Interhemispheric Sleep EEG Asymmetry in the Rat is Enhanced by Sleep Deprivation

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INTRODUCTION

The electroencephalogram (EEG) represents a unique method to investigate sleep regulation in both humans and animals. EEG slow-wave activity (SWA, EEG power between 0.75 and 4.0 Hz) is a reliable index of non-rapid eye movement (NREM) sleep intensity. It is determined by the amount of prior waking and sleep and may serve as a marker of sleep homeostasis (Borbély 1982). The enhancement of SWA by prolonged waking and its subsequent monotonic decline during sleep was documented for humans (Borbély et al. 1981) and several animal species (Tobler and Borbély 1986; Tobler and Jaggi 1987; Huber et al. 2000). These observations suggested that SWA might reflect a recovery process occurring during waking. Exposing the right hand of human subjects to a standardized vibration stimulus during waking resulted in a larger increase of SWA in the brain region contralateral to the stimulated hand during the subsequent NREM sleep episode (Katter et al. 1994). The effect was limited to the derivation overlying the somatosensory cortex and was restricted to the first hour of sleep. An analogous result was obtained in the rat where unilateral vibrissae stimulation induced an interhemispheric shift of low-frequency EEG power in NREM sleep explored in the temporal domain, the spatial domain (i.e., topographic differences in the sleep EEG) was only recently examined.

EEG power within the delta band showed a frontal predominance in human NREM sleep, a feature that was most prominent in the first part of the night (Werth et al. 1996) and was enhanced by sleep deprivation (SD) (Cajochen et al. 1999). Also in rodents, a 6-h SD period gave rise to a larger SWA rebound in the frontal derivation than in the occipital derivation (mouse: Huber et al. 2000; rat: Schwierin et al. 1999). Spindle frequency activity (SFA; power in the 12- to 15-Hz band in NREM sleep), another marker of human sleep regulation (Achermann and Borbély 1998; Dijk et al. 1993), also exhibited a specific topographic pattern (Finelli et al. 2001). After prolonged waking in humans, it showed the largest decrease in the centro-parietal region. In the rat, prolonged waking reduced EEG power in the spindle frequency range (10.25–16.0 Hz) more in the frontal derivation than in the occipital derivation (Schwierin et al. 1999).

In addition to the regional differences along the anteroposterior axis, interhemispheric asymmetries in the sleep EEG may be of functional significance. Spectacular examples are dolphins which exhibit “deep” slow wave sleep only in one hemisphere at a time (Mukhametov et al. 1977). State-related interhemispheric EEG asymmetries favoring the right hemisphere in NREM sleep and the left hemisphere in REM sleep have been reported for humans, cats, and rabbits (Goldstein et al. 1972). In a recent human study, power in the 4- to 8-Hz band of the centro-parietal derivation was higher on the right side in NREM sleep and on the left side in REM sleep (Roth et al. 1999). Power within the spindle frequency range (11–15 Hz) in NREM sleep exhibited a left-hemispheric predominance.

To examine the functional significance of EEG asymmetries during sleep, one hemisphere was selectively activated during waking. Exposing the right hand of human subjects to a standardized vibration stimulus during waking resulted in a larger increase of SWA in the brain region contralateral to the stimulated hand during the subsequent NREM sleep episode (Katter et al. 1994). The effect was limited to the derivation overlying the somatosensory cortex and was restricted to the first hour of sleep. An analogous result was obtained in the rat where unilateral vibrissae stimulation induced an interhemispheric shift of low-frequency EEG power in NREM sleep.

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toward the contralateral cortex (Vyazovskiy et al. 2000). We showed recently that a prolongation of waking was sufficient to induce EEG asymmetry in human sleep (Achermann et al. 2001). The enhancement of the anterior predominance of delta activity was present only in the left hemisphere. This observation shows that the challenge of extended waking enhances the manifestation of regional differences of the sleep EEG in humans.

A phylogenetic approach to sleep regulation has proved useful for elucidating basic mechanisms such as sleep homeostasis (Tobler 1995, 2000). Whereas functional brain asymmetries are an ubiquitous feature in vertebrates, their role in sleep regulation is still unclear (Vallortigara et al. 1999). To further investigate this aspect in an animal model, the sleep EEG of the rat was recorded from both hemispheres under baseline conditions and after enhancing sleep pressure by a short period of SD.

METHODS

Animals

Adult male albino rats of the Sprague-Dawley strain (total n = 16) with a mean body weight 274.3 ± 5.0 (SE) g were used. The animals were kept individually in Macrolon cages (53 × 34 × 37 cm) with food and water available ad libitum and maintained on a 12-h light-12-h dark cycle (light from 8.00–20.00 h; 7 W OSRAM Dulux EL energy saving lamp, approximately 30 lux). Mean ambient temperature during recording days was 21.7 ± 0.7°C. Under deep pentobarbital anesthesia (pentobarbital sodium, Nembutal, 80 mg/kg ip, volume approximately 0.5 ml), the rats were implanted with gold-plated miniature screws (0.9-mm diam), which served as EEG electrodes. The electrodes were implanted over the parietal cortex (5.5 mm lateral to the midline, 2.5 mm posterior to the bregma) and the frontal cortex (1.5 mm lateral to the midline and 1.5 mm anterior to the bregma) in both the left and right hemisphere. The common reference electrode was placed above the cerebellum (2 mm posterior to the lambda, on midline). Two gold wires (diameter: 0.2 mm) inserted into the neck muscles served to record the electromyogram (EMG). The electrodes were connected to stainless steel wires that were fixed to the skull with dental cement. At least 10 days were allowed for recovery.

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<th>Vigilance states for baseline and the two sleep deprivation conditions</th>
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Means ± SE of waking, non-rapid eye-movement (NREM) sleep and rapid eye-movement (REM) sleep expressed as percentage of recording time for rats which were subjected to sleep deprivation (SD) during the light (n = 8) and dark period (n = 8) for 6-h intervals (baseline, 6-h SD, and recovery). Effect of SD within a group: * P < 0.01; † P < 0.05 (2-tailed paired t-test).

Experimental protocol and data acquisition

The EEGs and the EMG were recorded during a 24-h baseline day, a 6-h sleep deprivation (SD) period starting either at light onset (SDL, n = 8) or dark onset (SDD, n = 8), and an 18-h recovery period. SD was performed by introducing a variety of objects (e.g., nesting material, pieces of wood, paper, tissue, PVC boxes with holes) into the cage and by tapping on the cage whenever the animal appeared drowsy or the EEG exhibited slow-waves. The rats were never disturbed during feeding and drinking. The EEG and the EMG signals were amplified (amplification factor: approximately 2,000), conditioned by analog filters (high-pass filter: −3 dB at 0.016 Hz; low-pass filter: −3 dB at 40 Hz, less than −35 dB at 128 Hz) sampled with 512 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20–50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for 4-s epochs by a fast Fourier transform (FFT) routine. Adjacent 0.25-Hz bins were averaged into 0.5-Hz (0.25–0.5 Hz) and 1.0-Hz (0.25–0.5 Hz) bins, and those above 25 Hz were omitted. The EMG was full-wave rectified and integrated over 4-s epochs, and ambient temperature inside the cage was sampled at 4-s intervals.

Vigilance states and analyses

The three vigilance states, NREM sleep, REM sleep and waking were scored for 4-s epochs as described previously (Tobler et al. 1997). Briefly, the vigilance states were determined off-line by visual inspection of the parietal EEG and EMG recordings and the values of EEG power in the slow-wave range (0.75–4.0 Hz). Epochs containing EEG artifacts in any of the four derivations were excluded from spectral analysis in all derivations (27.7 ± 1.9% of total recording time; 93.1% of all EEG artifacts occurred in the waking spectra). Those frequency bands that differed significantly between derivations were selected for further analyses. Differences in EEG spectra between derivations or hemispheres and effects of SD were tested with two- or three-way ANOVAs for repeated measures (rANOVA), factors “derivation” (frontal, parietal) or “hemisphere” (left, right), “day” (baseline, recovery), and “time interval.” Contrasts were tested by post hoc two-tailed t-tests (for equal variances).

RESULTS

Vigilance states

The vigilance states for baseline, SD, and recovery are shown for the two experimental conditions [i.e., SD in light...
Anterior-posterior gradients in the NREM sleep EEG

The effect of SD on the NREM sleep EEG showed regional differences. In Fig. 2, power of the right hemisphere in the frontal derivation is expressed relative to power in the parietal derivation for the first two 3-h intervals of recovery from SDL (top) and SDD (bottom). To eliminate differences in absolute power between individual animals, a normalization procedure was applied. Prior to computing the intrahemispheric differences, power in each derivation was normalized relative to its 24-h baseline EEG power in each frequency bin served as reference. Horizontal lines below the curves indicate those bins which differed significantly between the frontal and parietal derivation (2-tailed paired t-test on log-transformed values).

Power in the range beyond 6.25 Hz was reduced, an effect that was largest in the last two 2-h intervals.
Left-right asymmetry in the NREM sleep EEG

Comparing the EEG recorded from the left and right hemisphere revealed during baseline systematic interhemispheric variations in the parietal derivations but not in the frontal derivations (Fig. 3). They were statistically significant in the low-frequency range (1.25- to 7.0-Hz range). An almost symmetrical left-right distribution of power in the first 6-h interval of the light period changed to a right predominance in the second 6-h interval. Then it reverted back toward zero in the first half of the dark period and ended with a left predominance in the second half. The overall daily left-right changes were in the range of 6%.

The time course of the interhemispheric distribution of low-frequency power (1.25–7.0 Hz) in the NREM sleep EEG during baseline is depicted in Fig. 4, top. Prior to computing the interhemispheric asymmetries, power in each derivation was normalized relative to its mean 24-h baseline value for each frequency bin. In the parietal derivation, a left predominance was present in the first 1.5-h interval of the light period. The ensuing shift toward the right hemisphere reached its maximum in the last interval of the light period, and then reversed. The right-left trend in the dark period persisted, reaching maximal left predominance 3 h before light onset. Power in the frontal derivations exhibited a symmetrical distribution throughout the 24 h.

SDL induced in the parietal derivation a shift from a right predominance during baseline (Fig. 4) to a left-hemispheric predominance of low-frequency power in the corresponding 2-h intervals (Fig. 5). This effect reached a tendency in the first 2-h interval (P = 0.08), was significant in the second and third 2-h interval (P < 0.05) as well as over the first 6-h interval of recovery (9.8% ± 4.0 left predominance over right hemisphere; P = 0.043, paired t-test) and was no longer present in the subsequent dark period. Seven of eight animals showed this change. SD did not affect the interhemispheric distribution of power in the frontal derivation. SDD did not have a significant effect on hemispheric dominance in the parietal or the frontal EEG.
A correlation analysis served to further explore the relationship between power in the low-frequency range and hemispheric asymmetry. EEG power (1.25–7.0 Hz) computed for 2-h intervals was positively correlated with the left-right ratio of power (mean of \( n = 8 \) rats) both for baseline and recovery after SDL (Pearson product-moment correlation; \( r^2 = 0.81 \) and 0.86, respectively, \( P < 0.001 \) for both).

**Left-right differences in the REM sleep EEG**

Figure 6 shows the interhemispheric distribution of EEG power for REM sleep and waking during baseline in the parietal derivation. Significant differences were seen only in REM sleep where power within the theta band and in the lowest bin of the delta band exhibited a right predominance. Eleven of the 16 animals exhibited a right predominance of parietal theta power. No asymmetry in REM sleep occurred in the frontal derivation. The parietal interhemispheric difference was present during almost the entire 24-h period and, in contrast to low-frequency power in NREM sleep, exhibited no clear changes over time (Fig. 4, bottom). Moreover, SD did not affect the interhemispheric asymmetry in REM sleep (not shown).

The right-hemispheric predominance of parietal theta power during baseline appeared immediately after the transition from NREM sleep to REM sleep (Fig. 7). In NREM sleep, a symmetrical distribution of power prevailed during the entire 2 min before the transition. The symmetry prevailed also during the surge of theta power immediately before the transition. Such a surge had been reported previously in the rat by Trachsel et al. (1988) for a parietal derivation and was attributed to the manifestation of spindle-like activity in the 6.25- to 15-Hz range. The overall changes at the NREM-REM sleep transition were similar at the frontal derivation, and no asymmetry was present.

With the exception of the parietal derivation in REM sleep (Fig. 6), the 24-h baseline spectra of NREM sleep and REM sleep showed no significant interhemispheric differences. In waking, the frontal derivation exhibited a left predominance in the 8.25- to 23.0-Hz range (not shown).

**DISCUSSION**

The low-frequency range of the NREM sleep EEG is of particular interest because it is a reliable marker of homeostatic sleep regulation and an index of sleep intensity (Borbély 1982). The present study confirmed that low-frequency power declines progressively during the light period, the rat’s main sleep period (Franken et al. 1991; Trachsel et al. 1988). That this trend reflects sleep pressure is shown by the marked enhancement of low-frequency power by sleep deprivation. The novel aspect of the present study is the demonstration that these changes do not occur uniformly over the entire cortex but differ along the antero-posterior and left-right axes. The results are relevant for the tenet of “local sleep” (Krueger and Obid 1993; Krueger et al. 1999), which postulates that in addition to being a global brain phenomenon, sleep has also a local aspect. Regional differences in the sleep EEG are assumed to reflect different intensities of local sleep.

The frontal predominance of the baseline EEG at the beginning of the light period was enhanced further under increased sleep pressure. Thus the increase in low-frequency power in the NREM sleep EEG encountered after SD exhibited a prominent antero-posterior gradient. The increase in power was larger at the frontal derivation than at the parietal derivation. This result is in agreement with previous observations in the rat (Schwierin et al. 1999) and mouse (Huber et al. 2000). In the latter study, a subdivision of slow-wave activity into a higher and a lower frequency band revealed further regional differences in their time course during recovery sleep. This heterogeneity in the low frequency range was evident in the present study also on a regional scale where a low-frequency component (around 2 Hz) was less affected by SD (Fig. 2).

The frontal predominance of low-frequency power is also a striking feature of the human EEG, both for baseline sleep (Finelli et al. 2001; Werth et al. 1996) and for the recovery period after sleep deprivation (Cajochen et al. 1999; Finelli et al. 2001). These findings could indicate that anterior neocorti-
cal areas are particularly susceptible to sleep loss. Their particular role is underlined by positron emission tomography (PET) scans showing that in NREM sleep the prefrontal cortex undergoes a large reduction of relative regional cerebral blood flow relative to waking (Finelli et al. 2000).

The similar electrophysiological results in rat and humans may rely on common features of cortical morphology. The density of neurons in the neocortex does not vary substantially across a wide range of species, including humans, and the basic features of cortical morphology are relatively invariant (Rockel et al. 1980). The medial prefrontal cortex of the rat, which is located in the vicinity of the frontal recording site, is considered to contain a homologue of the primate associative prefrontal area (Birrel and Brown 2000). In humans “prefrontal cortex-oriented” cognitive tasks are more vulnerable to a sleep deficit than other tasks (Harrison et al. 2000).

Whereas the frontal EEG showed a large change in response to SD, there was no difference in recordings from the left and right frontal derivation. In contrast, the EEG recorded over the parietal cortex exhibited substantial interhemispheric differences of approximately 20% in REM sleep and to a lesser extent (6–10%) in NREM sleep. The differences in the NREM sleep EEG occurred in the low-frequency range. Power in this range is a principal marker of sleep homeostasis. The declining sleep pressure in the light period was associated with a progressive shift of power from the left to the right hemisphere. A shift in the opposite direction occurred in the dark period when sleep pressure increases. The left predominance of low-frequency power was enhanced after a 6-h SD in the light period. The SD in the first 6 h of the dark period was less effective probably because the rat normally sleeps little during this interval. The progressive changes of the interhemispheric asymmetry as sleep pressure dissipated during baseline, as well as the asymmetry increase after SD when sleep pressure was enhanced, and the positive correlation between EEG power in the low frequencies and left-hemispheric predominance constitute strong evidence that a homeostatic regulatory process determines not only the amount of low-frequency power but also its interhemispheric distribution. An analogous observation was recently made in humans where the SD-induced enhancement of the antero-posterior gradient in the delta band was limited to the left hemisphere (Achermann et al. 2001).

These asymmetries may be related to behavioral and morphological factors. Preferential activation of the left hemisphere during waking may lead to a higher local sleep intensity during sleep. This has been demonstrated experimentally by unilateral stimulation of whiskers in the rat (Vyazovsky et al. 2000) and of the dominant hand in humans (Kattler et al. 1994). Spontaneous waking activities may also entail a differential activation of the hemispheres. Distinct aspects of spatial learning were associated with the left or right hemisphere in the rat (LaMendola and Bever 1997). The asymmetries were observed in complex behaviors such as acquiring a novel foraging pattern, where the left hemisphere was associated with map representation while the right hemisphere was mainly involved in route representation (LaMendola and Bever 1997). A number of other behavioral lateralizations in the rat have been described (Crowne et al. 1992; Grabowski et al. 1991; Sherman et al. 1980; Tang and Verstynen 2002). In humans, handedness is only one of the many factors for a differential hemispheric activation. On a morphological basis, there are various indications of asymmetries in the rat brain (Dowling et al. 1982; Kolb et al. 1982; Sherman and Galaburda 1984).

Another major finding of the present study was the state-related interhemispheric difference in the sleep EEG. Whereas during high-intensity NREM sleep low-frequency power showed a left-hemispheric predominance, power in the theta band, the hallmark of REM sleep EEG in rodents, exhibited a remarkable right-hemispheric predominance of approximately 20%. It appeared within seconds after the transition from NREM to REM sleep, persisted throughout the 24 h, and was not affected by SD. The 6-h SD may be an insufficient challenge for REM sleep mechanisms to induce more pronounced EEG asymmetries. The magnitude of the asymmetry in REM sleep, which exceeded the one in NREM sleep, may indicate a larger involvement of the right hemisphere in the REM sleep regulatory mechanisms. It has been suggested that EEG power within the theta frequencies may represent an intensity component of REM sleep (Borbély et al. 1984; Tobler and Borbély 1986). However, a physiological relevance of its right hemispheric predominance has not been demonstrated. It is known that paw preference is lateralized in rodents (Tang and Verstynen 2002). Therefore it is possible that such behavioral asymmetries may lead to anatomical brain asymmetries, which may be reflected in the sleep EEG.

Our study presents for the first time evidence for an opposite EEG asymmetry in high-intensity NREM sleep and REM sleep in the rat. State-related interhemispheric variations of the EEG associated with the NREM-REM sleep cycle have been reported for humans (Goldstein et al. 1972; Roth et al. 1999; Shannahoff-Khalsa et al. 2001).

In summary, the present findings provide further support for the notion of use-dependent local sleep. The anterior predominance of low-frequency activity in the NREM sleep EEG during high sleep pressure may reflect the increased vulnerability of the frontal cortex to a sleep deficit and its higher need for recovery during sleep. The left predominance at the parietal cortex may be due to a preferential unilateral sensori-motor activation during spontaneous waking. It is known that there is a higher occurrence of right “handedness” in rodents (Tang and Verstynen 2002). In addition, the state-related alternation of hemispheric dominance between the major EEG markers of NREM sleep and REM sleep could indicate that the NREM-REM sleep cycle modulates the functional relations between hemispheres.

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