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TITLE
TWNETY-FOUR-HOUR PLASMA CORTISOL AND PROLACTIN IN HUMAN AFRICAN TRYPANOSOMIASIS
PATIENTS AND HEALTHY AFRICAN CONTROLS

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TWENTY-FOUR-HOUR PLASMA CORTISOL AND PROLACTIN IN HUMAN AFRICAN TRYPANOSOMIASIS PATIENTS AND HEALTHY AFRICAN CONTROLS

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Abstract. We have previously demonstrated that human African trypanosomiasis (sleeping sickness) at the stage of meningoencephalitis results in a major disruption of the circadian rhythmicity of sleep and wakefulness that is proportional to the severity of the disease. This paper examines the corresponding 24-hourly secretion in cortisol and prolactin and compares it with the hourly distribution of sleep composition in infected patients and healthy African subjects. The secretion of cortisol in humans follows a circadian rhythm relatively independent of the sleep-wake cycle, whereas that of prolactin exhibits fluctuations over the 24-hr day that are strongly related to the sleep-wake cycle. After the clinical classification of the patients according to the severity of the disease, hourly blood samples were taken over 24 hr via an indwelling catheter. Plasma cortisol and prolactin were analyzed by radioimmunoassay, and the variations in the hourly concentrations were analyzed for the presence of a potential 24-hr rhythm (circadian). All of the healthy African subjects showed significant circadian rhythms in both cortisol and prolactin secretion, similar to data on humans from temperate regions, and a sleep-related anamnestic afternoon peak of prolactin. Major disruptions in the circadian rhythms of plasma cortisol and prolactin were found in the three patients with the most severe illness, in contrast to the four who were less severely ill and the healthy controls. Thus, it appears that as the disease progresses in severity, major disruptions begin to occur in body circadian rhythms, not only in the sleep-wake cycle as reported elsewhere, but also in cortisol and prolactin secretion, suggesting that sleeping sickness affects the circadian timing system.

Human African trypanosomiasis, or sleeping sickness, is caused by the inoculation of *Trypanosoma brucei gambiense* by *Glossina palpalis* (tse-tse fly) in western and central Africa. Patients suffering from this disease have been described in the past as sleeping during the day and restless at night.¹ Although this is suggestive of a disturbance in the circadian organization of the sleep-wake cycle, the first 24-hr polysomnographic recording of a patient with sleeping sickness meningoencephalitis was made by Buguet and others in 1989, who found a major disruption in the sleep-wake distribution rather than a hypersomnia.² Alterations in sleep-wake episodes were found to occur equally throughout the day and night, with the disappearance of the normal 24-hr distribution of sleep and wakefulness observed in healthy Africans.³ Subsequently, in a more extensive investigation of infected humans, a relationship between the intensity of sleep-wake disturbances and the stage of advancement of the disease was found.⁴ Thus, it would appear that sleeping sickness at the stage of meningoencephalitis manifests itself as a significant disturbance in the circadian rhythm of sleep and wake episodes throughout the 24-hr day. Buguet and others postulated that this disruption in the 24-hr sleep-wake cycle in infected patients may be due to a disease-induced disturbance of the circadian timing system.⁴

To further examine the extent of the circadian disruption in infected patients, we measured the 24-hr patterns of cortisol and prolactin in the same patients as Buguet and others.⁴ Normal cortisol secretion follows an endogenous circadian rhythm that is independent of shifts in the sleep-wake cycle, with the lowest concentrations occurring several hours

preceding and following sleep onset, followed by major secretory episodes occurring during the latter half of a night's sleep and early morning.^{5,6} Prolactin secretion is an example of a primarily sleep-related rhythm with large secretory episodes during normal nocturnal sleep.^{5,7-9} Twenty-four-hour patterns of cortisol and prolactin secretion were measured in sleeping sickness patients at various degrees of progression of the disease as well as in a control group of similar ethnic origin and from the same geographic area. The relationship of the circadian rhythmicity of these hormones to the distribution of sleep over 24 hr and to the severity of the disease was then examined.

POPULATION, MATERIALS, AND METHODS

Patients and healthy subjects. Eight patients (five men and three women, 17-58 years of age) diagnosed as exhibiting the clinical features of sleeping sickness were selected by a medical surveillance team from two villages located in an endemic area near Daloa and subsequently examined at the Clinical Research Project on Trypanosomiasis at Daloa in Cote d'Ivoire. Informed consent to participate in the study was obtained from the patients, as well by agreement with the Ivorian authorities. *Trypanosoma brucei gambiense* was detected in the blood or a lymph gland puncture and in the cerebrospinal fluid of all study patients by a serologic immunofluorescence test. The patients were then examined by two neurologists and ranked according to the clinical and biological severity of their illness (Table 1).¹⁰ The principal clinical and laboratory criteria used to determine the severity

TABLE 1
*Clinical and laboratory criteria used to determine the severity of the disease**

	Patients						
	P1	P2	P3	P4	P6	P7	P8
Age (years)	25	35	16	25	58	33	23
Sex	M	M	F	M	M	F	F
Headache	Present	Present	Present	Present	Present	Present	Absent
Intermittent fever	Present	Present	Present	Present	Present	Present	Absent
Swollen lymph nodes	Present	Present	Present	Present	Present	Absent	Absent
Daytime somnolence	While active	At rest	At rest	At rest	Absent	Absent	Absent
Hyperpathia	Present without vibratory sense	Present without vibratory sense	Present with vibratory sense	Present with vibratory sense	Absent	Absent	Absent
Primitive reflexes	Present	Present	Present	Present	Absent	Absent	Absent
Deep tendon reflexes	General hyperreflexes	Brisk	Present	Present	Present	Present	Present
Psychiatric disorders	Confused	Confused	None	None	None	None	None
Pruritis	Present	Absent	Present	Present	Absent	Absent	Absent
Tremor	Present	Present	Present	Present	Absent	Absent	Absent
CSF cells/ μ l	288	772	180	56	6	8	84

* Patients are ranked from P1 (most severely sick) to P8 (least severely sick). CSF = cerebrospinal fluid.

of the disease included the presence of headache, intermittent fever, swollen lymph nodes, daytime somnolence, sensory disturbances with uncomfortable diffuse superficial or deep sensations (hyperpathia), presence of primitive reflexes (palm-mental reflex, sucking reflex), exaggerated deep tendon reflexes, psychiatric disorders (confusion, mood swings, agitation, aggressive behavior, euphoria, mutism, indifference), pruritis, with or without skin lesions, tremor (fine and diffuse without any myoclonic jerk at rest or during movement), and an abnormal number of cells (lymphocytes on microscopic examination) ($> 5/\mu$ l) in the cerebrospinal fluid. The patients did not present any Babinski sign, or any alteration in muscle tone or numbness. They had no objective sensory deficit. All patients were at the stage of meningocnephalitis. The most severely ill patient was designated as P1 and the least severe one as P8. The duration of the illness prior to selection for the study could not be established with any degree of certainty. No cases were seropositive for human immunodeficiency virus type-1 or human T cell lymphotropic virus type-1. Once the patients had been clinically classified, they were included in the study. Subsequently, one of the patients (P5) was excluded due to the presence of severe diabetes. Specific treatment of the patients with melarsopol was undertaken immediately after completion of the sleep recordings.

Six healthy African volunteers (six men 20–25 years of age) were selected from an Ivorian population in the city of Abidjan and examined at the University Hospital of Yopougon to serve as controls living in this tropical climate. Due to logistical and cultural reasons, ages could not be matched in the two groups. Controls also tested negative for human immunodeficiency virus-1 and human T cell lymphotropic virus type-1.

In order not to disturb the sleep of the patients and subjects during the study, they were kept in an air-conditioned room (ambient temperature = 24°C) adjacent to the recording room to which equipment leads and catheters were passed through an opening in the adjoining wall. Patients and subjects remained at bed rest during the 24-hr experi-

mental period and all blood samples were taken while patients were in a supine position to eliminate any variations in plasma concentrations due to changes in posture.

Sleep-wake analysis. Polysomnographic recordings were taken continuously throughout the 24-hr period.⁴ These included electroencephalogram, electrooculogram, and electromyogram, following a standard technique.¹¹ The sleep traces were scored at 20-sec intervals and the temporal distribution of sleep and wakefulness throughout the 24 hr was expressed as the hourly percent of wakefulness, stages 1 + 2, slow-wave sleep, stages 3 + 4, and rapid-eye movement (REM) sleep. The sleep patterns of the patients have been reported elsewhere.⁴

Blood sampling and plasma analysis. The patients and subjects were catheterized in the median basilic vein. The end of the catheter was accessible on the other side of the wall via a three-way valve where a syringe could be attached for withdrawal of blood. Ten-milliliter blood samples were removed each hour over a 24-hr period and transferred to tubes containing 10.5 mg of EDTA per tube. Each sample was centrifuged immediately in a refrigerated centrifuge (CR 1000; Jouan, Nantes, France) and the separated plasma was frozen and stored at -60°C .

Hormones were measured in duplicate by radioimmunoassay, with the 24 samples from each patient being analyzed at the same time to minimize any effect of interassay variability. Plasma cortisol was analyzed with the GammaCoat[®] (¹²⁵I) Cortisol Radioimmunoassay kit (INCSTAR Corp., Stillwater, MN) (sensitivity = 0.21 $\mu\text{g/L}$, intra-assay coefficient $< 7.0\%$). Prolactin was analyzed with a double antibody assay (Diagnostic Products Corp., Los Angeles, CA) (sensitivity = 1.4 ng/ml, intra-assay coefficient $< 4.0\%$).

Circadian and statistical analysis. The 24-hr plasma cortisol and prolactin levels were tested for the presence of potential circadian rhythmicity by applying the cosinor analysis technique, in which the span of the data over the 24-hr period is represented by the best fitting cosine function.¹² The properties of this cosine curve can be characterized by the following three parameters: the mesor (mean 24-hr value

TABLE 2
*Circadian rhythm characteristics of plasma cortisol and prolactin in control subjects and patients with sleeping sickness**

Subjects	Cortisol				Prolactin			
	M nmol/L	A nmol/L	Φ hr	<i>P</i> †	M μ g/L	A μ g/L	Φ hr	<i>P</i> †
Healthy controls								
Mean of six controls	215.0 ± 22.2	112.4 ± 8.5	7.9 ± 0.3	0.001	8.1 ± 1.0	2.5 ± 0.6	1.6 ± 1.1	0.001
Patients with sleeping sickness								
P1	198.2 ± 13.6	—	—	NS	7.4 ± 0.5	—	—	NS
P2	266.5 ± 9.5	—	—	NS	3.9 ± 0.3	—	—	NS
P3	299.4 ± 21.5	—	—	NS	23.3 ± 1.4	—	—	NS
P4	213.0 ± 19.1	74.2	7.4	0.01	9.2 ± 1.3	6.1	4.9	0.001
P6	204.0 ± 11.9	68.7	8.7	0.001	2.6 ± 0.4	2.2	2.7	0.001
P7	225.4 ± 9.4	47.5	7.8	0.001	3.4 ± 0.3	1.2	4.6	0.001
P8	209.5 ± 17.1	64.0	7.8	0.025	5.6 ± 0.5	2.0	1.5	0.01
Mean of patients P4, P6, P7, and P8	213.0 ± 4.5	63.6 ± 5.8‡	7.9 ± 0.3	0.001	5.2 ± 1.5	2.9 ± 1.1	3.5 ± 0.8	0.001

* Values, where indicated, are the mean ± SEM. M = mesor; A = amplitude; Φ = acrophase. — = the values of A and Φ for P1, P2, and P3 could not be calculated because no significant cosine curve was found for cortisol and prolactin in these patients.

† Indicates the significance of the sinusoidality of the cosine curve. NS = not significant.

‡ *P* < 0.001 versus healthy control value.

around which the curve oscillates), amplitude of the cosine curve (half of the difference between the highest and lowest calculated 24-hr values in a complete cycle), and acrophase (the clock time at which the highest calculated value encountered in the cycle occurs). The sinusoidality of the cosine curves was tested according to the method of Nelson and others.¹² Statistical comparisons were made using a one- or two-factor analysis of variance and the Student's *t*-test. All results are the mean ± SEM.

RESULTS

The results of the cosinor analysis of both patients' and controls' 24-hr variations in plasma cortisol and prolactin are shown in Table 2. Since all of the control subjects demonstrated a typical significant circadian rhythmicity in both hormones, their values were averaged and only the mean for the six controls is shown. Each of the four less severely ill patients (P4, P6, P7, and P8) also demonstrated significant cosinor rhythms in both hormones, but no significant circadian rhythmicity in either cortisol or prolactin could be detected in the three most severely ill patients (P1, P2, and P3). Statistical comparison of the mean of the circadian values for the four patients with significant rhythms in hormonal levels to the mean control values revealed no significant differences in the mesor or acrophase of cortisol and prolactin. However, a significant decrease in the amplitude of cortisol (*P* < 0.01) was observed in these four patients. The amounts of cortisol and prolactin were comparable in patients and controls, except for patient P3, a 17-year old female, who had an unexplained hyperprolactinemia.

Figure 1A shows the 24-hr variations in mean cortisol and prolactin levels for the healthy African subjects along with their sleep patterns. The classic increase in cortisol occurred during the latter half of the sleep period, with a peak in the early morning hours, followed by a nadir in the late afternoon. Prolactin levels increased immediately upon sleep onset, remained elevated during the night, and decreased on

awakening. An additional peak in prolactin was observed in the afternoon. The control group showed a characteristic distribution of sleep stages throughout the night, similar to that normally seen in a tropical climate for slow-wave sleep and REM sleep.^{3,13} Four of the control subjects napped in the afternoon during the experiment, although all of the experimental subjects were accustomed to napping in the afternoon.

The mean curves for the four patients with significant cosinor rhythmicity in both hormones are shown in Figure 1B. Both cortisol and prolactin increased during the sleep period, but the maximal increase in prolactin was shifted until later in the sleep period when compared with the control group (Figure 1A). Although the four less severely ill patients slept primarily during the night, the fragmentation of sleep into the daylight hours was becoming evident, with the occurrence of scattered daytime sleep episodes between 8:00 AM and 6:00 PM. Furthermore, a high proportion of slow-wave sleep was present throughout the nocturnal period, while slow-wave sleep was only predominant at the beginning of the night in the control subjects.

Figure 2 shows that the 24-hr variations of cortisol and prolactin in the three most severely ill patients showed no significant circadian rhythmicity. The patterns of variations in the concentrations of these hormones consisted of the occurrence of multiple peaks throughout the 24-hr period. Sleep patterns were severely disturbed and sleep episodes were distributed throughout the 24-hr day, with no apparent day-night pattern of variation. In addition, increased secretions of prolactin seemed to coincide with sleep episodes during the corresponding hours.

DISCUSSION

Disturbances in the sleep-wake cycle in sleeping sickness meningoencephalitis have been described by Buguet and others,⁴ who found a relationship between the intensity of the sleep-wake disturbances and the degree of evolution of

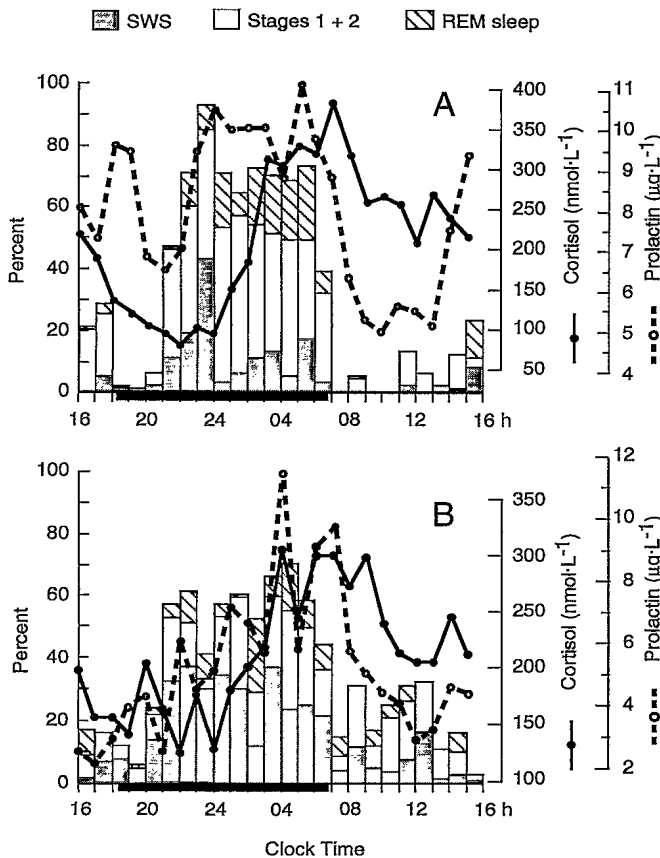


FIGURE 1. Mean hourly time course of plasma cortisol (nmol/L, solid line) and prolactin ($\mu\text{g/L}$, dashed line) in the six control subjects (A) compared with the means of the four patients (P4, P6, P7, and P8) fitting the cosinor 24-hr sine model (B), along with hourly proportions of sleep stages 1 + 2 (blank bars), slow-wave sleep (SWS, dotted bars), and rapid-eye movement (REM) sleep (lined bars). The difference from the 100% value represents the proportion of wakefulness in the given hour. The horizontal black bar above the time scale represents the night period. h = hours.

the disease. They also demonstrated that despite its appellation, sleeping sickness is not a hypersomnia. This study examined plasma cortisol and prolactin variations in healthy African subjects and in a group of sleeping sickness patients to determine 1) whether a well-established circadian rhythm (cortisol)^{5,6} would be disturbed by sleeping sickness, thus confirming the possible alteration of the circadian timing system by the disease, and 2) whether the internal relationship of prolactin with the sleep-wake cycle would still persist, despite the alteration of the timing of sleep and wakefulness. It is re-emphasized that the patients were first classified according to their clinical symptoms prior to a study of their sleep and hormonal rhythms, and any changes in circadian rhythms were not a criteria for ranking the progression of the disease.

Our healthy volunteers showed a hormonal secretion pattern similar to that of Caucasians living in temperate conditions, demonstrating that healthy Africans exposed to the equatorial light-dark cycle have similar 24-hr rhythm fluctuations of cortisol and prolactin as controls living in northern temperate latitudes.⁶⁻⁹ The afternoon peak in prolactin

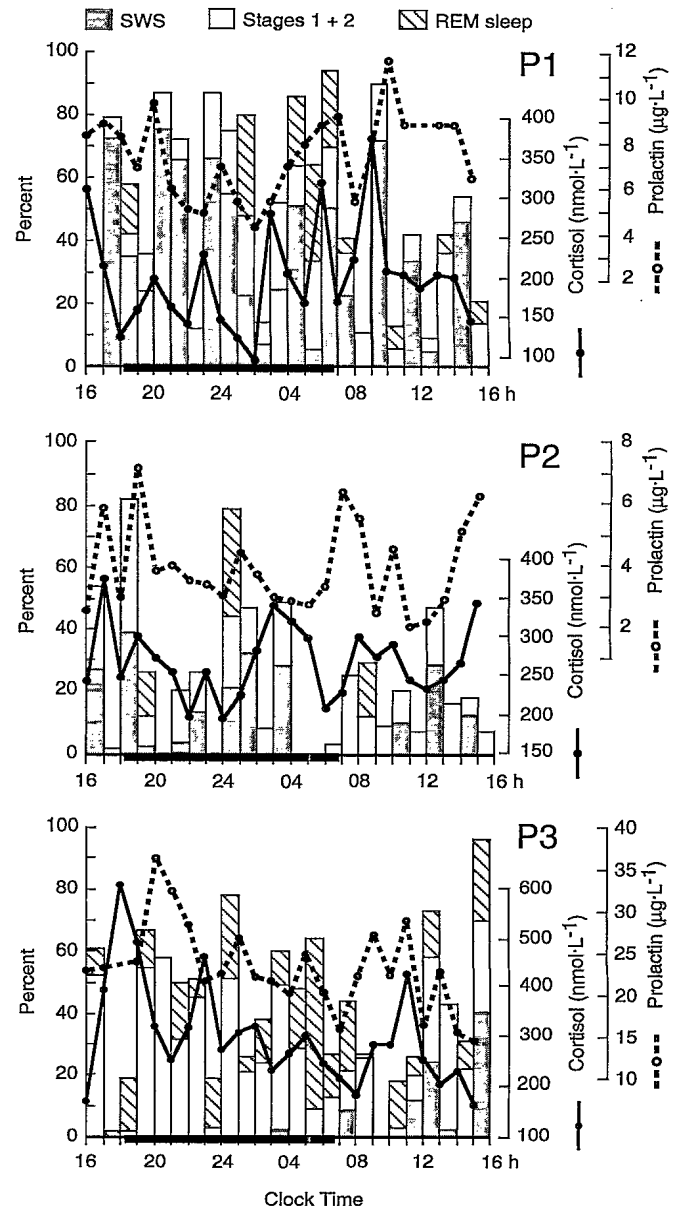


FIGURE 2. Mean hourly time course of plasma cortisol (nmol/L, solid line) and prolactin ($\mu\text{g/L}$, dashed line) in the three patients (P1, P2, and P3) whose data points did not fit the cosinor 24-hr sine model, with hourly proportions of sleep stages 1 + 2 (blank bars), slow-wave sleep (SWS, dotted bars), and rapid-eye movement (REM) sleep (lined bars). The difference from the 100% value represents the proportion of wakefulness in the given hour. The horizontal black bar above the time scale represents the night period. h = hours.

has already been described in Caucasians who take an afternoon nap.⁷ In our study, this afternoon increase occurred even in the absence of any nap. This observation is in agreement with the description of an amnesic peak of prolactin secretion at the time of habitual sleep by Desir and others¹⁴ after displaced sleep and by Spiegel and others¹⁵ during a sleep deprivation night. Our control subjects were habituated to taking an afternoon nap, which is common practice in tropical climates.¹⁶

The finding of a circadian rhythm disorganization in cortisol and prolactin secretion in the most severely ill patients and its independence of the light-dark cycle, along with the findings of disrupted sleep-wake cycles by Buguet and others⁴ on the same patients, supports the suggestion that the loss of the temporal organization of various body rhythms is proportional to the degree of severity of the clinical symptoms. However, as the mean 24-hr secretion of cortisol in the most severely ill patients (P1, P2, and P3) was in the range of values observed in the other patients, it does not appear that the disease-induced disturbances in the circadian timing system affected the actual secretory function of the hypothalamo-hypophyso-adrenocortical axis. The fact that the amplitude of the 24-hr secretion of cortisol was significantly smaller in the less affected patients (P4, P6, P7, and P8) compared with the controls may be reflective of initial disturbances in circadian regulation and may be, therefore, an early clinical sign of the neurologic progression of the disease. Similar disappearances of circadian rhythms have been observed in animals after the development of a lesion of the hypothalamic suprachiasmatic nucleus, which is thought to be the generator of endogenous circadian rhythms,¹⁷ and, in fact, a lymphoplasmacytic infiltration of the hypothalamus by *T. brucei brucei* has been described in animals.¹⁸ Thus, the disturbances observed in our severely infected humans appear to mimic the effects of lesions of the suprachiasmatic nucleus in animals.

Since the early work of Sassin and others¹⁹ and Parker and others,²⁰ the circadian rhythm of prolactin has been regarded as being highly correlated with the sleep component of the sleep-wake cycle in humans. Although this concept has subsequently been challenged,^{21,22} plasma prolactin does increase shortly after sleep onset, remains high throughout the night with an acrophase between 3:00 AM and 4:00 AM,²³ and decreases following awakening.⁸ This relationship persists in subjects examined under entrained conditions or following a phase shift in the sleep-wake cycle due to transmeridian jet travel.¹⁴ This is further confirmed by the fact that sleep deprivation is accompanied by a disappearance of the sleep-dependent prolactin increase,²⁴ and that naps have also been shown to coincide with increases in plasma prolactin.¹²

The major difference found between our controls and patients was the suppression of any circadian rhythmicity in the fluctuations of prolactin secretion in the most severely ill patients, along with a highly disturbed sleep-wake cycle. Although the secretion of prolactin in the most severely ill patients seemed to be related to sleep episodes, the existence of a close temporal relationship with the sleep cycles could not be determined with certainty because of the long intervals between blood samples compared with published data.^{25,26} However, patient P3, who had an elevated prolactinemia, also had 18 REM sleep phases, while the other patients with normal mesor values of prolactin had between three and 13 phases. Our patients had sleep-onset REM periods that are classically observed in patients with narcolepsy. However, Higuchi and others found that circadian rhythmicity in cortisol persisted in narcolepsy as well as the nocturnal increase of plasma prolactin in three of their four narcoleptic patients.²⁶

In conclusion, patients with severe sleeping sickness dem-

onstrate a unique relationship between disruptions in the distribution of sleep-wake episodes and fluctuations in prolactin and cortisol secretion over the 24-hr day. These results are consistent with a dysfunction of the circadian modulation of sleeping and waking, and of aspects of hormonal secretion in the hypothalamo-hypophysio-corticoadrenal axis.

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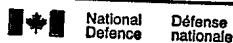
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