

The Functional Anatomy of Sleep-dependent Visual Skill Learning

Matthew P. Walker¹, Robert Stickgold², Ferenc A. Jolesz³ and Seung-Schik Yoo³

¹Sleep and Neuroimaging Laboratory, Center for Sleep and Cognition, Department of Psychiatry, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA, ²Center for Sleep and Cognition, Department of Psychiatry, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA and ³Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

Learning of procedural skills develops gradually, with performance improving significantly with practice. But improvement on some tasks, including a visual texture discrimination task, continues in the absence of further practice, expressly during periods of sleep and not across equivalent waking episodes. Here we report that the brain activation revealed significantly different patterns of performance-related functional activity following a night of sleep relative to 1 h post-training without intervening sleep. When task activation patterns after a night of sleep were compared with activation patterns without intervening sleep (1 h post-training), significant regions of increased signal intensity were observed in the primary visual cortex, the occipital temporal junction, the medial temporal lobe and the inferior parietal lobe. In contrast, a region of decreased signal intensity was found in the right temporal pole. Corroborating these condition differences, correlations between behavioural performance and brain activation revealed significantly different patterns of performance-related functional activity following a night of sleep relative to those without intervening sleep. Together, these data provide evidence of overnight bi-directional changes in functional anatomy, differences that may form the neural basis of sleep-dependent learning expressed on this task.

Keywords: memory, plasticity, sleep, visual skill learning

Introduction

The process of learning procedural skills is a gradual one. While practice has long been known to trigger improvement during training, recent studies demonstrate that performance on certain tasks continues to improve in the absence of any further practice exclusively during sleep, and not across equivalent periods of time awake (for reviews, see Smith, 1995; Maquet, 2001; Stickgold *et al.*, 2001; Walker, 2004).

Evidence of sleep-dependent learning has now been established across a wide range of visual (Karni *et al.*, 1994; Gais *et al.*, 2000; Stickgold *et al.*, 2000a,b), auditory (Atienza *et al.*, 2004; Gaab *et al.*, 2004) and motor skills (Smith and MacNeill, 1994; Walker *et al.*, 2002, 2003a,b; Fischer *et al.*, 2002; Korman *et al.*, 2003; Robertson *et al.*, 2004). Within the visual domain, several behavioural studies using a visual texture-discrimination task (TDT) have identified a delayed, sleep-dependent learning component which develops following initial training. Karni *et al.* (1994) have established that learning of the TDT, which does not benefit from 4–12 h of wake following acquisition (Stickgold *et al.*, 2000b), improves significantly following a night of sleep. Furthermore, they demonstrated that selective disruption of rapid eye movement (REM) sleep, but not non-REM (NREM) sleep, results in a loss of these performance gains (Karni *et al.*, 1994). Gais *et al.* (2000) have selectively deprived subjects of early sleep (normally dominated by slow-wave sleep;

SWS) or late-night sleep (normally dominated by REM and stage-2 NREM) following learning of the TDT, concluding that consolidation of this task is initiated by SWS-related processes, while REM sleep then promotes additional enhancement.

Using the same task, Stickgold *et al.* (2000b) have shown that these enhancements are specifically sleep-dependent, with the overnight improvements correlating positively with the amount of both early night SWS and late night REM sleep. It was also demonstrated that these delayed performance benefits are absolutely dependent on the first night of sleep following training (Stickgold *et al.*, 2000a). In addition, they have also shown that short daytime sleep episodes can restore performance decrements caused by repeated practice on this task (Mednick *et al.*, 2002), and that 60–90 min sleep epochs, containing both REM and SWS, can produce performance enhancements equivalent to a normal night of sleep (Mednick *et al.*, 2003). Yet while the sleep-dependent nature of this TDT has been extensively investigated, the underlying neural correlates of sleep-dependent learning remain largely unexplored.

Using neuroimaging techniques, Maquet *et al.* (2003) have specifically investigated the effects of sleep deprivation on memory consolidation and functional brain anatomy using a visuo-motor task. Subjects were trained on the task and then retested during an fMRI scan 72 h later. Half of the subjects underwent total sleep-deprivation across the first night after training, followed by two recovery nights of sleep, while the remaining subjects slept all three nights. At the later 72 h retest, subjects in the sleep deprivation condition demonstrated a significantly altered pattern of brain activation relative to control subjects. Moreover, these differences in functional brain activation were also accompanied by significant learning impairments in the sleep-deprived group. Nevertheless, no study has yet investigated the beneficial effect of a night of sleep on skill learning and brain plasticity without employing sleep deprivation techniques.

In a seminal study, Schwartz *et al.* (2002) have recently examined changes in brain activity using the TDT, comparing task-dependent activation without training ('untrained'), relative to performance following training ('trained') at a later 24 h retest, which included a night of sleep. When the two conditions were compared, significantly greater signal intensity was evident in the primary visual cortex in the trained condition relative to the untrained condition, indicative of a low-level plastic change associated with task learning. However, these findings could not determine whether such changes occurred during training, across post-training wake, or across a subsequent night of sleep.

Regarding the brain basis of visual perceptual discrimination, the highly specific characteristics of this form of learning (Sagi

and Tanne, 1994; Karni and Bertini, 1997) have led to the hypothesis that orientation discrimination should be reflected in neural changes early in the visual cortex (Karni and Sagi, 1991; Karni, 1996; Gilbert *et al.*, 2001). While some experimental evidence has confirmed these predictions (Gilbert *et al.*, 2001), other reports have not been successful in identifying substantial differences in either the selectivity or responsivity of neurons in V1 or V2 (Crist *et al.*, 2001; Schoups *et al.*, 2001; Ghose *et al.*, 2002). While the reasons for this discrepancy remain unclear, it has led to the possibility that visual perceptual learning may be mediated by changes downstream of these early visual processing regions. For example, a recent study by Yang and Maunsell (2004) has demonstrated that orientation discrimination is associated with plasticity in later stages of the visual cortex, specifically area V4, indicating that perceptual learning can cause overt changes in the tuning of neurons outside of V1. Therefore, visual perceptual learning, during both training and potentially following sleep, may be related to distinct neural changes throughout several visual processing regions.

Using the sleep-dependent TDT, we now report a functional magnetic resonance imaging (fMRI) study demonstrating changes in human regional brain activity exclusively across a night of sleep, relative to a condition without intervening sleep but following equivalent amounts of training. Learning of this task is both retinotopically and monocularly specific (Karni and Sagi, 1991). That is to say, learning to discriminate these texture targets is specific to the portion of the visual field in which subjects are trained, and the learning achieved with the trained eye does not transfer to the untrained eye. Based on the visual specificity of this TDT, we were therefore able to investigate, in the same subjects, during the same scanning session, at the same circadian time and after equivalent amounts of training, how functional brain activity and learning seen during retesting differed following a night of sleep, compared with retesting without intervening sleep.

Based on previous neurophysiological and functional imaging studies of perceptual learning, we hypothesized that intervening sleep, and the associated learning benefit, would result in significantly greater signal intensity within the corresponding primary visual cortex. Moreover, considering that orientation discrimination learning may also trigger corresponding neural changes downstream of the primary visual cortex, we also expected additional regions of increased activation in later visual processing areas following a night of sleep.

Materials and Methods

The study was approved by the local human studies committee and participants provided written informed consent.

Participants

Subjects ($n = 18$; 10 females, 8 males, mean age \pm SD = 25.7 \pm 2.9) had no prior history of drug or alcohol abuse, neurological, psychiatric or sleep disorders, and agreed to be drug, alcohol and caffeine free for 24 h prior to and during the study period. All subjects maintained a standard sleep schedule for 1 week prior to the study. On the intervening night of sleep during the experimental phase, subjects obtained an average 8.3 \pm 0.78 h sleep, as measured by sleep-log diaries.

Experimental Protocol

All training and retesting on the TDT was performed monocularly. Subjects were trained monocularly on one eye at 9 P.m. on day 1. The following morning, at 9 a.m., subjects were trained monocularly using the other eye. One hour later, subjects were then retested monocularly using each eye separately, during the fMRI scan (Fig. 1*a*). Retesting of

the eye with sleep and without sleep was counterbalanced with regard to first and second retests. Assignment of the condition of sleep or without sleep to either the left or right eye, and the dominant or non-dominant eye, was also counterbalanced across subjects. In both the training sessions and at the subsequent fMRI retest sessions, target stimuli were presented in the lower right quadrant of the visual field.

Thus, each eye received identical amounts of training, but differed at retest based on the presence or absence of intervening sleep between training and the fMRI retest session (assigned as the 'SLEEP' or 'WITHOUT-SLEEP' conditions respectively; Fig. 1*a*).

TDT Task

The TDT was composed of a series of trials, organized into successive blocks. Each 3 s trial consisted of 500 ms of fixation followed by a target screen presented for 17 ms (Fig. 1*b*). Each target screen containing either a 'T' or 'L' at the fixation point and a horizontal or vertical array of three diagonal bars in the lower-right quadrant of the visual field. The background was always a field of horizontal bars. After a blank interstimulus interval (ISI) screen of varying length (80–500 ms), a mask was presented for 17 ms followed by a blank screen (1966–2386 ms) producing a constant total trial length of 3 s. During the final blank screen, subjects indicated with a button press which letter was presented at the fixation point, and then whether the three diagonal line segments in the lower-right quadrant were arranged in a horizontal or vertical array. The central fixation cross was then redisplayed as the next trial began. Monocularity was achieved using a custom cotton eye patch worn during training, taped to the subject's forehead, completely occluding vision from the patched eye.

Training

Monocular training consisted of 10 blocks, with decreasing ISIs (increasing difficulty) of 500, 400, 300, 250, 200, 160, 140, 120, 100 and 80 ms. Each block began with 24 s of central fixation, followed by five sets of eight TDT trials (24 s per set) separated by 24 s period of central fixation, and ending with a final 24 s of fixation. During training, subjects sat at 35–40 cm from a 15" CRT screen. The target diagonal array was 2.58° in width and was located 58° from the fixation point.

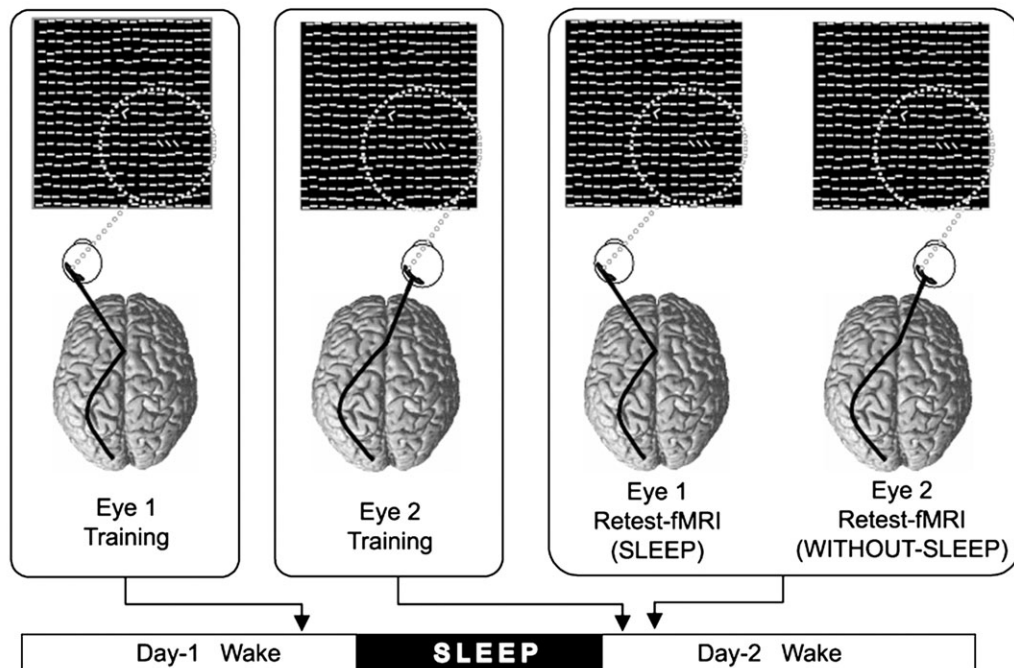
fMRI Retest

Monocular retesting of each eye during the fMRI session involved a single block (40 trials as described above) of the TDT at an ISI of 250 ms. This ISI was identified in pilot testing as providing a manageable difficulty level across conditions, while still remaining challenging enough to identify subtle differences. During retesting inside the MRI scanner, subjects monocularly viewed the stimuli using a mirror box inside the scanner. Stimuli (448 square resolution) were projected by an SVGA resolution LCD projector on a central screen located 270 cm from the subject, at a projected image size of 82 cm, resulting in a viewing angle of 17°. Monocularity during the MRI retest was again achieved using the same custom cotton eye patch as worn during training, taped to the subjects forehead above the eye. Between each scan session, an experimenter gently flipped up one eye patch and placed down the other eye patch (from behind the subjects head in the magnet bore), to occlude the opposite eye, preventing the need for subject head movement or change of position. The shift in the center-of-view due to monocularity was small, <1°, and was also compensated by adding the crosshair for the visual fixation, preventing any effect on hemispheric stimulation by shifting eye patch.

Measurement of Skill Level

Behavioural performance during both training and retesting was measured as the discrimination accuracy within each ISI block. For the training sessions, target discrimination accuracy was plotted against ISI, and the detection threshold defined as the interpolated ISI which produced 80% accuracy. The threshold value (ms) thus reflects the time required for cortical discrimination of the visual target from its background of horizontal bars. For the fMRI retest sessions, containing one block of trials per eye, a single accuracy percentage was calculated. The number of correct discriminations made during the single-block retest of each eye in the scanner similarly reflects cortical processing efficiency at the set ISI.

A PROTOCOL



B TRIAL

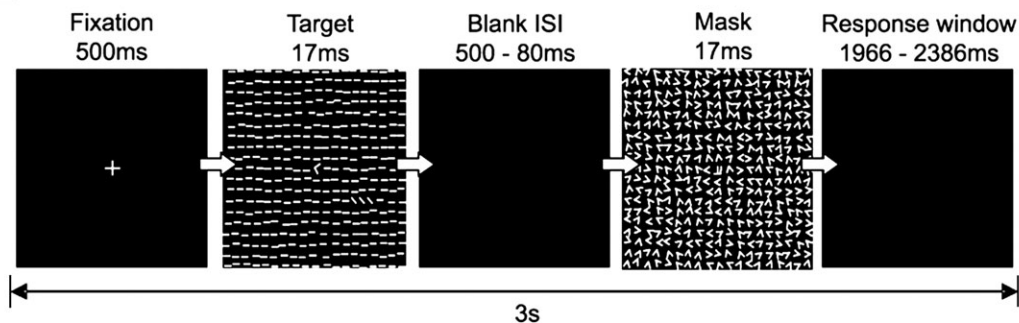


Figure 1. Texture discrimination task and experimental protocol. (a) Subjects training monocularly in the lower right visual quadrant on day 1 (9 p.m.). After an intervening night of sleep, subjects trained monocularly in the same visual quadrant using the other eye (9 a.m.). Training of the left eye versus right eyes was counterbalanced across subjects and conditions, as was eye dominance. Each eye was then retested monocularly on one single block (ISI = 250 ms) in the visual quadrant during fMRI scanning. Retesting as either the first or second scanning session was also counterbalanced across each eye condition. (b) Each 3 s trial consisted of a series of stimulus screens, including a target stimulus containing either a 'T' or 'L' letter at the fixation point, and a horizontal or vertical array of three diagonal bars in the lower-right quadrant of the visual field. During the blank response screen, subjects indicated with a button press which letter was presented at the fixation point, and then whether the three diagonal line segments in the lower-right quadrant were arranged in a horizontal or vertical array. Monocular training consisted of 10 blocks containing 40 trials, with decreasing ISIs: 500, 400, 300, 250, 200, 160, 140, 120, 100 and 80 ms.

MRI Scanning

MRI data were acquired with a 1.5 T GE Signa system (GE Medical Systems, WI). Structural anatomical images were acquired using a 3D-Spoiled Gradient Recalled (SPGR) sequence, covering the whole brain volume with 1.5 mm sagittal slices ($T_i/T_r = 6/35$ ms, flip angle = 75° , field of view = 24 cm, matrix = 256×256). Functional MRI images were acquired using a gradient echo-planar T2*-sequence sensitive to the blood-oxygenation level-dependent (BOLD) contrast. Functional image volumes consisted of 28 oblique-axial slices (thickness = 5 mm, matrix = 64×64 , $T_r/T_i = 2500/50$ ms, flip angle = 90°) covering the whole brain volume.

fMRI Data Analysis

fMRI data were analyzed using the SPM99 software package (www.fil.ion.ucl.ac.uk/spm/spm99.html). Each set of axial images for each subject were realigned to the first functional image (following removal of the first four initial functional images used to achieve steady-state equilibrium), co-registered with the corresponding T1-weighted data

set, spatially normalized to the SPM99 template, and smoothed with an isotropic Gaussian kernel (6 mm full-width at half-maximum).

Condition and subject effects were estimated using a general linear model (Friston *et al.*, 1995). The effects of global differences in scan intensity were removed by scaling each scan in proportion to its global intensity, and low-frequency drifts were removed using a temporal high-pass filter with the default cutoff of 110 s.

Individual contrast images were first produced by comparing task-dependent activation in the SLEEP condition relative to the WITHOUT-SLEEP condition for each subject separately (therefore controlling for within-subject variance). This condition contrast was then entered into a second level, group analysis using a random-effects model. A one-sample *t*-test was performed across the set of individual contrast images to identify brain areas in which cerebral activity differed between the SLEEP and WITHOUT-SLEEP conditions in each direction (SLEEP > WITHOUT-SLEEP and SLEEP < WITHOUT-SLEEP), controlling between-subject variances. To control for type I error through multiple comparisons, regions of significant difference were identified using

a corrected joint expected probability distribution of extent ($P < 0.05$) and height ($P < 0.001$).

In addition to the group subtractions, within each condition we explored the relationship between behavioural performance (percentage correct responses for 'horizontal' and 'vertical' discrimination; accuracy) and brain activation across subjects using a correlation analysis. The behavioural retest accuracy score was therefore used as a specified covariant in the design matrix, and correlated with brain activation using the regression tool in SPM99 (Jansma *et al.*, 2000; Peigneux *et al.*, 2003; Pleger *et al.*, 2003; Madden *et al.*, 2004). Regions of significant correlation were again identified using a corrected joint expected probability distribution of extent ($P < 0.05$) and height ($P < 0.001$) to control for type I error through multiple comparisons.

Results

Behavioural

During the initial training sessions, no significant difference in threshold was observed between the two eyes [eye-SLEEP 110.5 ms versus eye-WITHOUT-SLEEP 107.56 ms; paired t -test; $t(17) = 0.77$, $P = 0.45$], confirming equal performance by both eyes and an absence of learning transfer between eyes (Fig. 2). Thus, both eyes experienced identical amounts of training and both eyes appeared to benefit equally from the initial training session.

During the fMRI retest session, significantly better visual discrimination was expressed by the eye in the SLEEP condition compared with the eye in the WITHOUT-SLEEP condition [96.8 versus 92.7% respectively, paired t -test; $t(17) = 2.93$, $P = 0.009$]. Thus, the night of intervening sleep produced a significant learning benefit, consistent with previous reports of overnight sleep-dependent enhancement of TDT performance (Karni *et al.*, 1994; Gais *et al.*, 2000; Stickgold *et al.*, 2000a,b).

fMRI Group Comparisons

Group analysis of retest brain activation maps demonstrated a region of significantly greater activation in the left primary visual cortex (V1) in the SLEEP condition, relative to the WITHOUT-SLEEP condition (SLEEP > WITHOUT-SLEEP; Fig. 3) — an area that corresponds to the trained visual field and also supports the achievement of successful monocularly. This finding of increased activation in V1 is consistent with previous fMRI findings using the TDT (Karni *et al.*, 1995; Schwartz *et al.*, 2002) and is consonant with the suggested low-level specificity of the task. However,

there were additional lateralized regions of significantly increased activity beyond the primary visual cortex following sleep. These included portions of the ventral visual processing stream — the left temporal-occipital junction and the left posterior temporal lobe, as well as the left inferior parietal region of the dorsal visual processing stream (Ungerleider and Mishkin, 1982; Ungerleider and Haxby, 1994; Yantis and Serences, 2003).

In the opposite group contrast (SLEEP < WITHOUT-SLEEP), there was a region of decreased signal intensity in the right temporal pole (Fig. 3). Anatomical coordinates for all these regions of difference, and the corresponding z -scores, are provided in Table 1.

Together, these data indicated clear differences in activation between the two conditions, suggesting an influence of sleep on task related function brain activation. However, since behavioural skill level was significantly higher in the SLEEP condition, these data alone were not able to dissociate the possibility that better performance skill, rather than a specific sleep-dependent influence *per se*, formed the basis of these differences.

To address this issue, a correlation analysis was carried out between behavioural performance and functional activation patterns within each of the SLEEP and WITHOUT-SLEEP conditions separately. Since there was a range of performance values across subjects within each condition (i.e. both high and low level performance scores), this offered the ability to investigate whether superior accuracy in the SLEEP condition correlated with the same or different brain regions, relative to superior accuracy in the WITHOUT-SLEEP condition. If it were the case that sleep, rather than skill level, determined differences in brain activation between the two conditions, then better performance would be expected to correlate with different brain regions in each of these conditions.

Performance Correlations

When behavioural task performance within each condition was correlated with functional brain activation, clear differences in the patterns of both positive and negative performance-related brain activity emerged.

In the SLEEP condition, positive correlations with task performance were identified bilaterally in the posterior temporal lobes, largely inferior, as well as a region of the right

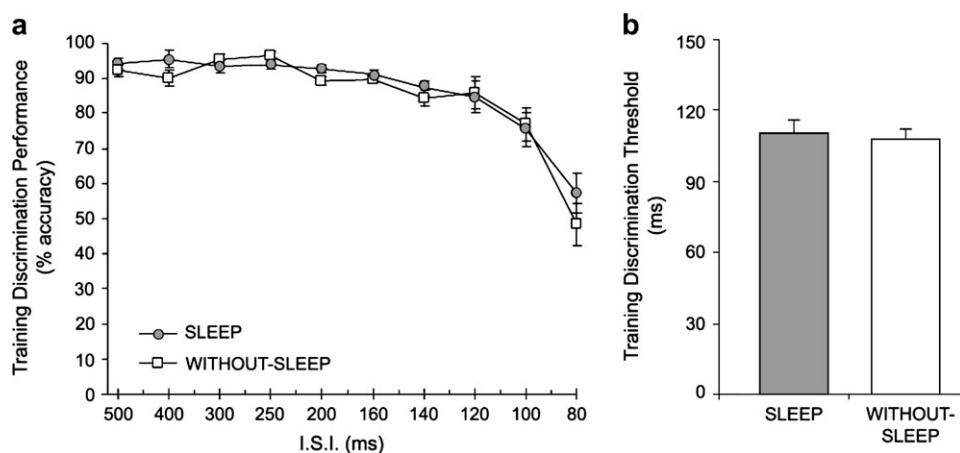


Figure 2. Monocular training curves and initial discrimination thresholds. (a) Training curves for performance in the SLEEP and WITHOUT-SLEEP conditions across the 10 blocks of decreasing ISI (increasing difficulty, resulting in decreasing performance accuracy). (b) The detection thresholds, defined as the interpolated ISI which produced 80% accuracy, for the SLEEP and WITHOUT-SLEEP conditions.

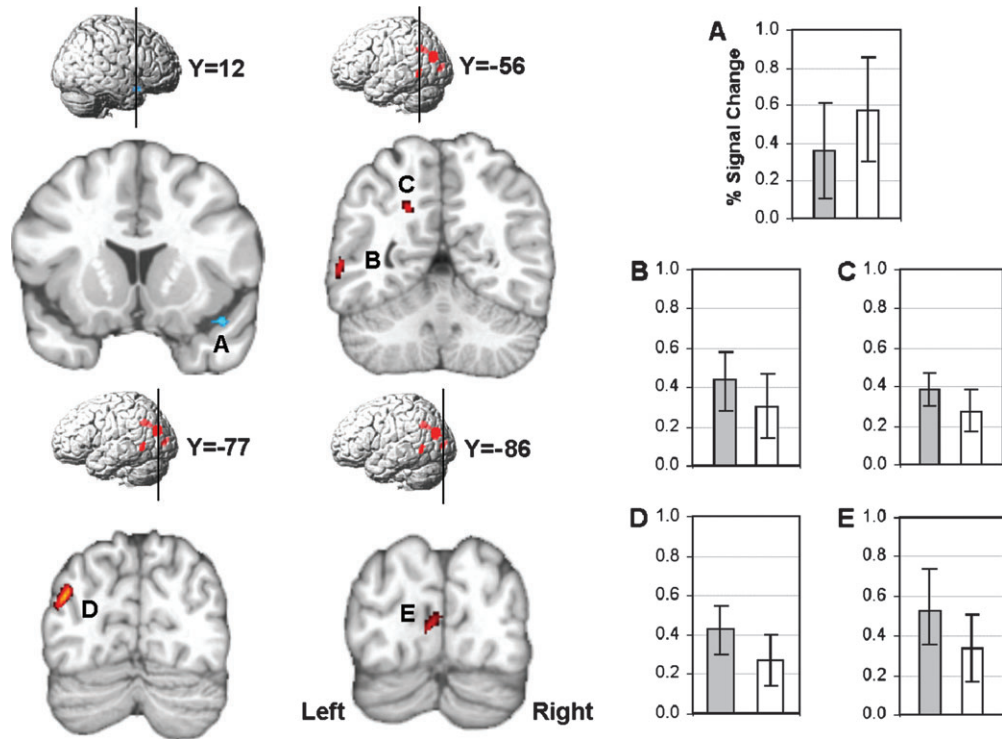


Figure 3. fMRI group (condition) effects. Increased fMRI signal activity (in red) and decreased signal activity (in blue) during task retest following training with sleep, relative to training without sleep. Corresponding coronal sections (A-E) in ICBM₁₅₂ (International Consortium for Brain Mapping) space are provided, with averaged BOLD signal change (in % with respect to the baseline signal) for each condition (gray: trained & SLEEP; white: trained & WITHOUT-SLEEP).

Table 1
Anatomical coordinates for significant clusters of activation for the main group (condition) comparisons

Region (Brodmann's area)	x	y	z	Cluster size (mm ³)	Peak z-score
SLEEP > WITHOUT-SLEEP					
Primary visual area (striate cortex; BA 17)	-2	-90	10	280	3.82
Occipital-temporal junction (BA 39)	-42	-74	22	1048	4.85
Inferior parietal lobe (BA 7)	-20	-56	38	656	3.91
Inferior/middle temporal lobe (BA 21)	-58	-56	2	288	3.86
WITHOUT-SLEEP > SLEEP					
Temporal pole (BA 38)	42	14	-20	312	4.31

The x-y-z coordinates are given in ICBM₁₅₂ space. The Brodmann's area location is identified according to the atlas of Talairach and Tournoux (1988).

medial frontal lobe [Brodmann's area (BA) 6/8] (Fig. 4a). In addition to these positive correlations, significant negative correlations were identified in the left medial dorsal and pulvinar nuclei of the thalamus (Fig. 4a).

In contrast to the diffuse regions of correlation in the SLEEP condition, there was only one region of positive correlation with behavioural performance in the WITHOUT-SLEEP condition, restricted to the right posterior medial temporal lobe (Fig. 4b). Furthermore, no negative correlations in any brain regions were found in the WITHOUT-SLEEP condition.

Anatomical coordinates for these regions of correlation in both the SLEEP and WITHOUT-SLEEP conditions, together with the corresponding z-scores and r-values, are provided in Table 2.

Taken as a whole, these results demonstrated that skill level in the SLEEP condition (ranging from low to high) correlated with a different pattern of brain activation compared with skill level in the WITHOUT-SLEEP condition (also ranging from low to high). It does not therefore appear that skill level alone determined differences in brain activation between the two conditions. If this were true, better performance should have correlated with the same brain regions in both of the conditions. Instead, better performance in each group correlated with quite different patterns of brain activation, indicating the existence of distinct performance-related networks with or without-sleep, rather than skill level *per se*.

Discussion

By comparing brain activity with and without a night of sleep following training on a visual TDT, we have identified regionally specific increases and decreases in functional activation, associated with sleep-dependent learning. Moreover, when behavioural performance measures were correlated with brain activation, significantly different patterns of covariance were evident between the two conditions; indicative of a unique performance-related brain network, post-sleep.

It should be noted that these differences cannot be explained either by (i) variations in alertness due to differences in circadian test times, since both conditions were measured at the same time of day in the same scanning session; or (ii) differences in training, since the two eyes, although trained separately, underwent identical training regimens. Furthermore, each eye attaining near identical training thresholds,

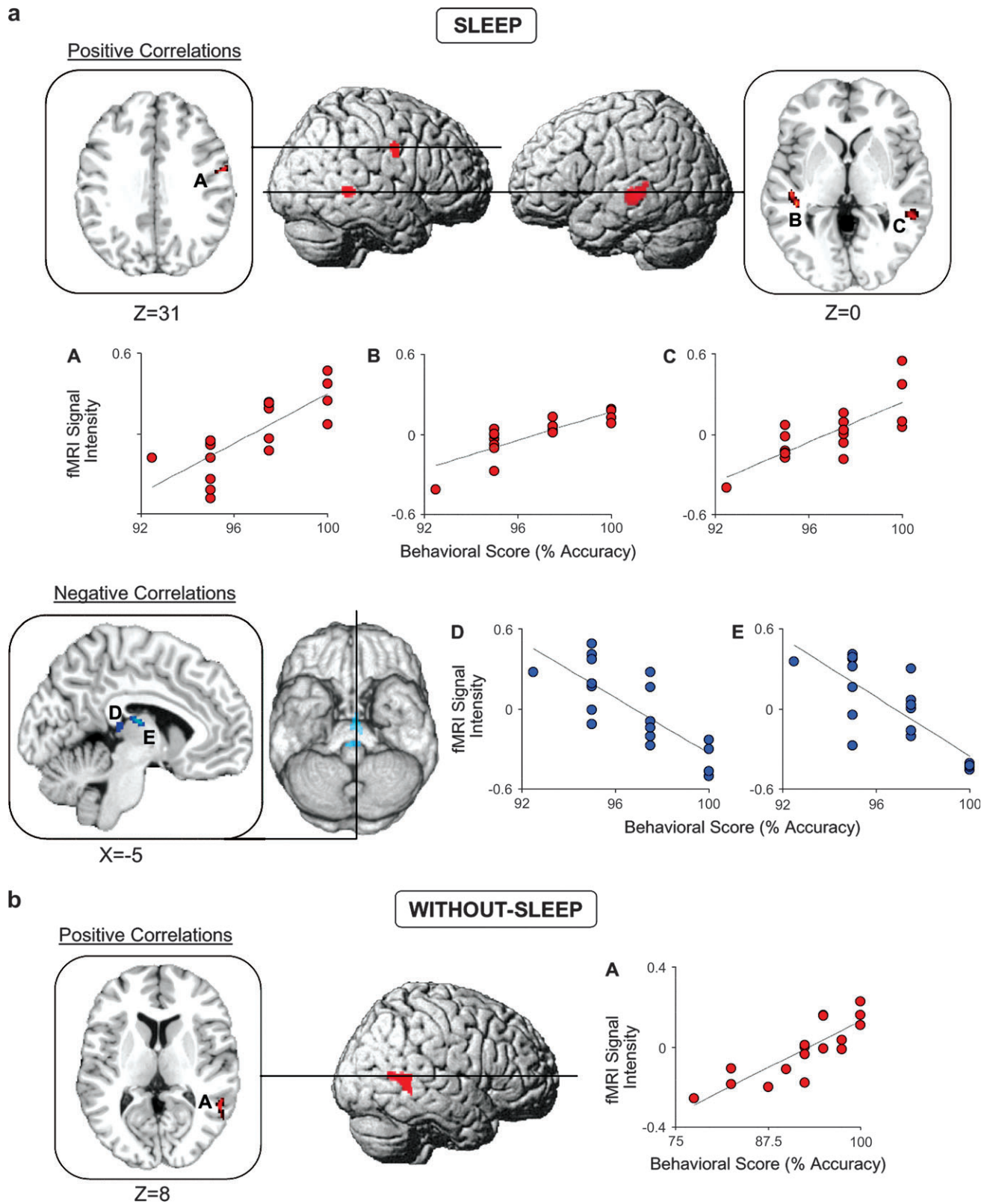


Figure 4. Correlational analyses between behavioural task performance and functional brain activity within each group (condition). Positive correlations between task performance and increased fMRI signal activity (in red) and negative correlations (in blue) within each of the experimental conditions of (a) SLEEP and (b) WITHOUT-SLEEP. Corresponding axial and sagittal sections in ICBM₁₅₂ space are provided, together with correlational plots (positive in red, negative in blue) between peak signal intensity in each region and the associated behavioural task performance score.

Table 2

Anatomical coordinates for regions of activity correlating significantly with behavioural performance score within each condition

Region (Brodmann's area)	x	y	z	Cluster size (mm ³)	Peak z-score	r-value
SLEEP						
Positive correlations						
Middle temporal gyrus (BA 21)	-42	-33	3	1032	4.10	0.814
Middle temporal gyrus (BA 21)	51	-41	-1	520	3.77	0.773
Precentral gyrus (BA 6)	57	-2	31	472	3.74	0.770
Negative correlations						
Thalamus, medial dorsal nucleus	-4	-15	14	728	3.84	-0.783
Thalamus, Pulvinar	-6	-29	11	496	3.75	-0.771
WITHOUT-SLEEP						
Positive correlations						
Middle temporal gyrus (BA 21)	53	-46	8	744	4.37	0.842

The x-y-z coordinates are given in ICBM₁₅₂ space. The Brodmann's area location is identified according to the atlas of Talairach and Tournoux (1988).

suggesting that the initial practice-dependent improvements were similar.

We cannot, however, rule out the possibility that differences observed between the two conditions are simply a factor of time rather than sleep, since the two conditions differed not only in the presence or absence of intervening sleep, but also in the amount of total intervening time between training and retest — an important limitation of this study. According to Karni *et al.* (1994), a small percentage of subjects (1–2 of 11 subjects) did express some performance gains without sleep. Nevertheless, to verify that an intervening time period awake did not confer any performance improvement on this modified version of the task, an additional group of subjects ($n = 10$; 8 females, 2 males, mean age 23.6 ± 1.7) were trained monocularly (10 blocks) in the morning (9 a.m. \pm 30 min) and 12h later, after waking, were retested monocularly (10 blocks) without scanning, offering a behavioural wake control. Consonant with previous studies, no significant improvement was expressed at the later retest without sleep [training threshold = 116.3 ms versus retest threshold = 114.2; $t(9) = 0.37$, $P = 0.71$], indicating that the behavioural characteristics of this modified task are similar to those of previous task versions (Karni *et al.*, 1994; Stickgold *et al.*, 2000a,b; Mednick *et al.*, 2002, 2003) and that an intervening time period awake did not confer any learning enhancements, in contrast to improvements expressed overnight.

This evidence, together with data from prior investigations demonstrating that performance on this task does not improve across waking episodes of 60 min, 90 min, 3 h, 6 h, 9 h or even 12 h (Karni *et al.*, 1994; Stickgold *et al.*, 2000a,b; Mednick *et al.*, 2002, 2003), leads us to consider that differences in functional activation patterns between each condition are mostly likely explained by intervening overnight sleep. Nevertheless, it will be vital for future studies to clarify that similar neural changes to those identified overnight do not occur across equivalent time periods awake, truly confirming the sleep-dependency of such brain plasticity.

Group Difference between Conditions

The retinotopic and monocular specificity of the TDT task suggests that at least part of the underlying neural substrates of this form of learning reside early in the visual processing pathway (Karni and Sagi, 1991; Karni, 1996; Gilbert *et al.*,

2001; but see Crist *et al.*, 2001; Schoups *et al.*, 2001; Ghose *et al.*, 2002). Neuroimaging studies that have compared brain activation between trained and untrained conditions using this task have demonstrated increased activation in the primary visual cortex (V1) (Karni *et al.*, 1995; Schwartz *et al.*, 2002), findings that are in accordance with the known cell selectivity for stimulus orientation in this region (Hubel and Wiesel, 1959).

However, since learning on this task continues to develop across sleep, independent of further practice, the plastic changes associated with overnight improvement may rely on a different functional anatomy to those changes which mediate practice-dependent learning. This possibility is supported by evidence of neural changes in later stages of the visual system associated with orientation discrimination learning, indicating that a more distributed system can support perceptual learning, beyond V1 (Yang and Maunsell, 2004). It is also of note that increased proficiency of visual object recognition has been related to changes in downstream regions of the visual processing pathway (Tanaka *et al.*, 1991; Sakai and Miyashita, 1994; Sakai *et al.*, 1994; Zohary *et al.*, 1994), although these tasks have usually involved visual stimuli of a more complex nature. Thus, additional overnight improvement leading to enhanced visual discrimination accuracy may not only be evident in low-level processing regions of the primary visual regions (as may be the case during training), but may extend beyond this region (Gilbert *et al.*, 2001).

Our findings describe a region of increased activation within the primary visual cortex following a night of sleep, relative to without sleep, supporting previous neuroimaging data comparing untrained to trained performance using the TDT (Karni *et al.*, 1995; Schwartz *et al.*, 2002). In this sense, the overnight changes in functional brain activation (and the associated improved task performance) reported here appear, in part, to be a continuance of the neural changes associated with initial practice-dependent learning. However, our data also demonstrate further changes that extended beyond V1, into later visual processing areas, including the inferior temporal and inferior parietal regions. One interpretation of these findings is that overnight, sleep-dependent learning produces orientation-specific changes exclusively in V1; plastic changes that then drive increased responsivity in associated downstream regions involved in object and spatial recognition, but which do not themselves undergo a plastic change. An alternative interpretation is that the entire collection of cortical regions which show greater activation represent a genuine large-scale, systems-level, plastic change that takes place throughout several posterior visual processing areas, as well as in V1. In this respect, sleep would be considered to trigger a modification in the locus of the memory representation beyond the primary visual cortex (Gilbert *et al.*, 2001). It is also possible that regions of increased activation in later visual processing areas, particularly in the parietal lobe, reflect an overnight adjustment of top-down attentive modulation on the primary visual cortex, thus improving object recognition (Kastner and Ungerleider, 2000). This is especially pertinent considering the fact that perceptual learning appears to require a degree of conscious attentive involvement (Hochstein and Ahissar, 2002). However, based on the temporal resolution capabilities of fMRI, the current study is not able to differentiate between these possibilities.

Nevertheless, these data do argue for a potential dissociation between initial training-dependent plastic changes, relative to the subsequent delayed, overnight changes, which may recruit

greater involvement of later visual processing regions (Karni *et al.*, 1995; Schwartz *et al.*, 2002). This dissociation is supported by behavioural models which propose unique stages of memory processing during initial practice and across the subsequent brain states of wake and sleep (for review, see Walker, 2004), and recent molecular studies describing the temporal stage progression of plasticity-associated *zif268* gene expression across intervals of wake, slow-wave sleep and REM sleep (Ribeiro *et al.*, 2002).

In association with the increased activations in the SLEEP condition, there was also a region of decreased signal in the right temporal pole, an area considered to be a higher order visual structure (Nakamura and Kubota, 1996), potentially representing a reduced visual memory load. Interestingly, Lane *et al.*, have reported greater temporal pole activation specifically related to the processing of visual stimuli with strong emotional meaning (Lane *et al.*, 1999). In this sense, it would not be surprising if processing demands related to the emotional burden of the task decreased during retesting following sleep due to greater ease of task performance (because of the sleep-dependent learning benefit), thereby reducing activity in the temporal pole.

Performance Correlations

While the main group contrasts revealed significant differences between the sleep and WITHOUT-SLEEP conditions, an alternative explanation was that superior skill level in the SLEEP condition, rather than the intervening sleep itself, was the cause of these differences. That is to say, differences in activation were more learning related rather than sleep related. To explore this possibility further, behavioural performance was correlated with brain activation within each group separately.

Contrary to a simple learning-related explanation, behavioural performance in the SLEEP condition, which ranged from high to low across individual subjects, correlated with a very different pattern of brain activity relative to performance in the WITHOUT-SLEEP condition, which also ranged from high to low across subjects. Specifically, in the SLEEP condition, performance scores correlated positively with brain activity in bilateral anterior temporal lobe regions, as well as in the right prefrontal area (BA6/8), while negative correlations were evident throughout the thalamus. In contrast, only a unilateral positive correlation in the right temporal lobe was observed in the WITHOUT-SLEEP condition. This would suggest that skill level across subjects does not correlate with the same brain regions in each condition, but instead, distinct performance-related brain networks had developed in the two conditions, determined by the presence or absence of intervening sleep.

It is interesting to note that processing of complex stimuli along the ventral stream, particularly in the inferior temporal (IT) regions, appears to play a crucial role in object recognition (Tanaka, 1996), and that the tuning properties of these cells can be modified by practice (Logothetis *et al.*, 1994; Booth and Rolls, 1998; Kobatake *et al.*, 1998). Neuroimaging studies in humans have largely confirmed these findings, reporting a similar human homologue in the temporal lobe (Malach *et al.*, 1995; Grill-Spector *et al.*, 2001), and Grill-Spector *et al.* (2001) have termed this human ventral processing area the lateral occipital complex (LOC). Moreover, Grill-Spector *et al.* (2000) have found that training on a visual object recognition task improves behavioural performance considerably, and that the degree of LOC activation shows a strong positive correlation with in-

creased recognition ability. Thus the LOC appears to play a functional role in object familiarity and hence enhanced visual stimulus recognition.

Our finding that bilateral temporal lobe regions (which conform to the LOC) correlated with better task performance in the SLEEP condition may therefore be a reflection of improved visual stimulus recognition post-sleep, although it should be noted that these previous studies have utilized much more complex visual stimuli. While the characteristics and neural underpinnings of these different forms of visual learning (basic orientation discrimination and complex object identification) are somewhat different, it may indicate that sleep-dependent learning offers a greater degree of stimulus familiarity and recognition; with those subjects who benefit most from the sleep-dependent process (characterized by better performance at retest), demonstrating proportionally more intense and diffuse (bilateral) LOC activation.

Finally, there was also a region of positive correlation in the SLEEP condition within BA6, located in the area of the frontal-eye fields (FEF) (Paus, 1996). Considering that the FEF have been consistently associated with visual selection, even in the absence of eye movements (Muggleton *et al.*, 2003; O'Shea *et al.*, 2004), we speculate that sleep-dependent learning is accompanied by a proportional overnight increase within this functional region, allowing the ability for improved visual target discrimination (Muggleton *et al.*, 2003; O'Shea *et al.*, 2004).

In parallel with these changes, a significant negative correlation developed in the SLEEP condition in the left medial dorsal and pulvinar nuclei of the thalamus — a region involved in visual attention (Grieve *et al.*, 2000). In this respect, the improved recognition abilities of the LOC following sleep might allow for reduce attentional demands and thus decreased thalamic involvement, consonant with the notion that sleep-dependent learning promotes task automation (Atienza *et al.*, 2004; Kuriyama *et al.*, 2004).

In summary, we have demonstrated overnight, bi-directional changes in functional brain activity during retesting on a visual skill task which lead to specific increases in activity within both the dorsal and ventral visual processing streams following sleep, together with a region of reduced activity in the temporal pole post-sleep. Furthermore, task performance after a night of sleep correlated with a significantly different pattern of functional brain activation relative to activation without intervening sleep. These findings provide evidence of overnight changes in brain activation and may represent the neural substrate of sleep-dependent visual skill learning.

Notes

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Address correspondence to Seung-Schik Yoo, Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA. Email: yoo@bwh.harvard.edu.

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