Cyclic alternating pattern in narcolepsy patients and healthy controls after partial and total sleep deprivation

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Highlights
- Cyclic alternating pattern (CAP) rates are lower in narcolepsy patients in comparison to healthy controls.
- CAP rates decrease in both narcolepsy patients and healthy controls during morning recovery sleep after partial and total sleep deprivation.
- Our findings suggest that NREM sleep homeostasis although altered, is functional in narcolepsy patients.

Abstract
Objective: To investigate the regulation NREM sleep at baseline and in morning recovery sleep after partial and total sleep deprivation (SD) in narcolepsy–cataplexy (NC) using cyclic alternating pattern (CAP).
Methods: Daytime sleep under either increased (no sleep in the previous night) or decreased sleep pressure (allowing 4 h of sleep, 23:00–3:00 h) was recorded in ten drug-free, HLA-positive, hypocretin deficient NC patients and ten age, gender and body mass index matched healthy controls. Baseline sleep was also recorded and used for comparison purposes. CAP parameters were scored and analyzed for each subject.
Results: Narcolepsy patients had significantly lower CAP rate, CAP index, CAP time, number of CAP cycles, A1 index and number of A1 cycles in comparison to healthy controls at baseline as well as after partial and total SD. In both narcolepsy patients and healthy control subjects there was a significant decrease in these parameters after partial and total SD but the changes followed a similar pattern.
Conclusion: The persistence of baseline differences in CAP parameters between narcolepsy patients and healthy controls and their similar behavior after partial and total SD suggests similar homeostatic NREM sleep regulation but on a different level.
Significance: CAP analysis demonstrates that NREM sleep homeostasis although altered, is functional in narcolepsy patients.

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1. Introduction

Narcolepsy–cataplexy (NC) is a disabling life-long sleep-wake disorder, characterized by excessive daytime sleepiness (EDS) and sudden loss of muscle tone triggered by emotions (cataplexy) (Bassetti and Aldrich, 1996). Additional symptoms include sleep paralysis, hypnagogic hallucinations and fragmented nighttime sleep. Cataplexy is specific for narcolepsy and its presence allows the clinical diagnosis of NC. Sleep studies in NC, in particular the occurrence of two or more sleep onset REM periods (SOREMPs) in the multiple sleep latency test (MSLT) is characteristic though not specific for NC (American Academy of Sleep Medicine (AASM), 2005). Two biological markers are tightly associated with cataplexy: there is a strong association to the HLA system (Lin et al., 2001) and a loss of the hypothalamic neuropeptide hypocretin (also called orexin) in the cerebrospinal fluid (CSF) (Baumann and Bassetti, 2005; Mignot et al., 2002). The genetic marker HLA DQB1*0602 is found in 98% of narcolepsy patients with cataplexy compared to 35% of healthy controls (Lin et al., 2001). Hypocretin is involved in various basic functions such as sleep-wake regula-
tion, feeding, reward and drug-seeking behavior. In NC the loss of CSF hypocretin is tightly associated with REM sleep abnormalities such as sleep onset REM periods and dissociated REM phenomena as well as with NREM sleep abnormalities (Mochizuki et al., 2004).

Cyclic alternating pattern (CAP) is a way to describe the arousability state during NREM sleep. Different subtypes have been defined: subtypes A1 – slow frequency high-voltage EEG patterns (sequences of K-complexes and delta-bursts), subtypes A2 – rapid low-amplitude EEG patterns preceded by or mixed with slow high-voltage waves and subtypes A3 – phases with fast low-voltage EEG patterns (Terzano et al., 2002). The A1 subtypes dominate during the build up of deep NREM sleep and are indicative of neural mechanisms for NREM sleep maintenance while subtypes A2 and A3 are the expression of NREM sleep disruption (Parrino et al., 2001; Terzano et al., 2005). A2 and A3 subtypes are more prevalent before the onset of and during REM sleep, suggesting an association with REM-on mechanisms, active during the last part of a NREM sleep episode and during REM sleep (Terzano et al., 2005).

In narcolepsy patients reduced CAP time, CAP rate, number of CAP cycles and phase A subtypes (especially subtypes CAP A1) were observed during baseline night time sleep in comparison to healthy controls (Terzano et al., 2006; Ferri et al., 2005b).

Total CAP time, CAP rate in slow wave sleep and all CAP rates in healthy subjects were lower in night-time recovery sleep (De Gennaro et al., 2002; Parrino et al., 1993) and higher in morning recovery sleep after sleep deprivation (Parrino et al., 1993). Parrino et al. studied morning and night-time recovery sleep in two different sets of subject.

The aim of the present study was to investigate the regulation of NREM-sleep at baseline sleep and in morning recovery sleep after partial and total sleep deprivation in patients with NC in comparison to age and gender matched healthy controls. For the purpose we planned to analyze CAP parameters, including CAP rate, CAP index, CAP time, number of CAP cycles, A1, A2 and A3 index as well as number of A1, A2 and A3 cycles. Considering previous publication and the proposed functions of CAP and its subtypes we expected a decrease in CAP rates after partial and especially total sleep deprivation. Regarding A1 subtypes a possible increase and regarding A2 and A3 subtypes a possible decrease was expected. As previously reported CAP parameters were expected to be lower in narcolepsy patients than in healthy controls. Although as we planned to record morning recovery sleep circadian effects could influence the data and lead to an increase of different CAP parameters.

2. Patients and methods

Ten patients with NC (three men, seven women; aged 29.6 ± 2.5 years; body mass index (BMI), 29.6 ± 2.7; disease duration, 12.1 ± 2.7 years; mean ± SEM) and 10 healthy control subjects matched for age (30.1 ± 2.5), gender, and BMI (28.4 ± 2.3) participated in the study. All patients had a history of classic narcolepsy with cataplexy and findings typical for narcolepsy on polysomnography (SOREMPs in 10/10 patients), MSLT (> 2 SOREMPs in 8/8), and both polysomnography and MSLT. All patients were HLA DQB1*0602 positive and 7 of 7 who were tested were hypocretin deficient. CSF hypocretin-1 levels were determined in a single radioimmunoassay, as has been previously described (Baumann et al., 2004). The patients had high scores on the Epworth Sleepiness Scale (mean ± SEM, 15.2 ± 1.5). A score of at least 14 is suggestive of narcolepsy (Sturzenegger and Bassetti, 2004). The Ullanlinna Narcolepsy Scale score was 22.5 ± 2.7. A score of at least 14 is suggestive of narcolepsy (Hublin et al., 1994). The Swiss Narcolepsy Scale score was 46.2 ± 10.3; a score less than 0 is suggestive of narcolepsy (Sturzenegger and Bassetti, 2004). The Stanford Cataplexy Scale (Anic-Labat et al., 1999) score was 66.1% ± 8.7%. The mean values ± SEM, including age, BMI, and various scores of the patients and control subjects are presented in Table 1.

Six patients were treatment naive or drug free for years; the other four patients stopped taking narcolepsy-specific medication 14 days prior to the sleep studies. Concomitant medications prior to the study included modafinil (n = 3, in 2 patients as a monotherapy), fluoxetine (n = 1, in combination with modafinil), and ephedrine (n = 1).

The study was approved by the local ethics committee, and written informed consent was obtained from all subjects. All participants were asked to maintain regular sleep patterns and to avoid day-time sleep prior to each sleep recording, which was controlled with sleep diary and actigraphy. No adaptation night was included in the study. Baseline night-time sleep (23:00-07:00 h), which served also as a screening night, and daytime sleep under either increased (no sleep in the previous night) or decreased sleep pressure (allowing 4 h of sleep from 23:00-03:00 h in the previous night) was recorded in all participants on non-consecutive occasions. Partial and total sleep deprivation was performed in the sleep lab on two separate nights at least 7 days apart in order to allow full recovery from the previous sleep deprivation. The participants were randomly assigned to begin with either partial or total sleep deprivation. For sleep deprivation all participants arrived in the lab at 19:00 h and were kept awake either until 07:00 h the next morning (total sleep deprivation, increased sleep pressure) or were allowed 4 h of sleep (23:00-03:00 h, partial sleep deprivation, decreased sleep pressure). Morning recovery sleep was recorded from 07:00 h until latest 15:00 h. During baseline night video-PSG consisted of eight channel EEG (F3/A2, F4/A1, C3/A2, C4/A1, P3/A2, P4/A1, O1/A2, O2/A1), left and right electrooculography (EOG), submental electromyography (EMG), electrocardiography (ECG), respiratory flow (using a nasal cannula) and effort (using Medcare XactTrace respiratory inductive plethysmography (RIP) technology belts), pulse-oximetry and left and right anterior tibialis EMG. During morning recovery sleep after total and partial sleep deprivation in the previous night sleep scoring was based on EEG, EOG, EMG and ECG. All recordings were done on Medcare Somnologica Studio. Sleep stages, periodic limb movements and arousals during baseline night were scored manually according to international criteria (AASM, 2005; Rechtschaffen and Kales, 1968; American Sleep Disorders Association (ASDA), 1992). Apleas/hypopneas were scored according to the recommendations of the American Academy of Sleep Medicine (AASM, 1999) with the difference that a desaturation of 4% instead of 3% was needed when the amplitude decrease did not reach 50%. Sleep onset was defined as the first epoch of either NREM 2 or REM sleep. CAP parameters were scored and analyzed for each subject according to international rules (Terzano et al., 2002).

Significant differences in CAP parameters were estimated by repeated measures analysis of variance (rANOVA) (general linear model), with one between-subject factor “group” (patients and controls) and one within-subject factor “sleep episode” (baseline, night, day, recovery).
after partial and after total night-time sleep deprivation). A Greenhouse-Geisser correction of degrees of freedom was applied when data sphericity was violated but the original degrees of freedom are reported. Additionally, if appropriate, post hoc t-tests were performed. Bonferroni correction was applied to pair wise comparisons across the set of baseline, partial and total sleep deprivation. For statistical analysis the SPSS Version 15.0 was used.

### 3. Results

The macrostructural measures of sleep in NC patients and controls are presented in Table 2. The analysis of the visual sleep scoring is a part of another study (E. Werth, in preparation). As our narcolepsy subjects were overweight and we matched the control subjects for body mass index, it is important to note that sleep apnea was excluded in all subjects during the baseline night.

#### 3.1. Patients and controls

According to repeated measures ANOVA NC patients had significantly lower CAP rate, CAP index, CAP time, number of CAP cycles, A1 index and number of A1 cycles in comparison to healthy controls (significant main effect for “group”, see Table 3 and Fig. 1). A2 and A3 subtypes were not significantly different between patients and controls.

#### 3.2. Evolution of CAP parameters

CAP parameters decreased significantly after partial and total sleep deprivation (significant main effect for “sleep episode” in the repeated measures ANOVA analysis for CAP rate, CAP time, CAP index, CAP cycles, A1 cycles, A1 index, A2 cycles, A3 cycles and A3; A2 index was in the trend range and not significantly different). Most of these changes followed a similar pattern after partial and total SD in both groups as there was no significant interaction between “group” and “sleep episode”, except for A2 subtypes (A2 cycles and A2 index). In order to clarify which of the 3 conditions (baseline, partial or total SD) was significantly different, post hoc t-tests were applied. As there was no interaction between the factors “group” and “sleep episodes” patients and controls were collapsed together. Bonferroni correction was applied to the pair wise comparisons and significance was set at \( p = 0.016 \). CAP rate, CAP time, CAP index, CAP cycles, A1 index and A3 cycles were significantly lower after both partial and total SD in comparison to baseline but did not differ significantly from each other, except for CAP time, which was lower after partial in comparison to total SD. A1 cycles were significantly lower after partial SD in comparison to baseline and to total SD. Regarding baseline and total SD the difference was not significant. A3 index was significantly lower after total SD in comparison to baseline but not after partial SD. Total and partial SD did not differ significantly.

Although A2 subtypes did not differ between patients and controls (no main effect for “group”) they changed after SD (significant main effect for “sleep episode”, for A2 index only in the trend range) and the changes were group dependent (significant interaction between “group” and “sleep episode”). The graphic representation of the results (Fig. 1) shows a flat curve in NC patients, whereas in controls subjects there is a slight decrease after partial and an increase after total SD. Post hoc t-tests showed no significant differences within the patients' group, whereas in the control group only the decrease after partial SD in comparison to baseline was significant (\( p = 0.016 \)).

### 4. Discussion

Our study showed lower CAP rate, CAP index, CAP time, number of CAP cycles, A1 index and number of A1 cycles in NC patients in comparison to healthy controls, which is consistent with two previous reports in narcolepsy patients (Terzano et al., 2006; Ferri et al., 2005b). There was a reduction in these parameters during morning recovery sleep after partial and total night-time SD (except for A1 cycles, which were significantly lower only after partial SD) and the changes followed similar pattern in patients and in controls. A3 subtypes did not differ between patients and controls initially but they decreased significantly after total SD, after partial SD the decrease was significant only for A3 cycles but not A3 index. A2 index and cycles did not differ between the two groups but followed different pattern of changes after SD.

### 4.1. Differences between patients and controls

Reduced arousability has been suggested as a possible explanation for the lower CAP rate, CAP index, CAP time and number of CAP cycles in narcolepsy patients (Terzano et al., 2006). Reduced strength of the arousal system has been attributed to hypocretin deficiency in NC (Mignot, 2001; Sakurai, 2005). The reduction of A1 subtypes in narcolepsy translates to an altered development of the sleep profile (Terzano et al., 2006) as A1 subtypes are similar to the very slow wave sleep oscillations (Ulbert et al., 2004; Vanhatalo et al., 2004), and promote synchrony among neural groups. The drive towards deep NREM sleep is paced by phase A1 subtypes which are able to increase progressively the EEG slow wave activity (Ferrillo et al., 1997; Ferri et al., 2005a). A lower central arousal level in narcolepsy has been hypothesized based on a significant decrease in beta activity in

### Table 2

Sleep parameters in the NC patients and the controls, mean ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients, baseline nighttime sleep</th>
<th>Controls, baseline nighttime sleep</th>
<th>Patients, 4 h nighttime sleep within partial SD</th>
<th>Controls, 4 h nighttime sleep within partial SD</th>
<th>Patients, daytime sleep after partial SD</th>
<th>Controls, daytime sleep after partial SD</th>
<th>Patients, daytime sleep after total SD</th>
<th>Controls, daytime sleep after total SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTA (min)</td>
<td>454 ± 7.3</td>
<td>472 ± 3.8</td>
<td>239.5 ± 0.4</td>
<td>240.3 ± 0.7</td>
<td>403.7 ± 17.5</td>
<td>371.7 ± 24.2</td>
<td>449.4 ± 12.8</td>
<td>466.7 ± 10.3</td>
</tr>
<tr>
<td>TST (min)</td>
<td>392.4 ± 8.3</td>
<td>406.4 ± 11.7</td>
<td>210.4 ± 4.5</td>
<td>213.7 ± 6.6</td>
<td>343.3 ± 16.4</td>
<td>303.4 ± 30</td>
<td>407.3 ± 14.6</td>
<td>428 ± 17.7</td>
</tr>
<tr>
<td>SL (min)</td>
<td>5.6 ± 0.8</td>
<td>28.8 ± 7.2</td>
<td>7.7 ± 1.8</td>
<td>14.7 ± 1.9</td>
<td>3.3 ± 0.8</td>
<td>8.2 ± 1.2</td>
<td>2.8 ± 0.5</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>REM-SL (min)</td>
<td>11.3 ± 11.1</td>
<td>99.8 ± 11.4</td>
<td>30.2 ± 10.1</td>
<td>77.7 ± 9.1</td>
<td>8.6 ± 8</td>
<td>29 ± 6.7</td>
<td>35 ± 3.5</td>
<td>54 ± 7.3</td>
</tr>
<tr>
<td>SE (%)</td>
<td>87.2 ± 1.8</td>
<td>91 ± 1.6</td>
<td>90.8 ± 1.8</td>
<td>94.7 ± 2.5</td>
<td>85.4 ± 2.1</td>
<td>81.1 ± 3.2</td>
<td>90.7 ± 1.7</td>
<td>91.7 ± 2.3</td>
</tr>
<tr>
<td>NREM1 (%)</td>
<td>17 ± 1.7</td>
<td>7.4 ± 0.9</td>
<td>15.4 ± 2.1</td>
<td>5.5 ± 1.1</td>
<td>21.6 ± 2.2</td>
<td>6.7 ± 0.7</td>
<td>17 ± 2.5</td>
<td>5.3 ± 0.7</td>
</tr>
<tr>
<td>NREM2 (%)</td>
<td>40.8 ± 2.1</td>
<td>50.1 ± 1.6</td>
<td>46.3 ± 2.8</td>
<td>50.1 ± 2.4</td>
<td>30.6 ± 1.6</td>
<td>40.9 ± 2.7</td>
<td>34 ± 3</td>
<td>45 ± 1.8</td>
</tr>
<tr>
<td>SWS (%)</td>
<td>12.9 ± 1.7</td>
<td>15.9 ± 1.8</td>
<td>16 ± 2.6</td>
<td>27.8 ± 2.8</td>
<td>11.3 ± 1.4</td>
<td>9.4 ± 1.3</td>
<td>18.7 ± 1.9</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>REM (%)</td>
<td>16.5 ± 1.9</td>
<td>17.6 ± 1.6</td>
<td>13.2 ± 1.2</td>
<td>11.2 ± 2.4</td>
<td>22 ± 1.3</td>
<td>24 ± 1.7</td>
<td>19.7 ± 1.2</td>
<td>18.5 ± 1.2</td>
</tr>
<tr>
<td>AH/h</td>
<td>1.3 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>PLMSI/h</td>
<td>4.2 ± 3.6</td>
<td>1.3 ± 1.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

TTA, total time analyzed; TST, total sleep time; SL, sleep latency; REM-SL, REM sleep latency; SE, sleep efficiency; SWS, slow wave sleep; AH/h, apneahypopnea index; PLMSI, periodic limb movements in sleep index; SD, sleep deprivation.
comparison to healthy controls (Mukai et al., 2003). Insufficient NREM sleep intensity with rapid decline of SWA and increased sleep fragmentation during the second sleep cycle in NC has been recently suggested (Khatami et al., 2007). Forty hours of sleep deprivation consolidated sleep and postponed sleep fragmentation in NC patients (Khatami et al., 2008). As A1 subtypes are linked to REM-off mechanisms, the significantly lower A1 subtypes in NC can also be associated with insufficient NREM sleep intensity in NC. On the contrary, A2 and A3 subtypes, which are influenced by REM-on mechanisms, were unchanged, which suggests that REM-on mechanisms are operational in NC.

4.2. Partial and total SD

A decrease in CAP rate, CAP index, CAP time, number of CAP cycles and A3 subtypes was observed after partial and total SD. These findings are in line with previous reports, showing a decrease in CAP parameters after SD during night-time recovery sleep (De Gennaro et al., 2002). It is important to note, that a decrease in CAP rate was also reported in a second consecutive night-time recording in normal subjects after an adaptation night and the finding was interpreted as a “first-night” effect (Moser et al., 2010). Such an effect is unlikely in our study, as sleep recordings
were not performed on consecutive nights/days. However De Gennero et al. included an adaptation night and still found a significant decrease in CAP parameters after SD (De Gennero et al., 2002). We could not confirm the findings of an earlier work, which showed an increase in CAP parameters in morning recovery sleep (Parrino et al., 1993). However the authors of this study performed morning and night-time recovery sleep in two different sets of subjects, which could have contributed to the inconsistent results.

In our experiment the changes in the two groups followed a similar pattern, which suggests that sleep homeostasis in narcolepsy is functional, still on a different level compared to controls. We also found a decrease in A1 subtypes after partial and total SD in both narcolepsy patients and controls instead of the predicted decrease. As A1 subtypes are considered to promote deep NREM sleep, probably after SD less effort is needed to consolidate and reach slow wave sleep in both groups. To the other hand circadian effects might have influenced these findings. The higher sleep consolidation after SD is also supported by the decrease in A3 subtypes, which correspond to the ASDA arousals classification (De Gennaro et al., 2001, 2002; ASDA, 1992).

A2 subtypes did not differ between narcolepsy patients and controls. However they changed after partial and total SD and the changes were group dependent. The graphic representation of the results showed a flat curve in narcolepsy patients, whereas in control subjects there was a slight decrease after partial and an increase after total SD, post hoc t-tests revealed that only the decrease after partial SD was significant. As A2 subtypes are influenced by REM-on mechanisms, this can be explained with the shortening of REM sleep latency in control subjects after partial sleep deprivation. The participants were allowed to sleep only during the first half of the night, when usually deep NREM sleep occurs. This leads to a decrease in NREM sleep pressure and an increase in REM sleep pressure. After the occurrence of REM sleep in the beginning of the sleep episode, less REM-on dependent A2 subtypes were generated. In narcolepsy patients REM sleep latency was short, independent of SD. These findings support the suggested alteration of REM/NREM interaction in narcolepsy patients (Khatami et al., 2007; Khatami et al., 2008).

As our protocol aimed mainly at manipulating REM-sleep pressure, sleep deprivation was performed at night and sleep was recorded in the morning, which resulted in a circadian shift. This is certainly a limitation of the study as we could not rule out circadian influences. In spite of the high sleep pressure the maximum wake propensity in the morning might have influenced our results, especially regarding A1 subtypes, where a decrease, instead of the predicted increase was observed. Possible circadian influences should be examined by further studies.

In conclusion, the persistence of baseline differences between CAP parameters and their similar behavior after partial and total sleep deprivation suggests similar homeostatic NREM regulation in narcolepsy patients and healthy controls but on a different level.

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