Selective REM sleep deprivation during daytime:
II. Muscle atonia in nonREM sleep

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Abstract

One of the hallmarks of REM sleep is muscle atonia. Here we report extended epochs of muscle atonia in nonREM sleep (MAN). Their extent and time course was studied in a protocol, which included a baseline night, a daytime sleep episode with or without selective REM sleep deprivation, and a recovery night. The distribution of the latency to the first occurrence of MAN was bimodal with a first mode shortly after sleep onset and a second mode 40 minutes later. Within a nonREM sleep episode, MAN showed a U-shaped distribution with the highest values before and after REM sleep. Whereas MAN was at a constant level over consecutive 2-h intervals of nighttime sleep, MAN showed high initial values when sleep began in the morning. Selective daytime REM sleep deprivation caused an initial enhancement of MAN during recovery sleep. It is concluded that episodes of MAN may represent a REM sleep equivalent and that it may be a marker of homeostatic and circadian REM sleep regulating processes. MAN episodes may contribute to the compensation of a REM sleep deficit.

Key words: REM sleep; sleep deprivation; sleep regulation; EMG
Abbreviations

FIR: finite impulse response; MAN: muscle atonia in NREM sleep; NREM sleep: non-rapid eye movement sleep; PGO waves: ponto-geniculo-occipital waves; rANOVA: ANOVA for repeated measures; REM sleep: rapid eye movement sleep; SOMAN: sleep onset MAN (occurring within the first 15 min after sleep onset (stage 2)); SOREMS: sleep onset REM sleep; SWA: power in the 0.75-4.5 Hz range of the sleep EEG; TST: total sleep time
Introduction

The characteristics of human REM sleep include three physiological components: Desynchronized EEG pattern, muscle atonia with phasic twitches, and rapid eye movements. In animals, the occurrence of ponto-geniculo-occipital (PGO) waves is an additional phenomenon that is typical for REM sleep (16).

The various REM sleep components do not always appear in synchrony. Marked dissociations occur during REM sleep parasomnias and as a consequence of pharmacological treatment (see (13) for a recent review). Thus cataplexy may represent an inappropriate intrusion of REM sleep atonia into wakefulness, while a loss of REM sleep atonia may be induced by antidepressant medication. Drug-induced dissociations of REM sleep components have been also observed in animals. For example, serotonin depletion by parachlorophenylalanine causes PGO spikes to occur in all vigilance states, and not only in REM sleep (6).

During physiological sleep the REM sleep components do not always occur synchronously. In early human studies, submental muscle atonia was reported to precede the typical EEG changes of REM sleep, and to persist during the initial part of the subsequent NREM sleep episode (4). These authors have attempted to quantify this phenomenon by the reset rate of the integrated EMG signal. They observed that the drop of the EMG level began about 5 min before the onset of a REM sleep episode, and that a gradual increase occurred in the 20-min interval following the REM sleep episode. This gradual rise of the tonic EMG level during a NREM sleep episode was also reported by Brunner et al. (5) who used the statistical variance of the digitized signal for quantifying the EMG. These authors found that unlike in the later episodes, a decrease of the EMG level occurred in the first NREM sleep episode. The regulatory aspects of
sleep were not considered in these previous studies. The present analysis is based on a data set that includes a selective REM sleep deprivation period as well as sleep at different circadian phases. It was prompted by the recognition of episodes of muscle atonia in NREM sleep (MAN). This raised the question whether they represent REM sleep components occurring outside REM sleep and whether they could serve as a marker of homeostatic and circadian influences.

**Method**

**Subjects and Protocol**

Twelve male subjects (mean 24±0.17 years), recruited from the student population, participated in a selective REM sleep deprivation study (19). The work fully conforms to the guiding principles for research involving animals and human beings (American Physiological Society). Only 11 subjects were analyzed (see Statistics). The recruitment, screening, subject selection and procedural details are described in the companion paper (19). In brief, a two-session protocol was used. The first session consisted of baseline sleep (23:00-7:00h; B1), a waking episode of 24 h followed by daytime sleep (7:00-15:00h; E1), and recovery sleep (23:00-7:00h; R1). During E1 subjects were repeatedly awakened to prevent REM sleep. The second session served as a control for the first session, and had the same structure (B2, E2, R2) except that daytime sleep (E2) was undisturbed. Total sleep time (TST) of E2 (consolidated sleep) was restricted to match individually TST of E1.
Polygraphic Recordings

The electroencephalogram (EEG), electrooculogram (EOG), submental electromyogram (EMG) and electrocardiogram (ECG) were recorded by a polygraphic amplifier (PSA24, Braintronics Inc., Almere, The Netherlands), digitized and transmitted via a fiber-optic cable to a personal computer. Data were sampled with a frequency of 512 Hz, digitally filtered (EMG: band-pass FIR filter, -3 dB points at 15.6 and 54 Hz), and stored on a PC with a resolution of 128 Hz. For further details see the companion paper (19). For the ECG, consecutive RR intervals (time interval between consecutive R-waves) and one value of heart rate every 20 s (beats per minute, as calculated from the mean RR interval) were stored. An R-wave was detected whenever the rectified first derivative of the ECG exceeded 40% of the maximal value.

Vigilance states were visually scored for 20-s epochs according to standard criteria (15). Sleep latency was defined as the interval between lights off and first appearance of stage 2. REM sleep latency was defined as the interval between sleep onset and REM sleep. A REM sleep episode with latency shorter than 15 min was defined as a sleep onset REM (SOREM) sleep episode.

Muscle atonia

Epochs of muscle atonia were identified when the variance of the EMG (i.e. total power) was below the 90th percentile level of the EMG variance in REM sleep epochs (Fig. 1). The EMG variance was calculated for consecutive 20-s epochs. Since the EMG was occasionally contaminated by ECG artifacts, 30 data points before and after the occurrence of an R-wave (i.e. ± 0.23 s) were excluded for calculating the variance. The
threshold was determined separately for each sleep episode. Muscle twitches or short arousals were excluded before determining the threshold level. Therefore, REM sleep epochs in which EMG variance exceeded twice the median value of all REM sleep epochs, were not used for defining the 90\textsuperscript{th} percentile threshold level. All threshold levels were verified by visual inspection. In 13 of 64 recordings the signal-to-noise ratio of the EMG changed in the course of the sleep episode and the threshold had to be adjusted. In 2 recordings the threshold had to be slightly increased. Twenty-second epochs of NREM sleep in which the EMG variance was below threshold, were defined as epochs with muscle atonia in NREM sleep (MAN). Atonia latency was defined as the interval from sleep onset to the first appearance of MAN. Episodes of MAN with a latency shorter than 15 min were considered as a sleep onset MAN episodes.

**Statistics**

Two-way or one-way ANOVA for repeated measures (rANOVA) with Huynh-Feldt correction were performed (for details see text). Post-hoc comparisons were performed with two-tailed paired t-tests. For comparing REM sleep and MAN latencies Wilcoxon signed-rank tests were applied.

One subject was excluded from the analysis because both baseline recordings showed very low sleep efficiency (<75\%). Thus 11 subjects contributed to the present analysis. Due to technical problems one recording during selective REM sleep deprivation (E1) was lost. Due to the loss of an EMG electrode the EMG of one recording during R2 could not be analyzed. In three subjects the first baseline recording (B1) was not used due to sleep efficiency below 80\% (2 subjects) and stomach problems (one subject).
These recordings were either not included in the analysis (Fig. 2 and 3) or were replaced with B2 for performing rANOVAs (Tab. 1).

**Results**

**Muscle tone in NREM sleep and REM sleep**

In addition to the typical low EMG level in REM sleep, epochs with muscle atonia in NREM sleep (MAN) were observed (Fig. 1). In the hypnogram of Figure 1 sleep stages with muscle atonia are indicated in black. In this daytime sleep episode MAN was present during the initial long stage 4 episode and intermittently during several short stage 2 episodes.

EMG variance in NREM sleep was significantly higher than in REM sleep (NREM sleep: $3.26 \pm 0.36 \mu V^2$, REM sleep: $0.19 \pm 0.01 \mu V^2$; $p<0.001$, 2-tailed paired t-test). The values for REM sleep and MAN were very similar but nevertheless differed significantly ($0.19 \pm 0.01 \mu V^2$ vs $0.20 \pm 0.02 \mu V^2$; $p<0.001$).

The time course of MAN during the first NREM sleep episode and the following episodes (average of NREM sleep episodes 2-5) is illustrated in Figure 2. To compute the average values, each NREM sleep episode was subdivided into 7 equal intervals and for each interval the MAN epochs were expressed as percentage of all NREM sleep epochs. For episodes 2-5, MAN showed a U-shaped distribution with the high values before and after REM sleep. A similar pattern was present in the first NREM sleep episode where the percentage of MAN epochs was higher after sleep onset than in the middle of the episode.
Atonia latency and REM sleep latency

The latency to the first appearance of MAN showed a bimodal distribution with modes at 2.5 min and 42.5 min and a trough between 10 and 20 min (Fig. 3). The atonia latencies showed a bimodal distribution with the first mode centered at sleep onset and the second mode situated in the proximity of REM sleep onset. Two sleep onset REM (SOREM) sleep episodes occurred (one in E2 and one in R1; Tab. 1), and one skipped first REM sleep episode was observed (in B2).

The latencies for the different experimental conditions are indicated in Table 1. While the latency to MAN did not differ between baseline (B1) and daytime sleep with selective REM sleep deprivation (E1), it was shortened in the subsequent recovery sleep (R1). In contrast to the REM sleep deprivation schedule, atonia latency in undisturbed daytime sleep (E2) was shorter than in baseline sleep, while the following recovery night (R2) did not differ from baseline. The frequency of occurrence of sleep onset MAN was highest in R1 and E2. REM sleep latencies showed no significant differences.

Time course of MAN in the different experimental conditions

The upper panel of Fig. 4 illustrates the time course of MAN in the different experimental conditions. For comparison, the amount of NREM sleep and REM sleep is indicated in the middle and lower panel, respectively. During baseline sleep, MAN episodes were at a rather constant level of 10-15 %, while REM sleep showed the typical increasing trend (lower left). The repeated interventions to prevent REM sleep
(E1) led to a gradual decrease of MAN and to the reduction of NREM sleep in the third 2-h interval compared to baseline. In the recovery night (R1), MAN was enhanced in the first interval and decreased below baseline in the third interval. Undisturbed daytime sleep (E2) showed in the first 2-h interval much higher MAN values than in baseline sleep. In the following nighttime sleep episode (R2) its level did not differ from baseline.

**Discussion**

For the first time, the present report systematically documents epochs of muscle atonia in NREM sleep (MAN). Although their most frequent occurrence is in proximity to REM sleep, they are present throughout a NREM sleep episode. This gives rise to a U-shaped pattern. The present observations are in accordance with previous reports that epochs with a low EMG level occur in the part of the NREM sleep that precedes and follows REM sleep (4, 5, 12). These findings indicate that a REM sleep episode is not sharply delimited but that it has antecedents during NREM sleep and that it vanishes gradually in the succeeding NREM sleep episode. Also in animals it was observed that transitions from NREM sleep to REM sleep are not always sharply delimited, but premonitory signs appear prior to the state change. Benington and Heller (3) reported that during a NREM sleep episode brief REM sleep episodes occurred with increasing frequency, leading finally to a sustained REM sleep episode. In view of the U-shaped distribution of MAN episodes it is unlikely that they are analogous events. However, there is evidence from animal studies that typical electrophysiological changes occur prior to the onset of REM sleep (e.g. (18)).
The notion that REM sleep components permeate NREM sleep was advocated by Nielsen (14). In his recent review of mentation in sleep, he argued in favor of covert REM sleep processes in NREM sleep, which occur predominantly before and after a REM sleep episode. This pattern corresponds to the occurrence of MAN in the present study. The proximity of MAN to REM sleep suggests that it is functionally related to this sleep state rather than being an epiphenomenon of NREM sleep. MAN may also indicate that the two sleep states are not as clearly demarcated as the sleep scoring rules suggest. In the interesting case of the echidna components of REM sleep and NREM sleep are present within a single state (17).

The distribution of MAN within the first NREM sleep episode was of particular interest, since it supports the presence of an early ‘REM sleep window’. Brunner et al. (5) reported a gradual decline of the EMG level from sleep onset to the first occurrence of REM sleep. In the present study a first mode of atonia was seen in the first two bins (Fig. 2). The bimodal distribution was also evident from the atonia latencies (Fig. 3). An early ‘REM sleep window’ close to sleep onset has been postulated previously and was incorporated into a model (1). Both, the distribution of MAN within NREM sleep episodes and the distribution of MAN latency suggests that it may reflect a REM sleep process.

In previous studies, the level of the EMG was studied only under baseline conditions. We report for the first time the response of an EMG variable to a circadian and homeostatic challenge. When sleep was scheduled to begin in the morning hours after an extended waking episode, the initial level of MAN was three times higher than during nighttime sleep. In the early morning hours, the circadian REM sleep propensity is known to be high (7). Therefore the high level of MAN could reflect REM sleep
propensity. Interestingly, REM sleep itself was not elevated at this time. This could be due to the fact that a prolonged waking episode enhances NREM sleep propensity, which during sleep gives rise to a high initial NREM sleep intensity as reflected by EEG SWA (19, Fig. 3). This in turn is known to exert a suppressing action on REM sleep. Thus MAN may be a more faithful marker of REM sleep propensity than REM sleep itself. Let us consider in this light the response to a selective REM sleep deprivation.

In the initial interval of nighttime recovery sleep (R1) MAN was markedly enhanced and then declined (Fig. 4). This pattern was seen only after REM sleep deprivation. When the nighttime sleep episode followed upon uninterrupted daytime sleep (R2), MAN was at a uniform level throughout the night similar to baseline.

In contrast to the manifestation of REM sleep itself, MAN seems to be little influenced by changes in NREM sleep propensity. Neuropharmacological studies are consistent with the notion that atonia is a particularly sensitive marker of the REM sleep process. Thus when carbachol was microinjected into pontine nuclei of the cat, only atonia was induced by low doses, whereas the full REM sleep pattern was elicited at larger doses (11, 16).

If MAN is not only a marker of REM sleep propensity but a REM sleep equivalent, the question arises whether it could play a functional role in REM sleep regulation. Puzzling aspects of REM sleep regulation such as the partial and delayed compensatory response after a deficit may be explained, if functionally analogous states can occur outside REM sleep. We have previously speculated that REM sleep compensation could occur during waking (8; see also 10). The present results suggest that this may be the case also in NREM sleep. Thus MAN could contribute to the functional compensation of a REM sleep deficit. Although the atonia episodes in NREM sleep do
not fully compensate the loss incurred during a REM sleep deprivation, they may represent a partial compensatory mechanism. Further investigations must explore the possible functional significance of a reduced muscle tone.

In summary, the present observations support the proposition of Nielsen (14) that sleep stages are fluid and interactive rather than discrete and independent. In fact, even the waking state contains physiological markers of sleep propensity (i.e. theta activity in humans, delta activity in rodents) that have been suggested to reflect sleep regulatory processes (2, 9). The present data indicate that MAN may represent a particular feature of NREM sleep that reflects circadian as well as homeostatic REM sleep propensity and may be even involved in REM sleep regulation. A reconsideration of the state concept could lead to a new understanding of the regulatory processes underlying sleep and waking.
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References


**Figure 1:** Time course of EMG variance (i.e. total power) and sleep profile of a daytime sleep episode in one subject. EMG variance is plotted on a logarithmic scale and the horizontal line indicates the threshold level that was predetermined to identify episodes of muscle atonia (see Methods). Sleep stages with muscle atonia are indicated in black, all others in grey. The raw signals (EEG, EOG, EMG) of three 4-s epochs (see arrows in hypnogram) are enlarged to illustrate the state dependent differences in muscle tone.

**Figure 2:** Average time course of muscle atonia in NREM sleep (MAN). Individual NREM sleep episodes were subdivided into 7 equal intervals. For each interval the percentage of epochs with atonia was determined. Mean values + 1 SE (n=11) are plotted for the first NREM sleep episode and for subsequent episodes. All experimental conditions (B1, B2, E2, R1, R2) were pooled and an average was calculated in each subject prior to calculating the mean across subjects. Not all recordings had up to 5 NREM sleep episodes. 51 individual episodes contributed to NREM sleep episode 1 and 181 to episodes 2 to 5.

**Figure 3:** Distribution of the latency to the first occurrence of muscle atonia in NREM sleep (MAN). Only MAN episodes that occurred prior to the first REM sleep episode were included (n=58). In three cases no MAN was observed prior to the first REM sleep episode. Data of E1 were excluded because of the experimental manipulation. The zero minute mark delimits sleep onset as defined by the first stage 2 epoch. The negative values correspond to stage 1.
Figure 4: Muscle atonia in NREM sleep (MAN; epochs of MAN as a percentage of NREM sleep), amount of NREM sleep (NREMS) and REM sleep (REMS) in consecutive 2-h intervals. Mean values + 1 SE are plotted for baseline mean (Bm, n=11), daytime sleep (E1, n=10; E2, n=11), and recovery sleep (R1, n=11; R2, n=10). * significant difference from corresponding Bm value; § significant difference from corresponding value of control condition; ° significant difference to following 2-h interval (p<0.05, two-tailed paired t-test). p-values above bars (ns, not significant) indicate significance of factor ‘interval’ of a 1-way rANOVA.
Table 1. Latencies to muscle atonia in NREM sleep (MAN) and to REM sleep

<table>
<thead>
<tr>
<th></th>
<th>Bm (baseline)</th>
<th>E (experimental sleep)</th>
<th>R (recovery sleep)</th>
<th>session</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAN latency (min)</td>
<td>38.70±5.08</td>
<td>33.93±6.00</td>
<td>15.88±5.94°*</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.09±6.60 °</td>
<td>41.27±7.08</td>
<td>2</td>
</tr>
<tr>
<td>REMS latency (min)</td>
<td>63.61±3.83</td>
<td>51.70±2.85</td>
<td>51.27±5.94</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>51.76±6.26</td>
<td>60.50±3.07</td>
<td>2</td>
</tr>
<tr>
<td>SOMAN episodes</td>
<td>5/19</td>
<td>3/10</td>
<td>7/11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>7/11</td>
<td>2/10</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>SOREMS episodes</td>
<td>0/19</td>
<td>0/10</td>
<td>1/11</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1/11</td>
<td>0/10</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Latencies are mean values ± SE; sleep onset episodes are number of episodes per number of recordings analyzed. Session 1 is the selective REM sleep deprivation condition (deprivation in E1), and session 2 the control condition (total sleep time in E2 matched with E1).
Bm: baseline mean; MAN: muscle atonia in NREM sleep; REMS: REM sleep; SOMAN: sleep onset MAN (occurring within the first 15 min after sleep onset (stage 2)); SOREMS: sleep onset REMS; * difference to control (session 2); ° difference to baseline mean (p<0.05, Wilcoxon signed rank test). REMS latencies in E1 and R2 differ slightly from those reported in Tab. 1 of (19) since EMG analysis was only possible in 10 subjects.
Fig. 1
Fig. 2

Interval

MAN (% of NREMS)

NREMS episode 1

NREMS episodes 2-5

Interval

1 2 3 4 5 6 7

1 2 3 4 5 6 7
Fig. 3