

Challenging Sleep Homeostasis in Narcolepsy-Cataplexy: Implications for Non-REM and REM Sleep Regulation

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Study objectives: We recently proposed insufficient non-rapid eye movement sleep (NREMS) intensity to contribute to disturbed nocturnal sleep in patients with narcolepsy-cataplexy (NC). To test this hypothesis, we investigated the effect of physiologically intensified NREMS in recovery sleep following sleep deprivation.

Design: Nocturnal baseline and recovery sleep architecture, and the sleep electroencephalogram (EEG) before and after 40 hours of sustained wakefulness were compared between 6 drug-free patients with NC (age range: 19-37 years) and 6 individually matched, healthy control subjects (18-43 years).

Measurements: Sleep and sleep EEG power spectra (C3A2 derivation). The dynamics of the homeostatic Process S were estimated from the time course of slow-wave activity (SWA, spectral power within 0.75-4.5 Hz) across consecutive NREMS episodes.

Settings: Sleep research laboratory.

Results: In baseline, SWA decreased across consecutive NREMS episodes in patients with NC and control subjects. The build-up of SWA, however, was attenuated in NC in the second episode ($P = 0.01$) due to a higher number of short wake periods ($P = 0.02$). Prolonged wakefulness increased initial SWA in both groups ($P = 0.003$) and normalized the baseline differences between patients and control subjects in the

time course of SWA in NREMS. The changed dynamics of SWA in the patients in recovery sleep when compared with baseline were associated with reduced numbers of intermittent wake periods in the first ($P = 0.01$) and second ($P = 0.04$) NREMS episodes. All patients, but no control subjects, showed a sleep-onset rapid eye movement period (SOREMP) in both baseline and recovery sleep. Sleep deprivation increased SOREMP duration ($P = 0.03$).

Conclusions: Increased SWA after sleep deprivation indicates that sleep homeostasis is functional in NC. Increased NREMS intensity in recovery sleep postpones sleep fragmentation, supporting our concept that sleep fragmentation is directly related to insufficient NREMS intensity in NC. The persistence of SOREMP despite enhanced NREMS pressure suggests an abnormal interaction between NREMS and REMS regulatory processes.

Keywords: sleep homeostasis; slow-wave activity; sleep fragmentation; sleep-onset REM sleep; excessive daytime sleepiness

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SINCE THE FIRST DESCRIPTION OF THE NARCOLEPTIC TETRADE (EXCESSIVE DAYTIME SLEEPINESS [EDS], CATAPLEXY, HYPNAGOGIC HALLUCINATIONS and sleep paralysis) by Yoss and Daly in 1957,¹ it has been increasingly recognized that sleep-maintenance insomnia is a major problem in many patients with narcolepsy-cataplexy (NC).^{2,3} Nocturnal sleep in NC is characterized by short sleep latency, the presence of sleep-onset rapid eye movement periods (SOREMPs) (in up to 50% of patients⁴), frequent brief awakenings, long periods of intermittent wakefulness, or a combination of these. Despite sleep fragmentation, patients with NC usually feel refreshed after awakening in the morning, but sleepiness will rapidly evolve within hours and becomes irresistible ("sleep attacks"). Although sleep attacks and voluntary daytime

sleep are typically short (< 30 min) and refreshing,⁵ sleepiness returns after a few hours of wakefulness. The mechanisms underlying sleep fragmentation and the highly refreshing character of even short naps in NC are largely unknown. Coexisting primary sleep disorders such as obstructive sleep apnea, periodic limb movements in sleep, and parasomnias may be present in NC, yet their contribution to disturbed nocturnal sleep and EDS appears to be relatively small.⁶ Thus, sleep fragmentation and EDS usually persist despite successful treatment of these comorbidities.

It has been hypothesized that patients with NC are unable to consolidate wakefulness and sleep because of abnormal sleep-wake regulation. The processes underlying wakefulness and sleep are conceptualized in the 2-process model of sleep-wake regulation.^{7,8} It is widely accepted that sleep homeostasis (Process S) and the endogenous circadian clock (Process C) interact to regulate the timing and stability of both wakefulness and sleep. Although circadian rhythms have been described to be functional in NC,^{9,10} homeostatic sleep regulation, as estimated from the time course of electroencephalogram (EEG) slow-wave activity (SWA; power within 0.75-4.5 Hz) over consecutive non-rapid eye movement sleep (NREMS) episodes may be altered in NC.^{10,11} More specifically, a faster dissipation of SWA from the first to the second NREMS episode has been consistently found in patients with NC, when compared with healthy control subjects.¹⁰⁻¹² We recently suggested that the steep de-

Disclosure Statement

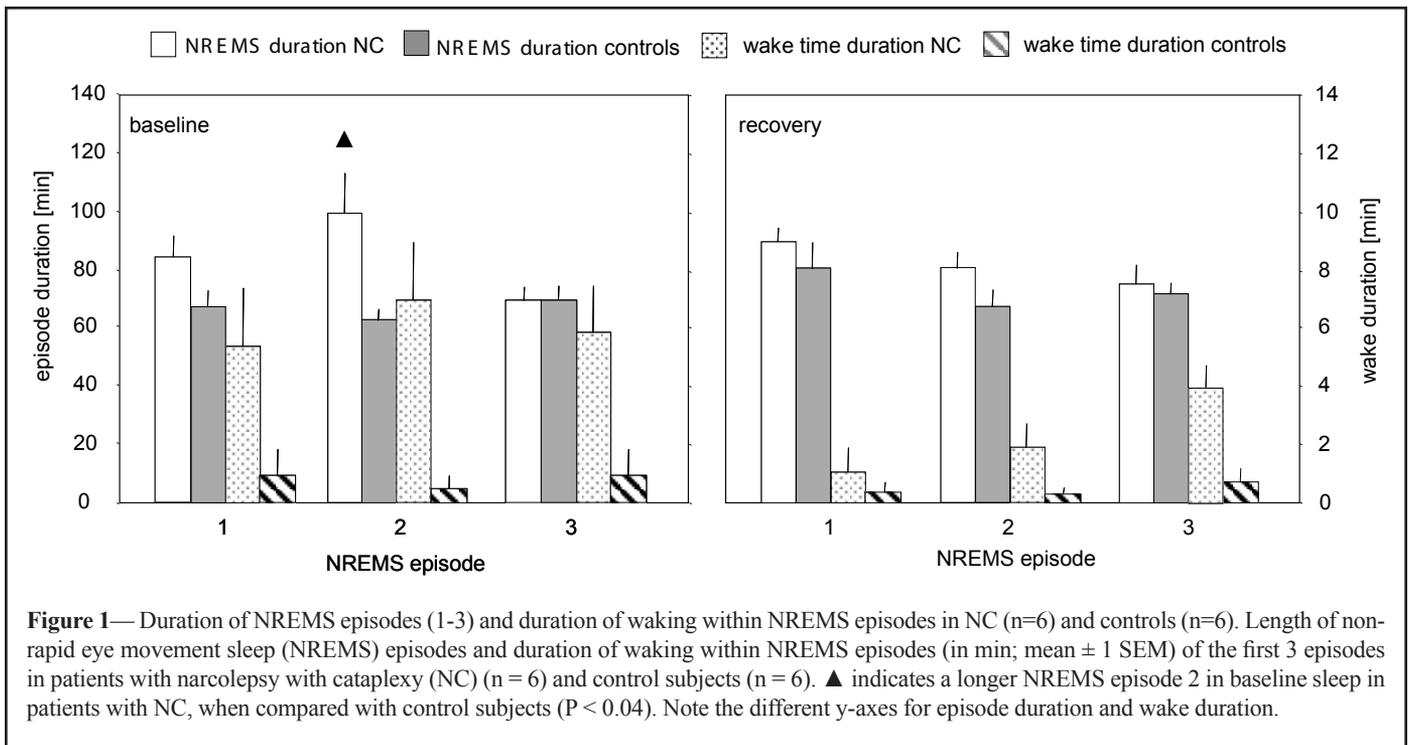
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cline of SWA from the first to the second NREMS episode is due to a disturbed build-up of SWA in the second cycle.¹² Sleep in the second cycle is interrupted by an increased number and a longer duration of short wake periods in NC. We proposed the changes in the time course of SWA to reflect threshold-dependent insufficient NREMS intensity. That is, nocturnal sleep fragmentation occurs after dissipation of sleep pressure in the first sleep cycle, i.e., when NREMS intensity has decayed below a certain threshold.

Sleep deprivation is a powerful tool to evaluate the integrity of homeostatic sleep regulation. So far, only 1 sleep-deprivation study consisting of 24 hours of prolonged wakefulness has been performed in NC.¹¹ In this study, sleep deprivation increased initial SWA in both NC and control subjects, compared with baseline values, and SWA dissipated in both groups exponentially with a similar time course in NREMS. Nevertheless, similar to baseline nights, SWA enhancement was most prominent in the first sleep cycle and attenuated in the second cycle in NC. This study is limited by the fact that baseline and recovery sleep were scheduled at different circadian times. Circadian factors may have contributed to sleep and sleep EEG changes in the recovery night. In a second study, Nobili and colleagues¹⁰ tested the effects of 16 hours of wakefulness (i.e., wakefulness during the daytime, starting in the morning and lasting until the evening of the same day), which was followed by a 32-hour continuous bedrest, with sleep permitted ad libitum. Patients with NC showed increased SWA and an exponential decline of SWA during sleep. In the night following prolonged bedrest, the exponential decline of SWA was no longer present and the time course of SWA showed a 4-hour periodicity. These findings suggest the presence of an intact homeostatic sleep regulation under conditions of high sleep pressure and ultradian periodicity in SWA, which is unmasked under low homeostatic sleep pressure. Although 16 hours of continuous wakefulness may have enhanced sleep pressure in NC, this duration of wakeful-

ness is not sufficient to increase sleep pressure in healthy control subjects.

With the aim to further investigate sleep homeostasis in NC, we employed a 40-hour sleep-deprivation protocol, which allowed us to compare baseline sleep and recovery sleep at the same circadian time. We expected that increased NREMS intensity after sleep deprivation would consolidate sleep in the recovery night and provide us with the opportunity to estimate Process S from the time course of undisturbed SWA. We hypothesized that sleep homeostasis in NC is functional. We predicted that patients with NC would show increased SWA at the beginning of recovery sleep, in comparison with baseline sleep, and an exponential decline of SWA over consecutive NREMS episodes in the recovery night. Furthermore, we expected to find a reduced number of SOREMP in patients with NC in recovery sleep, when compared with baseline.

METHODS

Subjects and Study Protocol

Seven patients with NC and 7 healthy control subjects, individually matched for sex and age, participated in a 40-hour extended-waking protocol. Because 1 patient could not be kept awake for the entire sleep-deprivation period and fell asleep after 23 hours of wakefulness, the analyses are based on the data of 6 patients (men: 3, women: 3; mean age: 29 years; range: 18-36 years) and their control subjects (mean age: 30 years; range: 18-43 years). The baseline data of the study participants were included in a recent publication.¹² Diagnosis of NC was made according to standard criteria (American Academy of Sleep Medicine, 2005) based upon clinical symptoms and sleep studies (polysomnography; multiple sleep latency test [MSLT]). The mean of the patients' Epworth Sleepiness score¹³ was 15.5 ± 3.1; the Ullanlinna Narcolepsy Scale,¹⁴ 22.3 ± 5.3;

Table 1—Sleep Variables Derived from Visual Scoring

	Narcolepsy-cataplexy (n = 6)				Controls (n = 6)				ANOVA		
	Baseline		Recovery		Baseline		Recovery		Group	Condition	Group x Condition
	mean	SD	mean	SD	mean	SD	mean	SD	P	P	P
TST	425.2	26.4	446.2	12.3	451.7	7.4	463.1	8.2	0.00	0.02	0.47
Sleep latency	3.8	2.3	1.7	0.9	8.1	4.8	4.5	1.8	< 0.01	0.01	0.88
WASO	39.5	26.3	22.3	12.4	11.2	5.6	4.7	5.7	0.00	0.07	0.40
Stage 1	88.9	29.3	57.7	12.7	38.9	9.4	24.5	8.9	0.00	< 0.01	0.25
Stage 2	180.6	13.8	167.9	30.6	229.4	43.8	214.0	31.6	0.00	0.29	0.92
SWS	71.9	34.0	127.1	17.7	91.2	26.2	136.1	19.5	0.18	< 0.01	0.62
REMS	83.8	27.0	93.6	13.1	92.6	14.1	88.5	25.3	0.86	0.72	0.44
sleep efficiency	88.7	5.5	93.0	2.5	94.1	1.6	96.5	1.7	0.00	0.02	0.47
MT	10.2	3.6	9.8	2.3	8.6	4.7	7.7	3.5	0.23	0.68	0.86
REM sleep latency	0.7	1.8	0.0	0.0	75.7	16.2	90.6	44.0		0.08 ^a	
SOREMP ⁺	16.5	10.1	25.6	7.7	0.0	0.0	0.0	0.0		< 0.03 ^b	

All values are in minutes, except sleep efficiency (total sleep time [TST] expressed as a percentage of time in bed). Controls refers to age- and sex-matched healthy control subjects; Baseline, baseline night; Recovery, recovery night; sleep latency, time from lights-off to the first occurrence of rapid eye movement (REM) sleep or stage 2; WASO, wakefulness after sleep onset; SWS, slow-wave sleep (non-REM sleep stages 3 & 4); MT, movement time; REMS, REM sleep. All patients with narcolepsy-cataplexy (NC) entered baseline and recovery sleep with a sleep-onset REM sleep period (SOREMP); SOREMP⁺ refers to the duration of the SOREMP sleep episode.

^aBaseline compared with recovery; Wilcoxon test (control subjects only).

^bBaseline compared with recovery; Wilcoxon test (patients with NC only).

the Swiss Narcolepsy scale,⁵ -48 ± 21.7 ; and the Stanford Catalepsy scale,¹⁵ 73.3 ± 25.1 . HLA-DQB1*0602 was positive in all 6 patients. Hypocretin measurements from cerebrospinal fluid were available in 2 patients, and hypocretin was not detectable in these patients.

Two patients never received medical treatment for narcolepsy. In the remaining 4 patients, the pharmacologic treatment was discontinued at least 5 half-life times of the respective substances before the patients entered the experiment.

Controls were recruited among the students of the University of Zürich and ETH Zürich and by advertisement in the local newspaper. Controls were paid for participation. Their eligibility for study participation and the preexperimental phase and verification of compliance with the prestudy instructions of all participants has been described previously.¹² All participants gave written informed consent to participate in the study after reviewing the protocol, which was approved by the local ethics committee. The experiment included an adaptation night prior to the baseline night, which was followed by an extended waking period (40 hours, i.e., participants stayed awake for 2 days and skipped 1 night of sleep) until bedtime of the recovery night. Participants were allowed to sleep as long as they could during the recovery night but had to stay in the sleep laboratory until at least 12:00 noon.

During the waking period, patients and control subjects were under continuous supervision. In particular, each patient underwent 1-to-1 supervision by members of the research team and was constantly engaged in social interactions (e.g., playing games and taking outside walks) to prevent unintended sleep.

Polysomnographic Recordings

Polysomnographic recordings included EEG (data from C3A2 derivation are reported here), electrooculogram, and

electromyogram (EMG) using a polygraphic amplifier (PSA24, Braintronics, Inc., Almere, The Netherlands). Pre-experimental instructions and recording procedures, and the definition of NREMS-rapid eye movement sleep (REMS) cycles have been described previously.¹² The EEG frequency bins and bands are indicated by the encompassing frequency range (i.e., the 1.0-Hz bin denotes 0.875-1.125 Hz). In 1 narcoleptic patient, the first REMS episode following a SOREMP, and, in 1 control, the first REMS episode was skipped. A skipped REMS episode was defined according to previously proposed criteria.^{16,17} Thus, a REMS episode was scored as skipped when a clearly descending SWA slope of the first NREMS episode was followed by a new SWA surge rather than a REMS episode. In both cases, an epoch of movement time was used as the criterion for completion of the first cycle.

Statistics and Data Analysis

The visually scored sleep variables, the durations of NREMS and REMS episodes, and the EEG power spectra in NREMS (stages 2, 3, and 4) and REMS were analyzed. Significant differences in sleep stages were calculated by repeated-measures analysis of variance (rANOVA), with the between-factor “group” (patients vs control subjects) and the within-factor “condition” (baseline vs recovery night). Nonparametric tests were used for analyzing SOREMP duration and REMS latency. The length of NREMS episodes, SWA, number of short wake episodes, and power values in consecutive NREMS episodes were analyzed by 3-way rANOVAs with the between-subject factor “group” (patients vs control subjects) and the within-subject factors “condition” (baseline vs recovery) and “NREMS episode” (1-3). To approximate a normal distribution, absolute power densities and ratios of power densities were log-transformed prior to statistical tests. For factors with more than 2

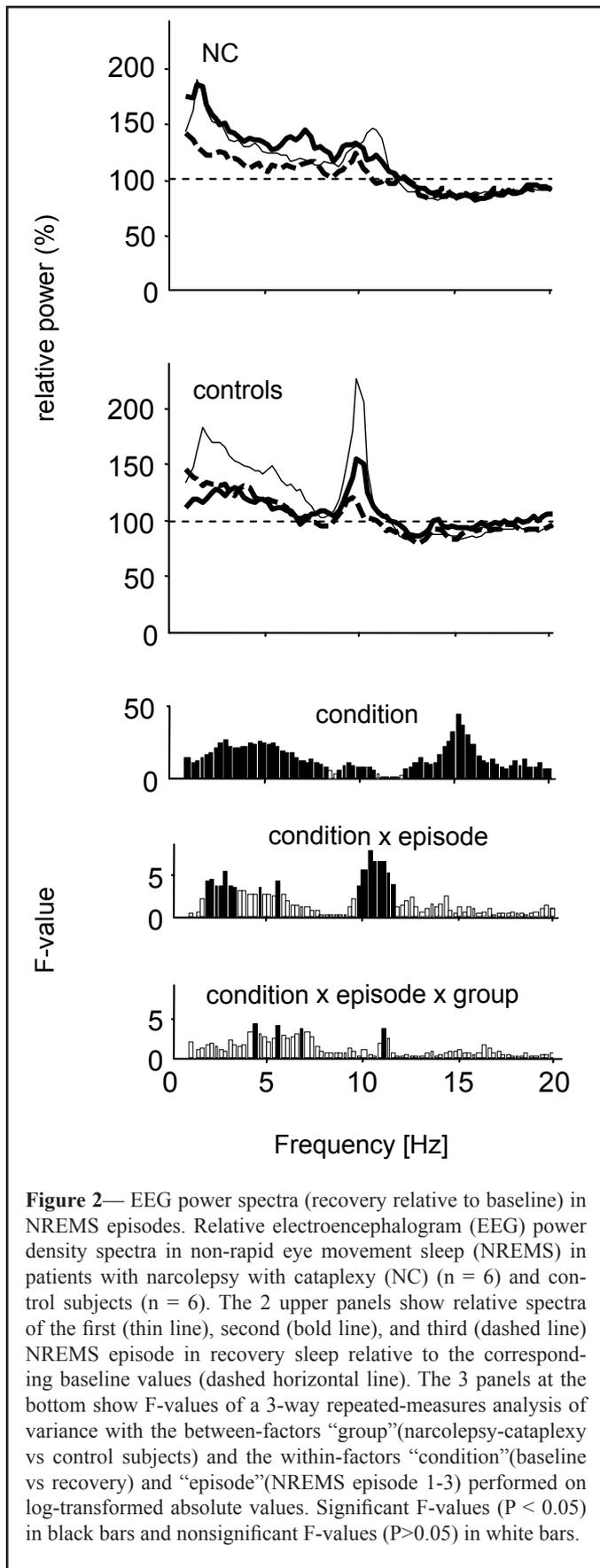


Figure 2— EEG power spectra (recovery relative to baseline) in NREMS episodes. Relative electroencephalogram (EEG) power density spectra in non-rapid eye movement sleep (NREMS) in patients with narcolepsy with cataplexy (NC) ($n = 6$) and control subjects ($n = 6$). The 2 upper panels show relative spectra of the first (thin line), second (bold line), and third (dashed line) NREMS episode in recovery sleep relative to the corresponding baseline values (dashed horizontal line). The 3 panels at the bottom show F-values of a 3-way repeated-measures analysis of variance with the between-factors “group” (narcolepsy-cataplexy vs control subjects) and the within-factors “condition” (baseline vs recovery) and “episode” (NREMS episode 1-3) performed on log-transformed absolute values. Significant F-values ($P < 0.05$) in black bars and nonsignificant F-values ($P > 0.05$) in white bars.

within the 2 groups, unpaired or paired, 2-tailed t -tests were performed. To estimate the possible associations between the length of NREMS episodes and the duration of short awakenings occurring within NREMS episodes, Pearson product-moment correlation coefficients (r) were calculated. The significance level was set at $\alpha < 0.05$. Only significant values are reported, unless otherwise mentioned. For statistical analyses, SPSS Version 15.0 and SAS Version 8.02 (SAS Institute Inc., Cary, NC) were used.

RESULTS

Sleep Variables and NREMS Episode Length

The analyses of sleep variables of baseline and recovery nights were restricted to the first 480 minutes of time in bed. The results are summarized in Table 1. During the baseline night, the expected typical sleep abnormalities were found in patients with NC, when compared with control subjects. The patients had lower sleep efficiency, decreased sleep latency, and higher amounts of NREMS stage 1, wake time after sleep onset, and movement time. Noteworthy, all patients with NC, but no control subjects, initiated sleep with a SOREMP in both baseline and recovery nights. There were no group differences in the amount of REMS or slow-wave sleep. Sleep deprivation shortened sleep latency; increased sleep efficiency, total sleep time, and slow-wave sleep; and reduced NREMS stage 1. The amount of REMS remained unaffected. There was no significant “group” \times “condition” interaction in any sleep variable, indicating that patients with NC and control subjects responded similarly to sleep deprivation. Remarkably, the duration of the SOREMP was significantly longer in the recovery night, when compared with the baseline night ($P < 0.03$; Wilcoxon matched-pair signed-rank test).

The length of the NREMS episodes and the duration of brief intervening awakenings occurring within NREMS episodes are illustrated in Figure 1. The second NREMS episode during the baseline night was longer in patients with NC than in control subjects (rANOVA for baseline nights: “cycle” \times “group” interaction: $F_{2,20} = 5.15$, $P = 0.02$, NREMS episode 2: 102.7 ± 14.4 min [mean \pm SEM] vs 63.4 ± 4.7 min, unpaired t -test: $P = 0.04$). In contrast, the length of NREMS episodes 1 and 3 did not differ between the groups (NREMS episode 1: 84.2 ± 8.7 min vs 68 ± 7.2 min, unpaired t -test: ns; NREMS episode 3: 71.0 ± 6.7 min vs 71.5 ± 4.5 min, unpaired t -test: ns). A positive correlation between the length of NREMS episodes 1 to 3 and the duration of intermittent wakefulness occurring in these episodes was observed in patients with NC ($r = 0.49$, $P = 0.04$; Pearson product moment correlation), yet not in control subjects ($r = 0.14$, $P = \text{ns}$). After sleep deprivation, NREMS episode lengths were similar in both groups (no significant “cycle” or “group” main effects or “cycle” \times “group” interaction). Moreover, the duration of brief intervening awakenings was reduced in both groups and, in NC, was no longer related to the NREMS episode length (NC: $r = 0.1$, ns; control subjects: $r = 0.13$, ns).

Effect of sleep deprivation on EEG power spectra in NREMS

Prolonged wakefulness induced distinct changes in EEG power spectra (0.75-20 Hz) in NREMS (stages 2, 3, and 4) and

levels and in which data sphericity was violated, Huynh-Feldt correction of degrees of freedom was applied, but the original degrees of freedom are reported. For comparisons between and

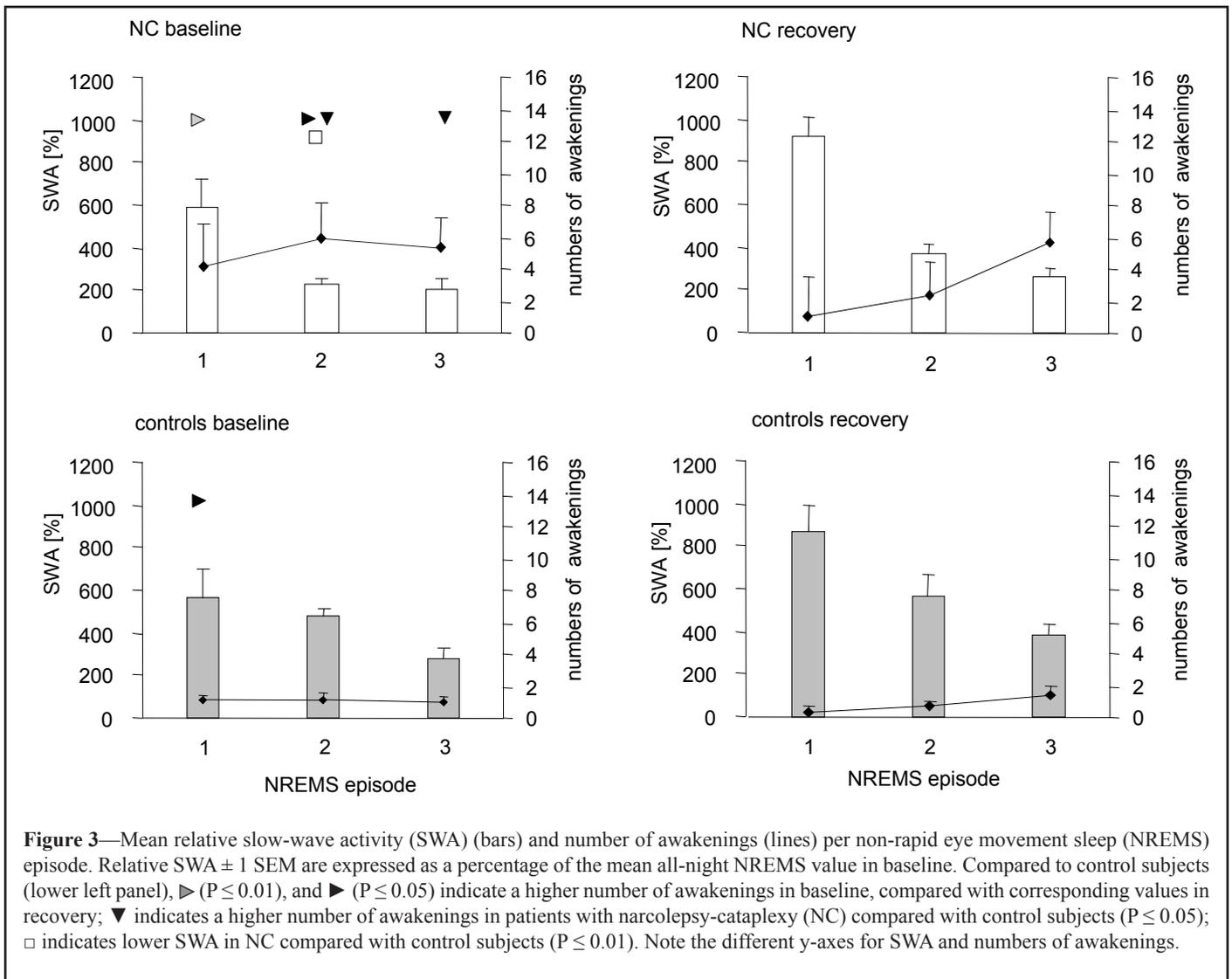


Figure 3—Mean relative slow-wave activity (SWA) (bars) and number of awakenings (lines) per non-rapid eye movement sleep (NREMS) episode. Relative SWA \pm 1 SEM are expressed as a percentage of the mean all-night NREMS value in baseline. Compared to control subjects (lower left panel), \blacktriangleright ($P \leq 0.01$), and \blacktriangle ($P \leq 0.05$) indicate a higher number of awakenings in baseline, compared with corresponding values in recovery; \blacktriangledown indicates a higher number of awakenings in patients with narcolepsy-cataplexy (NC) compared with control subjects ($P \leq 0.05$); \square indicates lower SWA in NC compared with control subjects ($P \leq 0.01$). Note the different y-axes for SWA and numbers of awakenings.

in REMS. In NREMS, power increased in delta, theta, and alpha frequencies (0.75-8.25 Hz, 8.5-11 Hz) and decreased in sigma and beta frequencies (14-17.5 Hz and 18 Hz) in both groups during the recovery night. No differences between NC and control subjects and no “condition” \times “group” interactions were observed in any frequency bin between 0.75 and 20 Hz, indicating that sleep deprivation affected the all-night EEG similarly in NC and control subjects. In REMS, power density increased in the delta and theta frequency band (1-7 Hz) and decreased in the 13.75- to 14-Hz and 14.5- to 15-Hz bands. A significant main effect of “group” (2-2.75 Hz) and a significant “group” \times “condition” interaction (0.75-1 Hz, 2-2.25 Hz, and 3.25 Hz) were present in single frequency bins within the delta range.

The effect of sleep deprivation on EEG power spectra in consecutive NREMS episodes is illustrated in Figure 2. Compared to baseline, prolonged wakefulness affected power in nearly the entire frequency range (except in 8.25- to 8.50-Hz and 10.75- to 12.25-Hz bands). The waking-induced increase in delta (1.5-3 Hz) and alpha (9.5-11.25 Hz) ranges was most prominent in the first NREMS episode (“episode” \times “condition” interaction: minimum $F_{2,20} = 3.55$, $P \leq 0.05$ and minimum $F_{2,20} = 3.46$, $P \leq 0.05$, respectively). Except for some single bins within the delta, theta, and alpha ranges (Figure 2), the dynamics of sleep

deprivation-induced changes in the NREMS power spectrum were similar in NC and control subjects.

Wake episodes per NREMS episodes and dynamics of SWA

The number of brief intervening awakenings per NREMS episode, defined as at least one 20-second epoch of wakefulness after sleep onset, differed between patients with NC and control subjects (Figure 3; lines; “group”: $F_{1,10} = 7.5$, $P = 0.02$). During baseline, the difference was significant in the second and third, yet not in the first, NREMS episode (“group” \times “NREMS episode” interaction: $F_{2,20} = 9.7$, $P = 0.001$) (episode 2: NC 6.0 ± 5.2 vs 1.2 ± 1 , $P = 0.02$, t-test; episode 3: 5.3 ± 4.7 vs 1.0 ± 0.9 , $P < 0.05$ t-test). Sleep deprivation reduced the number of awakenings, yet did so differently in consecutive NREMS episode (“condition” \times “NREMS episode”: $F_{2,20} = 3.66$, $P = 0.04$). In NC, the reduction in comparison with baseline was significant in the first (1.0 ± 1.6 vs 4.2 ± 4.2 , $P = 0.01$, paired t-test) and second NREMS episode (2.3 ± 1.2 vs 6 ± 4.3 , $P = 0.04$) but not in the third NREMS episode (5.7 ± 3.3 vs 5.3 ± 4.7). In the control subjects, the number of wake episodes was reduced only in the first NREMS episode (0.3 ± 0.8 vs 1.2 ± 0.8 , unpaired t-test $P = 0.04$). Note that, after sleep deprivation, the number of

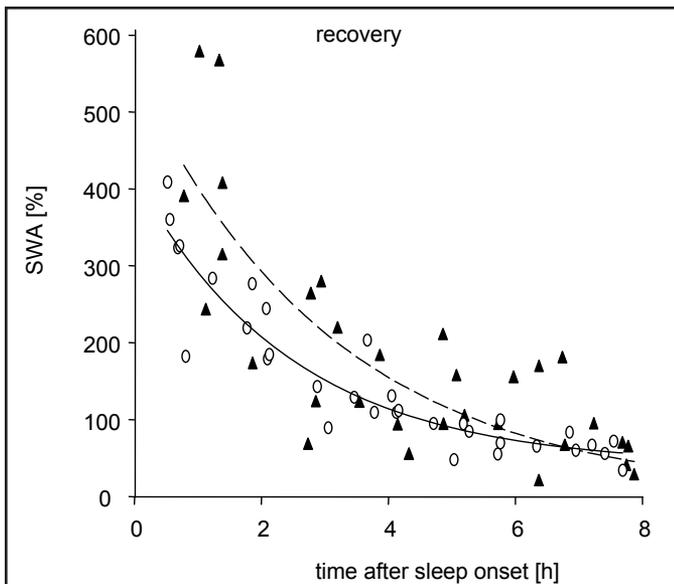


Figure 4—Time courses of relative slow-wave activity (SWA) across non-rapid eye movement sleep (NREMS) episodes in the recovery nights. Individual SWA values per NREMS episode (expressed as percentage of all-night SWA) were plotted at episode midpoint relative to sleep onset (either NREMS stage2 or rapid eye movement sleep) and pooled for patients with narcolepsy-cataplexy (NC) ($n = 6$, filled triangles) and control subjects ($n = 6$, open circles). All available NREMS episodes were pooled. The lines represent exponential functions for patients with NC (dotted line) and healthy control subjects (solid line) fitted to the data: $SWA(t) = SWA_0 \times e^{-t/\tau} + SWA_\infty$, where t is time, τ the time constant, $(SWA_0 + SWA_\infty)$ the initial value and SWA_∞ the asymptote.

awakenings remained higher in patients with NC than in control subjects (“group” \times “condition”: $F_{1,10} = 0.71$, ns; baseline NC: 5.2 ± 4.3 vs control subjects: 1.1 ± 0.8 , t-test: $P < 0.01$; recovery NC: 3 ± 2.9 vs control subjects: 0.8 ± 1.1 , t-test: $P < 0.01$). The “group” \times “condition” \times “NREMS episode” interaction did not yield significance, indicating that the differences in the number of awakenings between NC and control subjects across NREMS episodes was not altered by sleep deprivation.

Relative SWA per NREMS episode expressed as a percentage of the mean all-night value in NREMS is shown in Figure 3 (bars). In baseline and recovery nights, SWA was highest in both groups in the first NREMS episode and declined across consecutive NREMS episodes. Sleep deprivation increased SWA in both groups (main effect for “condition”: $F_{2,20} = 14.78$, $P = 0.003$; “group” \times “condition” interaction: $F_{1,10} = 1.24$, ns). Significant interactions, however, indicated that SWA evolved differently in baseline and recovery nights (“NREMS episode” \times “condition”: $F_{2,20} = 8.91$, $P = 0.002$), and between the 2 groups (“NREMS episode \times group” interaction: $F_{2,20} = 6.31$, $P = 0.01$). The effects of sleep deprivation on the time course of SWA, however, were similar in both groups (no significant “group” \times “condition” \times “NREMS episode” interaction). Posthoc analyses further revealed that the different time course of SWA was due to lower relative SWA in NC than in control subjects in the second NREMS episode during baseline ($224.9\% \pm 71\%$ [mean \pm SD] vs $482.6\% \pm 172.3\%$, $P = 0.01$, unpaired t-tests). In contrast, relative SWA in the first and third NREMS episodes in

baseline (episode 1 NC: $585.8\% \pm 324.6\%$ vs control subjects: $564.5\% \pm 200.1\%$; $P = 0.9$; episode 3 NC: $206.9\% \pm 111\%$ vs control subjects: $282.6\% \pm 133.1\%$; $P = 0.3$), as well as in NREMS episodes 1 to 3 in recovery did not differ between the groups (episode 1 NC: $918.9\% \pm 218.3\%$ vs control subjects: $874\% \pm 295.7\%$; $P = 0.8$; episode 2 NC: $371.2\% \pm 100.4\%$ vs control subjects: $568.3\% \pm 239.1\%$; $P = 0.1$, episode 3 NC: $263.6\% \pm 100.9\%$ vs control subjects: $386.5\% \pm 121.4\%$; $P = 0.1$). As compared with baseline conditions, sleep deprivation induced an increase in SWA in the first and the second NREMS episodes in patients with NC (NREMS episode 1: $585.8\% \pm 324.7\%$ vs $918.9\% \pm 218.3\%$, $P = 0.04$; NREMS episode 2: $224.8\% \pm 71\%$ vs $371.2\% \pm 100.4\%$, $P = 0.02$) but not in the third episode ($206.9\% \pm 111\%$ vs $263.7\% \pm 100.9\%$, $P = 0.33$; paired t-test).

Exponential functions fitted to all available individual SWA data during the recovery nights (i.e., not restricted to the first 3 NREMS episodes) are shown in Figure 4. The decline of SWA was similar in both groups, as indicated by the overlapping 95% confidence intervals (95%CI) of the time constants (NC vs patients: 103.3 ± 37.4 min, 95%CI [59.9 - 373.9 min] and control subjects: 147.4 ± 32.4 min, 95%CI [101.9 - 265.9 min]).

The evolution of SWA in the first 30 minutes of the first 2 NREMS episodes

To examine whether sleep deprivation affected the build-up rate of SWA within the first 30 minutes of NREMS episodes, the rise rate of SWA in the first and second NREMS episodes was calculated from the median slopes of adjacent 2-minute epochs (Figure 5). The rise rate of SWA was steeper in the first, compared with the second, NREMS episode during baseline and recovery nights (“NREMS episode”: $F_{1,10} = 23.56$, $P < 0.001$). The “NREMS episode” \times “group” interaction yielded significance ($F_{1,10} = 10.54$, $P = 0.01$), indicating a different build-up of SWA across NREMS episodes between the 2 groups. Posthoc analyses with t-tests revealed that the difference was due to an attenuated build-up of SWA in NC in the second NREMS episode during baseline (10.8 ± 3 [mean \pm SEM] $\mu V^2/\text{min}$ vs 33.8 ± 8.8 $\mu V^2/\text{min}$; $P = 0.03$, unpaired t-test). Prolonged wakefulness normalized the build-up of SWA in the second NREMS episode in NC. More specifically, after sleep deprivation, the rise rate in this episode became similar in both groups (22.2 ± 4.4 $\mu V^2/\text{min}$ vs 43 ± 9.4 $\mu V^2/\text{min}$, $P = 0.1$ unpaired t-test; “NREMS episode” \times “group” \times “condition” interaction: $F_{1,10} = 0.0$, $P = 1.0$). No difference between the 2 groups in the build-up of SWA was found in the first NREMS episode in either baseline or in recovery nights (Figure 5).

DISCUSSION

The present study examined the effects of 40 hours of extended wakefulness on sleep and the sleep EEG in patients with NC. The major findings are that sleep deprivation in patients with NC (1) increased SWA, similarly to that of healthy control subjects; (2) postponed sleep fragmentation during the recovery night; and (3) prolonged the duration of SOREMP. These findings suggest that homeostatic NREMS regulation is normal, yet insufficient NREMS intensity contributes to fragmented sleep

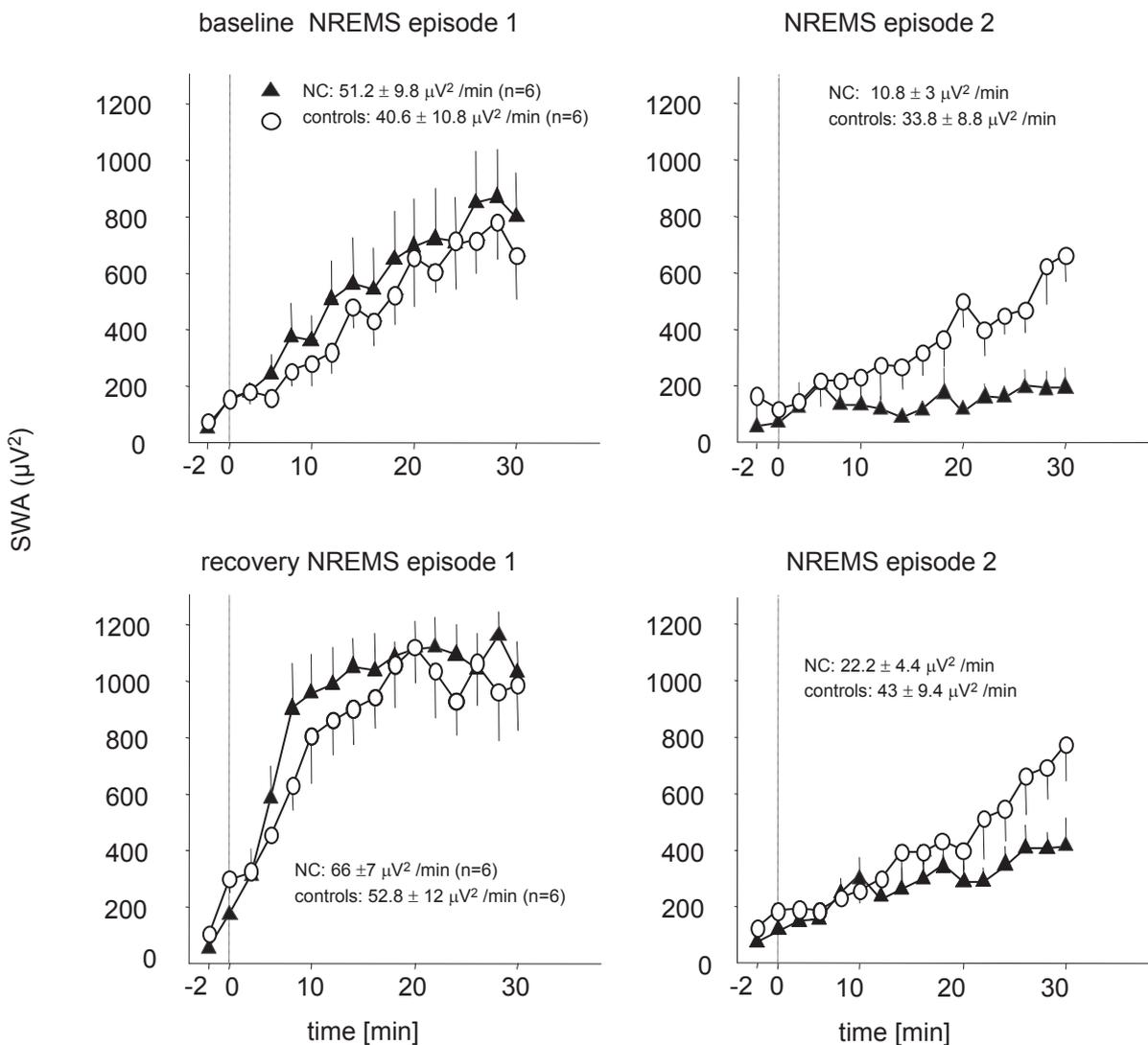


Figure 5—Build-up of slow-wave activity (SWA) in the first 30 minutes of the first 2 non-rapid eye movement sleep (NREMS) episodes for patients with narcolepsy-cataplexy (NC) (n = 6, filled triangles) and control subjects (n = 6, open circles). The rise rate of SWA was calculated as the median slope of adjacent 2-minute epochs over 30 min. Absolute values (mean \pm 1 SEM) are plotted for consecutive 2-min epochs for the time interval of -2 min to +30 min in each NREMS episode. The dashed vertical line indicates the beginning of the NREMS episode

in patients with NC. Moreover, we conclude that an impaired interaction between NREMS and REMS underlies the occurrence of frequent SOREMP in NC.

Homeostatic NREMS Regulation in NC

The fast decline from the first to the second NREMS episode in the baseline condition is in line with the results of previous studies in patients with NC^{10,11,18-20} and appears to represent a polysomnographic characteristic of the disease. We found, in a previous analysis of baseline sleep episodes with more subjects, that the strong decline of SWA from the first to the second NREMS episode is related to impaired build-up of SWA in the second cycle due to an increased number and longer duration of short intervening wakefulness.¹² These data prompted us to propose 2 hypotheses. First, the time course of SWA in NC is affected by sleep fragmentation and, therefore, may not permit the accurate estimation of the dynamics of Process S in baseline conditions. Second, sleep fragmenta-

tion occurs when NREMS intensity decays below a certain threshold level. In other words, the inability to consolidate sleep would be associated with threshold-dependent insufficient NREMS intensity. The present study adds essential new data to both hypotheses.

Altered Dynamics of Process S?

With respect to the question whether different dynamics of Process S underlie the altered time course of SWA in NC,¹¹ we expected that more consolidated sleep after sleep deprivation would provide us with the opportunity to estimate Process S from undisturbed SWA values. In both groups, we found a rebound, as well as an exponential dissipation of SWA, in recovery sleep after sleep deprivation. Because the 2 groups consist of only 6 individuals and statistical power is limited, we are cautious in interpreting these results as firm evidence for an absence of differences in sleep homeostasis between patients and control subjects. Nevertheless, the strong decline

of SWA from the first to the second NREMS episode was no longer present in NC when sleep was more consolidated in the recovery night. Therefore, attenuated SWA in the second NREMS episode during baseline appears to reflect increased sleep fragmentation¹² rather than a different dynamics of Process S.

Sleep Fragmentation Related to Insufficient NREMS Intensity?

The intent of sleep deprivation was to enhance NREMS intensity and to consolidate nocturnal sleep in NC. We predicted that, if nocturnal sleep fragmentation is related to reduced NREMS intensity, the occurrence of intervening wake episodes (which disturbed the build-up of SWA within NREMS episodes with the beginning of the second NREM-REMS cycle in baseline¹²) will be prevented or at least delayed by the increase of NREMS intensity due to sleep deprivation. Indeed, total sleep deprivation successfully increased sleep pressure, reduced nocturnal sleep fragmentation, and shortened NREMS-REMS cycles in NC. Sleep was more consolidated in the recovery night, with markedly reduced number wake episodes and duration of wakefulness, especially in the first and the second NREMS episodes. In contrast, in the third NREMS episode of the recovery night, fragmentation increased and was comparable with that of baseline sleep. These data fit the assumption that increased NREMS intensity postpones the occurrence of sleep fragmentation. Taken together, the effects of sleep deprivation on sleep consolidation support our hypothesis of a threshold-dependent insufficient NREMS intensity to explain the inability to maintain nocturnal sleep in NC.

REMS Regulation in NC

It is remarkable that recovery sleep started with a SOREMP in all patients with NC, even after substantially NREMS pressure was increased by the preceding 40 hours of wakefulness. The persistence of SOREMP after sleep deprivation is consistent with the results of 2 earlier studies, in which SOREMP occurred in patients with NC after 16 and 24 hours of prolonged wakefulness.^{10,11}

Circadian and homeostatic factors, as well as the NREMS-REMS interaction, are known to influence the timing and duration of REMS.^{7,21-22} Because recovery sleep started in the morning at a time when endogenous REMS propensity is high,²²⁻²⁴ the SOREMP following 24 hours of sleep deprivation in a previous study may be attributed to strong circadian REMS pressure.¹¹ In our study, baseline and recovery sleep started at the same circadian time of low endogenous REMS propensity. We therefore consider it unlikely that an abnormal circadian signal underlies the occurrence of SOREMP after sleep deprivation in NC.

Alternatively, an excessively strong homeostatic drive for REMS may account for the occurrence of SOREMP in NC. Two findings argue against enhanced homeostatic REMS pressure as the main source for SOREMP. First, all-night REMS duration during the recovery night was not increased, compared with corresponding baseline values. This finding is consistent with the results of studies in healthy subjects using selective REMS²⁴⁻²⁵ or total-sleep-deprivation protocols.²⁶ These studies

show that REMS rebound is absent or mild when sleep is not deprived for more than 1 night. Second, homeostatic REMS regulation was found to be normal in NC in a study using selective REMS deprivation during 2 consecutive nights followed by undisturbed recovery sleep.²⁷ Although the number of interventions to prevent REMS was higher in NC compared to control subjects in both REMS-deprivation nights, the increase of interventions from the first to the second deprivation night was similar in both groups. Neither group had a REMS rebound during the recovery night. Therefore, we conclude that the need to compensate for the loss of REMS following sleep deprivation is not increased in patients with NC, when compared with healthy subjects.

A plausible explanation for SOREMP in NC is an impaired NREMS-REMS interaction upon sleep onset. High NREMS pressure is known to inhibit REMS,^{26,28-30} suggesting priority of slow-wave sleep over REMS in recovering from sleep deprivation.^{7,31} Conversely, reduced NREMS pressure at sleep onset could disinhibit REMS and account for SOREMP.^{7,22,28} In our patients with NC, NREMS pressure after sleep deprivation was comparable to that of control subjects, rendering it unlikely that SOREMP are caused by a low NREMS pressure at the beginning of the sleep episode. It is of particular interest that SOREMP were even longer after 40 hours of wakefulness, compared with the baseline condition. The longer SOREMP duration is consistent with the results of a MSLT study in patients with NC³² and may result from abnormal NREMS and REMS interaction. Tafti et al³² showed that the occurrence of SOREMP during daytime naps on Multiple Sleep Latency Tests was related to sleep latency, i.e., the more rapidly patients with NC fell asleep, the more SOREMP occurred. Interestingly the number of SOREMP during MSLT was independent of prior nocturnal sleep or waking. This finding is consistent with our results that the occurrence of SOREMP is independent of homeostatic NREMS regulation. Thus, sleep onset and the interplay of NREMS and REMS mechanisms appear to be relevant for SOREMP in NC. Further research is needed to characterize these mechanisms at a more basic level.

Summary and Conclusions

We present the first study employing a 40-hour, prolonged, waking protocol in patients with NC, to challenge homeostatic sleep regulation without the confound of circadian influences. We found that NREMS homeostasis is functional in NC, as evidenced by increased SWA after sleep deprivation and an exponential decline of SWA during baseline and recovery sleep. The increased sleep intensity after prolonged wakefulness postponed the occurrence of sleep fragmentation, as compared with baseline. Following the assumption that SWA reflects NREMS intensity, our findings support the hypothesis that sleep fragmentation in NC is associated with insufficient NREMS intensity. The mechanisms underlying the inappropriate wake intrusions into sleep, however, remain unclear. Similarly, the reasons why all patients with NC start sleep with a SOREMP and why the duration of these episodes was even longer in recovery sleep, when compared with baseline sleep, need further investigation.

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