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Sleep and memory consolidation: Motor performance and proactive interference effects in sequence learning

Guillermo Borragán [a,*], Charline Urbain [a,b,c], Rémy Schmitz [a,d], Alison Mary [a], Philippe Peigneux [a,*]

[a] UR2NF – Neuropsychology and Functional Neuroimaging Research Group at CRCN – Center for Research in Cognition and Neurosciences, Université Libre de Bruxelles (ULB) and UNI – ULB Neurosciences Institute, Brussels, Belgium
[b] Department of Diagnostic Imaging, The Hospital for Sick Children, Toronto, Canada
[c] Neuroscience & Mental Health Program, The Hospital for Sick Children Research Institute, Toronto, Canada
[d] LABNIC – Laboratory for Neurology and Imaging of Cognition, Department of Neurosciences, Campus Biotech, University of Geneva (UNIGE), Geneva, Switzerland

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ABSTRACT

That post-training sleep supports the consolidation of sequential motor skills remains debated. Performance improvement and sensitivity to proactive interference are both putative measures of long-term memory consolidation. We tested sleep-dependent memory consolidation for visuo-motor sequence learning using a proactive interference paradigm. Thirty-three young adults were trained on sequence A on Day 1, then had Regular Sleep (RS) or were Sleep Deprived (SD) on the night after learning. After two recovery nights, they were tested on the same sequence A, then had to learn a novel, potentially competing sequence B. We hypothesized that proactive interference effects on sequence B due to the prior learning of sequence A would be higher in the RS condition, considering that proactive interference is an indirect marker of the robustness of sequence A, which should be better consolidated over post-training sleep. Results highlighted sleep-dependent improvement for sequence A, with faster RTs overnight for RS participants only. Moreover, the beneficial impact of sleep was specific to the consolidation of motor but not sequential skills. Proactive interference effects on learning a new material at Day 4 were similar between RS and SD participants. These results suggest that post-training sleep contributes to optimizing motor but not sequential components of performance in visuo-motor sequence learning.

1. Introduction

Motor learning is at the root of many daily activities, requiring coordination between afferent multimodal stimulations and the production of appropriate efferent motor commands (Wolpert, Ghahramani, & Flanagan, 2001). Successful motor learning is a long-term process whereby a rapid improvement of performance is observed within the first trials, then followed by slower performance gains achieved through sustained repetition (Karni et al., 1998). Motor schemas progressively become more stable and resistant to interference with practice, disclosing a memory consolidation process (Krakauer & Shadmehr, 2006). Besides, motor memories continue to be consolidated after actual practice has ended, i.e. during so-called offline periods. In particular, post-learning sleep might contribute to the consolidation of novel motor representations, eventually leading to performance stabilization or improvement (Stickgold & Walker, 2007). However, this assumption is disputed by studies suggesting that post-training sleep and wakefulness periods might equally benefit the consolidation of motor skills (Al-Sharman & Siengsukon, 2014; Nemeth et al., 2010; Song, Howard, & Howard, 2007), or alternatively that sleep-related improvements in motor memory consolidation might be due to confounding factors such as massive practice and circadian confounds (Rickard, Cai, Rieth, Jones, & Ard, 2008).

Delineating how and to what extent sleep contributes to consolidating novel motor representations is complicated by several factors. First, sleep might actually subtend stabilization or improvement of performance for specific components of motor memories, or in definite contexts of acquisition. Accordingly, sleep-related improvement or stabilization in performance has been repeatedly reported using a motor finger-tapping task (FTT) in which subjects continuously reproduce the same short sequence of five-finger movements (Albouy et al., 2013; Debas et al., 2010; Doyon et al., 2009; Wilhelm et al., 2011). Visuo-motor sequence

* Corresponding authors at: UR2NF – Neuropsychology and Functional Neuroimaging Research Unit, Université Libre de Bruxelles, Avenue F.D. Roosevelt 50, B-1050 Brussels, Belgium. Fax: +32 (0)2 650 2209.
E-mail addresses: gborraga@ulb.ac.be (G. Borragán), philippe.peigneux@ulb.ac.be (P. Peigneux).

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learning tasks feature both progressive visual and motor adaptation to the settings of the task and the integration of sequential regularities embedded in the material (e.g. a repeated sequence of button presses corresponding to the stimuli's locations on a screen in the paradigmatic serial reaction time task [SRT; Nissen & Bullemer, 1987]). In these kind of tasks, sleep-dependent consolidation was reported for the goal-related, but not for the movement-related components of the learned sequences (Cohen, Pascual-Leone, Press, & Robertson, 2005) or when a cue indicates the presence of the sequence (Robertson, Pascual-Leone, & Press, 2004). In contrast, several studies found equal performance after sleep or wakefulness in sequence learning tasks when the succession of elements was embedded in noise (ASRT) or in probabilistic sequence learning paradigms, thus making sequence learning essentially implicit (Nemeth et al., 2010; Song et al., 2007). These latter results are in apparent contradiction with other studies having showed sleep-related performance changes (Cajochen et al., 2004), neuronal reactivation during post-learning sleep (Maquet et al., 2000; Peigneux et al., 2003) and sleep-dependent plasticity processes (Urbain et al., 2013) for implicitly learned sequences.

In a related domain, performance improvement on non-sequential motor learning tasks such as continuous tracking (Siengsukon & Al-sharman, 2011; but see Maquet, Schwartz, Passingham, & Frith, 2003) or motor adaptation to deviated trajectories was claimed to be non-sleep-dependent (Debas et al., 2010; Doyon et al., 2009). Alternatively, sleep was found to help to prevent a decrease of performance as compared to the end of learning (Albouy, Sterpenich, et al., 2013). Although these studies may suggest that post-training sleep is not beneficial for the consolidation of simple motor adaptation skills, high-density EEG data in adults disclosed a local increase in slow wave activity (SWA) during NREM sleep after task practice in learning-related areas, that was correlated with performance improvement (Huber, Ghilardi, Massimini, & Tononi, 2004). Also in children in whom it was claimed that sleep does not benefit procedural learning at all (Wilhelm, Diekelmann, & Born, 2008), sleep-dependent consolidation for the same motor adaptation task was exhibited looking at proactive interference effects (Urbain, Houyoux, Albouy, & Peigneux, 2014). In this latter study, although performance for the learned motor deviation was identical after an episode of sleep or of wakefulness, like in prior studies (Al-Sharman & Siengsukon, 2014; Siengsukon & Al-sharman, 2011), the presentation of the opposite motor deviation resulted in markedly higher proactive interference effects on performance in participants having slept after learning than in the wake condition. This suggests that the learned deviation was in fact more automatized after the post-training sleep episode, paradoxically resulting in more difficulties to adapt to a novel, opposite motor deviation (Urbain et al., 2014).

Hence, proactive interference effects may be useful markers of behavioral changes. Notwithstanding, only few studies have used interference (Korman et al., 2007; Urbain et al., 2014; Walker, Brakefield, & Hobson, 2003) or transfer (Witt, Margraf, Bieber, Born, & Deuschl, 2010) effects to index or modulate consolidation processes for simple motor tasks, and to the best of our knowledge none tested this effect in the context of visuomotor sequence learning. In the present study, we hypothesized that sleep-dependent memory consolidation processes in visuomotor sequence learning, and more specifically in a SRT paradigm, could be reflected through proactive interference effects. We surmised that even in a case when performance for the learned sequential material seemingly benefits to the same extent from post-training sleep and wakefulness, qualitative reorganization and structuration processes might benefit more from post-training sleep. This would eventually lead to an increased automatization of the learned sequence, which would be expressed by higher proactive interference effects when learning a novel material. In other terms, in line with Schneider and Shiffrin's (1977) assumption that "once learned, an automatic process is difficult to suppress, to modify, or to ignore", consolidation of motor memories can be measured as a function of the extent to which a consolidated sequence "A" proactively interferes with the learning of a novel sequence "B" (Ghilardi, Moisello, Silvestri, Ghez, & Krakauer, 2009). To test this hypothesis, we administered a tactile adaptation of the deterministic Serial Reaction Time (SRT) task on two different days. On day 1, participants learned a sequence "A". Half of them had a normal night of sleep after learning, whereas the other half was deprived of sleep. After two recovery nights, all participants were first tested on the learned sequence, allowing for the measurement of potential sleep-dependent changes in motor and sequential components of the learned sequence. They had to learn a novel sequence "B", allowing for the testing of proactive interference effects due to the possible sleep-dependent consolidation of the previously learned sequence "A".

2. Methods

2.1. Participants

Thirty-three young healthy adults (28 women, 5 men; mean ± SD age 21.8 ± 3.36 years) gave their written informed consent to participate in this study conducted in agreement with the Declaration of Helsinki and approved by the Faculty Ethics committee. No participant reported any history of neurological, psychiatric condition or sleeping disorder. Participants were randomly distributed in two groups. There were no significant differences for age ($p > .6$) or sex (Chi-square: .12, $p > .7$) between the two groups.

2.2. Experimental task (tactile SRT)

We used a tactile screen version of the deterministic serial reaction time (SRT) task initially developed by Nissen and Bullemer (1987). Participants were instructed to respond as quickly as possible to the appearance of a stimulus at one of the four screen locations (i.e. corners, see Fig. 1a), by pressing on the stimulus location using a finger of their dominant hand. The non-dominant hand was used irrespective of the participant's laterality because motor dexterity (i.e. precision and speed) is less developed with this hand than with the dominant hand (Francis & Spirduso, 2000), leaving more room for performance improvement. Unbeknownst to participants, the sequence of locations at which successive stimuli appeared was manipulated. A fixed 8-elements sequence was repeated throughout successive blocks of trials, except for one block during which the sequence followed a different order (see Procedure). In the SRT task, reaction time (RT) typically decreases with repeated presentation of a sequence, but presentation of a novel sequence (transfer) elicits slower RTs, indicating anticipation of the next elements in successive trials and successful learning of the trained sequence. Stimuli (i.e. the drawing of a car) were presented using the E-Prime software (Psychology Software Tools) on a computer screen (16 in.; refresh rate 60 Hz) adapted for tactile responses (Magic Touch Add-On Touch Screen, KeyTec, INC). Each stimulus lasted on screen upon subject's response for a maximum of 3000 ms, after which the next stimulus was displayed (response stimulus interval [RSI] 250 ms). Four different sequences were used in this experiment (see Procedure): learning sequences L1 (locations 4 2 1 3 2 4 3 1) and L2 (locations 1 3 4 2 3 1 2 4), reverses of each other (Schmitz, Pasquali, Cleeremans, & Peigneux, 2013) and transfer sequences T1 (locations 2 1 4 3 4 1 2 3) and T2 (locations 3 4 1 2 1 4 3 2). Each SRT block comprised 8 repetitions of the same sequence (i.e. 64 trials). To confirm correct understanding of

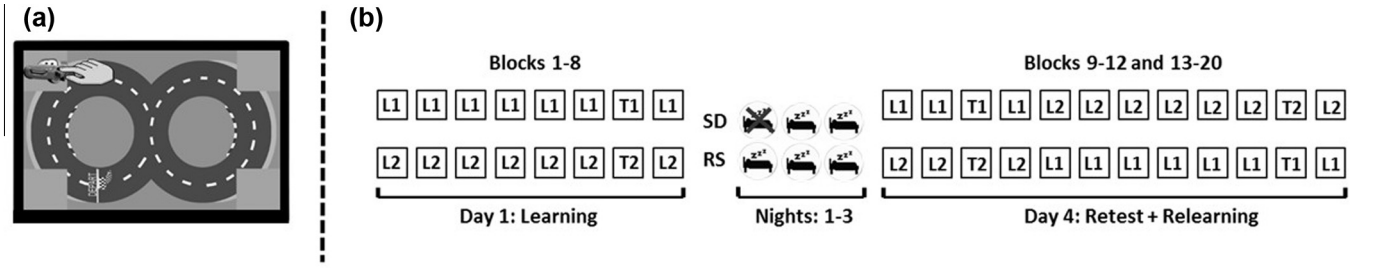

Fig. 1. (a) Illustration of the tactile adaptation of the deterministic visuo-motor adaptation SRT task. Participants are instructed to respond as quickly as possible by pressing with the index finger on the stimulus appearing at one of the four corners on a tactile screen. (b) Experimental design. L1 and L2 sequences are counterbalanced. Half the participants in each condition are trained on sequence L1 and the other half is trained on sequence L2. To allow participants in the SD condition to recover from the sleep deprivation night, all participants are allowed two supplementary nights of normal sleep before retest on Day 4. Day1: Learning phase sequence L1 (resp. L2). Day 4: Retest on sequence L1 (resp. L2) following by Relearning on sequence L2 (resp. L1).

the experimental instructions, a practice block was administered at the beginning of the experiment, comprising four trials presented along the four different locations. These responses were not analysed.

2.3. Procedure

An overview of the experimental design is illustrated in Fig. 1b. Participants were pseudo-randomly distributed across two post-training sleep conditions in a between-groups design: Regular Sleep (**RS**; $n = 16$; 14 right-handed participants, 2 men, mean age ± standard deviation 22.4 ± 4.4 years) and Sleep Deprivation (**SD**; $n = 17$; 14 right-handed participants, 3 men, mean age 21.6 ± 2.0 years).

Before entering the protocol, participants completed the Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, et al., 1989) providing an index of the average sleep quality over the last month. Cut-off exclusion global score for bad sleep quality was set at value > 7. They were also administered the morningness–eveningness questionnaire (Horne & Ostberg, 1976) aimed at determining the circadian chronotype. Extreme morning (>70) and extreme evening (<31) chronotypes were not included in the experiment.

Participants were requested to maintain regular sleep habits during the entire experiment. Compliance was assessed using actimeters (Daqtometer, Daqtix GbR, Oetzen, Germany) measuring variations in physical activity and ambient light. Actimeters were worn at the wrist of the non-dominant hand for a total of 7 days starting 4 days before the experimental manipulation (recording rate 2 Hz). In parallel, participants completed an adapted version of the St-Mary Hospital questionnaire (Ellis et al., 1981) every morning, assessing subjective sleep quality and duration of sleep in the preceding night.

On the first experimental day (Day 1; Learning phase), participants were administered 6 SRT blocks (B1–B6) using one of the two learning sequences (e.g. L1), followed by a transfer bloc (B7; e.g. T1) in which the succession of stimuli followed a different order of presentation, and then a final block (B8) using the learned sequence again (e.g. L1). L1 and L2 sequences were counterbalanced across participants and conditions. In each condition (RS or SD) half of the participants were administered sequence L1 and the other half sequence L2 (Fig. 1b).

Following the learning session on Day1, participants were either allowed to go home for a normal night of sleep (RS, Regular Sleep) or kept in the laboratory for a night of total sleep deprivation (SD) under the supervision of the principal investigator (GB). During the sleep deprivation night, participants remained quietly seated as much as possible. They were allowed quiet activities such as talking, watching movies or playing board games and had access to their personal computers. Stimulant drinks and tobacco were forbidden. To allow participants in the SD condition recovering from the effects of the sleep deprivation before retesting, all participants were allowed two supplementary nights of normal sleep at home before retesting on Day 4.

On Day 4, participants were administered the tactile SRT task under two different conditions. First, we tested retention of the sequence learned at Day 1 (Retest phase). Participants were administered the trained sequence (e.g. L1) on blocks B9, B10 and B12, whereas the transfer sequence was presented at block B11 (e.g. T1). Second, participants had to learn a novel sequence (e.g. L2) under the same condition than on Day 1 (Relearning phase). That is, they were administered 6 SRT blocks (B13–B18) using the novel sequence (e.g. L2), followed by a transfer block (B19; e.g. T2), then a final block (B20) using the same sequence as on blocks B13–B18 (Fig. 1b).

To control for circadian confounds on performance, all participants were tested between 17:00 and 20:00 both on Day 1 and Day 4, in the same quiet experimental room.

3. Results

3.1. Sleep and vigilance measures

3.1.1. Habitual sleep quality and chronotype

Habitual sleep quality and chronotype of the participants exhibited normal distribution (Kolmogorov test $ps > .20$) and homogeneous variance (Levene test $ps > .85$). Morningness–Eveningness Questionnaire (MEQ; Horne & Ostberg, 1976) scores did not differ between experimental groups (mean ± standard deviation RS = 46.4 ± 9 vs. SD = 46.2 ± 8.3; $T(31) = 0.04$, $p > .96$) and scored in the range of neutral chronotype. Likewise, average sleep quality over the month prior to the experiment was below the study cut-off score (<8) and similar between groups (PSQI score RS = 5.81 ± 1.9 vs. SD = 5 ± 2.23; $T(31) = 1.12$, $p > .27$, $p > .47$).

3.1.2. Sleep quality during the experimental protocol

A repeated measure ANOVA was conducted on daily reported subjective sleep quality scores (from 1 [bad] to 6 [good]) and sleep duration (hours) as derived from the St-Mary Hospital Questionnaire (Ellis et al., 1981) for the 4 days prior to the sleep manipulation, with within-subject factor Night (4 levels) and between-subjects factor Group (2 levels: RS vs. SD). Neither analysis disclosed any significant main or interaction effect (all $ps > 0.1$), indicating that participants had a similar stable sleep quality prior to the experimental manipulation (see Table 1).

Similarly, a separate repeated measure ANOVA was conducted on sleep quality score and sleep duration for the 2 days after the RS/SD manipulation, with within-subject factor Night (2 levels)

Table 1
Self-reported sleep quality (score) and sleep duration (hours) from the St-Mary Hospital Questionnaire (questions 4 and 5). Mean ± standard deviation.

Group	Night.1	Night.2	Night.3	Night.4	Night.5	Night.6	Night.7
Sleep duration							
RS	7.8 ± 1.3	7.5 ± 1	7.9 ± 1.1	8.1 ± 0.9	8.2 ± 0.6	8.4 ± 0.9	8.1 ± 0.6
SD	7.5 ± 0.9	7.8 ± 1	7.4 ± 1.1	7.9 ± 0.9		10 ± 1.3	8.4 ± 0.9
Sleep quality							
RS	4.2 ± 0.9	4 ± 1	3.9 ± 0.8	4.4 ± 0.6	3.7 ± 0.7	4 ± 0.8	4 ± 0.7
SD	4 ± 0.9	4.4 ± 1.3	3.8 ± 0.8	3.9 ± 0.8		5.1 ± 0.7	3.6 ± 0.9

and between-subjects factor Group (RS vs. SD). Analyses conducted on sleep duration disclosed significant main effects of Group ($F_{(1, 31)} = 14.17$, $p < .001$) and Night ($F_{(1, 31)} = 26.59$, $p < .001$) and a significant interaction effect ($F_{(1, 31)} = 14.96$, $p < .001$). Post-hoc HSD Tukey tests displayed a longer sleep duration in the SD than in the RS group on the first night after sleep deprivation ($p < .001$) but not on the night after ($p > .8$), showing the expected sleep rebound after sleep deprivation then normalisation (Peigneux, Urbain, & Schmitz, 2011). Similar analyses conducted on sleep quality scores disclosed a main effect of Night ($F_{(1, 31)} = 13.97$, $p < .001$) and a Night by Group interaction effect ($F_{(1, 31)} = 27.74$, $p < .001$). Again, post hoc analyses revealed a better sleep quality for participants in the SD group during the first night after the sleep deprivation ($p < .01$). Sleep duration and quality differences disappeared during the second night following sleep deprivation ($p > .8$; See Table 1).

3.1.3. Sleep-wake cycle: Actigraphy recordings

All participants wore their actimeter during the entire experiment to ensure that they respected normal sleep schedules. Actimetric motor activity and ambient light recorded every 30 s during the entire protocol were hourly averaged over Day (16 h) and Night (8 h) periods. Actimetric data of 5 participants in the RS group cannot be included in the analyses due to technical data storage problems.

Motor activity values for the 3 days and nights prior to the sleep manipulation were entered in a repeated measures ANOVA with within-subject factors Day (3 levels) and Cycle (Night vs. Day period) and a between-subjects factor Group (2 levels: RS vs. SD). The analysis disclosed a significant main effect of Cycle ($F_{(1, 24)} = 272.7$, $p < .001$) with higher activity scores during the day than during the night (arbitrary scores mean ± standard deviation; Night: 503.8 ± 47.36 versus Day: 1657 ± 80.29) and a significant Cycle by Group interaction ($F_{(1, 24)} = 7.2$, $p < .05$; mean ± standard deviation RS Night 621 ± 76 and Day 1586 ± 129 versus SD Night 386 ± 55.7 and Day 1727.5). However, Tukey post hoc tests conducted on the Cycle by Group interaction failed to reveal significant differences (all $ps > .7$). Any other interaction or main effect was non-significant, indicating that participants respected normal sleep schedules for the days prior to the experimental procedure.

Finally, a repeated measure ANOVA was conducted on motor activity values for the 3 days after the Learning Phase (Day 1) with within-subject factor Day (3 levels) and Cycle (Night vs. Day period) and a between-subjects factor Group (RS vs. SD). Results indicated a main effect of Cycle ($F_{(1, 24)} = 73$, $p < .001$) and a Group by Day interaction ($F_{(2, 48)} = 6.3$, $p < .005$). Post-hoc analysis revealed the expected higher motor activity on Day 1 (Deprivation Night) in the SD than in the RS group ($p < .05$; mean ± standard deviation SD = 1376 ± 90 vs. RS = 886 ± 123). The Day by Cycle by Group interaction was not significant. Hence, results confirm that subjects in the SD group regained their usual activity – rest cycle after the sleep deprivation night (Table 2).

3.2. Experimental SRT task

Errors were defined as absent response or responses given outside of the screen area in which the current stimulus was presented (a 5 × 6 cm square at each corner of the screen). Mean accuracy scores per block (64 trials) ranged 93.1–99.1%. Since error rate per block was similar between groups and constant across blocks (mean error rate ± standard deviation **RS** = 1.23 ± 0.46% vs. **SD** = 1.08 ± 0.34%; $ps > 0.4$), analyses were conducted on mean reaction times (RTs) per block computed on correct responses only (raw data are displayed in Fig. 2a). Disproportionately slow responses > 3 standard deviations from the mean were rejected as likely due to fatigue or distraction.

3.2.1. Learning session (Day 1)

A repeated measure ANOVA conducted on RTs with between-subject factor Group (2 levels: RS vs. SD) and within-subject factor Block (6 levels: B1 to B6) disclosed a main effect of Block ($F_{(5, 155)} = 81.45$, $p < .001$; all other effects $ps > .2$), showing a similar evolution of motor performance in SD and RS participants during practice of the learned sequence. Learning of the sequential regularities was assessed computing the percentage of RT increase during the transfer block B7, as compared to the adjacent blocks B6–B8, averaged (i.e. $[(B7 - (B6 + B8/2))/B7] * 100$). *T*-tests against the 0 value were significant, disclosing a robust transfer effect in both Groups ($ps < .001$; Cohen's $d > 1$; Table 3 and Fig. 2a). Unexpectedly, transfer effects were higher in the RS than in the SD group ($p < .01$).

3.2.2. Sleep-dependent memory consolidation for the Learned Material
3.2.2.1. Sleep-dependent motor improvement. A repeated measure ANOVA computed on RTs on the last SRT block of Day 1 (B8) and the first SRT block of Day 4 (B9) with within-subject factor Consol-

Table 2
Motor activity (arbitrary values) recorded using wrist actigraphy during 7 days, 4 before the beginning of the experiment on day 4. Subjects in SD condition remained awake during night 5.

Group	Night.1	Night.2	Night.3	Night.4	Night.5	Night.6	Night.7
RS							
Day	1994.89	1874.77	1420.77	1464.88	1430.44	1611.55	1500.22
Night	444.78	759.89	593.67	510	341.89	460.11	397.89
SD							
Day	1482.82	1689.82	1689.9	1782.88	1607.82	1500.35	1468
Night	504.29	364.29	431.47	363.59	1144.88	268.82	541.47

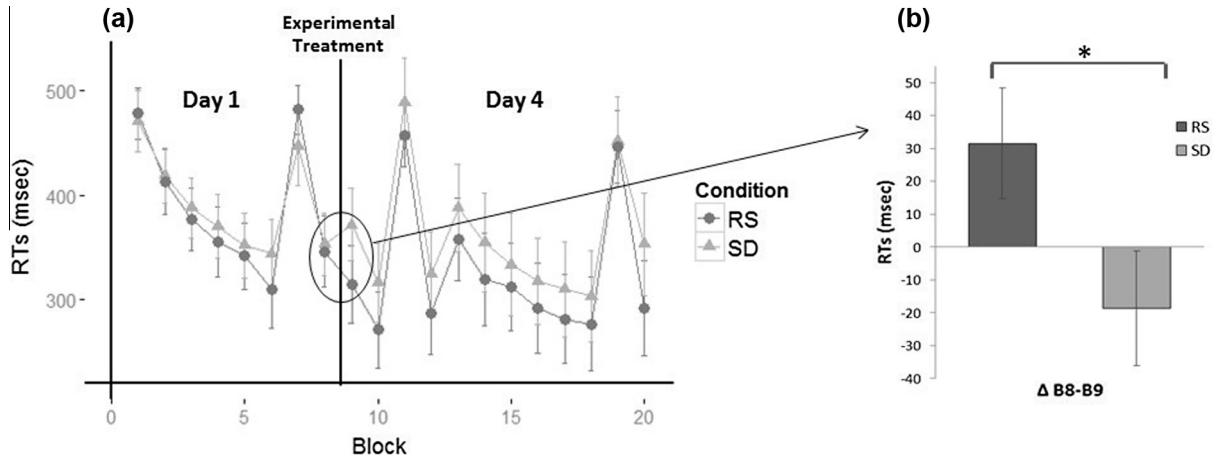

Fig. 2. (a) Mean reaction times (ms) by Group during the three sessions. The vertical solid line indicates the separation between the Learning phases (Blocks 1–8) and the Retest (Blocks 9–12) and Relearning (Blocks 13–20) phases. Post-training sleep deprivation (SD) or Regular Sleep (RS) took place on Day 1 after Learning, followed by two recovery nights. (b) Focus: Evolution of RTs in motor performance expressed as Delta scores computed between B8 and B9. Error bars represent one standard error of the mean.

Table 3
Sequential learning effect at Learning, Retest and Relearning phases, calculated as the percentage of RT increase during the transfer block as compared to the two adjacent blocks. *P* values are computed against the expected 0 value; *d* = Cohen effect size, sd = standard deviation to the mean. RS: Regular Sleep; SD: Sleep Deprivation.

	RS			SD		
	% (sd)	*p*	*d*	% (sd)	*p*	*d*
Learning	32.30% (10.15)	0.01	2.17	21.12% (11.66)	0.01	1.81
Retest	39.69% (10.51)	0.01	3.19	34.10% (12)	0.01	2.84
Relearning	36.42% (16.75)	0.01	1.89	26.64% (15.63)	0.01	1.70

idation (Day 1 vs. Day 4) and between-subjects factor Group (RS vs. SD) disclosed a significant interaction effect ($F_{(1,\ 31)} = 4.25$, MSE = 2434; $p < .05$, $\eta^2 = 0.12$). Post-hoc tests disclosed faster RTs in block 9 for RS than SD participants (Mean ± Sd = 314.8 ± 71.6 vs. 371.9 ± 71.7 ms, $p < .05$; Cohen's $d = 0.9$), and a trend for faster RTs on Day 4 than Day 1 in the RS condition ($p = .08$; Cohen's $d = 1.2$), whereas it remained stable or decreased in a non-significant manner in the SD condition ($p > .25$; Fig. 2a). A confirmatory analysis computed on RT differences from Day 1 to Day 4 (mean RT on block B8 minus B9: Δ B8-B9) disclosed a higher consolidation-related improvement in the RS than in the SD condition (Mean ± Sd = 31.51 ± 67.3 ms vs. −18.56 ± 71.9 ms; $T_{31} = 2.06$, $p < .05$, Cohen's $d = 0.71$; see Fig. 2b).

3.2.2.2. Sleep-dependent improvement in sequence learning. Transfer effects were computed for each group as the percentage of RT increase during the transfer block as compared to the adjacent blocks (i.e. Retest Day 4 ([B11 vs. [averaged] B10–12)]/B11] ∗ 100)). *T*-test against the 0 value disclosed significant transfer effects at the Retest phase (*ps* < .001; Cohen's $d > 1.7$) like in the Learning phase (see above). A repeated measure ANOVA conducted on transfer effects with Consolidation (Day1 vs. Day 4) as within-subject factor and Group (RS vs. SD) as between-subject factor disclosed a main Consolidation effect (transfer effect at Learning = 26.7% versus Retest = 36.9%; $F_{(1,\ 31)} = 28$, MSE = 1711; $p < .001$) and a main Group effect (transfer effect in RS = 36% vs. SD = 26.7%; $F_{(1,\ 31)} = 6.17$, MSE = 1160; $p < .05$). The interaction effect between Group and Consolidation was non-significant ($p > .15$).

These results indicate a sleep-dependent improvement in motor performance on the SRT task, independently of the learning of the sequential regularities.

3.2.3. Sleep-dependent proactive interference

As a reminder, we hypothesized a higher proactive interference effect in participants that were allowed to sleep after learning, as a result of higher competition between the newly and the previously learned sequences, thus reflecting sleep-dependent automatization of the firstly learned sequence. A repeated measure ANOVA conducted on RTs with between-subject factor Group (2 levels: RS vs. SD) and within-subject factors Block (6 levels: B1 to B6 or B13 to B18) and Phase (Learning at Day 1 vs. Relearning at Day 4) disclosed a main effect of Block ($F_{(5,\ 155)} = 48$, MSE = 3899; $p < .001$; $\eta^2 = 0.61$) a main effect of Phase ($F_{(1,\ 31)} = 82$, MSE = 826; $p < .001$; $\eta^2 = 0.73$), with higher RTs for Learning; Mean ± Sd = 365 ± 41 vs 339 ± 45) and a Block by Phase interaction ($F_{(5,\ 155)} = 7.9$, MSE = 725; $p < .001$; $\eta^2 = 0.20$), but no interaction with the Group condition (and all other effects non-significant *ps* > .15), indicating that proactive interference from the earlier learned sequence was not modulated by the post-learning sleep condition on Day 1.

We also computed the size of the transfer effect in each group (i.e. the percentage of RT increase during the transfer block as compared to the adjacent blocks; [B19 vs. [averaged] B18–20)]/B19] ∗ 100). *T*-Tests against the 0 value displayed significant and robust transfer effects both in the RS and SD groups (all *ps* < .001 and Cohen's $d > 1.7$; Table 3). Transfer effects were similar in the RS and SD groups ($p > .1$), indicating similar learning of the sequential regularities in the novel material.

Additionally, we conducted a repeated measure ANOVA on the size of the transfer effect with between-subjects factor Group (2 levels: RS vs. SD) and within-subject factor Transfer Phase (3 levels: Learning [Day 1] vs. Retest [Day 4] vs. Relearning [Day 4]). This analysis disclosed significant main effects of Group ($F_{(1,\ 31)} = 6$, MSE = 1942; $p < .05$; $\eta^2 = 0.16$; RS = 36.16% vs. SD = 27.29%) and Transfer Phase ($F_{(2,\ 62)} = 9.13$, MSE = 856; $p < .001$; $\eta^2 = 0.23$), but no Phase by Group interaction ($F_{(2,\ 62)} = 0.75$, $p = .48$). Post-hoc tests on the Transfer Phase effects (Learning = 26.7%, Retest = 36.9% and Relearning = 31.5%) disclosed the existence of significant differences between Learning and Retest interference phases ($p < .001$) evidencing a general increase of interference in the Retest as compared to the Learning phase (as already reported above). Moreover, a trend for significance was also observed between Retest and Relearning interferences (Retest > Relearning; $p = .067$). Finally, Pearson correlation analyses disclosed high and positive coefficient correlations between transfer effects (percentage of RT

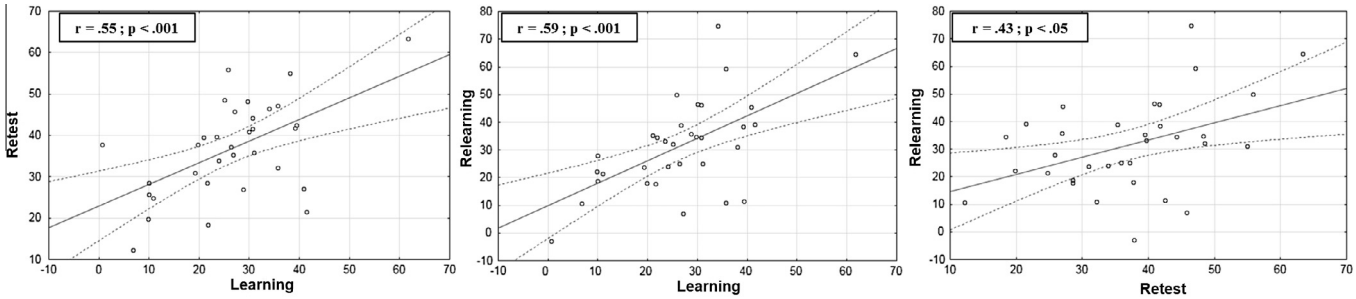

Fig. 3. Correlations between transfer effects at Learning, Retest and Relearning phases. Transfer effects are computed as the percentage change in RTs (slowing) due to the presentation of the transfer sequence, as compared to the learned sequence. Confidence interval = 0.95.

increase from transfer to adjacent blocks) and the different phases (Learning vs. Retest $r = .55$, $p < .001$; Retest vs. Relearning $r = .59$, $p < .001$; Learning vs. Relearning $r = .43$, $p < .05$), indicating that the amplitude of the transfer effects was similar across Learning, Retest and Relearning phases within individuals (see Fig. 3).

4. Discussion

The present study aimed at investigating the impact of post-training sleep on off-line consolidation processes sustaining visuo-motor sequence learning. Off-line improvements are generally computed in terms of performance improvements rather that sensitivity to interference (Krakauer & Shadmehr, 2006). Nonetheless, these two measures of consolidation may evolve differently within off-line periods (Krakauer & Shadmehr, 2006), especially when there is a period of sleep after training (Walker et al., 2003). Our study sheds new light on this issue by using a proactive interference paradigm for the first time in the context of visuomotor sequence learning. According to the postulate that the consolidation of sequential components in a serial motor learning task would benefit from sleep (Cohen et al., 2005; Siengsukon & Al-sharman, 2011), we hypothesized that a learned sequence consolidated during post-training sleep would eventually hamper, or at least slow down the relearning of a potentially competitive novel sequence after sleep. In other words, proactive interference caused by the first sequence upon the acquisition of the second one should be more pronounced for subjects in the post-training sleep condition, positing that they benefitted from sleep-dependent consolidation after training. Additionally, in line with this first proposal, we also predicted a more pronounced increase of interference caused by the presentation of the block transfer during Retest for participants having benefitted from a night of regular sleep after learning.

Contrary to our predictions, we found that the relearning of a new sequence on day 4 did not differ between groups. This result suggests that sleep did not modulate the amplitude of proactive interference effects. We assumed that sleep-related consolidation of sequence components in the RS condition would lead to an increased resistance in the learning of a new sequence, in line with the results of a prior study in which we showed that the presentation of opposite, unlearned motor information elicited significantly higher interference effects after post-training sleep than wakefulness in children (Urbain et al., 2014). A possible explanation for a lack of sleep-related proactive interference in the present study may be the nature of the SRT task. Indeed, interference effects were observed using a motor adaptation task in Urbain et al. (2014), without any sequential component, whereas interference was measured here on the sequential component of learning in a visuo-motor serial reaction time (SRT) paradigm. Alternatively, differences between studies might be related to the fact that children were tested in Urbain et al. (2014) whereas we tested young adults. Of note, these results are in agreement with a recent animal study showing that sleep promotes the formation of dendritic spines after learning, and that distinct motor experiences actually do not overlap but are discretely represented in the brain (Yang et al., 2014). If new spines are formed on different sets of dendritic branches in response to different learning tasks, protecting them from elimination during sleep when multiple tasks are learned, then sleep-related proactive interference effects might be less likely, as different motor representations are actually less in competition than we initially thought.

On the other hand, we found that participants having benefitted from regular sleep after learning were significantly faster for the previously learned material at the start of Day 4, as compared to those who were sleep deprived after learning. Looking at raw performance data (see Fig. 2a), RTs improved for participants in the RS group, but slightly deteriorated (albeit non-significantly) in the SD group. These results suggest that sleep may favor the consolidation of motor memories, possibly independently of their sequential components.

4.1. Does sleep benefit the consolidation of the sequential components presented in a deterministic SRT task?

It still remains unclear how and to what extent sleep impacts on the consolidation of distinct motor and sequential components in a deterministic SRT paradigm. Although prior studies have related sleep-dependent consolidation processes with the sequential component of the SRT task (Siengsukon & Al-sharman, 2011), the absence of transfer blocks did not allow to differentiate between sequential-based and motor-based increases in performance. In the present study, the use of transfer sequences and of a proactive interference protocol allowed us to address this question more directly. As stated above, we found an off-line performance improvement of motor performance in the post-training sleep condition, which fits with the results obtained in the rather similar Siengsukon & Al-sharman's SRT study (2011). Nevertheless, the fact that transfer and interference effects similarly evolved between groups within Learning and Retest conditions, as well as the absence of a sleep-dependent proactive interference effect at the Relearning phase, suggest that post-training sleep mostly benefits the motor constituents subtending performance in the SRT task, more than the sequential components. Accordingly, patients with obstructive sleep apnea impairing sleep quality do not exhibit motor specific-improvement after a night of sleep, as compared to controls (Csabi, Varszegi-Schulz, Janacsek, Malecek, & Nemeth, 2014).

That post-training sleep participates in the consolidation of motor-based memories has been demonstrated already using e.g. motor adaptation (Huber et al., 2004; Maquet et al., 2003;

Urbain et al., 2014) and pursuit rotor (Maquet et al., 2003; Smith & MacNeill, 1992; but see Siengsukon & Al-sharman, 2011) tasks. Other studies suggested that post-training sleep mostly acts in the stabilization of visuo-motor adaptation components, as performance on motor adaptation decreased over wakefulness but remained at the same level after sleep, again suggesting a sleep-related consolidation of motor memories (Albouy, Sterpenich, et al., 2013). Interestingly, neuroimaging studies demonstrated the sequence-specific reactivation of learning-related neuronal ensembles during rapid eye movement (REM) sleep using a probabilistic SRT task, and found learning performance levels to correlate with increased regional cerebral blood flow (CBF) during post-training REM sleep (Maquet et al., 2000; Peigneux et al., 2003). Furthermore, using the same probabilistic SRT task, sleep-related changes in neural activity were shown to not be systematically paralleled by behavioral changes (Urbain et al., 2013). Also, using simpler paradigms such as the finger-tapping task (FTT) in which a short sequence of finger movements is repeatedly executed, many studies found sleep-dependent improvement of performance (Debas et al., 2010; Doyon et al., 2009) indicating that a sequential component in itself does not prevent a beneficial effect of post-training sleep. In this respect, a defining feature of FTT, motor adaptation and other simple motor paradigms is that they rely on a process of automatization whereby increases in performance rely on constant repetition (Anderson, 1988). In this respect, tasks involving short sequences would actually be akin to simple motor tasks in that measurement of speed of execution is prioritized over measurement of sequence acquisition (Krakauer & Shadmehr, 2006; Orban et al., 2011).

Finally, we did not specifically investigate in this study the level of awareness that participants gained about the practiced sequence. Prior studies suggest that post-training sleep might particularly benefit the consolidation of explicitly learned sequences (Robertson et al., 2004). Also, hippocampal recruitment during explicit motor sequence learning was associated with subsequent performance improvement over sleep (Albouy et al., 2008), and functional dissociations in consolidation processes have been evidenced between striatum-based learning, supporting memory stabilization in a time-dependent manner, and hippocampal-based learning associated with sleep-dependent memory enhancement (Albouy, Sterpenich, et al., 2013; Albouy et al., 2013). It is possible that learning was essentially implicit in the present study. Indeed, even when participants are able to notice stimuli repetitions in the sequence, they are often unable to explicitly reproduce the learned pattern (Destrebecqz & Cleeremans, 2001). Hence, despite partial knowledge developed by participants, learning might be based on implicit representations (Destrebecqz & Cleeremans, 2001; Robertson, 2007), which would be in agreement with an absence of sleep-related benefits for the consolidation of implicitly learned sequences (Robertson et al., 2004). Further studies should investigate this issue.

5. Conclusion

In the present study, we hypothesized that sleep would promote the consolidation of sequential components embedded in a deterministic SRT task, eventually leading to increased proactive interference effects when learning a novel, potentially competitive sequence. Surprisingly, results showed no differences in proactive interference between post-training sleep and sleep deprivation conditions. However, we found a sleep-related improvement of performance for the motor but not the sequential components of visuomotor sequence learning. This suggests that sleep benefits the off-line consolidation of motor components in skill learning.

Conflicts of interest

The authors declare no conflicts of interest in this work.

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