Effects of Sleep Fragmentation on the Arousability to Resistive Loading in NREM and REM Sleep in Normal Men

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Study Objective: In healthy subjects, arousability to inspiratory resistive loading is greater during rapid eye movement (REM) sleep compared with non-REM (NREM) sleep but is poorest in REM sleep in patients with sleep apnea. We therefore examined the hypothesis that sleep fragmentation impairs arousability, especially from REM sleep.

Design: Two blocks of 3 polysomnographies (separated by at least 1 week) were performed randomly. An inspiratory-loaded night followed either 2 undisturbed control nights (LN) or 2 acoustically fragmented nights (LF).

Setting: Sleep laboratory.

Participants: Sixteen healthy men aged 20 to 29 years.

Interventions: In both loaded nights, an inspiratory resistive load was added via a valved facemask every 2 minutes during sleep and turned off either when arousal occurred or after 2 minutes.

Measurements and Results: During LN, arousability remained significantly greater in REM sleep (71% aroused within 2 minutes) compared with stage 2 (29%) or stage 3/4 (16%) sleep. After sleep fragmentation, arousability was decreased in stage 2 sleep (LN: 29%; LN: 38%; p < .05) and low in early REM sleep, increasing across the night (p < .01). In stage 3/4 sleep, neither an attenuation nor a change across the night was seen after sleep fragmentation.

Conclusions: Mild sleep fragmentation is already sufficient to attenuate arousability in stage 2 sleep and to decrease arousability in early, compared with late, REM sleep. This means that sleep fragmentation affects the arousal response to increasing resistance and that the effects are different in stage 2 and REM sleep. The biologic reason for this increase in the arousability response in REM sleep across the night is not clear.

Keywords: Arousal, inspiratory resistive loading, sleep apnea, sleep stage dependent

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INTRODUCTION

AROUSAL IS BELIEVED TO BE AN ESSENTIAL PROTECTIVE MECHANISM AGAINST LIFE-THREATENING ASPHYXIA DURING SLEEP AND CONTRIBUTES TO DAILY SLEEPINESS AND BLOOD PRESSURE ELEVATION in patients with obstructive sleep apnea-hypopnea syndrome (OSAHS). Apneas are mostly, although not exclusively, terminated by arousals. A major consequence of repetitive arousals is sleep fragmentation, which is marked in patients with OSAHS. Sleep fragmentation causes increases in the arousal threshold to acoustic stimuli and is associated with subsequent daytime sleepiness.

Many stimuli can cause arousal. Although auditory arousal in the absence of apnea and hypoxemia is associated with significant nighttime blood pressure elevation, only respiratory arousals lead to sustained daytime hypertension in dogs after 3 months. As mechanical respiratory stimuli seem to be most relevant in the context of OSAHS, inspiratory resistive loading was used in this study to determine the arousal threshold.

Disclosure Statement
This was not an industry supported study. Dr. Bassetti has participated in a speaking engagement supported by ResMed. Dr. Douglas has received research support from ResMed Ltd; and chairs for the international medical advisory board of ResMed. Drs. Zavodny, Roth, Mathis, and Gugger have indicated no conflicts of interest.

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fragmentation affects the sleep-time–dependent evolution of the arousal response within a sleep stage.

METHODS

Subjects

Sixteen healthy men aged 24 ± 0.7 years (mean ± SEM) were studied. Subjects were recruited from a student population by advertisement on notice boards in our hospital and in the Technical College in Biel. Only subjects with no sleep complaints assessed by our sleep questionnaire were accepted. All were nonobese (body mass index: 22 ± 0.3 kg/m²), nonsmokers, and regular nocturnal sleepers without any sleep disturbance or habitual snoring, and none were taking any medication. They had an Epworth Sleepiness Scale score of 6 ± 0.8. A screening night served to exclude any subject with sleep-disordered breathing, and only subjects with a respiratory disturbance index (RDI) less than 10 qualified for the study. All subjects gave written informed consent to participate in the study and were paid for participation. Ethical permission for the study was obtained from the ethics committee of the Faculty of Medicine of the University and the Canton of Berne.

Study Protocol

The study protocol is summarized in Figure 1. The screening night, preceding the start of the study by at least 3 days, served to familiarize subjects with the equipment. Subjects wore a face mask for acclimatization, but no load was applied. They had an RDI of 2.9 ± 0.5 and a sleep efficiency of 88% ± 2.6%. Following the screening night, subjects spent 6 study nights in the sleep laboratory, and all 7 nights were studied using standard polysomnography. The study nights were divided into 2 blocks of 3 nights, the blocks separated from each other by at least a week. The subjects were randomly allocated to start with either 2 undisturbed control nights or 2 acoustically fragmented nights. During the third night of each block (LN: loaded night), an inspiratory resistive load was applied via a facemask. All studies were performed between 10:00 PM and 7:00 AM, and the study time was matched to the usual time in bed of each subject. After the study nights, the subjects left the sleep laboratory and continued their normal daily routine activities. They had to refrain from caffeinated beverages and from alcohol 1 day before and throughout the 3 days of each study block apart from 1 cup of coffee in the morning. Naps were not allowed, and compliance with this instruction was verified by continuous recording of wrist activity (Activwatch Cambridge Neurotechnology, Cambridge, UK).

The instrumentation was restricted to the minimum required for investigating the questions outlined in the introduction because the sleep-disturbing effects of equipment often impair data collection, particularly in REM sleep. To reduce the sleep-disturbing effect of the performed load applications, the occlusion of the inspiratory airflow was not complete. Only a single level of resistance was used to decrease the number of possible comparisons and to be able to compare the results between the different sleep stages.

All measurements were recorded with a computerized recording system (Embla; Flaga hf. Medical Devices, Reykjavik, Iceland). Sleep state was recorded using the standard placements of electroencephalogram (F3/A2, F4/A1), left and right electro-oculograms, and submental electromyogram. Nasal airflow was monitored using a pressure transducer airflow sensor (PTAF2; Pro-Tech Services Inc., Woodinville, WA, USA) and nasal prong devices (Pro-Tech Services Inc.), thoracic and abdominal respiratory effort using a piezo-electric sensor (Ultima DL Effort Sensor; Braebon Medical Corporation, Kanata, ON, Canada). Arterial oxygen saturation was recorded using pulse oximetry (Oximeter Flex Sensor 8000J; Nonin Medical Inc., Minneapolis, MN, USA). During LN, airflow was monitored via a sealed facemask (modified full facemask; ResMed Ltd, North Ryde, NSW, Australia) with built-in inspiratory and expiratory valves serving to apply the inspiratory resistance. The mask dead space was approximately 60 mL, depending on individual face configuration. A leak detector, consisting of a perforated polyethylene catheter connected to a capnometer (OxiCap 4700; Ohmeda, Louisville, CO, USA), was attached to the circumference of the facemask cushion and was set at maximum gain.18

Sleep stages and awakenings were scored for 30-second epochs using the criteria of Rechtschaffen and Kales.19 Arousal was defined as an episode lasting 3 seconds or longer, according to the criteria of the American Sleep Disorders Association.20 Figure 2 shows an example of an arousal during REM sleep. Apneas and hypopneas were defined as an event lasting 10 seconds or longer according to the recommendations of the American Academy of Sleep Medicine.21 Flow-limitation events were defined as any series of 2 or more breaths (lasting at least 10 seconds) that had a flattened or nonsinusoidal appearance on the inspiratory nasal-cannula flow signal and ended abruptly with a return to breaths with sinusoidal shape.22

Sleep Fragmentation

Sleep was fragmented on 2 consecutive nights because, in a

![Figure 1](https://example.com/figure1.png)
preliminary study, 1 night of sleep fragmentation combined with partial sleep deprivation might have been insufficient to affect arousal. Therefore, every 2 minutes (interseries interval) from the onset of undisturbed stage 2, stage 3/4, or REM sleep, tone series of 1000 Hz generated by an audiometer (GSI 16; Grason-Stadler Inc., Littleton, MA) were presented via 2 loudspeakers positioned beside the subject’s head until an arousal response was obtained. The duration and volume of tones to produce arousal were increased because normal subjects may acclimatize to arousing stimuli. The first tone was applied for 5 seconds at 60 dB. If no arousal was noted within 15 seconds (intertone interval), a second tone was sent at 60 dB, but this time lasting 10 seconds. If no response was observed, tone duration was kept constant (10 seconds), and sound intensity was augmented in 10-dB increments every 15 seconds until 90 dB. The next 3 tones were set at a maximal decibel level of 95 dB. The first 8 tones were frequency-modulated sine waves (warble tones). The ninth tone was a narrow band noise at 90 dB and was repeated twice if no arousal occurred. The intertone intervals were 15 seconds, and the inter-series intervals were 2 minutes of undisturbed stage 2, stage 3/4, or REM sleep. Each tone series was terminated by the technicians upon signs of electroencephalographic arousal or after a total of 11 tones were presented.

**Inspiratory Resistance**

In accordance with previous studies, sustained resistive loading was accomplished by the addition of a 25-cm H2O per liter per second linear resistive load to the inspiratory line by turning a 3-way tap in the inspiratory line. The tap was turned only during expiration to avoid disruption of the breathing cycle. The tap was in a different room from the subject, and careful attention was paid to avoid any noise or contact of the tubing that might have disturbed sleep. The resistance was added every 2 minutes from the onset of undisturbed stage 2, stage 3/4, or REM sleep. After spontaneous sleep-stage changes, the resistance was not applied for at least 1 minute. In case of an occasional respiratory event, the resistance was not applied for at least 1 minute after termination of the event. If no arousal or awakening (> 15 seconds) occurred during the resistive breathing, the resistance was switched off after 2 minutes and again not reapplied for at least 2 minutes. If arousal or awakening occurred, the resistance was switched off. “Time to arousal” was defined as the time between the start of loading and the occurrence of an arousal within the intervention. If no arousal occurred within the 2 minutes of loading, it was defined as “nonarousal”.

**Control Periods**

Control periods of 2 minutes were selected on the control nights to look at arousals during them. These periods were retrospectively marked in the recordings during stage 2, stage 3/4, and REM sleep, according to the inspiratory loading protocol and regardless of arousal during these control periods. The number of arousals occurring within these periods was compared with the number of arousals occurring within 2 minutes of the application of the inspiratory resistance.

**Data Analysis**

The results are presented as mean ± SEM. The data were first analyzed with a 2-way analysis of variance for repeated measures (rANOVA) using the SAS General Linear Model procedure with Greenhouse-Geisser correction. In case of significant effects, contrasts were further tested by the 2-tailed Wilcoxon signed-rank test and the Friedman test, respectively. All tests were paired. The pooled numbers of arousals and “nonarousals” were compared between the different sleep stages within a loaded night and between loaded nights (all-night data and per sleep stage) with 2 × 2 contingency tables and χ2 tests of independence. Results with a p value < .05 were considered significant.

The total number of arousals includes all detected arousals. They include arousals in which retrospective analysis showed that the stimulus was not applied strictly according to the study protocol, as well as additional arousals within the intervention interval. These arousals, which were 2% of all arousals, were not included in any further analysis.

Sleep variables, as well as number of arousals of the 2 control nights, were averaged to compare the control night (CN) mean with the loaded and fragmented nights, respectively. In order to take into account the numerous “nonarousals” when analyzing “time to arousal”, “time to arousal” was ranked, and the highest rank was assigned in event of a “nonarousal”.

To represent the sleep-time-dependent evolution of the arousal response to inspiratory loading, the data obtained on the loaded nights were broken into thirds of the night, and the first third was compared with the last third of the night, as in a study of Philip et al. To avoid an uneven distribution of the data in the different sleep stages (since sleep stages are not equally distributed throughout the night), we compared the results obtained with the first third of load applications to the last third in each sleep stage.

**RESULTS**

**Effectiveness of Sleep Fragmentation**

To fragment sleep, 67.6 ± 3.6 tone series with an average of 3.9 ± 0.4 tones were applied in the first fragmented night (FN1), of which 66.0 ± 3.9 (97% ± 1%) resulted in arousals. In the second fragmented night (FN2) there were 69.1 ± 2.9 tone series with a mean of 6.0 ± 0.4 tones and 61.2 ± 3.9 (88% ± 3%) induced an arousal. The number of tone series was similar in both fragmented nights, but the number of tones per series was significantly higher in FN2 than in FN1 (p < .0001). In FN2, significantly fewer
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Table 1—Sleep Variables and Arousals on Fragmented, Control, and Loaded Nights

<table>
<thead>
<tr>
<th>Sleep stage percentage, %</th>
<th>FN1</th>
<th>FN2</th>
<th>CN</th>
<th>LN_F</th>
<th>LN_C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.8 ± 1.5*</td>
<td>11.8 ± 0.6*</td>
<td>9.2 ± 0.6</td>
<td>11.1 ± 0.7*</td>
<td>13.3 ± 1.2*</td>
</tr>
<tr>
<td>2</td>
<td>61.3 ± 1.4*</td>
<td>53.6 ± 1.6</td>
<td>51.0 ± 1.3</td>
<td>50.0 ± 1.3</td>
<td>51.1 ± 1.5</td>
</tr>
<tr>
<td>3/4</td>
<td>6.3 ± 1.2*</td>
<td>13.9 ± 1.5*</td>
<td>20.1 ± 1.4</td>
<td>19.9 ± 1.7</td>
<td>17.7 ± 1.0</td>
</tr>
<tr>
<td>REM</td>
<td>13.6 ± 1.3*</td>
<td>20.8 ± 1.3</td>
<td>19.7 ± 0.9</td>
<td>19.0 ± 1.1</td>
<td>18.0 ± 1.1*</td>
</tr>
<tr>
<td>TST, min</td>
<td>383.7 ± 17.0*</td>
<td>443.3 ± 8.6</td>
<td>438.9 ± 5.8</td>
<td>433.3 ± 6.9</td>
<td>412.7 ± 9.6*</td>
</tr>
<tr>
<td>Movement time, min</td>
<td>5.9 ± 0.8</td>
<td>7.3 ± 1.2</td>
<td>7.3 ± 0.8</td>
<td>6.7 ± 0.8</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>WASO, min</td>
<td>72.5 ± 14.9*</td>
<td>23.9 ± 5.6</td>
<td>18.8 ± 3.6</td>
<td>25.2 ± 4.0</td>
<td>47.6 ± 8.4*</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>81.2 ± 3.3*</td>
<td>92.4 ± 1.4</td>
<td>92.1 ± 1.0</td>
<td>91.2 ± 1.2</td>
<td>86.6 ± 1.9*</td>
</tr>
<tr>
<td>Sleep latency, min</td>
<td>10.6 ± 2.4</td>
<td>5.4 ± 1.3*</td>
<td>11.3 ± 1.8</td>
<td>9.8 ± 1.9</td>
<td>11.1 ± 1.8</td>
</tr>
<tr>
<td>REM sleep latency, min</td>
<td>191.2 ± 21.2*</td>
<td>94.9 ± 12.3</td>
<td>91.1 ± 8.5</td>
<td>80.2 ± 7.7</td>
<td>125.6 ± 18.2*</td>
</tr>
<tr>
<td>Arousals, no./h of sleep</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16.9 ± 1.2*</td>
<td>13.2 ± 0.9*</td>
<td>10.0 ± 0.9</td>
<td>11.2 ± 0.7</td>
<td>11.7 ± 0.8</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>5.9 ± 1.0</td>
<td>4.6 ± 0.5*</td>
<td>7.5 ± 0.8</td>
<td>7.4 ± 0.6</td>
<td>7.4 ± 0.6</td>
</tr>
</tbody>
</table>

Values are presented as means ± SEM (n = 16). FN1 refers to first fragmented night; FN2, second fragmented night; CN, mean of the 2 control nights; LN_F, loaded night after FN; LN_C, loaded night after CN; TST, total sleep time: non-rapid eye movement sleep stages 1, 2, 3, 4, and rapid eye movement (REM) sleep between sleep onset and lights on; WASO, wake after sleep onset; sleep efficiency, total sleep time as a percentage of bedtime; sleep latency, time from lights off to the first epoch of stage 2, 3, 4, or REM sleep; REM sleep latency, time from sleep onset to first epoch of REM sleep.

Table 2—Number of Load Applications and Induced Arousals per Sleep Stage

<table>
<thead>
<tr>
<th>Sleep stage</th>
<th>LN_F</th>
<th>N</th>
<th>Loading</th>
<th>Arousal</th>
<th>Nonarousal</th>
<th>LN_C</th>
<th>N</th>
<th>Loading</th>
<th>Arousal</th>
<th>Nonarousal</th>
</tr>
</thead>
<tbody>
<tr>
<td>REM</td>
<td>16.6 ± 1.5</td>
<td>11.4 ± 1.1</td>
<td>5.1 ± 0.9</td>
<td>15.2 ± 1.3</td>
<td>10.5 ± 1.0</td>
<td>4.7 ± 0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>42.2 ± 1.7</td>
<td>12.0 ± 1.3</td>
<td>30.2 ± 1.8*</td>
<td>38.9 ± 2.2</td>
<td>14.7 ± 1.8</td>
<td>24.2 ± 2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>19.3 ± 1.7*</td>
<td>2.9 ± 0.5</td>
<td>16.4 ± 1.7*</td>
<td>15.6 ± 0.8</td>
<td>2.4 ± 0.3</td>
<td>13.3 ± 0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are shown as means ± SEM (n=16). Number of load applications and induced arousals per sleep stage for both loaded nights. LN_F refers to loaded night after 2 fragmented nights; LN_C, loaded night after 2 control nights; REM, rapid eye movement.

*Significant difference between LN_F and LN_C (p < .05; 2-tailed paired Wilcoxon signed-rank test).

Sleep variables were altered by sleep fragmentation compared with CN (Table 1). Sleep efficiency was significantly decreased in LN_F (p < .01) but remained unchanged in FN2. The percentage of stage 1 was significantly higher in both fragmented nights (FN1: p < .0001, FN2: p < .05). Wake after sleep onset increased significantly in FN1 (p < .01) but remained unchanged in FN2. Percentage of stage 2 sleep increased significantly in FN1 (p < .01) but remained unchanged in FN2. The percentage of slow-wave sleep was significantly lower on both fragmented nights (FN1: p < .0001, FN2: p < .01). The percentage of REM sleep decreased significantly in FN1 (p < .01) but remained unchanged in FN2. Sleep latency remained unchanged in FN1 but was significantly lower in FN2 (p < .01). REM sleep latency increased significantly in FN1 (p < .01) but remained unchanged in FN2.

Number of Arousals

The total number of arousals and the number of spontaneous arousals per hour slept on both LN and CN are presented in Table 1. Inspiratory loading caused no change in either the total number of arousals or in the number of arousals per hour slept on both LN. The number of spontaneous arousals per hour slept on both...
LN was the same as on CN.

The number of arousals occurring within 2 minutes of the application of the inspiratory resistance as a percentage of the total number of load applications are shown in Figure 3. The comparison between stage 2, stage 3/4, and REM sleep showed a significant difference between these sleep stages in both loaded nights (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001). There was a significantly higher percentage of arousals in REM sleep compared with stage 2 sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001) and compared with stage 3/4 sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001). There was a significantly higher percentage of arousals in LN\textsubscript{c} compared with LN\textsubscript{F} (all-night data: 40% to 34%; p < .05). Looking at individual sleep stages, there was a significantly higher percentage of arousals only in stage 2 sleep (p < .05, 1-tailed paired Wilcoxon signed-rank test).

In order to check the results (obtained from the individual arousal frequencies in each sleep stage) for any bias caused by doing a disproportionate number of tests in each subject in each sleep stage, we also looked at the pooled data from all subjects. The analysis of the pooled data showed the same changes. There was a significantly higher percentage of arousals in REM sleep (LN\textsubscript{c}: 69%; LN\textsubscript{F}: 69%) compared with stage 2 sleep (LN\textsubscript{c}: 38%, p < .0001; LN\textsubscript{F}: 28%, p < .0001) and compared with stage 3/4 sleep (LN\textsubscript{c}: 15%, p < .0001; LN\textsubscript{F}: 15%, p < .0001). There was a significantly higher percentage of arousals in LN\textsubscript{c} compared with LN\textsubscript{F} (all-night data: 40% to 34%; p < .05). Looking at individual sleep stages, there was a significantly higher percentage of arousals only in stage 2 sleep (p < .0003).

During CN, there was a significantly higher percentage of arousals within control periods in REM sleep (28%) compared with stage 2 sleep (19%; p < .05) and compared with stage 3/4 sleep (14%; p < .001). During both loaded nights, the percentage of arousals in REM sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001) and stage 2 sleep (LN\textsubscript{c}: p < .0003; LN\textsubscript{F}: p < .01) was significantly higher than in CN, whereas, in stage 3/4 sleep, there was no difference between the loaded nights and CN.

**Figure 3**—Number of arousals within 2 minutes of load application as a percentage of the total number of load applications in the different sleep stages (rapid eye movement [REM], stage 2, stage 3/4) for both loaded nights (LN\textsubscript{c}, loaded night after 2 control nights; LN\textsubscript{F}, loaded night after 2 fragmented nights). Values are shown as means ± SEM (n=16).

† Significant difference between LN\textsubscript{c} and LN\textsubscript{F} (p < .05; 1-tailed paired Wilcoxon signed-rank test).

***Significant difference between sleep stages (p < .0001; paired Friedman test performed for each night).

During both loaded nights, the probability of arousal in stage 2 and REM sleep was highest within the first 30 seconds after the start of loading, whereas, in stage 3/4 sleep, the arousal frequency was equally distributed in 30-second time bins within 2 minutes of loading (Figure 4). During CN, the arousal frequency was equally distributed in 30-second time bins within control periods in the NREM sleep stages 2 and 3/4 and REM sleep (Figure 4). There was no difference in the “time to arousal” between the loaded nights. Figure 5 shows significant differences in the “time to arousal” between REM sleep, stage 2 sleep, and stage 3/4 sleep for both nights (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001). The “time to arousal” was significantly shorter in REM sleep compared with stage 2 sleep (LN\textsubscript{c}: p < .0003; LN\textsubscript{F}: p < .0001) and stage 3/4 sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001), respectively.

Considering only the “time to arousal” of the arousals within 2 minutes of the application of the inspiratory load (not ranked and “nonarousals” not included), the subjects aroused faster from REM sleep (LN\textsubscript{c}: 36 seconds; LN\textsubscript{F}: 43 seconds) than from stage 2 sleep (LN\textsubscript{c}: 45 seconds; LN\textsubscript{F}: 49 seconds) and from stage 3/4 sleep (LN\textsubscript{c}: 55 seconds; LN\textsubscript{F}: 54 seconds) in both loaded nights.

**First Third Versus Last Third of Load Applications**

Figure 6 presents the percentage of induced arousals on each loaded night in the first third and in the last third of load applications for stage 2, stage 3/4, and REM sleep. On LN\textsubscript{c}, from the first to the last third, percentage of arousals increased significantly in REM sleep (p < .01), while decreasing significantly in stage 2 sleep (p < .05). On LN\textsubscript{F}, comparing the first and the last third, stage 2 showed a decrease, as on LN\textsubscript{c}, but did not reach statistical significance (p = .06), while there was no significant change in REM sleep from the first to the last third. From the first to the last third, stage 3/4 sleep remained unchanged on both loaded nights. However, there was a significant difference between stage 2, stage 3/4, and REM sleep in the first (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001) and the last (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001) third on both loaded nights. There was no significant difference between the loaded nights for the first and the last third in each sleep stage.

**Figure 4**—Distribution of all induced arousals during LN (pooled data from both loaded nights) and CN (pooled data from both control nights). Shown is the percentage of induced arousals within 30-s time bins in the different sleep stages (rapid eye movement [REM], 2, and 3/4).

**Time to Arousal**

The analysis of the pooled data showed the same changes. There was a significantly higher percentage of arousals in REM sleep compared with stage 2 sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001) and compared with stage 3/4 sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001). There was a significantly higher percentage of arousals in LN\textsubscript{c} compared with LN\textsubscript{F} (all-night data: 40% to 34%; p < .05). Looking at individual sleep stages, there was a significantly higher percentage of arousals only in stage 2 sleep (p < .05, 1-tailed paired Wilcoxon signed-rank test).

In order to check the results (obtained from the individual arousal frequencies in each sleep stage) for any bias caused by doing a disproportionate number of tests in each subject in each sleep stage, we also looked at the pooled data from all subjects. The analysis of the pooled data showed the same changes. There was a significantly higher percentage of arousals in REM sleep (LN\textsubscript{c}: 69%; LN\textsubscript{F}: 69%) compared with stage 2 sleep (LN\textsubscript{c}: 38%, p < .0001; LN\textsubscript{F}: 28%, p < .0001) and compared with stage 3/4 sleep (LN\textsubscript{c}: 15%, p < .0001; LN\textsubscript{F}: 15%, p < .0001). There was a significantly higher percentage of arousals in LN\textsubscript{c} compared with LN\textsubscript{F} (all-night data: 40% to 34%; p < .05). Looking at individual sleep stages, there was a significantly higher percentage of arousals only in stage 2 sleep (p < .0003).

During CN, there was a significantly higher percentage of arousals within control periods in REM sleep (28%) compared with stage 2 sleep (19%; p < .05) and compared with stage 3/4 sleep (14%; p < .001). During both loaded nights, the percentage of arousals in REM sleep (LN\textsubscript{c}: p < .0001; LN\textsubscript{F}: p < .0001) and stage 2 sleep (LN\textsubscript{c}: p < .0003; LN\textsubscript{F}: p < .01) was significantly higher than in CN, whereas, in stage 3/4 sleep, there was no difference between the loaded nights and CN.

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DISCUSSION

The aim of this study was to investigate the arousal response (in the sleep electroencephalogram) to an added inspiratory resistance during NREM and REM sleep in healthy subjects after 2 nights of prior sleep fragmentation. In addition, we examined whether sleep fragmentation affects the sleep-time-dependent evolution of the arousal response within a sleep stage.

There were 4 main findings: In REM sleep the arousal response to inspiratory resistive loading after sleep fragmentation remained significantly better (i.e., the arousals occurred faster and more frequent) compared with NREM sleep. Looking at the all-night data, the arousal response to inspiratory resistive loading was significantly decreased after sleep fragmentation, compared with the arousal response after undisturbed sleep. This effect was due to the significantly decreased arousal response in stage 2. There was a significant sleep-time-dependent increase in the arousal response to inspiratory resistive loading in REM sleep after sleep fragmentation that was not present after undisturbed sleep. Sleep fragmentation had no effect at all on the arousal response to inspiratory resistive loading in stage 3/4 sleep.

(1) The higher percentage of load-induced arousals in REM sleep, compared with NREM sleep, after sleep fragmentation is in agreement with previous studies without prior sleep fragmentation.\textsuperscript{27,29} The more common and brisker arousal response to external inspiratory resistive loading during REM, as compared with NREM sleep, in the healthy subjects studied is different from the pattern in patients with OSAHS, in whom breathing disorders are worst during REM sleep. However, 2 points have to be taken into consideration. First, the duration of prior sleep fragmentation is an important determinant of its effect on breathing during sleep. Even 1 night of sleep fragmentation and partial sleep deprivation has been shown to affect breathing during sleep.\textsuperscript{13,14,30} However, even though we did find significant effects of inspiratory resistive loading on arousability, 2 nights of sleep fragmentation might not have been enough to cause the same effects on the arousability in REM sleep, as the long-lasting sleep fragmentation seen in OSAHS might do. Second, different stimuli may evoke different responses. The work of Williams et al indicated that the electroencephalographic stage of sleep is not an invariant indicator of the responsiveness of the organism but is also stimulus dependent.\textsuperscript{31} Frederickson and Rechtschaffen demonstrated that arousal responses of sleeping rats to trigeminal nerve stimulation were unaffected by sleep deprivation.\textsuperscript{32} They pointed out that the changes in the arousal response might not be nonspecific but, rather, dependent on the class of peripheral stimuli. However, we assume that external inspiratory resistive loading has effects similar to those of the spontaneously occurring increase in “internal” airway resistance during OSAHS, although we acknowledge that this is not proven.

(2) Looking at all-night data, the arousal response to inspiratory resistive loading was significantly decreased after sleep fragmentation, compared with the response after normal sleep; looking at individual sleep stages, the arousal response was significantly decreased after fragmentation only in stage 2 sleep. These findings are partly in agreement with the work of Phillips and Bowes. They found decreased arousal responses to hypercapnia and hypoxia during both NREM and REM sleep after sleep fragmentation in 5 dogs.\textsuperscript{33,34} Several points have to be considered. First, our results are due to the effects of the decreased arousal response in stage 2 sleep, as the arousal response in both stage 3/4 and REM sleep did not change. Second, although REM sleep did not show an impairment of arousability, there was an effect when looking at the time course as there was a significant sleep-time-dependent increase in the arousal response to inspiratory resistive loading in REM sleep after sleep fragmentation. Third, species differences in the arousal responses between humans and animals are known, as has been demonstrated earlier by showing a low arousal threshold in REM sleep in humans, as compared with the high arousal threshold in animals.\textsuperscript{27,35}

During the loaded night after 2 control nights (LN\textsubscript{C}), awake time more than doubled compared with CN, with a decrease in total sleep time, and stage 1 sleep increased. The redistribution

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**Figure 5**—“Time to arousal” after start of loading as an average (± SEM; n=16) of the weighted mean ranks per night and subject in the different sleep stages (rapid eye movement [REM], 2, and 3/4) for both loaded nights (LN\textsubscript{C}: loaded night after 2 control nights; LN\textsubscript{F}: loaded night after 2 fragmented nights).

***Significant difference between sleep stages (p < .0001; paired Friedman test performed for each night).

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**Figure 6**—Number of arousals within 2 minutes of load application as a percentage of the first and the last third of load applications in the different sleep stages (rapid eye movement [REM], 2, and 3/4) for both loaded nights (LN\textsubscript{C}: loaded night after 2 control nights; LN\textsubscript{F}: loaded night after 2 fragmented nights). Values are shown as means ± SEM (n=16).

*Significant difference between the first and the last third of load applications (p < .05; 2-tailed paired Wilcoxon signed-rank test).

But there was a significant difference in the time course during REM sleep when comparing the difference from the first to the last third between the loaded nights (p < .05).
of sleep stages during LN, is presumably the consequence of ongoing sleep disruption induced by repetitive inspiratory loading. However, the loaded night after sleep fragmentation (LN) did not show these signs of sleep disruption due to the increased sleep pressure after sleep fragmentation. In LN, only stage 1 sleep was increased, compared with CN. During both loaded nights, after sleep fragmentation and after normal sleep, the total number of arousals per hour slept (arousal index) was similar, as compared with CN. This seems to be in agreement with the results of Desai et al., who found no different arousal index after sleep deprivation in 13 subjects with mild OSAHS and 16 subjects without OSAHS.36

(3) The sleep-time–dependent increase in the arousal response to inspiratory resistive loading in REM sleep after sleep fragmentation is, to our knowledge, a new finding. The decrease in the arousal response to inspiratory resistive loading across the night in stage 2 sleep might have reflected the effects of some sleep loss due to inspiratory resistive loading during the first hours of sleep and/or adaptation to the stimulus. This finding is in agreement with the study of Montserrat et al.37 They found an increase in the level of inspiratory effort (measured by the tension time index of the diaphragm) associated with apnea lengthening across the night in stage 2 sleep in subjects with OSAHS, suggesting that there is a blunting of the arousal response across the night. However, the sleep-time–dependent increase in the arousal response during REM sleep is intriguing. The results obtained in LN, in REM sleep as well as in stage 2 sleep are also in agreement with the findings of Roehrs et al.,38 who found a decreasing arousal threshold to acoustic stimulation in REM sleep and an increasing arousal threshold in stage 2 sleep when comparing the first and the last 3 hours of a night. However, they contrast to the findings of Philip et al.,39 who found an increasing arousal threshold to acoustic stimulation when comparing the first and third parts of the night for stage 2 and REM sleep. This seeming contrast may be explained by different definitions of the arousal threshold (decibel level versus percentage of interventions producing arousal). This notion is further supported by the finding of Roehrs et al.,38 who found an increasing arousal threshold from the first to the last 3 hours when the arousal threshold was measured by the number of tones necessary to produce an arousal instead of percentage of interventions producing arousal.

(4) The arousal response to inspiratory resistive loading in stage 3/4 sleep neither was attenuated nor did it vary across the night after sleep fragmentation (the power was about 90% to detect a difference between LN and LN of 10%, which corresponds to a large effect size of 0.8, with a type I error of 5%). In addition, the arousal threshold is highest in stage 3/4 sleep, compared with stage 2 and REM sleep, independent of prior sleep fragmentation. This is in accordance with OSAHS, in which longstanding ongoing sleep fragmentation does not affect the usually stable breathing during stage 3/4 sleep.40 These findings go along with the hypothesis of Younes that the likelihood for self-perpetuating cycling is reduced if arousals are delayed.41 When, with decreasing arousal threshold, the arousal response is high, “arousals preempt orderly compensation and, by augmenting the overshoot, perpetuate cycling. As arousal threshold rises, arousal is delayed, permitting an orderly compensation by reflex mechanisms.”42

In pervious studies measuring esophageal pressure deflection preceding arousal, sleep fragmentation was thought to be a major cause for the impairment of the arousal response to naturally occurring airway occlusion in patients with OSAHS.40,41 However, from our results, it cannot be excluded that the impaired arousal response in OSAHS, especially during REM sleep, could, at least in part, be the consequence of a primary defect in the arousal system, which might be accentuated by long-lasting sleep fragmentation and/or sleep deprivation, as occurs in sleep apnea.

Even though the sleep fragmentation was mild, it was sufficient to affect arousal in stage 2 sleep and to decrease arousability in early, compared with late, REM sleep. This means that sleep fragmentation affects the arousal response to increasing resistance and that the effects are different in stage 2 and REM sleep. However, the biologic reason or plausibility for the increase in the arousal response in REM sleep across the night is not clear.

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