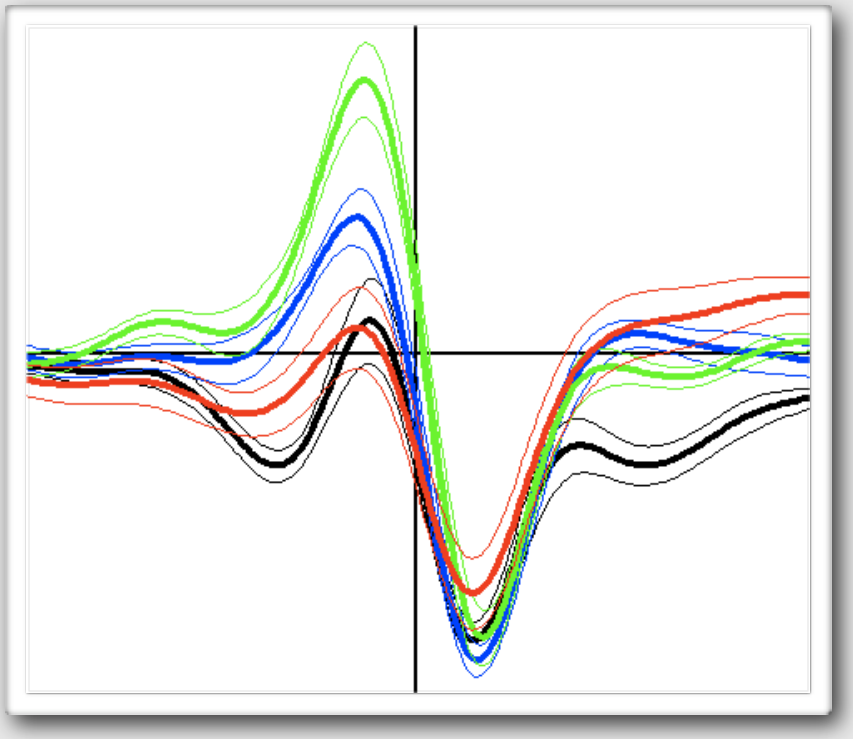
NARCOLEPSY: STATE-DEPENDENT AUTONOMIC REGULATION AND CIRCADIAN CONTROL OF BLOOD PRESSURE, HEART RATE, BODY CORE TEMPERATURE



Daniela Grimaldi MD

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NARCOLEPSY: STATE-DEPENDENT AUTONOMIC REGULATION AND CIRCADIAN CONTROL OF BLOOD PRESSURE, HEART RATE, BODY CORE TEMPERATURE

Presentata da: **Daniela Grimaldi**

Coordinatore Dottorato

Prof. Pasquale Montagna

Relatori

Prof. Pietro Cortelli

Dr. Giuseppe Plazzi

Ai miei genitori, Pippi e Paola,
per tutto l'amore di sempre.

INDEX OF CONTENTS

SCIENTIFIC BACKGROUND AND OBJECT OF THE STUDY

This study was inspired by the original results of a sleep research doctorate study conducted in the Neurological Sciences Department of Bologna University a few years ago. Lombardi and colleagues [1] studied patients with sleep-related breathing disorders (SRBD) and explored the relation between excessive daytime sleepiness (EDS), objectively determined, with indices of autonomic cardiac regulation, such as baroreflex sensitivity and heart rate variability (HRV), and polysomnographic (PSG) indices of SRBD severity and quality of sleep. The study demonstrated that patients with EDS had significantly lower baroreflex sensitivity and significantly higher low-to-high frequency power ratio of HRV during the different stages of nocturnal sleep than patients without EDS, thus indicating a derangement of cardiac autonomic control at night. The relevance of this finding lies in its pathophysiological but also practical clinical implications since an abnormal autonomic regulation is known to be associated with increased cardiovascular risk. Although the authors could not establish a causal link between these phenomena, they postulated a vicious circle of events whereby a deranged cardiac vagal modulation is related to EDS through a complex interaction between dysfunctions in cerebral regions responsible for sleep regulation, daytime vigilance and autonomic cardiovascular control.

The major objective of the present study was to study patient with narcolepsy, a sleep disease characteristically associated with EDS, to verify if the association between EDS and deranged cardiac autonomic control at night is specific to SRBD. We confined the study to patients with narcolepsy and cataplexy to have a subset of patients with homogeneous clinical and pathophysiological features [2]. In addition, given the strong evidence of hypothalamic involvement in the onset of narcolepsy with cataplexy [3], we also investigated circadian control of blood pressure, heart rate and body core temperature in these patients under controlled environmental conditions.

The study was undertaken from 2006 to 2008. The first year was dedicated to reviewing the existing literature on the topic, devising the study protocol and defining the inclusion criteria for the study population. The second year served for patient recruitment and investigation; the

third and last year was used for data analysis.

THESIS STRUCTURE

The thesis is organised in sections originating from a common study protocol and sharing the same study population of narcoleptic patients. Each module specifies the methods of data analysis and the characteristics of the control populations.

The sections are structured as follow:

1 **Sleep structure in a 32-hour bed-resting schedule**

2 **Analysis of cardiovascular parameters:**

I. autonomic control of the cardiovascular system during wakefulness

II. circadian rhythm of blood pressure and heart rate;

III. time and state dependent changes in blood pressure, heart rate, cardiac output stroke volume, total peripheral resistances;

IV. state-dependent autonomic regulation of heart rate and blood pressure

3 **Analysis of body core temperature:**

I. circadian rhythm, time and state dependent analysis

The thesis is written in English to allow international diffusion and to publish papers on the topics developed in each section.

STUDY POPULATION

Inclusion criteria

We considered for the study adult patients with narcolepsy and cataplexy meeting the ICSD II diagnostic criteria (Table 1) [2]. The exclusion criteria were cardiovascular and/or endocrinologic diseases and other neurological or mental disorders. Sleep-related breathing disorders were excluded by means of a dynamic polysomnographic study (MESAM; AHI < 10). Patients were drug-free except for substances to treat narcoleptic symptoms which were suspended at least one week before the study tests began.

Table 1 Narcolepsy with Cataplexy diagnostic criteria ICSD-II [2]
A. The patient has a complaint of excessive daytime sleepiness occurring almost daily for at least three months.
B. A definite history of cataplexy, defined as sudden and transient episodes of loss of muscle tone triggered by emotions, is present.
C. The diagnosis of narcolepsy with cataplexy should, whenever possible, be confirmed by nocturnal polysomnography followed by an MSLT; the mean sleep latency is less than or equal to eight minutes and two ore more SOREMPSs are observed following sufficient nocturnal sleep (minimum six hours) during the night prior to the test. Alternatively, hypocretin-1 levels in the CSF are less than or equal to 110 pg/ml or one third of mean normal control values.
D. The hypersomnia is not better explained by another sleep disorder, medical or neurological disorder, mental disorder, medication use, or substance use disorder.

Subjects

We enrolled ten patients with narcolepsy and cataplexy (age: 38 ± 12 years; BMI: 28 ± 4) whose major clinical features are summarised in Table 2.

STUDY PROTOCOL

Patients and controls were admitted to hospital and studied following a five-day protocol (Table 3). Subjects were admitted on Monday morning; in the afternoon they received an enema to prepare for rectal temperature monitoring and at night they underwent MESAM to exclude SRBD. From the following morning (between 11 and 12 a.m.), arterial blood pressure (BP), heart rate (HR), body core temperature (BcT°) and sleep-wake cycle were continuously monitored for 44 hours under controlled conditions (recording ending at 8 a.m. two days later). BP and HR were monitored beat-to-beat with a Portapres portable recorder, rectal temperature was monitored every 2 minutes by a Mini-loggerTM portable device and the sleep-wake cycle was monitored by an ambulant polygraphic recorder (Colleague) recording electroencephalogram (EEG: C3-A2, C4-A1), right and left electro-oculogram (EOG), electrocardiogram (ECG), and electromyogram (EMG) of mylohyoideus and left and right tibialis muscles. During the study patients lived in a temperature (24 ± 1°C) and humidity (40-50%) controlled room, lying in bed except when eating, in a light-dark schedule (dark

period: 11 p.m.-7 a.m.). They were allowed to sleep ad libitum. The subjects were placed on a 1.800 kcal/day diet divided into three meals (8 a.m., 12 a.m., 6 p.m.) and three snacks (10 a.m., 4 p.m., 10 p.m.). From midnight preceding the monitoring, subjects were instructed to avoid alcohol, caffeinated beverages and to abstain from smoking. On the morning of the fourth day, the Portapres was removed and subjects underwent multiple sleep latency test (MSLT) maintaining sleep-wake activity and BcT monitoring. On the morning of the fifth and last day, subjects underwent cardiovascular reflexes tests in a temperature controlled (23 ± 1°C) laboratory. Autonomic reflex screening included measurement of BP and HR response to tilt test, Valsalva ratio and BP variation after the Valsalva manoeuvre with respect to the basal value (overshoot), BP response to handgrip test, and HR response to cold face test.

Table 2 Narcolepsy patients characteristics

ID	Age (yr)	Sex	BMI	Age of onset (yr)	Disease duration (yr)	ESS	MSLT min (n° of sleep onset REM periods)	HLA (DQB1*0602)	CSF Hypocretin dosage (pg/ml)	Current Medication	Medication withdrawn (days before hospital admission)
1	30	M	25	15	15	18	3.8 (5)	+	92	none	
2	38	M	27.6	32	6	18	5.4 (5)	+	ND	Anafranil 50mg/die, Modafinil 200mg/die	14
3	39	M	26.6	32	7	14	5.4 (4)	+	ND	none	
4	40	M	33.7	16	24	11	2.4 (5)	+	ND	Modafinil 200mg/die	14
5	32	M	28	17	15	15	2.8 (4)	+	*	Modafinil 100mg/die	7
6	65	F	28	46	19	19	4.1 (2)	+	ND	none	
7	30	M	25.8	18	12	11	5.4 (3)	+	168	Modafinil 400mg/die	14
8	39	M	30	27	12	16	2.2 (4)	+	ND	Modafinil 200mg/die	10
9	33	M	22.7	24	9	19	3.6 (3)	+	30	Modafinil 200mg/die	10
10	29	M	34	12	17	18	4.6 (5)	+	576	Modafinil 300mg/die	7

ND = not detectable; ESS: Epworth Sleepiness Scale; MSLT: multiple sleep latency test: mean of sleep latency (min) of five naps; sleep onset was defined as the latency to the first epoch of any sleep stage [4]; * = patient declined lumbar puncture

Table 3 Study Protocol

	Day 1	Day 2	Day 3	Day 4	Day 5
Time					
07:00		Routine blood chemistry; EKG			
08:00	Hospital patient admission			**48-hours circadian rhythms monitoring ending**	Cardiovascular Reflexes Tests
09:00				MSLT with BcT monitoring	
11/12:00		**48-hours circadian rhythms monitoring starting**			
14:00	Enema				
18:00	MESAM				Patient discharge

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4. Littner MR, Kushida C, Wise M, Davila DG, Morgenthaler T, Lee-Chiong T, Hirshkowitz M, Daniel LL, Bailey D, Berry RB, Kapen S, Kramer M **Standards of Practice Committee of the American Academy of Sleep Medicine Practice parameters for clinical use of the multiple sleep latency test and the maintenance of wakefulness test** Sleep 2005;28:113-121.

Section 1

SLEEP STRUCTURE IN NARCOLEPTIC PATIENTS WITH CATAPLEXY: A 32-HOUR STUDY UNDER BEDREST CONTROLLED CONDITIONS

SCIENTIFIC BACKGROUND

Narcolepsy with cataplexy is characterized by excessive daytime sleepiness (EDS), cataplexy (a sudden bilateral loss of voluntary muscle tone provoked by emotions) and possibly, by other rapid eye movement (REM) sleep phenomena such as sleep paralysis and hallucinations (narcoleptic tetrad). Sleep structure and distribution in humans with narcolepsy differ from normal subjects, being characterized by frequent occurrence of REM sleep onset during daytime and by numerous awakening fragmenting nocturnal sleep [1]. Deprivation and bed rest study protocols showed that the homeostatic regulation of sleep is preserved in narcoleptics [1-3]. In particular, the prolonged sleep deprivation compact nocturnal sleep structure in narcoleptic patients with an overall enhancement of slow wave activity (SWA) that decays exponentially during sleep with a time-constant similar to normal subjects [3, 4, 5]. Nevertheless, a disequilibrium between homeostatic regulation and sleep-wake regulatory mechanisms is suspected in narcoleptic patients on the basis of an enhanced probability of REM sleep onset, longer NREM/REM cycles and a less progressive increase in REM duration through the night.

Several studies investigated the nocturnal sleep architecture of narcoleptic patients [6] and their polysomnographic (PSG) findings showed that patients with narcolepsy present a total sleep time (TST) similar to controls but with reduced sleep efficiency, shortened sleep latency and latency to the first REM episode. Night-time sleep in narcolepsy is highly fragmented, interrupted by numerous awakenings with an increased amount of sleep stage 1 at the expense mainly of sleep stage 2, which is reduced. Slow wave sleep (SWS) can be preserved or reduced and the amount of REM sleep is comparable to that of the normal population. Some studies [2, 3, 7] found out a normal amount of sleep over 24 hours in narcoleptic patients compared to control subjects however used widely different methodological approaches and data analysis.

The present study investigated sleep structure and wake-sleep cycle fragmentation in a sample of narcoleptic patients with cataplexy studied for 32 hours in controlled environmental conditions

METHODS

Subjects

Narcoleptic patients were compared with ten healthy control subjects (7 men; age: 44.9 ± 12 years; BMI: 27 ± 4.7). Subjects and controls had a regular life-style living in particular with regard to the wake-sleep cycle and they did not complain of life-stress events that could interfere with sleep.

Data analyses

The 32-h sleep-wake cycle was visually scored according to the standardized criteria of Rechtschaffen and Kales [8] in 30 s epochs. Total sleep time (TST), sleep efficiency (SE: time spent asleep out of recording time: TRT), duration (minutes) and percentage of TST of NREM stages 1, 2, 3 and 4 and of REM sleep, were calculated for each subject over the 24-hour period (**24h**: from 8 a.m. of the second day of PSG recording), the light period (**daytime**) from 7 a.m to 11 p.m. of the second day of PSG recording and the dark period from 11 p.m. to 7 a.m. of the two nights (**night 1** and **night 2**).

Wake-Sleep fragmentation was analysed in each subject and subsequently in the two groups, by calculating a frame-shift index indicating the number of 30 s sleep stage shifts occurring every 15 minutes throughout the 32 hours.

Statistics

For statistical analysis of sleep variables between and within groups, unpaired or paired 2-tailed *t*-tests were performed. Data of wake-sleep fragmentation expressed as frame-shift indexes and not normally distributed were analysed using the Mann-Whitney rank-sum test for between group analysis of the 24-h and the Wilcoxon matched-pair signed rank test for the within group analysis of night 1 *vs* night 2. All statistical analyses were performed with STATA 9.0 and significance was set at $p \leq 0.05$.

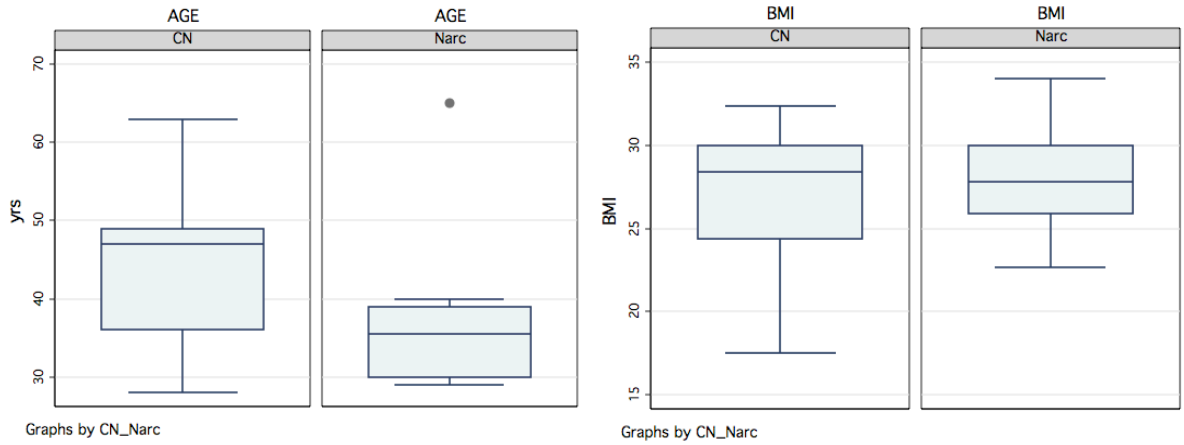
Data are expressed as mean ± SD.

RESULTS

Subjects

Narcoleptic patients and controls did not differ significantly for age (unpaired *t*-test: p=0.16) or BMI (p= 0.56) (Figure 1)

FIGURE 1 AGE AND BODY MAX INDEX IN PATIENTS WITH NARCOLEPSY AND CONTROLS



CN: controls; Narc: patients with narcolepsy; yrs: years; BMI: body mass index; bars represent mean value; error bars represent standard deviations

Sleep architecture

The 24h period

Sleep variables over the 24h are shown in Table 1.

Narcoleptic patients slept significantly more than controls (SE: 43.5% ± 8.6% *vs* 27.2 ± 9.4) and showed more NREM stage 1 and REM sleep. There were no group differences in the amount of NREM stage 2 and SWS although the amount of NREM stage 4 was higher in narcoleptic patients. The analysis of the percentage of sleep phases out of TST (Figure 2) showed significant differences between the two groups in the representation of NREM stage 1 that was higher in narcoleptic patients and of NREM stages 2 and 3 that were higher in the control group.

Night 1 and Night 2

Sleep variables over the two nights are summarized in Table 2.

The within groups analysis did not disclose significant differences in any of the variables considered either in narcoleptic patients or controls.

The between groups analysis showed that TST, SE, the amount of NREM stages 2 and 4 and of REM sleep was comparable in the two groups on nights 1 and 2. SWS was reduced in narcoleptic patients only in night 1 because of the significative reduction of NREM stage 3, not confirmed on night 2.

The analysis of the percentage of sleep phases out of TST (Figure 3 and 4) showed more NREM stage 1, less NREM stage 3 on nights 1 and 2 in narcoleptic patients. However, the prevalence of SWS was significantly reduced in narcoleptic patients only on night 1. NREM stages 2 and 4 and of REM sleep were comparable in the two groups on nights 1 and 2.

Daytime

Sleep variables over the light-period are shown in Table 3.

Narcoleptic patients slept significantly more than controls (SE: 29.6 % ± 10.7 % *vs* 6.3 ± 7.7) and showed a higher amount of every sleep stage.

The analysis of the percentage of sleep phases out of TST (Figure 5) showed more NREM stage 1 and REM sleep in narcoleptic patients whereas NREM stages 2, 3, 4 and SWS were comparable in the two groups.

Wake-sleep fragmentation

The 24h period

The analysis of wake-sleep fragmentation over 24h (Figure 6, Table 4) disclosed an overall increase of the frame-shift index in the narcolepsy group which remained significantly higher than controls especially during the light period from 8:45 a.m to 8:45 p.m, except for short periods corresponding to meal time. The two groups showed a comparable wake-sleep fragmentation from 8:45 p.m until 11:30 p.m. Considering the dark-period, differences between the two groups were less prominent in the first part of the night.

Night 1 vs Night 2

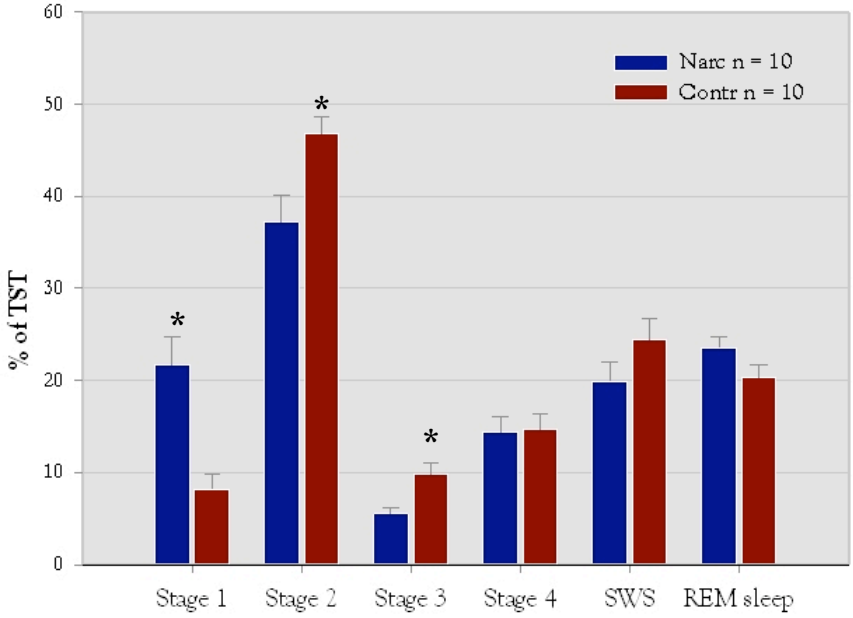
The within group analysis of sleep fragmentation on night 1 *vs* night 2 showed no significant differences in the narcolepsy group. In the control group we detected only isolated differences between the two nights at h 11:30 p.m., h 12:45 p.m and h 3:00 a.m. (Figure 7).

Table 1 Sleep structure over the 24h period in patients with narcolepsy and controls

	Patients with narcolepsy (n=10)		Controls (n=10)		p values
	min (SD)	% of TST (SD)	min (SD)	% of TST (SD)	
TST	626.4 (124.7)		391.8 (135.7)		<0.01
SE %	43.5 (8.6)		27.2 (9.4)		<0.01
Stage 1	119.6 (38.5)	21.7 (9.5)	30.6 (23.6)	8.2 (5.6)	<0.01
Stage 2	237.55 (87.3)	37.2 (9.3)	184.9 (77.6)	46.8 (5.7)	ns
Stage 3	33.7 (12.4)	5.5 (2)	39.2 (19)	9.8 (4)	ns
Stage 4	87.3 (30.9)	14.4 (5.5)	55.8 (20.8)	14.7 (5.4)	0.01
SWS	121.1 (38.6)	19.9 (6.8)	95 (31.5)	24.5 (6.9)	ns
REM sleep	148.2 (39.7)	23.6 (3.8)	81.2 (37.7)	20.3 (4.6)	<0.01

TST: total sleep time; SE: sleep efficiency (time spent asleep over total recording time); SWS: slow wave sleep; p values refer to sleep stages duration in minutes; *ns*: not significant

Figure 2 Sleep structure over 24h



TST: total sleep time; SWS: slow wave sleep; * $p \leq 0.01$: p values refer to sleep stages percentage of TST

Table 2 Sleep structure over night 1 and night 2 in patients with narcolepsy and controls

	Night 1			Night 2		
	Patients with narcolepsy N=10	Controls N=10		Patients with narcolepsy N=10	Control subject N=10	
	min (SD)	min (SD)	p values	min (SD)	min (SD)	p values
TST	362.4 (67.4)	373.3 (64.4)	ns	359.7 (63.7)	334.8 (94.4)	ns
SE %	75.5 (14)	77.7 (13.4)	ns	74.9 (13.2)	69.7 (19.6)	ns
Stage 1	62.8 (23.9)	24.8 (22.9)	**<0.01**	55.8 (19.1)	21.9 (14.5)	**<0.01**
Stage 2	151.4 (50.7)	170.25 (45.4)	ns	136.3 (44)	153.3 (56.5)	ns
Stage 3	19.1 (9.8)	38.2 (19.9)	**0.01**	24.5 (10.9)	35.4 (17)	ns
Stage 4	43.5 (21.6)	51.6 (21.3)	ns	51.4 (19.4)	49.7 (20.5)	ns
SWS	62.7 (28.8)	89.8 (27.5)	**0.04**	76 (25.5)	85.2 (30.9)	ns
REM sleep	85.5 (29.8)	92 (26.3)	ns	91.6 (34.7)	74.35 (26.2)	ns

TST: total sleep time; SE: sleep efficiency (time spent asleep out of total recording time); SWS: slow wave sleep; ns: not significant

Figure 3 Sleep structure over night 1

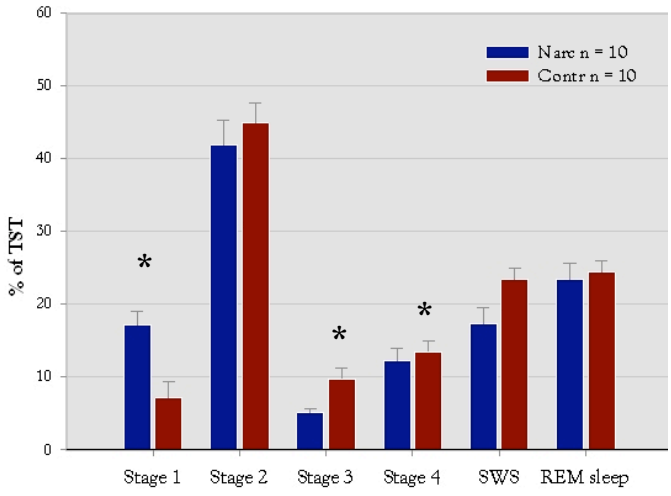
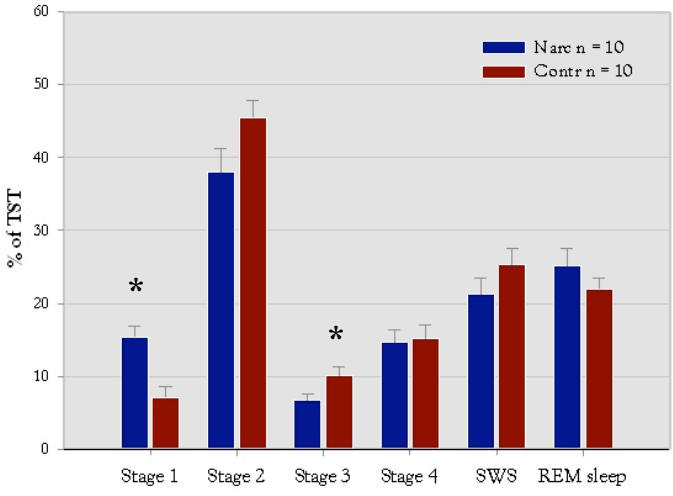


Figure 4 Sleep structure over night 2



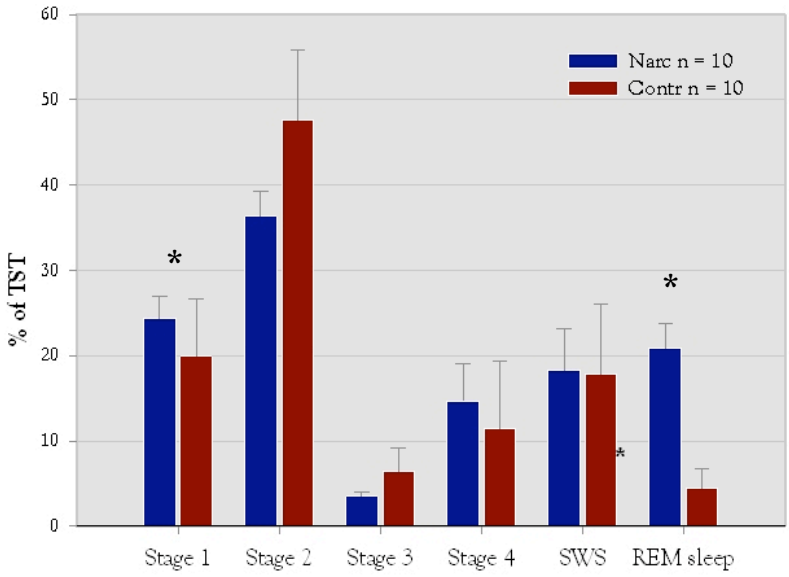
TST: total sleep time; SWS: slow wave sleep; * $p \leq 0.01$: p values refer to sleep stages percentage of TST; Row data are not reported in Table 2

Table 3 Sleep structure over daytime in patients with narcolepsy and controls

	Patients with narcolepsy (n=10)		Controls (n=10)		p values
	min (SD)	% of TST (SD)	min (SD)	% of TST (SD)	
TST	266,6 (97,1)		56,95 (69,4)		<0.01
SE %	29.6 (10.7)		6.3 (7.7)		<0.01
Stage 1	63.8 (32.3)	24.4 (8.3)	8.7 (12.2)	19.9 (21.7)	<0.01
Stage 2	101.2 (53.2)	36.3 (9.4)	31.6 (38.2)	47.6 (26.2)	<0.01
Stage 3	9.1 (4.9)	3.5 (1.7)	3.7 (4.2)	6.4 (9)	0.01
Stage 4	35.9 (28.7)	14.7 (14)	6 (10)	11.5 (24.8)	<0.01
SWS	45 (31.5)	18.3 (15.2)	9.8 (10.8)	17.9 (26.1)	<0.01
REM sleep	56.6 (28.2)	20.9 (8.9)	6.8 (17)	4.5 (7.5)	<0.01

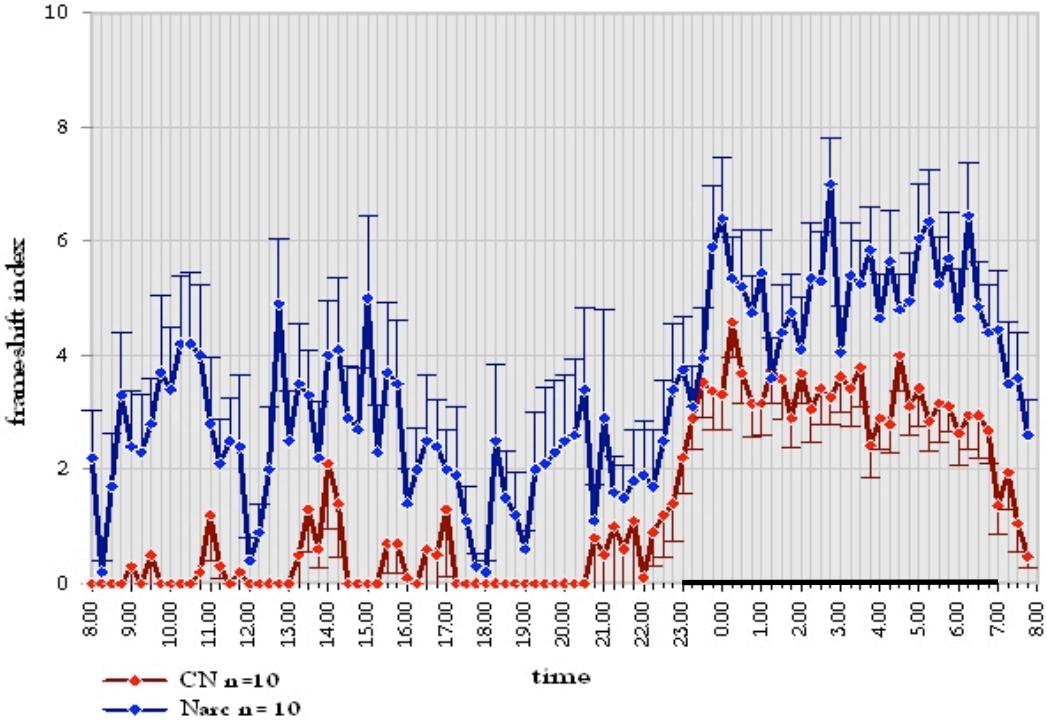
TST: total sleep time; SE: sleep efficiency (time spent asleep out of total recording time); SWS: slow wave sleep; p values refer to sleep stages duration in minutes

Figure 5 Sleep structure over daytime



TST: total sleep time; SWS: slow wave sleep; * $p \leq 0.01$; *p* values refer to sleep stages percentage of TST

FIGURE 6 SLEEP FRAGMENTATION THROUGHOUT THE 24H



Each point from 8:00 to 23:00 represents the mean ± SE of 10 narcolepsy patients and 10 controls; each point during night-time (23:00 - 7:00) represents the mean of 20 values (night 1 and night 2) ± SE of 10 narcolepsy patients and 10 controls; dark bar represents dark period from lights off to lights on

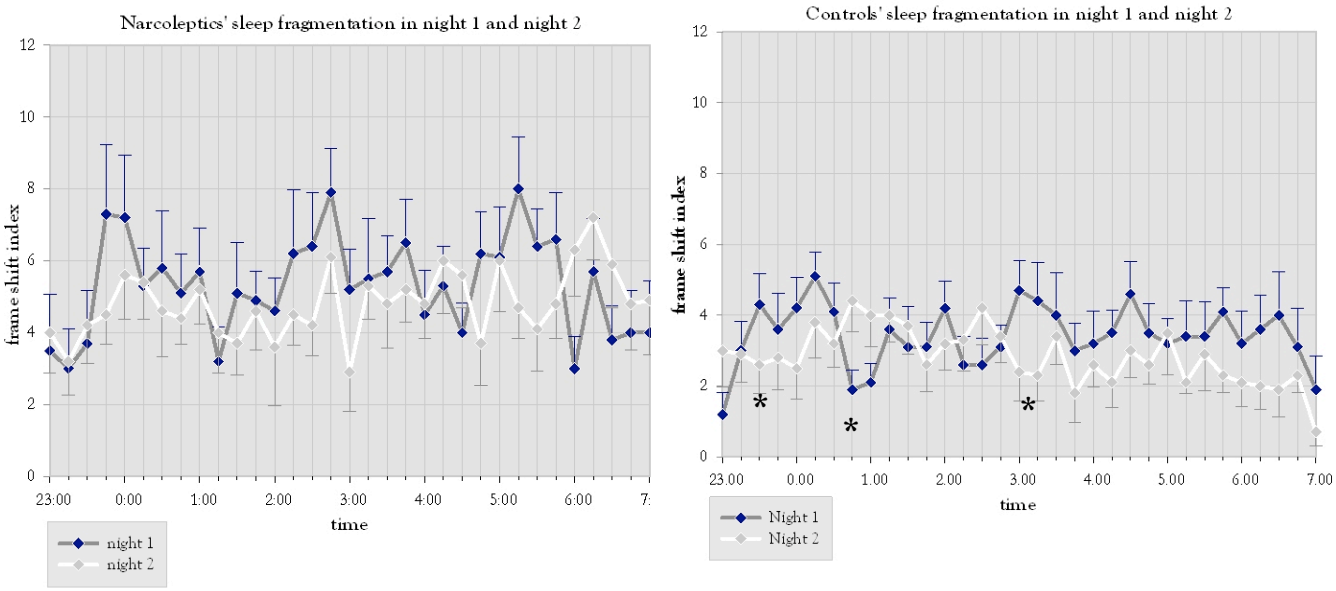
Table 4 Frame-shift index values obtained every 15 minutes throughout the 24h in narcolepsy patients and controls

Time	CN (n=10)		Narc (n =10)		p
	Frame Shift Index		Frame Shift Index		
	mean	SD	mean	SD	
08:00	0	0	2,2	2,7	<0.01
08:15	0	0	0,2	0,6	
08:30	0	0	1,7	3,0	
08:45	0	0	3,3	3,5	<0.01
09:00	0,3	1	2,4	3,1	0.03
09:15	0	0	2,3	3,2	0.04
09:30	0,5	2	2,8	2,5	0.01
09:45	0	0	3,7	4,3	<0.01
10:00	0	0	3,4	3,4	<0.01
10:15	0	0	4,2	3,7	<0.01
10:30	0	0	4,2	3,9	<0.01
10:45	0,2	1	4	3,9	<0.01
11:00	1,2	3	2,8	3,7	
11:15	0,3	1	2,1	2,4	0.02
11:30	0	0	2,5	2,4	<0.01
11:45	0,2	1	2,4	4,0	0.05
12:00	0	0	0,4	1,3	
12:15	0	0	0,9	1,5	
12:30	0	0	2	3,5	0.01
12:45	0	0	4,9	3,6	<0.01
13:00	0	0	2,5	2,8	<0.01
13:15	0,5	2	3,5	3,3	<0.01
13:30	1,3	2	3,3	2,5	0.02
13:45	0,6	1	2,2	3,2	
14:00	2,1	4	4	3,0	
14:15	1,4	3	4,1	4,0	0.01
14:30	0	0	2,9	2,9	<0.01
14:45	0	0	2,7	3,4	<0.01
15:00	0	0	5	4,6	<0.01
15:15	0	0	2,3	2,6	<0.01
15:30	0,7	2	3,7	3,9	0.03
15:45	0,7	2	3,5	3,5	0.02
16:00	0,1	0	1,4	1,9	0.06
16:15	0	0	2	2,3	<0.01
16:30	0,6	2	2,5	3,6	0.05
16:45	0,5	2	2,4	2,6	0.01
17:00	1,3	4	2	2,2	
17:15	0	0	1,9	3,8	0.01
17:30	0	0	1,1	1,9	
17:45	0	0	0,3	0,7	
18:00	0	0	0,2	0,6	
18:15	0	0	2,5	4,2	
18:30	0	0	1,5	2,6	
18:45	0	0	1,2	2,4	0.03
19:00	0	0	0,6	1,1	0.03
19:15	0	0	2	3,2	0.01
19:30	0	0	2,1	4,3	0.03
19:45	0	0	2,3	4,0	0.03
20:00	0	0	2,5	3,7	0.01
20:15	0	0	2,6	4,2	<0.01
20:30	0	0	3,4	4,6	<0.01
20:45	0,8	3	1,1	2,0	
21:00	0,5	2	2,9	6,0	
21:15	1	3	1,6	2,0	
21:30	0,6	2	1,5	1,8	
21:45	1,1	3	1,8	2,8	
22:00	0,1	0	1,9	3,0	
22:15	0,9	2	1,7	3,1	
22:30	1,2	2	2,5	3,3	
22:45	1,4	2	3,4	3,6	

Time	CN (n=10)		Narc (n =10)		p
	Frame Shift Index		Frame Shift Index		
	mean	SD	mean	SD	
23:00	2,21	3	3,8	4,2	
23:15	2,89	2	3,1	3,1	
23:30	3,53	3	4,0	4,0	
23:45	3,37	3	5,9	4,8	0.03
00:00	3,32	3	6,4	4,7	0.03
00:15	4,58	3	5,4	3,2	
00:30	3,68	2	5,2	4,5	
00:45	3,16	3	4,8	2,9	0.02
01:00	3,16	2	5,5	3,4	0.01
01:15	3,74	3	3,6	3,2	
01:30	3,58	3	4,4	3,7	
01:45	2,89	2	4,8	2,9	0.02
02:00	3,68	2	4,1	4,1	
02:15	3,05	3	5,4	4,4	
02:30	3,42	3	5,3	3,9	
02:45	3,26	2	7,0	3,6	<0.01
03:00	3,63	3	4,1	3,6	
03:15	3,42	3	5,4	4,2	0.05
03:30	3,79	3	5,3	3,5	
03:45	2,42	3	5,9	3,4	<0.01
04:00	2,89	2	4,7	3,4	
04:15	2,79	2	5,7	4,0	0.01
04:30	4	3	4,8	2,8	
04:45	3,11	2	5,0	3,8	
05:00	3,42	3	6,1	4,3	0.04
05:15	2,84	2	6,4	4,0	<0.01
05:30	3,16	3	5,3	3,6	0.03
05:45	3,11	2	5,7	3,6	0.01
06:00	2,63	3	4,7	3,8	0.05
06:15	2,95	3	6,5	4,2	<0.01
06:30	2,95	3	4,9	3,5	0.05
06:45	2,68	3	4,4	3,8	
07:00	1,37	2	4,5	4,6	<0.01
07:15	1,95	3	3,5	4,8	
07:30	1,05	2	3,6	3,6	<0.01
07:45	0,45	1	2,6	2,8	<0.01

Each value from 8:00 to 23:00 represents the frame-shift index mean ± SE of 10 narcoleptic patients and 10 controls; the night-time (23:00 – 7:00) values represent the mean of 20 values ± SE (nigh1 and night 2) of 10 narcoleptic patients and 10 controls

FIGURE 7 SLEEP FRAGMENTATION IN NIGHT 1 AND NIGHT 2 IN NARCOLEPSY PATIENTS AND CONTROLS



Each point during night-time (23:00 – 7:00) represents the mean of 10 values of 10 narcolepsy patients and 10 controls; *: $p < 0.05$; error bars represent standard errors

DISCUSSION

This study investigated sleep structure and wake-sleep cycle fragmentation of ten narcoleptic patients with cataplexy and ten healthy control subjects following a 32 hour schedule of bedrest under controlled conditions. The results showed that the amount of the 24 hour sleep in narcoleptic patients was significantly higher than controls, mainly due to the higher amount of NREM stage 1 and of REM sleep. The total amount of SWS was nevertheless comparable in the two groups. Since TST did not differ in the two groups on nights 1 and 2, this suggests that there was more daytime sleep (light period) in narcoleptic patients. This result is in line with previous findings of Volk et al. [9] who demonstrated that narcoleptic patients under continuous bedrest slept two to threefold more during the day than patients who were sitting at a table, suggesting the influence of body position and the related somatic activity on the amount of sleep but also on its internal architecture. In fact, REM sleep was suppressed by the sitting position. Furthermore, the study by Volk et al. found that daytime sleep had a selective

influence on subsequent nocturnal sleep mainly represented by a decreased amount of SWS in the bed group which displayed some SWS also during the day. This finding supported the assumption of a homeostatic regulation of SWS in narcoleptic patients [1, 3, 5].

The 24h total amount of SWS in our study was comparable in narcoleptic patients and controls and although narcoleptic patients displayed a significant increase in SWS also during the light period, this was associated with a reduced amount of SWS only on night 1.

Our study confirmed previous findings [6] that night sleep in narcoleptic patients is similar to that of control subjects in terms of TST, amount of SWS (slightly reduced on night 1) and REM sleep, being mainly characterized by a higher amount of NREM stage 1, suggestive of a more fragmented sleep. In our bed-rest schedule, narcoleptic patients slept significantly more than controls during the daytime displaying all sleep stages, but sleep structure mainly comprised NREM stage 1 and REM sleep. As expected, the analysis of wake-sleep fragmentation expressed as frame-shift index values revealed a higher fragmentation in the narcolepsy group especially during the daytime indicative of an ongoing fluctuation of vigilance. Interestingly, there was a period of about three hours from 8:45 p.m to 11:45 p.m (time preceding nocturnal sleep) during which the two groups behaved similarly. During the dark-period, the differences between narcoleptic patients and controls appeared to be less impressive and continuous in the first part of the night. This might support previous findings [5] suggesting that nocturnal sleep fragmentation occurs in narcoleptic patients after dissipation of sleep pressure in the first sleep cycle which is followed by cycles interrupted by an increased number and longer duration of short wake periods. The within group analysis of sleep fragmentation of night 1 vs night 2 showed a comparable frame-shift index pattern in narcoleptic patients and controls thereby supporting evidence that in this bed-rest schedule, patients and controls behaved similarly on the two consecutive nights of PSG recording in terms of both sleep architecture and sleep fragmentation.

Further information will be added to the present study by the analysis of sleep and REM sleep latency, arousal and PLMS indexes.

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Section 2

Analysis of cardiovascular parameters

I. AUTONOMIC CONTROL OF THE CARDIOVASCULAR SYSTEM DURING WAKEFULNESS IN PATIENTS WITH NARCOLEPSY AND CATAPLEXY

SCIENTIFIC BACKGROUND

An autonomic nervous system (ANS) impairment has been suggested in narcoleptic patients on the basis of abnormalities found in pupillometry [1] ejaculatory [2] and cardiovascular [3, 4] functions. However, the evidence has been considered inconclusive [5]. A few studies evaluated cardiovascular reflex during wakefulness in patients with narcolepsy with conflicting results. Sachs and Kaijser [3] found that the increase in heart rate (HR) and resting arm blood flow during isometric handgrip exercise in unmedicated narcoleptic patients ("with clinically well-defined narcolepsy") was less than in control subjects. In addition, narcoleptic patients showed a decreased incidence of sinus arrhythmia to Valsalva manoeuvre. The same authors subsequently confirmed these results in a sample of nine drug-naïve narcoleptic patients with cataplexy who also had a significantly lower Valsalva ratio than controls [4]. They concluded that cardiovascular reflex abnormalities are characteristic of narcolepsy caused by a defect in central nervous system (CNS) excitatory mechanisms. This hypothesis, however, was not subsequently supported by Hublin et al [6] who failed to find cardiovascular reflex deficits in 22 unmedicated narcoleptic patients, suggesting that the abnormalities reported in earlier studies may have reflected differences or insufficient standardization in test conditions.

Based on the hypothesis that the discrepancy among all these studies could be a consequence of vigilance fluctuation characteristic of narcoleptic patients, Ferini-Strambi et al. [7] investigated ANS activity in patients with narcolepsy. They analysed heart rate variability during sleep since the repetitive changes in autonomic variables are constant and not influenced by patients' alertness or degree of cooperation in this condition. The study revealed decreased power in the low frequency (LF) during sleep compared with wakefulness and a significantly increased LF/HF ratio (HF: high frequency) during wakefulness before sleep in patients with narcolepsy compared with controls. They excluded a primary disturbance of cardiac autonomic nervous system control in narcolepsy but suggested an impaired circadian autonomic function in these patients.

More recently, Fronczek et al. [8] investigated HRV and blood pressure variability (BPV) in narcoleptic patients and healthy subjects during wakefulness, finding higher power in all

frequency bands in narcolepsy patients associated with a comparable LF/HF ratio, this being interpreted as indicative of a reduced sympathetic tone. Since ANS activity changes have been implicated in triggering cataplexy [9], Donadio et al. 10] investigated the time course of sympathetic and cardiovascular activities during cataplexy by means of microneurography. Their findings did not show significant changes in autonomic activity prior to cataplexy onset, thus ruling out a triggering role of the ANS. However, cataplexy was associated with activation of sympathetic and parasympathetic autonomic systems, a pattern reminiscent of that reported during the vigilance reaction in animals.

The present study tested the autonomic control of the cardiovascular system in narcoleptic patients with cataplexy and healthy control subjects under controlled conditions.

METHODS

Subjects

Narcoleptic patients were compared with 18 healthy control subjects (2 women; age: 41.6 ± 12 years; BMI: 26.5 ± 2).

All subjects were tested in the morning between 8 and 12 a.m. Before the tests, they were allowed to drink water but otherwise had to fast overnight. All had to abstain from smoking or drinking alcohol on the day before the study. They were asked not to sleep or talk during the study.

Cardiovascular reflexes

Patients were studied in a temperature-controlled clinical investigation room (23 ± 1°C). Systolic and diastolic blood pressure (SBP, DBP; Portapres model 2, TNO-TPD Biomedical Instrumentation, Delft, the Netherlands), heart rate (HR; Grass 7P511 [Astro-Med West Warwick, RI, USA] and Light Work Station for digital RR quantification), oronasal and abdominal breathing (Grass DC preamplifier 7P1) were monitored continuously.

After 30 min of supine rest, the head-up tilt test (10 min at 65°, HUTT), Valsalva manoeuvre (40 mmHg for 15 s), deep breathing (6 breaths/min), cold face test (60 s of application of cold compresses at 0–1°C to the forehead) and sustained handgrip (30% of maximal effort for

5 min) were performed using standard procedures [11]. The correct execution of tests was checked automatically by an electronic device which displays the time and execution of the manoeuvre. Tests were repeated until they were performed with an error <5% with respect to the expected procedure. The manoeuvres were carried out in the sequence described, allowing a period of rest required to reach basal BP and HR values in between investigations. The results of each test were automatically obtained by means of home-developed software. Finger pressure detected by Portapress can be measured between 10 and 300 mmHg and the internal accuracy of the instrument is 1.5% of full scale.

Basal values of SBP, DBP and HR were obtained calculating the mean value of the last five minutes of supine rest preceding HUTT and tilt values were obtained calculating the mean value of the last of ten minutes of HUTT.

During Valsalva manoeuvre, the following indices of autonomic activity were considered: the ratio between HR in phases II and IV (VR) and the overshoot during phase IV (difference between the highest SBP after the expiratory effort and the basal value), along with the baroreflex sensitivity index (BRSI) calculated during the release phase of the manoeuvre according to Goldstein *et al.* [12].

At deep breathing, the sinus arrhythmia (calculated in beats per minute using the 10 longest R-R intervals during expiration and the 10 shortest R-R intervals during inspiration) and the I/E ratio (ratio between the mean of higher HR values during 10 deep inspirations and the mean of the lower HR values during expirations) were calculated. At cold face test, the changes with respect to the basal value of SBP, DBP and HR were computed after 60 s of application of cold compresses (0–1°C) to the forehead. At sustained handgrip, the changes with respect to the basal value of SBP, DBP and HR were calculated after 5 min of isometric effort.

Analysis of heart rate variability

HRV was analysed in the time and frequency domains using dedicated HRV Analysis Software 1.1 for Windows developed by the Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland (http://venda.uku.fi/research/biosignal) [13].

HRV analysis was performed in each subject on 512 consecutive RR-interval series chosen in the last 6 minutes at the end of supine rest and between 4th and 9th minute of HUTT.

Time domain analysis

The time-domain parameters were calculated directly from the raw RR intervals [14]. We considered as statistical measures: **mean** and **standard deviation** of the selected **RR intervals series** (s) and **heart rate series** (bpm), the first describing the overall variation in the RR interval signal and the second the short-term variation; **RMSSD** (ms), the square root of the mean squared differences of successive RR intervals; **NN50** (count), the number of consecutive RR intervals differing more than 50 ms; **pNN50** (%), the percentage value of consecutive RR intervals differing more than 50 ms.

Frequency domain analysis

Autoregressive power spectral analysis (PSA) of HR variability (HRV) during supine rest and HUTT was performed using standard procedures [15]. Power spectral density was calculated using both a non-parametric (**Fast Fourier Transform**: FFT) and parametric algorithms (**autoregressive model**: AR) to allow the comparison of our results with the existing published data [7].

We considered a high-frequency (HF) component, reflecting mostly vagal activity, centred for each subject to his/her respiratory frequency determined by power spectral analysis, and a low frequency (LF) component (centred at ~0.1 Hz, range: 0.04-0.15 Hz), reflecting mostly sympathetic activity. The oscillatory components < to 0.04 Hz (very low frequency: VLF) were considered to be DC noise. We also calculated the total power spectrum (ms^2) resulting from the sum of VLF power, LF power and HF power. The LF/HF ratio was used as an index of sympathovagal balance and was calculated on the normalized units of LF and HF power (LF_nu, HF_nu), obtained dividing each power component by the total power less the DC component. Owing to its inherent nonlinearity, the signal from the nasal thermistor was used only to assess the main respiratory frequency of the period considered for the evaluation of HRV.

Statistics

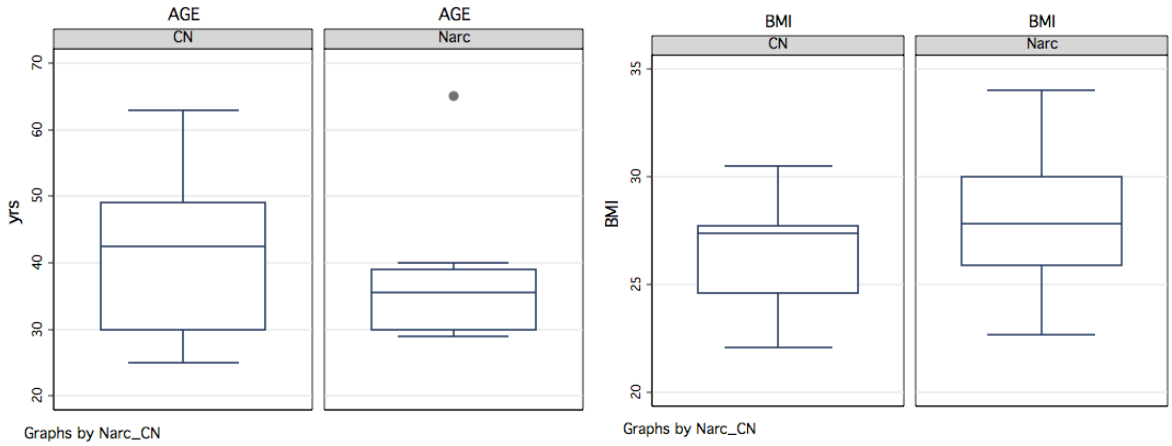
Data are reported as mean ± SD. Data normally distributed were compared using Student's unpaired *t*-test. RMMSD, NN50, pNN50, RR triangular index and data of PSA of HRV expressed as normalized unit, not normally distributed, were analysed using the Mann-Whitney rank-sum test for between groups analysis and the Wilcoxon matched-pair signed rank test for the within group analysis. All statistical analyses were performed with STATA 9.0 and significance was set at $p \leq 0.05$.

RESULTS

Subjects

Narcoleptics and controls did not differ significantly for age (unpaired *t*-test: $p=0.36$) or BMI ($p=0.13$) (Figure 1).

FIGURE 1 AGE AND BODY MAX INDEX IN NARCOLEPSY PATIENTS AND CONTROLS



Contr: controls; Narc: narcoleptic patients; yrs: years; BMI: body mass index; bars represent means; error bars represent standard deviations

Cardiovascular reflexes

All subjects correctly performed all the autonomic tests. The basal values of SBP and DBP were comparable in the two groups whereas HR values were significantly higher in narcoleptics (Table 1).

After 10 min of HUTT (Table 2), SBP, DBP and HR showed a trend to a mild increase with respect to the basal values in the two groups without reaching statistical significance.

The cardiovascular responses to Valsalva manoeuvre, BRSI, cold face, deep breathing and isometric handgrip were normal and similar in narcoleptics and controls (Table 3).

Analysis of heart rate variability

Time domain analysis

The time-domain parameters calculated at rest and after 10 min of HUTT are reported in Table 4. HR was significantly higher in narcoleptic patients at rest whereas it was comparable in the two groups after HUTT. RMMSD, NN50 and pNN50 were similar in narcoleptic patients and controls at rest and during HUTT.

Frequency domain analysis

Autoregressive model (AR)

Data of spectral analysis of HRV obtained with the AR are summarised in Table 5.

Respiratory frequency in supine position was significantly higher in narcoleptic patients whereas during HUTT the two groups showed comparable values.

Total power in HRV spectrum was similar in the two groups at rest and during HUTT.

LF_nu and HF_nu components of the PSA of HRV differed in narcoleptic patients and controls during supine rest, the LF_nu component being higher and the HF_nu component lower in the narcoleptic patients (Figure 2). Consequently, the LF/HR ratio at rest was significantly higher in the narcoleptic group ($p < 0.01$).

During HUTT, the LF_nu and HF_nu components were similar in patients and controls (Figure 1). However, while the expected changes in PSA components were observed in controls (significant increase in the LF component ($p< 0.01$) and significant reduction of the HF component ($p< 0.01$)), this was not detected in the narcoleptic patients who showed values of LF_nu and HF_nu during tilt comparable to those in the rest condition. The LF/HF ratio during HUTT was in fact similar in the two groups.

Fast Fourier Transform model (FFT)

Data of spectral analysis of HRV obtained with the FFT are summarised in Table 6.

Total power in the HRV spectrum was higher in the control group at rest whereas narcoleptics and controls behaved similarly during HUTT. LF_nu and HF_nu components at rest and during HUTT showed a pattern similar to that observed in AR analysis characterised by an increase in the LH_nu component and a reduction of the HF_nu component at rest in narcoleptic patients and by comparable values of LF_nu and HF_nu during HUTT (Figure 3). The within group analysis again showed a significant increase ($p<0.01$) in the LF_nu component and a significant reduction ($p<0.01$) of the HF_nu component during HUTT in controls, absent in the narcoleptic group. Similarly, the LF/HF ratio was significantly higher in narcoleptic patients at rest and comparable in the two groups during HUTT.

Table 1 Cardiovascular findings (raw data) at supine rest in narcoleptic patients and controls

Rest supine	**Narcoleptic patients** (n = 10)	**Controls** (n = 18)	p values
SBP (mmHg)	122 ± 16	119 ±11	ns
DBP (mmHg)	65 ± 9	62 ± 6	ns
HR (beats/min)	71 ± 9	64 ± 6	**0.02**

Basal values (mean ± SD) obtained averaging all the values from the last 5 min before head-up tilt, after 30 min of supine rest; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; ns: not significant

Table 2 Cardiovascular findings (raw data) at 10TH min of head-up tilt test in narcoleptic patients and controls

Head-up tilt 10 min	Narcoleptic patients (n = 10)	Controls (n = 18)	p values
SBP (mmHg)	128 ± 20	131 ± 8	ns
DBP (mmHg)	73 ± 10	75 ± 7	ns
HR (beats/min)	84 ± 9	77 ± 9	ns

Values (mean ± SD) after 10 min of head-up tilt test; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; ns: not significant

Table 3 Cardiovascular responses to Valsalva manoeuvre, deep breathing, cold face and handgrip in narcoleptic patients and controls

	Valsalva manoeuvre			Deep breathing	
	VR	**Overshoot (mmHg)**	**BRSI (ms/mmHg)**	**I/E**	**ΔHR (beats/min)**
Narcoleptics patients (n = 10)	1.69 ± 0.35	44.2 ± 22	4.7 ± 2.8	1.4 ± 0.16	24 ± 8
Controls (n = 18)	1.64 ± 0.35	41 ± 17.7	5.5 ± 4.5	1.37 ± 0.13	21 ± 6
	Cold face			**Isometric handgrip**	
	ΔSBP (mmHg)	**ΔDBP (mmHg)**	**ΔHR (beats/min)**	**ΔSBP (mmHg)**	**ΔDBP (mmHg)**	**ΔHR (beats/min)**
Narcoleptics patients (n = 10)	21.1 ± 16.2	6.2 ± 8.8	-15.6 ± 10.3	31.2 ± 15.4	17.5 ± 7	10.4 ± 4.7
Controls (n = 18)	19.7 ± 15.7	9.3 ± 8.9	-13.6 ± 12.5	37.1 ± 15	20 ± 7.9	11.3 ± 11

VR: Valsalva ratio; BRSI: baroreflex sensitivity index; I/E: inspiratory–expiratory ratio; Δ: change with respect to the basal value; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR, heart rate

Table 4 Time-domain parameters of heart rate variability calculated in 512 consecutive RR intervals at supine rest and during head-up tilt test in narcoleptic patients and controls

Time-domain parameters	**Narcoleptic patients** (n = 10)	**Controls** (n = 18)		**Narcoleptic patients** (n = 10)	**Controls** (n = 18)	
	REST		p values	**TILT**		p values
Mean RR ± SD (sec)	0.87 ± 0.12	0.95 ± 0.09	ns	0.72 ± 0.08	0.78 ± 0.08	ns
Mean HR ± SD (bpm)	69.7 ± 8.9	63.3 ± 6.6	3	83.9 ± 10	77 ± 9	ns
RMSSD± SD (ms)	29 ± 13	37 ± 27	ns	16 ± 9	18 ± 7	ns
NN50 ± SD (count)	48 ± 41	78 ± 94	ns	8,7 ± 10	12,3 ± 17	ns
pNN50 ± SD (%)	10 ± 9	15 ± 8	ns	1,6 ± 1,9	2,4 ± 3,5	ns

RR: interval between consecutive R peaks; HR: heart rate; SD: standard deviation; RMMSD: square root of the mean squared differences of successive RR intervals; NN50: number of consecutive RR intervals that differ more than 50 ms; pNN50: percentage value of consecutive RR intervals that differ more than 50 ms; ns: not significant

Table 5 Power spectral analysis (PSA) of heart rate variability (HRV) with autoregressive model at supine rest and during head-up tilt test in narcoleptic patients and controls

PSA of HRV		**Narcoleptic patients** (n = 10)	**Controls** (n = 18)	p values
Respiratory frequency (Hz)	Rest	0.29 ± 0.05	0.24 ± 0.04	**0.03**
	Tilt	0.28 ± 0.06	0.27 ± 0.05	ns
Total Power Spectrum ± SD (ms2)	Rest	867385 ± 532914	1765267 ± 1883242	ns
	Tilt	836836 ± 1187942	2217624 ± 4918801	ns
LF_nu (Hz ± SD)	Rest	61 ± 19 (0.1 ± 0.01)	51 ± 15.7 (0.09 ± 0.01)	**< 0.01***
	Tilt	68.8 ± 21 (0.08 ± 0.01)	75.9 ± 17.8 (0.12 ± 0.1)	ns *
HF_nu (Hz ± SD)	Rest	24 ± 12.6 (0.28 ± 0.05)	39 ± 14.2 (0.25 ± 0.04)	**< 0.01***
	Tilt	21.2 ± 14 (0.27 ± 0.06)	18.6 ± 16.1 (0.26 ± 0.04)	ns *
LF/HF	Rest	3.7 ± 3	1.6 ± 1	**< 0.01***
	Tilt	7 ± 6	9 ± 9	ns*

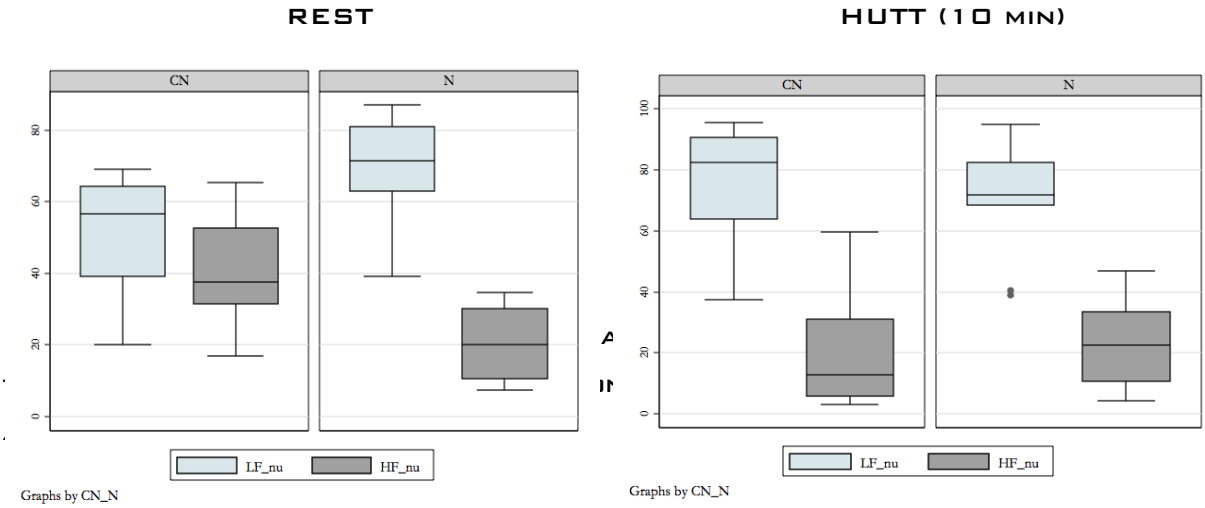
Rest: values obtained in supine rest position; tilt: values obtained during head-up tilt test; total power spectrum: sum of VLF power, LF power and HF power; LF_nu: LF normalized unit = percentage obtained by dividing the low frequency spectral component (LF), centred at 0.1 Hz by the total power less the DC noise; HF_nu: HF normalized unit = percentage obtained by dividing the high frequency spectral component (HF) centred at subject's respiratory frequency by the total power, less the DC noise; *: significance refers to statistical analysis on LF_nu and Hf_nu components; ns: not significant

Table 6 Power spectral analysis (PSA) of heart rate variability (HRV) with Fast Fourier Transform model at supine rest and during head-up tilt test in narcoleptics and controls

PSA of HRV		**Narcoleptics** (n = 10)	**Controls** (n = 18)	p values
Total Power Spectrum ± SD (ms2)	Rest	136600 ± 82673	247065 ± 131781	**< 0.01**
	Tilt	58583 ± 33264	86077 ± 42658	ns
LF_nu	Rest	73.1 ± 17.2	73.8 ± 7.2	**0.05**
	Tilt	74.4 ± 18.6	78.6 ± 2.3	ns
HF_nu	Rest	21.7 ± 4.6	26 ± 7.2	**0.05**
	Tilt	19.9 ± 4.9	21.3 ± 2.3	ns
LF/HF	Rest	3.7 ± 3	1.6 ± 1	**0.05**
	Tilt	7 ± 6	9 ± 9	ns

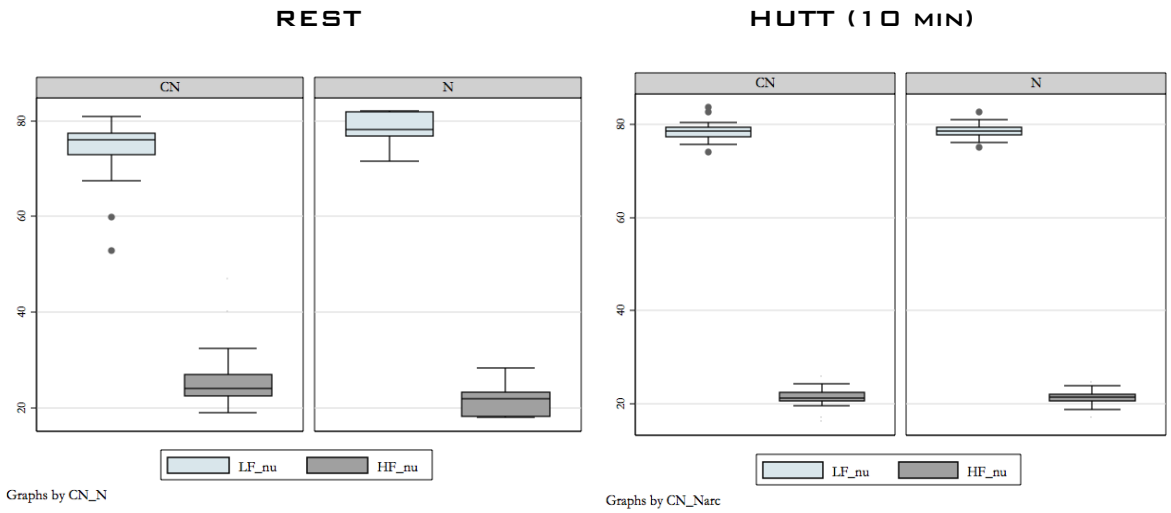
Rest: values obtained in supine rest position; tilt: values obtained during head-up tilt test; total power spectrum: sum of VLF power, LF power and HF power; LF_nu: LF normalized unit = percentage obtained by dividing the low frequency spectral component (LF), centred at 0.1 Hz by the total power less the DC noise; HF_nu: HF normalized unit = percentage obtained by dividing the high frequency spectral component (HF) centred at subject's respiratory frequency by the total power, less the DC noise; *ns*: not significant

Figure 2 LF and HF normalised unit at supine rest and during head-up tilt test in narcoleptic patients and controls with autoregressive analysis



CN: controls; N: narcoleptic patients; LF_nu: LF normalized unit; HF_nu: HF normalized unit; bars represent means; error bars represent standard deviations

Figure 3 LF and HF normalised unit at supine rest and during head-up tilt test in narcoleptic patients and controls with Fast Fourier Transform analysis



CN: controls; N: narcoleptic patients; LF_nu: LF normalized unit; HF_nu: HF normalized unit; bars represent means; error bars represent standard deviations

DISCUSSION

We investigated the autonomic control of cardiovascular reflexes in controlled conditions in ten unmedicated narcoleptic patients with cataplexy and 18 healthy control subjects. We also studied power spectral analysis of HRV in supine rest condition and during HUTT.

Our findings excluded abnormalities in cardiovascular responses to Tilt test, Valsalva manoeuvre, BRSI, cold face, deep breathing and isometric handgrip in the narcoleptic group, thus supporting the latest findings of Hublinc et al. [6]. It is probable that the lack of uniformity in the narcoleptic study population and in control subjects and the insufficient standardization in test conditions may have influenced the different results of Sachs and Kaijser's earlier studies [3,4].

Narcoleptic patients in our study showed higher values of HR at rest with respect to control subjects, however the HR variations at HUTT, cold face, deep breathing and isometric handgrip were similar in the two groups.

Spectral analysis of HRV yielded the most impressive result of this study, showing an enhanced LF power component during supine rest in narcoleptic patients, comparable to that observed during HUTT. FFT and AR analysis on LH and FH normalised components, showed (more evident in AR analysis) that in rest supine position the LF/HF ratio was significantly higher in narcoleptic patients due to the increased LF component and the reduced HF component. During HUTT, the physiological PSA changes in HRV observed in controls (significant increase in the LF component and significant reduction of the HF component) were only slight in narcoleptic patient, their sympathetic tone probably being nearly at its maximum in the supine position. This finding is compatible with the increased mean HR that we found in the narcolepsy group during supine rest. Since the LF/HF ratio is used as a measure of the autonomic parasympathetic-sympathetic balance [14], it is suggestive of an increased sympathetic tone in narcoleptic patients in rest conditions.

Our data clash with the findings of Fronczek et al. [8] who studied the PSA of HRV with FFT in 9 unmedicated narcoleptic patients with cataplexy lying in rest supine position. They found an increased total power in the HR spectrum of narcoleptic patients with respect to controls associated with a comparable LF/HF ratio. They interpreted these findings as suggestive of a reduced sympathetic tone in narcoleptic patients.

To compare our results with Fronczek et al.'s study, we performed PSA of HRV also with FFT, finding that in supine position the total power spectrum of HR was greater in the control group, being comparable in the two groups during HUTT. However, this result was not confirmed by AR analysis that showed comparable values in supine rest and during HUTT. Furthermore, Fronczeck et al. calculated the LF/HF ratio considering the absolute value of LF and HF power (ms^2) and not their normalized value, as we did. The normalized value is more representative of the controlled and balanced behaviour of the two branches by the autonomic nervous system and minimizes the effect on the values of LF and HF components of the changes in total power [14].

An increased LF/HF ratio in narcoleptic patients compared with controls has been described during wakefulness preceding sleep [7] but not confirmed during the awake state far from sleep.

However, the study provided no data on subjects' body position and activity during the awake state.

Various animal studies have shown that the hypocretin system is heavily involved in neuroendocrine and autonomic control [16]. By inducing c-*fos* expression, intracerebroventricular injection of orexine-A in rats can activate neurons in hypothalamic and brainstem cardiovascular centers (e.g. paraventricular nucleus, raphe, locus coeruleus, dorsal motor nucleus of the vagus) [17] and stimulate sympathetic outflow with an increase in HR and BP [18]. However, the exact site of action of the orexins to stimulate autonomic function remains unknown. Further, while orexine microinjection into the commissural nucleus tractus solitarius (NTS) may determine a rapid, dose-dependent increase in BP and HR [19], orexine microinjection into the caudal dorsolateral and medial regions of the same nucleus may evoke the opposite effects of hypotension and bradycardia [20], suggesting that orexines may act on different autonomic control regions exerting different, even opposite, effects.

Thus, our findings on a sample of narcoleptic patients with an orexin deficit suggest the proper functioning of the autonomic brainstem effectors centres regulating cardiovascular reflexes and a selective imbalance in the autonomic control of HRV in favour of an enhanced sympathetic

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Section 2

Analysis of cardiovascular parameters

II. THE CIRCADIAN RHYTHM OF BLOOD PRESSURE AND HEART RATE IN NARCOLEPSY WITH CATAPLEXY

SCIENTIFIC BACKGROUND

Blood pressure (BP) and heart rate (HR) show circadian and short-term fluctuations resulting from changes in body posture, daily activity and neurohumoral activity. In particular, the day-night oscillation of BP is strongly linked to the sleep-wake circadian rhythm and plays a dominant role in the observed 24-h BP variation characterised by BP decline to its lowest levels during nigh-time sleep (dipper pattern) [1].

Although sleep is the most important and consistent source of circadian BP variation, the exact mechanism mediating its influence on circadian BP rhythm is not completely understood [2]. Patients with fatal familial insomnia, a rare prion disease characterised by the total and sustained disruption of the sleep-wake cycle [3, 4], maintain the 24-h pattern of BP variability until the terminal stage of the disease. When the sleep deprivation is accompanied by a total loss of the nocturnal BP fall, nocturnal bradycardia is still preserved. These data suggest that circadian rhythms in autonomic nervous system (ANS) function may exist independently of the sleep/wake cycle and that the 24-h variations of HR reflect modulatory influences different from those controlling the BP pattern.

Independently of sleep abnormalities, circadian changes in BP depend on the proper functioning of the sympathetic nervous system. In fact, patients with multiple system atrophy (MSA) often present sleep abnormalities (e.g. sleep fragmentation, frequent awakenings) in the presence or absence of autonomic failure, but only MSA patients with autonomic failure exhibited a nocturnal BP dysregulation [2]. The importance of studying endogenous circadian cardiovascular rhythms also lies in their clinical implications. It is well documented that cardiovascular incidents follow a daily rhythm with maximum risk in the early morning [5], which cannot be totally explained by a circadian rhythm in exogenous factors such as activity and body position. This could be attributed rather to circadian changes in BP, vascular tone, catecholamines, platelet aggregation and HR [6]. In addition, recent findings suggest a disorganization of the 24-h and ultradian BP rhythm in non-dipper hypertensive subjects [7, 8].

Endogenous circadian rhythms in mammalian physiology and behaviour are known to be regulated by the suprachiasmatic nucleus (SCN) in the anterior hypothalamus, “the biological

clock". Lastly, recent anatomical evidence documented an impairment of SCN functioning in patients with essential hypertension [9].

Accumulating data in animals and humans support an endogenous circadian regulation of HR and heart rate variability (HRV) [10]. Sheer et al [11] demonstrated that lesioning the SCN in rats abolished the HR circadian rhythm and resting HR levels were between the day and night values of intact rats. The same study demonstrated a multisynaptic connection from the SCN to the autonomic subdivision of the paraventricular nucleus of the hypothalamus suggesting that circadian control of the cardiac cycle is mediated by a neural mechanism, at least in rats.

There is strong evidence of a hypothalamic involvement in the onset of narcolepsy with cataplexy, characterised by the loss of hypothalamic neurons containing hypocretin. Patients with narcolepsy and cataplexy show excessive daytime sleepiness and cataplexy as clinical hallmarks. In addition, their sleep structure and distribution differ from those in normal subjects, being characterized by frequent REM sleep onset during daytime and numerous awakenings fragmenting nocturnal sleep. Aim of this study was to evaluate the circadian 24-h rhythm of BP and HR in a sample of patients with narcolepsy and cataplexy under controlled conditions in a bed-rest protocol.

METHOD

Subjects

Patients with narcolepsy were compared to ten healthy control subjects described in Section 1

Circadian rhythm of blood pressure and heart rate analysis

Rhythmicity was analysed evaluating the time series for systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR according to the single cosinor method using a computerized procedure [12]. This procedure will determine whether or not there is a rhythm within a 24-hour period ($P < 0.05$) and evaluates the following parameters with their 95% confidence limits: (1) mesor (Midline Estimating Statistic of Rhythm): rhythm adjusted 24-hour average; (2) amplitude: (AMP) the difference between the maximum value measured at the acrophase and the mesor in the cosine curve; (3) acrophase: (ACR) lag between reference time (12 p.m.) and

time of highest value of the cosine function used to approximate the rhythm. For each subject we analysed the 24-hour rhythmicity of two consecutive days, the first starting between 11 a.m and 12 a.m. and the second starting at 8 a.m.

Statistics

The between group differences were analyzed using Student's unpaired t-test. The within group analysis to test reproducibility of the rhythm parameters between day 1 and day 2 was made using the Student's paired t-test. Variables were considered significantly different if $p \leq 0.05$.

RESULTS

Results of circadian rhythms of SBP, DBP and HR are reported in Table 1.

The within group analysis did not show significant differences in any of the rhythm parameters considered between day 1 and day 2, both in narcoleptics and controls.

Systolic blood pressure

A significant circadian rhythm of SBP was detected in narcoleptics and controls. The two groups showed similar mesor and ACR whereas AMP was significantly reduced in narcoleptics ($p=0.05$).

Diastolic blood pressure

A significant circadian rhythm of DBP was detected in narcoleptics and controls. The two groups did not differ for mesor, AMP or ACR values.

Heart rate

A significant circadian rhythm of HR was detected in narcoleptics and controls. Narcoleptic patients showed a higher mesor and phase-advanced ACR values with respect to controls whereas AMP was similar in the two groups.

Table 1 Results of circadian rhythm analysis of SBP, DBP and HR in narcoleptic patients and controls

Circadian rhythm of SBP	**Narcoleptics** (n = 10*)	**Controls** (n = 10*)	p values
Mesor (mmHg) Mean ± SD	122.1 ± 11.1	121.3 ± 11.1	ns
Amplitude Mean ± SD	6 ± 3.1	8.1 ± 3.6	0.05
Acrophase (h, min) Mean ± SD	14.3 ± 3.1	14.3 ± 2.1	ns
Circadian rhythm of DBP			
Mesor (mmHg) Mean ± SD	71.7 ± 6.5	70.4 ± 5.3	ns
Amplitude Mean ± SD	3.9 ± 1.7	4.9 ± 2.2	ns
Acrophase (h, min) Mean ± SD	14.6 ± 4.1	14.2 ± 2	ns
Circadian rhythm of HR			
Mesor (bpm) Mean ± SD	73.2 ± 8.7	63.7 ± 10.9	< 0.01
Amplitude Mean ± SD	6.9 ± 3.5	6.3 ± 3.8	ns
Acrophase (h, min) Mean ± SD	16,17 ± 2.21	15 ± 1.3	0.05

SBP: systolic blood pressure, DBP: diastolic blood pressure; HR: heart rate; * each value represents the mean of 20 values (day 1 and day 2) of 10 narcoleptics and 10 controls

DISCUSSION

The results of this study show that under controlled conditions the circadian rhythmicity of BP and HR was preserved in patients with narcolepsy and cataplexy compared to healthy control subjects. Although patients with narcolepsy showed a significantly higher fragmentation of the wake-sleep cycle throughout the 24 hours (see Section 1), this did not seem to influence the circadian rhythmicity of these cardiovascular parameters. However, the analysis of SBP rhythm

disclosed reduced AMP values in patients with narcolepsy with respect to controls but with comparable ACR and mesor values. All DBP rhythm parameters were similar in the two groups. These data overall suggest that endogenous circadian regulation of BP is preserved in patients with narcolepsy and cataplexy.

The analysis of HR rhythm was the more interesting result of the study. Patients with narcolepsy had a higher HR mesor and a slightly phase-advanced ACR compared to controls. The higher HR mesor in narcolepsy patients ($p<0.01$) reinforces the data we found in autonomic tests exploring cardiovascular reflexes and heart rate variability (Section 2), indicative of an imbalanced cardiac autonomic tone in narcolepsy patients, in favour of an enhanced sympathetic tone. The slight phase-advancing of ACR values ($p=0.05$) in narcolepsy patients may suggest a selective derangement of HR circadian rhythm timing in narcolepsy with cataplexy, but this should be considered with caution as our sample of 10 patients was relatively small, thereby increasing the chance of type II errors. Furthermore, Vandevalle G et al [13] recently demonstrated robust endogenous variations in HR and HRV in humans in the absence of the masking effect of sleep, general activity, postural changes and light. They also found that extended sleep opportunity combined with exogenous melatonin, phase-advanced the HR and HRV endogenous rhythm presumably acting on a common pacemaker, the SCN. Thus, the extended sleep opportunity intrinsic to our protocol resulting in extended sleep/rest episodes in narcolepsy patients may have induced the significant phase-shift advancing in HR rhythm in our patients with narcolepsy.

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Section 2

Analysis of cardiovascular parameters

III. TIME AND STATE-DEPENDENT MODULATION OF BLOOD PRESSURE AND HEART RATE IN NARCOLEPSY WITH CATAPLEXY

SCIENTIFIC BACKGROUND

Many studies evaluated the 24h variations of blood pressure (BP) and heart rate (HR) in humans, showing that they decrease significantly during the nyctohemeral phase [1, 2]. However, while recent studies indicated that the fall in HR during sleep is largely due to an endogenous circadian rhythm [3], the fall in BP is considered almost entirely dependent on the sleep process [4] .

Studies investigating sleep in humans have confirmed that there is a fall in BP and HR during non-rapid eye movement (NREM) sleep compared with relaxed wakefulness. Blood pressure may also be lower in slow wave sleep (SWS) than stage 2. During rapid eye movement (REM) sleep, BP and in some studies HR, return to waking levels. Other studies found that HR remains below waking but above NREM sleep levels. During phasic REM sleep, BP and HR both show pulsatile increases in activity [5]. The changes in HR and BP during sleep appear to be mediated primarily by changes in autonomic circulatory control. During NREM sleep stages 1 and 2 the increase observed in vagal activity may or may not parallel the development of SWS (NREM stages 3 and 4) and a reduction in total sympathetic vascular tone. During REM sleep these two changes are reversed[5].

Most studies have investigated the sleep effects on autonomic control of the cardiovascular system, starting from the assumption that stage effects are independent of time during the sleep period. Trinder et al [6] found that BP and the low frequency (LF) and high frequency (HF) of heart rate variability (HRV) were not affected by time during the sleep period, suggesting that time of night effects are due to the changing distribution of sleep stages. By contrast, HR changed over time within each sleep stage supporting previous findings of a combined sleep and circadian influence over this variable. In addition, these authors found few non significant differences in autonomic activity between NREM stage 2 and SWS, suggesting that the degree of cortical desynchronisation has little effect on cardiac activity during sleep.

The BP decline during night-time has major clinical implications. During nocturnal sleep BP is lowered by 10-20% compared to daytime mean featuring the so-called normal dipper pattern [7, 8]. The absence of this normal decline in BP during sleep, typically observed in non-dipper

hypertensive subjects, is considered one of the most sensitive predictors of cardiovascular mortality (stroke, acute myocardial infarct and sudden cardiac death) [9] since BP is elevated and impacts target tissues and organs for a prolonged amount of time during the 24h. Moreover, the non-dipper pattern is predictive of cardiovascular mortality even in normotensive subjects [10]

One study on narcoleptic patients with cataplexy and healthy control subjects [11] evaluated the distribution over night-time of mean systolic blood pressure (SBP) and compared mean SBP values between each sleep stages. No differences were found in SBP measurements throughout the night or between sleep stages in the two groups whereas a mild non significant BP increase in REM sleep compared with stage 3-4 NREM sleep was detected in narcoleptic patients.

The present study evaluated time and state-dependent 24h variations of BP, HR and other parameters of autonomic cardiac control in a sample of narcoleptic patients with cataplexy compared to healthy control subjects evaluated under controlled conditions.

METHODS

Subjects

Narcoleptics patients were compared to the ten healthy control subjects described in Section 1.

Time-dependent analysis

Data were obtained by two consecutive days of polysomnographic (PSG) recording, the first starting at about 12 a.m and the second at 8 a.m.

For each subjects, we analysed the distribution over the 24h period of the mean value of the beat-to-beat values of SBP, diastolic blood pressure (DBP), HR, stroke volume (SV), cardiac output (CO) and total peripheral resistances (TPR) calculated every 15 min. SV, CO and TPR were calculated by portapress modelflow method which uses a three-element model of aortic input impedance to compute a flow pulsation from an arterial pressure pulsation [12].

Day-night pattern

To evaluate the nocturnal decline of SBP, DBP and HR we calculated a daytime SBP, DBP and HR mean value (from 9 a.m. to 9 p.m) and a night-time mean value (from 12 p.m to 6 a.m) for each subject [13, 14]. The difference between night-time and daytime values (ΔSBP, ΔDBP, ΔHR) was then calculated and also expressed as % decline of night-time values over daytime values.

Statistics

The between group analysis of ΔSBP, ΔDBP and ΔHR was performed using Student's two-tailed *t* test. Significance level was set at $p < 0.05$.

State-dependent analysis

The analysis was conducted on the 32 consecutive hours starting from the first night of PSG recording at 11 p.m (lights off). For each subject we calculated the mean value of SBP, DBP, HR, SV, CO and TPR during wakefulness (W) and each sleep stage (NREM stage 1, 2, SWS and REM sleep) and expressed data considering the 24h period (24h; from 7 a.m), the daytime period (daytime; from 7 a.m. to lights off at 11. pm) and the night-time period (night-time; from 11 p.m. to lights on at 7 a.m.)

During daytime and night-time we also calculated in each subject the difference (Δ) between the mean value of each cardiovascular parameter in W, NREM stage 1, 2, SWS and REM sleep and the corresponding mean value during daytime W (ΔSBP, ΔDBP, ΔHR, ΔSV, ΔCO, ΔTPR). This allowed us to express the state-dependent variability during daytime and night-time of each parameter when compared with a reference value (subjects' daytime W mean value).

Wake-sleep structure of patients with narcolepsy and controls during 24h, daytime and night-time is described in Section 1.

Statistics (thanks to the collaboration with Prof. Patrizia Agati, Dipaertment of Statistical Sciences, University of Bologna)

Significance analyses were performed by fitting a mixed model (a model with fixed and random effects) where the factors were wake-sleep phases (W, NREM stage 1 and 2, SWS, REM) and group (controls *vs* narcolepsy subjects). Significance level was set at $p < 0.05$. All the analyses were performed using Stata 9. Data are reported as mean ± SD.

RESULTS

Time-dependent analysis

The analysis of the 24-h distribution of SBP and DBP (Figures 1 and 2) showed a similar pattern in narcolepsy subjects and controls characterized by the physiological BP decline during night-time. However, narcoleptics exhibited a diminished nocturnal decline of SBP and DBP. The 24h distribution of HR mean values was similar in the two groups but patients with narcolepsy showed higher HR values throughout the 24h (Figure 3).

CO, SV and TPR showed a similar 24h pattern in narcolepsy subjects and controls (Figures 4, 5 and 6). SV, which represents the strength of heart contraction, it was increased in the narcolepsy group throughout the 24h. Since CO is a positive function of HR and SV, both increased in narcolepsy subjects, it resulted increased in narcolepsy group throughout the 24h. Finally, the modelflow method computed TPR as a negative function of CO. CO being increased in narcoleptics, TPR was reduced throughout the 24h in the narcolepsy group.

Day-night pattern

ΔSBP and ΔDBP were significantly ($p < 0.01$) higher in controls than in narcolepsy subjects. Controls showed a night-time SBP decline of -14 ± 7 mmHg and a SBP % decline of 11% ± 4.8%. Patients with narcolepsy showed a night-time SBP decline of - 7 ± 5 mmHg and a SBP % decline of 5% ± 3.9%.

The night-time DBP decline in controls was of - 8.4 ± 4.5 mmHg with a DBP % decline of 11 % ± 4.5%. The night-time DBP decline in narcoleptics was of – 4.5 ± 3.3 mmHg with a DBP % decline of 6.2 % ± 4.4%.

One patient with narcolepsy showed an inverted nocturnal BP pattern (reverse dipping) characterized by an increase in SBP and DBP nocturnal values with respect to daytime values (SBP night-time % increase: +1.9% and DBP night-time % increase: + 2.1%). Another patient with narcolepsy displayed a similar night-time pattern only in DBP values (DBP night-time % increase: + 0.3%)

ΔHR was comparable in the two groups (p=0.8) being of – 10.6 ± 7 bpm in controls and -10 ± 6 bpm in narcoleptic patients. HR % decline in controls and narcoleptic patients was of 15.8 ± 9% and 12.2 ± 6.5 % respectively.

State-dependent analysis

Data of SBP, DBP, HR, ΔSBP, ΔDBP, and ΔHR in wake-sleep phases are shown in Table 1.

Systolic blood pressure

Controls and patients displayed a significant physiological modulation of SBP values through sleep phases during the 24h, daytime and night-time. In the 24h, the two groups had comparable SBP mean values during W but narcoleptic patients showed higher SBP values during NREM stage 1 ($p< 0.01$), NREM stage 2 ($p= 0.01$), SWS ($p= 0.04$) and REM ($p<0.01$), as compared to controls. An identical SBP pattern was detectable during daytime.

In the night-time (Figure 7), narcoleptic patients and controls showed comparable SBP mean values during W, NREM stage 1, NREM stage 2 and SWS whereas SBP in REM was higher in the narcolepsy group ($p= 0.02$).

In daytime (Figure 8), patients with narcolepsy showed significantly reduced ΔSBP values ($p=0.02$) in all sleep phases compared to controls. In the night-time (Figure 9), patients with narcolepsy showed a trend ($p=0.06$) towards reduced ΔSBP values in all sleep phases, but significance was detected only during REM sleep ($p=0.02$).

Diastolic blood pressure

Controls and patients displayed a significant physiological modulation of DBP values through sleep phases during the 24h, daytime and night-time. In the 24h, the two groups had comparable DBP mean values during W, NREM stage 1, NREM stage 2 and SWS but not in REM when narcoleptic patients showed higher DBP values (p= 0.03). During daytime, DBP mean values were comparable in the two groups during W and SWS but they were higher in narcoleptics in NREM stage 1 (p=0.04), NREM stage 2 (p=0.04) and REM (p=0.01).

In the night-time, narcoleptics and controls showed comparable SBP mean values during all sleep phases. In daytime, patients with narcolepsy showed significantly reduced ΔDBP values (p=0.04) in all sleep phases compared to controls. In the night-time ΔDBP was comparable in the two groups in all sleep phases.

Heart rate

Controls and patients displayed a significant physiological modulation of HR values through wake-sleep phases during the 24h, daytime and night-time. The narcolepsy group was characterized by a significant increase in HR values during W that remained stable in all sleep phases in the 24h (p= 0.01), daytime (p= 0.01) and night-time (p= 0.02). ΔHR values were comparable in the two groups in all sleep phases in daytime and night-time.

Data of CO, SV, TPR, ΔCO, ΔSV, and ΔTPR in wake-sleep phases are shown in Table 2.

Cardiac Output

Controls and patients displayed a significant modulation of CO values through sleep phases during the 24h, daytime and night-time. The narcolepsy group was characterized by a significant increase in CO values during W that remained stable in all sleep phases in the 24h (p<0.01), daytime (p<0.01) and night-time (p<0.01). ΔCO values were comparable in the two groups in all sleep phases in daytime and night-time.

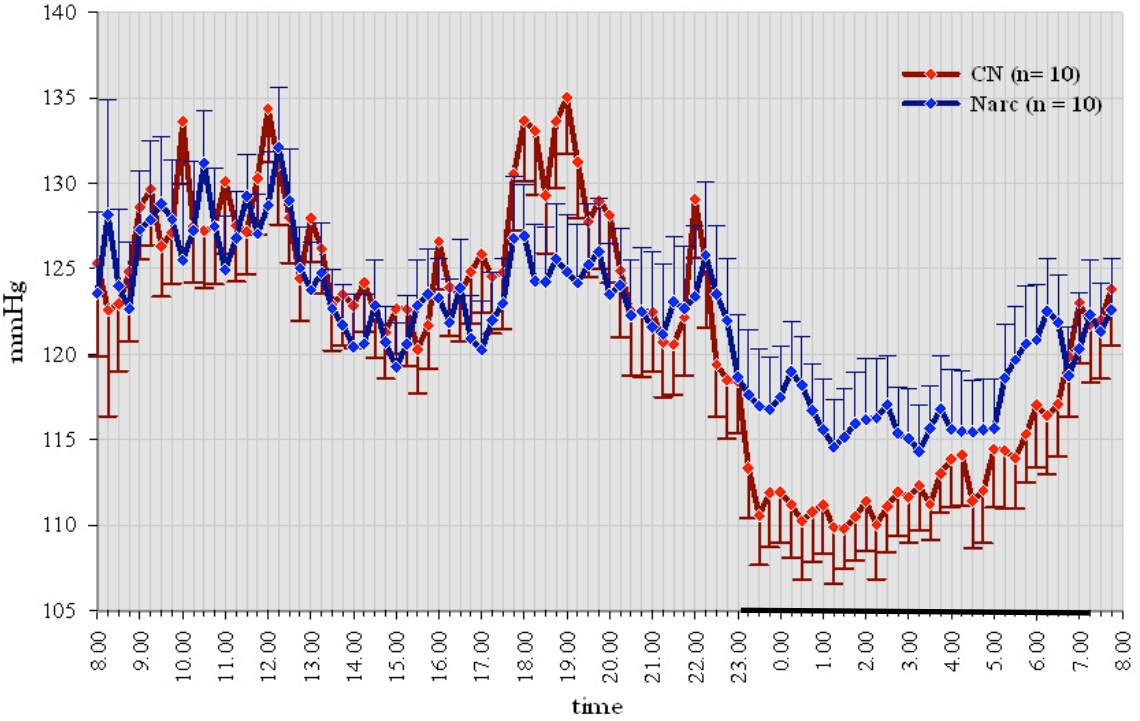
Stroke Volume

Controls and patients displayed a significant modulation of SV values through sleep phases during the 24h, daytime and night-time. The narcolepsy group was characterized by a significant increase in SV values during W that remained stable in all sleep phases in the 24h ($p=0.03$) and daytime ($p=0.04$). In night-time, SV values were comparable in the two groups in all sleep phases with the exception of SWS during which narcoleptics showed higher SV values ($p=0.02$) ΔSV values did not differ in the two groups in daytime in any sleep phases while in night-time they were increased in SWS in narcoleptic patients.

Total Peripheral Resistances

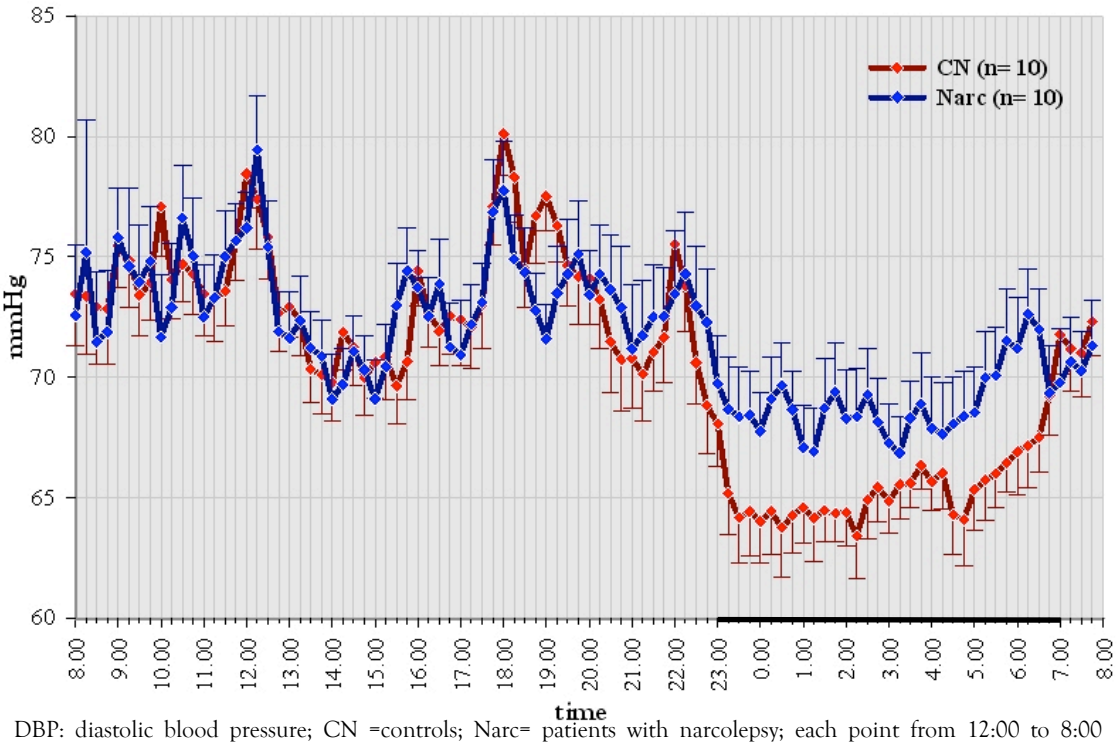
Controls and patients displayed a significant modulation of TPR values through sleep phases during daytime. In the 24h and night-time a significant reduction of TPR ($p= 0.02$) was detectable only during transition from W to NREM stage 1. The narcolepsy group was characterized by a significant decrease of TPR values during W that remained stable in all sleep-phases in the 24h ($p<0.01$), daytime ($p<0.01$) and night-time ($p< 0.02$). ΔTPR values were comparable in the two groups in all sleep phases in daytime and night-time.

Figure 1 Mean SBP values over the 24h in patients with narcolepsy and controls



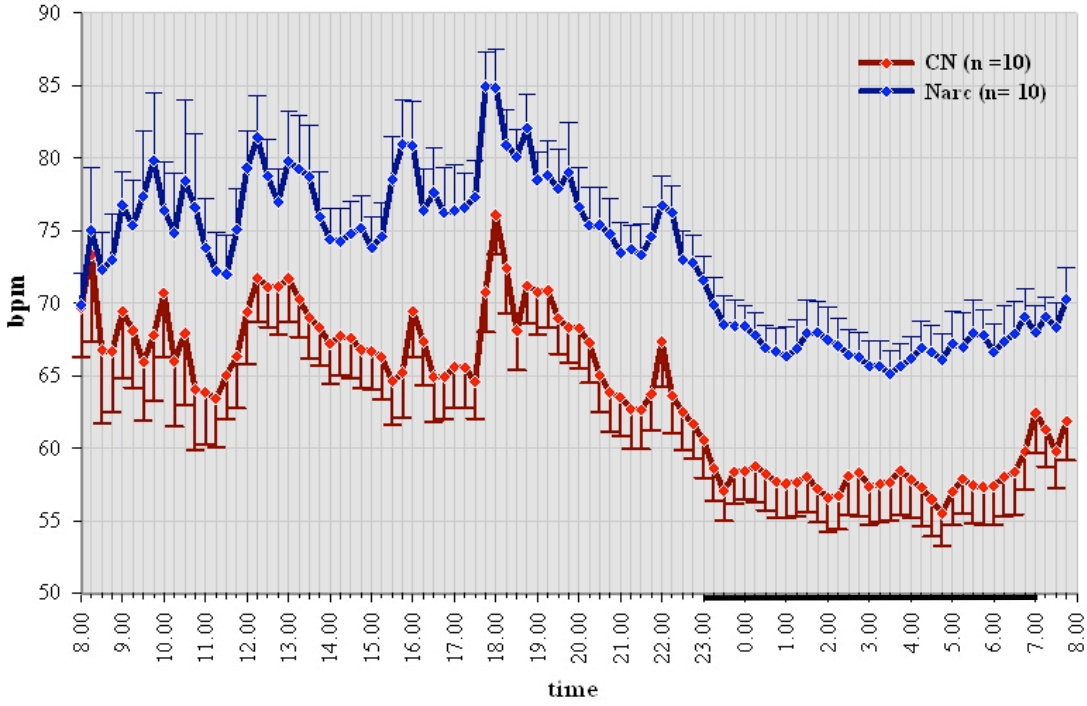
SBP: systolic blood pressure; CN= controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of SBP values of day 1 and 2 while each point from 8:00 to 12:00 represents the mean every 15 min ± SE of SBP values of day 2; dark bar represents the dark period (from lights off to lights on)

Figure 2 Mean DBP values over the 24h in patients with narcolepsy and controls



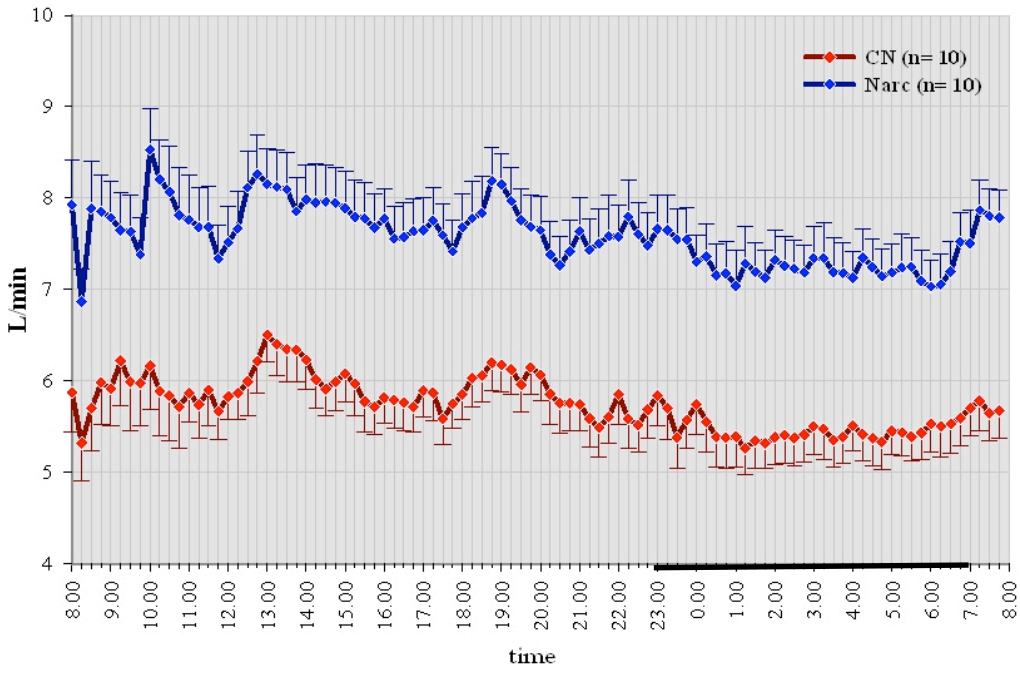
DBP: diastolic blood pressure; CN =controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of DBP values of day 1 and 2 while each point from 8:00 to 12:00 represent the mean every 15 min ± SE of DBP values of day 2; dark bar represents the dark period (from lights off to lights on)

Figure 3 Mean HR values over the 24h in patients with narcolepsy and controls



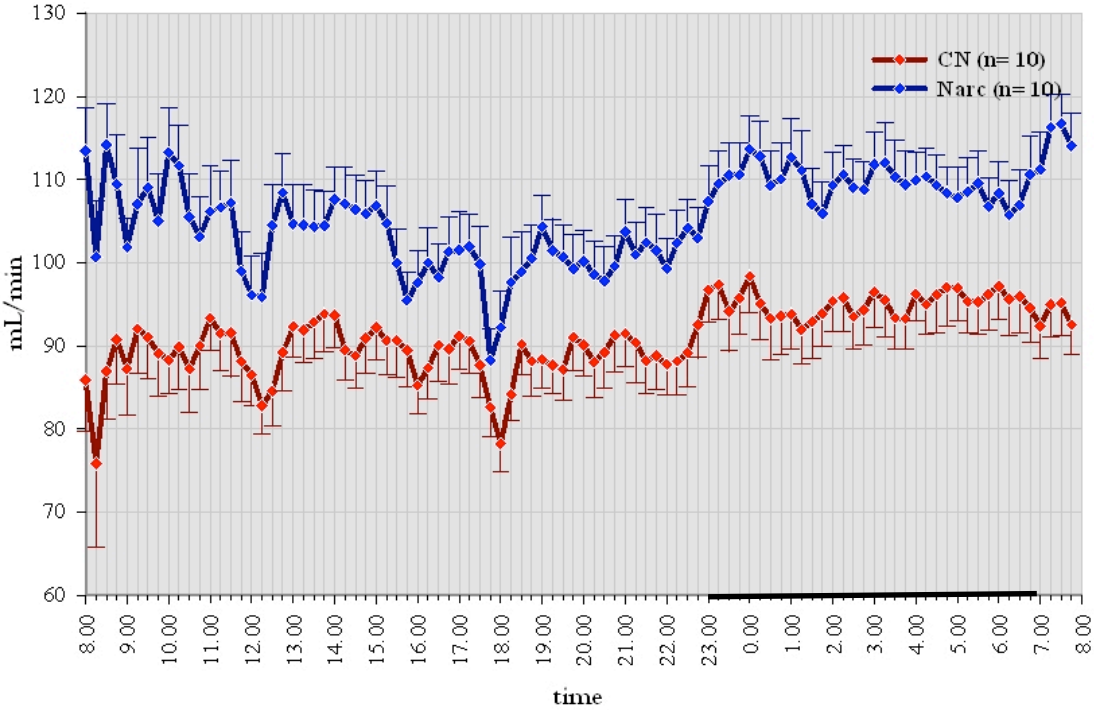
HR: heart rate; CN =controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of HR values of day 1 and 2 while each point from 8:00 to 12:00 represents the mean every 15 min ± SE of HR values of day 2; dark bar represents the dark period (from lights off to lights on)

Figure 4 Mean cardiac output values over the 24h in patients with narcolepsy and controls



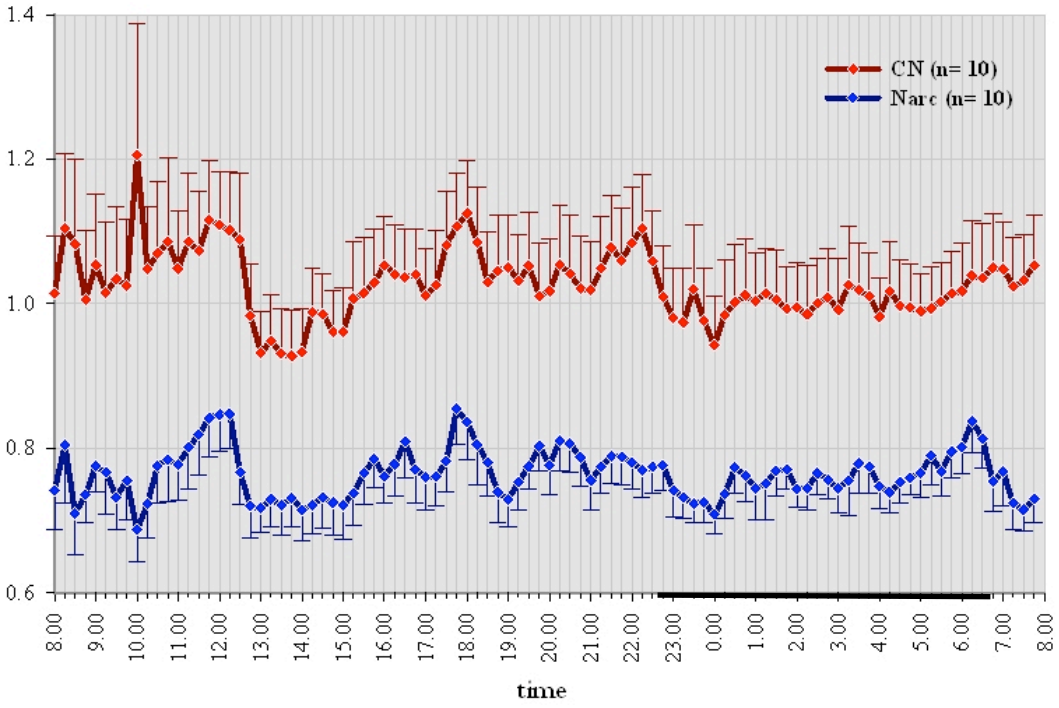
CN =controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of cardiac output (CO) values of day 1 and 2 while each point from 8:00 to 12:00 represents the mean every 15 min ± SE of CO values of day 2; dark bar represents the dark period (from lights off to lights on)

Figure 5 Mean stroke volume values over the 24h in patients with narcolepsy and controls



CN =controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of stroke volume (SV) values of day 1 and 2 while each point from 8:00 to 12:00 represents the mean every 15 min ± SE of SV values of day 2; dark bar represents the dark period (from lights off to lights on)

Figure 6 Mean total peripheral resistances values over the 24h in patients with narcolepsy and controls



CN =controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of total peripheral resistances (TPR) values of day 1 and 2 while each point from 8:00 to 12:00 represents the mean every 15 min ± SE of TPR values of day 2; dark bar represents the dark period (from lights off to lights on)

Table 1 Mean values and Δ values of SBP, DBP and HR in wake-sleep phases in patients with narcolepsy and controls

	Patients with Narcolepsy (n=10)					Controls (n=10)				
	SBP (mmHg)					**SBP** (mmHg)				
	W	**Ph 1**	**Ph 2**	**SWS**	**REM**	**W**	**Ph 1**	**Ph 2**	**SWS**	**REM**
24-h mean (SD)	127.5 (12.2)	121.15 (12.3)	119.1 (12.8)	116.2 (14.4)	124.2 (12.5)	127.2 (12.3)	115.2 (14.5)	112.5 (12.4)	108.8 (10.3)	115.5 (14.7)
Daytime * mean (SD)	128.7 (12)	124.7 (12.8)	124 (12.8)	119.3 (14.3)	127.6 (13.6)	128.2 (12.2)	118.4 (15.2)	118.7 (13.6)	109.9 (10.5)	110.5 (18.51)
Night-time mean (SD)	120.5 (12.2)	116.5 (11.8)	114.4 (12.4)	112.4 (13.7)	121 (13.5)	118 (13.1)	113.9 (12.7)	110.7 (10.3)	108.6 (9.5)	114.4 (12.9)
	Δ SBP (mmHg)					**Δ SBP** (mmHg)				
Daytime * mean (SD)	0	-4 (4.5)	-4.6 (6.4)	-9.3 (6.5)	-1 (9.4)	0	-10.8 (8.7)	-10.5 (7.3)	-16.9 (7.4)	-14.3 (4.7)
Night-time mean (SD)	-5.4 (4.7)	-9.4 (5.1)	-11.5 (6)	-13.6 (8.8)	-5 (6.7)	-8.6 (5.1)	-12.7 (5.2)	-15.8 (5.6)	-18 (7.5)	-12.1 (7.4)
	DBP (mmHg)					**DBP** (mmHg)				
24-h mean (SD)	75.6 (7.2)	70 (7.2)	69.1 (7.4)	67.4 (8.6)	71.5 (7.3)	74.1 (5.8)	65.9 (7.5)	64.4 (6.2)	62.7 (5.7)	66.6 (7.1)
Daytime * mean (SD)	76.4 (7.1)	71.2 (7.7)	70.7 (8.3)	68.7 (8.6)	73.1 (7.7)	74.7 (5.8)	65.8 (8.6)	65.4 (7.5)	61.8 (5.1)	66.2 (9.8)
Night-time mean (SD)	71.2 (7.4)	68.4 (7.2)	67.4 (7.4)	65.5 (8.1)	70.3 (7.7)	68.5 (6.3)	66 (6.1)	64.1 (4.8)	62.8 (5.1)	65.7 (6.1)
	Δ DBP (mmHg)					**Δ DBP** (mmHg)				
Daytime * mean (SD)	0	-5.1 (3.3)	-5.6 (4.3)	-7.6 (4.4)	-3.3 (4.8)	0	-9.2 (5.5)	-9.5 (4.7)	-12.1 (5)	-8.6 (3.7)
Night-time mean (SD)	-3.6 (2.7)	-6.4 (3.5)	-7.4 (4.5)	-9.2 (6.2)	-4.5 (3.8)	-5.2 (3.3)	-7.7 (3.6)	-9.6 (3.7)	-10.9 (4.7)	-8 (4.1)
	HR (bpm)					**HR** (bpm)				
24-h mean (SD)	79 (9.7)	67.4 (7.4)	66.4 (7.4)	67.4 (7.1)	67.3 (6.9)	67.6 (12.2)	57.7 (10.6)	55.8 (10.3)	55.6 (10)	57.8 (10.8)
Daytime * mean (SD)	79.9 (10.2)	69.4 (8.8)	68.8 (9.2)	69.4 (8.9)	70.5 (9.2)	68.1 (12.4)	57.7 (10.1)	56.8 (10)	56.4 (10.4)	57.4 (18.6)
Night-time mean (SD)	73.2 (8.7)	66.1 (6.6)	64.8 (6.8)	66 (7)	65.8 (6.6)	63.6 (10.6)	57.4 (10.4)	55.3 (10.2)	55.7 (10)	57.3 (10)
	Δ HR (bpm)					**Δ HR** (bpm)				
Daytime mean (SD)	0	-10.5 (5.7)	-11.1 (6.2)	-10.5 (5)	-9.4 (5.8)	0	-8.6 (2.8)	-9.5 (3.9)	-11.5 (4.7)	-8.9 (4.1)
Night-time mean (SD)	-6 (5.5)	-13.1 (6.5)	-14.4 (6.8)	-13.2 (6.9)	-13.3 (6.5)	-3.9 (4.1)	-10.1 (5.8)	-12.2 (6.3)	-11.8 (6.5)	-10.2 (6.9)

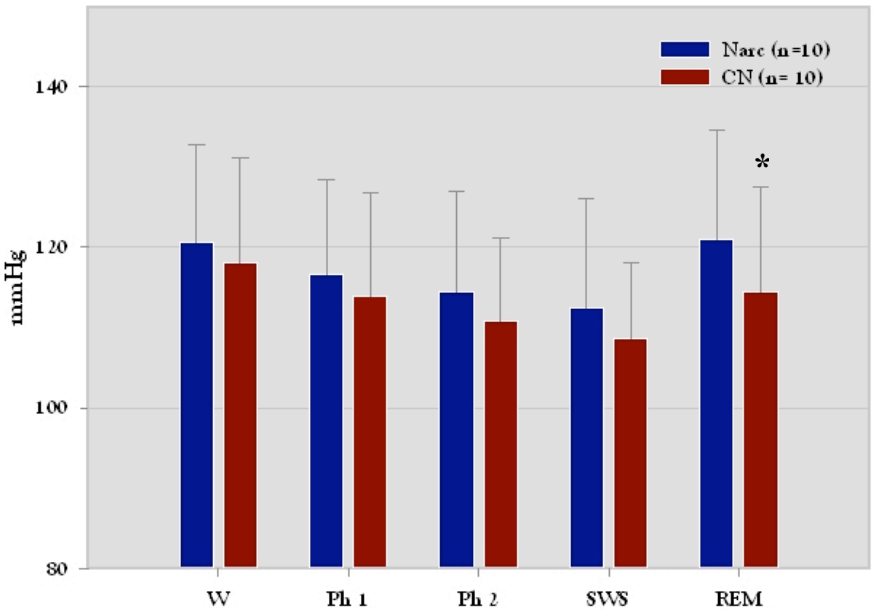
SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; W: wake; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; ΔSBP, ΔDBP and ΔHR represent the difference between the mean value of each cardiovascular parameter in W, NREM stage 1, 2, SWS and REM sleep and the corresponding mean value during daytime-W; * the 10 controls subjects (CN) and the 10 narcoleptic patients (narc) contributed to daytime sleep as follow: Ph1: 10 narc and 7 CN; Ph2: 10 narc and 7 CN; SWS: 9 narc and 6 CN, REM: 10 narc an 1 CN

Table 2 Mean values and Δ values of CO, SV and TPR in wake-sleep phases in patients with narcolepsy and controls

	Patients with Narcolepsy (n=10)					Controls (n=10)				
	CO (L/min)					**CO** (L/min)				
	W	**Ph 1**	**Ph 2**	**SWS**	**REM**	**W**	**Ph 1**	**Ph 2**	**SWS**	**REM**
24-h mean (SD)	7.9 (1.3)	7.6 (1.4)	7.4 (1.5)	7.6 (1.4)	7.6 (1.3)	5.9 (1.3)	5.5 (1.3)	5.4 (1.2)	5.3 (1.3)	5.4 (1.2)
Daytime * mean (SD)	7.9 (1.3)	7.8 (1.6)	7.7 (1.7)	7.7 (1.7)	7.7 (1.5)	5.9 (1.3)	5.7 (1.4)	5.7 (1.4)	5.4 (1.5)	4.4 (1.5)
Night-time mean (SD)	7.7 (1.4)	7.2 (1.3)	7.1 (1.4)	7.4 (1.6)	7.3 (1.4)	5.9 (1.3)	5.4 (1.2)	5.2 (1.2)	5.2 (1.3)	5.4 (1.2)
	Δ CO (L/min)					**Δ CO** (L/min)				
Daytime * mean (SD)	0	-0.1 (0.5)	-0.2 (0.6)	-0.2 (0.6)	-0.2 (0.4)	0	0.06 (0.4)	0.03 (0.5)	-0.3 (0.8)	-0.4 (0.2)
Night-time mean (SD)	-0.08 (0.5)	-0.5 (0.6)	-0.7 (0.5)	-0.3 (0.8)	-0.4 (0.6)	0.06 (0.4)	-0.4 (0.7)	-0.6 (0.8)	-0.6 (0.9)	-0.4 (0.8)
	SV (mL/beat)					**SV** (mL/beat)				
24-h mean (SD)	102.2 (14.2)	113.2 (14.8)	111.9 (16.2)	113 (15.3)	113.7 (13.9)	89.1 (14.9)	97.4 (17.3)	97.2 (17.3)	96.3 (19.9)	94.5 (16)
Daytime * mean (SD)	102.2 (14.3)	113 (16.5)	111.7 (16.8)	110.8 (19.1)	109.8 (15.4)	88. 3 (14.9)	100.6 (21.9)	102.1 (22.6)	96.5 (23.4)	79 (19.3)
Night-time mean (SD)	106.9 (16.2)	110.3 (15.4)	109.5 (16.5)	113.1 (17.6)	111.8 (16.2)	95 (17.3)	96 (17.9)	95.6 (17)	95.2 (17.9)	95.5 (16.5)
	Δ SV (mL/beat)					**Δ SV** (mL/beat)				
Daytime* mean (SD)	0	+12.1 (9.1)	+10.8 (9.8)	+9.9 (10.6)	+8.9 (7.8)	0	+12.9 (9.8)	+14.3 (9.9)	+10.4 (10.9)	+3.8 (5.5)
Night-time mean (SD)	+6.7 (4.9)	+10.2 (8.9)	+9.3 (8.7)	+13 (9.8)	+11.7 (11.2)	+6.1 (4.9)	+7.1 (6.8)	+6.8 (5.9)	+6.4 (7.9)	+6.6 (7.3)
	TPR					**TPR**				
24-h mean (SD)	0.77 (0.1)	0.75 (0.1)	0.76 (0.1)	0.71 (0.1)	0.75 (0.1)	1 (0.3)	1 (0.3)	1 (0.2)	0.98 (0.2)	1 (0.2)
Daytime * mean (SD)	0.78 (0.1)	0.75 (0.1)	0.76 (0.1)	0.73 (0.1)	0.77 (0.1)	1.06 (0.3)	0.98 (0.3)	0.96 (0.3)	0.97 (0.3)	1.2 (0.3)
Night-time mean (SD)	0.75 (0.1)	0.76 (0.1)	0.77 (0.1)	0.71 (0.1)	0.77 (0.1)	0.98 (0.3)	1.02 (0.3)	1.01 (0.2)	0.98 (0.2)	1 (0.2)
	Δ TPR					**Δ TPR**				
Daytime * mean (SD)	0	-0.03 (0.05)	-0.02 (0.07)	-0.05 (0.05)	-0.01 (0.06)	0	-0.12 (0.1)	-0.13 (0.1)	-0.12 (0.2)	-0.08 (0.1)
Night-time mean (SD)	-0.03 (0.07)	-0.02 (0.08)	-0.01 (0.08)	-0.07 (0.1)	-0.01 (0.09)	-0.07 (0.09)	-0.03 (0.1)	-0.03 (0.1)	-0.06 (0.1)	-0.04 (0.1)

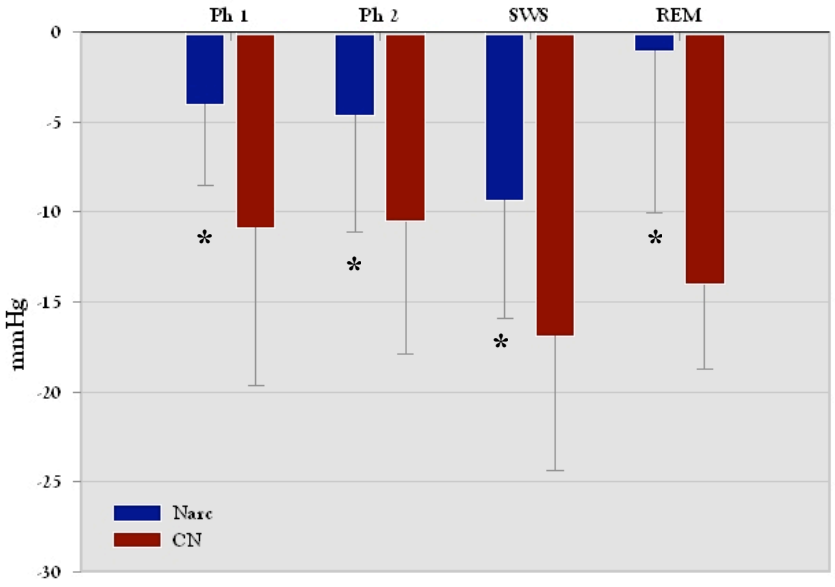
CO: cardiac output; SV: stroke volume; TPR: total peripheral resistances; W: wake; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; ΔCO, ΔSV and ΔTPR represent the difference between the mean value of each cardiovascular parameter in W, NREM stage 1, 2, SWS and REM sleep and the corresponding mean value during daytime-W; * the 10 controls subjects (CN) and the 10 narcoleptic patients (narc) contributed to daytime sleep as follow: Ph1: 10 narc and 7 CN; Ph2: 10 narc and 7 CN; SWS: 9 narc and 6 CN, REM: 10 narc an 1 CN.

Figure 7 State-dependent variation of SBP during night-time in patients with narcolepsy and controls



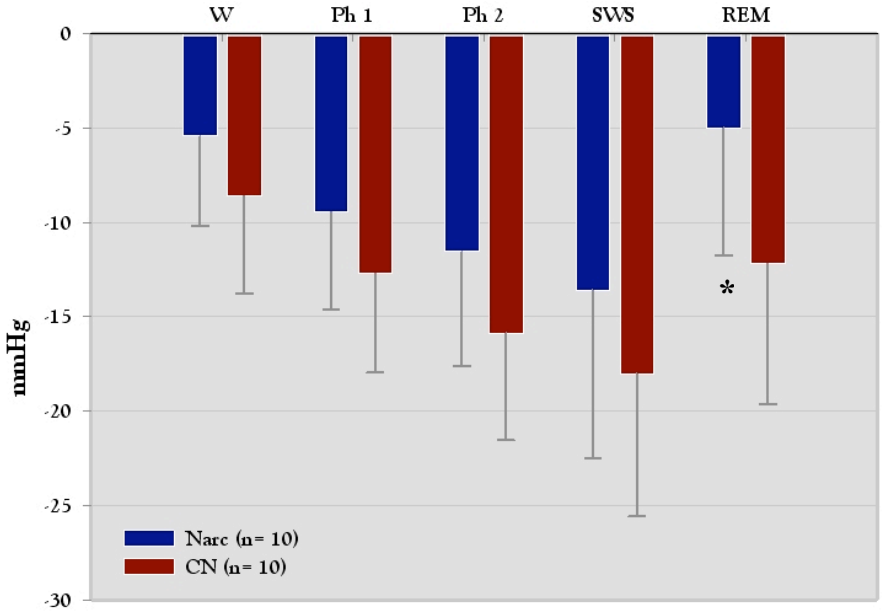
SBP: diastolic blood pressure; W: wake; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; Narc: patients with narcolepsy; CN: controls; *: P= 0.02

Figure 8 State-dependent SBP decline during daytime in patients with narcolepsy and controls



SBP: systolic blood pressure; W: wake; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; Narc: patients with narcolepsy; CN: controls; the 10 CN and the 10 Narc contributed to daytime sleep as follow: Ph1: 10 narc and 7 CN; Ph2: 10 narc and 7 CN; SWS: 9 narc and 6 CN, REM: 10 narc an 1 CN. Row data are reported in Table 1; *:p=0.02

Figure 9 State-dependent SBP nocturnal decline in patients with narcolepsy and controls



SSBP: systolic blood pressure; W: wake; W: wake; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; Narc: patients with narcolepsy; CN: controls; Row data are reported in Table 1; *: p=0.02

DISCUSSION

This study investigated the time and state-dependent variations of BP, HR and other cardiovascular parameters in a sample of patients with narcolepsy and cataplexy compared to healthy subjects, under controlled conditions.

The time-dependent analysis allowed us to investigate the effects exerted by the circadian endogeneous rhythms of BP and HR and by the wake-sleep cycle on the 24h variations of cardiovascular parameters (SBP, DBP, HR, SV, CO and TPR).

The time-dependent analysis of HR showed higher HR values throughout the 24h in the narcolepsy group associated with higher values of CO and SV and reduced TPR values compared to controls. Under physiological conditions, the increase in HR and SV is mediated by the increase in sympathetic nervous system activity. Compensatory mechanisms warrant the maintenance of normal BP values by reducing TPR. Thus, our results suggest the existence of

an imbalanced cardiac autonomic tone in narcolepsy patients, in favour of an enhanced sympathetic tone.

The analysis of the day-night pattern of SBP and DBP yielded the most impressive result of the study showing a significant reduction of the nocturnal BP decline in the narcolepsy group. Narcoleptics failed to show the normal 10-20% decline in SBP/DBP during nocturnal sleep, as observed in the control group, and exhibited a 5% in SBP values and 6% in DBP values. Furthermore, two patients with narcolepsy showed a reverse dipping pattern with nocturnal SBP/DBP values higher during the nigh-time period.

These data may be of great clinical relevance. Some evidence has shown that a non-dipping nocturnal pattern in subjects with hypertension is a strong independent risk factor for cardiovascular mortality. A recent prospective cohort study (the Ohsama study) [14], extended this concept to subjects without hypertension demonstrating that there was a linear and inverse relationship between cardiovascular mortality and the nocturnal decline in blood pressure, independent of the overall blood pressure load during 24h and other cardiovascular risk factors. Overall, each 5% increment in the systolic or diastolic night/day ratio was associated with a 20% increase in the risk of cardiovascular death even when 24h BP was within the normotensive range. According to these findings, the reduced nocturnal BP fall that we found in patients with narcolepsy, albeit within the normotensive range, should expose them to a significantly increased cardiovascular risk. In this regard, another study [15] recently showed that sympathetic activation plays a significant role in the non-dipper pattern of hypertensive subjects and that there is a close inverse association between the degree of sympathetic activation and the magnitude of the night-time fall in SBP or DBP.

Interestingly, in our study HR maintained a comparable nocturnal decline in narcoleptic patients and controls strengthening previous findings that the 24h variations of HR reflect modulatory influences different from those controlling the BP pattern.

The analysis of sleep structure showed that during daytime narcolepsy subjects slept significantly more than controls and showed a higher amount of every sleep stage (Section 1, Table 3). During night-time, the total amount of sleep and its architecture were similar in the two groups, although narcoleptic patients showed a longer NREM stage 1 and a slightly shorter SWS

(Section 1, Table 2). Furthermore, wake-sleep fragmentation was greater in the narcolepsy group throughout the 24h.

Given that the autonomic control of the cardiovascular system differs during W, NREM and REM sleep, we investigated whether changes in the state-dependent modulation of cardiovascular parameters could differentiate narcoleptic patients from controls.

The state-dependent analysis in the 24h, daytime and night-time showed a physiological modulation of BP and HR through sleep phases in narcoleptic patients and controls, characterised by the BP and HR fall during NREM sleep compared with relaxed wakefulness and an increase during REM sleep compared to NREM sleep.

During daytime, NREM and REM sleep were characterized by a lower SBP and DBP decline in the narcolepsy group with respect to controls. HR values were significantly higher in NREM and REM sleep in narcoleptic patients compared to controls even if the degree of this increase was not affected by the sleep phase. Similarly, during NREM and REM sleep, CO and SV values were increased and TPR reduced by the same amount in the narcolepsy groups as compared controls.

During night-time, NREM sleep was characterized by comparable values of SBP and DBP in the two groups. During REM sleep, DBP values were similar in narcoleptics and controls while SBP values were significantly increased in narcolepsy group ($p=0.02$). Furthermore, the narcoleptic group showed a trend towards a reduction of SBP fall during NREM sleep without reaching statistic significance ($p=0.06$), that was instead detectable in REM sleep ($p=0.02$).

During night-time, NREM and REM sleep were also characterized by higher HR and CO values and lower TPR values in the narcolepsy group compared to controls, although the extent of their variation from controls was constant in NREM and REM sleep. SV values were similar in the two groups in NREM stages 1 and 2 and REM sleep, whereas in SWS narcoleptics exhibited higher SV values ($p=0.02$)

Taken together, the results of the state-dependent analysis, suggest that a phase-specific impaired modulation of BP during REM sleep resulting in higher SBP values, may contribute the non-dippers BP pattern we found in patients with narcolepsy.

REM sleep in human is characterized by a great variability of BP and HR together with breathing irregularities, depending directly on central changes in the regulation of the autonomic outflow that activated peripherally controlled variables. BP rises are sudden, irregular and often wide. During REM, muscle sympathetic nerve activity increases above the levels recorded during wakefulness and this increase is more pronounced than the changes in HR and BP [16, 17].

The key brain structure for generating REM sleep is the brainstem, particularly the pons and adjacent portions of the midbrain. However, at a higher level, sleep promoting neurons, which are scattered in the vicinity of the central autonomic network and its connection (i.e the preoptic-anterior hypothalamic region and nucleus tractus solitarius) along with cholinergic "REM-on" and catechoalminergic "REM-off" cells in the pontomesencephalic junction and pons, control NREM and REM sleep cycles. Hypocretin neurons, located in the hypothalamus, contribute to the regulation of the activity of norepinephrine, serotonin, histamine and acetycholine cell groups implicated in REM sleep regulation.

The loss of hypothalamic neurons containing hypocretin characterizing narcolepsy with cataplexy, might determine an unbalanced hypothalamic activity which in turn may result in an impaired autonomic cardiovascular control in favour of an enhanced sympathetic tone, particularly during REM sleep.

Our results contrast with what should have been expected on the basis of the scant data available in literature that suggested a reduced sympathetic activity secondary to orexinergic depletion. However, both the results of the time-dependent analysis showing a reduced night-time decline of SBP and DBP in patients with narcolepsy in association with higher HR and SV values stable throughout the 24h, and the state-dependent analysis showing higher SBP during REM sleep in narcolepsy group, clearly point in the opposite direction, i.e. an increased sympathetic tone in narcolepsy with cataplexy.

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Section 2

Analysis of cardiovascular parameters

IV. SLEEP-DEPENDENT CHANGES IN THE COUPLING BETWEEN HEART PERIOD AND BLOOD PRESSURE IN NARCOLEPSY WITH CATAPLEXY

SCIENTIFIC BACKGROUND

Daytime sleepiness (DS) is a symptom that has recently acquired increasing clinical relevance since several epidemiologic studies have shown a relation between self-reported sleepiness and cardiovascular diseases. The Cardiovascular Health Study, found that DS was the only sleep disturbance symptom associated with incident myocardial infarction, heart failure, overall cardiovascular morbidity and mortality [1]. Some studies also showed that people with sleep-related breathing disorders (SRBD), a common cause of excessive daytime sleepiness (EDS), are at increased risk for developing hypertension and other cardiovascular diseases [2, 3]. However, only a small proportion of patients with SRBD and an elevated apnea-hypopnea index (AHI), complain of significant DS [4].

Lombardi and colleagues [5] studied patients with sleep-related breathing disorders (SRBD) and explored the relation between excessive daytime sleepiness (EDS), objectively determined, with indices of autonomic cardiac regulation, such as baroreflex sensitivity and heart rate variability (HRV), and polysomnographic (PSG) indices of SRBD severity and quality of sleep. The study demonstrated that patients with EDS had significantly lower baroreflex sensitivity and significantly higher low-to-high frequency power ratio of HRV during the different stages of nocturnal sleep than patients without EDS, thus indicating a derangement of cardiac autonomic control at night. Although the authors could not establish a causal link between these phenomena, they postulated a vicious circle of events whereby a deranged cardiac vagal modulation is related to EDS through a complex interaction between dysfunctions in cerebral regions responsible for sleep regulation, daytime vigilance and autonomic cardiovascular control.

Another study [6] has recently strengthened this finding, showing that the association of SRBD with hypertension was stronger in individuals who reported DS than in those who did not.

EDS is also one of the clinical hallmarks of narcolepsy. Hence, the aim of this study was to study the state-dependent autonomic cardiac modulation in a sample of narcoleptic patients with cataplexy to establish whether the association between EDS and a deranged cardiac autonomic control at night is specific for SRBD. The existing data on cardiac autonomic regulation in narcolepsy are confined to animal studies. In particular two studies [7, 8] demonstrated that under basal conditions orexine deficient mice have baroreflex parameters similar to wild type mice but showed a suppressed reflex bradycardia during the defence response

The present study evaluated state-dependent autonomic cardiac modulation in narcoleptic patients and healthy control subjects using the cross-correlation function (CCF) computed on spontaneous fluctuation of heart period (HP) and systolic blood pressure (SBP) [9], and an analysis of baroreflex sequences.

METHODS

Subjects

Narcoleptic patients were compared with 15 control subjects (7 women, age 44 ± 3 yrs, BMI 23.9 ± 0.8) free of drugs. Cardiac endocrine, metabolic and renal diseases were excluded by history-taking, physical examination and routine laboratory tests. Obstructive sleep apnoea syndrome was excluded by a dynamic polysomnographic study (apnea-hypopnea index < 10). Subjects did not report subjective DS. Control subjects underwent the same study protocol as the narcoleptic patients described in the introduction except for multiple sleep latency test (MSLT).

Sleep scoring

For both narcoleptic patients and controls the sleep states were visually scored on 30 s epochs according to the standard Rechtschaffen and Kales criteria [10] as light (stages 1 and 2) NREMS, deep (stages 3 and 4) NREMS, and REMS. For the analysis we considered two consecutive nights of PSG recording (from lights-off:11p.m to lights on: 7 a.m.).

Time series of cardiovascular signals (thanks to the collaboration with Dr. A. Silvani, Depaertment of Human and General Physiology, University of Bologna)

The data analysis was performed with MATLAB and its signal processing toolbox (the MathWorks) on all episodes of light NREMS, deep NREMS, or REM lasting ≥5 min. The analysis was also performed on episodes of quite wakefulness (QW, with subjects lying in bed), selected in the hour before the start and end of the sleep period. HP values were computed as the intervals between adjacent QRS complexes that were due to sinus node depolarizations. Beat-to-beat values of SBP and diastolic blood pressure (DBP) were computed. Portapres calibration and occasionally subject movement in QW caused artifacts in the raw blood pressure signal identified as disruptions of pulse wave morphology. When such artifacts affected the pressure signal for a time interval < 4 s, beat-to-beat SBP data in that interval were

reconstructed by piecewise cubic spline interpolation to allow the analysis of the CCF between HP and SBP, whereas they were excluded from the analysis performed with the sequence technique. The time-intervals longer than 4 s with artifacts in the pressure signal were excluded from all of the analyses.

Cross-correlation analysis

For each subject and wake-sleep state the CCF between HP and SBP, HP variance and SBP variance were averaged over consecutive data subsets of 5 min duration overlapped for 4 min [11]. Data subsets were included in the analysis if they were free of ectopic beats and if artifacts in the pressure signal had individual duration < 4 s and cumulative duration < 30 s. The time series of HP and SBP were resampled at 4 Hz by linear interpolation. The CCF was computed at time shifts between -25 s and 25 s and normalized so that that the autocorrelations at 0 time shift were identically 1. The CCF analysis yielded the linear correlation coefficient between HP and SBP as a function of the time shift between these variables [12] whose sign indicates whether HP fluctuations precede (positive sign) or are preceded by (negative sign) those of SBP (Figure 1). For each subject and wake-sleep state, the maximum positive and the minimum negative correlation coefficients of the average CCF were retained for analysis together with the corresponding time shifts.

These computations were repeated after low-pass filtering the resampled time series of HP and SBP below the breathing rate (<0.15 Hz, 10 pole Butterworth filter). Data analysis was performed on low-filtered time series of HP and SBP, having previously demonstrated [9] that the CCF pattern was independent of the tight coupling between oscillations of SBP and HP at the breathing rate.

Analysis with the sequence technique

The sequence technique was implemented with criteria that have been validated in animal models and applied in human subjects [13, 14]. SBP ramps were identified as sequences of ≥ 3 consecutive beats with monotonic changes of SBP ≥ 1mmHg/beat. The baroreflex sequences were identified as SBP ramps linearly correlated (squared correlation coefficient > 0.85) with monotonic changes in HP, which had amplitude ≥ 5ms/beat, the same direction as the SBP changes (e.g., hypertension-bradycardia), and a delay of 0, 1 or 2 heart beats [13]. The occurrence of these sequences reflects a physiological rather than a chance coupling in human subjects [14] and is dramatically reduced by sinoaortic denervation in cats [13].

The cardiac baroreflex sensitivity (BRS) was computed as the average slope of the regression lines between HP and SBP values in each baroreflex sequence [13].

Statistics

Statistical tests were performed with the SPSS software (SPSS) and were significant at $p < 0.05$.

CCF coefficients and BRS values were analyzed by ANOVA (GLM procedure with mixed-model design) to test the effect of the wake-sleep factor (state), the effect of group (narcoleptics *vs* controls) and the state x group interaction effect. The between group difference of BRS values in different wake-sleep states was also analyzed with the Mann-Whitney rank-sum test.

The time shifts corresponding to the CCF maxima and minima in each wake-sleep state were analyzed with the binomial single-sample sign test (cut-off value of 0; probability parameter of 0.5) to evaluate whether they significantly clustered at negative or positive values. The median value and interquantile range of these time shifts are shown in Table 1. The remaining tables and the text report the data as means ± SE.

RESULTS

Sleep structure

The sleep structure of narcoleptic patients is shown in Section 1. Control subjects spent 54 ± 3%, 24 ± 3% and 22 ± 1% of the total sleep time in lights NREMS, deep NREMS and REMS respectively [9].

Cross-correlation analysis

According to visual analysis of the time series (Figure 2), the average CCF between HP and SBP in narcoleptic patients and controls showed a positive peak at negative time shifts in deep NREMS, which became progressively less evident in light NREMS and REMS. However, while during QW in controls such a peak was reduced to a hump of the CCF which showed a negative correlation between HP and both previous and subsequent SBP values, in narcoleptic patients it still occurred as a small positive peak.

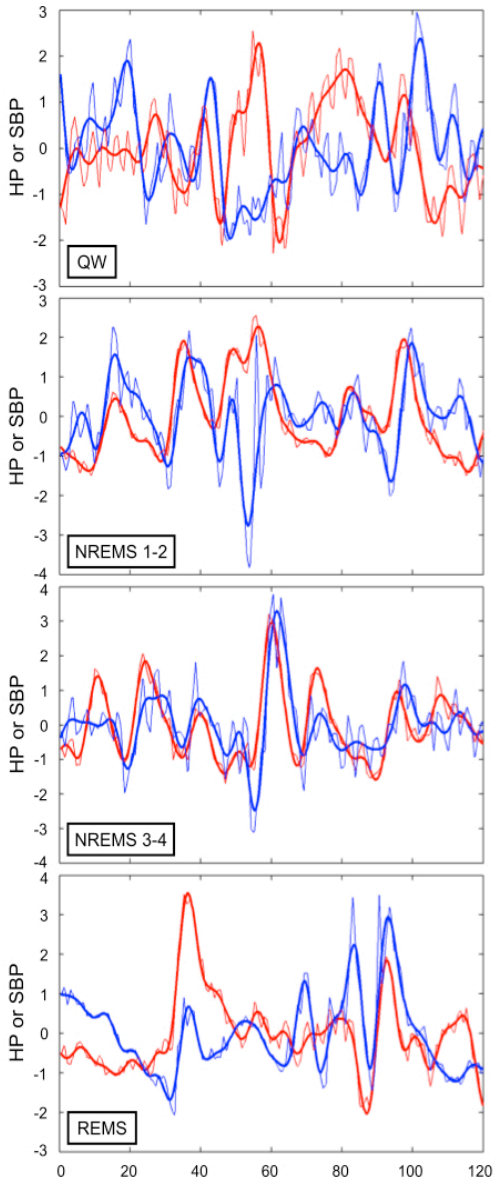
Time shifts corresponding to the CCF maxima and minima are shown in Table 1. The time shifts corresponding to the CCF maxima clustered at negative values in all sleep states except QW in controls and in all wake-sleep states except REMS in narcoleptic patients. The time shifts corresponding to the CCF minima clustered at positive values in all wake-sleep states in controls and in all wake-sleep states except REMS in narcoleptic patients.

The maximum and minimum CCF values are shown in Table 2. The ANOVA analysis of maximum values of CCF displayed a significant state effect ($p<0.01$) but not significant group ($p= 0.6$) or state x group interaction ($p= 0.1$) effects. Similarly, the analysis of minimum values of CCF showed a significant state effect ($p<0.01$) but not significant group ($p=0.1$) or state x group interaction ($p=0.1$) effects.

Analyses with the sequence technique

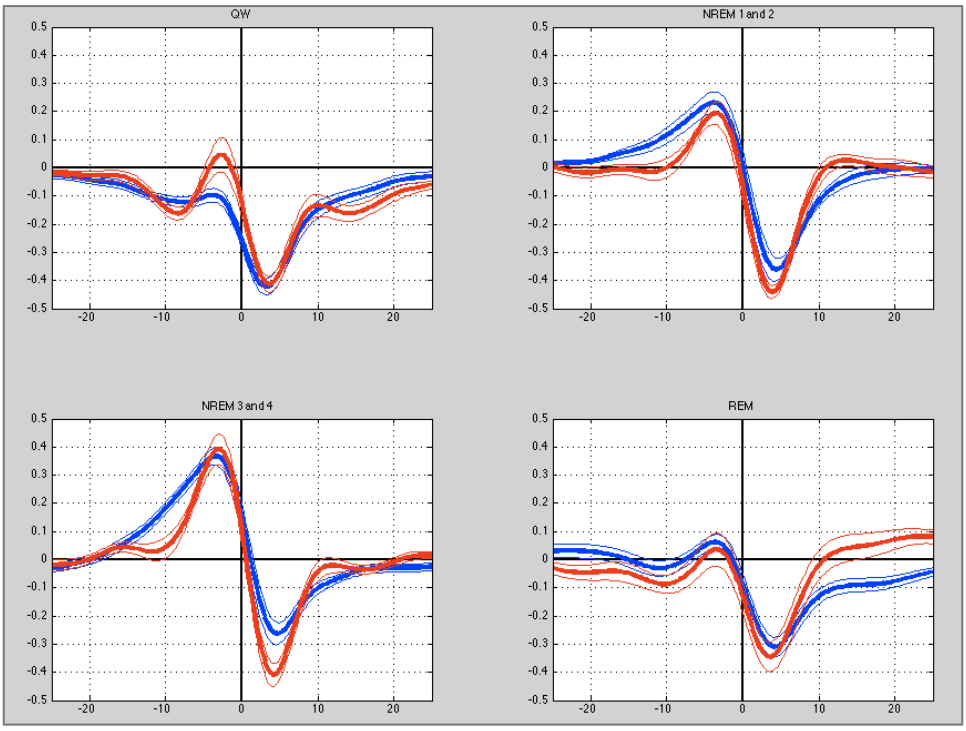
BRS values are shown in table 3. The ANOVA analysis displayed a significant state effect ($p< 0.01$) but not significant group ($p=0.3$) or state x group interaction ($p=0.2$) effects. The between group difference of BRS values in different wake-sleep states did not show significant results (QW: $p=0.2$, NREMS 1 and 2: $p=1$; NREMS 3 and 4: $p=0.9$; REMS: $p=0.9$).

FIGURE 1



Representative time series of heart period (HP, blue lines) and systolic blood pressure (SBP; red lines). Thick lines indicate data low-pass filtered below 0.15 Hz. Data were obtained during quiet wakefulness (QW), stages 1 and 2 and 3 and 4 of non rapid-eye-movement sleep (NREMS), and rapid-eye-movement sleep (REMS) in the same subject. Values are in standardized units. Adapted from Silvani et al [9]

Figure 2



Cross-correlation functions between low-pass filtered (< 0.15 Hz) fluctuations of heart period and SBP during quite wakefulness (QW), NREM stages 1 and 2, 3 and 4, and REM sleep in patients with narcolepsy (red lines) and controls (blu lines). Data are mean ± SE.

Table 1 Time shifts corresponding to the maximum and minimum values of the cross-correlation functions between heart period and systolic blood pressure in patients with narcolepsy and controls

	QW	NREMS 1 and 2	NREM 3 and 4	REMS
Narcoleptic patients				
LF-τ_{MAX} s	-2.8 (22.5)*	-3.2 (2.6)*	-3 (0.8) λ	15.1 (28)
LF-τ_{MIN} s	3.6 (0.6) λ	3.8 (1) λ	4 (0.8) λ	3.5 (5)
Controls				
LF-τ_{MAX} s	-16.2 (35)	-4 (3) λ	-3.5 (2.2) λ	-2.7 (17.5)*
LF-τ_{MIN} s	3.2 (0.7) λ	4 (2) λ	4.2 (1.7) λ	3,7 (2) λ

Data are expressed as median values (interquartile range). LF-τ_{MAX} s and LF-τ_{MIN} s, time shifts corresponding to the maximum and minimum values of the CCF between the low-pass filtered (<0.15 Hz) time series of HP and SBP; * $p<0.05$; λ $p<0.01$

Table 2 Maximum and minimum values of the cross-correlation function between heart period and systolic blood pressure in patients with narcolepsy and controls

	QW	NREMS 1 and 2	NREM 3 and 4	REMS
Narcoleptic patients				
LF-ρMAX	0.08. ± 0.1	0.21 ± 0.11	0.39. ± 0.16	0.18. ± 0.09
LF-ρMIN	-0.41 ± 0.08	-0.45 ± 0.06	-0.42 ± 0.11	-0.37 ± 0.13
Controls				
LF-ρMAX	0.02. ± 0.03	0.26 ± 0.13	0.38. ± 0.11	0.13 ± 0.07
LF-ρMIN	-0.42 ± 0.1	-0.38 ± 0.14	-0.29 ± 0.13	-0.32 ± 0.12

Data are expressed as means ± SE. LF-ϱMAX and LF-ϱMIN, maximum and minimum values of the CCF between the low-pass filtered (<0.15 Hz) time series of HP and SBP

Table 3 Results of the analysis of baroreflex sequences in patients with narcolepsy and controls

	QW	NREMS 1 and 2	NREM 3 and 4	REMS
BRS, ms mmHg-1				
Narcoleptic patients	7.2 ± 0.83	10.3 ± 1.1	9.4 ± 1.1	10.1 ± 0.9
Controls	10.7 ± 1.5	12.6 ± 2	11.1 ± 1.5	11.8 ± 1.9

Data are expressed as means ± SE. BRS, baroreflex sensitivity

DISCUSSION

We tested the hypothesis that the relative contribution of the baroreflex and central commands to the control of HP among wake-sleep states differs between healthy subjects without DS and patients with narcolepsy and cataplexy and EDS objectively determined by MSLT.

We estimated BRS values among wake-sleep states by means of the sequence technique [13] to allow comparisons with previous studies [5, 7, 8]. However, BRS indexes show several limitations since they do not take into account the effects exerted on spontaneous cardiovascular fluctuations in addition to the baroreflex by multiple control mechanisms including central autonomic commands [16] and reflexes from cardiac and aortic walls [17]. Thus, we completed the analysis computing the CCF between HP and SBP to evaluate the relative contribution among wake-sleep states of feed-forward mechanisms due to central autonomic commands and negative feed-back mechanisms due to baroreflex.

Analysis with both the sequence technique and the CCF yielded congruent results showing a significant state-dependent autonomic modulation of the coupling between HP and BP without a significant effect determined by the group (controls *vs* narcolepsy patients). In fact, BRS mean values did not differ between groups in any wake-sleep state. Furthermore, the visual analysis of the CCF showed a similar state-dependent pattern in controls and patients characterized by the peak CCF value in deep NREMS, entailing the highest feedback contribution of the baroreflex, progressively less evident in light NREMS and REMS. A negative correlation between HP and the subsequent SBP values was also observed in each state consistent with the mechanical feed-forward action of HP on SBP and with central autonomic commands.

Overall these data suggest that autonomic cardiac modulation through wake-sleep states is preserved in narcolepsy with cataplexy, in line with the results obtained from studies on animals [7, 8]. However, this study investigated autonomic cardiac modulation in narcolepsy subjects under controlled conditions and alterations emerging during behavioural reactions, such as the defense response, as observed in animals cannot be ruled out.

Most importantly, this study allowed demonstrated that EDS, objectively demonstrated by MSLT in our narcolepsy sample, is not associated per se with impaired autonomic cardiac

regulation. Only the association of EDS and SRBD is accompanied by impaired autonomic cardiac regulation whereas cardiac regulation is normal in narcolepsy patients with comparable EDS.

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Section 3

BODY CORE TEMPERATURE REGULATION IN NARCOLEPSY WITH CATAPLEXY

SCIENTIFIC BACKGROUND

The idea that temperature and sleep are interrelated is based on evolutionary history [1]. Mammalian sleep developed in association with endothermy, and all species usually sleep during the circadian trough of their body core temperature (BcT) rhythm. The circadian pattern of BcT results from the balance between heat production and heat loss, the latter being relevant for sleep induction. Sleep under entrained conditions is typically initiated on the declining portion portion of the BcT curve when its rate of change and body heat loss is maximal. Body heat loss before lights off, via selective vasodilatation of distal skin regions, promotes sleepiness and the rapid onset of sleep.

Experimental evidence indicates that the medial preoptic/anterior hypothamalic area (POA), the dorsomedial nucleus of the hypothalamus (DMH) and different brainstem regions (the nucleus raphe pallidus, periaqeductal gray matter) play a critical role in thermoregulation [2]. The exact mechanisms of thermoregulation remain unsettled, but the sympathetic nervous system probably play a crucial role by mediating the vasoconstriction and vasodilatory skin responses implicated in heat-production and heat loss mechanisms.

The characteristics of thermoregulatory control vary significantly between sleep phases (NREM sleep, REM sleep) and wake and with time of the day, being modulated by the circadian system and sleep control mechanisms [3]. Body temperature is regulated at a lower level during NREM sleep than during wakefulness NREM sleep being characterized by downward resetting of the thermostat, and a decrease in body temperature and metabolism. During NREM sleep thermoregulation mechanisms are operative and the ambiental thermal load variations are balanced. This homeothermy is controlled by POA integrative mechanisms that drive subordinate brainstem and spinal somatic and visceral mechanisms. In contrast, the transition from NREM sleep to REM sleep is characterized by a disruption of ongoing thermoregulation and a marked inhibition of thermoregulation with changes in body temperature occurring passively in relation to the heat environmental load.

Evidence has accumulated showing that REM sleep propensity follows an endogenous circadian rhythm which is closely coupled to the BcT rhythm [4]. In fact, peak REM sleep propensity

occurs on the rising slope of the average BcT curve, coinciding with the phase of peak sleep tendency. This coupling between the rhythm of BcT and REM sleep propensity has raised the possibility that the wake-sleep disturbance of narcolepsy might be related to an impairment in the circadian BcT rhythm.

Mosko et al [5], studied the 24h BcT rhythm in 12 narcoleptic patients under normal conditions. The cosinor analysis showed a significant 24h circadian rhythm but the mesor of narcoleptics was elevated in comparison with control subjects and the temperature nadir was phase-advanced in nine narcoleptic patients who exhibited sleep-onset REM (SOREM) compared with three without SOREM. Using a different method, the analysis of variance, the authors found a temperature nadir anticipated in narcoleptic patients irrespective of SOREM, and a mesor elevated only those with SOREM.

Pollak et al., subsequently investigated the circadian BcT rhythm in six narcoleptic patients in a time-isolation condition for about 20 days founding that the levels, periods, amplitudes and phases of the circadian temperature rhythms of patients and controls did not significantly differ. The increases in temperature that normally follow the main sleep periods were smaller in narcoleptic subjects, and narcoleptic naps, which were involuntary, were heralded and accompanied by small decreases of mean temperature [6].

Mayer et al., studied 15 narcoleptic patients under normal conditions for 36h [7]. Their major findings were that patients with narcolepsy and controls did not differ in cosinor analysis of BcT, whereas the time-dependent analysis of BcT obtained by averaging the 3 min interval recordings showed a dampening of temperature amplitude, a reduced temperature curve rise in the morning and an anticipation of maximal temperature decline in patients with SOREM with respect to control subjects. The authors interpreted these results as suggestive of a defect in temperature-locked REM triggering in narcoleptic subjects.

A different approach to the study of temperature regulation in narcolepsy has been recently proposed. Starting from the evidence that skin temperature modulates neural activity in the sleep-regulating brain area and might thus promote falling asleep [8], Fronczeck et al. [9] investigated skin temperature in eight narcolepsy with cataplexy patients and found that the distal-proximal skin temperature gradient (DPG) during multi sleep latency test (MSLT) was

higher in narcoleptic patients than controls due to increased distal skin temperature and decreased proximal skin temperature, and it was related to a shorter subsequent sleep-onset latency. To support the potential therapeutic implications of this finding, the same study group subsequently demonstrated that the maintenance of wakefulness in narcolepsy was promoted manipulating core body and skin temperature within the natural range of the diurnal cycle [10].

The present study investigated the circadian rhythm and the state-dependent variations of BcT in narcolepsy with cataplexy patients, under controlled environmental conditions.

METHODS

Subjects

Patients with narcolepsy were compared to the 10 control subjects described in Section 1

Circadian rhythm of BcT analysis

The analysis of rhythmicity was performed evaluating the time series for BcT° according to the single cosinor method, using a computerized procedure as described in Section 2.II.

For each subject we analysed the 24 hour rhythmicity of two consecutive days, the first starting between 11 a.m and 12 a.m. and the second starting at 8 a.m.

Statistics

The between group differences were analyzed using Student's unpaired t-test. Variables (mesor, amplitude: AMP and acrophase: ACR), were considered significantly different if $p \leq 0.05$).

Time-dependent analysis

Data were obtained by two consecutive days of polysomnographic (PSG) recording as for cosinor analysis. For each subject, we analysed the distribution over the 24h period of the mean BcT value calculated every 15 min.

State-dependent analysis

The analysis was conducted on the 32 consecutive hours starting from the first night of PSG recording at 11 p.m (lights off). For each subject we calculated the mean value of BcT during wakefulness (W) and each sleep stage (NREM stage 1, 2, SWS and REM sleep) and expressed data considering the 24h period (24h; from 7 a.m), the daytime period (daytime; from 7 a.m. to lights off at 11.pm) and the night-time period (night-time; from 11 p.m. to lights on at 7 a.m.)

During daytime and night-time we also calculated in each subject the difference (Δ) between the mean value of BcT in W, NREM stage 1, 2, SWS and REM sleep and the mean value during daytime-W (Δ BcT). This allowed us to express the state-dependent variability during daytime and night-time of BcT when compared with a reference value (subjects' daytime BcT mean value).

Statistics (thanks to the collaboration with Prof. Patrizia Agati, Dipaertment of Statistical Sciences, University of Bologna)

Significance analyses were performed by fitting a mixed model (a model with fixed and random effects) where the factors were wake-sleep phases (W, NREM stage 1 and 2, SWS, REM) and group (controls vs narcolepsy subjects). Significance level was set at $p < 0.05$.

All the analyses were performed using Stata 9. Data are reported as mean ± SD.

RESULTS

For technical reasons BcT could not be recorded for 1 day in one patient with narcolepsy and two control subjects.

Circadian rhythm of BcT analysis

A significant circadian rhythm of BcT was detected in patients with narcolepsy and controls. We did not find differences of mesor, AMP or ACR between the two groups (Table 1).

Time-dependent analysis

The 24-h distribution of BcT (Figure 1), showed a similar day-night pattern in patients with narcolepsy and controls.

State-dependent analysis

Data of BcT and ΔBcT in wake-sleep phases are shown in Table 2. Patients with narcolepsy and control showed a significant physiological modulation of BcT between W, NREM and REM sleep in 24h, daytime and night-time. In the 24h, during daytime and night-time (Fig 2) the two groups had comparable BcT values during W, NREM and REM sleep.

During daytime, narcoleptics and controls exhibited a similar ΔBcT pattern through sleep phases characterized by a significant reduction of BcT in REM sleep ($p=0.01$). However a significant phase-specific effect in NREM stage 1 was detected in the narcolepsy group during which patients showed a significant increase in BcT fall compared to controls ($p<0.01$), and in REM sleep during which patients showed a reduced BcT fall compared to controls ($p= 0.01$).

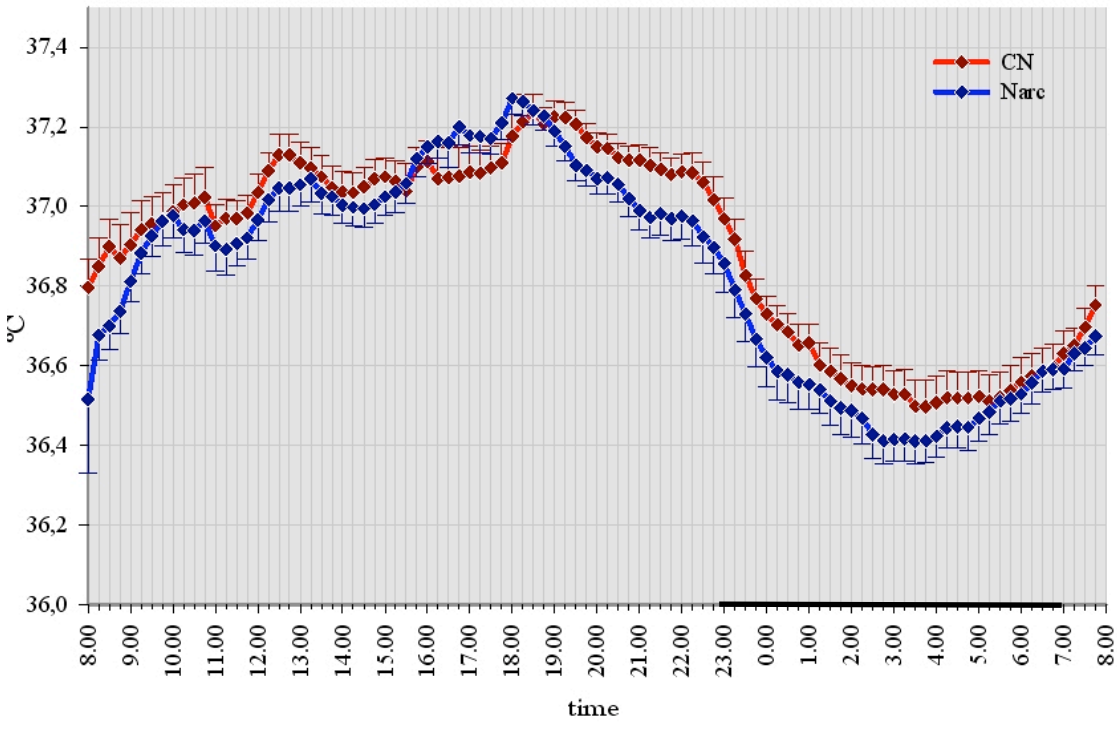
During night-time the BcT decline was comparable during NREM and REM sleep in narcoleptic patients and controls (Figure 3).

TABLE 1 RESULTS OF CIRCADIAN RHYTHM ANALYSIS OF BCT IN PATIENTS WITH NARCOLEPSY AND CONTROLS

Circadian rhythm of BcT	**Narcoleptics** (n = 10*)	**Controls** (n = 10*)	p values
Mesor (°C) Mean ± SD	36.8 ± 0.12	36.9 ± 0.13	ns
Amplitude Mean ± SD	0.36 ± 0.14	0.31 ± 0.15	ns
Acrophase (h, min) Mean ± SD	16.45 ± 1.31	16.43 ± 1.03	ns

Each value represents the mean of 19 values in narcoleptics (night 1 and night 2) and 18 values in controls (night 1 and night 2)

FIGURE 1 MEAN BCT VALUES OVER THE 24-H IN PATIENTS WITH NARCOLEPSY AND CONTROLS



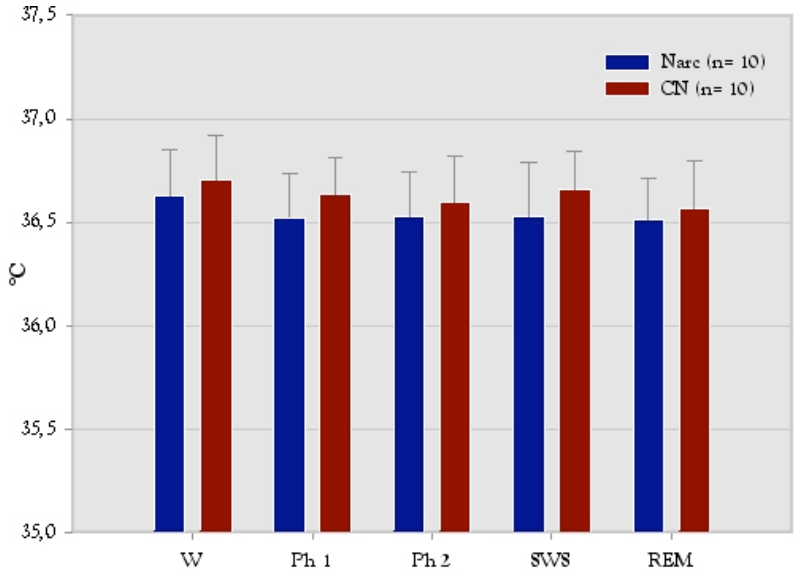
BcT: body core temperature; CN =controls; Narc= patients with narcolepsy; each point from 12:00 to 8:00 represents the mean every 15 min ± SE of BcT values of day1 and 2 while each point from 8:00 to 12:00 represent the mean every 15 min ± SE of BcT values of day 2; dark bar represent dark period from lights off to lights on

Table 2 BcT mean values and Δ values in wake-sleep phases in patients with narcolepsy and controls

	Patients with Narcolepsy (n=10)					**Controls** (n=10)				
	BcT (°C)					**BcT (°C)**				
	W	**Ph 1**	**Ph 2**	**SWS**	**REM**	**W**	**Ph 1**	**Ph 2**	**SWS**	**REM**
24-h mean (SD)	37 (0.12)	36.71 (0.17)	36.69 (0.18)	36.67 (0.18)	36.65 (0.2)	37 (0.16)	36.73 (0.16)	36.68 (0.22)	36.73 (0.2)	36.63 (0.22)
Daytime * mean (SD)	37 (0.12)	36.94 (0.18)	37 (0.21)	37 (0.17)	36.94 (0.24)	37.1 (0.17)	37 (0.21)	36.99 (0.2)	36.95 (0.18)	36.65 (0.17)
Night-time mean (SD)	36.63 (0.22)	36.52 (0.21)	36.53 (0.21)	36.53 (0.26)	36.51 (0.2)	36.7 (0.22)	36.63 (0.17)	36.59 (0.22)	36.66 (0.19)	36.56 (0.24)
	Δ BcT (°C)					**Δ BcT (°C)**				
Daytime * mean (SD)	0	-0.12 (0.12)	-0.06 (0.15)	-0.15 (0.15)	-0.11 (0.19)	0	-0.10 (0.11)	-0.12 (0.14)	-0.15 (0.18)	-0.36 (0.16)
Night-time mean (SD)	-0.39 (0.23)	-0.5 (0.22)	-0.5 (0.21)	-0.5 (0.25)	-0.51 (0.2)	-0.36 (0.25)	-0.43 (0.21)	-0.47 (0.23)	-0.41 (0.18)	-0.5 (0.25)

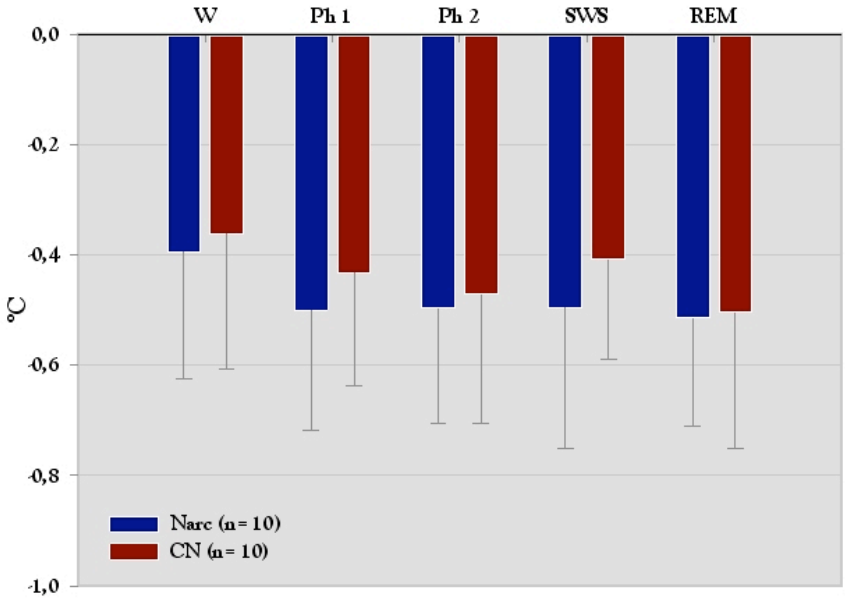
BcT: body core temperature; W: wake; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; Delta-BcT represent the difference between the mean value of BcT in W, NREM stage 1, 2, SWS and REM sleep and the mean value during daytime-W; * the 10 controls subjects (CN) and the 10 narcoleptics (Narc) contributed to daytime sleep as follow: Ph1: 10 narc and 7 CN; Ph2: 10 narc and 7 CN; SWS: 9 narc and 6 CN, REM: 10 narc an 1 CN

Figure 2 State-dependent variation of BcT during night-time in patients with narcolepsy and controls



BcT: body core temperature; W: wake;; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; Narc: patients with narcolepsy; CN: controls; Raw data are reported in Table 2

Figure 3 State-dependent night-time decline of BcT in patients with narcolepsy and controls



BcT: body core temperature; W: wake;; Ph1: NREM stage 1, Ph2: NREM stage 2; SWS: slow wave sleep; Narc: patients with narcolepsy; CN: controls; Raw data are reported in Table 2

DISCUSSION

This study analysed the 24h circadian rhythm and the state-dependent modulation of BcT in narcolepsy patients with cataplexy and healthy subjects under controlled environmental conditions.

The cosinor analysis of BcT exhibited comparable values of mesor, AMP and ACR in the two groups, at variance from the results of Mosko et al. [4] who found higher mesor values in narcoleptic subjects.

The controlled conditions under which subjects were investigated in our study contrast with those of previous studies [4, 6] that investigated subjects in their daily routine. The influence exerted on BcT rhythm by external factors such as room temperature, humidity, body position, light-dark cycle, food intake and sleep is well documented [7]. Thus, one merit of this study was that of reducing the effects induced by masking components on BcT circadian rhythm that may have influenced previous findings.

The state-dependent analysis of BcT showed that narcoleptic patients and controls displayed a physiological modulation of body temperature between wake, NREM and REM sleep during the 24h, daytime and night-time. BcT was in fact reduced during NREM sleep with respect to wakefulness and further reduced during REM sleep.

During NREM and REM sleep, BcT values were comparable in narcoleptic patients and controls during daytime and night-time. During daytime, the narcolepsy group exhibited a reduction of BcT fall in REM sleep and an increased BcT fall during NREM stage 1 compared to the control group. Nevertheless, the contribution to daytime sleep-phases was unbalanced between the two groups (see Section 1, Table 3) as narcolepsy subjects slept significantly more than controls during the daytime showing a higher amount of every sleep stage. In particular REM sleep that was experienced by all patients with narcolepsy was recorded in only one control subject.

In Section1 we discussed the differences occurring in the sleep structure during the 24h, daytime and night-time between narcoleptic patients and controls and stressed that despite an increased total sleep time during daytime, narcoleptics slept similarly to controls during night-

time. Sleep fragmentation was increased in narcolepsy patients throughout the 24h (Section 1, Figure 6). We might have expected that the higher sleep fragmentation in narcoleptic patients could alter the state-dependent modulation of BcT since duration of sleep may interfere with body temperature changes and vice versa but this was not confirmed in our study.

Unfortunately, we cannot compare our results with other studies since this is the first investigation to explore the state-dependent variations of BcT in narcolepsy.

Overall, our data suggest that the endogenous BcT circadian rhythm is preserved in narcolepsy with cataplexy. The suprachiasmatic nucleus, in the anterior hypothalamus, serves as the brain's "master clock". Its projections to the relay neurons in the dorsal supraventricular zone (dSPZ) are crucial for rhythms of body-core temperature. Cycles of body temperature are maintained by dSPZ projections back to the medial preoptical area. [11].

The relation between the orexinergic system and BcT regulation is complex and still controversial, at least in humans. Intracerebroventricular injection of orexin A raises body temperature in animal. In addition, orexin may affect body temperature through direct autonomic effects on sympathetic nervous system. Overall, orexin facilitates heat generation as a result of behavioural and/or sympathetic activation. Unexpectedly, a study on orexin knockout mice with a narcolepsy-like phenotype, showed a blunted body temperature fall during sleep in orexin deficient mice as compared to wild-type mice [12]. This result was in part confirmed [5] and in part rejected in [6, 7] in human studies.

The results of the present study suggest that the orexin deficit in narcolepsy patients with cataplexy is not associated with an impaired BcT regulation in terms either of circadian rhythmicity or state-dependent modulation.

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CONCLUSIONS

In the present study we investigated narcoleptic patients with cataplexy and healthy control subjects under controlled environmental conditions to evaluate the 24h circadian rhythm of blood pressure (BP), heart rate (HR) and body core temperature (BcT), their time and state-dependent modulation during daytime, night-time and the 24h and the state-dependent autonomic cardiac modulation during night-time sleep.

Following a 32h schedule of bed-rest, patient with narcolepsy exhibited a significant increase of total sleep time (TST) as compared to control subjects that was mainly due to a higher amount of daytime sleep. During night-time the two groups behaved similarly showing a comparable TST and a similar sleep internal architecture. Nevertheless, narcolepsy group was characterized by an increased sleep fragmentation throughout the 24h.

The analysis of cardiovascular reflexes during wakefulness did not disclose differences between narcolepsy patients and controls, suggesting the proper functioning of the autonomic brainstem centres regulating cardiovascular reflexes. However, the analysis of the heart rate variability at rest supine displayed an imbalance of the autonomic control in favour of an enhanced sympathetic tone in narcolepsy group.

The physiologic circadian rhythmicity of BP and HR was preserved in patients with narcolepsy with a blunted systolic blood pressure (SBP) amplitude and a higher HR mesor compared to controls.

The time and state-dependent analysis of BP and HR yielded the most impressive results of this study.

Interestingly although patients with narcolepsy remained within the normotensive range, they failed to show the normal 10-20% decline in SBP/DBP during nocturnal sleep, as we observed in the control group. The importance of these results is underlied by the Ohsama study that demonstrated a linear and inverse relationship between cardiovascular mortality and the nocturnal decline in blood pressure independently of the overall blood pressure load during 24h and other cardiovascular risk factors. Thus, patients with narcolepsy might be exposed to a

significant cardiovascular risk and epidemiologic studies looking at cardiovascular mortality in narcoleptic subjects are needed to clarify this issue.

The time-dependent analysis showed higher HR values in the narcolepsy group throughout the 24h compared to controls, confirming the results of the HR circadian rhythm analysis. The night-time fall of HR was however similar in the two groups strengthening previous findings that the 24h variations of HR reflect modulatory influences different from those controlling the BP pattern.

The state-dependent analysis of BP displayed an impairment of SBP regulation during REM sleep in narcoleptic patients who showed higher SBP values with respect to controls. This altered control of BP during REM sleep may contribute to the non-dippers BP pattern of patients with narcolepsy.

HR was higher in patients with narcolepsy during wakefulness, NREM sleep and REM sleep with respect to controls, but its state-dependent variations were comparable in the two groups.

This study demonstrated also that state-dependent modulation of the autonomic cardiac control during relaxed wakefulness, NREM stages 1 and 2, NREM stages 3 and 4 and REM sleep, evaluated with the cross correlation function and with the analysis of baroreflex sequences, did not differ between the narcolepsy and the control group. These results demonstrate that EDS is not associated per se with an impaired autonomic cardiac regulation but only when it is secondary to SRBD, as suggested by previous studies.

Finally this study showed that the circadian control of BcT and its state-dependent modulation were preserved in patients with narcolepsy when evaluated under controlled conditions.

In conclusion, this study showed that narcoleptic subjects have an unbalance autonomic cardiovascular regulation in favour of an enhanced sympathetic tone.

Further studies are needed in order to clarify the evident contrast between our results and the reduced sympathetic activity that several animal studies have suggested as the autonomic effect of the orexinergic depletion that underlies narcolepsy with cataplexy.