Neuroendocrinology Letters Volume 36 No. 7 2015
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Hippocampal but not amygdalar volume loss in narcolepsy with cataplexy

Veronika Němcová [1], Jan Krásenský [2], David Kemlink [3], Pavel Petrovický [1], Manuela Vaněčková [2], Zdeněk Seidl [2], Aaron Rulseh [4,2], Jitka Bušková [5,3], Marek Susta [3,6], Karel Šonka [3]

1 Department of Anatomy, Charles University in Prague, First Faculty of Medicine, Czech Republic
2 Department of Radiology, Charles University in Prague, First Faculty of Medicine and General University Hospital, Prague, Czech Republic
3 Department of Neurology and Centre of Clinical Neuroscience, Charles University in Prague, First Faculty of Medicine and General University Hospital, Prague, Czech Republic
4 Department of Radiology, Na Homolce Hospital, Prague, Czech Republic
5 National Institute of Mental Health, Klecany, Czech Republic
6 Department of Public Healthcare, St Elisabeth University, Bratislava, Slovakia

Correspondence to: Prof. Karel Šonka, MD., DSc.
Sleep and Wake Disorders Center
Department of Neurology and Center for Clinical Neurosciences
First Faculty of Medicine, Charles University and General University Hospital
Kateřinska 30, 120 00 Praha 2, Czech Republic.
TEL: +420 2 2496 5568 (50); FAX: +420 2 2492 2678; E-MAIL: ksonka@lf1.cuni.cz

Submitted: 2015-11-14 Accepted: 2015-12-04 Published online: 2015-12-28

Key words: **narcolepsy with cataplexy; narcolepsy without cataplexy; amygdala; hippocampus; MRI volumetry; Freesurfer**

Neuroendocrinol Lett 2015; **36**(7):682–688 **PMID:** 26859591 NEL360715A07 © 2015 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVE: Narcolepsy with cataplexy (NC) and narcolepsy without cataplexy (NwoC) are lifelong neurological disorders characterized primarily by excessive daytime sleepiness. Emotional events such as laughter are a trigger of cataplexy in NC.

METHODS: We compared the volumes of key limbic structures, the amygdala and hippocampus, in 53 NC, 23 NwoC and 37 control subjects. MRI volumetry was performed in FreeSurfer (FS) and by manual delineation.

RESULTS: We found no differences in amygdalar volume in the three groups, however, hippocampal volume was significantly smaller in the NC group than in other groups. Amygdalar and hippocampal volumes assessed by FS were significantly greater, but strong positive correlation between manual and FS results were observed. Thus, both methods are suitable for amygdalar and hippocampal volumetry.

INTRODUCTION

Narcolepsy with cataplexy (NC) and narcolepsy without cataplexy (NwoC) are lifelong neurological disorders characterized primarily by excessive daytime sleepiness (EDS). Despite sharing the common manifestation of EDS, the etiologies of NC and NwoC differ and thus, according to the international classification of sleep disorders, 2nd edition (ICSD-2; American Academy of Sleep Medicine, 2005) are different nosological entities.

Aside from EDS, NC symptoms include cataplexy (short lasting symmetrical loss of muscle tone caused by an emotional trigger), and in roughly half of patients, sleep paralysis and hypnagogic hallucinations. Night sleep in NC is dis-

turbed, including the occurrence of REM sleep within the first 15 minutes of sleep, termed sleep onset REM period (SOREMp), and the occurrence of REM sleep behavior disorder (RBD). EDS must be objectively measured by the multiple sleep latency test (MSLT). Low or undetectable levels of hypocretin-1 (orexin-1) in the cerebrospinal fluid (CSF) is a crucial finding in NC, due to destruction of hypocretin-producing neurons in the lateral hypothalamus (Dauvilliers *et al.* 2007). Although the exact mechanism of hypocretin deficiency is unknown, evidence from the past 20 years strongly favors an immune-mediated or autoimmune attack, specifically targeting hypocretin neurons in genetically predisposed individuals (Liblau *et al.* 2015). The hypothesis that a targeted immune-mediated or autoimmune attack causes the specific degeneration of hypocretin neurons arose mainly through the discovery of genetic associations, first with the HLA DQB1*06:02 allele (Mignot, 1998) and then with the T-cell receptor α locus (Hallmayer *et al.* 2009). It has been suggested that a specific autoimmune process is triggered by different environmental stimuli such as streptococcal infection, anti-H1N1 vaccination or H1N1 infection itself in genetically predisposed subjects (Partinen *et al.* 2014). The age of onset varies from early childhood to the fifth decade, with a bimodal distribution including a large peak around 15 years of age and a smaller peak around 36 years of age (Dauvilliers *et al.* 2001). NC manifestations generally remain stable throughout life.

NwoC is characterized by EDS and the absence of cataplexy. MSLT diagnostic criteria are the same as for NC including the number of SOREMPs. CSF hypocretin-1 is within the normal range. The etiology of NwoC is unknown and the prevalence is lower compared to NC. Furthermore, it has been suggested that NwoC is not a homogenous nosological entity (Šonka *et al.* 2015).

The amygdaloid body (or amygdala) is part of the limbic system. It plays a key role in the detection and processing of emotional stimuli. Connections to sensory, motor and autonomic output systems provide the anatomical basis for adaptive responses to stimuli that signal fear (Amaral & Price 1984; Le Doux, 1992). Although the amygdala appears to play a more extensive role in negative emotion, it is involved in positive emotion as well. Activation of the right amygdala has been detected by positron emission tomography with the presentation of stimuli that elicit positive emotions (Hamann *et al.* 2002).

Emotions are important as the trigger of cataplexy in NC (Dauvilliers *et al.* 2007) and abnormal activity in the amygdala during the processing of humorous stimuli has been detected by functional magnetic resonance imaging (fMRI; Swartz *et al.* 2008; Nakamura *et al.* 2012). It has been reported that NC patients do not show any increase in amygdalar response on fMRI in emotional learning (Ponz *et al.* 2010), although changes have been detected by proton resonance spectroscopy in the amygdala and the hypothalamus suggesting metabolic dysfunction in NC (Poryazova *et al.* 2009). Direct inhibitory output from amygdala to the pontomesencephalic tegmental areas activates (desinhibits) the glycinergic inhibitory neurons of oblongate and thus it causes the muscle atonia during the REM sleep (Burgess *et al.* 2013). In narcoleptic dogs, two populations of amygdalar cells showed a significant change in activity within cataplexy. A population of sleep active cells localized to the central and basal nucleus of the amygdala increased discharges prior to and during cataplexy. A population of wake active cells localized to the cortical nucleus of amygdala decreased activity prior to and during cataplexy. These cell populations play a role in mediation or modulation of cataplexy through interactions with mesopontine regions controlling muscle atonia (Gulyani *et al.* 2002). In hypocretin knock-out mice, cataplexy (frequency and duration of cataplexy bouts) was increased by stimuli associated with positive emotions and an amygdalar lesion in the central nucleus decreased cataplexy (Burgess *et al.* 2013). These studies suggest a key role of the amygdala in the generation of cataplexy. The amygdala has rich reciprocal interconnections with the hypothalamic nuclei.

The hippocampal formation (hippocampus) is also part of the limbic system. The hippocampus participates in learning and memory, in the regulation of emotional behavior, in certain aspects of motor control, and in the regulation of some hypothalamic functions (Duvernoy *et al.* 2005). The hippocampus is highly interconnected with the hypothalamus (Swanson & Cowan, 1975; Stranahan *et al.* 2007) and the amygdala (Aggelton, 1986; Fudge *et al.* 2012). The hippocampus consists of the subiculum on the upper surface of the parahippocampal gyrus, the dentate gyrus in the depth of the sulcus hippocampi and cornu Ammonis bulging into the temporal horn of the lateral ventricle.

Our preliminary results (Brabec *et al.* 2011) showed reduced amygdalar volume in NC, thus we elected to measure and compare the volumes of the amygdala and hippocampus in NC, NwoC, and healthy subjects.

METHODS

Participants

Fifty-three subjects diagnosed with NC, 23 subjects diagnosed with NwoC, and 37 control subjects were included. Control subjects were interviewed to exclude a history of neurological, psychiatric, cardiovascular and metabolic disease. The evaluation of all patients was conducted at our center specializing in sleep disorders and included a detailed clinical history, complete physical examination including anthropometric measurements such as weight, height and BMI, and the Epworth Sleepiness Scale (ESS). Only patients with an unambiguous diagnosis of NC or NwoC without any other brain disease were included in the study. The

study was approved by the local ethics committee, and all participants provided signed, informed consent. Demographic data are presented in Table 1.

The diagnoses of NC and NwoC were made according to the ICSD-2 (American Academy of Sleep Medicine, 2005). The clinical diagnostic criteria of NC included the presence of EDS lasting ≥3 months and typical cataplexy not explained by other medical or psychiatric disorders. The clinical criteria of NwoC were EDS duration of ≥3 months and the absence of typical cataplexy. The diagnosis was confirmed by night video-polysomnographic recording (EEG, EOG, mental and tibialis anterior EMG, ECG, nasal-oral air flow, thoracic and abdominal effort, respiration noises and saturation of peripheral blood by oxygen) followed by MSLT the next day. The MSLT criteria were identical for the diagnosis of NC and NwoC (mean sleep latency ≤8 minutes and ≥2 SOREMPs). Night polysomnography and MSLT were performed in drug-naïve patients or patients abstaining from medications that affect sleep and mood for two or more weeks. HLA typing for DQB1*06:02 was additionally performed (unavailable in one NC subject), and positivity was found in 96% of NC patients and 48% of NwoC patients.

Magnetic resonance imaging (MRI)

All participants were examined in the same MRI scanner 1.5 T (Gyroscan, Philips Medical Systems, Best, The

Tab. 1. Demographic information.

				p-value		
	NC	**NwoC**	**controls**	**NC vs. NwoC**	**NC vs. controls**	**NwoC vs. controls**
N	53	23	37			
Men/women (N), chi-square	28/25	14/9	16/21	n.s.	n.s.	n.s.
Age, years (mean, SD)	39.6 (16.9)	40.4 (14.8)	36.3 (8.8)	n.s.	n.s.	n.s.
Disease duration, years (mean, SD)	18.5 (15.3)	14.7 (11.8)	NA	n.s.	NA	NA
Previous treatment by antidepressants (N, %)	20 (38%)	3 (13%)	NA	0.030 (chi-square)	NA	NA
Previous treatment by stimulants (N, %)	24 (45%)	8 (35%)	NA	n.s. (chi-square)	NA	NA
Previous treatment by sodium oxybate(N, %)	5 (9%)	NA	NA	NA	NA	NA
ESS (mean, SD)	17.4 (3.6)	15.2 (5.0)	NA	0.048	NA	NA
BMI (mean, SD)	29.6 (5.6)	27.3 (4.5)	NA	n.s.	NA	NA
nPSG SE, % (mean, SD)	83.6 (10.2)	87.7 (5.8)	NA	n.s. (MWU)	NA	NA
nPSG awakenings, N (median, quartil range)	34 (27–51)	25 (20–27)	NA	0.002 (MWU)	NA	NA
Subjects with nPSG SOREM (%)	56.0	25.0	NA	0.019 (chi square)	NA	NA
PLMI (mean, SD)	20.9 (28.6)	5.8 (13.7)	NA	0.002 (MWU)	NA	NA
AHI (mean, SD)	9.0 (18.3)	3.7 (3.9)	NA	n.s. (MWU)	NA	NA
MSLT SL min (mean, SD)	2.3 (1.7)	5.1 (2.3)	NA	<0.001 (MWU)	NA	NA
MSLT SOREM (median N, quartil range)	4 (3–4)	3 (2–4)	NA	0.013 (MWU)	NA	NA

The intergroup comparisons were made by T-test (2-sided, with different sample variances), nonparametric data by the Mann-Whitney U-test (indicated), and percentage by chi-square test (indicated). N, number; ESS, Epworth sleepiness scale; BMI, body mass index; nPSG, night polysomnography; SE, sleep efficiency; SOREM, sleep onset REM period; PLMI, periodic limb movements index; AHI, apnoe/hypopnoe index; MSLT, multiple sleep latency test; MWU, Mann-Whitney U-test; NA, not applicable.

Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

Netherlands). Images were acquired using a T1-weighted 3-dimensional fast field echo sequence (TR=25 ms, TE=5 ms, flip angle=30°, matrix size=256×256, slice thickness/gap=1/0 mm, field of view (FOV)=256 mm), axial and coronal images T1-weighted IR (inversion recovery); TR=2820 ms, TE=15 ms, TI=400 ms, matrix size=410×512, flip angle=90°, slice thickness/gap=2/0 mm, FOV=200/156 mm) in transversal plain.

Images were analyzed by two methods. An automated analysis was performed in Freesurfer (FS) version 4.5.0 (http://surfer.nmr.mgh.harvard.edu) and ScanView.cz. Manual delineation was performed by an experienced neuroanatomist (V.N.) blinded to the diagnosis and the process is described in detail below.

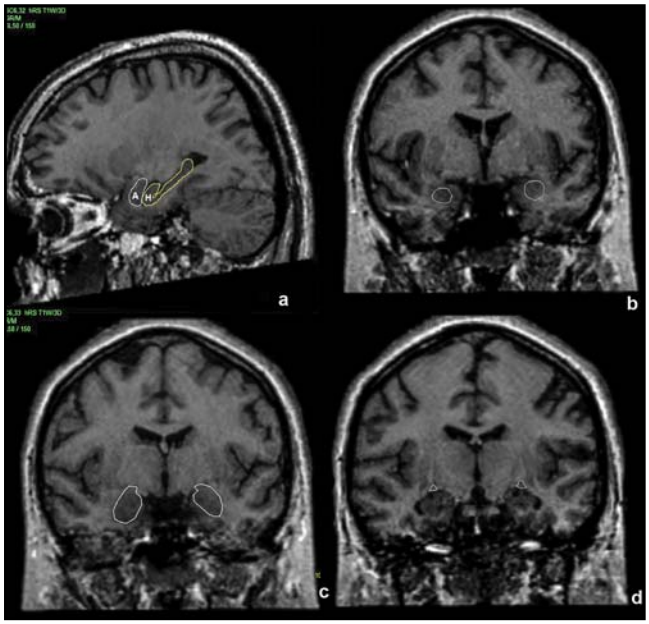

Fig. 1. Manual delineation of the amygdala and hippocampus on T1-weighted images. a) in the sagittal plane, b) rostral amygdalar border on coronal section, c) middle part of the amygdala on coronal section, d) caudal end of the amygdala on coronal section. A, amygdala; H, hippocampus.

Manual delineation

Manual measurements were performed using ScanView software (scanview.cz). First, non-normalized brain volume was measured using T1-weighted images thresholded above 4,000 artificial units. The delineation of structures of interest was performed in coronal (amygdala) and sagittal (hippocampus) orientations on 1 mm isotropic T1-weighted images (Figures 1 and 2). Coronal, sagittal and horizontal views were available to aid in the delineation. Each 1 mm^2 pixel was divided into 16 subpixels 0.25×0.25 mm. All subpixels inside the delineated area were counted and divided by 16. The volume of delineated structures was assessed by counting delineated areas, as the slice thickness was 1 mm. The volume of structures of interest was related to the total brain volume from automated image analysis obtained by ScanView.cz.

Manual amygdalar delineation

We measured the volume of the "classic amygdala" (Brabec *et al.* 2010), which includes the subnucleus centralis, medialis, corticalis, basalis accessorius, basalis lateralis, intercalares, area amygdalaris anterior, area cortico-amygdalaris transitiva, amygdalo-striatica transitiva and the sublenticular part of the extended amygdala as it continues to the substantia innominata under the globus pallidus. The area preamygdalaris (anterior to the amygdala) was not included in the measurement.

The anterior pole of the amygdala was particularly difficult to differentiate from the surrounding gray matter (piriform cortex, periamygdalar area and preamygdalar claustrum). Therefore, sagittal and horizontal views were used in its definition (Figure 1). Other parts of the amygdala were more clearly visible. The lateral amygdalar border (lateral and basolateral subnuclei) is orientated toward the external capsule, which divides the amygdala from the claustrum. Dorsomedially, the cortical nuclei of the amygdala form the semilunar gyrus; this part of the amygdala is on the surface of the hemisphere. Ventromedially, the amygdala bor-

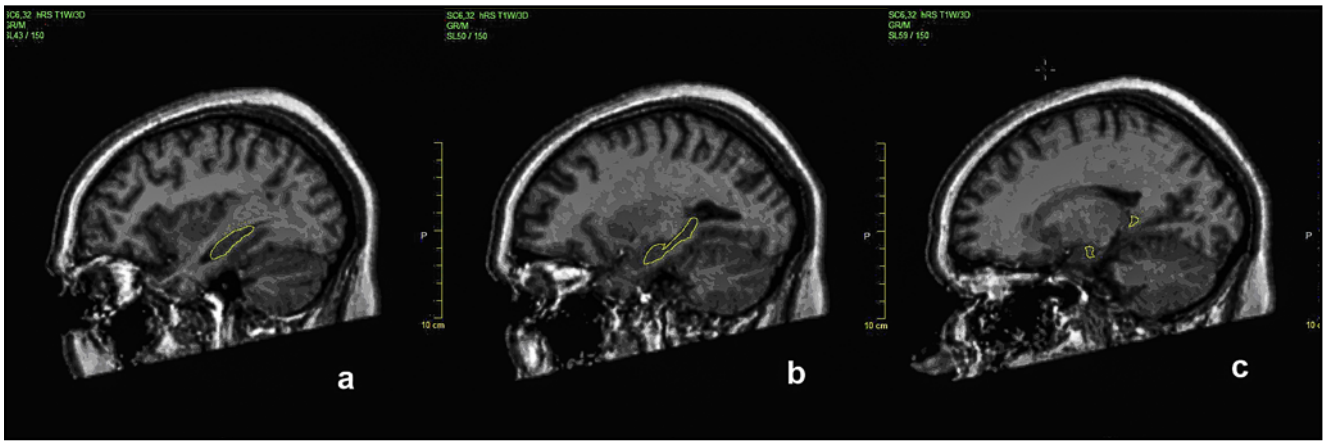

Fig. 2. Delineation of the hippocampus on T1-weighted images in the sagittal plane laterally, b) in the middle and c) medially.

Tab. 2. Whole-brain, amygdalar and hippocampal volumes.

	NC		NwoC		Controls		
	Mean (cm^3) SD	Relative volume (‰)	Mean (cm^3) SD	Relative volume (‰)	Mean (cm^3) SD	Relative volume (‰)	*p*-value ANCOVA
Brain volume	1216.8 (154.0)		1239.0 (164.6)		1270.4 (151.3)		F(2, 107)=1.23, *p*=0.296
Amy R man	1.13 (0.16)	0.94	1.09 (0.12)	0.89	1.14 (0.19)	0.92	F(2, 106)=1.33, *p*=0.269
Amy L man	1.15 (0.17)	0.95	1.15 (0.14)	0.93	1.17 (0.18)	0.94	F(2, 106)=0.28, *p*=0.759
Hip L man	2.40 (0.31)	1.99	2.54 (0.30)	2.07	2.67 (0.36)	2.12	F(2, 106)=6.08, *p*=0.003
Hip R man	2.40 (0.32)	1.99	2.54 (0.33)	2.06	2.64 (0.35)	2.11	F(2, 106)=5.05, *p*=0.008
Amy_L FS	1.47 (0.19)	1.21	1.53 (0.19)	1.24	1.58 (0.25)	1.24	F(2, 106)=1.49, *p*=0.231
Amy_R FS	1.56 (0.18)	1.29	1.63 (0.22)	1.33	1.62 (0.22)	1.28	F(2, 106)=0.95, *p*=0.389
Hip. L FS	4.31 (0.44)	3.57	4.54 (0.45)	3.69	4.68 (0.9)	3.69	F(2, 106)=2.63, *p*=0.077
Hip R FS	4.25 (0.40)	3.52	4.45 (0.51)	3.61	4.57 (0.62)	3.62	F(2, 106)=3.33, *p*=0.040

Results are presented as mean (±standard deviation) in cm^3, and as per mille (±standard deviation) of whole-brain volume in the amygdala and hippocampus. L, left; R, right; man, manual delineation; FS, FreeSurfer assessment; Amy, amygdala; Hip, hippocampus.

ders the white matter of the ambient gyrus. The sublenticular part of the extended amygdala continues to the substantia innominata under the globus pallidus. More caudally, the optic tract forms the medial amygdalar border. The ventral amygdalar border is formed by the temporal horn of the lateral ventricle on caudal sections, by the hippocampus more rostrally, and by the white matter of the temporal lobe rostrally.

Manual hippocampal delineation

The cornu Ammonis, dentate gyrus and subiculum were delineated as the hippocampus. The alveus and fimbria fornicis were excluded from measurements (Figure 2). The hippocampus may be divided to three parts along the longitudinal axis: the head, body and tail (Duvernoi *et al.* 2005). The head (digitationes hippocampi) is anteriorly bordered by the amygdala and the uncal recess of the temporal horn of the lateral ventricle. The body of the hippocampus is dorsolaterally covered by the alveus. The ambient cistern is medial to the body of the hippocampus. The tail is covered medially by the fimbria fornicis and the ambient cistern and ends with the subsplenial gyrus. The splenium corporis callosi is localized above the tail of the hippocampus and the white matter of the temporal lobe is found laterally. The inferior border of the hippocampus is formed by the white matter of the parahippocampal gyrus.

Statistical analyses

All statistical tests were performed using STATISTICA software version 12 (statsoft.com). Volumetric data were found to be normally distributed by the Shapiro-Wilk's W test and are presented by both uncorrected in cm^3 and as per mille portion of the total brain volume. Intergroup comparisons for raw volumetric data were performed by ANCOVA using total brain volume as a covariate, with post-hoc comparison by the HSD test. Other parameters were compared either by T-test (2-sided, with different sample variances), the Mann-Whitney U test, or chi square test where appropriate. Correlation in continuous variables was evaluated by Pearson correlation. The Bonferroni correction for multiple comparisons was applied, and *p*-values <0.05 were considered significant.

RESULTS

Amygdalar and hippocampal volumes obtained by manual delineation and FS analysis are displayed in Table 2. The post hoc HSD test showed only significant differences between NC and control groups, for hippocampus manually measured in was $p<0.001$ for both sides, for automatical methods $p<0.01$.

No clinically relevant differences in right and left amygdalar or hippocampal volumes were found. Absolute amygdalar and hippocampal volumes were larger in males than in females, but after correction for whole-brain volume no sex differences were found.

There were no correlations between amygdalar and hippocampal volume and percentage of sleep stages during night polysomnography after correction for multiple comparisons. Only relative volume of the right

Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • www.nel.edu

amygdala positively correlated with age after correction for multiple comparisons in NC patients. In NwoC patients, all hippocampal parameters positively correlated with age at MRI examination and age at disease onset.

Automated amygdalar and hippocampal volume measurements were larger than manually delineated volumes ($p<0.001$). However, the results of both methods were highly correlated (Table 3) and hippocampal values had significantly higher correlation coefficient than amygdalar volumes ($p<0.05$), as r-to-Fisher-z transformation. Correlation of automated and manually assessed amygdalar and hippocampal volume is presented in Figure 3 for the right side. The correlation parameters of the both left sided structures were same as on the right side.

Tab. 3. Correlation of manual and automated volumetric assessment in the amygdala and hippocampus.

Manual versus automatic	correlation coefficient	*p* - Pearson correlation	*p* - paired t-test
Amy R	0.5485	$p<0.001$	$p<0.0001$
Amy L	0.5177	$p<0.001$	$p<0.0001$
Hip R	0.7230	$p<0.001$	$p<0.0001$
Hip L	0.7207	$p<0.001$	$p<0.0001$

Amy, amygdala; Hip, hippocampus; L, left; R, right.

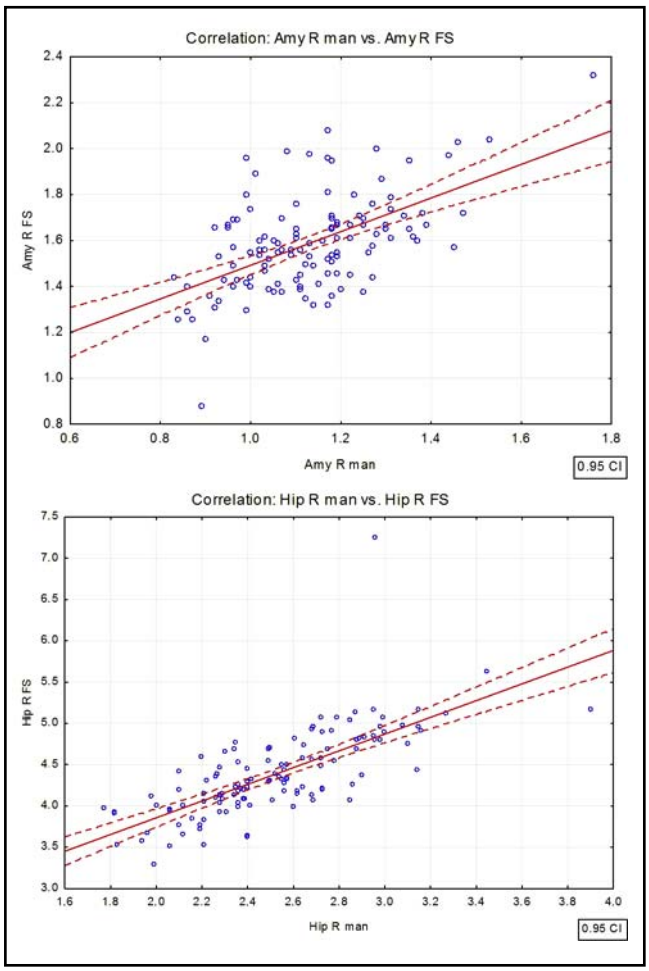

Fig. 3. Correlation between manual and Freesurfer assessed volumes of the right amygdala and right hippocampus. Amy, amygdala; Hip, hippocampus; R, right, man, manual delineation; FS, FreeSurfer assessment.

DISCUSSION

The most significant finding of the present study is the demonstration of very high correlation between manual and FS measurements in the hippocampus and amygdala, although absolute sizes differed. Both methods are useful in these two structures with the knowledge that FS provides greater volumes. We found that FS includes the preamygdalar area as part of the amygdala, thus the final volume is larger. In the hippocampus, FS includes the alveus, which leads to greater hippocampal volume. In manual volumetry, the alveus is not part of the measured hippocampal formation. To our knowledge, this is the first such comparison in these structures.

Following correction for whole-brain volume, we detected smaller hippocampal volume in NC subjects than in controls, while no differences in amygdalar volume between NC patients and controls were found. We did not discover any differences in amygdalar or hippocampal volumes in NwoC subjects in comparison to NC subjects and controls. In NC subjects the volume of the right amygdala delineated manually correlated with age and disease duration and the volume of the left hippocampus also correlated with disease duration. The importance of our amygdalar and hippocampal volume measurements is overshadowed by a recently published study by Kim *et al.* (2015). They compared drug-naïve NC patients (similar group size in comparison to the present study) and controls. Higher resolution images were acquired at 3 tesla and processed manually, which allowed the authors to evaluate amygdalar and hippocampal subfield volumes and correlate them with clinical data. They found bilateral hippocampal atrophy localized mainly in the CA1 region and amygdalar reduction in the area of the centromedial nucleus and laterodorsal groups. Hippocampal CA1 atrophy and amygdalar centromedial atrophy were associated with a longer duration of EDS and shorter mean REM sleep latency. Amygdalar centromedial atrophy was associated with a longer duration of cataplexy (Kim *et al.* 2015). Our work confirms correlation between NC duration and amygdalar volume, but only on the right and only by automated measurement. There are some differences between patient´s population studied by Kim *et al.* and by us. The age at onset of NC was 15 years in the study by Kim *et al.*, as opposed to 21 years in our study. Some of our patients were already treated before they had undergone MRI and disease duration was longer in our patients (18 vs. 12 years). We also detected atrophy of the hippocampus, albeit without detailed division. In contrast to the results of Joo *et al.*

(2012), we have not found lower hippocampal volume in NC subjects related to sleep and REM sleep latencies (Joo *et al.* 2012). In the present study, the volume of the right amygdala positively correlated with age at NC onset. In NwoC, a positive correlation between age and disease onset (and age at MRI) with bilateral hippocampal volume (manual and automated) was detected.

We did not detect any laterality differences in hippocampal volume by manual or automated methods in any subjects (controls, NC, NwoC), in contrast to reports in healthy subjects (Lucarelli *et al.* 2012; Woolard & Heckers, 2012).

The results of the present study differ from our previous study (Brabec *et al.* 2011), in which decreased amygdalar volume was detected in narcolepsy. This discrepancy may be related to the use of higher-resolution data in the present study (1 mm isotropic) and by the greater number of NC participants (53 in the present study, 11 in the previous study). Interestingly, the amygdalar volumes in the previous study were more homogenous (smaller standard deviation) while the differences between NC subjects and controls more pronounced. In the present study we detected a similar trend, however the differences did not reach significance.

CONCLUSION

Amygdalar and hippocampal volumes obtained by manual and automated methods differ (automated resulted in greater volumes), however, we observed strong positive correlation between both methods. Thus, both methods are suitable for amygdalar and hippocampal volumetry. We found smaller hippocampal volumes in NC subjects in comparison to controls. We did not detect any differences in amygdalar volume between NC subjects and controls, nor between NC and NwoC subjects. No clinically important correlations between hippocampal or amygdalar volumes and symptoms of narcolepsy were detected.

ACKNOWLEDGEMENT

The study was supported by the Czech Ministry of Health grant NT 13238-4/2012 and by Charles University grant PRVOUK P26/LF1/4.

REFERENCES

1 Aggleton JP (1986). A description of the amygdalo–hippocampal interconnections in the macaque monkey. Experimental Brain Res. **64**: 515–526.
2 Altman J, Das GD (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol. **124**: 319–335.
3 Amaral DG, Price JL (1984) Amygdalo-cortical projections in monkey (Macaca fascicularis). J Comp Neurol. **230**: 465–496.
4 American Academy of Sleep Medicine (2005) International classification of sleep disorders, 2nd ed: Diagnostic and coding manual. Westchester, Illinois: American Academy of Sleep Medicine.
5 Brabec J, Rulseh A, Hoyt B, Vizek M, Horinek D, Hort J, *et al.* (2010). Volumetry of the human amygdala - An anatomical study. Psychiatry Res. **182**: 67–72.
6 Brabec J, Rulseh A, Horinek D, Pala A, Guerreiro H, Buskova J, *et al.* (2011). Volume of the amygdala is reduced in patients with narcolepsy – structural MRI study. Neuro Endocrinol Lett. **32**: 652–656.
7 Burgess CR, Oishi Y, Mochizuki T, Peever JH, Scammell TE (2013). Amygdala lesions reduce cataplexy in orexin knock-out mice J Neurosci. **33**: 9734–9742.
8 Dauvilliers Y, Arnulf I, Mignot E (2007). Narcolepsy with cataplexy. Lancet. **369**: 499–511.
9 Dauvilliers Y, Montplaisir J, Molinari N (2001). Age at onset of narcolepsy in two large populations of patients in France and Quebec. Neurology. **57**: 2029–33.
10 Duvernoy HM (2005). The human hippocampus: Functional anatomy, vascularization and serial sections with MRI. Berlin Heidelberg: Springer Verlag.
11 Fudge JL, Decampo DM, Becoats KT (2012). Revisiting the hippocampal-amygdala pathway in primates: association with immature-appearing neurons. Neuroscience. **212**: 104–119.
12 Guyliani S, Wu MF, Nienhuis R, John J, Siegel JM (2002). Cataplexy related neurons in the amygdala of the narcoleptic dog. Neroscience. **112**: 335–365.
13 Hallmayer J, Faraco J, Lin L. *et al.* (2009). Narcolepsy is strongly associated with T-cell receptor alpha locus. Nat Genet. **41**: 708–711.
14 Hamann SB, Ely TD, Hoffman JM, Kilts CD (2002). Ecstasy and agony: Activation of the human amygdala in positive and negative emotion. Psychological Science. **13**: 135–141.
15 Joo EY, Kim SH, Kim ST, Hong SB (2012). Hippocampal volume and memory in narcoleptics with cataplexy. Sleep Med. **13**(4): 396–401.
16 Kim H, Suh S, Joo EY,Hong SB (2015). Morphological alterations in amygdalo-hippocampal substructures in narcolepsy patients with cataplexy Brain Imaging and Behav. **7**: 1–11.
17 Le Doux JE (1998). The emotional brain. New York. Simon and Schuster.
18 Liblau RS, Vassalli A, Seifinejad A, Tafti M (2015). Hypocretin (orexin) biology and the pathophysiology of narcolepsy with cataplexy. Lancet Neurol. **14**: 318–328.
19 Lucarelli RT, Peshock RM, McColl R, Hulsey K, Ayers C, Whittemore AR *et al.* (2013). MR imaging of hippocampal asymmetry at 3T in a multiethnic, population-based sample: results from the Dallas Heart Study. AJNR Am J Neuroradiol. **34**: 752–757.
20 Mignot E. (1998). Genetic and familial aspects of narcolepsy. Neurology. **50** (Suppl 1): 16–22.
21 Nakamura T, Matsui T, Utsumi A, Yamazaki M, Makita K, Tanabe HC, *et al.* (2012). The role of the amygdala in the proces of humour appreciation. CogSci Proceedings 2012: 797–802.
22 Partinen M, Kornum BR, Plazzi G, Jennum P, Julkunen I, Vaarala O (2014). Narcolepsy as an autoimmune disease: the role of H1N1 infection and vaccination. Lancet Neurol. **13**: 600–613.
23 Poryazova R, Schnepf B, Werth E, Khatami R, Dydak U, Meier D, *et al.* (2009). Evidence for metabolic hypothalamo-amygdala dysfunction in narcolepsy. Sleep. **32**: 607–613.
24 Ponz A, Khatami R, Poryazova R, Werth E, Boesiger P, Bassetti CL, Schwartz S (2010). Abnormal activity in reward brain circuits in human narcolepsy with cataplexy. Ann Neurol. **67**: 190–200.
25 Schwartz S, Ponz A, Poryazova R, Werth E, Boesiger P, Khatami R, *et al.* (2008). Abnormal activity in hypothalamus and amygdala during humour processing in human narcolepsy with cataplexy. Brain. **131**: 514–522.
26 Swanson LW, Cowan WM (1975). Hippocampo-hypothalamic connections: origin in subicular cortex, not ammons horn. Science. **189**: 303–304.
27 Šonka K, Šusta M, Billiard M (2015). Narcolepsy with and without cataplexy, idiopathic hypersomnia with and without long sleep time: a cluster analysis. Sleep Med. **16**: 225–231.
28 Woolard AA, Heckers S (2012). Anatomical and functional correlates of human hippocampal volume asymmetry. Psychiatry Res. **201**: 48–53.

Copyright © 2015 Neuroendocrinology Letters ISSN 0172–780X • **www.nel.edu**