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Temporal distribution of sleep onset REM periods and N3 sleep in the MSLT and night polysomnogram of narcolepsy type 1 and other hypersomnias

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ABSTRACT

Introduction: The presence of ≥2 sleep onset REM periods (SOREMP) in the Multiple Sleep Latency Test (MSLT) and the previous night polysomnogram (PSG) is part of the diagnostic criteria of narcolepsy, with every SOREMP having the same diagnostic value, despite evidence suggesting that time of SOREMP appearance and their preceding sleep stage might be relevant. We studied the temporal distribution of SOREMPs and associated sleep stages in the MSLT of patients with narcolepsy type 1 (NT1) and other hypersomnias (OH).
Methods: We reviewed consecutive five-nap MSLTs and their preceding PSG from 83 untreated adult patients with hypersomnolence and ≥1 SOREMPs. Wake/N1(W/N1)-SOREMPs, N2-SOREMPs, and N3 sleep presence and time of appearance were analyzed.
Results: Thirty-nine patients had NT1 and 44 OH. There were 183 (78%) SOREMPs in patients with NT1 and 83 (31%) in OH. Sixty-seven percent of SOREMPs in NT1 were from W/N1, and 20% -none from wake-in OH ($p < 0.001$). Most patients (94%) with ≥2 W/N1-SOREMPs had NT1 (specificity 95%, sensitivity 82%). In patients with NT1 but not in OH, W/N1-SOREMPs decreased throughout the day (from 79% in the 1st nap to 33% in the preceding night, $p < 0.001$), whereas N2-SOREMPs did not change. N3 sleep frequency in the 5th nap was higher in NT1 than in OH (28% versus 7%, p:0.009). Nocturnal-SOREMP plus ≥4 daytime SOREMPs, Wake-REM transitions, and REM followed by N3 were only seen in NT1.
Conclusion: Measuring the sleep stage sequence and temporal distribution of SOREMP helps to identify patients with narcolepsy in the MSLT.

1. Introduction

The Multiple Sleep Latency Test (MSLT) consists of four or five nap opportunities every 2 h during the day performed under soporific, standard conditions, following nocturnal polysomnography (PSG). The MSLT objectively measures the tendency to fall asleep and detects the presence of sleep onset REM periods (SOREMP) [1]. The test, which is part of the diagnostic criteria of narcolepsy type 1 (NT1) and type 2 (NT2), requires the presence of ≥2 SOREMPs and initially a mean sleep latency of <5 min [2], later adjusted to ≤8 min to increase sensitivity [3]. The diagnostic criteria were finally modified to include a SOREMP in the preceding nocturnal PSG as a substitute for one SOREMP in the MSLT [4,5]. These rules, however, do not consider the sleep stage preceding each SOREMP nor the time of the day when SOREMP appears, two aspects that could add diagnostic value to the test given that SOREMP may appear in conditions associated with hypersomnia other than narcolepsy [6,7] or in the general population [8]. There are two ways of entering REM sleep in the MSLT, one following N2 sleep (N2-SOREMPs) and another directly from Wake or N1 (W/N1) stage (W/N1-SOREMPs). Currently, both ways are given the same diagnostic value, even though W/N1 to REM sleep transitions are much more frequent in NT1 than in other hypersomnias (OH) associated with SOREMPs, such as sleep deprivation [9], [–] [13] although these studies did not describe how many of them occur in each patient and if there is a cut-off that could have clinical relevance to identify NT1 patients. Also, the time of the day when SOREMPs appear could have additional diagnostic relevance. For instance, a night-SOREMP, although occurring only in 51% of the NT1 patients, was very specific for this disorder (99%) [5]. In addition, SOREMP occurrence in the 4th nap of the MSLT was reported to be less frequent than in the other naps [14], particularly in patients with narcolepsy [15] or to be only present in patients with narcolepsy [16]. However, other studies did not find it [17]. To our knowl-

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edge, the temporal distribution of W/N1-SOREMPs and N2-SOREMPs has only been marginally mentioned in one study [9].

Finally, although one study reported that N3 sleep percentage in the MSLT is higher in NT1 than in other sleep disorders [12], the temporal distribution of N3 sleep in the MSLT has not been assessed.

We aimed to characterize the temporal distribution and number of the different SOREMP types and N3 sleep presence in the MSLT of patients with NT1 and OH. We hypothesized that the appearance of a specific SOREMP type or N3 sleep at a particular time of the day or a given number of W/N1-SOREMPs could have a diagnostic value for NT1.

2. Methods

This observational and retrospective study was conducted at the Sleep Center of the Department of Neurology, Hospital Clínic de Barcelona, Spain. The study protocol was approved by the Ethics Committee of this institution.

2.1. Patients

We analyzed five-nap MSLT studies showing ≥1 SOREMP of 83 consecutive patients who were not taking REM-influencing medication between 2013 and 2019. Diagnoses were NT1 (N = 39) or OH (n = 44). All patients had a 2-week actigraphy or sleep diary preceding an overnight video-PSG before the MSLT [18]. NT1 and OH were diagnosed according to the international classification of sleep disorders - 3rd edition [19]. For the diagnosis of insufficient sleep syndrome, the sleep extension resulting in the remission of sleepiness was not indispensable for the diagnosis if the clinical suspicion was high, it was not feasible for the patient and met all the other criteria. Insufficient sleep time was considered <7 h in adults and <8 h in adolescents. For long sleepers, sleep time was higher. The significant increase in sleep time on holidays or weekends was defined as >2 h and had to be documented in the actigraphy/sleep log. Delayed sleep phase was considered if bedtime was >1:00–2:00 a.m. Obstructive sleep apnea syndrome was diagnosed with a minimum of 10 apneas/hypopneas per hour. Depression was diagnosed by a psychiatrist. Demographic, clinical characteristics, and ancillary tests were reviewed from patient clinic charts.

2.2. PSG and MSLT

were recorded with a Deltamed-Coherence 7 system and analyzed with the BrainRT software. The nocturnal PSG and the MSLT were performed following the AASM protocol [18]. The previous night PSG started at 23:00 and ended at 7:30. The MSLT began 2 h later with the following nap schedule: 1st nap (9:30), 2nd (11:30), 3rd (13:30), 4th (15:30), and 5th (17:30). Sleep stage scoring was done manually in all naps and PSG following the AASM criteria [20]. We assessed in each MSLT nap and the previous nocturnal PSG the presence of SOREMP and its sleep stage sequence and the presence of N3 sleep in the first 15 min of sleep according to the AASM scoring criteria version 2014 [21]. The two patients with NT2 who were HLA DQB1*06:02-positive were lost to follow-up and restudied five and nine years later, one because of worsening sleepiness and the other as a result of this investigation. Only the data from their first MSLT was used for the analysis.

2.3. Statistical analysis

First, we analyzed the demographical, clinical, and ancillary tests data according to diagnosis (NT1 versus OH). Second, in an individual nap analysis (without considering the time of the day), we compared SOREMP types (all, W/N1-SOREMPs and N2-SOREMPs) and N3 sleep presence by diagnoses (NT1 vs. OH). Third, we compared the number of each SOREMP type in each MSLT according to diagnoses (NT1 vs. OH). For the three previous comparisons, we used the Chi-square test for nominal data and t-student for quantitative ones, and P values less than 0.05 were considered significant. Then, we tested if analyzing the two SOREMP subtypes (W/N1-SOREMPs and N2-SOREMPs) independently, we could identify a SOREMP value that was more specific than the current criteria without losing much sensitivity to discriminate NT1 from OH. Sensitivity, specificity, Youden Index, ROC curve, and positive predictive values (PPV) for NT1 were calculated. A sub-analysis of the MSLTs having two or three SOREMPs was also performed since this was the number of SOREMPs that more often had patients with other causes of sleepiness, trying to find clues associated with a diagnosis of NT1 (supplementary material). Fourth, in the temporal distribution analysis, we compared within each diagnostic group (NT1 or OH) SOREMP types and N3 sleep presence at each time of the day with the other times of the day. Here, we used the Holm's method to correct for multiple comparisons [22]. Once the temporal distribution trends of NT1 and OH were calculated, we described the differences observed. Data were reported as a number, percentage, mean, standard deviation, and range. Even though the PSG study was performed the night before the MSLT, for the sake of clarity, the previous nocturnal PSG results are presented in the figures with a broken time bar at the end of the MSLT naps.

3. Results

3.1. Demographical and clinical data

The mean age of the 83 patients was 39 ± 15 (range 14–80) years, and 46 (55%) were male (Table 1). Thirty-nine patients had NT1 and 44 OH, including three NT2, 23 insufficient sleep syndrome (ISS), 6 obstructive sleep apnea (OSA), 1 depression, 1 idiopathic hypersomnia, and 10 multifactorial sleepiness (4 ISS + OSA, 2 ISS + delayed sleep phase, 1 ISS + depression, 2 ISS + shift work and 1 ISS + periodic leg movements of sleep fragmenting sleep). Of the 33 patients with ISS with or without other sleep disorders, 15 were able to extend their sleep duration and presented a remission of the symptoms, whereas the remaining 18 were unable to change their daily sleep amount.

3.2. Sleep stage sequence preceding SOREMP

3.2.1. Individual nap analysis

415 MSLT daytime naps and 83 nocturnal PSG (498 naps/PSG) were analyzed. SOREMPs occurred 266 times: in 8 (3%) naps arising directly from wake, in 131 (49%) from N1, and in 127 (48%) from N2 sleep. SOREMPs were more frequent in patients with NT1 than with OH (78% versus 31%, $p < 0.001$) and arose from wake/N1 more often in patients with NT1 (67% versus 20%, $p < 0.001$). Direct Wake-REM transitions occurred only in six patients (8 naps) with NT1.

3.2.2. MSLT analysis

The current criterion for narcolepsy of ≥2 SOREMPs (without characterizing their type) had 100% sensitivity, but only 45% specificity for NT1, and none of the patients with 1 daytime SOREMP had a nocturnal SOREMP to meet this criterion. In an exploratory analysis of how many MSLT SOREMPs of each type better discriminate NT1 (Table 2, ROC curves and Youden Index in Fig. S1), we found that there were different cut-offs for SOREMP types to differentiate all the patients with narcolepsy (either NT1 or NT2 HLA DQB1*06:02-positive) from the other sleep disorders. At least two W/N1-SOREMPs occurred in 34 narcolepsy patients: 32/34 (94%) had NT1 (95% specificity, 82% sensitivity, $p < 0.001$), and two had NT2, one with intermediate hypocretin levels (160 pg/ml), (Table S1). Similarly, ≥4 SOREMPs occurred in 30 patients with narcolepsy, 29 with NT1 (98% specificity but a lower sensitivity of 74%, $p < 0.001$), and 1 NT2. In contrast, N2-SOREMPs had always a sensitivity below 50%.

Table 1
Demographic clinical and ancillary tests of patients with narcolepsy type 1 (NT1) and other hypersomnias (OH) with ≥1 SOREMP in the MSLT. CSF: cerebrospinal fluid. TST: total sleep time. REM: rapid eye movement. AHI: Apnea-hypopnea index. CPAP: continuous positive airway pressure. PLMS: periodic limb movements of sleep. Data are presented as number, percentage, and mean ± standard deviation and range.

	NT1 (n = 39)	OH (n = 44)	P value
Demographic and clinical data			
Age (years)	37 ± 14 (14–73)	40 ± 14 (16–80)	0.5
Gender (male)	17 (44)	29 (66)	**0.05**
Age at reported onset (years)	20 ± 10 (5–45)	30 ± 18 (8–79)	0.06
Age at diagnosis (years)	34 ± 13 (14–73)	40 ± 16 (16–79)	0.06
Cataplexy	38 (97)	0	**<0.01**
Sleep paralysis	30 (77)	16 (37)	**<0.01**
Hypnagogic/hypnopompic hallucinations	26 (67)	8 (18)	**<0.01**
Poor sleep quality	24 (62)	10 (23)	**<0.01**
Epworth Sleepiness Scale	20 ± 3 (11–24)	16 ± 5 (1–21)	**<0.01**
Ancillary tests			
HLA DQB1*06:02	37/37 (100)	5/19 (26)	**<0.01**
Hypocretin-1 levels in CSF (pg/ml)	41 ± 44 (0–129)	186 (160–211)	NA
≤ 110 pg/mL	11/12 (92)	0	
> 110-<200 pg/mL	1/12 (8)	1 (50)	
≥ 200 pg/mL	0	1 (50)	
PSG			
TST (min)	423 ± 50 (294–495)	458 ± 37 (346–510)	**<0.01**
Sleep efficiency (%)	85 ± 9 (58–97)	90 ± 7 (60–97)	**0.01**
REM latency (min)	62 ± 96 (0–381)	69 ± 39 (4–179)	0.7
SOREMP	24 (65)	3 (6)	**<0.01**
AHI	5 ± 13 (0–67)	5 ± 7 (0–29)	1
<10	36 (92)	32 (73)	**0.02**
10–30	1 (3)	8 (18)	**0.03**
>30	2 (5)	0 (0)	0.1
CPAP treatment	2 (5)	4 (9)	0.7
PLMS Index	22 ± 30 (0–127)	9 ± 22 (0–147)	**0.02**
MSLT			
Mean sleep latency	1.8 ± 1.7 (0.2-8.4)	3.9 ± 2.4 (0.3-10.9)	**<0.01**
≤ 8 min	38 (97)	40 (91)	0.2
≤ 5 min	37 (95)	31 (70)	**<0.01**
Number of SOREMPs	4.1 ± 1.1 (2–5)	1.8 ± 0.9 (1–5)	**<0.01**
≥ 2	39 (100)	24 (55)	**<0.01**
Mean sleep latency ≤ 8 and ≥ 2 SOREMPs	38 (97)	22 (50)	**<0.01**

3.3. Temporal distribution of SOREMPs

(Tables S2–4). In patients with NT1, there was a decreasing trend of SOREMP frequency throughout the day, which was not seen in patients with OH (Fig. 1A). The PPV of a SOREMP for NT1 was highest in the night (89%), followed by the 4th nap (77%). However, in the night, there was a difference when considering patients with ≥4 daytime SOREMPs, where the PPV for NT1 was 100% (21/21), and patients with only 2 or 3 SOREMPs where the PPV for NT1 was only 50% (3/6), (Table S4). W/N1-SOREMP frequency had a decreasing trend throughout the day in patients with NT1 (Fig. 1B and D), whereas they were always similarly infrequent in patients with OH (Fig. 1B and E). The eight direct Wake-REM transitions occurred in six patients with NT1, on five occasions in the 1st nap. Comparing by diagnoses, W/N1-SOREMPs were at all times significantly more frequent in NT1 than in OH, and its PPV for NT1 was >90% in the 1st and the 4th naps and the night, and between 80 and 90% in the remaining naps. N2-SOREMPs in patients with NT1 had an opposite trend to that of W/N1-SOREMPs, with a minimum frequency in the 1st nap and a maximum in the 4th nap (Fig. 1C and D), whereas in patients with OH, the temporal distribution of N2-SOREMPs was similar in all daytime naps and decreased in the night (Fig. 1C and E). In patients with OH, N2-SOREMPs were significantly more frequent in the 1st nap (PPV for NT1 of only 25%), whereas they occurred more frequently in patients with NT1 in the 4th nap (PPV for NT1 65%) and the nocturnal PSG (PPV for NT1 85%). In patients with 2 or 3 daytime SOREMPs (n = 33), the presence of an N2-SOREMP in the 5th nap was only seen in six, all with OH (Table S4).

3.4. Temporal distribution of N3 sleep

(Tables S2–4): N3 sleep was recorded in 55/498 (11%) naps/PSG without differences comparing NT1 and OH (p: 0.8) (Fig. 1F). N3 sleep mainly occurred in the 4th and 5th naps and in the night (87%) and did not appear in the 1st nap. Notably, patients with NT1 had a significantly higher N3 sleep frequency in the 5th nap than OH (28% versus 7%, PPV for NT1 79%, p:0.009). In contrast to the 5th nap, N3 was more frequent in the night in OH than in NT1. Of note, there were only five naps, all from four NT1 patients, where REM sleep followed by N3 sleep was recorded, three occasions in the 5th nap and one each in the 3rd and 4th.

3.5. MSLT findings occurring only in narcolepsy

Three findings only occurred in NT1 patients. First, the presence of ≥4 SOREMPs in the MSLT plus a nocturnal SOREMP (n = 21); second, direct W-REM transitions (n = 6); and third, REM sleep followed by N3 sleep in the same nap (n = 4). Twenty-four out of 39 (62%) NT1 patients had at least one of these findings, and all the eight patients but one with either W-REM or REM-N3 transitions had ≥4 SOREMPs in the MSLT. Additionally, three findings appeared only in patients with NT1 or with NT2 HLA DQB1*06:02-positive. First, ≥4 SOREMPs in the MSLT of 30 patients, 29 (97%) with NT1 and the remaining with NT2. Second, ≥2 W/N1-SOREMPs in 34 patients, 32 (94%) with NT1 and two with NT2 (one with intermediate hypocretin levels). Third, a W/N1-SOREMP in the 4th nap in 16 patients, 15 (94%) with NT1 and one NT2. Thirty-eight out of 42 (90%) narcolepsy patients had at least one of these six findings.

4. Discussion

We have found that a detailed analysis of the SOREMP type and their temporal distribution in the MSLT and preceding PSG provides information that could help to better differentiate patients with NT1 from those with OH. A circadian variation in REM sleep propensity in narcolepsy and control subjects has been reported in several [14,15,23] [–] [25] but not all studies [17]. However, the temporal distribution of the different SOREMP subtypes was not assessed previously. We found that in patients with NT1, W/N1-SOREMPs and N2-SOREMPs have a different temporal distribution. Whereas W/N1-SOREMPs had a decreasing frequency throughout the day, from maximal in the morning to minimal in the previous night, N2-SOREMPs remained stable or slightly increased during the day. In OH, this decreasing tendency of W/N1 SOREMPs was also less evident. Consequently, W/N1-SOREMPs in the 1st nap were more frequent in NT1 than in OH, whereas the opposite was true for N2-SOREMPs, where the presence of an N2-SOREMP in the 1st nap had a PPV for NT1 of only 25%. The physiological mechanisms generating the two SOREMP subtypes and their different temporal distribution are presently unknown. It can be speculated that a higher REM sleep propensity would facilitate a direct wake or N1 to REM transition, whereas entering REM sleep from N2 could indicate a lower abnormal REM tendency. The presence of a SOREMP, particularly of the W/N1 subtype, when REM propensity is low (in the 4th nap and the night) would have an additional diagnostic value for NT1.

We also found that the presence of N3 sleep in the naps of the MSLT may be of diagnostic value for NT1, particularly if it occurred in the 5th nap (PPV of 79%) or if it appeared in the same nap after a SOREMP

Table 2
Sensitivity, specificity, and positive predictive value (PPV) for NT1 of SOREMPs in the MSLT. Percentage values of 100% are marked in dark green, 90–99% in medium green, and 80–89% in light green. The presence of ≥2 W/N1-SOREMPs had a high specificity and PPV (both >90%) and sensitivity of >80% for NT1, whereas ≥2 SOREMPs (without differentiating the subtypes) had 100% sensitivity but only 45% specificity. N2-SOREMPs had a low sensitivity regardless of their number (always below 50%).
*: HLA DQB1*06:02-positive NT2 patients. NT1: narcolepsy type 1. NT2: narcolepsy type 2. OH: other hypersomnias.

SOREMPs (n)	NT1 (n=39)	OH (n=44)	Sensitivity (%)	Specificity (%)	PPV (%)
ALL TYPES					
5	18	1*	46	98	95
≥ 4	29	1*	74	98	97
≥ 3	34	10	87	77	77
≥ 2	39	24	100	45	62
W/N1-SOREMPs					
5	5	0	13	100	100
≥ 4	14	1*	36	98	93
≥ 3	21	1*	54	98	95
≥ 2	32	2*	82	95	94
N2-SOREMPs					
5	3	0	8	100	100
≥ 4	3	0	8	100	100
≥ 3	8	4	21	91	67
≥ 2	14	18	36	59	44

SOREMPs (n)	NT1 (n=39)	OH (n=44)	Sensitivity (%)	Specificity (%)	PPV (%)
ALL TYPES					
5	18	1*	46	98	95
≥ 4	29	1*	74	98	97
≥ 3	34	10	87	77	77
≥ 2	39	24	100	45	62
W/N1-SOREMPs					
5	5	0	13	100	100
≥ 4	14	1*	36	98	93
≥ 3	21	1*	54	98	95
≥ 2	32	2*	82	95	94
N2-SOREMPs					
5	3	0	8	100	100
≥ 4	3	0	8	100	100
≥ 3	8	4	21	91	67
≥ 2	14	18	36	59	44

(PPV 100%). Murer et al. [12] reported more time in N3 sleep in NT1 in an MSLT study, but they did not assess its circadian variation. We have found that N3 prevalence oscillates during the day, confirming previous studies in healthy subjects [26]. In our study, very few patients presented N3 sleep in the morning, whereas most of the N3 sleep occurrences were in the 4th and 5th naps and the night, with maximal differences in N3 prevalence between NT1 and OH in the 5th nap. This finding is also novel and cannot be explained easily. Patients undergoing the MSLT may have an increased homeostatic need for N3 sleep accumulating throughout the day. Still, this peak disappears by the time of the 5th nap in OH, perhaps due to an end-of-test effect, but surprisingly, it persists in some patients with NT1. N3 sleep preceded by REM sleep was found only in four NT1 patients. This sequence has not been described previously in the MSLT and was found rarely in studies of sleep/wake cycles using experimental protocols of 30/60 min during the day in both NT1 and control subjects [23,24].

The MSLT diagnostic criteria of narcolepsy require the presence of ≥2 SOREMPs and a mean sleep latency of ≤8 min. This relatively wide range of values was shown to have a 70–92% sensitivity and high specificity for NT1 when compared with a healthy control population but has a lower specificity for the diagnosis of NT1 when assessing patients with hypersomnia of different causes, around 70–95% [5,7,27,28], which is even lower in our study as we included only OH with ≥1 SOREMP. This lack of specificity is accepted as one of the test's limitations. Still, it has prevented focusing on the value of other salient characteristics of the MSLT that could achieve a 100% PPV.

We have identified three findings that appeared specific for patients with NT1 and another three that were exclusively observed in narcolepsy patients of any type (present in 62% and 90% of NT1 or NT1 + NT2, respectively). The presence of these MSLT findings, if confirmed by other studies, would support the diagnosis of NT1 or narcolepsy with a higher degree of certainty. The presence of ≥4 SOREMPs in the MSLT plus a nocturnal SOREMP in the PSG, direct W-REM transi-

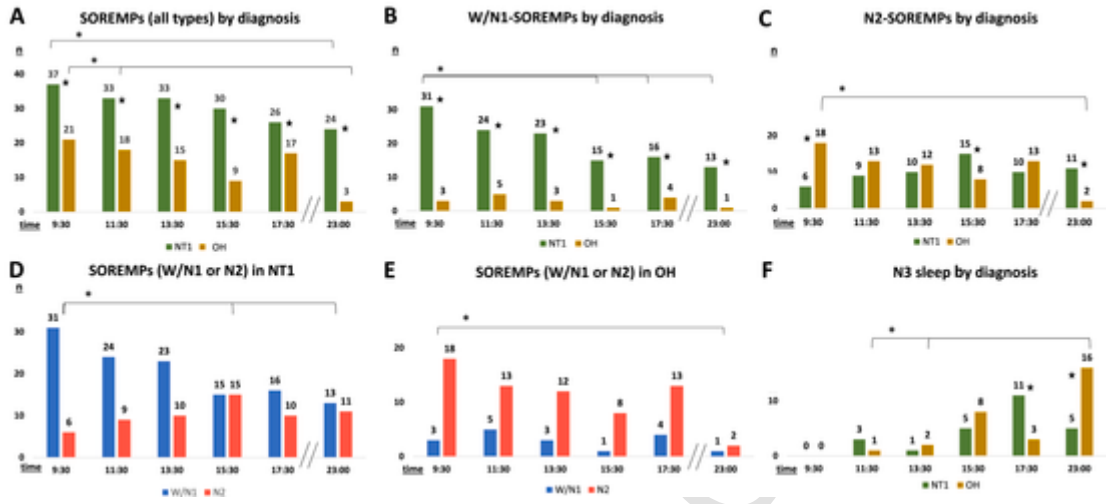

Fig. 1. Temporal distribution of SOREMPs and N3 sleep by diagnosis or sleep stage sequence
A. SOREMPs (all types). In patients with narcolepsy type 1 (NT1), SOREMP frequency decreased throughout the day. The 1st nap SOREMP frequency was higher than the night (horizontal bracket). In patients with other hypersomnias (OH), the night-SOREMP frequency was lower than the 1st and the 2nd naps (horizontal bracket). SOREMP frequency in NT1 was higher than OH in all naps.**B. Wake/N1 SOREMPs**: In patients with NT1, W/N1-SOREMP decreased throughout the day. W/N1-SOREMP frequency in the 1st nap was higher than the 4th, 5th, and the night (horizontal bracket). This pattern was not seen in patients with OH, where W/N1 SOREMPs were infrequent all day. W/N1-SOREMPs were significantly more frequent in patients with NT1 than with OH in all naps.**C. N2-SOREMPs**. In patients with OH, the previous night N2-SOREMP was less frequent than the 1st nap (horizontal bracket). This pattern was not seen in patients with NT1, where N2-SOREMP was minimal in the 1st nap and maximal in the 4th. N2-SOREMPs were more frequent in OH than NT1 in the 1st nap and more frequent in NT1 than in OH in the 4th nap and the night.**D. Type of SOREMPs (Wake/N1 or N2) in patients with NT1**. The ratio W/N1:N2-SOREMP decreased throughout the day. The 1st nap ratio (5.2) was higher than the 4th (1) and the night (1.18), (horizontal bracket)**E. Type of SOREMPs (Wake/N1 or N2) in patients with OH.** The ratio W/N1:N2-SOREMP in the night (0.5) was higher than the 1st nap (0.17), (horizontal bracket)**F. N3 sleep**. N3 sleep occurred predominantly in the 4th and 5th naps and the first 15 min of nocturnal sleep. N3 sleep was more frequent in NT1 than in OH in the 5th nap and more frequent in OH than NT1 in the night. In OH, N3 sleep frequency in the night was higher than the 2nd and 3rd naps (horizontal bracket).Green bars: NT1. Light brown bars: OH. Blue bars: W/N1-SOREMPs. Red bars: N2-SOREMPs.The different comparisons between different times of the day with statistically significant differences are indicated by brackets (*: $p < 0.0016$). All p-values above 0.0016 were considered non-significant according to Holm's method to correct for multiple comparisons. Stars: comparison between diagnoses in the same nap with significant differences ($p < 0.05$). Nonrelevant differences are not detailed.For the sake of clarity, the previous nocturnal PSG results are placed with a broken line in the time bar at the end of the MSLT naps. The values in the figure represent the number of naps having a SOREMP or N3 sleep each time, and their corresponding percentages are detailed in the results section. . (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

tions, and REM followed by N3 sleep in the same nap were observed only in NT1. In addition, the presence of ≥4 SOREMPs or ≥2 W/N1-SOREMPs in the MSLT or a W/N1-SOREMP in the 4th nap occurred only in NT1 or HLA DQB1*06:02-positive NT2 (in one case with intermediate hypocretin levels). Similar findings with higher specificity were previously reported in a four-nap MSLT study [12], where 96% of the patients with four SOREMPs had NT1 [15], and in a 5-nap MSLT study [17], with 100% of patients with five SOREMPs and 80% with four SOREMPs having NT1 or NT2 [13]. SOREMP number correlated inversely with hypocretin levels [29]. We also replicate the previous findings of the diagnostic value of the sleep stage sequence of SOREMPs [9–13]. In fact, we found that direct Wake-REM transitions occurred exclusively in patients with NT1. Marti et al. [13] reported for the first time the diagnostic relevance of the sleep stage sequence preceding REM, mentioning N1-REM transitions but not Wake-REM transitions. Subsequent studies found Wake-REM transitions more specific for NT1 (8–39.1%) or NT2 (2–8.5%), although it could also be present with variable frequency in other hypersomnias (0–3.2%) or Parkinson's disease (>20%) [9–12]. A recent study found that although the absolute number of W/N1-REM transitions was more frequent in NT1 and NT2 than in IH, OSA, and ISS, these differences disappeared when only naps with SOREMPs were analyzed [30]. However, in this study, the total number of SOREMPs was relatively low, probably because naps were not extended above 20 min to allow for 15 min of sleep after sleep onset. Discrepancies in the specificity of Wake-REM transitions might also be explained by other causes, including different sleep stage sequence scoring methods, inter-center, inter-rater variability, and ethnic differences. We think that direct Wake-REM transitions likely represent the highest degree of REM sleep propensity and probably are the most specific NT1 form of SOREMP.

Our results might also have implications for the identification of patients with NT2. Diagnosis of NT2 relies heavily on the MSLT findings. Still, the reproducibility of the results in repeated MSLT examinations is weak [30,31] and other sleep disorders may fulfill the standard criteria required, making it difficult to distinguish them from NT2 using current MSLT criteria. The prevalence of NT2 is probably lower than is usually considered. For instance, Baumann-Vogel et al. [32] encountered only six patients with NT2 in a series of 1392 consecutive patients with excessive daytime sleepiness, whereas there were NT1 (n = 91), insufficient sleep syndrome (n = 128), obstructive sleep apnea (n = 34), shift work (n = 34), and delayed sleep phase (n = 4). Similarly, in our cohort, only 3 out of 83 (4%) patients had NT2. Incorporating the findings reported in this study might help better identify genuine NT2 patients, as previously hypothesized [33]. We have found that two of our three patients diagnosed with NT2 had the same MSLT criteria as those with definite NT1, both were HLA DQB1*06:02-positive, and one had intermediate hypocretin levels.

Although the role of the nocturnal polysomnogram in the diagnosis of NT1 is important, it cannot substitute the MSLT. In line with previous studies [31,34], we also found that the night-SOREMP was never critical for reaching the second SOREMP required to fulfill the diagnostic criteria for NT1 and NT2. In all the patients with a nocturnal SOREMP, the MSLT already had ≥2 SOREMPs. In addition, although a nocturnal SOREMP was highly specific [5], in patients with four or five SOREMPs in the MSLT, in the group of patients with only two or three daytime SOREMP, the specificity for NT1 decreased drastically to 50%.

There is a tendency in the protocols of electrophysiological evaluation of hypersomnolence to expand the period of observation from 9 a.m. to 5 pm to up to >24 h [35–37]. We think that the standard MSLT preceded by actimetry/sleep diary excluding sleep deprivation still has an essential role in the evaluation of increased daytime sleepiness, particularly if attention to the sleep sequence and temporal distribution of SOREMP and N3 is taken into consideration. Our results support the need to routinely perform the 5th nap in the MSLT since the specificity for NT1 significantly increases when having four or five SOREMPs. On the other side, in the group of patients with only two or three SOREMPs, the presence of N3 sleep in the 5th nap suggests NT1, whereas the 5th nap with N2-SOREMP suggests OH.

Limitations of our study are the heterogeneity of the group with other hypersomnias and the relatively low number of patients in the different diagnostic categories, which could result in different results if these numbers were larger. However, we think that this constellation of diagnostic categories reflects the type of patients evaluated for hypersomnolence and having SOREMPs in the MSLT in clinical practice in a tertiary center [32,38]. It is difficult to recruit a comparable number of patients in each diagnostic category, although a sizeable amount of patients with insufficient sleep syndrome and multifactorial sleepiness is included. The low number of patients with NT2 has also been reported to occur in other studies with a larger number of patients [32]. The strength of this study is that we analyzed in detail the temporal distribution of the different SOREMP subtypes and N3 sleep in the MSLT performed with a homogeneous five-nap protocol.

In conclusion, our study shows that analyzing in detail the SOREMP subtype, and their temporal distribution as well as that of N3 sleep may be helpful in discriminating NT1 from other hypersomnias and better identifying NT2. If replicated, these findings could be incorporated into the MSLT routine to increase the diagnostic yield of the test.

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CRediT authorship contribution statement

Gerard Mayà : Formal analysis. **Joan Santamaria :** Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sleep.2022.12.018.

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