Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome

Marti, I; Valko, P O; Khatami, R; Bassetti, C L; Baumann, C R
Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome

Abstract

BACKGROUND: Short mean latencies to the first epoch of non-rapid eye movement sleep stage 1 (NREM1) and the presence of 2 sleep onset REM (SOREM) periods on multiple sleep latency test (MSLT) occur in both narcolepsy-cataplexy (NC) and behaviourally induced insufficient sleep syndrome (BIISS). It is not known whether specific MSLT findings help differentiate the two disorders. METHODS: We analyzed MSLT data including sleep latencies to and between different sleep stages of 60 age-, gender- and body mass index (BMI)-matched subjects (hypocretin-deficient NC, actigraphy-confirmed BISS, healthy controls: each 20). RESULTS: Mean latency (in minutes) to NREM1 sleep was significantly shorter in NC (1.8+/−1.5) than in BISS (4.7+/−2.1, p<0.001) and controls (11.4+/−3.3, p<0.001). Mean latency to NREM2 sleep was similar in NC (8.6+/−4.7) and BISS (8.1+/−2.7, p=0.64); latency to either NREM2 or rapid eye movement (REM) sleep (i.e., the sum of the sleep latency to NREM1 and the duration of the first NREM1 sleep sequence), however, was shorter in NC (4.4+/−2.9) than in BISS (7.9+/−3.5, p<0.001). Referring to all naps with SOREM periods, the sequence NREM1-REM-NREM2 was more common (71%) in NC than in BISS (15%, p<0.001), reflecting the shorter latency from NREM1 to NREM2 in BISS (3.7+/−2.5) than in NC (6.1+/−5.9, p<0.001). CONCLUSIONS: Our findings show that both sleepiness (as measured by NREM1 sleep latency) and REM sleep propensity are higher in NC than in BISS. Furthermore, our finding of frequent REM sleep prior to NREM2 sleep in NC is in line with the recent assumption of an insufficient NREM sleep intensity in NC. Together with detailed clinical interviews, sleep logs, actigraphy, and nocturnal polysomnography, mean sleep latencies to NREM1 2.5min, the presence of multiple SOREM periods, and the sequence NREM1-REM-NREM2 may be the best MSLT measures to discriminate NC from BISS.
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Article Type: Original Article

Keywords: narcolepsy-cataplexy;
behaviourally induced insufficient sleep syndrome;
multiple sleep latency test;
sleep stage sequencing;
sleep onset REM period

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Prof. C. Guilleminault  
Field editor  
Sleep Medicine  

Zurich, November 29th, 2008

re: Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome (Ms. No. SLEEP-D-08-00299)

Dear Prof. Guilleminault

We would like to resubmit our revised manuscript entitled „Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome.“ for consideration for publication as an Original Paper in ‘Sleep Medicine’.

We are thankful for your recommendations concerning the occurrence of SOREMPs in patients with sleep disordered-breathing and in healthy controls, and for the valuable comments of the three reviewers. We have completely revised the manuscript (particularly the “Discussion”) according to these comments.

Thank you in advance for considering this paper for publication.

Sincerely yours
Philipp O. Valko, MD

------------------------------------------------
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------------------------------------------------
Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome

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Abstract

Background: Short mean latencies to the first epoch of non-rapid eye movement sleep stage 1 (NREM1), and the presence of ≥2 sleep-onset REM (SOREM) periods on multiple sleep latency test (MSLT) occur in both narcolepsy-cataplexy (NC) and behaviourally induced insufficient sleep syndrome (BIISS). It is not known whether specific MSLT findings help differentiating the two disorders.

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Conclusions: Our findings show that both sleepiness (as measured by NREM1 sleep latency) and REM sleep propensity are higher in NC than in BIISS. Furthermore, our finding of frequent REM sleep prior to NREM2 sleep in NC is in line with the recent assumption of an insufficient NREM sleep intensity in NC. Mean sleep latencies to NREM1 ≤2.5 minutes, the presence of multiple SOREM periods, and the sequence NREM1-REM-NREM2 may be the best measures to discriminate NC from BIISS.
Introduction

Excessive daytime sleepiness (EDS) represents the leading symptom of narcolepsy, along with cataplexy, sleep paralysis, and hypnagogic hallucinations. The pathophysiological hallmark of narcolepsy with cataplexy (NC) is the loss of hypothalamic neurons that produce wake-promoting neuropeptides, the hypocretins (orexins). Besides the assessment of a detailed history, the multiple sleep latency test (MSLT) is the most important ancillary test for the diagnosis of NC, together with the measurement of cerebrospinal fluid hypocretin levels. The typical MSLT findings for narcolepsy include (1) a mean sleep latency of ≤ eight minutes and (2) the presence of ≥ 2 sleep onset rapid eye movement (SOREM) periods as an indicator of high REM sleep propensity [1-3]. However, these findings are not specific for narcolepsy, since other sleep-wake disorders with EDS such as sleep apnea syndrome or behaviourally induced insufficient sleep syndrome (BIISS; also referred to as chronic sleep deprivation) may present with similar MSLT results [2, 4].

There is considerable evidence of a continuous reduction in average sleep time by 20% over the past century [5]. Due to professional or social obligations, people often are not able or do not allow themselves to sleep enough. Accordingly, BIISS is a frequent and important differential diagnosis of NC in clinical practice: BIISS patients are often referred to sleep laboratories with the suspicion of narcolepsy. Besides EDS, BIISS may be associated with decreased neurocognitive functioning, fatigue, increased risk of motor vehicle crashes, negative mood states, and decreased motivation [6]. In addition, cataplexy-like symptoms, sleep paralysis, and hallucinations can be reported by patients with non-narcoleptic EDS [7]. EDS in BIISS patients is often as severe as in narcolepsy which can be confirmed by objective EDS measures such as low mean sleep latencies on MSLT (own observation). Polysomnography typically shows short sleep latency and high sleep efficiency [8]. It has been shown, furthermore, that NREM sleep is better preserved than REM sleep during sleep.
MSLT characteristics of NC and BIISS

restriction, possibly leading to high REM sleep propensity [9, 10]. Indeed, SOREM periods are also observed in BIISS patients [2, 4].

To better distinguish MSLT findings in NC and in BIISS, we aimed at characterizing the sleep latencies to and between different sleep stages in age-, gender- and BMI-matched patients with definite NC, BIISS, and normal controls.

Patients and methods

Subjects. Among patients referred to the Sleep Disorder Centre of the Neurology Department of the University Hospital of Zurich, we retrospectively selected 3 groups (NC, BIISS, and controls), each consisting of 20 age-, gender- and BMI-matched patients. EDS was present in all patients and was estimated by the Epworth sleepiness scale (ESS). In all NC patients, the diagnosis was confirmed by deficient cerebrospinal fluid hypocretin levels. In order to minimize concomitant BIISS, NC patients were instructed to pay regard to an optimal sleep hygiene including sufficient sleep amount, regular sleep-wake habits and repeated short daytime naps. Actigraphic recordings in NC patients confirmed the absence of chronic sleep restriction (i.e. reduced sleep time per 24 hours). The diagnosis of BIISS was made according to the international classification of sleep disorders, including improvement of subjective EDS after sleep prolongation as documented by sleep logs and actigraphy [3]. Patients with additional sleep-wake disorders (e.g. sleep apnea syndrome) were excluded. Control subjects did not report EDS (normal ESS scores), nor did they suffer from any kind of sleep-wake disorder. Patients and controls were free from drugs affecting the central nervous system, except for two narcoleptic patients (modafinil and sodium valproate, respectively), one BIISS patient (citalopram), and two controls (phenytoin/gabapentin and citalopram, respectively).

Sleep studies. All patients underwent videopolysomnographic recording in the night prior to the MSLT. MSLT was done according to standard guidelines [11]. Briefly, patients
underwent four or five naps, each lasting 20 minutes, at two hours interval over the day. If sleep occurred, tests were run for another 15 minutes to assess SOREM periods. The MSLT montage included central (C3 and C4) and occipital (O1 and O2) electrodes with auricular reference electrodes, two electrooculographic channels, two submental electromyographic channels, and electrocardiography (ECG). Sleep stages were scored using 30-second epochs according to standard criteria by Rechtschaffen and Kales [12].

We measured sleep latencies from lights-off to the first epoch of non-rapid eye movement stage 1 (NREM1), NREM2, and REM sleep. Furthermore, we assessed the duration of specific sleep stages. In naps without sleep, mean sleep latency was defined as 20 minutes.

Statistics. Statistical analyzes were performed using SPSS statistical analysis program (SPSS 12.0). Data are reported as means ± standard deviation if not otherwise indicated. Comparisons between NC, BIIS and controls were made using one way ANOVA. For direct comparisons between NC and BIIS, we used independent t-test and Chi-square statistics. Significance level was assumed for p values <0.05. Significant result on chi-square and t-tests are only presented when one-way ANOVA revealed differences between the three groups (F-values and p levels of ANOVA are not reported).

Results

Subjects. The demographic and clinical characteristics of the three groups are summarized in Table 1. Subjective daytime sleepiness (estimated by the ESS) was similar between NC and BIIS patients (16±4 and 15±3, respectively, p=0.2) and significantly lower in controls (7±4, p<0.001).

Sleep studies. On polysomnography, NC and BIIS had similar mean sleep latencies to NREM2 (6.7±4.7 and 9±4.9 minutes, respectively, p=0.14) and to REM sleep (44±86 and
82.6±48.3 minutes, respectively, $p=0.09$), but sleep efficiency was significantly lower in NC than in BIISS (86.8±11.9% vs. 94.8±4.7%, $p=0.008$); other polysomnographic data are presented in Table 2.

In BIISS patients, the actigraphy-based measure of sleep duration was 7h31min±54min, and 8h32min±1h17min after sleep extension ($p=0.02$).

On MSLT, mean sleep latencies to NREM1 were significantly shorter in NC (1.8±1.5 minutes) compared to BIISS (4.7±2.1 minutes, $p<0.001$) and controls (11.4±3.3 minutes, $p<0.001$) (Figure 1). A mean sleep latency ≤2.5 minutes was found in 16/20 (80%) NC patients compared to 3/20 (15%) BIISS patients ($p<0.001$). Mean sleep latencies to NREM2 were similar in NC and BIISS (8.6±4.7 minutes and 8.1±2.7 minutes, respectively, $p=0.64$), but significantly shorter than in controls (13.4±3.3 minutes, $p<0.001$). Measuring sleep latencies to either NREM2 or REM (e.g. the sum of the sleep latency to NREM1 and the duration of the first NREM1 sleep sequence), however, NC revealed significantly shorter latencies than BIISS (4.4±2.9 and 7.9±3.5 minutes, respectively, $p<0.001$).

Subanalysis of MSLT naps with SOREM periods. SOREM periods occurred in 62/85 naps (73%) in NC patients, compared to 13/89 naps (15%) in BIISS patients ($p<0.001$). SOREM periods were not observed in controls. Mean sleep latency below 8 minutes was found in each NC patient, but only 17 NC patients (85%) had ≥2 SOREM periods. Three BIISS patients (15%) had a mean sleep latency <8 minutes and ≥2 SOREM periods. When referring only to naps with SOREM periods, mean sleep latency from lights off to NREM1 did not differ between NC and BIISS (1.9±2.3 and 1.8±1.3 minutes, respectively; $p=0.96$), but mean sleep latency from lights off to REM sleep was significantly shorter in NC than in BIISS (5.4±3.8 and 10.0±4.0 minutes, respectively; $p<0.001$). Conversely, mean sleep latency from lights off to NREM2 was significantly shorter in BIISS than in NC (6.5±4.4 and 10.3±6.4 minutes, respectively; $p<0.001$). In NC, no difference was found when comparing
mean sleep latencies to NREM1 in naps with and without SOREM periods (1.9±2.3 and 1.8±1.3 minutes, respectively; \( p=0.85 \)). In BIIS patients, on the other hand, mean sleep latency to NREM1 was significantly shorter in naps with than without SOREM periods (1.8±1.3 and 5.2±3.4 minutes, respectively; \( p<0.001 \)) (Table 3). In NC, we found no association between number of SOREM periods and mean sleep latencies.

**Sleep stage sequencing in MSLT naps with SOREM periods.** In NC, REM sleep occurred immediately after NREM1 in 44/62 naps (71%), and was followed in 20/44 (45%) naps by NREM2; in 24/44 (55%) naps NREM2 was not achieved. In the remaining 18/62 naps (29%) the sequence NREM1-NREM2-REM was observed (\( p<0.001 \)). In BIIS, REM sleep immediately after NREM1 was found in only 2/13 (15%) naps, followed by NREM2 in one nap; in 11/13 (85%) naps the sequence NREM1-NREM2-REM was observed (\( p<0.001 \)) (Figure 2). Consecutively, NC had a significant longer NREM1-NREM2 latency than BIIS (6.1±5.8 and 3.5±2.5 minutes, respectively; \( p=0.001 \)). However, when referring only to naps without REM prior to NREM2, no difference in NREM1-NREM2 latency was found between NC and BIIS (2.4±1.9 and 3.6±2.1 minutes; \( p=0.14 \)).

**Discussion**

In our study, we found (1) shorter sleep latencies to NREM1 and REM in NC compared to BIIS, (2) but similar sleep latencies to NREM2 in both disorders, which is explained by the observation that (3) the sequence NREM1-REM-NREM2 is more prevalent in NC (71% of all naps with REM sleep) compared to BIIS with NREM1-NREM2-REM as the most common sequence (85%).

In general, sleepiness (as assessed with mean sleep latencies to NREM1) and REM sleep propensity (as assessed with the number of SOREM periods and the latency to REM sleep) were higher in NC than in BIIS patients. However, considering only naps with
SOREM periods, mean sleep latencies to NREM1 were similar between the two conditions. In other words, BIISS patients with SOREM periods had significantly shorter mean sleep latencies to NREM1 than BIISS patients without SOREM periods, which is in line with other studies [13]. This finding suggests that the presence of SOREM periods in BIISS is associated with a higher sleep pressure, leading to sleep latencies not discernible from narcoleptic patients. In NC, however, the occurrence of SOREM periods was not associated with shorter sleep latencies on MSLT naps. Furthermore, we could not confirm previous findings of a significant correlation between higher number of SOREM periods and lower mean sleep latencies in NC [14].

The combination of ≥2 SOREM periods and short mean sleep latency on MSLT has been shown to have limited sensitivity and specificity for the diagnosis of NC [2, 15]. Indeed, our finding of ≤1 SOREM periods in 15% of NC patients corroborates the assumption of suboptimal sensitivity of this particular test. Regarding specificity, on the other hand, ≥2 SOREM periods have been reported also in other sleep-wake disturbances and even in healthy subjects. Chervin et al. found ≥2 SOREM periods in almost 5% of 1145 consecutive patients with suspected or confirmed obstructive sleep apnea [16]. Aldrich et al. reported a frequency of ≥2 SOREM periods in 7% of 1251 patients with sleep-related breathing disorders [2]. Also, Guilleminault et al. observed multiple SOREM periods in patients suffering from upper airway resistance syndrome [15]. Furthermore, ≥2 SOREM periods have been observed in a high number of healthy controls. Bishop et al. observed a surprisingly high frequency (17%) of ≥2 SOREM periods among 139 young, drug-free subjects without medical, psychiatric or sleep-related complaints [17]. However, as the same authors acknowledge, no actigraphic recordings were performed in these subjects to confirm regular sleep schedules and to exclude BIISS as a potential explanation of the high frequency of multiple SOREM periods. Similarly, Mignot et al. found multiple SOREM periods in 13.1% of males and 5.6% of females of the community-based Wisconsin Sleep Cohort Study [4]. Conversely, in our healthy controls, in
whom BIISS was excluded by actigraphic recordings and sleep logs, we could not find any SOREM periods on MSLT. Other groups also failed to demonstrate multiple SOREM periods in healthy controls [18-21]. The reason for these discrepant findings is not clear at the moment and will warrant further studies, as we believe (based on our daily clinical work) that BIISS in the healthy population is frequent.

Until now, there are no systematic studies on the frequency of multiple SOREM periods in BIISS patients. In 1994, Rosenthal first suggested that ≥2 SOREM periods in patients without clinical symptomatology for NC might reflect chronic sleep deprivation [22]. We found the combination of ≥2 SOREM periods and mean sleep latency ≤2.5 minutes in 15% of BIISS patients.

Sleep stage sequencing is different in NC and BIISS, with NREM1-REM-NREM2 being the most frequent pattern in NC, contrary to NREM1-NREM2-REM in BIISS. This finding of frequent REM prior to NREM2 in NC may indicate that in NC both REM and NREM sleep regulation are altered. It has been hypothesized that REM sleep propensity is enhanced in NC, consecutively leading to phenomena which are believed to be fragments of REM sleep (atonia, dream-like states) [23]. Recently, Lu and colleagues identified REM-off neurons in the brainstem, which are densely innervated by the hypocretin neurons. A loss of hypocretin neurons, therefore, weakens the REM-off side of a REM-on-off flip-flop mechanism, giving way to enhanced REM pressure [24]. Less is known about NREM sleep regulation in NC. NREM sleep intensity may be insufficient in NC, allowing for frequent arousals during nocturnal sleep and REM sleep intrusions [25]. Thus, in accordance with earlier reports, the frequent observation of the sequence NREM1-REM-NREM2 might be explained by a dysregulation involving both REM and NREM sleep in NC [14]. We postulate that the interplay of NREM sleep and REM sleep mechanisms is relevant for SOREM periods in NC. An abnormal NREM-REM sleep interaction in NC would also explain why SOREM
episodes at sleep onset were even longer after 40 hours total sleep deprivation compared to baseline [26]. This abnormal NREM-REM sleep interaction, however, is not present in BIISS. These findings indicate that SOREM episodes are independent from homeostatic NREM sleep regulation in NC but not in BIISS. We therefore consider the preserved NREM1-NREM2-REM sequencing in BIISS to reflect sleep pressure cumulating with chronic sleep deprivation whereas enhanced REM sleep facilitation rather than increased (REM) sleep pressure may explain the NREM1-REM-NREM2 sequencing in NC.

The main limitation of this study is the small sample size. However, our three groups have been strictly matched for age, gender, and BMI. In addition, our study only analyzed narcolepsy patients with clear-cut cataplexy. Hence, our findings cannot be generalized to narcolepsy patients without cataplexy, although this subgroup particularly needs objective features that allow differentiating it from BIISS. Therefore, future studies will want to prospectively compare MSLT findings between BIISS patients and narcolepsy patients with and without cataplexy. Furthermore, we included two NC patients who were not free from drugs affecting the central nervous system (modafinil and sodium valproate, respectively). However, the clinical, polysomnographic and MSLT findings of these two patients did not significantly differ from the remaining NC patients, nor did the exclusion of these two patients alter the results of this study.

In conclusion, we aimed at analyzing differences of MSLT characteristics between NC and BIISS, two conditions whose clinical presentation may show substantial overlap. The need of objective parameters to differentiate between the two conditions is further reflected by the almost equal degree of subjective EDS (estimated by the ESS) in our study population. We found that several findings might serve as an indicator for NC and against BIISS: mean sleep latencies to NREM1 ≤2.5 minutes, the presence of multiple SOREM periods, and the sequence NREM1-REM-NREM2 may be the best discriminating factors.
References


Table 1 Demographic and clinical characteristics of patients with narcolepsy and cataplexy (NC) and behaviourally induced insufficient sleep syndrome (BIISS), and normal controls.

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>BISS</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD) [years]</td>
<td>39±18</td>
<td>36±13</td>
<td>40±15</td>
<td>0.78</td>
</tr>
<tr>
<td>Gender (men/women)</td>
<td>11/9</td>
<td>10/10</td>
<td>11/9</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI (mean±SD) [kg]</td>
<td>25±5</td>
<td>24±5</td>
<td>23±4</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*BMI : body mass index
SD: standard deviation
MSLT characteristics of NC and BIISS

Table 2  Polysomnographic data.

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>BIISS</th>
<th>controls</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time [min]</td>
<td>325±54</td>
<td>406±26</td>
<td>357±50</td>
<td>0.03</td>
</tr>
<tr>
<td>Sleep efficiency [%]</td>
<td>87±12</td>
<td>95±5</td>
<td>91±6</td>
<td>0.013</td>
</tr>
<tr>
<td>Sleep latency to NREM2</td>
<td>7±5</td>
<td>9±5</td>
<td>50±49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>REM sleep latency</td>
<td>44±86</td>
<td>83±48</td>
<td>98±39</td>
<td>0.02</td>
</tr>
<tr>
<td>NREM1 [%]</td>
<td>17±10</td>
<td>7±7</td>
<td>9±7</td>
<td>0.001</td>
</tr>
<tr>
<td>NREM2 [%]</td>
<td>37±11</td>
<td>51±9</td>
<td>49±9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SWS(^1) [%]</td>
<td>13±7</td>
<td>17±5</td>
<td>16±6</td>
<td>0.11</td>
</tr>
<tr>
<td>REM [%]</td>
<td>20±9</td>
<td>19±5</td>
<td>16±5</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* one-way ANOVA
\(^1\) slow wave sleep (NREM3+NREM4)
Table 3  MSLT: Mean sleep latencies (in minutes) to NREM1 in NC and BIISS.

<table>
<thead>
<tr>
<th></th>
<th>n (naps)</th>
<th>Mean sleep latency to NREM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC with SOREM</td>
<td>62</td>
<td>1.9±2.3</td>
</tr>
<tr>
<td>NC without SOREM</td>
<td>23</td>
<td>1.8±1.3</td>
</tr>
<tr>
<td>BIISS with SOREM</td>
<td>13</td>
<td>1.8±1.3</td>
</tr>
<tr>
<td>BIISS without SOREM</td>
<td>76</td>
<td>5.2±3.4</td>
</tr>
</tbody>
</table>

n=number of naps
Figure 1  Mean sleep latencies to NREM1, NREM2, and REM sleep in NC, BISS, and controls (including standard deviations).

msl = mean sleep latency
**Figure 2**  Different sleep stage sequencing in NC and BIISS patients in MSLT naps with SOREMs (NC: n=62/85 naps, BIISS: n=13/89 naps).

<table>
<thead>
<tr>
<th>Sleep stage sequencing</th>
<th>NC (%)</th>
<th>BIISS (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NREM1</td>
<td>71</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>REM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NREM2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NREM1</td>
<td>29</td>
<td>85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NREM2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REM</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

- **Lights-off**
- **Mean sleep latency**: 20 minutes
Reply to the reviewers

Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome (*Sleep Medicine*, No. SLEEP-D-08-00299)
Marti I, Valko PO, Khatami R, Bassetti CL, Baumann CR

Dear Prof. Guilleminault

Thank you very much to reconsider our above-mentioned revised manuscript for publication in *Sleep Medicine*. Based on your recommendations and the comments of the three reviewers we performed the following changes (changes in the manuscript are indicated with red color):

**Reviewer #1**

1.) **Clarify the difference in the data in the two following statements.**
   a) Mean sleep latencies to NREM2 were similar in NC and BIISS (8.6±4.7 minutes and 8.1±2.7 minutes, respectively, p=0.64), but significantly shorter than in controls (13.4±3.3 minutes, p<0.001).
   b) Measuring sleep latencies to either NREM2 or REM, however, NC revealed significantly shorter latencies than BIISS (4.4±2.9 and 7.9±3.5 minutes, respectively, p<0.001).
   **In the second sentence, can you specify if those values are NREM latencies or REM latencies?**
   We agree with the reviewer that these two sentences may be misleading. The main message is that NC and BIISS do not differ concerning mean NREM2 latencies. When, however, measuring the latency from lights off to the first sleep stage after the first NREM1 sleep stage sequence (irrespective whether this is NREM2 or REM), then there were significant differences between NC and BIISS patients. Sleep latencies to either NREM2 or REM reflect the sum of sleep latency to NREM1 and the duration of the first NREM1 sleep stage sequence. We have clarified this difference adding a specific explanation in the second sentence in order to make the intended meaning more clear (in both Abstract, paragraph “Results” on p.2, and on p.6, last sentence of the second paragraph).

2.) **Why didn't you exclude the two narcolepsy patients who were still on medications?**
   We agree with the reviewer that the inclusion of two NC patients, which were not free from drugs affecting the central nervous system, represents a limitation of our study. We have, therefore, added this point in a paragraph at the end of our discussion (“limitations of study”). On the other hand, however, the clinical, polysomnographic and MSLT findings of these two patients did not differ from the remaining NC patients, nor did the exclusion of these two patients alter the results of our study.
3.) *The sample size is low, which should be stated clearly as a limitation of the study.*

We agree with the reviewer that the small sample size of our study represents a limitation. We have stated this at the end of our discussion (“limitation of study”). On the other hand, however, the included patients have been carefully selected concerning the diagnosis of NC or BIISS, and they are age-, sex-, and BMI-matched.

4.) *There is a need to be able to have tools to distinguish narcolepsy without cataplexy from BIISS - but not as much NC because the presence of cataplexy is a clear distinguishing feature. However, if these results could be generalizable to narcolepsy without cataplexy, this would be a really interesting and useful finding. Discussion should include whether this conclusion can or cannot be made.*

Thank you very much for this comment. We fully agree with the reviewer that the differentiation between narcolepsy patients with and without cataplexy remains a challenge, and that therefore our findings would be of clinical relevance if they were generalizable also to narcolepsy patients without cataplexy. At the moment it remains unclear, whether our findings can be generalizable to narcolepsy patients without cataplexy. We have discussed this aspect in the penultimate paragraph of the “Discussion”, suggesting also that future studies will want to prospectively compare MSLT findings between BIISS patients and narcolepsy patients with and without cataplexy.

5.) *It would be important to know how sleep restricted the BIISS patients were. Is there any data showing how much sleep on average they were obtaining?*

We have added the actigraphic data of the BIISS patients (before and after sleep extension) in the third paragraph of the “Results” section.

6.) *How do we separate NC from NC + BIISS in the findings?*

We agree that NC patients may additionally have insufficient sleep due to NC-related insomnia, and often present irregular sleep-wake rhythms. Hence, an overlap between NC and BIISS cannot be totally excluded. However, our NC patients were emphatically instructed to pay regard to an optimal sleep hygiene including regular sleep-wake habits and repeated short daytime naps, in order to minimize concomitant BIISS. Hence, actigraphic recordings have shown that our NC patients did not suffer neither from chronic sleep restriction (i.e. BIISS) nor from hypersomnia sensu strictu (extended sleep duration per 24 hours). We therefore believe that the degree of confounding BIISS in our NC patients is not a major concern. This aspect has now been discussed under “Patients and Methods” (first paragraph).
Reviewer #2

1.) This article looked at an important point but the control group is small and the authors ignore many data in the literature that have lead to question the MSLT: Aldrich et al, Chervin et al, Guilleminault et al have shown that subjects with OSA and SDB (ie mild breathing problems particularly in young women) have SOREMP and very short sleep latency (less than 3 minutes), but the more important problem are the data from the Wisconsin sleep cohort (Mignot et al) and those from the Detroit group that show that SOREMP is common in normal subjects (up to 11 %) and there is no consideration, even in the discussion, of such issue. This is clearly a problem for the study.

Again we fully agree that our control group is small (however, age- and gender-matched). This limitation is now mentioned in the penultimate paragraph of the “Discussion” (see also Reviewer 1, point 3).

We added a new paragraph addressing the studies on SOREMP in patients with OSA, SDB, and healthy controls.

We agree that - considering the debate on the prevalence of narcolepsy without cataplexy - the knowledge about the frequency of SOREMP in normal subjects is important. We therefore incorporated a paragraph in the “Discussion” section about the different findings of frequencies of SOREMP in normal subjects, including in particular the results of the Wisconsin Sleep Cohort Study and of the Detroit group. Both studies were very important to delineate the prevalence of SOREMP in a general population, but both studies did not systematically (including actigraphy) screen for BIISS. On the other hand, however, in another study the same Detroit laboratory failed to demonstrate ≥2 SOREMP in any of the included 30 healthy, young control subjects [Folkerts M. et al., Biol Psychiatry 1996]. In healthy controls without BIISS (as shown by sleep logs and actigraphic recordings), we could not find SOREMPs on MSLT, a finding reported also by other groups. The reason for these discrepant findings is not clear at the moment and will warrant further studies, as we believe (based on our daily clinical work) that BIISS in the healthy population is frequent.

Reviewer #3

1. The main goal of this study (page 4) is to better characterize MSLT in narcolepsy-cataplexy (NC) and in behaviourally induced insufficient sleep syndrome (BIISS). This is of value given the possibility of SOREMPs in BIISS. The study is well conducted with appropriate and well described methods. Statistics are correct. Results are sound.

On the other hand the practical interest of the MSLT to differentiate NC from BIISS must not be overemphasized. First, the positive diagnosis of BIISS mainly rests on clinical approaches including a thorough interview and completing a sleep log. Second, the overlap between BIISS and NC is minor.

We agree that the diagnostic value of the MSLT in isolation is limited, and that the differentiation between NC and BIISS mostly relies on a thorough interview, sleep logs, and on actigraphic recordings. However, although the overlap between BIISS and NC is minor, we repeatedly observe patients assigned with suspected narcolepsy,
who meet MSLT criteria for narcolepsy but finally (after actigraphically documented sleep extension) are diagnosed to have BIISS.

**BIISS patients may have hypnagogic hallucinations and/or sleep paralysis but no cataplexy.**

We fully agree that clear-cut cataplexy does not occur in BIISS patients. Nevertheless, cataplexy-like symptoms may rarely occur in the setting of non-narcoleptic EDS and cause diagnostic difficulties. More importantly, differentiation between BIISS and narcolepsy without cataplexy remains a challenge. We have discussed this aspect in the penultimate paragraph of the “Discussion”, suggesting also that future studies will want to prospectively compare MSLT findings between BIISS patients and narcolepsy patients with and without cataplexy (see also paragraph 4 of Reviewer 1).

**Unwanted episodes of sleep are generally less abrupt and irresistible than those of NC.**

We agree, but occasionally the patient’s statements might be not enough precise to allow distinguishing NC and BIISS.

**Brief episodes of sleep are more refreshing in NC.**

We agree that brief naps of NC patients are typically more refreshing than naps in other sleep-wake disorders. However, compared to patients suffering from idiopathic hypersomnia, BIISS patients may experience their brief naps as very refreshing (e.g. power naps for sleep-deprived hospital doctors).

**Third, a mean sleep latency \(<8 \text{ min}\) and \(\geq 2\) SOREMPs is almost the rule in NC patients (in 17 out of 20 NC patients in the current study) and rather exceptional in BIISS patients (in 3 out of 20 patients in the current study).**

Although the combination of a mean sleep latency \(<8 \text{ min}\) and multiple SOREM periods is most characteristic for NC and rather unusual for BIISS, our results still suggest that occasionally this finding may lack in NC patients (15%) and occur in BIISS patients (15%). In our opinion, a frequency of 15% is quite noteworthy.

2. **Introduction: The clinical and polysomnographic features of NC are reminded. Not those of BIISS.**

We have added the clinical and polysomnographic features of BIISS in the second paragraph of the “Introduction”.

3. **The most interesting part is the subanalysis of MSLT naps with SOREMPs, and the finding that the mean sleep latency from lights off to NREM did not differ between NC and BIISS, while the mean sleep latency from lights off to NREM sleep was significantly shorter in NC than in BIISS. Discussion page 7, second paragraph. This paragraph is not a discussion, but a mere repetition of the subanalysis of MSLT naps with SOREMPs page 8, second paragraph, line 6. The REM on-off flip-flop model is less substantiated than the flip-flop model for reciprocal interaction between sleep and wake promoting regions (Saper et al. 2001). Consequently the hypothesis that a loss of hypocretin neurons would weaken the REM-off side of a REM-on-off flip-flop mechanism, giving way to enhanced REM pressure sounds a little speculative.**
The authors suggest that the frequent irruption of REM sleep prior to NREM2 sleep in NC is in line with their recent assumption of an insufficient NREM sleep intensity in NC. However other authors have found the homeostatic process to be normal in narcolepsy. Moreover an insufficient NREM sleep intensity would not fit with sudden SONREM episodes.

We thank you for this valuable comment. Our small study does not allow drawing definite conclusions on pathophysiological processes underlying BIISS and NC. Therefore, we agree that the underlying mechanisms of different sleep stage sequences in MSLT naps with SOREM between NC and BIISS patients remain speculative.

Thank you again for reconsidering our manuscript for publication.

With kind regards,

Philipp O. Valko, MD

Zurich, 29.11.2008
re: Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome (Ms. No. SLEEP-D-08-00299R1)

Dear Prof. Guilleminault

We would like to submit the second revision of our manuscript entitled "Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome" for consideration for publication as an Original Paper in 'Sleep Medicine'.

We have integrated the comments of our first revision into the manuscript (according to the recommendation of Reviewer 3) and revised it according to the remaining concerns of the reviewers.

Thank you in advance for considering this paper for publication.

Sincerely yours
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Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome

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Word count abstract: 292
Word count text: 2572
Tables: 3
Figures: 2
References: 25
Abstract

Background: Short mean latencies to the first epoch of non-rapid eye movement sleep stage 1 (NREM1), and the presence of ≥2 sleep-onset REM (SOREM) periods on multiple sleep latency test (MSLT) occur in both narcolepsy-cataplexy (NC) and behaviourally induced insufficient sleep syndrome (BIISS). It is not known whether specific MSLT findings help differentiating the two disorders.

Methods: We analyzed MSLT data including sleep latencies to and between different sleep stages of 60 age-, gender- and body mass index (BMI)-matched subjects (hypocretin-deficient NC, actigraphy-confirmed BIISS, healthy controls: each 20).

Results: Mean latency (in minutes) to NREM1 sleep was significantly shorter in NC (1.8±1.5) than in BIISS (4.7±2.1, \(p<0.001\)) and controls (11.4±3.3, \(p<0.001\)). Mean latency to NREM2 sleep was similar in NC (8.6±4.7) and BIISS (8.1±2.7, \(p=0.64\)), latency to either NREM2 or rapid eye movement (REM) sleep (i.e. the sum of the sleep latency to NREM1 and the duration of the first NREM1 sleep sequence), however, was shorter in NC (4.4±2.9) than in BIISS (7.9±3.5, \(p<0.001\)). Referring to all naps with SOREM periods, the sequence NREM1-REM-NREM2 was more common (71%) in NC than in BIISS (15%, \(p<0.001\)), reflecting the shorter latency from NREM1 to NREM2 in BIISS (3.7±2.5) than in NC (6.1±5.9, \(p<0.001\)).

Conclusions: Our findings show that both sleepiness (as measured by NREM1 sleep latency) and REM sleep propensity are higher in NC than in BIISS. Furthermore, our finding of frequent REM sleep prior to NREM2 sleep in NC is in line with the recent assumption of an insufficient NREM sleep intensity in NC. Together with detailed clinical interviews, sleep logs, actigraphy, and nocturnal polysomnography, mean sleep latencies to NREM1 ≤2.5 minutes, the presence of multiple SOREM periods, and the sequence NREM1-REM-NREM2 may be the best MSLT measures to discriminate NC from BIISS.
Introduction

Excessive daytime sleepiness (EDS) represents the leading symptom of narcolepsy, along with cataplexy, sleep paralysis, and hypnagogic hallucinations. The pathophysiological hallmark of narcolepsy with cataplexy (NC) is the loss of hypothalamic neurons that produce wake-promoting neuropeptides, the hypocretins (orexins). Besides the assessment of a detailed history, the multiple sleep latency test (MSLT) is the most important ancillary test for the diagnosis of NC, together with the measurement of cerebrospinal fluid hypocretin levels. The typical MSLT findings for narcolepsy include (1) a mean sleep latency of ≤ eight minutes and (2) the presence of ≥ 2 sleep onset rapid eye movement (SOREM) periods as an indicator of high REM sleep propensity [1-3]. However, these findings are not specific for narcolepsy, since other sleep-wake disorders with EDS such as sleep apnea syndrome or behaviourally induced insufficient sleep syndrome (BIISS; also referred to as chronic sleep deprivation) may present with similar MSLT results [2, 4].

There is considerable evidence of a continuous reduction in average sleep time by 20% over the past century [5]. Due to professional or social obligations, people often are not able or do not allow themselves to sleep enough. Besides EDS, BIISS may be associated with decreased neurocognitive functioning, fatigue, increased risk of motor vehicle crashes, negative mood states, and decreased motivation [6]. Accordingly, BIISS is a frequent and important differential diagnosis of NC in clinical practice: General practitioners and neurologists often refer BIISS patients to sleep laboratories with the suspicion of narcolepsy. For sleep specialists who are familiar with NC patients, differentiating between NC and BIISS often is possible on clinical grounds alone. First, clear-cut cataplexy is pathognomonic for NC and does not occur in BIISS patients. However, cataplexy-like symptoms, sleep paralysis, and hallucinations are occasionally found in the setting of non-narcoleptic EDS patients [7]. Second, BIISS-related EDS can be as irresistible as in NC (e.g. microsleep during car
driving), and short episodes of sleep usually also are refreshing (e.g. power naps for sleep-deprived hospital doctors). In terms of objective measures of EDS, very low mean sleep latencies on MSLT and polysomnography recordings are found in both BIISS and NC patients [8]. Last not least, NREM sleep is better preserved than REM sleep during sleep restriction, possibly leading to high REM sleep propensity [9, 10]. Indeed, SOREM periods are also observed in BIISS patients [2, 4].

To better distinguish MSLT findings in NC and in BIISS, we aimed at characterizing the sleep latencies to and between different sleep stages in age-, gender- and BMI-matched patients with definite NC, BIISS, and normal controls.

**Patients and methods**

**Subjects.** Among patients referred to the Sleep Disorder Centre of the Neurology Department of the University Hospital of Zurich, we retrospectively selected 3 groups (NC, BIISS, and controls), each consisting of 20 age-, gender- and BMI-matched patients. EDS was present in all patients and was estimated by the Epworth sleepiness scale (ESS). In all NC patients, the diagnosis was confirmed by deficient cerebrospinal fluid hypocretin levels. In order to minimize concomitant BIISS, NC patients were instructed to pay regard to an optimal sleep hygiene including sufficient sleep amount, regular sleep-wake habits and repeated short daytime naps. Actigraphic recordings in NC patients confirmed the absence of chronic sleep restriction (i.e. reduced sleep time per 24 hours). The diagnosis of BIISS was made according to the international classification of sleep disorders, including improvement of subjective EDS after sleep prolongation as documented by sleep logs and actigraphy [3]. Patients with additional sleep-wake disorders (e.g. sleep apnea syndrome) were excluded. Control subjects did not report EDS (normal ESS scores), nor did they suffer from any kind of sleep-wake disorder. Patients and controls were free from drugs affecting the central nervous system,
except for two narcoleptic patients (modafinil and sodium valproate, respectively), one BIISS patient (citalopram), and two controls (phenytoin/gabapentin and citalopram, respectively).

**Sleep studies.** All patients underwent videopolysomnographic recording in the night prior to the MSLT. MSLT was done according to standard guidelines [11]. Briefly, patients underwent four or five naps, each lasting 20 minutes, at two hours interval over the day. If sleep occurred, tests were run for another 15 minutes to assess SOREM periods. The MSLT montage included central (C3 and C4) and occipital (O1 and O2) electrodes with auricular reference electrodes, two electrooculographic channels, two submental electromyographic channels, and electrocardiography (ECG). Sleep stages were scored using 30-second epochs according to standard criteria by Rechtschaffen and Kales [12].

We measured sleep latencies from lights-off to the first epoch of non-rapid eye movement stage 1 (NREM1), NREM2, and REM sleep. Furthermore, we assessed the duration of specific sleep stages. In naps without sleep, mean sleep latency was defined as 20 minutes.

**Statistics.** Statistical analyzes were performed using SPSS statistical analysis program (SPSS 12.0). Data are reported as means ± standard deviation if not otherwise indicated. Comparisons between NC, BIISS and controls were made using one way ANOVA. For direct comparisons between NC and BIISS, we used independent *t*-test and Chi-square statistics. Significance level was assumed for *p* values <0.05. Significant result on chi-square and *t*-tests are only presented when one-way ANOVA revealed differences between the three groups (F-values and *p* levels of ANOVA are not reported).

**Results**

**Subjects.** The demographic and clinical characteristics of the three groups are summarized in Table 1. Subjective daytime sleepiness (estimated by the ESS) was similar
between NC and BIISS patients (16±4 and 15±3, respectively, \(p=0.2\)) and significantly lower in controls (7±4, \(p<0.001\)).

**Sleep studies.** On polysomnography, NC and BIISS had similar mean sleep latencies to NREM2 (6.7±4.7 and 9±4.9 minutes, respectively, \(p=0.14\)) and to REM sleep (44±86 and 82.6±48.3 minutes, respectively, \(p=0.09\), but sleep efficiency was significantly lower in NC than in BIISS (86.8±11.9% vs. 94.8±4.7%, \(p=0.008\)); other polysomnographic data are presented in Table 2.

In BIISS patients, the actigraphy-based measure of sleep duration was 7h31min±54min, and 8h32min±1h17min after sleep extension (\(p=0.02\)).

On MSLT, mean sleep latencies to NREM1 were significantly shorter in NC (1.8±1.5 minutes) compared to BIISS (4.7±2.1 minutes, \(p<0.001\)) and controls (11.4±3.3 minutes, \(p<0.001\)) (Figure 1). A mean sleep latency ≤2.5 minutes was found in 16/20 (80%) NC patients compared to 3/20 (15%) BIISS patients (\(p<0.001\)). Mean sleep latencies to NREM2 were similar in NC and BIISS (8.6±4.7 minutes and 8.1±2.7 minutes, respectively, \(p=0.64\)), but significantly shorter than in controls (13.4±3.3 minutes, \(p<0.001\)). Measuring sleep latencies to either NREM2 or REM (e.g. the sum of the sleep latency to NREM1 and the duration of the first NREM1 sleep sequence), however, NC revealed significantly shorter latencies than BIISS (4.4±2.9 and 7.9±3.5 minutes, respectively, \(p<0.001\)).

**Subanalysis of MSLT naps with SOREM periods.** SOREM periods occurred in 62/85 naps (73%) in NC patients, compared to 13/89 naps (15%) in BIISS patients (\(p<0.001\)). SOREM periods were not observed in controls. Mean sleep latency below 8 minutes was found in each NC patient, but only 17 NC patients (85%) had ≥2 SOREM periods. Three BIISS patients (15%) had a mean sleep latency <8 minutes and ≥2 SOREM periods. When referring only to naps with SOREM periods, mean sleep latency from lights off to NREM1 did not differ between NC and BIISS (1.9±2.3 and 1.8±1.3 minutes, respectively; \(p=0.96\), but
mean sleep latency from lights off to REM sleep was significantly shorter in NC than in BIISS (5.4±3.8 and 10.0±4.0 minutes, respectively; p<0.001). Conversely, mean sleep latency from lights off to NREM2 was significantly shorter in BIISS than in NC (6.5±4.4 and 10.3±6.4 minutes, respectively; p<0.001). In NC, no difference was found when comparing mean sleep latencies to NREM1 in naps with and without SOREM periods (1.9±2.3 and 1.8±1.3 minutes, respectively; p=0.85). In BIISS patients, on the other hand, mean sleep latency to NREM1 was significantly shorter in naps with than without SOREM periods (1.8±1.3 and 5.2±3.4 minutes, respectively; p<0.001) (Table 3). In NC, we found no association between number of SOREMs and mean sleep latencies.

Sleep stage sequencing in MSLT naps with SOREMs. In NC, REM sleep occurred immediately after NREM1 in 44/62 naps (71%), and was followed in 20/44 (45%) naps by NREM2; in 24/44 (55%) naps NREM2 was not achieved. In the remaining 18/62 naps (29%) the sequence NREM1-NREM2-REM was observed (p<0.001). In BIISS, REM sleep immediately after NREM1 was found in only 2/13 (15%) naps, followed by NREM2 in one nap; in 11/13 (85%) naps the sequence NREM1-NREM2-REM was observed (p<0.001) (Figure 2). Consecutively, NC had a significant longer NREM1-NREM2 latency than BIISS (6.1±5.8 and 3.5±2.5 minutes, respectively; p=0.001). However, when referring only to naps without REM prior to NREM2, no difference in NREM1-NREM2 latency was found between NC and BIISS (2.4±1.9 and 3.6±2.1 minutes; p=0.14).

Discussion

In our study, we found (1) shorter sleep latencies in MSLT to NREM1 and REM in NC compared to BIISS, (2) but similar sleep latencies to NREM2 in both disorders, which is explained by the observation that (3) the sequence NREM1-REM-NREM2 is more prevalent
MSLT characteristics of NC and BIISS

in NC (71% of all naps with REM sleep) compared to BIISS with NREM1-NREM2-REM as the most common sequence (85%).

In general, sleepiness (as assessed with mean sleep latencies to NREM1) and REM sleep propensity (as assessed with the number of SOREM periods and the latency to REM sleep) were higher in NC than in BIISS patients. However, considering only naps with SOREM periods, mean sleep latencies to NREM1 were similar between the two conditions. In other words, BIISS patients with SOREM periods had significantly shorter mean sleep latencies to NREM1 than BIISS patients without SOREM periods, which is in line with other studies [13]. This finding suggests that the presence of SOREM periods in BIISS is associated with a higher sleep pressure, leading to sleep latencies not discernible from narcoleptic patients. In NC, however, the occurrence of SOREM periods was not associated with shorter sleep latencies on MSLT naps. Furthermore, we could not confirm previous findings of a significant correlation between higher number of SOREM periods and lower mean sleep latencies in NC [14].

The combination of ≥2 SOREM periods and short mean sleep latency on MSLT has been shown to have limited sensitivity and specificity for the diagnosis of NC [2, 15]. Indeed, our finding of ≤1 SOREM periods in 15% of NC patients corroborates the assumption of suboptimal sensitivity of this particular test. Regarding specificity, on the other hand, ≥2 SOREM periods have been reported also in other sleep-wake disturbances and even in healthy subjects. Chervin et al. found ≥2 SOREM periods in almost 5% of 1145 consecutive patients with suspected or confirmed obstructive sleep apnea [16]. Aldrich et al. reported a frequency of ≥2 SOREM periods in 7% of 1251 patients with sleep-related breathing disorders [2]. Also, Guilleminault et al. observed multiple SOREM periods in patients suffering from upper airway resistance syndrome [15]. Furthermore, ≥2 SOREM periods have been observed in a high number of healthy controls. Bishop et al. observed a surprisingly high frequency (17%)
MSLT characteristics of NC and BIISS

of ≥2 SOREM periods among 139 young, drug-free subjects without medical, psychiatric or sleep-related complaints [17]. However, as the same authors acknowledge, no actigraphic recordings were performed in these subjects to confirm regular sleep schedules and to exclude BIISS as a potential explanation of the high frequency of multiple SOREM periods. Similarly, Mignot et al. found multiple SOREM periods in 13.1% of males and 5.6% of females of the community-based Wisconsin Sleep Cohort Study [4]. Conversely, in our healthy controls, in whom BIISS was excluded by actigraphic recordings and sleep logs, we could not find any SOREM periods on MSLT. Other groups also failed to demonstrate multiple SOREM periods in healthy controls [18-21]. The reason for these discrepant findings is not clear at the moment and will warrant further studies, as we believe (based on our daily clinical work) that BIISS in the healthy population is frequent.

Until now, there are no systematic studies on the frequency of multiple SOREM periods in BIISS patients. In 1994, Rosenthal first suggested that ≥2 SOREM periods in patients without clinical symptomatology for NC might reflect chronic sleep deprivation [22]. We found the combination of ≥2 SOREM periods and mean sleep latency ≤2.5 minutes in 15% of BIISS patients. This finding ultimately underscores the low specificity and limited reliability of the MSLT in differentiating NC from BIISS, indicating that the clinical interview remains most important in the diagnosis of these sleep-wake disorders. Furthermore, sleep logs, actigraphy, and nocturnal polysomnography are helpful in the distinction of the two disorders.

Sleep stage sequencing is different in NC and BIISS, with NREM1-REM-NREM2 being the most frequent pattern in NC, contrary to NREM1-NREM2-REM in BIISS. The finding of frequent REM prior to NREM2 in NC may primarily indicate that REM sleep regulation is altered in NC. In this line, it has been hypothesized that REM sleep propensity is enhanced in NC, consecutively leading to phenomena which are believed to be fragments of
REM sleep (atonia, dream-like states) [23]. Recently, Lu and colleagues identified REM-off neurons in the brainstem, which are densely innervated by the hypocretin neurons. A loss of hypocretin neurons, therefore, would weaken the REM-off side of a REM-on-off flip-flop mechanism, giving way to enhanced REM pressure, and finally to REM sleep periods occurring prior to the constitution of NREM2 [24]. However, our finding also might indicate that homeostatic NREM sleep regulation is altered in NC. In fact, little is known about NREM sleep regulation in NC. A polysomnography study suggested that NREM sleep intensity is insufficient in NC, with frequent interruptions and an impaired build-up of slow-wake activity during nocturnal NREM sleep [25]. Thus, in accordance with earlier reports, the frequent observation of SOREM episodes with the sequence NREM1-REM-NREM2 might be explained by a dysregulation involving both REM and NREM sleep in NC [14]. Although BIISS patients also often present with increased REM sleep pressure and with SOREM periods, this abnormal NREM-REM sleep interaction does not appear to be present in BIISS.

The main limitation of this study is the small sample size. However, our three groups have been strictly matched for age, gender, and BMI. In addition, our study only analyzed narcolepsy patients with clear-cut cataplexy. Hence, our findings cannot be generalized to narcolepsy patients without cataplexy, although this subgroup particularly needs objective features that allow differentiating it from BIISS. Therefore, future studies will want to prospectively compare MSLT findings between BIISS patients and narcolepsy patients with and without cataplexy. Furthermore, we included two NC patients who were not free from drugs affecting the central nervous system (modafinil and sodium valproate, respectively). However, the clinical, polysomnographic and MSLT findings of these two patients did not significantly differ from the remaining NC patients, nor did the exclusion of these two patients alter the results of this study.
In conclusion, we aimed at analyzing differences of MSLT characteristics between NC and BIISS, two conditions whose clinical presentation may show substantial overlap. The need of objective parameters to differentiate between the two conditions is further reflected by the almost equal degree of subjective EDS (estimated by the ESS) in our study population. Nevertheless, the diagnosis of NC cannot be made by means of the MSLT alone but still mostly relies on a thorough clinical interview, together with sleep logs, actigraphy, and nocturnal polysomnography. In cases with similar clinical presentation, however, MSLT results can additionally help differentiating between NC and BIISS. We found that several findings might serve as an indicator for NC and against BIISS: mean sleep latencies to NREM1 ≤2.5 minutes, the presence of multiple SOREM periods, and the sequence NREM1-REM-NREM2 may be the best discriminating factors.
References


**Table 1** Demographic and clinical characteristics of patients with narcolepsy and cataplexy (NC) and behaviourally induced insufficient sleep syndrome (BIISS), and normal controls.

<table>
<thead>
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<th>NC</th>
<th>BISS</th>
<th>Controls</th>
<th>(p)</th>
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<tbody>
<tr>
<td><strong>Age (mean±SD) [years]</strong></td>
<td>39±18</td>
<td>36±13</td>
<td>40±15</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Gender (men/women)</strong></td>
<td>11/9</td>
<td>10/10</td>
<td>11/9</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>BMI (mean±SD) [kg]</strong></td>
<td>25±5</td>
<td>24±5</td>
<td>23±4</td>
<td>0.14</td>
</tr>
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*BMI: body mass index
SD: standard deviation*
### Table 2  Polysomnographic data.

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<tr>
<th></th>
<th>NC</th>
<th>BISS</th>
<th>controls</th>
<th>(p^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sleep time [min]</td>
<td>325±54</td>
<td>406±26</td>
<td>357±50</td>
<td>0.03</td>
</tr>
<tr>
<td>Sleep efficiency [%]</td>
<td>87±12</td>
<td>95±5</td>
<td>91±6</td>
<td>0.013</td>
</tr>
<tr>
<td>Sleep latency to NREM2</td>
<td>7±5</td>
<td>9±5</td>
<td>50±49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>REM sleep latency</td>
<td>44±86</td>
<td>83±48</td>
<td>98±39</td>
<td>0.02</td>
</tr>
<tr>
<td>NREM1 [%]</td>
<td>17±10</td>
<td>7±7</td>
<td>9±7</td>
<td>0.001</td>
</tr>
<tr>
<td>NREM2 [%]</td>
<td>37±11</td>
<td>51±9</td>
<td>49±9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SWS(^1) [%]</td>
<td>13±7</td>
<td>17±5</td>
<td>16±6</td>
<td>0.11</td>
</tr>
<tr>
<td>REM [%]</td>
<td>20±9</td>
<td>19±5</td>
<td>16±5</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\(^*\) one-way ANOVA  
\(^1\) slow wave sleep (NREM3+NREM4)
**Table 3**  MSLT: Mean sleep latencies (in minutes) to NREM1 in NC and BIISS.

<table>
<thead>
<tr>
<th>Group</th>
<th>n (naps)</th>
<th>Mean sleep latency to NREM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC with SOREM</td>
<td>62</td>
<td>1.9±2.3</td>
</tr>
<tr>
<td>NC without SOREM</td>
<td>23</td>
<td>1.8±1.3</td>
</tr>
<tr>
<td>BIISS with SOREM</td>
<td>13</td>
<td>1.8±1.3</td>
</tr>
<tr>
<td>BIISS without SOREM</td>
<td>76</td>
<td>5.2±3.4</td>
</tr>
</tbody>
</table>

n=number of naps
Figure 1  Mean sleep latencies to NREM1, NREM2, and REM sleep in NC, BIIS, and controls (including standard deviations).

msl = mean sleep latency
**Figure 2** Different sleep stage sequencing in NC and BISS patients in MSLT naps with SOREMs (NC: n=62/85 naps, BISS: n=13/89 naps).

<table>
<thead>
<tr>
<th>Sleep stage sequencing</th>
<th>NC</th>
<th>BISS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NREM1-REM-NREM2</td>
<td>71%</td>
<td>15%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NREM1-NREM2-REM</td>
<td>29%</td>
<td>85%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Lights-off

Mean sleep latency 20 minutes
Reply to the reviewers (II.)

Multiple sleep latency measures in narcolepsy and behaviourally induced insufficient sleep syndrome (Sleep Medicine, No. SLEEP-D-08-00299)
Marti I, Valko PO, Khatami R, Bassetti CL, Baumann CR

Dear Prof. Guilleminault

Thank you very much to reconsider our above-mentioned, re-revised manuscript for publication in Sleep Medicine. We have integrated the comments of our first revision into the manuscript (according to the recommendation of Reviewer 3) and revised it according to the remaining concerns of the reviewers (changes in the manuscript are indicated with red color):

Reviewer #1:

Comments were well addressed. The discussion could be a little better in addressing some of the speculative issues. Overall, an interesting study with good ideas.

We agree that our paragraph dealing with the meaning of different sleep stage sequencing between NC and BIISS is not very convincing and rather speculative. Therefore, we have rewritten and reduced this section in a more prudent and less speculative way.

Reviewer #3:

The authors have carefully responded to all our remarks. However none of these responses have been transferred into the revised version, except for a better clinical and polysomnographic picture of BIIS.

If we accept that patients with BIIS may be repeatedly referred with suspected diagnosis of narcolepsy, it is not our experience that up to 15% of them do meet MSLT criteria for narcolepsy.

Even in the case they would meet such criteria, the differentiation between BIIS and narcolepsy must not rely only on MSLT features, but also and principally on a thorough clinical interview, on sleep logs and on all night polysomnography.

Besides, the authors themselves agree that the main challenge is not in differentiating between BIIS and narcolepsy with cataplexy, but between BIIS and narcolepsy without cataplexy, and the results of the current study can unfortunately not be generalized to narcolepsy without cataplexy.

In conclusion: The paper is of interest, but the authors should stress the ultimate value of clinical interview, sleep log and all-night polysomnography in the discussion and conclusion.

Thank you for your comments. We fully agree that in differentiating between narcolepsy with cataplexy and BIIS the MSLT cannot replace a thorough clinical interview, in conjunction with sleep logs and all-night polysomnography. We have therefore underscored in the
abstract, discussion and conclusion the indispensable value of the clinical interview, sleep logs and all-night polysomnography. However, it remains our experience that patients with BIISS-related excessive daytime sleepiness are often referred to our sleep clinic with the suspicion of narcolepsy. In some of these patients (15% in our study), MSLT findings (mean sleep latency, number of SOREMs) are indeed indiscernible from the MSLT findings in NC patients. Differentiating these BIISS patients from NC patients becomes possible when considering the remission of EDS after actigraphically documented sleep extension. Since narcolepsy patients may present without clear-cut cataplexy, BIISS patients with EDS and narcolepsy-like MSLT findings can initially be misdiagnosed with narcolepsy. In addition, we have integrated the comments of our first revision into the manuscript (second paragraph of the introduction).

Thank you again for reconsidering our manuscript for publication.

With kind regards,

Philipp O. Valko, MD

Zurich, 5.2.2009