Atrophy of the hippocampal CA1 subfield relates to long-term forgetting in focal epilepsy

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Abstract

Objective: The mechanisms underlying accelerated long-term forgetting (ALF) in patients with epilepsy are still under investigation. We examined the contribution of hippocampal subfields and their morphology to long-term memory performance in patients with focal epilepsy.

Methods: We prospectively assessed long-term memory and performed magnetic resonance imaging in 80 patients with focal epilepsy (61 with temporal lobe epilepsy and 19 with extratemporal lobe epilepsy) and 30 healthy controls. The patients also underwent electroencephalography recording. Verbal and visuospatial memory was tested 30 seconds, 10 minutes, and 1 week after learning. We assessed the volumes of the whole hippocampus and seven subfields and deformation of the hippocampal shape. The contributions of the hippocampal volumes and shape deformation to long-term forgetting, controlling for confounding factors, including the presence of interictal epileptiform discharges, were assessed by multiple regression analyses.

Results: Patients with focal epilepsy had lower intelligence quotients and route recall scores at 10 minutes than controls. The focal epilepsy group had smaller volumes of both the right and left hippocampal tails than the control group, but there were no significant group differences for the volumes of the whole hippocampus or other hippocampal subfields. Multiple regression analyses showed a significant association between the left CA1 volume and the 1-week story retention ($\beta = 7.76$; Bonferroni-corrected $P = 0.044$), but this was not found for the whole hippocampus or other subfield volumes. Hippocampal shape analyses revealed that atrophy of the superior-lateral, superior-central, and inferior-medial regions of the left hippocampus, corresponding to CA1 and CA2/3, was associated with the verbal retention rate.

Significance: Our results suggest that atrophy of the hippocampal CA1 region and its associated structures disrupts long-term memory consolidation in focal epilepsy. Neuronal cell loss in specific hippocampal subfields could be a key underlying cause of ALF in patients with epilepsy.

Keywords: accelerated long-term forgetting; memory consolidation; hippocampal subfield; FreeSurfer; shape analysis; long-term memory

Key points:

- We assessed the relationship between hippocampal subfield volumes and ALF in 80 patients with focal epilepsy
- The left CA1 was the only subfield markedly associated with worsening of verbal long-term memory after controlling for confounding factors
- Shape analysis showed atrophy of superior-lateral, -central, and inferior-medial regions of the left hippocampus was associated with ALF

Introduction

Many people with epilepsy suffer from not only seizures, but also various comorbidities, including cognitive disorders. Among these, memory dysfunction is one of the most common complaints. However, short-term memory loss is not always detected, even with subjective complaints. Recent studies have shown that patients may have a newly-described form of memory dysfunction, accelerated long-term forgetting (ALF), where there is a rapid loss of newly-acquired memories over days to weeks, despite normal retention at shorter intervals.[1] This impairment was first described in patients with transient epileptic amnesia,[2] but recent work has also reported ALF in other epilepsy syndromes,[3] presymptomatic Alzheimer's disease,[4,5] and traumatic brain injury.[6]

Various theories have considered which pathophysiological factors might contribute to ALF, highlighting mood disorders, medications, epileptiform activity, and structural brain abnormalities. The latter two are considered the most plausible causes of ALF,[7] and recent studies have emphasized the role of epileptic discharges. For instance, recurrent seizure or subclinical seizure activity has been shown to contribute to ALF.[8,9] Animal and human studies have suggested that interictal epileptic discharges (IED) disturb memory consolidation during non-rapid eye movement sleep.[10,11] However, structural factors could also underlie ALF, given that it is observed in conditions other than epilepsy.[4–6]

Previous work has investigated the relationship between ALF and structural abnormalities of the hippocampus, which plays a predominant role in memory processes. Studies have reported that the hippocampal volume only relates to early recall and not to ALF in patients with epilepsy.[12,13] The focus on the whole hippocampal volume may have obscured any subtle ALF-related structural differences, because the hippocampus comprises functionally distinct subfields. Recent animal and human studies have characterized the

temporal aspects of the hippocampal subfields' functional specialization in memory.[14] There is evidence that the cornu ammonis (CA) 3 subfield plays an important role in short-term to intermediate-term episodic memory, and the CA1 region in intermediate-term and long-term memory.[15–17] Patients with a specific type of hippocampal sclerosis with predominant neuronal loss in CA1 have been reported to show preserved short-term memory, unlike patients with other types of hippocampal sclerosis where there is diffuse neuronal loss across all subfields or predominant cell loss in CA4, CA3, and the dentate gyrus.[18] Consistent with this finding, we hypothesized that CA1 atrophy relates to the ALF observed in patients with epilepsy. To test this hypothesis, we conducted a prospective study with a larger sample size than in previous, similar studies to examine the impact of the hippocampal subfield volumes and hippocampal shape on long-term forgetting while controlling for several confounding factors.

Materials and methods

Participants

This prospective study recruited outpatients from two tertiary referral hospitals (Kyushu University Hospital and Fukuoka Sanno Hospital, Fukuoka, Japan) between October 2017 and July 2019. The inclusion criteria were as follows: (1) between 20 and 70 years of age, (2) clinically diagnosed with focal epilepsy, and (3) fluent in written and spoken Japanese. The exclusion criteria were as follows: (1) history of epilepsy surgery, (2) history of neurodegenerative or cerebrovascular disease, (3) psychiatric comorbidities requiring medical intervention, such as depression, (4) contraindications for MRI, and (5) intelligence quotient (IQ) less than 70.

Focal epilepsy was categorized as either temporal lobe epilepsy (TLE) or extratemporal lobe epilepsy (ETLE); we defined ETLE as having a seizure focus outside of the temporal lobe or at an undetermined location (see Supporting Methods “Details of participants”).

In total, data were analysed for 80 patients with focal epilepsy and 30 controls. The imaging data included 76 patients and 27 controls. The institutional review boards at Kyushu University Hospital and Fukuoka Sanno Hospital approved this study, and written consent was obtained from all participants in accordance with the Declaration of Helsinki.

Neuropsychological assessment

Neuropsychological assessment included the Wechsler Adult Intelligence Scale Third Edition short form, which included the Digit Symbol, Matrix Reasoning, Digit Span and Information subtests.[19] We also assessed the Japanese Standard Verbal Paired-Associate Learning Test,[20] Benton Visual Retention Test,[21] Test of Lexical Processing in Aphasia,[22] and Trail Making Tests A and B.[23] The Hospital Anxiety and Depression Scale (HADS)[24] was used to determine depression and anxiety levels, and self-ratings of everyday memory problems and spatial navigation ability were assessed using the Everyday Memory Questionnaire-Revised Version[25] and the Santa Barbara Sense of Direction Scale.[26]

We adopted the methods of Cassel *et al*.[27] to measure long-term forgetting rates, and developed a localized version with some modifications. The test consisted of prose passages for the verbal tasks and videos of routes for the visuospatial tasks, tested at 30 seconds, 10 minutes, and 7 days after learning. Details of the test procedure are presented in the Supporting Methods.

Hippocampal subfield volumetry

MR images were obtained using a Philips Achieva 3.0-T system at each hospital. 3D T1-weighted magnetization-prepared rapid acquisition gradient echo images (repetition time = 7.5 milliseconds, echo time = 3.5 milliseconds, flip angle = 8°, in-plane resolution = 0.47 × 0.47 mm^2, 0.5-mm slice thickness with no gap) and high-resolution T2-weighted images (repetition time = 6000 milliseconds, echo time = 78 milliseconds, flip angle = 90°, in-plane resolution = 0.43 × 0.43 mm^2, 2.0-mm slice thickness with no gap) were acquired for all subjects.

The estimated total intracranial, hippocampal, and hippocampal subregion volumes were calculated using FreeSurfer version 7.1.0 (Figure S1).[28,29] All MR images and segmentations were visually checked to ensure the quality of the analyses. The hippocampal volumetry analysis was hypothesis-driven, and we specifically selected the whole hippocampus and the CA1, CA2/3, CA4 and dentate gyrus subfields. We considered the subicular complex (subiculum, presubiculum and parasubiculum) as one structure because, in general, the inner subfields are functionally indistinctive. The whole hippocampal and subfield volumes were normalized using the estimated total intracranial volume using the residual method.[30] For the multiple regression analyses, the volumetric results were converted to z-scores and then entered into the model.

Shape analysis

To investigate local anatomical differences in the hippocampus, we applied a standard analysis pipeline for subcortical shape analysis developed by ENIGMA (http://enigma.ini.usc.edu).[31] Briefly, a mesh model was created for the hippocampal boundary using the segmentation data created using FreeSurfer. Its surface was then spherically inflated and registered to a template model with 2502 vertices by matching local

geometric features. The thickness, defined here as the distance from a vertex to the medial curve of the hippocampus, was extracted and used for the statistical analyses. We correlated the thickness with volumetry using volume-to-shape correlation analysis (see Supporting Methods).

Electroencephalography recording and evaluation

All patients underwent sleep EEG. Scalp electrodes were placed according to the International 10–20 system. EEG recordings were obtained just before the neuropsychological assessment. Patients were instructed to sleep during the recording. Two epileptologists independently annotated IEDs in the whole recording. The number of spikes was divided by the total recording time (minutes) to calculate the spike frequency. Examiners were blinded to all patient information (see Supporting Methods).

Statistical analysis

Statistical analyses were performed using R (v4.0.5, www.R-project.org). For between-group comparisons, categorical variables were analysed using Pearson's chi-squared tests, and continuous variables using two-sample t-tests or Mann–Whitney U tests. Multiple linear regression analyses were conducted to examine the effects of group, hippocampal and hippocampal subfield volumes, and hippocampal shape on the ALF test measures. The ALF test scores for 10-minute recall were used to evaluate initial retention; the retention rate between the 10-minute and 1-week recall tests (1-week retention) was used to evaluate long-term forgetting, which was calculated as follows: (1-week retention) = (1-week recall score) / (10-minute recall score) × 100. Because the 1-week story retention was truncated at zero, a Tobit (censored) regression model was used[32] with the R package VGAM.[33] For the other test measures, multiple linear regression analysis was used. Initially, group comparisons were

performed while adjusting for age, IQ and test set. Subsequently, in the volumetric analysis, which examined the effect of hippocampal and hippocampal subfield volumes in the focal epilepsy group, we controlled for age, IQ, test set, HADS score, medication and hippocampal sclerosis for the 10-minute recall score; for 1-week retention, we further controlled for seizures during the retention period and the presence of IEDs on scalp EEG. We analysed each structure separately, and Bonferroni correction was applied for multiple comparisons. Post hoc analyses were carried out if the volume of a structure showed a significant effect on ALF test measures. We extracted the subgroup of patients with focal epilepsy whose volume was below the lower quartile, and then performed pairwise comparisons between this subgroup and healthy controls. See the Supporting Methods for details of the subgroup and shape analyses.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Results

Baseline characteristics, clinical data and neuropsychological performance

The baseline characteristics, clinical characteristics and neuropsychological performance of the study population are shown in Table 1. In total, data from 80 patients with focal epilepsy (61 with TLE and 19 with ETLE) and 30 healthy controls were analysed. The mean age of patients with focal epilepsy was 36.9 (SD 11.8) years, including 32 male and 48 female

patients. Thirty-four patients (43%) were suspected to have a left seizure focus. No significant differences in age, sex or educational level were found between the two groups. However, the focal epilepsy group had a lower estimated full-scale IQ score than the controls ($P = 0.0096$). Patients with focal epilepsy had a higher HADS score than the controls ($P < 0.001$), and they tended to have a higher self-rating of their memory (Everyday Memory Questionnaire-Revised score), although this difference was not significant ($P = 0.07$). Short-term verbal memory (Standard Verbal Paired-Associate Learning Test) was impaired in some patients: 20% of patients with a bilateral seizure onset, 15% with a left seizure onset, 8% with a right seizure onset and 7% with an undetermined seizure onset. Fourteen patients (18%) were identified as having hippocampal sclerosis based on the MRI scans. Additional lesions detected using MRI, other than those in the hippocampus, are summarized in Table S1.

Long-term verbal and visuospatial memory performance

Long-term forgetting results for both the story and route tasks are shown in Table 1, Figure 1A and 1B. For the story task, all participants reached the 80% learning criterion. After adjusting for age, IQ and test set, there were no significant differences between the focal epilepsy group and the controls in terms of the number of trials required and the recall scores at different intervals. For the route task, four (5.5%) of the 73 patients with focal epilepsy did not reach the 80% learning criterion. The focal epilepsy group had significantly lower 10-minute recall scores than the controls ($P = 0.018$). All participants completed both tasks, but the route tasks were upgraded after the first seven participants, so these initial results were excluded. The focal epilepsy group had poorer 1-week retention for the story task than the controls, but this difference was not significant (adjusted difference in mean: 10.7, $P = 0.19$); there was no marked difference for the route task (1.19, $P = 0.82$).

MRI volumetric and EEG results

For the volumetric analyses, one patient and two healthy controls were excluded because they did not have MRI data; four additional participants (three patients and one control) were excluded because of poor segmentation results. The intraclass correlation coefficient of hippocampal subfield volumes among the healthy controls was 0.951, suggesting excellent reproducibility of the volumetry. The focal epilepsy group had significantly smaller volumes of both the right and left hippocampal tails than the healthy controls (right, $P = 0.0027$; left, $P = 0.0011$; Table 2 and Figure S2). There were no other significant differences between these two groups, including for the volume of the whole hippocampus and the other hippocampal subfields.

IEDs were observed in 36 (47%) of the 76 EEG recordings. Sixty-seven (88%) patients entered N2 sleep during the recordings.

Multivariate analyses assessing the contribution of the whole hippocampal and CA1 volumes to ALF

Multiple regression analyses were performed using the long-term forgetting scores as the outcome parameter to evaluate the specificity of the association between the memory scores (dependent variable) and the whole hippocampal and CA1 volumes, while controlling for confounding variables (independent variables). Other subfield volumes (CA2/3, CA4, dentate gyrus, molecular layer, subicular complex and tail) were also evaluated to examine subfield specificity. The results are shown in Table 3. The analyses showed a significant association between the left CA1 volume ($\beta = 7.76$; Bonferroni-corrected $P = 0.044$) and the 1-week story retention, but this was not found for the whole hippocampal or other subfield volumes. There were no significant relationships between the subfield volumes and memory scores for

the 10-minute story recall or route task tests. To determine the contribution of the left CA1 volume to verbal memory trajectories, we extracted a subgroup of patients whose left CA1 volume was below the lower quartile (n = 19), and compared them with healthy controls (Figure 1C). This subgroup showed significantly lower 1-week story retention (i.e., forgot faster) than healthy controls (P = 0.025).

Post hoc subgroup analyses

To explore the consistency of the relationship between the left CA1 volume and 1-week story retention in the subgroups of patients with focal epilepsy, we performed post hoc analyses for three subgroups: TLE with hippocampal sclerosis (TLE-HS), TLE without hippocampal sclerosis (TLE-no) and ETLE.

Baseline characteristics and neuropsychological performance

The baseline characteristics, clinical characteristics and neuropsychological performance of the three subgroups are summarized in Table S2. The estimated IQ was lower in all three subgroups than the controls, but this was only significant for the TLE-no subgroup (P = 0.033). Short-term verbal memory (Standard Verbal Paired-Associate Learning Test) was impaired in 21% of the patients with TLE-HS, 13% of those with TLE-no and 0% of those with ETLE. HADS was highest in the TLE-no subgroup (P < 0.001), followed by TLE-HS (P = 0.038) and then ETLE. Although there was no significant difference between the subgroups and controls on the ALF test story task, the route task 10-minute recall score was significantly lower in the TLE-HS and TLE-no subgroups (P = 0.008 and P = 0.016, respectively). The whole hippocampus and most hippocampal subfields volumes were significantly lower in the TLE-HS subgroup, but not in the others (Table S3 and Figure S2).

Left CA1 volume and 1-week story retention in different subgroups

Pearson correlation analyses revealed a significant relationship between the left CA1 volume and 1-week story retention in the TLE-HS subgroup (r = 0.78, P = 0.005, 95% CI = 0.33, 0.94), but not in the others (TLE-no: r = 0.12, P = 0.44, 95% CI = −0.18, 0.39; ETLE: r = 0.33, P = 0.19, 95% CI = −0.17, 0.69; Figure 2A). Statistical comparison of the correlations revealed that the TLE-HS subgroup had a significantly stronger correlation than the TLE-no subgroup (Zou's 95% CI = 0.14, 1.00; P = 0.016). Because 79% of TLE-HS subgroup had a seizure onset involving the left hemisphere, i.e., the laterality was suspected to be bilateral or left, we compared those with left-sided involvement in each subgroup. This analysis showed moderate to strong correlations between 1-week story retention and the left CA1 volume in all subgroups (TLE-HS: r = 0.82, P = 0.012, 95% CI = 0.28, 0.97; TLE-no: r = 0.51, P = 0.018, 95% CI = 0.10, 0.77; ETLE: r = 0.64, P = 0.17, 95% CI = −0.35, 0.96; Figure 2B). Furthermore, there were no significant group differences in the correlation strength.

Shape Analysis

We analysed the association between the hippocampal shape and both the story and route memory performances. No significant correlations were observed between the hippocampal thickness and the 10-minute recall scores (Figure 3A). For 1-week retentions, a significant correlation was observed between the 1-week verbal retention and the left hippocampal thickness, mainly in the superior-lateral, superior-central, and inferior-medial regions of the left hippocampus (Figure 3B). The volume-to-shape correlation analysis suggested that the thickness of these significant regions was most associated with CA1 volume in the superior-lateral part and CA2/3 volume in the remaining parts (Figure 3C and 3D). No significant correlations were found between the thickness and 1-week route retention.

Discussion

The present study found that the left CA1 volume in patients with focal epilepsy was associated with a verbal memory decline between 10 minutes and 1 week. While the focal epilepsy group did not forget more rapidly than healthy controls in the verbal memory task, a subgroup with a smaller left hippocampal CA1 volume did. The contribution of CA1 atrophy to verbal memory decline was most prominent in the TLE-HS subgroup, but this subgroup difference was no longer apparent when the analyses were restricted to patients with a left-lateralized seizure focus. The shape analyses revealed that the accelerated forgetting of verbal material was related to atrophy of the superior-lateral, superior-central, and inferior-medial regions of the left hippocampus, with the former corresponding to CA1. For the first time, we have revealed the contribution of hippocampal CA1 atrophy to ALF using rigorous statistical methods, controlling for many confounding factors.

Unlike previous studies, we found that 1-week retention of verbal material was not significantly different between the focal epilepsy and control groups. One possible explanation for this finding is that our study population may have had relatively preserved hippocampal function, unlike in past studies.[12,13,27] In line with this, there were no significant group differences for the baseline neuropsychological tests, except for the IQ. Furthermore, the focal epilepsy and control groups did not significantly differ in whole hippocampal or CA1 volumes; a difference was found for the TLE-HS subgroup, but these patients represented only 18% of our study population. Thus, the 1-week retention for the focal epilepsy group may not have been significantly impaired.

We found that patients had poorer visuospatial memory than controls at 10 minutes, unlike the verbal memory task. The rate of visuospatial forgetting from 10 minutes to 1 week did not differ significantly between the two groups. Four patients did not reach the 80%

learning criterion on the visuospatial task, which could have reduced the overall group scores. Other studies have reported a temporal discrepancy between verbal and visuospatial memory performance;[27,34] consistent with these studies, our participants with low 1-week story retention also tended to exhibit earlier forgetting on the route task. This suggests that short-term forgetting in this type of visuospatial task may be a sensitive marker of verbal ALF.[27]

Several previous studies have attempted to clarify the contribution of the hippocampus to ALF, but with no firm conclusions.[12,13,27,35–37] Two studies, one with patients with transient epileptic amnesia[12] and another with patients with TLE,[13] investigated the association between hippocampal volume and ALF, and revealed that reduced hippocampal volume was associated with initial verbal retention, but not with long-term delayed recall. Other studies have argued that hippocampal sclerosis contributes to earlier forgetting at 10 minutes[27] and that other hippocampal lesions contribute to memory loss after 30 minutes or up to 24 hours.[36,37] However, the sample sizes of these studies were too small to perform rigorous statistical analyses or the samples included postsurgical cases. Therefore, it is generally believed that hippocampal structural abnormalities are likely to underlie early forgetting, but not ALF.[35]

Our study clarified the association between volume reduction of the left CA1 and the rate of forgetting verbal material between 10 minutes to 1 week. Animal studies have reported that CA1 plays an essential role in long-term memory consolidation and retrieval.[15] Although controversy exists concerning the role of CA1 in human memory formation,[17,18] delayed verbal recall performance has been related to CA1 atrophy in older adults[38,39] and CA1 neuronal density in presurgical patients with TLE.[17,40] In another study, patients with transient global amnesia who had focal lesions only in CA1, as determined by MRI, showed impaired retrieval of remote episodic memory.[41] Our results provide additional evidence for

the principal role of CA1 in episodic memory. Our results may also explain why ALF is observed in individuals at risk of Alzheimer's disease[4,5] because the hippocampal atrophy in this disease begins in the CA1 region before spreading to other subfields.[42]

Because most of our study population had TLE, the generalizability of the results to focal epilepsy could be questioned. To address this issue, we analysed the data in separate patient subgroups: TLE-HS, TLE-no and ETLE. We found a significant contribution of CA1 to 1-week story retention in the TLE-HS subgroup, but not the other subgroups. However, when the analyses were restricted to patients with a seizure focus in the left hemisphere, all subgroups had a moderate to strong correlation between the CA1 volume and 1-week story retention. This implies that hippocampal damage due to seizures could underlie ALF. The results are compatible with previous studies[43,44] that indicate the specificity of the left hippocampus for verbal memory.

We conducted a shape analysis of the hippocampus to complement our volumetry findings using a priori segmentation. Verbal ALF was associated with reduced hippocampal thickness, mainly in the superior-lateral, superior-central, and inferior-medial regions of the left hippocampus. Although the presence of multiple subfields could have affected the thickness of each point in the shape analysis, the volume-to-shape correlation analysis showed that the superior-lateral region most likely corresponds to CA1 atrophy and the superior-central and inferior-medial regions correspond to CA2/3 atrophy. These findings were inconsistent with the volumetric analysis, which did not detect a significant effect of CA2/3. Because volumetric studies examine a subfield as a whole, shape analysis may be more sensitive than volumetric analysis to subtle atrophy restricted to part of a subfield. Therefore, we speculate that although CA1 is the primary subfield, CA2/3 is the secondary essential subfield for verbal memory retention in patients with focal epilepsy. The CA3-CA1

axis contributes to the generation and spreading of sharp wave-ripples, which is crucial for memory consolidation during sleep.[45] These results, therefore, are consistent with the current concept of memory consolidation, and the CA1 and CA2/3 regions may be associated with ALF in patients with epilepsy.

Although we controlled for the presence of IEDs, our results do not contradict the hypothesis that ALF is a disorder of memory consolidation during sleep caused by epileptic discharges.[12] IED may impair the hippocampal-cortical coupling during non-rapid eye movement sleep that facilitates memory consolidation,[46] as shown in a rat model and in patients with focal epilepsy who underwent stereoelectroencephalography.[10,11] Given that CA1 plays an essential role in memory consolidation during sleep as a generator of hippocampal sharp wave-ripples,[45] it is possible that patients with CA1 atrophy are more vulnerable to memory impairments caused by IED.

Our study has some limitations that should be noted. In the ALF assessment, we tested initial delayed recall at 10 minutes to ensure that the outpatient examinations were completed within a reasonable time, while most previous studies tested at 30 minutes. This factor should be considered when comparing our results with those of previous volumetric studies of ALF.[12,13] Rehearsal effects may have affected the 1-week recall scores, because we did not adopt methods to eliminate this. In addition, our results may have been caused by subtle acquisition or early consolidation deficits, rather than by late consolidation or retrieval. Additionally, sleep quality was not considered in the multivariate analysis, even though this factor may aggravate ALF. The absence of continuous EEG monitoring between the encoding and retrieval phases may have obscured any effects of IED, as determined through the multiple regression analyses. Lastly, our analyses did not include the side of the seizure focus, handedness, age at epilepsy onset, duration of epilepsy or seizure frequency as

explanatory variables because of the sample size, even though these may have contributed to 1-week story retention. Further studies using continuous EEG monitoring with larger sample sizes are needed to clarify the underlying mechanisms of ALF.

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Conflict of Interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Author Contributions

All coauthors were substantially involved in the study and the preparation of the manuscript. TM, TU, TA, KT, OT, and JK took part in the conception and design of the study. TM, TU, JY, TA, SY, NA, and HS acquired the data. TM, TU, JY, TO, and AS analyzed the data. TM, TU, HS, NI, and JK contributed to drafting the manuscript and preparing the figures.

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Supporting Information

Additional supporting information may be found in the online version of the article at the publisher's website.

Figure legends

Figure 1 Long-term forgetting assessment results. Comparison between the recall scores of all patients with focal epilepsy (FE, dotted blue line, n = 80) and healthy controls (HC, solid black line, n = 30) at 30 seconds, 10 minutes and 1 week for the story **(A)** and route **(B)** tasks. **(C)** Trajectories of story task scores for the HC and the subgroup of focal epilepsy patients whose left CA1 volume was below the lower quartile (Low, dash-dotted red line, n = 19). The Low group forgot significantly more rapidly than controls after controlling for age, IQ, and test set. Each recall score and SR is an unadjusted average value. For the route task, the chance level is shown as a dotted line. HC = healthy control; SR = 1-week story retention. *$p < 0.05$.

Figure 2 Scatter plots of the left CA1 volume and the 1-week story retention in each patient subgroup. Pearson's correlation analyses examined the association between the left CA1 volume and the 1-week story retention in each patient subgroup, while controlling for effects of age, IQ, test set, Hospital Anxiety and Depression Scale score, medication, seizures during the retention period and the presence of IED. Scatter plots for all three subgroups **(A)** and those with left-sided involvement **(B)** are shown separately. The Pearson's correlation coefficients and P values are shown in the same colour as the corresponding subgroup. The left CA1 volumes were transformed into z-values. ETLE = extratemporal lobe epilepsy; TLE-HS = temporal lobe epilepsy with hippocampal sclerosis; TLE-no = temporal lobe epilepsy without hippocampal sclerosis.

Figure 3 Hippocampal shape differences associated with story and route memory performances. (A) Right and left hippocampal morphological differences associated with the story and route 10-minute recall scores are shown from superior and inferior views. Z value maps on the left show deformations related to 10-minute recall scores, adjusted for age, IQ,

test set, medication and hippocampal sclerosis. **(B)** Right and left hippocampal morphological differences associated with 1-week story and route retentions. Z value maps on the left show deformations related to 1-week retentions, adjusted for age, IQ, test set, medication, Hospital Anxiety and Depression Scale score, hippocampal sclerosis, the presence of seizures and IED. A positive z value (shades of yellow) indicates a positive correlation with the 10-minute recall score (i.e., a negative correlation with the degree of short-term memory deficit) or 1-week retention (i.e., a negative correlation with the degree of long-term forgetting). P value maps for $p < 0.05$ (FDR corrected) are shown on the right. An approximation of the major hippocampal subfield boundaries is overlaid on the hippocampal surfaces. **(C)** The most highly correlated subfield volume to each vertex based on the left hippocampal shape. The correlation coefficient between the thickness and subfield volumetric measures was calculated for each vertex. Each vertex is coloured according to the subfield with the highest coefficient. **(D)** The most highly correlated hippocampal subfield volume at the vertices in three main regions of the p value map of hippocampal shape difference associated with story 1-week retention (i.e., the overlap of the second panel of B and C). Bar graphs show the number of vertices at which each subfield shows a maximum r value. DG = dentate gyrus; ML = molecular layer; sub = subicular complex.

Supporting Information for "Atrophy of the hippocampal CA1 subfield relates to long-term forgetting in focal epilepsy"

Supporting Methods

Details of participants

Ninety-four patients were initially included in the study, but 14 failed to fulfil the IQ criterion and were thus excluded. For 28% of the patients, the ictal epileptiform activity was captured during routine EEG or video EEG monitoring; for the rest of the patients, only interictal EEG abnormalities were observed. Hippocampal sclerosis was diagnosed based on the MRI scans. The clinical information was collected by an epileptologist (T.M.), and decisions concerning the inclusion/exclusion of patients, the seizure location and laterality, and the presence of hippocampal sclerosis were discussed by a group of epileptologists (T.M., T.U., N.A., H.S.). For the determination of the seizure location and laterality, the semiology, EEG and imaging findings were required to be consistent, and any cases with a discrepancy were classified as 'undetermined'. Thirty healthy controls were recruited from the local community, none of whom had a history of seizures or neurologic disorders. They were matched to the patients in terms of age, sex and educational level. All of the participants were paid for taking part in the study. Data from several participants were excluded from the multiple regression analyses: one patient who did not undergo MRI because of a sudden, unexpected death; two controls who did not undergo MRI; and four participants with poor quality hippocampal segmentation.

Assessment of accelerated long-term forgetting

PowerPoint (Microsoft Corp., WA, USA) was used to play the pre-recorded stories and videos.

The story task consisted of three prose passages, each with 10 cued recall questions (one set of questions is shown in Table S4). Their presentation order was counterbalanced using a Latin square design. Participants were required to listen to each story through a speaker. After listening to the first story, participants were asked to complete a calculation sheet as a distractor task and were then asked the cued recall questions. If they did not reach the 80% learning criterion at the 30-second delay, the story and questions were presented again; this process was repeated a maximum of three times. The remaining two stories were presented the same number of times as the first, and recall was tested at 10 minutes and 7 days after learning. During the 10-minute delay, participants completed neuropsychological questionnaires. A 10-minute delay was chosen because accelerated forgetting is most evident within the first 10 minutes in hippocampal amnesia.[1,2] For the 1-week test, participants received a pre-arranged phone call during which they were asked cued recall questions about the story. All participants were told not to rehearse the task during the 1-week interval, and there was no debriefing. For each cued question, zero to two points were given for each answer, based on predetermined rules, resulting in a possible total of zero to 20 points for the test.

Routes in three towns were filmed from the front of a moving car using a SONY action camera. Video clips were modified such that each had a duration of 150 seconds; pauses were inserted at spatial decision points and salient landmarks in the environment during the presentation. Each video contained five spatial decision points and five landmarks. Participants were asked to watch the videos on a 23-inch display and to remember the directions and the characteristics of the landmark points. For the tests, still images of each of the five spatial decision points were shown in sequential order, and participants were asked to state which direction the car proceeded in (two-alternative forced choice method) or the subsequent landmark (cued recall; Figure S3). The test procedure was the same as for the story task.

Again, if the 80% learning criterion was not reached, there was a repeat presentation, but the maximum number of trials was two. During the 10-minute delay, participants completed the Trail Making Test and the Test of Lexical Processing in Aphasia. Seven days after the tests, participants received a pre-arranged phone call to test cued recall. An envelope containing a copy of the still images was sent to their homes, arriving a day before the test, and was used for the route task. Participants were asked not to visualize or rehearse the task during the 1-week interval, and there was no debriefing. Zero to two points were given for each of the five cued recall items, based on predetermined rules, as well as for each of the five two-alternative forced choice items. Thus, the maximum and chance-level scores were 20 and 5, respectively.

Electroencephalography recording and evaluation

We exclusively used Nihon Kohden equipment for recording of EEG (Nihon Kohden Corporation, Tokyo, Japan). Sleep EEG recordings typically started at 1 pm. No sleep medication was used. The technicians were asked to record N2 sleep according to the following protocol: (1) When the total N2 sleep duration reached 20 minutes, the recording was discontinued; and (2) If the recording was not satisfactory, it was continued for a total duration of 60 minutes. IEDs in the whole recording were independently annotated by two epileptologists, and a third epileptologist checked any discrepancies of more than 10%. The number of spikes was divided by the total recording time (minutes) to calculate the spike frequency; this calculation used the highest of the two raters' numbers. An experienced technician evaluated sleep macrostructural scoring for 30-second epochs.

Statistical analysis

In the volumetric analysis, the volumes were controlled for age, IQ, test set, HADS score, medication (mono/polytherapy) and hippocampal sclerosis (yes/no) for the 10-minute recall score; for 1-week retention, we further controlled for seizures during the retention period (yes/no) and the presence of IEDs on scalp EEG (yes/no). These variables were selected based on the participants' characteristics, past studies and our study hypothesis.[3–7]

Because the patients with focal epilepsy were heterogeneous, we investigated the consistency of the findings for the different patient subgroups: those with TLE-HS, those with TLE-no and those with ETLE. These different subgroups were compared with the healthy controls. Significant results that had been identified for the whole group of patients were assessed for each subgroup separately. As the subgroup sample sizes were inadequate for multivariate analysis, we first removed the effects of the confounder variables (age, IQ, test set, HADS score, medication, seizures during the retention period and the presence of IEDs in scalp EEG) from the memory test measures using the same models as in the main analyses. Then, Pearson correlation analyses were run between the residuals and the hippocampal structure volumes for each subgroup. To determine whether the strength of the correlations differed significantly between the subgroups, Fisher's z tests and Zou's 95% confidence intervals (CI) were calculated using the R package cocor.[8]

For the shape analyses, we tested the association between the ALF test measures and the thickness at each vertex on the hippocampal mesh for the focal epilepsy group. The covariate variables were the same as for the volumetric analyses. Correction for multiple comparisons was carried out using the false discovery rate (FDR) correction at $P < 0.05$. The z and p values were mapped onto a template surface using in-house Matlab (MathWorks Inc., USA) scripts. The Pearson correlation analyses were also performed to clarify the association between subfield volumetric measures and hippocampal shape (volume-to-shape correlation analysis). We calculated the correlation coefficient between each subfield volume and the thickness at

each vertex of hippocampal shape. Then, a winner-takes-all map was created in which each vertex was assigned to the subfield showing the highest correlation coefficient at that vertex.

Supporting Figures and Tables

Table S1. Lesions outside of the hippocampus detected using MRI

Temporal lobe epilepsy	FLAIR hyperintensity suspected to be focal cortical dysplasia or a tumour in the left amygdala (n = 1) Diffuse cerebral atrophy (n = 1) Multiple FLAIR hyperintensities suspected to be a transmantle sign in the subcortical white matter (n = 1) FLAIR hyperintensity suspected to be a ganglioglioma in the left temporal lobe (n = 1) Right amygdala enlargement (n = 1) A cavernous angioma in the right temporal lobe (n = 1)
Extratemporal lobe epilepsy	Slight atrophy in the bilateral parietal and occipital lobes (n = 1) A cyst in the left cornu inferius ventriculi lateralis (n = 1) A cavernous angioma in the base of the left frontal lobe (n = 1)

Lesions that are also seen in age-related changes, such as chronic ischemic changes, are not included in the list.

FLAIR = fluid-attenuated inversion recovery

Table S2. Demographics and neuropsychological performance of the different patient subgroups and control participants

		TLE-HS (n = 14)		TLE-no (n = 47)		ETLE (n = 19)		HC (n = 30)	
Demographics and Clinical Characteristics									
Age	mean (SD)	42.8	(11.2)	35.6	(11.5)	35.7	(12.3)	35.1	(6.5)
Sex (male)	n (%)	6	(43%)	19	(40%)	7	(37%)	13	(43%)
Dominant hand (right)	n (%)	12	(86%)	42	(89%)	17	(89%)	27	(90%)
Education (>12 years)	n (%)	13	(92%)	39	(83%)	14	(73%)	27	(90%)
Age at onset	mean (SD)	19.2	(16.1)	25.9	(14.4)	20.6	(16.7)	-	
Duration of epilepsy	mean (SD)	23.6	(12.0)	9.7	(8.6)	17.4	(12.3)	-	
Laterality of suspected seizure focus	L/R/Bi/Un	10/2/1/1		17/8/4/18		7/2/0/10			
Seizure frequency	n (%)								
Daily		1	(7.1%)	2	(4.3%)	1	(5.3%)	-	
Weekly		0	(0%)	2	(4.3%)	0	(0%)	-	
Monthly		4	(29%)	13	(28%)	3	(16%)	-	
Yearly		2	(14%)	12	(26%)	9	(47%)	-	
Seizure free (>1 year)		7	(50%)	18	(38%)	6	(32%)	-	
Medication (Monotherapy)	n (%)	2	(14%)	21	(45%)	10	(53%)	-	
Seizure focus	T/F/O/Un	14/0/0/0		47/0/0/0		0/5/3/11			
FIAS during 1-week interval	n (%)	2	(14%)	6	(13%)	0	(0%)	-	
Any type of seizure during interval	n (%)	3	(21%)	13	(28%)	4	(21%)	-	
Neuropsychological Performance									
Estimated Full Scale IQ	mean (SD)	93.1	(13.3)	91.0	(12.1)*	91.3	(11.4)	98.1	(11.1)
S-PA									
Normal	n (%)	9	(64%)	30	(64%)	15	(79%)	25	(83%)
Borderline	n (%)	2	(14%)	11	(23%)	4	(21%)	3	(10%)
Abnormal	n (%)	3	(21%)	6	(13%)	0	(0%)	2	(6.7%)
BVRT	mean (SD)	8.4	(1.0)	8.4	(1.4)	8.1	(1.2)	8.4	(1.3)
TMT A (sec)	mean (SD)	29.2	(7.5)	29.7	(10.5)	27.2	(9.6)	26.3	(7.3)
TMT B (sec)	mean (SD)	59.7	(19.9)	61.5	(19.7)	56.3	(14.3)	60.5	(19.4)
Object Naming (TLPA)	mean (SD)	38.2	(2.5)	38.0	(1.6)	38.9	(1.4)	38.4	(1.5)
Questionnaire Measures									
Anxiety and Depression (HADS)	mean (SD)	15.6	(5.1)*	16.5	(5.9)***	13.6	(5.4)	11.2	(4.9)
Everyday Memory Problems (EMQ-R)	mean (SD)	11.4	(9.5)	13.3	(9.0)	13.7	(11.3)	9.5	(7.0)
Spatial Navigation (SBSOD)	mean (SD)	51.9	(22.5)	49.6	(18.6)	45.9	(16.3)	56.1	(18.7)
Long-term memory performance									
Story task									
Number of trials	median (IQR)	1.0	(1.0–2.25)	1.0	(1.0–2.0)	2.0	(1.0–2.0)	1.0	(1.0–1.25)
30 sec recall	median (IQR)	18.0	(16.5–18.25)	18.0	(17.0–19.0)	19.0	(18.0–20.0)	18.0	(17.8–20.0)
10 min recall	median (IQR)	14.0	(11.75–16.5)	16.0	(14.0–18.0)	16.0	(14.0–18.0)	16.0	(14.0–19.0)
1 week recall	median (IQR)	6.0	(1.5–10.0)	9.0	(4.0–14.0)	10.0	(6.0–16.0)	11.5	(6.0–16.0)
Retention rate (%)	median (IQR)	43.8	(10.0–67.4)	52.6	(22.2–85.7)	55.0	(33.3–94.7)	74.2	(35.3–90.6)
Route task[a]									
Number of trials	median (IQR)	1.0	(1.0–1.0)	1.0	(1.0–1.0)	1.0	(1.0–2.0)	1.0	(1.0–1.0)
30 sec recall	median (IQR)	18.0	(17.0–20.0)	18.0	(16.0–20.0)	18.0	(17.5–20.0)	20.0	(18.0–20.0)
10 min recall	median (IQR)	16.0	(13.0–17.0)**	16.0	(14.0–18.0)*	19.0	(18.0–20.0)	18.0	(16.0–20.0)
1 week recall	median (IQR)	8.0	(7.5–16.0)	11.5	(8.0–15.0)	12.0	(9.0–14.25)	13.0	(10.0–14.5)
Retention rate (%)	median (IQR)	72.7	(50.0–84.4)	71.0	(56.7–100.0)	60.0	(50.0–80.8)	71.1	(56.1–89.3)

[a]Seven patients were excluded (n = 73).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, group comparison with healthy controls.
Bi = bilateral; BVRT = Benton visual retention test; EMQ-R = everyday memory questionnaire - revised version; ETLE = extra-temporal lobe epilepsy; F = frontal lobe; FE = focal epilepsy; FIAS = focal impaired awareness seizure; HADS = hospital anxiety and depression scale; HC = healthy control; IQ = intelligence quotient; IQR = interquartile range; L = left; O = occipital lobe; R = right; SBSOD = Santa Barbara sense of direction scale; SD = standard deviation; S-PA = standard verbal paired-associate learning test; T = temporal lobe; TLE-HS = temporal lobe epilepsy with hippocampal atrophy; TLE-no = temporal lobe epilepsy without hippocampal atrophy; TLPA = test of lexical processing in aphasia; TMT = trail making test; Un = undetermined

Table S3. Volumes of hippocampal subfields and EEG results in patient subgroups and control participants

Hippocampal Subfield of Interest[a]		FE (n = 76)		TLE-HS (11/76)		TLE-no (47/76)		ETLE (18/76)		HC (n = 27)	
		Mean (SD)									
R WHP	mm^3	3437	(414)	3018	(637)***	3507	(329)	3509	(305)	3526	(238)
L WHP	mm^3	3247	(465)	2565	(588)***	3393	(304)	3396	(347)	3384	(208)
R CA1	mm^3	675	(97)	589	(145)**	686	(79)	699	(84)	690	(55)
L CA1	mm^3	627	(106)	481	(133)***	648	(74)	660	(88)	644	(49)
R CA2/3	mm^3	222	(36)	201	(47)	225	(33)	229	(31)	223	(29)
L CA2/3	mm^3	200	(39)	154	(49)***	204	(30)	217	(34)	206	(22)
R CA4	mm^3	258	(38)	228	(60)*	264	(32)	260	(30)	264	(22)
L CA4	mm^3	239	(46)	169	(61)***	249	(29)	255	(35)	250	(18)
R DG	mm^3	316	(49)	277	(73)**	323	(43)	320	(37)	322	(27)
L DG	mm^3	294	(58)	205	(75)***	306	(37)	315	(44)	306	(22)
R molecular layer	mm^3	501	(66)	455	(107)*	504	(53)	520	(57)	507	(47)
L molecular layer	mm^3	453	(66)	366	(86)***	467	(51)	469	(45)	465	(33)
R subicular complex[b]	mm^3	750	(102)	647	(128)**	773	(94)	751	(64)	750	(61)
L subicular complex[b]	mm^3	768	(111)	634	(139)***	799	(90)	768	(84)	765	(74)
R tail	mm^3	569	(73)*	503	(98)***	579	(61)	583	(68)	619	(70)
L tail	mm^3	542	(79)*	437	(78)***	562	(64)	556	(66)	600	(70)
EEG											
IEDs during recording	n (%)	36	(47%)	5	(45%)	25	(53%)	6	(33%)	N/A	
Spike frequency (/min)	median (IQR)	0.05	(0.00–0.61)	0.04	(0.00–0.27)	0.07	(0.00–0.73)	0.009	(0.00–0.37)	N/A	
Sleep stage 2 (>0 min)	n (%)	67	(88%)	9	(82%)	42	(89%)	16	(89%)	N/A	

[a]The volumes of the whole hippocampus and hippocampal subfields were normalized for each subject using the estimated total intracranial volume.

[b]The subicular complex included the subiculum, presubiculum and parasubiculum.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, in comparison with healthy controls.

CA = cornu ammonis; DG = dentate gyrus; EEG = electroencephalography; ETLE = extra-temporal lobe epilepsy; FE = focal epilepsy; HC = healthy control; IED = interictal epileptic discharge; IQR = interquartile range; L = left; N/A = not available; R = right; SD = standard deviation; TLE-HS = temporal lobe epilepsy with hippocampal atrophy; TLE-no = temporal lobe epilepsy without hippocampal atrophy; WHP = whole hippocampus

Table S4. Example of a prose passage from the story task (translated from Japanese).

“Yesterday afternoon, a robber broke into a bank in Aomori City, took 5 million yen, and fled in a car. On the way, the car had a flat tyre and stopped, so he ran to a nearby shed. He hid in a pile of straw but was almost bitten by a snake; he ran out of the shed and was arrested.”

Item	Question	Answer
1	Where was the bank located?	2: Aomori 1: Place names beginning with “A”
2	Who broke into the bank?	2: Robber, thief, burglar 1: -
3	When did the robbery take place?	2: Yesterday (afternoon) 1: Mention of "afternoon" only, in which case "late afternoon" or "evening" is acceptable as long as it means between noon and midnight
4	How much did the robber get away with?	2: 5 million yen 1: Half a million yen
5	What kind of vehicle did the robber use to escape?	2: Car 1: When the meaning is close to car, such as vehicle
6	How did the car get stuck in the middle of the road?	2: Because of the flat tyre 1: If the flat tyre is not mentioned, but that "it broke down" is mentioned
7	Where did the robber run to when the police chased him?	2: Shed, barn, storehouse 1: Buildings other than the above, such as warehouses
8	What did the robber hide in?	2: A pile of straw 1: shed, barn, storehouse
9	What tried to bite the robber when he was hiding?	2: Snake 1: -
10	What happened when he jumped out of the straw?	2: He was arrested 1: The police found him

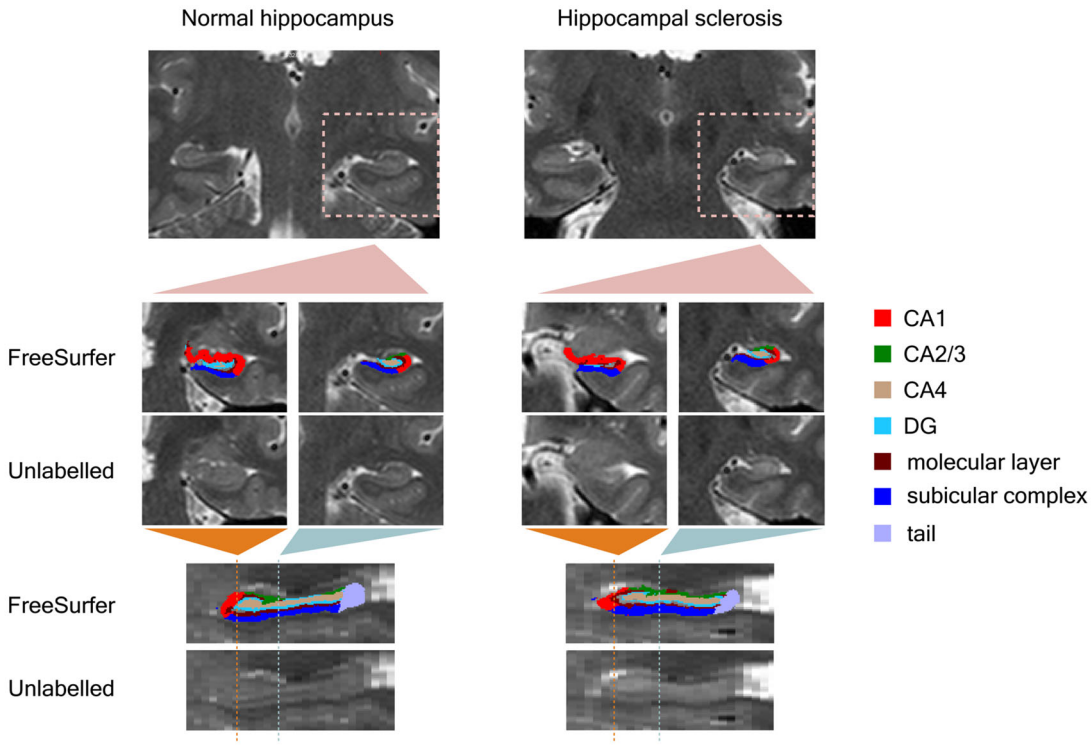

Figure S1. Automated segmentation of hippocampal subfields with FreeSurfer v7.1.0. Example of automated segmentation with FreeSurfer v7.1.0. Sagittal and coronal slices are shown for two representative subjects (left: one healthy control subject, right: one temporal lobe epilepsy patient with left hippocampal sclerosis). The subicular complex is a combined volume of the subiculum, presubiculum and parasubiculum from the original segmentation. CA = cornu ammonis; DG = dentate gyrus.

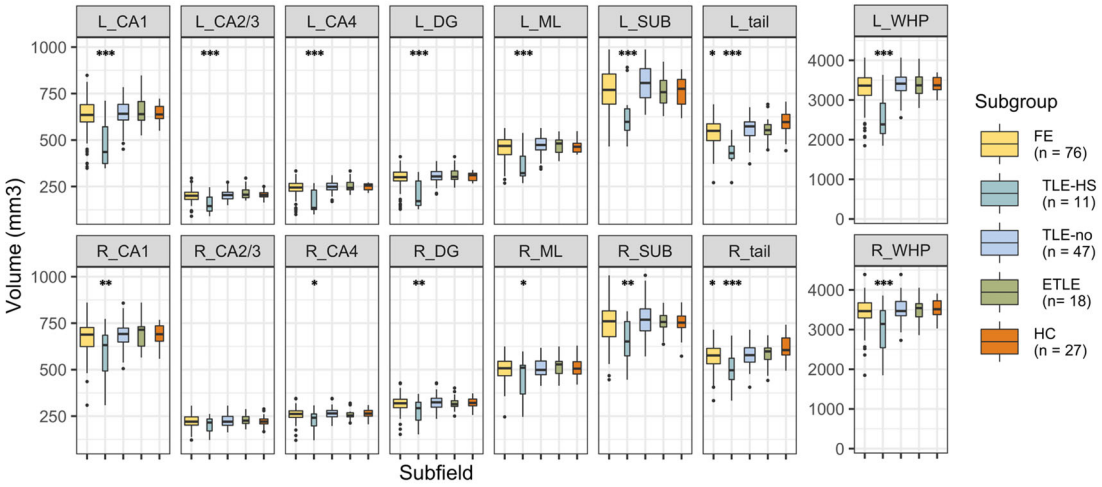

Figure S2. Volumes of hippocampal subfields results. The volumes of the whole hippocampus and hippocampal subfields were normalized for each subject using the estimated total intracranial volume. CA = cornu ammonis; ETLE = extratemporal lobe epilepsy; DG = dentate gyrus; FE = focal epilepsy; HC = healthy control; L = left; ML = molecular layer; R = right; SUB = subicular complex; TLE-HS = temporal lobe epilepsy with hippocampal sclerosis; TLE-no = temporal lobe epilepsy without hippocampal sclerosis; WHP = whole hippocampus. *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$, in comparison with healthy controls.

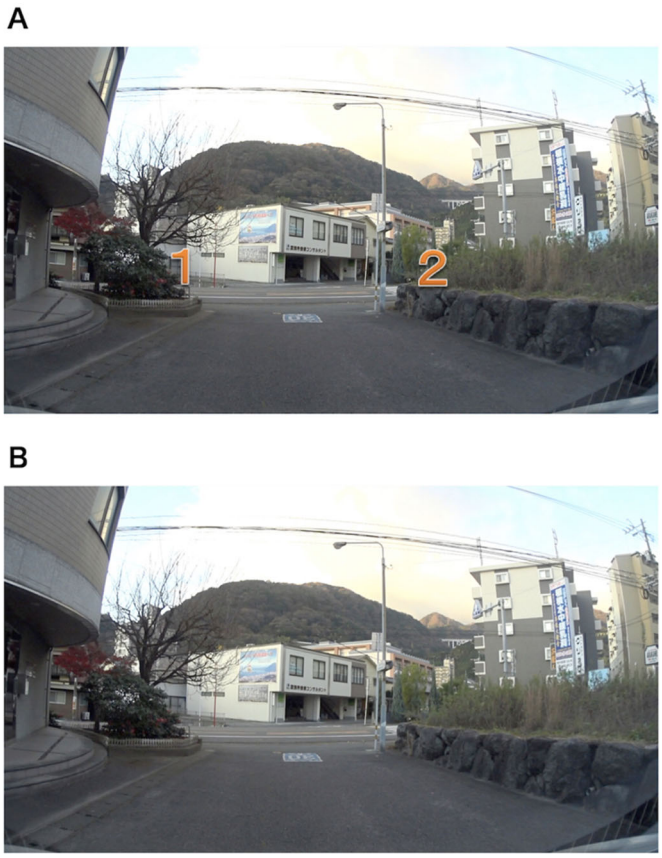

Figure S3. Example of route task. (A) Route task forced choice recognition. Two numbers were superimposed on the picture, and participants were asked to choose the number indicating the correct direction at each intersection. **(B)** Landmark cued recall. Participants were asked the name or characteristics of the landmark that appeared after each intersection. One route task consisted of five questions each, with a total of 10 questions.

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Supporting Information for "Atrophy of the hippocampal CA1 subfield relates to long-term forgetting in focal epilepsy"

Supporting Methods

Details of participants

Ninety-four patients were initially included in the study, but 14 failed to fulfil the IQ criterion and were thus excluded. For 28% of the patients, the ictal epileptiform activity was captured during routine EEG or video EEG monitoring; for the rest of the patients, only interictal EEG abnormalities were observed. Hippocampal sclerosis was diagnosed based on the MRI scans. The clinical information was collected by an epileptologist (T.M.), and decisions concerning the inclusion/exclusion of patients, the seizure location and laterality, and the presence of hippocampal sclerosis were discussed by a group of epileptologists (T.M., T.U., N.A., H.S.). For the determination of the seizure location and laterality, the semiology, EEG and imaging findings were required to be consistent, and any cases with a discrepancy were classified as 'undetermined'. Thirty healthy controls were recruited from the local community, none of whom had a history of seizures or neurologic disorders. They were matched to the patients in terms of age, sex and educational level. All of the participants were paid for taking part in the study. Data from several participants were excluded from the multiple regression analyses: one patient who did not undergo MRI because of a sudden, unexpected death; two controls who did not undergo MRI; and four participants with poor quality hippocampal segmentation.

Assessment of accelerated long-term forgetting

PowerPoint (Microsoft Corp., WA, USA) was used to play the pre-recorded stories and videos.

The story task consisted of three prose passages, each with 10 cued recall questions (one set of questions is shown in Table S4). Their presentation order was counterbalanced using a Latin square design. Participants were required to listen to each story through a speaker. After listening to the first story, participants were asked to complete a calculation sheet as a distractor task and were then asked the cued recall questions. If they did not reach the 80% learning criterion at the 30-second delay, the story and questions were presented again; this process was repeated a maximum of three times. The remaining two stories were presented the same number of times as the first, and recall was tested at 10 minutes and 7 days after learning. During the 10-minute delay, participants completed neuropsychological questionnaires. A 10-minute delay was chosen because accelerated forgetting is most evident within the first 10 minutes in hippocampal amnesia.[1,2] For the 1-week test, participants received a pre-arranged phone call during which they were asked cued recall questions about the story. All participants were told not to rehearse the task during the 1-week interval, and there was no debriefing. For each cued question, zero to two points were given for each answer, based on predetermined rules, resulting in a possible total of zero to 20 points for the test.

Routes in three towns were filmed from the front of a moving car using a SONY action camera. Video clips were modified such that each had a duration of 150 seconds; pauses were inserted at spatial decision points and salient landmarks in the environment during the presentation. Each video contained five spatial decision points and five landmarks. Participants were asked to watch the videos on a 23-inch display and to remember the directions and the characteristics of the landmark points. For the tests, still images of each of the five spatial decision points were shown in sequential order, and participants were asked to state which direction the car proceeded in (two-alternative forced choice method) or the subsequent landmark (cued recall; Figure S3). The test procedure was the same as for the story task.

Again, if the 80% learning criterion was not reached, there was a repeat presentation, but the maximum number of trials was two. During the 10-minute delay, participants completed the Trail Making Test and the Test of Lexical Processing in Aphasia. Seven days after the tests, participants received a pre-arranged phone call to test cued recall. An envelope containing a copy of the still images was sent to their homes, arriving a day before the test, and was used for the route task. Participants were asked not to visualize or rehearse the task during the 1-week interval, and there was no debriefing. Zero to two points were given for each of the five cued recall items, based on predetermined rules, as well as for each of the five two-alternative forced choice items. Thus, the maximum and chance-level scores were 20 and 5, respectively.

Electroencephalography recording and evaluation

We exclusively used Nihon Kohden equipment for recording of EEG (Nihon Kohden Corporation, Tokyo, Japan). Sleep EEG recordings typically started at 1 pm. No sleep medication was used. The technicians were asked to record N2 sleep according to the following protocol: (1) When the total N2 sleep duration reached 20 minutes, the recording was discontinued; and (2) If the recording was not satisfactory, it was continued for a total duration of 60 minutes. IEDs in the whole recording were independently annotated by two epileptologists, and a third epileptologist checked any discrepancies of more than 10%. The number of spikes was divided by the total recording time (minutes) to calculate the spike frequency; this calculation used the highest of the two raters' numbers. An experienced technician evaluated sleep macrostructural scoring for 30-second epochs.

Statistical analysis

In the volumetric analysis, the volumes were controlled for age, IQ, test set, HADS score, medication (mono/polytherapy) and hippocampal sclerosis (yes/no) for the 10-minute recall score; for 1-week retention, we further controlled for seizures during the retention period (yes/no) and the presence of IEDs on scalp EEG (yes/no). These variables were selected based on the participants' characteristics, past studies and our study hypothesis.[3–7]

Because the patients with focal epilepsy were heterogeneous, we investigated the consistency of the findings for the different patient subgroups: those with TLE-HS, those with TLE-no and those with ETLE. These different subgroups were compared with the healthy controls. Significant results that had been identified for the whole group of patients were assessed for each subgroup separately. As the subgroup sample sizes were inadequate for multivariate analysis, we first removed the effects of the confounder variables (age, IQ, test set, HADS score, medication, seizures during the retention period and the presence of IEDs in scalp EEG) from the memory test measures using the same models as in the main analyses. Then, Pearson correlation analyses were run between the residuals and the hippocampal structure volumes for each subgroup. To determine whether the strength of the correlations differed significantly between the subgroups, Fisher's z tests and Zou's 95% confidence intervals (CI) were calculated using the R package cocor.[8]

For the shape analyses, we tested the association between the ALF test measures and the thickness at each vertex on the hippocampal mesh for the focal epilepsy group. The covariate variables were the same as for the volumetric analyses. Correction for multiple comparisons was carried out using the false discovery rate (FDR) correction at $P < 0.05$. The z and p values were mapped onto a template surface using in-house Matlab (MathWorks Inc., USA) scripts. The Pearson correlation analyses were also performed to clarify the association between subfield volumetric measures and hippocampal shape (volume-to-shape correlation analysis). We calculated the correlation coefficient between each subfield volume and the thickness at

each vertex of hippocampal shape. Then, a winner-takes-all map was created in which each vertex was assigned to the subfield showing the highest correlation coefficient at that vertex.

Supporting Figures and Tables

Table S1. Lesions outside of the hippocampus detected using MRI

Temporal lobe epilepsy	FLAIR hyperintensity suspected to be focal cortical dysplasia or a tumour in the left amygdala (n = 1) Diffuse cerebral atrophy (n = 1) Multiple FLAIR hyperintensities suspected to be a transmantle sign in the subcortical white matter (n = 1) FLAIR hyperintensity suspected to be a ganglioglioma in the left temporal lobe (n = 1) Right amygdala enlargement (n = 1) A cavernous angioma in the right temporal lobe (n = 1)
Extratemporal lobe epilepsy	Slight atrophy in the bilateral parietal and occipital lobes (n = 1) A cyst in the left cornu inferius ventriculi lateralis (n = 1) A cavernous angioma in the base of the left frontal lobe (n = 1)

Lesions that are also seen in age-related changes, such as chronic ischemic changes, are not included in the list.

FLAIR = fluid-attenuated inversion recovery

Table S2. Demographics and neuropsychological performance of the different patient subgroups and control participants

		TLE-HS (n = 14)		TLE-no (n = 47)		ETLE (n = 19)		HC (n = 30)	
Demographics and Clinical Characteristics									
Age	mean (SD)	42.8	(11.2)	35.6	(11.5)	35.7	(12.3)	35.1	(6.5)
Sex (male)	n (%)	6	(43%)	19	(40%)	7	(37%)	13	(43%)
Dominant hand (right)	n (%)	12	(86%)	42	(89%)	17	(89%)	27	(90%)
Education (>12 years)	n (%)	13	(92%)	39	(83%)	14	(73%)	27	(90%)
Age at onset	mean (SD)	19.2	(16.1)	25.9	(14.4)	20.6	(16.7)	-	
Duration of epilepsy	mean (SD)	23.6	(12.0)	9.7	(8.6)	17.4	(12.3)	-	
Laterality of suspected seizure focus	L/R/Bi/Un	10/2/1/1		17/8/4/18		7/2/0/10			
Seizure frequency	n (%)								
Daily		1	(7.1%)	2	(4.3%)	1	(5.3%)	-	
Weekly		0	(0%)	2	(4.3%)	0	(0%)	-	
Monthly		4	(29%)	13	(28%)	3	(16%)	-	
Yearly		2	(14%)	12	(26%)	9	(47%)	-	
Seizure free (>1 year)		7	(50%)	18	(38%)	6	(32%)	-	
Medication (Monotherapy)	n (%)	2	(14%)	21	(45%)	10	(53%)	-	
Seizure focus	T/F/O/Un	14/0/0/0		47/0/0/0		0/5/3/11			
FIAS during 1-week interval	n (%)	2	(14%)	6	(13%)	0	(0%)	-	
Any type of seizure during interval	n (%)	3	(21%)	13	(28%)	4	(21%)	-	
Neuropsychological Performance									
Estimated Full Scale IQ	mean (SD)	93.1	(13.3)	91.0	(12.1)*	91.3	(11.4)	98.1	(11.1)
S-PA									
Normal	n (%)	9	(64%)	30	(64%)	15	(79%)	25	(83%)
Borderline	n (%)	2	(14%)	11	(23%)	4	(21%)	3	(10%)
Abnormal	n (%)	3	(21%)	6	(13%)	0	(0%)	2	(6.7%)
BVRT	mean (SD)	8.4	(1.0)	8.4	(1.4)	8.1	(1.2)	8.4	(1.3)
TMT A (sec)	mean (SD)	29.2	(7.5)	29.7	(10.5)	27.2	(9.6)	26.3	(7.3)
TMT B (sec)	mean (SD)	59.7	(19.9)	61.5	(19.7)	56.3	(14.3)	60.5	(19.4)
Object Naming (TLPA)	mean (SD)	38.2	(2.5)	38.0	(1.6)	38.9	(1.4)	38.4	(1.5)
Questionnaire Measures									
Anxiety and Depression (HADS)	mean (SD)	15.6	(5.1)*	16.5	(5.9)***	13.6	(5.4)	11.2	(4.9)
Everyday Memory Problems (EMQ-R)	mean (SD)	11.4	(9.5)	13.3	(9.0)	13.7	(11.3)	9.5	(7.0)
Spatial Navigation (SBSOD)	mean (SD)	51.9	(22.5)	49.6	(18.6)	45.9	(16.3)	56.1	(18.7)
Long-term memory performance									
Story task									
Number of trials	median (IQR)	1.0	(1.0–2.25)	1.0	(1.0–2.0)	2.0	(1.0–2.0)	1.0	(1.0–1.25)
30 sec recall	median (IQR)	18.0	(16.5–18.25)	18.0	(17.0–19.0)	19.0	(18.0–20.0)	18.0	(17.8–20.0)
10 min recall	median (IQR)	14.0	(11.75–16.5)	16.0	(14.0–18.0)	16.0	(14.0–18.0)	16.0	(14.0–19.0)
1 week recall	median (IQR)	6.0	(1.5–10.0)	9.0	(4.0–14.0)	10.0	(6.0–16.0)	11.5	(6.0–16.0)
Retention rate (%)	median (IQR)	43.8	(10.0–67.4)	52.6	(22.2–85.7)	55.0	(33.3–94.7)	74.2	(35.3–90.6)
Route task[a]									
Number of trials	median (IQR)	1.0	(1.0–1.0)	1.0	(1.0–1.0)	1.0	(1.0–2.0)	1.0	(1.0–1.0)
30 sec recall	median (IQR)	18.0	(17.0–20.0)	18.0	(16.0–20.0)	18.0	(17.5–20.0)	20.0	(18.0–20.0)
10 min recall	median (IQR)	16.0	(13.0–17.0)**	16.0	(14.0–18.0)*	19.0	(18.0–20.0)	18.0	(16.0–20.0)
1 week recall	median (IQR)	8.0	(7.5–16.0)	11.5	(8.0–15.0)	12.0	(9.0–14.25)	13.0	(10.0–14.5)
Retention rate (%)	median (IQR)	72.7	(50.0–84.4)	71.0	(56.7–100.0)	60.0	(50.0–80.8)	71.1	(56.1–89.3)

[a]Seven patients were excluded (n = 73).

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, group comparison with healthy controls.
Bi = bilateral; BVRT = Benton visual retention test; EMQ-R = everyday memory questionnaire - revised version; ETLE = extra-temporal lobe epilepsy; F = frontal lobe; FE = focal epilepsy; FIAS = focal impaired awareness seizure; HADS = hospital anxiety and depression scale; HC = healthy control; IQ = intelligence quotient; IQR = interquartile range; L = left; O = occipital lobe; R = right; SBSOD = Santa Barbara sense of direction scale; SD = standard deviation; S-PA = standard verbal paired-associate learning test; T = temporal lobe; TLE-HS = temporal lobe epilepsy with hippocampal atrophy; TLE-no = temporal lobe epilepsy without hippocampal atrophy; TLPA = test of lexical processing in aphasia; TMT = trail making test; Un = undetermined

Table S3. Volumes of hippocampal subfields and EEG results in patient subgroups and control participants

Hippocampal Subfield of Interest[a]		FE (n = 76)		TLE-HS (11/76)		TLE-no (47/76)		ETLE (18/76)		HC (n = 27)	
		Mean (SD)									
R WHP	mm^3	3437	(414)	3018	(637)***	3507	(329)	3509	(305)	3526	(238)
L WHP	mm^3	3247	(465)	2565	(588)***	3393	(304)	3396	(347)	3384	(208)
R CA1	mm^3	675	(97)	589	(145)**	686	(79)	699	(84)	690	(55)
L CA1	mm^3	627	(106)	481	(133)***	648	(74)	660	(88)	644	(49)
R CA2/3	mm^3	222	(36)	201	(47)	225	(33)	229	(31)	223	(29)
L CA2/3	mm^3	200	(39)	154	(49)***	204	(30)	217	(34)	206	(22)
R CA4	mm^3	258	(38)	228	(60)*	264	(32)	260	(30)	264	(22)
L CA4	mm^3	239	(46)	169	(61)***	249	(29)	255	(35)	250	(18)
R DG	mm^3	316	(49)	277	(73)**	323	(43)	320	(37)	322	(27)
L DG	mm^3	294	(58)	205	(75)***	306	(37)	315	(44)	306	(22)
R molecular layer	mm^3	501	(66)	455	(107)*	504	(53)	520	(57)	507	(47)
L molecular layer	mm^3	453	(66)	366	(86)***	467	(51)	469	(45)	465	(33)
R subicular complex[b]	mm^3	750	(102)	647	(128)**	773	(94)	751	(64)	750	(61)
L subicular complex[b]	mm^3	768	(111)	634	(139)***	799	(90)	768	(84)	765	(74)
R tail	mm^3	569	(73)*	503	(98)***	579	(61)	583	(68)	619	(70)
L tail	mm^3	542	(79)*	437	(78)***	562	(64)	556	(66)	600	(70)
EEG											
IEDs during recording	n (%)	36	(47%)	5	(45%)	25	(53%)	6	(33%)	N/A	
Spike frequency (/min)	median (IQR)	0.05	(0.00–0.61)	0.04	(0.00–0.27)	0.07	(0.00–0.73)	0.009	(0.00–0.37)	N/A	
Sleep stage 2 (>0 min)	n (%)	67	(88%)	9	(82%)	42	(89%)	16	(89%)	N/A	

[a]The volumes of the whole hippocampus and hippocampal subfields were normalized for each subject using the estimated total intracranial volume.

[b]The subicular complex included the subiculum, presubiculum and parasubiculum.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, in comparison with healthy controls.

CA = cornu ammonis; DG = dentate gyrus; EEG = electroencephalography; ETLE = extra-temporal lobe epilepsy; FE = focal epilepsy; HC = healthy control; IED = interictal epileptic discharge; IQR = interquartile range; L = left; N/A = not available; R = right; SD = standard deviation; TLE-HS = temporal lobe epilepsy with hippocampal atrophy; TLE-no = temporal lobe epilepsy without hippocampal atrophy; WHP = whole hippocampus

Table S4. Example of a prose passage from the story task (translated from Japanese).

“Yesterday afternoon, a robber broke into a bank in Aomori City, took 5 million yen, and fled in a car. On the way, the car had a flat tyre and stopped, so he ran to a nearby shed. He hid in a pile of straw but was almost bitten by a snake; he ran out of the shed and was arrested.”

Item	Question	Answer
1	Where was the bank located?	2: Aomori 1: Place names beginning with “A”
2	Who broke into the bank?	2: Robber, thief, burglar 1: -
3	When did the robbery take place?	2: Yesterday (afternoon) 1: Mention of "afternoon" only, in which case "late afternoon" or "evening" is acceptable as long as it means between noon and midnight
4	How much did the robber get away with?	2: 5 million yen 1: Half a million yen
5	What kind of vehicle did the robber use to escape?	2: Car 1: When the meaning is close to car, such as vehicle
6	How did the car get stuck in the middle of the road?	2: Because of the flat tyre 1: If the flat tyre is not mentioned, but that "it broke down" is mentioned
7	Where did the robber run to when the police chased him?	2: Shed, barn, storehouse 1: Buildings other than the above, such as warehouses
8	What did the robber hide in?	2: A pile of straw 1: shed, barn, storehouse
9	What tried to bite the robber when he was hiding?	2: Snake 1: -
10	What happened when he jumped out of the straw?	2: He was arrested 1: The police found him

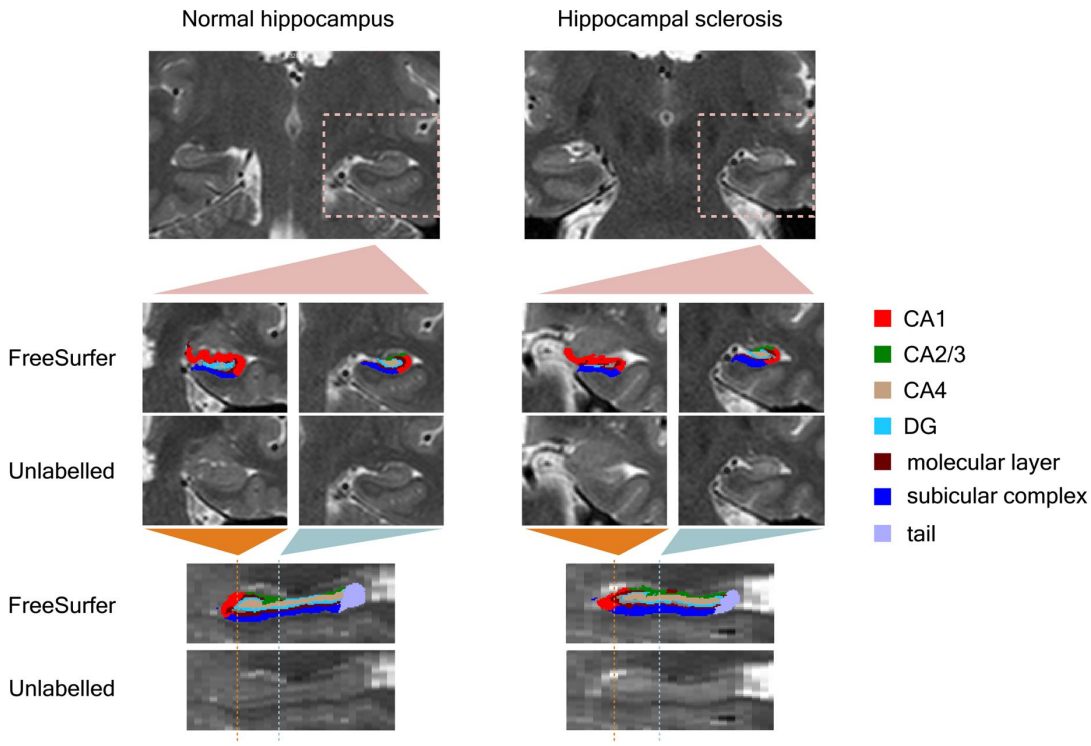

Figure S1. Automated segmentation of hippocampal subfields with FreeSurfer v7.1.0. Example of automated segmentation with FreeSurfer v7.1.0. Sagittal and coronal slices are shown for two representative subjects (left: one healthy control subject, right: one temporal lobe epilepsy patient with left hippocampal sclerosis). The subicular complex is a combined volume of the subiculum, presubiculum and parasubiculum from the original segmentation. CA = cornu ammonis; DG = dentate gyrus.

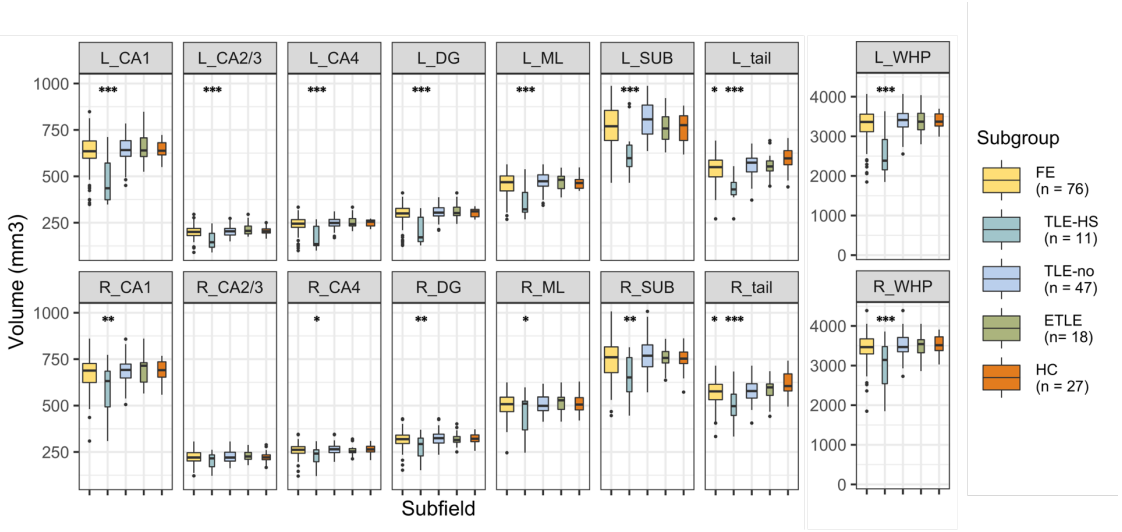

Figure S2. Volumes of hippocampal subfields results. The volumes of the whole hippocampus and hippocampal subfields were normalized for each subject using the estimated total intracranial volume. CA = cornu ammonis; ETLE = extratemporal lobe epilepsy; DG = dentate gyrus; FE = focal epilepsy; HC = healthy control; L = left; ML = molecular layer; R = right; SUB = subicular complex; TLE-HS = temporal lobe epilepsy with hippocampal sclerosis; TLE-no = temporal lobe epilepsy without hippocampal sclerosis; WHP = whole hippocampus. *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$, in comparison with healthy controls.

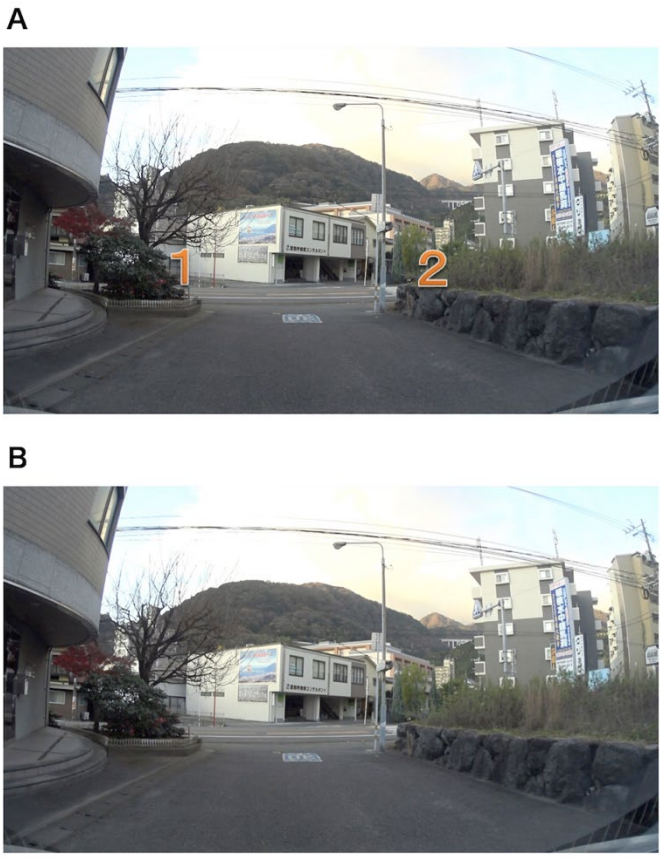

Figure S3. Example of route task. (A) Route task forced choice recognition. Two numbers were superimposed on the picture, and participants were asked to choose the number indicating the correct direction at each intersection. **(B)** Landmark cued recall. Participants were asked the name or characteristics of the landmark that appeared after each intersection. One route task consisted of five questions each, with a total of 10 questions.

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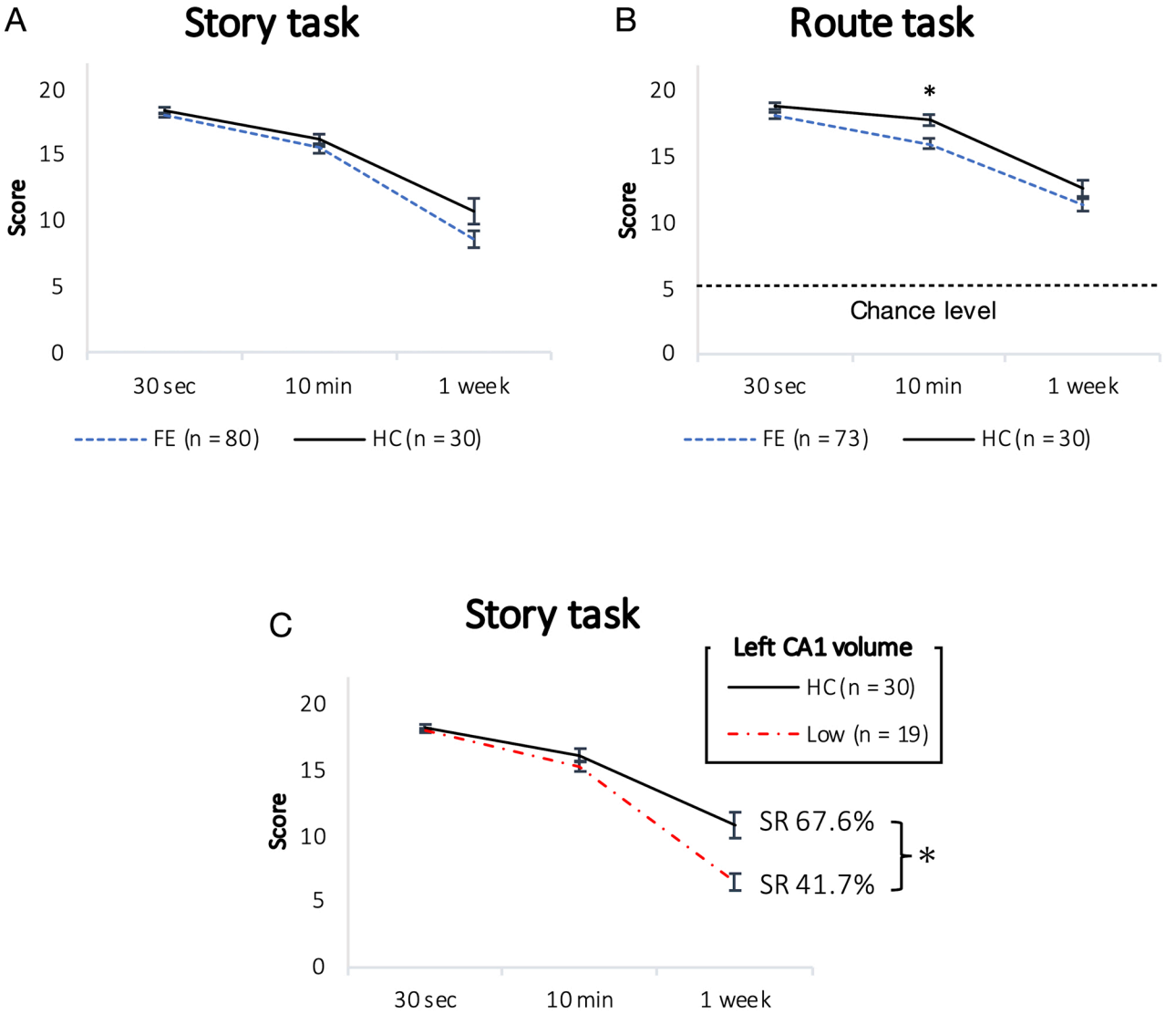
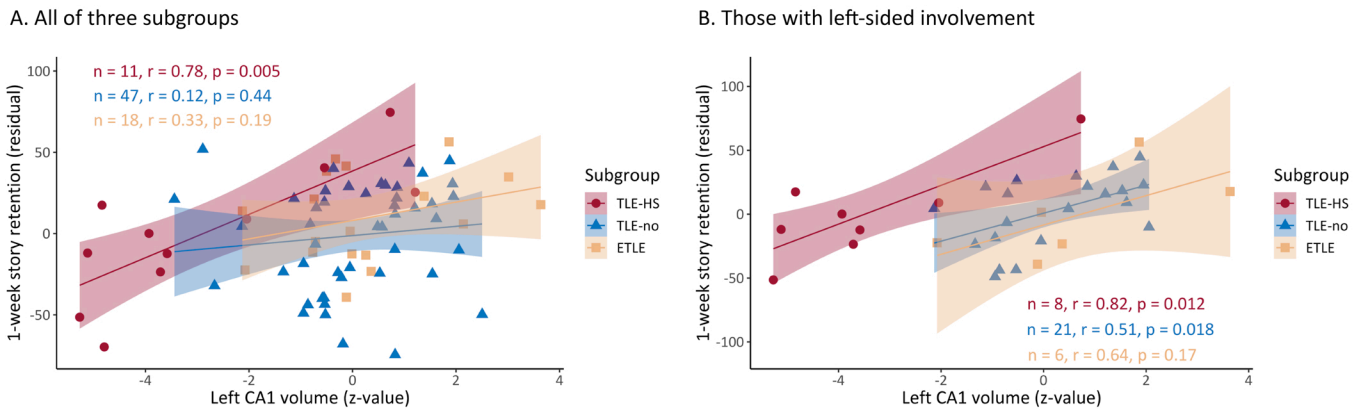
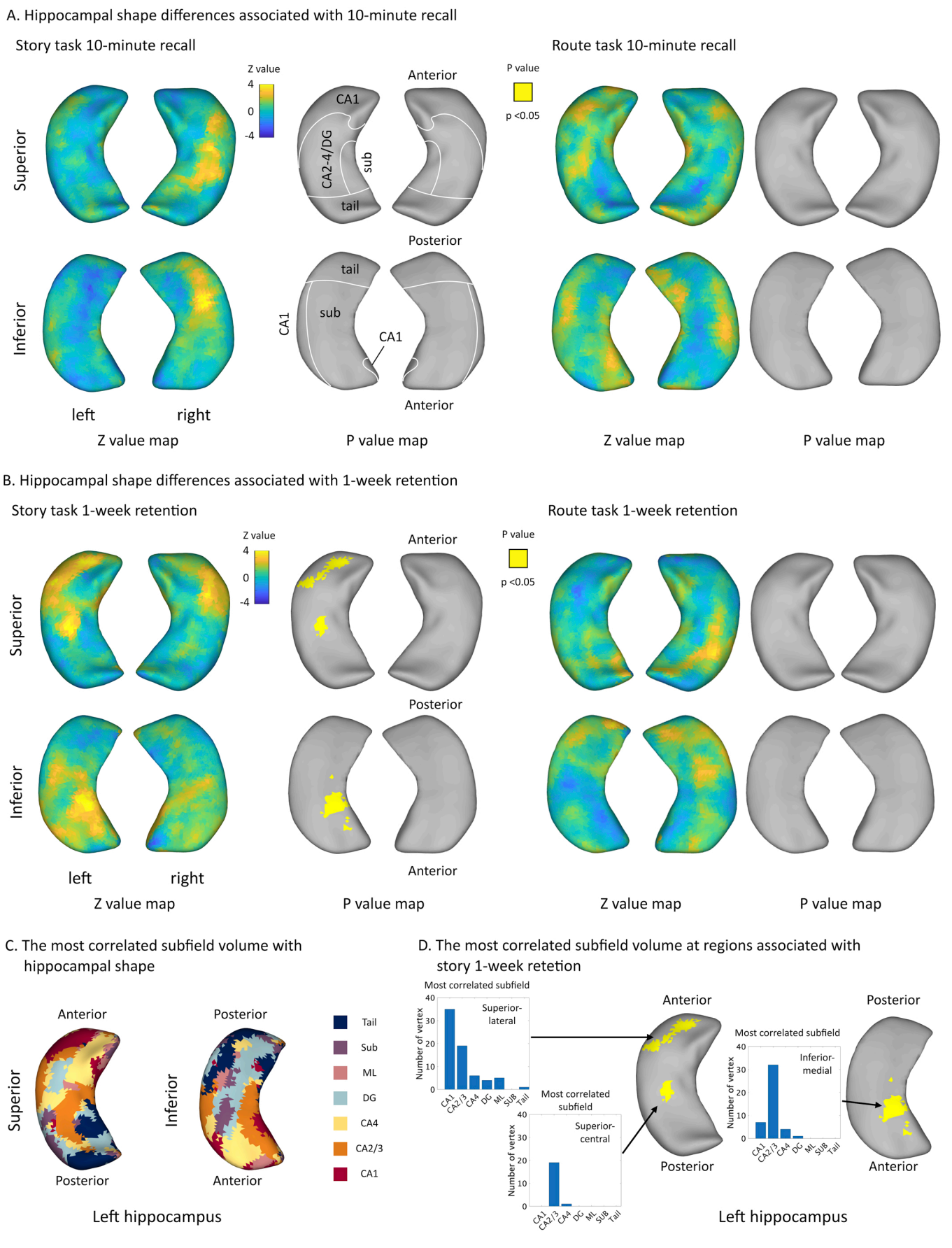

A
Story task
Score
20
15
10
5
0
30 sec
10 min
1 week
FE (n = 80)
HC (n = 30)
B
Route task
*
Chance level
FE (n = 73)
HC (n = 30)
C
Story task
Left CA1 volume
HC (n = 30)
Low (n = 19)
SR 67.6%
SR 41.7%
*


A. All of three subgroups
n = 11, r = 0.78, p = 0.005
n = 47, r = 0.12, p = 0.44
n = 18, r = 0.33, p = 0.19
1-week story retention (residual)
Left CA1 volume (z-value)
Subgroup
TLE-HS
TLE-no
ETLE
B. Those with left-sided involvement
n = 8, r = 0.82, p = 0.012
n = 21, r = 0.51, p = 0.018
n = 6, r = 0.64, p = 0.17


A. Hippocampal shape differences associated with 10-minute recall
Story task 10-minute recall
Route task 10-minute recall
Z value
4
0
-4
P value
p <0.05
Superior
Inferior
Anterior
Posterior
CA1
CA2-4/DG
sub
tail
left
right
Z value map
P value map
Z value map
P value map
B. Hippocampal shape differences associated with 1-week retention
Story task 1-week retention
Route task 1-week retention
Z value
4
0
-4
P value
p <0.05
Superior
Inferior
Anterior
Posterior
left
right
Z value map
P value map
Z value map
P value map
C. The most correlated subfield volume with hippocampal shape
Anterior
Posterior
Superior
Inferior
Posterior
Anterior
Tail
Sub
ML
DG
CA4
CA2/3
CA1
Left hippocampus
D. The most correlated subfield volume at regions associated with story 1-week retetion
Most correlated subfield
Superior-lateral
Number of vertex
CA1
CA2/3
CA4
DG
ML
SUB
Tail
Most correlated subfield
Superior-central
Anterior
Posterior
Most correlated subfield
Inferior-medial
Posterior
Anterior
Left hippocampus