl). After each daytime condition, participants were tested on a hand laterality judgment task to assess changes in the sensorimotor representation of the hands, followed by the measure of cortical excitability over both primary motor cortices (M_1). Polysomnography recordings were also monitored overnight to investigate the sleep-related consolidation effects. Data revealed that MI practice prevented *i*) the slow-down in the sensorimotor representation of hands, *ii*) the cortical excitability depression over the M_1 contralateral to arm-immobilization, *iii*) the sleep spindles reduction over both M_1s, and *iv*) extended the time spent in REM sleep. Altogether, these results provide strong evidence that implementing MI within the context of limb-

immobilization contributes to substantially limit the deleterious effect of disuse, and may further promote recovery of motor function.

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

Highlights

- MI prevents impaired sensorimotor representation caused by immobilization
- M_1 excitability contralateral to immobilization is preserved with mental practice
- REM sleep duration is prolonged following MI practice
- The impact of immobilization on spindles occurrence over both M_1s is counteracted by MI practice

A period of immobilization has been extensively shown to impair both musculoskeletal motor functions, associated with substantial modifications at the brain level [1]. Yet, several studies provided experimental evidence that short-term immobilization of the upper-limb (10 hr to 24 hr) *i*) modulated the cortical excitability in both primary motor cortices (M_{1s}) [2], *ii*) reorganized the sensorimotor system [3], *iii*) altered the sensorimotor representation of the nonuse hand [4, 5], and *iv*) affected sleep characteristics [6]. This better understanding of the multifaceted deleterious effects resulting from arm-immobilization contributes to design appropriate interventions to optimize and promote functional recovery.

From a practical viewpoint, previous research established that a stream of sensory input during immobilization may contribute to maintain intact brain organization [7, 8]. For instance, recent data showed that proprioceptive vibration (80 Hz) delivered onto the immobilized arm contributed to prevent the depression of excitability over contralateral M_1 [8]. Spurred by this finding, we postulate that it might be possible to induce comparable benefits during arm-immobilization using motor imagery practice (MI), which is the mental rehearsal of an action through visual and proprioceptive inputs without any overt body movement [9]. In the clinical context, accumulated evidence demonstrated that MI is a reliable, cost-effective and easy feasible substitute for executed movement as a means to activate motor networks, which in turn prompted and promoted functional recovery [10, 11]. In case of upper limb immobilization, however, contrasting results on MI efficiency have been reported, hence precluding from drawing general conclusions. Interestingly, MI practice during arm-immobilization was found to attenuate strength loss [12], prevent the impairment of movement preparation time [13], and counteract the slowdown in 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 disentangle whether MI practice might prevent the deleterious effects caused by short-term immobilization.

In contrast to the motor learning context, the effect of short-term immobilization has further been studied in the sleeping brain. Notably, Huber and collaborators [6] observed a reduction of slow wave activity (1-4 Hz) and spindles activity (12-15 Hz) over contralateral M_1 to the immobilized arm, during the first NREM sleep period (< 1 hr). The authors reported that brain plasticity after immobilization may be linked to local sleep regulations mirroring those induced after motor learning, most especially during the first cycle of NREM sleep [6, 17, 18]. Yet, it has been established that motor consolidation was also reported after MI, much like for actual motor practice, and yielded subsequent motor improvement [19, 20]. Yet, little attention has been paid to the specific stages or features of sleep that are critical for discrete steps in motor-memory consolidation following MI. While Debarnot et al. [21] emphasized the importance of NREM sleep for effective MI consolidation, Speth and Speth [22] rather underlined the key role of REM sleep in mediating MI. The peculiar context of limb immobilization, while being a common clinical concern, might be a suitable context to explore the daytime effect of MI practice during immobilization on sleep features.

Spurred by the findings mentioned above, the present study aimed to investigate whether MI training during arm-immobilization might counteract the adverse effects induced during an arm-immobilization. Specifically, we addressed, in a randomized crossover design, the effectiveness of MI practice during arm immobilization, and examined how it may trigger the subsequent night of sleep characteristics. We expected that MI practice

during arm-immobilization would *i*) compensate for the slowdown of the sensorimotor representation, *ii*) preserve cortical excitability over M_{1s}, and *iii*) maintain sleep features.

RESULTS

In a within-subjects design, fourteen healthy participants (mean age ± SD = 25.43 ± 2.56, nine women) were tested after three different daytime conditions of 12 hr, which were separated by 1 week (Figure 1). First, participants were examined after 12 hr of normal daily routine (i.e. no-immobilization, NoImmo condition) using a multimodal assessment including the sensorimotor representation of both hands through a mental rotation task (i.e. hand laterality judgment task), the cortical excitability of both M_1s by means of transcranial magnetic stimulation (TMS), and finally sleep polysomnographic-recordings. Then, all participants were randomly assigned either to an immobilization of the right (dominant) arm along with MI treatment (15 min each two hours; ImMI condition) or to a control condition without MI (ImCtrl condition). In both conditions, they were tested with the similar multimodal assessment as that used for the NoImmo condition. More details about the procedure can be found in Methods.

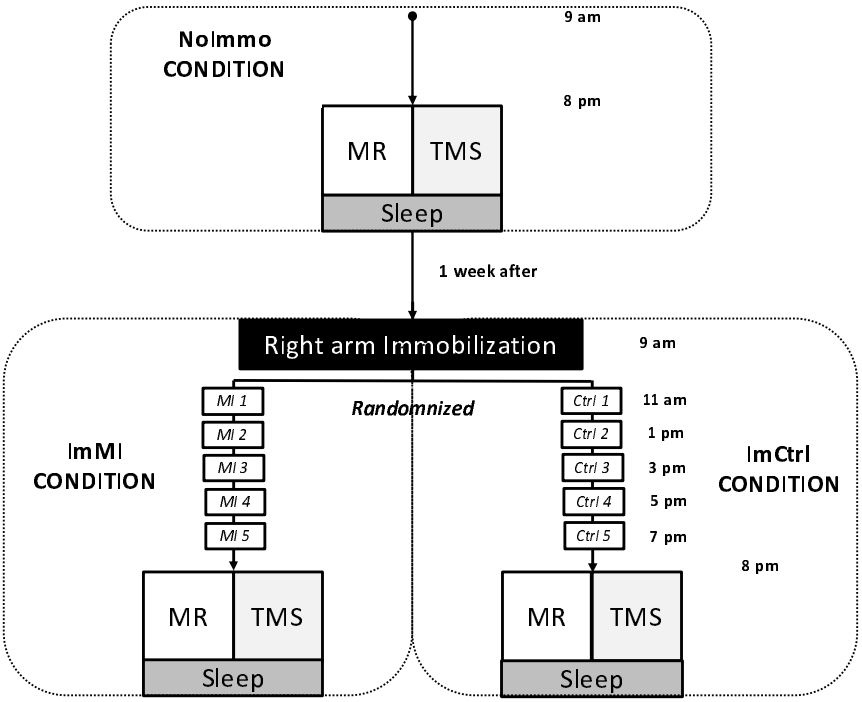

Figure 1: Experimental design. Participants performed three experimental conditions, separated by a week, to compare the effect of arm immobilization (12 hr) after MI treatment (ImMI condition) including 5 MI sessions, or a control condition without MI (ImCtrl Condition), with respect to a normal day without immobilization (NoImmo 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, polysomnographic data were recorded after each daytime condition.

Preliminary note

The mean score of the Stanford Sleepiness Scale assessed at 8 pm (i.e. before starting the multimodal assessment) after NoImmo (1.92 ± .64), ImMI (1.83 ± .55) and ImCtrl (2.23 ± .89) conditions were similar ($F(2, 24) = 1.74$, $p = .12$). All participants complied with the given instructions regarding the right-arm immobilization procedure, as confirmed by the MET scores. Accordingly, an analysis of variance for repeated measures (ANOVARM) revealed a significant effect of CONDITION ($F(2, 52) = 37.99$, $p < .001$, $\eta_p^2 = .59$), a main HAND effect ($F(1, 26) = 135.29$, $p < .001$, $\eta_p^2 = .83$), 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 NoImmo

condition, physical activity with the right arm was higher relative to the left ($p < .01$), which corresponded to subject's right hand dominance. Importantly, the activity of the right immobilized arm significantly decreased in both ImMI and ImCtrl conditions compared to NoImmo (each $p < .001$), and were significantly lower compared to the left arm activity ($p < .001$ in both ImMI and ImCtrl conditions). Surprisingly, activity of the left arm during NoImmo was equivalent to those in both ImMI and ImCtrl conditions ($p \geq .32$), hence suggesting that there was no overused of the left non-immobilized arm during right-arm disuse. Regarding the mean of MI performance across the 5 MI sessions, participants reported achieving easily (4.15 on a 1 to 5 scale) and vivid imagery (3.87; $p > .1$ for all comparisons between MI sessions).

Mental rotation tasks

*Hand laterality judgment*The ANOVARM performed on the percentage of correct responses yielded 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 three dimensional (95.31 ± 1.81) compared to the two dimensional (92.11 ± 1.99) configurations, and that left hand stimuli (96.14 ± 1.71) were more correctly identified rather than the right ones (91.96 ± 2.09), regardless the experimental conditions.

A different pattern of results was observed when considering mean response times. The ANOVARM did not reveal a main effect of LATERALITY ($F(1,52) = .45$, $p = .50$, $\eta_p^2 = .01$), but yielded main CONFIGURATION ($F(1,52) = 4.54$, $p < .05$, $\eta_p^2 = .08$) and CONDITION ($F(2,104) = 24.05$, $p < .001$, $\eta_p^2 = .32$) effects. Bonferroni post-hoc tests revealed that participants were faster to recognize both left and right hand stimuli after the ImMI condition compared to NoImmo

and ImCtrl conditions ($p < .001$ for each condition comparison with ImMI; Figure 2), thus attesting that MI exercises during arm-immobilization had a beneficial impact on hand representations.

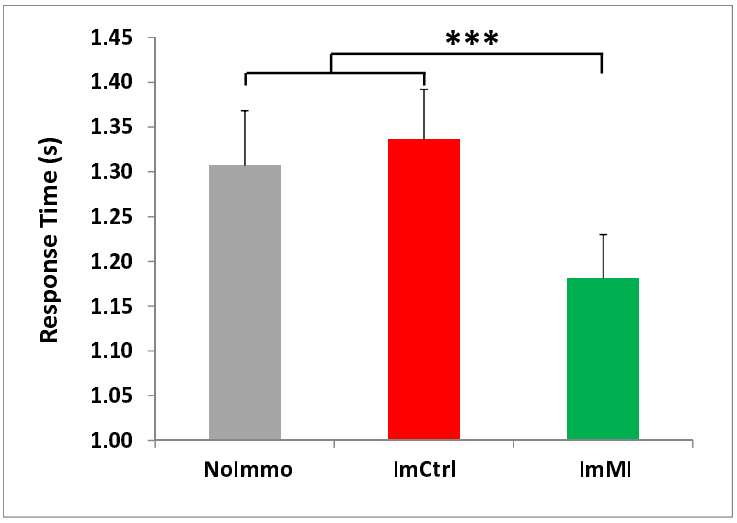

Figure 2: Response times on the mental rotation task. Independently of hand laterality, response times after the ImMI conditions were significantly faster compared to NoImmo and ImCtrl conditions. The pattern of results for right-hand stimuli suggests that learning may have occurred in the control condition but was blocked in the immobilization condition. Error bars indicate the standard error of the mean. *** p < .001.

Alphanumeric normal-mirror judgment task (control task)

The ANOVARM on the percentage of correct responses 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 ANOVARM did not yield a main effect of CONDITION ($F(1,52) = 0.44$, $p = .06$, $\eta_p^2 = .01$), but a CONDITION X CONFIGURATION interaction ($F(2,52) = 3.88.71$, $p < .0001$, $\eta_p^2 = .65$). *Post hoc* analysis only showed that normal stimuli were better identity after the ImMI compared to the NoImmo ($p < .05$) condition.

Similarly, the ANOVARM on response time 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 ($F(2,52) = .28$, $p = .75$, $\eta_p^2 = .01$).

Transcranial Magnetic stimulation

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

Corticomotor excitability

Left M_1 (Contralateral to the immobilized arm)

Analysis of the resting motor threshold (RMT, Supp. Table 1) in left M_1 showed a main CONDITION effect ($F(2,24) = 3.57$, $p < .05$, $\eta_p^2 = .23$), and post-hoc analysis revealed a higher RMT in the ImMI condition compared to the NoImmo ($p < .01$). 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 analysis did not show difference at 5% and 10% of the maximum stimulator output (MSO) among conditions, while significant changes appeared at higher intensity. As compared to NoImmo, the motor evocated potential (MEP) amplitude recorded in the ImCtrl condition was significantly lower at 15% ($p < .01$), 20% ($p < .001$) and 25% ($p < .001$) of MSO intensity. A different pattern of result was obtained in the ImMI condition as no difference appeared until 25% of the MSO relative to the NoImmo ($p < .05$). Furthermore, lower MEP at 20% ($p < .01$) and 25% ($p < .001$) were observed between the ImCtrl and the ImMI conditions, hence providing further evidence of the positive impact of MI training during arm immobilization. Most importantly, ANOVARM performed on slope of left M_1 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, hence allowing no difference with the NoImmo condition (p = .06, Figure 3B). In contrast, immobilization reduced the slope of left M_1 after the ImCtrl condition relative to NoImmo (p <.001), and a tendency compared with ImMI condition (p =.06). No significant correlation between the RC (at 15%, 20% and 25%) and the slopes of ImCtrl and ImMI conditions (relative to NoImmo values) were found with response time data on the mental rotation task (i.e independently of the hand stimuli, as no effect was previously found).

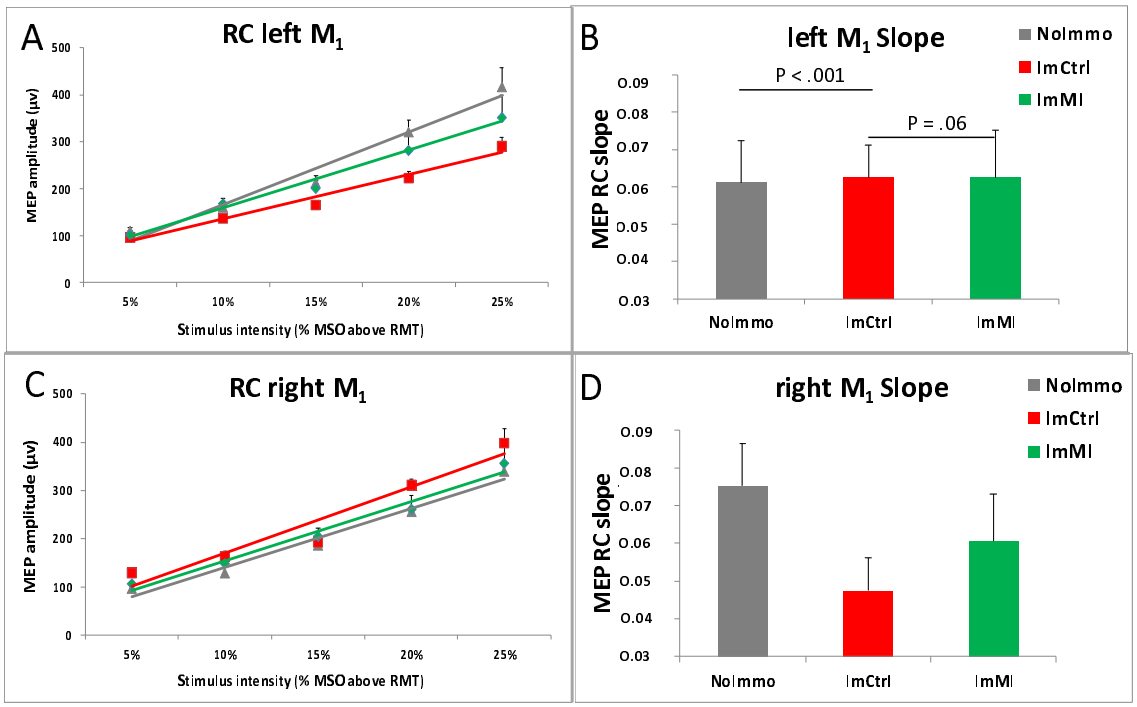

Figure 3: Decrease of cortical excitability over left M_1 after MI. In the superior panels, data of left M_1, contralateral to the immobilized arm are illustrated. (A) Relative to noImmo, MEP amplitude reduced in the ImCtrl condition at 15%, 20% and 25% of MSO intensity, but only at 25% in the ImMI condition. Lower MEP at 20% and 25% appeared between ImCtrl and ImMI conditions. (B) Slope data showed a significant reduction in the cortical excitability over left M_1 after immobilization alone (ImCtrl) relative to the no-immobilization condition, while MI practice limited the deleterious effect of impairment. In the inferior panels, data of the Right M_1, ipsilateral to the immobilized arm are illustrated. No difference between conditions was observed with respect to MEP amplitude over right M_1 (C), and slope data

(D). Key: RC, recruitment curve; MEP, motor evocated potential; MSO, maximum stimulator output; RMT, resting motor threshold; M_1, primary motor cortex.

Right M_1 (ipsilateral to the immobilized arm)

First, the RMT of right M_1 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 ($F(4, 38) = 33.02$, $p < .001$, $\eta^2 = .77$), and a main effect of CONDITION ($F(2, 76) = 3.36$, $p < .05$, $\eta^2 = .08$), but no INTENSITY X CONDITION interaction ($F(8, 76) = .51$, $p = .85$, $\eta^2 = .05$; Figure 3C). Moreover, the ANOVARM on the slope of right M_1 did not show any difference between conditions (Figure 3D) (NoImmo vs. ImCtrl: $p = .89$; NoImmo vs. ImMI: $p = .83$; ImCtrl vs ImMI: $p = .62$).

POLYSOMOGRAPHIC DATA

Three subject were excluded from spindles and spectral power analysis due to poor EEG signal quality (n = 11), while sleep architecture remained possible on three experimental nights (n = 14).

SLEEP ARCHITECTURE

Participants showed normal sleep patterns across the three recorded nights with no difference in time in bed, total sleep period (TSP), total sleep time nor sleep latency and sleep efficiency (Supp. Table 2). Analysis of the sleep architecture during the 1st sleep cycle revealed a significant interaction between conditions and proportion of time spent in each sleep stage (%TSP; $F(2, 26) = 7.37$; $p = .003$), due to an increase in the time spent in REM after ImMI treatment (10.73% ± 2.02%), as compared to ImCtrl (12.92 ± 1.29, $p = .02$) and NoImmo (10.78% ± 1.43%, $p = .003$) conditions (Figure 4A). A similar analysis of 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 ImMI condition (27.17% ± 1.69%) relative to NoImmo condition (22.57% ± 1.22%, $p < .01$), but not ImCtrl (26.42% ± 1.14%, $p = 1.00$, Figure 5A). No correlation emerged between time spent on REM (1st cycle and whole night) and RT on the mental rotation task nor on both [ImMI -NoImmo] and [ImCtrl – NoImmo] slopes (all $p < .05$)

Spindles during the 1st NREM sleep period

We performed an ANOVARM on NREM spindle (N2+N3) density using condition (Immo, ImMI, ImCtrl) as within-subject factors. On spindle detected during the 1st NREM cycle, we did not found significant main effect of condition for sleep spindles density over Cz ($F(2,24) = 2.03$, $p = .15$), but we did over the left M1 ($F(2,24) = 7.66$, $p < .01$), and right M1 ($F(2,24) = 9.32$, $p < .01$) (Supp. Table 3). Post-hoc tests with Bonferroni correction revealed a lower density in the ImCtrl condition over both M_1s as compared to NoImmo (Left M_1 $p < .01$ and Right M_1 $p < .01$) and ImMI (Left M_1 $p < .05$ and Right M_1 $p < .05$, Figure 4 B and C). There was no differences between NoImmo and ImMI condition over both M_1s ($p = 1.0$ for each M_1). Similar findings were found for number of spindles (N2+N3) with no difference between conditions for Cz ($F(2,24) = 2.48$, $p = .10$), and significant effects over left M_1 ($F(2, 24) = 6.74$, $p < .01$), and right M_1 ($F(2, 24) = 8.32$, $p < .01$). Post-hoc tests with Bonferroni correction only revealed a lower number of spindles after the ImCtrl condition relative to the NoImmo condition over M_1s compared to the NoImmo condition (Left M_1 $p < .01$ and $p < .01$ right M_1). No difference in duration, amplitude or frequency were found between condition (all $p<0.05$). No correlation between the density or number of 1st cycle spindles and RT on the mental rotation task was observed.

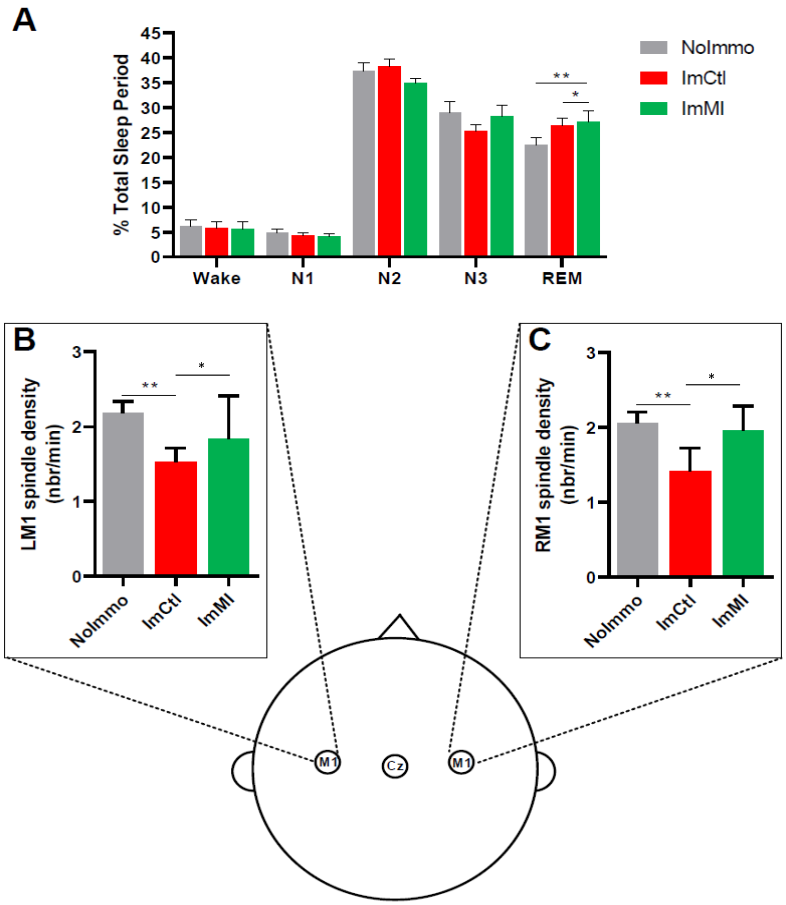

Figure 4: Sleep architecture and spindles density over both M1 during the 1st cycle of sleep after each condition (NoImmo, ImMI, ImCtrl). (A) Percentage of total sleep period for each sleep phase and condition. REM sleep was longer in the ImMI condition relative to both NoImmo and ImCtrl. (B) Lower spindle density over the Left M_1 and (C) Right M_1 in ImCtrl compared to the two other conditions. Key: NoImmo, without immobilization, ImMI, immobilization with motor imagery; ImCtrl, immobilization with control condition.

Spindles during the NREM of the whole night

Almost similar pattern of result between conditions were found on spindles density during the whole NREM sleep over Cz ($F(2,24) = 5.82$, $p < .01$), left M_1 ($F(2,24) = 10.94$, $p < .001$), and right M_1 ($F(2,24) = 12.29$, $p < .001$) (Supp. Table 4), as well as for number of spindles over Cz ($F(2,24) = 3.69$, $p < .05$), left M_1 ($F(2, 24) = 12.69$, $p < .001$), and right M_1 ($F(2, 24) = 14.61$, $p < .001$). Bonferroni post-hoc showed that the spindles density after ImCtrl were lower over each channels relative to both NoImmo ($p < .05$ Cz, $p < .001$ Left M_1 and $p <$

.001 right M_1), and ImMI ($p < .05$ Cz, $p < .05$ Left M_1 and $p < .01$ right M_1, Figure 5 B and C). As far the number of spindles was considered, ANOVA$_{RM}$ showed an effect of condition over Cz ($F(2, 24) = 3.69$, $p < .05$), Left M1 ($F(2, 24) = 12.69$, $p < .001$) and Right M1 ($F(2, 24) = 14.51$, $p < .001$). Post-doc showed that the number of spindles in the ImCtrl was lower relative over all channels relative to the NoImmo condition ($p < .05$ Cz, $p < .001$ Left M1 and $p < .001$ Right M1). Number of spindels did not change in the ImMI condition relative to NoImmo over Cz ($p = .47$) and Right M1 ($p = .11$), while it was lower in the Left M1 ($p < .05$). No correlation was observed between whole night NREM spindles density and RT on the mental rotation task.

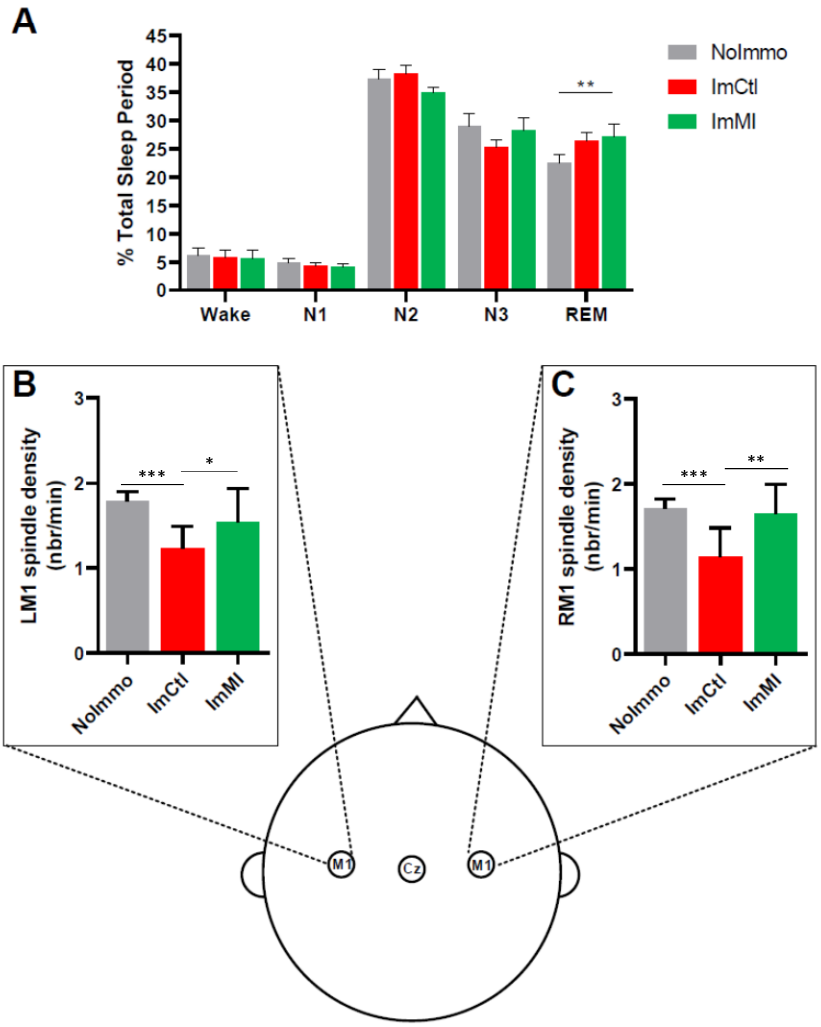

Figure 5: Sleep architecture and spindles density over both M1 during whole night of sleep after each condition (NoImmo, ImMI, ImCtrl). (A) Percentage of total sleep period for each sleep phase and condition. REM sleep remained longer in the ImMI condition relative to the NoImmo. (B) Lower spindle density over the Left M_1 and (C) Right M_1 in ImCtrl compared to the two other conditions. Key: NoImmo, without immobilization, ImMI, immobilization with motor imagery; ImCtrl, immobilization with control condition.

EEG Spectral analysis

ANOVARM performed separately on CZ and both M_1s 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 CONDITION ($p \geq .11$ for each channel and phase). Similarly, analysis performed on the EEG spectrum of the total night of sleep showed an effect of FREQUENCY BANDS ($p < .001$ on each channel).

DISCUSSION

The present study was designed to investigate the effects of MI practice administered during 12 hr 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 slow-down of sensorimotor representation regardless of hand laterality. Likewise, MI practice allowed maintenance of the cortical excitability over left M_1 contralateral to the arm-immobilization. Furthermore, the time spent in REM sleep was significantly longer during the 1st cycle of sleep following the ImMI condition compared to that after NoImmo and ImCtrl conditions. Finally, data revealed that the number of spindles was preserved after the ImMI condition over both M_1s, during the 1st cycle of sleep.

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 ImMI but not the ImCtrl condition, regardless the laterality of the

stimulus. The lack of task-repetition benefit after the ImCtrl condition for both right and left hand stimuli confirm and extend the findings by Toussaint et al. [23] after 48h of non-dominant hand immobilization. These authors suggested that the peripheral alteration affected the sensorimotor representation of the hand, which became less effective or more difficult to stress. Performance gains observed following the ImMI 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 to their initial level of functioning. In the present study, participants spent much more time to practice MI (75 min vs. 15 min), and the last MI session was performed 2 hr before the splint removal. Present results therefore support that practicing MI several times during the 12 hr of arm-immobilization may have promoted not only an acute reactivation of the sensorimotor processing, but its maintenance at a normal functioning level at least during 2 hr before the splint removal. Interestingly, a recent neuroimaging study conducted by Burianova et al. [3] showed that 24 hr of hand immobilization affected the neural correlates of MI, specifically over the sensorimotor areas contralateral to the limb disused. Therefore, it seems possible that reactivation of the sensorimotor system by means of MI practice early after immobilization would contribute 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 promote 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 left M_1 caused by the contralateral arm-immobilization. As

expected, we found a significant reduction in slope of RC data after the ImCtrl condition, hence reflecting the typical local synaptic depression induced by unilateral short-term immobilization [2, 6, 8, 27]. Importantly, such downregulation effect of left M_1 was not observed after the ImMI condition, hence suggesting that MI interventions during immobilization contributed to preserve activity of the sensorimotor network to an equivalent magnitude of that recorded in the absence of immobilization (NoImmo). 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 content of MI intervention, notably the nature and dose of MI exercises. 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, using 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 postulate that the posology of our MI intervention was thorough and sufficient to promote significant M_1 (re)activation, hence protecting the motor planning routines against the loss of sensorimotor information. This assumption is supported by strong evidences in the motor learning domain stating that MI increases corticospinal excitability in M1 and that the increase is specific to the representation of the limb involved during imagery [28, 29] .

Effect of MI practice on NREM and REM sleep

A main innovative finding of this study is that MI practice during immobilization elicited an increase in the time spend in REM sleep. Furthermore, and relative to NoImmo, the spindle number over both M_1s after the ImMI condition remained stable, while it decreased in the ImCtrl condition. Importantly, these effects were more pronounced during the 1st cycle of sleep. It is usually assumed that the motor system activation of REM sleep [30-32] corresponds to imaginative rather than actual movement [22, 33, 34]. 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 [35, 36]. It is therefore likely that practicing MI exercises during arm-immobilization might have potentiated REM sleep content in inducing more rehearsed movements, which was reflected in longer duration. As an alternative or complementary explanation, REM sleep has been found to contribute to motor memory consolidation [37-39], although this causal role in motor skill formation is still debated [40]. Based on the neurofunctional equivalence between physical and MI practice [41, 42], it is likely that MI during arm-immobilization might have been consolidated during REM sleep. As expected and consistent with the finding of Huber and collaborator [6], we found that 12 hr of immobilization caused a decrease in the density of spindles over both M_1s after the ImCtrl condition, during the 1st NREM sleep cycle. Importantly, the number of spindles over motor regions after the ImMI condition were preserved as compared to NoImmo. Yet, accumulating evidence in the motor learning domain has established that spindles play a critical role in the reprocess of newly formed memories in reactivating cortical and subcortical regions recruited during procedural learning [43, 44]. Accordingly, Debarnot et al. [21] reported that both short (10 min NREM) and long (NREM and REM) naps facilitated MI consolidation rather than a wake state interval of time. As no difference in performance gains appeared after the two nap conditions, NREM

sleep, and particularly spindles activity, were suspected to underpin the MI consolidation process. In accordance with this finding, present data revealed, for the first time, that MI practice during arm-immobilization prevented the loss of spindles over both M_1s.

To conclude, present results provided strong evidence that MI practice contributed to preclude the slowdown of the sensorimotor representation, and protected against the corticomotor depression induced by arm-immobilization. For the first time, data further revealed the triggering effects of MI practice during NREM sleep (limitation of the reduction in spindle number), as well as REM sleep (longer duration of this phase). Overall, MI contributed to reactivate injured sensorimotor networks, hence protecting for the maladaptive neuroplasticity occurring during the consolidation process after immobilization. These findings have strong fundamental and practical implications in both sleep/motor memory and (neuro)rehabilitation research domains. Notably, the benefits elicited by the early integration of MI in the context of limb disused, or in the classical course of physical therapy, might help to promote recovery of lost motor functions. 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 M_1 to the limb disused, and thereby increase the opportunity for the patient to benefit from brain computer interface-based interventions.

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, Oldfield, 1971); (2) have a Pittsburg Sleep Quality Index < 5 (Buysse et al., 1989) ; (3) have an

intermediate circadian typology score from 42 to 58 (Horne & Östberg, 1976); (4) have Epworth Sleepiness Scale score < 11; (5) have a Becks 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, Williams et al., 2012), 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.

Mental rotation task

Participants seated on a chair at a distance of 50 cm in front of 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. They performed two different mental rotation tasks, namely a hand laterality judgment task and an alphanumeric normal-mirror judgment task (control task). In the hand laterality judgment task, they were asked to identify whether a hand stimulus was a left or a right hand. In the alphanumeric normal-mirror judgment task, they had to determine whether the letter “R” and number “2” were presented in a normal or mirror view. Hand stimuli were either in two-dimensional (dorsum or palm view from four 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);

alphanumeric stimuli were only orientated in 0°, 90°, 180°, or 270°. Simple hand and alphanumeric stimuli (size: 13 cm in height, 7 cm in width) were equally presented either in clockwise or counterclockwise orientations.

Participants were instructed to identify stimuli displayed in the center of the computer screen as accurately and quickly as possible. 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 the number ''2'' and the letter "R" or their mirror image, respectively). Response times (RTs) were recorded using a microphone connected to the computer. The 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 NoImmo 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 half of left and right hands, respectively presented in a random order. Then, participants were 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 half normal and mirror alphanumeric items.

The hand mental rotation task was always performed before the alphanumeric mental rotation task, as Toussaint and Meugnot [23] demonstrated that arm immobilization-

induced effects are hidden when participants first perform a non-body mental rotation task. During each test session (NoImmo, ImMI and ImCtrl), participants thus completed a total of 64 trials of hand laterality judgment task and 32 trials of alphanumeric normal-mirror judgment task. During the ImMI and Control 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

Using TMS, we tested the cortical excitability of left M_1 (contralateral to the immobilized-arm) and then right M_1 (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 (Magstim Compagny), connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK) was placed tangentially on the scalp over M_1s; 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 remained 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 M_1, 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 the average MEP amplitude was taken as MEP size. The same RMT and RC procedure was then performed over right M_1.

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 (Biopac systems, Goleta, CA, USA) 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

Brain activity was recorded in all participants during three nights of sleep following NoImmo, ImMI and ImCtrl conditions, using 11 scalp EEG electrodes, three electrooculogram electrodes, and two chin electromyogram electrodes (sampling rate: 250 Hz). Precisely, EEG montage was composed by 11 electrodes placed according to the International 10–20 system (LM1, CZ, RM1, F3, FZ, F4, P3, PZ, P4, A1, A2). The three nights of sleep were monitored using a V-Amp 16 system recorder (Brain Products, Gilching, Germany), filtered between 0.05 and 50Hz and EEG signals were referenced against the mastoids.

Sleep data analysis

Polysomographic analyses were conducted using 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. 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) as well as sleep efficiency (TST/time in bed*100) and number of cycles. The epochs that were affected by artefacts were excluded from further quantitative EEG analysis after visual inspection by a blinded expert scorer. The EEG spectrum power average (30 s of time resolution with artefact excluded) 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: SOs (0.4-1 Hz), delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (11.5-15 Hz), spindle power ranges (12-15 Hz), and beta (15-30 Hz). Following the procedure of Ray and colleagues [46], spindles were detected automatically using the PRANA software. Sleep spindles were separated according to their main frequency (i.e. maximum power amplitude within the 12–15 Hz range), in line with the global standards used in sleep research [43, 47]. The detection was performed on each channel: 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. Based on these criteria, an experienced scorer visually supervised all detected spindle for each night of each participant.

Immobilization procedure

During the Immobilization conditions, participants were requested to not move the right arm (from the fingers to the shoulder) for 12 hr from the morning (9:00 am) until the beginning of the motor task (9:00 pm). The duration of the arm sensorimotor deprivation was chosen as it was recently demonstrated to modify M_1s excitability [27], with additional local changes during the subsequent night of sleep (Huber et al., 2006). 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 12 hr of 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. An accelerometer was set up in a multisensory actigraph (ActigraphwGT3X-BT) on their left and right forearms. The multisensory actigraph recorded the cumulative amount of time spent (in minutes) during physical activity at a certain level of energy expenditure by means of mathematical algorithms. Data were sampled at 30 Hz. The measure expressing the energy cost of physical activity was measured in metabolic equivalent of task (METs) i.e. one MET equals the resting metabolic rate obtained during quiet sitting [48]. Besides these bimanual movements recording, we also verified that all subjects maintained a regular sleep-wake schedule with a minimum of 7 hr of sleep, at least three days before each experimental day. Compliance to the schedule was assessed using both sleep diaries and wrist actigraphy measures. Finally, all participants filled out a subjective measure of alertness and fatigue using the Stanford Sleepiness Score [49]. This is an introspective measure of sleepiness in which participants

rate their alertness each hour during the 3 experimental days, using an 8-point scale. This assessment provided a subjective measure of alertness before each MI intervention (and control session), and right before performing the mental rotation and TMS procedure.

Motor imagery sessions

During the ImMI condition, participants performed 5 sessions (15 min each) of mental training with the immobilized arm. Each two hours, participants were 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 including monoarticular movements rehearsed mentally during the first and the second sessions, and polyarticular movement repeated during the three other MI sessions. Precisely, 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, they performed five different and complex "polyarticular" movements (e.g. throwing a ball) during 3 min each following the three other MI sessions, 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. It is worth noting that movements performed during the 3 last MI sessions were closer to daily live actions while emphasis on the immobilized right arm was always requested to the participants. For each exercise, the experimenter gave precise instructions about the movement to be performed and asked participants either to sit or stand during MI in order to facilitate the postural congruency related to the movement (e.g. finger tapping was performed sat while performing arm circle was executed up). Then, participants, if needed, could first practice the movement with the non-immobilized arm in order to have a sensory-experience prior to MI rehearsal with the immobilized arm [50]. They were instructed to say "go" when they started the mental

simulation of movement with the actual immobilized arm. They were asked to imagine themselves performing movements using a combination of visual and kinaesthetic imagery, i.e. imagining movement from within one's body and perceiving the induced proprioceptive sensations. 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 a Liker-type scale (from 1 = very difficult/poor mental representation to 5 = very easy/vivid mental representation).

Data analysis

For the mental rotation task, we analyzed two dependent variables, namely the percentage of correct responses and the 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 ANOVARM with HAND (left, right) and CONFIGURATION (simple vs. complex) as within factors. Likewise, an ANOVARM was performed for the alphanumeric normal-mirror judgment task, with CONDITIONS (NoImmo, ImMI and ImCtrl), CONFIGURATION (normal, mirror) and ORIENTATION (0°, 90°, 180°, or 270°) as within factors.

To evaluate the effect of immobilization on cortical excitability, RMT of left and right M_1 were compared for each condition by means of separated ANOVA. RC values were analyzed separately for each hemisphere by means of an ANOVARM with CONDITIONS (NoImmo, ImMI and ImCtrl) as between-subjects factor and INTENSITY (5%, 10%, 15%, 20%, and 25% of MSO above RMT) as within-subjects factors. Then, we further evaluated the differences between the slopes of the 3 regression lines obtained by pooling together data in

the different experimental conditions (NoImmo, ImMI and ImCtrl), separately for each M_1. Paired *t* test were performed on these data to examine whether the linear part of the RC was modify by the right-arm immobilization with MI treatment or without (ImCtrl condition). We further examined relationships between the arm-immobilization, with or without MI treatment, on cortical excitability outputs (RC and slope), and mental rotation performance (sensorimotor representation of both arm) by performing several Pearson's correlations. We conducted similar correlations between mental rotation performance and sleep features, when the latter were significant between conditions in independent and prior analysis.

To examine whether daytime conditions (NoImmo, MI, ImCtrl) influence the sleep architecture, several ANOVARM were performed on each sleep parameters. Then, we analysed spindle features (count, density, duration, peak amplitude and mean frequency) by means of ANOVARM with conditions as between-subjects factor. Similar analysis were performed on each power spectrum bands and on each channel as within-subjects factor and condition as between-subject factor. All sleep analysis (sleep architecture, spindles features and spectral power data) were examined separately during both 1st cycle and whole night of sleep.

Both the Stanford Sleepiness Scale and MET scores for the NoImmo, ImMI, ImCtrl conditions were evaluated by means of ANOVARM.

When 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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