Hypocretin deficiency : neuronal loss and functional consequences

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Hypocretin (Orexin) Loss in Parkinson's Disease

CHAPTER 2

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Hypocretin (Orexin) Loss in Parkinson's Disease

Objective **The hypothalamic hypocretin (orexin) system plays a central role in the regulation of various functions, including sleep/wake regulation and metabolism. There is a growing interest in hypocretin function in Parkinson's disease (PD), given the high prevalence of non-motor symptoms such as sleep disturbances in this disorder. However, studies measuring cerebrospinal fluid hypocretin levels yielded contradictory results so far.**

Methods **In PD patients and controls we (1) estimated the number of hypocretin neurons in post-mortem hypothalami using immunocytochemistry and an image analysis system and (2) quantified hypocretin levels in post-mortem ventricular cerebrospinal fluid (CSF) and (3) prefrontal cortex using a radioimmunoassay. Furthermore, presence of Lewy bodies was verified in the hypothalamic hypocretin cell area.**

Results **Data are presented as median (25^{th}-75^{th} percentile). We showed a significant decrease between PD patients and controls in (1) the number of hypocretin neurons (PD: 20,276 (13,821 – 31,229); controls: 36,842 (32,546 – 50,938); p = 0.016), (2) the hypocretin-1 concentration in post-mortem ventricular CSF (PD: 365.5 pg/ml (328.0 – 448.3); controls: 483.5 (433.5 – 512.3); p = 0.012) and (3) the hypocretin-1 concentrations in prefrontal cortex (PD: 389.6 pg/g (249.2 – 652.2); controls: 676.6 (467.5 – 883.9); p = 0.043).**

Conclusion **Hypocretin neurotransmission is affected in PD. The hypocretin-1 concentration in the prefrontal cortex was almost 40% lower in PD patients, while ventricular CSF levels were almost 25% reduced. The total number of hypocretin neurons was almost half compared to controls.**

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder in which motor symptoms such as hypokinesia, tremor and rigidity are the most well-known. However, there is a growing interest in the non-motor symptoms, such as autonomic dysfunction and cognitive disturbances.[1] Of these, sleep disorders are one of the most striking.[2-4] Sleep disturbances occur often in patients with PD and can even precede the motor symptoms. Excessive daytime sleepiness with frequent naps and so-called 'sleep-attacks' have been reported in 15-50% of patients.[5,6] Furthermore, there are clear nighttime sleep disturbances, such as fragmented nocturnal sleep, REM-sleep behavior disorder and periodic leg movements, as well as daytime sleep-onset REM periods.[3,4,7] The

combination of these symptoms suggest a common etiology with narcolepsy.[8]

Narcolepsy is a primary sleep-wake disorder characterized by excessive daytime sleepiness and REM-sleep dissociation phenomena such as cataplexy. Moreover, there are core symptoms of narcolepsy that resemble the nighttime sleep disturbances commonly seen in PD, most notably fragmented nocturnal sleep and REM-sleep behavior disorder.[9] Furthermore, sleep-onset REM periods form one of the neurophysiological characteristics of narcolepsy. Narcolepsy is caused by a loss of hypocretin (orexin) producing neurons, reflected in undetectable cerebrospinal fluid (CSF) levels.[10] Hypocretin neurons are exclusively located in the lateral hypothalamus and project widely throughout the central nervous system,[11] where they have an excitatory effect on several autonomic, metabolic, neuro-endocrine and arousal systems.[12]

In PD, there is a progressive and irreversible degeneration of dopaminergic neurons projecting from the substantia nigra to the striatum. In addition, there are degenerative changes in many other parts of the brain, including the hypothalamus.[13] Lewy bodies, the pathophysiological hallmark of PD, have been found in various brain regions, again including the hypothalamus.[14] These observations suggest that there are hypothalamic changes in PD, thus possibly involving the hypocretin system.
Several studies have been conducted to detect damage to the hypocretin system in PD. However, these only assessed CSF hypocretin levels. Moreover, results have been conflicting. One study reported decreased levels in ventricular CSF in late stage PD patients,[15] but three other groups found normal concentrations in spinal CSF.[16-18]

Here we used a combination of approaches in three brain compartments to detect whether the hypocretin system is affected in PD. First, we measured hypocretin levels in post-mortem ventricular CSF. Second, hypocretin content was determined in peptide extracts from cerebral cortex, as this has been shown to be a more sensitive technique compared to CSF measurements.[10] Third, we directly counted the total number of hypocretin neurons in the lateral hypothalamus of PD patients versus matched controls.

Materials and Methods

Post Mortem Material

Hypothalami and ventricular CSF were provided by The Netherlands Brain Bank. Frozen prefrontal cortex tissue was obtained from the Leiden PD Brain Bank. Permission was obtained for a brain autopsy and for the use of human material and clinical information for research purposes. The controls were matched for age, sex and Alzheimer Braak stage (for both groups ≤2).[19] Clinicopathological details are given in tables 1 and 2. Exclusion criteria for control subjects were use of corticosteroids and primary neurological or psychiatric disease, unless stated otherwise. This was verified by a systematic neuropathological analysis.[20,21] In all PD patients the clinical diagnosis was confirmed by a systematic neuropathological examination;[20] all patients were late-stage PD. No direct mentioning of the occurrence of EDS, sleep onset REMs or cataplexy could be found in the medical records of PD patients.

Table 2.1 Clinicopathological details of subjects used for hypocretin cell count and ventricular CSF measurement

	NBB no.	Age	Sex	PMD (hours)	Fixation time (days)	Cause of death	Hcrt-1 cell number x10^3	CSF (pg/ml)
PD	00-102	56	M	5.08	37	Malignant neuroleptic syndrome	N.D.	404
	02-057	62	M	9.25	43	Combined kidney and liver failure	22.1	327
	93-064	73	F	41.00	34	Massive pneumonia of the left lung	40.6	N.A.
	94-092	77	F	9.67	24	Cachexia	14.2	327
	02-013	80	F	5.50	30	Cachexia	18.4	331
	98-043	81	F	4.17	43	Pneumonia	28.9	377
	00-034	86	M	8.50	45	Unknown	12.5	463
	01-122	86	M	5.58	32	Aspiration pneumonia	13.7	354
	02-064	87	M	7.33	36	Respiratory insufficiency	32.0	486
Median		80.0		7.33	36.0		20.3	366
Percentiles (25th-75th)		67.5-86.0		5.12-9.45	31.0-43.0		13.8-31.3	328-448
Controls	98-127	56	M	5.42	35	Myocardial infarction	49.1*	554
	99-101	69	M	19.25	41	Pneumonia, unexpected death in sleep	39.2	468
	97-156	77	F	2.67	47	Unknown	32.4	495
	93-139	78	F	6.42	32	Respiratory insufficiency	17.4	472
	00-142	82	F	5.50	36	Myocardial infarction	35.5	368
	00-022	83	F	7.75	34	Myocardial infarction	53.2	N.A.
	95-106	74	M	8.00	60	Myocardial infarction	33.5	422
	94-076	78	M	8.42	24	Cardiac arhythmia	65.9	495
	00-072	78	M	18.00	45	Kidney failure, dehydration	44.2	518
Median		78.0		7.75	36.0		38.2	484
Percentiles (25th-75th)		71.5-80.0		5.45-13.20	33.0-46.0		32.9-51.2	434-512

NBB no, Netherlands Brain Bank number; PMD, postmortem delay; M, male; F, female; N.D., not determined; N.A., not available; Hcrt-1, Hypocretin-1.
* This control was matched with the incomplete PD patient and thus excluded from the group analysis.

Table 2.2 Subjects used for hypocretin brain tissue measurement

	LBB no	Age	Sex	Cause of death	Hcrt-1 tissue content (pg/g)
Controls	91-124	37	M	Myocardial infarction	996
	04-117	46	M	Cardiac arhythmia	364
	92-062	48	M	Myocardial infarction	665
	93-194	52	M	Stroke	688
	04-072	60	F	Pneumonia	433
	04-063	63	F	Leptomeningeal metastasis	737
	04-118	64	M	Myocardial infarction	1368
	00-064	67	F	Aortic Dissection	858
	00-041	68	M	Pneumonia	697
	93-306	70	M	Metastasized carcinoma	1282
	04-071	71	M	Cardiomyopathy	573
	00-067	74	M	Metastasized carcinoma	243
	00-083	76	M	Aortic Dissection	638
	93-303	79	F	Stroke	892
	04-054	83	M	Leptomeningeal metastasis	358
	00-061	86	M	Myocardial infarction	570
Median		68			677
Percentiles (25th-75th)		54-76			468-884
PD	95-008	62	M	Unknown	198
	96-145	70	M	Gastrointestinal Bleeding	534
	87-329	74	F	Unknown	296
	89-215	75	F	Pneumonia	202
	98-057	77	F	Lung Carnicoma	390
	04-055	80	F	Cardiac Asthma	906
	98-136	80	M	Pneumonia	771
	89-199	83	M	Myocardial infarction	304
	89-032	84	M	Cachexia	432
Median		77			390
Percentiles (25th-75th)		72-82			249-653

LBB no, Leiden Brain Bank number; M, male; F, female; N.D., not determined; N.A., not available; Hcrt-1, Hypocretin-1.

Immunocytochemistry

Hypothalami from nine PD patients and nine matched controls were used. Tissues were fixed in 10% PBS (pH 7.4) formalin at room temperature and were paraffin-embedded and serially sectioned at 6 µm from rostral to caudal. Every 100th section in the expected hypocretin cell area, from the level where the fornix touches the paraventricular nucleus to the level where the fornix reaches the corpora mammillaria, was stained using a hypocretin-1 antibody (Phoenix Pharmaceuticals, Inc., Belmont, CA; catalog no. H-003-30). The specificity of this antibody was confirmed in a previous study.[22] Antibody binding was visualized according to the avidin-biotin complex method using diaminobenzidinenickel solution to finish the staining as described previously by Goldstone et al.[23] If these sections did not cover the whole hypocretin area, extra sections were added at equal distances, both rostral

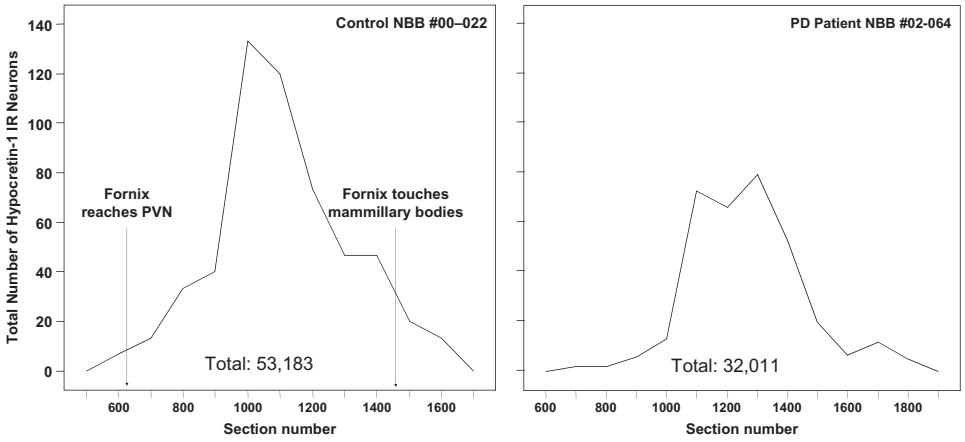

Figure 2.1 **Sample distribution patterns**
Sample distribution patterns of a PD patient (NBB #02-064, left) and a control subject (NBB #00-022, right). For each slide, the total number of hypocretin neurons is shown. The total number of cells is determined by calculating the total area under the curve. Only when the complete hypocretin cell area is contained within the measured slides, a subject can be included.hypothalamic area containing these neurons.

and caudal, until no more hypocretin cells were present.

For each subject, three sections were taken from the middle of the verified hypocretin cell area and double-stained for hypocretin-1 and Lewy Bodies, using a cocktail of the aforementioned hypocretin-1 antibody and an alpha-synuclein antibody (Zymed, Carlsbad, CA; catalog no. 32-8100). Hypocretin-1 antibody binding was visualized using the avidin-biotin complex method described above, while alpha-synuclein antibody binding was visualized using the alkaline-phosphatase blue method.[24,25]

Immunocytochemistry quantification

An estimate of the total number of hypocretin-1 immunoreactive (IR) cells was made using an image analysis system (ImagePro version 5.1, Media Cybernetics, Silver Spring) connected to a camera (JVC KY-F55 3CCD) and plane objective microscope (Zeiss Axioskop with Plan-NEOFLUAR Zeiss objectives, Carl Zeiss GmbH, Jena, Germany). Randomly selected fields were counted in every section, covering in total 15% of a manually outlined area containing the hypocretin-1 IR cells. This was done by one person while blinded for the diagnosis. To prevent influence of cell size, only positively stained cell profiles containing a nucleolus (~2 µm) were counted. This counting procedure, which was judged to be the best for the thin (6 µm) sections used, is based on the principle that nucleoli can be considered as hard particles that will not be sectioned by a microtome knife but, instead, are pushed either in or out of the paraffin when hit by the knife.[26-28] Calculation of the total number of hypocretin-1 IR neurons was performed by a conversion program based upon multiplication of the neuronal counts by sample frequency of the sections, as was described previously by Goldstone *et al.*[23] Mean (±SD) number of sections needed to cover the complete hypocretin area was 9.7±3.1 per subject. The coefficient of variation (SD/mean x 100%) of this method

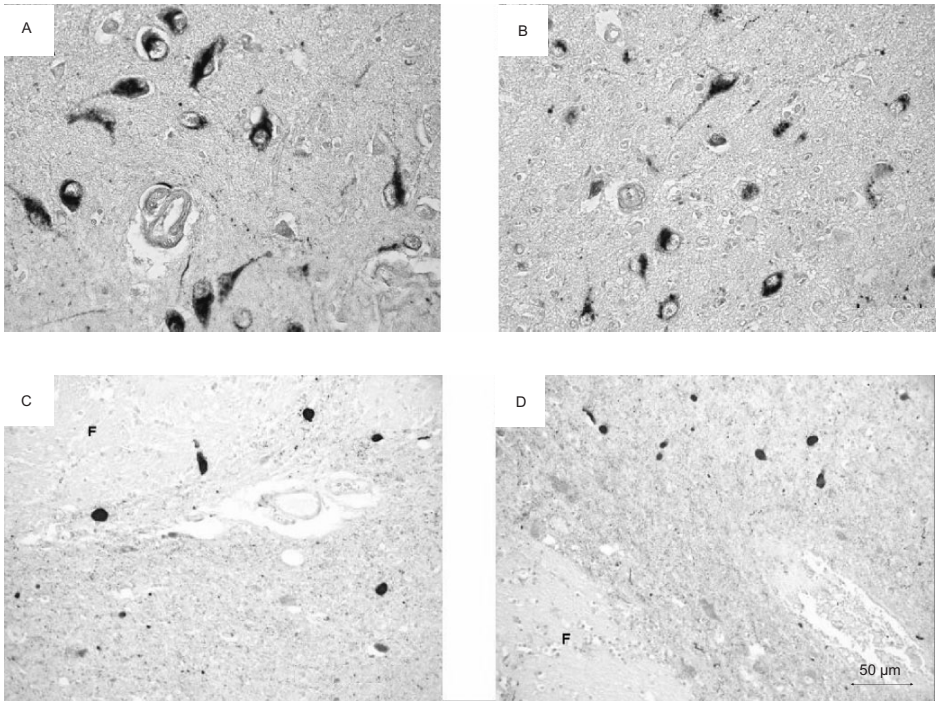

Figure 2.2 Example of staining

Examples of hypocretin-1 cell bodies in the lateral hypothalamus of a control subject NBB #94-191 (A) and a PD patient NBB #91-272 (B). There was no significant difference in the intensity of staining and the distribution pattern of hypocretin neurons. (C, D) Lewy bodies (alpha-synuclein) in the perifornical region of the lateral hypothalamus of two PD patients NBB #01-122 and NBB #94-092. 'F' indicates the fornix.

was 7.3% (calculated by counting one complete subject five times). Reliability and completeness of the cell counting was further confirmed by graphically presenting the actual numbers of neurons counted in every section from rostral to caudal to review the distribution pattern (see sample control and patient in Figure 2.1).

Hypocretin-1 measurements in cortex and CSF

One gram of frozen pre-frontal cortex was used from nine (5 male) PD patients and sixteen (4 male) healthy controls. We used the most rostral part of the pre-frontal cortex, as this cortical region is densely innervated by hypocretin neurons resulting in high hypocretin concentrations.[10] Diced tissue samples were boiled for 10 minutes in 10.0 ml of MilliQ water, cooled to room temperature, acidified using glacial acetic acid and HCl (final concentration: 1.0 M and 20.0 mM respectively), homogenized and centrifuged. The supernatant was acidified again with an equal volume of 0.1% trifluoracetic acid (TFA) and vacuum dried. Samples were re-suspended in 500 µl of RIA buffer before measurements.

Ventricular CSF was available from eight Parkinson's patients and eight matched controls (all these subjects were also included in the immunocytochemistry study).

After collection, ventricular CSF was centrifuged at 2500rpm for 10 minutes and the supernatant immediately stored at -80°C until measurements.

Hypocretin-1 levels were measured using a commercially available radioimmunoassay (RIA) (Phoenix Pharmaceuticals, Belmont, USA). All measurements were conducted in duplicate 100 µl aliquots in a single assay run. The detection limit was 50 pg/ml and intra-assay variability was less than 5%. We used a validated reference sample to adjust levels to previously reported values.[10]

Statistics

All data are given as median (25^{th} – 75^{th} percentile). Group differences were analyzed using the Mann-Whitney U and the chi-square test. Correlations between hypocretin-1 tissue concentration, CSF concentration, cell number, post-mortem delay, fixation time and age were evaluated using Spearman correlation. All reported p-values are two-sided, with 0.05 as the significance threshold.

Results

Hypocretin-1 histochemistry

The location and staining intensity of the hypocretin-1 IR cell bodies was similar in controls and PD patients (Figure 2.2A, B). Hypocretin-1 IR neurons showed the same distribution pattern as described before:[22] cell bodies were restricted to the perifornical region of the lateral hypothalamus. On the level where the fornix crosses the paraventricular nucleus, some hypocretin neurons started to appear in the supraoptic area. In the subsequent levels, the fornix migrated to the corpora mammillaria while passing through an area with a high number of hypocretin cells. When the fornix reached the corpora mammillaria, there were still many hypocretin-1 IR cell bodies visible. In all PD patients Lewy bodies were abundantly present in the perifornical region of the lateral hypothalamus (Figure 2.2C, D), while only a few Lewy bodies could be discerned in one control patient (NBB #00-320). However, hypocretin neurons

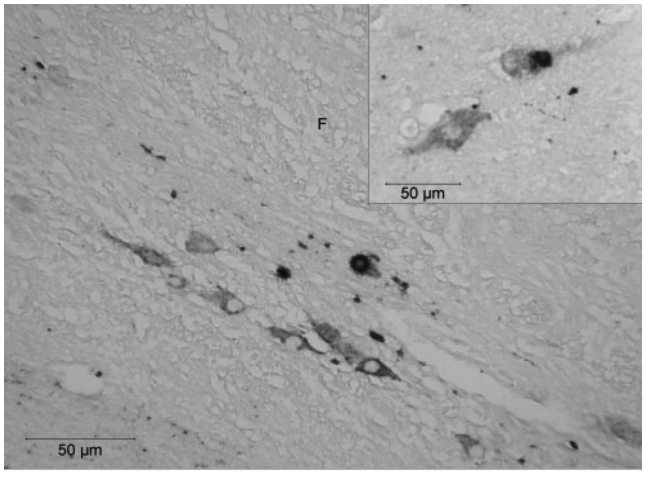

Figure 2.3
Example of Staining
Example of hypocretin neurons (DAB staining in grey) that contain a Lewy body (alpha-synuclein, AP-blue staining, in black) in PD patients NBB #01-280 and #94-245 (insert). Several hypocretin neurons that do not show this colocalization can be seen as well. 'F' indicates the fornix.

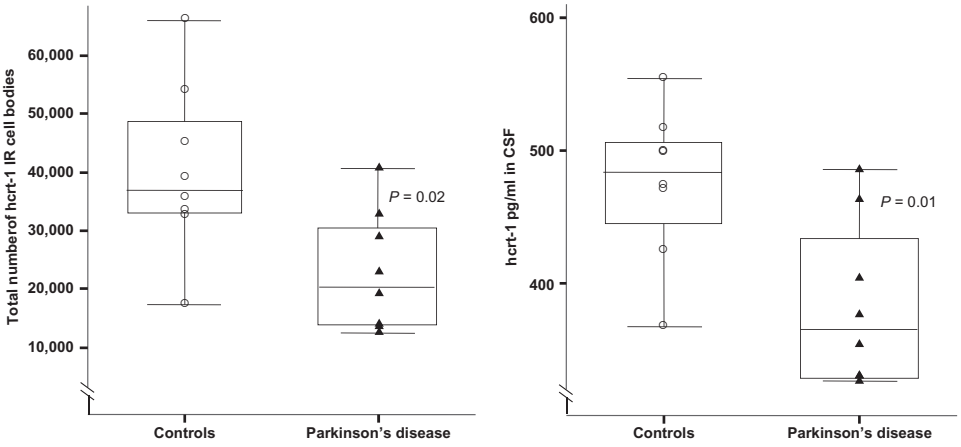

Figure 2.4 Boxplots showing total number of hypocretin neurons and CSF concentration

Boxplots showing the median, 25th-75th percentiles and the range of the number of Hypocretin neurons (A) and the hypocretin-1 concentration in post-mortem ventricular cerebrospinal fluid (B) in PD patients (right) and controls (left). Open circles represent controls, closed triangles represent PD patients.

that contained a Lewy body (Figure 2.3) were rare and only 1-2 double-stained neurons could be discerned in sections that contained numerous hypocretin neurons.

In one PD patient (NBB #00-102, table 1) the area showing hypocretin-1 IR cell bodies was not completely present in the available hypothalamic material. Therefore, the total counts of this patient and the matched control (NBB #98-127) were not included in the final analysis.

Hypocretin-1 cell number

There were no significant differences in age, sex, post-mortem delay (PMD) and fixation time between groups (all $p > 0.43$). Furthermore, there was no significant correlation of these variables with hypocretin-1 cell number in PD patients (all $p > 0.55$), controls (all $p > 0.14$), or the combined group (all $p > 0.33$).

In PD, the total number of hypocretin neurons was almost half compared to controls (PD: 20,276 (13,821 – 31,229); controls: 36,842 (32,546 – 50,938); $p = 0.016$, Figure 2.4).

Ventricular CSF Hypocretin-1 Content

Post-mortem ventricular CSF was not available for one PD patient (NBB #93-064) and one control (NBB #00-022). There were no significant differences in age, sex, PMD and fixation time between groups (all $p > 0.51$). Furthermore, there was no significant correlation of these variables with hypocretin-1 CSF content in PD patients (all $p > 0.13$), controls (all $p > 0.44$), or the combined group (all $p > 0.44$).

There was a significant reduction in hypocretin-1 ventricular CSF content in PD patients compared to controls (PD: 365.5 pg/ml (328.0 – 448.3); controls: 483.5 (433.5 – 512.3); $p = 0.012$, Figure 2.4).

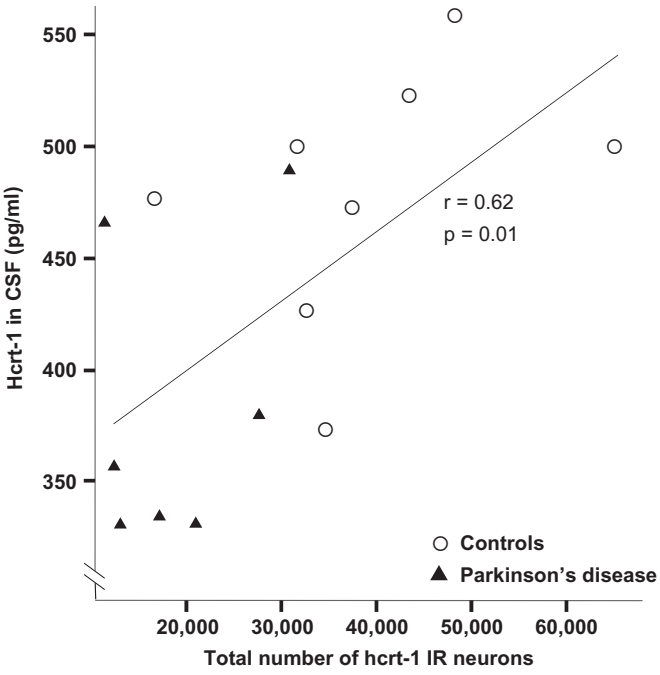

Figure 2.5
Correlation
Correlation between hypocretin-1 IR cell number and post-mortem ventricular CSF hypocretin-1 content. Open circles represent controls, closed triangles represent PD patients.

Relation between hypocretin cell number and CSF levels
As hypocretin cell counts and CSF levels were available in the same subjects, we were able to correlate these two variables directly. There was a significant correlation between cell number and ventricular CSF content in the combined group (n=15, r=0.62, P=0.010, Figure 2.5), but not within the separate groups (controls: p = 0.23; PD: p = 0.70).

Hypocretin-1 concentration in prefrontal cortex
There was no significant correlation between age or sex and hypocretin-1 concentrations in PD patients (all p > 0.19), controls (all p > 0.37), or the combined group (all p > 0.34). The effect of Braak grade could not be evaluated, since all PD subjects were late-stage. Hypocretin-1 concentration in controls was 676.6 (467.5 – 883.9) pg/gram of wet brain tissue, comparable to previously reported values.[10] Hypocretin levels were almost 40% lower in PD patients (389.6 pg/g (249.2 – 652.2); p = 0.042; Figure 2.6).

Discussion

In this study we show that the hypocretin system is affected in PD by examining three brain compartments. Hypocretin-1 tissue concentrations in the prefrontal cortex were almost 40% lower in PD patients, while ventricular CSF levels were almost 25% reduced. The total number of hypocretin neurons was almost half compared to controls. Lewy bodies were abundantly present in the perifornical hypothalamus as a sign of an active disease process in that region. Hypocretin neurons that contained a Lewy body were discernable in every PD patient, but the majority of hypocretin neurons did not

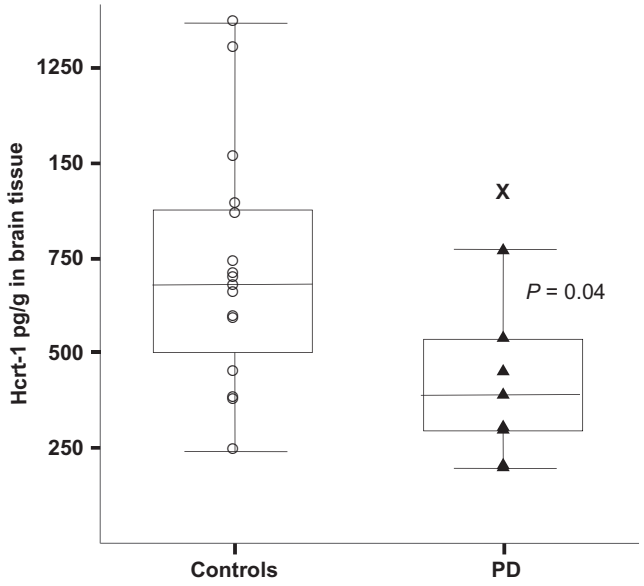

Figure 2.6
Boxplot
Boxplot showing the median, 25th-75th percentiles and the range of the hypocretin-1 concentration in the prefrontal cortex of PD patients (right) and controls (left). The 'X' indicates an out-of-range value. Open circles represent controls, closed triangles represent PD patients.

show this colocalization.

These results convincingly show that the hypocretin system is damaged in PD and are thus in line with and extend upon one of the previous CSF studies, in which low hypocretin-1 levels were found in ventricular CSF in patients with late-stage PD.[15] In that study an inverse correlation between hypocretin-1 levels and disease severity was reported. We could not correlate hypocretin concentrations with disease severity, since all our subjects were late-stage PD.

Studies using spinal CSF have all found normal hypocretin-1 levels. Even PD patients who were selected because of clear sleep abnormalities did not show lowered hypocretin-1 concentrations in spinal CSF.[16,18] The discrepancies between the measurements in spinal CSF and ventricular CSF could be due to the fact that the results of Drouot et al. were obtained in much more advanced PD patients than the studies using spinal CSF. Another explanation could be that CSF hypocretin-1 concentrations are more representative in the area around the hypothalamus, where hypocretin-1 is produced and released by fibers protruding into the lumen of the ventricles, as was shown in the rat.[29] However, in one human study, hypocretin-1 was measured in six subsequent fractions of spinal CSF, using up to 12 ml and no clear gradient between ventricular and spinal CSF levels was found.[17]

It has been shown that hypocretin levels in spinal CSF can be decreased in subjects with acute brain pathology, such as head trauma or a vascular event.[30] Two of the control subjects included in the brain tissue measurement in our study died of stroke (#93-303 and #93-194). However, the hypocretin concentrations in their prefrontal cortex were well within the control range .

There was one control subject with a relatively low number of hypocretin neurons (#93-193). As a specific HLA subtype (DQB1*0602) is an almost invariably necessary factor to develop the sporadic form of narcolepsy with cataplexy. Therefore, an intriguing explanation for this finding could be HLA DQB1*0602 positivity of this subject. Regrettably, we could not obtain frozen brain tissue of the subjects that were included in the cell counts. It was thus not possible for us to determine HLA subtypes. However, HLA DQB1*0602 positivity could be a factor involved in a lower hypocretin cell number, even in the normal population, and this should be explored in future studies.

We used the same hypocretin-1 RIA that has been used by many authors.[16-18,31] It is a well-known fact that the inter-assay variability of this particular RIA is not optimal. However, the intra-assay variability is very low. This stresses the importance of running all samples in a single assay in these types of study, which we did. To compare values with previous reported results, we included a standard reference sample to correct for inter-assay variability.[31] In all tested samples, hypocretin-1 levels were well above the detection limit, and therefore measured reliably.

Hypocretin measurements in CSF have been widely used as a reflection of hypocretin function. However, the relation between actual hypocretin cell number and CSF concentrations was not known as of yet. In a recent rodent study, lesioning about 15% of hypocretin cells did not alter CSF hypocretin-1 levels, but a loss of more than 70% of neurons resulted in a 50% decline in CSF levels.[32] Apparently, in young adult rats it is possible to loose a substantial number of hypocretin cells without changes in CSF levels. This is the first human study that shows a possible correlation between hypocretin cell number and ventricular CSF levels.

Whether our findings fully explain the sleep symptoms in PD remains an intriguing question. Due to the retrospective character of this brain bank study, we had no clinical data on sleep disturbances. However, between one third and half of all PD patients have been reported to experience excessive daytime sleepiness and during sleep registrations even a narcolepsy-like phenotype, including sleep-onset REM periods and fragmented nocturnal sleep, is found on a regular basis.[2-4] This implicates that a significant proportion of the cases we studied would have suffered from sleep disturbances. In a recent rodent study, microinjection of prepro-hypocretin short interfering RNA's (siRNA) in the perifornical hypothalamus resulted in a 60% reduction of prepro-orexin mRNA and a persistent increase in the amount of REM-sleep.[33] In the aforementioned rodent study by Gershchenko, where 70% of hypocretin neurons were lesioned, an increase in REM-sleep was seen as well.[32] Although these results were obtained in rodents, it is not improbable that the reduction in hypocretin neurotransmission found in our human study contributes to the sleep problems commonly seen in PD.

It is likely that the loss of hypocretin neurons is not limited to this cell group in the hypothalamus. Many cell types are affected in PD throughout the brain, but vulnerability seems to be different. To gain more insight into the specificity of the reduction in hypocretin neurons, it would be of interest to count melanin concentrating hormone (MCH) neurons in the peri-fornical region in future studies. Deficiencies

in other neurotransmitters besides hypocretin have been proposed as an explanation for the sleepiness in PD. For example, Rye et al. mention of the possible involvement of midbrain dopaminergic and noradrenergic neurons that influence sleep/wake state through thalamocortical pathways.[3] Both the loss of dopamine and hypocretin neurons can thus contribute to sleep disturbances in PD.

Although sleep-onset REM and REM-sleep behavior disorder are described frequently in PD,[8] there are no reports about cataplexy. Cataplexy is the essential feature of narcolepsy with cataplexy, which is characterized by REM sleep abnormalities and undetectable levels of hypocretin in the spinal CSF.[9] In contrast, hypocretin is usually detectable in narcolepsy without cataplexy, where REM sleep disturbances occur without cataplexy, comparable to the findings in PD. It has been proposed that narcolepsy without cataplexy may be caused by a milder form of hypocretin deficiency compared to the almost complete loss of hypocretin in narcolepsy with cataplexy.[34] Indeed, Thannickal et al. described the highest number of surviving hypocretin neurons in the brain of a narcoleptic patient that did not suffer from cataplexy.[35] Our findings may support this hypothesis, since we found a reduction in number of hypocretin neurons but not a complete loss in PD, possibly leading to REM sleep disturbances and sleep/wake abnormalities, but not to cataplexy.

To conclude, our data shows that the disease process in PD also affects the hypothalamic hypocretin system. It is now important to establish the correlation between hypocretin impairment and the occurrence of the various sleep disturbances. Furthermore, our findings implicate that in the future, hypocretin agonists may have a place in the treatment of PD.

Acknowledgements

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