

Twenty-Four-Hour Disruption of the Sleep-Wake Cycle and Sleep-Onset REM-Like Episodes in a Rat Model of African Trypanosomiasis

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Study Objectives: Patients with human African trypanosomiasis (sleeping sickness) due to the inoculation of *Trypanosoma brucei gambiense* or *rhodesiense* show a major disruption of the 24-hour sleep-wake distribution, accompanied by the occurrence of sleep-onset rapid-eye-movement (REM) sleep episodes, proportional to the severity of the illness. Although animal models of human African trypanosomiasis have been developed to understand the pathogenic mechanisms leading to immune alterations, the development of an animal model featuring the alterations of endogenous biologic rhythms remains a necessity.

Animals: Sprague-Dawley rats (N = 10) entrained to a 12:12-hour dark-light regimen.

Interventions: Rats were infected with *Trypanosoma brucei brucei* AnTat 1.1E and instrumented with electrocorticographic and electromyographic electrodes. Polysomnography was recorded continuously from 2 days before infection until the animal's death.

Measurements and Results: The analysis of the spontaneous sleep-wake architecture revealed an increased proportion of slow-wave sleep (SWS) and a decreased amount of wakefulness 2 days before death. Considerable sleep fragmentation was observed in the infected rats, with

numerous changes in sleep-wake stages and an increased number of episodes of wakefulness and SWS. Infected rats presented a fragmented pattern of SWS and a marked reduction in the mean paradoxical-sleep (PS) latency, resulting in a considerable disruption of the PS-SWS sequences. Abnormal transitions, particularly the appearance of sleep-onset REM episodes, marked the disruption of the internal sleep structure. The electrocorticogram traces were modified during SWS, with the occurrence of abnormal hypersynchronous slow waves and a disappearance of spindles.

Conclusion: The *Trypanosoma brucei brucei*-infected rat is a good model of the syndrome seen in human African trypanosomiasis, ie, the 24-hour disruption of the sleep-wake cycle and the occurrence of sleep-onset REM-like sleep episodes.

Key Words: 24-hour rhythms; sleep-wakefulness cycle; parasitic infections of the nervous system; experimental African trypanosomiasis

Citation: Darsaud A; Bourdon L; Mercier S et al. Twenty-four-hour disruption of the sleep-wake cycle and sleep-onset REM-like episodes in a rat model of African trypanosomiasis. *SLEEP* 2004;27(1):42-6.

INTRODUCTION

HUMAN AFRICAN TRYPANOSOMIASIS (HAT), KNOWN AS SLEEPING SICKNESS, HAS REEMERGED IN 36 COUNTRIES THROUGHOUT SUB-SAHARAN AFRICA AND IS AN IMPORTANT CAUSE OF HUMAN MORTALITY AND MORBIDITY.¹ It results from the infectious bite of tsetse flies (genus *Glossina*), which inoculate protozoan parasites of the *Trypanosoma* species. The East African form is provoked by *Trypanosoma brucei* (*T. b.*) *rhodesiense*; the West African form by *T. b. gambiense*. It is estimated that about 50 million people worldwide are at risk of being infected.²

Both diseases affect the central nervous system, with *T. b. rhodesiense* causing an acute form of neurologic disease, and *T. b. gambiense*, a more chronically evolutive meningoencephalitis.³ In HAT, the involvement of the nervous system occurs at a late stage of the disease, with the disease being invariably fatal if untreated.

The meningoencephalitic stage of HAT is characterized by a number of distinct neurologic symptoms. These include altered sleep patterns and diverse neuropsychiatric disorders including dysesthesia, extrapyramidal motor disturbances, mood disturbances, etc.^{4,5}

Humans suffering from African trypanosomiasis at the stage of meningoencephalitis exhibit 2 specific disturbances: (1) a major disruption of the 24-hour sleep-wake distribution, which worsens with the severity of the disease⁶⁻⁹ and (2) the occurrence of sleep-onset rapid-eye-movement (REM) sleep episodes (SOREM), proportional to the severity of the illness.^{8,10} A recent report¹¹ postulated that both alterations might be triggered by a serotonergic dysfunction impacting the circadian timing system.

Experimental animal infection has been used to investigate the neuropathologic alterations of African trypanosomiasis. However, the correlation with clinical symptoms remains to be clarified, especially regarding sleep and wake alterations. In order to understand such disturbances, a few experimental studies have been conducted in rats.¹²⁻¹⁵ Continuous 24-hour recordings have revealed a considerable sleep fragmentation during the second week following infection¹²; changes in sleep-wake stages were numerous, the number of wakefulness and slow-wave sleep (SWS) episodes increased, and the infection produced a progressive disruption of the sleep-wake cycle. However, the extent of the disturbances observed in patients with HAT has not been attained in the rat model. More recently, a considerable fragmentation of SWS and a consequent disruption of the SWS-paradoxical sleep (PS) sequence were described 3 weeks after infection.¹³⁻¹⁵

The purpose of this study was to specify the time course of the disturbances in the sleep-wake cycle and that of the alteration of sleep architecture in infected rats. Electrophysiologic recordings were per-

Disclosure Statement

This work was partly supported by a grant from the region Rhône-Alpes (thématiques prioritaires santé, 2000-2002 n° 01 018675 01). The first author received a research grant from the European Community (FSE objective 3) and the Conseil Régional du Limousin.

Submitted for publication June 2003

Accepted for publication September 2003

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formed continuously in rats 2 days before infection with *T. b. brucei* and were maintained until the animal's death.

METHODS

The institutional ethics committee for animal care and use for research purposes approved the experimental protocol. Efforts were focused on limiting pain and the number of animals included in the study.

Animals

Ten adult male albino Sprague-Dawley rats (IFFA-CREDO, Lyon, France) were used. They weighed 200 to 220 g at the beginning of the investigation. The rats were individually housed in Plexiglas cages (36 × 20 × 35 cm) placed in an individual, sound-attenuated chamber to which they had been adapted since at least 7 days before the experiments (ambient temperature 21°–22°C). A 12:12-hour light-dark cycle was provided. The animals had unlimited access to food and water.

Operations

Operations were performed under deep pentobarbital sodium anesthesia (pentobarbital, 35 mg·kg⁻¹ intraperitoneally). All rats were placed in a stereotaxic frame (ASI Instruments, Houston, Tex, USA) and surgically equipped with electrodes to achieve conventional polysomnographic recordings. Four stainless steel-plated, round-tipped miniature screws (1.19 mm in diameter, Phymep, Paris) served as electrodes to record the electrocorticogram (ECoG). They were screwed into the skull through small trepanation holes drilled at the level of the fronto-occipital cortex and secured to the calvarium with acrylic dental cement (Hesadon n°31, Import dentaire, Paris). The electrodes were positioned in relation to the bregma, lambda, and midline sutures¹⁶: (1) left and right frontal cortex (2.0 mm lateral to the midsagittal line, 1.5 mm anterior to the bregma) and (2) left and right occipital cortex (2.0 mm lateral to the midsagittal line, 3.5 mm posterior to the bregma). The midsagittal-line reference electrode was implanted 1.5 mm posterior to the lambda. Three stainless steel wire electrodes were inserted beneath the neck muscles to record the electromyogram (EMG). The electrode electrical wires were soldered to a miniconnector (Sonepar Electronique France, Palaiseau) anchored to the skull with the acrylic dental cement.

One week was allowed for recovery from the surgical procedure. The animals were then acclimated to the recording conditions, particularly to the flexible counterbalanced recording leads connecting the Medilog 9000 II recorder (Oxford Instrument, Abington, UK) through a light cable and a turning commutator (Air Précision, Le Plessis Robinson, France). The commutator allowed the rat to move freely and prevented cable tangling. Recordings began 7 to 10 days later, and 2 days of stable baseline data were obtained.

Trypanosome and Injection Procedure

Trypanosoma brucei brucei AnTat 1.1E was used as previously described.^{12, 17} The protozoa is not pathogenic to human. The strain was a pleiomorphic clone derived from an EATRO cryostablate (East African Trypanosomiasis Research Organization) 1125, isolated in 1966 from the

blood of the *Trailephagus scriptus* in Uganda and kindly provided by Prince Leopold Institute of Tropical Medicine at Antwerp, Belgium. Briefly, the cryostablate was rewarmed by mixing it in 0.9% NaCl containing 1% glucose (1:5 ratio) before use. The mobility of the trypanosomes was controlled under an optic microscope, and their concentration adjusted to 10⁴ per mL.

After 2 consecutive 24-hour stable baseline recordings, the rats were infected through an intraperitoneal inoculation of 200 µL of the above solution containing approximately 2,000 trypanosomes. This procedure

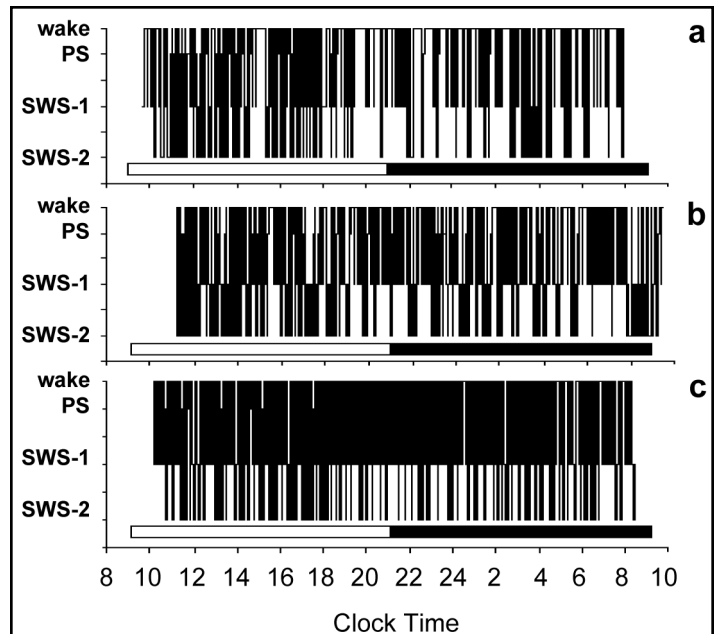


Figure 1—Hypnograms of 1 rat showing the distribution of wakefulness (wake, W), slow-wave sleep (SWS-1, SWS-2), and paradoxical sleep (PS) during the baseline (control) period (a), at the end of the 2nd week of infection by *Trypanosoma brucei brucei* (b, 14th day after the inoculation), and on the day preceding death (c, 21st day after the inoculation). After infection, W and SWS tend to be equally distributed during the dark (black bar underneath the hypnogram) and light (white bar) periods.

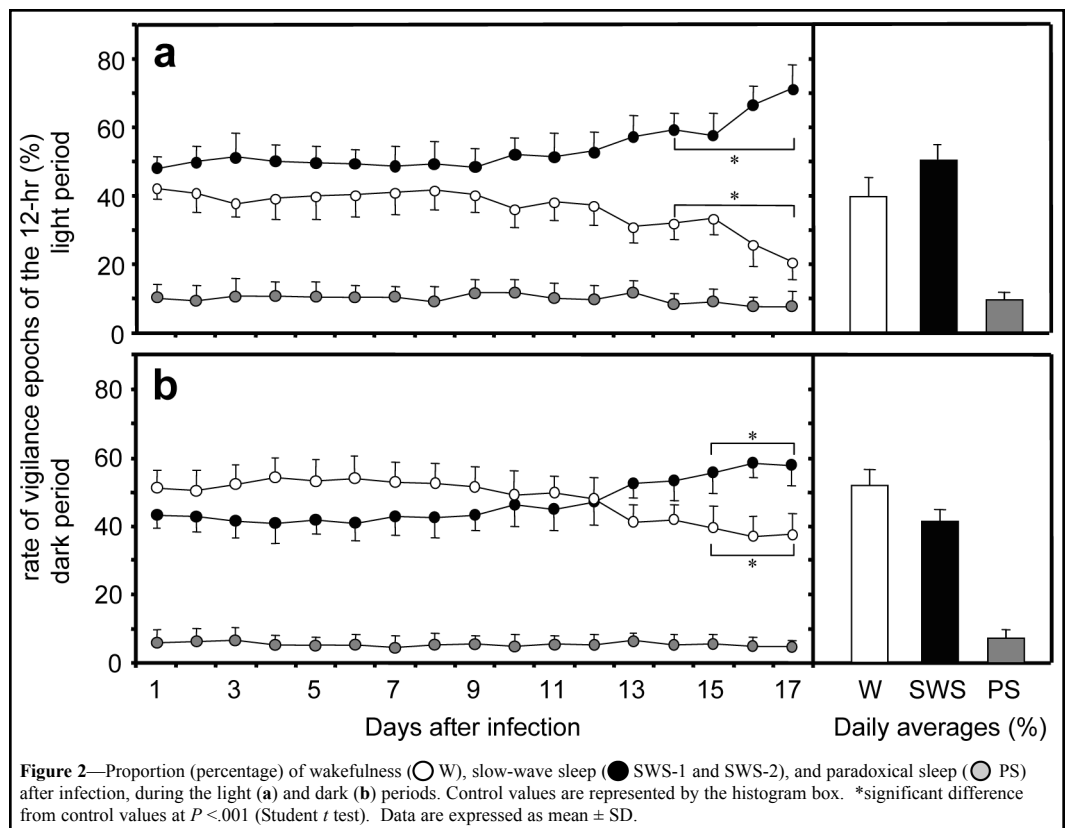


Figure 2—Proportion (percentage) of wakefulness (○ W), slow-wave sleep (● SWS-1 and SWS-2), and paradoxical sleep (○ PS) after infection, during the light (a) and dark (b) periods. Control values are represented by the histogram box. *significant difference from control values at $P < .001$ (Student t test). Data are expressed as mean $\pm$ SD.

was always conducted between 9:00 AM and 9:30 AM. The parasitemia was checked in blood sampled from the tail 10 days after infection, by observation under microscope.

Polysomnographic Recordings and Sleep-Wake Stage Scoring

The ECoG and EMG were recorded continuously in 24-hour periods: 2 baseline 24-hour periods preceding infection, and then daily until the animal's death. The ECoG and EMG signals were recorded on analog FM magnetic tape (Medilog 9000 II, Oxford Instruments, Abington, UK) and transcribed into digital format using the Vision software (Oxford) with a sampling frequency of 128 Hz and a 12-bit amplitude resolution.

The polysomnographic recordings were visually scored by 20-second epochs into 4 sleep-wake states according to conventional criteria¹⁸ using the Prana software (PhiTools, Strasbourg, France). The sleep-wake states were identified as follows: wakefulness (desynchronized ECoG, low ECoG amplitude, high to medium EMG levels); SWS separated in 2 components, SWS-1 or light SWS (synchronized ECoG with low to

medium amplitude and low EMG levels) and SWS-2 or deep SWS (high-amplitude synchronized ECoG and low EMG levels); and PS (desynchronized ECoG with predominant theta rhythm of 6-9 Hz, low to medium ECoG amplitude, and very low EMG levels).

Several sleep measures were calculated: (1) total amount of sleep stages (percentage of total sleep time); (2) number and duration of the different sleep episodes; (3) interval from the end of a SWS episode to the beginning of the next one; (4) mean PS latency, defined as the mean duration of SWS that preceded a PS episode¹⁴; (5) number of stage changes; (6) number of SOREM-like episodes defined as PS episodes that follow two 20-second epochs or more of wakefulness without any interruption by an episode of SWS. Sleep measures were averaged over 12-hour epochs corresponding to the succession of the light and dark conditions.

Statistical Analysis

Statistical analysis was performed using the StatView® package (SAS Institute inc, Cary, NC, USA). Because no differences were observed among individual data for the 10 rats during the baseline condition, the data were pooled. A 2-way analysis of variance (ANOVA) (factors: Time and Period, ie dark vs light) with repeated measures was performed to determine the influence of Time and Period on the sleep-wake measures. Post hoc simple-effects analyses were applied to multiple comparisons, with Bonferroni α adjustments when necessary. All *P* values less than .05 were considered to be significant. The mean $\pm$ SD was used to express group data.

RESULTS

The major finding obtained in the rats infected with *T. b. brucei* confirm that this model represents a good replica of the syndrome observed in humans: 24-hour disruption of the sleep-wake cycle on the one hand, sleep architecture disorder with the occurrence of sleep-onset PS episodes (SOREM-like episodes) on the other hand. These changes were supported by the quality of baseline recordings, representative of normal sleep patterns in rats (Figure 1 and Figure 2). In these conditions, sleep was predominant in the daytime ($39.9 \pm 3.6\%$ wakefulness and $60.1 \pm 4.5\%$ sleep; $P < .05$), wakefulness at nighttime ($52.5 \pm 4.5\%$ wakefulness and $47.5 \pm 3.6\%$ sleep; $P < .05$). For each rat, the 24-hour distribution of sleep and wake episodes was represented as hypnograms, of which Figure 1 is a representative example. After infection, the rats died in 18 ± 2.8 days.

The 24-Hour Sleep-Wake Cycle Disruption

Disturbances of the 24-hour distribution of sleep-wake states were observed at a late stage of the experimental disease. They occurred only a few days before death, independently of the individual animal's survival. Similarly, the proportion of sleep-wake states did not change until this time.

During the last 2 days of the animal's life, amounts of SWS increased and wakefulness decreased during both light and dark conditions (Table 1 and Figure 2). The proportion of PS did not change until immediately before death (Table 1). Once again, this sequence did not depend upon the survival time of the animal.

Changes in the depth of SWS were also observed. The proportion of SWS-1 increased 5 days before death, at the expense of SWS-2 (Table 1 and Figure 3). The number of SWS-1 and SWS-2 episodes increased, beginning on Day 15 (15th day after infection) during the dark period of the nyctohemeron and on day 14 during the light period ($P < .01$), whereas the mean duration of episodes decreased from Day 15 in both illumination conditions ($P < .001$) (Figure 4).

After infection, the mean duration of the wakefulness episodes decreased at approximately the same time (Figure 4; comparison to control: $P < .01$ on Day 15 during the dark period; $P < .01$ on Day 14 during the light period). On Day 14, the

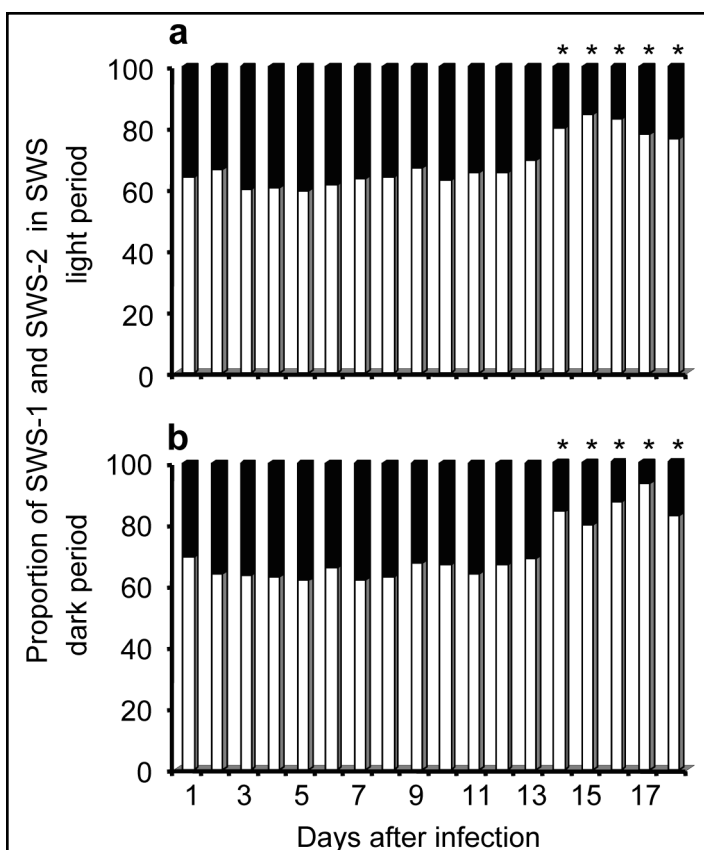


Figure 3—Respective proportion of the 2 components of slow-wave sleep (SWS), SWS-1 (open bars) and SWS-2 (filled bars) after infection, during the light (a) and dark (b) conditions. *significant difference from control values at $P < .001$ (Student *t*-test). Data are expressed as mean $\pm$ SD.

Table 1—Proportion (%) of wakefulness (W), slow wave sleep (SWS-1 and SWS-2), and paradoxical sleep (PS) during the light and dark periods for baseline (before infection), 12 days after infection by *T. b. brucei*, and two days prior to death. Data are expressed as mean $\pm$ SD; for statistical differences, see the results section.

	Control (n=10)		D12 (n=10)		Two days prior to death (n=10)	
	light	dark	light	dark	light	dark
W	39.9 $\pm$ 3.6	52.5 $\pm$ 4.5	38.4 $\pm$ 3.1	51.7 $\pm$ 4.6	20.4 $\pm$ 2.5	35.9 $\pm$ 3.9
SWS	49.4 $\pm$ 3.4	40.7 $\pm$ 3.1	51.4 $\pm$ 3.6	41.8 $\pm$ 3.7	70.4 $\pm$ 5.4	58.3 $\pm$ 5.1
SWS-1	31.5 $\pm$ 2.8	28.4 $\pm$ 2.6	33.8 $\pm$ 2.3	28.7 $\pm$ 2.5	44.9 $\pm$ 4.4	38.5 $\pm$ 4.7
SWS-2	17.9 $\pm$ 2.3	12.4 $\pm$ 1.9	17.6 $\pm$ 2.1	13.1 $\pm$ 1.5	25.5 $\pm$ 2.8	19.7 $\pm$ 2.6
PS	10.6 $\pm$ 1.2	6.7 $\pm$ 0.97	10.2 $\pm$ 1.1	6.5 $\pm$ 0.7	9.1 $\pm$ 1.4	5.9 $\pm$ 1.1

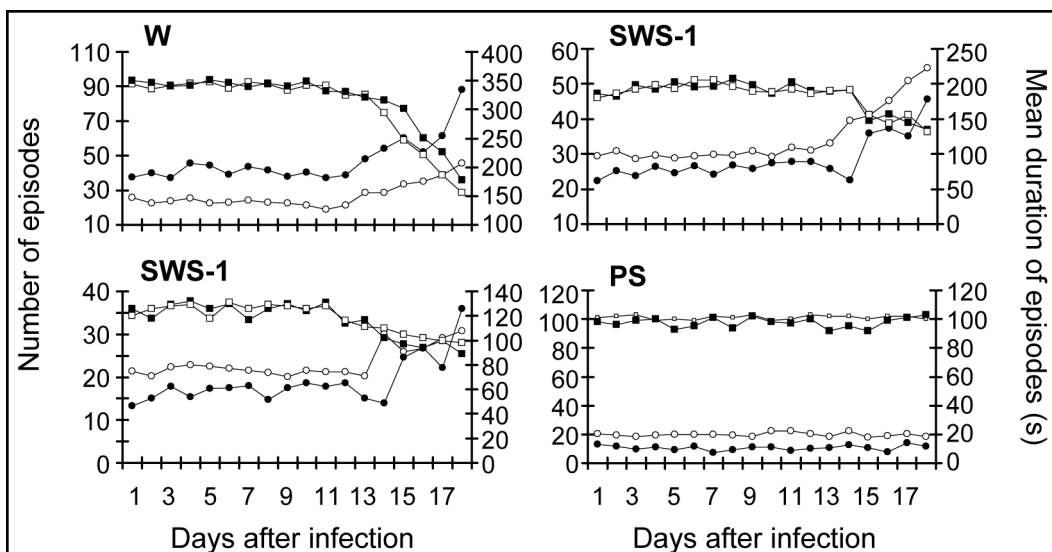


Figure 4—Number (circle) and mean duration (square) of the episodes of wakefulness (W), slow-wave sleep (SWS-1, SWS-2), and paradoxical sleep (PS) after infection, during the dark (filled symbols) and light (open symbols) environments. * significant difference from control values at $P < .001$ (Student t test). Data are expressed as mean $\pm$ SD.

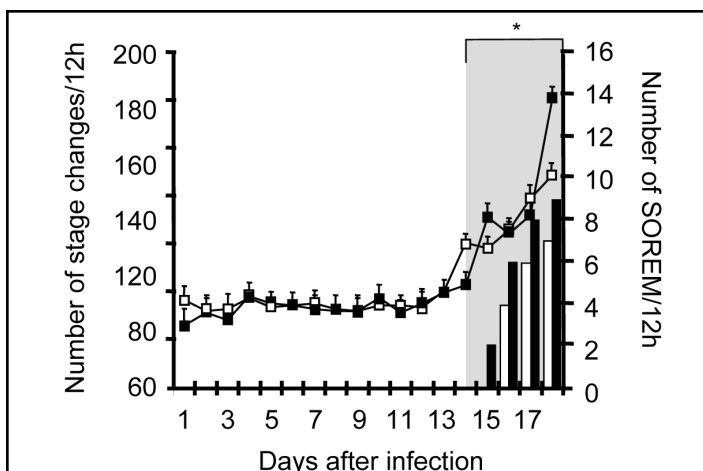


Figure 5—Number of state changes (squares) and sleep-onset rapid-eye-movement episodes (SOREM) (histograms) throughout the post-infection period with differentiation between the 12-hour light (open symbols) and dark (filled symbols) exposures. *significant difference from control values at $P < .001$ (Student t test). Data are expressed as mean $\pm$ SD.

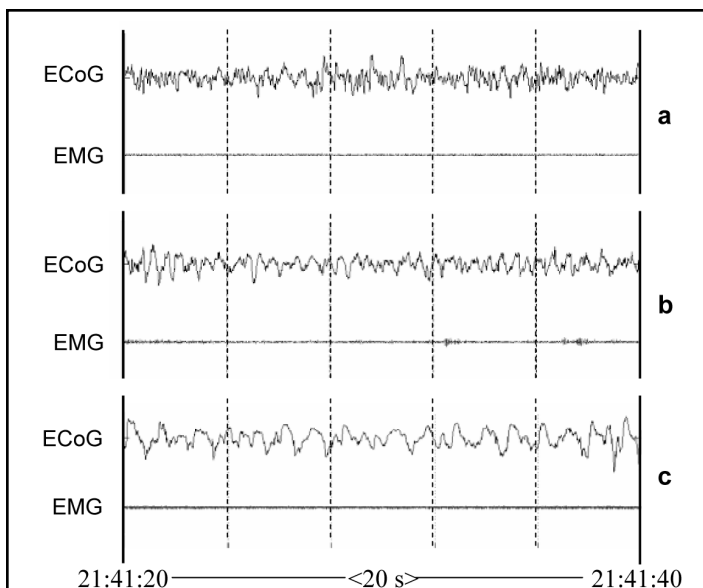


Figure 6—Morphologic electrocorticographic (ECoG) modifications of slow-wave sleep (SWS) during the baseline (control) period (a), on the 14th day after infection (b), and on the day preceding death (c, 21st day after infection). EMG refers to electromyogram.

number of wakefulness episodes increased ($P < .001$), resulting in a decrease in the duration of the intervals between SWS episodes, which became significant on Day 15 (5.26 ± 0.19 minutes for control values vs 3.82 ± 0.23 min; $P < .01$). At this time, the structural changes of synchronized sleep were readily evident on the hypnograms (SWS fragmentation in infected animals; Figure 1). This is also demonstrated by the increased number of state changes at the end of the second week after infection (Figure 5).

The above changes were also reflected in the morphology of the ECoG tracing during SWS: hyper-synchronous slow waves appeared (Figure 6) in all animals 3 to 4 days before death and totally invaded the ECoG trace 24 hours before death.

Sleep spindles characterizing SWS-1 progressively decreased, disappearing 1 day before death. The sleep tracing was then very monotonous.

Sleep-onset PS episodes (SOREM-like episodes)

Contrary to SWS, the number and duration of PS episodes did not vary throughout the progression of the disease (Figure 4). However, the mean PS latency was significantly reduced on Day 14 (6.68 ± 0.37 minutes for control value vs 3.96 ± 0.24 minutes, $P < .001$). The internal structure of sleep episodes was then also disturbed, exhibiting noticeably abnormal transitions from wakefulness to PS, ie, sleep-onset PS episodes (Figure 5).

DISCUSSION

The present study, performed on the basis of continuous polysomnographic recordings, demonstrates that the inoculation of *T. b. brucei* in the rat leads to the development of a neurologic disease, characterized by 2 main symptoms: a 24-hour disruption of the sleep-wake rhythm occurring 2 weeks after infection, and an alteration of the internal sleep structure marked by the occurrence of sleep-onset PS episodes. These changes were accompanied by a progressive invasion of the SWS ECoG by abnormal slow waves during both the daytime and nighttime. The reported sleep and wakefulness changes therefore parallel those observed in patients with HAT.^{7,8,10}

Our approach using continuous polysomnographic recordings is similar to that of 1 previous investigation¹² and 2 other reports conducted with iterative short-lasting recordings.^{14,15} The use of such short-lasting recordings may explain the discrepancies between our data and the results reported. Studying rats infected with *T. b. brucei* between days 19 and 21 after infection showed an increased relative proportion of wakefulness and a decreased synchronized sleep during their resting daytime period.^{14,15}

The sleep-wake cycle of nocturnal rodents is highly polyphasic, comprising wakefulness, SWS, and PS sequences, with a prevalence of sleep episodes during the daytime and that of wakefulness in the dark condition.¹⁹⁻²¹ In our study, the amounts of the sleep-wake states were stable during the first 10 days in every animal, with changes being observed only during the last days preceding death.

The changes observed in rats infected with *T. b. brucei* are comparable to the now well-established syndrome described in humans infected with *T. b. gambiense*^{7,8} and *T. b. rhodesiense*.²² Rats did indeed show a disruption of the 24-hour sleep-wake pattern with the sole basic²³ ultradian rhythmicity characterized by short periodicities in the alternation of

the sleep-wake states. Noticeably, the rats also exhibited definite sleep-onset PS episodes (SOREM-like episodes). Clearly, the definition of SOREM-like phenomena is more difficult to define in rats than in humans. To show these events, we used very strict criteria, defining a sleep-onset PS episode as occurring immediately after an episode of wakefulness of at least 40 seconds' duration, uninterrupted by SWS. The shortening of the PS latency that was also observed in this investigation is supportive of the occurrence of SOREM-like episodes in our rats. In humans, SOREM episodes are defined as REM-sleep episodes occurring after either no non-REM sleep episode or a very short one (less than 20 minutes).²⁴

The most important problem encountered in HAT is the diagnosis of the disease stage, ie, the precise detection of the time at which the trypanosome penetrates the central nervous system. The knowledge of this instant is crucial in determining the treatment that the patient should receive. Although medications that are active on the first hemolymphatic stage are relatively well tolerated, treating the meningoencephalitic stage is essentially done with an arsenical, melarsoprol, which may lead to the development of reactive encephalitis.²⁵ In our rat model, the breaking point between the 2 stages seems to occur at the end of the second week after infection. All the changes observed in sleep measures occurred precisely at this time. The present observation is in agreement with a previous study on rats infected with *T. b. brucei*.¹² In the same animal model, a recent clinical follow-up study revealed a dramatic decrease in daily food intake and weight gain also occurring at the end of the second week of infection.²⁶ Thereafter, the observed fluctuations were not related to sleep changes.

The main difference between the rat model and HAT resides in the sleep-wake-cycle disruption that occurs in the rat concomitantly with SOREM-like episodes. In humans with trypanosomiasis, the 24-hour disturbances, compared to the occurrence of SOREM episodes, seem to be delayed, and we believe the latter to represent a potent marker of the penetration of trypanosomes into the central nervous system.²⁷

The rat model also responds similarly to patients with HAT, regarding the ECoG alterations. In patients with late-stage meningoencephalitis, sleep spindles disappear and abnormal delta-wave bursts occur during SWS, along with paroxysmal hypnopompic hypersynchronous events.^{28,29} Such alterations were also present in our animals.

Therefore, it can be concluded that rats infected with *T. b. brucei* represent a good model of the sleep-wake disturbances observed in patients with HAT. Moreover, the breaking point occurring a few days before death may constitute a good marker of the instant at which trypanosomes penetrate the brain. It could therefore represent a good standard to which biologic and immunologic tests can be compared to develop simple tests that would be usable in field trials and investigations.^{30,31} Our model can also be useful as a tool to further analyze the dysfunction in the central nervous system that accompanies the sleep-wake disturbances.

ACKNOWLEDGMENTS

The authors are grateful to A. Roux and D. Popieul for their skilled technical assistance.

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