Daytime sleep condenses the time course of motor memory consolidation

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Two behavioral phenomena characterize human motor memory consolidation: diminishing susceptibility to interference by a subsequent experience and the emergence of delayed, offline gains in performance. A recent model proposes that the sleep-independent reduction in interference is followed by the sleep-dependent expression of offline gains. Here, using the finger-opposition sequence–learning task, we show that an interference experienced at 2 h, but not 8 h, following the initial training prevented the expression of delayed gains at 24 h post-training. However, a 90-min nap, immediately post-training, markedly reduced the susceptibility to interference, with robust delayed gains expressed overnight, despite interference at 2 h post-training. With no interference, a nap resulted in much earlier expression of delayed gains, within 8 h post-training. These results suggest that the evolution of robustness to interference and the evolution of delayed gains can coincide immediately post-training and that both effects reflect sleep-sensitive processes.

Skill acquisition can be characterized by at least two distinct phases: a fast, within-session phase of performance improvement, and a delayed, latent, time-dependent improvement phase occurring between sessions[1–7]. The latter phase has been conceptualized as reflecting memory consolidation processes[1,3,8,9]. The classical notion of memory consolidation relates to a process whereby the memory of a given experience is transformed into a robust and enduring form[10,11]. During the post-learning phase, the establishment of long-term memory can be blocked by several means: chemical, electrical or behavioral interference[11]. Retrograde behavioral interference refers to the disruptive effect of a later experience on the consolidation in memory of a prior training experience[2,10–13]. With the passage of time after the training experience, however, this interference effect is first reduced and then lost in about 5–6 h post-training[2,13]. During the interference time window, experience-dependent gene expression and protein synthesis may lead to long-lasting changes in synaptic efficacy—that is, synaptic consolidation[11]. Although molecular consolidation processes are mostly referred to in connection with declarative (medio-temporal lobe dependent) memory, recent animal studies suggest that procedural memory consolidation may also depend on *de novo* synthesis of proteins (for example, in motor cortex)[11,14,15].

The second behavioral correlate of consolidation processes, the emergence of delayed gains in performance after a latent phase of at least several hours duration, has been described in many tasks following an effective training experience[1,8,9,16,17]. In motor sequence learning, enhanced performance on post-training re-testing has been shown to depend on the first post-training night's sleep[9,18–22].

A recent model[3] proposes that the two aspects of procedural memory consolidation in skill learning reflect separate processes, with the evolution of resistance to interference (stabilization) depending on time after training *per se*, whereas the evolution of delayed gains requires sleep. However, the effect of sleep on interference has not been directly addressed, as the relevant data and current models deal with the role of overnight sleep, a sleep interval which extends beyond the time window of interference. A protocol allowing a direct test of both interference and the evolution of delayed gains, the two measures proposed to reflect procedural memory consolidation, in a shared time window, is lacking. Here, we tested the hypothesis that the two measures of procedural memory consolidation are functionally related to each other and can be similarly affected by sleep. We trained participants to carry out a given five-element finger-to-thumb opposition sequence with their nondominant left hand (**Fig. 1a**). Experiment I tested whether a given interference experience could affect the evolution of delayed gains (**Fig. 1b**). After establishing a clear interaction between interference and delayed gains, we tested, in Experiment II, the effects of a post-training nap on the interference effects, the expression of the delayed performance gains, and their interaction during the post-training day (**Fig. 1c**).

RESULTS
Experiment I
Two groups of subjects carried out interference training on sequence B at either 2 or 8 h after the initial training on sequence A, to test the effect of interference on the expression of the delayed gains during 24 h post-training. The third, control group, was not trained with an interfering sequence. For the group given interference training on the reversed sequence 2 h after the initial training session (group 2hInt), the average numbers of correct sequences immediately post-training

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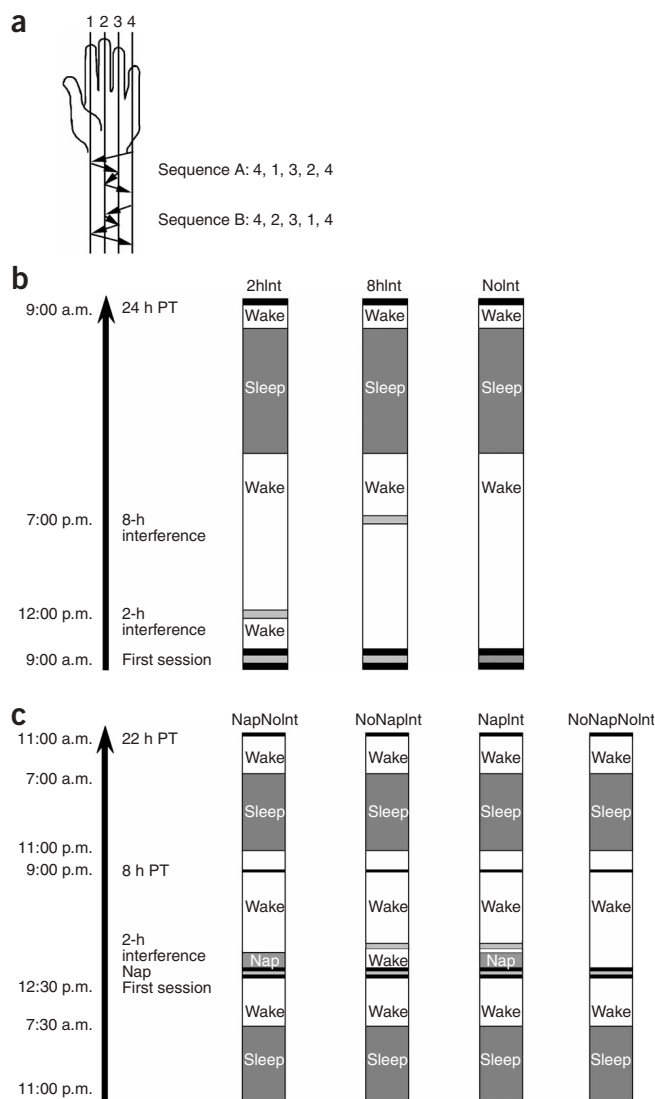
Figure 1 The finger-to-thumb opposition task and protocols. (**a**) Finger-to-thumb opposition sequences. The sequences were matched for number of movements per digit and mirror-reversed in relation to each other (in terms of order). (**b**) Protocol of Experiment I. Times of the first session (including pre-test, training on sequence A and immediate post-training performance test), training on interference sequence B, as well as re-test of sequence A at 24 h post-training (24 h PT) are shown. (**c**) Protocol of Experiment II. Times of the first session (including pre-test, training on sequence A and immediate post-training performance test), nap, training on interference sequence B, as well as re-tests of sequence A at 8 and 22 h post-training (8 h PT and 22 h PT) are shown.

A repeated measures GLM analysis showed no significant learning effect in terms of the absolute number of errors ($P = 0.685$, pre-test, 0 h post-training, 24 h post-training) in all three groups (**Fig. 2a–c**, lower panels, slopes of regression lines fitted to all data points are around zero). However, the absolute number of errors made at 24 h post-training tended to decrease relative to the number made at 0 h post-training (from 1.2 to 1, 1.09 to 0.6 and 2.2 to 1; average number of errors produced at 0 h post-training and 24 h post-training for groups 2hInt, 8hInt and NoInt, respectively). The correlation between the number of correct sequences and the number of errors made at pre-test, 0 h post-training and 24 h post-training was negative and strong ($R^2 = 0.54$) for the NoInt group, and negative and weak for the 8hInt and the 2hInt groups ($R^2 = 0.18$ and $R^2 = 0.03$, respectively), indicating that there was no trade-off between speed and accuracy.

Experiment II

The effects of daytime sleep on the evolution of delayed gains and the susceptibility of the gains to post-training interference were tested in four groups of participants, who spent 3 consecutive days in the laboratory (**Figs. 1c** and **3a–d**). Polysomnographic recordings confirmed that all participants slept well during the adaptation night preceding the training on the finger sequence task. On average, subjects slept 373.6 ± 7.6 (mean ± s.e.m.) min, with a sleep efficiency of 93.51 ± 1.34% (stage 1, 2.5 ± 0.47%; stage 2, 48.95 ± 1.41%, slow wave sleep (SWS), stages 3 and 4, 28.68 ± 1.36%; rapid eye movement sleep (REM), 19.84 ± 0.89%. All of the participants also slept well during the post-training night: sleep time of 392.82 ± 6.29 min, with a sleep efficiency 95.01 ± 0.69% (stage 1, 2.41 ± 0.46%; stage 2, 45.98 ± 1.4%; SWS, 27.85 ± 1.34%; REM, 23.8 ± 0.87%).

Polysomnographic analysis showed that all participants assigned to the nap groups slept during the nap time. On average, participants of the NapNoInt group slept 78.92 ± 3.63 min, with a sleep efficiency of 88.93 ± 4.74% (stage 1, 17.02 ± 7.8%; stage 2, 43.02 ± 5.07%; SWS, 28.81 ± 5.68%; REM, 12 ± 2.94%). One participant had no REM sleep during the nap, but nevertheless showed improved speed at both 8 h post-training and 22 h post-training (**Fig. 3b**, open circles). During the 90-min nap, participants of the NapInt group slept on average 74.35 ± 6.24 min, with a sleep efficiency of 86.28 ± 7.18 (stage 1, 8.4 ± 3.6%; stage 2, 52.11 ± 8.26%; SWS, 29.35 ± 7.9%; REM, 9.91 ± 4.03%). Three subjects had no REM sleep during the nap, but nevertheless two of them showed improved speed by 8 h post-training and all three had delayed gains by 22 h post-training.

Training on a given sequence of movements without interference and without the nap (NoNapNoInt group) resulted in both early within-session and delayed gains in performance speed (**Fig. 3a**). The delayed improvements in performance were expressed only following the post-training night's sleep, replicating previous results[9,19,21]. A repeated measures GLM analysis showed a robust significant learning effect for speed ($F_{3,7} = 30.6$, $P < 0.001$; pre-test, 0 h post-training, 8 h post-training and 22 h post-training). Significant gains occurred only

(0 h) and at 24 h post-training were 17.5 ± 2.1 and 17.9 ± 2.0, respectively (mean ± s.e.m.; **Fig. 2a**). In contrast, the introduction of interfering experience at 8 h post-training (**Fig. 2b**, 8hInt group) did not block the expression of the delayed gains in performance: 17.5 ± 1.4 and 19.5 ± 1.6 sequences at 0 h and 24 h post-training, respectively (mean ± s.e.m., $F_{1,7} = 21.63$, $P < 0.05$). In the control group (**Fig. 2c**, NoInt group) the average numbers of correct sequences at 0 h and at 24 h post-training were 17.6 ± 1.2 and 22.1 ± 0.9, respectively (mean ± s.e.m.; $F_{1,8} = 25.12$, $P < 0.05$), indicating robust delayed gains in performance expressed by 24 h post-training. A repeated measures general linear model (GLM) analysis (with three groups, 2hInt, 8hInt and NoInt; two time points, 0 h and 24 h post-training) showed a significant group × time points interaction ($F_{2,46} = 8.42$, $P < 0.05$), with the offline gains of the 2hInt group being the lowest (practically zero). The delayed gains in the 8hInt group were on average smaller than the gains accrued in training without interference (**Fig. 2b,c**); however, this difference was nonsignificant ($P = 0.362$) as a result of high variance of gains in the NoInt group. Thus, our results indicate that the interference experienced at 2 h post-training completely abolished the evolution of delayed gains, but at 8 h post-training interference was practically ineffective.
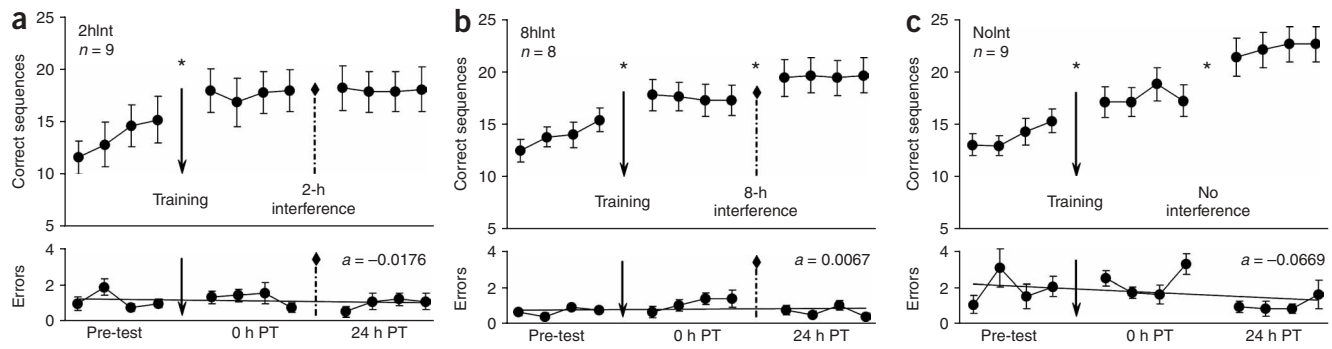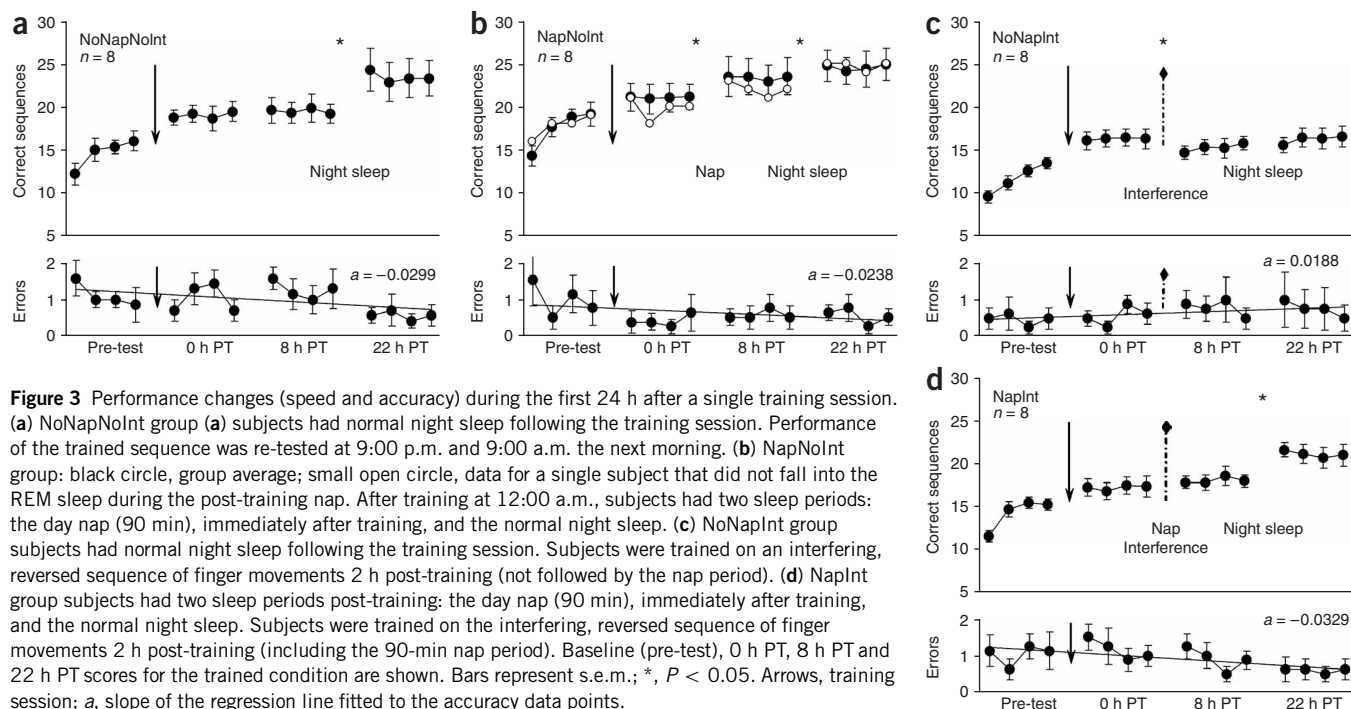
Figure 2 Performance changes (speed and accuracy) during the first 24 h after a single training session, followed 2 h (**a**) and 8 h (**b**) later by training on an interfering condition. Data for the control group that had no interference training is shown in (**c**). Baseline (pre-test), immediate post-training (0 h PT), and 24 h PT scores for the trained condition are shown. Bars represent s.e.m.; *, $P < 0.05$. Arrows, training session; stripped arrows, interference training; a, slope of a regression line fitted to the accuracy data points.

in the within-session interval and over the night-time interval (pre-test, 0 h post-training, $F_{1,7} = 98.5$, $P < 0.001$; 8 and 22 h post-training, $F_{1,7} = 21.8$, $P < 0.001$), but not during the first 8 h post-training interval ($P = 0.234$, 0 and 8 h post-training). Altogether, although the number of errors made was very small (performance at almost perfect accuracy), there was an overall decrease in the number of errors made as reflected by the negative slope of the regression line fitted to all data points (**Fig. 3a**, lower panel). A repeated measures GLM analysis showed nonsignificant learning effect for accuracy ($P = 0.087$, pre-test, 0, 8 and 22 h post-training).

The corresponding results for the participants of the NapNoInt group, who were given a 90-min nap immediately after the end of the training session, underscore the contribution of the daytime sleep (nap) to the post-training gains in performance (**Fig. 3b**). The pattern of results was qualitatively different from that of the NoNapNoInt group. Overall, there was a significant improvement in performance speed ($F_{3,21} = 32$, $P < 0.001$, pre-test, 0, 8 and 22 h post-training), as

well as an overall small decrease in the absolute number of errors made, as reflected by the negative slope of the fitted regression line (**Fig. 3b**). A repeated measures GLM analysis showed no significant learning effect for accuracy ($P = 0.143$, pre-test, 0, 8 and 22 h post-training). However, the significant overall improvement in speed in the NapNoInt group was not only due to within-session ($F_{3,21} = 49.54$, $P < 0.001$, pre-test, 0, 8 and 22 h post-training) and overnight gains ($F_{1,7} = 8.4$, $P < 0.05$, 8 and 22 h post-training), but also due to significant delayed gains evolving in the first 8 h post-training, the interval including the nap ($F_{1,7} = 18.4$, $P < 0.05$, 0 and 8 h post-training).

Training on the interfering task 2 h after the initial training session (NoNapInt group), prevented the expression of any delayed gains by 22 h post-training (**Fig. 3c**). The overall significant learning effect ($F_{3,21} = 30.6$, $P < 0.001$, pre-test, 0, 8 and 22 h post-training) was only due to the within-session improvement in performance. The interference training experience induced a small, but significant, decrease in speed at 8 h post-training compared with 0 h post-training ($F_{1,7} = 14.5$, $P < 0.05$,

Figure 3 Performance changes (speed and accuracy) during the first 24 h after a single training session. (**a**) NoNapNoInt group (**a**) subjects had normal night sleep following the training session. Performance of the trained sequence was re-tested at 9:00 p.m. and 9:00 a.m. the next morning. (**b**) NapNoInt group: black circle, group average; small open circle, data for a single subject that did not fall into the REM sleep during the post-training nap. After training at 12:00 a.m., subjects had two sleep periods: the day nap (90 min), immediately after training, and the normal night sleep. (**c**) NoNapInt group subjects had normal night sleep following the training session. Subjects were trained on an interfering, reversed sequence of finger movements 2 h post-training (not followed by the nap period). (**d**) NapInt group subjects had two sleep periods post-training: the day nap (90 min), immediately after training, and the normal night sleep. Subjects were trained on the interfering, reversed sequence of finger movements 2 h post-training (including the 90-min nap period). Baseline (pre-test), 0 h PT, 8 h PT and 22 h PT scores for the trained condition are shown. Bars represent s.e.m.; *, $P < 0.05$. Arrows, training session; a, slope of the regression line fitted to the accuracy data points.
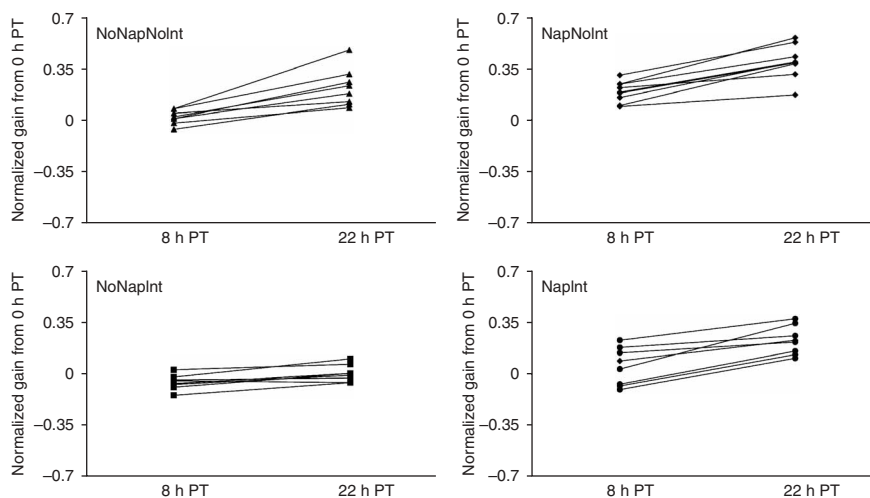
Figure 4 Individual normalized gains in performance speed. The difference in each participant's average performance at 8 and 22 h PT from his or her own average 0 h PT performance speed, normalized to the 0 h PT performance. Data for participants in the four groups, NapNoInt, NapNoInt, NoNapNoInt and NoNapInt, are shown. Positive values indicate delayed gains in the task performance, whereas negative values correspond to the slowing down of the performance speed relative to the immediate post-training level.

0 and 8 h post-training) and there was no significant improvement at 22 h post-training, even across the ensuing normal night's sleep ($P = 0.82$, 8 and 22 h post-training). Thus, the results of the 2hInt group of Experiment I (**Fig. 2a**) were replicated, with the interference training experience arresting the evolution of delayed gains. Note also, that the absolute number of errors produced at 22 h post-training tended to increase (as evidenced by the positive slope of the regression line, **Fig. 3c**), although given the small number of errors overall, there was no significant change in the number of errors throughout the experiment ($P = 0.447$, pre-test, 0, 8 and 22 h post-training).

In contrast, the participants of the NapInt group showed no blocking of the evolution of delayed gains in speed at 22 h post-training (**Fig. 3d**), despite training on the interfering sequence. Overall, there were significant post-acquisition improvements in speed of performance ($F_{3,21} = 36.4$, $P < 0.001$, pre-test, 0, 8 and 22 h post-training). Also, the number of errors made tended to decrease, as indicated by the negative slope of the regression line (**Fig. 3d**). Thus, despite the interference training, accuracy did not deteriorate when a post-training nap was afforded. A repeated measures GLM analysis showed that there was no significant change ($P = 0.449$, pre-test, 0, 8 and 22 h post-training) in accuracy. The main step in the delayed improvement occurred across the following night's sleep: that is, the improvement between 8 to 22 h post-training ($F_{1,7} = 29.3$, $P < 0.05$, 8 and 22 h post-training). The relative improvement from the baseline by 22 h post-training was comparable to that of the NapNoInt group (around 80% from the pre-test). The improvement from 0 to 8 h post-training was not significant ($P = 0.279$, 0 and 8 h post-training).

Our results show that the daytime sleep and the interference training experience interact in the time window of 2 h post-training on the initial finger movement sequence (**Fig. 4**).

The delayed post-training improvements occurred in several steps, depending on presence of daytime sleep and the interference training experience. The 90-min daytime nap, following training, contributed to an earlier expression of the delayed gains, compared with an equal interval of time spent entirely in the awake state. In the NapNoInt group, 8/8 participants showed delayed gains by 8 h post-training. Moreover, in most participants these were relatively large gains. Also, even when interference training followed the nap, as in the NapInt group, 5/8 participants showed large delayed gains by 8 h post-training. When no nap was afforded, interference training experienced 2 h into the consolidation period prevented the expression of the delayed gains by 22 h post-training. Negative delayed gains were expressed at 8 h post-training in 7/8 participants of the NoNapInt group, and there were no significant gains at 22 h post-training (**Fig. 4**). The post-training daytime nap, in contrast, had a remarkable effect on the sensitivity of the novel memory trace to the interference. When napping was allowed, the delayed gains in task performance for the NapInt group were on the order of the gains in the NapNoInt group by 22 h post-training, although delayed gains at 8 h post-training were apparent only for 5/8 subjects.

For direct between-groups statistical comparisons, the normalized data (each participant's performance and number of correct sequences, normalized to his or her performance in the initial test block) was analyzed using a repeated measures GLM analysis with four groups as categorical values, and with three post-training retests time points (0, 8 and 22 h post-training) and four blocks as within-subject factors. There was a significant main effect for time points ($F_{2,28} = 65.7$, $P < 0.001$) with, however, a significant group × time points interaction ($F_{6,84} = 8.7$, $P < 0.001$), indicating that the gains attained at the different time points were group dependent. To characterize the effects of nap and interference during the course of consolidation, a pair-wise comparison between groups was conducted for two time windows, overday (0 h post-training versus 8 h post-training) and overnight (8 h post-training versus 22 h post-training) (**Table 1**). The nap had a significant effect on the gains attained in both time windows (significant group × time

Table 1 The group × gains interaction between pairs of groups for performance in two time–windows: 0 h PT vs. 8 h PT (over–day) and 8 h PT vs. 22 h PT (over–night)

	Overday (0 h post-training versus 8 h post-training)			Overnight (8 h post-training versus 22 h post-training)		
	NapNoInt	NoNapInt	NapInt	NapNoInt	NoNapInt	NapInt
NoNapNoInt	$P = 0.010$ $F_{1,14} = 8.8$	$P = 0.004$ $F_{1,14} = 11.9$	$P = 0.507$	$P = 0.004$ $F_{1,14} = 11.4$	$P = 0.002$ $F_{1,14} = 14.2$	$P = 0.962$
NapNoInt	X	$P = 0.000$ $F_{1,14} = 34.0$	$P = 0.373$	X	$P = 0.933$	$P = 0.019$ $F_{1,14} = 7.1$
NoNapInt	X	X	$P = 0.024$ $F_{1,14} = 6.4$	X	X	$P = 0.010$ $F_{1,14} = 9.0$

PT, post training.

points interaction in comparing the NoNapNoInt and the NapNoInt groups, $F_{1,14} = 8.8$, P = 0.01; **Table 1**), as most of the gains in performance speed were expressed overday rather than overnight in the nap group. The interference had a significant effect as well (NoNapNoInt and NoNapInt groups, $F_{1,14} = 8.8$, P = 0.004; **Table 1**), with loss of performance speed overday and no effective delayed gains overnight in the interference group. However, there was no difference in the gains attained either overday or overnight when groups NoNapNoInt and NapInt were compared. The latter result suggests that the nap effectively prevented the overday loss of performance induced by interference and, moreover, fully restored the expression of overnight delayed gains. There was no significant difference in the overday gains in the two nap groups (NapNoInt and NapInt, P = 0.373; **Table 1**), reflecting the fact that even in the NapInt group most participants showed gains by 8 h post training ($F_{1,14} = 8.8$, P = 0.01) (**Fig. 4**) and irrespective of the nonsignificant gain in the mean group performance (**Fig. 3d**). The group data reflects the fact that 3/8 participants expressed 'negative gains' in performance at 8 h post-training. The delayed gains, in these 3 participants, occurred only overnight. No correlation between sleep parameters and overday/overnight gains was found to explain the discrepancy (in our relatively small groups).

Additionally, a direct comparison between groups NapInt and No-NapNoInt (0 h post-training versus 22 h post-training) showed no significant difference in the gains attained by 22 h post-training (significant main effect for time point, $F_{1,14} = 75.3$, P < 0.001; no significant main effect for group, P = 0.928, and no significant group × time point interaction, P = 0.186), indicating a nonlinearity in the combination of sleep and interference. Similarly, no significant difference in the gains attained by 22 h post-training were found between groups NapNoInt and NoNapNoInt (0 h post-training versus 22 h post-training, significant main effect for time point, $F_{1,14} = 78.5$, P < 0.001; no significant main effect for group and no significant group × time point interaction, P = 0.278) indicating that the overall gains were similar despite the earlier expression of the gains in the NapNoInt group.

There was a substantial positive correlation between the delayed gains expressed at 8 h post-training and the relative amount of time spent in stage 2 sleep during the nap in the NapNoInt group ($R^2 = 0.786$), but not between the delayed gains at 8 h post-training and the relative time in SWS ($R^2 = 0.009$) and REM ($R^2 = 0.109$) stage, nor to total sleep (nap) time ($R^2 = 0.185$). No substantial correlations were found between the relative time spent in the different sleep stages during the post-training night and the delayed gains expressed by 22 h post-training in the NapNoInt group and the NoNapNoInt group.

DISCUSSION

The results of Experiment I showed that training on the second sequence of finger opposition movements (interference) 2 h after the initial training prevented the expression of any delayed gains at 24 h post-training. However, when 8 h were allowed to elapse before training on the second sequence, robust delayed gains were expressed at 24 h post-training. The latter result provides an upper boundary for retrograde interference, by the training on the second sequence, on the evolution of delayed gains for the first trained sequence. Similar time windows for behavioral interference to motor learning have been reported previously[2,3,14]. In Experiment II, the results showed that, when a nap was allowed immediately after the initial training session, substantial delayed gains in performance were expressed by 8 h after the termination of training in the no interference group. Moreover, a daytime nap prevented the expression of any interference effects by a second sequence introduced 2 h after training on the initial sequence.

Thus, participants who were allowed to nap expressed robust delayed gains by 22 h post-training, even in the face of interference training. The time course of motor memory consolidation processes was condensed by a 90-min daytime nap, both in terms of the time to expression of delayed gains and in terms of the time window for susceptibility to interference by a subsequent training experience. Altogether, our results suggest that the consolidating influence of sleep on motor memory extends to the protection against post-training interference.

It is not clear how, when and under what conditions the stability of a memory trace is achieved[3,10,11]. Although much of the human evidence pertaining to this issue is concerned with medio-temporal lobe–dependent declarative ('what') memory, there is compelling evidence for memory consolidation processes in procedural ('how to', 'habits') memory[3,6,23]. The notion of memory consolidation, in relation to the acquisition and retention of skills, has been studied using two different measures. First, in terms of interference, it has been studied in reference to the putative transformation of the training experience–dependent memory trace from a susceptible to a more robust memory form. Up to a few hours after the training experience, physical and pharmacological manipulations, and importantly, subsequent experience, can substantially interfere with the establishment of memory. The second measure refers to the delayed, offline, post-training improvements in performance. Delayed performance gains are expressed hours after the termination of the training experience, evolving in a latent manner in the interval following training[3,24]. Both of these aspects share the notion of procedural memory consolidation as a time-dependent reorganization of the representation of experience, at least in part structural at the synaptic level, triggered by an effective training experience[2,3,8–11,21,25–28]. A recent model suggests that these two aspects of procedural memory consolidation (referred to as 'stabilization' and 'enhancement', respectively) relate to different neuronal mechanisms, on the basis of the finding that although enhancement is often sleep dependent, memory stabilization is not[3,13,21,22]. The model refers to the post-training night's sleep, as a necessary condition for enhancement, although there is data indicating that a nap may suffice[29]. Our results underscore a role for sleep not only for the development of the delayed, offline gains in performance, but also for the process of memory stabilization *per se*. Our results provide evidence indicating, for the first time, that the two measures of memory consolidation, in motor sequence learning, may be functionally related to each other.

The results of the Experiment I showed that behavioral interference experienced 2 h, but not 8 h, into the consolidation period could prevent the expression of the delayed gains by 24 h post-training. There were, however, no retroactive interference effects. Thus, the effects of interference were expressed in terms of the (arrested) delayed gains and not as impairment of retention for the within-session improvements, as the model would suggest on the basis of the interference data on learning manipulandum movements in a force field[2]. This difference in interference effects may help to resolve the debate on whether memory consolidation (in terms of stabilization) occurs only in the case of dynamic motor tasks, such as force field learning, but not in motor sequence learning[30]. Our results provide clear evidence for the consolidation of knowledge in a nondynamic motor task, in the classical meaning of sensitivity to interference.

The interference effects occurred in the awake state, suggesting that the generation of delayed gains was critically dependent on a time-dependent process that occurred after the termination of training in the awake state. These results are consistent with our recent proposal[9] that sleep may be critical to the timing of the behavioral expression of the delayed gains in motor learning. Thus, the transition of the products of

the training experience to a stable memory form coincided with a critical period for the emergence of the delayed performance gains, suggesting the parsimonious notion that the two effects are not independent and may be subserved by common physiological mechanisms. An important question, therefore, is whether time spent in specific brain states (awake, a night's sleep, a nap) following a training session can simultaneously affect both measures of memory consolidation.

The results of Experiment II showed that a daytime nap, after training, facilitated both aspects of motor memory consolidation compared with spending a similar time interval in the awake state. The nap resulted in a clear acceleration of the expression of delayed gains (NapNoInt compared with NoNapNoInt). Thus, when a nap was afforded, significant delayed gains were accrued by 8 h post-training. In terms of the susceptibility to behavioral interference, a 90-min nap allowed the development of normal post-acquisition gains; that is, it facilitated the expression of robust delayed gains, as if no interference had been experienced. The nap countered the interference effect. By 22 h post-training, the nap-mediated gains in performance in the groups NapInt and NapNoInt were qualitatively and quantitatively similar. Without a nap, interference resulted in the absolute suppression of the expected delayed gains in performance (NoNapInt group). The data from the groups NapInt and NapNoInt also suggests that the expression, and presumably the evolution, of delayed gains can occur in several steps after a given training experience, specifically within 8 h post-training following the nap, and again during the subsequent interval of 14 h that included a night's sleep. Further support for this notion comes from the finding that, in the NapInt group, the nap, given before the interference training, allowed the expression of robust overnight delayed gains, but in some participants no delayed gains were expressed at 8 h post-training. Thus, sleep can affect the stabilization aspect of procedural memory consolidation during the classical interference time window, and the consolidating memory trace can be secured in the face of post-nap interference.

Substantial delayed gains were expressed earlier in the NapNoInt group than in the NoNapNoInt group, but with overall similar gains at 22 h post-training. These results are consistent with the recent findings[29] that, in a similar motor sequence learning task, a daytime nap mediated the expression of earlier performance improvements at the expense of subsequent overnight learning. There is also evidence, from a perceptual learning task, that in conditions of training-related decreases in performance, naps may provide a restitution of performance, and even add to subsequent sleep-dependent improvements, as measured by 24 h post-training[31].

Several recent studies suggest that delayed gains (offline learning) are correlated with the amount of non-REM sleep following training[24,32–34]. Others[5,35,36] reported a correlation between improvement in performance and the time spent in REM during post-training sleep. Whether sleep is a necessary condition for the evolution of delayed gains in all procedural memory tasks is currently being debated[17,34,37–39]. We found a correlation between the amount of stage 2 daytime sleep and the delayed gains. Other correlations between performance changes and time spent in specific stages during both daytime and night-time post-training sleep were not substantial. Individuals who failed to reach REM sleep during daytime sleep nevertheless showed significant overday and overnight delayed gains.

The standard model of consolidation, in relation to declarative memory[10], posits that long-term memories are initially registered in the hippocampal system and the relevant neocortex. The post-training stabilization of these internal representations is assumed to involve synaptic consolidation, a process requiring protein synthesis, which may take up to a few hours. In parallel, or as a consequence of, a process

conceptualized as system consolidation is initiated, characterized by a much slower time course, whereby the representation of the learned experience is reorganized over a period of days and weeks, shifting the burden of retention to the cortex. Synaptic plasticity is also involved in this process. In a partial analogy, we propose that both synaptic and system consolidation processes might be involved in the expression of delayed gains in some types of procedural learning, specifically, in motor sequence learning. System consolidation in procedural memory can be conceptualized as the processes whereby the burden of representation and retention of the skill is shifted within and between different cortical and subcortical areas that are relevant for task performance; in the cortex, perhaps, to successively lower levels of representation[9,23,28,40–44]. Thus, although synaptic consolidation is always involved, not all types of skill learning may require system consolidation. In tasks where system consolidation occurs, sleep might be an important brain state that determines the time of expression of delayed gains[45]. It may also be the case that sleep is necessary whenever the system consolidation–related circuitry critically involves the basal ganglia, but not when the cerebellar contributions dominate[42]. An alternative, perhaps additional, proposal is that sleep may be needed whenever synaptic changes induced by the training experience and subsequent wakefulness result in a net increase in synaptic strength in the task-specific activated brain circuits. The role of sleep may be to modulate the weights of inhibitory and excitatory synaptic inputs in the activated network; for example, to downscale synaptic strength to baseline levels[46–48]. The amount and structure of the training experience may also partly determine the sleep dependency of the consolidation phase gains[46]. Synaptic and system consolidation processes may occur in parallel during the awake state, but also as the current results suggest, during sleep.

Taken together, our results suggest that the two aspects of motor memory consolidation in the finger-opposition sequence–learning task, susceptibility to interference and delayed gains, do not necessarily reflect distinct and successive stages of novel motor memory formation, but rather reflect two behavioral manifestations of a complex process of slow learning[1] that may be contemporaneous and physiologically linked. We propose that daytime sleep facilitates both memory stability and the expression of delayed gains. Thus, the provision of a post-training nap should be considered for facilitating the learning of skills.

METHODS

Participants. Sixty-seven young adults (18–34 years, 24.2 ± 3.9, mean ± s.d., 39 females) were paid to train on a finger-opposition sequence–learning task. Participants were right-handed (as assessed by the Edinburgh Handedness Inventory[49]), nonsmoking, morning- to moderate-morning–type persons, had no medical conditions that could impair fine motor performance, reported >6 h of regular sleep per night and had no sleep-wake cycle disruptions. Subjects were not allowed to ingest caffeine or alcohol 2 d before the experiment and on the days of the experiment. Musicians and professional typists were excluded. Informed consent was obtained before the experiment. The study was approved by the Ethics Committee of the Chaim Sheba Medical Center.

Finger sequence task. Participants were trained on carrying out a given five-element finger-to-thumb opposition sequence with their nondominant left hand (**Fig. 1a**). They performed the instructed movements while lying supine. The hand was positioned on the subject's chest with the elbow flexed in a direct view (palm facing) of a video camera, to allow recording of all digit finger movements. Visual feedback was not afforded.

In the initial session, each participant underwent a pretraining performance test, a training session and an immediate post-training performance test. A training session consisted of 160 repetitions of the assigned sequence A, which were divided into 10 training blocks. During training, the initiation of

each sequence was cued by an auditory signal at a rate of 0.4 Hz (2.5 s per sequence). For performance testing, participants were provided only with an initial cue and were instructed to continuously tap the sequence as rapidly and accurately as possible until given a stop signal. Each test and retest block consisted of four trials of 30 s spaced by a 50-s rest period between sessions. Participants were instructed that occasional errors should not be corrected and to continue with the task without pause as smoothly as possible. In a previous study[9], a control experiment showed no differences in baseline production of the sequences A and B.

Experiment I. To assess whether the sensitivity to interference and the evolution of delayed performance gains were related to each other, 26 participants were trained in a single session on sequence A. Three groups were tested (**Fig. 1b**). Each participant underwent a pretraining performance test, a training session and an immediate post-training performance test starting at 9:00 a.m. The participants of groups 2hInt and 8hInt were also trained on the reversed sequence B (the interfering task), which was composed of identical component movements, 2 or 8 h, respectively, after the initial training on sequence A. No additional training in either sequence was given to the NoInt group. All participants reported a normal, >7 h sleep that night and no naps during the experimental day and on the 7 d before the experiment. All participants were retested at 24 h following the training on the performance of the initially trained sequence. Subjects were not allowed to nap during the experimental days.

Experiment II. To investigate the effects of daytime sleep on the evolution of delayed gains and the susceptibility of the gains to post-training interference, 31 participants were tested during 3 consecutive days (**Fig. 1c**). All participants were screened for sleep-related disorders during the first adaptation and screening night in the sleep laboratory before the training session. Polygraphic recordings that included electroencephalogram (EEG recorded between electrode pairs C3-A2 and C4-A1), electro-oculogram, electromyogram (chin submental and anterior tibialis), electro-cardiogram, nasal airflow, chest and abdominal breathing movements, snoring, position and pulse oxymetry were conducted during the adaptation and screening night. During the nap and the post-training night, polygraphic recordings that included electroencephalogram, electro-oculogram, electromyogram (chin submental) and electro-cardiogram were conducted. Polysomnographic data of the nocturnal sleep recordings and daytime nap were scored by trained sleep technicians and analyzed according to standard criteria[50] using the Somnologica Digital sleep data system (Somnologica Studio 3.3.1.1529, Embla Recording Systems). To facilitate napping, the time schedule of the experiment was set so as to time the nap interval into the early afternoon. Thus, half an hour after lunch, at 12:30 p.m., each participant underwent a pretraining performance test, a training session, and then an immediate post-training performance test. Performance of the trained sequence was retested at 9:00 p.m. of the same day and again at 9:00 a.m. the next morning. Two subjects were excluded as a result of sleep abnormalities (sleep apnea and periodic limb movement disorder indices above thresholds). All subjects spent the experimental days in the laboratory rooms, where light, temperature and meal times were controlled. Subjects were not allowed to train on sequence production between the test sessions.

Participants were divided into four groups. In the NapInt group, participants were allowed to nap for 90 min (with full polysomnographic recording) immediately after training. Interference training (the reversed sequence) was given after the nap within a 2-h period post-training. The NapNoInt group underwent baseline training, was allowed to nap for 90 min and was retested 8 h later without interference training. The NoNapInt and NoNapNoInt groups were trained and re-tested as were the first two groups, respectively, but were not allowed to nap after the baseline training.

In both experiments the data were analyzed in a within-subjects design using repeated measures GLM analysis with time points (for example, pre-test, 0, 8 and 22 h post-training) and blocks as variables for either speed (number of correct sequences) or accuracy (number of sequencing errors) separately. To directly compare the delayed gains between-groups in Experiment II, we normalized each participant's performance to his or her performance in the initial test block ([mean of each test – mean of 0 h post-training] / [mean of 0 h post-training], calculated for each individual in all groups). A repeated measures GLM analysis with the number of groups as categorical values, and the number of post-training retests time points (0, 8 and 22 h post-training) and four blocks as within-subject factors, was used.

AUTHOR CONTRIBUTIONS

M.K. designed and conducted the experiments and data analysis and wrote the manuscript; J. Doyon provided theoretical input and edited the manuscript; J. Doljansky analyzed sleep recordings; J.C. provided technical input and edited the manuscript; Y.D. supervised the sleep-related aspects of the project; A.K. designed and supervised the project and wrote the manuscript.

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