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.—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 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.

sleep regulation; polysomnography; cycles; rapid eye movement; non-rapid eye movement; homeostasis

ALTERNATION OF RAPID EYE MOVEMENT (REM) sleep with non-REM (NREM) sleep across the night is a common characteristic of practically all studied homeothermic species. The number and duration of these NREM-REM “cycles” are likely to be genetically determined and vary from species to species, with a strong interspecies correlation betwee cycle duration and brain weight (58). In young and middle-aged human sleep, two to seven cycles of NREM-REM sleep have been observed (12, 13, 26, 31, 36, 49), and the cycle frequency has been shown to be normally distributed across individuals (36). The functional significance of this alternation and the relationship between the two sleep states remain two of the major mysteries of sleep.

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 not been clearly 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-
ingly vital process that has survived evolution despite its apparent maladaptive characteristics.

A new element was introduced in the debate on the REM-NREM alternation process from studies performed on monkeys (56), cats (51), rats (8, 53), and humans (6). They showed that the duration of a NREM sleep episode is positively associated with the duration of the preceding REM sleep episode (except, of course, in the first cycle). The most widely accepted interpretation of finding is that REM sleep determines the amount of the immediately consecutive NREM sleep episode. This observation has revived interest in the alternative view that REM sleep is essentially related to the expression of NREM sleep (19, 21, 22) and that a strong relationship exists between both states (6, 8).

According to this theory, the cycles formed by each successive NREM and REM sleep episode could be considered elementary sleep units, a miniature representation of the sleep process. There is no need for an ultradian oscillator in this approach, which posits that REM sleep propensity is accumulated essentially during NREM sleep and that its "pressure" increases progressively until some substance or process reaches a threshold and triggers the next REM sleep episode. In turn, REM sleep would facilitate the appearance of NREM sleep by an opposite mechanism. A similar conception of charge and discharge between REM and NREM sleep during the night is proposed in a revised version of the two-process model (1) as well as in the "cortical homeostasis" hypothesis (18), which posits that REM sleep is a stimulating state counterbalancing the potentially dangerous decrease in vigilance that occurs during NREM sleep. For a general discussion on this topic, see Benington and Heller (9).

The positive association between the duration of a NREM sleep episode and the duration of the preceding REM sleep episode can be interpreted differently, however, because the duration of a NREM sleep episode is also, by definition, the delay to the next REM sleep episode. Thus REM sleep might determine this delay simply for reasons proper to its own "short-term" (within the night) homeostasis, such as a negative feedback mechanism in which long REM sleep episodes are followed by long latencies to the next one and vice versa (17). Looking at the data this way, no particular relationship between NREM sleep and REM sleep would be inferred (53). Studies on REM and NREM sleep duration alone probably cannot solve this debate.

The present study examines the frequency of NREM-REM sleep cycles, a cardinal characteristic of the alternation process, to help disentangle the issue. In a recent paper (34), we confirmed that the number of cycles per night, like many other biological variables, was normally distributed in healthy controls and thus could be used as a continuous variable in parametric comparisons. We also demonstrated high intraclass correlations between 4 consecutive nights for this variable, which indicates that the cycle frequency varies much more from subject to subject than from night to night, at least in the reported study conditions.

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 weekend 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 electroocul-
logram electrodes, a chin and two inferior limb electromyo-
gram electrodes, thoracic and abdominal gauges for respira-
tory movements, thermoreceptors around the mouth and
ose, a finger oximeter, and a microphone for detection of
noring. Subjects went to bed at their usual sleep time and
connected the wires, in a very straightforward procedure, to
leep analyzer Alice (Respironics, Pittsburgh, PA). When
ubjects decided to go to sleep, they launched the polyson-
ography 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 80 poly-
sonograph 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 as each REM sleep episode and the NREM sleep
immediately preceding it, going back to sleep onset (first
NREM-REM 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
ight.

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 regres-
sion analyses were performed to analyze the respective con-
tributions 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
(SPPS, Chicago, IL). The graph was created by using Stat-
view 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 ques-
ionnaire and physician interviews were causes for
clusion 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 tech-
nical 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 polysonograph 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 dura-
tions by cycle frequency (n = 78, 26 subjects over 3
ights). 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 only by 105%. When
measured in percentages of total sleep time (TST),
REM sleep was 19.8% (6 cycles/night) vs. 6.3% (2 cy-
cles/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 previ-
ously across nights in the same subjects (34), we aver-
aged 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 val-
ues 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>
<thead>
<tr>
<th>Cycle Frequency, cycles per night</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>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>18, 3</td>
<td>16, 3</td>
<td></td>
<td></td>
<td></td>
<td>17.2</td>
<td>2.3</td>
</tr>
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<td>3</td>
<td>9</td>
<td>17, 1</td>
<td>17, 7</td>
<td>26, 5</td>
<td></td>
<td></td>
<td>22.6</td>
<td>5.6</td>
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<tr>
<td>4</td>
<td>35</td>
<td>12, 8</td>
<td>19, 9</td>
<td>22, 5</td>
<td>20, 7</td>
<td></td>
<td>19.8</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>8, 8</td>
<td>20, 2</td>
<td>22, 4</td>
<td>21, 4</td>
<td>20, 8</td>
<td>19.6</td>
<td>4.5</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>7, 8</td>
<td>12, 8</td>
<td>17, 8</td>
<td>21, 7</td>
<td>9, 3</td>
<td>7, 5</td>
<td>1.6</td>
</tr>
</tbody>
</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.
Data analysis. The correlation between the mean REM/NREM sleep ratio and mean cycle frequency was highly significant \((r = 0.590, P = 0.001)\). Although age was not correlated with the number of cycles, it was partialed out for its well-known association with NREM sleep. Also, because the number of cycles could be biassed by total sleep duration, mean time in bed (TIB), sleep period time (SPT), and TST were partialed out individually. The correlations remained highly significant (controlling for TIB: \(r = 0.554, P = 0.005\); controlling for SPT: \(r = 0.524, P = 0.009\); controlling for TST: \(r = 0.526, P = 0.008\)). Elimination of one, two, three, and four outliers still provided strong correlations between cycle frequency and the REM/NREM sleep ratio. The associations between the REM/NREM sleep ratio and the cycle frequency were consequently examined for the mean number of cycles and the REM/NREM sleep ratio on each night. The correlations remained significant only in men, with a correlation between the number of cycles and REM/NREM sleep ratio of \(r = 0.772 (P = 0.005)\). As seen in the overall sample, regression analysis of the male subgroup showed only mean REM sleep to be significantly associated to the mean number of cycles \((F = 15.2, df = 1, P = 0.002)\). Examination of individual nights in men again showed the number of cycles to be associated with the REM/NREM sleep ratio on night 2 \((r = 0.709, P = 0.010)\), night 3 \((r = 0.610, P = 0.035)\), and night 4 \((r = 0.678, P = 0.015)\).

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 sleep deprivation (39). Comparisons between sham-lesioned rats and rats with lesions of their hypothalamic 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 states 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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REFERENCES