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Report

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

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Summary

Visual perceptual learning is defined as performance enhancement on a sensory task and is distinguished from other types of learning and memory in that it is highly specific for location of the trained stimulus. The location specificity has been shown to be paralleled by enhancement in functional magnetic resonance imaging (fMRI) signal in the trained region of V1 [1–3] after visual training. Although recently the role of sleep in strengthening visual perceptual learning has attracted much attention, its underlying neural mechanism has yet to be clarified. Here, for the first time, fMRI measurement of human V1 activation was conducted concurrently with a polysomnogram *during* sleep with and without preceding training for visual perceptual learning. As a result of predetermined region-of-interest analysis of V1, activation enhancement during non-rapid-eye-movement sleep after training was observed specifically in the trained region of V1. Furthermore, improvement of task performance measured subsequently to the post-training sleep session was significantly correlated with the amount of the trained-region-specific fMRI activation in V1 during sleep. These results suggest that as far as V1 is concerned, only the trained region is involved in improving task performance after sleep.

Results

Recently, the results of a number of studies have suggested that sleep plays a role in improving performance of a texture-discrimination task (TDT) repeatedly performed before sleep [2, 4–8]. Using fMRI, previous studies compared the blood-oxygen-level-dependent (BOLD) signal while subjects were awake and performing TDT before and after sleep after being trained on the TDT and found that the BOLD signal in the region in the low-level visual cortex corresponding to the trained stimulus location was enhanced after sleep [2, 7].

In order to address the question regarding what processing occurs for improvement of visual perceptual learning during sleep, one must train subjects on a visual perceptual learning task and then measure brain activation *during* sleep by using fMRI, which provides sufficient localization ability. The present study constitutes the first attempt to do so. We also conducted concurrent polysomnogram (PSG) monitoring (Figure S1 in the Supplemental Data available online) to determine precisely the asleep or awake status of the subject during fMRI measurement. Primarily, we tested the hypothesis that the sleep consolidation process in visual perceptual learning occurs specifically in the trained region. Note that, with regards to V1, activation has been found to be modulated during training on the TDT (i.e., while the subjects are awake), but only in the trained location [1, 2, 7]. Thus, using ROI (region-of-interest) analysis, we specifically tested whether only the trained region of V1 or other regions of V1 as well as the trained region are activated during sleep after training. The ROI analysis is an important starting point for extending future research to involve whole-brain processing analysis [9].

Our main experiments involved pre-training and post-training fMRI sessions (Figure 1). BOLD signal in humans were measured mostly *during* sleep (although some wakefulness periods were included), before (pre-training) and after (post-training) training of the TDT. See Supplemental Data for a more detailed account of the procedure. PSG indicated that the subjects slept more than 80% of the time during both the pre- and post-training sleep sessions (see Supplemental Results section 1, Figure S2, and Table S1).

V1 Activity during Sleep and Performance Improvement after Sleep: ROI Analysis

It has been found that in V1, the enhanced activation is observed specifically in the trained location while subjects are conducting the task after learning the TDT [1, 2, 7]. To test the hypothesis that a consolidation process in perceptual learning occurs specifically in the trained region during sleep, we used ROI analysis that targeted V1. We identified a detailed retinotopic representation in the visual cortex, including V1, for each subject by using a retinotopic mapping technique [1, 10] to localize trained and untrained locations of low-level visual cortical areas. These areas were functionally demarcated for four quadrants of the visual field within 5° eccentricity (see Supplemental Data). We defined sleep activation as the BOLD signal measured while the PSG indicated that the subject was awake subtracted from the BOLD signal measured while the PSG indicated that the subject was in non-rapid-eye-movement (NREM) sleep during the pre-training and post-training fMRI sessions (see Supplemental Data for more detail). During the pre-training fMRI session, sleep activation levels were the same in the trained and untrained regions of V1. However, during the post-training fMRI session, the sleep activation in the trained region of V1

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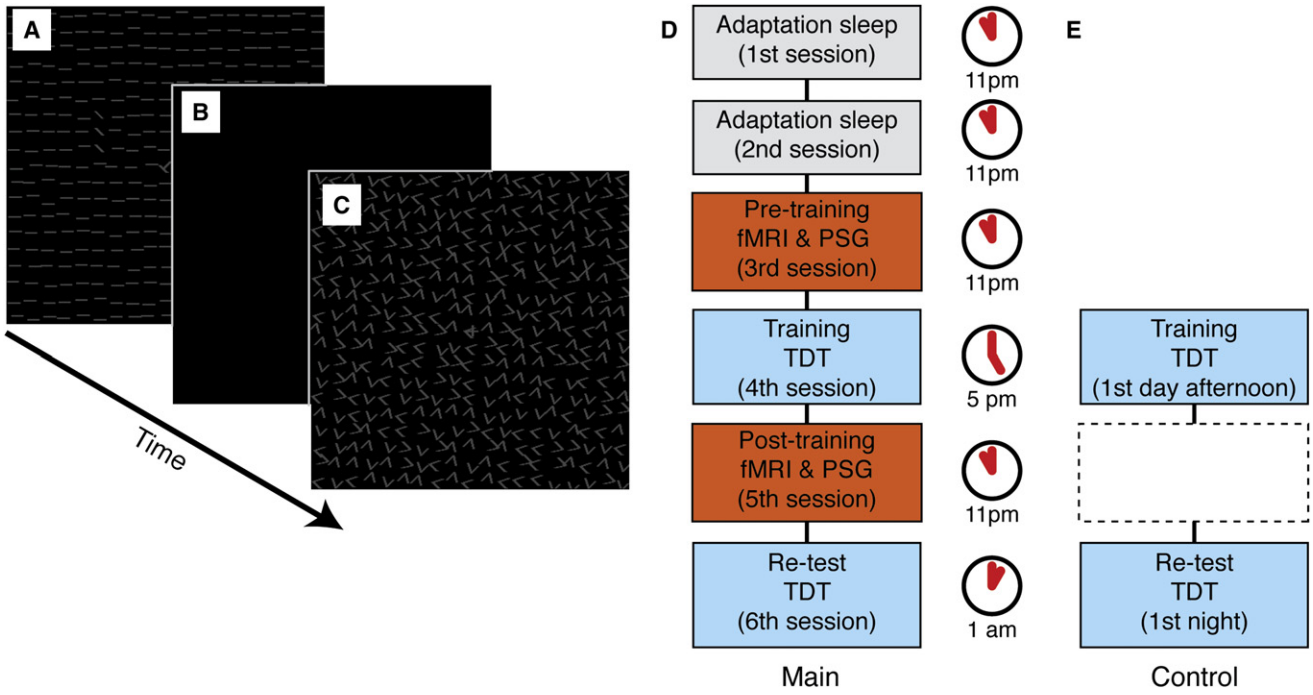

Figure 1. Experimental Procedure

See Supplemental Data for additional details.

(A–C) Texture discrimination task (TDT). During each trial, the subjects were presented with a target display (A) that contained a triplet of diagonally oriented bars within the upper-left quadrant (n = 4) or lower-right quadrant (n = 3) (counter-balanced across subjects) and a letter ("T" or "L") presented at the center fixation point of the display. The target and letter were presented against a background consisting of horizontally oriented bars. The target display was followed by a blank display (B) whose duration varied from trial to trial, and this was followed by a masking noise display (C). In each trial, subjects were asked to report whether the central letter was a "T" or "L" and then whether the orientation of the triplet was vertical or horizontal.

(D) Main experiment. The subjects were asked to spend the first night of adaptation sleep (first session) in a mock scanner that physically mimics an actual MRI scanner. The subjects were then asked to spend the second night of adaptation sleep (second session) in an actual MRI scanner while scanning was conducted. All electrodes required for the real measurements were attached during the adaptation periods. On the night of the third session, a pre-training fMRI session was conducted for 90 min, during which polysomnogram (PSG) was concurrently obtained with fMRI. On the late afternoon of the fourth day (fourth session), the subjects were trained on the TDT. Approximately 6 hr after the training session, a post-training fMRI session (fifth session) was conducted with the procedure identical to that of the pre-training fMRI session. After the post-training fMRI session, a retest of the TDT was conducted (sixth session).

(E) Control experiment. Here, only the training and retest sessions were conducted with a new group of subjects (see Supplemental Data). The onset times of these sessions were about the same as those of the training and retest sessions in the main experiment.

was significantly higher than in the untrained regions ($p < 0.018$, Wilcoxon signed-rank test). In addition, the amount of sleep activation in the untrained region of V1 subtracted from that in the trained region was significantly larger during the post- than during the pre-training fMRI sessions (Figure 2, Wilcoxon signed-rank test, $p < 0.018$).

In learning of a visuomotor task, it has been reported that the activation observed during the visuomotor training persisted covertly in the specific brain region during wakefulness after the training [11]. Thus, we tested whether the difference in activity between the trained and untrained regions of V1 was specific to the status of sleep in TDT perceptual learning. Immediately before sleep onset in both the pre-training and post-training fMRI sessions, we presented checkerboard patterns (see Supplemental Data) and measured the subjects' brain activation via fMRI. The responses to the checkerboard patterns (as compared to those to the fixation point) in the trained and untrained regions of V1 were not significantly different from each other during either the pre-training or the post-training fMRI session (see Supplemental Results section 2 and Figure S3). This result suggests that excitability or general responsiveness in the trained region of V1 was equivalent to that in the untrained region while the subjects were awake with the lights on, which occurred during the post-training fMRI session and that the difference that we found in the V1 activity in the trained and untrained regions in the post-training sleep fMRI session might be specific to the post-training sleep period (see Supplemental Discussion and Figure S4 for more detail regarding V1 activation associated with TDT).

The mean correct response during the last two blocks of the re-test was significantly higher than during the corresponding two blocks of the training session (red circles in Figure 3A, Wilcoxon signed-rank test, $p < 0.018$). In addition, we obtained the threshold time interval between the onset of the test stimulus and the onset of the masking noise pattern, that is, stimulus-to-mask onset asynchrony, SOA, which is another measure of learning: the shorter the interval, the more difficult the task becomes (see [12]). The threshold time interval for 80% correct responses was significantly shorter during the re-test session than during the training session (black bar in Figure 3B, Wilcoxon signed-rank test, $p < 0.03$). These results indicate that performance improved after sleep subsequent to training.

Importantly, sleep activation in the trained region of V1 was highly correlated with the level of performance improvement ($r = 0.82$ for threshold SOA reduction, $r = 0.83$ for correct response increase; see Supplemental Results section 3 and

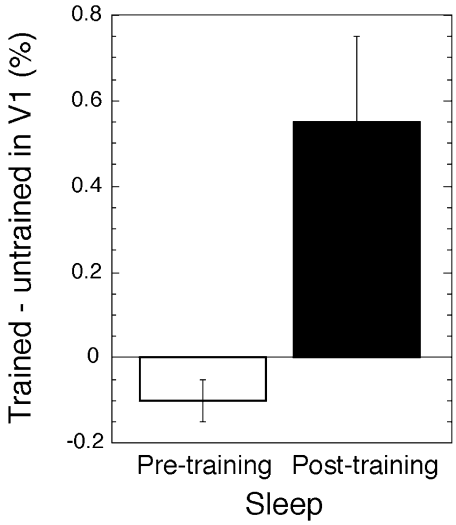

Figure 2. Sleep Activation

Mean subtraction (±1 SEM) of sleep activation in the untrained region from that in the trained region of V1 for the pre- and post-training fMRI sessions.

Figure S5). However, sleep activation in the untrained region of V1 was not significantly correlated with the level of performance improvement. These results indicate that this trained-region-specific activation of V1 during sleep after training is highly involved in the performance improvement observed after sleep and reflects processing specifically for improving learning rather than residual activation after training while subjects were awake. Taken together, the results indicate that a highly localized activation specific to the trained region of V1 is involved in improving PL during sleep.

Discussion

The present study is the first to use concurrent fMRI and PSG recordings to measure brain activation during sleep with and without preceding training of a visual task. PSG allows for precise identification of on-going sleep status. The results revealed significantly increased activation specifically in the trained location of V1 during NREM sleep after subjects were trained on a visual task. This increased activation in the trained region was specific to the sleep status. Notably, the amount of activation in the trained location of V1 during sleep in the post-training fMRI session was highly correlated with performance improvement after sleep. These results indicate that activity enhancement specifically in the trained location of V1 during sleep reflects processing that improves visual perceptual learning, and they are in accord with the hypothesis that consolidation of learning occurs during sleep after training.

Two models have been proposed to account for performance enhancement after sleep subsequent to training for learning in general: the synaptic homeostasis model [13] and the reactivation model [14–16]. The reactivation model proposes that neurons that are involved in learning acquisition are covertly reactivated during sleep to strengthen neuronal connections [14–16]. For example, firing-rate patterns during training of episodic memory in cortical areas including the hippocampus and the medial prefrontal cortex in rats [17, 18]. In addition, it has been shown that the brain regions that were recruited during the training showed enhanced brain activation during sleep in human PET studies [19, 20]. The reactivation model predicts activation in the area highly related to trained memory and learning during sleep and is in accord with the present findings of BOLD signal enhancement in the trained area of V1 during sleep and performance enhancement after the sleep. The synaptic homeostasis model [13] indicates that slow-wave activity, which is prominent during early NREM sleep, plays a role in scaling down synapses, including those that are excessively increased or strengthened by a learning-acquisition process during wakefulness. This model is supported by increased slow-wave activity near the motor and parietal areas in the right hemisphere during sleep after implicit motor learning [21]. Although this model is highly intriguing, from our results, which are not based on any spectral analysis, it is difficult to judge the validity of the homeostasis model. If a downscaling requires an active molecular process (cf. [22, 23])

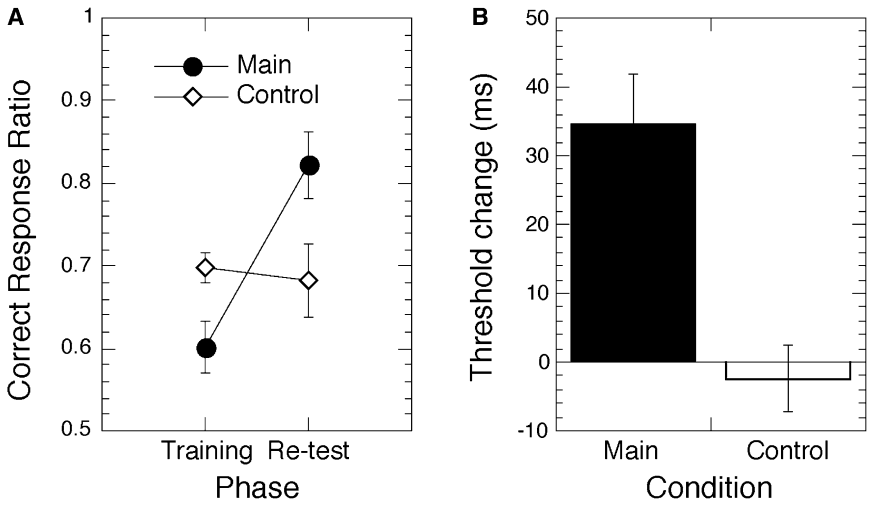

Figure 3. Mean Performance Changes during the Main and Control Experiments

(A) Mean correct response changes (±1 SEM) for the corresponding two blocks (SOAs) for each subject in the training and re-test sessions (with the same SOAs) in the main experiment (with sleep) and the control experiment (without sleep, Figure 1E; see Supplemental Experimental Procedures for a detailed explanation of the procedure for this control group as well as Supplemental Results section 5). In the main experiment, the correct response in the retest session was significantly higher than in the training session, whereas in the control experiment, the correct responses in the training and retest sessions were not significantly different.

(B) A threshold SOA change (mean ± 1 SEM) is defined as subtraction of the threshold SOA in the retest session from that in the training session. Thus, a positive change in threshold SOA indicates that the threshold SOA became shorter and that performance improved in the retest session. Significant performance improvement was found in the main condition, whereas no significant improvement was found in the control condition.

resulting in increased metabolism, the present result would be in accordance with the synaptic homeostasis model.

In the current study, we had an a priori anatomical hypothesis that in V1 the sleep consolidation process in visual perceptual learning occurs specifically in the trained region. To test the hypothesis, we made a predetermined ROI analysis that targeted V1 and obtained results supporting the hypothesis. Although the result was in accord with our hypothesis, note that this does not indicate that other areas are not involved in consolidation of sleep. Some suggest that post-sleep changes on this task have been identified in the later visual cortical regions, including within the occipital, temporal, and parietal areas [7]. In addition, the dramatic connectivity changes during NREM sleep [9, 24] suggest that a learned representation during wakefulness might lead to plasticity processes not only in the trained location but also in reciprocal areas connected to it. For example, Schwartz et al. indicate that connectivity observed between other areas, including the left frontal cortex, and V1 at the beginning of training disappeared 24 hr after training of TDT in visual perceptual learning [2]. Thus, examining connectivity during sleep after training would constitute an important future study. Because the location of the target presentation was counterbalanced across the subjects in the present experiment, this design is not suitable for analysis of multiple brain areas by averaging all subjects. However, sleep activation in the whole cortex is shown in Figure S6. This shows that enhanced activity occurring in the trained region of V1 was found to be significant when pre-determined ROI analysis was applied as aforementioned. Although less clearly, some activity in the left dorsolateral prefrontal area was also observed (see Supplemental Results section 4 for the limitation of this analysis to our data). A future study with an experimental design suitable for analysis of multiple areas would clarify whether activation of the left dorsolateral prefrontal area is significantly involved in consolidation during sleep.

In the present study, we did not investigate brain activation during REM sleep. Although we found trained-region-specific brain activation in V1 associated with consolidation of visual perceptual learning during NREM sleep, it is possible that REM sleep also plays a role in the consolidation of visual perceptual learning [14, 25, 26]. Previous studies have indicated the involvement of REM sleep in consolidation of the visual task used in the present study [4, 6]. PET studies in humans have also shown involvement of REM sleep: activation in the brain regions that were recruited for the visuomotor training before the sleep was enhanced [19, 27], and the connectivity between the frontal and parietal regions changed [28] during REM sleep. Thus, it is possible that the trained-region-specific activation we found in V1 during NREM sleep is just a part of multiple stages of consolidation processing during sleep, which usually lasts several hours in adults [9]. Testing this possibility will require future studies.

In the present study, we measured and compared the BOLD signal during sleep with and without preceding visual training and concurrently used PSG to measure subjects' wakefulness. For the first time, we observed that a significant amount of activation occurred specifically in the trained region of V1 during sleep after training of a visual task and was highly correlated with performance increase after sleep. In this initial study, we utilized ROI analysis and concentrated on examining activation in V1. Future studies should clarify whether brain areas other than V1 are also involved and how cortical connectivity of the trained area of V1 to other areas might change during sleep after training, as well as how REM sleep is involved in sleep consolidation.

Supplemental Data

Supplemental Data include detailed Experimental Procedures, additional Results, six figures, and one table and can be found online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01249-4.

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