A Molecular Window on Sleep: Changes in Gene Expression between Sleep and Wakefulness

CHIARA CIRELLI
Department of Psychiatry
University of Wisconsin–Madison

Sleep is thought to be "by the brain and for the brain," but despite decades of behavioral and neurophysiologic research, we still do not know why the brain actually needs to sleep. Recently, gene expression studies have allowed researchers to investigate the molecular correlates of sleep and wakefulness and to gain new insights into the benefits that sleep may bring at the cellular level. In the latest series of studies, a genome-wide screening of brain gene expression was performed in rats that had been asleep, spontaneously awake, or sleep deprived for 8 hours. It was found that of ~15,000 transcripts expressed in the cerebral cortex, about 5% change their expression levels depending on behavioral state but independently of time of day. Half of the modulated genes increase in wakefulness and half in sleep. Moreover, wakefulness-related and sleep-related transcripts belong to different functional categories. Waking-related transcripts are involved in energy metabolism, excitatory neurotransmission, transcriptional activation, synaptic potentiation and memory acquisition, and the response to cellular stress. Sleep-related transcripts are involved in brain protein synthesis, synaptic consolidation/depression, and membrane trafficking and maintenance, including cholesterol metabolism, myelin formation, and synaptic vesicle turnover. NEUROSCIENTIST 11(1):63–74, 2005. DOI: 10.1177/1073858404270900

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Introduction: The Problem of Sleep

Sleep must be exceedingly important judging from the time spent in this state and from its ubiquitous occurrence in all animals studied so far, from humans to fruit flies (Tobler 2000). The drive for sleep is manifested in sleep-deprivation experiments in which sleep pressure becomes overwhelming and the maintenance of waking is virtually impossible. Like hunger or thirst, the drive for sleep appears to satisfy an elementary need. However, unlike eating and drinking, the purpose of sleep remains obscure: Sleep is the one major biological process whose functions have not yet been specified.

All the available evidence suggests that it is the brain, rather than the body, that needs to sleep. Sleep-deprived subjects tend to take longer to respond to stimuli, particularly when tasks are monotonous and low in cognitive demands. In fact, sleep deprivation produces more than just decreased alertness. Tasks that emphasize higher cognitive functions, such as logical reasoning, encoding, decoding, and parsing complex sentences, complex subtraction tasks, and tasks requiring divergent thinking, such as those involving a flexible thinking style and the ability to focus on a large number of goals simultaneously, are significantly affected even after one night of sleep deprivation. Similarly, tasks requiring sustained attention, such as those including goal-directed activities, can be impaired by even a few hours of sleep deprivation. Thus, sleep loss causes attention deficits, decrease in short-term memory, speech impediments, perseveration, and inflexible thinking (Harrison and Horne 2000).

Impairment in cognitive performance is observed not only after total sleep deprivation but also after sleep restriction, for instance, when subjects limit their daily amount of sleep to 4 hours for 2 weeks (Dinges and others 1997; Doran and others 2001; Van Dongen and others 2003). The detrimental effects of prolonged waking on cognition are not only dose dependent but also cumulative and can subside only after a period of recovery sleep but not after a period of restful wakefulness. Thus, the muscular fatigue caused by strenuous exercise, as well as the subjective sleepiness associated with sleep loss, can be reverted by a rest break of a few hours, but cognitive performance returns to baseline levels only after a nap or a full night of recovery sleep (reviewed in Horne 1988; Rogers and others 2003). In fact, if sleep loss was sustained, two full nights of recovery sleep are required (Dinges and others 1997).

The cognitive impairment caused by sleep loss has striking practical consequences. Each year, errors due to sleep deprivation and sleepiness cause 25,000 deaths, cause 2.5 million disabling injuries, and cost more than $56 billion in the United States alone (National Commission on Sleep Disorders Research). The National Highway Traffic Safety Administration esti-

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Address correspondence to: Chiara Cirelli, MD, PhD, University of Wisconsin–Madison, Department of Psychiatry, 6001 Research Park Blvd., Madison, WI 53719 (e-mail: ccirelli@wisc.edu).

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mates that every year, 4% of all fatal motor vehicle crashes are caused by drowsy driving.

But why does the brain need to sleep? Some evidence indicates that sleep may represent a favorable time for brain protein synthesis (Ramm and Smith 1990; Nakanishi and others 1997). Another possibility, suggested by behavioral studies (Stickgold and others 2001; Walker and others 2002; Huber and others 2004), is that sleep may improve the performance of tasks acquired during the previous waking period. It is also widely thought that the functions of sleep may ultimately relate to cellular and molecular aspects of neural function (Moruzzi 1972; Rechtschaffen 1998; Tononi and Cirelli 2001; Steriade and Timofeev 2003; Tononi and Cirelli 2003). It has been suggested that sleep is needed to maintain the synaptic efficacy of the neural circuits not frequently used during waking (Krueger and others 1995; Kavanau 1997). Alternatively, sleep may be required to downscale synapses whose number and/or weight have increased during waking (Tononi and Cirelli 2003). Several of these hypotheses are not mutually exclusive, and sleep may serve more than one important function.

The rationale underlying our research is that an understanding of the benefits that sleep may bring at the cellular level requires an extensive analysis of its molecular correlates. The identification of all the genes whose expression changes in the brain between sleep and wakefulness may suggest why brain cells need to sleep and why their functions are impaired if they are prevented from doing so during sleep deprivation.

Early Studies: Global Changes of Brain RNA and Protein Content Related to Sleep and Sleep Deprivation

A few years ago, most scientists agreed that sleep and wakefulness differ significantly in terms of metabolism, electrophysiological activity, and behavior, just to name a few examples, but the idea that they could also differ at the level of gene expression was not widespread. Yet the typical duration of sleep/waking states and the time constants of their regulation are in the range of minutes and hours rather than seconds or milliseconds. These time constants, which apply to humans as well as to animals as different as birds, rodents, and fruit flies, make it plausible that gene expression may be subject to significant modulations in the course of sleep and waking. Moreover, as summarized below, several studies had at least suggested that global changes in brain gene expression occur between sleep and waking.

Early experiments did not focus on specific genes but examined overall changes in RNA content or synthesis, as well as global changes in protein synthesis in relation to sleep and waking or sleep deprivation. In a series of pioneering studies, Giuditta and colleagues examined whether sleep could influence the synthesis of RNA in the brain (Vitale-Neugebauer and others 1970; Giuditta and others 1980). After the injection of [^{3}H]-orotate intraventricularly, they measured its incorporation into newly synthesized RNA and correlated the accumulation of labeled RNA with the amount of sleep during the period of incorporation. They found that in the fraction of cerebral cortex containing large nuclei, the relative content of radioactive RNA was increased in sleep with respect to waking, suggesting that during sleep, nuclei accumulate newly synthesized RNA at a faster rate. Interestingly, the effect was present in both the neuronal and the mixed fraction, indicating that RNA synthesis could take place also in the glial compartment. Panov (1982) found variations in protein and RNA content in individual neurons and glial cells of some brain stem nuclei after 1 to 4 days of total or selective REM sleep deprivation. Bobillier and colleagues (1971) reported a generalized decrease of [^{3}H]-amino acid incorporation into the proteins of telencephalon and brain stem after 3 hours of total sleep deprivation in rats. Conversely, a striking increase of labeled proteins was found in rats that were allowed to sleep for 1.5 hours after 1.5 hours of total sleep deprivation. Ramm and Smith (1990) found that the rate at which labeled leucine was incorporated into the rat brain was positively correlated with the occurrence of non–rapid eye movement (NREM) sleep but not with that of either wakefulness or REM sleep. The positive correlation between protein synthesis rate and NREM sleep was present in the brain as a whole and in several discrete brain regions, although none of them were particularly striking. In a later study in which leucine incorporation was measured in the brain of rhesus monkeys, Nakanishi and colleagues (1997) also found that in most brain regions, protein synthesis rate was positively correlated with slow-wave sleep. Finally, a recent preliminary proteomic analysis using SELDI-Mass Spectrometry (a method to perform a large-scale profiling of hundreds of brain proteins) found a general decrease in protein levels in the cerebral cortex of sleep-deprived mice relative to sleeping mice (Ding and others 2004). Thus, more than 20 years ago, there was already evidence that significant changes in gene expression could occur between sleep, wakefulness, and sleep deprivation. Moreover, several experiments had suggested that sleep may favor protein synthesis, whereas sleep deprivation may have a negative impact on it. The early studies, however, did not address the question of how many and which genes change their expression in a state-dependent manner.

Changes in the Expression of Immediate Early Genes between Sleep and Wakefulness

A series of studies in the mid-1990s examined the effects of sleep and wakefulness on the expression of immediate early genes (IEGs) such as *c-fos*, *NGFI-A, c-jun*, and *junB*. IEGs share the property that their transcription is induced via preexisting cell proteins without requiring de novo protein synthesis. This property is analogous to that of the IEGs of some viruses and bacteriophages, which are expressed immediately after the

infection of the cell in the absence of cellular protein synthesis. In fact, the gene *fos* was first identified as a retroviral gene (*v-fos*) present in the *F*inkel-Biskis-Jinkins *os*teosarcoma virus, an oncogene that has transforming ability when overexpressed. *c-Fos* is the normal cellular gene (or proto-oncogene) from which *v-fos* evolved. A significant number of IEGs, such as *c-fos* and other members of the *fos* and *jun* families, encode transcription factors like Fos, which, by binding to DNA regulatory regions, can control the expression of many other target genes (e.g., Sheng and Greenberg 1990; Herrera and Robertson 1996).

Several laboratories (reviewed in Cirelli and Tononi 2000b) examined IEG expression with targeted approaches such as in situ hybridization and immunocytochemistry using probes specific for the mRNA and/or the protein product of these genes. Our studies (Pompeiano and others 1994; Cirelli and others 1995; Pompeiano and others 1997) showed that the expression of *c-fos* and *NGFI-A* is low or absent in most brain regions if the animals had spent most of the previous 3 to 8 hours asleep, whereas it is high if the animals had been either spontaneously awake or sleep deprived for a few hours before sacrifice (Fig. 1). In awake animals, for instance, the expression of *c-fos* is high in most regions of the neocortex and allocortex, including frontal, motor, parietal, temporal, occipital, cingulate, insular, piriform, and entorhinal areas. During waking, *c-fos* expression is also high in several hypothalamic areas (medial and lateral preoptic areas, posterior hypothalamic area, supramammillary nuclei), septum, amygdala, and thalamus (paraventricular, rhomboid, reunions nuclei, and intralaminar nuclei), as well as in the brain stem (superior and inferior colliculi, central gray, dorsal raphe, locus coeruleus, and parabrachial nuclei). Interestingly, even in areas with a high level of expression, *c-fos* is not uniformly expressed in all neurons. In the cerebral cortex, for instance, Fos-positive cells are scattered across all layers, but they represent a small fraction of the cells present in a given section. The expression of *c-fos* is not strictly proportional to the amount of prior waking, as indicated by studies of sleep deprivation ranging from 3 to 24 hours. In most brain regions, and in particular in the cerebral cortex, the overall levels of *c-fos* are in fact higher after 3 than after 24 hours of sleep deprivation. After long-term sleep deprivation lasting 5 to 14 days, only a few scattered Fos-positive cells are present in the cerebral cortex, with no specific localization to any cortical area or cortical layer (Cirelli and Tononi, unpublished results). This suggests that the main determinant of *c-fos* expression during waking is not the duration of waking per se. In fact, a series of experiments in our laboratory, reviewed in a later section, demonstrated that a major reason why *c-fos*, as well as *NGFI-A*, *P-CREB*, and several other genes involved in synaptic plasticity, is expressed at higher levels during waking than during sleep is the activity of the noradrenergic system of the locus coeruleus (LC). LC cells are active during waking and much less so or not at all during sleep (Aston-Jones and Bloom 1981a).

Whole-Genome Analysis of Gene Expression in Sleep and Waking

The finding that sleep and waking are associated with very different levels of expression of Fos, NGFI-A, and P-CREB implied that widespread transcriptional changes may occur when the brain transitions from one behavioral state to another. As mentioned before, this is because Fos, NGFI-A, and P-CREB are transcription factors, and therefore their up- or down-regulation can trigger changes in the pattern of expression of many other genes.

Over the years, our laboratory has performed a gene expression profiling of the sleeping and awake brain using different techniques ranging from mRNA differential display and nylon membrane arrays (Cirelli and Tononi 1998, 2000c) to GeneChip technology (Cirelli and others 2004). In all cases, the experimental paradigm was designed to distinguish between changes in gene expression related to sleep and waking per se, as opposed to circadian time or to the sleep-deprivation procedure. As shown in Figure 2, brain gene expression was compared between spontaneously asleep (S), sleep-deprived (SD), and spontaneously awake (W) rats. S rats were killed at 6 p.m. during their usual sleep period, SD rats were killed at the same time of day after having been kept awake for 8 hours, and W rats were killed at 6 a.m. during their usual waking period. As expected, the electrocorticogram (EEG) of awake rats (W and SD groups) was characterized by low-voltage/high-frequency patterns, whereas the EEG of sleeping rats (S group) was dominated by higher voltages/lower frequencies (slow-wave activity) and characteristic sleep rhythms such as spindles and slow waves (Fig. 2). Thus, at the time of sacrifice, S rats had been predominantly asleep, whereas W and SD rats had been predominantly awake for several hours. Because S and SD rats were sacrificed at the same time of day but in an opposite behavioral state, and because SD and W rats were sacrificed 12 hours apart but in the same behavioral state, day/night and sleep/wakefulness effects could be dissociated.

Our analysis focused on the cerebral cortex, the brain area that generates the characteristic electrical rhythms of sleep (Steriade and Timofeev 2003) and responds to prolonged wakefulness with clear signs of increasing sleep pressure, such as an increase in slow-wave activity during NREM sleep (Borbély and Achermann 1999). The cerebral cortex is also responsible for the cognitive defects observed after sleep deprivation, which increase progressively as a function of prior time awake (Van Dongen and others 2003). Thus, the cerebral cortex was chosen as the most informative brain region to examine the cellular consequences of sleep and wakefulness.

The first finding of this systematic study was that up to ~5% of the transcribed sequences tested in the cere-

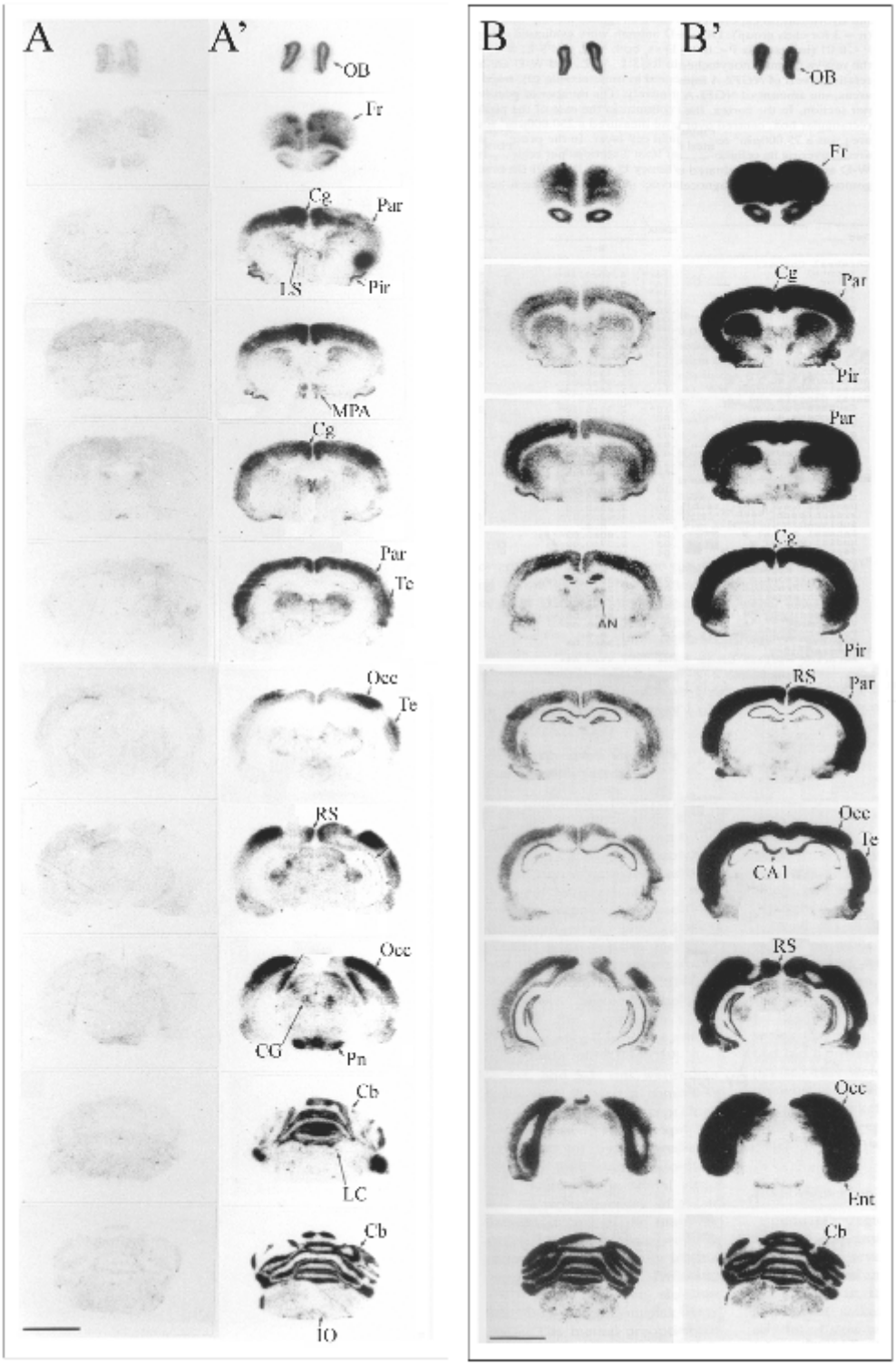

Fig. 1. Expression of *c-fos* and *NGFI-A* in sleep and wakefulness. In situ hybridization shows the differential expression of *c-fos* (*A*, *A'*) and *NGFI-A* (*B*, *B'*) in brain sections of a rat sacrificed after 5 hours of spontaneous sleep (*A*, *B*) and of a rat sacrificed after 5 hours of sleep deprivation (*A'*, *B'*). *c-Fos* and *NGFI-A* mRNA levels are low in the sleeping rat, whereas they significantly increase in most brain regions after a few hours of sleep deprivation. Scale bar = 5 mm. CA1 = field CA1 of Ammon's horn; Cb = cerebellum; Cg = cingulate cortex; CG = central gray; Ent = entorhinal cortex; Fr = frontal cortex; IO = inferior olive; LC = locus coeruleus; LS = lateral septum; MPA = medial preoptic area; OB = olfactory bulb; Occ = occipital cortex; Par = parietal cortex; Pir = piriform cortex; Pn = pontine nuclei; RS = retrosplenial cortex; Te = temporal cortex.

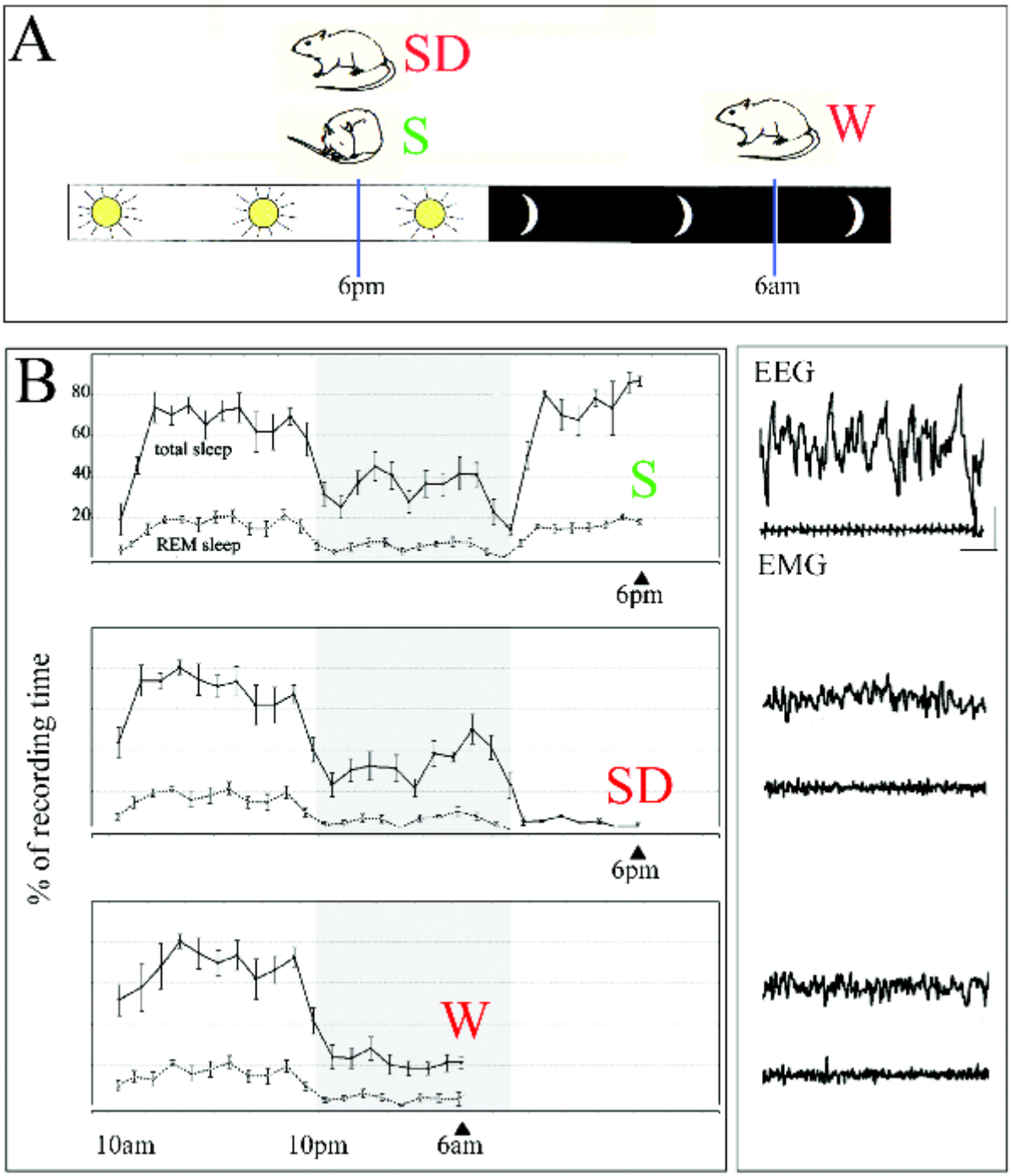

Fig. 2. The three experimental groups (S = sleep; SD = sleep deprivation; W = wakefulness) selected to identify gene expression changes associated with behavioral state as opposed to time of day. *A*, Schematic of the experimental paradigm. Rats are nocturnal and therefore spontaneously asleep for most of the light period and spontaneously awake for most of the dark period. S rats were sacrificed 8 hours after lights-on (6 p.m.) after spending at least 75% of the previous 8 hours asleep. W rats were killed 8 hours after lights-off (6 a.m.) after spending at least 70% of the previous 8 hours awake. SD rats were killed at 6 p.m. as S rats, but they were kept awake for the previous 8 hours by introducing novel objects in their recording cages. *B* (*left panels*), Total sleep (rapid eye movement sleep [REM] + non-REM sleep [NREM]) and REM sleep in S, SD, and W rats during the last 20 to 32 hours before sacrifice (indicated by an arrowhead). Represented values are mean ± SEM for the 6 rats/group used for microarray analysis. *B* (*right panels*), Representative examples of the prevailing electrocorticogram (EEG) activity during the last 8 hours before sacrifice. The low-voltage, fast-activity cortical EEG of SD and W rats is associated with high electromyographic (EMG) activity, whereas the slow waves in the cortical EEG of S rats are associated with low EMG activity. Scale bars: *x*-axis = 1 second, *y*-axis = 50 μV. It should be mentioned that this protocol was not designed to distinguish between the effects on gene expression of different sleep stages (NREM vs. REM sleep). As expected, however, NREM sleep episodes make up the majority of sleep time in S rats.

bral cortex (752, 4.9% of 15,459) are up- or down-regulated in rats that had slept for 8 hours relative to rats that had been spontaneously awake or sleep deprived for a similar period of time. These sequences included both known, fully-annotated transcripts as well as expressed sequence tags (ESTs; i.e., cDNA subsequences that are quickly generated to inventory the transcribed components of a genome; several ESTs can correspond to the same full-length transcript). Interestingly, a similar number of transcribed sequences (808, 5.2% of 15,459) were found to change their expression in the cerebral cortex of S, SD, and W rats because of time of day rather than because of behavioral state. Thus, day/night time and sleep/wakefulness appear to influence gene expression in the cerebral cortex to a similar extent. This finding has an important practical implication, namely, that changes in behavioral state should be taken into account in gene expression studies involving behavior. Most array studies that use nocturnal animals such as rats and mice compare gene expression between an "experimental" group, which is asked to perform, learn, and move during the day, when it would normally sleep, and a "control" group, which is left undisturbed and thus sleeps most of the time. The potential confounding effect of comparing animals in different behavioral states is also evident in studies specifically aimed at identifying genes regulated by the circadian clock, in which animals are sacrificed every 4 hours across the 24-hour cycle. These studies have recently identified hundreds of transcripts cycling in the brain and in peripheral tissues of mice (Akhtar and others 2002; Panda and others 2002; Storch and others 2002; Ueda, Chen, and others 2002) and flies (Claridge-Chang and others 2001; McDonald and Rosbash 2001; Ceriani and others 2002; Lin and others 2002; Ueda, Matsumoto and others 2002) as a function of circadian time. Because these studies did not control for behavioral state, it is possible that at least some of the identified changes were due to differences in sleep and wakefulness rather than to time of day per se. Interestingly, the cycling genes as identified in these studies are involved in extremely diverse biological functions, from protein synthesis and immune response to metabolism, some of which, as we will see, are also associated with the sleep-related and wakefulness-related transcripts.

A second finding of our systematic analysis of gene expression in S, SD, and W rats was that the number of known transcripts up-regulated during wakefulness (waking-related genes) is similar (~100) to the number of transcripts up-regulated during sleep (sleep-related genes). Thus, although sleep is a state of behavioral inactivity, it is associated with the increased expression of many genes in the brain. Importantly, the increased expression in the brain during sleep is specific because transcripts that are sleep-related in the brain are not sleep-related in other tissues such as liver and skeletal muscle (Fig. 3).

Another finding was that many (~40%) of the genes that were waking related in the cerebral cortex were also waking related in the cerebellum. Similarly, many (50%)

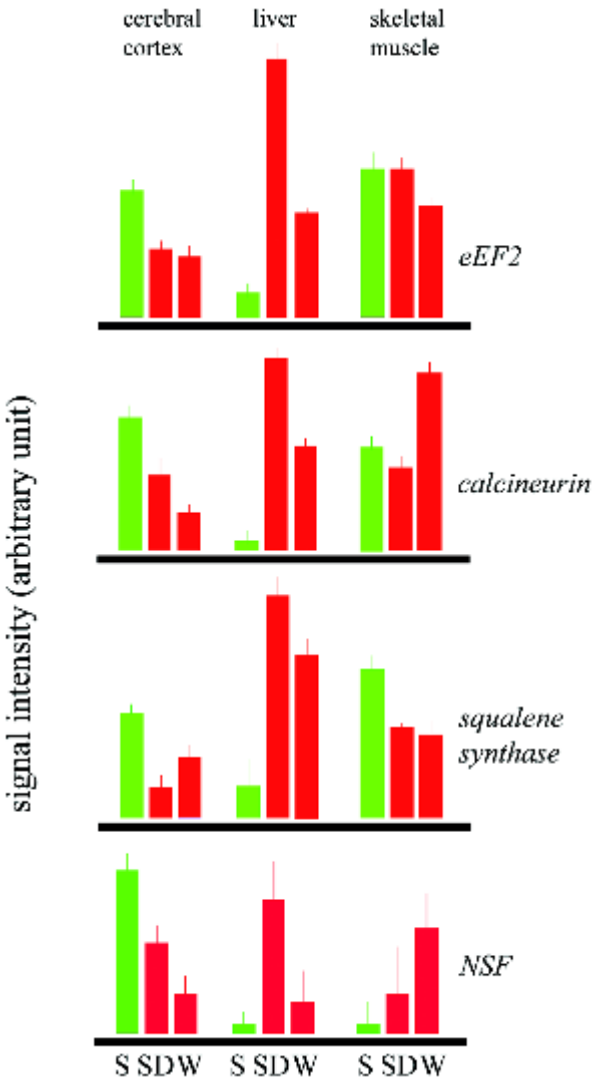

Fig. 3. Examples of transcripts whose expression is sleep related in the cerebral cortex but not in the liver or skeletal muscle. Transcripts include the eukaryotic elongation factor 2 (eEF2), the phosphatase calcineurin, squalene synthase, and the N-ethylmaleimide sensitive factor (NSF). Graphs refer to real-time quantitative polymerase chain reaction experiments (the signal intensity units are arbitrary and are meaningful only for comparisons within the same graph). The wakefulness-related increase of all examined transcripts in the liver is probably related to feeding because eating and drinking occur mostly at night in rats kept on a 12:12 light/dark schedule. S = spontaneously asleep; SD = sleep deprived; W = spontaneously awake.

of the cortical sleep-related genes were also sleep related in the cerebellum. The fact that molecular correlates of sleep and wakefulness are found in the cerebellum is intriguing because this brain area is not involved in the generation of the classical EEG markers of sleep such as spindles and slow waves. Thus, this suggests that functions associated with sleep may take place whether or not electrographic signs of sleep can be recorded. Moreover, it also suggests that at least some of the changes in gene expression observed between sleeping

and awake animals may depend on changes in the activity of neuromodulatory systems with diffuse projections. The LC system mentioned above is a case in point and will be discussed later.

Wakefulness-Related and Sleep-Related Transcripts

One of the most important findings of our study was that sleep-related and wakefulness-related transcripts belong to different functional categories, suggesting that sleep and wakefulness may favor different cellular processes (Fig. 4). Perhaps not surprisingly, many wakefulness-related transcripts are involved in energy metabolism (mitochondrial genes, *GLUT1*), excitatory neurotransmission (*Narp*, *Vesl/Homer*), transcriptional activation (*Per2*, *NGFI-A*, *NGFI-B*, *CHOP*), memory acquisition (*Arc, NGFI-A*, *BDNF*), and cellular stress (*HSPs*, *Bip*). Sleep-related transcripts, on the other hand, include a two-pore domain potassium channel controlling resting membrane potential (*TREK-1*), key components of the translational machinery (*translation elongation factor 2*, *initiation factor 4AII*), and genes involved in depotentiation, depression, and consolidation of long-term memory (e.g., *calcineurin*, *calmodulin-dependent protein kinase IV*). A large number of sleep-related transcripts are also involved in membrane trafficking and maintenance, including synaptic vesicle turnover (*Rab genes*, *NSF*; *ARF1*, *ARF3*) glia/myelin function (*MOBP*, *MAG*, *plasmolipin*, *carbonic anhydrase II*), and synthesis and transport of glia-derived cholesterol (e.g., *HMG-CoA synthase*, *squalene synthase*), the limiting factor for synapse formation and maintenance. Thus, wakefulness-related transcripts may help the brain to face high-energy demands, high synaptic excitatory transmission, high transcriptional activity, the need for synaptic potentiation in the acquisition of new information, and the cellular stress that may derive from one or more of these processes. What about sleep-related transcripts? Do they suggest hypotheses about what sleep is for?

Sleep-related transcripts support an involvement of sleep in protein synthesis and suggest that this function is specific for the brain rather than for peripheral tissues such as liver and skeletal muscle (Fig. 3). As summarized in the second section of this article, a positive correlation between sleep and protein synthesis had already been suggested by several studies. Another study (Drucker-Colín and others 1975) analyzed perfusates obtained from the mesencephalic reticular formation and hippocampus of freely moving cats by means of a push-pull cannula and found a higher protein concentration during REM sleep than during waking. Reich and others (1967) reported a two- to threefold increase in the incorporation of inorganic orthophosphate ^{32}P into phosphoprotein fraction of the brains of 20-day-old rats during sleep, and this increase was confirmed in adult rats (Reich and others 1973). Other authors have reported an increased protein metabolism during pharmacologically-induced sleep (Voronka and others 1971). Preliminary data from our laboratory suggest that a link between sleep and protein synthesis also exists in the djungarian hamster, in which mRNA levels of the elongation factor EF2 are higher after 4 hours of sleep relative to 4 hours of sleep deprivation (Cirelli C, Deboer T, Tobler T, unpublished results). As mentioned above, recent preliminary data in mice also suggest that sleep deprivation is associated with a global decrease in the levels of several brain proteins (Ding and others 2004). Whether sleep favors protein synthesis globally or whether it enhances the synthesis of specific classes of proteins is still unclear.

A second group of transcripts whose mRNA levels are higher in sleep than in waking include *calmodulin-dependent protein kinase IV*, a gene that has been specifically involved in the consolidation of long-term memory as well as in synaptic depression (e.g., Kang and others 2001), and other genes that have been associated with synaptic depression and depotentiation, such as *calcineurin*, *FK506 binding protein 12*, *inositol 1,4,5-trisphosphate receptor*, and *amphiphysin II*. Thus, although wakefulness is the appropriate time for memory acquisition, as indicated by the up-regulation of genes involved in neural plasticity and long-term potentiation such as *Arc*, *NGFI-A*, and *BDNF*, sleep may favor complementary aspects of plasticity, such as synaptic consolidation and/or downscaling. An involvement of sleep in such processes is suggested by behavioral and physiological experiments showing that sleep improves the performance of different learning tasks acquired during the previous waking period (Stickgold and others 2001; Walker and others 2002; Huber and others 2004). At this stage, however, the mechanism by which sleep enhances performance is still debated. Some researchers think that by allowing the rehearsal of previously acquired information (e.g., Lee and Wilson 2002), sleep may further strengthen those specific synapses that have been potentiated during waking (Sejnowski and Destexhe 2000; Steriade and Timofeev 2003). Others instead think that sleep benefits the brain by producing a global synaptic downscaling, which reduces the energetic cost of synaptic activity, removes the weak and ineffective synapses, and increases the signal-to-noise ratio (Tononi and Cirelli 2003).

A large group of sleep-related transcripts is involved in membrane trafficking and maintenance. Some of these transcripts are involved in exocytosis and neurotransmitter release (*SV2B*, *complexin II*, *Rab3a*, *neuronal calcium sensor-1*), others in synaptic vesicle recycling (*Rab5*, *amphiphysin II*, *endophilin I*), tethering/docking of vesicles to their target organelles (*Rab4*, *Rab5*, *Rab11*, *Rab14*, *Rab GDI*), dissociation of the SNARE core complex (*NSF*), recruitment of coat proteins (*ARF1*, *ARF3*, *alpha-centaurin*), and cycling between trans-Golgi network and plasma membrane (*MG160*, *TGN38*). Another large group of genes with higher mRNA levels during sleep are important for the synthesis/maintenance of membranes in general and of myelin in particular (Kramer and others 2001), such as oligodendrocitic genes coding for myelin structural proteins (*MOBP*, *MAG*, *plasmolipin*, *CD9*), myelin-related

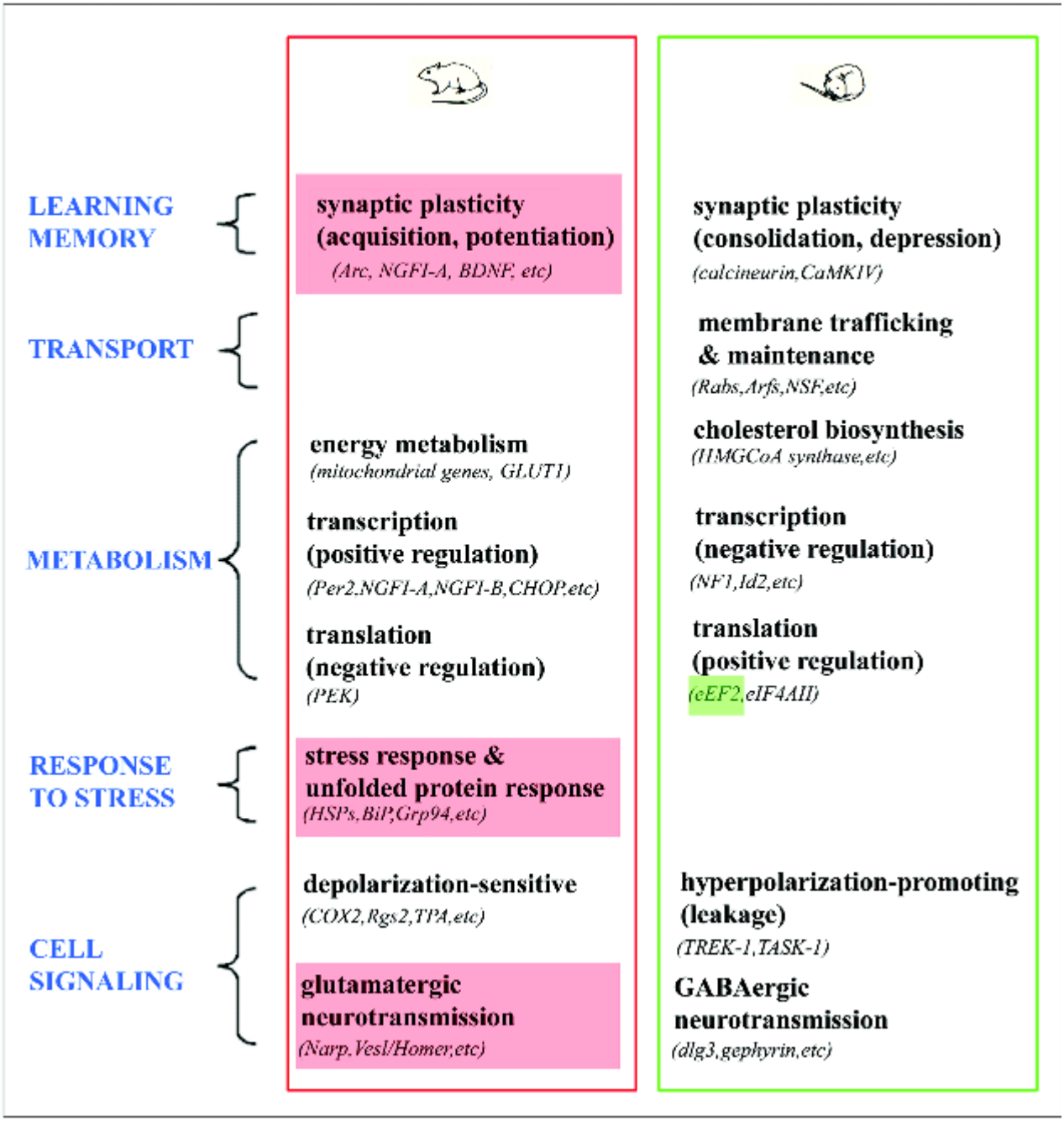

Fig. 4. Biological functions associated with transcripts with higher expression in wakefulness (*left*) and sleep (*right*). Solid boxes indicate transcripts whose state-dependent modulation of expression depends on the activity of the noradrenergic system of the locus coeruleus. They include wakefulness-related transcripts that are involved in synaptic plasticity, such as *Arc*, *NGFI-A*, *BDNF*, *Narp*, and *Homer*, in the cellular response to stress, such as heat shock proteins (HSPs) and chaperones (*Bip*) as well as the sleep-related transcript coding for the eukaryotic elongation factor eEF2.

receptors (*insulin-like growth factor binding protein 2*), and enzymes (*2′:3′-cyclic nucleotide-3′-phosphodiesterase*, *Na/K ATPase subunit alpha2*, *methionine adenosyltransferase*, *carbonic anhydrase II*). Finally, transcripts with higher expression in sleep code for enzymes involved in the synthesis and transport of cholesterol, a major constituent of myelin and other membranes (*thiolase*, *3-hydroxy-3-methylglutaryl-Coenzyme A synthase*, *squalene synthase*, *lanosterol 14 alpha-demethylase*). In agreement with these data, circadian studies in flies and mice also found that the expression of several genes related to the synthesis of cholesterol (Ceriani and others 2002; Panda and others 2002) and to synaptic vesicle recycling (Claridge-Chang and others

2001) peaks during the resting phase. Sleep is abundant early in life, at a time in which synaptogenesis and myelinization are prominent, and appears to be important for circuit formation and plasticity (Frank and others 2001; Shaffery and others 2002). Recent data indicate that glia-derived cholesterol may be the limiting factor for synapse formation and maintenance (Mauch and others 2001). Moreover, depletion of cholesterol/sphingolipid leads to instability of surface AMPA receptors and gradual loss of synapses and dendritic spines (Hering and others 2003). Thus, the link between sleep, membrane trafficking, and cholesterol synthesis, on one hand, and the link between sleep, protein synthesis, memory consolidation, and synaptic homeostasis, on the other hand, may not be unrelated.

Gene Expression and Neuromodulatory Systems

What are the mechanisms that underlie the widespread changes in cortical gene expression between sleep and wakefulness? One possibility is that they result from the action of neuromodulatory systems with diffuse projections whose firing rate is high in wakefulness and low in sleep. Two such systems are the noradrenergic nucleus of the LC and the serotonergic nucleus of the raphe dorsalis (RD). LC and RD cells are tonically active during wakefulness, reduce their firing rate during NREM sleep, and cease firing during REM sleep (McGinty and Harper 1976; Aston-Jones and Bloom 1981a). Moreover, the firing rate of LC neurons, but not that of RD neurons, increases phasically in response to salient events (Aston-Jones and Bloom 1981b; Rasmussen and others 1986) and in relation to the decision to act (Rajkowski and others 2004).

We first focused on the role of LC (Figs. 5*A*, *B*), and in one series of experiments in rats, we used a local injection of 6-hydroxydopamine to selectively destroy the LC of one side and thus to deplete noradrenaline from the ipsilateral side of the brain (Fig. 5*C*). In these animals, the raw EEG and its power density spectrum were not significantly different between the lesioned and the intact side 1 to 2 weeks following the lesion. Moreover, as expected, after a few hours of waking, *Arc*, *Fos*, *NGFI-A*, *P-CREB*, and *BDNF* levels on the intact side were high and comparable to those observed in normal awake animals. However, the expression of these genes was significantly decreased in cortical areas and hippocampus on the lesioned side (Fig. 5*C*). In another series of experiments, rats were treated systemically with DSP-4, a neurotoxin that selectively ablates noradrenergic axon terminals originating from LC (Fritschy and Grzanna 1989). After DSP-4 treatment, noradrenergic fibers were almost entirely and bilaterally destroyed in the cerebral cortex, hippocampus, thalamus, tectum, cerebellum, and spinal cord. Like after unilateral LC lesions, waking behavior and waking EEG were normal but *c-fos*, *NGFI-A*, *BDNF*, *and P-CREB* expression during waking was significantly reduced with respect to control rats that were injected with saline (Cirelli and others 1996; Cirelli and Tononi, 2000a). In a complementary experiment in mice (Salbaum and others 2004), the activity of the LC of one side was increased using a conditional transgenic approach. This manipulation resulted in an increased ipsilateral expression of *NGFI-A* in cortical and subcortical target areas (Fig. 5*D*). Thus, LC activity plays a major role in the high expression during wakefulness of five genes involved in synaptic plasticity, *Arc*, *BDNF*, *c-fos*, *NGFI-A*, and *P-CREB*. The significant effect of the noradrenergic system appears to be specific because diffuse lesions of cortical serotoninergic fibers do not affect the expression of these genes during waking (Tononi and others 2000).

Does LC affect other wakefulness-related or sleep-related transcripts? If so, what are the genes, and what are their functions? Are they only plasticity-related genes? To answer these questions, we recently used microarrays to measure the expression of ~5000 transcripts in the cerebral cortex of awake rats pretreated with saline or DSP-4 (Cirelli and Tononi 2004). Cortical levels of noradrenaline were reduced by ~90% in DSP-4 treated animals. We took advantage of our database of gene expression changes associated with wakefulness and sleep (Cirelli and others 2004) and performed a conjunction search to determine which state-dependent genes are sensitive to noradrenaline depletion. We found that the expression of ~20% of all known wakefulness-related transcripts was significantly decreased in awake rats previously treated with DSP-4 relative to awake rats previously injected with saline. Perhaps not surprisingly, given the established role of noradrenaline in neural plasticity, most of these transcripts are involved in synaptic plasticity and include *Vesl/Homer* and *Narp* in addition to *Arc*, *BDNF*, *c-fos*, *NGFI-A*, and *P-CREB* (Fig. 4). A second group of wakefulness-related transcripts whose expression is positively modulated by noradrenaline is involved in the cellular response to stress and includes heat shock proteins and chaperones. By contrast, the transcript for the translation elongation factor 2 was the only known sleep-related transcript whose expression increased after cortical noradrenaline depletion. Thus, LC activity during wakefulness modulates neuronal transcription to favor synaptic potentiation and memory acquisition and to counteract cellular stress, whereas LC inactivity during sleep may play a permissive role in enhancing brain protein synthesis.

Conclusions and Future Directions

Old and new evidence indicates that extensive and divergent changes in gene expression occur in the brain between sleep and wakefulness. Transcripts differentially expressed in sleeping and awake rats belong to diverse and often complementary functional categories, suggesting that sleep and wakefulness favor different cellular processes. Wakefulness-related transcripts may help the brain to face high-energy demand, high synaptic excitatory transmission, high transcriptional activity, the need for synaptic potentiation in the acquisition of new information, as well as the cellular stress associated with

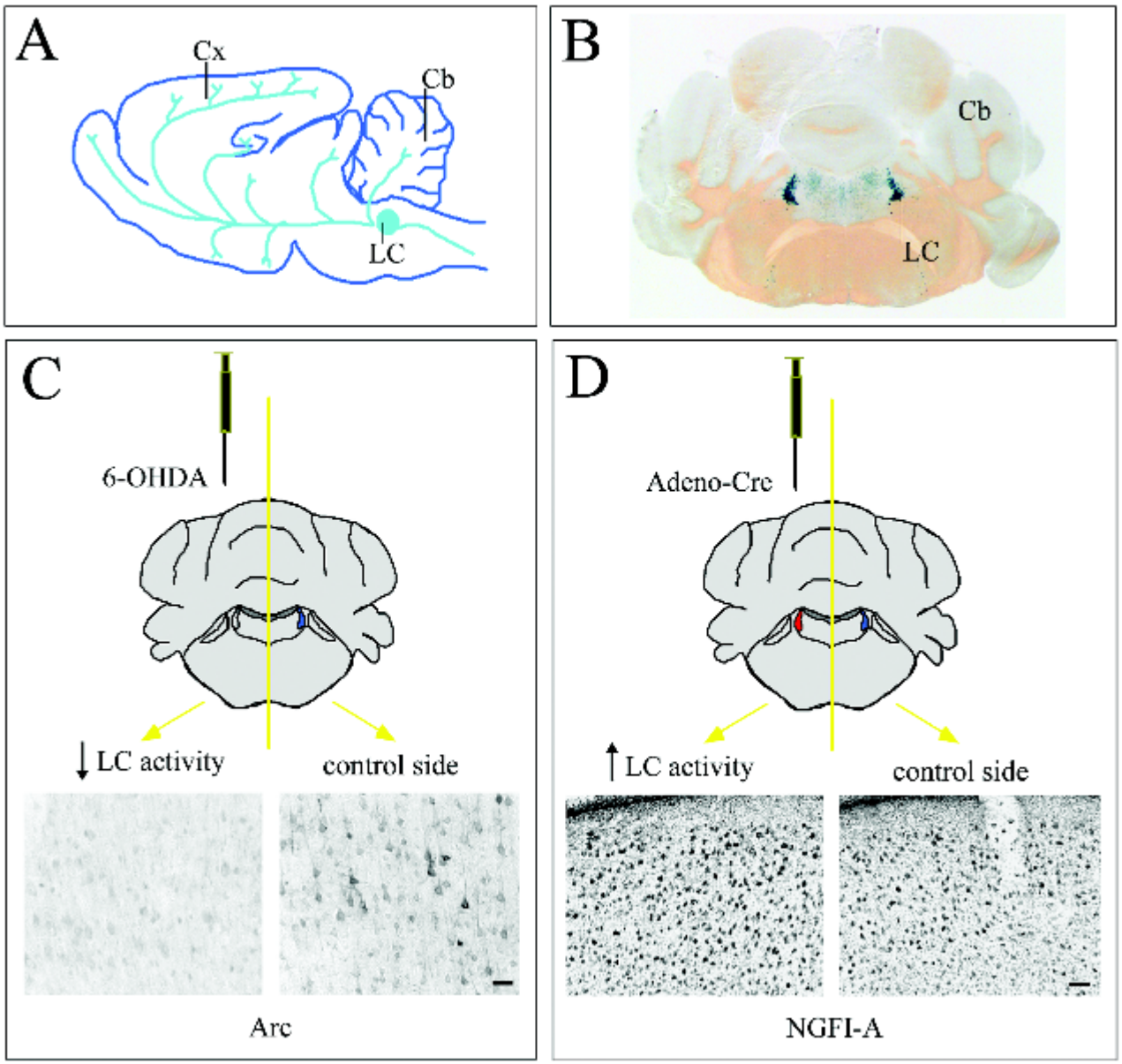

Fig. 5. Locus coeruleus (LC) control of state-dependent gene expression. *A*, LC neurons, which are located in the medial pontine tegmentum, innervate most brain regions, including the cerebral cortex (Cx) and the cerebellum (Cb). *B*, Frontal section of a mouse brain at the level of LC. LC cells are stained in blue. *C*, A pharmacological approach to lesion the LC of one side (*left*). LC cells were destroyed by a local injection of 6-hydroxydopamine (6-OHDA), which results in an almost complete ipsilateral depletion of noradrenaline in LC target areas, such as the cerebral cortex. Two weeks after, the lesioned rats were sleep deprived for 3 hours and then sacrificed. Frontal sections of the parietal cortex were reacted with an antibody against Arc. Arc expression is high on the intact side (*right*) after 3 hours of sleep deprivation, but it is as low as in sleep on the side where the noradrenergic innervation had been destroyed (*left*). Scale bar = 100 μm. *D*, A mouse transgenic approach to stimulate LC in a highly selective manner for prolonged periods of time. The mouse carries a *Chlorotoxin* transgene under the control of the promoter of the rat *dopamine-beta hydroxylase*, to allow specific expression in LC. Chlorotoxin is a component of scorpion venom that partially blocks small conductance chloride channels. The injection of Adeno-Cre virus in the LC of one side (*left*) induces the expression of *Chlorotoxin*, with subsequent increase in LC activity. Four weeks after the adenoviral injection, mice were sleep deprived for 4 to 6 hours and then sacrificed. Frontal sections of cerebral cortex were reacted with an antibody against NGFI-A. NGFI-A staining is higher on the left side, which receives input from the LC in which *Chlorotoxin* was induced, relative to the right, uninfected side. Scale bar = 100 μm.

these processes. Sleep-related transcripts suggest that sleep is far from being a quiescent state of global inactivity and may play a positive role in brain protein synthesis and in complementary aspects of neural plasticity such as synaptic consolidation and downscaling. Sleep-related transcripts also suggest that sleep may be involved in membrane trafficking and maintenance.

Ongoing and future experiments will extend our understanding of the molecular correlates of sleep and wakefulness by using two complementary approaches.

The first approach is to perform systematic gene-expression analyses in other animal species such as fruit flies, mice, and birds, to name a few. Because sleep is present and tightly regulated in all animals studied so far (Tobler 2000), it is reasonable to assume that at least some of the basic functions of sleep are conserved across phylogeny. Thus, one should expect to find some interspecies similarities among the functional categories of wakefulness-related and sleep-related transcripts. The second approach is to examine changes at the protein, rather than at the mRNA level. After all, proteins, rather than DNA or RNA, are the ultimate carriers of most cellular functions. Thus, if sleep really benefits the brain by causing, for example, synaptic downscaling or by increasing cholesterol synthesis and membrane maintenance, one should expect to find some supporting evidence when measuring levels and activity of specific proteins involved in these processes.

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