Structural brain changes in migraine and cluster headache

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The anterior hypothalamus in cluster headache

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Abstract

Objective:

To evaluate the presence, localization, and specificity of structural hypothalamic and whole brain changes in cluster headache and chronic paroxysmal hemicrania (CPH).

Methods:

We compared T1-weighted magnetic resonance images of subjects with cluster headache (episodic n=24; chronic n=23; probable n=14), CPH (n=9), migraine (with aura n=14; without aura n=19), and no headache (n=48). We applied whole brain voxel-based morphometry (VBM) using two complementary methods to analyze structural changes in the hypothalamus: region-of-interest analyses in whole brain VBM, and manual segmentation of the hypothalamus to calculate volumes. We used both conservative VBM thresholds, correcting for multiple comparisons, and less conservative thresholds for exploratory purposes.

Results:

Using region-of-interest VBM analyses mirrored to the headache side we found enlargement ($p<0.05$, small volume correction) in the anterior hypothalamic gray matter in subjects with chronic cluster headache compared to controls, and in all participants with episodic or chronic cluster headache taken together compared to migraineurs. After manual segmentation, hypothalamic volume (mean±SD) was larger ($p<0.05$) both in subjects with episodic (1.89±0.18 ml) and chronic (1.87±0.21 ml) cluster headache compared to controls (1.72±0.15 ml) and migraineurs (1.68±0.19 ml). Similar but non-significant trends were observed for participants with probable cluster headache (1.82±0.19 ml; $p=0.07$) and CPH (1.79±0.20 ml; $p=0.15$). Increased hypothalamic volume was primarily explained by bilateral enlargement of the anterior hypothalamus. Exploratory whole brain VBM analyses showed widespread changes in pain-modulating areas in all subjects with headache.

Interpretation:

The anterior hypothalamus is enlarged in episodic and chronic cluster headache and possibly also in probable cluster headache or CPH, but not in migraine.

Introduction

Cluster headache is a relatively rare headache syndrome typically characterized by frequent, highly disabling attacks of 15-180 minutes of intense, unilateral headache accompanied by ipsilateral features of facial autonomic dysfunction.[1] In *episodic* cluster headache (≈85% of patients), attacks come in periods of several weeks to months, alternating with complete attack-free periods of several months to years. In *chronic* cluster headache, attacks continue to recur without intermittent attack-free periods for at least a year.[2] In *probable* cluster headache, attacks fulfill all but one of the diagnostic criteria for cluster headache, usually by lasting longer than 3 hours.[2;3] Chronic paroxysmal hemicrania (CPH) is very similar to cluster headache except for shorter attack duration (2-30 minutes), much higher attack frequency (up to 30 per day), and often excellent response to indomethacin. Both cluster headache and CPH belong to the trigeminal autonomic cephalalgias (TACs).[4]

The etiology of cluster headache is unknown. The circadian timing of the attacks, the circannual clustering of attacks typically during autumn and spring, and the hormonal changes during attacks all point at involvement of the hypothalamus,[5;6] in particular the suprachiasmatic nucleus, the endogenous biological clock.[5;7] Studies using functional magnetic resonance imaging (MRI) and positron emission tomography have suggested hypothalamic activation during attacks of cluster headache.[8-11] One highly cited MRI-study using voxel-based morphometry (VBM) found increased gray matter volume of the posterior inferior hypothalamus in cluster headache patients,[12] but this could not be confirmed in four other studies.[13-16] These latter studies, however, did find structural changes in brain areas which are structurally or functionally connected with the hypothalamus[17;18] and which are involved in emotional handling and nociception and other types of sensory processing.[13-16]

In the present study we wanted to assess (i) whether there are structural changes in the hypothalamus of subjects with typical episodic and chronic cluster headache; (ii) whether there are structural changes in other brain areas in these subjects; and (iii) whether these changes are specific to typical cluster headache or can also be found in other episodic headache disorders such as probable cluster headache, CPH, and migraine (which is not a TAC). To this end, we applied

state-of-the art whole brain VBM using two complementary methods to assess structural hypothalamic changes: region-of-interest analyses in whole brain VBM, and manual segmentation of the hypothalamus.

Methods

Study population

We included 112 subjects with either episodic (n=25), chronic (n=27) or probable (n=16) cluster headache, CPH (n=9), or migraine with (n=16) or without aura (n=19). Patients were diagnosed by two experienced headache experts (GGS, JAvV) according to the diagnostic criteria (2nd edition) of the International Headache Society.[1] All patients also fulfilled the 3rd edition of the diagnostic criteria (2). Subjects with episodic cluster headache were in remission for at least three months. Participants with probable cluster headache fulfilled all but one of the diagnostic criteria for typical cluster headache: in n=13, the attack duration was longer than 180 minutes, and in n=3, autonomic symptoms and a sense of restlessness or agitation were absent. For comparison, 50 otherwise healthy volunteers (recruited by advertisement) without a history of headache were included as control subjects. The local medical ethics committee approved the study and all subjects gave written informed consent.

Neuroimaging and image post processing

Structural 3D T1-weighted turbo field echo brain images (repetition /echo time of 7.4/3.4 ms; 160 axial 1.0 mm continuous slices) were acquired using a 1.5 Tesla MRI system (NT-ACS, Philips, Best, The Netherlands). All MRIs were checked for structural brain abnormalities (including confluent white matter hyperintensities; small, punctate white matter hyperintensities were not considered an exclusion criterion) or artifacts that could interfere with further automatic image post-processing.

Voxel-based morphometry

To localize regional volumetric gray and white matter differences between patient groups and controls, MR images were processed with voxel-based morphometry (VBM), applying dif-

feomorphic anatomical registration exponentiated lie algebra (DARTEL; using default parameters),[19] in SPM8 (Statistical Parametric Mapping, Wellcome-Department of Cognitive Neurology, London, United Kingdom, http://www.fil.ion.ucl.ac.uk/spm) on a MATLAB platform (The MathWorks Inc., Natick, MA, USA; version 7.5). The VBM-DARTEL procedure involved (i) segmentation of MR images into gray matter, white matter, and cerebrospinal fluid, (ii) creation of a DARTEL template derived from nonlinear deformation fields for the aforementioned segmentation procedure, and (iii) registration of all individual deformations to this DARTEL template. This registration step included modulation, which preserved the absolute amount of local gray and white matter volumes in spatially normalized images by scaling by Jacobian determinants (i.e. a correction for the distance over which a voxel had to be stretched or compressed to fit into standard space). Finally, (iv) modulated normalized gray and white matter segments were smoothed with an isotropic 8 mm full width at half maximum Gaussian-kernel for statistical comparison.

Assessment of hypothalamic volumes

Using FSLView v3.0 (http://www.fmrib.ox.ac.uk/fsl/), hypothalami (including mammillary bodies) were segmented manually in the original MR images blinded for diagnosis, by one rater (E.B.A.), using a validated segmentation procedure with predefined borders described in detail elsewhere.[20] The anterior border of the hypothalamus is formed by the lamina terminalis. At this level, the upper and lower borders of the hypothalamus are demarcated by the anterior commissure and the optic chiasm. The medial border is demarcated by the third ventricle, and laterally the hypothalamus approximates the substantia innominata. Slightly more caudally, additional borders are formed by the fornix and the genu of the internal capsule. The infundibular stalk forms the inferior margin of the hypothalamus in this area. Further posteriorly, the upper border is formed by the posterior limb of the internal capsule and the telodiencephalic fissure. In mediolateral direction the hypothalamus lies between the third ventricle and the posterior limb of the internal capsule, approximating the globus pallidus. The posterior part of the hypothalamus, including the mammillary bodies, was segmented using the third ventricle, the telodiencephalic fissure, the mammillothalamic tract and the cerebral exterior as delineations. Hypothalamic volumes were calculated for comparison between groups. Applying intraclass correlation coefficient,

both intrarater (0.84, assessed by segmenting hypothalami in ten brains twice) and interrater reliability (0.82, assessed by segmenting ten hypothalami by a second rater) was good. To localize differences between controls and subjects with headache, segmented hypothalami were registered to the Montreal Neurological Institute (MNI) standard template provided in FSL using the 12-parameter linear registration algorithm of FLIRT.[21]

Statistical analyses

Voxel-based morphometry

A general linear model was used to compare gray and white matter segments voxelwise between the six groups of subjects with headache and controls, implementing age, sex and total parenchymal volume as covariates. Total parenchymal volume was chosen instead of total intracranial volume because of faulty segmentations of cerebrospinal fluid in some subjects. Total parenchymal and intracranial volume can be used interchangeably in VBM analyses because of their strong correlation.[22] As expected, hypothalamic volume was smaller with increasing age and larger with increasing total parenchymal volume (exploratory analysis at $p<0.001$, uncorrected for multiple comparisons, data not shown). Sex was included to adjust for sex-related differences, but regional grey matter volume in and surrounding the hypothalamus was not different between males and females (exploratory analysis at $p<0.001$, uncorrected for multiple comparisons). Although smoking may influence trigeminal pain processing on supraspinal and brainstem levels, it did not influence our statistical model substantially and was therefore not included. Possible interactions between the covariates did not affect the statistical model and were not included either. To exclude false positives in non-gray or non-white matter tissue, voxelwise comparisons were masked with explicit optimal threshold masks created using the SPM Masking Toolbox.[23] Statistical comparisons were thresholded at $p<0.05$, corrected for multiple comparisons (family-wise error, FWE), which is standard in neuroimaging data.[24] For additional exploratory whole-brain analyses, statistical parametric maps were subsequently thresholded at $p<0.001$, uncorrected for multiple comparisons, with a minimal cluster size of 100 voxels. The locations of significant voxels were identified using two detailed atlases in consensus.[25;26]

Based on our a priori hypothesis, we applied small volume corrections (pFWE-SVC<0.05, no cluster size threshold) applied in a region encompassing the complete hypothalamus with wide margins (x=[-12 12], y=[6 -18], z=[0, -20]) to compare headache patient groups to non-headache controls, and in addition, compare all subjects with typical cluster headache versus controls and versus all migraineurs. Some researchers believe that mirroring of hemispheres is essential for neuroimaging studies in TACs to preclude false negatives,[27] as cluster headache and CPH are lateralized disorders. The effect of mirroring in VBM-DARTEL analyses is unknown, however. When using the standard procedure, an asymmetrical study-specific DARTEL template is created upon the asymmetrical MNI template.[28] Therefore, mirroring hemispheres (even before creating the study-specific DARTEL template) may introduce non-existing structural differences in stereotactic space because of hemispheric asymmetry, causing false positives to occur. Therefore, we only report results from mirrored and unmirrored analyses for the ROI analyses of the hypothalamic region. For these lateralized analyses, MRIs of subjects with predominantly right-sided headaches during attacks were mirrored in the midline, as if all participants effectively had left-sided headache attacks.

Other statistical analyses

The Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA; version 16.0.2) was used for statistical analysis. To compare demographic, clinical and headache characteristics, one-way ANOVA (for continuous variables) and chi-squared tests (for categorical variables) were applied. For comparison of hypothalamic volumes, linear regression models were corrected for age, sex, and total intracranial volume (estimated with SIENAX[29] part of FSL[30]) with diagnoses included as an independent variable. Possible interactions between the covariates did not affect regression models and were not included. Additional analyses investigating the influence of perceived headache side, headache history and attack frequency were carried out. Standardized regression coefficients (β values) representing the relative contribution of headache diagnosis to the linear regression models and corresponding p-values were calculated.

Results

Study sample

Eleven subjects were excluded (n=7 with episodic [n=1], chronic [n=4] and probable [n=2] cluster headache; n=2 with migraine with aura and n=2 controls) because of incomplete MRI acquisition (n=3), MRI artifacts (n=4) or unexpected structural brain abnormalities (n=4; all outside of the hypothalamus region) as these might have affected further automatic image post-processing. Table 3.1 summarizes the demographic, clinical and specific headache characteristics of the included subjects in each diagnostic group (n=151). Sex was unevenly distributed, reflecting the normal sex distribution of the disorders. Current smoking was more prevalent in subjects with cluster headache or migraine with aura. Other demographic and clinical characteristics were similar across the diagnostic groups.

Hypothalamus

Voxel-based morphometry

In mirrored ROI analyses, locally increased gray matter volume in the anterior hypothalamus was observed when comparing participants with chronic cluster headache to controls (ipsilateral to headache side; T 3.80; Z 3.70; cluster of 34 voxels, pFWE-SVC=0.021) and when comparing all subjects with typical cluster headache to migraineurs (contralateral to headache side; T3.61; Z 3.52; cluster of 37 voxels, pFWE-SVC=0.036) (Figure 3.1), but not in other subanalyses. In unmirrored analyses, hypothalamic gray matter volume did not differ between non-headache controls and subjects with cluster headache or any of the other headache groups either.

Manual segmentation

Bilateral hypothalamic volumes were larger in subjects with cluster headache compared to controls (standardized β=0.253; p=0.003) and migraineurs (standardized β=0.272; p=0.009), both for episodic cluster headache (p=0.009 [standardized β=0.212] versus controls; p=0.015 [standardized β=0.228] versus migraineurs) and chronic cluster headache (p=0.021 [standardized β=0.184] versus controls; p=0.030 [standardized β=0.199] versus migraineurs).

Table 3.1 Subject characteristics (n=151)

		Trigeminal autonomic cephalalgias					Migraine			
	Controls n=48	Total CH n=47	eCH n=24	cCH n=23	pCH n=14	CPH n=9	Total n=33	MA n=14	MO n=19	p-value
Demographic										
Female	30 (63%)	8 (17%)	4 (17%)	4 (17%)	8 (57%)	6 (67%)	31 (94%)	13 (93%)	18 (95%)	0.000
Age	47 (12)	47 (9)	45 (9)	48 (9)	50 (10)	48 (9)	47 (8)	47 (9)	47 (8)	0.840
Headache characteristics										
Predominant headache side:										
Left	-	24 (51%)	11 (46%)	13 (57%)	7 (50%)	1 (11%)	6 (18%)	2 (14%)	4 (21%)	-
Right	-	23 (49%)	13 (54%)	10 (44%)	7 (50%)	8 (89%)	12 (36%)	4 (29%)	8 (42%)	-
Bilateral	-		-	-	-	-	15 (45%)	8 (57%)	7 (37%)	-
Number of attacks (/day for TACs, /month for migraine)	-	3.6 (2.9)	3.8 (3.5)	3.4 (1.8)	2.6 (1.9)	14.0 (6.6)	2.7 (1.7)	2.5 (1.2)	2.8 (1.9)	-
Headache history (in years)	-	15 (10)	16 (12)	14 (9)	19 (10)	15 (7)	28 (13)	28 (10)	29 (15)	-
Other clinical characteristics										
Systolic blood pressure (mmHg)	134 (18)	136 (13)	136 (13)	137 (13)	135 (15)	146 (17)	133 (21)	133 (17)	134 (17)	0.577
Diastolic blood pressure (mmHg)	88 (13)	87 (8)	88 (8)	87 (8)	86 (11)	89 (12)	86 (12)	85 (13)	87 (11)	0.942
Hypertension	6 (13%)	10 (21%)	5 (21%)	5 (22%)	5 (36%)	4 (44%)	6 (18%)	3 (21%)	3 (16%)	0.292
Current smoker	7 (15%)	35 (74%)	17 (71%)	18 (78%)	8 (57%)	1 (11%)	7 (21%)	6 (43%)	1 (5%)	0.000
Ever smoker	25 (52%)	43 (91%)	21 (88%)	22 (96%)	13 (93%)	5 (56%)	19 (58%)	11 (79%)	8 (42%)	0.000
Number of cigarettes (or equivalent) per day	14 (10)	20 (13)	14 (8)	26 (15)	18 (7)	3 (-)	12 (8)	13 (8)	3 (-)	0.021
Ever use of (medication) treatment										
Triptans	-	37 (79%)	17 (71%)	20 (87%)	13 (93%)	6 (67%)	30 (91%)	12 (86%)	18 (95%)	-
Ergotamine	-	11 (23%)	3 (13%)	8 (35%)	3 (21%)	2 (22%)	14 (42%)	6 (43%)	8 (42%)	-
Indomethacin	-	2 (4%)	0 (0%)	2 (9%)	0 (0%)	9 (100%)	2 (6%)	2 (14%)	0 (0%)	-
Lithium	-	10 (21%)	2 (8%)	8 (35%)	1 (7%)	1 (11%)	0 (0%)	0 (0%)	0 (0%)	-
Verapamil	-	32 (68%)	13 (54%)	19 (83%)	6 (43%)	5 (56%)	0 (0%)	0 (0%)	0 (0%)	-
Oxygen	-	16 (34%)	8 (33%)	8 (35%)	6 (43%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	-

For continuous variables denotation is mean (SD); for categorical variables denotation is number (%); CH: cluster headache; eCH: episodic CH; cCH: chronic CH; pCH: probable CH; CPH: chronic paroxysmal hemicrania; MA: migraine with aura; MO: migraine without aura. P-values for one-way ANOVA in continuous variables, for chi-squared test in categorical variables between controls and headache subtypes.

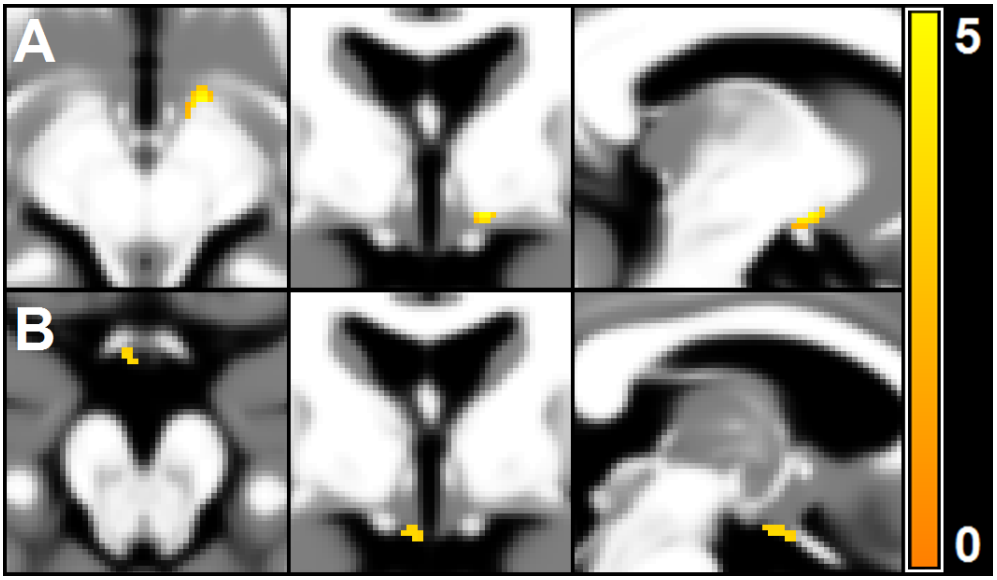

Figure 3.1 Results of region-of-interest voxel-based morphometry analyses. Axial, coronal and sagittal images (left to right) zoomed in on the area around the hypothalamus showing focal increases in anterior hypothalamic gray matter volume when comparing subjects with chronic cluster headache to controls (row A, ipsilateral to headache side, T 3.80, Z 3.70, cluster of 34 voxels, pFWE-SVC=0.021, x -10/y -3/z -8) and when comparing participants with cluster headache to migraineurs (row B, contralateral to headache side, T3.61, Z 3.52, cluster of 37 voxels, pFWE-SVC=0.036, x 3/y -3/z -15). Images displayed in radiological convention (left side of the image is right side of the brain).

Also when analysing only males with cluster headache (n=39) compared to male controls (n=18), hypothalamic volume was larger (standardized β=0.328, p=0.015 [episodic cluster headache: standardized β=0.319, p=0.026; chronic cluster headache: standardized β=0.280, p=0.046]). In the small group of females with cluster headache (n=8) compared to female controls (n=30), volumes were not significantly larger (standardized β=0.114, p=0.297). The volume differences did not lateralize after stratification by usual headache side (data not shown). Hypothalamic volumes were not significantly larger in the smaller subgroups of subjects with probable cluster headache (p=0.071 [standardized β=0.132] versus controls; p=0.065 [standardized β=0.144] versus migraineurs) and CPH (p=0.152 [standardized β=0.101] versus controls; p=0.131 [standardized β=0.111] versus migraineurs). Between groups with CPH or episodic, chronic and probable cluster headache there were no statistically significant differences in hypothalamic volume (p>0.584 for every comparison).

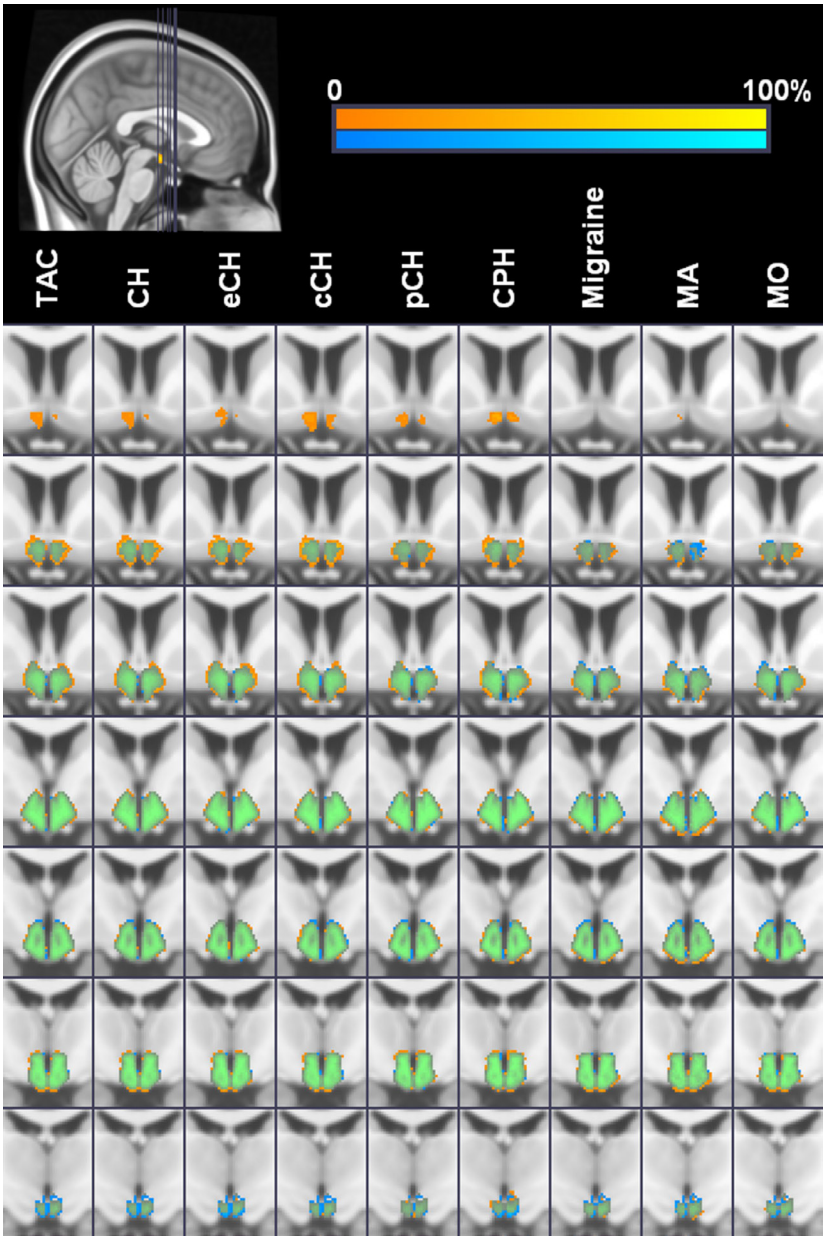

Figure 3.2 Results of manual segmentation. Mean hypothalami in subjects with headache (TAC: trigeminal autonomic cephalalgia; CH: cluster headache; eCH: episodic CH; cCH: chronic CH; pCH: probable CH; CPH: chronic paroxysmal hemicrania; MA: migraine with aura; MO: migraine without aura; in yellow/orange) and control subjects (in blue) projected on top of each other (overlap in green). Images displayed in radiological convention with from top-to-bottom coronal slices through the hypothalamus (from anterior to posterior) as displayed on sagittal slice.

Table 3.2 Mean volume in millilitres of manually segmented hypothalami

		Trigeminal autonomic cephalalgias						Migraine	
	Controls	Total	CH	eCH	cCH	pCH	CPH	Total	MA	MO
	n=48	n=70	n=47	n=24	n=23	n=14	n=9	n=33	n=14	n=19
Total	1.72 (0.15)	1.85 (0.20)[a,b]	1.88 (0.20)[a,b]	1.89 (0.18)[a,b]	1.87 (0.21)[a,b]	1.82 (0.19)	1.79 (0.20)	1.68 (0.19)	1.68 (0.18)	1.65 (0.19)
Left	0.88 (0.08)	0.95 (0.11)[a,b]	0.97 (0.11)[a,b]	0.97 (0.10)[a,b]	0.96 (0.11)[a]	0.95 (0.11)	0.93 (0.12)	0.87 (0.10)	0.87 (0.09)	0.86 (0.11)
Right	0.83 (0.07)	0.90 (0.10)[a,b]	0.90 (0.10)[a,b]	0.91 (0.09)[a,b]	0.91 (0.10)[a,b]	0.88 (0.09)	0.86 (0.08)	0.81 (0.09)	0.82 (0.09)	0.79 (0.08)

[a]p<0.05, compared to controls; [b]p<0.05, compared to migraineurs. CH: cluster headache; eCH: episodic CH; cCH: chronic CH; pCH: probable CH; CPH: chronic paroxysmal hemicrania; MA: migraine with aura; MO: migraine without aura.

When all participants with typical and probable cluster headache and CPH were considered one group of subjects with different forms of TACs (n=70), the hypothalamic volume was larger than in non-headache controls (standardized β=0.252; p=0.002) and migraineurs (standardized β=0.269; p=0.006). Hypothalamic volume was larger in male subjects with TACs (n=48), compared to controls (n=18; standardized β=0.289, p=0.014), and also in female subjects with TACs (n=22) compared to female controls (n=30; standardized β=0.212, p=0.064) and migraineurs (n=31; standardized β=0.289, p=0.026). Hypothalamic volume was not significantly related to disease duration (in years) (standardized β=0.153; p=0.181) or attack frequency (standardized β=0.219, p=0.228). After normalization by registration of the segmented hypothalamic volumes to standard space, qualitative analysis revealed that the volume differences between subjects with cluster headache, migraineurs and controls were present in the whole hypothalamus, but most prominent in the anterior part of the hypothalamus (Figure 3.2). No differences were found between migraineurs and controls (Table 3.2).

Whole brain voxel-based morphometry

In whole brain analyses, gray matter volume was decreased in subjects with migraine without aura in the right lingual gyrus (p=0.035, FWE-corrected). Other increases and decreases in gray and white matter that were found when applying less stringent thresholds (p<0.001, uncorrected for multiple comparisons, minimal cluster size 100 voxels) are presented in Table 3.3 and 3.4.

Table 3.3 Voxel-based morphometry: increases and decreases in gray matter volume in subjects with headache, compared to control subjects (p<0.001, uncorrected for multiple comparisons, cluster extend threshold 100 voxels)

	BA	L/R	DARTEL coordinates			k_E	Z-score
			x	y	z		
Gray matter increases							
Episodic cluster headache							
-	-	-	-	-	-	-	-
Chronic cluster headache							
Middle frontal gyrus	8	L	-42	15	43	285	3.69
Probable cluster headache							
Middle frontal gyrus	10	L	-26	54	-7	226	3.99
Chronic paroxysmal hemicrania							
Precuneus, parietal lobe	19	L	-3	-76	37	401	4.31
Parahippocampal gyrus	30	L	-12	-37	-10	427	4.08
Superior parietal lobule	7	R	12	-50	62	443	3.94
Cuneus, occipital lobe	17	L	-11	-83	7	215	3.43
Parahippocampal gyrus	28	L	-19	-17	-18	124	3.42
Migraine with aura							
Inferior occipital gyrus	18	R	32	-93	-4	174	3.70
Migraine without aura							
-	-	-	-	-	-	-	-
Gray matter decreases							
Episodic cluster headache							
Putamen	-	R	29	-13	-10	457	3.78
Middle temporal gyrus	37	R	55	-41	-8	265	3.71
Chronic cluster headache							
-	-	-	-	-	-	-	-
Probable cluster headache							
Amygdala	-	R	21	-9	-10	216	3.55
Superior frontal gyrus	8	R	6	25	50	304	3.42
Chronic paroxysmal hemicrania							
-	-	-	-	-	-	-	-
Migraine with aura							
Medial frontal gyrus	8	R	3	22	43	1205	4.42
Migraine without aura							
Lingual gyrus	18	R	19	-83	-3	520	4.61[a]
Postcentral gyrus	2	L	-35	-26	40	730	4.05
Postcentral gyrus	3	R	44	-20	53	641	3.95
Anterior cingulate cortex	32	R	7	23	-7	197	3.61
Angular gyrus	39	R	35	-66	27	207	3.30

BA: Brodmann area; L:left; R:right; k_E:cluster size. [a]p<0.05, family-wise error-corrected.

Discussion

By using two complementary MRI post-processing techniques, we found that the *anterior* part of the hypothalamus is bilaterally enlarged in typical episodic and chronic cluster headache, and possibly also probable cluster headache and CPH. In migraine, another episodic (but non-TAC)

Table 3.4 Voxel-based morphometry: increases and decreases in white matter volume in subjects with headache, compared to control subjects (p<0.001, uncorrected for multiple comparisons, cluster extend threshold 100 voxels)

	L/R	DARTEL coordinates			k_E	Z-score
		x	y	z		
White matter increases						
Episodic cluster headache						
Medial frontal WM	R	14	41	7	133	3.31
Chronic cluster headache						
-	-	-	-	-	-	-
Probable cluster headache						
Precuneal WM	R	8	-54	48	349	3.93
Anterior cingulate WM	R	9	32	2	319	3.34
Frontal WM	L	-24	47	3	144	3.24
Chronic paroxysmal hemicrania						
-	-	-	-	-	-	-
Migraine with aura						
-	-	-	-	-	-	-
Migraine without aura						
-	-	-	-	-	-	-
White matter decreases						
Episodic cluster headache						
-	-	-	-	-	-	-
Chronic cluster headache						
Parietal WM	R	22	-41	54	146	3.46
Probable cluster headache						
-	-	-	-	-	-	-
Chronic paroxysmal hemicrania						
Precentral gyral WM	R	48	-14	32	438	3.64
Cerebellar WM	R	13	-69	-34	160	3.34
Migraine with aura						
Cingulate WM	R	9	21	39	272	4.09
Migraine without aura						
Precentral gyral WM	R	34	-22	52	399	3.64

L: left; R:right; k_E: cluster size.

headache syndrome, we did not find changes in hypothalamic volume compared to controls. We were unable to identify structural changes in brain areas other than the hypothalamus that might distinguish between typical cluster headache and the other episodic headache syndromes. Implications, relevance and validity of these findings are discussed below.

Several nuclei in the anterior part of the hypothalamus might explain the larger hypothalamic

volumes in typical cluster headache. Functional disturbances of the suprachiasmatic nucleus, the endogenous biological clock, might cause the striking circadian and circannual rhythms of cluster headache attacks and periods.[31] Another anteriorly located hypothalamic nucleus, the paraventricular hypothalamic nucleus, has been suggested to modulate or trigger TACs by mediating the regulation of nociceptive and autonomic input.[32] Several processes affecting the local MRI T1-signal may explain the increase in gray matter volume. These include an increase in the number or size of neurons or glial cells, increases in synaptic plasticity, fluid shifts between intra- and extracellular space due to homeostatic imbalance, and presence of gliosis. High-field MRI, MR spectroscopy, and molecular imaging studies are probably better suited to differentiate between these possible causes.

We have been the first to use manual segmentation to study volume changes in the hypothalamus in typical cluster headache. This showed a major limitation of VBM; even with mirrored ROI-VBM-analyses we were almost unable to detect the bilateral enlargement of the (anterior) hypothalamus in participants with typical cluster headache revealed by manual segmentation. Perhaps these volume changes were too widespread or too small to be picked up by state-of-the art VBM. The region of the hypothalamus is characterized by large T1-signal intensity differences in a relatively small volume of brain tissue which makes this delicate area extra susceptible to effects of normalisation and smoothing procedures in VBM, leading to loss of information.

Our findings suggest the possibility of false negative findings of previous VBM studies in cluster headache negative for structural change in the hypothalamus (13-16).
In line with these 'negative' studies however, we could not reproduce the previously reported increased volume of the posterior inferior hypothalamus in cluster headache (12) with either one of the two complementary post-processing techniques. The sample size in our study was sufficiently large for obtaining stable results in neuroimaging studies (n=15-20)[33;34] and we, therefore, believe that the statistical power to reproduce earlier findings of increased posteroinferior hypothalamic volume should have been sufficient.[12] Moreover, we did find changes but in a different area of the hypothalamus. In general, differences in methodology may explain why VBM-studies in cluster headache found different (positive and negative) results, including accurate evaluation of MR and

post-processed images for structural abnormalities and artefacts,[12;14] the use of registration algorithms superior[19] to those used in earlier studies[12;14] and differences in smoothing, modulation, covariate implementation in statistical models and statistical thresholding.

We were not able to confirm previous findings of structural changes in the *posterior* hypothalamus with our complementary manual delineation of the hypothalamus either. We might accidentally not have included this area in our manual segmentation procedure, because the posterior border of the hypothalamus is much more difficult to delineate than the anterior part, even on high-resolution T1-weighted images. An alternative explanation for non-inclusion is that the area of structural hypothalamic changes as reported in cluster headache,[12] is located in the midbrain tegmentum rather than in the posterior hypothalamus.[35]

Our study has some limitations. First, to obtain patient and control groups that are truly matched is a major challenge in cluster headache research. Consequently, sex was unequally distributed between headache patient groups, which might suggest that this interfered with our main results, as previously larger regional hypothalamic volumes were found in men compared to women.[36]

However, we were unable to detect an effect of sex in the hypothalamus in our VBM analyses. Moreover, manually segmented hypothalamic volumes were also larger when comparing male subjects with cluster headache with male controls, which affirms that our results were not majorly affected by this unequal sex distribution. We cannot exclude that regional grey matter volume has been influenced by the use of prophylactic medical treatment, such as lithium,[37] or by history of smoking,[38] although we are unaware of any effect of these agents on regional hypothalamic volume.

Second, the patient groups with probable cluster headache and CPH were quite small, and the changes in hypothalamic volume failed to reach statistical significance in these subgroups, probably due to lack of power. Our current findings however do suggest that enlargement of the anterior hypothalamus is not specific to cluster headache, but might also apply to other TACs. This should be confirmed in future studies also including episodic paroxysmal hemicrania and

short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing (SUNCT).

In line with previous studies, we found plasticity changes in cortical gray matter and connecting white matter pathways which might be due to repetitive pain[27] or behavioural and emotional responses to pain.[39] These changes are not specific to TACs, as they are also found in migraine. To prevent obscuration by plasticity changes in a maladaptive pain modulatory network, future studies searching for structural brain changes that are specific for episodic headache syndromes might benefit from implementing only patients who recently developed headache symptoms. Drug naivety in these patients may be an additional advantage, as it is unknown in what way acute or prophylactic drug treatment in headache patients influences neuroplasticity.

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