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Local sleep and learning

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Human sleep is a global state whose functions remain unclear. During much of sleep, cortical neurons undergo slow oscillations in membrane potential, which appear in electroencephalograms as slow wave activity (SWA) of <4 Hz[1]. The amount of SWA is homeostatically regulated, increasing after wakefulness and returning to baseline during sleep[2]. It has been suggested that SWA homeostasis may reflect synaptic changes underlying a cellular need for sleep[3]. If this were so, inducing local synaptic changes should induce local SWA changes, and these should benefit neural function. Here we show that sleep homeostasis indeed has a local component, which can be triggered by a learning task involving specific brain regions. Furthermore, we show that the local increase in SWA after learning correlates with improved performance of the task after sleep. Thus, sleep homeostasis can be induced on a local level and can benefit performance.

To investigate local aspects of sleep regulation, we asked subjects to perform a motor learning task just before going to sleep. In this task, subjects reach for visual targets using a handheld cursor while unconsciously adapting to systematic rotations imposed on the perceived cursor trajectory (Fig. 1a; see also ref. 4). This rotation adaptation task was chosen because it is an implicit learning paradigm; it is suitable for extended sessions; it permits accurate parameterization of both performance improvement and noise reduction; it can be contrasted with a no-rotation task that has the same kinematic requirements and is subjectively indistinguishable; and in contrast with the no-rotation task, it activates circumscribed brain regions (that is, right parietal areas 40 and 7 (ref. 4)). Over a number of movements, all our subjects adapted to the imposed rotation by progressively reducing the directional error of their trajectory as well as its variance (Fig. 1b).

Immediately after the rotation adaptation task, we recorded the sleep electroencephalogram (EEG) using a 256-channel system (Electrical Geodesics) inside a soundproofed room. As whole-night recordings with the high-density EEGs are not comfortable, the cap was removed after 2 h and subjects were then allowed to sleep undisturbed for the rest of the night (all reported satisfactory, restful sleep). As a control condition, 1 week earlier or later, subjects performed the no-rotation task, where the requirements were kinematically identical but the cursor was not rotated[4,5]. High-density recordings after both tasks showed the usual progression of sleep stages. Values (mean ± s.e.m.) for rotation and no-rotation tasks were, respectively: sleep latency, 6.1 ± 0.8 and 6.5 ± 1.4 min; wakefulness, 14.4 ± 4.8% and 14.2 ± 3.8%; rapid eye movement (REM) sleep, 2.3 ± 0.9% and 2.9 ± 1.2%; non-REM sleep (stages 2–4), 65.2 ± 6.4% and 65.7 ± 7.0% (% of recording time).

Average power spectra of consecutive 4-s epochs during non-REM sleep showed that SWA was prevalent in anterior regions, in accord with previous studies[2,6], and that the topographic pattern was highly reproducible in both the rotation and no-rotation conditions (Fig. 2a). However, when we compared the two conditions, we found that the rotation condition produced a local increase in SWA at a cluster of six right parietal electrodes, which

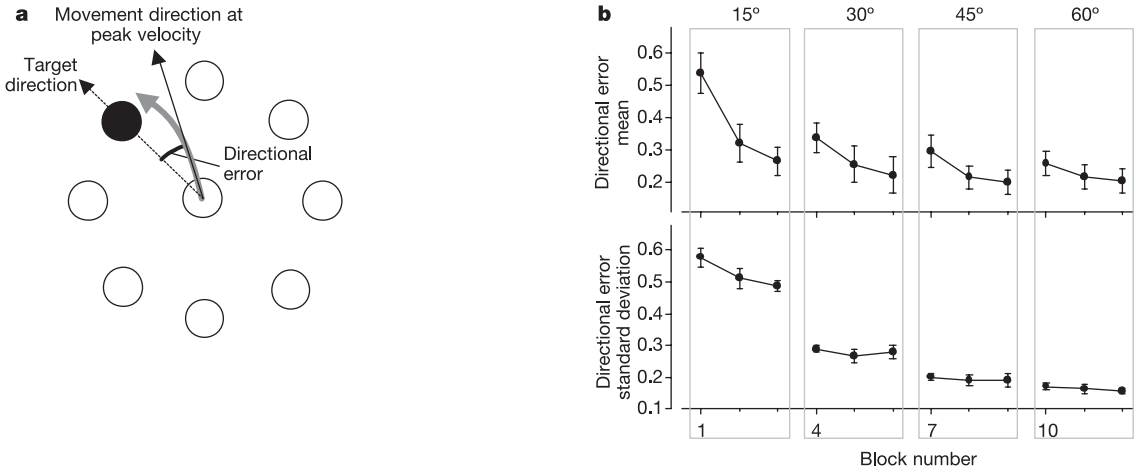

Figure 1 Rotation adaptation. **a**, The tasks. Subjects moved a handheld cursor on a digitizing tablet, executing out-and-back movements from a central starting point to one of eight targets (distance of 4.2 cm) displayed on a computer screen together with the cursor position. An opaque shield prevented subjects from seeing their arm and hand at all times. Targets were randomly highlighted at regular 1-s intervals. In the rotation adaptation task, unbeknown to the subjects, the cursor position was rotated anticlockwise relative to the hand position by a fixed angle. In a separate session, one week earlier or one week later, subjects performed the same task without any imposed rotation (no-rotation task). As shown, we computed the directional error for each movement as the angle between the line from the initial hand position to the position of the target (dotted line) and the line to the position of the hand at the peak outward velocity (solid line)[4]. The grey line represents the hand trajectory. Other movement parameters such as movement time were also computed[6]. **b**, Learning curves for the rotation adaptation task. To measure the time course of rotation adaptation, directional errors were normalized by the angle of the imposed rotation. There were four incremental steps of 15°, up to a maximum of 60° total, with short breaks in between; three blocks per step of 90 movements each (indicated at the top of the diagram). The mean directional error and the mean standard deviation for each block of 90 movements are plotted in the top and bottom panels, respectively. Points are means across subjects and bars represent standard errors. Both the mean and the standard deviation of the directional error decreased during the training blocks.

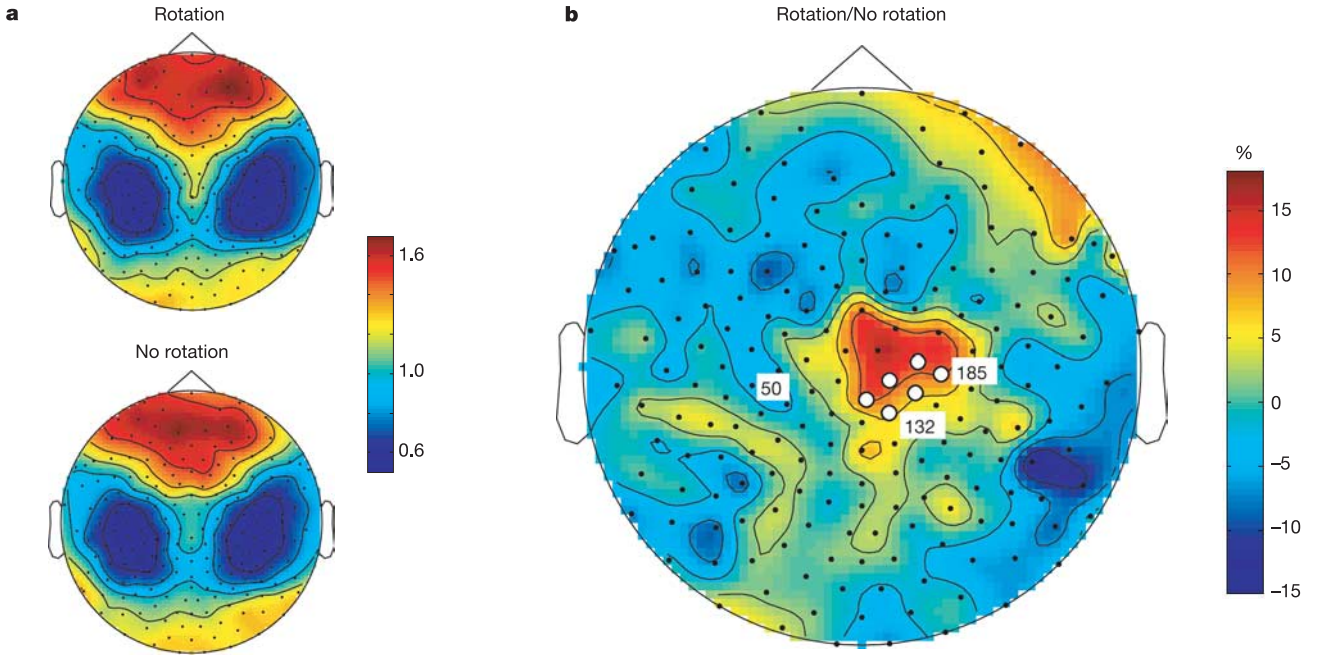

Figure 2 Local SWA homeostasis during sleep after rotation adaptation. **a**, Topographic distribution of SWA after the rotation and no-rotation tasks. Average EEG power density at 1–4 Hz (SWA, $n = 11$ subjects) for the first 30 min of non-REM sleep. EEG signals (average reference) were digitized at 500 Hz together with electromyogram and electro-oculogram, filtered (0.5–50 Hz), artefact-rejected, and sleep-staged[6]. Values (colour bar) were normalized by total power for the recording and plotted at the corresponding position on the planar projection of the scalp surface, and interpolated (bi-harmonic spline) between electrodes (dots). **b**, Topographic distribution of the percentage change in SWA during non-REM sleep between the rotation and the no-rotation condition. White dots indicate the cluster of six electrodes showing increased SWA after rotation adaptation ($P < 0.01$, statistical nonparametric mapping, supra-threshold cluster test controlling for multiple comparisons[7]).

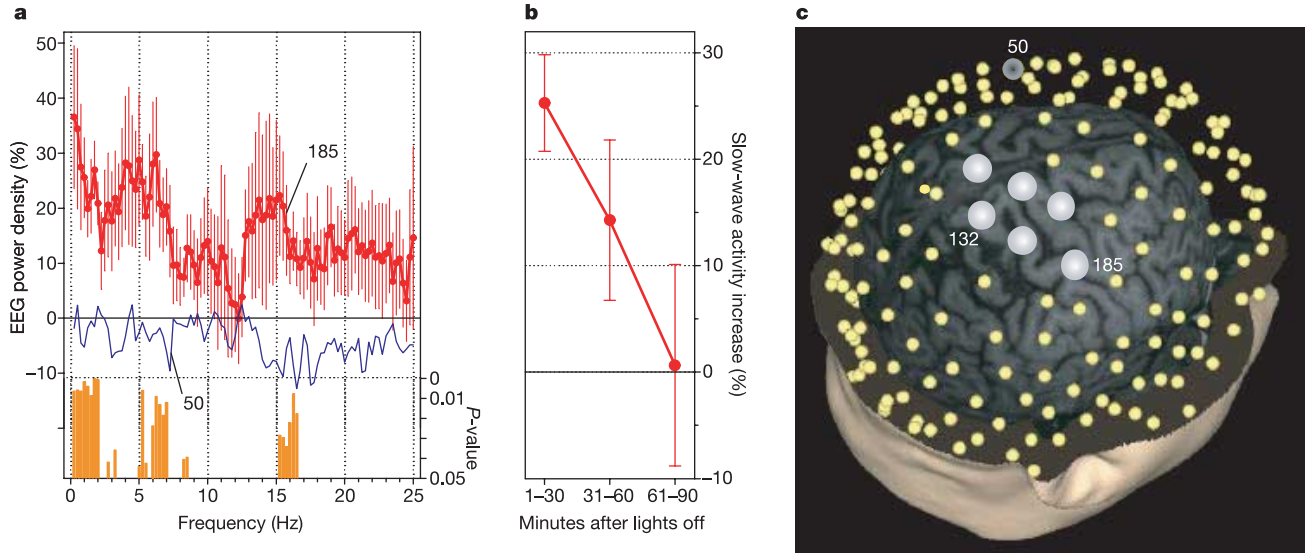

Figure 3 Frequency specificity, time course and anatomical localization of local SWA homeostasis. **a**, Frequency specificity of power changes. EEG power density spectrum for the first 30 min of non-REM sleep. Values represent the percentage change of the rotation adaptation task with respect to the no-rotation task (mean ± s.e.m. for 0.25-Hz bins, $n = 11$ subjects). Red curve, average power change across subjects for the electrode yielding the peak SWA increase for each subject in the region surrounding electrode 185; blue curve, average power change at a contralateral position, symmetric to electrode 185 (electrode 50). Bottom bars indicate frequency bins for which power in the rotation condition differed significantly from the no-rotation condition (paired t-test). **b**, Time course of SWA changes after the rotation adaptation task. The change in average EEG power in the 1–4-Hz band was calculated for three consecutive 30-min intervals during the first non-REM sleep episode (mean ± s.e.m.). As in **a**, we selected the electrode yielding the peak SWA increase for each subject in the region surrounding electrode 185. The decline in power across the three 30-min intervals was significant ($P < 0.05$, analysis of variance). **c**, Anatomical localization of electrodes showing a significant difference in SWA during the first 30 min of non-REM sleep between the rotation and the no-rotation conditions. All 256 electrodes (yellow dots) were digitized and co-registered with the subject's magnetic resonance images. The cluster of six electrodes showing an increase in SWA is marked with white dots. For reference purposes, the electrode at the right anterior border of the cluster (185) projects onto area 40 (Talairach coordinates: $x = 50$, $y = -34$, $z = 41$), the electrode on the right posterior border of the cluster (132) projects onto area 7 (24, −68, 48).

averaged 13.1 ± 6.1% and was statistically significant[7] ($P < 0.01$, Fig. 2b). The peak SWA increase, whose precise location varied slightly between subjects, amounted to 25.3 ± 4.6%. Thus, the rotation adaptation task elicited a response in the sleep EEG, and this response was local rather than global.

We then examined whether the local EEG response after rotation adaptation shared key features with the global homeostatic response observed in the sleep EEG after prolonged wakefulness[2,6]. The EEG signature of sleep pressure is an increase in power predominantly in the frequency range of SWA (1–4 Hz)[2,6] and a slight reduction in power in the sigma band (12–15 Hz). Figure 3a shows the percentage changes observed in sleep EEG power after the rotation adaptation task compared with the no-rotation condition. Consistent with a homeostatic response, there was an increase in power predominantly in the SWA frequency range, as well as a slight decrease in power in the sigma band. The increase was especially evident within the low delta band (<2 Hz) and at frequencies corresponding to the slow oscillation (<1 Hz)[1]. Period amplitude analysis revealed that the increase of SWA at the site of the peak response was primarily due to an increased amplitude of slow waves (22.1 ± 9.7% at 1–2 Hz), whereas their period was unchanged. Another feature of the global homeostatic response of SWA is its decline within and across consecutive sleep cycles[2]. The local SWA response also revealed a decreasing trend across the first 90 min after lights off (Fig. 3b). Thus, both the spectral signature and the time course of local sleep changes resembled the global homeostatic response of sleep.

To localize the region of SWA increase, we used a positioning system (Nexstim) to digitize the 256 electrodes and co-register them with each subject's magnetic resonance images. The increase of SWA was localized to regions of the right parietal lobe encompassing Brodmann areas 40 and 7 (Fig. 3c). These regions corresponded to those highlighted by positron emission tomography (PET) subtractions between the rotation and no-rotation conditions in subjects scanned after training[4]. Both areas 40 and 7 receive converging visual and proprioceptive inputs, and are involved in processing sensory information relevant for spatial attention[8]. Area 7 is a station in the dorsal visual pathways and processes spatial aspects of vision related to skilled actions[8]. The right hemisphere specificity in both EEG and PET experiments is in line with the right hemisphere specialization for spatial tasks[9] and for spatial coordinate transformation[8].

Recent work has shown that performance in certain perceptual, motor and categorization tasks improves after a night of sleep[10–18]. These studies have demonstrated that the improvement is specifically due to sleep rather than to the mere passage of time or to circadian factors[11–13]. We therefore asked whether performance in the rotation adaptation task would also be enhanced by sleep. As shown in Fig. 4a, when subjects were tested after a night of sleep, the directional error had decreased further, corresponding to a performance enhancement of 11.1 ± 3.0% above and beyond the level achieved at the end of awake training. By contrast, an independent group of subjects who trained in the morning and were re-tested after 8 h of wakefulness showed no such improvement (Fig. 4a). Because other movement parameters that also improved with training, such as movement time, did not differ between the two groups, the improvement in directional error was probably due to sleep and not to circadian differences. In summary, the EEG changes observed by comparing sleep after rotation adaptation with sleep after the kinematically identical no-rotation task were: (1) local, suggesting a cellular substrate; (2) characterized by an increase in SWA that behaved as expected of homeostatic changes in the sleep EEG; (3) localized to cortical regions known to be involved in learning rotation adaptation; and (4) followed the next day by an enhancement of performance.

Finally, we asked whether the post-sleep enhancement of performance and the increase of SWA in right parietal areas 40 and 7 were correlated. We found that the decrease of directional error was positively correlated with the peak increase in SWA (Fig. 4b; $r = 0.86$, $P < 0.005$). The positive correlation of post-sleep performance enhancement with the local increase in EEG power was specific to the SWA frequency range (1–4 Hz, data not shown). By contrast, there was no correlation between the improvement in rotation adaptation and SWA changes at other electrodes, or

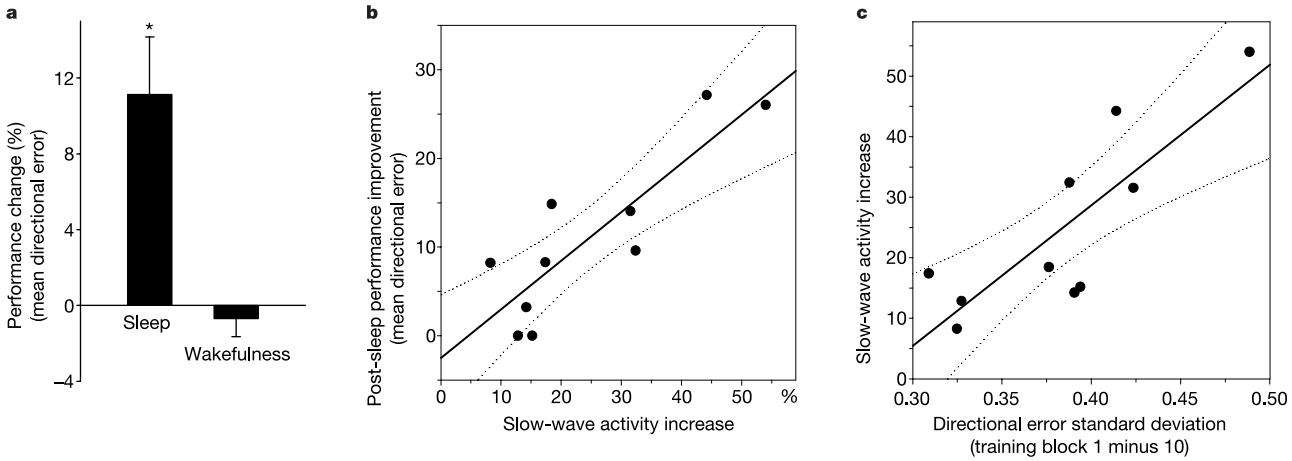

Figure 4 Enhancement of performance after sleep and its relationship to SWA. **a**, Post-sleep performance improvement. Two groups of subjects (sleep group and wakefulness group) underwent rotation adaptation as described in Fig. 1. The extent of rotation adaptation was tested 10 min after the end of training using an imposed rotation of 60°. Subjects in the sleep group ($n = 11$, mean age 25.8 ± 1.8 yr) trained in the evening and were re-tested after 7–8 h of sleep (one subject did not complete the re-test and was dropped from the analysis). Subjects in the wakefulness group ($n = 10$, mean age 28.2 ± 2.9 yr) trained in the morning and were re-tested after being engaged in their normal waking activities for 8 h. A repeated measure analysis of variance and post-hoc tests showed that the two groups had similar performance when tested immediately after training; however, at re-test approximately 8 h later, mean directional error was significantly reduced in the sleep group ($P < 0.006$) but not in the wakefulness group. The two groups differed significantly in the extent of test–re-test performance change ($P < 0.002$). **b**, Positive correlation between peak SWA increase during sleep within the six electrode cluster and decrease of mean directional error after sleep ($n = 10$ subjects). There was no correlation between the decrease of the mean directional error after sleep and the amount of total sleep or individual sleep stages during the first sleep cycle. Dotted lines indicate the 95% confidence interval. **c**, Positive correlation between the change in standard deviation of directional error during rotation adaptation (block 1 minus block 10; see Fig. 1b) and the peak SWA increase in the subsequent non-REM sleep episode ($n = 10$ subjects).

between the local increase of SWA and changes in other movement parameters such as movement time. Therefore, local SWA homeostasis in right parietal areas 40 and 7 is probably related to local neural processes specific to rotation adaptation and to their post-sleep enhancement. We did not find any correlation between the increase in SWA and the mean decrease of directional error at the end of the training session. Instead, we found a high correlation between the increase in SWA and the standard deviation of directional error at the beginning minus the end of training (Fig. 4c; $r = 0.83$, $P < 0.005$). Therefore, subjects who began rotation adaptation with much more variability or 'noise' in directional error compared to the end of training not only displayed a marked local increase in SWA during sleep but also benefited most from sleep.

These findings provide compelling evidence that the electrophysiological marker of sleep homeostasis, SWA, can be selectively induced in circumscribed regions of the cerebral cortex. Thus, they make a strong case for the local regulation of sleep[2,6,19–22] and support a role for sleep at the cellular level[3,23,24]. Moreover, they show that local SWA induction is triggered by a learning task, suggesting that local plastic changes associated with learning may be involved, directly or indirectly. Finally, they show that local SWA homeostasis is strongly correlated with improved performance in the task after sleep. This suggests that SWA homeostasis may be related to cellular processes underlying learning rather than to metabolic fatigue or depletion. Thus, together with evidence from intracellular studies[25], our results support the notion that slow oscillations might help synaptic consolidation[25–30] or produce synaptic downscaling and increase signal-to-noise ratios in relevant neural circuits[3]. Most importantly, these results connect two fields that had thus far remained separate—the study of sleep homeostasis and that of sleep and plasticity. □

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1. Steriade, M. Corticothalamic resonance, states of vigilance and mentation. *Neuroscience* **101,** 243–276 (2000).
2. Borbély, A. A. & Achermann, P. in *Principles and Practice of Sleep Medicine* (eds Kryger, M. H., Roth, T. & Dement, W. C.) 377–390 (W. B. Saunders, Philadelphia, 2000).
3. Tononi, G. & Cirelli, C. Sleep and synaptic homeostasis: a hypothesis. *Brain Res. Bull.* **62,** 143–150 (2003).
4. Ghilardi, M. F. *et al.* Patterns of regional brain activation associated with different forms of motor learning. *Brain Res.* **871,** 127–145 (2000).
5. Ghilardi, M. F., Eidelberg, D., Silvestri, G. & Ghez, C. The differential effect of PD and normal aging on early explicit sequence learning. *Neurology* **60,** 1313–1319 (2003).
6. Finelli, L. A., Borbély, A. A. & Achermann, P. Functional topography of the human nonREM sleep electroencephalogram. *Eur. J. Neurosci.* **13,** 2282–2290 (2001).
7. Nichols, T. E. & Holmes, A. P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* **15,** 1–25 (2001).
8. Cohen, Y. E. & Andersen, R. A. A common reference frame for movement plans in the posterior parietal cortex. *Nature Rev. Neurosci.* **3,** 553–562 (2002).
9. Jonides, J. *et al.* Spatial working memory in humans as revealed by PET. *Nature* **363,** 623–625 (1993).
10. Mednick, S. C. *et al.* The restorative effect of naps on perceptual deterioration. *Nature Neurosci.* **5,** 677–681 (2002).
11. Stickgold, R., James, L. & Hobson, J. A. Visual discrimination learning requires sleep after training. *Nature Neurosci.* **3,** 1237–1238 (2000).
12. Walker, M. P., Brakefield, T., Morgan, A., Hobson, J. A. & Stickgold, R. Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron* **35,** 205–211 (2002).
13. Fenn, K. M., Nusbaum, H. C. & Margoliash, D. Consolidation during sleep of perceptual learning of spoken language. *Nature* **425,** 614–616 (2003).
14. Maquet, P., Schwartz, S., Passingham, R. & Frith, C. Sleep-related consolidation of a visuomotor skill: brain mechanisms as assessed by functional magnetic resonance imaging. *J. Neurosci.* **23,** 1432–1440 (2003).
15. Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J. & Sagi, D. Dependence on REM sleep of overnight improvement of a perceptual skill. *Science* **265,** 679–682 (1994).
16. Fischer, S., Hallschmid, M., Elsner, A. L. & Born, J. Sleep forms memory for finger skills. *Proc. Natl Acad. Sci. USA* **99,** 11987–11991 (2002).
17. Gais, S., Plihal, W., Wagner, U. & Born, J. Early sleep triggers memory for early visual discrimination skills. *Nature Neurosci.* **3,** 1335–1339 (2000).
18. Smith, C. Sleep states and memory processes. *Behav. Brain Res.* **69,** 137–145 (1995).
19. Vyazovskiy, V., Borbély, A. A. & Tobler, I. Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. *J. Sleep Res.* **9,** 367–371 (2000).
20. Kattler, H., Dijk, D.-J. & Borbély, A. A. Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. *J. Sleep Res.* **3,** 159–164 (1994).
21. Huber, R., Deboer, T. & Tobler, I. Topography of EEG dynamics after sleep deprivation in mice. *J. Neurophysiol.* **84,** 1888–1893 (2000).
22. Krueger, J. M. & Obál, F. Jr A neuronal group theory of sleep function. *J. Sleep Res.* **2,** 63–69 (1993).
23. Tononi, G. & Cirelli, C. Some considerations on sleep and neural plasticity. *Arch. Ital. Biol.* **139,** 221–241 (2001).
24. Cirelli, C., Gutierrez, C. M. & Tononi, G. Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron* **41,** 35–43 (2004).
25. Steriade, M. & Timofeev, I. Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron* **37,** 563–576 (2003).
26. Maquet, P. The role of sleep in learning and memory. *Science* **294,** 1048–1052 (2001).
27. Hoffman, K. L. & McNaughton, B. L. Sleep on it: cortical reorganization after-the-fact. *Trends Neurosci.* **25,** 1–2 (2001).
28. Stickgold, R., Hobson, J. A., Fosse, R. & Fosse, M. Sleep, learning, and dreams: off-line memory reprocessing. *Science* **294,** 1052–1057 (2001).
29. Benington, J. H. & Frank, M. G. Cellular and molecular connections between sleep and synaptic plasticity. *Prog. Neurobiol.* **69,** 71–101 (2003).
30. Walker, M. P. A refined model of sleep and the time course of memory formation. *Behav. Brain Sci.* (in the press).

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Resilient circadian oscillator revealed in individual cyanobacteria

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Circadian oscillators, which provide internal daily periodicity, are found in a variety of living organisms, including mammals, insects, plants, fungi and cyanobacteria[1]. Remarkably, these biochemical oscillators are resilient to external and internal modifications, such as temperature and cell division cycles. They have to be 'fluctuation (noise) resistant'[2] because relative fluctuations in the number of messenger RNA and protein molecules forming the intracellular oscillators are likely to be large. In multicellular organisms, the strong temporal stability of circadian clocks, despite molecular fluctuations, can easily be explained by intercellular interactions[3–5]. Here we study circadian rhythms and their stability in unicellular cyanobacteria *Synechoccocus elongatus*. Low-light-level microscopy has allowed us to measure gene expression under circadian control in single bacteria, showing that the circadian clock is indeed a property of individual cells. Our measurements show that the oscillators have a strong temporal stability with a correlation time of several months. In contrast to many circadian clocks in multicellular organisms, this stability seems to be ensured by the intracellular biochemical network, because the interactions between oscillators seem to be negligible.

Cyanobacteria *S. elongatus* sp. PCC7942, chosen for the present study, is among the simplest organisms showing circadian behaviour[6]. Recent investigations of large populations of these prokaryotic cells have shown that the expression of most of their genes is under the control of a circadian clock[7]. To measure circadian gene