Association of CSF Orexin-A levels and nocturnal sleep stability in patients with hypersomnolence

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Abstract

Objective

To evaluate the associations between CSF orexin-A (ORX) levels and markers of nocturnal sleep stability, assessed by polysomnography.

Methods

Nocturnal polysomnography data and ORX levels of 300 drug-free participants (55% men, 29.9±15.5 years, ORX level 155.1±153.7 pg/mL) with hypersomnolence were collected. Several markers of nocturnal sleep stability were analyzed: sleep and wake bouts and sleep/wake transitions. Groups were categorized according to ORX levels, in 2 categories (deficient ≤110; >110), in tertiles (≤26, 26–254, >254), and compared using logistic regression models. Results were adjusted for age, sex, and body mass index.

Results

We found higher number of wake bouts (43 vs 25, $p < 0.0001$), sleep bouts (43 vs 25.5, $p < 0.0001$), and index of sleep bouts/hour of sleep time, but lower index of wake bouts/hour of wake time (41.4 vs 50.6, $p < 0.0001$), in patients with ORX deficiency. The percentage of wake bouts <30 seconds was lower (51.3% vs 60.8%, $p < 0.001$) and of wake bouts ≥1 minutes 30 seconds higher (7.7% vs 6.7%, $p = 0.02$) when ORX deficient. The percentage of sleep bouts ≤14 minutes was higher (2–5 minutes: 23.7% vs 16.1%, $p < 0.0001$), and of long sleep bouts lower (>32 minutes 30 seconds: 7.3% vs 18.3%, $p < 0.0001$), when ORX deficient. These findings were confirmed when groups were categorized according to ORX tertiles, with a dose–response effect of ORX levels in post hoc comparisons, and in adjusted models.

Interpretation

This study shows an association between ORX levels and nocturnal sleep stabilization in patients with hypersomnolence. Sleep and wake bouts are reliable markers of nighttime sleep stability that correlate with CSF ORX levels in a dose-dependent manner.

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Glossary

AHI = apnea–hypopnea index; **BMI** = body mass index; **EDS** = excessive daytime sleepiness; **ICSD-3** = International Classification of Sleep Disorders, 3rd ed; **IH** = idiopathic hypersomnia; **MSLT** = multiple sleep latency test; **NREM** = non-REM; **NT1** = narcolepsy type 1; **NT2** = narcolepsy type 2; **ORX** = orexin; **PLMS** = periodic leg movements during sleep; **PSG** = polysomnography.

The orexin (ORX)/hypocretin system contributes to sleep–wake regulation by sustaining long periods of wakefulness in humans and animals. Although ORX neurons are a restricted group of cells localized exclusively in the lateral hypothalamus, their projections are widely distributed through the brain. The densest projections are in the locus ceruleus, the raphe, and the tuberomammillary nuclei.[1] In the flip-flop model of reciprocal interactions between sleep- and wake-promoting brain regions,[2,3] aminergic regions promote wakefulness through direct excitatory effects on the cortex and inhibition of sleep-promoting neurons of the ventrolateral preoptic nucleus. ORX neurons increase the activity of aminergic neurons, thus supporting the inhibition of sleep-promoting and REM sleep-promoting neurons.

A defect of the orexinergic tone leads to wake and sleep instability, as observed in narcolepsy type 1 (NT1).[4] In NT1, the irreversible loss of ORX neurons, probably due to an autoimmune process, results in irresistible transitions to sleep (especially REM sleep) during the day, difficulties in maintaining long wakefulness periods, fragmented nighttime sleep with frequent shifts between sleep stages, and arousals.[5] Although cataplexy (sudden loss of muscle tone while awake) is the pathognomonic symptom of this rare sleep disease, the main symptom is excessive daytime sleepiness (EDS). Other typical symptoms are hypnagogic or hypnopompic hallucinations and sleep paralysis,[6,7] and many patients with NT1 also complain of disturbed and fragmented nocturnal sleep.[8] The neurobiology of other central hypersomnolence disorders (such as idiopathic hypersomnia [IH] and narcolepsy type 2 [NT2]) is unknown; however, ORX levels are normal in these disorders.[6,7]

As disrupted ORX signaling leads to a narcoleptic phenotype in different species, animal data may be translated to humans. Indeed, behavioral state instability caused by ORX deficiency has been well studied in rodents. Compared with wild-type mice, ORX knock-out mice have normal amounts of sleep and wake, but wake and non-REM (NREM) sleep bouts are very brief, with many more transitions between behavioral states.[9–11] Survival analysis of wake bouts showed that ORX is necessary for the maintenance of long wake bouts, and that ORX deficiency has little impact on wake bouts <1 minute.[12] In narcoleptic mice, sleep and wakefulness are less distinct and less stable.[13] In humans, several studies have reported an increased number of transitions between wake, NREM, and REM sleep stages during nighttime polysomnography (PSG) in patients with NT1 compared with other conditions.[14–17] Few studies assessed the nocturnal fluctuation between sleep and wake in function of ORX-A levels.[16,17] They showed that low ORX-A levels are associated with higher instability of wakefulness and REM-NREM sleep.[16] To our knowledge, no study has analyzed sleep bouts and wake bouts in patients with hypersomnolence to evaluate the nocturnal sleep stability as a function of ORX levels.

We assessed the associations between CSF ORX-A levels and markers of nocturnal sleep stability assessed by PSG (sleep and wake bouts, sleep/wake transitions) in a large sample of drug-free participants referred for suspected central hypersomnolence disorders.

Methods

Standard protocol approvals, registrations, and patient consents

This study was approved by local ethics committees (Comité de Protection des Personnes, France: Constitution of a cohort and of a clinical, neurophysiologic and biological bank of rare hypersomnolence disorders–NARCOBANK PHRC AOM07-138). Consent was provided by all participants prior to participation, written consent by adults, and by both parents for minors.

Participants

Data on 300 consecutive individuals referred for evaluation of a hypersomnolence complaint at the French National Reference Center for Narcolepsy and Rare Hypersomnia were collected for this study. All participants underwent a standardized clinical evaluation, video-PSG recording in the sleep laboratory, followed by a multiple sleep latency test (MSLT) and lumbar puncture on the second day. No participant was taking medications at evaluation time. Patients with relevant psychiatric and medical (especially significant neurologic, metabolic-endocrine, or immunologic) conditions or comorbidities that could explain hypersomnolence were not selected for the study.

Age, sex, body mass index (BMI), age at symptom onset, EDS assessed with the Epworth Sleepiness Scale,[18] and disturbed nocturnal sleep (assessed with a single item of the validated self-questionnaire Narcolepsy Severity Scale[19]) were collected for all participants. On the MSLT, mean sleep latency and number of sleep-onset REM periods were recorded. According to ICSD-3 criteria,[20] 167 patients had a diagnosis of NT1, 51 of NT2, 35 of IH, and 47 participants had EDS without central disorder of hypersomnolence (no objective sleepiness on the MSLT or during prolonged PSG monitoring).

Polysomnography analysis

All participants underwent a video-PSG recording from 11:00 PM (light-off) to 7:00 AM (light-on). Sleep was scored by experienced sleep experts, in 30-second epochs, based on the standard method,[21] and then analyzed with the Natus Coherence software. The following usual nocturnal sleep data were collected: time spent in bed (i.e., total time between light-off and light-on), sleep latency, total sleep time, sleep efficiency, apnea–hypopnea index (AHI), periodic leg movements during sleep (PLMS) and microarousal indexes per hour of sleep, and proportions of each sleep stage. Total wake time after sleep onset was defined as the total duration of wake after the beginning of sleep onset, including the wake epochs at the end of the recording, if any, before light-on.

CSF analysis

All participants were hospitalized in the sleep laboratory for 48 hours and benefited from the same standardized evaluation procedure. The lumbar puncture was performed following the last nap (the 5th) of the MSLT, between 5:00 PM and 7:00 PM. After centrifugation, CSF samples were collected and aliquots frozen and stored immediately at −80°C. CSF ORX-A level was determined in duplicate using the I^{125} radioimmunoassay kit from Phoenix Pharmaceuticals, Inc. (Belmont, CA), according to the manufacturer's recommendations. All values were back-referenced to the Stanford reference samples (Stanford University Center for Narcolepsy, Palo Alto, CA). CSF ORX-A levels below 110 pg/mL (i.e., the established threshold of ORX deficiency) were considered low.[20,22,23]

Markers of nocturnal sleep fragmentation: wake bouts, sleep bouts, transitions

Wake bouts

A wake bout was defined as a continuous sequence of wake epochs, occurring after the beginning of sleep onset until the end of the night. The shortest duration of a wake bout is 30 seconds and other durations are multiples of 30 seconds. The total number and median duration of all wake bouts were first calculated per participant. Then the wake bout length was categorized according to distribution in the sample as wake bout of 30 seconds, 1 minute, 1 minute 30 seconds, 2 minutes, or >2 minutes (50th, 60th, 80th, and 90th percentile in the entire wake bout set).

Sleep bouts

A sleep bout was defined as any sleep epoch, or any continuous sequence of sleep epochs (any sleep stage). Like for wake bout, the shortest sleep bout duration is 30 seconds, and other durations are multiples of 30 seconds. The total number and median duration of all sleep bouts were calculated for each participant. Three different sleep bout subtypes were described: (1) sleep bout with NREM sleep only, (2) sleep bout with REM sleep only, and (3) sleep bout with REM and NREM sleep. Sleep bout length was categorized as 30 seconds, 1 minute–1 minute 30 seconds, 2 minutes–5 minutes, 5 minutes 30 seconds–14 minutes, 14 minutes 30 seconds–32 minutes 30 seconds, and >32 minutes 30 seconds (10th, 25th, 50th, 75th and 90th percentile of the entire sleep bout set).

Sleep–wake transitions

For each participant, the number of transitions was described (1) from sleep to wake: REM sleep to wake and NREM sleep (NREM1, NREM2, NREM3) to wake and (2) from wake to sleep: wake to REM sleep and wake to NREM sleep (NREM1, NREM2, NREM3). The instability of a sleep stage was defined as the number of transitions to wake during that stage reported to the time spent in that stage (i.e., a high index indicates high instability for that stage).

Statistical analysis

Categorical variables were presented as percentages, quantitative variables as medians with ranges. Participants were categorized according to their CSF ORX-A levels in 2 categories (≤110, >110 pg/mL) and in tertiles (≤26, [26–254], >254 pg/mL). The subgroup of participants with intermediate ORX-A levels (between 110 and 200 pg/mL) was too small to be individualized. The participants' characteristics in the 2 ORX-A categories (ORX deficiency vs ORX nondeficiency) were compared using logistic regression models (univariate analysis). Then, the associations between ORX-A levels, wake bouts, sleep bouts, and transition parameters were adjusted for the characteristics found to be significant in the univariate analysis ($p < 0.10$). The same methodology was used to detect associations between ORX-A levels divided in tertiles, wake and sleep bouts, and transition parameters using multinomial regression models. When comparisons were significant in the 3 ORX-A groups, 2-by-2 comparisons were carried out, by using the Bonferroni method to correct for multiple comparisons. The Pearson correlation coefficient was used to determine associations between continuous variables. Significance was set at $p < 0.05$. Analyses were performed with SAS statistical software (version 9.4; SAS, Cary, NC).

Data availability

Anonymized data not provided in the article because of space limitations will be shared by request from any qualified investigator.

Results

Characteristics of the population

Among the 300 participants, 55% were men, and 23.67% (n = 71) were children (<18 years old). Their mean age was 29.89±15.55 years. All participants were drug-free, and 180 (60%) were drug-naive (never exposed to medication against hypersomnolence) while the others discontinued treatments with an effect on sleep at least 2 weeks before the PSG recording. The mean CSF ORX-A level was 155.12 ± 153.75 pg/mL. It was below 110 pg/mL in 164 patients, among whom 68 had undetectable levels (≤10 pg/mL). ORX levels were intermediate (between 110 and 200 pg/mL) only in 13

patients, with 3 of them having typical cataplexy. Compared with patients with normal and intermediate ORX-A levels (n = 136), patients with low ORX-A levels were younger ($p = 0.02$), more frequently men ($p = 0.04$), with higher BMI ($p = 0.03$), shorter total sleep time ($p = 0.02$), and longer total wake time after sleep onset ($p < 0.0001$). The clinical and electrophysiologic characteristics of the study population categorized according to the ORX-A level tertiles are described in table 1.

Association between wake bouts and CSF ORX-A levels

The total wake bout number was higher and their duration longer in participants with ORX deficiency than in the others (table 2). The shortest wake bouts (30 seconds) were the most numerous in all groups, but their percentage (relative to all wake bouts) was lower in the ORX deficiency group. Conversely, the percentage of wake bouts ≥1 minute 30 seconds

Table 1 Characteristics of the study population, categorized according to tertiles of CSF orexin-A (ORX-A) levels, pg/mL

Variables	ORX-A ≤26 group 1, n = 100	ORX-A (26–254) group 2, n = 100	ORX-A >254 group 3, n = 100	*p* Value	Post hoc, groups
Clinical characteristics					
Sex, men[a]	63 (63.00)	56 (56.00)	46 (46.00)	0.05	—
Age, y[a]	22.00 (5.00–76.00)	26.50 (5.00–77.00)	27.50 (6.00–78.00)	0.19	—
Age, y[b]					
<20	45 (45.00)	26 (26.00)	23 (23.00)	0.006	1 > 2 > 3
20–35	27 (27.00)	41 (41.00)	37 (37.00)		
≥35	31 (31.00)	33 (33.00)	40 (40.00)		
BMI, kg/m^2[a]	25.73 (16.80–43.42)	23.51 (15.61–35.92)	22.70 (13.67–40.80)	0.004	1 > 2 > 3
BMI, overweight/obese[a]	58 (59.79)	42 (42.00)	25 (28.09)	<0.0001	1 > 2, 3
Disturbed nocturnal sleep[c]					
No	14 (23.33)	16 (33.33)	16 (61.54)	0.002	1 < 3
Slightly	14 (23.33)	18 (37.50)	6 (23.08)		
Moderate/severe	32 (53.33)	14 (29.17)	4 (15.38)		
Duration of sleepiness, y[a]	4.00 (0.00–58.00)	5.00 (0.00–65.00)	6.00 (0.00–61.00)	0.10	—
ESS score[a]	18.50 (6.00–24.00)	18.50 (5.00–24.00)	16.00 (6.00–23.00)	0.001	1, 2 < 3
Polysomnography and MSLT					
Total sleep time, h:min[a]	7:21 (1:54–10:05)	7:32 (4:37–8:43)	7:29 (4:36–9:21)	0.02	1 < 2
Sleep efficiency, %[a]	85.26 (24.39–96.50)	90.14 (51.88–98.23)	91.74 (57.14–98.96)	<0.0001	1 < 2 < 3
T-WASO, min:s[a]	75:15 (16:30–309:00)	47:00 (6:30–252:00)	29:00 (6:00–185:00)	<0.0001	1 > 2 > 3
T-WASO, min[b]					
<34	7 (7.00)	34 (34.00)	57 (57.00)	<0.0001	1 > 2 > 3
34–71	37 (37.00)	36 (36.00)	28 (28.00)		
≥71	56 (56.00)	30 (30.00)	15 (15.00)		
AHI/h[a]	2.03 (0.00–62.20)	2.16 (0.00–46.80)	1.71 (0.00–52.37)	0.38	—
Index PLMS/h[a]	3.24 (0.00–60.49)	1.10 (0.00–42.98)	0.00 (0.00–49.40)	0.002	1 > 2 > 3
Microarousal index/h of sleep[a]	14.25 (0.00–68.57)	12.70 (2.30–34.10)	10.74 (0.00–55.66)	0.0006	1 > 2 > 3
MSLT mean sleep latency, min[a]	2.80 (0.00–14.67)	5.80 (0.60–18.20)	8.40 (0.80–20.00)	<0.0001	1 < 2 < 3
Number of SOREMPs (nocturnal and MSLT)[a]	5.00 (1.00–6.00)	3.50 (0.00–6.00)	2.00 (0.00–5.00)	<0.0001	1 > 2 > 3

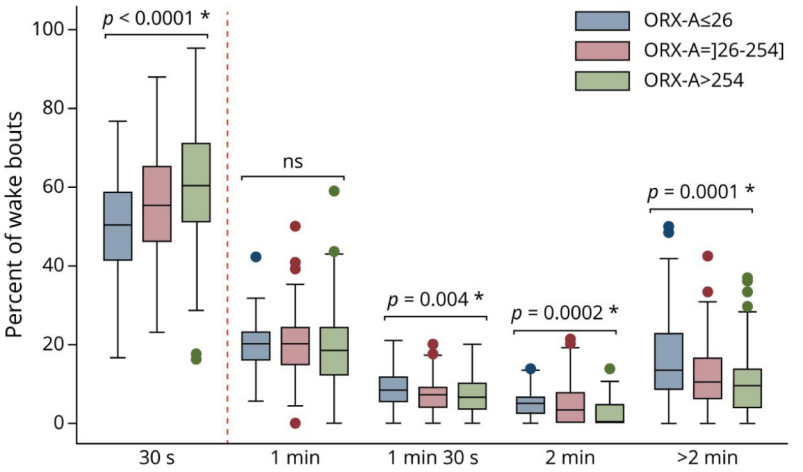
Abbreviations: AHI = apnea–hypopnea index; BMI = body mass index; ESS = Epworth Sleepiness Scale; MSLT = multiple sleep latency test; NREM = non-REM; PLMS = periodic leg movements during sleep; SOREMP = sleep-onset REM period; T-WASO = total wake time after sleep onset.
[a] Values are n (%) or median (minimal value–maximal value) (continuous variables).
[b] Tertiles of the whole sample (n = 300).
[c] Assessed with item 15 of the Narcolepsy Severity Scale.

Table 2 Associations between wake bouts (WBs) during polysomnography and CSF orexin-A (ORX-A) levels, pg/mL

WB measurement	ORX-A ≤110, n = 164 median (min–max)	ORX-A >110, n = 136 median (min–max)	*p*[a]	ORX-A ≤26 group 1, n = 100 median (min–max)	ORX-A (26–254) group 2, n = 100 median (min–max)	ORX-A >254 group 3, n = 100 median (min–max)	*p*[a]	Post hoc, groups
Total WBs, n	43.00 (16.00–99.00)	25.00 (8.00–79.00)	<0.0001[b,c]	45.00 (16.00–99.00)	33.00 (9.00–75.00)	25.00 (8.00–79.00)	<0.0001[b,c]	1 > 2 > 3
Index of WBs/hours of T-WASO	41.44 (3.11–107.14)	50.56 (9.81–114.55)	<0.0001[b]	39.59 (3.11–94.55)	47.03 (8.33–107.14)	51.47 (9.81–114.55)	<0.0001[b]	1 < 2, 3
WB duration, s	30.00 (30.00–165.00)	30.00 (30.00–90.00)	<0.0007[b]	37.50 (30.00–165.00)	30.00 (30.00–90.00)	30.00 (30.00–90.00)	0.001[b]	1 > 3
Number of WBs of 30 s	21.00 (4.00–53.00)	15.50 (3.00–55.00)	<0.0001[b,c]	22.00 (4.00–53.00)	19.00 (3.00–41.00)	16.00 (3.00–55.00)	<0.0001[b,c]	1 > 2 > 3
% Among all WBs	51.29 (16.67–88.00)	60.81 (16.00–95.24)	<0.0001[b]	50.29 (16.67–76.67)	55.36 (23.08–88.00)	60.30 (16.00–95.24)	<0.0001[b]	1 < 2, 3
Number of WBs of 1 min	9.00 (0.00–21.00)	5.00 (0.00–17.00)	<0.0001[b,c]	9.00 (2.00–21.00)	6.00 (0.00–17.00)	5.00 (0.00–14.00)	<0.0001[b,c]	1 > 2 > 3
% Among all WBs	20.13 (0.00–42.11)	18.52 (0.00–58.82)	0.52	20.13 (5.56–42.11)	20.00 (0.00–50.00)	18.49 (0.00–58.82)	0.91	—
Number of WBs of 1 min 30 s	3.00 (0.00–15.00)	2.00 (0.00–6.00)	<0.0001[b,c]	4.00 (0.00–15.00)	2.00 (0.00–15.00)	1.50 (0.00–6.00)	<0.0001[b,c]	1 > 2 > 3
% Among all WBs	7.69 (0.00–20.97)	6.67 (0.00–20.00)	0.02[b]	8.33 (0.00–20.97)	7.06 (0.00–20.00)	6.50 (0.00–20.00)	0.004[b]	1 > 2, 3
Number of WBs of 2 min	2.00 (0.00–9.00)	0.00 (0.00–4.00)	<0.0001[b,c]	2.00 (0.00–8.00)	1.00 (0.00–9.00)	0.50 (0.00–4.00)	<0.0001[b,c]	1 > 2 > 3
% Among all WBs	4.49 (0.00–21.21)	0.00 (0.00–20.00)	<0.0002[b,c]	5.03 (0.00–13.89)	3.39 (0.00–21.21)	0.63 (0.00–13.79)	0.0003[b,c]	1, 2 > 3
Number of WBs >2 min	6.00 (0.00–25.00)	2.00 (0.00–21.00)	<0.0001[b,c]	6.00 (0.00–25.00)	4.00 (0.00–16.00)	2.00 (0.00–21.00)	<0.0001[b,c]	1 > 2 > 3
% Among all WBs	13.18 (0.00–50.00)	9.20 (0.00–36.84)	<0.0001[b]	13.42 (0.00–50.00)	10.39 (0.00–42.31)	9.60 (0.00–36.84)	<0.0001[b]	1 > 2, 3

Abbreviation: T-WASO = total wake time after sleep onset.
[a] Adjustment for age, sex, and body mass index.
[b] Still significant after adjustment for all the covariates in model 0 plus total sleep time.
[c] Still significant after adjustment for all the covariates in model 0 plus T-WASO, when applicable.

Figure 1 Percentages of wake bouts of different durations categorized according to tertiles of CSF orexin-A (ORX-A) levels



*Still significant after adjustment for age, sex, and body mass index. ORX-A levels are in pg/mL. ns = nonsignificant.

was higher in this group (table 2). These results were unchanged after adjustment for age, sex, and BMI. To investigate a potential dose–response effect, the wake bout parameters were then compared in participants categorized in tertiles of CSF ORX-A. Participants in the lowest tertile had more wake bouts, and of longer duration, than participants classified in the other 2 tertiles, with a significant dose–response effect in post hoc comparisons (table 2). Similarly, the percentage of wake bouts ≥1 minute increased progressively with the reduction of CSF ORX-A concentration (table 2). Compared with the other 2 tertiles, the percentage of very short wake bouts (30 seconds) was lower and that of wake bouts ≥1 minute 30 seconds was higher in the lowest tertile (figure 1). All results remained significant after adjustment for age, sex, and BMI. The results remained almost unchanged when analyzing the index of wake bouts per hour of total wake time after sleep onset, and after adjusting the wake bout parameters for total sleep time or wake time after sleep onset (table 2). Wake bout results were comparable in patients in the lowest tertile and in those with undetectable CSF ORX-A levels. Results were unchanged also after excluding alternatively adults, children, patients with PLMS index >5/h and AHI >5/h, and nontreatment naive patients. A negative correlation was found between wake bout number and CSF ORX-A levels ($r = -0.51$, $p < 0.0001$), and was significant only in the ORX deficiency group. We also compared the wake bout parameters according to the diagnostic groups (NT1, NT2, IH, and EDS without central disorder of hypersomnolence) and found differences between the groups for sex, BMI, total sleep time, sleep efficiency, and wake time after sleep onset (table e-1, doi.org/10.5061/dryad.bg79cnp85). After adjustment for sex and BMI, or sex, BMI, and total sleep time, between-group differences were found for the number and duration of wake bouts, with patients with NT1 having increased number of wake bouts compared to other groups (table e-1, doi.org/10.5061/dryad.bg79cnp85). No wake bout differences were found between other groups.

Association between sleep bouts and CSF ORX-A levels

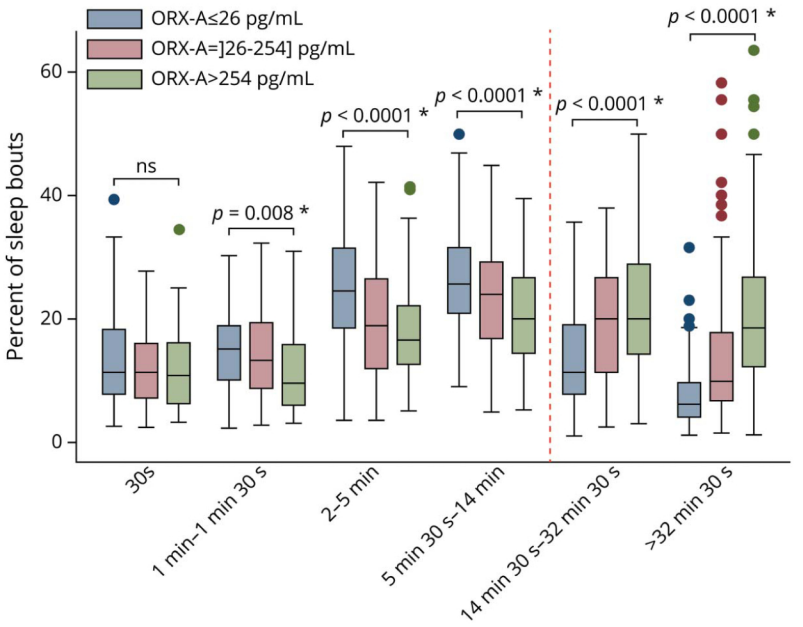
The total sleep bout number was higher and their duration shorter in participants with ORX deficiency. All sleep bout subtypes (i.e., NREM, REM, and NREM-REM sleep bout) were more frequent in the ORX-A <110 pg/mL category, particularly NREM sleep bouts (table 3). The percentage of sleep bout ≤14 minutes was higher in patients with ORX deficiency, with opposite results found for longer sleep bout (table 3). These results remained unchanged after adjustment for age, sex, and BMI. Sleep bout parameters were then compared in groups categorized in tertiles of ORX-A levels. The total sleep bout number was higher and their duration shorter in the lowest tertile, with a dose–response effect in post hoc analyses (table 3). This dose–response effect was also observed when sleep bouts were divided according to their duration (table 3). The percentage of sleep bouts ≤14 minutes was higher and that of sleep bouts >14 minutes was lower in the lowest ORX tertile than in the other 2 (figure 2). All results remained significant after adjustment for age, sex, and BMI. The results remained almost unchanged when analyzing the index of sleep bouts per hour of total sleep time, and after adjusting the sleep bout parameters for total sleep time or wake time after sleep onset (table 3). Results were comparable in patients in the lowest tertile and in those with undetectable ORX levels. They remained unchanged in the sensibility analyses to assess the different subpopulations, as previously described for wake bouts. A negative correlation was found between sleep bout number and CSF ORX-A levels ($r = -0.51$, $p < 0.0001$), but was significant only in the ORX deficiency group. After adjustment for sex and BMI, or sex, BMI, and total sleep time or wake time after sleep onset, the number and duration of sleep bouts differed between groups, with patients with NT1 having increased number and decreased duration of sleep bouts compared to other groups (table e-1, doi.org/10.5061/dryad.bg79cnp85). No sleep bout differences were found between other groups.

Table 3 Associations between sleep bouts (SBs) of different durations and types and CSF orexin-A (ORX-A) levels, pg/mL

	ORX-A ≤110, n = 164	ORX-A >110, n = 136		ORX-A ≤26 group 1, n = 100	ORX-A (26–254) group 2, n = 100	ORX-A >254 group 3, n = 100		
SB measurement	**median (min–max)**	**median (min–max)**	***p*[a]**	**median (min–max)**	**median (min–max)**	**median (min–max)**	***p*[a]**	**Post hoc, groups**
Total n	43.00 (16.00–100.0)	25.50 (9.00–80.00)	<0.0001[b,c]	45.50 (16.00–100.00)	34.00 (9.00–75.00)	26.00 (9.00–80.00)	<0.0001[b,c]	1 > 2 > 3
Index of SBs/hour of TST	5.98 (2.43–21.54)	3.45 (1.07–12.75)	<0.0001[c]	6.45 (2.45–21.54)	4.41 (1.07–15.00)	3.46 (1.18–12.75)	<0.0001[c]	1 > 2 > 3
SB duration, min:s	5:30 (1:00–24:30)	10:00 (1:15–38:45)	<0.0001[b,c]	5:00 (1:00–21:00)	7:45 (1:30–38:45)	10:00 (1:15–36:30)	<0.0001[b,c]	1 < 2 < 3
Number of SBs of NREM sleep	29.00 (5.00–88.00)	16.00 (1.00–72.00)	<0.0001[b,c]	31.00 (5.00–88.00)	24.00 (4.00–69.00)	16.00 (1.00–72.00)	<0.0001[b,c]	1 > 2 > 3
Duration, min:s	3:30 (1:00–30:00)	5:48 (1:00–59:30)	<0.0001[b,c]	3:08 (1:00–30:00)	4:30 (1:00–59:30)	5:53 (0:30–34:00)	0.0004[b]	1 < 2, 3
Number of SBs of REM sleep	3.00 (0.00–19.00)	0.00 (0.00–12.00)	<0.0001[b,c]	3.00 (0.00–17.00)	1.00 (0.00–19.00)	1.00 (0.00–12.00)	<0.0001[b,c]	1 > 2, 3
Duration, min:s	4:30 (1:00–29:30)	5:38 (1:00–28:45)	0.06	4:23 (0:30–28:00)	4:30 (0:30–29:30)	5:00 (0:30–28:45)	0.10	—
Number of SBs of NREM and REM	9.00 (2.00–30.00)	7.00 (2.00–38.00)	0.0002[b,c]	9.00 (2.00–30.00)	8.00 (2.00–22.00)	7.00 (2.00–38.00)	0.002[b,c]	1, 2 < 3
Duration, min:s	12:30 (3:00–45:30)	24:23 (4:15–114:15)	<0.0001[b,c]	11:08 (3:00–45:30)	17:08 (4:15–114:00)	25:00 (4:10–75:45)	<0.0001[b,c]	1 < 2 < 3
Number of SBs of 30 s	5.00 (0.00–29.00)	2.00 (0.00–20.00)	<0.0001[b]	5.00 (1.00–29.00)	4.00 (1.00–20.00)	3.00 (1.00–20.00)	<0.0001[b]	1 > 2, 3
% Among all SBs	11.37 (2.38–39.44)	10.71 (2.94–34.48)	0.01[c]	11.32 (2.56–39.44)	11.32 (2.38–27.78)	10.81 (3.28–34.48)	0.07	—
Number of SBs 1 min–1 min 30	5.00 (0.00–30.00)	2.00 (0.00–20.00)	<0.0001[b]	6.00 (1.00–30.00)	4.00 (1.00–24.00)	3.00 (1.00–18.00)	<0.0001[b]	1 > 2 > 3
% Among all SBs	14.00 (2.27–32.00)	10.00 (3.03–32.26)	0.003[b]	15.07 (2.27–30.30)	13.24 (2.78–32.26)	9.65 (3.03–31.03)	0.01[b]	1 > 3
Number of SBs 2 min–5 min	10.00 (0.00–43.00)	4.00 (0.00–27.00)	<0.0001[b,c]	12.00 (1.00–43.00)	7.00 (1.00–25.00)	4.00 (1.00–27.00)	<0.0001[b,c]	1 > 2 > 3
% Among all SBs	23.68 (3.45–47.95)	16.13 (4.55–42.11)	<0.0001[b,c]	24.59 (3.57–47.95)	18.92 (3.45–42.11)	16.67 (5.00–41.30)	<0.0001[b,c]	1 > 2, 3
Number of SBs 5 min 30–14 min	11.00 (2.00–23.00)	6.00 (0.00–21.00)	<0.0001[b,c]	12.00 (3.00–23.00)	8.00 (1.00–20.00)	5.00 (1.00–21.00)	<0.0001[b,c]	1 > 2 > 3
% Among all SBs	25.00 (4.88–50.00)	20.00 (5.26–39.53)	<0.0001[b,c]	25.65 (9.00–50.00)	24.00 (4.88–44.83)	20.00 (5.26–39.53)	0.0007[b,c]	1 > 3
Number of SBs 14 min 30–32 min 30	6.00 (1.00–14.00)	6.00 (1.00–12.00)	0.19	6.00 (1.00–12.00)	6.00 (1.00–14.00)	5.50 (1.00–12.00)	0.12	—
% Among all SBs	13.33 (1.01–37.93)	20.00 (3.08–50.00)	<0.0001[b,c]	11.34 (1.01–35.71)	20.00 (2.50–37.93)	20.00 (3.08–50.00)	<0.0001[b,c]	1 < 2, 3
Number of SBs >32 min 30	3.00 (0.00–7.00)	5.00 (1.00–8.00)	<0.0001[b,c]	3.00 (1.00–7.00)	3.00 (1.00–8.00)	5.00 (1.00–8.00)	<0.0001[b,c]	1 < 2 < 3
% Among all SBs	7.32 (1.18–36.84)	18.35 (1.25–63.64)	<0.0001[b,c]	6.13 (1.18–31.58)	9.88 (1.47–58.33)	18.52 (1.25–63.64)	<0.0001[b,c]	1 < 2 < 3

Abbreviations: NREM = non-REM; TST = total sleep time.
[a] Adjustment for age, sex, and body mass index.
[b] Still significant after adjustment for all the covariates in model 0 plus TST, when applicable.
[c] Still significant after adjustment for all the covariates in model 0 plus total wake time after sleep onset.

Figure 2 Percentages of sleep bouts of different durations categorized according to tertiles of CSF orexin-A (ORX-A) levels



*Still significant after adjustment for age, sex, and body mass index. ORX-A levels are in pg/mL. ns = nonsignificant.

Sleep–wake transitions and stability of nighttime sleep

The number of sleep to wake and wake to sleep transitions was higher in patients with CSF ORX-A levels ≤110 pg/mL than >110 pg/mL in crude and adjusted models. The number of transitions from all sleep stages (i.e., REM sleep, NREM sleep, NREM 1, NREM 2, NREM 3) to wake was higher in the ORX deficiency category (table 4), particularly for transitions from NREM 2 to wake. The sleep instability indexes (any sleep stage) were higher in participants with ORX deficiency (table 4). When the population was categorized according to the tertiles of CSF ORX-A concentration, the number of sleep–wake transitions and the instability indexes (any sleep stage) were higher in participants in the low than in the median and high tertiles, with a dose–response effect of ORX-A levels in post hoc comparisons (table 4). All results were significant after adjustment for age, sex, and BMI, and almost unchanged after adjusting for total sleep time or wake time after sleep onset. They were confirmed in the sensibility analyses, as described for sleep and wake bouts. After adjustment for sex and BMI, or sex, BMI, and total sleep time or wake time after sleep onset, the sleep–wake transitions differed between groups, with patients with NT1 having increased sleep–wake transitions compared to other groups.

Discussion

Using reliable PSG markers, our study shows ORX-A stabilizing effect on nocturnal sleep in a large clinical sample of patients with hypersomnolence and different CSF ORX-A levels. The following main findings were obtained: (1) the number and frequency of wake bouts, sleep bouts, and sleep–wake transitions were higher in participants with than without ORX deficiency (i.e., CSF ORX-A concentration ≤110 pg/mL); (2) wake bout duration was longer and sleep bout shorter in the group with ORX deficiency; (3) the percentage of very short wake bouts (30 seconds) was lower, whereas the percentage of wake bouts ≥1 minute 30 seconds was higher in the ORX deficiency category; and (4) the percentage of sleep bouts ≤14 minutes was higher and that of longer sleep bouts was lower among patients with than without ORX deficiency. The subsequent analyses performed in the population categorized according to the tertiles of CSF ORX-A concentration confirmed all these findings, with a dose–response effect of ORX-A levels in post hoc comparisons.

Sleep is conventionally scored in humans using 30-second epochs, and most methods for assessing sleep quality focus on the time spent in sleep stages throughout the night, and on other typical parameters of nocturnal sleep, such as total sleep time, sleep efficiency, microarousal indexes, and wake time after sleep onset.[21] In this study, we propose an objective method to quantify the dynamic processes during sleep: the analysis of the number, duration, and proportion of sleep and wake bouts, and of transitions between sleep and wake. These parameters allow an accurate evaluation of sleep continuity, stability, and fragmentation. Sleep and wake bout analysis has been used previously in animal studies, whereas its use in humans remains limited. Few studies have described methods for modeling bout distribution,[24,25] and some authors used them to investigate the characteristics of disturbed nighttime sleep in fibromyalgia and insomnia,[26,27] and the effect of aging on sleep continuity.[28,29] To our knowledge, no study has used this method in patients with hypersomnolence disorders.

Table 4 Transitions from sleep to wake, wake to sleep, and instability of sleep stages as a function of CSF orexin-A (ORX-A) levels, pg/mL

Transitions measurement	ORX-A ≤110, n = 164 median (min–max)	ORX-A >110, n = 136 median (min–max)	p^a	ORX-A ≤26 group 1, n = 100 median (min–max)	ORX-A (26–254) group 2, n = 100 median (min–max)	ORX-A >254 group 3, n = 100 median (min–max)	p^a	Post hoc, groups
Transitions sleep → wake, n	43.00 (16.00–99.00)	25.00 (8.00–79.00)	<0.0001[b,c]	45.00 (16.00–99.00)	33.00 (9.00–75.00)	25.00 (8.00–79.00)	<0.0001[b,c]	1 > 2 > 3
Transitions REM sleep → wake, n	8.00 (0.00–28.00)	5.00 (0.00–39.00)	<0.0001[b,c]	9.00 (1.00–28.00)	6.00 (0.00–26.00)	5.00 (1.00–39.00)	<0.0001[b,c]	1 > 2, 3
REM sleep instability index	1.96 (0.00–89.03)	0.90 (0.00–23.03)	<0.0001[b]	2.01 (0.19–89.03)	1.39 (0.00–8.89)	0.93 (0.15–23.03)	<0.0001[b]	1 > 2, 3
Transitions NREM → wake, number	32.00 (9.00–88.00)	19.00 (2.00–72.00)	<0.0001[b,c]	35.00 (11.00–88.00)	26.00 (6.00–71.00)	19.00 (2.00–72.00)	<0.0001[b,c]	1 > 2 > 3
NREM sleep instability index	5.75 (1.61–23.01)	3.21 (0.31–13.19)	<0.0001[b,c]	6.43 (2.22–23.01)	4.56 (1.14–19.32)	3.28 (0.31–13.19)	<0.0001[b,c]	1 > 2 > 3
Transitions NREM 1 → wake, n	10.00 (0.00–53.00)	3.00 (0.00–34.00)	<0.0001[b,c]	10.00 (1.00–53.00)	6.00 (0.00–34.00)	3.00 (0.00–34.00)	<0.0001[b,c]	1 > 2 > 3
NREM 1 instability index	15.60 (0.00–48.00)	11.18 (0.00–38.18)	<0.0001[b]	15.94 (2.40–48.00)	13.58 (0.00–45.52)	11.63 (0.00–38.18)	0.0003[b]	1, 2 > 3
Transitions NREM 2 → wake, n	18.00 (4.00–63.00)	13.00 (0.00–63.00)	<0.0001[b,c]	19.00 (4.00–63.00)	15.50 (4.00–42.00)	12.50 (0.00–63.00)	0.0002[b]	1 > 2, 3
NREM 2 instability index	5.48 (1.31–21.14)	3.51 (0.00–13.90)	<0.0001[b,c]	5.97 (1.56–21.14)	4.37 (1.29–19.43)	3.47 (0.00–13.90)	<0.0001[b,c]	1 > 2 > 3
Transitions NREM 3 → wake, n	3.00 (0.00–12.00)	2.00 (0.00–7.00)	0.0004[b,c]	3.00 (0.00–12.00)	2.50 (0.00–11.00)	2.00 (0.00–7.00)	0.008[b,c]	1 > 3
NREM 3 instability index	1.94 (0.00–8.00)	1.63 (0.00–6.76)	0.0005[b,c]	2.13 (0.00–8.00)	1.66 (0.00–6.84)	1.66 (0.00–6.76)	0.0005[b,c]	1 > 2, 3
Transitions wake → sleep, n	43.00 (16.00–100.0)	25.50 (9.00–80.00)	<0.0001[b,c]	45.50 (16.00–100.00)	34.00 (9.00–75.00)	26.00 (9.00–80.00)	<0.0001[b,c]	1 > 2 > 3
Transitions wake → REM, n	5.00 (0.00–22.00)	2.00 (0.00–15.00)	<0.0001[b,c]	5.00 (0.00–21.00)	3.00 (0.00–22.00)	2.00 (0.00–15.00)	<0.0001[b,c]	1 > 2 > 3
Transitions wake → NREM, n	36.00 (13.00–91.00)	23.00 (4.00–73.00)	<0.0001[b,c]	38.00 (13.00–91.00)	31.00 (8.00–72.00)	22.50 (4.00–73.00)	<0.0001[b,c]	1 > 2 > 3

Abbreviation: NREM = non-REM.
Continuous variables are expressed as median (minimal value; maximal value). Instability index of a stage of sleep = number of transitions from this stage to wake/time spent in this stage.
[a] Adjustment for age, sex, and body mass index.
[b] Still significant after adjustment for all the covariates in model 0 plus total sleep time.
[c] Still significant after adjustment for all the covariates in model 0 plus total wake time after sleep onset.

The ORX effect on sleep stability in humans has been rarely studied, because ORX level is measured in CSF, which can be obtained only by lumbar puncture, an invasive procedure. We found a remarkably robust dose–response effect of ORX-A levels on markers of nocturnal sleep fragmentation (i.e., sleep and wake bouts and sleep/wake transitions). Nighttime sleep was more fragmented and unstable in patients in the middle and particularly low tertiles of ORX-A concentration. Our results are in agreement with a previous study showing the influence of ORX-A levels on the sleep–wake transitions.[16] On the basis of the current knowledge about the neurobiological processes underlying sleep and wake regulation,[3] our results suggest that ORX promotes wakefulness, but also stabilizes wakefulness and NREM and REM sleep. Although NT1 is often considered as a "pathology of dysregulation of REM sleep," this cannot explain the presence of sleepiness during the day, the short latency of both NREM and REM sleep during nocturnal and nap recordings, and the abnormal distribution of slow-wave sleep observed during the nocturnal recording.[5] We report that patients with low ORX-A levels also presented less consolidated NREM sleep than controls, with increased number of sleep bouts in both REM and NREM sleep, which confirms previous results.[5,8,14,15]

Low CSF ORX-A level is the gold standard (highly specific and sensitive) for the diagnosis of NT1,[22] but the threshold of 110 pg/mL remains questionable in some situations. Although all values need to be back-referenced to the Stanford reference samples, the large fluctuating radioactivity between radioimmunoassay batches prevents validation of the CSF ORX-A threshold (i.e., 110 pg/mL) established for NT1 diagnosis with high accuracy. Therefore, in this study, we categorized patients according to this threshold and also according to the tertiles of CSF ORX-A level. CSF ORX-A concentrations lower than 1/3 of the mean value obtained in normal participants with the same standardized assay is another accepted diagnostic criterion of NT1 according to the ICSD-3.[20] Normal or intermediate ORX-A level is one of the current diagnostic criteria for NT2; however, it has been suggested that NT2 could be, in some cases, caused by partial loss of ORX neurons.[30] Moreover, some patients with narcolepsy and typical cataplexy may have normal or intermediate ORX-A levels (i.e., 3 patients in the present study), and some without cataplexy may have low ORX-A levels.[7] Thus, whether NT2 and NT1 are separate disease categories or share a common pathophysiology remains unclear.[7] The subgroup of patients with CSF ORX-A levels between 110 and 200 pg/mL was too small to be individualized for further statistical analysis. We compared the sleep and wake bouts and sleep–wake transitions according to the diagnostic groups (NT1, NT2, IH, and EDS without central disorder of hypersomnolence) and found differences between NT1 and the other groups. These differences were expected as NT1 was associated with low CSF ORX-A levels. In contrast, we found no difference between the other groups; however, our sample of patients without NT1 is small, especially when subdivided by patient and age groups. IH is an orphan disorder and little is known about its pathophysiology. It might also partially overlap with NT2.[7,31] Whether sleep and wake bouts (markers of nighttime sleep stability and fragmentation) may be used as diagnostic tools to better characterize and differentiate central disorders of hypersomnolence (NT2, NT2, IH, and others) independently of ORX levels remain unknown. In a recent study, an artificial intelligence algorithm could discriminate patients with narcolepsy by analyzing their nocturnal sleep features.[32] Other studies showed the reliability of nocturnal sleep dynamics for the differential diagnosis of central disorders of hypersomnolence.[14] We plan to further test the specificity and sensitivity of the different features (number, duration, proportion) of sleep and wake bouts and sleep–wake transitions in a larger population affected by different hypersomnolence conditions, selecting enough patients by pathology and age group. The main goals will be to better identify biomarkers of NT2 and IH (with and without long sleep time) relative to NT1 and nonspecified hypersomnolence disorders and to assess their associations with complaints of disturbed nighttime sleep, EDS, sleep inertia, and overall severity.[19]

Currently, ORX peptides are receiving much attention as endogenous, potent, arousal-promoting neurotransmitters. Some ORX-receptor antagonists have recently become available to treat insomnia,[33] one of the most frequent sleep disorders, which affects around 100 million people worldwide.[34] Several preclinical studies have explored the effect of these antagonists on sleep.[35] A recent study quantified wake bouts in patients treated with suvorexant, one of these antagonists.[36] The authors showed that patients with insomnia taking suvorexant returned to sleep from their longest awakening more than twice faster than those on placebo. Moreover, the number and time spent in long wake bout (>2 minutes) were reduced in the treated group. The authors suggested that suvorexant reduces wake time after sleep onset by decreasing long wake bouts. This finding could be in accordance with our observation that wake bouts >2 minutes were more numerous in participants in the lowest tertile of ORX-A compared with participants in median and higher tertile, with a dose-dependent effect. This suggests that the markers of nocturnal sleep stability we studied here could be used to test the effect of drugs in patients with NT1, especially ORX-based therapies that are expected in the near future.[37] However, in a mouse model of narcolepsy, the proportion of brief wake bouts (<1 minute) increased and the proportion of long wake bouts decreased upon loss of ORX neurons.[38] Such discrepancy with our current results might be explained by sleep structure differences between mice and humans, and the exclusive analysis of nighttime and not daytime sleep in our study.

We acknowledge some limitations in our study. As participants were recruited in the National Reference Center for Narcolepsy and Rare Hypersomnias, they all presented with an initial complaint of hypersomnolence. However, performing lumbar puncture to measure ORX-A level in healthy controls and patients with insomnia would have been very difficult for ethical reasons. All lumbar punctures were

performed at the same time of the day, and ORX-A was measured in a single laboratory using radioimmunoassay, currently the standard method for its quantification. However, this technique presents some variability between batches, low accuracy due to fluctuating radioactivity even if the values were back-referenced to the Stanford reference samples, and cross-reactions with matrix constituents generating interference.[39] These limitations justified the categorization of CSF ORX-A levels according to the threshold of 110 pg/mL but also into tertiles. However, the participants' categorization as a function of the ORX-A tertiles individualized patients with different hypersomnolence disorders, especially in the median tertile (ORX levels ranging from 26 to 254 pg/mL), in contrast to the group with values below 26 pg/mL, which included by definition only patients with NT1. However, sleep bout, wake bout, and sleep/wake transition results were almost comparable when patients were categorized in 4 groups (undetectable CSF ORX-A levels, n = 68; low levels, n = 96; intermediate levels, n = 13; and normal levels, n = 123). In addition, no differences were found for sleep bout, wake bouts, and sleep/wake transition results between patients in the lowest tertile (≤26 pg/mL, n = 100) and in those with undetectable CSF ORX-A (≤10 pg/mL, n = 68). Overall, with this global categorization, we found a dose–response effect of ORX-A levels on sleep and wake bout frequency and duration. Additional studies are needed to determine whether these markers of nocturnal sleep fragmentation can efficiently discriminate different hypersomnolence disorders and their severity. The large variabilities of total sleep time and wake time after sleep onset between participants, and the fact that these variables are highly correlated with sleep and wake bouts (multicollinearity of these parameters), make the adjustment on these variables questionable. However, most of the results remained unchanged before and after these adjustments.

We followed a strict methodology in which patients' evaluation was standardized, at a single site, with a comprehensive systematic examination by sleep experts, and PSG recording in standardized sleep laboratory conditions. We recruited a large population of 300 patients with hypersomnolence of various etiologies, different CSF ORX-A levels, drug-free at the time of PSG recording and lumbar puncture. The robustness of our findings was confirmed by several statistical approaches.

This study shows an association between ORX levels and nocturnal sleep stabilization in patients with hypersomnolence. Sleep and wake bouts are reliable markers of nocturnal sleep stability that correlate with CSF ORX-A levels in a dose-dependent way. These promising PSG biomarkers could be used in clinical and research settings.

Acknowledgment

The authors thank the study participants and the collaborators at the Sleep Disorders Center of Montpellier, National Reference Network for Narcolepsy, France.

Study funding

No targeted funding reported.

Disclosure

Y. Dauvilliers received funds for seminars, board engagements, and travel to conferences by UCB Pharma, Jazz, Theranexus, Flamel, and Bioprojet. R. Lopez received funds for speaking by UCB Pharma and Shire. L. Barateau, A.-L. Rassu, S. Chenini, S. Scholz, J.P. Cristol, M. Lotierzo, and I. Jaussent report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Publication history

Received by *Neurology* January 10, 2020. Accepted in final form June 25, 2020.

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References

1. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998;18:9996–10015.
2. Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001;24:726–731.
3. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. Nature 2005;437:1257.
4. Dauvilliers Y, Arnulf I, Mignot E. Narcolepsy with cataplexy. Lancet 2007;369: 499–511.
5. Dauvilliers Y, Billiard M, Montplaisir J. Clinical aspects and pathophysiology of narcolepsy. Clin Neurophysiol 2003;114:2000–2017.
6. Scammell TE. Narcolepsy. N Engl J Med 2015;373:2654–2662.
7. Bassetti CLA, Adamantidis A, Burdakov D, et al. Narcolepsy: clinical spectrum, aetiopathophysiology, diagnosis and treatment. Nat Rev Neurol 2019;15:519–539.
8. Roth T, Dauvilliers Y, Mignot E, et al. Disrupted nighttime sleep in narcolepsy. J Clin Sleep Med 2013;9:955–965.
9. Trachsel L, Tobler I, Achermann P, Borbély AA. Sleep continuity and the REM-nonREM cycle in the rat under baseline conditions and after sleep deprivation. Physiol Behav 1991;49:575–580.
10. Franken P, Malafosse A, Tafti M. Genetic determinants of sleep regulation in inbred mice. Sleep 1999;22:155–169.
11. Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. Behavioral state instability in orexin knock-out mice. J Neurosci 2004;24:6291–6300.
12. Diniz Behn CG, Kopell N, Brown EN, Mochizuki T, Scammell TE. Delayed orexin signaling consolidates wakefulness and sleep: physiology and modeling. J Neurophysiol 2008;99:3090–3103.
13. Diniz Behn CG, Klerman EB, Mochizuki T, Lin S-C, Scammell TE. Abnormal sleep/wake dynamics in orexin knockout mice. Sleep 2010;33:297–306.
14. Pizza F, Vandi S, Iloti M, et al. Nocturnal sleep dynamics identify narcolepsy type 1. Sleep 2015;38:1277–1284.
15. Schoch SF, Werth E, Poryazova R, Scammell TE, Baumann CR, Imbach LL. Dysregulation of sleep behavioral states in narcolepsy. Sleep 2017;40:doi: 10.1093/sleep/zsx170.
16. Hansen MH, Kornum BR, Jennum P. Sleep-wake stability in narcolepsy patients with normal, low and unmeasurable hypocretin levels. Sleep Med 2017;34:1–6.
17. Sorensen GL, Knudsen S, Jennum P. Sleep transitions in hypocretin-deficient narcolepsy. Sleep 2013;36:1173–1177.
18. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540–545.
19. Dauvilliers Y, Beziat S, Pesenti C, et al. Measurement of narcolepsy symptoms: the narcolepsy severity scale. Neurology 2017;88:1358–1365.
20. AASM: American Academy of Sleep Medicine. ICSD-3: International Classification of Sleep Disorders, 3rd ed. Darien, IL: American Academy of Sleep Medicine; 2014.
21. Iber C, Ancoli-Israel S, Chesson A, Quan S; for the American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, 1st ed. Westchester, NY: American Academy of Sleep Medicine; 2007.
22. Mignot E, Lammers GJ, Ripley B, et al. The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. Arch Neurol 2002;59:1553–1562.
23. Dauvilliers Y, Baumann CR, Carlander B, et al. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions. J Neurol Neurosurg Psychiatry 2003;74:1667–1673.
24. Lo C-C, Chou T, Penzel T, et al. Common scale-invariant patterns of sleep-wake transitions across mammalian species. Proc Natl Acad Sci USA 2004;101:17545–17548.
25. Norman RG, Scott MA, Ayappa I, Walsleben JA, Rapoport DM. Sleep continuity measured by survival curve analysis. Sleep 2006;29:1625–1631.
26. Roth T, Bhadra-Brown P, Pitman VW, Roehrs TA, Resnick EM. Characteristics of disturbed sleep in patients with fibromyalgia compared with insomnia or with pain-free volunteers. Clin J Pain 2016;32:302–307.
27. Roth T, Bhadra-Brown P, Pitman VW, Resnick EM. Pregabalin improves fibromyalgia-related sleep disturbance. Clin J Pain 2016;32:308–312.
28. Klerman EB, Davis JB, Duffy JF, Dijk D-J, Kronauer RE. Older people awaken more frequently but fall back asleep at the same rate as younger people. Sleep 2004;27: 793–798.
29. Klerman EB, Wang W, Duffy JF, Dijk D-J, Czeisler CA, Kronauer RE. Survival analysis indicates that age-related decline in sleep continuity occurs exclusively during NREM sleep. Neurobiol Aging 2013;34:309–318.
30. Thannickal TC, Nienhuis R, Siegel JM. Localized loss of hypocretin (orexin) cells in narcolepsy without cataplexy. Sleep 2009;32:993–998.
31. Baumann CR, Mignot E, Lammers GJ, et al. Challenges in diagnosing narcolepsy without cataplexy: a consensus statement. Sleep 2014;37:1035–1042.
32. Stephansen JB, Olesen AN, Olsen M, et al. Neural network analysis of sleep stages enables efficient diagnosis of narcolepsy. Nat Commun 2018;9:5229.
33. Herring WJ, Snyder E, Budd K, et al. Orexin receptor antagonism for treatment of insomnia: a randomized clinical trial of suvorexant. Neurology 2012;79:2265–2274.
34. Morin CM, Drake CL, Harvey AG, et al. Insomnia disorder. Nat Rev Dis Primers 2015;1:15026.
35. Brisbare-Roch C, Dingemanse J, Koberstein R, et al. Promotion of sleep by targeting the orexin system in rats, dogs and humans. Nat Med 2007;13:150–155.
36. Svetnik V, Snyder ES, Tao P, et al. Insight into reduction of wakefulness by suvorexant in patients with insomnia: analysis of wake bouts. Sleep 2018;41:doi: 10.1093/sleep/zsx178.
37. Barateau L, Dauvilliers Y. Recent advances in treatment for narcolepsy. Ther Adv Neurol Disord 2019;12:1756286419875622.
38. Branch AF, Navidi W, Tabuchi S, et al. Progressive loss of the orexin neurons reveals dual effects on wakefulness. Sleep 2016;39:369–377.
39. Hirtz C, Vialaret J, Gabelle A, Nowak N, Dauvilliers Y, Lehmann S. From radioimmunoassay to mass spectrometry: a new method to quantify orexin-A (hypocretin-1) in cerebrospinal fluid. Sci Rep 2016;6:25162.