surrounding genes.
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16. When mutators and nonmutators competed, mutator bacteria generally won [T. C. Gibson, M. L. Scheppe, E. C. Cox, *Science* **169**, 686 (1970); L. Chao and E. C. Cox, *Evolution* **37**, 125 (1983)].
17. Three of 12 cultures, continuously propagated over 10,000 generations, displayed a mutator phenotype; defective alleles of *mutS* in one and of *uvrD* in two of the cultures were responsible [P. D. Sniegowski, P. J. Gerrish, R. E. Lenski, personal communication].
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27. Overnight cultures in brain-heart infusion (BHI) broth were plated on Luria broth (LB) agar containing rifampicin (100 μg/ml), spectinomycin (100 μg/ml), or nalidixic acid (20 μg/ml). For reference, *E. coli* K12 (W3110) values were Rif^R, 1.5 ± 0.3; Spc^R, 0.2; and Nal^R, 1.3 ± 0.7.
28. Strains were transformed with multicopy plasmid clones harboring the wild-type gene for *mutH* (pGW1899), *mutL* (pGW1842), *mutS* (pGW1811), and *uvrD* (pGT26). pGW plasmids are described by P. P. Pang, A. S. Lundberg, and G. C. Walker [*J. Bacteriol.* **163**, 1007 (1985)], and pGT26 plasmids are described by G. Taucher-Scholz and H. Hoffmann-Berling [*Eur. J. Biochem.* **137**, 573 (1983)]. Mutators C396 and SL78 were transformed by phage P22 HT *int* transducing particles carrying plasmid clones [M. J. Orbach and E. N. Jackson, *J. Bacteriol.* **149**, 985 (1982)]. Other mutators were transformed by electroporation with the use of a Bio-Rad Gene Pulser apparatus and protocol supplied by the manufacturer.
29. The primers used were R3, 5′-TTACCTGAGTGCCTACGCC; F2, 5′-CTGGCGGATAAAAGCTCCC; S8, 5′-GCCCATGATGCAGCAGTAT; and L119, 5′-TGCATCTCGATGCACTGGAG. Long PCR was done with the Perkin Elmer XL PCR kit and 30 cycles of 1 min at 94°C, 1.5 min at 55°C, and 5 min at 68°C.
30. The authors thank M. J. Bessman, H. Ochman, R. M. Schaaper, P. I. Tarr, and T. S. Whittam for supplying bacterial strains and E. F. Boyd, E. C. Cox, P. E. Hartman, R. E. Lenski, P. Modrich, and P. D. Sniegowski for helpful discussions and comments.

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Neuronal Gene Expression in the Waking State: A Role for the Locus Coeruleus

Chiara Cirelli, Maria Pompeiano, Giulio Tononi*

Several transcription factors are expressed at higher levels in the waking than in the sleeping brain. In experiments with rats, the locus coeruleus, a noradrenergic nucleus with diffuse projections, was found to regulate such expression. In brain regions depleted of noradrenergic innervation, amounts of c-Fos and nerve growth factor–induced A after waking were as low as after sleep. Phosphorylation of cyclic adenosine monophosphate response element–binding protein was also reduced. In contrast, electroencephalographic activity was unchanged. The reduced activity of locus coeruleus neurons may explain why the induction of certain transcription factors, with potential effects on plasticity and learning, does not occur during sleep.

In most brain regions, the expression of some transcription factors that could mediate long-term changes in neural function, such as the immediate-early genes (IEGs) encoding c-Fos and nerve growth factor–induced A (NGFI-A) (*1*), is much greater during the waking state than during sleep (*2–4*). The reasons why the expression of these genes is high during waking and low during sleep are not known. A key factor might be the activity level of neuromodulatory systems with diffuse projections to many regions of the brain, such as the noradrenergic locus coeruleus (LC) (*5*). During sleep, LC neurons fire regularly at very low levels, whereas during waking, LC neurons fire regularly at higher levels and emit phasic, short bursts of action potentials when triggered by salient events (*6*). These neurons release norepinephrine (NE), which can modify neural activity and excitability (*7*) as well as the expression of certain genes, including IEGs (*8*).

Does the LC play a role in the increased expression of transcription factors that occurs during the waking state? To analyze this possibility, we implanted electrodes in rats for electroencephalographic (EEG) and electromyographic recordings; we then administered a unilateral injection into the LC of 6-hydroxydopamine (6-OHDA), a neurotoxin that destroys catecholaminergic neurons (*9*). In rats in which the LC was not lesioned, we observed little or no Fos protein expression after 3 hours of sleep ($N = 7$; Fig. 1A), but there was a marked bilateral expression of Fos in cerebral cortex, hippocampus, and other brain areas after 3 hours of waking induced by sleep deprivation ($N = 8$; Fig. 1A) (*10*). In contrast, in sleep-deprived rats in which the LC of one side had been lesioned ($N = 9$), Fos expression was almost abolished in cortical areas and hippocampus on the lesioned side, whereas on the intact side, Fos levels were high and comparable to those observed in normal animals after periods of waking (Fig. 1B) (*11*). The extent and effectiveness of LC lesions were determined by examining quantitatively the disappearance of NE cell bodies in the LC and of NE fibers in target cortical regions (Fig. 1B) (*12*). On average, in cortical areas in which NE fibers were reduced by more than 80%, Fos expression after waking was reduced by 76 ± 8% (mean ± SEM, $P < 0.001$, Wilcoxon signed-rank test for matched pairs) with respect to the intact side (*13*). In all animals examined, Fos immunoreactivity decreased in close spatial correspondence with the disappearance of NE innervation, even at the level of individual NE fibers. The reduction of Fos protein on the lesioned side was accompanied by a comparable decrease of c-Fos mRNA as revealed by in situ hybridization ($N = 3$) (*14, 15*).

Because there is evidence that the release of NE may be responsible for the increased levels of IEG expression that are observed after stressful manipulations (*16*), we also examined Fos expression in rats with unilateral LC lesions that had been spontaneously awake for 3 hours in the dark without any external intervention. The rats were killed at least 2 to 3 weeks after surgery, when percentages of recording time spent in different behavioral states had returned to control values. As in animals killed after sleep deprivation in the light, Fos levels were high on the intact side but very low or absent in cortical areas depleted of NE fibers (77 ± 1% reduction, $N = 6$, $P < 0.001$) (Fig. 2, A and B). Thus, the expression of Fos in cortex and hippocampus was due to the waking state per se rather

The Neurosciences Institute, 10640 J. J. Hopkins Drive, San Diego, CA 92121, USA.

*To whom correspondence should be addressed. E-mail: tononi@nsi.edu

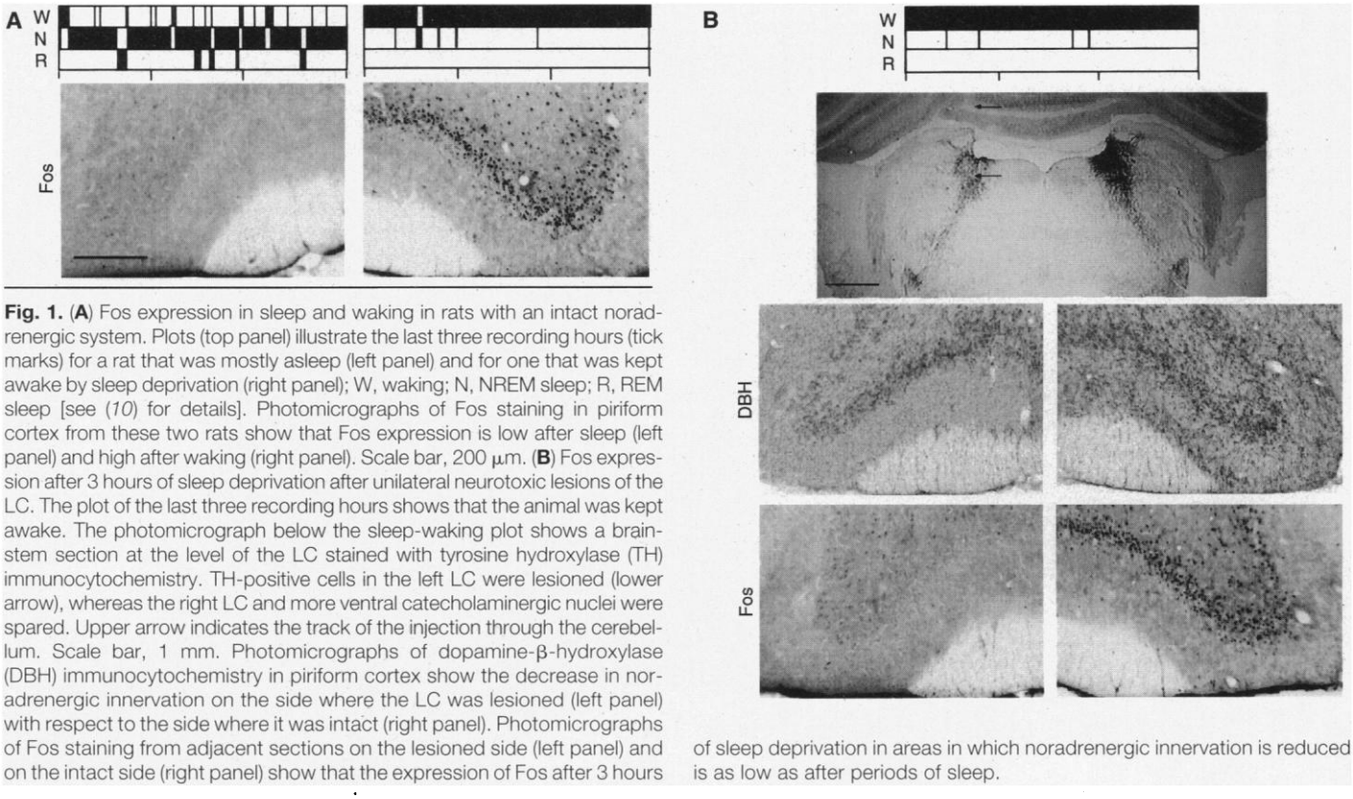

Fig. 1. (**A**) Fos expression in sleep and waking in rats with an intact noradrenergic system. Plots (top panel) illustrate the last three recording hours (tick marks) for a rat that was mostly asleep (left panel) and for one that was kept awake by sleep deprivation (right panel); W, waking; N, NREM sleep; R, REM sleep [see (*10*) for details]. Photomicrographs of Fos staining in piriform cortex from these two rats show that Fos expression is low after sleep (left panel) and high after waking (right panel). Scale bar, 200 μm. (**B**) Fos expression after 3 hours of sleep deprivation after unilateral neurotoxic lesions of the LC. The plot of the last three recording hours shows that the animal was kept awake. The photomicrograph below the sleep-waking plot shows a brainstem section at the level of the LC stained with tyrosine hydroxylase (TH) immunocytochemistry. TH-positive cells in the left LC were lesioned (lower arrow), whereas the right LC and more ventral catecholaminergic nuclei were spared. Upper arrow indicates the track of the injection through the cerebellum. Scale bar, 1 mm. Photomicrographs of dopamine-β-hydroxylase (DBH) immunocytochemistry in piriform cortex show the decrease in noradrenergic innervation on the side where the LC was lesioned (left panel) with respect to the side where it was intact (right panel). Photomicrographs of Fos staining from adjacent sections on the lesioned side (left panel) and on the intact side (right panel) show that the expression of Fos after 3 hours of sleep deprivation in areas in which noradrenergic innervation is reduced is as low as after periods of sleep.

than to stress; furthermore, an intact LC was essential for such expression (*17*).

Differences in IEG expression between sleep and waking are not limited to c-Fos (*17, 18*). The quantities of NGFI-A, another IEG that has higher basal levels of expression than c-Fos and that is induced more rapidly in response to physiological stimuli (*19, 20*), are also much higher in waking than in sleep (*17, 18*). We examined the expression of NGFI-A by immunocytochemistry on sections from animals with LC lesions adjacent to those used for Fos. We found that, as with Fos, NGFI-A concentrations in animals that had been awake were high on the intact side but were lower in cortical areas depleted of NE fibers (Fig. 2C). In contrast to Fos, a moderate amount of NGFI-A staining persisted after LC lesions, which is in accord with the persistence of a basal expression of this gene during sleep (*17, 18*).

Although there are a number of mechanisms by which NE release during waking might facilitate the induction of IEGs, an important pathway involves the phosphorylation of cyclic adenosine monophosphate response element–binding protein (CREB), which can act on the promoters for c-Fos and NGFI-A (*21*). Using an antibody specific for the phosphorylated Ser^{133} residue of CREB (P-CREB), we examined the amount of P-CREB in brain sections from rats in which the LC had been lesioned on one side. We found that P-CREB concentrations were considerably reduced in cortical areas depleted of NE fibers (Fig. 2D), in close correspondence with the decrease in Fos (Fig. 2B) and NGFI-A staining (Fig. 2C). Given the similar distribution of Fos expression during waking and of β-adrenergic receptors in the cortex (*22*), this result suggests that NE released by LC terminals during waking may induce Fos by

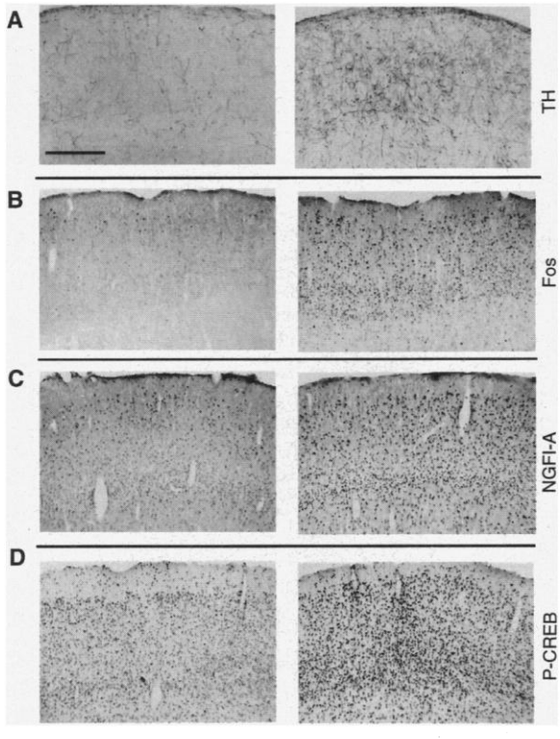

Fig. 2. Expression of IEGs after 3 hours of spontaneous waking following unilateral neurotoxic lesions of the LC. (**A**) TH immunocytochemistry of a rat with a lesion of the left LC that was killed after 3 hours of spontaneous waking in the dark. The panels show left and right parietal cortex, respectively. Scale bar, 200 μm. (**B**) Expression of Fos. Photomicrographs from adjacent sections show that Fos staining after spontaneous waking is high on the intact side (right) and almost absent on the lesioned side (left). (**C**) Expression of NGFI-A. NGFI-A staining is also higher on the intact side (right) than on the lesioned side (left). (**D**) Expression of P-CREB. The number of P-CREB–positive cells is lower on the side where the noradrenergic innervation has disappeared (left).

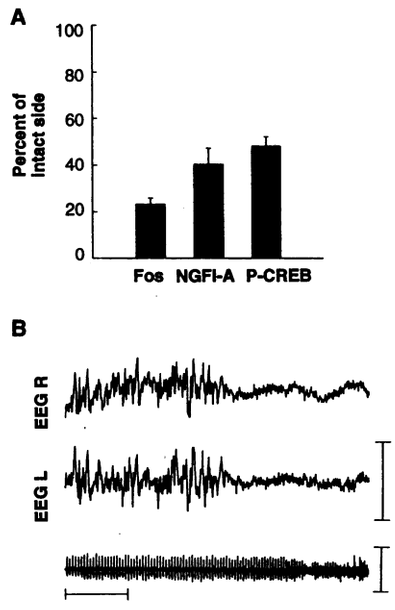

Fig. 3. Dissociation between the effects of lesions of the noradrenergic system on gene expression and on EEG activation. (**A**) Fos, NGFI-A, and P-CREB immunoreactivity in waking decreases after unilateral neurotoxic lesions of the LC. Mean percentage of stained cells on the lesioned side (intact side = 100%) in rats killed after sleep deprivation or after spontaneous waking where there was a widespread unilateral decrease in cortical NE innervation. Values refer to cortical areas where noradrenergic innervation was reduced by >80%. The decrease on the lesioned side was significant for Fos, NGFI-A, and P-CREB ($N = 9$, $P < 0.001$, Wilcoxon signed-rank test for matched pairs). (**B**) Raw EEG patterns are not modified. Raw EEGs were recorded from the intact (EEG R) and lesioned (EEG L) sides of the rat whose brain sections are shown in Fig. 2. The EEG (scale bar, 500 μV) and the electromyogram [scale bars, 150 μV (vertical) and 1 s (horizontal)] correspond to the transition between sleep and waking.

acting through a pathway that leads from β-adrenergic receptors to CREB phosphorylation (*23*).

Finally, we asked whether the reduction in IEG expression after lesions of the LC could be a result of the disappearance of the normal EEG activity seen in the waking state, because IEG expression is known to be activity dependent (*20, 24*). Thus, we examined the EEG of rats in which LC lesions had produced a widespread decrease of NE fibers in one hemisphere (Fig. 3). There was a relative decrease in the number of Fos-, NGFI-A–, and P-CREB–stained cells on the lesioned side in these animals (Fig. 3A). Despite this decrease, we did not detect differences between the raw EEG on the intact and lesioned side in any of these animals (one example is shown in Fig. 3B).

Because patches of spared NE fibers and volume conduction effects could have partially masked a change in the EEG, another group of rats received a systemic injection of *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) to destroy NE innervation of the cortex bilaterally and diffusely; they were killed 8 to 10 days later after 3 hours of waking. This neurotoxin selectively destroys NE fibers originating from the LC with a mechanism of action different from that of 6-OHDA (*25*). In all animals in which NE fibers in the cerebral cortex were bilaterally reduced (>90%, $N = 5$), there was a bilateral reduction of Fos (Fig. 4A), NGFI-A, and P-CREB staining with respect to controls that was similar in magnitude to that observed unilaterally after LC lesions with 6-OHDA. Both the raw EEG and its power density spectrum were not significantly different before and after (8 to 10 days) injection of DSP-4 (Fig. 4B) (*26*). Thus, in the absence of an intact NE system, waking behavior accompanied by low-voltage fast activity patterns is not sufficient for induction of IEGs. Conversely, it is likely that IEG expression is low during sleep because of the low level of LC activity rather than because of the presence of high-voltage slow activity per se. The low level of activity during sleep of other neuromodulatory systems with diffuse projections, such as the serotoninergic and the histaminergic systems, may also play a role [compare with (*5*)].

These results demonstrate a dissociation between the role of the LC in EEG activation and in activation of IEGs during the waking state. The activation of the EEG corresponds to the transition from a pattern of high voltages and low frequencies during sleep to one of low voltages and high frequencies during waking (*27*). As we have shown, although LC lesions have no persistent effects on EEG patterns of waking (*28*), they have striking and persistent effects on the expression of IEGs, which is reduced to the levels found in sleep. On the other hand, neural activation has also been defined as a readiness to react to external,

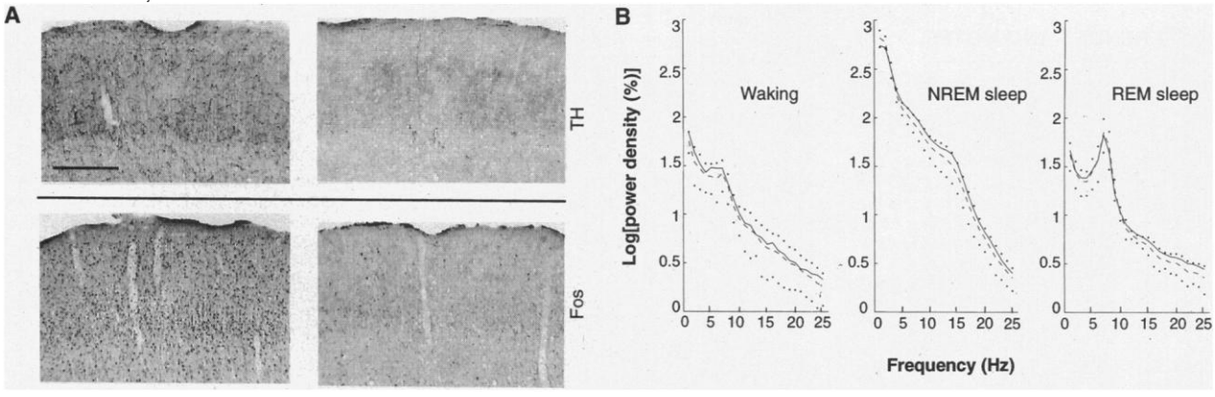

Fig. 4. (**A**) Reduction of Fos expression after 3 hours of sleep deprivation following bilateral lesions of LC terminals as a result of DSP-4 injections. TH immunocytochemistry in parietal cortex of a control animal (left panel) and of an animal that had received an injection of DSP-4 leading to a decrease in noradrenergic innervation (right panel). Photomicrographs are of Fos staining from adjacent sections from the control animal (left panel) and the animal injected with DSP-4 (right panel). Both animals were sleep-deprived for 3 hours. Fos concentrations are high in the control and low in the animal injected with DSP-4. Scale bar, 200 μm. (**B**) EEG power density is not modified. Mean power density of the EEG for different behavioral states recorded before (solid line) and after (dashed line) DSP-4 injections, resulting in a >90% decrease in noradrenergic innervation with respect to matched controls. Power densities expressed as percentage of the mean value during NREM sleep for each animal are plotted on a logarithmic scale. Dots indicate the standard deviation of power density values before the injections ($N = 5$) for each frequency bin. Despite the marked bilateral reduction in Fos, NGFI-A, and P-CREB staining in cortex and hippocampus in these animals, the mean spectrum after DSP-4 injection was within 1 SD of the values observed before DSP-4 injection, and no significant difference was found for any frequency bin (Wilcoxon signed-rank test for matched pairs).

salient signals (29), and NE release, which is high during waking, can enhance the readiness of neurons to respond to such signals by increasing their signal-to-noise ratio (7). It is possible that, by modifying second messenger concentrations, protein phosphorylation (30), or synaptic activity in ways not detected by the EEG, the release of NE by LC neurons may also lead to the activation of certain transcription factors during waking and permit the animal to respond to salient extrinsic signals with long-term changes in neural function. The expression of P-CREB and of IEGs has been linked to phenomena such as learning, developmental plasticity, and long-term potentiation (31). Likewise, several lines of evidence indicate that a sufficient quantity of NE is required for these phenomena to occur (32). A difference in the amount of NE release may explain why learning and conditioning, inasmuch as they require the activation of transcription factors, occur more readily during waking than during sleep (33). This might also explain why events occurring during rapid eye movement (REM) sleep are generally not remembered (34), given that in this state, although the EEG pattern is similar to that in waking, LC cells are not firing. If differences in the induction of transcription factors similar to those observed between waking and sleep also occur between other conditions associated with high LC activity, such as orienting to novel stimuli, associative learning, and exploratory behavior, and with low LC activity, such as grooming and consummatory behavior (6), then the firing of the LC may well be a key factor in determining whether neural activity is accompanied by neural plasticity.

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10. These animals included normal controls, rats with 6-OHDA injections that missed the LC, and rats with vehicle injections [saline containing ascorbic acid (1 mg/ml)] in the LC of one side (N = 15). The amount and distribution of Fos staining after sleep and after sleep deprivation in each of these cases were comparable to that reported in previous studies (4). Sleeping rats were killed after spending at least 80% of the previous 3 hours asleep, at the end of an uninterrupted period of sleep of 45 min or longer. Sleep-deprived rats never showed slow-wave activity for longer than 10 s. Both groups of animals were killed during the light hours at the same circadian time (13:00) and at least 2 to 3 weeks after surgery. Rats were anesthetized with pentobarbital and transcardially perfused with 0.9% cold saline (50 ml) followed by 4% cold paraformaldehyde in 0.1 M phosphate buffer (350 ml, pH 7.4). Brains were removed, fixed for 5 hours at 4°C, cryoprotected (in 20% sucrose), and frozen. Frontal sections (40 μm thick) of the entire brain were cut on a cryostat. Fos protein amounts were analyzed by immunocytochemistry as described (3), except that free-floating instead of mounted sections were used. Polyclonal antibodies from Cambridge Research Biochemicals (CRB) (OA-11-824, 1:2000 dilution) and Oncogene (ab 2, 1:5000 dilution) were used. Both antibodies were raised against the NH_2-terminal region of Fos protein and gave similar results [G. E. Hoffman, M. S. Smith, M. D. Fitzsimmons, *Neuroprotocols* **1**, 52 (1992)]. Double-labeling experiments with antibodies against Fos (CRB, 1:1000) and glial fibrillary acidic protein [GFAP (Sigma), 1:400] or microtubule-associated protein 2 [MAP-2 (Sigma), 1:250] performed on a subset of sections showed that Fos-positive cells were neurons because they stained for MAP-2 but not for GFAP.
11. These animals showed no obvious behavioral alteration or changes in the sleep-waking cycle. Two weeks or more after surgery, percentages of recording time spent in different behavioral states [light hours: waking, 32 ± 2%; non-REM (NREM) sleep, 52 ± 2%; REM sleep, 16 ± 1%; dark hours: waking, 67 ± 3%; NREM sleep, 28 ± 2%; REM sleep, 5 ± 1%] were not significantly different from those of age-matched controls. Details of polysomnographic recordings and analysis are given in (3). In rats, intracerebroventricular injections of 6-OHDA associated with an almost complete bilateral decrease of cortical NE content caused only a transient decrease in waking [E. Hartmann and R. Chung, *Nature* **233**, 425 (1971); P. Lidbrink, *Brain Res.* **74**, 19 (1974)]. Bilateral LC lesions with 6-OHDA had no effects on the sleep-waking cycle [B. Roussel, J. F. Pujol, M. Jouvet, *Arch. Ital. Biol.* **114**, 188 (1976)].
12. NE cell bodies and fibers were identified by incubating free-floating sections of the entire brain with a monoclonal antibody to tyrosine hydroxylase [TH (Boehringer), 1:1000 dilution] or, to ensure specificity, with a polyclonal antibody to dopamine-β-hydroxylase (Eugene, 1:1000 dilution). Immunoreactivity was detected with the avidin-biotin immunoperoxidase system (Vector) and nickel-enhanced diaminobenzidine. An effective unilateral LC lesion was scored if there was an almost complete disappearance of TH immunoreactivity in at least one sector of the LC of the injected side for at least four consecutive sections, whereas the amount of TH immunoreactivity in the LC of the intact side was comparable to that of control animals. The critical variable in determining the effectiveness of the injection was its site rather than its volume. It has been reported that 6-OHDA injected into the LC can affect other noradrenergic groups [A. H. Engelbrecht *et al.*, *J. Neurosci. Methods*, **52**, 57 (1994)]. Although this risk was reduced by the long injection times used in this study, the number of TH-positive cells in A5 and A7 as well as in A1 and A2 was counted in all animals, and TH and dopamine-β-hydroxylase staining in the hypothalamus were quantified by densitometry with an Image-1/Metamorph imaging system (Universal Imaging). Except for one animal with a slight reduction in the number of TH-positive cell bodies in A7 on the side of the injection, these values did not differ from those of control animals. Lesions of the dopaminergic system were also ruled out because the number of TH-positive cells in mesencephalic groups A8 and A10 was not modified. Decreases in TH and dopamine-β-hydroxylase staining in the cerebral cortex were also quantified by densitometry. Regions showing the strongest decrease in TH and dopamine-β-hydroxylase staining differed slightly from animal to animal, depending on the exact location of the 6-OHDA lesion within the LC. Decreases in TH and dopamine-β-hydroxylase staining were evident ipsilaterally to the LC lesion, consistent with the observation that only a small fraction of NE fibers arise from the contralateral LC [B. D. Waterhouse, C.-S. Lin, R. A. Burne, D. J. Woodward, *J. Comp.*

Neurol. **217**, 418 (1983)]. Previous studies have shown that, in brain regions depleted of NE innervation from the LC by neurotoxic lesions, NE quantities measured by high-performance liquid chromatography were correspondingly depleted [J.-M. Fritschy and R. Grzanna, *Prog. Brain Res.* **88**, 257 (1991)]. After 6-OHDA injections, no evidence of neuronal degeneration was found in cortical regions with cresyl violet staining, in agreement with previous reports [(J. C. Hedreen and J. P. Chalmers, *Brain Res.* **47**, 1 (1972)].

13. Cell counting was performed with the Image-1/Metamorph imaging system as described (*3*) by observers blind to the origin of the sections. In rats with unilateral lesions of the LC killed after 3 hours of sleep, the amounts of Fos staining were uniformly low and indistinguishable from those observed in sleeping controls. We did not observe differences between the effects of right and left LC lesions. In two animals, presumably because of a ventricular injection, as suggested by the position of the cannula track, NE cell bodies in the LC and NE fibers in the cortex were depleted bilaterally. In these animals, Fos staining after sleep deprivation was bilaterally low.
14. C. Cirelli, M. Pompeiano, G. Tononi, data not shown.
15. To detect c-Fos mRNA by nonradioactive in situ hybridization, we quickly removed brains and froze them without perfusion. Sections were mounted on slides, fixed, incubated with predigested Pronase, washed in phosphate-buffered saline, and then incubated overnight at 55°C with a c-Fos digoxigenin-labeled RNA probe (400 ng/ml). The probe was prepared by in vitro transcription of the c-Fos exon 4 mouse cDNA (bases 2437 to 2187) in pTRIPLEscriptTM vector (Ambion) with T7 RNA polymerase (Promega). After hybridization, sections were rinsed and incubated with an antibody to digoxigenin tagged with alkaline phosphatase (Boehringer); a color reaction was developed by using nitroblue tetrazolium as substrate [for details see C. Cirelli, M. Pompeiano, P. Arrighi, G. Tononi, *Arch. Ital. Biol.* **133**, 143 (1995)].
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17. A systematic quantification of Fos and NGFI-A expression in the brain after waking induced by sleep deprivation during the light hours or after spontaneous waking during the dark hours is found in (*4*) and (*3*), respectively.
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19. J. Milbrandt, *Science* **238**, 797 (1987); K.-H. Schlingensiepen, K. Lüno, W. Brysch, *Neurosci. Lett.* **122**, 67 (1991). NGFI-A mRNA concentrations also respond to manipulations of the NE system [G. Bing, D. Filer, J. C. Miller, E. A. Stone, *Mol. Brain Res.* **11**, 43 (1991); R. V. Bhat and J. M. Baraban, *Eur. J. Pharmacol.* **227**, 447 (1992); P.-J. Shen, T. C. D. Burazin, A. L. Gundlach, *Mol. Brain Res.* **28**, 222 (1995)].
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22. Like β-adrenergic receptors, Fos-positive cells were most numerous in layers II, III, and VI [compare with J. M. Palacios, W. S. Young III, M. J. Kuhar, *Science* **208**, 1378 (1980); T. C. Rainbow, B. Parsons, B. B. Wolfe, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1585 (1984); C. Aoki, T. H. Joh, V. M. Pickel, *Brain Res.* **437**, 264 (1987)].
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24. K. M. Rosen, M. A. McCormack, L. Villa-Komaroff, G. D. Mower, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5437 (1992). Consistent with these results, in a rat in which the trigeminothalamic tract was accidentally lesioned, we observed a marked reduction of Fos, NGFI-A, and P-CREB staining localized to the contralateral somatosensory cortex in the presence of normal NE innervation (*14*).
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26. Two gold-plated, round-tipped miniature screw electrodes implanted over the frontal cortex (2 mm anterior to the bregma and 2 mm lateral to the midline) and over the occipital cortex (4 mm posterior to the bregma and 3.8 mm lateral to the midline) were used to record the cortical EEG. EEG signals were low-pass filtered (−3 dB at 30 Hz, 24 dB per octave), analog-to-digital converted (sampling rate, 128 Hz), and subjected to spectral analysis. EEG power density values were computed for successive 4-s periods (24 hours of recording for each animal) in the frequency range from 0.25 to 25 Hz (collapsed into 0.5-Hz bins between 0.25 and 5 Hz and into 1-Hz bins between 5.25 and 25 Hz). Recording epochs showing EEG artifacts were not used for spectral analysis.
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28. Because the percentages of recording time spent in different behavioral states as well as overall EEG patterns of waking and sleep 8 to 10 days after lesioning of LC terminals were indistinguishable from those before the lesions, our results confirm that other reticular-activating systems in addition to the NE system are sufficient for maintaining long-term EEG activation [B. E. Jones, S. T. Harper, A. E. Halaris, *Brain Res.* **124**, 473 (1977); T. E. Robinson, C. H. Vanderwolf, B. A. Pappas, *ibid.* **138**, 75 (1977); P. Riekkinen Jr., M. Riekkinen, A. Valjakka, P. Riekkinen, J. Sirviö, *ibid.* **570**, 293 (1992)]. Short-term changes in LC activity, however, have definite effects on the EEG. Acute bilateral, but not unilateral, inactivation of the LC is associated with slowing of the EEG [R. Cespuglio, M. E. Gomez, H. Faradji, M. Jouvet, *Electroencephalogr. Clin. Neurophysiol.* **54**, 570 (1982); C. W. Berridge, M. E. Page, R. J. Valentino, S. L. Foote, *Neuroscience* **55**, 381 (1993)], and neurochemical activation of the LC is followed by EEG activation [C. W. Berridge and S. L. Foote, *J. Neurosci.* **11**, 3135 (1991)].
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