Temporal clustering of sleep spindles for motor skill consolidation and generalizability following learning by physical practice, motor imagery and action observation

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Abstract

Sleep benefits the consolidation of motor skills learned by physical practice, mainly through periodic thalamocortical sleep spindle activity. However, motor skills can be learned without overt movement, either through motor imagery or action observation. Here, we investigated whether sleep spindle activity also supports the consolidation of non-physically learned movements. Forty-five electroencephalographic sleep recordings were collected during a daytime nap after motor sequence learning by physical practice, motor imagery or action observation. Our findings revealed that irrespective of the modality of practice, spindles tend to cluster in trains on a low-frequency time scale of about 50 seconds, and during which spindles iterate every 3-4 seconds. However, despite this apparent modality-unspecific temporal organization of sleep spindles, different behavioral outcomes were elicited. We show that a daytime nap offers an early sleep window that promotes the retention of the learned motor skill following PP and MI practice, and its generalizability towards the transfer of skill from one effector to another after AO practice. Altogether, we demonstrated that the temporal cluster-based organization of sleep spindles may be a general mechanism for effective memory reprocessing.

Keywords: Sleep; spindles; memory consolidation; motor imagery; action observation; EEG

Introduction

Repeated practice is critical for the learning and mastering of motor skills. Training procedures encouraging the ability to exploit the features of a learned skill for its transfer from one situation to another is fundamental across diverse contexts, such as in sports sciences and rehabilitation (Robertson, 2018). Although the common way to learn a movement is by performing the task physically (see (Doyon et al., 2018; Krakauer et al., 2019) for reviews), other forms of practice can contribute to motor-skill learning. Compelling evidence shows that motor skills can be learned in the absence of overt movement, through motor imagery (MI) or action observation (AO) (Bird et al., 2005; Boutin et al., 2010; Di Rienzo et al., 2016; Ladda et al., 2021). While MI refers to the process of mentally rehearsing a movement without physically performing it, AO consists in observing another actor performing the movement. Numerous neuroimaging studies reported that a set of common neural structures is activated during MI, AO, and physical practice (PP), thus providing evidence of a relative "functional equivalence" between practice modalities (Hardwick et al., 2018; Hétu et al., 2013; Jeannerod, 2001).

Both covert modalities of practice (MI, AO) engage a cognitive demand upon sensorimotor networks, hence boosting activity-dependent neuroplasticity which leads to enhanced motor performance and learning (Bird et al., 2005; Boutin et al., 2010; Debarnot et al., 2011). Traditionally, to evaluate the beneficial effects of MI and AO practice on motor skill learning, participants observe a model or imagine themselves performing the motor task before being evaluated on a post-training test requiring overt practice of the movement (Boutin et al., 2010; Debarnot et al., 2015). It is commonly reported that both MI and AO practice lead to enhanced motor performance and learning, albeit to a lesser degree than PP (Avanzino et al., 2015; Badets et al., 2018; Deakin and Proteau, 2000; Gentili et al., 2010). However, motor learning is not limited to task-specific learning but also concerns the ability to transfer or generalize the newly acquired skill to another one or another effector (i.e., inter-limb transfer), which may depend on the modality of practice (Robertson, 2018). For instance, PP has been shown to mainly rely on an effector-specific representation of the motor skill, thus leading to impaired inter-limb skill transfer (Boutin et al., 2011). In contrast, MI and AO practice have mostly been revealed to develop an effector-unspecific representation of the learned motor task, thus allowing effective skill transfer from one limb to another (Amemiya et al., 2010; Boutin et al., 2010; Wohldmann et al., 2008). Therefore, the representation of a motor skill acquired through PP, MI and AO practice relies on distinct coding systems for movement production,

leading to specific skill learning and transfer capacities (Amemiya et al., 2010; Gruetzmacher et al., 2011; Ingram et al., 2016).

Although the repeated practice of a motor skill is crucial for its initial acquisition, the development of an effective movement representation is not only a result of practice (Doyon et al., 2018). The newly formed memory continues to be processed "offline" over several temporal scales, from a rapid form of consolidation at a microscale of seconds (Bönstrup et al., 2020) to longer forms of consolidation occurring primarily during the waking and sleeping hours following practice (Censor et al., 2012; Doyon et al., 2018). This offline period offers a privileged time window for memory consolidation, which relates to the process whereby newly acquired and relatively labile memories are transformed into more stable and even enhanced memory traces (Dudai et al., 2015). The memory trace is thought to be dynamically maintained during wakefulness and actively reprocessed during a subsequent sleep period. Both a night of sleep and a daytime nap have been shown to play a crucial role in the strengthening and transformation of motor memory representations developed through PP during consolidation (see (Boutin and Doyon, 2020) for a review), which behaviorally results in either performance stabilization or improvement (Robertson, 2012). In the same way, a few studies reported sleep-dependent consolidation of motor skills learned through MI practice (Debarnot et al., 2015, 2012, 2011, 2009) or AO (Van Der Werf et al., 2009) and it has been recently emphasized that the stability of the newly formed motor memory, through consolidation processes during wake and sleep episodes, may modify its generalizability (Robertson, 2018). However, whether similar consolidation mechanisms are engaged during sleep following PP, MI and AO practice, and how sleep affects the generalizability of motor skills remains to be determined.

At the brain level, and in the context of PP, motor memory consolidation is thought to be mediated by transient thalamocortical sleep spindle activity – an electrophysiological hallmark of non-rapid eye movement stage 2 (NREM2) sleep involving brief 0.3-2 s bursts of waxing and waning 11-16 Hz oscillations. Sleep spindles have been suggested to support the offline reactivation of newly acquired motor memories, resulting in post-night and post-nap motor memory improvements (Barakat et al., 2013; Boutin et al., 2018; Fogel et al., 2017; Fogel and Smith, 2006; Nishida and Walker, 2007). Boutin and Doyon (2020) recently proposed a theoretical framework for motor memory consolidation following PP that outlines the essential contribution of the clustering and hierarchical rhythmicity of spindle activity during this sleep-dependent process. They posited that the rhythmic expression of sleep spindles over task-relevant cortical and subcortical brain regions is critical for the efficient reprocessing and consolidation of motor memory traces following PP. Specifically, it is suggested that the

occurrence of sleep spindles follows two periodic rhythms: an infra-slow rhythm that corresponds to a 0.02 Hz periodicity of spindle-enriched periods, called spindle trains, and in which spindles iterate at an intermediate rhythm of about 0.2-0.3 Hz. Current theoretical models posit that this 0.2-0.3 Hz rhythmic occurrence of spindles during trains defines the sequential alternance of spindles and associated refractory periods, thus regulating the cyclic reinstatement and interference-free reprocessing of memory traces for their efficient consolidation (Antony et al., 2019, 2018; Boutin et al., 2022; Boutin and Doyon, 2020; Schönauer, 2018). However, whether a similar temporal cluster-based organization of sleep spindles underlies the consolidation of motor skills acquired by MI and AO practice remains to be addressed.

Hence, by combining behavioral and electroencephalographic (EEG) sleep measures, the aim of the present study was twofold: (i) to determine whether similar consolidation mechanisms are engaged during sleep following PP, MI and AO practice, and (ii) to investigate the specific contribution of sleep spindle activity and its rhythmic organization in motor skill consolidation and transfer, depending on the modality of practice.

Results

Behavioral data. Fig. 1 illustrates the changes in performance associated with motor skill acquisition, consolidation and transfer capacity. A one-way ANOVA with the between-participant factor MODALITY (PP, MI, AO) was first performed on the RT data during the pre-test to ensure that the three groups did not differ at baseline performance. The analysis failed to detect a significant MODALITY effect ($F(2, 42) = 2.05$, $p = 0.14$), revealing no performance differences between groups on the pre-test. Additional analyses on the RT data can be found in supplementary material (*Appendix A,* Fig. A.1.). Then, one-way ANOVAs with the between-participant factor MODALITY (PP, MI, AO) were applied separately for motor skill acquisition, consolidation and transfer. For motor skill acquisition, the analysis revealed a main effect of MODALITY ($F(2, 42) = 7.52$, $p = 0.002$, $\eta^2_p = 0.26$), with better performance gains for the PP group (M = 32.3%) in comparison to the MI group (M = 17.0%, $p = 0.001$). No significant difference in performance gains was found between the AO group (M = 23.3%) and the other two groups ($p = 0.06$ and $p = 0.12$ respectively for the PP and MI groups). For motor skill consolidation, the analysis failed to detect a significant MODALITY effect ($F(2, 42) = 2.50$, $p = 0.09$). No significant difference in post-nap performance gains was found between the PP group (M = -0.2%), the MI group (M = 7.9%) and the AO group (M = 4.0%). For motor skill transfer, the analysis revealed a significant main effect of MODALITY ($F(2, 42) = 5.10$, $p = 0.01$, $\eta^2_p =$

0.20), with greater performance decreases for the PP group (M = -36.1%) than the MI group (M = -17.1%, p = 0.03) and the AO group (M = -13.4%, p = 0.01), which did not significantly differ from each other (p = 0.63).

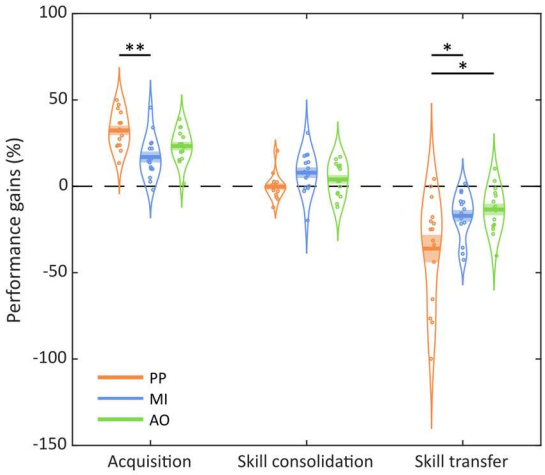

Fig. 1. Behavioral results. Performance gains (in percentages) on the motor sequence task during acquisition (from the pre-test to the post-test), sleep-related skill consolidation (from the post-test to the retention test), and inter-manual skill transfer (from the retention test to the transfer test) for each of the physical practice (PP; n = 15), motor imagery (MI; n = 15) and action observation (AO; n = 15) groups. The curved lines indicate the distribution of data, the dark bars represent the mean of the distribution, and the lighter area surrounding the mean represent the standard error of the means. Individual data points are displayed as colored circles. * $p < 0.05$, ** $p < 0.01$.

Sleep and spindle characteristics. For all groups, sleep architecture and spindle metrics are respectively summarized in Table 1 and Table 2. Additional analyses for other scalp derivations (F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2, Oz) can be found in supplementary material (*Appendix A*, Fig A.2. and A.3). One-way ANOVAs with the between-participant factor MODALITY (PP, MI, AO) were applied separately for each spindle metric. *Spindle clustering.* The analysis did not reveal any significant main effect of MODALITY for the total amount of sleep spindles (F(2, 42) = 0.22, p = 0.80), the amount of grouped spindles (F(2, 42) = 0.12, p = 0.89) and isolated spindles (F(2, 42) = 0.81, p = 0.45), as well as for the total amount of spindle trains (F(2, 42) = 0.24, p = 0.80). *Spindle rhythmicity.* No significant main effect of MODALITY was found for the inter-spindle interval (ISI) within trains (F(2, 42) = 0.03, p = 0.97) and inter-train interval (ITI) metrics (F(2, 42) = 0.69, p = 0.51). *Spindle density.* The analysis did not reveal any significant main effect of MODALITY for both the global density (F(2, 42) = 1.28, p = 0.29) and local density metrics (F(2, 42) = 0.80, p = 0.46).

	PP		MI		AO		ANOVAs	
	mean	SEM	mean	SEM	mean	SEM	F	p
WAKE (min)	26.0	3.9	28.8	4.3	41.6	5.3	2.82	0.07
NREM1 (min)	17.5	1.8	14.0	2.0	12.2	2.0	1.98	0.15
NREM2 (min)	24.7	2.1	29.8	3.3	25.3	3.4	1.03	0.37
NREM3 (min)	17.1	2.5	11.5	3.4	9.3	2.2	2.19	0.13
REM (min)	2.6	1.6	2.4	1.2	1.0	0.6	0.54	0.59

Table 1. Sleep architecture in all experimental groups. Sleep architecture (mean time and standard errors of the means; in minutes) during the 90-min daytime nap for each of the physical practice (n = 15), motor imagery (n = 15) and action observation (n = 15) groups. PP: Physical practice, MI: Motor imagery, AO: Action observation, NREM: Non-Rapid Eye Movement sleep, REM: Rapid Eye Movement sleep.

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	PP		MI		AO		ANOVAs	
	mean	SEM	mean	SEM	mean	SEM	F	p
Total amount of spindles	174.5	21.1	185.3	21.5	164.7	22.8	0.22	0.80
Grouped spindles	145.5	19.3	151.3	17.8	138.3	19.2	0.12	0.89
Isolated spindles	29.0	3.2	34.0	5.0	26.4	4.4	0.81	0.45
Number of trains	36.9	4.4	38.7	4.0	34.4	4.8	0.24	0.80
Inter-spindle interval (ISI)	3.4	0.1	3.5	0.1	3.5	0.1	0.03	0.97
Inter-train interval (ITI)	44.8	4.2	46.0	2.4	41.1	2.2	0.69	0.51
Global density	7.1	0.4	6.3	0.4	6.5	0.3	1.28	0.29
Local density	8.8	0.5	8.1	0.4	8.6	0.4	0.80	0.46

Table 2. Sleep spindle characteristics in all experimental groups. Means and standard errors of the means (SEM) are reported for the total amount of spindles, grouped spindles, isolated spindles, the number of trains, inter-spindle interval (ISI), inter-train interval (ITI), global density (number of spindles per minute), and local density (number of spindles within a spindle-centered 60-s sliding window) of NREM2 sleep spindles at the Pz electrode (midline parietal) during the 90-min daytime nap for each of the physical practice (n = 15), motor imagery (n = 15) and action observation (n = 15) groups. PP: Physical practice, MI: Motor imagery, AO: Action observation.

Modality-specific role of NREM2 sleep upon skill consolidation and transfer. To assess the role of NREM2 sleep in motor skill consolidation and generalizability, the duration of NREM2 sleep periods was respectively correlated with the magnitude of skill consolidation and transfer separately for each group. Pearson correlation analyses revealed a significant positive relationship between the duration of NREM2 sleep and post-nap performance gains for both the PP group ($r = 0.53$, $p = 0.041$) and the MI group ($r = 0.62$, $p = 0.015$), but not for the AO group ($r = 0.01$, $p = 0.98$). Interestingly, however, a positive relationship between the duration of NREM2 sleep and the magnitude of skill transfer was found in the AO group ($r = 0.54$, $p = 0.038$), but not in the PP ($r = -0.20$, $p = 0.47$) and MI groups ($r = -0.20$, $p = 0.48$), pointing towards modality-specific effects of sleep upon skill consolidation and transfer.

Relation between sleep spindles and skill consolidation. To investigate the modality-specific contribution of sleep spindle activity in motor skill consolidation, we performed correlation analysis between NREM2 sleep spindle characteristics and post-nap performance gains separately for each group. For both the PP and MI groups, correlation analyses revealed that the magnitude of skill consolidation correlated positively with the amount of sleep spindles ($r_{PP} = 0.60$, $p = 0.019$; $r_{MI} = 0.62$, $p = 0.014$) (Fig. 2A), with the total amount of grouped spindles ($r_{PP} = 0.59$, $p = 0.022$; $r_{MI} = 0.64$, $p = 0.010$) (Fig. 3A and 3B), as well as with the amount of spindle trains ($r_{PP} = 0.62$, $p = 0.013$; $r_{MI} = 0.55$, $p = 0.034$). In contrast, no significant correlations were found in the AO group between the magnitude of skill consolidation and the amount of sleep spindles ($r = 0.06$, $p = 0.83$) (Fig. 3A), the amount of grouped spindles ($r = 0.09$, $p = 0.76$) (Fig. 3C), and the amount of spindle trains ($r = 0.02$, $p = 0.95$). It is noteworthy, however, that no significant correlations were found between skill consolidation and the amount of isolated spindles for all groups ($r_{PP} = 0.40$, $p = 0.14$; $r_{MI} = 0.39$, $p = 0.15$; $r_{AO} = -0.06$, $p = 0.83$). Additional multiple regression analyses can be found in supplementary material (*Appendix A*, Note A.4.). Correlation analyses are in favor of a greater involvement of grouped rather than isolated spindles in the memory consolidation process following PP and MI. Altogether, these findings suggest that sleep spindle activity underlies the consolidation of the practiced motor sequence following PP and MI.

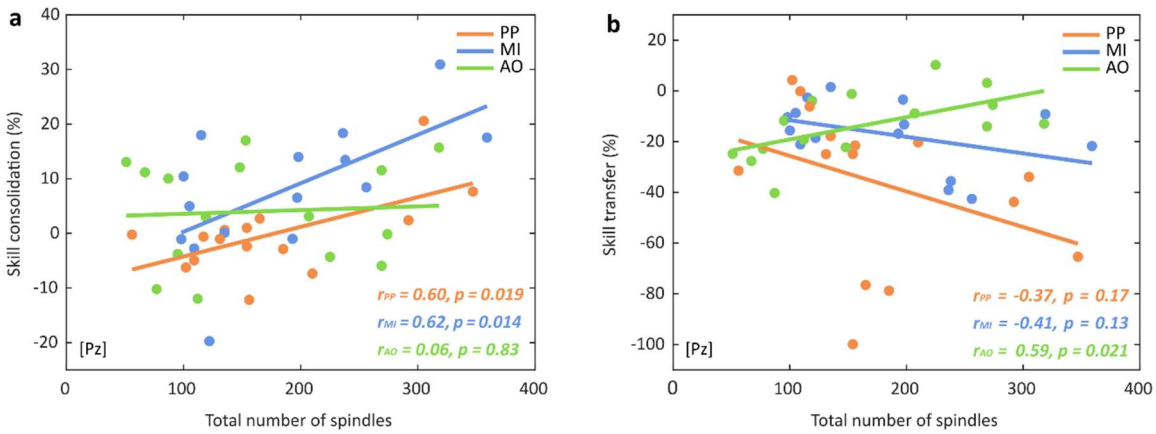

Fig. 2. Sleep spindles in relation with motor skill consolidation and transfer. (a) Relationship between skill consolidation (percentage of performance gains across the sleep retention interval, from the post-test to the retention test) and the total amount of NREM2 spindles for each of the physical practice (PP; n = 15), motor imagery (MI; n = 15) and action observation (AO; n = 15) groups. **(b)** Relationship between skill transfer (percentage of performance gains from the retention test to the inter-manual transfer test) and the total amount of NREM2 spindles for each practice group. Scatter plots and linear trend-lines are provided. Pearson correlation coefficients (*r*) and associated p-values are reported for each correlation.

Relation between sleep spindles and skill transfer capacity. To investigate the modality-specific role of sleep spindles in motor skill generalizability, we performed correlation analyses between NREM2 sleep spindle characteristics and the magnitude of skill transfer. For the AO group only, significant positive correlations were found between the magnitude of skill transfer and the amount of sleep spindles ($r = 0.59$, $p = 0.021$) (Fig. 2B), the amount of grouped spindles ($r = 0.58$, $p = 0.022$) (Fig. 3F), as well as the amount of spindle trains ($r = 0.56$, $p = 0.029$). In contrast, no significant correlations were found in the PP and MI groups between the magnitude of skill transfer and the amount of sleep spindles ($r_{PP} = -0.37$, $p = 0.17$; $r_{MI} = -0.41$, $p = 0.13$) (Fig. 2B), the amount of grouped spindles ($r_{PP} = -0.38$, $p = 0.16$; $r_{MI} = -0.40$, $p = 0.14$) (Fig. 3D and 3E), and the amount of spindle trains ($r_{PP} = - 0.29$, $p = 0.30$; $r_{MI} = -0. 31$, $p = 0.26$). For all groups, no significant correlations were found between the magnitude of skill transfer and the amount of isolated spindles ($r_{PP} = -0.16$, $p = 0.57$; $r_{MI} = -0.35$, $p = 0.19$; $r_{AO} = 0.50$, $p = 0.06$). Additional multiple regression analyses can be found in supplementary material (*Appendix A,* Note A.4.). Correlation analysis are in favor of a greater involvement of grouped rather than isolated spindles in motor skill transfer capacity following AO. Altogether, these findings suggest that sleep spindle activity promotes the consolidation of an effector-unspecific

representation (i.e., transfer to the unpracticed hand) of the learned motor sequence following AO.

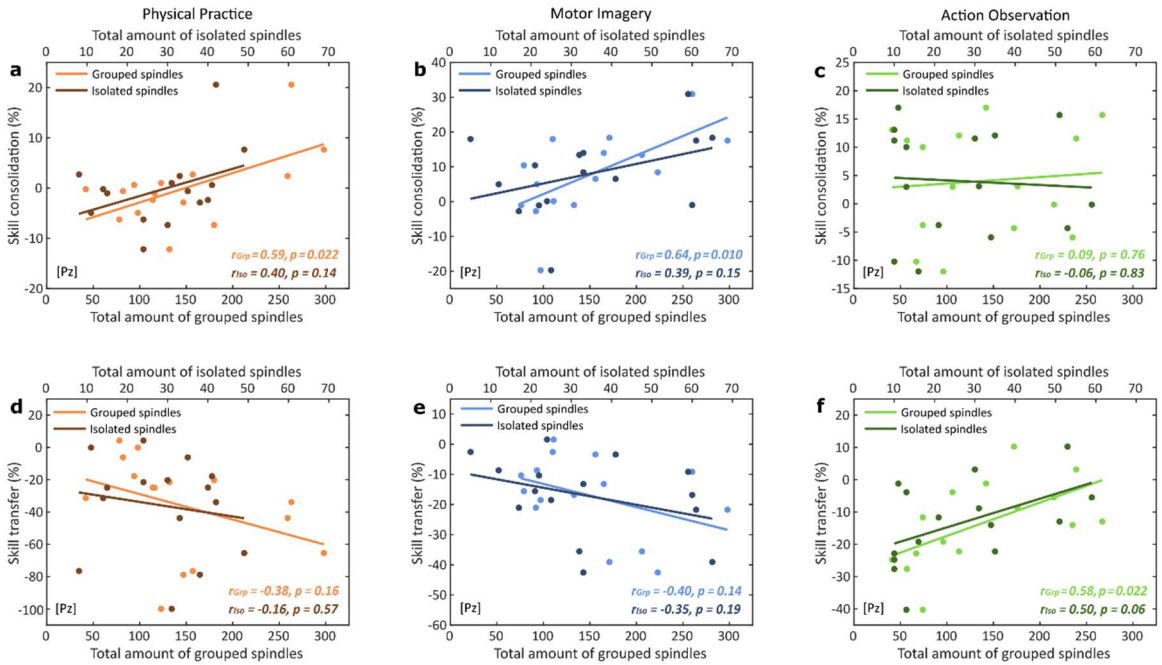

Fig. 3. Sleep-related motor skill consolidation and transfer following physical practice, motor imagery and action observation. *Upper row.* Graphical illustration of the relationship between the magnitude of skill consolidation (percentage of performance gains across the sleep retention interval, from the post-test to the retention test) and the total amount of grouped (lighter points) or isolated spindles (darker points) in the **(a)** physical practice (red; n = 15), **(b)** motor imagery (blue; n = 15), and **(c)** action observation (green; n = 15) groups. *Lower row.* Graphical illustration of the relationship between the magnitude of skill transfer (percentage of performance gains from the retention to the inter-manual transfer test) and the total amount of grouped (lighter points) or isolated spindles (darker points) in the **(d)** physical practice (red; n = 15), **(e)** motor imagery (blue; n = 15), and **(f)** action observation (green; n = 15) groups. Scatter plots and linear trend-lines are provided. Pearson correlation coefficients (*r*) and associated p-values are reported for each correlation.

Time-frequency maps. Fig. 4 depicts the grand average TF maps zoomed in on -6 s and +6 s around spindle onsets during NREM2 sleep for the PP (left panel), MI (middle panel) and AO (right panel) groups. TF analysis confirmed the clustering and rhythmic nature of spindle events in all groups. The group-averaged TF map illustrates the significant periodic power increases in the spindle frequency band every 3-4 seconds (~0.2-0.3 Hz), irrespective of the practice mode. Noteworthy, TF maps also reveal a rhythmic pattern of power increases in the theta frequency band (4-8 Hz), which accords with current trends suggesting that cross-frequency interactions

between sleep spindles and theta waves may be relevant for sleep-related memory consolidation (see also (Schreiner et al., 2015)).

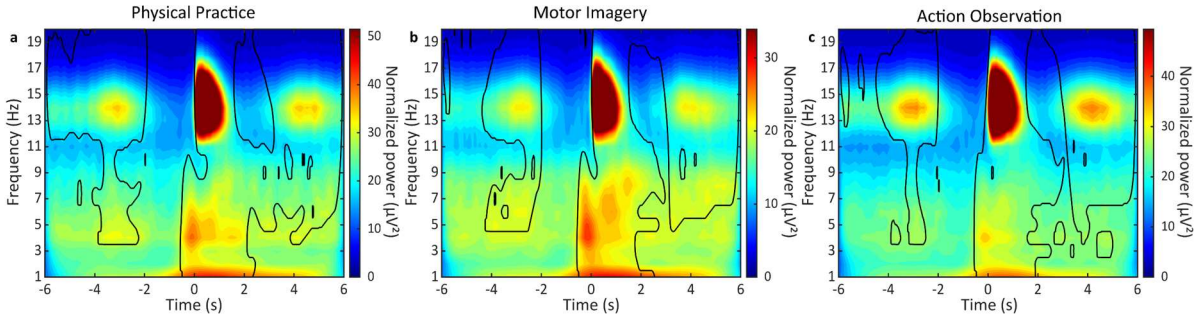

Fig. 4. Time-frequency decomposition of NREM2 sleep spindles following physical practice, motor imagery and action observation. Grand average time-frequency maps across participants for spindle events occurring at scalp electrode Pz, using epoch windows ranging from -6 s to +6 s around spindle onsets, and illustrating the 0.2-0.3 Hz spindle rhythmicity within trains during NREM2 sleep periods following physical practice (left panel; n = 15), motor imagery (middle panel; n = 15) and action observation (right panel; n = 15). The color bar reflects normalized (1/f compensation) spectral power values (µV²). Contour lines indicate regions where power is significantly higher than the baseline (-2 s to -0.5 s) for each frequency bin after correction for multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (N_{test} = 60020). NREM: Non-Rapid Eye Movement.

Discussion

In the current study, we examined (i) whether similar consolidation mechanisms are engaged during sleep following PP, MI and AO practice, and (ii) to assess the contribution of sleep spindles in motor skill consolidation and transfer, depending on the modality of practice. Our findings confirmed that participants acquired the motor sequence through PP, MI and AO practice, with an advantage for PP. Our results further revealed that sleep, and more specifically the time spent in NREM2 sleep, is crucial for motor skill consolidation following PP and MI practice and for motor skill transfer following AO practice, hence pointing towards potential modality-specific effects of sleep upon skill consolidation and its generalizability. In addition, we found that spindles occurring in trains during NREM2 sleep are more involved in the sleep consolidation process than isolated ones, leading to enhanced skill retention following PP and MI practice, and to improved skill transfer following AO practice. Finally, our results revealed that irrespective of the modality of practice, spindles tend to cluster in trains on a low-frequency time scale of about 50 s (~0.02 Hz), and during which spindles iterate every 3-4 s (~0.2-0.3 Hz). Therefore, and for the first time, our results provide evidence of a modality-unspecific organization of sleep spindles during the consolidation of motor skills.

During training, and as expected, the rehearsal of the motor sequence induced an increase in performance for all practice groups (Avanzino et al., 2015; Bird et al., 2005; Boutin et al., 2010; Gentili et al., 2010, 2006; Heyes and Foster, 2002; Ladda et al., 2021; Osman et al., 2005). As expected though, participants in the PP group expressed higher performance improvements than their MI group counterparts (Avanzino et al., 2015; Gentili et al., 2010, 2006; Land et al., 2016). Traditionally, it is also assumed that PP leads to greater practice-related gains than AO (Badets et al., 2018; Boutin et al., 2010; Deakin and Proteau, 2000). Albeit not significant, a clear tendency emerged in our results in accordance with this latter assumption. Previous studies have shown that AO learners may reach similar levels of performance than PP learners when only a few physical practice trials are provided (Boutin et al., 2010; Deakin and Proteau, 2000). Here, we can thus assume that motor-related information during AO may have been partly obtained through actual execution during the pre-test, leading to the encoding of a sequence representation calibrated with prior physical execution and not only because of additional physical practice during the test blocks since similar results would have emerged in the MI practice condition.

In relation to motor skill consolidation, our findings first corroborated previous findings in showing significant over-nap performance gains for all groups (see *Appendix A*, Fig A.1.) (Doyon et al., 2009; Fogel et al., 2017; Korman et al., 2007; Nishida and Walker, 2007; Walker et al., 2002). However, at the group level, no difference in skill consolidation was found between practice groups. To better examine the modality-specific effects of sleep upon motor skill consolidation, we evaluated the relationship between the magnitude of post-nap performance changes and sleep characteristics in the PP, MI and AO groups. We found a positive relationship between the time spent in NREM2 sleep and motor skill consolidation for participants in the PP and MI groups. This finding accords with previous studies depicting the crucial role of NREM2 sleep in motor memory consolidation following PP (Boutin and Doyon, 2020; Fogel et al., 2017; Nishida and Walker, 2007; Walker et al., 2002). To the best of our knowledge, this is the first demonstration of the role of NREM2 sleep in the consolidation of motor skills following MI practice, albeit theoretically assumed (Debarnot et al., 2021, 2011). In addition, we also found a positive relationship between the amount of NREM2 sleep spindles and the magnitude of motor skill consolidation following PP and MI practice, emphasizing the role of sleep spindles in motor memory consolidation, even in the absence of overt movement. In contrast, however, our findings did not reveal any relationship between NREM2 sleep or spindle activity with the magnitude of skill consolidation following AO practice. These results are at first sight difficult to reconcile with the seminal study of (Van Der Werf et al., 2009) who

demonstrated that an early sleep window following AO is crucial for the emergence of offline performance gains. However, in their study, the benefits of sleep were only observed on behavioral performance at the group level, and the absence of polysomnographic monitoring prevented any analysis of sleep architecture or spindle activity in relation to motor skill consolidation. Thus, our results rather accord with studies suggesting that AO practice may trigger different consolidation processes than those triggered by PP, leading to different behavioral outcomes (Badets et al., 2018; Trempe et al., 2011).

In this way, and very interestingly, our results confirmed the potential engagement of distinct consolidation mechanisms during sleep following PP, MI and AO. Indeed, our findings revealed a positive relationship between the time spent in NREM2 sleep and inter-manual skill transfer for participants in the AO group only. We further found a positive correlation between the amount of NREM2 sleep spindles and the magnitude of skill transfer following AO practice, revealing the critical role of sleep spindle activity in the memory consolidation process following AO. Hence, we conjecture that sleep differently affects the representation of a motor skill depending on prior training experience. This assumption is also supported by transfer performance at the group level, since we showed greater post-nap skill transfer following both MI and AO in comparison to PP. This latter finding accords with previous studies showing better skill transfer following MI and AO in comparison to PP (Boutin et al., 2011, 2010; Gruetzmacher et al., 2011; Land et al., 2016; Wohldmann et al., 2008). It is now well accepted in the literature that PP of long complex sequences would mainly engage an encoding of the motor task in visuo-spatial coordinates (i.e., effector-unspecific learning) (Boutin et al., 2010, p. 201; Doyon et al., 2018; Kovacs et al., 2009b). In contrast, shorter sequences would mainly be encoded in motor coordinates (i.e., effector-specific learning) (Doyon et al., 2018; Kovacs et al., 2010, 2009a). Therefore, physical practice of a short 5-element motor sequence in our study may have led to the development of an effector-specific learning of the motor skill, as reflected by impaired transfer performance. In contrast, the higher inter-manual skill transfer capacity found for the MI and AO groups indicates that both non-physical practice conditions may rather have led to the development of a motor skill representation at an effector-unspecific level (see also (Boutin et al., 2010; Land et al., 2016)).

Our behavioral results also accord with previous neuroimaging findings showing more consistent brain activations during MI and AO in comparison to PP, in an effector-unspecific manner within a predominant premotor-parietal network (Caspers et al., 2010; Hardwick et al., 2018; Hétu et al., 2013; Mizuguchi et al., 2014). In a recent neuroimaging meta-analysis, Hardwick and colleagues (Hardwick et al., 2018) compared the pattern of brain activations

following PP, MI and AO, and identified brain areas involved in both the simulation of actions (MI and AO) and actual motor execution (PP) through a cortical-dominant network. This network comprises essentially premotor, parietal, and sensorimotor regions, albeit to a lesser extent for MI and AO. Hence, while a set of common neural structures is activated during PP, MI and AO, leading to enhanced motor performance and learning, the additional and specific recruitment of brain regions with respect to the modality of practice may be responsible for the distinct encoding strategies and behavioral outcomes observed during the post-training, retention and transfer tests.

Finally, we investigated the organization of sleep spindle activity in all practice groups, and its potential contribution to motor skill consolidation and transfer. Interestingly, time-frequency analyses revealed a clear clustering and temporal occurrence of sleep spindles every 3 to 4 seconds in all practice groups. Hence, such a cluster-based organization of sleep spindles in trains may be an endogenous, modality-unspecific mechanism critical for consolidating newly-formed motor memories during sleep. In addition, and as previously shown for PP-based motor learning (Antony et al., 2018; Boutin et al., 2022; Fernandez and Lüthi, 2020; Lecci et al., 2017), our analyses confirm that spindles tend to cluster on an infraslow time scale of about 50 s (~0.02 Hz) during NREM2 sleep, with a rhythmic occurrence of spindles during trains that follows a time scale of about 3-4 s (~0.2-0.3 Hz), irrespective of the practice mode. In order to assess the relevance of this temporal organization, sleep spindles were either categorized as grouped or isolated according to whether or not they were belonging to spindle trains, respectively (see section 2. Material and methods). Correlation analyses revealed significant positive relationships between grouped spindles and skill consolidation following PP and MI, but not AO. In contrast, a significant positive relationship was found between grouped spindles and skill transfer in the AO practice group only. No significant correlations were found between isolated spindles and the magnitude of skill consolidation and transfer for all groups. These results extend recent findings towards overt and covert practice, by showing that grouped spindles are playing a more critical role than isolated ones in the consolidation and transfer of motor skills acquired through physical and non-physical practice (Boutin et al., 2022, 2018; Boutin and Doyon, 2020; Debarnot et al., 2011, 2009; Trempe et al., 2011). Altogether, our findings demonstrate a different involvement of spindle activity in the sleep consolidation and transfer of motor skills during a daytime nap, despite an apparent modality-unspecific and cluster-based organization of NREM2 sleep spindles.

It should be noted, however, that we failed to found significant relationships between the magnitude of skill consolidation and transfer with both global and local spindle density

metrics, which are commonly used in the memory consolidation and sleep research domains (Boutin et al., 2018). This is likely due to a difference in spindle activity between a nap and a night of sleep. Indeed, there are substantial variations in the sleep EEG spectra (in the spindle frequency band in particular) depending on melatonin rhythms and endogenous circadian phases of sleep consolidation (Dijk et al., 1997; Dijk and Czeisler, 1995) that could potentially explain the absence of significant correlations between spindle density metrics and post-nap performance in our study. A density calculation over such a short sleep time may therefore not be adequate to quantify the direct relationship between sleep spindles and motor memory consolidation (see (Nishida and Walker, 2007)).

To conclude, physical practice, motor imagery and action observation are effective practice modalities to learn a motor skill, with the development of an effective memory representation during practice that undergoes further modification during subsequent sleep. Interestingly, our findings underline the fundamental and modality-specific role of NREM2 sleep spindle activity in motor skill consolidation and transfer. Despite an apparent similar cluster-based organization of NREM2 spindles during the post-learning nap in all groups, different behavioral outcomes are elicited during retention and transfer performance. We show that a daytime nap offers an early sleep window that promotes the consolidation and retention of the learned motor skill following PP and MI practice, while an effector-unspecific sequence representation favoring skill transfer is consolidated after AO practice. Altogether, we demonstrate that PP per se is not a pre-requisite for sleep-related consolidation of motor skills, and that the clustering of sleep spindles in trains may be a critical mechanism for effective skill consolidation and transfer, depending on the modality of practice. Finally, given the non-physical nature of MI and AO practice, and their sleep-related skill consolidation and transfer opportunities, our findings may encourage the development of non-physical training protocols for the learning of new motor skills, as well as for the designing of innovative rehabilitation interventions such as for patients with motor deficits having to remaster skills following physical or brain injury.

Material and methods

Participants

Forty-five healthy volunteers (18 females, mean age: 23.7 ± 4 years) were recruited by local advertisements and were randomly and equally assigned to either a PP group (8 females, mean age: 22.8 ± 4 years), MI group (6 females, mean age: 24.5 ± 4 years) or AO group (4

357 females, mean age: 23.9 ± 5 years). All participants met the following inclusion criteria: aged
358 between 18 and 35 years, right-handed (Edinburgh Handedness Inventory (Oldfield, 1971)),
359 medication-free, without history of mental illness, epilepsy or head injury with loss of
360 consciousness, sleep or neurologic disorders and no recent upper extremity injuries. The
361 experimental protocol was approved by the "Université Paris-Saclay" Local Ethics Committee
362 (CER-Paris-Saclay-2019-057-A1) and conformed to relevant guidelines and regulations. All
363 participants gave written informed consent before inclusion. Participants were asked to
364 maintain a regular sleep-wake cycle and to refrain from all caffeine- and alcohol-containing
365 beverages 24h prior to the experimentation. Participants in the MI group were also required to
366 complete the French version of the Movement Imagery Questionnaire-Third version (MIQ-3f)
367 (Robin et al., 2020) before starting the experimental protocol. The MIQ-3f is a twelve-item self-
368 report questionnaire, in which participants are asked to perform a given movement followed by
369 its mental execution either by external visual imagery, internal visual imagery or kinesthetic
370 imagery. Participants rate the difficulty with two 7-point scales respectively for visual or
371 kinesthetic imagery, ranging from 1 (very hard) to 7 (very easy). A higher average score
372 represents a greater imagery capacity.
373
374 **Experimental design and motor sequence task**
375 Participants sat on a chair at a distance of 50 cm in front of a computer screen, equipped
376 with a 64-channel EEG cap. The motor task consisted of performing as quickly and as
377 accurately as possible a 5-element finger movement sequence by pressing the appropriate
378 response keys on a standard French AZERTY keyboard using their left, non-dominant hand.
379 The sequence to be performed (C-N-V-B-C, where C corresponds to the little finger and N to
380 the index finger) was explicitly taught to the participant prior to training.
381 Physical practice blocks consisted of 16 repetitions of the 5-element sequence (i.e., a total
382 of 80 keypresses). Each block began with the presentation of a green cross in the center of the
383 screen accompanied by a brief 50-ms tone. In case of occasional errors, participants were asked
384 "not to correct errors and to continue the task from the beginning of the sequence" (see (Gabitov
385 et al., 2017) for a similar procedure). At the end of each block, upon completion of the 80
386 keypresses, the color of the green-colored imperative stimulus turned red, and participants were
387 then required to look at the fixation cross during the 30-s rest period. This protocol controlled
388 for the number of movements executed per block to ensure that the same amount of practice
389 with the task was afforded across participants during a particular session. Stimuli presentation

and response registration were controlled using the MATLAB R2016b software from The MathWorks (Natick, MA) and the Psychophysics Toolbox extensions (Kleiner et al., 2007).

The study started at 1.00 pm to minimize the putative impact of both circadian and homeostatic factors on individual performance levels and sleep characteristics (Dijk et al., 1997; Dijk and Czeisler, 1995; Dijk and von Schantz, 2005). The experimental procedure was composed of seven main phases: familiarization, pre-test, training, post-test, 90-minute nap, retention and transfer (Fig. 5). Hence, prior to training, participants underwent a brief familiarization phase during which they were instructed to repeatedly and slowly perform the 5-element sequence until they accurately reproduced the sequence three consecutive times. This familiarization was intended to ensure that participants understood the instructions and explicitly memorized the sequence of movements.

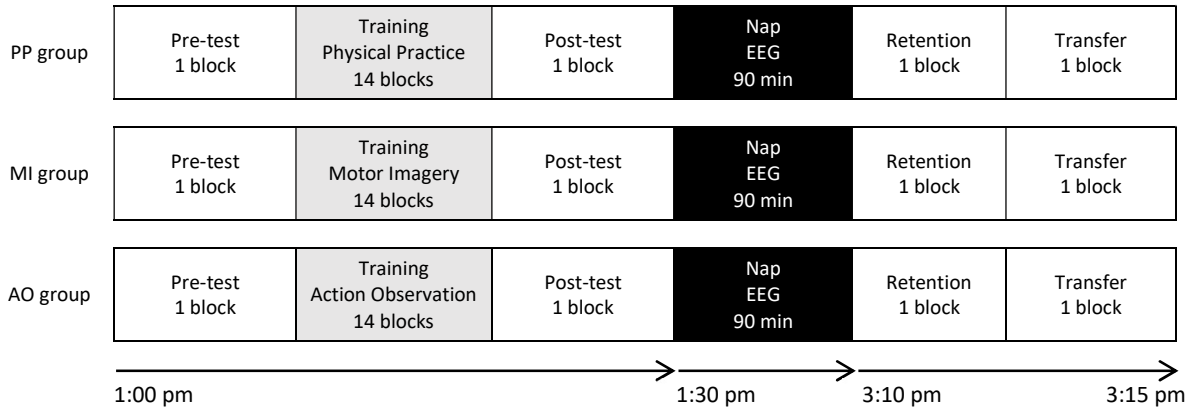

Fig. 5. Experimental design. Participants (n = 45) were trained on a 5-element finger movement sequence either by physical practice (PP), motor imagery (MI) or action observation (AO). Sleep EEG recordings were acquired during a 90-min daytime nap following training. Participants were subsequently tested on the same motor sequence during retention and inter-manual transfer tests. During the pre-test, post-test, retention and transfer tests, all participants were required to physically performed the motor sequence. EEG: Electroencephalography.

During the pre-test, all participants physically performed one block of the 5-element motor sequence. The ensuing training phase consisted of 14 blocks performed with physical, observational or mental practice. Participants in the PP group were asked to physically execute the sequence task with their left-hand fingers, as previously described. Participants in the AO group were instructed to keep their fingers on the corresponding response keys. Following the imperative green-cross stimulus and audio cue, a video of a model performing the motor task was displayed on the screen. The model was depicted so that the observers could see both the finger movements of the model and the green cross appearance on the screen. This viewing angle was adopted in order to closely match the perspective view of the AO participants. An

additional window inset zooming on the left-hand fingers of the model was implemented so that participants could precisely watch fine finger movements. Participants in the AO group were free to observe both perspectives in an active and conscious manner while avoiding any concomitant muscular execution of the movement (controlled online by electromyography (EMG) recording electrodes placed on the left flexor digitorum superficialis; see section 2.3. EEG-EMG data acquisition and pre-processing for details). Based on previous motor sequence learning findings (Boutin et al., 2018), performance improvements of the model across training blocks followed the power-law of practice (mean response time (RT) between consecutive keypresses ranging from RT_{Block1} = 616 ms to $RT_{Block14}$ = 223 ms). Participants in the MI group were instructed to keep their left-hand fingers on the corresponding response keys and their thumb on the keyboard's space bar. When they heard the imperative audio cue, they had to imagine themselves performing the sequence using a combination of internal visual and kinesthetic imagery, while avoiding any associated overt movements (controlled online by similar EMG procedure as for the AO group). After completing each mentally rehearsed sequence, they were asked to press the space bar with their thumb to objectively control for the amount of MI practice. Each block was composed of 16 mental repetitions of the motor sequence.

Approximately five minutes after completion of the training phase, all participants underwent a post-test phase identical to the pre-test. This session was briefly preceded by a physical warm-up phase (i.e., slow-paced production of the sequence three consecutive times) for all groups. Following the post-test, all participants were administered a 90-minute nap. Ten minutes after awakening, all participants were asked to perform again a physical warm-up phase (i.e., three slow-paced repetitions of the trained sequence) before completing the retention and transfer test blocks. The retention test was done first and consisted of one practice block performed with the non-dominant left hand. An inter-manual transfer test was then carried out. Participants were instructed to perform one block on the original motor sequence but with the unpracticed, dominant right hand.

During each test block, RT was measured as the time interval between two consecutive keypresses. Also, since participants were asked to start over from the beginning of the sequence if they made any error during task production, RTs from error trials (i.e., erroneous key presses) were excluded from the analyses. Across all test blocks, the PP group performed 14.6 (± 1.6) accurate sequences, 14.1 (± 1.7) for the MI group, and 14.6 (± 1.7) for the AO group (out of a maximum of 16 sequences). To better reflect individual performance on the motor sequence task, we computed mean RT performance on accurately typed sequences (Bönstrup et al., 2019;

Vahdat et al., 2017). Individual RTs were then averaged to obtain an overall estimation of the performance for each practice block. Motor skill acquisition was assessed by analyzing the RT performance gains (in percentages) from the pre-test to the post-test block. Motor skill consolidation was assessed by analyzing the post-nap retention performance gains, and indexed by the percentage of RT changes from the post-test to the retention test. Finally, inter-manual skill transfer capacity was assessed by computing the difference (in percentages) between RT performance on the retention and transfer test blocks. Note that negative values reflect impairments in skill transfer.

EEG-EMG data acquisition and pre-processing

EMG recordings. Two bipolar Ag-AgCl electrodes with 10 kΩ safety resistor were placed at a distance of 3 cm from each other along the belly of the left flexor digitorum superficialis. During training, the EMG signal was monitored and controlled online to ensure the absence of micro finger movements for the MI and AO groups. If the experimenter detected any muscle activity, participants were instructed to immediately relax their hand.

EEG recordings. EEG was acquired using a 64-channel EEG cap (actiCAP snap BrainProducts Inc.). The EEG cap included slim-type electrodes (5kΩ safety resistor) suitable for sleep recordings, with FCz and AFz being, respectively, the reference and ground electrodes. For reliable sleep stage scoring, we also added electrooculography (EOG) and EMG recordings using bipolar Ag-AgCl electrodes. We recorded the vertical EOG component by placing pairs of electrodes above and below the left eye. EMG bipolar electrodes were placed over the chin. All EEG, EMG and EOG data were recorded using two battery-powered 32-channel amplifiers and a 16-channel bipolar amplifier (respectively, BrainAmp and BrainAmp ExG, Brain Products Inc.). All signals were recorded at a 5-kHz sampling rate with a 100-nV resolution. Electrode-skin impedance was kept below 5 kΩ using Abralyt HiCl electrode paste to ensure stable recordings throughout all experimental phases.

EEG data were bandpass filtered between 0.5 and 50 Hz to remove low-frequency drift and high-frequency noise, down-sampled to 250 Hz, and re-referenced to the linked mastoids (i.e., TP9 and TP10). EOG and EMG data were respectively bandpass filtered between 0.3-35 Hz and 10-100 Hz.

EEG analysis

Spindle detection. The artefact-free EEG signal was sleep-stage scored according to AASM guidelines (Iber et al., 2007). Each 30-second epoch was visually scored as either

NREM stages 1-3, REM, or wake (Table 1). The detection of spindle events was conducted using all artefact-free NREM2 sleep epochs over the parietal site (electrode Pz), as expression of spindles has been shown to predominate over this region following motor sequence learning (Boutin et al., 2022, 2018; Fogel et al., 2017; Laventure et al., 2016). Discrete sleep spindle events (i.e., onset and offset) were automatically detected using a wavelet-based algorithm (see (Boutin et al., 2018) for further details). Spindles were detected at the Pz derivation by applying a dynamic thresholding algorithm (based on the median absolute deviation of the power spectrum) to the extracted wavelet scale corresponding to the 11-16 Hz frequency range and a minimum window duration set at 300 ms (Boutin et al., 2022, 2018; Lustenberger et al., 2018) (see (Boutin and Doyon, 2020) for a review). Events were considered sleep spindles only if they lasted 0.3-2 seconds, occurred within the 11-16 Hz frequency range and with onsets during NREM2 sleep periods.

Spindle clustering. As recently proposed by Boutin and Doyon, (2020), sleep spindles may be split into two categories: clusters of two or more consecutive and electrode-specific spindle events interspaced by less than or equal to 6 seconds were operationalized as trains, in comparison to those occurring in isolation (i.e., more than 6 seconds between two consecutive spindles detected on the same electrode). Hence, for convenience, spindles belonging to trains were categorized as *grouped spindles*, and those occurring in isolation were categorized as *isolated spindles*. Several variables of interest were considered: the total amount of spindles, three metrics related to spindle clustering (total number of grouped and isolated spindles, total number of trains), two measures of spindle rhythmicity (inter-spindle interval [ISI] within trains and inter-train interval [ITI]; in seconds), and two measures of spindle density (global density [number of spindles per minute] and local density [number of spindles within a spindle-centered sliding window of 60 seconds]).

Time-frequency maps. To evaluate the temporal organization of NREM2 sleep spindles, we conducted time-frequency (TF) analyses. For each NREM2 sleep spindle detected, we extracted a 12-second time window centered on the spindle onset. We applied for each epoch a TF decomposition across the 1-20 Hz frequency range using complex Morlet Wavelet. Spectral resolution of the wavelet was defined with a central frequency set at 1 Hz, and temporally with the full-width at half-maximum (FWHM) set at 3 seconds. TF maps were normalized by multiplying the power at each frequency bin with the frequency value (1/f compensation). Finally, to assess statistical significance of the power variation at each frequency bin over time, we used the student's *t*-test against a predefined baseline window for each practice group. The baseline was set from -2 seconds to -0.5 seconds before spindle onsets. Given the *a priori*

hypothesis that power increases are expected in the spindle frequency band in comparison to the baseline, we performed a one-tailed *t*-test with a significant threshold set at 0.05. Statistical maps were then corrected for multiple comparisons using the Benjamini-Hochberg procedure to control the false discovery rate (Ntest = 60020) (Benjamini and Hochberg, 1995). All analyses were done using the MATLAB R2019b software from The MathWorks (Natick, MA) and the open-source Brainstorm software (Tadel et al., 2011).

Code availability

Sleep EEG data were processed using the MATLAB R2019b software from The MathWorks (Natick, MA) and the open-source Brainstorm and EEGLAB software. The codes for the detection and clustering of sleep spindles are available at the following GitHub repositories: https://github.com/arnaudboutin/Spindle-detection and https://github.com/arnaudboutin/Spindle-clustering. The codes used to perform other analyses are available from the corresponding author upon reasonable request.

Data availability

The data that support the results of this study are available from the corresponding author upon reasonable request and under a formal data sharing agreement.

Author Contributions

Author contributions: A.C., A.B. and U.D. conceived the experiment; A.C. collected the data; A.C. and A.B. analyzed the data and discussed the results; A.C., A.B. and U.D. wrote the manuscript; All authors revised the manuscript.

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Appendix A

Figure A.1. Behavioral results. Illustration of the RT performance of all groups for each test block. To assess changes in performance at the end of the acquisition phase depending on the initial practice mode, we performed a mixed ANOVA using a MODALITY (PP, MI, AO) x BLOCK (pre-test, post-test) factorial design, with repeated measures on the factor BLOCK. The analysis revealed significant main effects of MODALITY ($F(2,42) = 5.25$, $p = 0.009$, $\eta^2_p = .13$) and BLOCK ($F(1,42) = 128$, $p < 0.001$, $\eta^2_p = .26$), but failed to detect a significant MODALITY X BLOCK INTERACTION ($F(2,42) = 2.02$, $p = 0.15$). For the main effect of MODALITY, Holm post-hoc comparisons revealed that participants in the PP group (RT = 341 ms) outperformed participants in both the MI (RT = 425 ms, $p = 0.017$) and AO groups (RT = 419 ms, $p = 0.020$). For the main effect of BLOCK, post-hoc comparisons revealed lower RTs during the post-test relative to the pre-test (from $RT_{pretest} = 450$ ms to $RT_{posttest} = 339$ ms, $p < 0.001$).

In addition, to assess sleep-dependent changes in performance depending on the initial practice mode, we performed a mixed ANOVA using a MODALITY (PP, MI, AO) x BLOCK (post-test, retention) factorial design, with repeated measures on the factor BLOCK. The analysis revealed significant main effects of MODALITY ($F(2,42) = 8.85$, $p < 0.001$, $\eta^2_p = .28$) and BLOCK ($F(1,42) = 9.18$, $p = 0.004$, $\eta^2_p = .11$), but failed to detect a significant MODALITY X BLOCK INTERACTION ($F(2,42) = 2.76$, $p = 0.075$). For the main effect of MODALITY, Holm post-hoc comparisons revealed that participants in the PP group (RT = 273 ms) outperformed participants in both the MI (RT = 367 ms, $p = 0.001$) and AO groups (RT = 354 ms, $p = 0.003$). For the main effect of BLOCK, post-hoc comparisons revealed lower RTs during retention relative to the post-test (from $RT_{posttest} = 339$ ms to $RT_{retention} = 322$ ms, $p = 0.004$).

Finally, to assess the transfer capacity depending on the initial practice mode, we performed a mixed ANOVA using a MODALITY (PP, MI, AO) x BLOCK (retention, transfer) factorial design, with repeated measures on the factor BLOCK. The analysis revealed significant main effects of MODALITY ($F(2,42) = 3.54$, $p = 0.038$, $\eta^2_p = .11$) and BLOCK ($F(1,42) = 61.0$, $p < 0.001$, $\eta^2_p = .14$), but failed to detect a significant MODALITY X BLOCK INTERACTION ($F(2,42) = 2.18$, $p = 0.13$). For the main effect of MODALITY, Holm post-hoc comparisons revealed that participants in the PP group (RT = 316 ms) outperformed participants in the MI group (RT = 380 ms, $p = 0.049$) but not in the AO group (RT = 368 ms, $p = 0.097$). For the main effect of BLOCK, post-hoc comparisons revealed higher RTs during transfer test relative to retention (from $RT_{retention} = 323$ ms to $RT_{transfer} = 387$ ms, $p < 0.001$).

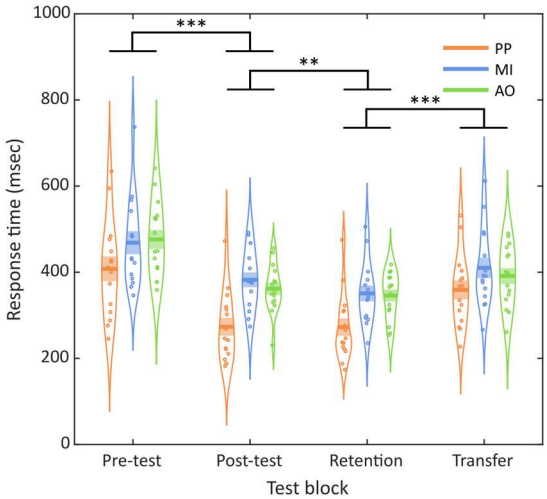

Fig. A.1. Behavioral results. Mean response time (RT) on each test block according to the modality of practice in the physical practice (PP), motor imagery (MI) and action observation (AO) groups. The curved lines indicate the distribution of data, the dark bars represent the mean of the distribution, and the lighter area surrounding the mean represent the standard error of the means. Individual data points are displayed as colored circles (n = 15 for each group at each test block). ** $p < 0.01$, *** $p < 0.001$.

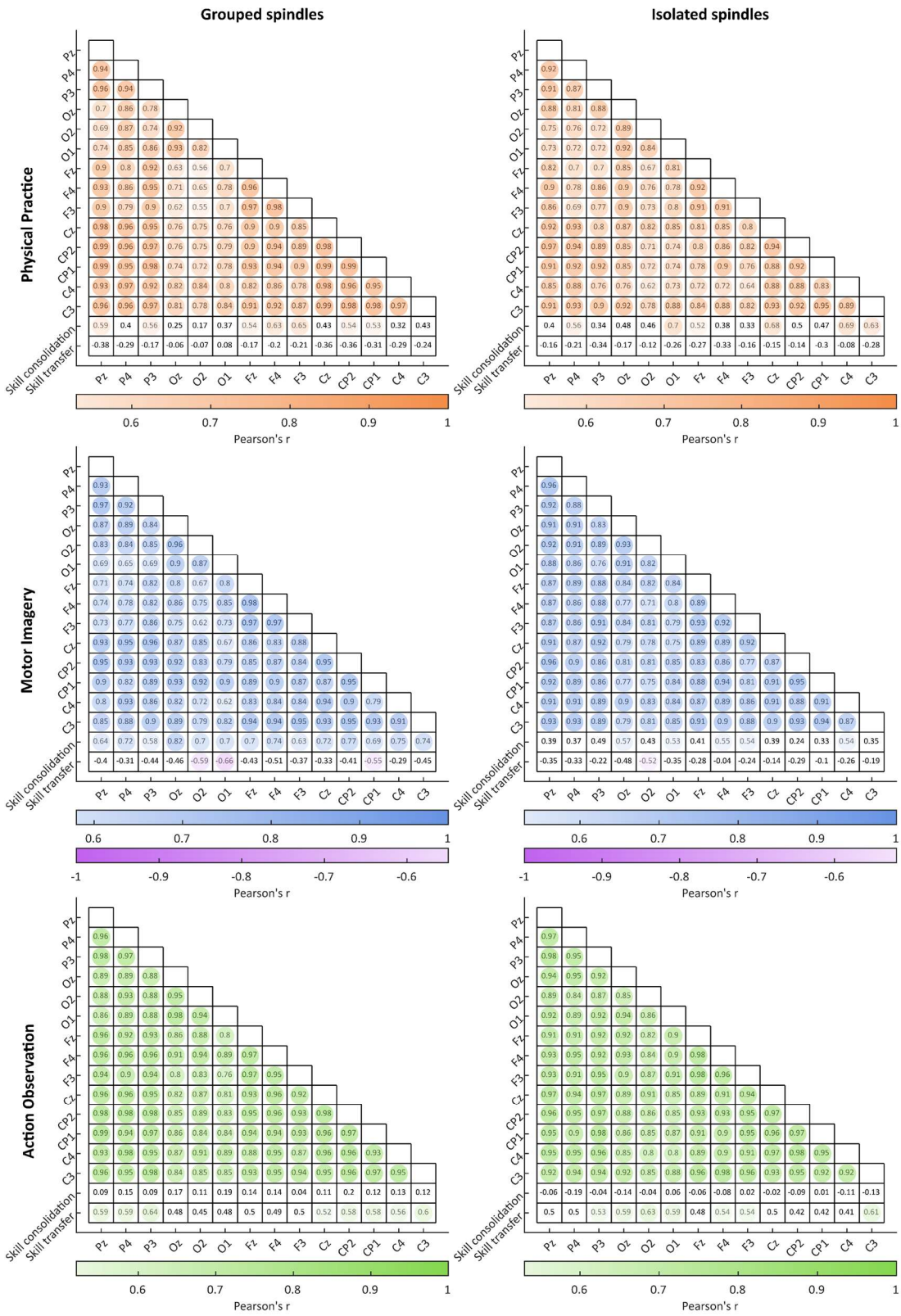

Fig. A.2. Grouped and isolated spindles in relation with skill consolidation and transfer. Relationship between the number of grouped spindles (left column) and isolated spindles (right column) detected over main scalp derivations with the magnitude of skill consolidation and transfer following physical practice (orange, first row), motor imagery (blue, second row) and action observation (green, third row). Pearson correlation coefficients (*r*) are reported for each correlation. Colored circles correspond to significant p-value ($p < 0.05$).

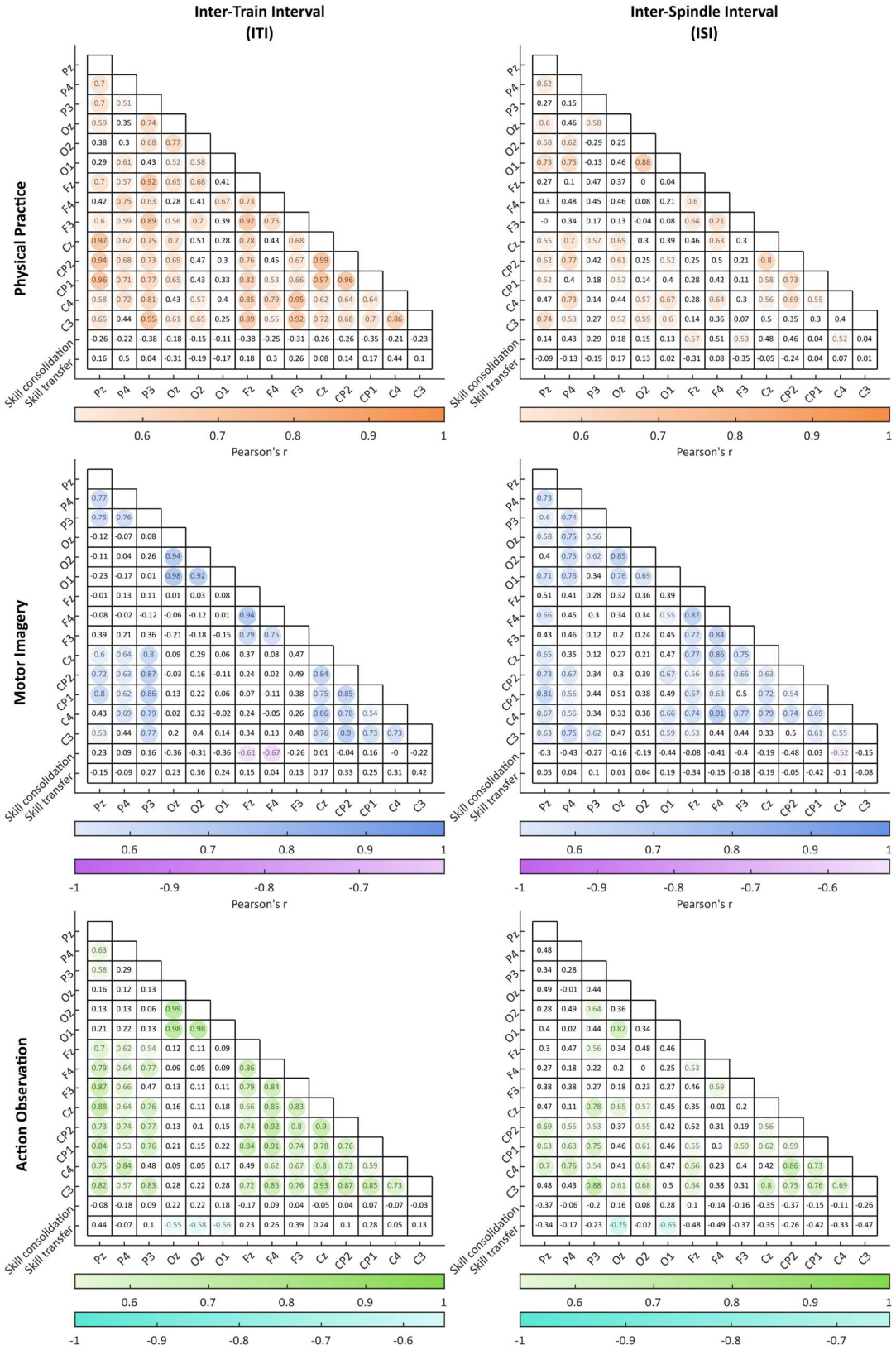

Fig. A.3. Sleep spindle clustering and rhythmicity in motor skill consolidation and transfer. Relationship between the inter-train interval (left column) and inter-spindle interval (right column) detected over main scalp derivations with the magnitude of skill consolidation and transfer following physical practice (orange, first row), motor imagery (blue, second row) and action observation (green, third row). Pearson correlation coefficients (*r*) are reported for each correlation. Colored circles correspond to significant p-value ($p < 0.05$).

Note A.4. Multiple regression analyses of grouped and isolated spindles regarding skill consolidation and transfer. Multiple regression analyses were separately performed for each group to determine whether grouped or isolated sleep spindles predict motor skill consolidation, with respect to the modality of practice. The multiple regression model significantly predicted skill consolidation for the MI group only ($F(2,12) = 4.24$, $p = 0.04$, $R^2 = 0.41$). More specifically, only the factor grouped spindles was found to significantly contribute to the prediction of the model ($t_{Grp} = 2.32$, $p = 0.039$; $t_{Iso} = -0.35$, $p = 0.74$), thus revealing that the higher the amount of grouped spindles during the nap following MI practice, the better the offline performance gains during sleep consolidation. The multiple regression model failed to significantly predict skill consolidation in the PP group ($F(2,12) = 3.32$, $p = 0.07$, $R^2 = 0.36$) and the AO group ($F(2,12) = 0.32$, $p = 0.73$, $R^2 = 0.05$). Additional multiple regression analyses were separately performed for each group to determine whether grouped or isolated sleep spindles predict motor skill transfer capacities, with respect to the modality of practice. The multiple regression model failed to predict skill transfer capacity in the PP ($F(2,12) = 1.03$, $p = 0.39$, $R^2 = 0.15$), MI ($F(2,12) = 1.24$, $p = 0.32$, $R^2 = 0.17$), and AO groups ($F(2,12) = 3.21$, $p = 0.08$, $R^2 = 0.35$).