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Rapid eye movement sleep behaviour disorder in patients with narcolepsy is associated with hypocretin-1 deficiency

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Rapid eye movement sleep behaviour disorder is characterized by dream-enacting behaviour and impaired motor inhibition during rapid eye movement sleep. Rapid eye movement sleep behaviour disorder is commonly associated with neurodegenerative disorders, but also reported in narcolepsy with cataplexy. Most narcolepsy with cataplexy patients lack the sleep–wake, and rapid eye movement sleep, motor-regulating hypocretin neurons in the lateral hypothalamus. In contrast, rapid eye movement sleep behaviour disorder and hypocretin deficiency are rare in narcolepsy without cataplexy. We hypothesized that rapid eye movement sleep behaviour disorder coexists with cataplexy in narcolepsy due to hypocretin deficiency. In our study, rapid eye movement sleep behaviour disorder was diagnosed by the International Classification of Sleep Disorders (2nd edition) criteria in 63 narcolepsy patients with or without cataplexy. Main outcome measures were: rapid eye movement sleep behaviour disorder symptoms; short and long muscle activations per hour rapid eye movement and non-rapid eye movement sleep; and periodic and non-periodic limb movements per hour rapid eye movement and non-rapid eye movement sleep. Outcome variables were analysed in relation to cataplexy and hypocretin deficiency with uni- and multivariate logistic/linear regression models, controlling for possible rapid eye movement sleep behaviour disorder biasing factors (age, gender, disease duration, previous anti-cataplexy medication). Only hypocretin deficiency independently predicted rapid eye movement sleep behaviour disorder symptoms (relative risk = 3.69, $P = 0.03$), long muscle activations per hour rapid eye movement sleep (ln-coefficient = 0.81, $P < 0.01$), and short muscle activations per hour rapid eye movement sleep (ln-coefficient = 1.01, $P < 0.01$). Likewise, periodic limb movements per hour rapid eye movement and non-rapid eye movement sleep were only associated with hypocretin deficiency ($P < 0.01$). A significant association between hypocretin deficiency and cataplexy was confirmed ($P < 0.01$). In a sub-analysis, hypocretin deficiency suggested the association of periodic limb movements and rapid eye movement sleep behaviour disorder outcomes (symptoms, non-periodic short and long muscle activity) in rapid eye movement sleep. Our results support the hypothesis that hypocretin deficiency is independently associated with rapid eye movement sleep behaviour disorder in narcolepsy. Thus, hypocretin deficiency is linked to the two major disturbances of rapid eye movement sleep motor regulation in narcolepsy: rapid eye movement sleep behaviour disorder and cataplexy. Hypocretin deficiency is also significantly associated with periodic limb movements in rapid eye movement and non-rapid eye movement sleep, and provides a possible pathophysiological link between rapid eye movement sleep behaviour disorder and periodic limb movements in narcolepsy. The study supports the hypothesis that an impaired hypocretin system causes a general instability of motor regulation during wakefulness, rapid eye movement and non-rapid eye movement sleep in human narcolepsy.

Keywords: narcolepsy; cataplexy; rapid eye movement sleep behaviour disorder; periodic leg movements; hypocretin-1

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Abbreviations: HLA = human leukocyte antigen; ICSD-2 = International Classification of Sleep Disorders, 2nd edition; RBD = rapid eye movement sleep behaviour disorder; REM = rapid eye movement

Introduction

Rapid eye movement (REM) sleep behaviour disorder (RBD) is characterized by dysregulation of REM sleep: sleep-disruptive or injurious dream-enacting behaviour in association with polysomnographic EMG evidence of REM sleep with limb movements or without muscle atonia (American Academy of Sleep Medicine, 2005). RBD is commonly associated with parkinsonian neurodegenerative disorders (Schenck and Mahowald, 2002), but has also been reported in narcolepsy (Schenck and Mahowald, 1992; Mayer and Meier-Ewert, 1993; Nightingale *et al.,* 2005; Dauvilliers *et al.,* 2007*b*; Mattarozzi *et al.,* 2008; Mayer *et al.,* 2008). Patients with narcolepsy present not only symptoms of unstable sleep–wake regulation (daytime sleep attacks, nightly multiple awakenings), but also symptoms of REM sleep dysregulation: cataplexy (loss of muscle tone during wakefulness), sleep paralysis and hypnagogic hallucinations (American Academy of Sleep Medicine, 2005). Moreover, RBD and narcolepsy patients both display another motor disturbance during sleep—periodic leg movements.

Narcolepsy with cataplexy is associated with an almost complete loss of hypocretin neurons in the lateral hypothalamus (Peyron *et al.,* 2000; Thannickal *et al.,* 2000) and corresponding low levels of hypocretin-1 in the CSF (CSF hypocretin-1) (Baumann and Bassetti, 2005; Bourgin *et al.,* 2008). The hypocretin neuropeptides (hypocretin-1 and hypocretin-2, also called orexin-A and orexin-B) are believed to act as main stabilizers and coordinators of sleep–wake states and REM sleep-associated muscle tone, through dense projections to the recently discovered neuronal sleep–wake flip–flop switch and the REM flip–flop switch (Saper *et al.,* 2001; Lu *et al.,* 2006). Hypocretin deficiency could therefore be responsible for the symptoms of narcolepsy with cataplexy. The pathogenesis is less clear in narcolepsy without cataplexy, where only 10% of patients have low CSF hypocretin-1 (Mignot *et al.,* 2002).

It is not known whether hypocretin deficiency is associated with RBD in narcolepsy. However, RBD is common in narcolepsy with cataplexy but rarer in narcolepsy without cataplexy (Nightingale *et al.,* 2005), indicating that cataplexy and RBD could reflect a general instability of REM sleep motor regulation due to hypocretin deficiency in narcolepsy. As the association between cataplexy and hypocretin deficiency is not complete, we hypothesized that there are two likely explanations for RBD in narcolepsy: (i) RBD is primarily associated with cataplexy or (ii) RBD is primarily associated with hypocretin deficiency.

The aim of the study was to evaluate the association between cataplexy, hypocretin deficiency, RBD symptoms and the number of muscle activations (periodic and non-periodic) during REM and non-REM sleep in patients with narcolepsy.

Materials and methods

Participants

Patients with narcolepsy seen at the Danish Center for Sleep Medicine over a six-year period (2001–07) were consecutively included after giving their written informed consent. Exclusion criteria were additional neurological or psychiatric disorders. All patients except one (who had severe cataplexy) were free of antidepressants and stimulants 7–14 days before inclusion. The study (KA03119) was approved by the Danish Ethical Committee.

Narcolepsy diagnosis

The diagnosis of narcolepsy was based on International Classification of Sleep Disorders (ICSD)-2 criteria (American Academy of Sleep Medicine, 2005). Narcolepsy history was obtained by a semi-structured interview based on the Stanford Sleep Inquiry (Anic-Labat *et al.,* 1999). All patients were evaluated by neurological examination, determination of routine blood characteristics, polysomnography, the multiple sleep latency test, human leukocyte antigen (HLA) typing and determination of CSF hypocretin-1 levels.

Symptoms of RBD

All patients took part in a semi-structured interview covering the following questions.

While dreaming, have you ever:

(i) acted so you hurt yourself or others?
(ii) moved or verbalized (enacted your dream)?
(iii) acted, resulting in awakening?

Examples were required from each patient to confirm typical RBD episodes. Patients were subsequently contacted by telephone if clarification of the original information was needed.

The reported RBD symptoms were body/limb movements (kicking, punching, pushing, arm flailing, reaching out, grimacing, turning/twisting as if in agony, sitting up, getting out of bed) and/or verbalization (talking, arguing, shouting, screaming, swearing, crying, laughing, moaning). Harmful/injurious behaviour consisted of hitting body parts against solid parts of the bed, falling out of bed or hitting their bed partner. Two patients with narcolepsy with cataplexy reported dangerous RBD episodes: the first sustained a shoulder injury after leaping out of bed, while the second woke up by a window out of which he was preparing to climb.

Procedures

Polysomnography recordings

The polysomnography recordings consisted of the following signals: electroencephalography (C3–A2, C4–A1), vertical and horizontal electro-oculography, surface EMG of the submentalis and tibialis anterior muscles, electrocardiography, nasal air flow, thoracic respiratory effort and oxygen saturation. Recordings were made with the Embla System (Embla System Inc, Broomfield, CO, USA). EMG recordings were calibrated and filtered similarly (sampling rate 200 Hz, filter 100 Hz, time constant 0.03). The EMG impedance was below 10 kΩ. Sleep was scored according to standard criteria (Rechtschaffen and Kales, 1968), although REM sleep was scored regardless of EMG muscle activity.

Detection and analysis of muscle activity

We analysed the muscle activity in one tibialis anterior muscle, because limb twitches and movements are typically reported in RBD. The non-dominant leg (left) was chosen to ensure increased sensitivity

(Frauscher *et al.*, 2008). Muscle activity was automatically detected by a computer-based system (Somnologica version 3, Embla Systems Inc.) and subsequently edited by sleep technicians, blinded to the diagnosis. The threshold for muscle activity was dynamically calculated by Somnologica throughout the polysomnography recording using a smoothed version of the baseline EMG activity before a given muscle activity occurred. Muscle activity was defined as (i) an amplitude greater than a threshold twice that of baseline EMG activity and (ii) a duration of 0.1–0.49 s (short muscle activity: twitches) or of 0.5–15 s (long muscle activations: movements). These muscle activity definitions are known to reflect the pathophysiology of RBD (Eisensehr *et al.*, 2003*a*). Long muscle activations were further analysed as non-periodic limb movements and periodic leg movements according to ICSD-2 criteria for leg movement periodicity (American Academy of Sleep Medicine, 2005). Outcomes were calculated as short muscle activations per hour in REM and non-REM sleep, long muscle activations per hour in REM and non-REM sleep (subdivided into non-periodic and periodic leg movements), and short and long muscle activations per hour REM/non-REM sleep index.

CSF hypocretin-1 measurements

CSF was collected between 7 and 10 a.m. Hypocretin-1 was analysed in crude CSF by radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA, USA). All samples were measured blindly as duplicates, and the means were calculated. Intra-assay variability was <5% and inter-assay variability was avoided by use of three internal control CSF samples (representing low, intermediate and normal CSF hypocretin-1 levels). Low CSF hypocretin-1 (hypocretin deficiency) was defined as <129 pg/ml (30% of the normal mean value) (American Academy of Sleep Medicine, 2005).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS version 16.0) was used for statistical analyses. Results are reported as the mean ± SEM

Table 1 Demographic and clinical data

	Narcolepsy with cataplexy *N* = 48	Narcolepsy without cataplexy *N* = 15	*P*-value
Demography			
Gender (male), *n* (%)	21 (43.8)	7 (46.7)	1.00
Age (years), mean ± SEM	36.60 ± 2.38	28.73 ± 2.88	0.10
Age at disease onset (years), mean ± SEM	19.08 ± 1.19	15.83 ± 1.61	0.14
Disease duration (years), mean ± SEM	17.52 ± 2.13	12.90 ± 2.66	0.44
HLA-DQB1*0602-positivity, *n* (%)	41 (95.3) *n* = 43	4 (28.6) *n* = 14	<0.01
CSF hypocretin-1 ⩽129 pg/ml, *n* (%)	40 (87.0) *n* = 46	3 (21.4) *n* = 14	<0.01
Sleepiness			
Daytime sleepiness, *n* (%)	48 (100)	15 (100)	1.00
Epworth Sleepiness Scale (sum), mean ± SEM	18.74 ± 0.49	15.87 ± 1.02	0.01
Age at onset (years), mean ± SEM	19.57 ± 1.33	15.83 ± 1.61	0.17
Cataplexy, *n* (%)			
Severity[a]			
Grade 0	0 (0)	14 (93.3)	
Grade 1	2 (4.2)	1 (6.7)	
Grade 2	4 (8.3)	0 (0)	<0.01
Grade 3	4 (8.3)	0 (0)	
Grade 4	17 (35.4)	0 (0)	
Grade 5	21 (43.8)	0 (0)	
Age at onset (years), mean ± SEM	22.97 ± 1.62 (*n* = 47)	18 (*n* = 1)	–
Hypnagogic hallucinations			
Hypnagogic hallucinations, *n* (%)	42 (87.5)	8 (53.3)	<0.01
Age at onset (years), mean ± SEM	21.63 ± 1.42 (*n* = 42)	16.62 ± 1.62 (*n* = 8)	0.10
Sleep paralysis			
Sleep paralysis, *n* (%)	36 (75.0)	5 (33.3)	<0.01
Age at onset (years), mean ± SEM	22.82 ± 1.56 (*n* = 36)	13.25 ± 2.63 (*n* = 4)	0.03
Automatic behaviour			
Automatic behaviour, *n* (%)	17 (39.5) (*n* = 43)	6 (42.9) (*n* = 14)	0.83
Disrupted night sleep[b]			
Awakenings per night, mean ± SEM	5.84 ± 0.66	2.75 ± 1.07	<0.01

[a]Cataplexy severity 0 indicates no cataplexy. Cataplexy severity 1–5 indicates cataplexy attacks ⩽1/year, 1/month, 1/week, several times per week or 1 or several/day, respectively.
[b]Number of awakenings on polysomnography recording.

Table 2 RBD symptoms and polysomnography data (muscle activity during REM and non-REM sleep)

	Narcolepsy-with cataplexy N = 48	Narcolepsy without cataplexy N = 15	*P*-value	Low CSF hcrt-1 N = 43	Normal CSF hypocretin-1 N = 17	*P*-value
Symptoms of RBD[a]						
Prevalence, *n* (%)	34 (70.8)	6 (40.0)	0.06	31 (72.1)	7 (41.2)	0.04
Dream-enacting movements[b]	29 (85.3)	6 (100.0)	1.00	27 (87.1)	7 (100.0)	1.00
Verbalization while dreaming[b]	32 (94.1)	5 (83.3)	0.39	30 (96.8)	5 (71.4)	0.81
Interrupting sleep[b]	29 (85.3)	3 (50.0)	0.08	26 (83.9)	4 (57.1)	0.15
Harmful behaviours[b]	20 (58.8)	2 (33.3)	0.38	19 (61.3)	2 (28.6)	0.21
Frequency[c], *n* (%)						
Grade 0	14 (30.4)	9 (60.2)		12 (29.3)	10 (58.9)	
Grade 1	2 (4.3)	3 (20.0)		1 (2.4)	3 (17.6)	
Grade 2	1 (2.2)	1 (6.7)	<0.01	0 (0)	2 (11.8)	<0.01
Grade 3	2 (4.3)	2 (13.3)		3 (7.3)	1 (5.9)	
Grade 4	13 (28.3)	0 (0)		11 (26.8)	1 (5.9)	
Grade 5	14 (30.4)	0 (0)		14 (34.1)	0 (0)	
Age at onset (years), mean ± SEM	21.26 ± 2.51	18.50 ± 3.50	1.00	20.97 ± 2.80	21.00 ± 3.21	0.49
Polysomnography muscle activity during REM[d]	N = 36	N = 12		N = 33	N = 14	
Short muscle activity (number/h REM), mean ± SEM	17.51 ± 2.94	9.90 ± 2.33	0.18	19.29 ± 3.06	7.74 ± 2.00	<0.01
Long muscle activity (number/h REM), mean ± SEM	43.91 ± 4.95	22.67 ± 4.31	0.01	47.11 ± 5.07	20.70 ± 3.67	<0.01
Periodic legs movements (number/h REM), mean ± SEM	21.46 ± 3.95	7.73 ± 2.75	0.05	23.59 ± 4.13	6.21 ± 2.46	<0.01
Non-periodic legs movements (number/h REM), mean ± SEM	22.45 ± 1.97	14.93 ± 1.91	0.04	23.53 ± 2.07	14.49 ± 1.43	<0.01
Polysomnography muscle activity during non-REM[d]	N = 36	N = 12		N = 33	N = 14	
Short muscle activity (number/h non-REM), mean ± SEM	6.41 ± 1.08	1.96 ± 0.45	<0.01	6.86 ± 1.14	1.80 ± 0.40	<0.01
Long muscle activity (number/h non-REM), mean ± SEM	34.62 ± 3.54	13.15 ± 1.50	<0.01	36.90 ± 3.60	12.53 ± 1.44	<0.01
Periodic legs movements (number/h non-REM), mean ± SEM	21.26 ± 3.07	4.78 ± 1.13	<0.01	23.27 ± 3.14	3.70 ± 0.94	<0.01
Non-periodic legs movements (number/h non-REM), mean ± SEM	13.36 ± 1.09	8.37 ± 0.78	<0.01	13.63 ± 1.61	8.83 ± 0.83	0.19*
Polysomnography muscle activity index REM/non-REM[d]	N = 36	N = 12		N = 33	N = 14	
Short muscle activity (number/h), mean ± SEM	3.69 ± 0.58	14.42 ± 1.01	0.28	3.92 ± 0.65	12.50 ± 8.69	0.55
Long muscle activity (number/h), mean ± SEM	1.75 ± 0.25	1.72 ± 0.29	0.60	1.77 ± 0.27	1.76 ± 0.28	0.55

a: CSF hypocretin-1 levels available from 60/63 patients (two narcolepsy with cataplexy and one narcolepsy without cataplexy did not want lumbar puncture).
b: Patients with RBD symptoms.
c: RBD frequency 0 indicates no RBD symptom. RBD frequency 1–5 indicates RBD episodes ⩾1/year, 1/month, 1/week, several times per week, or every night, respectively.
d: Polysomnography data available from 48 patients, CSF hypocretin-1 levels available from 47/48 patients.
*Significant ($P < 0.01$) in the univariate regression model, but non-significant ($P = 0.19$) in the multivariate regression model.

unless otherwise stated. Values of $P < 0.05$ were considered statistically significant. Data presented in Tables 1 and 2 were analysed by non-parametric Mann–Whitney and Fisher's exact tests, or univariate and backwards stepwise multivariate linear regression modelling. Those presented in Table 3 were analysed by univariate and backwards stepwise multivariate linear or logistic regression. Continuous data were natural log-transformed to meet the criteria for linearity, normal distribution and variance homogeneity of residuals. Data in Figs 1 and 2 were analysed by Fisher's exact test. Those presented in Fig. 7 were analysed by non-parametric one-way ANOVA, while those in Figs 8–11 were analysed by Spearman's non-parametric correlation test (as the variance of the residuals was not homogeneous). The study outcome conclusions were not influenced by the inclusion or exclusion of three patients with mild restless legs syndrome (see Results section), so these were retained in the study population.

Results

Narcolepsy demography

It was possible to include 63 (48 narcolepsy with cataplexy and 15 narcolepsy without cataplexy) of the 77 eligible patients because they fulfilled the ICSD-2 criteria for narcolepsy, exhibited normal responses to a neurological examination, and had normal results in routine blood and CSF tests. Patients were excluded for the following reasons: secondary narcolepsy (1/14), refusal to be included (12/14) and death (1/14). The distributions of age, gender and prevalence of cataplexy did not differ between excluded and included patients (data not shown).

Occasional mild restless legs syndrome (American Academy of Sleep Medicine, 2005) was reported by two hypocretin-deficient patients included with narcolepsy with cataplexy and one patient with narcolepsy without cataplexy and normal CSF hypocretin-1. Their clinical and polysomnography profiles did not differ from the remainder included in the study population (see statistical data).

Table 1 shows demographic and clinical data. Patients with narcolepsy with cataplexy had a significantly higher prevalence of HLA-DQB1*0602-positivity (41/43, 95.3%) and low CSF hypocretin-1 levels (40/46, 87.0%) than patients with narcolepsy without cataplexy (4/14, 28.6% HLA-DQB1*0602-positivity, 3/14, 21.4% low CSF hypocretin-1) ($P < 0.01$). 6/46 patients with narcolepsy with cataplexy had normal CSF hypocretin-1;

Table 3 RBD symptoms and polysomnography data (univariate and multivariate regression analyses)

	Univariate	P-value	Multivariate	P-value
RBD symptoms				
Age, per year	1.02	0.22	1.08	0.09
RR (95% CI)	(0.99 to 1.06)		(0.99 to 1.17)	
Gender, female	0.71	0.52		
RR (95% CI)	(0.25 to 2.02)			
Disease duration, per year	1.01	0.73	0.93	0.11
RR (95% CI)	(0.97 to 1.05)		(0.85 to 1.02)	
Previous anti-cataplexy	3.05	0.18		
medication, yes	(0.60 to 15.55)			
RR (95% CI)				
Cataplexy, yes	3.64	**0.04**		
RR (95% CI)	(1.09 to 12.21)			
Low hcrt-1, yes	3.69	**0.03**	3.69	**0.03**
RR (95% CI)	(1.14 to 11.93)		(1.14 to 11.93)	
Long muscle activations (number/hour REM sleep)[a]				
Age, per year	0.000	0.98		
Coefficient (95% CI)	(−0.016 to 0.015)			
Gender, female	−0.277	0.23		
Coefficient (95% CI)	(−0.737 to −1.183)			
Disease duration, per year	−0.001	0.90		
Coefficient (95% CI)	(−0.018 to 0.016)			
Previous anti-cataplexy	0.247	0.42		
medication, yes				
Coefficient (95% CI)	(−0.368 to 0.861)			
Cataplexy, yes	0.645	**0.01**		
Coefficient (95% CI)	(0.148 to 1.142)			
Low hypocretin-1	0.809	**<0.01**	0.809	**<0.01**
Coefficient (95% CI)	(0.372 to 1.246)		(0.372 to 1.246)	
Short muscle activations (number/hour REM sleep)[a]				
Age, per year	−0.004	0.69		
Coefficient (95% CI)	(−0.026 to 0.017)			
Gender, female	−0.588	0.06	−0.569	0.05
Coefficient (95% CI)	(−1.208 to 0.031)		(−1.139 to 0.001)	
Disease duration, per year	−0.005	0.64		
Coefficient (95% CI)	(−0.028-0.018)			
Previous anti-cataplexy	0.801	0.06		
medication, yes	(−0.017 to 1.619)			
Coefficient (95% CI)				
Cataplexy, yes	0.602	0.10		
Coefficient (95% CI)	(−0.109 to 1.313)			
Low hypocretin -1	1.010	**<0.01**	1.012	**<0.01**
Coefficient (95% CI)	(0.374 to 1.646)		(0.396 to 1.628)	

a: Variable is ln-transformed to fulfil normality and variance homogeneity assumptions.
CI = confidence interval; RR = relative risk. Bold values indicate significant values ($P<0.05$).

4/5 (80%) of these were HLA-DQB1*0602-positive (one HLA-type was unavailable). All patients with narcolepsy without cataplexy and low CSF hypocretin-1 were HLA-DQB1*0602-positive. Patients with narcolepsy with cataplexy had a greater prevalence of excessive daytime sleepiness, hypnagogic hallucinations, sleep paralysis and awakenings than patients with narcolepsy without cataplexy ($P<0.01$).

Symptoms of RBD

RBD symptoms in relation to cataplexy and CSF hypocretin-1 status

Table 2 shows the RBD symptoms. These were present in 40/63 (63.4%) patients. There was no significant difference in incidence between genders: 21/35 (60.0%) female and 19/28 (67.9%) male ($P=0.52$). When patients were grouped by cataplexy status, RBD symptoms tended to be more prevalent ($P=0.06$) and were significantly more frequent in patients with narcolepsy with cataplexy ($P<0.01$). However, when the patients were grouped with respect to CSF hypocretin-1 status, RBD symptoms were both significantly more prevalent and frequent in patients with low CSF hypocretin-1 than in those with normal CSF hypocretin-1 levels ($P=0.04$; $P<0.01$, Fisher's exact test). This was confirmed by the univariate and multivariate regression analyses of factors known or suspected to influence RBD (Table 3). In the univariate analysis (Table 3), and further illustrated by Figs 1 and 2, cataplexy and low CSF hypocretin-1 significantly predicted the prevalence of RBD symptoms, but in the final multivariate

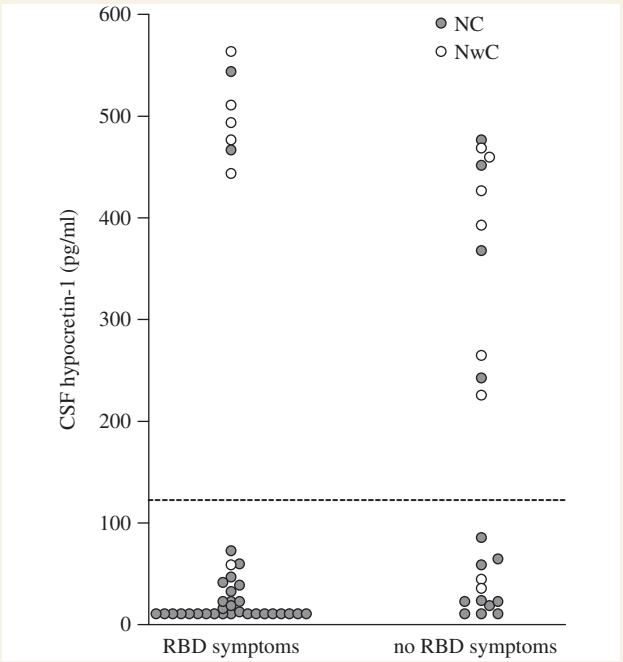

Figure 1 Relationship between RBD symptoms and CSF hypocretin-1 levels. Dotted line indicates cut-off limit for low CSF hypocretin-1 levels. Prevalence of low CSF hypocretin-1 versus RBD symptoms, $\chi 2 = 0.025$. NC = narcolepsy with cataplexy; NwC = narcolepsy without cataplexy.

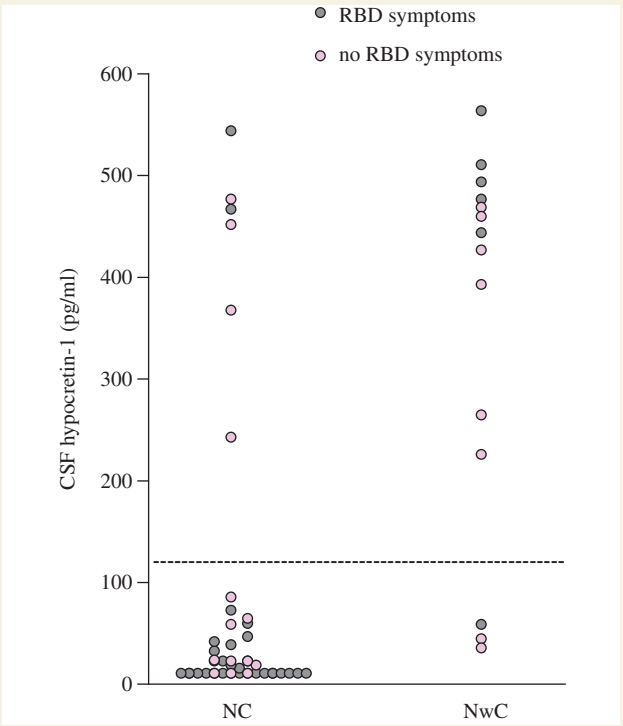

Figure 2 Relationship between cataplexy and CSF hypocretin-1 levels. Dotted line indicates cut-off limit for low CSF hypocretin-1 levels. Prevalence of cataplexy versus RBD symptoms, $\chi 2 = 0.03$; prevalence of cataplexy versus low CSF hypocretin-1, $\chi 2 < 0.01$. NC = narcolepsy with cataplexy; NwC = narcolepsy without cataplexy.

analysis (Table 3) only low CSF hypocretin-1 remained a significant predictor.

Polysomnography data

Polysomnography recordings from 48/63 patients were analysed. The remainder were excluded for the following reasons: 12/15 lacked an EMG recording from the tibialis anterior muscle, 2/15 had electrode artefacts and 1/15 (a hypocretin-deficient patient with narcolepsy with cataplexy) exhibited severe sleep apnoea.

Polysomnography data in relation to cataplexy and CSF hypocretin-1 status

Table 2 shows the polysomnography data. Patients with narcolepsy with cataplexy presented significantly more long muscle activations per hour REM ($P = 0.01$), short muscle activations per hour non-REM ($P < 0.01$) and long muscle activations per hour non-REM ($P < 0.01$) than narcolepsy without cataplexy patients. There was no significant difference in short muscle activity per hour REM between patients with narcolepsy, with or without cataplexy. When the patients were grouped by CSF hypocretin-1 status, all muscle activity (short and long) in REM and non-REM sleep was significantly more frequent in patients with low CSF hypocretin-1 ($P < 0.01$). These relationships are illustrated by Figs 3–6, in which both patients with narcolepsy, with and without cataplexy, with normal CSF hypocretin-1 levels, displayed fewer muscle activations per hour REM and non-REM sleep than hypocretin-deficient patients with narcolepsy with or without cataplexy. This was confirmed by the multivariate linear regression analysis (Table 3), in which only low CSF hypocretin-1 remained a significant predictor of short and long muscle activity per hour REM sleep ($P < 0.01$). None of the possible biasing factors (age, gender, disease duration, previous anti-cataplexy medication) nor HLA-DQB1*0602-positivity (data not shown) predicted any of the RBD outcomes presented in Table 3.

When long muscle activity was analysed with respect to periodic and non-periodic limb movements, patients with narcolepsy with cataplexy showed a trend towards more periodic leg movements per hour REM sleep ($P = 0.05$) and presented significantly more non-periodic limb movements per hour REM ($P = 0.04$), non-periodic limb movements per hour non-REM and periodic leg movements per hour non-REM sleep ($P < 0.01$) than patients with narcolepsy without cataplexy (Table 2). When the patients were grouped by CSF hypocretin-1 status, all muscle activity (non-periodic and periodic leg movements) in REM and non-REM sleep was significantly more frequent in patients with low CSF hypocretin-1 ($P < 0.01$) (Table 2). The multivariate linear regression model (variables: age, gender, disease duration, previous anti-cataplexy medication, low CSF hypocretin-1, cataplexy) confirmed that the number of periodic leg movements per hour REM, periodic leg movements per hour non-REM and non-periodic limb movement per hour REM sleep were only predicted by low CSF hypocretin-1 ($P < 0.01$; $P = 0.02$; $P < 0.01$, respectively) (detailed multivariate regression data not shown). Non-periodic limb movements per hour non-REM sleep were

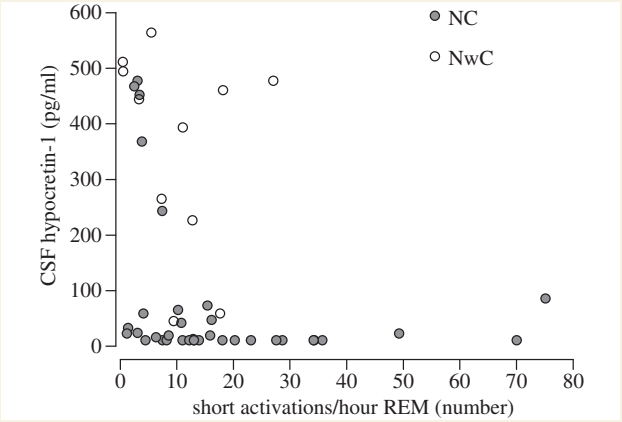

Figure 3 Number of short muscle activations per hour REM sleep in relation to CSF hypocretin-1 levels and cataplexy status. Hypocretin deficient patients have significantly more muscle activations than patients with normal CSF hypocretin-1 levels ($P < 0.01$). NC = narcolepsy with cataplexy; NwC = narcolepsy without cataplexy.

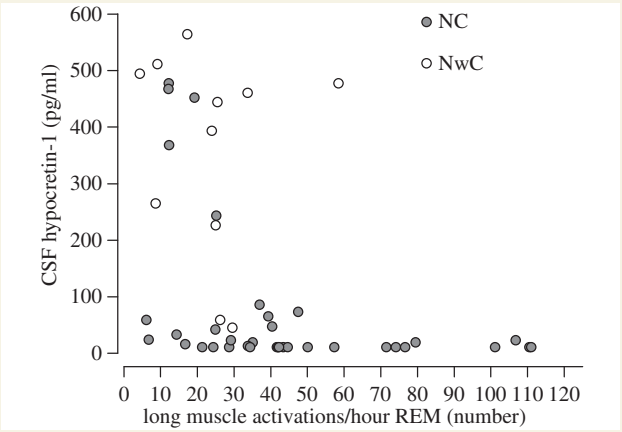

Figure 4 Number long muscle activations/h REM sleep in relation to CSF hypocretin-1 levels and cataplexy status. Hypocretin deficient patients have significantly more muscle activations than patients with normal CSF hypocretin-1 levels ($P < 0.01$). NC = narcolepsy with cataplexy; NwC = narcolepsy without cataplexy.

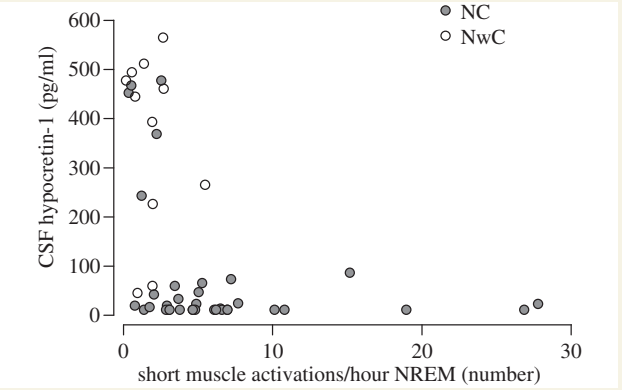

Figure 5 Number of short muscle activations/h non-REM sleep in relation to CSF hypocretin-1 levels and cataplexy status. Hypocretin deficient patients have significantly more muscle activations than patients with normal CSF hypocretin-1 levels ($P < 0.01$). NC = narcolepsy with cataplexy; NwC = narcolepsy without cataplexy.

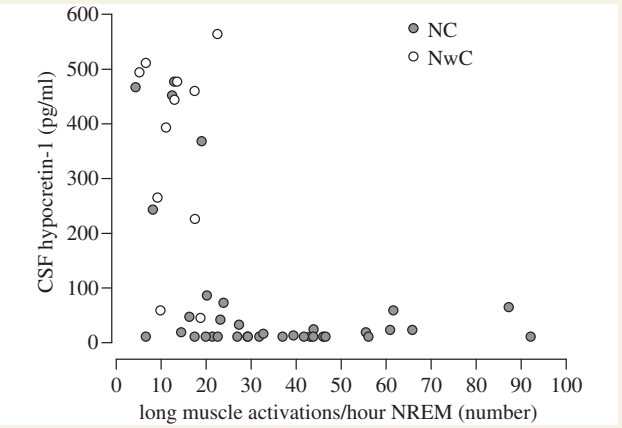

Figure 6 Number of long muscle activations/h non-REM sleep in relation to CSF hypocretin-1 levels and cataplexy status. Hypocretin deficient patients have significantly more muscle activations than patients with normal CSF hypocretin-1 levels ($P < 0.01$). NC = narcolepsy with cataplexy; NwC = narcolepsy without cataplexy.

predicted by age ($P < 0.01$) and weakly predicted by prior anti-cataplexy medication ($P = 0.048$) in the multivariate analysis; although this may be unspecific, since 5/6 variables were predictors in the univariate analysis.

Polysomnography data in relation to RBD symptoms

A total of 18/48 (38%) of patients with available polysomnography recordings reported no RBD symptoms, while the remaining 30/48 (62%) patients did report them. The latter group had significantly more long muscle activations per hour REM sleep (45.81 ± 5.31) than patients without RBD symptoms (26.79 ± 5.37) ($P < 0.01$), but not significantly different numbers of short muscle activations per hour REM sleep (16.18 ± 2.69 versus 14.64 ± 4.35, respectively; $P = 0.34$), long muscle activations per hour non-REM sleep (28.86 ± 3.53 versus 29.92 ± 5.52, respectively; $P = 0.75$) or short muscle activations per hour non-REM sleep (5.28 ± 1.03 versus 5.31 ± 1.55, respectively; $P = 0.75$) (data not shown).

Periodic leg movements in relation to RBD outcomes and CSF hypocretin-1 status

Since periodic leg movement (Table 2) and RBD outcomes (symptoms, short and long muscle activity) (Table 3) were exclusively predicted by hypocretin deficiency, the internal relationship between these variables was analysed further. Figure 7 shows the periodic leg movement data in relation to RBD symptoms and CSF hypocretin-1 status. Within the hypocretin-deficient

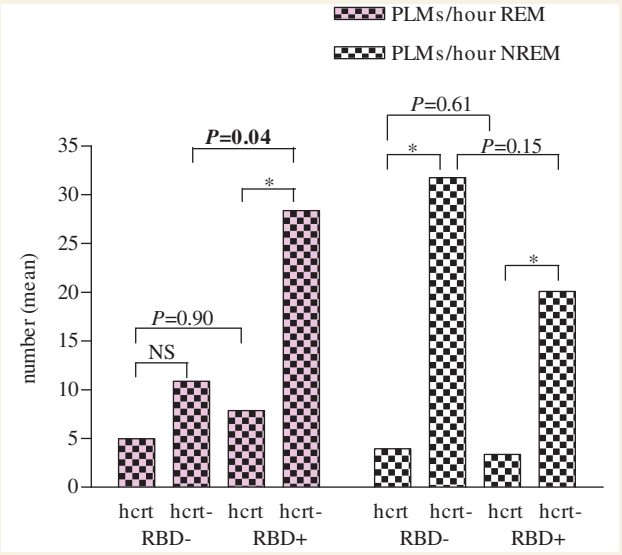

Figure 7 Relationship between periodic leg movements (PLM)/h REM and non-REM sleep, RBD symptoms and low CSF hypocretin-1. RBD− indicates no RBD symptoms, RBD+ indicates RBD symptoms, hcrt indicates normal CSF hypocretin-1 levels; hcrt− indicates low CSF hypocretin-1 levels. In REM sleep, hypocretin-deficient patients (hcrt−) with RBD symptoms have significantly more periodic leg movements than hypocretin-deficient patients without RBD symptoms ($P = 0.04$). Asterisk indicates significant differences between groups with low and normal CSF hypocretin-1 levels.

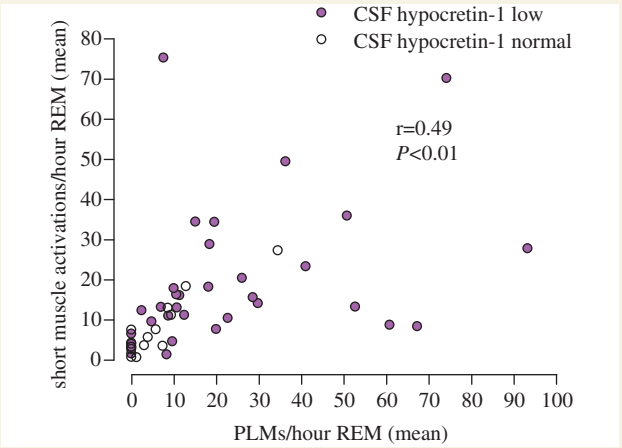

Figure 8 Correlation between periodic leg movements (PLM) and short muscle activations per hour REM sleep. Correlation in hypocretin-deficient patients: $r = 0.49$, $P < 0.01$ (marked).

group, patients with RBD symptoms had more periodic leg movements per hour REM sleep ($P = –0.04$), but not a significantly different number of periodic leg movements per hour non-REM sleep ($P = 0.15$), when compared with patients without RBD symptoms. Within the normal CSF hypocretin-1 patient group, the presence of RBD symptoms was not significantly related to the number of periodic leg movements per hour REM sleep ($P = 0.90$) and non-REM sleep ($P = 0.61$).

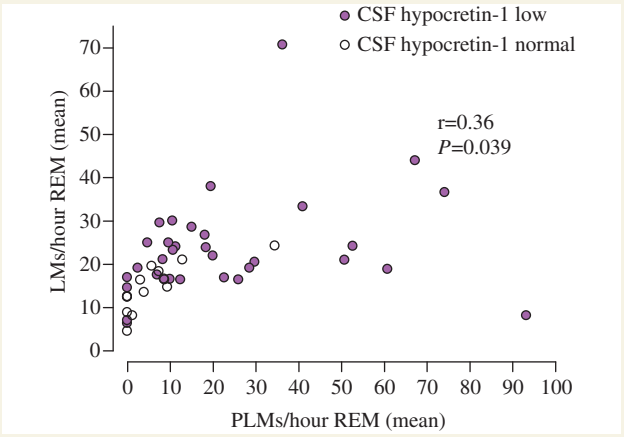

Figure 9 Correlation between periodic leg movements (PLM) and non-periodic leg movements (LM) per hour REM sleep. Correlation in hypocretin-deficient patients: $r = 0.36$, $P = 0.039$ (marked).

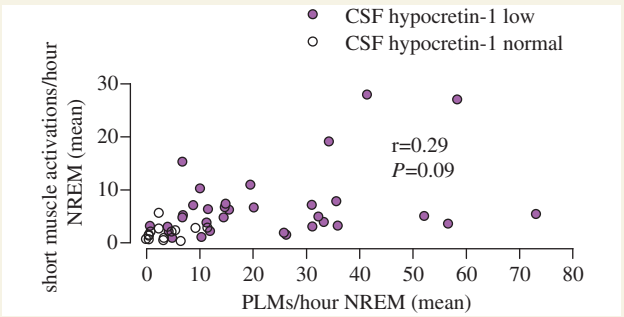

Figure 10 Correlation between periodic leg movements and short muscle activations per hour non-REM sleep. Not correlated in hypocretin deficient patients: $r = 0.29$, $P = 0.09$ (marked).

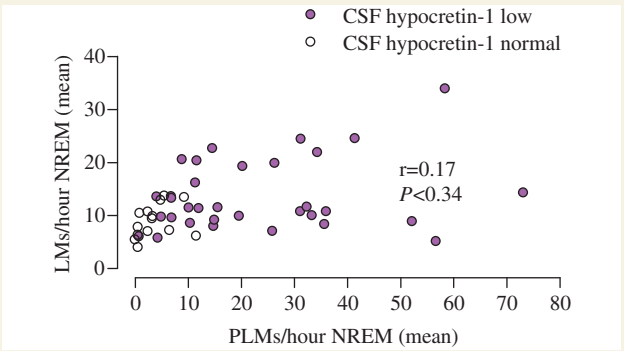

Figure 11 Correlation between periodic leg movements (PLM) and non-periodic leg movements (LM) per hour non-REM sleep. Not correlated in hypocretin-deficient patients: $r = 0.17$, $P < 0.34$ (marked).

Figures 8–11 show the correlations between periodic leg movement data and short and long muscle activity with respect to CSF hypocretin-1 status. As periodic leg movements are a subset of long muscle activations in this study, these two variables are inevitably correlated. Instead, correlation analysis between periodic leg movement and the long non-periodic muscle activity (non-periodic limb movement) was performed. In REM sleep (Figs 8 and 9), the number of periodic leg movements per hour was significantly positively correlated with the frequency of short muscle activity per hour and non-periodic limb movements per hour, within the combined CSF hypocretin-1 patient group (short: $r=0.73$, $P<0.01$; non-periodic limb movements: $r=0.64$, $P<0.01$), within the hypocretin-deficient group (short: $r=0.49$, $P<0.01$; non-periodic limb movements: $r=0.36$, $P=0.039$), and the normal CSF hypocretin-1 patient group (short: $r=0.76$, $P<0.01$; non-periodic limb movements: $r=0.85$, $P<0.01$). In non-REM sleep (Figs 10 and 11), the number of periodic leg movements per hour was significantly positively correlated with frequency of short muscle activity per hour and non-periodic limb movements per hour in the combined CSF hypocretin-1 group (short: $r=0.59$, $P<0.01$; non-periodic limb movements: $r=0.43$, $P<0.01$), but neither within the separate hypocretin-deficient group nor within the normal CSF hypocretin-1 patient group.

Discussion

The study shows for the first time that RBD is associated with hypocretin deficiency in narcolepsy, independent of cataplexy status and other factors. The association between narcolepsy with cataplexy and hypocretin deficiency is well established (Baumann and Bassetti, 2005; Bourgin *et al.,* 2008) and is confirmed in the present study. Thus, the two major disturbances of REM sleep motor regulation in narcolepsy—cataplexy and RBD—are both independently linked to hypocretin deficiency.

RBD, narcolepsy and hypocretin deficiency

The hypocretin neuropeptides are believed to play a central role as stabilizers of the neuronal REM flip–flop switch in the brainstem, which regulates the onset of REM sleep and associated REM sleep atonia. This switch consists of mutually inhibitory REM-ON and REM-OFF neuron centres with connections to the motor neurons of the ventral spinal cord (Lu *et al.,* 2006). Hypocretin neurons have been shown to excite mainly REM-OFF neurons (Mileykovskiy *et al.,* 2005; Lu *et al.,* 2006). Lesions of REM-OFF neurons result in sleep onset REM periods, sleep paralysis and cataplexy-like phenomena in rodents (Lu *et al.,* 2006). The hypocretin neuropeptides are also main stabilizers of consolidated wakefulness, via excitatory projections, to wake-active neurons of the neuronal sleep–wake flip–flop switch that are also localized in the brainstem (Saper *et al.,* 2001). Consequently, the hypocretin neurons are thought to stabilize and coordinate the sleep–wake and the REM flip–flop switches in the intact brain, thereby, for example, preventing REM sleep from appearing inappropriately during wakefulness (Saper *et al.,* 2001; Lu *et al.,* 2006). This could explain why hypocretin deficiency may result in REM sleep atonia during wakefulness (cataplexy). It has recently been shown that hypocretin neurons also project directly to the motor neurons in the spinal cord (Yamuy *et al.,* 2004) and send excitatory projections to one subset of REM-on neurons (sublaterodorsal nucleus) of the REM flip–flop switch (Brown *et al.,* 2008). While EEG activation in REM sleep is dependent on another subset of REM-on neurons (preceruleus nucleus) (Lu *et al.,* 2006), lesion studies indicate that the sublaterodorsal nucleus is crucial for maintenance of REM sleep atonia and that a dysfunctional sublaterodorsal nucleus results in RBD (Boeve *et al.,* 2007). Hence, hypocretin deficiency might result in discoordination between the subsets of REM-on neurons, causing REM sleep without atonia/RBD in narcolepsy. Moreover, weakening of either side of the REM flip–flop switch and the sleep–wake flip–flop switch produces multiple state transitions between REM and non-REM sleep (Lu *et al.,* 2006), and between sleep and wakefulness (Saper *et al.,* 2001), respectively.

Overall, it seems plausible that hypocretin deficiency could cause a general instability of sleep–wake regulation and REM sleep motor regulation, which would explain not only the sleep attacks and multiple awakenings, but also the coexistence of cataplexy and RBD in narcolepsy.

Hypocretin deficiency has not previously been studied in relation to RBD in narcolepsy, but others have studied RBD in HLA-DQB1*0602-positive patients with narcolepsy with cataplexy, the majority of whom may be expected to be hypocretin deficient (Mignot *et al.,* 2002), as confirmed by the present study.

Our finding of a high prevalence of RBD symptoms in hypocretin-deficient patients (72.2%) is supported by the presence of RBD symptoms in 61% of HLA-DQB1*0602-positive patients with narcolepsy with cataplexy (Mattarozzi *et al.,* 2008) and 68% of patients with narcolepsy with cataplexy and only 14% of patients with narcolepsy without cataplexy) of unknown HLA-type (Nightingale *et al.,* 2005), although exactly the opposite was found in four studies with RBD symptoms in 0–19% of HLA-DQB1*0602-positive patients with narcolepsy with cataplexy (Mayer and Meier-Ewert, 1993; Mayer *et al.,* 2002; Dauvilliers *et al.,* 2007*a, b*). The wide variation in the study results is possibly due to differences in the study designs, RBD definitions, the use or not of anti-cataplexy medication and the selection of patient populations.

Our observation of more EMG muscle activations during REM and non-REM sleep in hypocretin-deficient narcolepsy patients cannot be directly compared with the results of other studies due to differences in the EMG analysis. However, in previous studies, increased muscle activity during REM sleep was consistently found in HLA-DQB1*0602-positive patients with narcolepsy with cataplexy compared with patients with rare cataplexy (Mattarozzi *et al.,* 2008) or normal controls (Dauvilliers *et al.,* 2007*b*; Mayer *et al.,* 2008). Indications of increased muscle activity (mean muscle tone) during non-REM sleep were found in HLA-DQB1*0602-positive patients with narcolepsy with cataplexy compared with normal controls, although the difference did not reach significant levels (Mayer *et al.,* 2008).

Periodic leg movements, narcolepsy and hypocretin deficiency

In the present study, hypocretin deficiency was also independently associated with an increased number of periodic leg movements in REM and non-REM sleep in narcolepsy.

To our knowledge, periodic leg movements have not previously been studied in narcolepsy patients with known CSF hypocretin-1 levels, but the present results are supported by more numerous periodic leg movements per hour sleep in HLA-DQB1*0602-positive than in HLA-DQB1*0602-negative patients with narcolepsy with cataplexy (Hong *et al.,* 2000), as well as the increased number of periodic leg movements per hour REM and non-REM sleep in HLA-DQB1*0602-positive patients with narcolepsy with cataplexy compared with normative data (Dauvilliers *et al.,* 2007*a, b*; Ferri *et al.,* 2008; Mattarozzi *et al.,* 2008). In accordance with our results, the number of periodic leg movements per hour REM and non-REM sleep did not differ (Dauvilliers *et al.,* 2007*a,b*; Ferri *et al.,* 2008; Mattarozzi *et al.,* 2008).

It has been proposed that periodic leg movements arise from a dysfunction of the dopamine system, mainly on the basis of the periodic leg movement association to restless legs syndrome (who are treated with dopamine agonists) (Rye, 2004). In narcolepsy, dopamine agonists also greatly reduce periodic leg movements (Boivin *et al.,* 1993), which supports this hypothesis. Hypocretin neurons have been demonstrated to excite dopaminergic nuclei (Korotkova *et al.,* 2003) through dense projections (Peyron *et al.,* 1998). Periodic leg movements during sleep have been observed in narcoleptic dogs with a mutation in the gene for hypocretin receptor-2 (*hcrtR-2*) (Okura *et al.,* 2001). Moreover, these *hcrtR-2*-mutated dogs and hypocretin-deficient narcoleptic dogs (Okura *et al.,* 2004), as well as HLA-DQB1*0602-positive narcolepsy patients (Eisensehr *et al.,* 2003*b*), display an altered number of dopamine receptors that is significantly correlated with increased sleepiness (Eisensehr *et al.,* 2003*b*; Okura *et al.,* 2004), aggravation (Okura *et al.,* 2004) and frequency of cataplexy (Eisensehr *et al.,* 2003*b*). The present study indicates that hypocretin deficiency, possibly through secondary dopamine dysfunction, also plays an important role in the pathogenesis of periodic leg movements in narcolepsy. The equal distribution of periodic leg movements in REM and non-REM sleep in the present study is a further evidence that hypocretin deficiency causes general instability of motor regulation during sleep in narcolepsy.

RBD in narcolepsy versus neurodegenerative disorders

The present study demonstrated that the majority of hypocretin-deficient narcoleptic patients fulfil ICSD-2 RBD criteria, but several lines of evidence suggest that RBD in narcolepsy may be different from that associated with neurodegenerative disorders.

Long-term prospective studies provide increasing evidence that the currently defined 'idiopathic RBD' should almost always be considered as an early manifestation of the RBD associated with neurodegenerative disorders (especially Parkinson's disease, multiple system atrophy, dementia with Lewy bodies; for review, see Iranzo *et al.,* 2009). In these neurodegenerative disorders, progressive destruction of REM-ON and other brainstem nuclei (e.g., substantia nigra) leading to pronounced RBD/loss of REM sleep atonia and parkinsonism has been proposed as a main pathogenesis for RBD (Boeve *et al.,* 2007); the increased motor activity/loss of atonia is almost completely restricted to REM sleep (Eisensehr *et al.,* 2003*a*; Dauvilliers *et al.,* 2007*b*); and the typical RBD patient is an elderly male with long degenerative disease duration and injurious RBD (Schenck and Mahowald, 2002).

In contrast, brainstem destruction has not been found in narcolepsy, narcoleptic patients do not develop symptoms or signs of neurodegenerative disorders (Stiasny-Kolster *et al.,* 2007), and the frequency of muscle activations were greater equally in REM and non-REM sleep associated with hypocretin deficiency in the present study, implying a more general instability of motor regulation during sleep. Furthermore, we found that RBD did not precede the onset of narcolepsy (Table 2), and that RBD was not related to gender, age or disease duration in accordance with recent RBD studies of patients with narcolepsy with cataplexy (Nightingale *et al.,* 2005; Dauvilliers *et al.,* 2007*b*; Stiasny-Kolster *et al.,* 2007; Mattarozzi *et al.,* 2008; Mayer *et al.,* 2008), although male predominance was observed in two early narcolepsy with cataplexy studies (Schenck and Mahowald, 1992; Mayer and Meier-Ewert, 1993). Finally, RBD symptoms were not severe in the present narcolepsy population, as the majority fulfilled the ICSD-2 RBD criteria through disruption of sleep rather than because they exhibited injurious behaviour. Approximately one-third of the patients were confirmed as exhibiting 'harmful' RBD behaviour, but these mostly consisted of non-injurious slapping of the bed partner or the bed, in accordance with other narcolepsy with cataplexy studies (Mayer and Meier-Ewert, 1993; Mayer *et al.,* 2008).

RBD and periodic leg movements

Hypocretin deficiency also predicted the association between periodic leg movements per hour REM sleep and the RBD outcomes (symptoms and non-periodic muscle activations per hour REM sleep) in the sub-analyses, suggesting that periodic leg movements and RBD are pathophysiologically related motor disturbances in narcolepsy, with dysfunction of the hypocretin system being a plausible common cause. In contrast, the number of periodic leg movements are selectively increased or almost exclusively found during REM sleep in RBD associated with the neurodegenerative disorders (Fantini *et al.,* 2002; Dauvilliers *et al.,* 2007*b*) and levels of CSF hypocretin-1 are within the normal range (Baumann *et al.,* 2005).

RBD in the setting of neurodegenerative disease was found closely correlated with reduced dopamine binding in patients even prior to parkinsonian symptoms (Eisensehr *et al.,* 2003*a*) suggestive of a causal relationship, but the role of dopamine dysfunction in the pathogenesis of RBD in these patients is still unclear. For example, not all patients with Parkinson's disease develop RBD (Gagnon *et al.,* 2002). Moreover, parkinsonian disorders are characterized by widespread brain pathology including several brainstem areas involved in sleep–wake and motor

regulation (Iranzo *et al.,* 2009), so the pathogenesis of RBD in these disorders is likely to be complex.

RBD and partial hypocretin deficiency

Interestingly, two recent post-mortem studies proposed that partial hypocretin deficiency might be involved in the pathogenesis of Parkinson's disease. Up to 45–62% hypocretin cell loss was demonstrated in post-mortem Parkinson's patients compared with controls (Fronczek *et al.,* 2007; Thannickal *et al.,* 2007), and neuron loss was correlated with pathophysiological disease progression (Braak stages) (Thannickal *et al.,* 2007) and reduced hypocretin-1 levels in brain tissue and ventricular CSF, although the latter was still within normal range (Fronczek *et al.,* 2007). Whether parkinsonism is associated with narcolepsy-like features is still a matter of discussion, as 39% of patients in one study (Arnulf *et al.,* 2000), but only 17% in another, fulfilled multiple sleep latency test criteria for narcolepsy and did not exhibit cataplexy (Baumann *et al.,* 2005).

Importantly, partial hypocretin neuron loss was recently also demonstrated a post-mortem patient with narcolepsy without cataplexy (Thannickal *et al.,* 2009), indicating that a normal CSF hypocretin-1 level in narcolepsy does not necessarily imply that the hypocretin system is intact. This is in accordance with an animal model in which destruction (73%) of the hypocretin neurons caused sleep disturbance but not cataplexy, and only decreased CSF hypocretin-1 levels to 50% (Gerashchenko *et al.,* 2003) which would not be considered hypocretin deficient levels in humans (American Academy of Sleep Medicine, 2005).

In the present study, a normal CSF hypocretin-1 level ruled out neither the presence of cataplexy nor RBD symptoms, and in spite of a lower frequency of muscle activation, the muscle activity REM/non-REM sleep indices were not significantly different from those in patients with low CSF hypocretin-1 (Table 2), indicating that the same mechanisms of dysregulation (hypocretin deficiency) may be present in both groups, although to a lesser degree in the 'normal' CSF hypocretin-1 group.

The observed differences between narcolepsy and neurodegenerative disorders in RBD severity (mild versus severe) and motor activity (general versus restricted to REM sleep) could therefore represent the totally dysregulated but intact motor and sleep–wake modulating nuclei due to hypocretin deficiency in narcolepsy, versus a partially reduced hypocretin system combined with localized brainstem lesions in neurodegenerative disease, respectively.

Finally, it can be speculated that narcolepsy patients with 'normal' CSF hypocretin-1 levels may present characteristics of a partially hypocretin-deficient narcoleptic phenotype. Different degrees of hypocretin neuron destruction might even explain the clinical variation observed within the group with low CSF hypocretin-1 levels, for example the presence or not of RBD.

Study limitations

There are some limitations of our study that should be borne in mind when interpreting the results. We used standard ICSD-2 RBD criteria (Eisensehr *et al.,* 2003*a*; Mayer *et al.,* 2008). RBD symptoms were evaluated by semi-structured interview, and examples of the behaviour ensured typical RBD episodes. In endeavouring to avoid all known RBD-aggravating factors the analyses controlled for the effects of age, gender, prior anti-cataplexy medication and disease duration. The RBD prevalence depended on self-reporting, so RBD symptoms may have been underestimated. However, patients with RBD symptoms had significantly more EMG muscle activations in REM sleep (and not in non-REM sleep) so semi-structured interviews seem likely to be reliable for documenting the presence of RBD episodes. There are no quantitative ICSD-2 EMG criteria for RBD, or definitions of which muscles should be used for analysis (American Academy of Sleep Medicine, 2005). We analysed EMG activity in the tibialis anterior muscle to reflect RBD of the limbs directly. The sensitivity of this muscle is lower than that of the submentalis muscle (Frauscher *et al.,* 2008), which increases the risk of type 2 error, but this is less likely as several studies have confirmed significant activation of the tibialis anterior muscle in RBD (Eisensehr *et al.,* 2003*a*; Frauscher *et al.,* 2008).

The sub-analyses within the low and normal CSF hypocretin-1 groups (Figs 7–11) are mainly hypothesis-generating (due to small subgroups), and the results should be confirmed in future larger populations. Finally, the study did not include a normal control group, so it is not possible to predict normative EMG data.

Conclusion

In conclusion, hypocretin deficiency was found to be independently associated with the prevalence of RBD outcomes (symptoms and muscle activation) during REM sleep in narcolepsy. The finding of low CSF hypocretin-1 in narcoleptic patients should therefore be followed by an evaluation of coexistent RBD. Hypocretin deficiency also predicted a greater number of periodic leg movements in REM and non-REM sleep, and suggested an association between periodic leg movements and RBD outcomes in REM sleep. The association between hypocretin deficiency and cataplexy was confirmed.

The study supports the hypothesis that hypocretin deficiency in narcolepsy causes a general instability of motor regulation during sleep and wakefulness.

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References

American Academy of Sleep Medicine. The International Classification of Sleep Disorders (ICSD)—Diagnostic and Coding Manual 2nd edn, Westchester, IL, 2005.

Anic-Labat S, Guilleminault C, Kraemer HC, Meehan J, Arrigoni J, Mignot E. Validation of a cataplexy questionnaire in 983 sleep-disorders patients. Sleep 1999; 22: 77–87.

Arnulf I, Bonnet AM, Damier P, et al. Hallucinations, REM sleep, and Parkinson's disease: a medical hypothesis. Neurology 2000; 55: 281–8.

Baumann CR, Bassetti CL. Hypocretins (orexins) and sleep–wake disorders. Lancet Neurol 2005; 4: 673–82.

Baumann CR, Ferini-Strambi L, Waldvogel D, Werth E, Bassetti C. Parkinsonism with excessive daytime sleepiness. A narcolepsy-like disorder? J Neurol 2005; 253: 139–45.

Boeve BF, Silber MH, Saper CB, et al. Pathophysiology of REM sleep behaviour disorder and relevance to neurodegenerative disease. Brain 2007; 130: 2770–88.

Boivin DB, Lorrain D, Montplaisir J. Effects of bromocriptine on periodic limb movements in human narcolepsy. Neurology 1993; 43: 2134–6.

Bourgin P, Zeitzer JM, Mignot E. CSF hypocretin-1 assessment in sleep and neurological disorders. Lancet Neurol 2008; 7: 649–62.

Brown RE, McKenna JT, Winston S, et al. Characterization of GABAergic neurons in rapid-eye-movement sleep controlling regions of the brainstem reticular formation in GAD67-green fluorescent protein knock-in mice. Eur J Neurosci 2008; 27: 352–63.

Dauvilliers Y, Pennestri MH, Petit D, Dang-Vu T, Lavigne G, Montplaisir J. Periodic leg movements during sleep and wakefulness in narcolepsy. J Sleep Res 2007a; 16: 333–9.

Dauvilliers Y, Rompre S, Gagnon JF, Vendette M, Petit D, Montplaisir J. REM sleep characteristics in narcolepsy and REM sleep behavior disorder. Sleep 2007b; 30: 844–9.

Eisensehr I, Linke R, Tatsch K, et al. Increased muscle activity during rapid eye movement sleep correlates with decrease of striatal presynaptic dopamine transporters. IPT and IBZM SPECT imaging in subclinical and clinically manifest idiopathic REM sleep behavior disorder, Parkinson's disease, and controls. Sleep 2003a; 26: 507–12.

Eisensehr I, Linke R, Tatsch K, et al. Alteration of the striatal dopaminergic system in human narcolepsy. Neurology 2003b; 60: 1817–9.

Fantini ML, Michaud M, Gosselin N, Lavigne G, Montplaisir J. Periodic leg movements in REM sleep behavior disorder and related autonomic and EEG activation. Neurology 2002; 59: 1889–94.

Ferri R, Franceschini C, Zucconi M, et al. Searching for a marker of REM sleep behavior disorder: submentalis muscle EMG amplitude analysis during sleep in patients with narcolepsy/cataplexy. Sleep 2008; 31: 1409–17.

Frauscher B, Iranzo A, Hogl B, et al. Quantification of electromyographic activity during REM sleep in multiple muscles in REM sleep behavior disorder. Sleep 2008; 31: 724–31.

Fronczek R, Overeem S, Lee SY, et al. Hypocretin (orexin) loss in Parkinson's disease. Brain 2007; 130: 1577–85.

Gagnon JF, Bedard MA, Fantini ML, et al. REM sleep behavior disorder and REM sleep without atonia in Parkinson's disease. Neurology 2002; 59: 585–9.

Gerashchenko D, Murillo-Rodriguez E, Lin L, et al. Relationship between CSF hypocretin levels and hypocretin neuronal loss. Exp Neurol 2003; 184: 1010–6.

Hong SC, Hayduk R, Lim J, Mignot E. Clinical and polysomnographic features in DQB1*0602 positive and negative narcolepsy patients: results from the modafinil clinical trial. Sleep Med 2000; 1: 33–9.

Iranzo A, Santamaria J, Tolosa E. The clinical and pathophysiological relevance of REM sleep behavior disorder in neurodegenerative diseases. Sleep Med Rev 2009.

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci 2003; 23: 7–11.

Lu J, Sherman D, Devor M, Saper CB. A putative flip–flop switch for control of REM sleep. Nature 2006; 441: 589–94.

Mattarozzi K, Bellucci C, Campi C, et al. Clinical, behavioural and polysomnographic correlates of cataplexy in patients with narcolepsy/cataplexy. Sleep Med 2008; 9: 425–33.

Mayer G, Kesper K, Peter H, Ploch T, Leinweber T, Peter JH. [Comorbidity in narcoleptic patients]. Dtsch Med Wochenschr 2002; 127: 1942–6.

Mayer G, Kesper K, Ploch T, et al. Quantification of tonic and phasic muscle activity in REM sleep behavior disorder. J Clin Neurophysiol 2008; 25: 48–55.

Mayer G, Meier-Ewert K. Motor dyscontrol in sleep of narcoleptic patients (a lifelong development?). J Sleep Res 1993; 2: 143–8.

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: 1553–62.

Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 2005; 46: 787–98.

Nightingale S, Orgill JC, Ebrahim IO, de Lacy SF, Agrawal S, Williams AJ. The association between narcolepsy and REM behavior disorder (RBD). Sleep Med 2005; 6: 253–8.

Okura M, Fujiki N, Kita I, et al. The roles of midbrain and diencephalic dopamine cell groups in the regulation of cataplexy in narcoleptic Dobermans. Neurobiol Dis 2004; 16: 274–82.

Okura M, Fujiki N, Ripley B, et al. Narcoleptic canines display periodic leg movements during sleep. Psychiatry Clin Neurosci 2001; 55: 243–4.

Peyron C, Faraco J, Rogers W, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000; 6: 991–7.

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.

Rechtschaffen A, Kales A. A manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects. Los Angeles: Brain Information Service/Brain Research Institute; 1968.

Rye DB. Parkinson's disease and RLS: the dopaminergic bridge. Sleep Med 2004; 5: 317–28.

Saper CB, Chou TC, Scammell TE. The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24: 726–31.

Schenck CH, Mahowald MW. Motor dyscontrol in narcolepsy: rapid-eye-movement (REM) sleep without atonia and REM sleep behavior disorder. Ann Neurol 1992; 32: 3–10.

Schenck CH, Mahowald MW. REM sleep behavior disorder: clinical, developmental, and neuroscience perspectives 16 years after its formal identification in SLEEP. Sleep 2002; 25: 120–38.

Stiasny-Kolster K, Clever SC, Moller JC, Oertel WH, Mayer G. Olfactory dysfunction in patients with narcolepsy with and without REM sleep behaviour disorder. Brain 2007; 130: 442–9.

Thannickal TC, Lai YY, Siegel JM. Hypocretin (orexin) cell loss in Parkinson's disease. Brain 2007; 130: 1586–95.

Thannickal TC, Moore RY, Nienhuis R, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000; 27: 469–74.

Thannickal TC, Nienhuis R, Siegel JM. Localized loss of hypocretin (orexin) cells in narcolepsy without cataplexy. Sleep 2009; 32: 993–8.

Yamuy J, Fung SJ, Xi M, Chase MH. Hypocretinergic control of spinal cord motoneurons. J Neurosci 2004; 24: 5336–45.