Correlations using the NREM-REM sleep cycle frequency support distinct regulation mechanisms for REM and NREM sleep

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Le Bon, O., L. Staner, S. K. Rivelli, G. Hoffmann, I. Pelc, and P. Linkowski. Correlations using the NREM-REM sleep cycle frequency support distinct regulation mechanisms for REM and NREM sleep. J Appl Physiol 93: 141–146, 2002. First published February 22, 2002; 10.1152/japplphysiol.00917.2001.—Polysomnograms of most homeothermic species distinguish two states, rapid eye movement (REM) and non-REM (NREM) sleep. These alternate several times during the night for reasons and following rules that remain poorly understood. It is unknown whether each state has its own function and regulation or whether they represent two facets of the same process. The present study compared the mean REM/NREM sleep ratio and the mean number of NREM-REM sleep cycles across 3 consecutive nights. The rationale was that, if REM and NREM sleep are tightly associated, their ratio should be comparable whatever the cycle frequency in the night. Twenty-six healthy subjects of both sexes were recorded at their home for 4 consecutive nights. The correlation between the REM/NREM sleep ratio and the number of cycles was highly significant. Of the two sleep components, REM sleep was associated to the number of cycles, whereas NREM sleep was not. This suggests that the relationship between REM sleep and NREM sleep is rather weak within cycles, does not support the concept of NREM-REM sleep cycles as miniature units of the sleep process, and favors the concept of distinct mechanisms of regulation for the two components.

Crucial to understanding sleep function and dysfunction is whether the two states simply share the same general environment and compete for expression or whether the two components are closely interrelated, with precise interchanges between them.

In the classical view, REM and NREM sleep have different functions, are regulated independently of each other, and an ultradian oscillating process is responsible for their alternation. Hypotheses about the regulation and function of NREM sleep have been isolated from hypotheses about slow-wave sleep, one of its components, or of those of sleep in general. A nonexhaustive list of proposed functions of sleep includes 1) energy conservation (59); 2) restoration or rejuvenation of some process or substance accumulated during wakefulness, especially in the brain (10, 27); 3) time filling and protection during phases of diurnal cycle where no adaptive behavior can be performed (54); 4) a role in immune function (7); 5) temperature cooling (42); 6) avoidance of a permanent mixed state of “hypnovigilance” (38); 7) slow recovery and stabilization of synaptic processes (30, 32); and 8) removal of excess cerebral free radicals (45). REM sleep, for its part, has been postulated to 1) permit emotional adaptation (24), 2) discharge excess drive energy (15), 3) provide periodic endogenous stimulation (52), 4) prepare for wakefulness (48), 5) promote cerebral maturation (46), 6) protect infants when brain activity is high (46), 7) warm brain after NREM sleep cooling (55), 8) exercise binocular coordination (11), 9) upregulate catecholamine receptors (47), 10) rehearse genetically programmed behaviors (28), 11) facilitate memory consolidation (30), 12) protect neural circuitry of memory (16), 13) weaken useless memory traces (14), and 14) process off-line information (50). A discussion on these hypotheses would go beyond the scope of this paper. In an outstanding critical analysis on this topic (41), the general conclusion was that none of these hypotheses presented a sufficient comprehensive or parsimonious vision, and all probably still miss the primary, essential, functional core of sleep, a seem-

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The rationale for the present study was that, if the relationship between the two states is functionally close, there is no reason for the ratio between their respective durations to be a function of the cycle frequency in a night, and thus it would be independent of this number (null hypothesis). Conversely, a significant association would indicate that the ratio is not constant and that at least one of the components is a function of the number of cycles. In this case, the two states would behave differently, which would support the concept of discrete functions and regulations.

Analyses were performed by using a large group of carefully selected healthy subjects who were recorded at home and recruited prospectively for an ongoing study of sleep in healthy controls. This same group was previously examined in the above-mentioned study on number and duration on cycles (34), as well as in a study of the first-night effect (33).

METHODS

Subjects. Eighty-four volunteers, aged 15–45 yr (mean ± SD: 27.8 ± 9.7; n = 47 women), were recruited by advertisement and paid for participation. A comprehensive screening was conducted to ensure selection of individuals with no known existing or previous condition that might result in abnormal sleep. Volunteers first answered, by phone, a detailed questionnaire designed to elicit sleep history and to exclude subjects with sleep and psychiatric pathology. Those meeting questionnaire-based criteria were then given a structured interview (by O. Le Bon and G. Hoffmann) that used the American Sleep Disorders Association (3) criteria for sleep disorders. The more recent version of the International Classification of Sleep Disorders (4) could not be used because the study began before its introduction. Axis I DSM-IV (2) criteria were used for psychiatric diagnoses (except for sleep disorders).

Inclusion criteria were regular sleep schedules, absence of sleep-related complaints or regular naps, regular weekday work schedules or no employment, and no previous polysomnography. Exclusion criteria were DSM-IV axis I disorder, personal or first-degree familial affective disorder (because of potential implications on REM latency (23)), significant somatic condition, excessive daytime sleepiness, report by significant other of periodic limb movements, snoring or sleep apnea, sleep-apnea index of ≥5 on the first night of recording, periodic limb movement episodes on the first night of recording, routine consumption of more than 10 alcohol-containing (10 g units) drinks per week or consumption of illicit drugs, use of psychotropic drugs influencing sleep within 3 wk before the study, and transmeridian flights or shift work within 4 wk preceding the study. Subjects were requested not to drink alcohol for a week before entering the protocol and to change their life habits as little as possible during the time of the study.

The protocol was approved by the hospital’s ethics committee, and informed consent was obtained. The study was conducted in accordance with the rules and regulations for the conduct of clinical trials stated by the World Medical Assembly at Helsinki.

Methods. Recordings were made between Mondays and Fridays to avoid the more irregular weekend periods. A technician went to the subjects’ homes around 9 PM, explained the procedure, and answered questions. He then placed with each subject three pairs of electroencephalogram electrodes (FZp1-A1; C4-A1; O2-A1), one pair of electroocu-
logram electrodes, a chin and two inferior limb electromyogram electrodes, thoracic and abdominal gauges for respiratory movements, thermoresistors around the mouth and nose, a finger oximeter, and a microphone for detection of snoring. Subjects went to bed at their usual sleep time and connected the wires, in a very straightforward procedure, to sleep analyzer Alice (Respironics, Pittsburgh, PA). When subjects decided to go to sleep, they launched the polysomnography and turned out the light ("lights out"). When they spontaneously woke up in the morning, they stopped the recording ("end of night") and removed the electrodes. The same sequence was repeated for all 4 study nights.

Recordings were randomly analyzed by one of two well-trained technicians on a 21-in. screen displaying 30-s polysomnograph epochs. Classical sleep-stage scoring criteria were used (44). Interrater reliability measured in another recent protocol exceeded 0.90 for all variables (35). The REM-to-NREM sleep ratio was the REM sleep duration divided by the NREM sleep duration. NREM-REM sleep cycles were defined by each REM sleep episode and the NREM sleep immediately preceding it, going back to sleep onset (first NREM-REM sleep cycle) or to the limit of another REM sleep episode (from the second NREM-REM sleep cycle to the end of the night). The first NREM sleep episode began with the first epoch of stage 2. Each REM sleep episode began with the first epoch of REM sleep and ended when the last epoch of REM sleep was followed by at least 15 min of NREM sleep or the end of the night (20, 37). No minimal duration was demanded for REM sleep episodes. The NREM-REM sleep cycle frequency was expressed as the number of cycles per night.

Statistics. Data from the first night of recording were not included to minimize the impact of awakenings, which have been shown to be part of a first-night effect (33). Kolmogorov-Smirnov analyses were used for distribution testing. The relationships between continuous variables were evaluated with Pearson's product-moment correlation. Stepwise regression analyses were performed to analyze the respective contributions of several independent variables. Hypotheses tests were two sided and carried out at the 5% significance level. All statistics were computed with SPSS 10 for Power PC (SPSS, Chicago, IL). The graph was created by using Statview 5 (SAS Institute, Cary, NC).

RESULTS

Data description. Eighty-four subjects responded to our advertisement (mean age: 27.8 ± 9.7 yr; range: 15–45 yr; n = 47 women). Data from telephone questionnaire and physician interviews were causes for exclusion of 47 individuals (5 parasomnias, 5 irregular sleep schedules, 7 restless legs or suspicion of periodic limb movements, 10 snoring problems, 5 excessive daytime sleepiness, 9 anxiety disorders, 6 affective disorders). First-night polysomnography resulted in the exclusion of an additional six subjects (2 periodic limb movement and 4 apneic/hypopneic indexes over 5). Thirty-one subjects (36.9%) met inclusion criteria and were considered to be normal control subjects. Data from five subjects had to be excluded because of technical problems (two 800-Mb optical disks seriously damaged during storage for unknown reasons). Twenty-six subjects (mean age: 26.7 ± 9.8 yr; range: 15–45 yr; n = 12 women) completed all aspects of the study, and no missing polysomnography epochs were observed. The index of sleep respiratory disorders in the final 26 subjects was 2.8/h (SD = 1.49), and no episodes of periodic limb movement were observed. Previous reports on the same group of patients have shown no difference between bedtimes across the 4 nights (33) and no association between bedtime or waking time and cycle frequency (34).

Table 1 shows the distribution of REM sleep durations by cycle frequency (n = 78, 26 subjects over 3 nights). Cycle frequencies of three, four, and five cycles/night were normally distributed, with too few data points to allow testing for cycle frequencies of two and six cycles/night. The minimum duration for a REM sleep episode was 1 min, and the maximum was 65 min.

Table 2 shows selected sleep variables as functions of cycle frequency. There were both marked and gradual differences in REM sleep between nights with few cycles compared with those with many cycles, which contrasted with limited differences for NREM sleep. When comparing nights with six cycles to those with two cycles, mean REM sleep was longer by 311% and mean NREM sleep was longer by 105%. When measured in percentages of total sleep time (TST), REM sleep was 19.8% (6 cycles/night) vs. 6.3% (2 cycles/night) and NREM sleep was 78.1% vs. 89.7%. The mean REM sleep content per cycle was approximately constant, whereas the NREM sleep content per cycle was almost an inverse function of the number of cycles.

Given the high intraclass correlation found previously across nights in the same subjects (34), we averaged the data of nights 2, 3, and 4 for the relevant variables. This had the advantage of providing more stable numbers by subjects as well as noninteger values better suited for parametric comparisons. A normal distribution of the number of cycles was observed in each individual night in the set constituted by the pool of 3 nights and in the mean of 3 nights.

Table 1. Mean REM sleep duration by cycle frequency and sequential occurrence

<table>
<thead>
<tr>
<th>Cycle Frequency, cycles per night</th>
<th>n</th>
<th>REM 1</th>
<th>REM 2</th>
<th>REM 3</th>
<th>REM 4</th>
<th>REM 5</th>
<th>REM 6</th>
<th>Mean</th>
<th>SD</th>
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<tr>
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<td>3</td>
<td>18,3</td>
<td>16,3</td>
<td></td>
<td></td>
<td></td>
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<td>9</td>
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<td>17,7</td>
<td>26,5</td>
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<td></td>
<td>22.6</td>
<td>5.6</td>
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<tr>
<td>4</td>
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<td>19,9</td>
<td>22,5</td>
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</tr>
<tr>
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<td>17,8</td>
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<td>9,3</td>
<td>7,5</td>
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<td>1.6</td>
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</table>

n = No. of subjects. REM, rapid eye movement; cycle frequency, number of non-REM-REM sleep cycles per night; REM 1–REM 6, REM sleep duration per cycle; SD, standard deviation.
DISCUSSION

This study of a large sample of carefully selected young healthy subjects recorded across 4 consecutive nights in their homes showed a strong positive correlation between the REM/NREM sleep ratio and the number of cycles per night. This was observed for the means across 3 nights and was replicated in individual nights. Thus the ratio between the two states is not constant and varies as a function of the cycle frequency.

Interestingly, a dissociation was observed between the two components: REM sleep duration was found to be a function of the cycle frequency, whereas NREM sleep was not. Another recent study (40) also showed...
no correlation between the number of cycles and the lower frequencies of total spectral power per night, which represents slow wave sleep activity, an important component of NREM sleep.

As mentioned above, two conflicting interpretations can be given to the observed relationship between the duration of NREM sleep episodes and the immediately preceding REM sleep episodes: either it is an indication of a close association between the two sleep states forming a miniature unit of the sleep process (8) or it reflects short-term REM sleep homeostasis and is not markedly influenced by NREM sleep (53). The association observed in this study between the cycle frequency and REM sleep, but not NREM sleep, favors distinct regulation mechanisms for the two sleep states and hence is more compatible with the second interpretation.

Recent data also support the concept of distinct REM homeostasis. A lack of association was observed between REM and NREM sleep rebounds after long-term sleep deprivation (> 2 wk), where slow-wave sleep rebounds were reduced in contrast with huge rebounds of REM sleep in rats (43). Also in rats, a comparison of different durations of NREM sleep deprivation, while keeping fixed durations for REM sleep deprivation, showed that REM sleep rebounds were not affected by the duration of previous NREM deprivation (39). Comparisons between sham-lesioned rats and rats with lesions of their hypothalamus suprachiasmatic nucleus, the area responsible for the circadian propensity for REM sleep regulation, showed no difference in total amounts of REM sleep rebounds after selective REM sleep deprivation when the whole rest-activity cycle was taken into account. The circadian component was able to favor the expression of REM sleep at certain times of the day, but it did not influence the total amount, which resulted mostly from homeostatic influences (57).

A clear sex difference was noted in this study, as most analyses were more significant in the male subgroup than in the overall sample and as no correlation was found within the only marginally smaller female group. Increases in REM sleep duration (25) and very short REM latencies (5) have been reported in the midluteal phase. Thus other factors, such as the menstrual cycle, may cloud this relationship. The lack of data on hormonal cycles in our sample prevented us from exploring this point. Methodologically, the present findings depend on the definition of REM sleep episodes and of NREM-REM cycles. This is a sensitive matter because REM sleep is frequently interrupted by bouts of NREM sleep and because both sleep stages may be interrupted by awakenings with little predictability. Fortunately, in human studies, the Rechtschaffen and Kales criteria (44) for visually scored sleep definitions, including REM sleep duration, are not presently challenged. Similarly, the empirical 15-min rule as the maximum tolerable duration of NREM sleep and awakenings within a REM sleep episode, originally introduced by Feinberg and Floyd (20), was later confirmed to be a valid and robust choice (37) and is accepted as a gold standard in human sleep studies. It remains theoretically possible, however, that different scoring rules would influence the results. An additional limitation to this study is the lack of data on caffeine or tobacco habits.

To extend their validity, the present findings invite replications in other study groups, such as humans of different age or other mammals, for instance.

In conclusion, the present data show a strong positive correlation between the ratio of REM to NREM sleep and the cycle frequency in a night, at least in healthy men. This correlation undermines the concept of strong relationships between REM and NREM sleep within the cycles and supports the concepts of independent regulation and function of the two sleep states.

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