

Neuroendocrine Processes Underlying Ultradian Sleep Regulation in Man

CLAUDE GRONFIER, CHANTAL SIMON, FRANÇOIS PIQUARD, JEAN EHRHART,
AND GABRIELLE BRANDENBERGER

*Laboratoire des Régulations Physiologiques et des Rythmes Biologiques, 67085 Strasbourg Cédex,
France*

ABSTRACT

Sleep is not a uniform state but is characterized by the cyclic alternation between rapid eye movement (REM) and non-REM sleep with a periodicity of 90–110 min. This cycle length corresponds to one of the oscillations in electroencephalographic (EEG) activity in the delta frequency band (0.5–3.5 Hz), which reflect the depth of sleep. To demonstrate the intimate link between EEG and neuroendocrine rhythmic activities in man, we adopted a procedure permitting simultaneous analysis of sleep EEG activity in the delta band and of two activating systems: the adrenocorticotrophic system and the autonomic nervous system. Adrenocorticotrophic activity was evaluated by calculating the cortisol secretory rate in blood samples taken at 10-min intervals. Autonomic activity was estimated by two measures of heart rate variability: 1) by the ratio of low-frequency (LF) to high-frequency (HF) power from spectral analysis of R-R intervals; and 2) by the interbeat autocorrelation

coefficient of R-R intervals (rRR intervals between two successive cardiac beats).

The results revealed that oscillations in delta wave activity, adrenocorticotrophic activity, and autonomic activity are linked in a well-defined manner. Delta wave activity developed when cortisol secretory rates had returned to low levels and sympathetic tone was low or decreasing, as reflected by a low LF/HF ratio and by low levels in rRR. Conversely, the decrease in delta wave activity occurred together with an increase in the LF/HF ratio and in rRR. REM sleep was associated with a decrease in cortisol secretory rates preceding REM sleep onset, whereas the LF/HF ratio and rRR remained high.

These results demonstrate a close coupling of adrenocorticotrophic, autonomic, and EEG ultradian rhythms during sleep in man. They suggest that low neuroendocrine activity is a prerequisite for the increase in slow wave activity. (*J Clin Endocrinol Metab* 84: 2686–2690, 1999)

PULSATILE activity is characteristic of many neuroendocrine systems and has been traced to the intermittent discharge of hypothalamic releasing factors. In man, some well-defined rhythms have been identified, with periodicities averaging 90–110 min (1). This cycle length is of particular significance because it corresponds to the duration of rapid eye movement (REM) and non-REM (NREM) sleep cycles. Using electroencephalographic (EEG) spectral analysis, which provides a more detailed and dynamic description of sleep processes, authors have reported that delta wave activity, reflecting the depth of sleep, oscillates with a similar cycle duration of 90–110 min (2, 3). It is commonly admitted that the rhythmic oscillations in delta wave activity are a salient feature of good sleep quality; poor sleep is attributed to irregular and fragmented sleep cycles.

A variety of hormones have been reported to fluctuate in temporal association with EEG activity during sleep. For example, renin is considered to be a biological marker of sleep stage alternation (4), and GH and PRL exhibit positive association with delta wave activity (5–7). In contrast, TSH secretory episodes are inversely correlated with delta wave activity (8). Because of its circadian rhythm, cortisol presents

a rather complicated picture, displaying at the beginning of the night a period of low secretion, when delta wave activity oscillates with greatest amplitude. However, at the end of nocturnal sleep, when cortisol secretory pulses and oscillations in delta wave activity are simultaneously present, the two rhythms are synchronized in phase opposition (9). This coupling suggests that common control mechanisms may be involved in pulsatile hormone release and in the ultradian variations in EEG activity.

The aim of the present study was to demonstrate the intimate link between the oscillatory patterns of delta wave activity and of two activating systems: the adrenocorticotrophic system and the autonomic nervous system. We adopted a procedure permitting simultaneous analysis of EEG activity in the delta band and of cortisol secretion in blood taken at short time intervals. Concomitantly, we analyzed heart rate variability, in an attempt to quantify changes in the autonomic nervous system influences during sleep. Spectral analysis of R-R intervals, with its two regions of interest, provides indicators of sympathetic and parasympathetic activity: the low-frequency (LF) peak reflects predominant sympathetic activity, the high-frequency (HF) peak in the vicinity of the respiratory frequency is under parasympathetic control (10, 11), and the LF/HF ratio is commonly regarded as an index of sympathovagal balance. We also calculated the interbeat autocorrelation coefficient (rRR) derived from the Poincaré plot, which has been found to be closely related to variations in sleep EEG activity and to the profiles of LF/HF (12, 13).

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Address all correspondence and requests for reprints to: G. Brandenberger, Laboratoire des Régulations Physiologiques et des Rythmes Biologiques chez l'homme, Institut de Physiologie, 4 rue Kirschleger, 67085 Strasbourg Cédex, France. E-mail: brandenberger.gabrielle@medecine.u-strasbg.fr.

Subjects and Methods

Eight healthy male subjects, 21–28 yr old, gave their written informed consent to participate in this study. They had regular sleep-wake habits and did not take any medication. This study was approved by the local Ethics Committee. The experiments were carried out in a soundproof, air-conditioned sleep room. After a habituation night, the subjects underwent one experimental session during which sleep, cardiac recordings, and blood sampling were simultaneously carried out. Electrodes for polysomnographic recordings were applied 2 h before the beginning of the experiment. Lights were switched off at 2300 h, and the subjects were awakened at 0700 h.

Sleep analysis

Sleep recordings were performed using four EEG derivations (F3, C3, P3 *vs.* A2, and C4 *vs.* A1), one chin electromyographic derivation, and one diagonal electrooculographic derivation (upper canthus of one eye *vs.* lower canthus of the other eye). The recordings were visually scored at 30-sec intervals by use of standardized criteria (14). Although this procedure is useful for characterization of sleep architecture, it is inadequate for more refined, quantitative analysis of sleep EEG. For all-night spectral analysis, which provides a dynamic description of sleep EEG, the EEG signal (C3-A2 or C4-A1) was high-pass (0.3 Hz) and low-pass (35 Hz) filtered before being converted from analog to digital, with a sampling frequency of 128 Hz. Subsequently, spectra were computed for consecutive 2-sec periods with a fast Fourier transformation algorithm (15). To yield one mean spectrum every 10 min corresponding to the blood sampling interval, a median filter was applied for 300 consecutive 2-sec periods. The spectral parameter studied was the absolute power in the delta frequency band (0.5–3.5 Hz).

Blood sampling and plasma hormone measurements

Four hours before the beginning of the recordings, a catheter was inserted, under local anesthesia, into an antecubital vein, which was kept patent with a heparin-containing solution. Blood was sampled continuously for 8 h, from 2300 to 0700 h, by use of a peristaltic pump and was collected in EDTA-K2 tubes in an adjoining room over 10-min periods. Samples were immediately centrifuged at 4 °C, and the plasma was stored at –25 °C until assay. A maximum of 150 mL of blood was removed during the 8-h period.

Plasma cortisol was measured by RIA using commercial assay kits (Ciba Corning, Inc. Diagnostics, Cergy-Pontoise, France). The detection limit was 2 ng/mL. The intraassay coefficient of variation for the duplicate samples was 10% for levels less than 60 ng/mL and 4% for levels more than 60 ng/mL. All samples from a given subject were analyzed in the same assay. The secretory rate of cortisol for each 10-min interval was derived from the corresponding plasma level using deconvolution analysis based on a two-compartment model for distribution and degradation. As previously described (9), the short half-life was set at 5 min and the long half-life at 65 min, and the associated fraction of decay at 20%. The distribution volume was 5.3 L/m² body surface (16). The same metabolic parameters were used for all subjects. Determining the profile of cortisol secretory rates allows for a better understanding of the temporal variations of the hormone, avoiding the shouldering of plasma peaks, so that its relationships with other physiological variables can be accurately defined.

Heart rate analysis

The electrocardiogram signal was fed into a generator that produced a pulse at the rising phase of each R wave. The trigger event times were recorded with an accuracy of ± 1 msec, and the R-R intervals were calculated on a computer equipped with a data acquisition control board including a timer. Computers and polygraphs were precisely synchronized. Occasional ectopic or missing beats were identified and replaced with interpolative R-R interval data. Each R-R interval was plotted against the previous R-R interval, to produce a 10-min Poincaré plot (R-R_{n+1} *vs.* R-R_n). The interbeat autocorrelation coefficient of R-R intervals (rRR; *i.e.* Pearson's correlation coefficient between R-R_n and R-R_{n+1}) was calculated over 10-min periods.

Power spectral analysis of each consecutive 10-min recording was

performed in a sequential fashion with the use of a fast Fourier transform (based on a nonparametric algorithm using a Welsh window) after the ectopic-free data were detrended and resampled. A fixed resampling frequency of 1024 equally spaced points per 10-min period was used. The power densities in the LF band (0.04–0.15 Hz) and in the HF band (0.15–0.50 Hz) were calculated for each 10-min density spectrum by integrating the power spectral density into the respective frequency bands. The LF/HF ratio was calculated using the power in each band.

Statistical analysis

To assess the relationship among the profiles of delta wave activity, cortisol secretory rate, and the measures of heart rate variability, we selected the first NREM-REM-NREM sleep episode for each subject. During this NREM-REM-NREM sleep cycle, oscillations in delta wave activity were the largest in the course of the night, and intrasleep awakenings were less than 2 min. Because this first episode occurred during the quiescent period of cortisol secretion, an additional NREM-REM sleep cycle was selected during the last hours of the night corresponding to the pulsatile period of cortisol secretion, to achieve better insight into the relationship between delta wave activity and cortisol secretory rates. Only five of the eight subjects studied displayed such a NREM-REM sleep cycle at the end of the night, the other three subjects presenting at this time a waking period or fragmented sleep.

To compensate for the individual differences in the duration of the NREM and REM sleep episodes, the individual profiles were subdivided into equal parts according to the method of Achermann *et al.* (2). Individual data were subdivided: 1) into six parts from the beginning of the first NREM sleep episode to the maximum of delta wave activity, detected by a modified pulse analysis algorithm ULTRA (17), as previously described (9); 2) into four parts from the maximum of delta wave activity to REM sleep onset; 3) into four parts from REM sleep onset to REM sleep offset; and 4) and 5) again into six parts and four parts during the second NREM sleep episode. Data were averaged over subjects, and the SE was calculated for each interval.

Mean oscillations in delta wave activity, in cortisol secretory rate, and in the measures of heart rate variability were analyzed by one-way ANOVA for repeated measures with Greenhouse-Geisser correction, with time as a factor. The Student-Newman-Keuls test was employed for *post hoc* multiple comparisons between successive time points of delta wave activity, cortisol, and the measures of heart rate variability. The difference was considered to be significant if *P* was < 0.05.

Results

Figure 1 illustrates the mean profiles in delta wave activity, and the concomitant profiles of cortisol secretory rate, of the LF/HF ratio and of rRR, during the first normalized NREM-REM-NREM sleep episode in eight subjects. Delta wave activity significantly increased (from 56.9 ± 16.6 to 304.1 ± 30.4 μV²; *P* < 0.0001) and then decreased to reach a nadir during REM sleep (26.6 ± 1.3 μV²; *P* < 0.0001). The second oscillation in delta wave activity was of lower magnitude, but both the increase (from 29.5 ± 1.7 to 215.2 ± 76.6 μV²; *P* < 0.001) and the decrease (from 215.2 ± 76.6 to 49.8 ± 5.9 μV²; *P* < 0.05) were statistically significant.

The two oscillations in delta wave activity began during the quiescent period of cortisol secretion (levels below 18 μg·min⁻¹). A significant increase (*P* < 0.05) in cortisol secretory rate occurred during the second NREM sleep period, 10 min before the maximum of the second oscillation in delta wave activity, and peaked 20 min later (53.2 ± 12.3 μg·min⁻¹).

The LF/HF profile mirrored that of delta wave activity, with low or declining levels (around 1.0) when delta waves increased, and a significant rise (from 0.8 ± 0.1 to 1.6 ± 0.5; *P* < 0.01) when delta waves decreased. During REM sleep, LF/HF reached its maximum (2.1 ± 0.3) and decreased again

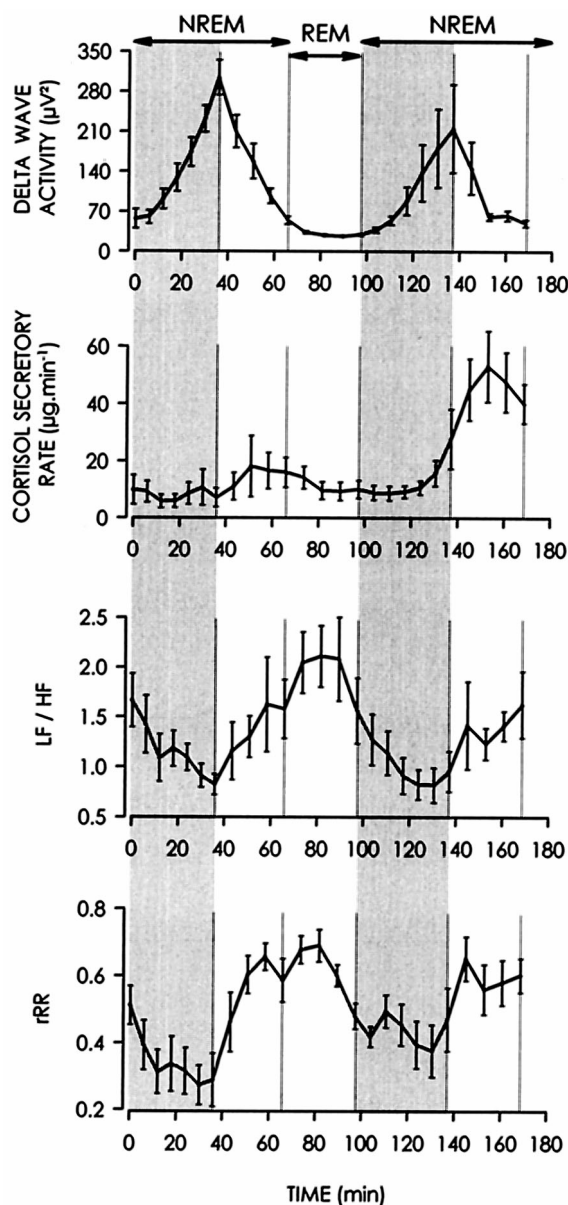


FIG. 1. Profile of delta wave activity (0.5–3.5 Hz), and concomitant profiles in cortisol secretory rates, of the LF/HF ratio and of rRR in eight subjects during the first normalized NREM-REM-NREM sleep cycle. LF/HF, Ratio of LF to HF power from spectral analysis of R-R intervals; rRR, interbeat autocorrelation coefficient of R-R intervals. Individual data were subdivided and averaged according to the method derived from Achermann *et al.* (2). The increasing phases of delta wave activity lie in the shaded areas. Vertical bars delineate initiation and termination of sleep events. The x-axis gives the real time of sleep events, averaged for eight subjects.

before the second oscillation in delta wave activity occurred, to reach a nadir of 0.8 ± 0.1 ($P < 0.01$). The rRR profile demonstrated a close temporal association with the LF/HF ratio. rRR displayed low or declining levels (around 0.3) when delta wave increased; and conversely, rRR increased (from 0.29 ± 0.08 to 0.66 ± 0.04 ; $P < 0.01$) when delta wave activity decreased. rRR reached the highest levels during REM sleep (0.69 ± 0.05). It decreased before the second oscillation of delta wave activity occurred (nadir: 0.37 ± 0.08)

and increased later during the descending phase of the second oscillation in delta wave activity.

To achieve better insight into the relationship between delta wave activity and cortisol secretory rates, the nocturnal cortisol profiles were analyzed during a NREM-REM sleep cycle occurring in five subjects, at the end of the night, during the pulsatile period of cortisol secretion (Fig. 2). Significant ($P < 0.001$) increase in delta wave activity occurred when the cortisol secretion rate was at low level (below $20 \mu\text{g}\cdot\text{min}^{-1}$). A significant increase in cortisol secretory rate occurred concomitantly with a decrease in delta wave activity (from 20.1 ± 5.0 to $64.7 \pm 9.5 \mu\text{g}\cdot\text{min}^{-1}$; $P < 0.001$). The decrease ($P < 0.001$) in cortisol preceded the initiation of REM sleep by 10 min, and the cortisol secretory rate reached low levels at the end of the REM sleep episode ($18.6 \pm 7.6 \mu\text{g}\cdot\text{min}^{-1}$).

Discussion

This study demonstrates a systematic link between the oscillations of slow wave activity, of adrenocorticotrophic activity, and of the autonomic nervous system activity. Using a method derived from Achermann *et al.* (2), which allows us to average data across NREM-REM-NREM sleep episodes of various lengths and to precisely delineate the initiation and termination of sleep events, we have defined the rules determining the relationship between neuroendocrine and EEG activities during sleep. The most important result is that slow waves begin to develop when adrenocorticotrophic activity and sympathetic influences are low. In no case do delta waves increase together with an increase in cortisol secretion and an increase in the LF/HF ratio. Thus, it seems that low adrenocorticotrophic activity and low sympathetic tone are a prerequisite for the increase of slow waves. Conversely, the disappearance of slow waves is linked to an increase in

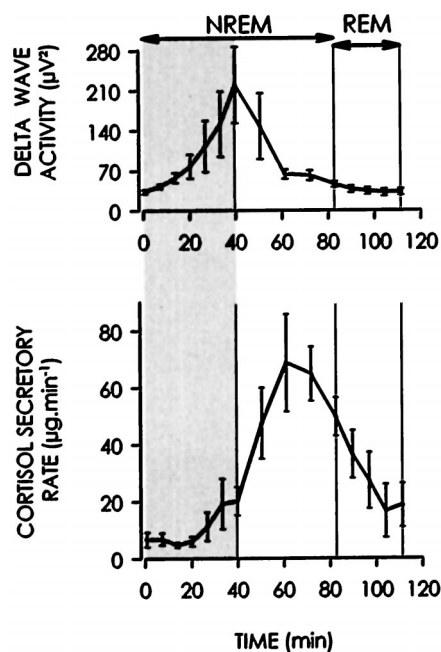


FIG. 2. Profile of delta wave activity (0.5–3.5 Hz) and the corresponding profile in cortisol secretory rates in five subjects during the last normalized NREM-REM sleep cycle occurring in the pulsatile period of cortisol secretion. Individual data were averaged as in Fig. 1.

cortisol secretion, at least during its pulsatile period, and a concomitant increase in sympathetic influences. REM sleep is associated with a decrease in cortisol secretory rate, preceding REM sleep onset. Sympathetic tone remains high, and its decrease occurs before the second increase in delta wave activity. These results suggest that neuroendocrine processes adjust in anticipation of sleep events. However, the reverse situation, described previously (18), whereby neuroendocrine processes are influenced by sleep events, cannot be excluded.

We recently reported (9) that cortisol and ACTH pulses were negatively correlated with delta wave activity (cortisol and ACTH changes preceding variations in delta wave activity by about 10 and 20 min, respectively). We noted that the two rhythmic variations of cortisol and delta waves were coupled in phase opposition at certain times of the night and dissociated at others. For example, oscillations in delta wave activity occurred at the beginning of the night without any concomitant cortisol pulses. Therefore, it has been postulated that the two rhythmic activities may be driven by two distinct and independent generators. These observations have been confirmed in the present study, in which the first oscillation in delta wave activity was observed during the quiescent period of cortisol secretion, whereas the second one occurred usually together with the first cortisol pulse linked to the circadian rhythm. Additional results, obtained in the last part of the night, revealed more precisely the relationship between cortisol secretory episodes and sleep events. They clearly demonstrate that cortisol decreases before the initiation of REM sleep, confirming previous results that establish an association between REM sleep and low adrenocortical secretion (19, 20). They also reveal the precise pattern of cortisol secretion during NREM sleep, with low cortisol secretion during the ascending phase of delta wave activity and an increasing cortisol level during the descending phase. The use of spectral analysis of the EEG thus allows for a more precise determination of the time courses of delta activity, in relation to cortisol secretory rate, and further contributes to previous studies based on the traditional scoring of sleep stages, which noted that the whole period of slow wave sleep is associated with low adrenocorticotrophic activity (20, 21). Finally, even if the relationship between cortisol and the NREM-REM sleep cycles seems to change over the course of the night [because of the changes from the quiescent period of cortisol secretion at the beginning of the night and the subsequent pulsatile period at the end of the night (22)], a single rule determines this association: delta wave activity cannot develop unless corticotrophic activity is low and never builds up in a period of high secretory activity.

In the present study, we focused on the changes in heart rate variability, in an attempt to continuously evaluate the autonomic influences during sleep. We calculated the power spectrum components of R-R intervals, because investigators frequently infer the sympathovagal balance from the ratio of LF to HF power (10, 11). We also calculated the autocorrelation coefficient of R-R intervals, recently reported to display coordinate variations with the overnight profiles of EEG mean frequency and of the LF/HF ratio, respectively, suggesting that rRR could be regarded as an index of sympathovagal balance during sleep in healthy men (12, 13). In the

present study, the LF/HF ratio and rRR had similar profiles across the normalized NREM-REM-NREM sleep cycle, with low levels when delta wave activity rose and increasing levels when delta waves disappeared, indicating a relationship between profound changes in sympathovagal balance and the oscillation of slow waves. These results further develop the findings of earlier studies reporting that REM sleep is characterized by a high LF/HF ratio, revealing increased relative sympathetic activity, and NREM sleep, by a predominance of parasympathetic activity (23–29). The fact that sympathetic activity precedes and possibly plays a role in the initiation of sleep events corroborates previous results from Bonnet and Arand (30), who reported that heart rate acceleration, usually considered to be associated with increased nervous system activity, precedes visually detected sleep events. This finding is also consistent with the results of Muzet and Michel (31) who found that spontaneous activation phases during sleep are preceded by an adjustment of heart rate, and with the results of Hobson (32) who demonstrated that postural changes occurred in slow wave sleep before the shift in REM sleep. Taken together, these results clearly support the theory that variations in sympathetic nervous system activity precede variations in EEG activity.

In conclusion, the continuous evaluation of sympathovagal balance, together with adrenocorticotrophic activity, suggests that neuroendocrine processes adjust in relation to sleep events. Both activating systems display oscillations intimately linked to sleep deepening and lightening and intervene for an harmonious coordination of ultradian processes to assure good sleep quality.

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