



## Sleep structure: a new diagnostic tool for stage determination in sleeping sickness

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### Abstract

Human African trypanosomiasis (HAT), due to the transmission of *Trypanosoma brucei* (*T. b.*) *gambiense* and *T. b. rhodesiense* by tsetse flies, is re-emerging in inter-tropical Africa. It evolves from the hemolymphatic Stage I to the meningo-encephalitic Stage II. The latter is generally treated with melarsoprol, an arseniate provoking often a deadly encephalopathy. A precise determination of the HAT evolution stage is therefore crucial. Stage II patients show: (i) a deregulation of the 24-h distribution of the sleep–wake alternation; (ii) an alteration of the sleep structure, with frequent sleep onset rapid eye movement (REM) periods (SOREMPs). Gambian HAT was diagnosed in eight patients (four, Stage II; three, Stage I; one, “intermediate” case) at the trypanosomiasis clinic at Viana (Angola). Continuous 48-h polysomnography was recorded on Oxford Medilog 9000-II portable systems before and after treatment with melarsoprol (Stage II) or pentamidine (Stage I and “intermediate” stage). Sleep traces were visually analyzed in 20-s epochs using the PRANA software. Stage II patients showed the complete sleep–wake syndrome, partly reversed by melarsoprol 1 month later. Two Stage I patients did not experience any of these alterations. However, the “intermediate” and one Stage I patients exhibited sleep disruptions and/or SOREMPs, persistent after pentamidine treatment. Polysomnography may represent a diagnostic tool to distinguish the two stages of HAT. Especially, SOREMPs appear shortly after the central nervous system invasion by trypanosomes. The reversibility of the sleep–wake cycle and sleep structure alterations after appropriate treatment constitutes the basis of an evaluation of the healing process.

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**Keywords:** Human African trypanosomiasis; Stage determination; Polysomnography; Angola; Melarsoprol; Pentamidine; SOREMP

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## 1. Introduction

Human African trypanosomiasis (HAT), sleeping sickness, is due to the transmission to humans of *Trypanosoma brucei* (*T. b.*) *gambiense* (Western and Central Africa) or *rhodesiense* (Eastern Africa) by tsetse fly bites. Estimates are that up to half a million individuals could be infected by this re-emerging disease, considered to rank among the greatest neglected diseases (Stich et al., 2003). The illness evolves in two stages, the hemolymphatic Stage I followed by the meningo-encephalitic Stage II (Dumas and Bisser, 1999). Stage I is considered to end when trypanosomes and/or mononuclear inflammatory cells appear in the cerebrospinal fluid (CSF), marking the beginning of the central nervous system (CNS) invasion. Among several neurological and psychiatric symptoms, daytime sleepiness is one of the most reported signs at Stage II. Untreated HAT evolves towards death in weeks (Rhodesian form) or months (Gambian form). The medications that treat successfully Stage I do not cross the blood–brain barrier in such an efficient amount as to affect CNS parasites. Stage II is generally treated with an arsenate, melarsoprol, which may provoke a reactive arsenical encephalopathy, occurring in 2–10% of the patients of whom, 50–75% may die. To treat patients appropriately, it is thus crucial to determine accurately the stage of evolution of the illness (Van Nieuwenhove, 1999).

However, there are no specific clinical signs nor blood tests for stage determination. Following the World Health Organization recommendations (1998), the diagnosis of Stage II is based on the CSF examination to search for: (i) the presence of trypanosomes, (ii) elevated white blood cell counts (proposed WHO cut-offs: Stage I < 5 cells/ $\mu$ L, “intermediate” stage with 5–20 cells/ $\mu$ L, early Stage II at 20 cells/ $\mu$ L), and (iii) determine total protein concentration (different cut-offs have been proposed and vary from 250 to 450 mg/L). The difficulty to find specific and reliable biological markers of stage determination has been stressed by recent exhaustive reports (Bisser et al., 2002; Lejon et al., 2003a, b).

Although since more than a century, patients with sleeping sickness have been reported as being sleepy during the daytime and restless at night, it is only since the second half of the 20th century that sleep and wakefulness can be objectively recorded by polysomnogra-

phy (electro-encephalogram, EEG; electro-oculogram, EOG; electromyogram, EMG). This technique represents the only objective means to distinguish between wakefulness, REM (rapid eye movement) sleep, and non-REM sleep and its four stages (Rechtschaffen and Kales, 1968). Polysomnographic recordings in sleeping sickness were firstly performed in Stage II patients at night and/or during afternoon naps (reviewed in Buguet et al., 2001). However, the first 24-h sleep recording was only performed in 1988 on a migrant Niger worker, who had contracted HAT in the Gagnoa focus of Côte d’Ivoire (Buguet et al., 1989). Our team later performed 120 polysomnographic recordings (24-h) in meningo-encephalitic patients, before and/or after treatment, which were compared to recordings in healthy African volunteers. Paper recordings were used at clinics (Buguet et al., 1989, 1993, 1999), ambulatory recordings at clinics and villages (Buguet et al., 1999). The investigations were conducted on Gambian HAT patients in Côte d’Ivoire (Abidjan, Daloa, Sinfra) and Congo (Brazzaville), and in two cases of Rhodesian HAT contracted in Rwanda and imported to France (Montmayeur et al., 1994).

The major findings obtained with polysomnography in meningo-encephalitic patients can be summarized by the occurrence of a polysomnographic syndrome made of two major disturbances (Buguet, 1999; Buguet et al., 2001). Firstly, the nycthemeral (night and day) alternation of sleep and wakefulness is altered in Stage II patients, proportionally to the severity of the disease. Short sleep episodes occur equally by day and night. The sleep–wake cycle alteration is reversed by melarsoprol treatment (Buguet et al., 1999). Secondly, the structure of sleep episodes is altered, with frequent sleep onset REM sleep periods (SOREMP), REM sleep episodes occurring soon after long wakefulness transitions with a latency shorter than 15 min (International Classification of Sleep Disorders, 1990). SOREMPs also recede or disappear after melarsoprol treatment (Buguet et al., 1999; Montmayeur et al., 1994). Unexpectedly, the disturbances observed in EEG morphology (Tapie et al., 1996) and evoked potentials (Tabaraud et al., 1992) were not found to be characteristic of HAT. The occurrence of SOREMP and/or sleep disruptions may therefore be determinant to diagnose the CNS involvement, especially in misclassified “intermediate” or Stage I patients, in whom clinical and laboratory means fail to determine with certainty the

passage from Stage I to II (Bisser et al., 2002; Buguet et al., 2001).

A group of eight patients (four at Stage II, three at Stage I, one at an “intermediate” stage) was therefore recorded with polysomnography before and after treatment at the Trypanosomiasis Clinic of Viana (Luanda) in Angola. Clinical and biological data were used to diagnose the stage of evolution of the illness. The recording session performed before treatment served to compare the clinical and biological ranking of the patients to the intensity of the polysomnographic symptoms. The post-treatment recordings were used as a follow-up to look at the effects of treatment on sleep and wake patterns.

## 2. Methods

### 2.1. Patients

Eight patients hospitalized at the Viana trypanosomiasis clinic volunteered to participate in the investigation, which was undertaken with the agreement of Angolan health and ethics authorities. Written informed consent to participate in the recording procedure was obtained from the patients or their families. The pa-

tients went through a thorough clinical and biological examination.

The principal clinical criteria used to determine the severity of the disease included: presence of lymph nodes, intermittent fever, pruritus with or without itchy skin lesions, daytime somnolence, headaches, sensory disturbances with 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, absent gaze, mutism, indifference), tremor (fine and diffuse without any myoclonic jerk at rest or during movement), and myoclonic jerks.

In all patients, *T. b. gambiense* was present in the blood or in the fluid collected from a cervical lymph gland puncture. After lumbar puncture, five patients presented trypanosomes in the CSF after double centrifugation of a 6-mL sample (only one trypanosome observed in P4). In four of them (P5–P8), the CSF cell counts, using microscopic examination realized with Kova cells (Hycor biomedical Inc., Gardengrove, CA, USA), showed an abnormal cell count (lymphocytes). Protein concentration in the CSF could not be analyzed in P1 and P8, but was elevated in patients P5, P6, and P7 (Table 1).

Table 1

Clinical and biological data obtained in the eight patients on their arrival at the Viana clinic, and complementary a posteriori biological data measured afterwards

|   | P1   | P2   | P3   | P4                         | P5     | P6     | P7  | P8     |
|---|------|------|------|----------------------------|--------|--------|-----|--------|
| Age (years)                                   | 13   | 11   | 10   | 21                         | 15     | 15     | 27  | 10     |
| Gender  | F    | M    | F    | F                          | F      | M      | F   | F      |
| General condition                             | Good | Good | Good | Good                       | Medium | Medium | Bad | Marasm |
| Pruritus                                      | –    | –    | –    | +                          | –      | –      | –   | +++    |
| Daytime somnolence                            | –    | –    | –    | Insomnia                   | +      | +      | ++  | ++     |
| Neurological signs                            | –    | –    | –    | Headache                   | ++     | ++     | +++ | +++    |
| Blood serology (CATT)                         | +    | +    | +    | +                          | +      | –      | +   | –      |
| CSF cells/ $\mu$ L                            | 2    | 0    | 2    | 7                          | 590    | 600    | 560 | 400    |
| CSF protein concentration (mg/L)              |      | 251  | 166  | 134                        | 414    | 930    | 648 |        |
| Trypanosomes in the CSF                       | 0    | 0    | 0    | 1 tryp. observed           | +      | +      | +   | +++    |
| Stage determination                           | I    | I    | I    | Intermediate (I may be II) | II     | II     | II  | II     |
| A posteriori biological criteria <sup>a</sup> |      |      |      |                            |        |        |     |        |
| Blood–brain barrier alteration                |      | –    | –    | –                          | –      | +      | +   |        |
| Intrathecal IgM synthesis                     |      | 0    | 0    | 0                          | +      | +      | +   |        |

Patients are ranked from the least severe hemolymphatic (Stage I) patient (P1) to the most severe meningo-encephalitic (Stage II) patient (P8). I: Stage I patient; II: Stage II patient; Intermediate: patient at an “intermediate” stage. Tryp.: trypanosome.

<sup>a</sup> The a posteriori biological tests did not change the classical stage determination.

The patients were ranked according to the clinical and biological severity of their illness (Table 1). Once the patients had been examined and ranked, their sleep–wake pattern was recorded for 48 h by polysomnography. All patients were treated immediately after this recording.

Plasma and CSF samples were frozen and further investigated at the Limoges University hospital. Proteins (IgG, IgM, and albumin) were analyzed to estimate blood–brain barrier integrity and intrathecal synthesis, following a recently published procedure (Reiber and Peter, 2001).

## 2.2. Treatment procedure

The patients were treated according to stage determination. Patients diagnosed as being at Stage II received melarsoprol (a combination of the trivalent arsenical melarsen with dimercaprol, or BAL; Arsobal<sup>®</sup>, presented in 5 mL ampoules as a 3.6% solution in propylene glycol) intravenously during 3 days (one slow injection until reaching a maximum of 3.6 mg/kg per day). Three to four similar treatment procedures were undertaken consecutively with 1-week intervals.

Patients diagnosed as being at Stage I or at the “intermediate” stage received seven intramuscular injections of pentamidine (20 mg/kg of Pentamidine Isethionate<sup>®</sup>), at the rate of one injection every 2 days.

## 2.3. Polysomnography

The first 48-h polysomnographic recording served to establish the patients’ sleep–wake patterns before treatment. In the patients ranked as being at Stage II, another 48-h recording was performed 1 month later, following the melarsoprol treatment. In the four remaining patients, the second 48-h recording session was scheduled after the 14-day pentamidine treatment. Therefore, all patients were recorded with polysomnography as

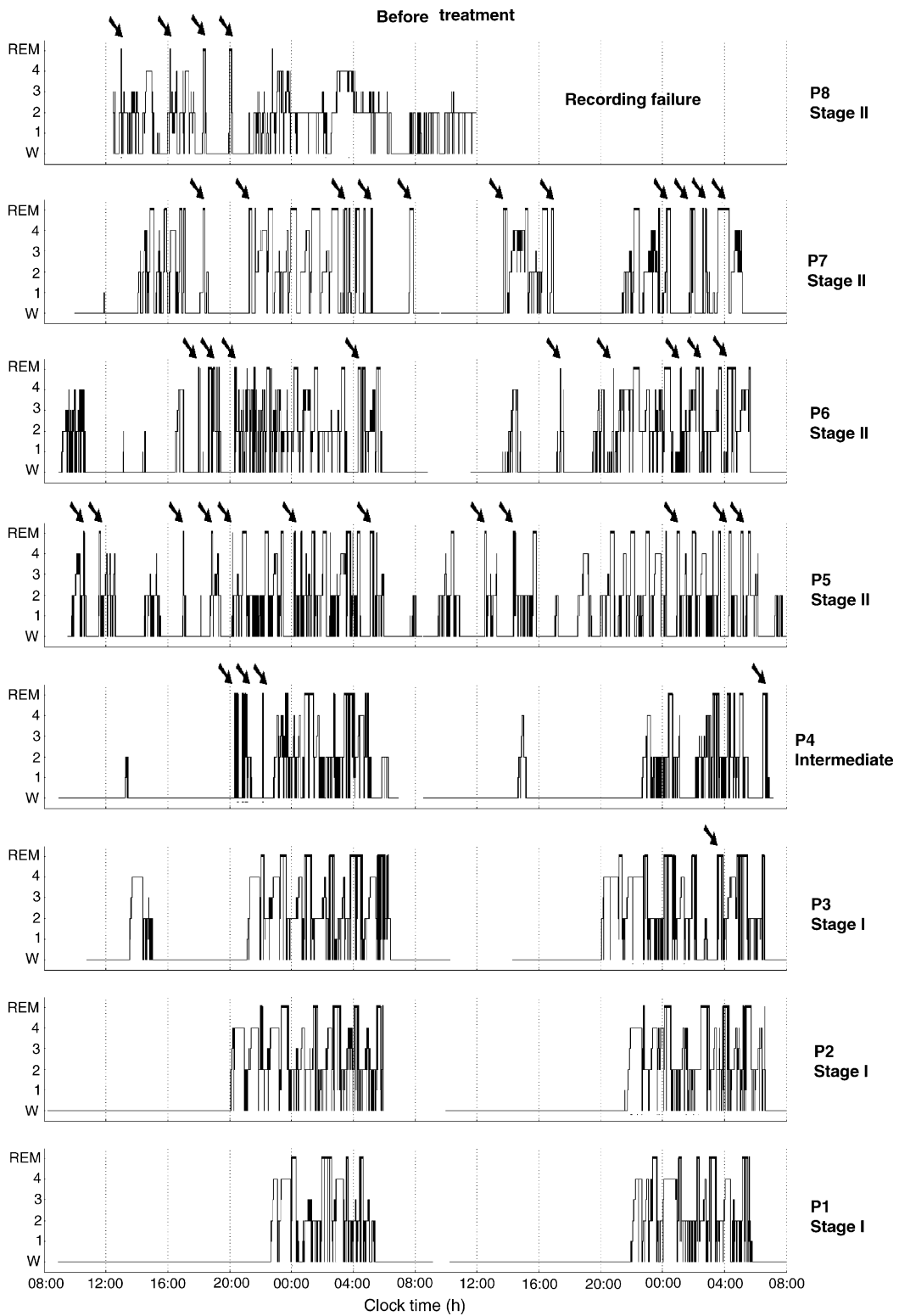
soon as the treatment procedure had been completed, in order to release the patients and their families as quickly as possible. In Africa, patients are entirely supported by their families, who have to accompany them to the hospital in order to take care of meals and other needs, often including nursing. During the ambulatory recordings, the patients had no restriction to sleep ad libitum.

Polysomnographic recordings (EEG, EOG, and EMG) were taken on one ambulatory eight-channel Oxford Medilog MR-9000 II<sup>®</sup> recorder (Oxford Instruments, Oxford, UK). The EEG was recorded with cup electrodes fixed with collodion on the scalp at sites C3, P3, C4, and P4, the electrodes being connected to the linked ears reference (A1–A2). The impedance of the electrodes was checked for signal quality (<100 k $\Omega$ ). Four montages were undertaken (C3–A2, P3–A2, C4–A1, P4–A1). The EOG was taken from two electrodes fixed at the outer canthi of the eyes, on diagonal to record both vertical and horizontal eye movements, and chin EMG from two electrodes at the tip of the chin. The analogical signals were stored on C-120 cassettes. Occasional technical problems led to premature recording interruptions, explaining missing data (Fig. 1).

After having been mailed to France, the cassettes were converted to digital with an amplitude resolution of 12 bits at a sampling frequency of 128 Hz, using the Oxford Instruments Vision<sup>®</sup> software. Polygraphic traces were interpreted on a PC screen in 20-s scoring epochs, blind as to patients and session, using the PRANA software (PhiTools<sup>®</sup>, Strasbourg, France) and following classical criteria (Rechtschaffen and Kales, 1968). The software generates automatically complete reports on sleep measures and calculations characterizing each patient’s sleep–wake cycle: recording time, sleep period time (SPT, from falling asleep to last awakening), awakenings occurring during SPT, total sleep time (TST = SPT minus awakenings), sleep

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Fig. 1. Hypnograms obtained from 48-h polysomnographic recordings of the eight patients before the instauration of the treatment procedure (W: wakefulness; 1, 2, 3, 4: Stages 1, 2, 3 and 4 of non-REM sleep; REM: REM sleep). Patients at Stage II (P5–P8, ranked in the order of illness severity) show the complete sleep–wake syndrome with: (i) a disturbance of the nycthemeral distribution of the sleep–wake cycle, proportional to the HAT severity appraised by clinical biological criteria; (ii) a disruption of the internal structure of sleep, with the frequent occurrence of sleep onset REM periods (SOREMP, arrows show clear examples of such events), REM sleep phases occurring soon after long episodes of wakefulness with a latency shorter than 15 min. Two Stage I patients (P1 and P2) did not experience any of these alterations. However, P4, the “intermediate” patient, and patient P3, classed as a Stage I patient on the basis of clinical and biological signs, already exhibited sleep disruptions and SOREMPs. However, the results of the polysomnographic data being unknown at that time, the patients were treated with pentamidine.



latency (from the beginning of the recording to the first stage of sleep). Latency to SWS (from sleep onset) and REM sleep latency (from the first stage of non-REM sleep) were measured during each sleep episode. Two sleep episodes were considered to be different when separated by at least 15 min of continuous wakefulness. Accordingly, two wake episodes differed when interrupted by more than 15 min of sleep. Two REM sleep phases differed when separated by more than 15 min.

In patients at Stage II of the illness, EEG readings are often difficult. The EEG traces are invaded by low-frequency, high-amplitude elements of the delta frequency band (0.5–4 Hz), with an impoverishment in K complexes (high-amplitude biphasic waves) and sleep spindles (12–16 Hz) (Tapie et al., 1996). The data were not treated statistically, due to the disparity of patients.

### 3. Results

Polysomnography was used to examine whether it could be considered as a means for diagnosing the stage of the disease (pre-treatment recording compared to clinical and biological staging) and whether it could be used to follow-up treatment efficacy (post-treatment recording compared to the first recording).

#### 3.1. Clinical and biological aspects

Clinical and biological symptoms upon arrival at the Viana clinic were used to sort the patients following the degree of severity of the illness, from the least severely ill (P1) to the most severely ill (P8; Table 1). Three

patients were diagnosed as being at Stage I (P1–P3), four at Stage II (P5–P8), one at an “intermediate” stage (P4). Patient P4 was nevertheless treated with pentamidine like Stage I patients, because he exhibited only a mild CSF reaction despite the observation of one trypanosome in the CSF (Table 1). The clinical and biological stage determination made at Viana was confirmed by a posteriori laboratory tests, which were positive in Stage II and negative in Stage I patients (Table 1). In the three meningo-encephalitic patients observed both before and after treatment (P5, P6, and P7), the clinical and biological symptoms were improved by melarsoprol treatment (Table 2). Pentamidine treatment did neither affect noticeably the health status nor the polysomnographic pattern of Stage I patients.

#### 3.2. Sleep–wake patterns

The hypnograms drawn after the scoring of sleep–wake states from the eight patients before treatment are given in Fig. 1. They represent the temporal distribution of sleep stages and wakefulness throughout the recording time. Three criteria were used to estimate polysomnographically the stage of evolution of the disease (Fig. 1, Table 3).

The first criterion relates to the 24-h distribution of sleep and wakefulness. The four meningo-encephalitic patients exhibited the disruption of the nycthemeral distribution of sleep and wakefulness episodes already described by Buguet et al. (1989, 1993, 1999). Stage I patients had an undisturbed sleep–wake distribution, sleeping at night and staying awake during the day. However, although the main sleep episode occurred at

Table 2  
Improvement of health condition after treatment in six of the patients

|                                  | P1           | P2           | P3           | P5          | P6          | P7          |
|----------------------------------|--------------|--------------|--------------|-------------|-------------|-------------|
| Treatment                        | Pentamidine  | Pentamidine  | Pentamidine  | Melarsoprol | Melarsoprol | Melarsoprol |
| General condition                | Good         | Asthenia     | Good         | Asthenia    | Medium      | Medium      |
| Pruritus                         | –            | –            | –            | –           | –           | –           |
| Daytime somnolence               | –            | –            | –            | –           | –           | ++          |
| Neurological signs               | –            | –            | –            | Headache    | +           | +           |
| CSF cells ( $\mu$ L)             | 0            | 5            | 1            | 34          | 25          | 300         |
| CSF protein concentration (mg/L) | <sup>a</sup> | <sup>a</sup> | <sup>a</sup> | 273         | 273         | 397         |
| Trypanosomes in CSF              | 0            | 0            | 0            | 0           | 0           | 0           |

The other two patients (P4 and P8) could not be followed-up because of logistical reasons.

<sup>a</sup> No post-treatment protein concentration analysis was performed in patients ranked as being at Stage I.

Table 3  
Polysomnographic features in the eight patients before treatment

|   | P1 | P2  | P3       | P4  | P5  | P6   | P7   | P8    |
|---|----|-----|----------|-----|-----|------|------|-------|
| Analyzed 24-h traces (number)                           | 2  | 2   | 2        | 2   | 2   | 2    | 2    | 1     |
| 24-h rhythm disturbance                                 |    |     |          |     |     |      |      |       |
| Polyphasic aspect                                       | —  | —   | —        | +   | +++ | +++  | +++  | ++++  |
| Sleep episodes (number, 1st 24 h)                       | 1  | 1   | 2        | 6   | 8   | 9    | 7    | 8     |
| Sleep episodes (number, 2nd 24 h)                       | 1  | 1   | 3        | 4   | 10  | 5    | 7    |       |
| SWS phases (number, 1st 24 h)                           | 6  | 9   | 7        | 5   | 13  | 13   | 9    | 18    |
| SWS phases (number, 2nd 24 h)                           | 6  | 8   | 4        | 5   | 10  | 11   | 5    |       |
| REM sleep phases (number, 1st 24 h)                     | 4  | 6   | 6        | 8   | 17  | 11   | 15   | 5     |
| REM sleep phases (number, 2nd 24 h)                     | 5  | 6   | 7        | 6   | 15  | 12   | 10   |       |
| Sleep structure   |    |     |          |     |     |      |      |       |
| Number of SOREMP (1st 24 h)                             | 0  | 0   | 0        | 3   | 7   | 4    | 5    | 4     |
| Number of SOREMP (2nd 24 h)                             | 0  | 0   | 1        | 1   | 5   | 4    | 6    |       |
| EEG morphological alterations                           |    |     |          |     |     |      |      |       |
| Wake  |    |     |          |     |     |      |      |       |
| Slowing down  | —  | —   | —        | —   | —   | +    | —    | +     |
| Microvoltage  | —  | —   | —        | —   | —   | —    | +    | —     |
| Periodic slow waves                                     | —  | —   | —        | —   | —   | —    | —    | ++    |
| Stage I   |    |     |          |     |     |      |      |       |
| Slowing down  | —  | —   | —        | —   | —   | +    | —    | +     |
| Periodic slow waves                                     | —  | —   | —        | —   | —   | —    | —    | —     |
| Stage II  |    |     |          |     |     |      |      |       |
| Slowing down  | —  | —   | —        | —   | —   | +    | +    | +++   |
| Periodic slow waves                                     | —  | —   | —        | —   | —   | —    | —    | —     |
| Altered K complexes                                     | —  | —   | —        | —   | —   | ++   | ++   | ++    |
| Sleep spindles  | N  | N   | N        | N   | N   | Rare | N    | Rare  |
| Slow-wave sleep   |    |     |          |     |     |      |      |       |
| Slow and altered delta waves                            | —  | —   | —        | —   | —   | —    | +++  | +++   |
| Hypersynchronous slow waves                             | —  | —   | —        | —   | —   | —    | —    | +     |
| Hypnopompic delta bursts                                | —  | —   | —        | —   | —   | —    | —    | +     |
| REM sleep   | N  | N   | N        | N   | N   | N    | N    | Short |
| Complementary biological criteria                       |    |     |          |     |     |      |      |       |
| Blood–brain barrier alteration                          |    | —   | —        | —   | —   | +    | +    |       |
| Intrathecal IgM synthesis                               |    | 0   | 0        | 0   | +   | +    | +    |       |
| Proteinorachia (mg/L)                                   |    | 251 | 166      | 134 | 414 | 930  | 648  |       |
| Proposed stage determination from polysomnographic data | I  | I   | Early II | II  | II  | II+  | II++ | II+++ |

A new classification of the evolutionary stage of the disease is proposed on the basis of sleep–wake alterations. Intensity of polysomnographic alterations: (+++): very strong; (++): strong; (+): present; N: normal.

night, the “intermediate” stage patient already showed slight disturbances of sleep maintenance.

The second criterion is represented by the altered sleep structure, SOREMPs occurring in one or several sleep episodes. SOREMPs were observed in all patients at Stage II. The “intermediate” patient exhibited three SOREMPs during the 48-h recording. Patient P3, ranked as being at Stage I clinically and biologically, exhibited one SOREMP in 48 h. The other

two patients at Stage I (P1 and P2) did not show any SOREMP.

The third criterion consists in EEG morphological alterations already described by [Tapie et al. \(1996\)](#). Three meningo-encephalitic patients (P6, P7, and P8) showed abnormal EEG events, such as slowing of the EEG or periodic slow-waves during wakefulness and sleep stages, altered K complexes, degraded spindles, and hypersynchronous slow waves and

Table 4  
Polysomnographic features in the six patients examined after treatment

|  | P1 | P2 | P3 | P5  | P6   | P7  |
|--|----|----|----|-----|------|-----|
| Analyzed 24-h traces (number)  | 2  | 2  | 1  | 2   | 2    | 1   |
| Number of days after treatment beginning                               | 14 | 14 | 13 | 30  | 30   | 30  |
| 24-h rhythm disturbance  |    |    |    |     |      |     |
| Polyphasique aspect  | –  | –  | –  | +++ | +++  | +   |
| “Concentration” of sleep to nocturnal period compared to pre-treatment |    |    | –  |     | +++  | ++  |
| Sleep episodes (number, 1st 24 h)                                      | 1  | 1  | 3  | 2   | 6    | 5   |
| Sleep episodes (number, 2nd 24 h)                                      | 1  | 1  |    | 3   | 5    |     |
| SWS phases (number, 1st 24 h)  | 5  | 6  | 8  | 6   | 11   | 3   |
| SWS phases (number, 2nd 24 h)  | 4  | 5  |    | 7   | 10   |     |
| REM sleep phases (number, 1st 24 h)                                    | 5  | 4  | 5  | 8   | 10   | 14  |
| REM sleep phases (number, 2nd 24 h)                                    | 5  | 6  |    | 8   | 7    |     |
| Sleep structure  |    |    |    |     |      |     |
| Number of SOREMP (1st 24 h)  | 0  | 0  | 0  | 2   | 4    | 7   |
| Number of SOREMP (2nd 24 h)  | 0  | 0  |    | 1   | 4    |     |
| EEG morphological alterations  |    |    |    |     |      |     |
| Wake   |    |    |    |     |      |     |
| Slowing down   | –  | –  | –  | –   | +    | –   |
| Microvoltage   | –  | –  | –  | –   | –    | +   |
| Periodic slow waves  | –  | –  | –  | –   | –    | –   |
| Stage I  |    |    |    |     |      |     |
| Slowing down   | –  | –  | –  | –   | +    | –   |
| Periodic slow waves  | –  | –  | –  | –   | –    | –   |
| Stage II   |    |    |    |     |      |     |
| Slowing down   | –  | –  | –  | –   | +    | +   |
| Periodic slow waves  | –  | –  | –  | –   | –    | –   |
| Altered K complexes  | –  | –  | –  | –   | ++   | ++  |
| Sleep spindles   | N  | N  | N  | N   | Rare | N   |
| Slow-wave sleep  |    |    |    |     |      |     |
| Slow and altered delta waves   | –  | –  | –  | –   | –    | +++ |
| Hypersynchronous slow waves  | –  | –  | –  | –   | –    | –   |
| Hypnopompic delta bursts   | –  | –  | –  | –   | –    | –   |
| REM sleep  | N  | N  | N  | N   | N    | N   |
| Improvement compared to pre-treatment                                  | =  | =  | =  | +++ | +++  | ++  |

N: normal; (–): no change; (++) and (+++): extent of the improvement.

hypnopompic delta bursts, both occurring in slow-wave sleep.

Table 3 shows how these criteria corresponded to the clinical classification established upon the admission of the patients to the clinic. It is obvious that if the sleep traces had been scored immediately, patient P4 would have been considered as being at Stage II and treated accordingly.

This assumption is reinforced by the examination of Fig. 2 and Table 4, which relate to the effects of treatment on the stage classification criteria. Unfortunately,

due to logistical reasons, one meningo-encephalitic patient (P8) and the “intermediate” stage patient (P4) could not be recorded after treatment. In meningo-encephalitic patients, sleep distribution improved after treatment with melarsoprol, sleep episodes tending to concentrate during the nocturnal part of the nycthemeron. Sleep structure also improved and the number of SOREMPs diminished. After having been treated with pentamidine during 14 days, patient P3 had a persistent sleep distribution during the first 24-h recording, although no SOREMP was observed throughout

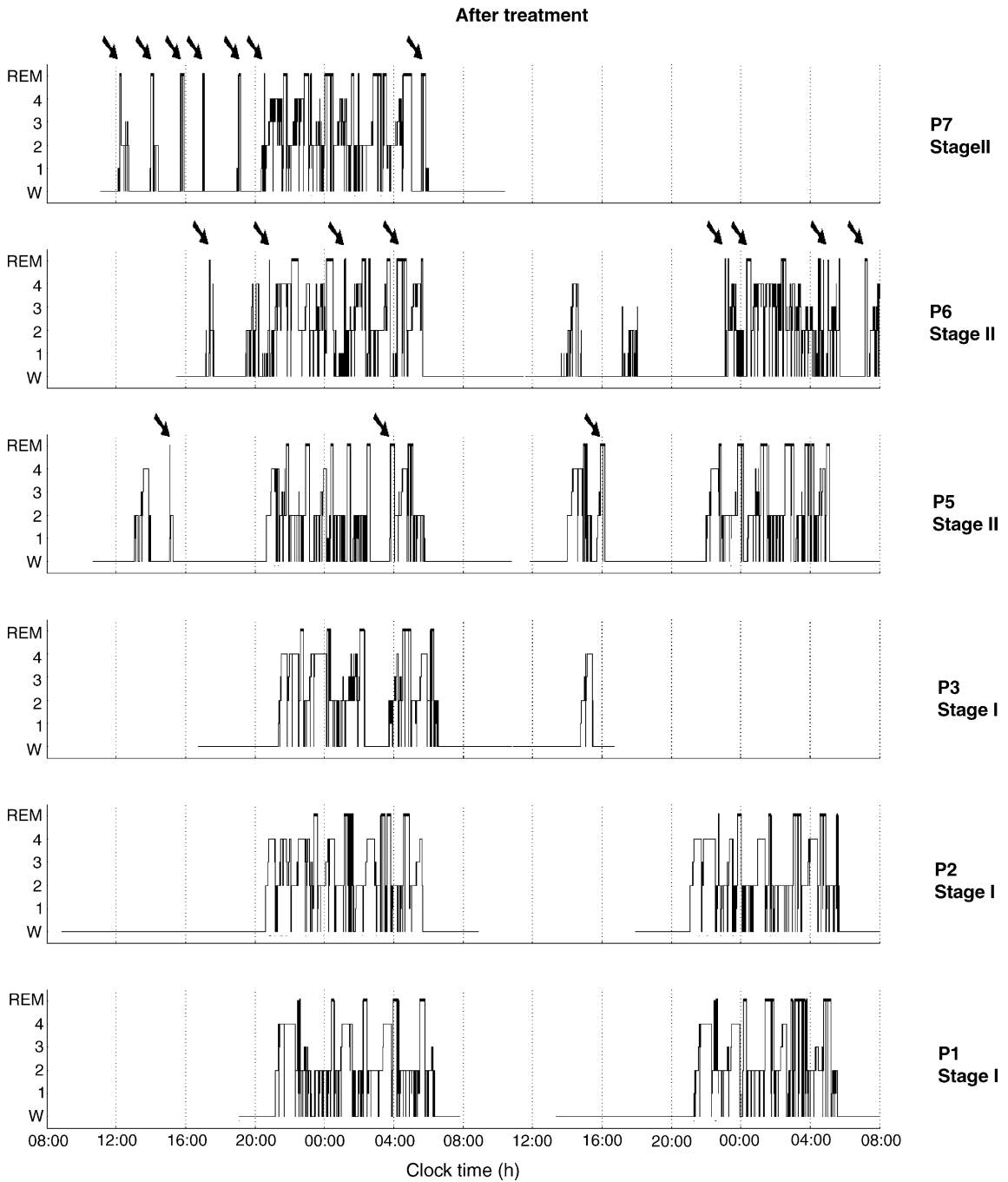


Fig. 2. Hypnograms obtained from 48-h polysomnographic recordings of six of the patients after the treatment procedure (W: wakefulness; 1, 2, 3, 4: Stages 1, 2, 3 and 4 of non-REM sleep; REM: REM sleep). The reversal of the HAT-induced sleep–wake disturbances starts to show up in Stage II patients (P5, P6, and P7). Patient P3, treated with pentamidine, still exhibits sleep–wake alterations, which are not observed in the Stage I patients P1 and P2, and which most probably mean that the penetration of trypanosomes into the central nervous system has occurred recently.

the recording. In comparison, sleep structure and distribution remained absolutely normal in the two pre-treatment symptom free Stage I patients.

#### 4. Discussion

The main finding of this investigation is that sleep–wake recordings may constitute a useful tool to diagnose the CNS intrusion by the parasite. Polysomnography may therefore be relevant for the HAT stage determination, especially in revealing the occurrence of SOREMPs.

The positive diagnosis of the CNS involvement in sleeping sickness is a necessity to insure appropriate treatment. Clinical and biological approaches are somewhat arbitrary and fail to classify with certainty the so-called “intermediate” stages of the disease into either first or second stages. Such an imprecision may put the patient’s life in danger if trypanosomes have already crossed the blood–brain barrier. As an example, in our investigation, stage diagnosis was only based on clinical and biological examination. Accordingly, patients P5–P8, diagnosed as being at Stage II were treated with melarsoprol. Patients P4, classified as “intermediate”, and P1–P3 (ranked as Stage I) were treated with pentamidine.

The polysomnographic data shown here in Stage II patients are in agreement with our previous work on sleep disturbances in HAT. The dual syndrome already described in meningo-encephalitic patients (Buguet et al., 2001) was also found in the four Stage II patients. This “polysomnographic” syndrome of Stage II HAT is made of: (i) a disturbance of the nycthemeral distribution of the sleep–wake cycle, proportional to the HAT severity appraised by clinical and laboratory criteria; (ii) a disruption of the internal structure of sleep with the frequent occurrence of SOREMP. Two Stage I patients (P1 and P2) did not show any of these symptoms. They slept only at night, exhibiting a normal sleep structure with a total absence of SOREMP. However, P4, the “intermediate” patient, and P3, ranked as Stage I, showed, respectively three and one SOREMP, along with disruptions in their nocturnal sleep–wake distribution.

The more sophisticated biological tests performed a posteriori in France did not change the ranking order of the patients obtained from “classical” clinical and bio-

logical stage determination performed at Viana. Similarly, the ranking order did not change with respect to stage determination proposed from polysomnographic data, which however, gave, six Stage II and two Stage I patients.

This investigation confirmed that treatment of meningo-encephalitic patients with melarsoprol leads to an amelioration of the sleep–wake distribution, sleep architecture and EEG morphology (Buguet et al., 1999). However, such an improvement may result from a slow-progression process, as seen in two patients followed polysomnographically during 11 months (Montmayeur et al., 1994). Unfortunately, the “intermediate” patient P4 was not recorded after treatment. Treatment with pentamidine did not alter the sleep patterns of patients P1 and P2 (Stage I), but P3 still showed sleep disturbances after the 13-day pentamidine treatment, indicating the latter may have been inappropriate. This statement is supported by the comparison with patients at early Stage II treated with melarsoprol, who were shown to restore sleep integrity as early as 2 weeks after the beginning of the treatment procedure (Buguet et al., 1999).

In conclusion, this study confirms that the sleep–wake cycle and sleep structure are totally disrupted in Stage II patients, these alterations being alleviated by treatment with melarsoprol. Such alterations may also be observed in some Stage I or “intermediate” patients, but more work has to be carried out before concluding that they may be considered as markers for the diagnosis between the two stages of the disease and/or for the follow-up of treatment efficacy.

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