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8. Both principal cells (excitatory pyramidal cells) and inhibitory interneurons were recorded during these sessions. Interneurons, identified on the basis of wave shape, firing rate, and spike interval characteristics, were not included in the study. Cross-correlations were normalized by spike counts [D. H. Perkel, G. L. Gerstein, G. P. Moore, *Biophys. J.* **7**, 419 (1967); G. L. Gerstein and D. H. Perkel, *Science* **164**, 828 (1969)].
9. Hippocampal EEGs from eight of the unit recording sites were continuously monitored. Sleep phases were predominantly characterized by intermittent SPW and ripple activity [J. B. Ranck Jr., *Exp. Neurol.* **41**, 461 (1973); J. O'Keefe and L. Nadel. *The Hippocampus as a Cognitive Map* (Oxford Univ. Press, Oxford, 1978), pp. 150–153; G. Buzsáki, *Brain Res.* **398**, 242 (1986)] with population bursts of variable duration (100- to 300-Hz band EEG, mean duration 74 m, mean inter-burst interval 1.7 s) with little or no REM sleep. The behavioral phase was dominated by theta activity (6- to 9-Hz modulation of EEG and unit discharge), which has been correlated with locomotion [C. H. Vanderwolf, *Electroencephalogr. Clin. Neurophysiol.* **26**, 407 (1969)].
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11. Figure 2 reveals a tendency for pairs that were negatively correlated (not overlapping) during behavior (see animals 1 and 2 in Fig. 3) to result in reduced correlation during the POST phase. This effect was small and statistically significant only for rat 1.
12. The time constant for decay of correlation was estimated by dividing the initial 10 min of the POST sleep phase into two 5-min periods and computing the mean correlation for overlapping cell pairs in each period. A third point obtained from the mean correlation of the PRE phase was assumed to be the asymptotic base line for the decay. The mean time constant was determined by the fitting of a single exponential to these three points. Rat 1 latency to sleep onset was 20 min, sleep duration was 18 min, and the time constant estimate was 15 min. Rat 2 latency was 10 min, duration was 20 min, and the time constant was 9 min. Rat 3 latency was 8 min, duration was 10 min, and the time constant was 13 min.
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Dependence on REM Sleep of Overnight Improvement of a Perceptual Skill

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Several paradigms of perceptual learning suggest that practice can trigger long-term, experience-dependent changes in the adult visual system of humans. As shown here, performance of a basic visual discrimination task improved after a normal night's sleep. Selective disruption of rapid eye movement (REM) sleep resulted in no performance gain during a comparable sleep interval, although non-REM slow-wave sleep disruption did not affect improvement. On the other hand, deprivation of REM sleep had no detrimental effects on the performance of a similar, but previously learned, task. These results indicate that a process of human memory consolidation, active during sleep, is strongly dependent on REM sleep.

Perceptual learning—the improvement of perceptual skills through practice—is a type of human learning that may serve as a paradigm for the acquisition and retention of procedural knowledge, "habits," or "how to" memories (*1*). Recent results suggest that when observers practice a simple texture discrimination task the large and consistent improvements that occur over the course of several consecutive daily sessions are subserved by discrete changes dependent on retinal input and within an early stage in the stream of visual processing (*2*). Psychophysical data implicate neuronal mechanisms of figure-ground segmentation at a stage in the processing pathway as early as the primary visual cortex in mediating (by becoming more efficient and faster) the learning of this basic visual skill (*2*, *3*). These results, as well as results from several other perceptual learning paradigms (*4*), suggest that different levels of visual processing may, under specific retinal input and task-defined conditions, undergo long-term, experience-dependent changes (functional plasticity) (*5*).

Recently, we and others have found that an improvement in perceptual performance occurs neither during nor immediately after practice but rather 8 to 10 hours after a training session has ended, suggesting a slow, latent process of learning (*6*). As the improved visual skills were not forgotten even

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after an interval of several years, we have proposed that this time course may reflect an active, time-consuming process underlying the consolidation of experience-dependent plasticity within the adult visual cortex. Our study here was designed to investigate the possibility that processes subserving the consolidation of human skills can be supported by mechanisms active during normal sleep. Because normal sleep (unlike the waking state) is parsed into several discrete stages, each with unique neurochemical and electrophysiological characteristics (*7*), the functional contributions of these brain states to the acquisition of procedural knowledge could be determined.

Six young adults (three females and three males, 17 to 22 years old) with normal or corrected to normal vision and no history of neurological or chronic illness took part in these experiments. Their task was to identify the shape of a small target texture composed of three diagonal line elements that differed only in orientation from a background texture of otherwise identical elements (Fig. 1A). Psychophysical measurements were made both before (initial training session) and after an interval that included either normal sleep or sleep disrupted at a specific sleep stage. An important property of our paradigm, a specificity of the learning for visual field location and background element orientation (*2*), enabled us to design within-subject comparisons across different sleep conditions, as well as to assess the differential effects of sleep-stage deprivation on performance on a novel stimulus configuration (learning) compared to that on a well-practiced one (control). The latter provided an independent measurement of visual discrimination performance to control for factors such as diurnal variation, stress, and fatigue (*8*), which would presumably affect performance on a previously learned, as well as on a new, configuration.

The initial night of each study phase was spent at the sleep laboratory with no recording or interference (night 1). This was followed by two consecutive nights of baseline recording during normal sleep (nights 2 and 3) and then by either one or two consecutive nights of deprivation of selected sleep stages when either rapid eye movement (REM) or slow wave (SW) sleep (stages 3 and 4) was systematically disrupted (night 4, or nights 4 and 5 of the study). These in turn were followed by one or two nights of recovery (rebound). Figure 1C depicts the course of one study phase during which REM sleep was disrupted. This was repeated after an interval of at least 1 week (mean = 6 weeks), and the complementary target sleep stage was disrupted. Selective deprivation of selected sleep stages was effected by forced arousal (through the ringing of an electric bell) after an epoch of the relevant sleep stage was recorded. Although this procedure allowed adherence to sleep stage scoring criteria (*9*), several epochs of the target sleep stage were of necessity included. Sleep stage scoring was done in real time. The recordings were later rescored independently. Two observers completed just one phase of the study (NK, REM sleep deprivation, and IN, SW sleep deprivation).

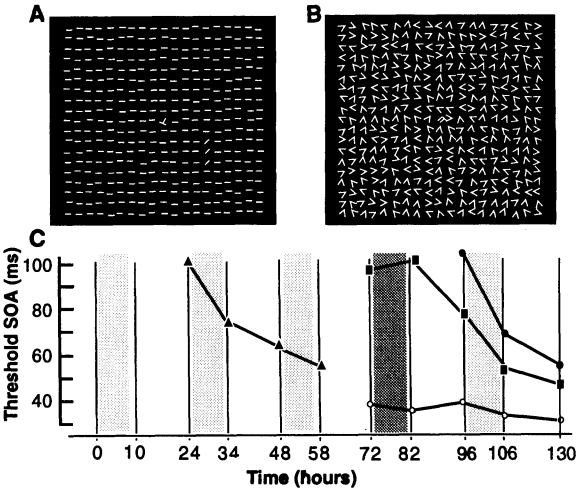

Fig. 1. (**A**) An example test stimulus with a small vertical target texture (three diagonal bars in the right lower quadrant of the display) embedded within a background of horizontal elements. A small rotated letter (either T or L) at the center served as the fixation target. The target texture's position was varied randomly from trial to trial but was always within a specific display quadrant and at 2.5° to 5° eccentricity from the center of the display. (**B**) Mask patterns made of randomly oriented V-shaped micropatterns, with a superimposed T and L micropattern in the center as the fixation letter's mask (*24*). (**C**) The sequence of events within a study phase (pilot study). Standard polysomnographic tracings of two-channel electroencephalograms [electrodes at T3-A2 and T4-A1 (10–20 international system)], three-channel electrooculogram monitoring, and electromyograms (surface electrodes under the chin) were recorded on four to six consecutive nights. Shading represents periods of sleep. Psychophysical testing (practice) sessions were administered twice each day at 9 to 10 p.m. and 7 to 8 a.m. (vertical lines). Representative data from a single participant are shown. Each data point corresponds to the threshold SOA (80% correct discrimination) interpolated from the psychometric curve for the corresponding session. Performance on several stimulus configurations was measured within each session. Filled triangles, learning across a normal night's sleep and during the following day (target in lower right field); filled squares, performance in a new visual quadrant, before and after an interval of REM-disrupted sleep and across the following day and night (target in lower left field); filled circles, performance across an interval of recovery (rebound) sleep (target in upper left field); empty circles, performance on a control, previously well trained, stimulus configuration (target in lower right field, but the orientation of the background elements was flipped to vertical).

Both REM and SW sleep deprivation required 20 to 60 arousals and awakenings, with test participants repeatedly reverting to the disrupted stage (Fig. 2). However, deprivation was quite effective, with REM (a total of seven nights) and SW (a total of six nights) sleep decreasing to 5.7 ± 1.6% (mean ± SD) and 8.1 ± 3.1% of total sleep time (corresponding to 19 ± 6 min in REM sleep and 30 ± 12 min in SW sleep) in the respective deprivation conditions. The difference between time spent in the two target stages during the respective deprivation nights was not significant (*t* test, $P = 0.16$). The mean length of uninterrupted REM or SW sleep was 30 s (a single epoch). Also, the difference between time spent in the awake state in each deprivation condition was not statistically significant (*t* test, $P = 0.27$), and the numbers of arousals and awakenings during the REM and the SW sleep deprivations were comparable (*t* test, $P = 0.34$) (*10*).

Learning, however, was found to be strongly dependent on the type of sleep (Fig. 3). Improvement occurred during normal sleep, with a performance gain of 23 ± 4 ms (mean ± SD) [with thresholds decreasing from 97 ± 18 ms to 74 ± 16 ms (paired *t* test, $P < 0.001$)], but no improvement occurred during a comparable interval with REM-disrupted sleep [performance gain of 0 ± 6 ms (mean ± SD), with thresholds changing from 85 ± 13 ms to 85 ± 18 ms (paired *t* test, $P = 0.42$)] (*11*). At the same time, performance on a previously learned stimulus configuration was unaffected by REM sleep deprivation. In contrast, significant improvements occurred for all observers after an interval of SW-deprived sleep [performance gain of 19 ± 4 ms (mean ± SD), with thresholds decreasing from 84 ± 13 ms to 66 ± 12 ms (paired *t* test, $P < 0.001$)]. Perceptual learning during the REM sleep deprivation condition compared to the gain during SW sleep deprivation was significantly less [$F(1,12) = 37.009$, $P < 0.001$]. On the other hand, compared to the REM sleep deprivation condition there was a small but significant detrimental effect of SW sleep deprivation on the already learned (control) task [$F(1,12) = 17.896$, $P = 0.001$]. This dissociation suggests that REM deprivation affected the consolidation of the recent perceptual experience, but not perceptual performance by itself, making it

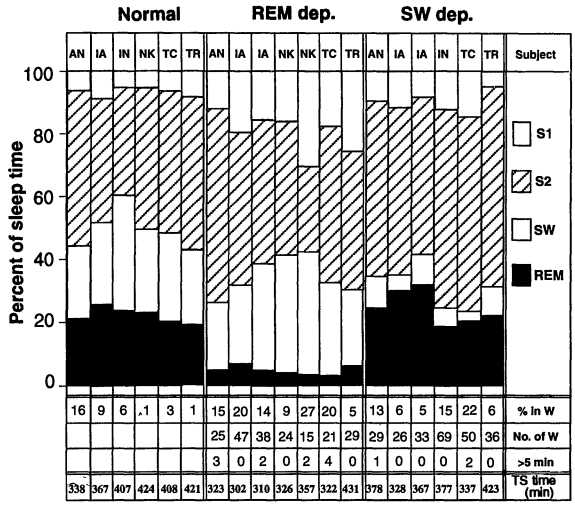

Fig. 2. Percent of total sleep time (TS time) spent in the various sleep stages during normal, REM deprivation, and SW deprivation sleep intervals. The total time in bed (polysomnographic recording time) was 415 ± 29 min (mean ± SD, 38 nights) for the six test participants shown. S1, sleep stage 1; S2, sleep stage 2; SW, sleep stages 3 and 4; % in W, percent of time in bed spent in the awake state; No. of W, number of forced arousals and awakenings during the sleep interval; >5 min, number of episodes of more than 5 min spent in the awake state. No participants had more than 1.5 min in SW stage 4 sleep during the SW deprivation phase. Two participants (IA and NK) underwent two consecutive nights of disruption of specific sleep stages (nights 4 and 5) at one or both experimental phases.

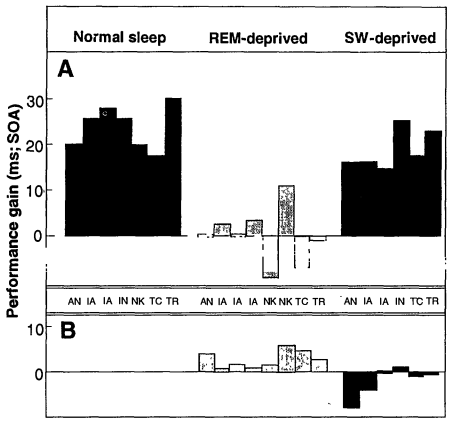

Fig. 3. Performance gain (in terms of threshold SOA) across the sleep intervals. (**A**) Performance gains on a novel stimulus configuration, first presented on the evening session before the sleep interval. (**B**) Performance on a previously well-practiced stimulus configuration (control task). Participant IA was tested during the first night of the REM deprivation phase on two novel stimulus configurations (that is, two independent measurements).

less likely that the effects we observed were nonspecific consequences of disturbed sleep.

Though it has long been hypothesized that memory-related processing, specifically the consolidation of long-term memory, may occur during REM sleep, early experiments designed to demonstrate this for human learning have provided equivocal results. Supporting evidence has come mainly from the work of Empson and colleagues (*12*). Others, however, have found no beneficial mnemonic effects related to REM sleep (*13*) [for recent reviews, see (*14, 15*)]. One reason for these conflicting results may have been the choice of learning paradigm. Previous studies were concerned with the effects of different sleep stages on the retention of material memorized before sleep (that is, the differential effects of selectively disrupted sleep on the rate of forgetting). Here, we examined how the evolving, time-dependent learning (that is, improvement, not loss) of a simple skill proceeds across both normal and deprived sleep. Thus, two factors may be critical for our results: (i) the fact that a nondeclarative memory system was probed [though it has been suggested that REM sleep may be important for the post-sleep recall of semantically well-integrated materials (*12, 14, 15*)] and (ii) the time course of perceptual learning—the finding that people perform much better on a later session than during, or even up to several hours after, the initial one—have provided a more direct measure of the consolidation process.

Our findings suggest that a mnemonic process occurs during normal sleep in the adult brain and that this process is critically dependent on the integrity of REM sleep (*16*). These results are consistent with several paradigms of animal learning in which post-learning REM sleep deprivation has impaired the acquisition and long-term retention of both perceptual and motor "habits" (*14, 17*). Two lines of evidence converge to suggest constraints on possible neuronal substrates that may underlie the learning of perceptual skills during sleep. (i) REM sleep has been shown to be strongly related to cholinergic activity (*18*). Cholinergic stimulation of the brainstem can elicit a state that is behaviorally and polygraphically indistinguishable from physiological REM sleep. Furthermore, desynchronized electroencephalogram activity (REM sleep as well as the waking state) is correlated with increased amounts of acetylcholine (ACh) in the neocortex, whereas REM deprivation is related to a reduction in the amount of ACh (*19*). (ii) Recent studies have demonstrated that a cholinergic input is a necessary requirement for the evolution of experience-dependent plasticity within the adult sensory cortex (*20*). Thus, a strong cholinergic input may be a critical factor for processes underlying the consolidation of some types of memory. As texture discrimination learning is determined by the specific retinal input presented during the pre-sleep practice session, the role of REM sleep may reside in providing a critical milieu (*21*) for the transformation of the activity-dependent neural change, presumably initiated during the pre-sleep session, into a more efficient and stable (consolidated) modification. A possible mechanism for such a process at the cellular level, suggested by Bear and Singer (*22*), may be for example the ACh-dependent phosphorylation of proteins involved in the long-term, structural modification of synaptic transmission.

We have previously shown that consolidation occurs during the waking state (*6*). Though parsimony would suggest a common process at the cellular level, the question whether the mnemonic process of REM sleep is qualitatively different from the waking state consolidation process remains open. Also open for empirical determination is the intriguing suggestion, raised by Smith and Butler, of specific, spaced REM "windows" occurring after the training session, when consolidation processes are presumably active (*23*). Finally, assuming that a limited repertoire of neuronal mechanisms underlies memory consolidation throughout the mammalian cortex, we conjecture that our results may be generalized to other types of human skill learning (for example, motor skill learning) and perhaps to the formation of some types of long-term association memory.

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11. NK, the only test participant to show some improvement across the REM deprivation interval (on his second deprivation night), had spent more than 5 hours of the interval in the awake state and more than 25% of his total sleep time in stage 1 sleep (see Figs. 2 and 3). Thus, the improvement may have resulted from the waking state consolidation process.
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Neutrophil and B Cell Expansion in Mice that Lack the Murine IL-8 Receptor Homolog

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Interleukin-8 (IL-8) is a proinflammatory cytokine that specifically attracts and activates human neutrophils. A murine gene with a high degree of homology to the two known human IL-8 receptors was cloned and then deleted from the mouse genome by homologous recombination in embryonic stem (ES) cells. These mice, although outwardly healthy, had lymphadenopathy, resulting from an increase in B cells, and splenomegaly, resulting from an increase in metamyelocytes, band, and mature neutrophils. Thus, this receptor may participate in the expansion and development of neutrophils and B cells. This receptor was the major mediator of neutrophil migration to sites of inflammation and may provide a potential therapeutic target in inflammatory disease.

IL-8 is a member of a family of proinflammatory cytokines that are related by a C-X-C motif, where X is any amino acid between two cysteines. IL-8 is a major factor in acute inflammation, being responsible for the activation of neutrophils and their chemotaxis to the site of acute injury (*1, 2*). Neutrophils destroy bacteria by phagocytosis and the release of superoxides and peroxides, providing the first line of defense in fighting infection; the response is rapid and is neither acquired nor antigen specific (*3*). Many cells produce IL-8 in vitro, and it has been implicated in neutrophil migration and, to a lesser extent, T-cell migration, to sites of IL-8 injection (*4*). Neither mouse nor rat IL-8 has been identified (*5*), but antibodies (Ab) to human IL-8 inhibit lung inflammation in rats (*6*), which suggests the presence of a similar molecule in rodents.

Two high-affinity human IL-8 receptors have been cloned and characterized (*7–9*). These receptors share 77% amino acid sequence identity and are members of the superfamily of seven transmembrane domain receptors that are coupled to GTP-binding proteins. We have cloned a murine homolog of the human IL-8 receptor by screening a mouse genomic library at reduced stringency with complementary DNA (cDNA) probes from both human IL-8 receptors (*7, 8*). DNA sequencing shows that the mouse receptor is encoded by a single exon (as are the two human receptors) containing a 350–amino acid open reading frame with 68% and 71% amino acid identity with human IL-8 receptors A and B (*10*). Using several different restriction enzymes and genomic DNA blots hybridized under low-stringency conditions, we found a single cross-hybridizing band (*10*), suggesting that unlike the human genome, the murine genome contains a single gene for the putative IL-8 receptor. We refer to this gene as the murine IL-8 receptor homolog (mIL-8Rh).

To determine the function of this receptor in inflammation, we used homologous recombination in ES cells to generate a mouse strain lacking this gene. We constructed a gene-targeting vector by deleting the single exon containing the open reading frame of the mIL-8Rh and replacing it with the neomycin resistance gene (*neo*). This ensures the complete elimination of the gene after homologous recombination (Fig. 1A). Of 814 individual ES clones screened by genomic blot hybridization, 7

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