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Hippocampal Substructural Vulnerability to Sleep Disturbance and Cognitive Impairment in Patients with Chronic Primary Insomnia: Magnetic Resonance Imaging Morphometry

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Study Objectives: Despite compelling evidence from animal studies indicating hippocampal subfield-specific vulnerability to poor sleep quality and related cognitive impairment, there have been no human magnetic resonance imaging (MRI) studies investigating the relationship between hippocampal subfield volume and sleep disturbance. Our aim was to investigate the pattern of volume changes across hippocampal subfields in patients with primary insomnia relative to controls.

Design: Pointwise morphometry allowed for volume measurements of hippocampal regions on T1-weighted MRI.

Setting: University hospital.

Patients: Twenty-seven unmedicated patients (age: 51.2 ± 9.6 y) and 30 good sleepers as controls (50.4 ± 7.1 y).

Interventions: N/A.

Measurements: We compared hippocampal subfield volumes between patients and controls and correlated volume with clinical and neuropsychological features in patients.

Results: Patients exhibited bilateral atrophy across all hippocampal subfields ($P < 0.05$ corrected). Cornu ammonis (CA) 1 subfield atrophy was associated with worse sleep quality (higher Pittsburgh Sleep Quality Index and higher arousal index of polysomnography) ($r < -0.45$, $P < 0.005$). The volume of the combined region, including the dentate gyrus (DG) and CA3-4, negatively correlated with verbal memory, verbal information processing, and verbal fluency in patients ($|r| > 0.45$, $P < 0.05$). Hemispheric volume asymmetry of this region (left smaller than right) was associated with impaired verbal domain functions ($r = 0.50$, $P < 0.005$).

Conclusion: Hippocampal subfield atrophy in chronic insomnia suggests reduced neurogenesis in the dentate gyrus (DG) and neuronal loss in the cornu ammonis (CA) subfields in conditions of sleep fragmentation and related chronic stress condition. Atrophy in the CA3-4-DG region was associated with impaired cognitive functions in patients. These observations may provide insight into pathophysiological mechanisms that make patients with chronic sleep disturbance vulnerable to cognitive impairment.

Keywords: chronic insomnia, hippocampus, memory, morphometry, sleep, surface analysis

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INTRODUCTION

Chronic primary insomnia, also termed psychophysiological insomnia, is a disorder characterized by chronically disturbed sleep and sleep loss, nonrefreshing sleep, and heightened arousal in bed; these symptoms have a profound effect on psychological well-being, as well as on social and cognitive functioning.[1] Patients with chronic primary insomnia commonly have daytime cognitive impairments and deficits in memory consolidation during sleep compared to good sleepers.[2,3] Negative association of disturbed sleep with performance on memory tasks[2,4,5] implicates hippocampal dysfunction in patients. The hippocampus consists of cytoarchitectonically distinctive and functionally specialized subfields,[6–8] the cornu ammonis sectors (CA1-4), the dentate gyrus (DG), and the subiculum. The hippocampus is a target for glucocorticoids and is thereby vulnerable to stress-induced responses that affect both the structure and function of the hippocampus.[9] Acute or prolonged psychosocial stress is associated with structural changes of the CA1 or CA3 neurons[10,11] as well as the DG in mammals.[12,13] Individual hippocampal subfields are responsible for distinct cognitive functions, and differentially vulnerable to neurological and neuropsychiatric conditions.[14–18] Experimental sleep deprivation and fragmentation affect neurogenesis and cell proliferation.[19,20] These studies have suggested that chronic psychological stress conditions driven by sleep fragmentation in patients with chronic insomnia may contribute to alterations in the flow of information through the hippocampal network because of an anomaly in the synaptic pathways, which is reflected by subfield-specific cytoarchitectonic damage, ultimately yielding a negative influence on cognition.[21]

Sophisticated neuroimaging techniques allow for the *in vivo* visualization of human brain anatomy with exquisite detail and the quantification of morphological changes. Recent chronic insomnia studies have analyzed hippocampal volume (HV) on magnetic resonance imaging (MRI),[22–24] but findings have been inconsistent. Although a first study observed bilaterally reduced

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HV in patients compared to good sleepers,[23] two recent studies did not replicate this finding.[22,24] Moreover, our group[22] found that memory impairments in patients with chronic insomnia are related to a decrease in HV. This discrepancy may be related not only to the low sensitivity of global hippocampal volumetry, but also the variability of the hippocampal segmentation between studies.[25] So far, there has been only one study that examined the regional specificity of hippocampal changes in patients and good sleepers, which revealed no significant difference between groups.[26] The automated subfield volumetry used in the study may not have been sufficiently sensitive to visualize hippocampal subfields at a low resolution MRI (1 mm^3). Alternatively, vertex (= point)-wise morphometry based on a surface extracted from the manual segmentation of the whole hippocampus[27–29] has been a surrogate to manual subfield volumetry, which can only be done on high-field (> 3 Tesla) and high-resolution MRI.[30–33] These surface-based approaches, which generate deformation maps that encode individual local shape variation relative to the template, have successfully identified hippocampal subfield pathology in various brain disorders.[27,28,34–36]

The objective of the current study is to investigate the pattern of volume changes across hippocampal subfields in patients compared to controls. Furthermore, we assessed the relationships between subfield volumes and clinical and neuropsychological parameters in patients. To this end, we quantified local volume changes using our previously developed surface-based framework. This procedure extracts the hippocampal boundary from manual labeling using spherical mapping to guarantee intersubject point correspondence.[37] Furthermore, it computes surface-based, point-wise jacobian determinants that represent local volume changes of a given hippocampus relative to the mean shape of all healthy hippocampi.[38] Compared to conventional surface shape analyses,[23,33] our technique, derived from other approaches,[37,39] allows the measurement of actual changes in volume independent of positional changes.[38,40]

METHODS

Forty patients with complaints of sleep onset and/or maintenance insomnia were recruited from a sleep disorder clinic in a university hospital setting (Samsung Seoul Hospital) located in Seoul, South Korea. Patients included in the current study must have met the following criteria: age 40–70 y, meet diagnostic criteria for chronic primary insomnia according to the International Classification of Sleep Disorders-2,[41] and have an insomnia duration of more than 1 y.

Good sleeper controls were recruited through an advertisement in the local community. Their age, sex, and education status did not differ from the patients with chronic insomnia ($P > 0.2$). All participants underwent a comprehensive clinical interview, during which a history of medical and sleep disorders was acquired. History of psychiatric disorders was assessed using the Structured Clinical Interview for Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR).[42] All participants completed a comprehensive physical and neurological examination, overnight polysomnography (PSG), neuropsychological tests, a sleep diary for at least 2 w, and a number of questionnaires assessing sleep quality and mood status.

Exclusion criteria were: (1) controls with an average nightly sleep time of less than 7 h over the recent 2 w based on sleep diaries; (2) obstructive sleep apnea (OSA, apnea-hypopnea index greater than 5/h); 3) moderate to severe periodic limb movement during sleep (PLMS, total PLM index > 25/h); (4) circadian rhythm sleep disorder determined by sleep-wake cycles of sleep diaries; (5) hypertension, diabetes, heart disease, or respiratory diseases; (6) history of cerebrovascular disease; (7) diagnosis of other neurological (neurodegenerative diseases, epilepsy, head injury) or psychiatric diseases (psychosis, current depression); (8) alcohol or illicit drug abuse or current intake of psychoactive medications; and (9) a structural lesion on brain MRI. To avoid the possible effect of medications on the results, four patients who had a history of taking antidepressants, anxiolytics, or sleeping pills were excluded. Four patients were excluded because of definite sleep disorders confirmed by PSG; three with moderate to severe OSA (apnea-hypopnea index, 17.5–40.2/h) and one with PLMS (total index 50.1/h, movement arousal index 10.5/h). Five patients who showed diffuse brain atrophy, white matter changes, or lacunar infarctions on brain MRIs were also excluded. Twenty-seven patients and 30 controls were included in the study. Of these subjects, 17 insomnia patients and 16 good sleepers were also included in a previous study.[22] Informed consent was obtained from all participants, and the institutional review board of Samsung Seoul Hospital authorized the study protocol and design.

Overnight Polysomnography

The day before the sleep study, subjects were asked to refrain from drinking alcohol or caffeinated beverages. Sleep studies were recorded using a Remlogic (Embla Systems, Denver, CO, USA). We have previously provided a detailed description of the test procedures.[39]

Neuropsychological Assessment

Subjects underwent a battery (2.5 h) 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.[22]

Self-Report Questionnaires

The Pittsburgh Sleep Quality Index (PSQI) measures the quality and disturbances of sleep during the past month.[44] A global score greater than 5 indicates poor sleep quality.

The Insomnia Severity Index (ISI) assesses subjective symptoms of insomnia, including the degree of distress caused by this particular sleep complaint.[45] A higher score represents greater insomnia severity. Scores above 14 are generally consistent with clinical levels of insomnia.[46]

The Beck Depression Inventory-II (BDI-II) measures the severity of depressive symptoms.[47] Higher scores reflect greater levels of depressive symptoms.

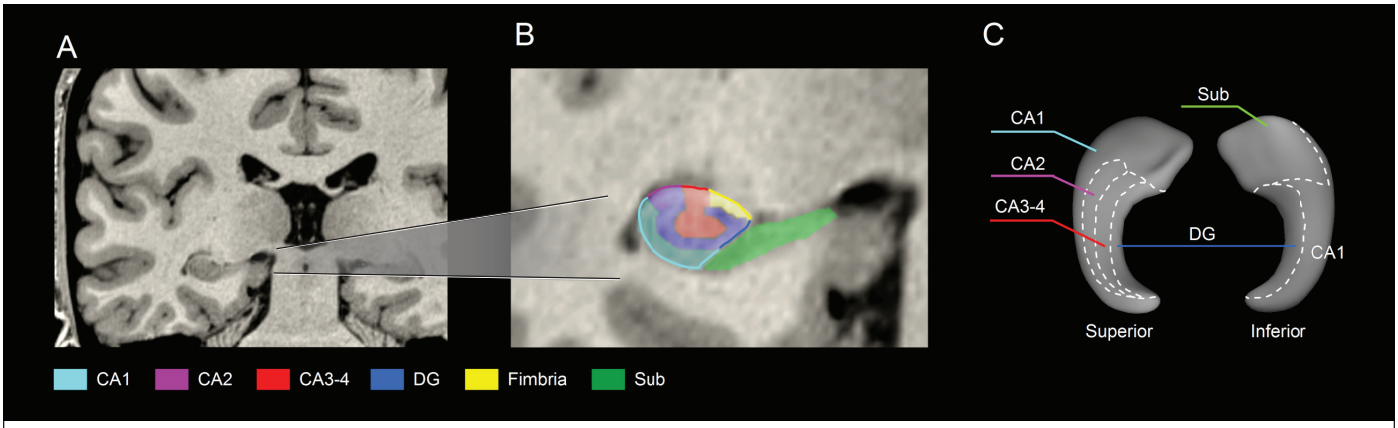

Figure 1—Surface-based subfield atlas. **(A, B)** Individual MRI on which hippocampal subfields are manually and schematically delineated based on Duvernoy atlas.[6] Our segmentation protocol excludes the subiculum at the level of hippocampal body and tail.[20] **(C)** Surface of the entire hippocampus for the same individual. By registering the given individual surface to the template representing healthy population, we obtain the borders between subfields (white dot lines) on the surface. CA, cornu ammonis; DG, dentage gyrus; Sub, subiculum

MRI Acquisition and Image Processing

MRI scanning was performed with a GE Signa 1.5-Tesla scanner (GE Medical Systems, Milwaukee, WI, USA). T1-weighted coronal spoiled gradient recalled (SPGR) MRI was performed with the following scanning variables: 1.6-mm thickness, no gap, 124 slices, repetition time (TR)/echo time (TE) = 30/7 ms, fractional anisotropy (FA) = 45°, number of excitation (NEX) = 1, matrix size = 256 × 192, and field of view = 22 × 22 cm. The voxel dimensions in the SPGR MRI images were 0.86 × 0.86 × 1.6 mm. Each image underwent automated correction for intensity nonuniformity and intensity standardization.[48]

To control for differences in brain volume, magnetic resonance images were registered into the the Montreal Neurological Institute/International Consortium of Brain Mapping standard brain template (MNI/ICBM) 152 nonlinear template[45] using a nine-parameter linear transformation.[50]

Labels of the hippocampus were obtained by manual segmentation on T1-weighted MRI according to a previous protocol[51] and used in an insomnia study,[23] which includes the DG and CA and excludes the subiculum (Figures 1A, 1B). We tested intrarater reliability on five randomly chosen control subjects. The agreement based on kappa statistics was excellent ($k = 0.94 \pm 0.02$), demonstrating that this protocol is highly reproducible.

Surface-Based Mapping of Mesiotemporal Atrophy

We used a previously validated surface-based approach[38] to measure local volume changes. This approach computes jacobian determinants on surface-based displacement vectors between a given subject and a template surface.[37] Briefly, manually segmented labels were first minimally smoothed and then converted to surface meshes for which a spherical parameterization (SPHARM) was computed using area-preserving, distortion-minimizing mapping (Figures 2A-2C). Based on a uniform icosahedron-subdivision of the SPHARM, we obtained a point distribution model (PDM), allowing shape-inherent point (1,002 vertices) correspondences across subjects (Figures 2D, 2E). 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 first order ellipsoid (Figure 2E).[52] Vertexwise displacement vectors were then calculated between each individual and the template (Figure 2F).[37] 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.[38] By projecting these jacobian determinants back onto the surface using trilinear interpolation and subtracting one from them, we obtained a metric of growth ($J > 0$) or shrinkage ($J < 0$) in a unit-size cube defined on each vertex (Figure 2F). We have previously shown that volume change at a vertex is driven by the structure underneath the surface.[38] Given that the surface-based analysis was performed in stereotaxic space, correction for differences in overall intracranial volume was unnecessary.

Statistical Analysis

To compare demographics, self-reported questionnaire 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 averaging them to represent each domain: working memory, executive functioning, verbal information processing, verbal memory, visual memory, and language processing.

Analyses to determine HV was performed using SurfStat toolbox[53] for Matlab (R2007a, The Mathworks, Natick, MA, USA). Global HV and surface-based local volumes were standardized relative to the distribution of controls using a Z-score transformation. Because no laterality of volume changes was expected in relation to insomnia,[23] we averaged the left and right volumes. In particular, local volumes were averaged at the same location between the left and right hemispheres. All the following statistical tests included BDI-II as a covariate because patients showed elevated depressive mood although this was clinically insignificant. (1) Group comparison: We compared differences in global volume between patients and controls using two-tailed *t*-tests. To assess local volume differences, we repeated the same test on vertex-wise jacobian determinants at each vertex. To assess a possible hemispheric

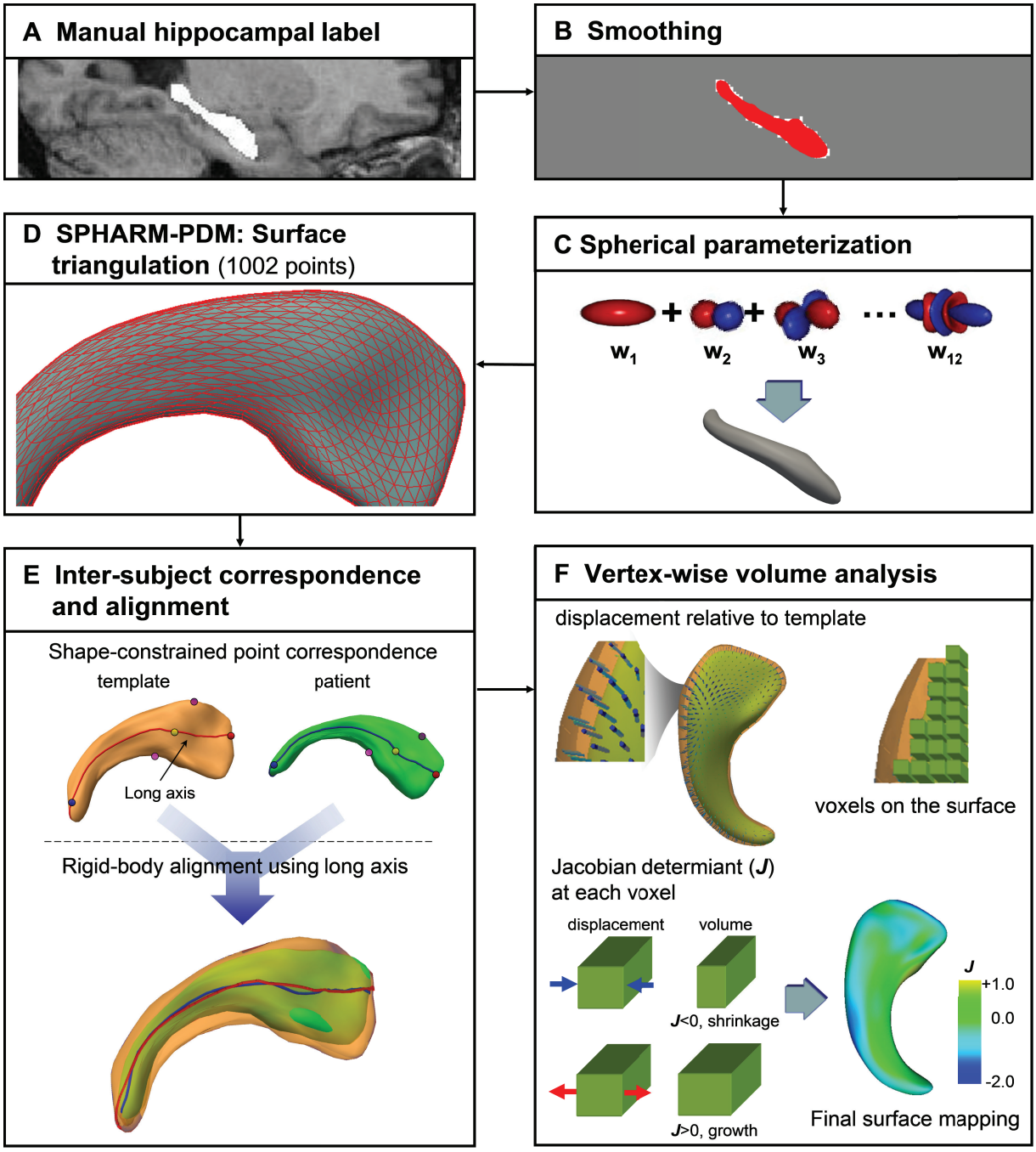

Figure 2—Flowchart of the surface-based analysis procedure. Manually segmented labels **(A)** are first minimally smoothed **(B)** and then converted to surface meshes for which a spherical parameterization (SPHARM) is computed using area-preserving, distortion-minimizing mapping **(C)**. Based on a uniform icosahedron-triangulation of the SPHARM, we obtain a point distribution model (PDM) **(D)**, allowing shape-inherent point (= vertices) correspondences across subjects **(E)** (top). Individual SPHARM-PDM surfaces are rigidly aligned to the template shape **(E)** (bottom). Vertex-wise displacement vectors are then calculated between each individual and the template **(F)** (top). Displacement vectors are diffused to voxels **(F)** (top) enclosed by the surface using a heat equation, yielding voxelwise vector field. To assess local volume changes, we calculate jacobian determinants (J) from this vector field. By projecting this metric back onto the surface using tri-linear interpolation, we finally obtain a metric of growth (J > 0) or shrinkage (J < 0) at each vertex **(F)** (bottom).

asymmetry in the changes in patients compared to controls, we first computed asymmetry in volume differences between hemispheres (i.e., 2 × (L − R) / (L + R) where L and R denote the left and right hemisphere) and further performed a *t*-test on this metric between patients and controls. (2) Reliability test: To test the reproducibility of our surface-based analysis, we performed a bootstrap analysis in which we iteratively selected random subsamples of the whole dataset (i.e., 10,000 times) and performed group comparisons at each iteration. To evaluate the consistency of patterns of volume change, we mapped the probability of having significant volume changes across all iterations on the template surface. (3) Association with clinical and neuropsychological parameters: To assess the association of volume changes with clinical demographics, PSG

and neuropsychological parameters, linear models were applied to compute Pearson correlation coefficient *r* while controlling for age, sex, and depressive mood. For local volume changes, the correlation was performed on individual measurements averaged within each significant cluster, as determined by group comparison. (4) Correction for multiple comparisons: In all vertex-wise surface analyses, significances were thresholded using the false discovery rate (FDR) procedure,[54] with FDR < 0.05. In the volumetric analysis, the Bonferroni adjustment was applied to control the family-wise error rate. (5) Localization of findings: We schematically outlined hippocampal subfields (Figure 1) on the surface template based on Duvernoy atlas[6] and its surface reconstructions that were previously published.[27,55] The segmentation protocol excluded the subiculum as the subiculum has been shown to be least affected by sleep deprivation in animal studies[19,20] and an MRI study of patients with posttraumatic stress disorder.[56] Furthermore, the one insomnia study that found significantly smaller HV among patients also excluded the subiculum.[23] We include the subiculum at the level of the hippocampal head because it was difficult to distinguish it from the hippocampus proper at our current spatial resolution. An approximate border was defined between the CA1 and DG on the inferomedial surface of the template. Although the hippocampal head contains all subfields, the portion of DG is relatively small and the DG and the CA2-4 situate in the middle.[6,57] Thus, we kept only the CA1 subfield on our surface atlas in the hippocampal head as in the previous atlases.[27,55]

Table 1—Characteristics of patients with chronic primary insomnia and controls

	Patients (n = 27)	Controls (n = 30)	P
Demographics			
Mean age, y	51.2 ± 9.6	50.4 ± 7.1	0.7
Female, n (%)	25 (93)	28 (93)	0.7
Duration of insomnia, y	8.4 ± 9.1	–	–
Body mass index, kg/m^2	25.5 ± 2.5	26.3 ± 1.3	0.1
Education, y	13.6 ± 1.9	14.4 ± 1.9	0.1
Beck Depression Inventory-II	10.1 ± 5.2	5.0 ± 4.1	< 0.001
Sleep questionnaires			
Pittsburgh Sleep Quality Index	14.9 ± 4.6	3.2 ± 0.9	< 0.001
Insomnia Severity Index	19.0 ± 5.2	2.4 ± 1.5	0.001
Overnight polysomnography			
Total sleep time, min	350.1 ± 60.0	409.4 ± 29.7	< 0.001
Sleep latency, min	25.5 ± 27.7	8.7 ± 6.2	0.002
REM latency, min	140.7 ± 77.3	83.1 ± 28.9	< 0.001
Sleep efficiency, %	80.8 ± 10.0	89.2 ± 5.4	< 0.001
WASO, min	59.2 ± 38.7	41.2 ± 25.3	0.04
Arousal index, /h	13.3 ± 6.6	9.4 ± 6.0	0.03
Apnea-hypopnea index, /h	2.5 ± 2.7	1.1 ± 1.6	0.02
PLMS index, /h	4.0 ± 6.0	1.8 ± 6.3	0.2
Movement Arousal Index, /h	1.0 ± 1.7	2.6 ± 7.6	0.3
N 1, %	13.3 ± 6.0	13.2 ± 6.0	1
N 2, %	62.7 ± 12.3	53.8 ± 6.0	0.001
N3, %	4.9 ± 6.8	9.0 ± 7.4	0.03
REM sleep, %	19.2 ± 9.0	23.6 ± 3.6	0.02

PLMS, periodic limb movement in sleep; REM, rapid eye movement; WASO, wakefulness after sleep onset.

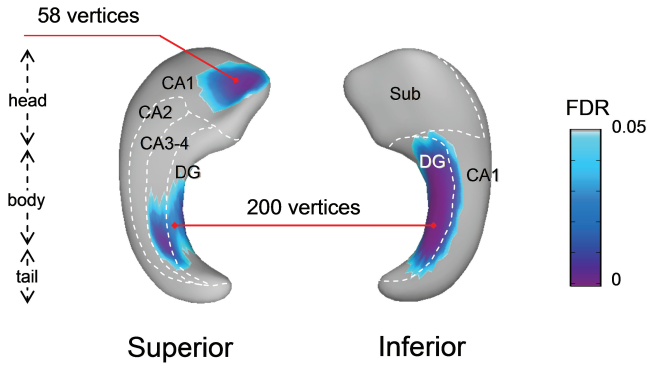

Figure 3—Vertexwise group comparison between patients with chronic primary insomnia and controls. Regions of volume decrease in patients relative to controls are shown. Significances are thresholded at FDR < 0.05.

RESULTS

Demographics, Self-Report Questionnaires, and Overnight Sleep Studies

All subjects were right-handed. There was no difference in the mean age or sex ratio between patients and controls (P > 0.7). Subjective sleep quality was determined by the PSQI, which was assessed during the 2 w prior to the study; as expected, the mean PSQI scores were significantly higher in patients than in the controls. Patients also reported severe symptoms and had significantly higher scores on the depressive mood index compared to controls (P < 0.001), although they reported no current or lifetime psychiatric disorders during the structured clinical interview. Detailed results are summarized in Table 1.

Neuropsychological Tests

Patients had significantly lower scores in working memory, verbal information processing, verbal memory, verbal fluency, and visual memory (P < 0.0001), but not executive functioning (P = 0.6) when compared to controls. The details of neuropsychological tests are summarized in Table 2.

Global and Local Volume Changes in Patients with Chronic Insomnia Relative to Controls

A two-tailed *t*-test uncovered a significant decrease in the HV of patients versus controls (left: 2980 ± 283 versus 3197 ± 337 mm^3; right: 3079 ± 298 versus 3247 ± 404 mm^3, P < 0.05). This change was hemispherically symmetric; asymmetry between patients and controls did not differ (P > 0.2, i.e., patients presented a 3% volume difference between the left and right hippocampi and controls also presented a similar asymmetry (2%). Results of vertex-wise comparisons between groups are shown in Figure 3. In patients, hippocampal atrophy was identified in all subfields. Vertex-wise asymmetry replicated this symmetric change (FDR > 0.1). We separately mapped regional volume changes in patients

Table 2—Neuropsychological analyses of patients with chronic primary insomnia and controls

	Patients (n = 27)	Good sleepers (n = 30)	P
Working memory composite score	-0.17 ± 0.42	0.36 ± 0.36	< 0.001
Digit span forward	7.6 ± 2.0	9.5 ± 2.0	
Digit span backward	5.4 ± 1.5	8.6 ± 2.1	
Corsi block forward	7.8 ± 1.9	10.7 ± 1.8	
Corsi block backward	7.5 ± 1.6	9.9 ± 0.9	
Trail-making test A	57.4 ± 52.4	34.8 ± 10.4	
Trail-making test B	125.9 ± 94.2	82.0 ± 30.1	
Executive functioning composite score	-0.05 ± 0.87	0.05 ± 0.49	0.6
Stroop word correct responses	112 ± 1.0	106.0 ± 14.9	
Stroop word correct responses time	112.7 ± 11.8	108.4 ± 12	
Verbal information processing composite score	-0.47 ± 1.03	0.4 ± 0.8	< 0.001
Digit symbol test	49.5 ± 12.5	65.2 ± 12.1	
Verbal memory composite score	-0.53 ± 1.05	0.48 ± 0.64	< 0.001
Korean California Verbal Test			
Total	45.4 ± 12.2	57.2 ± 7.4	
Short delay free recall	9.4 ± 2.8	12.5 ± 3.0	
Long delay free recall	9.7 ± 3.3	12.7 ± 2.7	
Recognition	14.4 ± 1.3	15.1 ± 1.3	
Verbal fluency composite score	-0.22 ± 0.83	0.20 ± 0.71	< 0.0001
Controlled Oral Word Association Test			
Phonetic word fluency	27.9 ± 11.9	32.1 ± 12.7	
Semantic word fluency	31.6 ± 8.2	36.2 ± 7.7	
Animal	15.2 ± 4.81	20.8 ± 5.1	
Supermarket	16.3 ± 5.2	15.4 ± 4.8	
Visual memory composite score	-0.41 ± 0.77	0.37 ± 0.62	< 0.001
Rey Complex Figure Test			
Copy	30.9 ± 6.82	34.6 ± 2.5	
Immediate recall	13.8 ± 7.7	21.6 ± 7.3	
Delayed recall	13.7 ± 7.8	20.8 ± 7.1	
Recognition	19.5 ± 1.9	20.8 ± 1.9	

compared to controls in left and right hippocampi (Figure S1, supplemental material). This confirmed that the pattern of regional atrophy was similar between the left and right hippocampi. The largest cluster of atrophy was detected at the level of the hippocampal body and tail (FDR < 0.005, 200 vertices) and located medially, mainly within the region corresponding to the combined region of CA2-4 and DG (Figure 1A). Atrophy at the level of the head was present on the superomedial surface corresponding to CA1 (FDR < 0.05, 58 vertices). There was no local volume increase in patients relative to controls (FDR > 0.1). Bootstrap reproducibility analysis revealed that the proposed surface-based analytic platform reproduced the same patterns of atrophy in more than 85% of the random subsampling iterations (Figure S2, supplemental material).

Association Between Hippocampal Volume, and Clinical and Neuropsychological Parameters

We found that age, sex, and education did not correlate with HV in patients or controls (P > 0.2). Depressive mood (BDI-II) was associated with smaller HV (r = -0.39, P < 0.05). In patients, hippocampal atrophy were associated with worse sleep quality (PSQI) (r = -0.40, P < 0.05) and a higher arousal index on the overnight PSG (r = -0.44, P < 0.05).

Surface-based analysis revealed that the degree of volume loss in the cluster located at CA1 subfield (Figure 3A) was associated with a higher arousal index (r < -0.50, P < 0.005) as well as higher PSQI (r = -0.45, P < 0.01, Figure 3B). No significant correlations were found between surface-based volume and insomnia duration or other PSG parameters (|r| < 0.23, P > 0.2).

The atrophy of DG and CA2-4 (Figure 4A) was associated with decreased verbal memory, processing, and fluency scores (r > 0.51, P < 0.005; Figure 4C). Volume asymmetry (left smaller than right) of this area was also associated with impaired verbal processing (r = 0.50, P < 0.005).

The CA1 atrophy of the hippocampal head (Figure 4A) did not correlate with any neuropsychological parameters (r < 0.30; P > 0.1).

DISCUSSION

The current study investigated hippocampal subfield volume changes in patients with chronic primary insomnia compared to controls, and the association of specific hippocampal subfield volume differences with clinical and neuropsychological parameters.

Reduced Hippocampal Volume in Patients with Chronic Primary Insomnia

Our study uncovered bilateral atrophy across subfields in patients. Surface-based analysis revealed that this atrophy mainly corresponded to the region CA2-4-DG of the hippocampal body and tail, and CA1 subfield of the hippocampal head.

Animal studies have reported that sustained sleep fragmentation reduced neurogenesis in hippocampal subfields, particularly in the DG.[19,20] In patients with chronic insomnia, sleep fragmentation correlates with higher cortisol levels in the evening and nighttime periods.[58] The hippocampus contains a higher density of glucocorticoid receptors, which make it more vulnerable to long-term stress than most other brain areas.[59] Chronic elevations in corticosterone have been shown to suppress synaptic plasticity in the DG.[60] Chronic restraint and stress-related steroids have been shown to cause atrophy of apical dendrites of CA3 pyramidal neurons[61,62] and result in the depletion and reorganization of synaptic vesicles in mossy fiber terminals in the CA3.[61] Supporting this notion, it has been reported that patients with Cushing disease[62] and those with posttraumatic stress disorder,[63] both of whom have increased levels of cortisol, have hippocampal atrophy.

Patients in our study suffered from long-term sleep disturbances (mean 8.4 y) and their PSG findings supported evidence

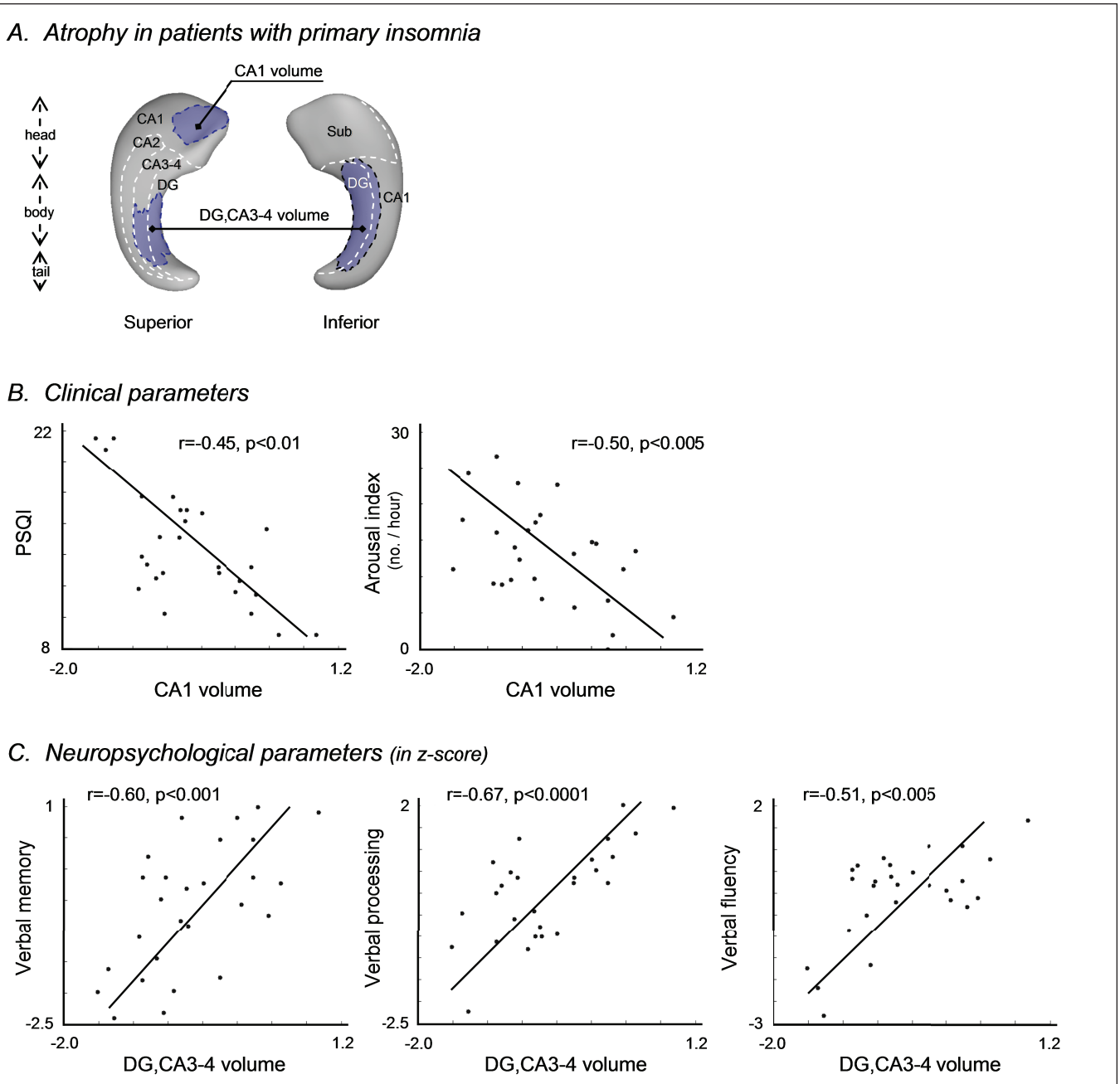

Figure 4—Association between hippocampal subfield volume and clinical **(B)** and neuropsychological **(C)** parameters in patients with chronic primary insomnia. For each cluster representing significant volume loss in patients relative to controls **(A)**, its mean volume is correlated with given clinical or neuropsychological parameter while controlling age, sex and depressive mood. Linear regression models are plotted for significant correlations. PSQI, Pittsburgh Sleep Quality Index.

of their sleep fragmentation, indexed by increased wakefulness after sleep onset, frequent arousals, and reduced sleep efficiency compared to controls. Higher arousal indices on PSG and PSQI scores were significantly associated with smaller CA1 subfield volumes in patients. It has been found that melatonin production is significantly diminished in chronic insomnia patients with sleep fragmentation.[23] Furthermore, melatonin has demonstrated protective effects in CA1 neuronal density in animal models.[64]

Our findings therefore suggest that a combined effect of disturbed sleep in patients and related chronic stress conditions may be related to the structural alteration of subfields of the hippocampal formation.

Relationship Between Hippocampal Atrophy and Cognitive Function in Patients

In the current study, patients showed cognitive impairment in verbal information processing, verbal memory, verbal processing, and visual memory compared to controls. These parameters correlated significantly with volume alterations in the cluster overlapping with the CA2-4-DG region.

The DG has been of particular interest in human cognition because dentate granule cells that are generated continuously in the adult mammalian brain[65,66] play an important role in regulating cognition[67] and storing new memories.[68] Sleep disturbance suppresses adult DG neurogenesis, especially with

prolonged sleep disruption, which may have implications for patients with chronic insomnia who have had long-term sleep disturbance.[19,20,69,70] An MRI study that performed volumetry in patients with posttraumatic stress disorder also showed a negative correlation between insomnia symptom severity and volumes of the CA3/DG subfields.[56] It is therefore tempting to speculate that structural alterations of specific hippocampal subfields, which are secondary to prolonged sleep disruption, may lead to the impairment of cognition in patients.

The relationship between hippocampal atrophy and cognitive impairment may also be explained by a disorganization of the hippocampal circuit. The hippocampal formation is organized as a mainly unidirectional circuit made up of multiple subregions — the entorhinal cortex, the DG, the CA1 and CA3 subfields, and the subiculum.[18] These subregions differ in their cellular organization and connectivity[71] and thus, potentially make distinct contributions to the known stages of memory processing.[62,72] A previous neuroimaging study has also demonstrated that early subregions in the hippocampal circuit (DG and CA2-3) are more selectively active during episodic memory creation, whereas the next subfield in the circuit (subiculum) is more involved in the retrieval process.[73] Atrophy found in our study may be macroscopic, which is indicative of cytoarchitectonic damage in the CA2-4-DG and therefore may impair the encoding process rather than the retrieval process. This hypothesis is consistent with patients with chronic insomnia complaining of difficulties with episodic memory.[4] An MRI volumetric study of patients with subjective memory impairment confirmed that performance in memory acquisition through training is related to the volume of a combined region of CA2-4 and DG in the hippocampus.[74]

Our analysis revealed that the volume atrophy of the CA3-4-DG subfield was particularly associated with verbal-specific domains (information processing, memory, and fluency). Notably, volume asymmetry (left smaller than right) of this region in patients was also associated with impaired verbal processing. Sleep loss impedes the spontaneous generation of words during verbal word fluency tasks and the articulation of speech during vocalized reading tasks in healthy subjects[75] and patients with chronic insomnia.[76] The hippocampus is a functional network hub for memory. Information is continuously exchanged in a network of brain areas centered at the hippocampus and including the neocortex and other key structures such as the amygdala and the striatum.[77] Verbal memory is processed asymmetrically between the cerebral cortices. Encoding of verbal memory preferentially relies on left hemispheric brain regions including the hippocampus.[78] Functional MRI study have confirmed that the left hippocampus is highly activated during verbal memory tasks.[79] Moreover, the hippocampus is integrally involved in the retrieval and binding of information, critical for language processing.[80] The extent of hippocampus remaining after an anterior temporal lobectomy in patients with left mesiotemporal sclerosis was significantly related to verbal fluency after surgery.[81] Therefore, our finding that left hippocampal atrophy is related to poor performance of verbal memory and verbal fluency in patients may be explained by a pathological disruption in the brain network involved in verbal-specific domain processing.

Comparison with Other Hippocampal Volumetry Studies

Manual volumetry has been considered a standard approach to assess hippocampal structural alterations in patients with chronic insomnia. Despite a generally accepted sensitivity of this technique, because of the precise delineation of the anatomy, inconsistent reports have been published. This inconsistency may caused by variations in which substructures were included in the delineation of the hippocampus. Our study and a previous study[23] excluded the subiculum from delineation and found significantly smaller HV among patients, whereas other studies[22,24] included the subiculum and did not uncover these differences. Moreover, we studied a large sample size compared to all previous studies except one.[82] The increased significance of our results may therefore be a consequence of increased statistical power due to our large database as well as the exclusion of the subiculum, which occupies more than 20% of the HV.[32,33] The subiculum is least likely to be affected by sleep deprivation, as suggested in animal studies[19,20] and an MRI study of patients with posttraumatic stress disorder in which low sleep quality was assessed.[56]

To the best of our knowledge, our study is the first to localize HV changes in patients with primary insomnia using a surface-based analytic model. This advanced approach, relying on mesh parameterization and the use of smoothness constraints, is known to enable the localization of volume changes as small as the voxel size.[36,37,83] Indeed, our surface mapping unveils region-specific volume reductions in the hippocampus related to chronic sleep disturbance. A vertex-wise correlation of subfield volumes with clinical and neuropsychological parameters allows us to find relationships among structural changes, insomnia severity, and cognitive impairments. However, our technique has a limitation in that it differs from manual hippocampal subfield volumetry.[30–33] It is difficult to distinguish subfields at the current image resolution (1 mm^3), thus we adopted a pointwise analytic platform and interpreted changes in patients by corresponding them to a schematic atlas of the subfields based on a histology study[6] as well as previously published surface-based atlases.[27,55] The localization of atrophy to subfields in patients was thus approximate.

CONCLUSION

Our study provides evidence of disturbed sleep in patients with primary insomnia affecting the hippocampal substructure. Our primary findings indicate that patients exhibit bilateral atrophy corresponding to the CA2–4-DG of the hippocampal body and tail and CA1 of the hippocampal head. A decrease in sleep quality was closely related to the degree of hippocampal CA1 atrophy. Furthermore, atrophy located in the CA2-4-DG region was associated with impaired cognitive functions in patients with PI. These observations may provide insight into the pathophysiological mechanisms that make patients with chronic sleep disturbances vulnerable to cognitive impairment.

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DISCLOSURE STATEMENT

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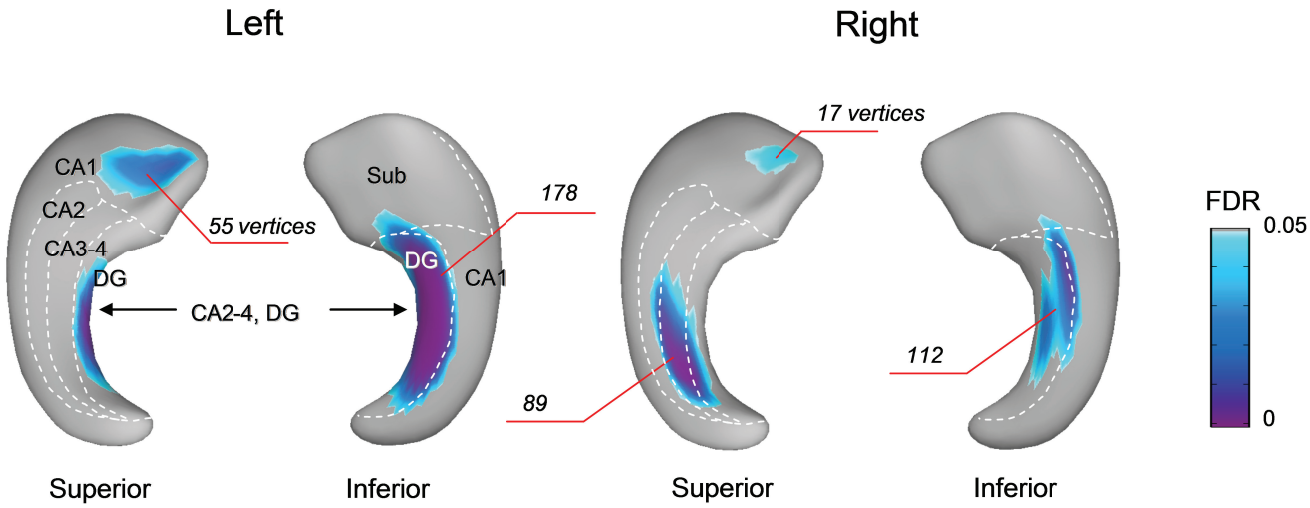

Figure S1—Vertexwise group comparison performed separately in each hemisphere. Pattern of atrophy was very similar between hemispheres and to that found when hemispheres were pooled (Figure 3).

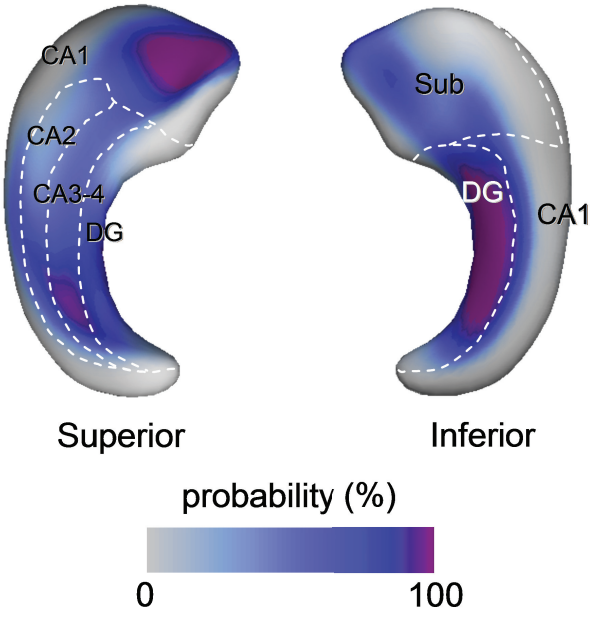

Figure S2—Reproducibility of our surface-based analytic framework. Bootstrap reproducibility analysis revealed that the proposed method highly reproduced the pattern of atrophy in Figure 3 as the same pattern was found in more than 85% of the random subsampling.