Learning by observation requires an early sleep window

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Numerous studies have shown that sleep enhances memory for motor skills learned through practice. Motor skills can, however, also be learned through observation, a process possibly involving the mirror neuron system. We investigated whether motor skill enhancement through prior observation requires sleep to follow the observation, either immediately or after a delay, to consolidate the procedural memory. Sequence-specific fingertapping performance was tested in 64 healthy subjects in a balanced design. Electromyography verified absence of overt or subliminal hand muscle activations during observation. The results show that immediate sleep is necessary for the enhancement of a motor skill through prior observation. Immediate sleep improved the speed of subsequent performance by 22 ± 11% (mean ± SEM) ($P = 0.04$) and reduced the error rate by 42 ± 19% ($P = 0.02$). In contrast, no performance gains occurred if sleep was initiated more than 12 h after observation. A second study on 64 subjects ruled out explicit familiarity with the sequence or the spatiotemporal rhythm of the sequence to underlie performance improvements. The sleep-dependent observational motor learning enhancement is at least similar to that previously reported for implicit and declarative memory. The apparent prerequisite of observing real movements indicates that subjects transfer experience obtained through observation of movements to subsequent self-initiated movements, in the absence of practice. Moreover, the consolidation of this transfer requires an early sleep window. These findings could improve learning new motor skills in athletes and children, but also in patients having to remaster skills following stroke or injury.

circadian rhythms | memory consolidation | mirror neurons | offline improvement

Recent studies have shown memory-enhancing effects of sleep, both preceding and following different types and stages of learning (1, 2). One of the most robust findings is the improvement of skills, or procedural memory, after a period of sleep following the initial acquisition phase (3–5). The benefit appears relatively independent of timing, as postponing sleep up to approximately 10 h after acquisition renders a similar skill memory enhancement as immediate sleep (6). The sparse evidence for enhancement of declarative memory, in contrast, appears to indicate that sleep favors enhancement most if it occurs within a limited time window after acquisition (7, 8). An explanation for a limited time window would be that during waking, the newly formed memories are susceptible to interference by competing memory traces. A second task administered immediately after learning the first abolishes sleep-dependent memory consolidation for the first task (9). Interference by stimuli competing with declarative memories is thought to arise more frequently during a waking day than interference by stimuli competing with a specific new motor skill, which would provide a possible explanation for the need for sleep to occur soon after learning declarative material, but not procedural material.

Sleep is believed to exert its effects by acting on memories formed during initial learning. For motor skills, memory formation is not necessarily completely dependent on the actual performance of the skill: motor memories can also be induced by observation of an action (10). The effect of sleep on observational learning has not previously been addressed. In this study, we investigated whether motor skill enhancement through prior observation requires sleep to follow the observation to consolidate the procedural memory; specifically, we tested whether sleep needs to occur immediately instead of after a delay.

In our first experiment (I), we investigated whether sleep aids learning by action observation, and whether the enhancement of the motor memory requires sleep to follow observation within a limited time window. Subjects ($n = 64$) initially viewed a video showing a hand performing one of three parallel fingertapping tasks, while we preempted the movements and the sensory feedback normally associated with the actual performance of the task, by requiring the subjects to press two of the fingers corresponding to those used in the fingertapping task on two spaced-apart keyboard keys. Subjects performed either the same (congruent) or a different (incongruent) sequence of the fingertapping task on a subsequent testing session that took place 12 or 24 h later and included either wakefulness and/or early or late sleep (Fig. 1). To explicitly control for time of day as a confounding factor with respect to sleep-dependent learning, we balanced the moment of testing across the subgroups. Thus we could not only investigate effects of sleep vs. wake, but also effects of immediate sleep vs. delayed sleep, while controlling for time-of-day effects on performance. In accord with previous studies, the initial performance speed and error rate at testing were taken as outcome measure (6, 9).

An important issue is that the mere observation of finger movements may result in unnoticed or inadvertent activation of the same muscles used for the actual movement. Absence of overt hand movements still leaves the possibility that hand muscle activation, subliminal for overt movement, might have occurred. If this were the case, our aim to study learning by observation could be confounded by mere learning by practice. In a second experiment (II), we therefore measured hand muscle grid electromyography (EMG) ($n = 7$) to investigate whether subliminal activation of the motor program involved in the task occurred during observation.

In a third experiment (III) ($n = 64$) we investigated the specificity of the sleep-dependent observational learning by testing whether memory enhancements could have been caused

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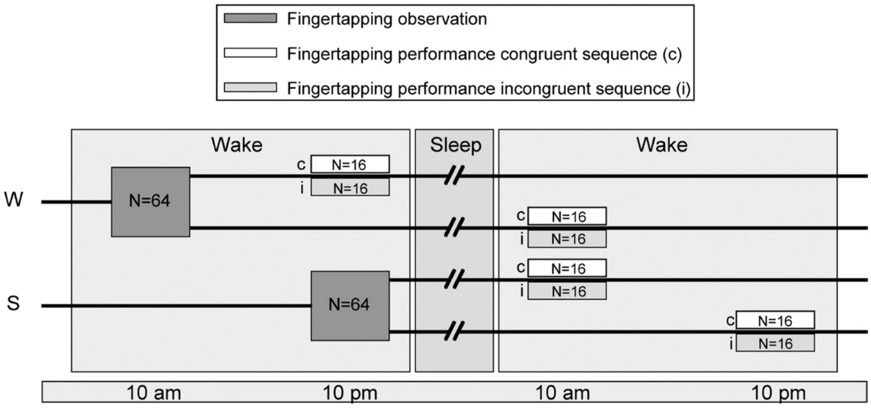

Fig. 1. Design Experiment I: sleep-dependent consolidation of observational memory. In a balanced design, subjects watched one of three parallel versions of the fingertapping video during a first session either in the morning or evening and were tested during a second session taking place either 12 or 24 h later, the following morning or the following evening. Testing consisted of the subjects performing either the same (congruent, c) or a different (incongruent, i) version of the fingertapping task during the test session. The 64 subjects participated twice and were allocated in eight sub-subgroups of 16 subjects each, such that they never encountered the same fingertapping sequence twice. The sub-subgroups differed on two dimensions: 1) (in) congruency of the observed relative to the performed sequence; 2) timing of observation relative to subsequent sleep, which was either immediate sleep (S) or sleep after wake (W), that is, sleep delayed until after a normal day of wakefulness. To balance for possible time-of-day effects on performance, in both the S and W subgroups, half of the subjects were assessed after a 12-h interval between observation and motor performance and the other half after a 24-h interval.

simply by (*a*) familiarity with the digit sequence or (*b*) familiarity with the spatiotemporal pattern of the task regardless of observing finger movements.

Finally, we investigated in a fourth experiment (IV) ($n = 32$) whether the performance gain in the original experiment could have been confounded by a differential immediate efficacy of learning through observation in the evening as compared to the morning. To do so, we investigated tapping performance immediately after seeing congruent versus incongruent videos in the morning and in the evening.

Examples of videos and stimuli used are shown in Fig. 2.

Results

Experiment I. Subjects who slept within the first 12-h interval after the video presentation of the fingertapping sequence (the immediate sleep subgroup, S) showed a 22 ± 11% (mean ± SEM) higher speed (congruent: 12.94 ± 0.92 sequences, incongruent: 10.60 ± 0.74 sequences; $z = 2.02$, $P = 0.04$) and a 42 ± 19% lower error rate (congruent: 0.09 ± 0.01; incongruent 0.15 ± 0.02; $z = -2.29$, $P = 0.02$) if the tapped sequence was congruent with the sequence previously observed, as compared to when a different sequence was previously observed (Fig. 3). This improvement occurred regardless of whether subjects in the immediate sleep sub-subgroups were tested in the morning or evening (i.e., there was no interaction between the time of day of testing and congruency) (sleep and sleep-wake sub-subgroups; all $P > 0.05$), in accord with previous findings (4, 9). Both the time interval between viewing and testing and the time of day of testing were balanced across the subgroups (Fig. 1).

The improvement was in strong contrast with the lack of benefit from observation in subjects who did not sleep within 12 h of the video presentation of the motor tapping sequence (delayed sleep subgroup, W). They showed an insignificant 4% increase in speed (congruent: 11.14 ± 0.63; incongruent: 10.70 ± 0.70; $z = 0.47$, $P = 0.64$) and a 0% difference in error rate (congruent: 0.14 ± 0.01; incongruent: 0.14 ± 0.02; $z = 0.00$, $P = 1.00$) for the congruent sequence as compared to the incongruent sequence. This lack of improvement in the delayed sleep subgroup occurred regardless of sub-subgroup; that is, there was no interaction between the time of day of learning and congruency (wake and wake-sleep sub-subgroups; all $P > 0.05$). In summary, sleep enhances learning after observation only if learning is followed by sleep within a limited time window.

Experiment II. We performed a separate control experiment in seven right-handed task-naïve subjects, of whom we measured the EMG of the left flexor digitorum muscle during the same observation and tapping conditions as used in Experiment I, using previously established methods to detect subliminal muscle activation (11).

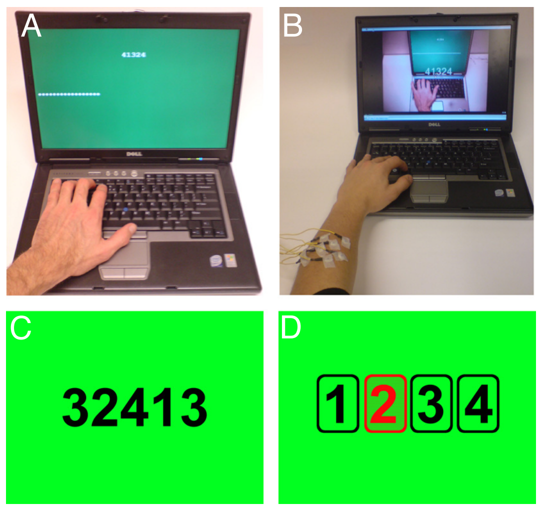

Fig. 2. Overview of procedures and stimuli used in the different experiments. (*A*) Performance of one of the three fingertapping task versions by a subject. The subject was required to tap the digit sequence indicated at the top of the screen. The number of keypresses appeared as a row incrementing from left to right in the middle of the screen during tapping. (*B*) A subject holding down the 'alt' and 'a' keys during observation of a fingertapping video, to prevent movements or practice. Note that the image on the screen corresponds to *A*. The subject has a grid of electrodes overlying the left flexor digitorum muscle, to measure any possible subliminal muscle activity during observation (Experiment II, otherwise setup is identical to Experiment I). (*C*) Video screen of the control study (Experiment III, sequence task) where subjects watched one of three digit sequences for the same duration and using the same setup as in Experiments I and II. (*D*) Video still of one of the three videos used for the second control study (Experiment III, spatiotemporal rhythm task), where digit locations were highlighted with the same speed and spatial pattern of alternation as the corresponding fingers in the fingertapping observation videos, according to each of the three digit sequences.

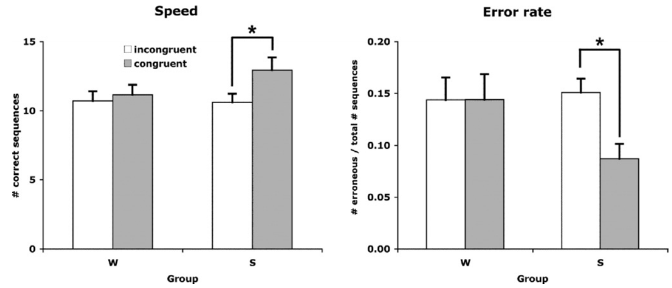

Fig. 3. Experiment I: Effects of immediate and delayed sleep intervals on performance. (*Left*) Speed (number of correct sequences tapped). (*Right*) Error rate (number of erroneous relative to total number of sequences). Graphs show the average values of the initial 6 epochs of motor performance after observation of a skill that was either congruent or incongruent with the skill to be performed. Subjects who slept within the first 12 h after viewing a congruent (c) sequence fingertapping video (S), improved performance relative to the incongruent (i) condition (22 ± 11% higher speed, $P = 0.04$ and 42 ± 19% lower error rate, $P = 0.02$) regardless of whether performance took place in the evening or morning. Subjects who did not sleep during the 12-h interval, or who initiated their sleep period more than 12 h after viewing (W) showed no performance gain from previous viewing of a congruent video (4% improvement in speed, 0% change in error rate, NS). Error bars, standard error of the mean; asterisks, significant subgroup-wise differences.

For each of the seven subjects, we determined the frequency with maximum spectral power in both the observation and tapping condition. There was no overlap in the peak frequency for the tapping (4.04 ± 0.66 Hz; mean ± SEM) and observational condition (14.95 ± 0.92 Hz; mean ± SEM) across the seven subjects (paired samples *t* test, $P < 0.001$; Fig. 4). In summary, muscle activation comparable to that seen during performance did not occur in the observation condition, ruling out subliminal activation as a source of learning.

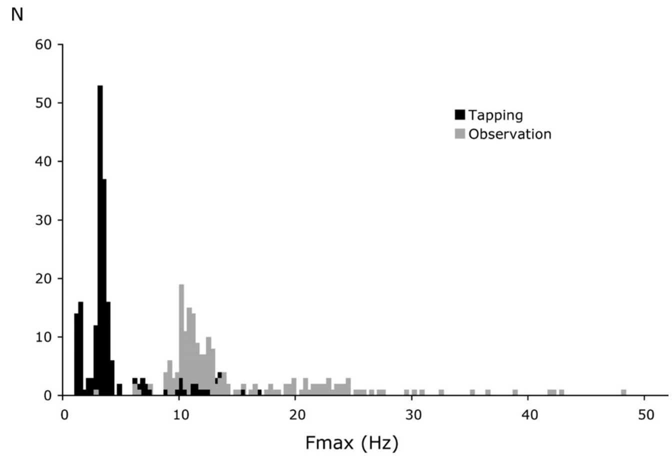

Fig. 4. Experiment II: Absence of subliminal hand muscle activation during observation. Seven healthy young right-handed subjects observed the fingertapping task and subsequently performed the same sequence themselves at the same speed, by synchronizing their movement to the video. During observation, they used their index and middle finger to press two keys of the pc keyboard. The figure shows the histograms resulting from the spectral analysis of the EMG data of the left flexor digitorum muscle. F_{max}, dominant frequency for a 3-s bin of EMG data (28 bins per condition per subject). N, number of instances that a particular frequency appeared as the dominant frequency. It can be appreciated that the distributions of F_{max} during tapping (black) and observation (gray) are non-overlapping, underscored by a paired *t* test on the average dominant frequencies per subject/condition. This analysis effectively rules out the possibility that observation leads to a subliminal muscle activation that could underlie motor learning rather than observational learning.

Experiment III. A next step was to investigate whether the observed sleep-dependent performance enhancement by observational learning could be explained by simply memorizing the digit sequence of the fingertapping task, or by a memory of the spatiotemporal distribution of the alternating locations. To this end, we recruited an additional 64 subjects to investigate whether immediate sleep (i.e., the same sleep- and sleep-wake subsubgroups from Experiment I) benefits performance on a fingertapping task if subjects are exposed to the congruent versus incongruent digit sequence or the spatiotemporal alternation of locations (but not finger movements) on a computer screen on the previous evening. Our results show that neither exposure to the congruent digit sequence, nor observing squares that lighted up in the same order and with the same speed across four locations on a screen as the finger movements in the fingertapping videos (Experiment I), resulted in any significant improvement in fingertapping speed (sequence: congruent 11.31 ± 0.80; incongruent 11.74 ± 0.68; rhythm: congruent 13.01 ± 1.04, incongruent 13.16 ± 1.02) or accuracy (sequence: congruent 0.14 ± 0.02; incongruent 0.13 ± 0.02; rhythm: congruent 0.12 ± 0.02, incongruent 0.10 ± 0.01) the next day (all $P > 0.38$). In summary, these results show that the sleep-dependent memory enhancement for observational learning found in Experiment I cannot be explained by sequence or rhythm (spatiotemporal) memory.

Experiment IV. The findings of Experiment I leave open the possibility that there is a time-of-day effect on immediate learning through observation. The results of Experiment I would in that case merely reflect the possibility that initial learning through observation only happens in the evening, but not in the morning. Experiment IV therefore investigated whether observation has an immediate beneficial effect on fingertapping performance, and if so, whether this immediate effect is stronger with observing and tapping in the evening than with observing and tapping in the morning.

We recruited another 32 subjects and tested their fingertapping performance after observing one of the three videos with a delay of 5 min. Again, the subjects were tested twice and we investigated the effects of congruent vs. incongruent videos and morning vs. evening. The results show that testing immediately after viewing does not result in a difference between the congruent and incongruent subgroups (speed: congruent 11.61 ± 1.03, incongruent 11.01 ± 1.00, z = 0.77, $P = 0.44$; error rate: 0.11 ± 0.02, incongruent 0.11 ± 0.02, z = −0.28, $P = 0.78$). In addition, no interactions occurred between congruency and time of day (z = 1.54, $P = 0.12$ for error rate, z = −1.26, $P = 0.21$ for speed).

Discussion

Our results demonstrate a type of sleep-dependent learning with an effect at least as large as reported for sleep benefits after motor skill practice. Finger tapping skills benefit from previous observation only if sleep occurred within 12 h; conversely, once sleep has consolidated the skill memory, the benefit remains unaltered regardless of whether the skill is assessed the next morning or the evening. The sleep-timing dependency contrasts with skill learning through motor practice, where the timing of the learning phase relative to the sleep phase seems less crucial, at least for delaying the sleep phase up to at least 10 h (12). Our findings seem to parallel declarative learning in showing enhancement only when sleep occurs soon after the acquisition (7), yet differ from that study in that the percentage of sleep-dependent improvement is considerably higher in our paradigm. Our complementary studies indicate that this learning is not due to learning subliminal hand movements (Experiment II), to memorizing the digit sequence to be tapped, or to learning the spatiotemporal alternation of locations used for the fingertapping (Experiment III). Experiment IV showed that the time of day of learning does not lead to differences in immediate performance that can confound the observed effect of sleep, that is, the results are not simply caused by subjects learning in the evening but not in the morning. The finding that there is no immediate benefit of tapping the congruent sequence as opposed to an incongruent sequence, morning or evening, argues against the possibility that the sleep-benefit observed in our original experiment could have been explained simply by a passive protection of information by sleep, i.e., by an absence of interference. Rather, the performance benefit, which occurs only after sleep, appears to be an observational memory enhancement depending on the time to sleep, indicating that it is the potential for sleep-related improvement that decays across the day.

These results of sleep-dependent observational learning resemble previous findings on both declarative and procedural sleep-dependent memory benefits, but differ from them in an important way: in our study, the learning does not entail practice of the movements or of the declarative material to be retrieved later. Therefore, the knowledge obtained through observation must be transferred to behavioral improvements through an as of yet unknown mechanism. Our subjects may have consciously mapped the observed actions onto learned perceptuo-motor associations (13); the memory-enhancing effect of sleep after motor skill performance is especially strong when the skill is explicitly encoded (14). Observation-related neural activity in the absence of overt motor practice appears sufficient to result in implicit motor learning (15, 16). It has been shown that imagined finger movements lead to cortical activity that in part resembles the activity seen during real movements (17). An intriguing possibility is that the mapping of observed movements onto one's own neural system for generating movement activates the so-called 'mirror neuron system', a system thought to be equivalent to the mirror neurons described in animals (18). Watching finger movements results in similar patterns of brain activation as performing those same movements in humans, as evidenced by studies using EEG (19), MEG (20), transcranial magnetic stimulation (21, 22), and fMRI (23). It is tempting to hypothesize that the brain mechanism of sleep-dependent consolidation of memories for observed actions involves the mirror-neuron system. A recent report describes that in songbirds passive exposure to a song 'tutor' causes sleep-dependent changes to the song structure the next day (24); to our knowledge this is the only report of non-practice dependent sleep-related learning. We here supply evidence that such a mechanism also exists in humans.

In conclusion, performance enhancement through observation depends critically on subsequent sleep and is sensitive to time spent awake between learning and sleep. The effects of sleep are as pronounced as previously reported for skill learning by practice and for explicit learning paradigms. The strong dependence on immediate sleep of the enhancement of skill learning by observation suggests that it provides a valuable paradigm to elucidate the brain processes involved in the role of sleep in consolidation and enhancement of prior learning.

These results could have implications for (re)learning movements in cases where practice is difficult or impossible, as in children, during rehabilitation following stroke or fractures, or in complex skill acquisition in, for example, sports or surgical techniques. An important recommendation in such circumstances would be to perform the observation just before sleep onset.

Methods

Experiment I: Observational Memory. ***Participants.*** To investigate whether performance of a novel task depends on previous observation of these same movements, we recruited 64 healthy, young right-handed subjects [32 M, 32 F, 25.7 ± 5.5 years of age (mean ± standard deviation), range 19–43 years] through local advertisement, in accordance with medical ethical committee guidelines; all respondents were screened for handedness, normal sleeping patterns, drug and medication use and psychiatric and neurological health. All subjects had completed primary school and were pursuing or had completed a university or a specialized degree. We excluded skilled musicians, such as piano players. Each subject participated twice, randomly allocated into different experimental subgroups resulting in a total of 128 datasets (Fig. 1). During the nights before and during the testing procedure, subjects were instructed to maintain normal habits and sleeping hours.

Procedure. Participants watched one of three different videos of a task-naïve right-handed person performing six 23-s epochs of a computerized finger-tapping task, as adapted by Walker et al. (9) from Karni et al. (25), with his left hand, spaced by 20-s waiting periods (Fig. 2*A*). The subject performing the task on the video showed a positive learning curve and made several errors throughout the task, making the observational learning of the task as naturalistic as possible. The three videos did not differ in terms of the skill acquisition speed and learning curve; they differed only in the sequence of fingers used (41324, 23142, or 32413). Subjects were simply instructed to watch the hand closely, without specific instruction for remembering the sequence. The digit sequence used for the finger tapping was shown on the screen throughout the tapping epochs. The hand in the video tapped when the background screen was green and stopped when it turned red. We prevented overt motor practice of the task during observation by requiring the subjects to press the 'alt' and 'a' keys on the computer keyboard with the index and middle finger of their left hand throughout the viewing procedure (Fig. 2*B*); if for any reason they let go of the keys, the video was interrupted and resumed only when the keys were pressed.

On the subsequent testing session, the subjects were asked to perform one of the three fingertapping tasks on a computer using Eprime software (Psychological Software Tools, Inc.). Subjects performed 12 trials of the fingertapping task, with the same duration of epochs as used in the video observed in the previous session; they tapped during the 23-s epochs when the screen was green and stopped when it turned red for 20 s. Before the start of each of the fingertapping epochs, a warning signal occurred allowing the subject to place his/her fingers on the one to four keys on the keyboard. To investigate the effect of immediate sleep on the enhancement of initial performance by prior observation of a congruent tapping sequence, video observation took place in the evening in half of the cases (immediate sleep, S) and in the morning in the other half of the cases (delayed sleep, W). Within both the immediate sleep and delayed sleep subgroups, half of the participants were assessed after a 12-h interval between observation and motor performance and the other half after a 24-h interval, thus balancing possible differences in performance due to time-of-day (morning versus evening) or interval duration (12 h versus 24 h). At each of these testing sessions half of the subjects tapped the sequence congruent with the sequence they previously observed, while the other half served as a reference control group tapping a sequence incongruent with the sequence previously observed (Fig. 1).

The three videos, the congruent-incongruent condition, the allocation of the S and W subgroups, and the order of participation for the two times each subject participated were randomized across the 64 subjects and orthogonalized to each other.

Experiment II: Verifying Absence of Motor Activity During Observation. Overt finger movements during observation in Experiment I were prohibited by requiring subjects to press two keyboard keys throughout viewing; This would still leave the possibility of muscle activations subliminal for eliciting an overt movement. Similar to Experiment I, we prevented overt motor practice of the task during observation in Experiment II, by requiring the subjects (3 F, 4 M, 22–36 y—same exclusion criteria as in Experiment I) to press the 'alt' and 'a' keys on the computer keyboard with the index and middle finger of their left hand throughout the viewing procedure; when they let go of the keys, the video was interrupted and resumed only when the keys were pressed. A bipolar arrangement of disposable electrodes (Medicotest, Ag/AgCl-electrodes, square 5 × 5 mm pick-up area) was attached with a center-to-center distance of 3 cm after cleansing and abrasion of the skin (Fig. 2*B*). The electrodes were positioned over the center of the muscle belly of the left flexor digitorum superficialis on the line from origin to insertion, as determined by palpation. EMG signals were sampled at 1,000 Hz (Porti5–16/ASD; 22 bits ADC recorder, TMS International) after band-pass filtering (0.5–450 Hz), and stored on a PC. Similar to experiment I, the observation condition (A) consisted of viewing the six 23-s epochs of one of the three fingertapping videos. The tapping condition (B) required the subjects to tap along to the hand shown on the screen for another six epochs. Any subliminal muscle activation during observation would result in epochs with spectral characteristics that resemble actual tapping (11). We discarded the first two epochs to obtain a stable performance suitable for spectral analysis, and to avoid movement start-up artifacts the first second of each of the remaining 4 epochs was also omitted. EMG records were band-pass (10–400 Hz) filtered using a bi-directional second-order Butterworth filter to reduce artifacts and, after whitening to remove autoregression components, full-wave rectified. A narrowband spectrogram was obtained using non-overlapping Gaussian window of 3,000 samples, resulting in a time-resolved frequency analysis of seven essentially independent time-frequency bins of 3 s $\times$ 0.33 Hz each, resulting in a total of 28 samples per subject per condition. A total of 150 frequency bands were examined (excluding frequencies below 1.0 Hz—that is, components containing DC offset and slow drift in amplitudes—and above 50.667 Hz—that is, components that did not designate tapping-related muscle activity). For each of the bins the dominant frequency (F_{max}) was determined and the distribution of F_{max} across the seven subjects $\times$ 28 bins is shown in Fig. 3. The mean of F_{max} was calculated for each subject, and a paired *t* test was used for statistical comparison of these subject means across conditions.

Experiment III: Sleep-Dependent Consolidation of Sequence or Rhythm. To investigate whether familiarity with the fingertapping digit sequence or learning the sequence of alternation of locations would result in sleep-dependent performance improvements, a second group of 64 healthy right-handed task-naive subjects [32 F, 32 M, 24.0 $\pm$ 4.4 years of age (mean $\pm$ standard deviation), range 18–43 years] were recruited using the same exclusion criteria as in Experiment I. This group of subjects observed, in the evening, either a digit sequence or a spatiotemporal rhythm video of one of the three sequences used in experiment I, that is, 41324, 23142, or 32413. The next morning or evening, they performed the fingertapping task as in Experiment I, being either the same sequence (congruent) or one of the other two sequences (incongruent). Subjects participated twice and performed a congruent fingertapping sequence on one and an incongruent sequence on the other occasion, in balanced order, again resulting in 128 data sets. In this way, every subject was exposed to each of the three sequences only once. We chose to implement only the Immediate sleep subgroup (S, consisting of the sleep and the sleep-wake sub-subgroups), as this was the condition that showed a significant sleep-dependent improvement in Experiment I. By using a partial within- and between subject paradigm with 64 subjects tested twice, we were sure to have the same statistical power for detecting possible sleep-dependent performance differences between the congruent and incongruent sessions, i.e., 16 subjects per testing sub-subgroup, see Fig. 1 (Note: the timing of the S subgroup was used for both the sequence and rhythm learning tasks).

The videos were identical to the videos used in Experiment I with respect to duration and number of blocks; the sequence videos showed the digit sequence (i.e., 41324, 23142, or 32413; Fig. 2*C*) on the screen for 23 s, interspersed with 20-s intervals in which the screen color changed to red and the digit sequence disappeared. The subjects were instructed to simply view the screen and to keep two fingers on two keys of the keyboard throughout viewing. The spatiotemporal rhythm videos showed four black square boxes on a horizontal row in the middle of a computer screen that would light up in the order of each of the sequences used (Fig. 2*D*). The speed of the sequential highlighting of the squares was identical to the speed of the fingers in the fingertapping videos used in Experiment I, and increased across the six 23-s viewing blocks in accord with the improvement of the hand in the videos.

Experiment IV: Performance Immediately After Viewing. We recruited 32 additional subjects [16 F, 16 M, 25.1 $\pm$ 5.0 years of age (mean $\pm$ standard deviation), range 19–43 years] to study performance on the fingertapping task immediately (5 min) after observation. Every subject participated twice, allocated to a different condition such that every subject performed an incongruent sequence and a congruent sequence once. In this way, every subject saw two different videos and performed two different versions of the task. We balanced morning vs. evening, video version and order of incongruent vs. congruent conditions across the subjects and orthogonalized them to each other. The videos and instructions to the subjects were identical to those used in Experiment I.

Statistical Analyses. We calculated initial performance level at the second testing session (i.e., post-observation) as the average speed (number of correct sequences tapped) and the average error rate (number of erroneous relative to total number of sequences) for the first six epochs of fingertapping of a total of 12 epochs, to avoid the effects of practice on performance but rather reflecting the immediately present sleep-related enhancement. In the analyses of the performance data, we made optimal use of the mixed design (i.e., all subjects tested twice in different experimental conditions) by using mixed effect model regression analyses (MLWIN 2.0 software, Centre for Multilevel Modeling, University of Bristol, Bristol, U.K.) with subject and testing session as hierarchical levels. The regression analyses estimated the performance gain by prior observation of a congruent sequence versus prior observation of an incongruent sequence either in the immediate sleep condition and the delayed sleep condition (Experiment I), or in the sequence and rhythm conditions (Experiment III), which were fully balanced with respect to the time of day of performance and the interval between observation and performance. Wald tests were applied to obtain the significance of estimated effects on speed and on error rate, with $P < 0.05$ regarded significant.

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