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Qualitative differences in offline improvement of procedural memory by daytime napping and overnight sleep: An fMRI study

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ABSTRACT

Daytime napping offers various benefits for healthy adults, including enhancement of motor skill learning. It remains controversial whether napping can provide the same enhancement as overnight sleep, and if so, whether the same neural underpinning is recruited. To investigate this issue, we conducted functional MRI during motor skill learning, before and after a short day-nap, in 13 participants, and compared them with a larger group (n = 47) who were tested following regular overnight sleep. Training in a sequential finger-tapping task required participants to press a keyboard in the MRI scanner with their non-dominant left hand as quickly and accurately as possible. The nap group slept for 60 min in the scanner after the training run, and the previously trained skill was subsequently re-tested. The whole-night sleep group went home after the training, and was tested the next day. Offline improvement of speed was observed in both groups, whereas accuracy was significantly improved only in the whole-night sleep group. Correspondingly, the offline increment in task-related activation was significant in the putamen of the whole-night group. This finding reveals a qualitative difference in the offline improvement effect between daytime napping and overnight sleep.

1. Introduction

For healthy adults, daytime napping offers various benefits such as relaxation, reduced fatigue, increased alertness, improved mood, and improved performance (Horne and Reyner, 1996), including faster reaction time (Taub et al., 1977) and better procedural memory (Cajochen et al., 2004). To understand the underlying mechanism, comparison with whole-night sleep is critical. In this article, we compared the improvement effects of daytime napping and whole-night sleep on procedural memory.

Motor skills are continuously developed after the end of practice (Robertson et al., 2004). For sequential motor skills, such as typing at a keyboard or playing the piano, numerous studies have suggested that performance robustly improves after post-training sleep (Fischer et al., 2002; Walker et al., 2002, 2003a,b; Nishida and Walker, 2007).

Overnight sleep is characterized by predominant slow wave sleep (SWS) during the first half of the night, and a high amount of rapid eye movement (REM) sleep during the second half (Weitzman et al., 1980). SWS is important for declarative memory (Gais and Born, 2004; Schabus et al., 2005), whereas REM sleep is more important for procedural memory consolidation (Smith et al., 2004). However, the effect of overnight sleep on memory consolidation is confounded by the circadian rhythm and many other factors such as cortisol, growth hormones, and the level of alertness (Van Cauter et al., 2000). On the other hand, daytime napping usually lacks the REM component because of its shorter duration (less than one hour) relative to whole-night sleep. REM sleep does not usually occur during the first hour of the sleep (Weitzman et al., 1980). These qualitative and quantitative (i.e., duration) differences may result in different effects on procedural memory enhancement.

It remains controversial whether daytime naps can provide the same procedural memory enhancement as overnight sleep, which

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aspect of the performance–speed or accuracy–is improved, and whether the same neural underpinnings are involved. Previous studies have reported robust behavioral offline improvements of both speed and accuracy (Fischer et al., 2002; Walker et al., 2003a,b; Rickard et al., 2008; Fischer and Born, 2009), or speed but not accuracy (Walker et al., 2002; Hotermans et al., 2006; Korman et al., 2007; Doyon et al., 2009) following post-training whole-night sleep. By contrast, a short-duration nap following motor training improves speed, but not accuracy (Nishida and Walker, 2007; Doyon et al., 2009). Offline increases in task-related neural activity in the putamen are more prominent when participants sleep after training than when they do not (Debas et al., 2010). A recent functional connectivity study also reported that the score of functional integration in the cortico-striatal network is altered during the offline interval after sequential motor skill training; this offline change in functional integration is particularly noticeable after overnight sleep (Debas et al., 2014). Therefore, the cortico-striatal system is important for both acquiring skills and overnight sleep-dependent skill consolidation. However, no detailed analysis of learning-related neural activity regarding the difference between daytime napping and overnight sleep has yet been performed.

To investigate this, we conducted functional MRI (fMRI) with 13 participants during motor skill learning before and after a 1-h nap, concomitantly with EEG monitoring. The data were compared with a larger group (n = 47) who performed the same learning task, but before and after regular overnight sleep and without EEG monitoring. All participants were trained in a sequential finger-tapping task (Walker et al., 2002, 2003a). In the whole-night sleep group, participants went home after the training and recalled the trained skill in the MRI scanner 24 h later. Meanwhile, participants in the nap group were equipped for polysomnography following the training and slept for 60 min in the MRI scanner. Immediately after napping, they recalled the trained skill while in the MRI scanner. We compared the offline changes in the behavioral performance (speed and accuracy) and the task-related activity between these two experimental groups.

2. Materials and methods

2.1. Participants

Sixty healthy volunteers participated in the study (21 females and 49 males; mean age = 21.63 ± 2.12 years). All participants were right-handed as measured by the Edinburgh's Laterality Quotient (Oldfield, 1971). None of the participants had a history of neurological, psychiatric, or sleep disorders. The study was conducted according to the Declaration of Helsinki's guidelines for research involving humans. Written informed consent, which was approved by the Ethical Committee of the National Institute for Physiological Sciences, Japan, was obtained from all volunteers before participation in the experiment.

2.2. Experimental procedures

2.2.1. Participant preparation

In order to compare the activity changes related to skill consolidation during nap and whole night sleep, participants were assigned to two groups according to post-training sleep conditions. In the nap group (n = 13), participants were instructed to sleep from 13:00 to 14:00 (60 min) in the scanner after receiving skill training. Throughout this period, fMRI and polysomnography recording were performed. Following the nap, participants were instructed to recall the previously trained skill at 16:00 on the same day. In the whole-night sleep group (n = 47), participants went home after the training run wearing an actigraphy device (A.M.I., USA) to measure their total duration of sleep during the night. The next day, as in the nap group they were asked to recall the previously trained skill in the MRI scanner. In the whole-night sleep group, the start time of training was at either 9:00, 13:00, or 16:00 depending on the availability of machine time; the training-retest interval was 24 h for all participants of the whole-night sleep group.

2.2.2. fMRI procedure

All participants were trained in the same sequential finger-tapping skill in the MRI scanner (Training run). Following training, they slept under different settings corresponding to their group (Sleep). After waking, participants were asked to recall the previously trained skill in the MRI scanner (Retest run, Fig. 1A). The fMRI study was performed in a 3.0T scanner (Verio; Siemens, Germany), using a gradient-echo-planar imaging (EPI) sequence (echo time [TE] = 30 ms, repetition time [TR] = 2500 ms; field of view = 192 × 192 mm^2; flip angle = 80°; matrix size = 64 × 64; 39 slices; slice thickness = 3 mm). The number of scans corresponded to 385 and 180 vols for the training and retest runs, respectively. A whole-brain high-resolution T1-weighted anatomical magnetization-prepared rapid-acquisition gradient echo (MP-RAGE) MRI was also acquired for each participant (TE = 2.97 ms; TR = 1800 ms; field of view = 256 × 256 mm^2; flip angle = 9°; matrix size = 256 × 256; slice thickness = 1 mm).

2.2.3. Setup

The participants lay in the MRI scanner with their heads immobilized by an elastic band and sponge cushions, and with their ears plugged. Stimulus presentation and response collection were conducted using the Presentation software (Neurobehavioral Systems, USA) implemented on a personal computer (dc7900; Hewlett-Packard, USA). An LCD projector (CP-SX12000; Hitachi, Japan) located outside and behind the scanner projected the stimuli through a waveguide to a translucent screen, which the participants viewed via a mirror placed in the MRI scanner. The spatial resolution of the projector was 1024 × 768 pixels, with a 60-Hz refresh rate. The distance between the screen and each of the participant's eyes was approximately 175 cm, and the visual angle was 13.8° (horizontal) × 10.4° (vertical). Participants held an MRI-safe optical four-button response box (Current Design, USA) with their non-dominant left hand during the scans.

2.2.4. Experiment design

The experiment consisted of two fMRI runs: a Training run followed by a Retest run. Each run started with a rest epoch of 30 s, alternating with a sequential finger-tapping execution epoch of 30 s. Rest and execution were repeated three times, generating a block. Participants in the whole-night sleep group repeated the block four times during the Training run (B1 to B4, Fig. 1B), and two times during the Retest run (B5 and B6, Fig. 1B). The last block (B6) consisted of two epochs. The nap group was treated identically, except that retest run included twelve epochs (four blocks, three epochs per block). Thus, both groups underwent identical tasks from B1 to B5, which were the target of the analysis.

2.2.5. Behavioral task

All participants trained on one sequence ("4-1-3-2-4"; numbers represent each finger: 1 = little, 2 = ring, 3 = middle, 4 = index; Fig. 1B and C). The sequential finger-tapping task required participants to press four buttons on the button box repeatedly with the fingers of their non-dominant (left) hand as quickly and accurately as possible for periods of 30 s (for details, see Walker et al., 2002, 2003a). Training and retesting consisted, respectively, of twelve and five 30-s tapping epochs interspersed with 30-s rest epochs (Fig. 1B). During the rest epoch, participants were instructed not to move any fingers and train in the sequence. Before the training run, all

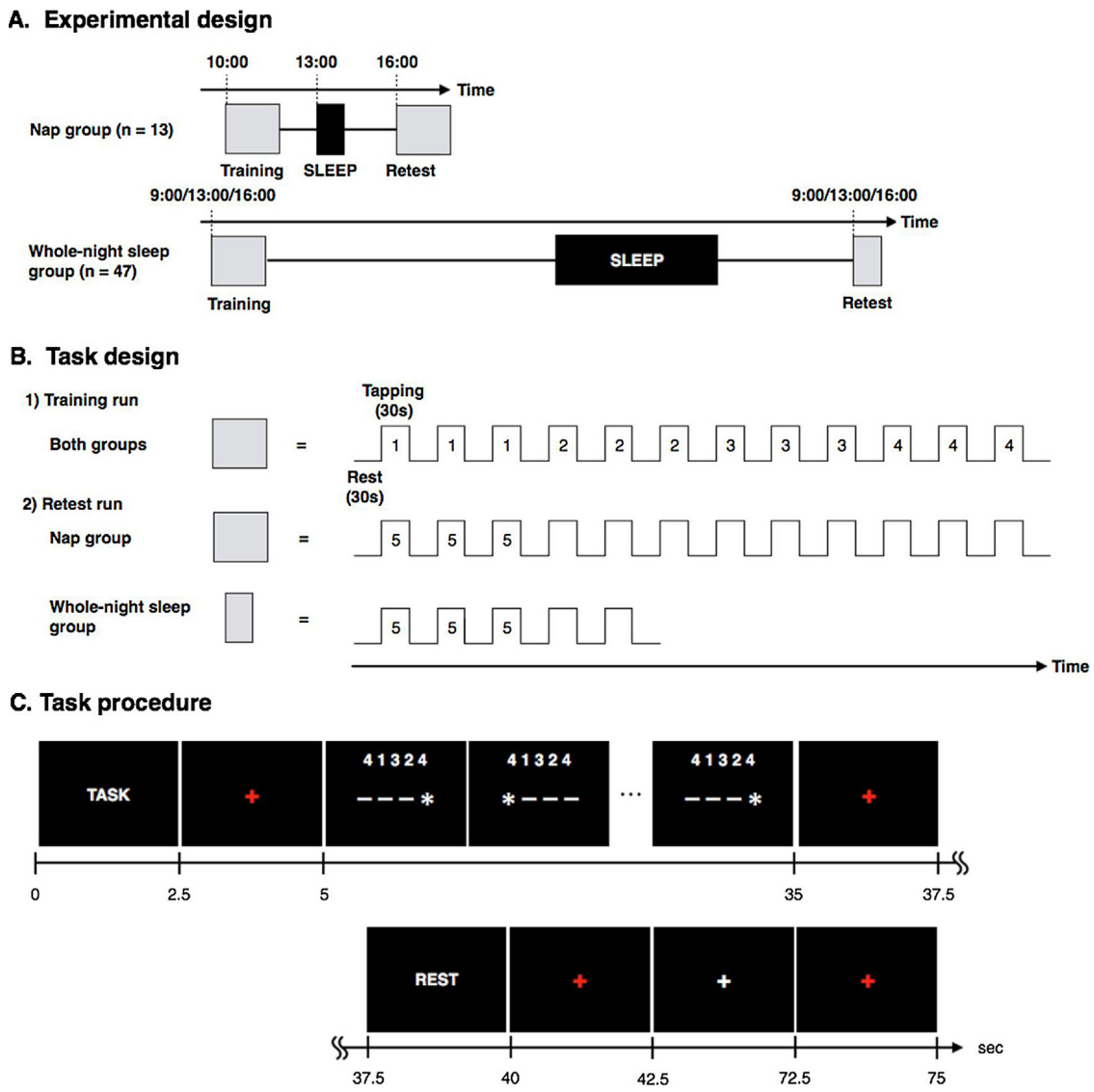

Fig. 1. Experimental procedures. (A) Participants were divided into two different groups and trained in the same sequential finger-tapping skill in the MRI scanner. They then slept under different settings corresponding to their group. After sleeping, participants were asked to recall the previous trained skill in the MRI scanner. (B) Training runs consisted of twelve epochs, whereas retest runs included twelve epochs for the nap group and five epochs for the whole-night sleep group. Because both groups underwent identical tasks from the first epoch of the training run to the third epoch of retest run, these 15 tapping epochs were grouped into five different blocks (B1–B5) for fMRI analysis. In this figure, the number indicates the block number. (C) During each task epoch, following the instructions and warning crosshair, a white asterisk appeared at one of four possible positions signaling the correct button. When the participant pressed the correct button, this asterisk moved on to next position, depending on the learned sequence ("4-1-3-2-4"), for 30 s. After the end of each task epoch, a rest instruction and warning crosshair were presented, and then the white crosshair appeared for 30 s.

participants performed a short practice of the behavioral task in order to familiarize themselves with the sequential finger-tapping task. Briefly, participants performed simple sequential movements ("1-2-3-4") for a 30-s epoch prior to the training run. Instructions as to whether the next epoch was a tapping or resting epoch were provided to participants via the words "TASK" or "REST" (in Japanese), which appeared for 2500 ms in white letters in the center of the screen. Following the presentation of the instruction terms, a red crosshair appeared in the center of the screen for 2500 ms to prepare the participants to respond (Fig. 1C). In the case of a tapping epoch, the sequence ("4-1-3-2-4") was displayed continuously at the top of the screen to exclude any working memory component. A white asterisk stimulus appeared on the screen at one of four possible positions within an equally spaced horizontal array to signal the correct button. Each of the four stimulus positions corresponded to one of the four buttons on the response button box. When participants pressed the correct button, the asterisk stimulus moved on to the next position of the learned sequence for 30 s. During the rest epoch, after the instruction words disappeared, a white crosshair was displayed in the center of the screen for 30 s. At the end of either the task or the rest epoch, the red crosshair appeared in the center of the screen for 2500 ms to signal the end of the epoch. Movement time and error rate were adopted as performance measures. Movement time (ms) was defined as the time required to perform one correct sequence, averaged over 30-s duration of the epoch. The error rate was estimated by the number of error sequences relative to the number of all sequences per epoch. The offline performance improvement following a nap or whole-night sleep was defined as the difference in mean performance between the last three tapping epochs of the training runs and the mean performance of the first three tapping epochs of the retest runs (Fig. 1B, Walker et al., 2002; Debas et al., 2010; Albouy et al., 2012).

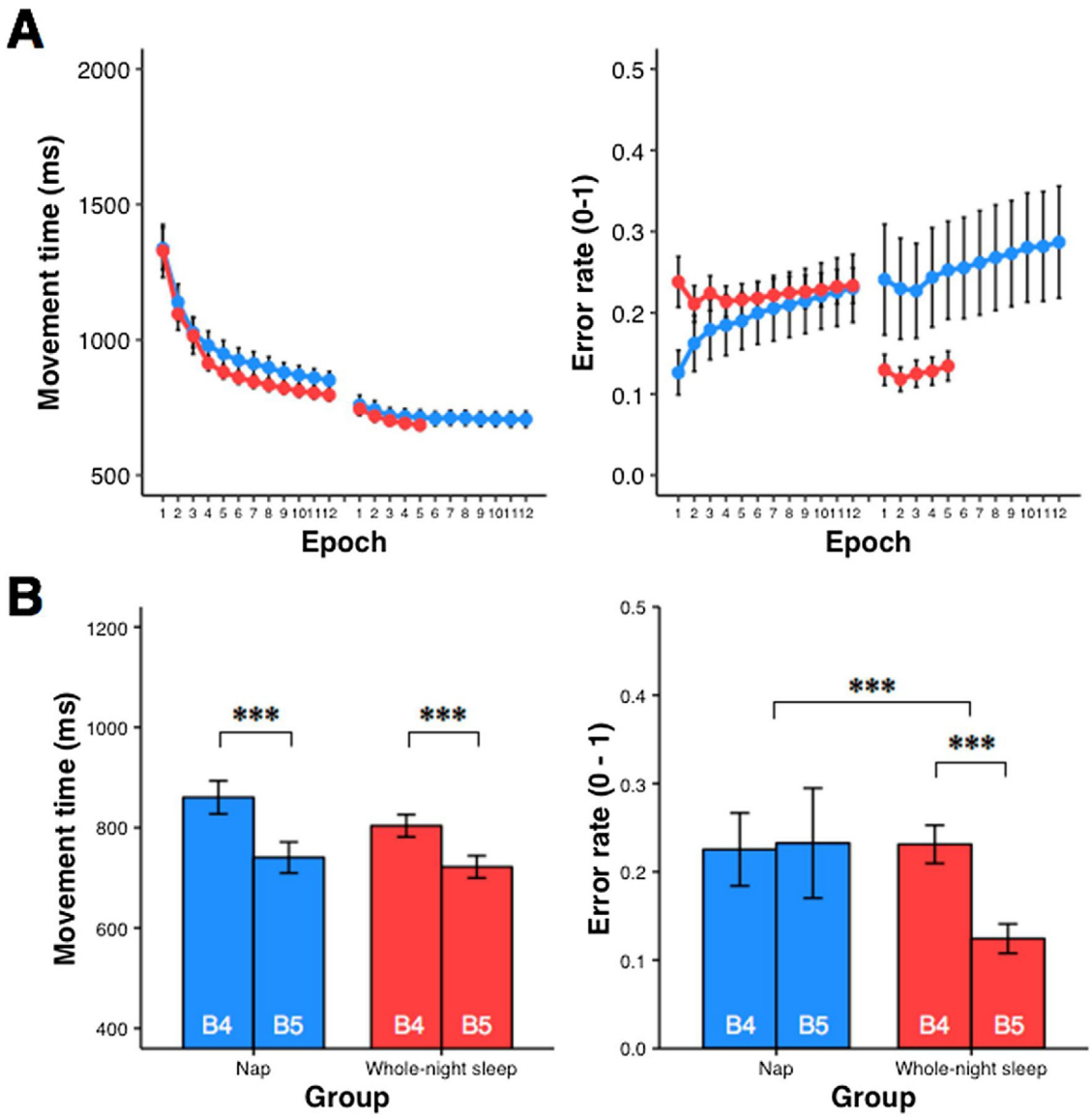

Fig. 2. Behavioral results. (A) Plots represent the mean movement time (left) and error rate (right) in each epoch and group. Movement time was defined as the time required to perform one sequence, averaged over each epoch. Error rate was estimated based on the number of error sequences relative to the number of all sequences per epoch. Blue and red points indicate the nap and whole-night sleep groups, respectively. (B) Offline improvements in movement time and error rate in each group. Offline improvement in movement time was observed in both groups, without significant differences. In regard to the error rate, however, the whole-night sleep group (red bars) surpassed the nap group (blue bars). Error bars indicate standard error of the mean. Asterisks indicate the significance level of mixed-design ANOVA and post-hoc pairwise *t*-tests (***$p < 0.001$). B4, last three epochs of training run; B5, first three epochs of retest run.

2.2.6. Sleep recording

In the nap group, 32-channel electroencephalograms (EEG), vertical and horizontal electrooculograms, electrocardiograms, and respirometer measurements were obtained using an MRI-safe amplifier (BrainAmp MR, Brain Products, Germany), an MRI-safe extended amplifier (BrainAmp ExG MR, Brain Products, Germany), and an electrode cap (Brain Products, Germany) with Ag/AgCl ring electrodes. A reference electrode was placed on the external occipital protuberance, and impedance was kept below 10 kΩ. A raw record was sampled at 5 kHz (bandpass filtered between 0.016 and 250 Hz) using a Brain Vision Recorder (Brain Products, Germany). To correct for gradient-induced artifacts and ballistocardiogram artifacts in the polysomnography data, the template-drift compensation method was adopted using the Brain Vision Analyzer2 software (Brain Products, Germany), offline.

In the whole-night sleep group, participants wore an actimetry device after the training run (Motionlogger, A.M.I., USA) to measure sleep duration and efficiency based on their physical motion, and were instructed to sleep at home as they usually did.

2.3. Data analysis

2.3.1. Demographic data and performance

We investigated group differences in the participants' age and sex using a two-sample *t*-test and chi-square test, respectively. No difference between groups was found in either age ($t[58] = 0.56$, $p = 0.58$) or gender ($\chi^2[1] = 0.91$, $p = 0.34$). For the behavioral performance, we first investigated the group difference of the learning curve during the training run. Mixed ANOVAs [2 (Group: Nap and Whole night sleep) × 12 (Epoch: 1–12)] were conducted for movement time and the error rate. To confirm the offline performance improvements by means of movement time and error rate (Debas et al., 2010) mixed ANOVAs [2 (Group: Nap and Whole night sleep) × 2 (Sleep: before and after sleep)] were performed for these

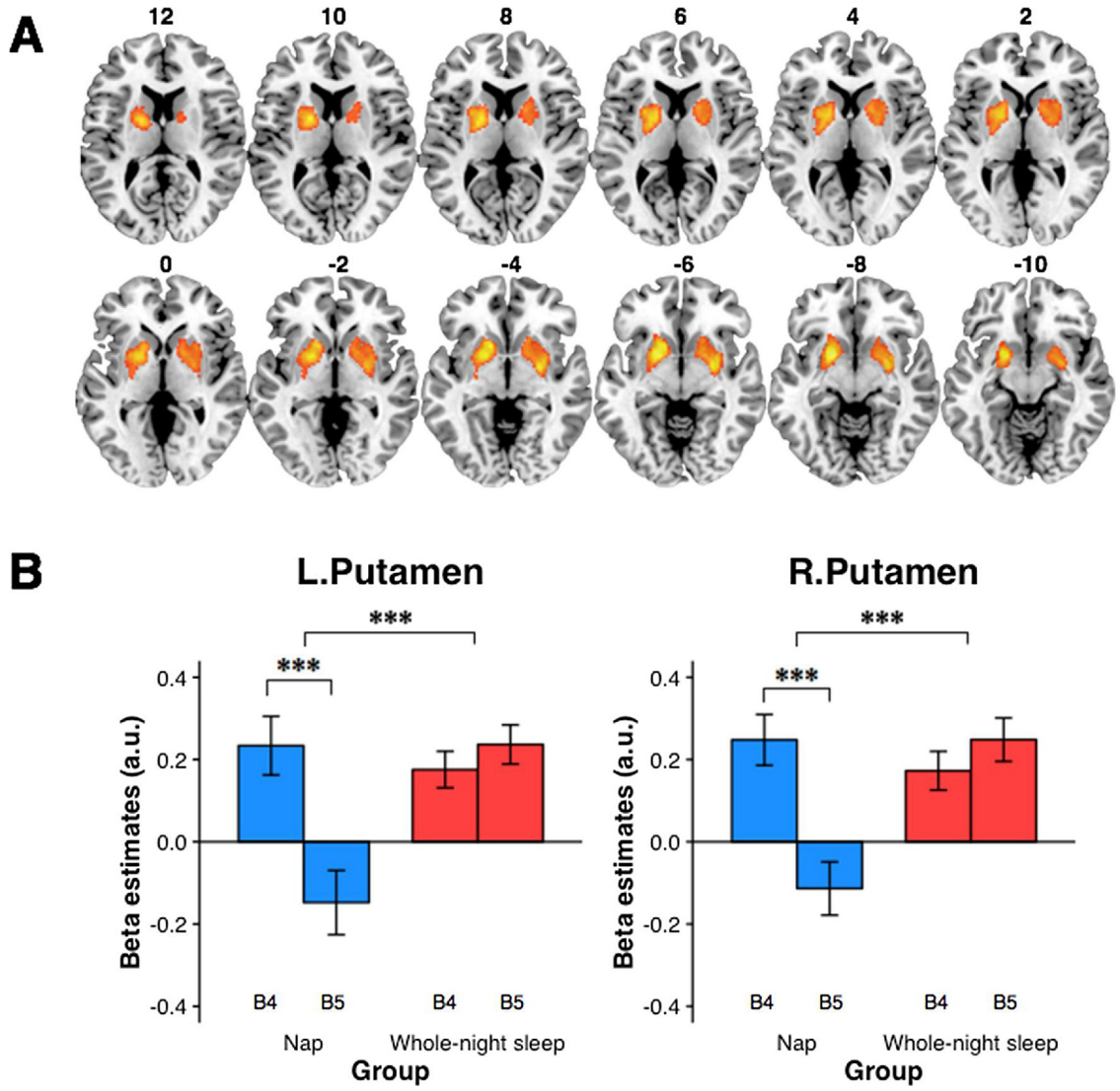

Fig. 3. Brain regions exhibiting differential brain activation between the nap and whole-night sleep groups. Contrast between training (B4) and retest (B5) runs is shown. (A) The putamen exhibited different patterns of offline changes in activity between the nap and whole-night sleep groups. (B) Graphs of the offline changes in task-related activity in the right and left putamen. Blue and red bars represent the nap and whole-night sleep groups, respectively. The error bar indicates the standard error of mean. Asterisks indicate the significance level of mixed-design ANOVA and post-hoc comparisons based on the Bonferroni correction (***$p < 0.001$).

performance measures. Post-hoc pair-wise comparisons were performed based on the Bonferroni correction. The statistical threshold for significance was set at 0.05 for all behavioral analyses.

2.3.2. Sleep data

Sleep recordings with EEG were visually scored according to standard criteria (Rechtschaffen and Kales, 1968; Iber et al., 2007) for each 30-s epoch. According to the criteria, an epoch was scored as the wakefulness stage when alpha rhythms (8–13 Hz) were detectable over the occipital regions in 50% of the epoch. Sleep stage 1 is defined by the attenuation of alpha waves accompanied by increased theta waves (4–7 Hz), the occurrence of slow eye movements, and the presence of a vertex wave. We defined the first epoch of sleep stage 1 as the onset of sleep.

In the whole-night sleep group, the duration (min) and efficiency of sleep during the night after the training were estimated from the physical activity measured by the actimetry device (Motionlogger, A.M.I., USA) using the Act Millennium software (A.M.I., USA). The data was analyzed with the AW2 software, which implements the sleep estimation algorithm of Cole et al. (1992).

2.3.3. fMRI data

We used the Statistical Parametric Mapping software (SPM8; The Wellcome Trust Centre for Neuroimaging, UK) in MATLAB 2012b (MathWorks, USA) to analyze functional images. The first two volumes of each fMRI run were discarded because the signal was unsteady. We conducted motion correction, performed slice-timing correction, co-registered the bias-corrected magnetization-prepared rapid-acquisition gradient-echo images with the mean EPI image, normalized the EPI images to the Montreal Neurological Institute (MNI) T1 template, and performed spatial smoothing with an isotropic Gaussian kernel (full-width-at-half maximum = 8 mm).

Statistical analysis of the fMRI data was conducted at two levels. At the first level in the whole-night sleep group, a general linear model (GLM) was fitted to the fMRI data for each participant (Friston et al., 1994; Worsley and Friston, 1995). The BOLD signal for tapping epochs in each block was modeled with boxcar functions convolved with the canonical hemodynamic response function. As mentioned above in the description of the experimental design, the individual GLM model consisted of training and retest runs with four (B1 to B4) and one task block (B5) regressors, respectively.

Table 1
Brain regions exhibiting different or common activation between nap and whole-night sleep groups at the end of training.

Cluster size (mm^3)	MNI coordinates			*Z* value	Hemi.	Anatomical Region
	x	y	z			
Nap (B4) > Whole night sleep (B4)						
	No significant clusters					
Nap (B4) < Whole night sleep (B4)						
	No significant clusters					
Conjunction between Nap (B4) and Whole-night sleep (B4)						
315032	34	−22	52	Inf	R	Primary motor cortex
	−38	−32	46	Inf	L	Postcentral gyrus
	48	−20	54	Inf	R	Postcentral gyrus
	−2	0	58	Inf	L	Supplementary motor area
	−56	2	32	Inf	L	Premotor cortex
	−30	−52	58	Inf	L	Superior parietal lobule
	28	−54	56	Inf	R	Superior parietal lobule
	−18	−52	−20	Inf	L	Cerebellum (Lobule VI)
	22	−56	−22	Inf	R	Cerebellum (Lobule VI)
	−20	−56	−50	Inf	L	Cerebellum (Lobule VIII)
	−2	−66	−18	Inf	L	Cerebellum (Vermis)
	46	−68	4	Inf	R	Middle temporal gyrus
	24	−80	−6	Inf	R	Fusiform gyrus

Table 2
Brain regions exhibiting different and common activation between nap and whole-night sleep groups in the sleep effect. Group differences in the contrasts between the end of training (B4) and the after-sleep retest (B5) are listed.

Cluster size (mm^3)	MNI coordinates			*Z* value	Hemi.	Anatomical Region
	x	y	z			
Whole night sleep (B5 > B4) > Nap (B5 > B4)						
9120	−26	6	6	4.17	L	Putamen
7728	26	−4	−6	4.58	R	Putamen
Whole night sleep (B5 > B4) < Nap (B5 > B4)						
	No significant clusters					
Conjunction between Whole night sleep (B5 > B4) and Nap (B5 > B4)						
	No significant clusters					
Conjunction between Whole night sleep (B5 < B4) and Nap (B5 < B4)						
	No significant clusters					

Additionally, the instructions preceding each block were modeled as a single regressor of no interest. Motion-related artifacts were modeled by incorporating the six parameters (three displacements and three rotations) from the rigid-body realignment stage and the additional regressor, describing intensity in the cerebrospinal fluid, into the GLM model. The time series for each voxel was high-pass filtered at 1/128 Hz. Assuming a first-order autoregressive model, the serial autocorrelation was estimated from the pooled active voxels with the restricted maximum likelihood procedure, and used to whiten the data (Friston et al., 2002). To calculate the estimated parameters, a least-squares estimation was performed on the high-pass filtered and pre-whitened data. To test the hypothesis about a regionally specific tapping effect, comparisons were made to the baseline rest condition by linear contrasts. The first-level analysis of the nap group was identical to that of the whole-night sleep group, except that retest run was modeled with twelve epochs (four blocks, three epochs per block) in the retest run.

The weighted sum of the parameter estimates in the individual analyses constituted contrast images that were used for the second-level analysis. Contrast images of the tapping-by-repetition effect (four training and one retest blocks per participant) from the individual analyses were incorporated into a flexible factorial design that modeled the subject effect, the six repetitions, and the group effect. To confirm that the degree of task-related activation at the end of training did not differ between two groups, we compared the task-related activation at the fourth training block (B4) between the two groups by estimating two contrast images (Nap > Whole night sleep and Nap < Whole night sleep). In addition, we investigated group-independent tapping-related activation using conjunction analysis (Friston et al., 2005) of the B4 contrasts from both groups. To evaluate the effect of sleep on task-related activation, we made the contrast [B5 > B4] in the whole-night sleep group and nap group separately. We then made a group comparison of this contrast to evaluate the group × sleep interaction, asking whether whole-night sleep influenced task-related activation differently from napping, and which areas were responsible for the difference. The resulting set of voxel values for each contrast constituted the SPM{*t*}, which was transformed into normal distribution units (SPM{*z*}). The threshold for SPM{*z*} was set at $Z = 3.09$ (equivalent to $p < 0.001$ uncorrected). The statistical threshold for the spatial extent test on the clusters was set at $p < 0.05$ and corrected for multiple comparisons over the search volume (Friston et al., 1996). MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/) was used to display activation patterns on T1- weighted MRI images.

3. Results

3.1. Sleep measurements

Sleep data from four participants in the whole-night sleep group were excluded from subsequent analysis due to measurement failure. Thus, final data from 56 participants (nap: $n = 13$; whole night sleep: $n = 43$) were analyzed. We compared the sleep durations between the two experimental groups to ensure that par-

ticipants in the whole-night sleep group slept more than the nap group. The observed sleep durations differed significantly between groups ($t[54] = 14.16$, two sample t-test, $p = 8.11 \times 10^{-20}$). Participants in the whole-night sleep group had significantly longer sleep (391.14 ± 13.49 min) than participants in the nap group (39.96 ± 4.35 min). In addition, according to the polysomnography data in the nap group, no participants exhibited any rapid-eye movement (REM) sleep periods.

3.2. Behavioral results

Learning curves during the training run were compared between the two groups (Fig. 2). Mixed ANOVAs [2 (Group: Nap and whole night sleep) × 12 (Epoch)] were performed for movement time and error rate. For movement time, the main effect of Epoch was significant ($F[11,638] = 20.75$, $p = 5.03 \times 10^{-7}$), but the main effect of Group ($F[1,58] = 0.74$, $p = 0.39$) and the Group × Epoch interaction were not ($F[11,638] = 0.10$, $p = 0.86$). For error rate, neither a main effect nor interaction was observed (Group: $F[1,58] = 0.44$, $p = 0.51$; Epoch: $F[11,638] = 3.09$, $p = 0.058$; Group × Epoch: $F[11,638] = 2.78$, $p = 0.076$).

To investigate the performance changes between the before- and after-sleep periods in the nap and whole-night sleep groups, mixed ANOVAs [2 (Group: Nap and whole night sleep) × 2 (Time: before and after sleep)] were conducted for movement time and error rate. For movement time, ANOVA revealed that the main effect of Time was significant ($F[1,58] = 31.28$, $p = 6.33 \times 10^{-7}$), but the main effect of Group and the Group × Time interaction were not (Group: $F[1,58] = 2.00$, $p = 0.17$; Group × Time: $F[1,58] = 1.73$, $p = 0.19$). This result indicated that the level of movement time decreased significantly between the before- and after-sleep periods in both groups (Fig. 2A; Nap: Mean ± SE = -133 ± 34 ms, $t[12] = 5.48$, paired t-test, Bonferroni corrected $p = 2.50 \times 10^{-4}$); Whole night sleep: Mean ± SE = -82 ± 18 ms, $t[46] = 4.33$, paired t-test, Bonferroni corrected $p = 2.40 \times 10^{-5}$). For the error rate, we observed a significant main effect of Time ($F[1,58] = 5.76$, $p = 0.020$), but no significant main effect of Group ($F[1,58] = 1.32$, $p = 0.26$). In contrast to the movement time, the Group × Time interaction was significant for the error rate ($F[1,58] = 6.87$, $p = 0.011$), indicating that the offline changes in error rate significantly differed between the two groups (Fig. 2B). Following ANOVA, to resolve the interaction of Group and Time, we carried out pairwise comparisons based on the Bonferroni correction. The results revealed that the error rates significantly decreased between the before- and after-sleep conditions in the whole-night sleep group (Mean ± SE = -0.11 ± 0.02, $t[46] = 5.20$, paired t-test, Bonferroni corrected $p = 1.00 \times 10^{-6}$), but not in the nap group (Mean ± SE = $+0.01 \pm 0.04$, $t[12] = 0.15$, paired t-test, Bonferroni corrected $p = 0.90$).

3.3. Task-related activity at the end of training

To confirm that the baseline task-related activities were comparable between the two groups, we compared task-related brain activity at the end of training between the nap and whole-night sleep groups. We did not find any brain region exhibiting significant differences in task-related activities between the two groups (Table 1). Conjunction analysis identified significant task-related activations in bilateral postcentral gyrus, superior parietal lobule, cerebellum lobule VI, left supplementary motor area, premotor area, cerebellum lobule VIII, cerebellum vermis, right primary motor cortex, middle temporal gyrus, and fusiform gyrus (uncorrected $p < 0.001$ at peak-level and family-wise error corrected $p < 0.05$ at cluster level), consistent with a visuo-motor task performed with the left hand.

3.4. Altered brain activity from the end of training to the retest

The purpose of this study was to determine whether the offline increment in the cortico-striatal system activity is associated with improved speed and/or accuracy in a sequential motor skill. Therefore, we examined the group differences in offline changes in task-related activation. The effect of sleep on task-related activation in the bilateral putamen was more prominent in the whole-night sleep group than the nap group (uncorrected $p < 0.001$ at peak-level and family-wise error corrected $p < 0.05$ at cluster level; Fig. 3A and B and Table 2). Post-hoc comparisons on the cluster basis showed that mean activation in this region decreased significantly from the end of training to the retest in the nap group (Left: $t[12] = 5.22$, paired t-test, Bonferroni corrected $p = 9.38 \times 10^{-7}$; Right: $t[12] = 5.16$, paired t-test, Bonferroni corrected $p = 2.20 \times 10^{-5}$). Meanwhile, bilateral putaminal activation after sleep tended to be increased in the whole-night sleep group, albeit not significantly (Left: $t[46] = 1.69$, paired t-test, Bonferroni corrected $p = 0.10$; Right: $t[46] = 1.79$, paired t-test, Bonferroni corrected $p = 0.071$). Conjunction analysis did not identify any commonly increased or decreased activations in the nap and whole-night sleep groups (Table 2).

4. Discussion

In the current study, we observed offline changes in striatal activity and offline performance improvements, in terms of both speed and accuracy, after whole-night sleep. These results are consistent with the predominant view that skill enhancement depends on sleep (Walker et al., 2005; Walker, 2005; Diekelmann and Born, 2010). Moreover, we demonstrated that the offline change in activity of the bilateral putamen was related to improved accuracy of performance.

4.1. Offline improvements in sequential motor skill induced by post-training sleep

In line with previous reports (Korman et al., 2007; Hotermans et al., 2006; Fischer et al., 2002; Fischer and Born 2009; Doyon et al., 2009; Nishida and Walker 2007; Rickard et al., 2008; Walker et al., 2002, 2003a,b), we found that the offline improvement in speed (recorded by movement time, or average time required to complete one correct sequence) was significantly increased by both whole-night sleep and napping. On the other hand, offline improvement in accuracy (measured by error rate) was observed only in the whole-night sleep group. These findings suggest that the consolidation process during post-training sleep differs qualitatively depending on the sleep modes.

4.2. What underlies the differences between overnight sleep and napping?

The first possibility is that the differences between the groups were due to the sleep stage. Earlier studies claimed that the consolidation of a sequential motor skill occurs during REM sleep (Smith, 1995; Maquet et al., 2000; Peigneux et al., 2001). Because participants in the nap group did not exhibit any period of REM sleep—in contrast to overnight sleep, during which REM sleep is very likely to occur—the increased striatal activity may have been related to REM sleep. However, pharmacological REM sleep suppression, by administration of selective serotonin or norepinephrine re-uptake inhibitors, has been shown to improve, rather than impair, skill memory (Rasch et al., 2009), indicating that REM sleep may not be required for skill-memory enhancement. However, unknown processes critical for memory enhancement that are associated with REM sleep could be spared from pharmacological suppression.

Therefore, the contribution of REM sleep to skill learning should be explored further.

The second possibility is that sleep duration, particularly non-REM sleep, may underlie the observed differences. Using procedures similar to those used in this study, Nishida and Walker (2007) demonstrated that a 60–90 min midday nap could enhance the speed of performance without changing its accuracy. Furthermore, they reported that the amount of offline improvement in terms of speed was significantly correlated with the duration of Stage-2 non-REM sleep. Given that 6 h sleep was shown to enhance both speed and accuracy of sequence performance regardless of the time of the day, i.e., daytime or nighttime (Fischer et al., 2002), the duration of sleep in our study (6 h for whole-night sleep and 1 h for napping) *per se* might have caused different effects on the performance.

The third possibility is that the differences may be due to an artifactual effect of an early performance boost (Hotermans et al., 2006). Recent studies indicated that sleep has a beneficial effect on procedural memory stabilization without enhancement (Hotermans et al., 2006; Rickard et al., 2008; Brawn et al., 2010; Nettersheim et al., 2015; Pan and Rickard, 2015). Specifically, the performance of a learned sequential skill significantly improves 5–30 min after the end of training, and then deteriorates during awake retention intervals before being restored after sleep (Hotermans et al., 2006; Brawn et al., 2010; Nettersheim et al., 2015). These reports suggest that the nap-induced speed enhancement observed in the present study may represent an early boost effect, perhaps also including recovery effect from the gradual build-up of fatigue over the course of the concentrated training that preceded the nap (Rickard et al., 2008). Because there was no significant difference in speed enhancement between the nap group and whole-night sleep group, the speed enhancement in both groups may have been related to the early boost effect (Hotermans et al., 2006; Nettersheim et al., 2015; Landry et al., 2016). This is consistent with the idea that sleep *per se* does not enhance performance in terms of speed (Rickard et al., 2008). However, it should be noted that accuracy, another aspect of the performance, was improved following whole-night sleep. This may be regarded as the "stabilization" process that results in the observed enhancement in accuracy, although the sleep parameters that are responsible for this improvement remain currently unknown.

4.3. Neural substrates of the enhancement in the sequential motor skill

In the present study, task-related activations in the bilateral putamen slightly increased from the end of training run to the first retest run in the whole-night sleep group. In the nap group, by contrast, putaminal activity decreased after the offline intervals. This is consistent with the findings of previous studies of the neural substrates of sleep-dependent enhancement of motor sequence learning (Walker et al., 2005; Albouy et al., 2008; Debas et al., 2010). Activities in this brain region at the end of training, before sleep, were comparable between the two groups. The offline improvement in speed was also comparable, whereas accuracy was more improved in the whole-night sleep group. Thus, the difference in the offline changes in task-related activation in the putamen may contribute to an offline improvement in accuracy. In this experiment, a control condition involving un-learned sequential motor control was not included. Thus, a non-specific motor effect, namely, higher movement efficiency in the after longer time interval, cannot be completely excluded.

Learning requires the online evaluation of performance, during which the error signal may be dealt with by the striatum, along with the anterior cingulate cortex (Báez-Mendoza and Schultz, 2016). We observed a significant decline in the error rate after whole-night sleep, but not after napping. This performance difference was reflected in the task-related activation of the striatum: the whole-night sleep group exhibited an increase in task-related activation, whereas the nap group exhibited a decline. This difference is unlikely to have been caused by the error signal *per se* because the striatal activation was enhanced in the whole-night group whereas the error rate declined. Thus, we inferred that the striatum may be related to the learning process, given the error rate as a measure of the accuracy which in turn the measure of the learning.

It remains debatable how the striatum contributes to motor sequence memory enhancement. In this study, the striatum was not active during the task (Table 1). Previous studies of sequential finger-tapping learning reported that the striatum is involved in late learning (over the range of several days), during which time the activity of the striatum increased (Penhune and Doyon 2002). This is consistent with the involvement of the striatum in performance of well-learned sequences (Doyon et al., 1996; Grafton et al., 1995; Rao et al., 1997; Rauch et al., 1997), particularly those related to the motor and perceptual timing (Rao et al., 1997; Harrington et al., 1998). Because performance is saturated in the later phase of training, the striatum may be involved in the performance of well-learned sequences. In rodents, the striatum represents the whole sequence (Jog et al., 1999; Jin et al., 2014). Moreover, a recent human neuroimaging study demonstrated that the sensorimotor territories of the striatum are involved in motor skill chunking, specifically in the concatenation of required movements (Wymbs et al., 2012). According to these data, it is plausible that the offline involvement of the sensorimotor striatum is related to enhanced chunk formation, resulting in more accurate execution of the task.

5. Limitations of the study

Due to resource limitations, we did not obtain EEG recordings during whole-night sleep. Thus, we are limited in our ability to determine which component of sleep causes the increment in striatal activity. In addition, the two groups differed in size, again due to limited resources for simultaneous EEG/fMRI measurements. Moreover, because a no-nap control group was not included, it is possible that the improvements in speed observed following nap may have been due to the amount of time elapsed, rather than an effect of napping *per se*. Future investigation with greater resources and more thorough equipment is warranted.

6. Conclusion

The qualitative difference in the offline improvement effect of sequential finger movement between overnight sleep and daytime napping may be related to the posterior striatum, in which sleep-related enhancement of task-related activity was correlated with improved accuracy. To our knowledge, this is the first study to clarify the link between the striatum and overnight sleep–specific enhancement of motor sequence learning.

Authors' contributions

S.K.S. and T.K. provided theoretical input and designed this study. S.K.S and H.K. designed the experimental tasks. S.K.S., T.K., H.K., K.M., H.K.T., E.N., and Y.H.H. conducted the experiments. S.K.S analyzed the behavioral and fMRI data. K.T. analyzed the sleep data in the nap group. S.K.S and N.S. wrote the manuscript. N.S. supervised the overall project.

Conflicts of interest

The authors declare no competing financial interests.

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