

Claude Gronfier, Florian Chapotot, Laurence Weibel, Christophe Jouny, François Piquard and Gabrielle Brandenberger
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Pulsatile cortisol secretion and EEG delta waves are controlled by two independent but synchronized generators

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Gronfier, Claude, Florian Chapotot, Laurence Weibel, Christophe Jouny, François Piquard, and Gabrielle Brandenberger. Pulsatile cortisol secretion and EEG delta waves are controlled by two independent but synchronized generators. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E94–E100, 1998.—We have previously described a temporal relationship between plasma cortisol pulses and slow-wave sleep and, more recently, an inverse significant cross-correlation between cortisol secretory rates and delta wave activity of the sleep electroencephalogram (EEG). The aim of this study was to observe ACTH, cortisol, and sleep delta wave activity variations throughout 24 h to get a better insight into their initiating mechanisms. Two groups of 10 subjects participated in a 24-h study, one group with a night sleep (2300–0700) and the other with a day sleep (0700–1500). Cortisol secretory rates were calculated by a deconvolution procedure from plasma levels measured at 10-min intervals. Delta wave activity was computed during sleep by spectral analysis of the sleep EEG. When delta waves and cortisol were present at the same time at the end of the night sleep as well as during the daytime sleep, they were negatively correlated, cortisol changes preceding variations in delta wave activity by ~10 min. Increases in delta wave activity occurred in the absence of cortisol pulses, as observed at the beginning of the night. Cortisol pulses occurred without any concomitant variations of sleep delta wave activity, as observed during wakefulness and intrasleep awakenings. In no case did delta wave activity increase together with an increase in cortisol secretory rates. In conclusion, cortisol secretion and delta wave activity have independent generators. They can oscillate independently from each other, but when they are present at the same time, they are oscillating in phase opposition.

hypothalamo-pituitary-adrenal axis; sleep electroencephalography; spectral analysis; pulsatility; oscillators

IN RECENT YEARS, it has been shown that numerous 24-h endocrine rhythms arise from a succession of secretory pulses of varying magnitude and frequency rather than from continuous release with superimposed fluctuations (35). In particular, it has been demonstrated that the cortisol secretory pattern results from modulated pulse amplitude without any change in the pulse frequency (35). This pattern is known to be relatively independent of sleep, because it is poorly affected by short-term manipulations of sleep such as sleep reversal, selective and total sleep deprivation, and abrupt shift in the sleep period. However, temporal relationships between cortisol and sleep have been found, but the effect of sleep on cortisol secretion still remains controversial. Sleep has been described as exerting an inhibitory effect on cortisol release, particularly in the first few hours of the night (3, 8, 28, 37). Nevertheless,

other studies have concluded, on the contrary, that sleep does not inhibit cortisol, because diurnal sleep failed to suppress cortisol secretion (24, 36). Despite contradictory results concerning the temporal relationships between cortisol and sleep structure, awakenings or light sleep periods (stage 1) are generally considered to be linked to increasing plasma levels (8, 12, 28, 36), whereas slow-wave sleep (SWS) is associated with low or decreasing cortisol release (12, 36). All of these results are based on conventional scoring of sleep stages. Using electroencephalographic (EEG) spectral analysis, which makes possible a more detailed and dynamic description of the sleep processes than the traditional visual scoring of sleep stages, authors have described the rhythmic profile of delta wave activity throughout the night (1, 2, 5). On the other hand, plasma cortisol levels are the reflection of the activity of the hypothalamo-pituitary-adrenal (HPA) axis, and corticotropin-releasing hormone (CRH) release is responsible, together with arginine vasopressin, through adrenocorticotropin (ACTH) secretion, for cortisol pulsatility, with a periodicity in a similar range to that of delta wave activity (13).

Using spectral analysis of the sleep EEG and deconvolution procedure for estimation of the cortisol secretory rates, we recently described an inverse relationship between cortisol secretory pulses and oscillations in delta wave activity during nocturnal sleep (17). However, at the beginning of the night, oscillations in delta wave activity were greatest at a moment of low cortisol secretion, i.e., during the quiescent period of cortisol secretion.

In the present study, we used a pulse-by-pulse analysis to accurately define the rules determining the temporal relationships between the ultradian rhythms of EEG activity in the delta band and of the HPA axis during sleep. An abrupt shift in the sleep period was effected, displacing the sleep period toward daytime, such that the oscillations in delta wave activity occurred together with numerous cortisol secretory pulses. ACTH was measured to determine whether central mechanisms are involved in the coupling of these widely differing physiological variables.

MATERIALS AND METHODS

Subjects and procedure. Two groups of ten healthy male subjects, aged 21–28 yr and having a body mass index of 22.1 ± 0.4 (SE) kg/m^2 for one group and of 22.7 ± 0.4 kg/m^2 for the other [a nonsignificant difference (NS)], participated in the study after medical examination. The two groups were aged matched (24.3 ± 0.6 vs. 23.3 ± 0.4 yr, NS). All had normal regular sleep-wake habits, and none were taking

medication in the 2 wk before and during the study. Subjects with signs of underlying disease and smokers were excluded from the study. Before their final enlistment, they took part in an experimental session to familiarize themselves with the new environment and with catheter insertion. Informed written consent was obtained from all subjects, and the experiment was approved by the local ethics committee.

The experiments were carried out in a sound-proof air-conditioned and electrically shielded sleep room. After an habituation night (*day 0-day 1*), all subjects underwent one experimental session. One group was studied during a normal 24-h sleep-wake cycle from 1800 (*day 1*) to 1800 (*day 2*), with sleep from 2300 to 0700, the other during a 24-h cycle with an abrupt sleep shift consisting of an 8-h delay in the sleep period (from 0700 to 1500). Throughout the whole experiment, the subjects remained supine, during both the night and the day portions of the 24-h studies. When awake, subjects were maintained in dim light (<100 lux) and were allowed to read or watch television. During the night of sleep deprivation, they were kept under continuous surveillance and conversed with a member of the laboratory staff. To avoid the influence of repeated meal intake, the subjects received continuous enteral nutrition, which began 4 h before blood sampling (Sondalis, ISO; Sopharga, Puteaux, France; 50% carbohydrate, 35% fat, and 15% protein; 378 kJ/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-s intervals by use of standardized criteria (25). For all-night spectral analysis, the EEG signal (C3-A2 or C4-A1) was converted from analog to digital with a sampling frequency of 128 Hz. Subsequently, spectra were computed for consecutive 2-s periods with a fast Fourier transformation algorithm. To yield one mean spectrum every 10 min corresponding to the blood sampling interval, a median filter was applied for each 300 consecutive 2-s periods. The spectral parameter studied was delta relative power (0.5–3.5 Hz) of the global EEG band (0.5–35 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 from 1800 (*day 1*) to 1800 (*day 2*) 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 250 ml of blood was removed during 24 h.

Plasma cortisol was measured by radioimmunoassay using commercial assay kits (Ciba Corning Diagnostics, Cergy-Pontoise, France). The detection limit was 2 ng/ml. The intra-assay coefficient of variation (CV) for the duplicate samples was 10% for levels <60 ng/ml and 4% for levels >60 ng/ml. ACTH was assayed using commercial assay kits (Nichols Institute Diagnostics, San Juan Capistrano, CA). The detection limit was 1 pg/ml. The intra-assay CV for the duplicate samples was 3% for levels <70 pg/ml and 4% for levels >70 pg/ml. All samples taken from a given subject were analyzed in the same assay.

Deconvolution of plasma cortisol and ACTH levels. The secretory rate of cortisol during each 10-min interval was derived from the corresponding plasma level using deconvolution analysis based on a two-compartment model for distribution and degradation. The short half-life was set at 5 min and the long half-life at 65 min with the associated fraction of

decay of 20% (34). The distribution volume was 5.3 l/m² body surface (20). The ACTH secretory rates were estimated using a two-compartment model. The short and the long half-lives used were 3 and 14 min, respectively; the fraction of decay associated with the long half-life was 33%, and the distribution volume was 40 ml/kg body weight (35). Preliminary calculations have indicated that no difference in the time of occurrence and the overall pattern of ACTH and cortisol pulses was observed by changing the half-life values in the range given in the literature. Thus, for cortisol and ACTH deconvolution, the same metabolic parameters were used for all subjects, depending on the hormone.

Determination of pulses of cortisol and ACTH secretory rates. To detect the significant pulses of hormonal secretory rates, the individual profiles of cortisol and ACTH secretory rates were subjected to the pulse detection algorithm ULTRA (33). The general principle of this algorithm is the elimination of all peaks for which either the increment and the decrement did not reach a certain threshold related to measurement error. Pulses of cortisol and ACTH were considered significant if their respective increments and decrements exceeded three times the CV. The statistical error propagation of the uncertainty in data measurements was taken into account in the determination of the secretory profile. For each significant pulse, the time of occurrence of the maximum level, the ascending phase, the descending phase, and the total duration was determined.

Determination of delta wave peaks. For quantification and characterization of the main delta wave activity peaks, the individual profiles were analyzed by a modification of the pulse analysis algorithm ULTRA (33). When the interindividual variability in the levels of delta wave activity was taken into account, the identification of the main peaks was achieved using a subject-adapted threshold for detection. This threshold was set at 20% of the maximum increment in delta relative power observed in the subject. A peak was considered significant if both the increase and the decrease exceeded this threshold. For each significant pulse, the time of occurrence of the maximum level, the ascending phase, the descending phase, and the total duration was determined.

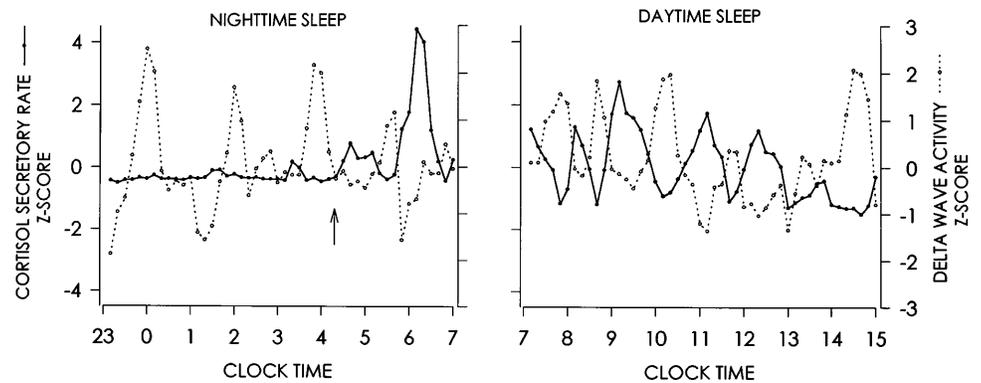
Cross-correlation analysis. The temporal relationship between the cortisol secretory rates and the delta wave activity profiles, starting with the onset of the first significant cortisol pulse for the nocturnal sleep and with the sleep onset for the diurnal sleep, was quantified using cross-correlation analysis (Box-Jenkins Time Series Analysis, BMDP Statistical Software, Los Angeles, CA). A natural logarithmic transformation for the spectral values (15) and a square root transformation for the cortisol secretory rates were retained. Cross-correlation coefficients were computed for lags (–3) to (+3) between the two chronological series, each lag corresponding to a 10-min blood sampling interval. For negative lags, cortisol secretory rates anticipated delta wave activity and, conversely, for positive lags, delta wave activity preceded cortisol secretory rates.

To obtain an average estimate of the correlation, the individual correlation coefficients were averaged for each sleep condition by use of Fisher's "z" transformation (9). This average coefficient calculation was allowed following a nonsignificant χ^2 homogeneity test (27) on the individual transformed coefficients.

Coincidence analysis. To accurately determine the temporal association between cortisol pulses and delta wave peaks, a two-step analysis was performed.

First, the significant ascending phases of cortisol secretion were detected. The concomitant delta wave activity was submitted to peak detection and classified into either ascend-

Fig. 1. Concomitant profiles of delta wave activity and cortisol secretory rates in 2 representative subjects, one during nighttime sleep (left), the other during daytime sleep (right). Both profiles were negatively cross-correlated for nighttime as well as for daytime sleep. Arrow, onset of pulsatile portion of cortisol secretory profile during nighttime sleep.



ing or nonascending phases. The association between ascending phases of cortisol secretory pulses and concomitant phases of delta wave activity was tested by χ^2 analysis, with care taken to account for the relative proportion of ascending and nonascending phases of delta wave activity throughout the sleep period studied, which started with the onset of the first significant cortisol pulse for nocturnal sleep and with the sleep onset for diurnal sleep.

Second, the significant ascending phases of delta wave activity were detected. The concomitant cortisol secretory rates were submitted to pulse detection and classified into either ascending or nonascending phases. The association between ascending phases of delta wave activity and concomitant phases of cortisol secretory rates was tested by χ^2 analysis, with care taken to account for the relative proportion of ascending and nonascending phases of cortisol secretory rates throughout the sleep period studied.

Mean pulse of ACTH and cortisol secretory rates and of delta wave activity. After pulse detection of ACTH and cortisol secretory rates and delta wave activity, a mean pulse was calculated for each parameter. For each subject, all individual pulses were aligned by their maximum and were averaged point by point, from (-4) to (+4) points around the maximum of the pulse. To obtain a mean pulse for the whole group of 10 subjects, the 10 individual mean pulses were expressed as percentages of the individual mean and were averaged point by point.

Mean pulse data were processed 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 ACTH, cortisol, and delta wave peaks.

Unless otherwise indicated, in all statistical analyses the difference was regarded to be significant if P was <0.05 .

RESULTS

Nighttime and daytime sleep profiles of cortisol secretory rates and of delta wave activity. Figure 1 illustrates the profiles of cortisol secretory rates and delta wave activity in two representative subjects, one during nocturnal sleep and the other during daytime sleep. For the two conditions, cortisol secretory rates were negatively correlated with delta wave activity. Cross-correlation coefficients were found to be highest for lags (-2), (-1), and (0), ranging between -0.244 and -0.519. For diurnal sleep, cross-correlation coefficients calculated from sleep onset were highest for lags (-2), (-1), and (0), ranging between -0.286 and -0.550 (Table 1). This indicated either that the two temporal series were concomitant or that the cortisol secretory profile antici-

pated the delta wave activity profile by 10–20 min. The average correlation coefficients between cortisol secretory rates and delta wave activity profiles were found to be significant for nighttime sleep as well as for daytime sleep (respectively, $r = -0.397$ and -0.403 , $P < 0.03$ in both cases). The homogeneity of the individual transformed correlation coefficients in each condition (respectively, $\chi^2 = 3.12$ and $\chi^2 = 7.54$; NS) authorized this calculation.

Cross-correlation analysis was conducted between plasma cortisol levels and delta wave activity, but no systematic relationship could be established. During both nocturnal and diurnal sleep, cross-correlation coefficients were found to be highest for lags (+3) to (-3), ranging between +0.324 and -0.669. They did not reach significance in 8 of 20 subjects.

Mean pulses of ACTH and cortisol secretory rates and delta wave activity. To determine accurately whether cortisol and ACTH secretory activities and delta relative power are temporally linked, a three-step analysis was performed.

First, significant cortisol pulses were detected and concomitant levels of delta relative power were taken

Table 1. Highest cross-correlation coefficients between cortisol secretory rates and delta wave activity profiles

Subject No.	Nocturnal Sleep		Diurnal Sleep	
	Lag	r	Lag	r
1	-1	-0.442*	-1	-0.549‡
2	0	-0.244	-1	-0.519‡
3	0	-0.489†	0	-0.550‡
4	-2	-0.519‡	-1	-0.286
5	0	-0.376*	0	-0.352*
6	0	-0.483*	0	-0.295*
7	-2	-0.328	-2	-0.308*
8	0	-0.316*	0	-0.252
9	-2	-0.465†	0	-0.411†
10	-1	-0.346*	0	-0.473‡
χ^2		3.12 NS		7.54 NS
Average r		-0.397‡		-0.403‡

Cross-correlations were computed from onset of the first significant pulse to 0700 for nocturnal sleep, and from sleep onset to 1500 for diurnal sleep (2 groups of 10 subjects). They were calculated from lag (-3) to lag (+3), each lag corresponding to 10-min blood sampling. For negative lags, cortisol secretion anticipated delta wave activity. NS, nonsignificance for the χ^2 test of homogeneity indicated that cross-correlation coefficients were homogenous and allowed the average r calculation. * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

into account. Figure 2 illustrates the mean cortisol pulse for the 10 subjects for both nighttime and daytime sleep. For nocturnal sleep as well as for diurnal sleep, the mean pulse analysis showed that rising cortisol secretory rates ($P < 0.001$) were temporally associated with significant declining delta wave activity ($P < 0.005$). The significant decreases in delta relative power preceded by 10 min the maximum of the mean pulse cortisol secretory activity (0). Individual pattern analysis revealed that 34 of 35 ascending phases of cortisol secretory pulses were temporally associated with nonascending phases of concomitant delta wave activity during nocturnal sleep (68% of nonascending phases of delta wave activity, $\chi^2 = 13.75$, $P < 0.0001$) and 30 of 33 ascending phases of cortisol secretory pulses during daytime sleep (59% of nonascending phases of delta wave activity, $\chi^2 = 14.00$, $P < 0.0001$).

Second, the major delta wave activity oscillations were detected with regard to the concomitant cortisol secretory rates. Figure 3 shows the mean major oscillations of delta wave activity and the concomitant cortisol secretory rates. The profile for nighttime sleep was similar to profiles of daytime sleep, indicating the same temporal relationship between the two parameters. Significant increases of delta wave activity ($P < 0.001$) were temporally linked to the lowest levels of cortisol secretory rates, indicating that delta wave oscillations occurred at a moment of low cortisol secretory activity. Coincidence analysis revealed that 16 of 19 ascending phases of delta wave activity were significantly associated with nonascending phases of cortisol pulses during nocturnal sleep (66% of nonascending phases of delta wave activity, $\chi^2 = 3.99$, $P < 0.05$) and 33 of 38 during diurnal sleep (59% of nonascending phases of delta wave activity, $\chi^2 = 14.36$, $P < 0.01$).

Third, ACTH was measured because changes in ACTH were assumed to reflect changes in the central

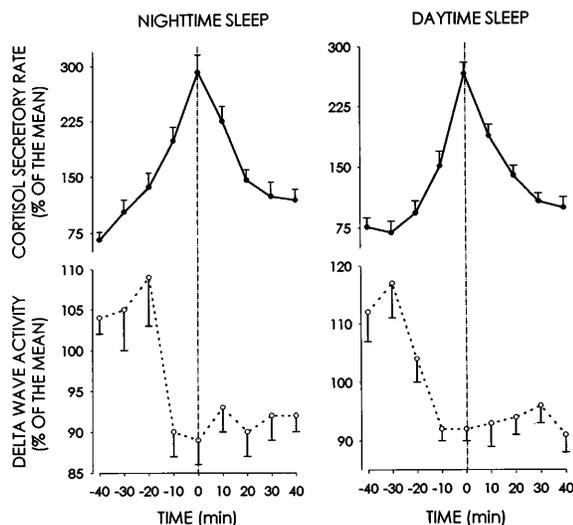


Fig. 2. Pulses (means \pm SE) of cortisol secretory rates and concomitant delta wave activity for nighttime and daytime sleep. *x*-Scale, time in min around maximum of cortisol pulse. Values of cortisol secretory rates and delta wave activity were expressed as percentages of mean values of individual pulses.

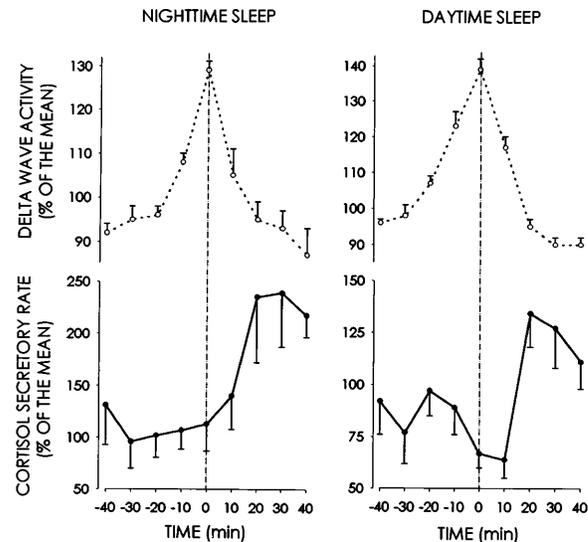


Fig. 3. Peaks (means \pm SE) of delta wave activity and concomitant cortisol secretion for nighttime and daytime sleep. *x*-Scale, time in min around maximum of delta wave activity peak. Values of cortisol secretory rates and delta wave activity were expressed as percentages of mean values of individual pulses.

regulation of the HPA axis much more closely than changes in cortisol. The mean pulse of ACTH was plotted together with the concomitant activities of cortisol secretory rates and of delta wave activity (Fig. 4). The significant increase in mean ACTH secretory levels ($P < 0.001$) preceded by ~ 10 min the significant increase in cortisol secretory rates ($P < 0.008$) and by 10–20 min the significant decrease in delta relative power ($P < 0.04$).

Twenty-four-hour profiles of cortisol secretory rates and delta wave activity. Figure 5 illustrates the 24-h profiles of cortisol secretory rates and delta wave activity in two subjects. To better illustrate the concomi-

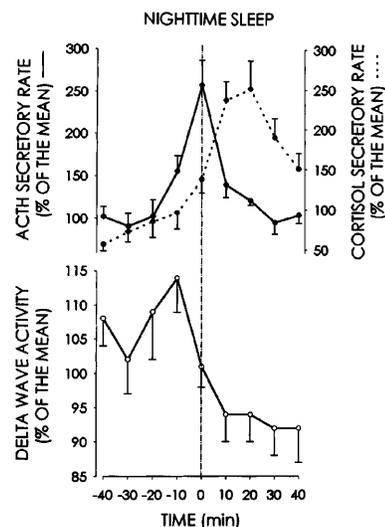
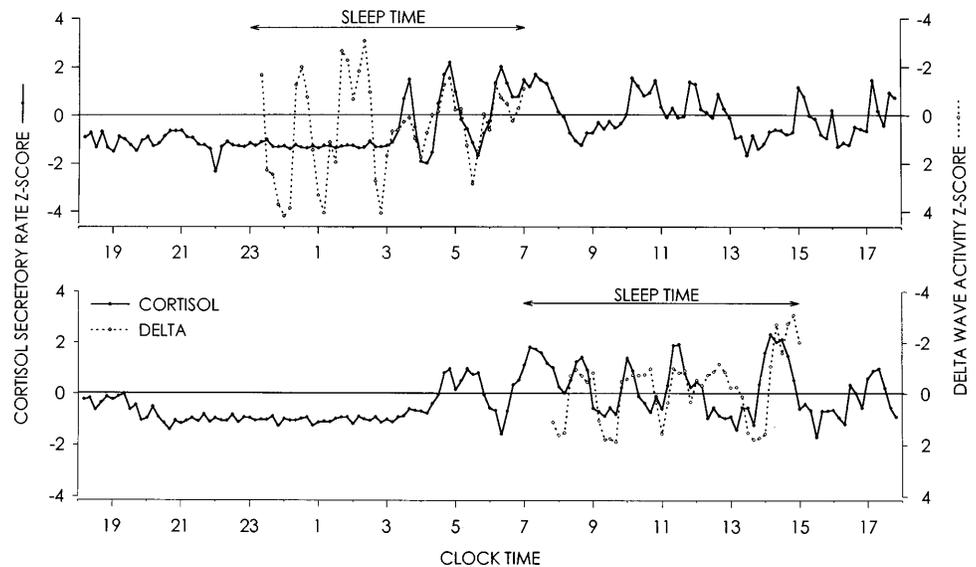


Fig. 4. Pulses (means \pm SE) of ACTH secretory rates and concomitant cortisol secretion and delta wave activity for nighttime and daytime sleep. *x*-Scale, time in min around maximum of ACTH pulse. Values of ACTH and cortisol secretory rates and delta wave activity were expressed as percentages of mean values of individual pulses.

Fig. 5. Twenty-four-hour profiles of cortisol secretory rates and delta wave activity in 2 subjects, one during nocturnal sleep (*top*), the other during diurnal sleep (*bottom*). To better illustrate concomitance of both phenomena, scale for delta wave activity was inverted. Variations in delta wave activity occurred in the absence of cortisol pulses, as observed at beginning of night. Cortisol pulses occurred in the absence of delta wave activity oscillations, as observed during waking periods. When delta waves and cortisol pulses were simultaneously present at the end of the night sleep as well as during diurnal sleep, they were negatively correlated.



tance of both phenomena, the scale for delta wave activity was inverted. Variations in delta wave activity occurred in the absence of cortisol pulses, as observed at the beginning of the night. Cortisol pulses occurred without any concomitant variations of sleep delta wave activity, as observed during normal waking period or during total sleep deprivation in the case of sleep shift. However, when delta waves and cortisol secretory pulses were simultaneously present, at the end of the night sleep as well as during the daytime sleep, they were synchronized in phase opposition.

DISCUSSION

This study demonstrates an inverse temporal relationship between cortisol secretion and delta wave activity, both during nocturnal and diurnal sleep, cortisol changes preceding the changes in EEG activity by ~10 min. The results clearly show that increases in cortisol secretory rates were associated with decreases in delta wave activity and, conversely, that increases in delta wave activity did not occur simultaneously with increases in cortisol secretion. As a consequence, when the 24-h cortisol pattern is considered, three important points should be emphasized: 1) oscillations in delta waves can occur without any concomitant cortisol pulses, as observed at the beginning of the night sleep; 2) cortisol pulses can occur without any oscillations in delta wave activity, as observed during the waking states and the intrasleep waking periods; 3) when cortisol secretory pulses and oscillations in delta wave activity are simultaneously present, as observed at the end of the nocturnal sleep and during daytime sleep, both rhythms are synchronized in phase opposition. Thereby, one can infer that these two rhythmic activities may be driven by two independent generators that may be coupled in phase opposition at certain times of the day.

Several hypotheses can be advanced to explain how the rhythmic activities of cortisol and delta waves can be coupled in an inverse relationship at certain times in

the 24 h and disjoined at others. The fact that the two activities are disjoined during waking periods or at the beginning of the night supports the existence of two distinct pacemakers or oscillators (21, 22). A first hypothesis is that the two distinct and independent generators responsible for the rhythmic activities of cortisol and delta wave activity, respectively, are conducted by a driver at a higher level, which could be the suprachiasmatic nucleus (SCN) in the anterior hypothalamus, which could synchronize them in phase opposition. A second hypothesis is that two distinct but nonindependent generators could explain the observed coupling between the EEG activity and the neuroendocrine system. They may operate in two different manners. 1) Delta wave activity or its generators, located in the cerebral cortex (30) with a substantial influence of the thalamus (31), may act on the HPA axis on the CRH pulse generator, which is likely to be located in the SCN and acts via the paraventricular nucleus (19, 26). 2) The HPA axis hormones, cortisol, ACTH, or CRH, or the central generators of the CRH pulses may have an inhibitory effect on delta wave activity. This inhibition could be exerted directly on the slow-wave activity generator or indirectly through an activating effect of one or more of the HPA axis hormones on particular cerebral structures implied in the waking networks (32). The time lag observed between cortisol and ACTH pulses, which precede oscillations in delta wave activity by 10 and 20 min, respectively, supports this latter hypothesis.

Cortisol administration has generally been described as exerting an activating effect on sleep, increasing wakefulness and light sleep and decreasing SWS (11, 16). However, other studies have reported an enhancing effect of glucocorticoids on the amount of SWS (6, 10, 14), which seems to be paradoxical. When administered, CRH has been described as inhibiting SWS (18), and cortisol, conversely, has been described as stimulating SWS (6, 14). This contradicts the assumption that the effects of CRH are mediated by stimulation of

cortisol. Cortisol, when elicited physiologically by CRH via ACTH, exerts effects opposite to those of exogenous cortisol (4, 7, 18). These data suggest that inhibition of SWS could be exerted by CRH and that the SWS-promoting effect of exogenous cortisol could be explained by a feedback inhibition of CRH (4, 29). Recent results in the Lewis rat, which possesses a gene defect that results in reduced secretion of CRH and spends less time awake and more time in SWS, support the hypothesis that CRH may be a modulator of waking and sleep (23).

How the steroid hormone signal is transmitted in the neural circuits that organize sleep behavior is unknown. The results from this study support the hypothesis of two or more distinct generators, one for the pathways for delta wave activity and others for the cortisol pathways. These generators could work independently from each other at certain times of the day, but when they are simultaneously active, cortisol, or rather its regulatory mechanisms, could modulate the delta wave activity in an inhibitory fashion.

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