Diagnosis, Assessment & Disease Monitoring

RESEARCH ARTICLE

Hippocampal subfield volumes are associated with verbal memory after first-ever ischemic stroke

Mohamed Salah Khlif[1] | Laura J. Bird[1] | Carolina Restrepo[1] | Wasim Khan[2,3] | Emilio Werden[1] | Natalia Egorova-Brumley[1,4] | Amy Brodtmann[1,5,6]

[1] The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia

[2] Department of Neuroscience, Central Clinical School, Monash University, Clayton, Victoria, Australia

[3] Department of Neuroimaging Institute of Psychiatry, Psychology, and Neuroscience (IoPPN), King's College London, London, UK

[4] Melbourne School of Psychological Sciences, University of Melbourne, Parkville, Victoria, Australia

[5] Department of Neurology, Austin Health, Heidelberg, Victoria, Australia

[6] Eastern Cognitive Disorders Clinic, Box Hill Hospital, Monash University, Box Hill, Victoria, Australia

Correspondence
Mohamed Salah Khlif, The Florey Institute of Neuroscience and Mental Health, 245 Burgundy Street, Heidelberg, VIC 3084, Australia.
E-mail: mohamed.khlif@florey.edu.au

Abstract

Introduction: Hippocampal subfield volumes are more closely associated with cognitive impairment than whole hippocampal volume in many diseases. Both memory and whole hippocampal volume decline after stroke. Understanding the subfields' temporal evolution could reveal valuable information about post-stroke memory.

Methods: We sampled 120 participants (38 control, 82 stroke), with cognitive testing and 3T-MRI available at 3 months and 3 years, from the Cognition and Neocortical Volume after Stroke (CANVAS) study. Verbal memory was assessed using the Hopkins Verbal Learning Test-Revised. Subfields were delineated using FreeSurfer. We used partial Pearson's correlation to assess the associations between subfield volumes and verbal memory scores, adjusting for years of education, sex, and stroke side.

Results: The left cornu ammonis areas 2/3 and hippocampal tail volumes were significantly associated with verbal memory 3-month post-stroke. At 3 years, the associations became stronger and involved more subfields.

Discussion: Hippocampal subfield volumes may be a useful biomarker for post-stroke cognitive impairment.

KEYWORDS
delayed recall, hippocampal subfields, immediate recall, stroke, verbal memory

1 | BACKGROUND

The hippocampus plays an important role in the learning and consolidation of information from short-term to long-term memory. Verbal and visual episodic memory, and spatial navigation, are critical cognitive functions known to be dependent upon the hippocampus. Atrophy of the hippocampus has been linked to cognitive impairment and dementia in many neurological and psychiatric disorders including Alzheimer's disease (AD), temporal lobe epilepsy, major depression, post-traumatic stress disorder, and schizophrenia.[1–5] In healthy elderly people without cognitive impairment, hippocampal atrophy is associated with memory performance—both verbal[6] and visual.[7]

The hippocampus is composed of several subfields with distinctive functions and characteristics. The hippocampal subfields including the cornu ammonis areas (CA1–4), dentate gyrus (DG), and the presubiculum–subiculum complex may be better predictors of

cognitive performance than whole hippocampal volume.[8] For instance, in a non-demented community-dwelling cohort, an association was found between subiculum atrophy, poorer cognition, and a higher risk of dementia.[9] Significant positive correlations were found between volumes of CA2/3, CA4–DG, and the subiculum complex and immediate and delayed recall in amnestic mild cognitive impairment (aMCI) and AD.[10] Another study of early AD found the number of synapses in the molecular layer to be highly correlated with delayed recall.[11] Strong histological associations have been found between Lewy pathology burden in CA1 regions and memory performance in people with dementia with Lewy bodies,[12] and also between CA1 neuronal density and immediate and delayed recall in verbal memory testing in people with left hippocampal sclerosis.[13] Baseline volumes of DG and right CA4 were implicated in the conversion of Parkinson's disease (PD) patients from normal to MCI,[14] and lower volumes of the subiculum complex predicted decline in delayed recall in PD patients.[15] Also, a significant association between lower DG volume and poorer memory performance was reported in participants with subjective memory complaints.[16]

Distinct hippocampal neuronal populations are selectively vulnerable to ischemic insults.[17] In older stroke patients, memory impairment and concomitant hippocampal atrophy are usually explained by an interaction of ischemia and a coexisting neurodegenerative factor such as an AD pathology. However, a study of hippocampal atrophy in young adult stroke survivors, where AD pathology is generally absent, suggests that ischemia is independently associated with remote hippocampal injury.[18] Also, *post mortem* studies have corroborated reduced pyramidal neuron volumes in post-stroke and vascular dementia populations, independent of AD pathology.[19] Up to one third of patients develop dementia years after the initial stroke incident[20] and those with smaller hippocampal volume were reported to have cognitive impairment.[19,21]

Post mortem studies reported that CA1 and CA2 neuronal volumes were positively correlated with global cognition and memory function in post-stroke people.[19,21] Episodic memory impairment has been reported in 25% to 46% of stroke survivors and contributes to functional disability.[22] Tests of episodic memory using free recall–based assessment were reported to be the most effective in the early AD stages.[23] Furthermore, investigation of early signs of dementia have proposed that episodic memory testing could potentially distinguish between normal aging and progression toward dementia up to 10 years prior to onset.[24]

The associations between hippocampal subfield volumes and verbal memory in stroke survivors remain largely undescribed. In a group of healthy individuals and left- and right-sided ischemic stroke participants, we sought to investigate these associations using in vivo magnetic resonance imaging (MRI) and tests of verbal memory. Guided by prior research, we hypothesized that:

- The associations between hippocampal volumes and verbal memory performance would be different in healthy controls and stroke survivors.

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed relevant literature from online sources (e.g., PubMed) using keywords including hippocampal subfields, episodic memory, and stroke. The association between hippocampal subfield volumes and verbal memory in various neurological disorders had been explored, but very limited information related to post–ischemic stroke survivors.

2. **Interpretation**: Consistent with findings in other neurological disorders, we found the association between hippocampal subfield volumes and verbal memory to be negative in healthy individuals, but positive in ischemic stroke survivors. We identified an association with the cornu ammonis areas CA2/3 and hippocampal tail in the left hippocampus and verbal memory status as early as 3 months post-stroke.

3. **Future directions**: Our findings suggest a systematic mechanism for the temporal evolution of volume–memory associations in both healthy and disease cohorts. Future works, conducted on larger cohorts, should refine the hippocampal volume ranges informative of normal and compromised verbal memory status.

HIGHLIGHTS

- Hippocampal volume–memory correlations were negative in control, positive in stroke.
- Left cornu ammonis areas 2/3 and tail volumes were associated with verbal memory at 3 months.
- At 3 years, more subfields in stroke became involved in volume–memory associations.
- Delayed recall associations were stronger than immediate recall.
- Volume–memory association is nonlinear: stroke memory decline is faster over time.

- In stroke, the subfield volumes would correlate better with verbal memory performance than whole hippocampal volume, particularly the CA areas.
- The associations between the left subfield volumes and delayed recall would be stronger than associations involving immediate recall and/or the right subfield volumes.
- The volume–memory associations would increase and/or become stronger at the 3-year timepoint in both control and stroke groups.

2 | METHODS

2.1 | Participants

Participants with available data at each timepoint were sampled from the Cognition and Neocortical Volume after Stroke (CANVAS) study.[25] Briefly, participants were recruited from three sites in Melbourne (Austin Health, Eastern Health, and Melbourne Health), with all MRI scans performed at The Florey Institute of Neuroscience and Mental Health, Austin Hospital. Ethical approval was granted by each hospital's human research ethics committee and all participants provided informed consent. Participants completed an interview (to collect demographic and medical history information), MRI scans, and neuropsychological assessments at four timepoints: baseline (within 6 weeks of index stroke), 3 months, 1 year, and 3 years post-stroke (post-baseline for controls). Patients diagnosed with primary hemorrhagic stroke, transient ischemic attack (TIA), or significant medical comorbidities precluding survival in the study were excluded. Age-matched healthy controls with no history of stroke or TIA were also recruited. No participants had a history of pre-existing dementia, intercurrent delirium, neurological disorders, major psychiatric illnesses, or substance abuse problems. Both first-ever and recurrent stroke participants were recruited into CANVAS, but only first-ever stroke patients were considered in this study.

2.2 | MR image processing

Whole brain MR images were acquired on a 3T Siemens 12-channel Tim Trio scanner using a T1-weighted 3D magnetization-prepared rapid-gradient sequence (160 sagittal slices, 1900 millisecond repetition time, 2.6 millisecond echo time, 900 millisecond inversion time, 9° flip angle, 1-mm isotropic voxel, 256 × 256 field-of-view). Volumetric segmentation was completed using longitudinal FreeSurfer (6.0) processing[26] including motion correction, Talairach transformation, segmentation of subcortical white matter and deep gray matter structures, intensity normalization, and tessellation of the gray matter–white matter boundary. An ex vivo ultra-high resolution (≈0.1 mm) probabilistic atlas[27] was used in the delineation of hippocampal subfields: CA1, CA2/3, CA4, subiculum, presubiculum, parasubiculum, DG, hippocampus-amygdala-transition-area (HATA), fimbria, molecular layer, hippocampal fissure, and hippocampal tail (Figure S1 in supporting information). FreeSurfer combines CA2 and CA3 due to unclear contrast between the two. A dice-overlap of ≈0.7 between manual and automated segmentation was reported for all subfields.[27] The sum of subfield volumes, excluding the hippocampal fissure, defined the hippocampus whole volume.

2.3 | Stroke lesion tracing

Stroke lesions were traced by an imaging analyst (MSK) and cross-checked by a stroke neurologist (AB) using high-resolution magnetization-prepared 3D fluid-attenuated-inversion-recovery (FLAIR) images: 160 1-mm-thick sagittal slices, 6000 millisecond repetition time, 380 millisecond echo time, 120° flip angle, and 512 × 512 field-of-view. A lesion overlap map is shown in Figure S2 in supporting information.

2.4 | Sociodemographic and clinical information

We gathered information about age; years of education; stroke and dementia family history; smoking (≥1 cigarette/day); alcohol consumption (high: >14 standard drinks/week); history of depression, hypertension, type-2 diabetes mellitus (T2DM), hypercholesterolemia, and atrial fibrillation (AF) based on physician diagnosis or medication use; and obesity (body mass index ≥30 kg/m^2).

We compiled data about stroke hemisphere, stroke severity (National Institute of Health Stroke Scale [NIHSS][28]) and subtype (Oxfordshire).[29] We used the modified Rankin Scale (mRS)[30] to estimate neurological disability and the validated Charlson Comorbidity Index (CCI)[31] to estimate general medical comorbidity. Venous blood was drawn for apolipoprotein E (APOE) genotype determination and individuals were categorized as APOE ε4 carriers or non-carriers.

2.5 | Neuropsychological testing

Neuropsychological testing was completed following the CANVAS study protocol.[25] The tests used for cognitive assessment were: Hopkins Verbal Learning Test-Revised (HVLT-R); computerized tests from the CogState battery (Detection, Identification, One-Back); tasks (Digit Span, Digit-Symbol) from the Weschler Adult Intelligence Scale–3rd edition; Verbal Fluency Task (FAS, Animals); Trail-Making Test A and B; Boston Naming Test; Clock Drawing Test; and Rey-Osterrieth Complex Figure. Test z-scores were computed using age-appropriate normative values.

An average composite z-score was computed for the five identified cognitive domains: attention (focused attention, working memory, processing speed), executive function, memory (verbal, visual), language, and visuospatial function. Other tasks, with no appropriate published normative data, were also completed including Star Cancellation and the 16-item Token Test using a cut-off score of 14 for aphasia.

The National Adult Reading Test (NART)[32] was used to estimate pre-morbid IQ. Screening for anxiety and depression was completed using the Generalized Anxiety Disorder-7 (GAD-7)[33] scale and Patient Health Questionnaire-9 (PHQ-9).[34] At 3 years, we also computed a weighted Global Clinical Dementia Rating (CDR).[35]

An evaluation panel was formed to assign a cognitive status to each participant based on their deidentified composite z-scores, mood and CDR scores, and information from clinical interviews. The panel included two cognitive and stroke neurologists, one clinical neuropsychologist, two research neuropsychologists, and one research psychologist. Participants were classified as cognitively normal if there was no evidence of cognitive impairment in any domain; cognitively impaired if a z-score was < −1.5 in at least one cognitive domain, without any

functional decline; or demented if the z-scores in at least two domains were < –1.5, in addition to a functional decline.

In this study, we were specifically interested in tests of verbal memory. We used HVLT-R[36] for assessing immediate and delayed recall, and raw scores were standardized (z-scores) using appropriate age-stratified normative data.[37]

2.6 | Statistical analysis

Statistical analyses were completed in MATLAB (Statistics Toolbox, R2019b, MathWorks). An alpha threshold of 0.05 was used to mark statistical significance.

2.6.1 | Demographic, clinical, neuropsychological, and imaging data

Comparisons between groups were conducted using: (1) two-sample t-test for age, cognitive testing (except visuospatial function), NART, and whole hippocampal volumes; (2) Fisher exact test for sex, clinical information (*APOE ε4*, depression, vascular risk factors), cognitive panel rating, aphasia, and Oxfordshire classes; and (3) Wilcoxon rank sum test for years of education, CCI, lesion volumes, visuospatial function, NIHSS, and mRS scores.

2.6.2 | Correlations between memory performance and hippocampal volumes

Partial Pearson's correlation analysis (partialcorr, MATLAB) was performed to test the association between standardized immediate and delayed recall scores and hippocampal whole and subfield volumes. Correlations were obtained separately for left and right volumes. We focused on the major subfields after excluding those with relatively small volumes, which we deemed more prone to segmentation inaccuracies: hippocampal fissure, fimbria, HATA, and parasubiculum. The FreeSurfer parcellations of the excluded subfields were shown to have higher reproducibility error compared to other subfields.[38] Sex and years of education were included as covariates for their known associations with hippocampal volume and/or memory. Stroke side was included as a covariate for its known different effects on ipsilateral and contralateral volumes. Because memory scores were standardized using appropriate age-stratified normative, age was not used as a covariate. The partial correlations across memory tests were corrected for multiple comparisons using a 5% false discovery rate.

Correlations between hippocampal volumes and verbal memory performance were assessed cross-sectionally at 3 months and 3 years. In addition, correlations were assessed for two distinct stroke subgroups: right-sided (n = 51) and left-sided (n = 31) stroke patients. New knowledge was gained from separately analyzing these subgroups (see section 3.3). A plot of average whole hippocampal volumes and recall scores at both timepoints (Figure 1) shows combined volume–memory states that are significantly different in volume and/or memory. Our
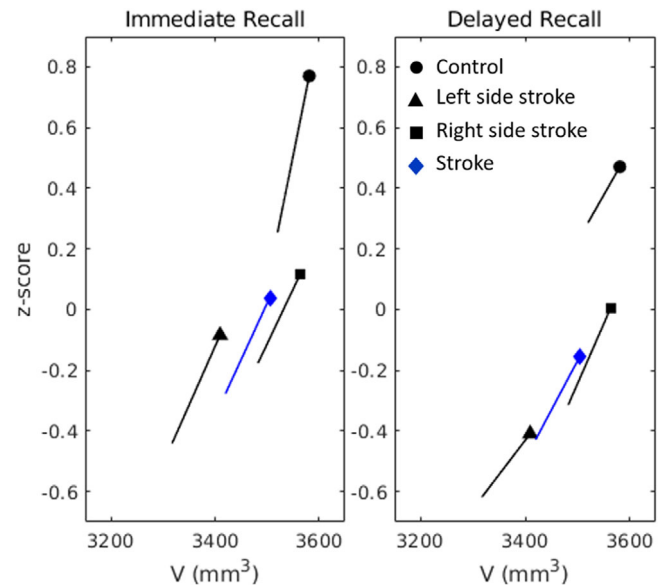

FIGURE 1 Hippocampal whole volumes and recall scores in control and stroke groups (markers indicate average volumes and recall scores at 3 months, lines show amounts of reduction in volumes and recall scores from 3 months to 3 years)

primary goal was to characterize the volume–memory associations for these distinct volume–memory states.

3 | RESULTS

This work was conducted on 38 (23 men, 68.7 ± 6.8 years) healthy individuals and 82 first-ever stroke patients (58 men, 66.7 ± 11.6 years). As shown in Figure 1, the reduction of hippocampal volume over time was accompanied by a reduction in verbal memory performance for both control and stroke groups; a general characteristic of volume–memory relationship in adults.

3.1 | Comparison of demographics, cognition, and clinical data between healthy controls and stroke patients

Demographics, vascular risk factors, z-scores for all five cognitive domains, general cognitive status (cognitive panel rating), and hippocampal volumes at 3 months are shown in Table 1. Comparisons between the groups at 3 years were generally similar. The general cognitive status at 3 years is also shown in Table 1.

There were no significant differences between groups in age and sex, but the stroke group had significantly less years of education than the control group. Also, there were no significant differences between healthy controls and stroke patients in terms of hippocampal volume, medical comorbidity score (CCI), or vascular risk factors except for AF. There were significantly more stroke patients with AF than healthy controls (P = .035). The left-sided stroke patients also showed significantly lower hippocampal volume at 3 months (P = .035) than the healthy controls.

TABLE 1 Comparison of demographics, cognition, and vascular risk factors of healthy controls and stroke patients at 3 months

Group	Control	Stroke	P
Number, N	38	82	
Sociodemographic			
Age, years, mean ± SD	68.7 ± 6.8	66.7 ± 11.6	.23[a]
Sex, men, no. (%)	23 (60.5)	58 (70.7)	.30[b]
Education, years, median (Q1, Q3)	17 (11, 18)	12 (10, 15)	<.001[c]
Clinical			
Charlson Comorbidity Index, median (Q1, Q3)	3 (2, 3)	3 (2, 4)	.45[c]
APOE ε4, no. (%)	4 (10.5)	15 (18.3)	.42[b]
Depression, no. (%)	4 (10.5)	8 (9.8)	>.99[b]
Hypertension, no. (%)	16 (42.1)	45 (54.9)	.24[b]
Hypercholesterolemia, no (%)	14 (36.8)	32 (39.0)	.84[b]
Type 2 diabetes mellitus, no. (%)	4 (10.5)	17 (20.7)	.21[b]
Atrial fibrillation, no (%)	1 (2.6)	14 (17.1)	.035[b]
Smoking, no (%)	7 (18.4)	18 (22.0)	.81[b]
Alcohol consumption, high, no (%)	6 (15.8)	7 (8.5)	.34[b]
Obese, no. (%)	6 (15.8)	23 (28.0)	.17[b]
Imaging			
Hippocampal volume (mm^3), mean ± SD	3581 ± 316	3505 ± 378	.25[a]
Cognitive—Verbal memory			
Immediate recall, z-score, mean ± SD	0.77 ± 1.0	0.04 ± 1.08	<.001[a]
Delayed recall, z-score, mean ± SD	0.47 ± 1.09	−0.15 ± 1.33	.008[a]
Cognitive—Other			
Dementia rating, cognitively impaired, no. (%)	1 (2.6)	21 (25.6)	.002[b]
Dementia rating at 3 years, no. (%) Cognitively normal (CN) Cognitively impaired (CI) Demented (D)	36 (94.7) 2 (5.3%) 0 (0)	66 (80.5) 14 (17.1) 2 (2.4)	.13[b]
NART-FSIQ, mean ± SD	118.7 ± 9.9	111.1 ± 11.4	<.001[a]
Aphasic, no. (%)	0 (0)	7 (8.5)	.10[a]
Attention, z-score, mean ± SD	0.27 ± 0.45	−0.26 ± 0.62	<.001[a]
Executive function, z-score, mean ± SD	0.02 ± 0.68	−0.62 ± 1.04	<.001[a]
Language, z-score, mean ± SD	0.48 ± 0.80	0.04 ± 0.81	.007[a]
Visual memory, z-score, mean ± SD	0.70 ± 1.13	−0.01 ± 1.14	.002[a]
Visuospatial function, z-score, median (Q1, Q3)	1.17 (0.75, 1.17)	0.62 (−0.21, 1.0)	<0.001[c]

Abbreviations: APOE, apolipoprotein E; NART-FSIQ, National Adult Reading Tests-Full Scale Intelligence Quotient; SD, standard deviation.
Note: Q1, Q3, 25th, 75th percentiles.
[a]Two-sample t-test.
[b]Fisher exact test.
[c]Wilcoxon rank sum test.

There was no significant difference in aphasia prevalence (P = .1) between control and stroke groups. However, the stroke patients scored significantly lower in all cognitive domains and in the general cognitive rating (P = .002) at 3 months. Over the period of 3 years, five cognitively impaired stroke patients became cognitively normal and two became demented. Thus, the difference in general cognitive status at 3 years between the control and stroke groups became insignificant (P = .13).

3.2 | Comparison of demographics, cognition, clinical data, and stroke characteristics between left-sided and right-sided stroke patients

Demographics, vascular risk factors, stroke characteristics, and cognition at 3 months for left-sided and right-sided stroke patients are shown in Table 2. Comparisons at 3 years remain similar.

TABLE 2 Demographics, cognition, and clinical and stroke characteristics of left-sided and right-sided stroke patients at 3 months

Group	Left stroke	Right stroke	P
Number, N	31	51	
Sociodemographic			
Age, years, mean ± SD	67.3 ± 11.2	65.8 ± 11.8	.59[a]
Sex, men, no. (%)	21 (67.7)	37 (72.6)	.80[b]
Education, years, median (Q1, Q3)	12 (10, 15.75)	12 (10, 15)	.72[c]
Clinical			
CCI, median (Q1, Q3)	3 (2, 4.75)	3 (2, 4)	.76[c]
APOE ε4, no. (%)	7 (22.6)	8 (15.7)	.56[b]
Depression, no. (%)	1 (3.2)	7 (13.7)	.25[b]
Hypertension, no. (%)	14 (45.2)	31 (60.8)	.18[b]
Hypercholesterolemia, no (%)	13 (41.9)	19 (37.3)	.82[b]
Type 2 diabetes mellitus, no. (%)	7 (22.6)	10 (19.6)	.78[b]
Atrial fibrillation, no (%)	5 (16.1)	9 (17.6)	>.99[b]
Smoking, no (%)	5 (16.1)	13 (25.5)	.41[b]
Alcohol consumption, high, no (%)	2 (6.5)	5 (9.8)	.70[b]
Obese, no. (%)	11 (35.5)	12 (23.5)	.31[b]
Imaging			
Hippocampal volume (mm^3), mean ± SD	3410 ± 340	3563 ± 392	.065[a]
Stroke lesion volume (mm^3), median (Q1, Q3)	1899 (503, 5762)	1522 (282, 6728)	.77[c]
Cognitive—Verbal memory			
Immediate recall, *z*-score, mean ± SD	−0.08 ± 1.26	0.11 ± 0.94	.45[a]
Delayed recall, *z*-score, mean ± SD	−0.41 ± 1.61	0 ± 1.12	.22[a]
Cognitive—Other			
Dementia rating, cognitively impaired, no. (%)	7 (22.6)	14 (27.5)	.80[b]
Dementia rating at 3 years, no. (%)	25 (80.7)	41 (80.4)	>.99[b]
Cognitively normal (CN)	5 (16.1)	9 (17.6)	
Cognitively impaired (CI)	1 (3.2)	1 (2.0)	
Demented (D)			
NART-FSIQ, mean ± SD	110.7 ± 9.5	111.3 ± 12.4	.81[a]
Aphasic, no. (%)	2 (6.5)	5 (9.8)	.70[b]
Attention, mean ± SD	−0.31 ± 0.60	−0.23 ± 0.64	.55[a]
Executive function, mean ± SD	−0.51 ± 0.84	−0.68 ± 1.15	.45[a]
Language, mean ± SD	−0.16 ± 0.88	0.16 ± 0.75	.11[a]
Visual memory, mean ± SD	0.07 ± 1.18	−0.05 ± 1.13	.64[a]
Visuospatial function, median (Q1, Q3)	0.71 (0.04, 1.07)	0.62 (−0.45, 0.93)	.28[c]
Stroke characteristics			
NIHSS score, median (Q1, Q3)	3 (1.25, 4.75)	2 (1, 4)	.58[c]
NIHSS severity, mild (0–7), no. (%)	27 (87.1)	48 (94.1)	.42[b]
mRS score, median (Q1, Q3)	1 (1, 1)	1 (1, 2)	.94[c]
mRS severity, mild (0–1), no. (%)	24 (77.4)	34 (66.7)	.33[b]
Oxfordshire, no. (%)	3 (9.7)	8 (15.7)	.84[b]
Lacunar infarct (LACI)	11 (35.5)	18 (35.3)	
Posterior cerebral infarct (POCI)	17 (54.8)	24 (47.1)	
Partial anterior cerebral infarct (PACI)	0 (0)	1 (2.0)	
Total anterior cerebral infarct (TACI)			

Abbreviations: APOE, apolipoprotein E; CCI, Charlson Comorbidity Index; NART-FSIQ, National Adult Reading Tests-Full Scale Intelligence Quotient; NIHSS,National Institutes of Health Stroke Scale; mRS, Modified Rankin Scale; SD, standard deviation.

Note: Q1, Q3, 25th, 75th percentiles.

[a]Two-sample *t*-test.

[b]Fisher exact test.

[c]Wilcoxon rank sum test.

There were no significant differences between the two groups in age, sex, years of education, medical comorbidity score, vascular risk factors, aphasia prevalence, z-scores in all five cognitive domains, or in general cognitive status.

The hippocampal volumes were relatively smaller in the left-sided stroke patients, compared to right-stroke group, although this difference was not statistically significant ($P = .065$). Also, the average stroke lesion volume was not significantly different between the two groups, nor was the severity of stroke (NIHSS) or neurological disability (mRS). Most of our participants had a mild stroke (87.1% in left-sided and 94.1% in right-sided patients). All stroke infarcts occurred remote to the hippocampi and were mostly posterior (POCI) and/or partial anterior (PACI) circulation infarcts. Most of the lesions were subcortical and occurred predominantly in areas away from known memory hubs such as the prefrontal cortex and medial temporal lobes.

3.3 | Associations between verbal memory performance and hippocampal volumes

We explored the linear associations between hippocampal whole and subfield volumes and verbal memory performance by computing the partial correlations between these variables after adjusting for sex, years of education, and side of stroke. Heat maps representing partial correlations, with significance values, are shown in Figure 2. The partial correlation coefficients ranged between –0.44 and +0.59 (see Table S1 in supporting information).

We found negative (i.e., inversely proportional) volume–memory correlations in healthy controls. These correlations were significant at 3 months for CA1 and whole hippocampal volumes, but not at 3 years. In the stroke group, we found positive (i.e., directly proportional) volume–memory correlations at 3 months, which were significant in the left hippocampus. At 3 years, these correlations were greater and included all hippocampal subfields except the presubiculum. No significant volume–memory correlations were seen in the right-sided stroke survivors at either timepoint.

The left-sided stroke participants had smaller volumes and lower memory performance, and we found strong correlations for both immediate and delayed recall with the left hippocampus in this group (see also Table S1). However, there were more significant associations involving delayed recall than immediate recall. At 3 months post-stroke, the left CA2/3 and hippocampal tail volumes were significantly associated with verbal memory in the left-sided stroke group.

Scatterplots of immediate and delayed recall as a function of hippocampal volume are shown in Figure 3. While Figure 2 is an illustration of the strength and significance of association between verbal memory and hippocampal volumes, Figure 3 is about the slope of best-fit regression line characterizing this association. In stroke, a reduction in hippocampal volume is mostly accompanied by a reduction in verbal memory performance. However, the slope of best-fit line is shown increasing with further volume reductions indicating a nonlinear relationship between the volume and memory variables in stroke, as well as in controls. Figure 3 shows that the slopes are increasing faster for certain

FIGURE 2 Heat maps—Partial correlations between hippocampal volumes and immediate and delayed recall after correcting for sex, years of education, and stroke side (IR, immediate recall; DR, delayed recall; 3 M, 3 months; 3Y, 3 years, *$P < .05$, **$P < .01$). The color shades reflect the sign and strength of volume–memory correlations (darkest red reflects correlation coefficients $\leq$–0.5, darkest green reflects correlation coefficients $\geq$0.5)

subfields (e.g., CA1, CA2/3, DG, hippocampal tail) than for whole hippocampus.

4 | DISCUSSION

We investigated the associations between hippocampal whole and subfield volumes and verbal memory performance (immediate and delayed recall) in healthy individuals and first-ever left-sided and right-sided stroke patients. At both 3-month and 3-year timepoints, verbal memory performance in healthy controls was significantly better than in stroke patients.
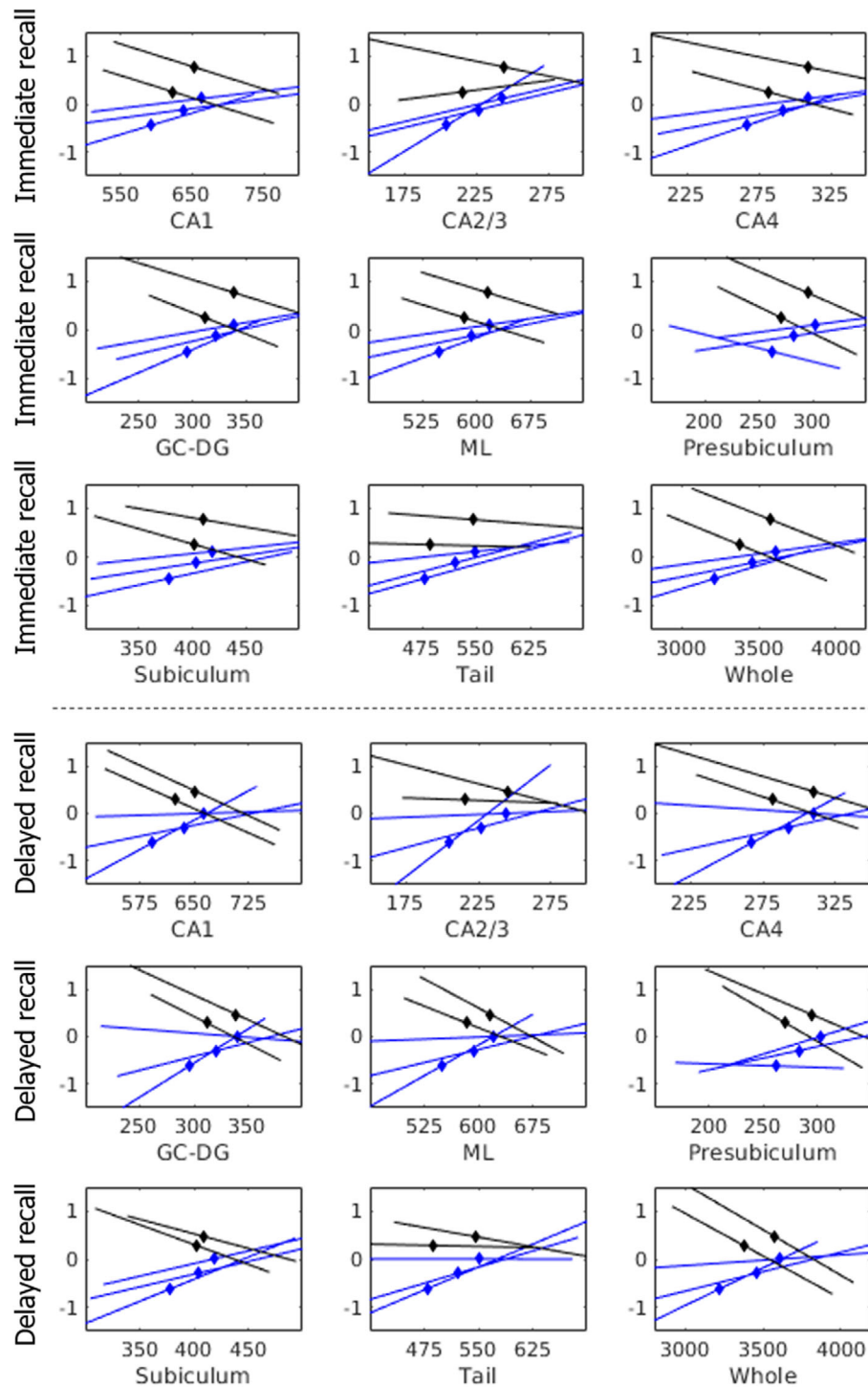
FIGURE 3 Scatterplots of recall performance as function of hippocampal volumes. For clarity, data points were omitted and replaced by averages (the markers) and best-fit regression lines (black = control, blue = stroke). The plotted averages represent distinct volume–memory states and they are, in descending order, (1) for control: average (volume, recall z-score) at 3 months and 3 years, (2) for stroke: average (volume, recall z-score) in right-sided patients at 3 months, average (volume, recall z-score) in all stroke patients at both 3-month and 3-year timepoints, and average (volume, recall z-score) in left-sided patients at 3 years. This figure exemplifies the dynamics of volume–memory association as hippocampal volumes are reduced. It also shows that these dynamics are different for the hippocampal subregions compared to the whole hippocampus

In healthy controls, we found a negative correlation between memory measures and hippocampal volumes at 3 months, but not at 3 years. Negative correlations between hippocampal volumes and verbal memory scores in healthy adults have been reported.[10] A meta-analysis of 33 studies investigating hippocampal volume and memory performance in healthy individuals across the lifespan reported little support for the "bigger-is-better" hypothesis—that is a larger volume predicts better performance. Instead, the study reported significant negative relationships between hippocampal volume and memory performance in children, adolescents, and young adults.[39] In studies of older adults, evidence for a positive relationship between hippocampal size and episodic memory ability was found to be weak.[39] A negative correlation may technically be explained by a relationship in which declining hippocampal volumes are not accompanied by alterations in memory performance, and/or by a compensatory volume increase to maintain memory. Adult hippocampal neurogenesis (AHN) happens throughout normal aging, up to the ninth decade of life, and is essential for memory, learning, and mood.[40,41] Impairment of AHN has been presented as a possible mechanism explaining memory deficits in AD,[40,41] and AHN impairment may underlie memory deficits in our stroke cohort.

We found positive volume–memory associations early after stroke—that is, larger hippocampal volumes in the left CA2/3 areas were associated with better delayed memory, and larger hippocampal volumes in the left hippocampal tail were associated with both better immediate and delayed memory in the left-sided stroke group. At 3 years post-stroke, we found significant associations with multiple left subfields including CA1 and CA4, DG, and molecular layer. These findings are supported by *post mortem* studies in which neuronal volumes in CA1 and CA2 were found positively correlated with global cognitive function and memory function in post-stroke subjects.[19,21] It is also worth noting that the association between whole hippocampal volume and delayed recall in the left-sided stroke group became significant only at 3 years. Our findings are in line with the view that the right hippocampus is particularly involved in the encoding of spatial relationships, with the left hippocampus more involved in episodic memory and storing of verbal information.[42]

The investigation of the slope of best-fit regression line revealed a nonlinear relationship between hippocampal volume and verbal memory in which a fixed-rate decrease in hippocampal volume predicted an accelerated decrease in verbal memory (Figure 3). The slopes for several subfields were increasing faster, compared to whole hippocampus, as average volumes dropped. In particular, the correlation slopes involving CA2/3 and hippocampal tail increased faster for both control and stroke groups. Therefore, the monitoring of left CA2/3 and hippocampal tail volumes may be used as a biomarker to detect post-stroke memory impairment, and perhaps for routine mental health check-ups in older adults. Future investigations, conducted on larger cohorts, ought to be able to finely define the hippocampal volume ranges informative of normal and compromised verbal memory status.

In a previous study[43] of a slightly larger group (n = 144) combining 40 healthy individuals and 104 stroke patients using a number of volumetric segmentation methods, we found an anatomical asymmetry (R > L, $P < .01$) between lateral hippocampal volumes. In this study, we again found an anatomical asymmetry (R > L, $P = .014$) between left and right whole hippocampal volumes at baseline (first session for controls and ≈27 days post-incident for stroke). In AD, the hippocampal volume asymmetry is even larger, perhaps suggesting the already smaller left hippocampus may be more vulnerable to the disease pathology than the right hippocampus. Thus, the smaller left hippocampal volumes have been found to be associated with poor verbal memory performance.[44] In ischemic stroke, we have shown the longitudinal atrophy of the hippocampus ipsilateral to the stroke infarct to be larger compared to atrophy of the contralateral hippocampus.[45,46] In the right-sided stroke participants, who had relatively higher volumes but less hippocampal volume asymmetry, there were no significant correlations between volumes and verbal memory in either hippocampus. Nevertheless, the characteristics of volume–memory association in this group still represented a clear departure from the association mechanism in healthy participants.

In the left-sided stroke participants, who already had smaller left volumes and more hippocampal asymmetry (R >> L), the correlations were stronger and significant between left volumes and delayed recall. However, there was a significant association between delayed recall and the right hippocampal tail volume. We posit the positive relationship of the right hippocampal tail with memory performance existed due to significant atrophy of this hippocampal subregion, and that further reductions to the right hippocampal volumes would be associated with verbal memory reductions. It is also possible that a functional lateral reorganization would be triggered by a compromised left hippocampus, but are unable to answer this question by our methods.

5 | CONCLUSIONS

At 3 months, reductions in hippocampal volumes in healthy individuals could not predict reductions in memory performance; for the relationship between the two was inversely proportional. In stroke, this relationship was directly proportional, mostly significant, and could predict verbal memory performance. In line with reports from studies of other neurodegenerative diseases, we found strong associations between the left subfield volumes and delayed recall. This may suggest that different disease pathologies influence the mechanism of association between hippocampal subfield volumes and episodic verbal memory in a similar manner.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Mohamed Salah Khlif https://orcid.org/0000-0002-0096-434X

REFERENCES

1. Frodl T, Schaub A, Banac S, et al. Reduced hippocampal volume correlates with executive dysfunctioning in major depression. *J Psychiatry Neurosci*. 2006;31(5):316-323.
2. Höller Y, Trinka E. What do temporal lobe epilepsy and progressive mild cognitive impairment have in common?. *Front Syst Neurosci*. 2014;8:58-58.
3. Meisenzahl EM, Seifert D, Bottlender R, et al. Differences in hippocampal volume between major depression and schizophrenia: a comparative neuroimaging study. *Eur Arch Psychiatry Clin Neurosci*. 2009;260:127-137.
4. Mueller SG, Schuff N, Yaffe K, et al. Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease. *Hum Brain Mapp*. 2010;31(9):1339-1347.
5. Woon FL, Sood S, Hedges DW. Hippocampal volume deficits associated with exposure to psychological trauma and posttraumatic stress disorder in adults: a meta-analysis. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010;34(7):1181-1188.
6. Reitz C, Brickman AM, Brown TR, et al. Linking hippocampal structure and function to memory performance in an aging population. *Arch Neurol*. 2009;66(11):1385-1392.
7. Zammit AR, Ezzati A, Zimmerman ME, et al. Roles of hippocampal subfields in verbal and visual episodic memory. *Behav Brain Res*. 2017;317:157-162.[CrossRef]
8. Uribe C, Segura B, Baggio HC, et al. Differential progression of regional hippocampal atrophy in aging and Parkinson's Disease. *Front Aging Neurosci*. 2018;10:325.
9. Evans TE, Adams HHH, Licher S, et al. Subregional volumes of the hippocampus in relation to cognitive function and risk of dementia. *Neuroimage*. 2018;178:129-135.
10. Carlesimo GA. Atrophy of presubiculum and subiculum is the earliest hippocampal anatomical marker of Alzheimer's disease. *Alzheimers Dement (Amst)*. 2015;1(1):24-32.
11. Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging*. 2006;27(10):372-384.
12. Adamowicz DH, Roy S, Salmon DP, et al. Hippocampal alpha-synuclein in dementia with lewy bodies contributes to memory impairment and is consistent with spread of pathology. *J Neurosci*. 2017;37(7):1675-1684.
13. Comper SM, Jardim AP, Corso JT, et al. Impact of hippocampal subfield histopathology in episodic memory impairment in mesial temporal lobe epilepsy and hippocampal sclerosis. *Epilepsy Behav*. 2017;75:183-189.
14. Foo H, Mak E, Chander RJ, et al. Associations of hippocampal subfields in the progression of cognitive decline related to Parkinson's disease. *Neuroimage Clin*. 2017;14:37-42.
15. Lim HK, Hong SC, Jung WS, et al. Automated segmentation of hippocampal subfields in drug-naive patients with Alzheimer disease. *AJNR Am J Neuroradiol*. 2013;34(4):747-751.
16. Cantero JL, Iglesias JE, Van Leemput K, et al. Regional hippocampal atrophy and higher levels of plasma amyloid-beta are associated with subjective memory complaints in nondemented elderly subjects. *J Gerontol A Biol Sci Med Sci*. 2016;71(9):1210-1215.
17. Nikonenko AG, Radenovic L, Andjus PR, Skibo GG. Structural features of ischemic damage in the hippocampus. *Anat Rec (Hoboken)*. 2009;292(12):1914-1921.
18. Schaapsmeerders P, van Uden IWM, Tuladhar AM, et al. Ipsilateral hippocampal atrophy is associated with long-term memory dysfunction after ischemic stroke in young adults. *Hum Brain Mapp*. 2015;36(7):2432-2442.
19. Gemmell E, Bosomworth H, Allan L, et al. Hippocampal neuronal atrophy and cognitive function in delayed poststroke and aging-related dementias. *Stroke*. 2012;43(3):808-814.
20. Mok VCT, Lam BYK, Wong A, et al. Early-onset and delayed-onset poststroke dementia [mdash] revisiting the mechanisms. *Nat Rev Neurol*. 2017;13(3):148-159.
21. Kliper E, Bashat DB, Bornstein NM, et al. Cognitive decline after stroke: relation to inflammatory biomarkers and hippocampal volume. *Stroke*. 2013;44(5):1433-1435.
22. Tatemichi TK, Desmond DW, Stern Y, Paik M, Sano M, Bagiella E. Cognitive impairment after stroke: frequency, patterns, and relationship to functional abilities. *J Neurol Neurosurg Psychiatry*. 1994;57(2):202-207.
23. Bäckman L, Jones S, Berger A-K, et al. Cognitive impairment in preclinical Alzheimer's disease: a meta-analysis. *Neuropsychology*. 2005;19(4):520-531.
24. Boraxbekk CJ, Lundquist A, Nordin A, et al. Free recall episodic memory performance predicts dementia ten years prior to clinical diagnosis: findings from the Betula Longitudinal Study. *Dement Geriatr Cogn Dis Extra*. 2015;5(2):191-202.
25. Brodtmann A, Werden E, Pardoe H, et al. Charting cognitive and volumetric trajectories after stroke: protocol for the Cognition and Neocortical Volume after Stroke (CANVAS) Study. *Int J Stroke*. 2014;9(6):824-828.
26. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage*. 2012;61(4):1402-1418.
27. Iglesias JE, Augustinack JC, Nguyen K, et al. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: application to adaptive segmentation of in vivo MRI. *Neuroimage*. 2015;115:117-137.
28. Ortiz GA, Sacco RL, National Institutes of Health Stroke Scale (NIHSS), In *Wiley StatsRef: Statistics Reference Online*. Iowa City: National Institutes of Health Stroke Scale (NIHSS).
29. Bamford J, Sandercock P, Dennis M, et al. A prospective study of acute cerebrovascular disease in the community: the Oxfordshire Community Stroke Project 1981-86. 1. Methodology, demography and incident cases of first-ever stroke. *J Neurol Neurosurg Psychiatry*. 1988;51(11):1373-1380.
30. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke*. 1988;19(5):604-607.
31. D'Hoore W, Sicotte C, Tilquin C. Risk adjustment in outcome assessment: the Charlson comorbidity index. *Methods Inf Med*. 1993;32(5):382-387.
32. HE N. National Adult Reading Test (NART). *For the Assessment of Premorbid Intelligence in Patients with Dementia: Test Manual 1982 NFER-Nelson*. Windsor, UK: National Adult Reading Test (NART).
33. Spitzer RL, Kroenke K, Williams JBW, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med*. 2006;166(10):1092-1097.
34. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606-613.
35. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology*. 1993;43(11):2412-2414.
36. Benedict RHB, Schretlen D, Groninger L, Brandt J. Hopkins verbal learning test—Revised: normative data and analysis of inter-form and test-retest reliability. *Clin Neuropsychol*. 1998;12(1):43-55.
37. Brandt J, Benedict RH. *Hopkins Verbal Learning Test–Revised: Professional Manual*: Psychological Assessment Resources; 2001.
38. Chiappiniello A, Tarducci R, Muscio C, et al. 22. Multicentric test-retest reproducibility of human hippocampal volumes with FreeSurfer 6.0: a

comparison between standard and longitudinal hippocampal subfields segmentation streams applied to 3D T1, 3D FLAIR and high-resolution 2D T2 neuroimaging. *Physica Med*. 2018;56:74-75.

39. Van Petten C. Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. *Neuropsychologia*. 2004;42(10):1394-1413.

40. Maruszak A, Pilarski A, Murphy T, et al. Hippocampal neurogenesis in Alzheimer's disease: is there a role for dietary modulation?. *J Alzheimers Dis*. 2014;38(1):11-38.

41. Moreno-Jiménez EP, Flor-García M, Terreros-Roncal J, et al. Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med*. 2019;25(4):554-560.

42. Burgess N, Maguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. *Neuron*. 2002;35(4):625-641.

43. Khlif MS, Egorova N, Werden E, et al. A comparison of automated segmentation and manual tracing in estimating hippocampal volume in ischemic stroke and healthy control participants. *Neuroimage Clin*. 2019;21:101581.

44. Muller MJ, Greverus D, Dellani PR, et al. Functional implications of hippocampal volume and diffusivity in mild cognitive impairment. *Neuroimage*. 2005;28(4):1033-1042.

45. Brodtmann A, Khlif MS, Egorova N, et al. Dynamic regional brain atrophy rates in the first year after ischemic stroke. *Stroke*. 2020;51(9):e183-e192.

46. Khlif MS, Werden E, Egorova N, et al. Assessment of longitudinal hippocampal atrophy in the first year after ischemic stroke using automatic segmentation techniques. *NeuroImage Clin*. 2019;24: 102008.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.