

# EUROBRAIN

## *Sleep*

### SLEEP

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#### **SLEEP IS A REGULATED PROCESS**

We know that we must sleep but we do not know why. The question about the function of sleep is one of the major challenges of biology.

Sleep can be characterised as a periodically occurring state of quiescence during which there is little or no interaction with the environment. However, sleep is more than switching off the waking state, it is a regulated process. Three major processes (fig. 1) are involved in sleep regulation: 1) A homeostatic process that is evident from the increase of sleep propensity in the course of waking and its dissipation during sleep; 2) a circadian process that is controlled by the circadian pacemaker and is not directly determined by prior sleep and waking; and 3) an ultradian process that is responsible for the alternation of nonREM sleep and REM (rapid-eye-movement) sleep within the sleep episode.

The decline of sleep intensity is reflected by the slow waves in the sleep electroencephalogram (EEG). These markers of sleep intensity of nonREM sleep predominate in the beginning of the sleep episode and then gradually decline as sleep becomes more shallow (fig. 1). The prevalence of slow waves can be quantified by subjecting the sleep EEG to spectral analysis and computing the power in the 0.75-4.5 Hz band (it is referred to as slow-wave activity). When waking is prolonged, the subsequent slow-wave activity is enhanced. In humans and other mammals, a close relationship between the duration of waking and slow-wave activity in sleep has been demonstrated. A sleep deficit elicits a compensatory response, which consists in an increase of sleep intensity as reflected by slow-wave activity. Excess sleep has the opposite effect. This regulatory facet of the sleep process is referred to as sleep homeostasis.



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# *Is sleep a local brain process?*



Alexander A. Borbély

## SLEEP DURING WAKING?

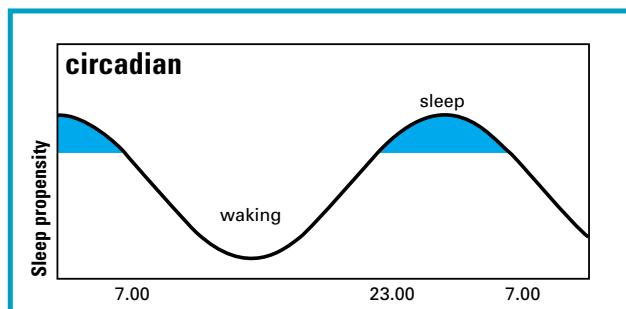
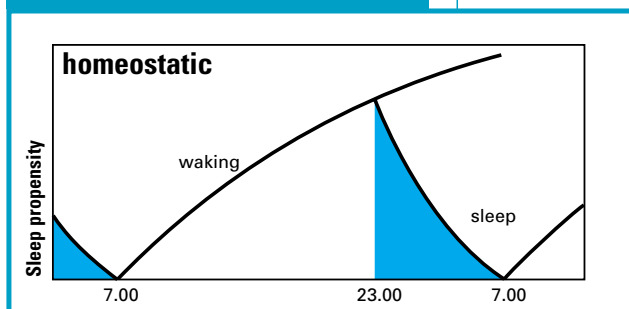
Is it possible to demonstrate the rise in sleep propensity during waking? To address this problem, we recorded with Luca Finelli and Peter Achermann the EEG intermittently during a sleep deprivation period. The waking EEG showed a rising trend of theta activity (5-8 Hz) reflecting the augmenting sleep pressure. When the subjects were allowed to sleep, the slow-wave activity in their nonREM sleep EEG was enhanced in comparison to baseline sleep. This is an indication of a deeper or more intense sleep. The main question in this study was whether the theta activity in waking was related to slow-wave activity during sleep. The comparison of individual records revealed a positive correlation between the rise rate of theta activity in the course of waking, and the enhancement of slow-wave activity in the beginning

of recovery sleep. In other words, the subject showing the steepest rise of theta activity during waking was the one who showed the largest increase of slow-wave activity during sleep. We concluded that the two markers of sleep propensity may reflect the same underlying homeostatic sleep process.

## A METABOLIC FUNCTION OF SLEEP?

Benington and Heller, two scientists from Stanford University, advanced in a paper in 1995 a provocative hypothesis to account for sleep homeostasis. They proposed that the increased metabolic demand of neurons during waking causes a transient deficit in cellular energy charge, which results in a higher concentration of adenosine (a breakdown product of the energy metabolism). According to their hypothesis, adenosine promotes slow waves, a

Fig. 1 – Three major regulatory processes of sleep



proposition that is in line with the known slow wave promoting effect of adenosine agonists. Due to the reduced metabolic demand during sleep, glycogen synthesis would be activated and replenish the glycogen stores in astrocytes. Therefore, the restorative function of sleep is assumed to be associated with energy metabolism in glia cells. There are indications from other groups that adenosine may indeed be involved in sleep regulation. Assaying the cerebral adenosine concentration by microdialysis in experimental animals revealed that its level rises during waking and declines during sleep.

### SLEEP AS A LOCAL BRAIN PROCESS

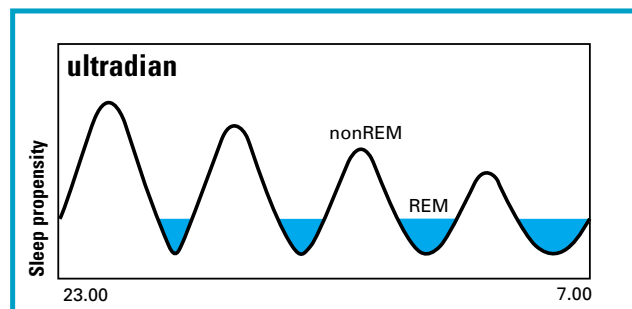
One of the interesting implications of the “metabolic restoration” hypothesis is that sleep is not only a global phenomenon involving the entire brain, but that it has

also local aspects. Krueger and Obál proposed in 1993 that sleep exhibits local, use-dependent features. The neuronal groups that are most active during waking, can be expected to exhibit the highest need for sleep. Together with Herbert Kattler and Derk-Jan Dijk we devised an experiment to test this proposition. Subjects were administered a prolonged vibratory stimulus to one hand to activate selectively the contralateral somatosensory cortex. The aim of the study was to see whether the cortical region that had been activated during waking, exhibits a different sleep EEG than the homologous contralateral area. A comparison of the EEG of the two hemispheres showed that the pre-sleep activation of the dominant hand induced a shift in the interhemispheric power ratio towards the contralateral hemisphere. This effect was significant in the low-

frequency range. It was limited to the central derivation overlying the stimulated cortex and was restricted to the first hour of sleep. This result supported the notion of sleep having a local, use-dependent facet.

Together with Vlad Vyazovskiy and Irene Tobler we tested the proposition in an animal model. Rats were subjected to unilateral sensory stimulation during waking. This was achieved by clipping their facial whiskers (*vibrissae*) on one side (fig. 2). Rats use their whiskers when exploring the environment and the sensory stimuli elicited by their movement activates the barrel field of the contralateral somatosensory cortex. In the experiment, the animals were kept awake for six hours in an enriched environment. In the subsequent period which the animals spent mostly asleep, an interhemispheric asymmetry in the sleep EEG was observed. Power in the low-frequency range (0.75-6.0 Hz) exhibited a shift towards the cortex that had been activated during waking (fig. 2). Thus also in animals, a regional, use-dependent facet of sleep can be demonstrated.

Can a regional specificity of sleep be demonstrated also for normal sleep episodes in humans? With Esther Werth and Peter Achermann we showed that frontal EEG derivations showed in the initial part of sleep more slow-wave activity than more posterior derivations. The dif-



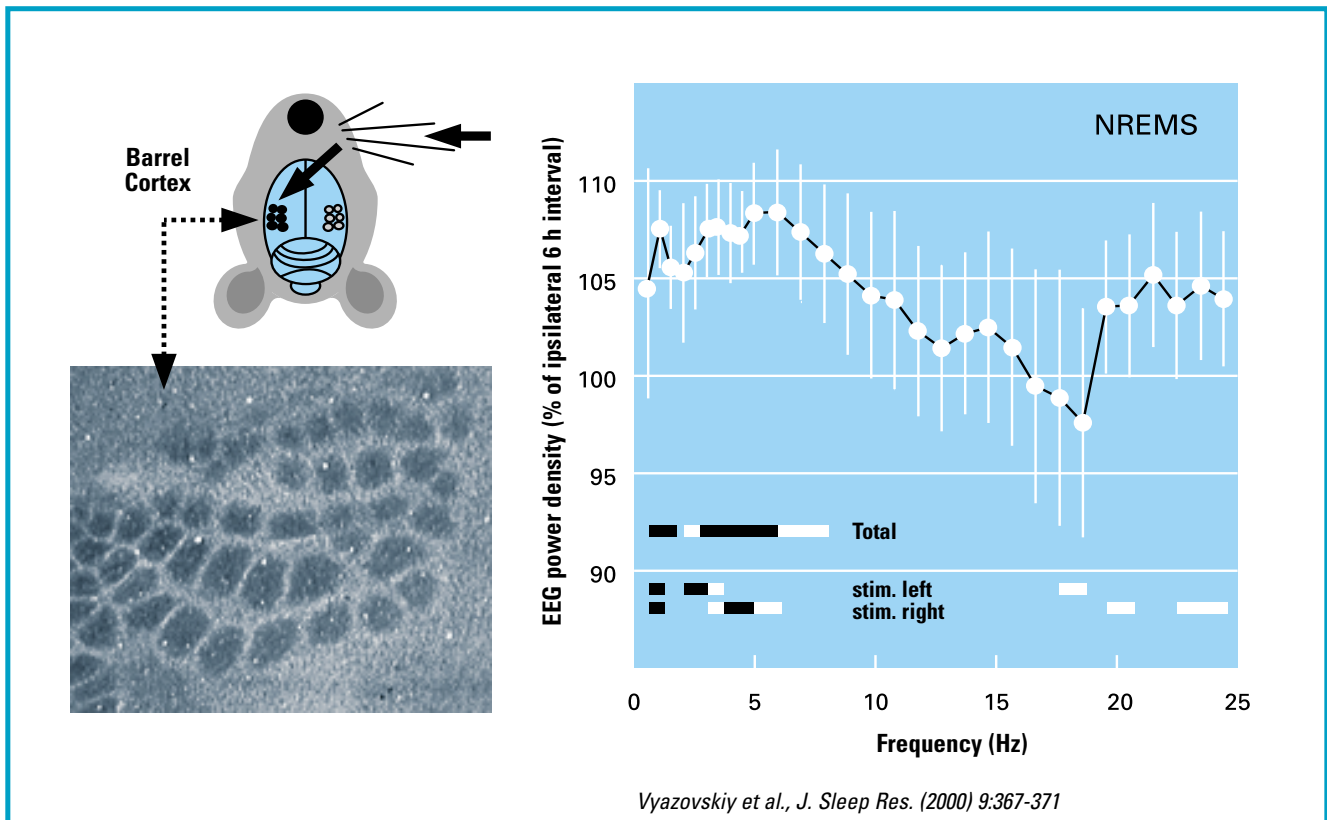


Fig. 2 – The effect of unilateral vibrissae stimulation during waking on the subsequent sleep EEG

ference vanished in later parts of sleep. This observation is interesting because Jim Horne in Loughborough had presented evidence that frontal brain regions are particularly sensitive to a sleep deficit. The predominance of frontal slow-waves may therefore reflect a higher need for recuperation of this area. Recently we performed an analysis of EEG topography with 27 derivations. Also this study confirmed the frontal predominance of low-frequency activity in the first part of sleep. Moreover, the enhancement of power in this frequency range by sleep deprivation was also most pronounced in the frontal areas. This result is in good correspondence with regional cerebral blood flow as determined by positron emission tomography (PET), which was shown to be lower during non-

REM sleep than during waking. It is tempting to speculate that the large rise of low-frequency power at frontal areas is a sign of an increased local, use-dependent sleep intensity, which could reflect a more intense recovery process.

If the concept of local sleep is substantiated in further studies, it may give rise to new experimental approaches in the quest for the functions of sleep.

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# Uncovering the molecular roots of narcolepsy



Recent findings suggest possible shortening of the length of time needed for an accurate diagnosis of narcolepsy and, eventually, potential relief for victims of both this debilitating sleep disorder and sleep problems in general. At the heart of these findings is a molecule called orexin.

The story began in 1999 when laboratory mice, under observation by researchers at the University of Texas Southwestern Medical Center in Dallas, started acting in a bizarre fashion. In the midst of normal activities, such as eating, drinking, and running, they would suddenly stop, fall down, and remain paralysed for minutes. Then, as if a switch had been flipped back on, they would resume their activities just as suddenly. "Initially, we thought it might be some type of *petit mal* seizure," explains Masashi Yanagisawa, a molecular geneti-

cist and Howard Hughes Medical Institute investigator who directs the laboratory. However, brain wave studies showed none of the sharp spikes in electrical activity characteristic of epileptic seizures. Instead, these mice instantly passed from wakefulness into rapid-eye-movement (REM) sleep, the deepest stage of sleep in which dreaming occurs, thus exhibiting a classic manifestation of narcolepsy.

Yanagisawa was excited because these mice were unusual in another way in that they lacked the gene to make a neuropeptide called hypocretin or orexin, a natural chemical that had already been shown to play a key role in appetite control. Might this same molecule also cause narcolepsy?

At roughly the same time, researchers led by Emmanuel Mignot at the Stanford

University School of Medicine made a parallel discovery: Narcoleptic dogs had mutations in the gene that codes for a particular receptor on the surface of brain cells. They concluded that in these dogs, narcolepsy was, in fact, caused by this single defective gene. The receptor encoded by this gene is the one that binds orexin.

The obvious next question was to see whether orexin-related genes play a central role in human narcolepsy, which affects an estimated 125,000 to 250,000 Americans. This debilitating disorder is characterised by excessive daytime sleepiness and other symptoms, such as cataplexy, a sudden weakness or collapse of muscles that can leave people incapacitated for minutes. Narcolepsy exacts a high physical and emotional toll,

contributing to impaired school and job performance, higher rates of driving accidents, marital stress, social inhibition, and untold embarrassment.

Subsequent DNA screening by several research groups showed that mutations in human genes coding for orexin or its receptor are very rare, according to Yanagisawa, affecting only about one out of hundred patients. Although the genes themselves appear to be intact, there is still a deficit in orexin production, as demonstrated in a *Lancet* study by Seiji Nishino, Mignot, and other Stanford researchers, who showed that, in marked contrast to normal human subjects, 80% of narcoleptic patients have no measurable orexin in the cerebrospinal fluid (CSF) that bathes the brain.

These findings suggest that measuring orexin levels in the CSF, by means of a spinal tap, could be important for the diagnosis of narcolepsy. According to Nishino, "The symptoms of narcolepsy usually appear in the early teens and it then typically takes seven years or more before a diagnosis is made. If a proper diagnosis can be made earlier, that could improve the education of these individuals as well as their quality of life." More tests are needed before this technique will see widespread use, he adds, but early results are promising.

Meanwhile, researchers are trying to understand the orexin deficit lying at the root of narcolepsy. Yanagisawa speculates that this sleeping disorder might result from an autoimmune disease that attacks the small cluster of neurons found in a brain region called the lateral hypothalamus that make the chemical, damaging or killing the neurons. More than 90% of narcolepsy

patients have genes for types of human leucocyte antigen that are commonly associated with autoimmune disorders, but the narcolepsy connection has yet to be proven.

In the latest experiments, Yanagisawa and his colleagues have shown that injection of orexin directly into the brains of mice can cure the animals of narcolepsy, at least until the body rids itself of the chemical. These results, which have not yet been published, make him think that narcolepsy might someday be cured by a pill that boosts the supply of orexin in the brain. Drug companies are already trying to develop an orexin analog to do this job, as the neuropeptide itself is too large to pass easily through the blood-brain barrier. Current treatments for narcolepsy, including stimulants, such as amphetamines, to combat daytime sleepiness and antidepressants to suppress cataplexy, "alleviate the symptoms without acting on the central, core mechanism," adds Seiji Nishino.

The push for a narcolepsy cure might, paradoxically, lead to the development of an ideal sleeping pill. If it turns out that insomnia is also orexin-related, a compound that blocks the orexin receptor could induce "truly natural sleep". According to Yanagisawa, "Available sleeping pills sedate you and interfere with REM sleep, so that you don't wake up feeling refreshed. Curing narcolepsy is certainly important, but this approach could, ultimately, be much bigger, since about 20% of the U.S. population complains about insomnia."

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