Author/s:
Khlif, MS;Werden, E;Bird, LJ;Egorova-Brumley, N;Brodtmann, A

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Date:
2022-07-01

Citation:
Khlif, M. S., Werden, E., Bird, L. J., Egorova-Brumley, N. & Brodtmann, A. (2022). Atrophy of Ipsilesional Hippocampal Subfields Vary Over First Year After Ischemic Stroke. Journal of Magnetic Resonance Imaging, 56 (1), pp.273-281. https://doi.org/10.1002/jmri.28009.

Persistent Link:
https://hdl.handle.net/11343/299237

Khlif Mohamed Salah (Orcid ID: 0000-0002-0096-434X)

Atrophy of Ipsilesional Hippocampal Subfields Vary Over First Year After Ischemic Stroke

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Mohamed Salah Khlif PhD[1], Emilio Werden PhD[1], Laura J. Bird PhD[1], Natalia Egorova-Brumley PhD[1,2], Amy Brodtmann FRACP MBBS PhD[1,3,4,5]

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

[2]Melbourne School of Psychological Sciences, University of Melbourne, Melbourne, Australia

[3]Department of Neurology, Austin Health, Heidelberg VIC, Australia

[4]Department of Neurology, Royal Melbourne Hospital, Parkville VIC, Australia

[5]Eastern Cognitive Disorders Clinic, Box Hill Hospital, Monash University, Box Hill VIC, Australia

Corresponding author: Mohamed Salah Khlif, 245 Burgundy Street, Heidelberg VIC 3084 Australia, +61435145568, mohamed.khlif@florey.edu.au

Acknowledgments: This work was supported by: NHMRC GTN1020526, GTN1045617 (AB), GTN1094974; Brain Foundation; Wicking Trust; Collie Trust; Sidney and Fiona Myer Family Foundation; ARC DE180100893 (NEB); Heart Foundation Future Leader Fellowship (100784, AB).

Data availability: Data supporting this study findings are available upon reasonable request.

Ethics: Ethical approval was granted by each hospital's Human Research Ethics Committee and all participants provided informed consent.

Clinical trial registration: http://www.clinicaltrials.gov. Unique identifier: NCT02205424

Running title: Hippocampal subfield atrophy after stroke

Abstract

Background

The structural integrity of hippocampal subfields has been investigated in many neurological disorders and was shown to be better associated with cognitive performance than whole hippocampus. In stroke, hippocampal atrophy is linked to cognitive impairment but it is unknown whether the hippocampal subfields atrophy differently.

Purpose

To evaluate longitudinal hippocampal subfield atrophy in first year post-stroke, in comparison with atrophy in healthy individuals.

Study Type

Cohort.

Subjects

92 ischemic stroke (age: 67±12 years, 63 men) and 39 healthy participants (age: 69±7 years, 24 men).

Field Strength/Sequence

3T/T1-MPRAGE, T2-SPACE, and T2-FLAIR.

Assessment

FreeSurfer (6.0) was used to delineate 12 hippocampal subfields. Whole hippocampal volume was computed as sum of subfield volumes excluding hippocampal fissure volume. Separate assessments were completed for contralesional and ipsilesional hippocampi.

Statistical Tests

A mixed-effect regression model was used to compare subfield volumes cross-sectionally between healthy and stroke groups and longitudinally between 3-month and 12-month timepoints. False discovery rate (FDR) at 0.05 significance level was used to correct for multiple comparisons. Also, a receiver operating characteristic (ROC) curve analysis was performed to assess differentiation between healthy and stroke participants based on subfield volumes.

Results

There were no volume differences between groups at 3 months, but there was a significant difference ($p = 0.027$) in whole hippocampal volume reduction over time between control and stroke ipsilesionally. Thus, the ipsilesional whole hippocampal volume in stroke became significantly smaller ($p = 0.035$) at 12 months. The hippocampal tail was the highest single-region contributor (22.7%) to ipsilesional hippocampal atrophy (1.19%) over nine months. The CA1 subfield volume reduction was minimal in controls and stroke contralesionally, but significant ipsilesionally ($p = 0.007$). CA1 volume significantly outperformed whole hippocampal volume ($p < 0.01$) in discriminating between stroke participants and healthy controls in ROC curve analysis.

Data Conclusion

Greater stroke-induced effects were observed in the ipsilesional hippocampus anteriorly in CA1 and posteriorly in the hippocampal tail. Atrophy of CA1 and hippocampal tail may provide a better link to cognitive impairment than whole hippocampal atrophy.

Keywords: Atrophy, Hippocampal subfields, FreeSurfer, Neurodegeneration, Stroke

INTRODUCTION

The hippocampus is a complex structure composed of interconnected subfields with distinctive histology and functions [1, 2]. Volume reductions of the whole hippocampus are a hallmark of most neurodegenerative diseases, and are associated with cognitive impairment and dementia [3-7]. However, the volumes of hippocampal subfields including the cornu ammonis areas (CA1 - 4), dentate gyrus (DG), and the presubiculum-subiculum complex are suggested as better predictors of cognitive performance than whole hippocampal volume [8-10].

For instance, the CA1 volume was found to be more sensitive than whole hippocampal volume in detecting structural changes at the pre-dementia stage in Alzheimer's disease (AD), mild cognitive impairment (MCI), and semantic dementia [9]. Histological studies in AD also confirmed particular neuronal loss in CA1, in addition to the subiculum [11, 12]. In patients with hippocampal sclerosis and temporal lobe epilepsy (TLE), preoperative CA1 and CA4 volumes were related to better surgical outcome after one year [13]. In Parkinson's disease, baseline DG and right CA4 volumes were more predictive of conversion to MCI than whole hippocampal volume [14]. Also, CA1 volume deficit was reported in schizophrenia cases [15]. Different hippocampal neuronal populations are remotely and selectively vulnerable to ischemic insults independent of the Alzheimer's pathology [16-19]. In a recent study of older people with vascular brain injuries, the volumes of white matter hyperintensities (WMH) were found associated with subiculum volumes [20]. The annualized rates of atrophy of the subiculum were also higher in people with highest rates of WMH progression, independent of demographic variables and vascular risk factors. In a study of acute ischemic stroke patients, increasing WMH volume was also selectively associated with decreased hippocampal-amygdala-transition-area volume [21]. Yet, another volumetric study of demented stroke patients found smaller volumes of DG and CA4, compared with normal controls and non-demented stroke individuals [22]. The vulnerability of selective hippocampal subfields was

also confirmed by a post-mortem study where CA1 and CA2 neuronal volumes were found positively correlated with global cognition and memory function in post-stroke survivors [17, 23].

Although AD is the leading cause of clinically diagnosed dementia, cognitive impairment and dementia of vascular origin are the second most common globally [24]. Up to a third of stroke survivors develop dementia years after the initial incident [25], and those with smaller hippocampal volumes were reported to have cognitive impairment [17, 23]. Despite this, brain volume changes after stroke have not been studied as intensely as atrophic patterns in other dementias. This is especially true of hippocampal atrophy which remains largely under-investigated. Particularly, little is known about the impact of stroke on the atrophy of hippocampal subfields in survivors with or at risk of vascular cognitive impairment or dementia. In view of this, this study sought to assess hippocampal subfield volume deficit early post-stroke (i.e., at 3 months) and to examine the longitudinal patterns of atrophy of the distinct hippocampal subfields in stroke in the period between 3 months and 1 year follow-up. Given that hippocampal subfields are differentially vulnerable to neuropathology in many neurological disorders, it is anticipated that the hippocampal subregions in stroke would also atrophy at different rates between 3 months and 12 months and that the ipsilateral subfields would show larger atrophy rates compared to contralateral subfields; given that a previous study has demonstrated greater reduction in whole ipsilesional hippocampal volume 12 months post-stroke [26].

MATERIALS AND METHODS

Participants

Participants were sampled from the Cognition And Neocortical Volume After Stroke (CANVAS) study [27]. Briefly, stroke participants were recruited from three sites in Melbourne (Austin Health, Eastern Health, and Melbourne Health), with all MRI scans held at

The Florey Institute of Neuroscience and Mental Health, Austin Hospital. Healthy controls were recruited from relatives of stroke participants and from the general public. Ethical approval was granted by each hospital's Human Research Ethics Committee and all participants provided informed consent. Participants completed an initial interview to collect demographic and medical history information, MRI scanning, and neuropsychological assessment at baseline (within 6 weeks post-incident for stroke), 3-month, and 1-year follow-up. The longitudinal CANVAS 3-month and 1-year data are available as part of the ENIGMA Stroke Recovery Working Group database collected across 18 research studies from 10 research institutes in 6 countries [28].

Patients diagnosed with primary hemorrhagic stroke, transient ischemic attack (TIA), venous infarction, or significant medical comorbidities were excluded from the study. Age- and sex-matched healthy controls had no history of stroke or TIA and both healthy and stroke participants had no pre-existing dementia, neurodegenerative disorders, major psychiatric illnesses, or substance abuse. This study was based on the assessment of 131 participants at 3 months (39 controls and 92 first-ever stroke patients) and 130 participants at 12 months. One control participant who had a stroke after the 3-month timepoint was excluded from the 12-month data subset.

MRI Acquisition and Processing

Brain images were acquired on a 3T Siemens Tim Trio Scanner (Erlangen, Germany). The MR images were obtained using a T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) sequence (160 sagittal slices, 1900 ms repetition time, 2.6 ms echo time, 900 ms inversion time, 9° flip angle, 1mm isotropic voxel, 256 × 256 field of view). Volumetric segmentation was completed using FreeSurfer 6.0 longitudinal processing pipeline [29] including motion correction, removal of non-brain tissue, Talairach transformation, segmentation of subcortical white and gray matter structures, intensity normalization, and

tessellation of the gray-white matter boundaries. The delineation of hippocampal subfields used a probabilistic ex-vivo ultra-high resolution (~0.1 mm) atlas [30]. A dice overlap of ~0.7 between manual tracing and automated segmentation based on the ex-vivo atlas was reported for all subfields [30]. Twelve hippocampal subregions were obtained: cornu ammonis areas (CA1, CA2/3, and CA4), subiculum, presubiculum, parasubiculum, DG, HATA, fimbria, molecular layer (ML), hippocampal fissure, and hippocampal tail. A whole hippocampal volume, V_{Whole} was defined as the sum of all subfield volumes after excluding the hippocampal fissure volume. The volumes from CA4, DG, and molecular layer subregions were combined and analysed as a single structure: CA4-DG-ML.

Stroke hippocampal whole and subfield volumes were clustered into ipsilesional and contralesional and each cluster was compared to hippocampal volumes in healthy controls. To ensure unbiased comparisons, weighted averages of left and right volumes in healthy controls were computed. The stroke group included 36% left-sided and 64% right-sided strokes. Accordingly, the ipsilesional volumes were compared to control averages weighted by a 36 : 64 left-right ratio, and the contralesional volumes were compared to averages weighted by a 64 : 36 left-right ratio. Finally, we note that the ischemic infarcts for all stroke participants occurred away from the hippocampus.

Statistical Analysis

Statistical analyses were completed in MATLAB (Release 2019a, The MathWorks Inc., Massachusetts, US). All analyses used a p-value of 0.05 to mark statistical significance.

Participants' demographics

Comparisons of group demographics were completed using the Two-sample *t*-test for age and total intracranial volume (TIV, estimated from FreeSurfer parcellation), the Fisher Exact test for sex, and the Wilcoxon Rank Sum test for years of education.

Estimation of hippocampal whole and subfield rates of atrophy

A linear mixed-effect regression model (*fitlme* function in MATLAB) with random intercept and slope was used to compare cross-sectional volumes between groups and to estimate longitudinal rates of atrophy between 3 and 12 months. Comparisons of differences in rates of atrophy between groups were also completed: ipsilesional and contralesional atrophy rates versus rates in healthy controls. The model included the fixed effects group, time, and the 'Group : Time' interaction, in addition to the covariates age, years of education, and TIV. Based on simulated likelihood ratio testing, sex was not included as a covariate. The covariates were checked for collinearity and the model residuals were checked for normality and heteroscedasticity. The random effects included uncorrelated intercept and slope terms. The *fitlme* coding is given by:

$V = \text{Group} * \text{Time} + \text{Education} + \text{TIV} + 1 \mid \text{Subject} + (\text{Time} - 1 \mid \text{Subject})$, where $V = \text{volume}$ and $\text{Group} * \text{TP} = \text{Group} + \text{TP} + \text{Group} : \text{TP}$.

Time was dummy coded as TP1 (3 months) and TP2 (12 months) since the follow-up interval (9 months) was comparable for both control and stroke groups. In the analyses, subfields with relatively small volumes were not included: HATA, parasubiculum, hippocampal fissure, and fimbria. Family-wise correction for multiple comparisons across the subfields was completed using a false detection rate at a 0.05 significance level.

Group discrimination using receiver operating characteristic (ROC) curve analysis

ROC curve analysis represented an alternative approach to assess differences in hippocampal volumes between stroke and control groups. A Random Forest classifier with stratified 10-fold cross-validation was used for this purpose, and the analyses were implemented using the WEKA 3.8 toolbox [32]. Results from ROC analyses based on 3-month and 12-month subfield volumes were comparable. Therefore, findings from just one ROC analysis based on combined volumes from both timepoints were reported.

ROC analysis based on regional and global hippocampal volumes estimated from FreeSurfer multi-model segmentation based on T1-MPRAGE and T2-SPACE (sampling perfection with application-optimized contrasts using different flip angle evolutions), and based on T1-MPRAGE and high-resolution T2-FLAIR (fluid-attenuated inversion recovery) scans were also performed. The T2-SPACE and T2-FLAIR scans were acquired using the following specifications:

T2: 176 sagittal slices, 1 mm isotropic, 3390 ms repetition time, 390 ms echo time, 120° flip angle, and 256 x 204 field of view.

FLAIR: 160 sagittal slices, 1 mm isotropic, 6000 ms repetition time, 388 ms echo time, 2100 ms inversion time, 120° flip angle, and 512 x 512 field of view.

RESULTS

Comparison of Demographic Variables

Comparisons of demographic variables between groups at 3 months are shown in Table 1. There were no statistical differences in age, sex, or TIV between stroke and control participants, but the healthy controls had significantly more years of education (median: 17 years for control vs 12 years for stroke, $p < 0.0004$).

Hippocampal Volumes and Atrophy Rates between 3 and 12 Months

Hippocampal whole and subfields volumes for control and stroke groups at 3 months are presented in Table 2. Significance of within-group volume changes between 3 and 12 months are also shown. Cross-sectional comparisons of volumes and atrophy rates between groups are shown in the last three columns of Table 2. The contralesional and ipsilesional rates of atrophy are graphically presented in Fig. 1.

Healthy Control Group

Between timepoints, the average reduction in whole hippocampal volume, Δ_{Whole} was 0.41% - not significant ($p = 0.14$). The largest regional contributions to whole hippocampal atrophy

came from subiculum (22.3% of Δ_{Whole}) and presubiculum (18.8% of Δ_{Whole}), while CA1 volume remained unchanged. The presubiculum ($\Delta = -0.95\%$) and subiculum ($\Delta = -0.80\%$) had the largest atrophy among the subfields in healthy controls, but these volumetric reduction rates were not significant.

Stroke Contralesional Volumes and Rates of Atrophy

As in controls, CA1 had very little change between 3 months and 12 months. However, whole ($\Delta = -0.62\%$, $p = 0.002$) and two subfield volumes were significantly reduced between timepoints including the presubiculum ($\Delta = -1.26\%$, $p = 0.003$) and hippocampal tail ($\Delta = -1.11\%$, $p = 0.007$), see Table 2. Though numerically smaller, none of the contralesional volumes were significantly different from control volumes at 3 months. Rates of atrophy were also not significantly different between the two groups; thus the volumes were not statistically different at 12 months either. The largest individual contributors to whole hippocampal contralesional atrophy were hippocampal tail (27.4% of Δ_{Whole}) and presubiculum (17.2% of Δ_{Whole}).

Stroke Ipsilesional Volumes and Rates of Atrophy

Whole ($\Delta = -1.19\%$, $p < 0.001$) and subfield volumes were significantly reduced (CA1: $\Delta = -0.71\%$, $p = 0.007$; CA2/3: $\Delta = -1.1\%$, $p < 0.001$; CA4-DG-ML: $\Delta = -1.15\%$, $p < 0.001$; presubiculum: $\Delta = -1.27\%$, $p = 0.003$; subiculum: $\Delta = -1.15\%$, $p < 0.001$; hippocampal tail: $\Delta = -1.8\%$, $p < 0.001$) between 3 and 12 months. Compared to controls, the ipsilesional atrophy rates were numerically larger especially for hippocampal tail, and significantly larger for the ipsilesional whole hippocampus (1.19% for stroke vs 1.15% for control, $p = 0.027$), see Table 2. The ipsilesional atrophy was also greater than contralesional atrophy; especially for whole hippocampus (1.19% ipsilesionally vs 0.62% contralesionally, $p = 0.023$). The reduction in ipsilesional whole hippocampus was three times that of controls and twice that of contralesional hippocampus. The largest single contribution to ipsilesional whole hippocampus

reduction came from the hippocampal tail (22.7% of Δ_{Whole}). Thus, the largest single subfield contribution to hippocampal atrophy was posteriorly from the hippocampal tail; both contralesionally and ipsilesionally. Figure 2 shows examples of ipsilesional hippocampal tail atrophy for left-sided and right-sided stroke patients.

Cross-sectionally, the ipsilesional volumes were numerically smaller compared to controls at 3 months; especially for CA4-DG-ML, presubiculum, and whole hippocampus. Due to faster volume reductions ipsilesionally, volume differences between control and stroke became larger at 12 months; especially for the ipsilesional whole hippocampus (3568 mm^3 for control vs 3433 mm^3 for stroke, $p = 0.035$).

Except for the presubiculum, Table 2 shows that contralesional and ipsilesional subfield volumes are comparable at 3 months. The presubiculum ipsilesional volume was significantly smaller than the contralesional volume at 3 months (281 mm^3 vs 292 mm^3, $p = 0.004$), and the two volumes were reduced by similar amounts between timepoints. To determine whether the deficit in subiculum ipsilesional volume was related to stroke, volumes at baseline (~27 days post-stroke) were compared. Again, the ipsilesional volumes were found significantly smaller ($p = 0.003$). In controls, the right subfield volumes were found to be larger, mostly significant, than the left subfield volumes except for the left presubiculum volume which was higher (313 mm^3 vs 293 mm^3, $p = 0.028$). Bilateral subiculum volumes in controls were nearly equal (left - 427 mm^3, right - 425 mm^3).

ROC Curve Analysis

Table 3 presents the results of ROC curve analysis of hippocampal volumes differentiating between healthy and stroke participants. Table 3 lists the sensitivity, precision, accuracy, and AUC (area under curve) measures for hippocampal subfields tested separately (single feature classification) and collectively using the ensemble of subfield volumes (multi-feature classification). Compared to classification based on whole hippocampal volume, the

classifications based on individual subfield volumes generally resulted in higher performance for most of the sensitivity, precision, accuracy, and AUC measures. CA1-based (sensitivity and accuracy: 0.79 vs 0.66, precision: 0.80 vs 0.65, AUC: 0.84 vs 0.50, $p < 0.001$ for all) and hippocampal tail-based (sensitivity and accuracy: 0.74 vs 0.66, $p < 0.05$; precision: 0.72 vs 0.65, $p < 0.05$; AUC: 0.69 vs 0.50, $p < 0.001$) classification performance was significantly better than whole hippocampus-based performance for all measures. The discrimination between healthy and stroke participants based on the ensemble of subfields (sensitivity and accuracy: 0.91 vs 0.66, precision: 0.91 vs 0.65, AUC: 0.95 vs 0.50, $p < 0.001$ for all) was as one would expect stronger than the discriminations based on whole hippocampus or even CA1 and hippocampal tail volumes; though the AUC for CA1 was larger for a false positive rate up to 5% (see Fig. 3). Multi-feature classification, using the ensemble of subfield volumes, resulted in 0.95 AUC and 0.91 for sensitivity, precision, and accuracy.

FreeSurfer multimodal segmentation based on T1-T2 and T1-FLAIR scan combinations resulted in slightly higher classification performance using the ensemble of subfield volumes: 0.97 for AUC (vs 0.95 AUC based on T1 alone) and approximately 0.92 for sensitivity, precision, and accuracy (vs 0.91 for all based on T1 alone). These were not significantly different.

DISCUSSION

We compared hippocampal subfield volumes and their reductions over a period of 9 months in ischemic stroke patients and healthy controls. Our findings showed accelerated atrophy of the ipsilesional hippocampus compared to normal controls and differential atrophy rates across the distinct subfields in both groups. None of the subfield volumes, nor the whole hippocampal volume were found to be significantly different from volumes in controls early after stroke (i.e., at 3 months). The assessed subfields did not include HATA, parasubiculum, hippocampal

fissure, and fimbria since FreeSurfer parcellation of these subfields were shown to have relatively higher reproducibility error compared to the rest of the subfields [31].

In the healthy group, there was no significant reduction of whole hippocampal volume between timepoints. But relatively larger regional atrophy in the subiculum and presubiculum were found. The subiculum-presubiculum complex constitutes about 20% of the whole hippocampal volume, yet it contributed more than 41% of total hippocampal volume reduction.

Over 9 months, all ipsilesional volume reductions were larger than their counterparts in the contralateral hemisphere, except for presubiculum where both contralesional and ipsilesional volumes were reduced by a similar amount. These findings suggest that, contrary to the rest of subfields, the presubiculum volume reduction, at least in the short term, may still be primarily driven by age-related neurodegeneration. Also, given a greater contralesional atrophy in the presubiculum compared to whole hippocampus, these findings suggest that the presubiculum may be the earliest remote contralesional hippocampal subregion to become significantly atrophic in the long term. This may be supported by findings in other neurological disorders. In AD, for instance, prevalent atrophy of the subiculum-presubiculum complex from the early stages of the disease have been reported where atrophy in the presubiculum-subiculum complex progressively worsened as patients transitioned from aMCI to AD [33]. Severe volume deficits in subiculum and presubiculum in AD have been attributed to isomorphic fibrillary gliosis resulting from early and severe degeneration of the perforant pathway from the entorhinal cortex to DG, via the subicular complex, and to a loss of pyramidal neurons in the subiculum [34]. Volume deficits have also been found in the left subiculum and presubiculum in patients with subcortical vascular MCI (svMCI) [18].

We noted that the ipsilesional presubiculum volume was significantly lower than contralesional volume at 3 months, even earlier at baseline. This can be explained by the fact that the right presubiculum volume was lower in controls and that our stroke group contained nearly twice

as many participants with right-side lesions. Furthermore, the high parity of presubiculum contralesional and ipsilesional atrophy rates suggests no added effects due to stroke in the first-year post-incident. A study of subfield atrophy over the adult lifespan (20-89 years) reported similar findings [35], where the right hippocampal subfield volumes were shown to be higher than the left volumes from early adulthood except for the presubiculum; suggesting a stronger age-effect on the right presubiculum.

At 3 and 12 months, there were no differences in CA1, CA2/3, and CA4-DG-ML volumes between stroke and healthy groups. While there were equivalent reduction rates in CA2/3 and CA4-DG-ML volumes between timepoints in both groups, there was no change in CA1 volume in controls and stroke contralesionally. However, the ipsilesional CA1 volume reduction over 9 months was significant which constituted the largest departure, among the CA areas, from normal age-related atrophy. Reports about volume deficits in the cornu ammonis areas, in other neurodegenerative disorders, vary among studies. For instance, no volume deficit was found in CA1 in early and late AD stages [33, 36], but CA2 and CA3 volumes were found to be significantly smaller [33]. However, CA1 was found in another AD study to be significantly atrophic [37]. In svMCI, no significant atrophy was observed in CA1 [18], and in schizophrenia, CA1 volume was reported to be selectively reduced in early-to-mid stages of the disease [15].

Reports on atrophy of the hippocampal tail are very limited. In a study of cognitively normal Parkinson's disease patients, time effects on the left hippocampal tail were found [8]. Here, we report a superadded atrophy in the hippocampal tail due to stroke; both contralesionally (0.83% more than age-related) and ipsilesionally (1.52% more).

The discriminative capacity of CA1 has been demonstrated in a prior study of aMCI where a ROC curve analysis concluded that CA1 volume was more accurate than whole hippocampal volume in distinguishing between patients and controls. In that study, 0.88 AUC based on CA1

was found compared to 0.76 AUC based on whole hippocampal volume [9]. We similarly found CA1 volume to be more accurate than whole hippocampal volume in discriminating between stroke and healthy participants. To a lesser degree, volumes of the hippocampal tail and presubiculum subregions were also more accurate than whole hippocampal volume in discriminating between healthy and stroke participants.

The hippocampus – as a cortical hub widely implicated in many cognitive functions including declarative memory, spatial navigation, emotions, and resilience – has abundant connections allowing it to coordinate large activity with parts of the anterior and posterior cortex [38]. The gradients of hippocampal functional connectivity were found to be along two organizational structural axes: one along the anterior-posterior (head-body-tail) subregional axis and another along the medial-lateral infolding subfield axis [38, 39]. The hippocampal anterior regions were found to have greater connectivity to default mode and limbic network while posterior regions were connected more to visual and attention networks in the posterior cortex [38]. Other studies have found the anterior hippocampal activities to be linked to general memory features and global (coarse) spatial representations while the posterior activities were linked to detailed memory features and local (fine-grained) spatial representations [40]. Evidence also points to greater posterior hippocampal activity and greater involvement of the hippocampal tail during spatial memory tasks [39, 41].

Event-related high-resolution fMRI analysis found that during memory encoding, all subfields were found active regardless of memory type (content, spatial, or associative being both content and spatial). However, during memory retrieval, hippocampal activity had shown an anterior-posterior gradient for all subfields and memory types [39]. While the hippocampal tail was more implicated in spatial memory processing, evidence from high-resolution fMRI analysis found that activity in CA1, more than any other subfield, displayed responses consistent with match-mismatch detection during memory encoding and retrieval. Additionally, CA1 was

sensitive to relevant and irrelevant behavioural changes - a key feature of an automatic activity comparator [42]. The accelerated atrophy in ipsilesional CA1 and hippocampal tail reported in this study represent a high risk for memory encoding and retrieval in stroke survivors.

Finally, the strengths of this study include longitudinal assessment of relatively large stroke sample, recruitment of age- and sex-matched healthy control sample, and acquisition of all MRI on a single scanner. Despite a short follow-up period, the results point to greater longitudinal hippocampal atrophy in stroke. Our cohort is still being followed and long-term effects of stroke on hippocampal subregions will be investigated.

Limitations

Segmentation of subfields using T1-weighted and/or ultrahigh-resolution (< 1 mm) T2-weighted scans was demonstrated to be better for classifying healthy and AD individuals compared to T1-based segmentation alone [30, 43]. Even in the absence of ultrahigh-resolution imaging, our findings suggest that studies could benefit from multi-model segmentation combining 1-mm T1 and T2 or FLAIR. However, due to the lack of ultrahigh resolution T1 and T2 scans, the atrophy in CA4, DG, and ML subregions couldn't be investigated separately, but rather as a cluster (i.e., CA4-DG-ML) with no significant findings reported. Secondly, most of patients used in this study suffered a mild stroke (as measured by the National Institute of Health Stroke Scale). Therefore, results may not be representative of the full scale of hippocampal subfield atrophy in more severe stroke cases.

Conclusion

We report accelerated first-year hippocampal atrophy especially in the subfields ipsilateral to the stroke infarct. The stroke-induced effects on ipsilesional hippocampus occurred both anteriorly and posteriorly with greater atrophy in CA1 and hippocampal tail. CA1 was more discriminative between healthy and stroke participants than whole hippocampus or any other

hippocampal subregion. Atrophy in the contralateral hippocampus was still driven by age with small effects due to stroke.

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TABLES

Table 1. Demographic variables. Comparison between stroke and control groups at the 3-month timepoint.

Variable	Control	Stroke	p
Number	39	92	
Age, years, mean (SD)	68.7 (6.7)	67.4 (12.1)	0.43[a]
Men, number (%)	24 (61.5)	63 (68.5)	0.54[b]
Education, years, median (Q1, Q3)	17 (11, 18)	12 (10, 15)	0.0004[c]
TIV (cm^3), mean (SD)	1532 (123)	1528 (167)	0.86[a]

Q1, Q3 = 25^{th}, 75^{th} percentiles; SD = standard deviation; TIV = total intracranial volume; [a] Two-sample *t* test; [b] Fisher exact test; [c] Wilcoxon Rank Sum test.

Table 2. Hippocampal volumes and atrophy rates in stroke and control groups (CA = cornu ammonis areas, DG = dentate gyrus, ML = molecular layer, Δ = percent within-group atrophy rate between 3-month (3M) and 12-month (12M) timepoints, V_{3M} = volume (mm^3) at 3 months, $p^{\dagger}$ = significance of comparison of volumes and atrophy rates between stroke and controls groups, SD = standard deviation).

Region	V_{3M} Mean (SD) (% of V_{Whole})	Δ Mean (SD) (% of Δ_{Whole})	p (Δ)	$p^{\dagger}$ (V_{3M})	$p^{\dagger}$ (V_{12M})	$p^{\dagger}$(Δ)
Stroke – Contralesional						
CA1	628 (59) (18.3)	-0.15 (0.02) (4.4)	0.64	0.77	0.64	0.92
CA2/3	223 (23) (6.5)	-0.73 (0.08) (7.7)	0.062	0.53	0.54	0.92
CA4-DG-ML	1177 (120) (34.3)	-0.52 (0.06) (29)	0.068	0.4	0.4	0.92
Presubiculum	292 (34) (8.5)	-1.26 (0.15) (17.2)	**0.003**	0.4	0.33	0.92
Subiculum	410 (44) (11.9)	-0.50 (0.06) (9.7)	0.13	0.4	0.33	0.92
Hippocampal tail	521 (53) (15.2)	-1.11 (0.12) (27.4)	**0.007**	0.77	0.55	0.54
Whole hippocampus	3436 (342)	-0.62 (0.07)	**0.002**	0.24	0.20	0.56
Stroke – Ipsilesional						
CA1	631 (69) (18.4)	-0.71 (0.09) (11)	**0.007**	0.43	0.33	0.54
CA2/3	228 (27) (6.6)	-1.1 (0.15) (6.1)	**< 0.001**	0.41	0.33	0.92
CA4-DG-ML	1179 (136) (34.3)	-1.15 (0.15) (33.1)	**< 0.001**	0.41	0.31	0.54
Presubiculum	281 (32) (8.2)	-1.27 (0.15) (8.7)	**0.003**	0.41	0.31	0.92
Subiculum	408 (44) (11.9)	-1.15 (0.14) (11.5)	**< 0.001**	0.41	0.33	0.92
Hippocampal tail	519 (60) (15.1)	-1.8 (0.23) (22.7)	**< 0.001**	0.41	0.33	0.54
Whole hippocampus	3433 (378)	-1.19 (0.15)	**< 0.001**	0.085	**0.035**	**0.027**
Control						
CA1	650 (43) (18.2)	0 (0) (0.2)	0.99			
CA2/3	234 (15) (6.6)	-0.73 (0.05) (11.2)	0.14			
CA4-DG-ML	1224 (78) (34.3)	-0.57 (0.04) (45.8)	0.13			
Presubiculum	303 (23) (8.5)	-0.95 (0.07) (18.8)	0.13			
Subiculum	426 (27) (11.9)	-0.80 (0.05) (22.3)	0.13			
Hippocampal tail	540 (37) (15.1)	-0.28 (0.02) (9.8)	0.68			
Whole hippocampus	3568 (226)	-0.41 (0.03)	0.14			

Table 3. ROC analysis. Performance measures for participant classification (as control or stroke) based on subfield volumes and the ensemble of subfield volumes in comparison with performance based on whole hippocampal volume.

Volume	Sensitivity	Precision	Accuracy	AUC
Whole hippocampus	0.66 (0.07)	0.65 (0.06)	0.66 (0.07)	0.50 (0.10)
Ensemble/Subfields	0.91 (0.05)***	0.91 (0.05)***	0.91 (0.05)***	0.95 (0.05)***
CA1	0.79 (0.06)***	0.80 (0.06)***	0.79 (0.06)***	0.84 (0.08)***
CA2/3	0.72 (0.06)	0.68 (0.07)	0.72 (0.06)	0.59 (0.11)
CA4-DG-ML	0.67 (0.06)	0.66 (0.06)	0.67 (0.06)	0.60 (0.11)*
Presubiculum	0.71 (0.05)	0.68 (0.06)	0.71 (0.05)	0.65 (0.10)**
Subiculum	0.66 (0.07)	0.62 (0.06)	0.66 (0.07)	0.55 (0.11)
Hippocampal tail	0.74 (0.05)*	0.72 (0.07)*	0.74 (0.05)*	0.69 (0.09)***

AUC = area under curve, CA = cornu ammonis areas, DG = dentate gyrus, ML = molecular layer; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ - corrected for multiple comparisons; (.) = standard deviation.

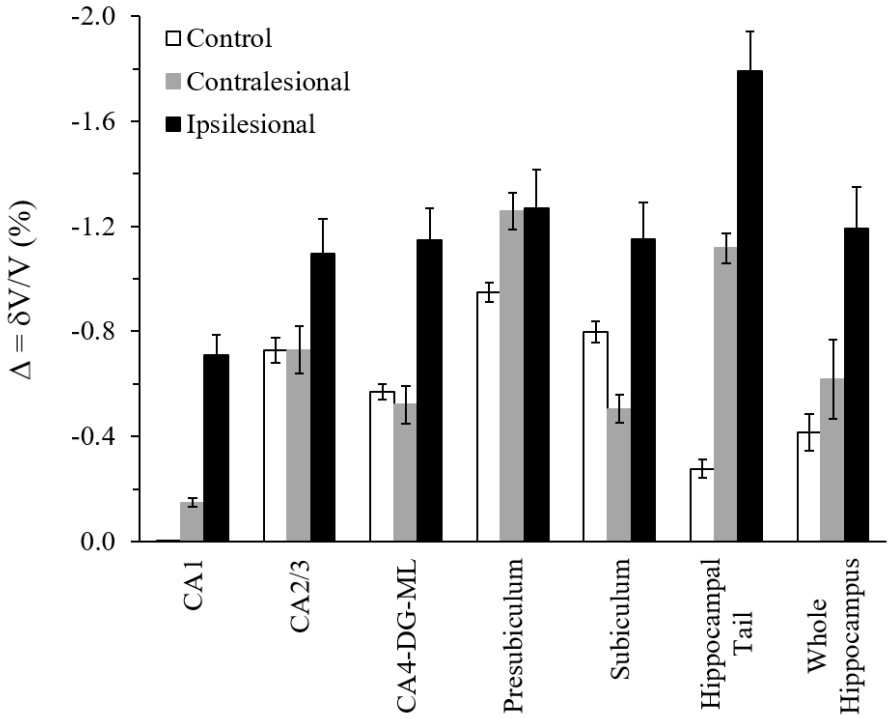
FIGURE LEGENDS

Figure 1. Hippocampal atrophy - Whole and subfield hippocampal atrophy rates between 3 and 12 months in healthy controls and stroke participants (CA = cornu ammonis areas, DG = dentate gyrus, ML = molecular layer, error bar = standard deviation, V = volume, Δ = atrophy rate).

Figure 2. Hippocampal tail atrophy - Examples of ipsilesional hippocampal tail atrophy between 3 and 12 months (L = left, R = right).

Figure 3. ROC curves - Classification performance based on CA1, hippocampal tail, and presubiculum subfield volumes, whole hippocampal volume, and the ensemble of subfield volumes.

FIGURES



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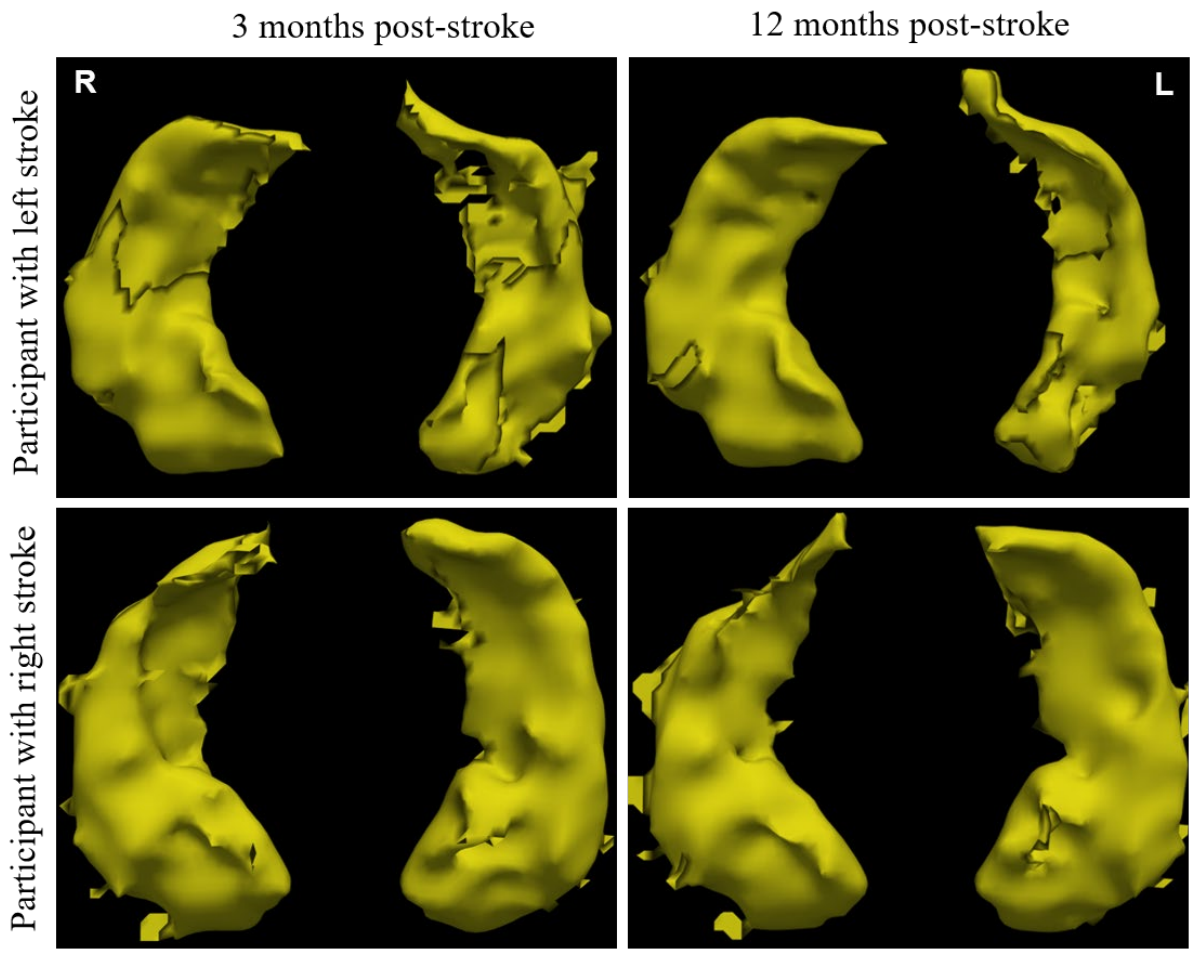

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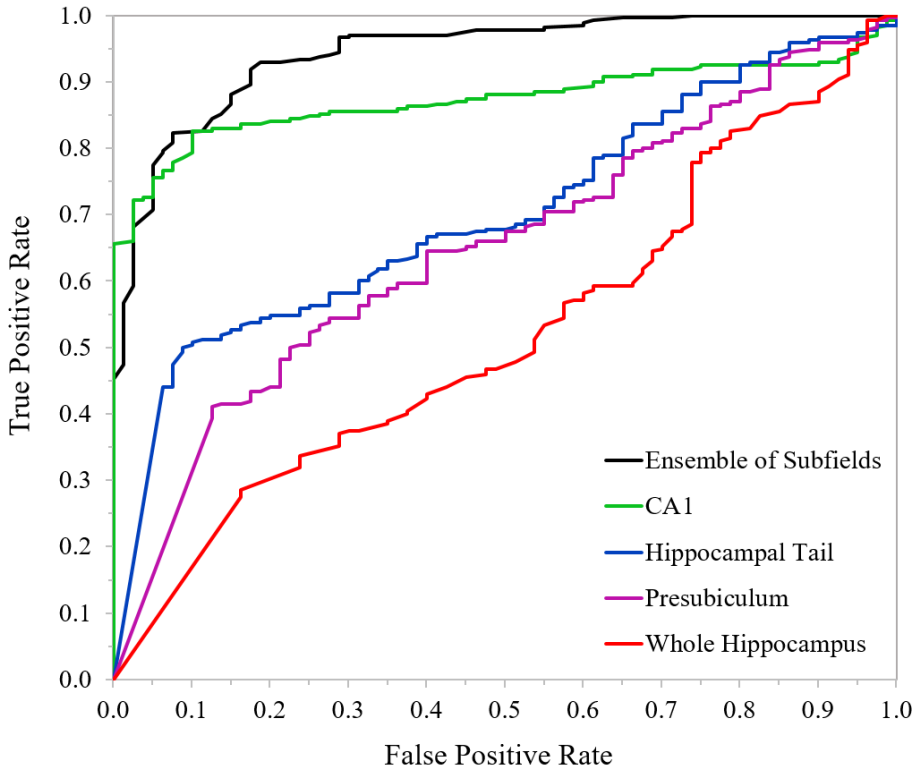
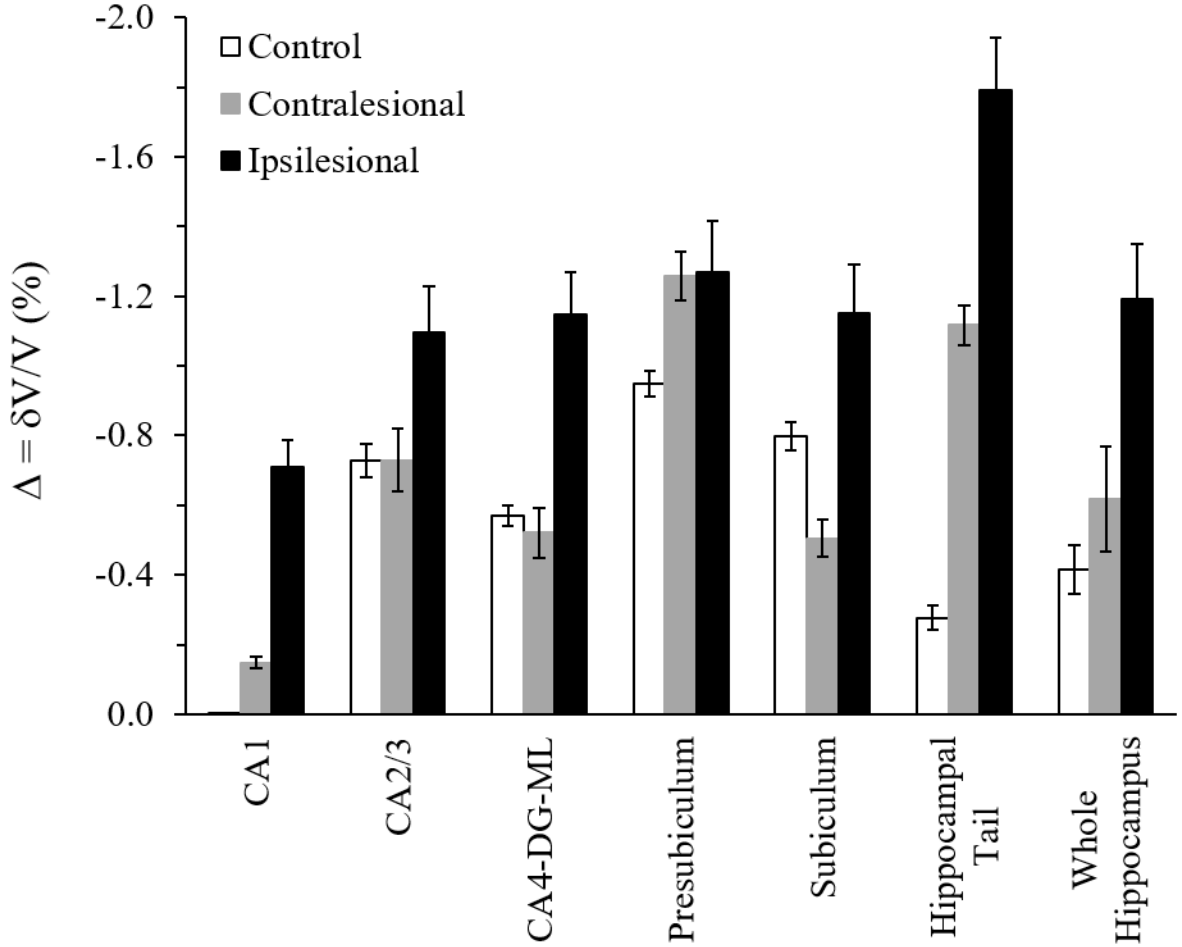


Figure 3. ROC curves. Classification performance based on CA1, hippocampal tail, and presubiculum subfield volumes, whole hippocampal volume, and the ensemble of subfield volumes.



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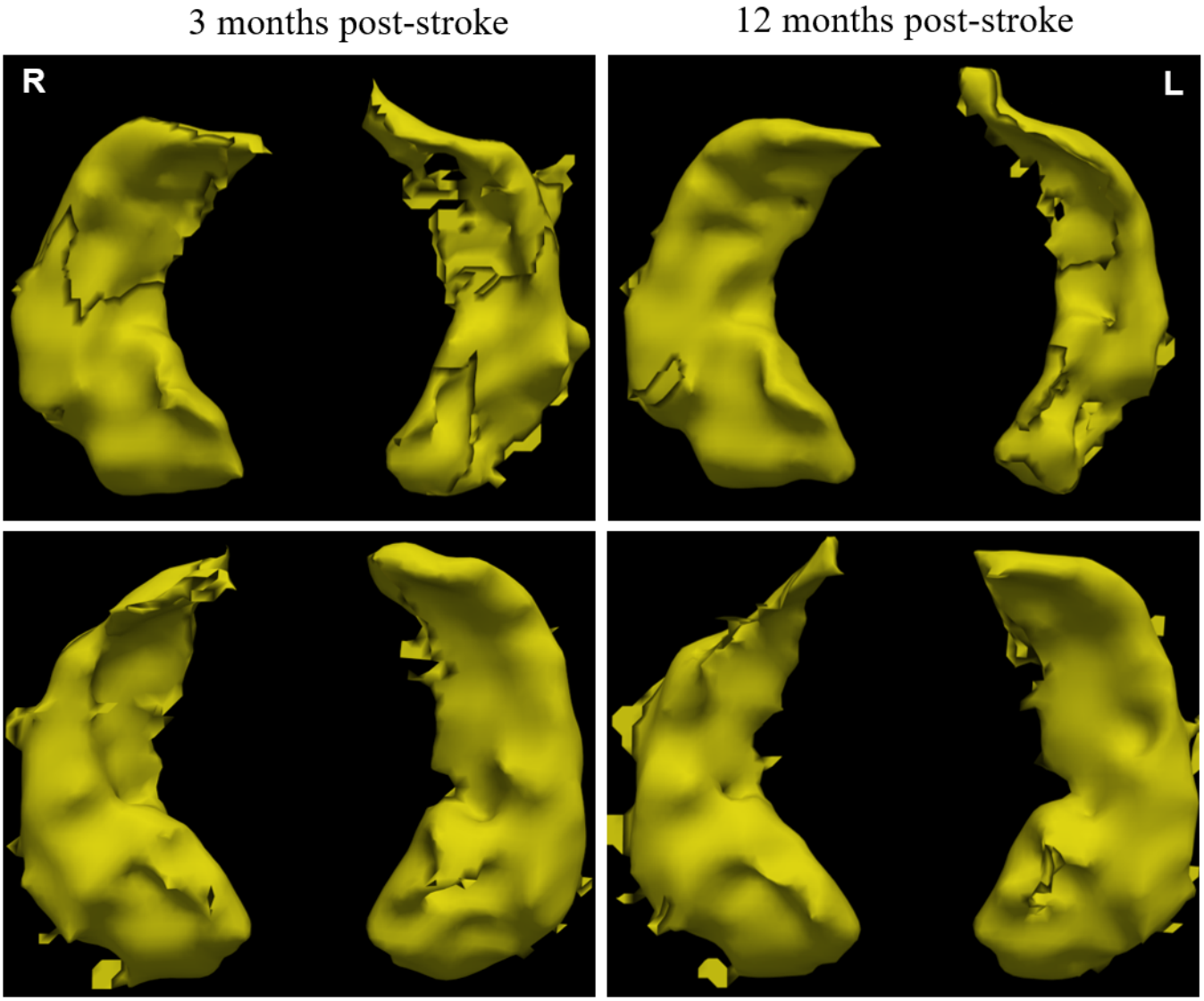

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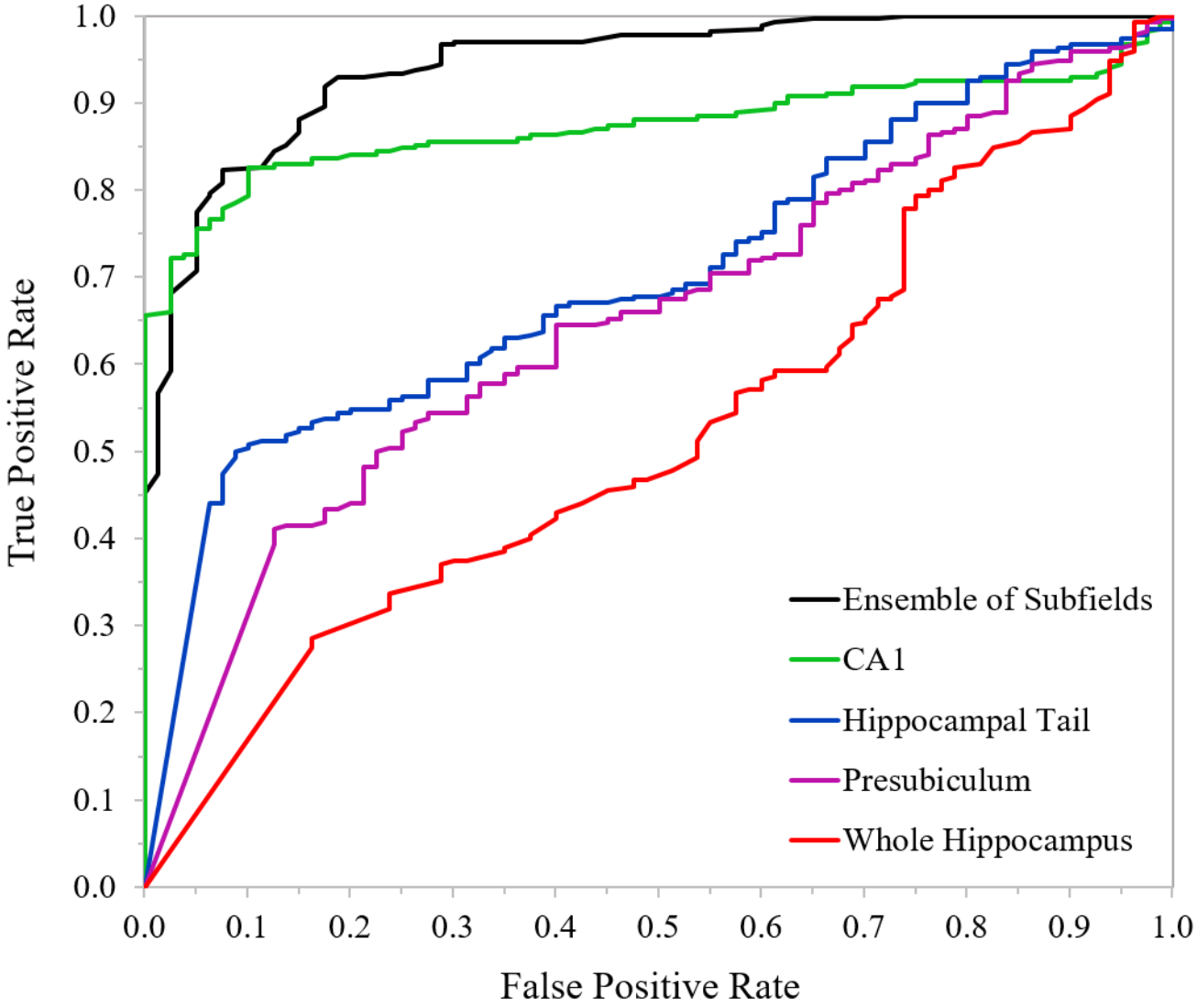

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