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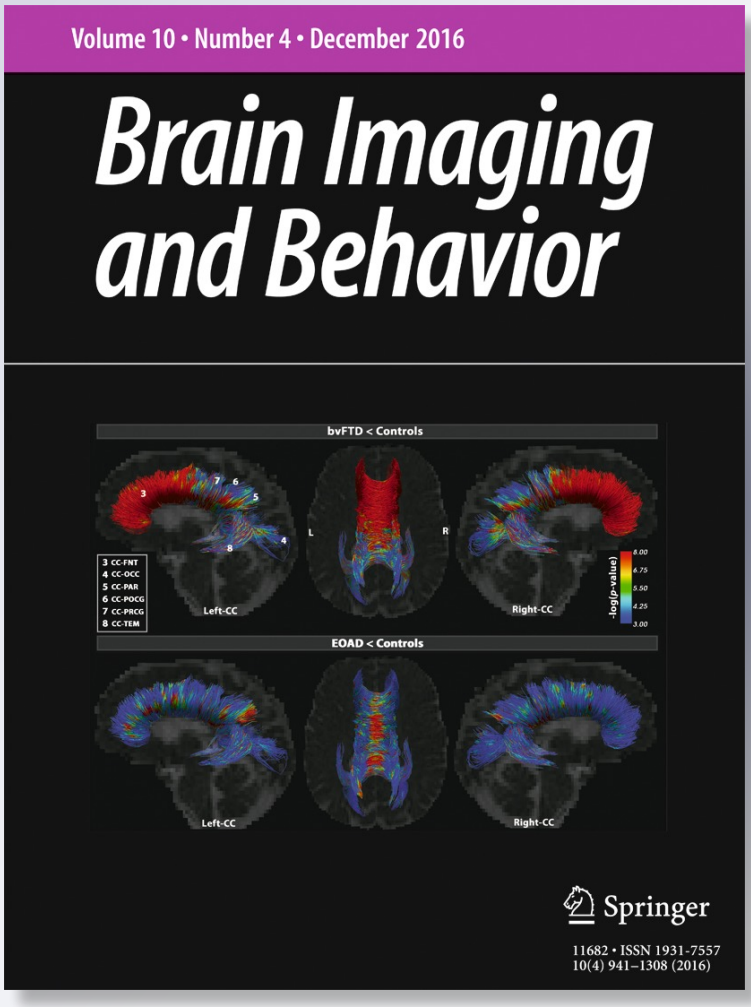

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ORIGINAL RESEARCH

Morphological alterations in amygdalo-hippocampal substructures in narcolepsy patients with cataplexy

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Abstract Although the role of hypocretin-mediated amygdalo-hippocampal dysfunction is hypothesized to be linked with narcolepsy, there have been no human MRI studies investigating the relationship between their regional volume and key symptoms of narcolepsy. To investigate the morphological changes of amygdalo-hippocampus and its relationship with clinical features in patients with narcolepsy, point-wise morphometry that allowed for measuring the regional volumes of amygdalo-hippocampus on T1-weighted MRI was applied. Participants were 33 drug-naïve patients and 35 age-/gender-matched controls (mean ± SD: 27±6 years). We compared hippocampal and amygdalar subfields volumes between patients and controls and correlated between volume and clinical and neuropsychological features in patients. Bilateral hippocampal atrophy (183 vertices) was identified mainly located within the CA1 subfield (FDR<0.05). Significant amygdalar volume reduction was found in the areas of the centromedial (102 vertices) and laterobasal nuclear groups (LB, 35 vertices). There was no volume increase in patients relative to controls (FDR >0.2). After controlling depressive mood, sleep quality, age, and gender, hippocampal CA1 atrophy and amygdalar centromedial atrophy were associated with longer duration of daytime sleepiness and shorter mean REM sleep latency ($|r|$ >0.44, p<0.01). The amygdalar centromedial atrophy was associated with longer duration of cataplexy ($|r|$ >0.47, p<0.005). Subfields atrophy of amygdalo-hippocampus in untreated patients with narcolepsy that was found relative to controls suggests that CA1 of the hippocampus and centromedial area of amygdala are closely related to the severity of narcolepsy and play a crucial role in the circuitry of cataplexy.

Keywords Narcolepsy · Amygdale · Hippocampus · Surface analysis · MRI volumetry

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Introduction

Narcolepsy is a chronic sleep disorder that is characterized by excessive daytime sleepiness (EDS), cataplexy (sudden loss of muscle tone precipitated by strong emotional stimuli), rapid eye movement (REM) sleep phenomena such as hypnogogic hallucinations occurring at sleep onset and sleep paralysis (Guilleminault and Dement 1977).

Emotional processing has been noted as important in narcolepsy patients as it is well known that sudden emotional reaction can provoke cataplectic episodes. Such dysfunction in the arousal system is known to be associated with hypocretin. The amygdala contains dense hypocretin/orexin fibers and receptors and is a primary site in the limbic system that is projected by hypocretin/orexin containing neurons (Siegel 1999). Accordingly, hypocretin-mediated amygdalar dysfunction may be linked with key symptoms of narcolepsy,

such as cataplexy episodes or REM-sleep dysregulation through the network with hypothalamic neurons (Bisetti et al. 2006). Such roles of amygdalar dysfunction is also hypothesized and tested in animal studies (Gulyani et al. 2002) as well as human studies (Khatami et al. 2007). Amygdalar dysfunction is also thought to be partly responsible for depressive symptoms and anxiety disorders, which is recognized with an increased incidence among patients with narcolepsy (Fortuyn et al. 2010).

Difficulty in cognitive function has been reported recently as another aspect of brain functional characteristics in narcolepsy (Hood and Bruck 1997). Multiple cortical structural alterations are known to be involved in cognitive functional deficits in narcoleptic patients (Bayard et al. 2011; Hood and Bruck 1997; Moraes et al. 2012). This calls for further studies assessing structural integrity of the hippocampus, which is considered a hub of memory processing, to allow for better understanding pathophysiological mechanisms underlying cognitive impairment in narcolepsy patients (Hood and Bruck 1997; Naumann et al. 2006).

Sophisticated magnetic resonance imaging (MRI) allows in vivo visualization of human brain anatomy with exquisite detail and quantification of morphological changes. In narcolepsy, studies using voxel-based morphometry (VBM) on structural MRI have identified gray matter loss in key projection sites of hypocretinergic neurons, including the thalamus and hypothalamus, nucleus accumbens, and frontotemporal cortices (Dang-Vu 2013). Cortical thinning is also identified in the prefrontal, cingulate, inferior parietal and temporal cortices in narcoleptic patients with cataplexy (Joo et al. 2011). However, studies that have investigated the structural integrity of the mesiotemporal lobe structures have been scarce, partly due to excessive manual work required for boundary delineation. Brabec et al. (2011), used VBM and identified amygdalar volume reduction in a small cohort of narcolepsy patients (n= 11) compared to controls. Joo et al. (2012) performed manual labeling of the hippocampus and reported bilateral volume reduction in narcolepsy patients when comparing 36 narcolepsy patients to controls. These studies have shown significant association of mesiotemporal lobar volume with clinical symptoms of narcolepsy patients.

Amygdalo-hippocampal structures consist of subfields or subnuclear divisions which are cytoarchitectonically and functionally distinctive. Encoding and decoding of memory and emotion in these structures are processed through different neural circuits that involve series of different subfields (Carpenter and Grossberg 1993; Chun and Phelps 1999). An in-depth study of amygdalar and hippocampal subdivisions may therefore provide novel insights into the pathophysiology of narcolepsy.

The objective of the current study was to investigate regional structural changes of the amygdalo-hippocampus, and its relationship with clinical and neuropsychological features in narcolepsy patients compared to healthy controls. To this end, we quantified local volume changes using our previously developed surface-based framework (Duvernoy 2005). Compared to conventional surface shape analyses (Styner et al. 2006b; Wang et al. 2010, 2011), our technique allows the measurement of actual changes in volume independent of positional changes and have shown sensitivity to subfield-level volume changes in various neurological disorders (Joo et al. 2014; Kim et al. 2013).

Methods

Participants

Thirty-three patients with narcolepsy with cataplexy were recruited from a sleep clinic in a university hospital setting (Samsung Seoul Hospital) located in Seoul, Korea. Patients underwent a sleep study consisting of one overnight polysomnography (PSG) followed by a Multiple Sleep Latency Test (MSLT). The MSLT consisted of five naps scheduled at 2-h intervals starting around 09:00. Subjects with a mean sleep latency of 8 min or less and two or more SOREMPs (sleep onset REM periods) were evaluated for human leukocyte antigen (HLA)-DQB1*0602 and DRB1*1501, which are the best genetic predictors of narcolepsy in humans (Chabas et al. 2003; Doherty et al. 1998). Detailed information, including the presence of sleep attacks, hypnagogic hallucinations, and sleep paralysis as well as a positive family history of narcolepsy, were obtained from patients and their families.

Thirty-one healthy controls were recruited through an advertisement in the local community, and were age and gender-matched to the patients. Each candidate control underwent a detailed clinical interview, sleep questionnaire, and PSG, and the results were evaluated and interpreted by a sleep specialist (Joo EY). If a control had an apnea-hypopnea index of 5 or greater or evidence of another sleep disorder (e.g., periodic limb movement disorder) on PSG, he or she was excluded from further participation.

Patients and controls were excluded if they exhibited any of the following : (a) controls with an average nightly sleep time of less than 7 h over the most recent 2 weeks based on sleep diary entries, (b) abnormal sleep-wake rhythms, (c) other sleep disorders apnea-hypopnea index≥5/h or period limb movement during sleep index more than≥15/h), (d) heart or respiratory disease, (e) history of cerebrovascular disease, (f) other neurologic or psychiatric diseases, (g) alcohol or illicit drug abuse or current intake of psychoactive medications, or (h) presence of a structural lesion on brain MRI. Accordingly we excluded 6 individuals from the initial group of 39 patients. Subsequently, we analyzed 33 patients and 31 controls.

All participants gave written informed consent before the study began. The Institutional Review Board at Samsung Medical Center authorized the informed consent form and the study protocol.

Neuropsychological assessment

Participants underwent a battery of neuropsychological tests and an individual standardized intelligence test. Neuropsychological tests assessed six broad domains: working memory (Digit Span test from the Wechsler Memory Scale-Revised and the Corsi Block tapping tests, forward and backward); executive functioning (Trail Making Tests A and B and the Stroop test), verbal information processing (Digit symbol test), verbal memory (Korean California Verbal Test), visual memory (Rey Complex Figure Test) and verbal fluency (controlled oral word association test). Information on the protocol of the neuropsychological assessments can be found elsewhere (Noh et al. 2012).

Self-report questionnaires

The Pittsburgh Sleep Quality Index (PSQI) measures the sleep quality during the past month (Buysse et al. 1989). A global score greater than 5 indicates poor sleep quality. The Beck Depression Inventory-II (BDI-II) measures the severity of depressive symptoms (Beck et al. 1987). Higher scores reflect greater levels of depressive symptoms.

MRI acquisition and image processing

MRI was performed using a Philips 3.0 Tesla scanner (Achieva, Philips Medical Systems, Best, the Netherlands) using a 16-channel head coil. All patients underwent 3-D T1- and T2-weighted, and fluid attenuated inversion recovery (FLAIR) imaging protocols. T1-weighted images were obtained using the following scanning variables: sagittal 0.5 mm thickness, 360 slices, no gap, repetition time/echo time=9.90/4.60 ms, flip angle=8°, number of excitations=1, sensitivity encoding factor=2.0, overcontiguous slices with 50 % overlap, matrix size of 480×480 over a field of view of 240×240 mm, and reconstructed voxel dimension of T1-weighted images=0.50×0.50×0.50 mm. Scans were visually checked for image quality. If an artifact was present, the scan was repeated.

Each image underwent automated correction for intensity non-uniformity correction (Sled et al. 1998) with a parameter of smoothing distance (40 mm) that optimally accounted for wave-length of 3 T MR non-uniformity fields as demonstrated in (Boyes et al. 2008; Zheng et al. 2009). We then normalized the MRI intensity to a range of 0–100 using the signal distribution within the brain region.

To control for differences in brain volume, MR images were registered into the MNI ICBM-152 nonlinear template (Fonov et al. 2011) using a 9 parameter linear transformation (Collins et al. 1994).

Labeling of the hippocampus (Hp) and the amygdala (Am) were manually performed by one rater (HK) on T1-weighted MRI (an example shown in Fig. 1) according to the protocol described in previous studies (Watson et al. 1992, 1997). We randomly selected 7 cases and evaluated intra-rater reproducibility. Mean Dice overlap indices (Kim et al. 2012) between the test-set and retest-set were 92 and 90 % for the Hp and Am, respectively, which was considered “highly reproducible”.

Surface-based mapping of amygdalo-hippocampal atrophy

We used a previously validated surface-based approach (Kim et al. 2008) that reliably measured local volume changes of amygdalo-hippocampal structures (Bernhardt et al. 2013; Kim et al. 2013). This approach computes Jacobian determinants on surface-based displacement vectors between a given subject and a template surface (Styner et al. 2006a). Briefly, manually segmented amygdalae and hippocampi were converted to surface meshes for which a spherical parameterization (SPHARM) was computed using area-preserving, distortion-minimizing mapping. Based on a uniform icosahedron-subdivision of the SPHARM, we obtained a point distribution model (PDM), allowing shape-inherent point (1002 points evenly sampled on the surface) correspondences across subjects. Each individual SPHARM-PDM surfaces were rigidly aligned to a template constructed from the mean surface of controls and patients with respect to the centroid and the longitudinal axis of the 1st order ellipsoid (Gerig et al. 2001). Vertex-wise displacement vectors were then calculated between each individual and the template (Styner et al. 2006a). Displacement vectors were diffused within the volume enclosed by the surface using a heat equation, yielding a displacement vector field. To assess local volume changes, we finally calculated Jacobian determinants from these vector fields (Kim et al. 2008). By projecting these Jacobian determinants back onto the surface using tri-linear interpolation and subtracting 1 from them, we obtained a metric of growth ($J>0$) or shrinkage ($J<0$) in a unit-size cube defined on each vertex. Given that the surface-based analysis was performed in a stereotaxic space, correction for differences in overall intra-cranial volume was thus unnecessary.

Statistical analysis

To compare demographics, self-report questionnaires scores, PSG data, and neuropsychological parameters between patients and controls, one-way analysis of variance was used. Six composite scores were computed from the neuropsychological tests by transforming all scores into standardized Z scores and then averaged to represent each domain: working memory,

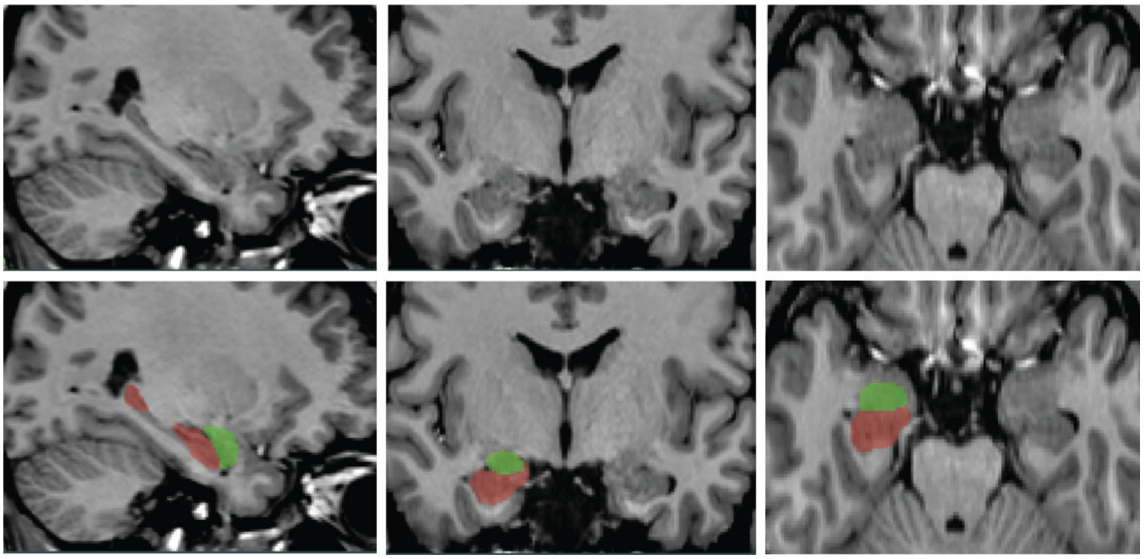

Fig. 1 Manual labeling of mesiotemporal structures. T1-weighted MR image on the three orthogonal planes (*top*) and labels of hippocampus (*red*) and amygdala (*green*) overlaid on the image (*bottom*)

executive functioning, verbal information processing, verbal memory, verbal fluency, and visual memory. These composite scores were used in a previous study (Joo et al. 2014).

Analyses with respect to global and local volumetry were performed using SurfStat toolbox (Chung et al. 2010) for Matlab (R2007a, The Mathworks, Natick, MA, USA). Global volumes and Surface-based local volumes of amygdalo-hippocampus were standardized relative to the distribution of controls using Z-score transformation. As no laterality of volume changes was expected in relation to narcolepsy (Joo et al. 2012), we averaged the normalized left and right volumes. In particular, local volumes were averaged at the same location between the left and right hemispheres. We however assessed a possible hemispheric asymmetry by computing asymmetry index (AI) as 2× (L - R) / (L + R) where L and R denote the left and right hemispheric volumes. All the following statistical tests included age, sleep quality (PSQI) and depressive mood (i.e., BDI-II) as covariates: (a) Group comparison: We assessed differences in global volume between patients and controls using two-tailed t-tests. To assess local volume differences, we repeated the same test on surface-based Jacobian determinants at each vertex (=surface point). To assess a hemispheric asymmetry in the changes in patients compared to controls, we performed a *t*-test on the AI between patients and controls. (b) Association with clinical and neuropsychological parameters: To assess the association of volume changes with clinical demographics, PSG, PSQI, and neuropsychological parameters, linear models were applied to compute Pearson's correlation coefficient r while controlling for age, sex and depressive mood. For local volume changes, this test was performed on individual measurements averaged within each cluster displaying significant changes in patients relative to controls in the group comparison. (c) Correction for multiple comparisons: In all vertex-wise surface analyses, significances were thresholded using the false discovery rate (FDR) procedure (Benjamini and Hochberg 1995), with FDR<0.05. In the volumetric analysis, Bonferroni-adjustment was applied to control the family-wise error rate. (d) Localization of findings: The amygdalar subdivisions were outlined based on a 3D cytoarchitectonic mapping on MRI (Amunts et al. 2005). The hippocampal subfields were schematically outlined on the surface template (Fig. 1) based on Duvernoy's atlas (Duvernoy 2005) and its surface reconstructions that were previously published (Joo et al. 2014).

Results

Self-report questionnaires, and overnight sleep studies

Patients and controls did not differ by age or gender. Patients had EDS onset at an average of 14.79 (±2.79) years, with an average EDS duration of 12.21 (±5.68) years. Average cataplexy onset was reported to be 17.70 (±4.57) years, with average cataplexy duration of 9.30 (±5.30) years. Patients had significantly higher depression scores and worse sleep quality compared to controls ($p<0.0001$). During the PSG, patients had significantly shorter sleep latency and REM latency, longer wake after sleep onset and total sleep time, and higher period limb movement during sleep index. Detailed results are summarized in Table 1.

Table 1 Demographic information of patients with narcolepsy and controls

	Patients (n=33)	Controls (n=31)	p-value
Age	27.00±5.88 (19–41)	27.16±5.72 (21–40)	0.91
Sex (% male)	21 (63.6 %)	19 (47.5 %)	.52
Beck depression index-II	9.58±6.47	4.74±2.97	<.0001*
Pittsburgh sleep quality index	6.42±3.11	2.46±1.30	<.0001*
Epworth sleepiness scale	14.85±3.65	4.29±1.84	<.0001*
Polysomnoraphy			
Sleep onset latency, min	3.96±3.68	9.69±7.17	<.0001*
Wakefulness after sleep onset, min	43.94±24.45	33.10±13.86	.03
Total sleep time, min	417.69±56.38	386.72±37.70	.01
Sleep efficiency, %	89.40±6.46	90.02±3.19	.63
Apnea-hypopnea index, per hour	1.53±1.45	1.35±1.09	.57
REM sleep latency, min	29.27±40.50	87.96±31.05	<.0001*
Arousal index, per hour	14.9±5.1	11.2±3.9	<.0001*
PLMD index, per hour	5.85±11.79	0.40±1.19	.01
For patients only			
EDS onset, year old	14.79±2.79	–	–
EDS duration, years	12.21±5.68	–	–
Cataplexy onset, year old	17.70±4.57	–	–
Cataplexy duration, years	9.30±5.38	–	–

Mean values ± standard deviation, Abbreviations: *REM* rapid eye movement, *PLMD* periodic limb movement disorder, *EDS* excessive daytime sleepiness. * p<0.05

Neuropsychological tests

Patients showed significantly lower scores on executive functioning (p=0.03) compared to controls. There were no differences in working memory, verbal information processing, verbal memory, verbal fluency, and visual memory between the two groups (Table 2).

Global and local volume changes in patients relative to controls

A two-tailed t-test uncovered only a trend of decrease (uncorrected p<0.05) in the hippocampal volume (HpV) and amygdalar volume (AmV) of patients compared to controls (HpV - left: 3240±236 vs. 3384±251; right: 3342±219 vs. 3457±208; AmV - left: 1996±159 vs. 2081±165; right: 2014±161 vs. 212±176). There was no hemispherical asymmetry either in HpV or AmV as a comparison of the AI between patients and controls did not find difference (p>0.4). Results of vertex-wise comparisons between groups are shown in Fig. 2. In patients, hippocampal atrophy was identified mainly within the region corresponding to the CA1 subfield. The largest cluster was located on the superolateral surface at the level of the hippocampal body and tail (FDR<0.005, 183/1002 vertices). Further analysis in vertex-wise asymmetry demonstrated no asymmetric change (FDR>0.3). Compared to controls, patients presented significant AmV reduction which was located in the regions corresponding to centromedial and laterobasal nuclear groups (FDR<0.05). The largest cluster (102 vertices) was located on the superoanterior surface within the centromedial subdivision. On the other hand, the smaller cluster (34 vertices) was detected on the inferoanterior surface within the laterobasal subdivision. There was no local volume increase in patients relative to controls (FDR>0.2).

Association between volume, and clinical and neuropsychological parameters

We found that age, gender, education and sleep quality did not correlate with HpV nor AmV in patients or controls (p>0.2). In patients, depressive mood (BDI-II) was associated with smaller HpV and smaller AmV (r=−0.41, p<0.05). After controlling depressive mood, sleep quality, age and gender, surface-based analysis revealed that the degree of volume loss in the cluster located at hippocampal CA1 subfield correlated negatively with the duration of EDS and positively with mean REM sleep latency (|r| >0.44, p<0.01; Fig. 3a). Volume reduction in Am centromedial subdivision was associated with longer duration of cataplexy, longer duration of EDS and decreased mean REM sleep latency (|r| >0.47, p<0.005, Fig. 3b). No significant correlations were found between surface-based volume and other clinical parameters (|r| <0.23, p>0.2). A smaller CA1 HpV in narcolepsy was associated with a lower performance in executive function (|r|= 0.54, p=0.001; Fig. 3a).

Table 2 Neuropsychological analyses of patients with narcolepsy and controls

	Patients (n=33)	Controls (n=31)	p-value
Working memory composite score	0.11 (±0.62)	0.38 (±0.49)	0.06
Digit span forward	9.52 (±2.21)	9.06 (±2.23)	
Digit span backward	8.39 (±2.01)	7.42 (±1.97)	
Corsi block forward	8.36 (±1.85)	10.06 (±11.22)	
Corsi block backward	8.30 (±1.48)	8.35 (±1.58)	
Executive functioning composite score	−0.10 (±0.41)	0.08 (±0.29)	0.03*
Stroop word correct responses	111.82 (±1.044)	111.97 (±.18)	
Stroop word response time	107.97 (±13.13)	111.26 (±4.07)	
Stroop color correct responses	107.82 (±9.04)	104.58 (±12.36)	
Stroop color response time	108.73 (±14.57)	113.65 (±6.93)	
Trail making test A	35.33 (±13.62)	32.10 (±12.54)	
Trail making test B	75.79 (±43.73)	82.29 (±30.95)	
Verbal information processing composite score	−0.09 (±0.78)	0.09 (±1.19)	0.46
Digit symbol test	62.03 (±12.27)	64.93 (±18.52)	
Verbal memory composite score	0.06 (1.09)	−0.06 (0.89)	0.62
Korean California verbal test			
Total	59.27 (10.38)	58.09 (8.48)	
Short delay free recall	13.39 (2.37)	12.51 (2.76)	
Long delay free recall	13.66 (2.32)	12.90 (2.66)	
Recognition	15.24 (1.09)	14.93 (1.36)	
Verbal fluency composite score	−0.03 (0.78)	0.10 (0.58)	0.26
Controlled oral word association test			
Phonetic word fluency	33.79 (±8.32)	38.00 (±13.84)	
Semantic word fluency	31.97 (±6.44)	35.65 (±6.83)	
Animal	18.52 (±6.08)	19.29 (±6.07)	
Supermarket	15.76 (±5.14)	15.81 (±4.72)	
Visual memory composite score	.09 (.71)	−.09 (.68)	0.27
Rey complex figure test			
Copy	35.69 (0.63)	35.48 (0.81)	
Immediate recall	24.90 (6.46)	24.30 (6.18)	
Delayed recall	25.03 (6.73)	23.85 (5.77)	
Recognition	20.87 (1.76)	20.51 (2.11)	

* p<0.05

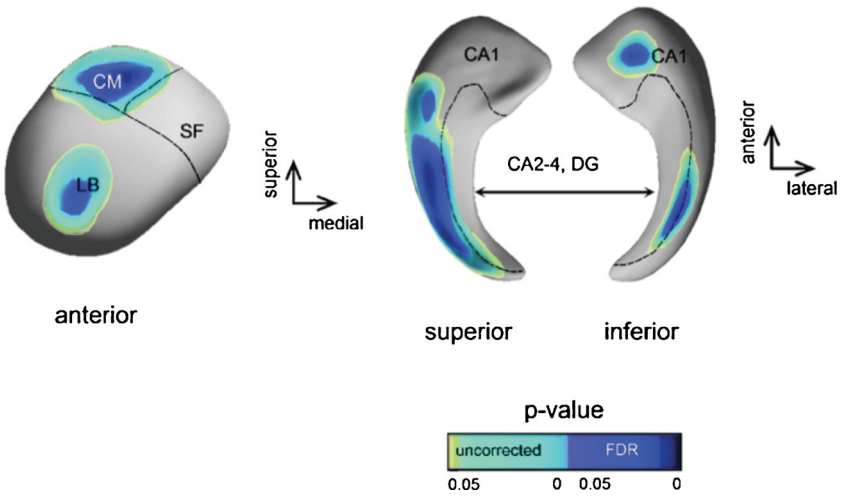

Fig. 2 Vertex (=surface point)-wise group comparison between patients with narcolepsy and healthy controls. Regions of volume decrease in patients relative to controls are shown: The identified atrophy was mapped mainly in hippocampal CA1 (A) and in amygdalar CM and LB subfields. A: Significances are thresholded at FDR<0.05. *Abbreviation*: *CA* cornu ammonis, *DG* dentate gyrus, *CM* centromedial, *SF* superficial, *LB* laterobasal

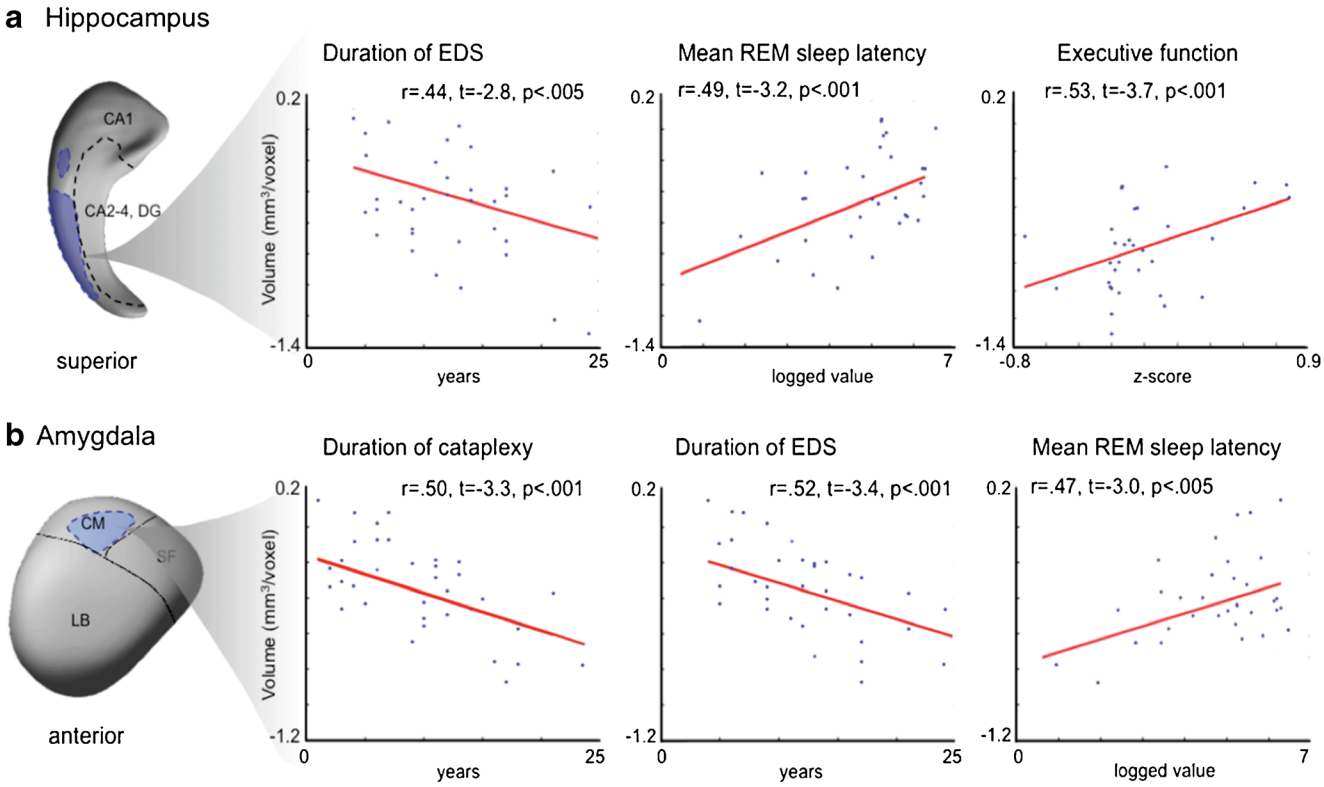

Fig. 3 Association between mesiotemporal local volume and clinical parameters and neuropsychological scores in patients with narcolepsy. For each cluster representing significant volume loss in patients relative to controls, the mean volume is correlated with a given clinical parameter while controlling age, sex, and depressive mood. Linear regression models are plotted for significant correlations. Abbreviations: *EDS*daytime sleepiness, *REM* rapid eye movement

Discussion

The current study investigated amygdalar and hippocampal subfield volume changes in narcolepsy patients compared to controls, and the association of specific subfield volume differences with clinical and neuropsychological parameters. To the best of our knowledge, this is the first study to localize mesiotemporal lobe subregional volume in patients with narcolepsy.

Volume reduction in the amygdala in narcolepsy patients

There is sufficient evidence that the amygdala is not a homogenous brain region, but consists of several sub-nuclei that differ in structure and function, and best regarded separately (Davis and Whalen 2001). Results from our study indicate that amygdalar volume decreases in narcolepsy were specifically mapped into centromedial and laterobasal nuclear subdivisions compared to controls. Our study is also consistent with a human MRI study that found a decrease of amygdalar volume in narcolepsy patients compared to controls (Brabec et al. 2011).

The amygdala plays a major role in the interpretation of emotionally significant stimuli, and has strong projections to the hypocretin areas and brainstem regions regulating muscle tone and the sleep/wake process (Sakurai et al. 2005). The amygdalar nuclei are bidirectionally connected to neurons in the hypothalamus, with hypocretin containing neurons that exert strong postsynaptic activation on neurons of the central and medial subnuclei of the amygdala and substantially contribute to major amygdalar output. Thus, it is conceivable that amygdalar atrophy and dysfunction is associated with key features of narcolepsy (Bisetti et al. 2006). This is consistent with our results in that volume reduction in amygdalar centromedial subdivision was associated with longer duration of cataplexy and decreased REM sleep latency of MSLT. Humourous stimuli elicited reduced hypothalamic response together with enhanced amygdala response in the event related functional MRI of narcolepsy patients, suggesting that suprapontine mechanisms of cataplexy involves a dysfunction of hypothalamic-amygdala interactions triggered by positive emotions (Schwartz et al. 2008).

Our study furthermore identified a decrease in amygdalar volume in the laterobasal nucleus in narcolepsy patients. It has been suggested that compared to other nuclear subregions of the amygdala, few spontaneously active cells can be recorded in the laterobasal nucleus (Fukuda et al. 1987). However, in relevance to sleep, one animal study found that projection cells of the lateral nucleus of the amygdala remains silent

during the sleep-wake cycle unless a complex stimulus is involved (Gaudreau and Pare 1996). In this study, 82 % of cells fired at <0.01 Hz during wake, slow wave sleep, and paradoxical sleep, but increased when animals were presented with complex sensory stimuli (Gaudreau and Pare 1996). Thus, it is presumed that abnormal processing of sensory stimuli may alter circuitry or contribute to an abnormal output from the laterobasal nucleus of amygdale could be associated with occurrence of cataplexy.

Hippocampal substructural vulnerability in narcolepsy patients

The main findings of this study indicate that narcolepsy patients display volume decreases in specific subfields of the hippocampus and amygdala. Specifically, narcolepsy patients showed hippocampal atrophy within the CA1 subfield compared to controls. Studying primary insomniacs (Joo et al. 2014), we previously found a significant association between CA1 atrophy and sleep quality. Our analysis in the current study showed that this was not the case in narcolepy, suggesting a different mechanism is involved in this disease population.

Hypocretin and histamine, the components in the brain wake-promoting system, are affected in narcolepsy and the hypocretinergic system controls arousal through the histaminergic system (Sundvik and Panula 2015). Studies using animal models have found that the majority of hypocretin-containing neurons provide dense innervation to the hippocampus via cholinergic and GABAergic projections (Wu et al. 2002). Hypocretin-1, in particular, modulates long-term synaptic plasticity in CA1 region of hippocampus (Selbach et al. 2010). Histamine also facilitates the induction of long-term potentiation in the CA1 region of rat hippocampal slices (Brown et al. 1995). Linked with reduced level of hypocretin and histamine in the cerebrospinal fluid in patients with narcolepsy (Kanbayashi et al. 2009), impaired hypocretin and histamine system in narcolepsy patients may be related to the hippocampus, especially CA1 atrophy in this study.

We found atrophy in the CA1 subfield correlated negatively with the duration of EDS and positively with REM sleep latency in narcolepsy patients. Hypocretin deficiency may disturb both the circadian control of melatonin release and its temporal association with sleep (Donjacour et al. 2012). Melatonin has protective effects in CA1 neuronal density in animal models (Cho et al. 1997). REM sleep plays a role in regulating both discharge rates and synchrony in the CA1 subfield of hippocampus (Grosmark et al. 2012) and narcolepsy has often been regarded as a disorder of REM sleep generation. Taken with these observations, our findings may suggest that the combined effect of hypocretin deficiency and related neuronal changes in narcolepsy may be responsible for the structural alteration of subfields of the hippocampal formation in patients.

Clinical and neuropsychological differences in narcolepsy patients compared to controls

The results from our study indicated narcolepsy patients displayed lower executive functioning compared to healthy controls, but no differences in other cognitive functions. This is consistent with a number of studies that have investigated cognitive function in narcolepsy have largely suggested that cognitive function other than executive function remains intact in this population, despite subjective complaints of memory problems (Hood and Bruck 1997).

Previous studies have suggested that narcoleptic patients accompany difficulties in subareas of executive function, including slower reaction times and decreased vigilance in comparison to controls (Delazer et al. 2011; Rieger et al. 2003). Narcolepsy patients have been found to have deficits in the executive attention network, with overall more slower and variable reactions compared to controls (Rieger et al. 2003). A study by Delazer et al. (2011) reported that while narcolepsy patients did not differ in accuracy, there was a slowing of responses in tasks under time pressure on the Go-NoGo task, which is a measure of response inhibition. Both the Go-No Go task used in Delazer's study and the Stroop task used in our study measure similar domains of attention and response inhibition (Barbarotto et al. 1998).

Additionally, a novel finding in our study was that smaller hippocampal volume in the CA1 subfield in narcolepsy patients was associated with a lower performance in executive function. The executive function is largely attributed to activation in the fronto-parietal lobes or more specifically in the prefrontal cortex, PFC (Alvarez and Emory 2006). Throughout literatures, it is widely accepted that the PFC is necessary but not solely sufficient for carrying out the executive function. The dopamine receptors in hippocampal–prefrontal cortical circuits are known to play a role in modulating the responsiveness of the executive function when a stimulus is given (Takahashi et al. 2008). Acquisition of new spatial memory as a type of executive function is known to be processed in CA1 and subiculum subfields (Mizumori et al. 1989). Rats with CA1 lesions indeed require longer durations in acquiring path-finding information (Floresco et al. 1996). It has been reported that integrity of the hippocampus influences on the function of prefrontal cortex (Tierney et al. 2004; Takahashi et al. 2007). Therefore, hippocampal atrophy may disrupt prefrontal functioning such as executive function by altering hippocampal-prefrontal connectivity. Significant association between hippocampal CA1 atrophy and the impaired executive function in human studies of dementia (Nagata et al. 2011) and Parkinson's disease (Beyer et al. 2013) supports our finding.

A study using an extensive neuropsychological test battery has found narcolepsy patients scored in the average in domains of selective attention, verbal fluency, and verbal working memory, which is consistent with the results of our study

(Delazer et al. 2011; Rieger et al. 2003). Despite the fact that narcolepsy patients subjectively complain about memory problems, it is possible that they inaccurately evaluate their memory abilities due to lowered self-efficacy for memory performance (Hood and Bruck 1997).

Conclusion

In conclusion, our work indicates that subfields atrophy in patients with relative to controls suggests that centromedial area of the amygdala and CA1 of the hippocampus are closely related to the severity of narcolepsy and play a crucial role in the circuitry of cataplexy. Additionally, structural atrophy of the CA1 region is also associated with impaired executive functioning, which has been consistently found in previous studies. Despite the relatively small sample size in current study, our results suggest that amygdalo-hippocampal subfield analysis may provide new perspectives how neural substrates are associated with key features in narcolepsy.

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Conflict of interest All authors (Kim H, Suh S, Joo EY, and Hong SB) declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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