Reduced hypothalamic gray matter in narcolepsy with cataplexy

J. Buskova[1], M. Vaneckova[2], K. Sonka[1], Z. Seidl[2] & S. Nevsimalova[1]

1. Department of Neurology, 1st Medical Faculty, Charles University, Prague, Czech Republic
2. Department of Radiology, 1st Medical Faculty, Charles University, Prague, Czech Republic

Correspondence to: Jitka Bušková, MD., PhD.
Department of Neurology, 1st Medical Faculty, Charles University
Katerinska 30, 128 08 Prague, Czech Republic
PHONE/FAX: +420-224922678
EMAIL: vankjit@seznam.cz

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Abstract

OBJECTIVE: Narcolepsy with cataplexy is associated with a loss of hypocretin. The question is, if there is an autoimmune or neurodegenerative process selectively killing the hypothalamic hypocretin-containing neurons or if these cells survive but fail to produce hypocretin. To support one of these hypothesis we aimed to detect structural changes in the hypothalamus of narcoletic patients.
MATERIAL AND METHODS: Nineteen narcoleptic patients were compared to 16 healthy controls. We used voxel-based morphometry (VBM), an unbiased MRI morphometric method with a high sensitivity for subtle changes in gray and white matter volumes to investigate hypothalamic region in this condition.
RESULTS: Classical MRI protocol revealed no structural abnormalities, but using VBM we found significant reduction in hypothalamic gray matter volumes between patients and controls.
CONCLUSIONS: VBM showed hypothalamic gray matter loss in narcolepsy with cataplexy. This suggest that functional abnormalities of hypocretin neurons in narcolepsy are associated with structural changes of hypothalamus.

ORIGINAL ARTICLE

Introduction

Narcolepsy with cataplexy is a homogeneous lifelong sleep disorder linked to the HLA-DQB1*0602 haplotype affecting approximately one in 2000 individuals [1,2]. It is characterized by excessive daytime sleepiness, disturbed nocturnal sleep, cataplexy and often also by sleep paralysis and hypnagogic hallucinations [3]. An important role in the pathophysiology of narcolepsy seems to play the loss of hypocretin-1 and -2, a hypothalamic neuropeptides involved in the regulation of arousal and appetite [4]. An unknown process markedly reduced the expression of hypocretin mRNA and immunoreactivity while sparing production of melanin-concentrating hormone in nearby cells [5,6]. Together with the known HLA association [7], these observations suggest that an autoimmune or neurodegenerative process may selectively kill the hypocretin neurons. However, it is possible, that the hypocretin neurons survive, yet fail to produce hypocretin [8]. The most of the recent studies report the reduction or absence of hypocretin neurons in the lateral hypothalamic region that widespread project to numerous areas of the brain including brainstem nuclei, thalamus and the cerebral cortex [9,10]. Postmortem studies showed undetectable hypocretin mRNA and even sings of gliosis in the

area where tho hypocretin neurons are located [6]. Also in animal models (narcoleptic dogs) has been observed the neuronal degeneration in brain areas receiving hypocretin input [11,12].

In the present study we investigated whether degeneration of hypocretin neurons in the hypothalamus is associated with macroscopic structural changes (resulting from cell loss and possible gliosis) using voxel-based morphometry (VBM), what would support the damage of the hypocretin neurons in narcolepsy. Recently, four groups used this technique to detect structural brain changes in narcolepsy, but unfortunately the results are inconsistent [13–16].

Material and methods

Subjects

Nineteen narcoleptic patients (10 males, 9 females, mean age 43.4 ± 13.8 years) and 16 healthy controls (9 males, 7 females, mean age 40.3 ± 10.9 years) were included in the study. Narcolepsy was diagnosed according to ISCD criteria [3], all patients were clinically evaluated by a neurologist experienced with narcolepsy (SN, KS) and had classical history of imperative sleepiness and cataplexy, while the presence of further symptoms was not unconditional. All patients had finding typical for narcolepsy on overnight polysomnography and a Multiple Sleep Latency Test (MSLT). HLA typing was not performed in five patients because of technical reasons. Hypocretin level in the CSF was not measured. None of the patients suffered from other sleep disorder including sleep apnea syndrome or REM sleep behaviour disorder, neither from any other neurological, psychological or endocrinological disorder. Ten of our patients were newly referred to our Sleep Disorders Center to make a diagnosis, that means they were drug-naive; 9 patients were medicated. The clinical characteristics of the patients are summarized in the Table 1.

The control group consisted of healthy volunteers reporting subjectively normal sleep habits, with no complaint of either insomnia or excessive daytime sleepiness, receiving no sleep and wake influencing drugs. All of them had normal MR images. All subjects gave informed consent before MR investigation.

Magnetic resonance protocol and statistics

MRI scans were performed on the Philips Gyroscan NT 15 (1.5 T). We made T1 weighted image/3D (T1WI/3D) in transversal section, with parameters: repetition time (TR) 10.50 ms, time to echo (TE) 3.33 ms, flip angle (FLIP) 20°, thickness of slices (THK/gap) 1.6/0 mm and T1 weighted image – inversion recovery (T1WI-IR) in transversal section, with parameters: TR 2000 ms, TE 13 ms, inversion time (TI) 350 ms, flip angle (FLIP) 90°. In addition, we used standard protocol T2WI and FLAIR in transversal sections and T2WI in coronal sections. These later sequences were performed in order to exclude other pathological findings which are not connected with narcolepsy.

Table 1. Clinical characteristics of patients with narcolepsy

N	Age (years)	Sex	Disease duration (yrs)	Cataplexy	HH	SP	HLA DQB1*0602	MSLT Mean SL	MSLT # SOREM	Medication
1	18	m	4	+	+	-	+	1.5	5	-
2	19	f	3	+	-	-	+	0.5	5	-
3	25	m	2	+	+	-	+	0.7	5	-
4	28	f	3	+	+	-	n.d.	4.2	4	-
5	31	m	3	+	-	-	+	4.8	3	-
6	33	f	2	+	-	+	+	3.2	3	-
7	33	m	2	+	+	+	+	1.4	4	-
8	34	f	4	+	+	+	+	3.1	3	-
9	36	f	9	+	+	+	+	4.6	3	sodium oxybate
10	47	m	25	+	+	+	n.d.	4.0	2	methylphenidate
11	51	f	32	+	+	-	n.d.	2.4	3	modafinil, escitalopram
12	51	m	1	+	-	-	+	3.2	3	-
13	52	f	22	+	+	+	+	2.0	4	methylphenidate
14	53	m	13	+	+	-	n.d.	1.3	5	methylphenidate
15	53	m	25	+	+	+	n.d.	0.6	5	modafinil
16	55	m	11	+	-	-	+	1.2	5	methylphenidate, CLO
17	57	m	3	+	+	-	+	2.4	3	-
18	59	f	40	+	+	-	+	0.5	4	methylphenidate
19	67	f	22	+	+	+	+	4.4	2	modafinil

HH=hypnagogic hallucinations, SP=sleep paralysis, SL=sleep latency, SOREM=sleep onset REM period, n.d.=not done, CLO=clomipramine

For voxel-based morphometry (VBM) we used sequence T1WI/3D. For each patient, each slice was normalized using the Talaraich coordinates. Voxel size was 1×1×1 mm. The structure of interest (hypothalamus) was traced by a mouse-driven cursor and computed by in-house developed software. Nonhomogeneity due to anizotrophy and nonhomogeneity of instrument rf coils was smoothed out in such way that white matter (WM) had the same intensity over the whole brain. This WM intensity was set to 5 000 relative units (RU). As the gray matter we considered the intensity in the range 4 150–4 700 RU. Finally, the density of gray matter was determined on normalized (Talaraich) brain, counting all voxels. This technique – voxel-based morphometry – enables an identification of slight differences in the brain morphology, which are not accessible in standard MR examination. A control group of patients was examined in the same manner [17]. All MRI scans were reviewed by an experienced neuroradiologist (MV, ZS).

Statistical analysis included the Student´s t-test. The normal distribution was evaluated by using the Shapiro-Wilk´s test. The significance level was set at $p<0.05$.

Results

We have found no MRI abnormalities between patients with narcolepsy-cataplexy and healthy controls using routine MRI protocol T2WI and FLAIR, but we detected a marked difference between hypothalamic region in narcoleptic patients and controls using voxel-based morphometry; patients suffering from narcolepsy showed significant decrease in hypothalamic gray matter concentration in compatison to healthy controls (0.696±0.92 vs. 0.798±0.93, t-value=3,22; df=33; p<0,01) as illustrates Figure 1. When we divided the patients group in two (first group consisted of ten drug-naive patients and the second group consisted of nine medicated patients) and compared these groups separately to the controls, we did not revealed significant results probably due to small number error. We did not concentrate on the hypocretin projection areas that could be pathophysiologicaly involved in narcolepsy.

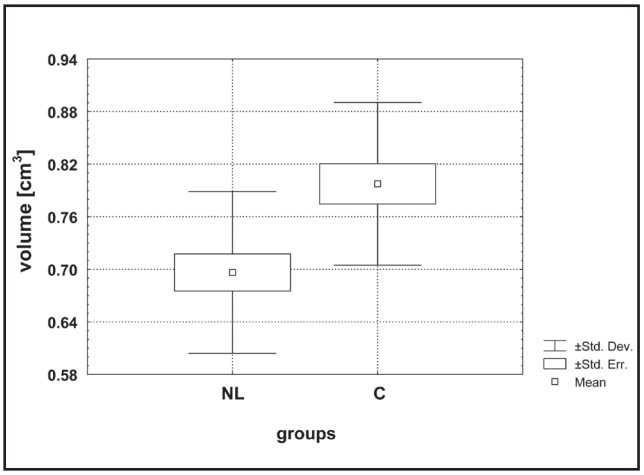

Figure 1. Box plots showing gray matter volume in a cm^3 voxel placed within the hypothalamus; NL=narcolepsy, C=controls

Discussion

The fact that most cases of human narcolepsy results from a deficiency of hypocretin production, which is probably caused by a secondary loss of hypocretin neurons in the lateral hypothalamus [6] has renew the interest in anatomical imaging studies in this condition. Although in general, no macroscopic neuroanatomical abnormalities have been found till 1990s, except for rare cases of so-called symptomatic narcolepsy typically linked to hypothalamic or rostral brainstem lesions [18] and also earlier imaging studies in idiopathic narcolepsy did not yield convincing results [19].

Together with development of novel technique for morphological comparison named voxel-based morphometry (VBM), appear four studies using this technique in patients suffering from narcolepsy [13–16]. Unfortunately, these works revealed controversial results, ranging from gray matter loss in multiple cortical areas – predominantly inferior temporal and inferior frontal [16] or in the right prefrontal and frontomesial cortex [14] to no morphometric changes at all [13]. One of these VBM studies reported gray matter loss in specific subcortical areas such as hypothalamus and accumens nucleus [15].

These discrepancies need to be discussed in the light of methodological and clinical aspects, especially different data processing, inhomogeneous patient groups and pretreatment.

Our results showed significant decrease of hypothalamic gray matter in patients suffering from narcolepsy with cataplexy using VBM (p<0,01), whereby confirm results this previous work on 29 narcoleptic patients [15], what is much larger group of subjects than in the other studies [13,14,16]. The number of subjects seems to be very important factor for statistical processing, inhomogenous patients group and small sample size could contributed to conflict results [13,14]. Simillarly, when we tried to divide our patient´s goup in two (drug-naive and medicated subjects), we revealed no significant results for this region of interest. Unfortunately, in the largest study were reported no details about clinical characteristics of the patients [15]. Our results are also in a good agreement with those confirming gray matter loss in hypocretin projection areas due to reduction of hypocretin input [14,16].

The benefit of our work in comparison with previous published studies is at first homogeneous group of patients, our subjects are uniform with respect to symptomatology (excessive daytime sleepiness and cataplexy) and the group of patiens is sufficiently extensive. Polysomnography findings including MSLT results unambiguously demonstrate the typical pattern for this

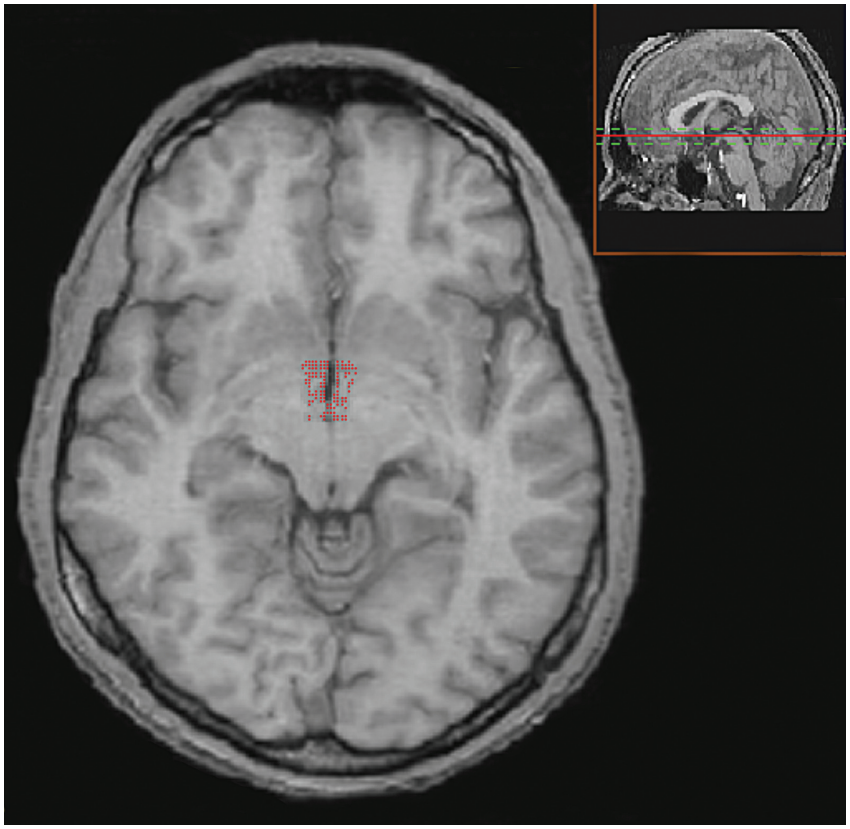

Figure 2. MRI scan showing the preselected structure of interest- hypothalamus.

disease. Accurancy of the diagnosis also confirm HLA DQB1*0602 positivity in majority of the patients. Our patients do not suffer from other sleep disorder. In an effort to minimalize potencial limitation of the technique the hypothalamic region was punctually manualy designated by one researcher in all subject double-blind.

In conclusion, the data contradict the etiological theory of a dysfunction of hypocretin-producing cells in a structurally normal hypothalamus and demonstate the precise anatomical location of the central nervous system lesion in narcolepsy.

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