Supplemental Data

Location-Specific Cortical Activation Changes during Sleep after Training for Perceptual Learning

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Subjects

A total of 21 subjects (14 females) ranging in age from 18 to 36 years participated in the experiments. Seven subjects (5 females) participated in the main sleep experiment, 5 subjects (2 females) in the first control experiment with no-sleep between the training and re-test sessions, 6 subjects (5 females) in a second control experiment with a longer re-test session, and 3 subjects (2 females) in the pilot study during adaptation nights (see 3. Adaptation sleep session). All subjects had normal vision and were naïve regarding the purpose of the experiments, under no medication, with a regular sleep-wake schedule and reported no sleep problems. Subjects refrained from alcohol and caffeine use from 24 hours before the onset of the experiment until the offset. All subjects gave informed written consent. The study was approved by the Institutional Review Board of Massachusetts General Hospital Human Research Committee.

Main Experiment

1. Experimental design

The complete experiment consisted of 6 sessions (see Fig. 1 in the main text). After the adaptation session the purpose of which was to familiarize seven subjects with the environment to alleviate the first night effect, known in human sleep studies [1-3], brain activation was measured twice (3rd and 5th sessions) by fMRI, which was polysomnographically monitored [4] with magnetic field compatible electrodes for 90 min [5, 6]. After brain measurement, subjects were moved to a quiet room to sleep for the rest of the night. Experimental days were not consecutive. There were 3-7 day intervals between them.

Several hours before the second brain measurement, subjects underwent an intensive training on a texture discrimination task [7] (TDT, see Fig. 1a-c in the main text). Importantly, TDT training leads to visual perceptual learning and brain activation changes are observed specific to the quadrant of V1 within which a trained target was retinotopically projected but not in other quadrants [7-9]. By taking advantage of the location specificity effect of TDT, in a single fMRI session we obtained and compared brain activation in the trained and untrained parts of the early visual cortex while subjects slept. A more detailed procedure description follows.

2. Definition of region of interests

We identified V1, V2, and V3 by a retinotopic mapping technique for each subject using fMRI. We used a block design, and presented checkerboard patterns at vertical and horizontal meridians [10-12] to localize the cortical representation of the upper and lower visual fields. In addition, we presented annulus stimuli of various sizes to localize eccentricity. Then, we localized 4 subregions of V1, V2, and V3, corresponding to 4 quadrants of the visual fields within 5-deg eccentricity. The eccentricity corresponds to the size of the trained visual field quadrant (see 5. Training session below for more detail). In this study, the trained region of V1 is

defined as the cortical quadrant, which retinotopically corresponds to the trained visual field quadrant, up to 5-deg eccentricity. The untrained region of V1 is defined as the remaining 3 cortical quadrants, up to 5-deg eccentricity.

For retinotopic mapping purposes, subjects were scanned in a 3T MR scanner (Trio, Siemens). A head coil was used throughout the experiments. For measurement of BOLD contrast, functional MR images were acquired using a gradient echo EPI sequence (TR = 2 sec, TE = 30 ms, Flip Angle = 90º). For retinotopic mapping, twenty-five contiguous slices (3 x 3 x 3.5 mm^3) orientated orthogonal to the calcarine sulcus were acquired covering the occipital to parieto-temporal cortices.

For retinotopic mapping, data were analyzed with FSFAST and FreeSurfer (http://surfer.nmr.mgh.harvard.edu) software. All functional images were motion corrected [13], spatially smoothed with a Gaussian kernel of 5.0 mm (FWHM), and normalized individually across scans. Functional data were registered to the individual reconstructed brain [14, 15], for which three T1-weighted MR images (MPRAGE) were acquired (TR = 2.531 sec, TE = 3.28 msec, flip angle = 7º, TI = 1100 msec, 256 slices, voxel size = 1.3 x 1.3 x 1.0 mm^3). The average signal intensity maps were then calculated for each subject in each condition. Voxel-by-voxel statistical tests were conducted by computing contrasts based on a univariate general linear model. Significance levels were projected onto the flattened cortex individually [14, 15]. Based on these maps, the entire region and subregions of V1, V2, and V3 were defined.

3. Adaptation sleep session

In sleep experiments in general, subjects often have difficulty falling asleep immediately on the first night in a novel environment with electrodes attached and under supervision of unfamiliar research staff. This phenomenon, called the first night

effect [2, 16, 17], has been well recognized in human sleep studies. To alleviate the situation, an adaptation protocol is often incorporated. In the protocol, dummy sleep measurements are conducted where subjects are asked to sleep with electrodes attached in an experimental room for 1 or 2 nights [1, 2, 18].

Machine noise produced by an MRI scanner while scanning is being conducted is thought to be another factor that can cause subjects not to fall asleep easily. Considering this factor as well, we used the following two-night procedure as the adaptation protocol in the present study. On the first night, subjects were asked to sleep inside a mock scanner, which mimics a true MRI scanner, but does not conduct scanning, with electrodes attached for 90 min at night. On the second night, the subjects were asked to sleep inside the actual MRI scanner with electrodes attached and earplugs inside both ears for 90 min during which real scanning was conducted with machine noise. After the adaptation sessions, the subjects were allowed to sleep in a quiet environment for the rest of the night with electrodes removed.

This 2-day adaptation schedule was determined on the basis of the results from a preliminary study. In the preliminary test, after this adaptation protocol, all subjects (n=3) reported falling asleep. The polysomnogram (PSG) corroborated the subjects' reports, showing non rapid eye movement (NREM) sleep patterns during the first 1 hour sleep period.

4. The pre- and post- training fMRI sessions

Upon confirmation that a subject could sleep inside the fMRI scanner during the adaptation sessions, s/he was asked to sleep inside the fMRI scanner in the pre- and post- training fMRI sessions for 90 min with electrodes attached (see below). After fMRI measurements were completed, the subject was asked to move to a quiet room and permitted to sleep for the rest of the night without the electrodes.

4-1. Polysomnography

To obtain PSG according to the standard criteria for the sleep scoring system [4], we measured electrocenphalogram (EEG; Cz and Oz), electrooculogram (EOG; left and right), and electromyogram (chin muscle), with high magnetic field compatible electrodes and motion sensors [5, 19, 20]. An electrode placed at the left mastoid served as reference. The polysomnographic recordings were conducted using a custom-made MRI-compatible system [5, 6, 21-23], which features high dynamic range (nominally 24 bits), and low noise with a sampling rate of 957 Hz. The system amplified electrophysiological signals, performed analog-to-digital conversion inside the high magnetic field, and was connected via a USB optical interface to a laptop (DELL Latitude C840) computer outside the magnet room. Data were displayed and recorded by the laptop computer, with LabView7.1 (National Instrument). EEG data were filtered with a 20 Hz low pass filter. Ballistrocardiogram was removed from the 2 EEG channels using the data from the motion sensors and an adaptive Kalman filter [5] with Matlab 7, and signal processing toolbox. After removal of motion and ballistocardiogram artifacts from EEG [5], sleep stages were visualized with Matlab plus EEGlab v4.515, and scored according to the standard criteria [4] for every 24 sec (hence, every 4 TRs, see below).

4-2. Calibration tasks

After the subjects entered the MRI booth, three calibration tasks were conducted before lights-out. First, the subjects were asked to open and close their eyes approximately every 12 seconds for a total of 1 min to test whether alpha waves could be obtained while the subject’s eyes were closed. This is a standard procedure to identify amplitudes and duration of subject’s alpha wave in a normal awake resting state [4]. The auditory or computer-voice instructions for subjects to open and close

their eyes were controlled by a Mac G4 computer and administered through plastic tubes located on the right and left sides in close proximity to the subjects, so that they could clearly hear the instructions. Second, checkerboard patterns were presented to measure visual responses. Black and white checkerboard patterns with a fixation point for 12 sec and a fixation point alone were presented for 12 sec alternately three times. Third, a small point was presented to the right of center of the screen for 2 sec, then to the left for 2 sec and so on. The subjects were asked to view the point through a mirror above their heads and to move their eyes by following the point for a total of 12 sec, to identify amplitude of their eye movements and to check for correct EOG monitor operation. The viewing distance, which is the sum of the distance between the mirror and the screen on which visual stimuli were projected and the distance between the mirror and eyes was 57 cm. After the eye movement task, subjects were instructed to fall asleep. After 90 min of sleep measurement, the tasks were repeated. Throughout the calibration tasks, fMRI and polysomgnographic measurements were conducted.

4-3. Functional MRI

The subjects were scanned in a 3T MR scanner (Trio, Siemens). A matrix coil was used. Functional images were collected with a gradient echo EPI sequence (TR=6sec, delay=3886ms, TE=30ms, Flip Angle=90º) for BOLD contrast. Sparse sampling was used to produce intact intervals of PSG free from the EPI scanning noise (see Figure S1). Thirty-five contiguous slices (3 x 3 x 3.5 mm^3) orientated parallel to the AC-PC plane were acquired, covering the entire brain. Functional data were motion corrected [13], spatially smoothed with a Gaussian kernel of 5.0 mm (FWHM), normalized by percent signal, and registered to the individual reconstructed brain as described above.

Based on sleep stage scoring with the PSG [4], a block-design analysis was applied. BOLD contrast was obtained between sleep and wakefulness stages, excluding REM sleep periods: We calculated the average BOLD signals during sleep (including sleep stages of 1, 2, 3, and 4), and wakefulness and computed percent signal changes of averaged BOLD signal during sleep relative to the averaged BOLD signal during wakefulness for each ROI.

5. Training session

5-1. Justification of timing of behavioral tests

As is shown in Fig.1 in the main text, the training session was conducted approximately 8 hours earlier than the re-test session. The interval between the initial and re-test session was determined based on previous studies [7, 24, 25]. The task used in this study was originally developed by Karni and Sagi [7]. They investigated the time course of learning on this task and found that it takes at least 8 hours for subjects to show improvement in performance of this task [25]. Based on this study [25], a previous paper by others [24] employed approximately 8 hours interval between test and re-test of sessions to investigate a role of sleep on this visual task. That study reported that memory formation of this task is prompted by the early part of sleep [24]. Thus, in our study, we decided to use the interval between the initial and re-test sessions to be comparable to those previous studies [7, 24, 25].

5-2. Detailed procedure of the training session

The following constitutes a detailed description of the training session procedure. Training of the TDT was based on the procedure for the task in the original study [7].

The training session lasted 70 to 90 min. Training was conducted in a dimly lit room as in our previous study [8]. Stimuli were generated on a Mac G4. The viewing

distance was 57cm. In each trial, a target stimulus was presented for 13 ms followed by a blank screen and a mask stimulus for 100 ms. While subjects were asked to fixate their eyes on the center of the display, they had to respond first to the fixational letter task ("L" or "T") and then to the orientation horizontal or vertical of a target array consisting of three diagonal bars. The location of a target was randomly determined from trial to trial within the given quadrant of the visual field in the training session. The trained visual field within which targets were presented was the upper left quadrant for 4 subjects and the lower right quadrant for the remaining 3 subjects.

There were 1760 trials in total. A block consisted of 40 trials, with each trial taking 2.5 sec. The temporal interval between the offset of a target stimulus and the onset of a mask stimulus (stimulus-to-mask-onset asynchrony, or SOA) varied from block to block. Trials within a block had the same SOA. A sequence of SOA for blocks was presented in a descending series, in which SOAs are 1000, 800, 460, 360, 220, 180, 160, 140, 120, 100, 80, and 60 ms. Two blocks were conducted for SOAs of 1000 ms and 800 ms, and 4 blocks were conducted for the remaining of SOAs. Subjects could take a break to rest every 40 trials. The percentage of correct responses in the orientation task was calculated for each SOA in order to construct a psychometric function for determining the threshold SOA for which subjects marked 80% correct response by interpolation.

The size of the entire visual stimulus subtended 14°-by-14° of visual angle. The position of each line segment was jittered slightly, by 0–0.05°, from trial to trial. Gray lines 0.43º-by-0.07º (32 cd/m^2) were presented against a black background (0.5 cd/m^2). The position of the target array also varied randomly from trial to trial, but was consistently presented within a specific quadrant and within a 3°–5° visual angle

from the center of the display. Auditory feedback was provided for the fixational letter task to facilitate subjects' fixation. No feedback was provided for the orientation task. Orientation task learning has been demonstrated previously in the TDT without feedback [7, 26].

No measures of subjects' alertness were utilized in this study.

6. Re-test session

The re-test session was conducted approximately 8 hours after onset of the training session in the main experiment, a total of 160 trials of the TDT were presented. To avoid occurrence of a learning effect, the trial numbers (and hence the number of blocks) for the re-test session was kept to a minimum. Otherwise, the procedure of the re-test session was similar to that of the training session. The re-test session consisted of 4 blocks of 40 trials each. Trials within the same block contained the same SOAs. A series of SOAs were presented in a descending order. To examine whether the correct response rate in the re-test session increased over the SOAs that were close to the threshold SOA in the initial training session, the range of SOAs in the re-test session was varied individually based on the estimated threshold SOA obtained in the initial training session, except for the first block (and SOA). The SOA of 800 ms in the first block was utilized to remind subjects how to conduct the task. The SOA for the second block of the re-test session was determined based on the individual threshold SOA in the initial training session. The second SOA in the re-test session was the shortest among the set of SOAs that were used and longer than the threshold SOA value determined individually in the training session. The third SOA was 20 ms shorter than the second SOA, and the fourth SOA was 20 ms shorter than the third SOA. The percentage of correct responses in the orientation task was calculated for each SOA in order to construct a psychometric function for determining

the threshold SOA for which subjects achieved 80% correct response by interpolation, which is a standard method used by most studies of TDT perceptual learning [7-9, 27-29]. Note that a few subjects showed correct response over 80% in all SOAs in the re-test session. In such cases, the threshold SOA in the re-test session fell outside the SOA range used in the re-test due to the interpolation.

Control experiment I (TDT with no sleep between the initial and retest sessions)

The purpose of control experiment I was to test whether performance improvement observed during the re-test session was due to sleep after training, or to the mere passage of several hours between the offset of the post-training fMRI session and the onset of the re-test session irrespective of subjects' physiological status of sleep or wakefulness. To address this question, we conducted this control experiment with a new group of subjects (n=5), where only the training and re-test sessions were conducted without sleep (see Fig. 1e in the main text). The time interval between these sessions, and the circadian timing were approximately the same as in the main sleep experiment but the subjects did not sleep during the interval. See page 16 in the Supplemental data for the results of this experiment.

Control experiment II (TDT with a longer re-test session)

The purpose of control experiment II was to test whether the number of trials of the re-test session affects performance when an adaptive method [30] is used in the TDT. We used this control experiment to exclude the possibility that increased performance in the re-test session in the main experiment was due to a reduced adaptation effect, which is known to decrease performance and is influenced by the number of trials in a session [30-32]. However, this adaptation effect is largely

reduced if an adaptive method is used in which the SOA for the initial block is close to the threshold SOA [30]. Since an adaptive method was used in the main experiment, control experiment II was conducted to confirm that the re-test session with longer trials would not make a huge difference in performance improvement compared to that with shorter trials in the main experiment if an adaptive method was used.

The procedure for the training and re-test sessions in this control experiment was identical to that in the main sleep experiment, except that the number of trials in the re-test session was almost identical to that of the training session and fMRI measurement was not conducted. As in the main experiment, the re-test session employed an adaptive method [7, 30], by which the starting SOA value in the re-test session was just above the threshold SOA determined in the previous training session. The average number of blocks and trials in the re-test session were 40.7 ± 3.3 (SD) and 1626.7 ± 133.33 (SD), respectively. See page 17 in the Supplemental data for the results of this experiment.

SUPPLEMENTAL RESULTS

1. Sleep structures obtained in the pre- and post-training fMRI sessions in the main experiment

The PSG results indicate that on average, the subjects were asleep 83.1% of the time (NREM sleep 82.1% and REM sleep 1.0%) during the pre-training fMRI session (after the light was turned off), and asleep 80.0% of the time (NREM sleep 78.8% and REM sleep 1.2%) during the post-training fMRI session (see Supplementary Table 1). The subjects were awake for the remainder of the time.

Since both the pre- and post- training fMRI measurement sessions were conducted during a 90 min period, the result that the observed sleep was mostly NREM sleep is consistent with previous studies (e.g., [4]).

Figure S2 summarizes the sleep structures obtained in the pre- and post-training sleep sessions. On rare occasions, a few subjects showed a brief period of REM sleep in the pre- and/or post- training sleep sessions but in no case were sleep onset REM periods [1] observed. As mentioned above, brain activity during such REM sleep intervals was excluded from the computation of sleep activity in this study.

A Wilcoxon signed-rank test did not reveal differences in any sleep parameters between the pre- and post- training sleep sessions. The time spent in any of the sleep stages 1, 2, 3, 4, and REM was not significantly different between the pre- and post-training fMRI sessions (Figure S2A). There was no significant difference between the sleep latency (the interval between lights-out and the first appearance of the sleep stage 1 in the pre- and post-training fMRI sessions). A sleep ratio was defined as the amount of sleep intervals divided by the total amount of measurement time (the total of sleep and awake periods). There was no significant difference between the sleep ratios in the pre- and post-training fMRI sessions (Figure S2B). The amount of slow wave sleep (SWS; sleep stages 3 and 4) in the pre- and post-training fMRI sessions was also calculated. There was a slight tendency for increased SWS during the post-training fMRI session (Figure S2C), although the difference was not statistically significant (Wilcoxon signed-rank test, $p=0.237$).

2. Brain activity in response to checkerboard patterns during wakefulness after training

To test whether the trained and untrained regions of V1 were equally activated when subjects were awake, BOLD signals were compared in the trained and untrained regions in V1 while checkerboard patterns were presented to BOLD signals while a fixation point was presented, as described in the second calibration task, both in the pre-training and post-training fMRI sessions (Figure S3). A Wilcoxon singed-rank test did not show differences between the amount of activation in the trained and untrained regions of V1 in either fMRI session. The amount of activation in the trained (Wilcoxon singed-rank test, $p=0.06$) and untrained (Wilcoxon singed-rank test, $p=0.06$) regions of V1 in the post-training fMRI session tended to be smaller than in the pre-training fMRI session, respectively. Note that brain response to the checkerboard averaged across the trained and untrained regions of V1 in the pre-training fMRI session was almost double that in the post-training fMRI session. However, this general trend did not reach statistical significance: When the trained and untrained regions are collapsed, there is a tendency for the response to the checkerboard in the pre-training fMRI session to be larger than that during the post-training fMRI session (Wilcoxon singed-rank test, $p=0.06$). The smaller response in the trained and untrained regions of V1 in the post-training fMRI session may reflect non-location specific overall fatigue/adaptation due to training.

3. Correlations between various parameters including the sleep activity, performance, and SWS

Correlations between the V1 activation and performance improvement were calculated. Performance improvements are highly and significantly correlated with activation in the trained region of V1 but not with that in the untrained region of V1. Figure S5 shows scatter plots for the correlation between sleep activation of the

trained region of V1 and reduction of threshold SOA (Figure S5A, r=0.82, p=0.02), between sleep activation of the trained region of V1 and increase in correct response rate (Figure S5B, r=0.83, p=0.02), between sleep activation of the untrained region of V1 and reduction of threshold SOA (Figure S5C, r=0.42, p=0.33) and between sleep activation of the untrained region of V1 and increase in correct response rate (Figure S5D, r=0.20, p=0.45).

Further whether the amount of SWS and sleep activity in the trained region of V1 were correlated and whether amount of SWS and performance were correlated were also tested. However, no strong evidence was found to support a significant correlation between SWS and sleep activity of the trained region of V1 or for SWS and performance improvement. The correlation coefficient between the SWS% and sleep activity in the trained region of V1 was 0.20 (p=0.46). Correlation coefficients of 0.41 (p=0.36) between amount of SWS and increase in correct response rate and 0.37 (p=0.42) between amount of SWS and reduction of threshold SOA were obtained.

The measures for increase in correct response rate (%), reduction of SOA (ms), and SWS% were arcsine-transformed. Bartlett's test [33] was used to check for homogeneity of variances. The results indicated the presence of homogeneity of variances (p=0.315).

4. Sleep activity map

The purpose of this paper was to test whether activation of the trained quadrant of V1 is modulated during sleep (i.e., whether sleep is involved in performance improvement in the trained visual field). To this end, the trained quadrant of V1 was counterbalanced across subjects and a pre-determined ROI

analysis was conducted. However, activation maps using the inflated and flattened formats of cortex were generated to overview other areas, since overviewing entire scanned regions is also informative to understand the nature of brain activation involved in consolidation of a visual task during sleep.

Due to counterbalancing of the trained quadrants of the visual field, in some subjects the cortical region retinotopically corresponded to the trained visual field was in the right hemisphere while in others it was in the left hemisphere. Thus, subjects' activation was averaged by trained visual field quadrant. Since the significant activation in V1 corresponded to the trained visual field quadrant, averaging all subjects would cancel out significant activation in V1. The averaged activation map by trained visual field quadrant showed dominant V1 activation in the cortical quadrant that corresponded to the trained visual field quadrant, irrespective of which visual field quadrant was trained.

Figure S6 shows the sleep activation maps ($p<0.01$) of the subjects, whose trained quadrant of the visual field was the left upper visual field quadrant for pre- and post-training sleep (n=4). SFigs. 6A (inflated views of whole brain) and 6B (flattened views including the occipital, temporal and parietal areas) show sleep activation in during pre-training sleep. SFigs. 6C and 6D show sleep activation during post-training sleep. It is shown clearly that dominant sleep activation is located in V1, which corresponds to the trained visual field quadrant, that is, the ventral part of V1 in the right hemisphere (Figure S6D, yellow circle). This pattern of activation is unlikely to be generated due to misalignment between the localized quadrants and the stimulus location, for activation caused by a misalignment (hence, noise) would have taken place along the border of quadrants.

In addition to activation of the trained region of V1, the left dorsolateral prefrontal cortex seemed to be activated. When an ROI analysis was conducted for the left dorsolateral prefrontal cortex from all of the subjects, sleep activation in the left dorsolateral prefrontal cortex was significantly higher during post-training than pre-training sleep (Wilcoxon signed-rank test, $p<0.018$). However, this statistical value would be too generous for an exploratory analysis. If there was not an *apriori* anatomical hypothesis and we were to explore the brain regions that are most responsive to sleep consolidation, a more stringent statistical criteria would have to be applied due to the multiple comparisons. Most of the significant activation shown in Figure S6 would disappear if more stringent statistical criteria were applied.

5. Control experiment I (TDT with no sleep between the initial and retest session)

The results of control experiment I showed that neither the correct response in the last 2 blocks of the re-test session (diamonds in Fig. 3A in the main text) or the mean threshold time interval for 80% performance of the re-test session (white bar in Fig. 3B in the main text) was significantly different from corresponding blocks of the training session in the control group. These results rule out the possibility that the observed performance improvement during the main sleep experiment was due to the mere passage of time from initial training. Rather, the results are in accord with the hypothesis that performance improvement found in the main sleep experiment was due to sleep that occurred during the 90-min period after training that was recorded via fMRI/PSG.

Note that the subjects were deprived of early night sleep prior to the re-test. Thus, the possibility that the control group subjects were sleepier at the re-test than

the sleep group subjects cannot be entirely ruled out, which could explain the difference. In addition, the results of this experiment do not directly address the question concerning of whether the relative increase in V1 activity in the trained region was sleep dependent or simply time dependent. However, the lack of significant activity difference between the trained and untrained regions of V1 to the checkerboard patterns immediately before the subject fell asleep in the post-training sleep session of the main experiment suggests that the V1 activity in the trained region was sleep dependent.

6. Control experiment II (TDT with a longer re-test session)

On average, threshold SOA was decreased in the re-test session by 24.2 ± 12.2 (SD) ms as compared to threshold SOA in the training session. Threshold SOA for the re-test session was significantly shorter than for the training session (Wilcoxon signed-rank test, $p<0.03$; Figure S4). Thus, we conclude that the increased performance found in the re-test session in the main experiment was likely due to increased performance per se, not a decreased adaptation effect resulting from the smaller number of trials.

SUPPLEMENTAL DISCUSSION

It has been suggested that excessive visual task practice leads to performance decrease rather than increase. This effect is called adaptation [30-32] or deterioration [29, 34, 35]. Adaptation within a training session tends to be stronger with an increased (larger) number of trials during a session [30, 31]. Note that in the first (main) experiment, the re-test session was significantly shorter than during the training session, in order to avoid eliciting learning during the former. Thus, it could

be said that the decreased threshold in the re-test session as compared with that in the training session was attributable to the reduced amount of adaptation rather than to a learning effect. However, we conclude that the decreased threshold is due to learning, not to adaptation/deterioration, for the following two reasons. First, we used an adaptive method [7, 30] in which the starting value of the SOA in a new session is adjusted just above the threshold SOA in the previous session. It has been reported that an adaptive method does not cause much adaptation in the TDT [30] and we confirmed that (see the procedure for Control Exp. II, Supplemental Result 6, and Figure S4). Second, it has been shown that performance deterioration is trained-region specific [29, 30] and accompanied by decreased BOLD signal in the trained region of V1 [35]. However, in our study, no such BOLD signal decrease was observed: The BOLD signal in the trained region of V1 in response to checkerboard patterns immediately before sleep onset but after intensive training did not decrease compared to the untrained region of V1 (Figure S3). Moreover, the BOLD signal in the trained region of V1 increased, compared to the untrained region of V1 during sleep in the post-training fMRI session. Collectively, this evidence strongly indicates that the observed performance increase in the re-test session relative to the training session is due to the resultant learning rather than adaptation or deterioration.

Table S1

Sleep stages at pre- and post-training fMRI sessions (mean and standard errors). In average, subjects slept 83.1% of the pre-training sleep period, and 80.0% of the post-training sleep period.

	Pre-training fMRI session	Post-training fMRI session
Wakefulness	16.8 ± 2.7%	20.0 ± 6.5%
NREM sleep	82.1 ± 3.7%	78.8 ± 6.4%
REM sleep	1.0 ± 1.0%	1.2 ± 0.9%
Unscored	0.2 ± 0.2%	0.0 ± 0.0%

SUPPLEMENTAL FIGURES AND LEGENDS

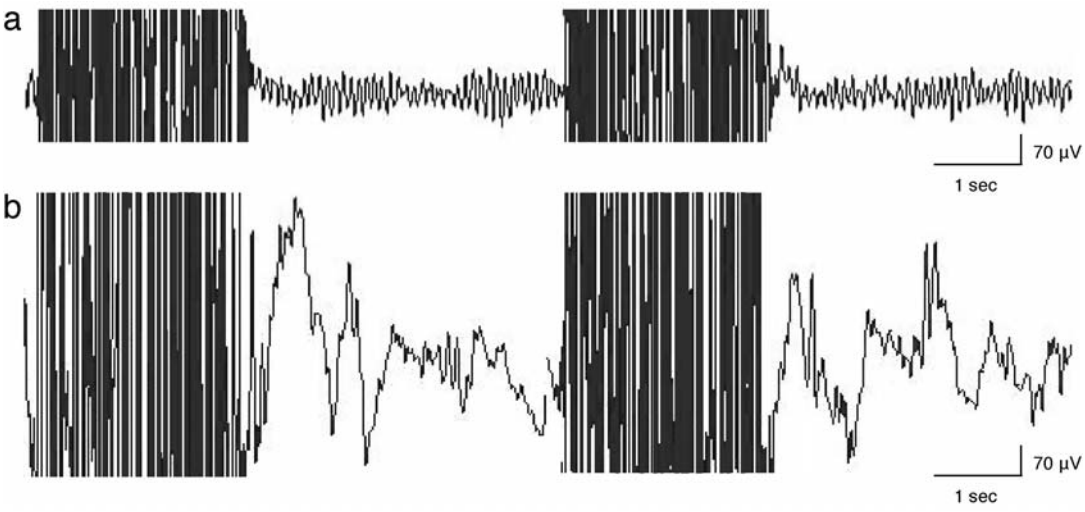

Figure S1. Examples of EEG measured concurrently with fMRI. Ballistocardiogram was removed while fMRI scan noise was maintained. (**a)** Alpha waves. (**b**) Slow waves.

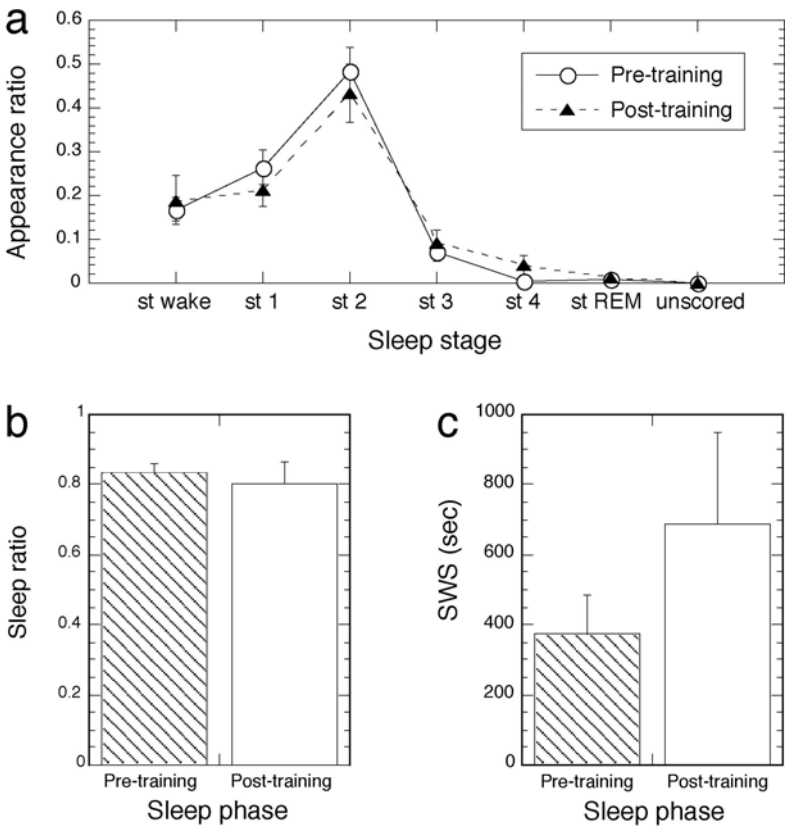

Figure S2. Comparison between sleep parameters during the pre- and post-training sleep sessions. (**a**) Mean ratio (±1 SEM) of the time for each sleep stage to the total amount of measurement time for the pre-training and post-training fMRI sessions. Sleep structures in both sessions are highly similar. (**b**) Mean ratio (±1 SEM) of sleep time to the total amount of measurement time. (**c**) Mean amount of time (±1 SEM) of slow wave sleep for the pre- and post-training sleep sessions.

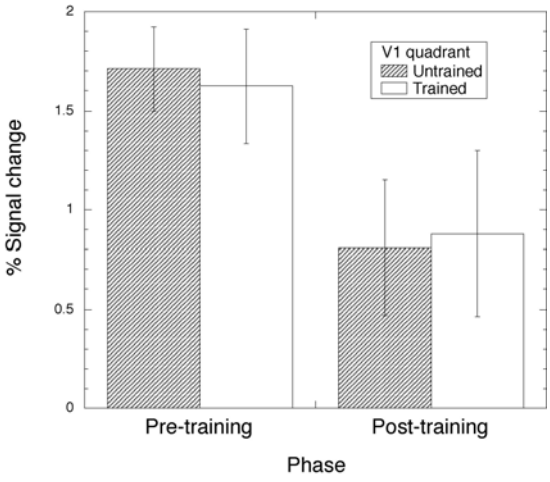

Figure S3. The mean (±1 SEM) amount of BOLD signal with a fixation point alone subtracted from that with checkerboard patterns with a fixation point in the trained and untrained regions of V1 during the pre-training and post-training sessions.

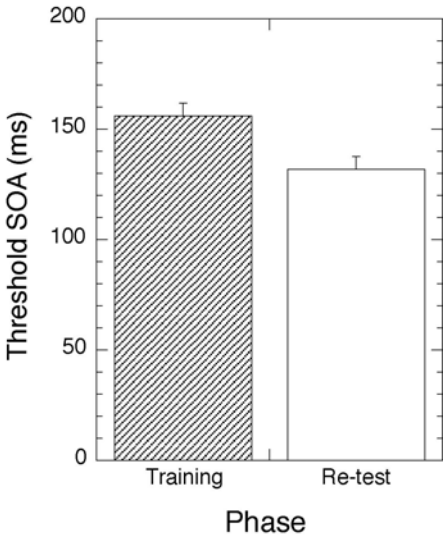

Figure S4. Threshold SOA (mean ± SEM) in the control experiment (see the procedure of control experiment II and Supplemental Result 6) in which the number of the re-test session was almost identical to that of the training session.

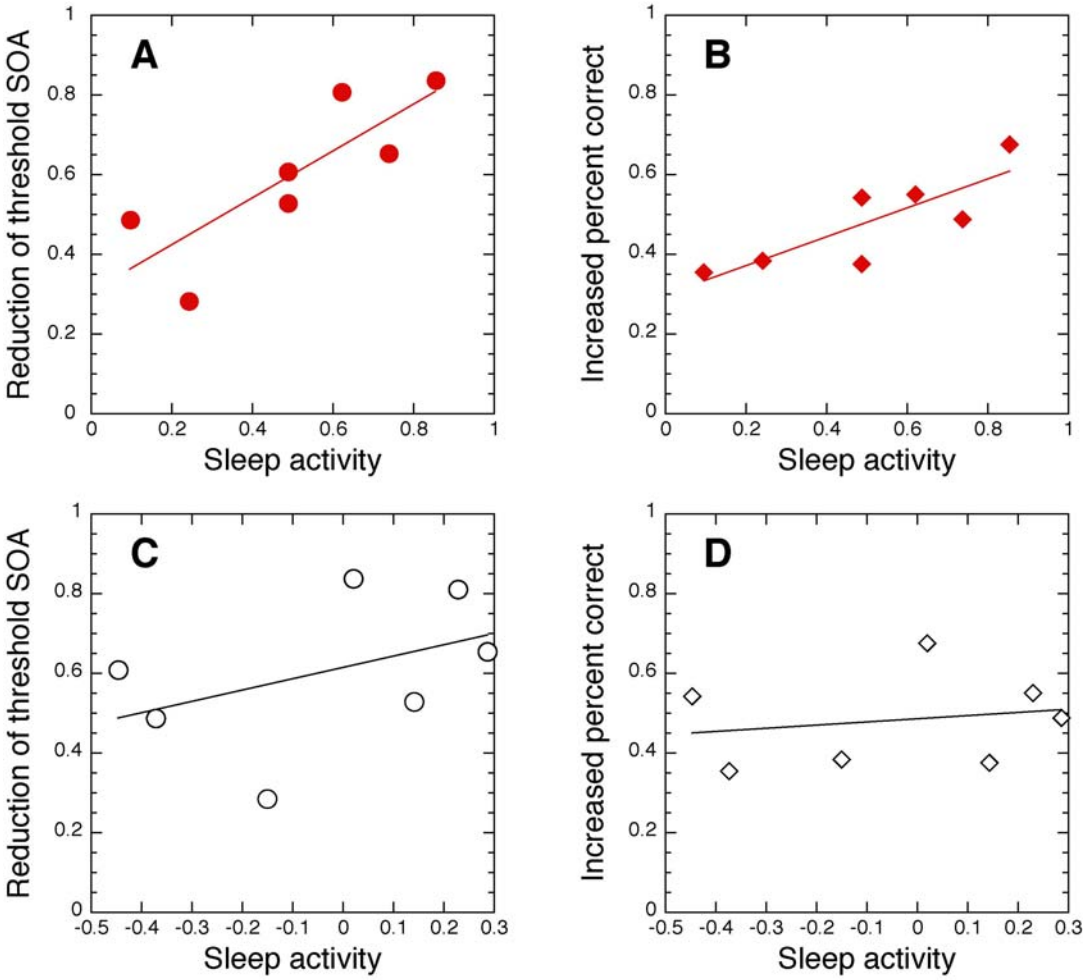

Figure S5. Scatter plots showing correlations between sleep activation in the trained/untrained regions of V1 during post-training sleep and performance improvement with best fit lines. Performance data (reduction of threshold SOA and increase in correct response rate) were arcsine-transformed. A: Sleep activation in the trained region of V1 and reduction of threshold SOA (r=0.82, p=0.02). B: Sleep activation in the trained region of V1 and increase in correct response rate (r=0.83, p=0.02). C: Sleep activation in the untrained region of V1 and reduction of threshold SOA (r=0.43, p=0.33). D: Sleep activation in the untrained region of V1 and increase in the correct response rate (r=0.20, p=0.45). Note that the data in the figures are not normalized by standard deviations.

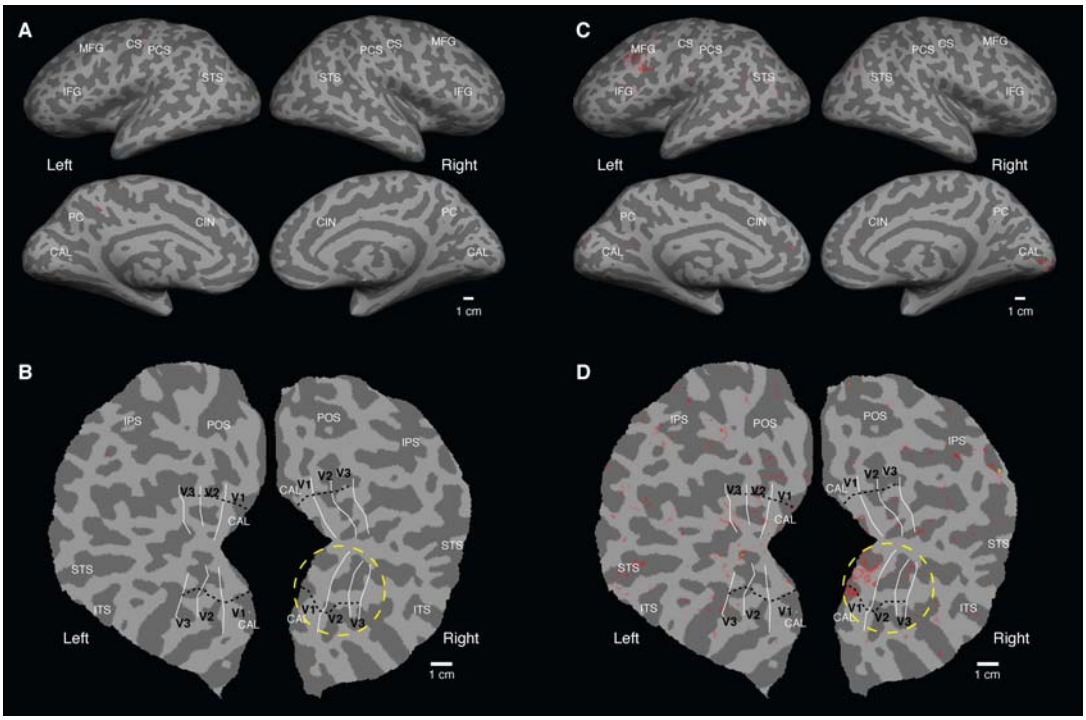

Figure S6. Pre- and post-training sleep activation comparisons in subjects with left upper visual field quadrant training (n=4). The averaged activation map was projected onto one subject's anatomical space. **A**: Lateral (upper rows) and medial (second rows) views of left and right inflated hemispheres during pre-training sleep. In the inflated brains, sulci are in darker gray and gyri are in lighter gray. Activation ($p<0.01$) is coded in red. Little activation was observed during pre-training sleep. A short horizontal white bar indicates 1 cm scale. MFG; middle frontal gyrus, IFG; inferior frontal gyrus, CS; central sulcus, PCS; post central sulcus, STS; superior temporal sulcus, CIN, cingulate cortex, PC; precuneus gyrus, CAL; calcarine sulcus. **B**: Sleep activation ($p<0.01$) during pre-training sleep is shown in inflated format of the posterior part of the brain for the left and right hemispheres. The activation maps in A and B are identical but the visualization format is different. Conceptually, a posterior part of an inflated brain (such as in A) is cut off and becomes a cone, which is flattened by scission along the calcarine sulcus (represented as CAL), and becomes a flattened cortex (such as in B). Borders between V1 and V2 and V2 and V3 are

shown by white lines. A yellow circle indicates the cortical region that corresponds to the to-be-trained visual field quadrant, that is, the left upper quadrant. The black dotted lines are the eccentricity of 5 deg, which corresponds to the size of the visual field where targets were presented. CAL; the calcarine sulcus, POS; the porieto-occipital sulcus, IPS; the intraparietal sulcus, STS; the superior temporal sulcus. **C**: Sleep activation ($p<0.01$) during post-training sleep is shown in the lateral and medial views of inflated brains. **D:** Sleep activation ($p<0.01$) during post-training sleep in the posterior part of inflated brains (C) is shown in the flattened format of cortex. Activation is primarily located in the trained region of V1 (inside the yellow circle).

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