



# Ongoing Secondary Degeneration of the Limbic System in Patients With Ischemic Stroke: A Longitudinal MRI Study

Muhammad E. Haque<sup>1\*</sup>, Refaat E. Gabr<sup>2</sup>, Khader M. Hasan<sup>2</sup>, Sarah George<sup>1</sup>, Octavio D. Arevalo<sup>2</sup>, Alicia Zha<sup>1</sup>, Susan Alderman<sup>1</sup>, Jerome Jeevarajan<sup>1</sup>, Manual F. Mas<sup>3</sup>, Xu Zhang<sup>4</sup>, Nikunj Satani<sup>1</sup>, Elliott R. Friedman<sup>2</sup>, Clark W. Sitton<sup>2</sup> and Sean Savitz<sup>1</sup>

<sup>1</sup> Institute for Stroke and Cerebrovascular Diseases, University of Texas Health Science Center at Houston, Houston, TX, United States, <sup>2</sup> Diagnostic and Interventional Imaging, University of Texas Health Science Center at Houston, Houston, TX, United States, <sup>3</sup> TIRR Memorial Hermann Rehabilitation and Research, Houston, TX, United States, <sup>4</sup> Biostatistics/Epidemiology/Research Design Component, Center for Clinical and Translational Sciences, McGovern Medical School at University of Texas Health Science Center at Houston (UTHealth), Houston, TX, United States

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### \*Correspondence:

Muhammad E. Haque  
muhammad.e.haque@uth.tmc.edu

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**Purpose:** Ongoing post-stroke structural degeneration and neuronal loss preceding neuropsychological symptoms such as cognitive decline and depression are poorly understood. Various substructures of the limbic system have been linked to cognitive impairment. In this longitudinal study, we investigated the post-stroke macro- and micro-structural integrity of the limbic system using structural and diffusion tensor magnetic resonance imaging.

**Materials and Methods:** Nineteen ischemic stroke patients (11 men, 8 women, average age  $53.4 \pm 12.3$ , range 18–75 years), with lesions remote from the limbic system, were serially imaged three times over 1 year. Structural and diffusion-tensor images (DTI) were obtained on a 3.0 T MRI system. The cortical thickness, subcortical volume, mean diffusivity (MD), and fractional anisotropy (FA) were measured in eight different regions of the limbic system. The National Institutes of Health Stroke Scale (NIHSS) was used for clinical assessment. A mixed model for multiple factors was used for statistical analysis, and  $p$ -values  $<0.05$  was considered significant.

**Results:** All patients demonstrated improved NIHSS values over time. The ipsilesional subcortical volumes of the thalamus, hippocampus, and amygdala significantly decreased ( $p < 0.05$ ) and MD significantly increased ( $p < 0.05$ ). The ipsilesional cortical thickness of the entorhinal and perirhinal cortices was significantly smaller than the contralesional hemisphere at 12 months ( $p < 0.05$ ). The cortical thickness of the cingulate gyrus at 12 months was significantly decreased at the caudal and isthmus regions as compared to the 1 month assessment ( $p < 0.05$ ). The cingulum fibers had elevated MD at the ipsilesional caudal-anterior and posterior regions compared to the corresponding contralesional regions.

**Conclusion:** Despite the decreasing NIHSS scores, we found ongoing unilateral neuronal loss/secondary degeneration in the limbic system, irrespective of the lesion location. These results suggest a possible anatomical basis for post stroke psychiatric complications.

**Keywords:** ischemic stroke, limbic system atrophy, secondary degeneration, chronic loss of gray matter, longitudinal neuroimaging study

## INTRODUCTION

Stroke research and clinical management have been predominantly focused on the acute phase of injury and little is known about the rates of ongoing chronic degeneration. Despite clinical improvement, stroke survivors often suffer from persistent hemiparesis, cognitive decline, depression, and anxiety. A number of neuropsychiatric studies have shown an association between ischemic injury and cognitive impairment, progressive brain atrophy, impaired neuronal connectivity, compromised hemodynamics, and secondary neuronal degeneration (1–8).

Chronic thalamic neuronal loss remote from the lesion was noted in an animal study (9). Later research validated this evidence, and further found that neuroinflammation was not limited to the lesion area, but globally impacted the ipsilesional hemisphere (10). Another longitudinal primate study demonstrated ipsilesional hemispheric atrophy and secondary neuronal degeneration in the lentiform nucleus (11). Nishio et al. (12) demonstrated association between ischemic injury and secondary hippocampal degeneration (12). Another study using a rodent unilateral common carotid occlusion model reported a correlation between hippocampal atrophy and vascular cognitive impairment (13). Furthermore, several cross-sectional studies and a handful of longitudinal studies suggested an association between post-stroke tissue loss in the limbic system and cognitive impairment (14–22). However, these studies reported only two or three substructures of the limbic system and in some cases the primary lesions were within the limbic system.

The limbic system, composed of interconnected gray and white matter structures, plays a vital role in mood, emotion and cognitive functions such as memory and learning (23, 24). Gray matter structures include the amygdala, hippocampus, thalamus, parahippocampus, entorhinal cortex, hypothalamic nuclei, mammillary bodies, and cingulate gyrus. The primary white matter structures include the fornix and the cingulum bundle. Hippocampal, parahippocampal, and entorhinal atrophy has been associated with impairment of verbal memory in Alzheimer's disease and was used as surrogate markers for disease progression (25, 26).

In a recent serial study, Bivard et al. (27) reported an association between global gray matter atrophy and cognitive decline in patients with transient ischemic stroke (27). Post-stroke neuronal degeneration in brain regions remote from the primary infarct may underlie subcortical atrophy and cognitive impairment (7, 28–31). In this prospective longitudinal neuroimaging study, we sought to investigate ongoing secondary

degeneration in eight substructures of the limbic system remote from the lesion using structural and diffusion tensor imaging (DTI) MRI to identify the most vulnerable substructures of the limbic system.

## MATERIALS AND METHODS

### Patient Enrollment and Clinical Assessment

This study was approved by our Institutional Review Board and written informed consent was obtained prior to enrollment. The study enrolled 19 patients (10 male and 9 female, age range, 18–75 years) with unilateral ischemic stroke. At the time of enrollment, the patients had lesion size 0–100 cm<sup>3</sup> and NIHSS ranges 1–25. Patients were excluded from the study if they were unable to undergo MRI examination, or if there was intracranial hemorrhage, cancer, seizure disorder, HIV, or pulmonary disease. Patients with lesions extending to any of the substructures within the limbic system were excluded from ipsi- and contralesional analysis.

The patients were imaged with a standard of care MRI protocol in the acute phase. Additional MRI exams were performed at 1 (1 M), 3 (3 M), and 12 (12 M) months of onset. The neurological assessment was done using the NIHSS at each follow-up visit. All patients underwent standard of care post-stroke rehabilitation.

### MRI Data Acquisition Protocol

All imaging experiments were performed on a whole-body 3.0 T Philips system (Philips Healthcare, Best, The Netherlands) using a multi-channel head coil. Structural MRI was obtained using 3D T1-weighted imaging (TE/TR = 3.66/8.2 ms, acquisition matrix = 256 × 256 × 170, FOV = 256 × 256 mm<sup>2</sup>). Anatomical localization of the lesion used Fluid-Attenuated Inversion Recovery (FLAIR) images (TE/TR/TI = 129 ms/4.8 s/1.6 s, acquisition matrix = 256 × 256 × 180, FOV = 256 × 256 mm<sup>2</sup>). DTI was used to assess microstructural integrity (TE/TR = 66 ms/7.1 s, acquisition matrix = 128 × 128, FOV = 256 × 256 mm<sup>2</sup>, slice thickness = 3 mm, no gaps; gradient scheme = 21 icosahedral, diffusion b-factor = 1,000 s/mm<sup>2</sup>) (32).

### Lesion Volume Measurements

A semi-automated algorithm in Analyze 12.0 (Analyze Direct Inc., KS, USA) was used to delineate lesion volume on FLAIR images by two raters. The rater selected two seed points within the hyper- and hypointense regions within the lesions and a region-growing algorithm automatically expanded the seed

points into the 3D space of the image. Manual editing of the lesion volume was done when necessary.

## Subcortical Volume and Cortical Thickness Analysis

Cortical thickness and subcortical volumes of the limbic structures were measured by automatic parcellation and segmentation of T1w images using FreeSurfer v5.3.0 (<http://surfer.nmr.mgh.harvard.edu/>) with default processing settings (33). Reconstructed segmented volumes and cortical thickness were visually inspected and manually edited, when necessary. All measurements were made in both the ipsi- and contralesional hemispheres. Contralesional measurements were used as control.

## Substructure Measurements of the Limbic System

Changes in volume, cortical thickness, mean diffusivity (MD), and fractional anisotropy (FA) were measured in eight substructures of the limbic system in each hemisphere. The volumetric measurements included the amygdala, hippocampus, and thalamus. Cortical thickness was measured in the entorhinal cortex, perirhinal cortex, parahippocampal, and cingulate cortex. The MD and FA were measured in the amygdala, hippocampus, thalamus, and cingulum. The cingulate cortex and cingulum measurements were divided into four segments: rostral-anterior, caudal-anterior, posterior, and isthmus cingulate. Two neuroradiologists

with a combined 22 years of experience identified the lesion locations. The volumetric measurements were normalized to the intracranial volume.

## Mean Diffusivity and Fractional Anisotropy Measurements

DTI preprocessing included volume realignment, non-brain tissue removal, and eddy current correction using FSL (<http://www.fmrib.ox.ac.uk/fsl/>). The diffusion tensor was calculated voxel-wise using multivariate linear fitting using DtiStudio ([www.DtiStudio.org](http://www.DtiStudio.org)), and MD and FA maps were generated. The T1w images, lesion mask, and b0 images (non-gradient DTI images) were registered using rigid-body transformation in SPM12 (<https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>).

MD and FA were measured by drawing a region-of-interest (ROI) containing ~25–30 voxels in gray matter (GM, thalamus, hippocampus, and amygdala) and white matter regions of the limbic system (WM, anterior, posterior, rostral, and caudal regions of the cingulum). The ROIs were placed in each region using the DTI color maps as a guide. Special care was given to ROI placement to ensure sampling of the same location at all the time points. The ROIs were overlaid on MD and FA maps to record the values.

## Statistical Analysis

Mixed model analysis evaluated effects of multiple factors and determined the correlation in repeated measurements (34). The fixed effects in the mixed model included stroke laterality

**TABLE 1** | Patient demographics.

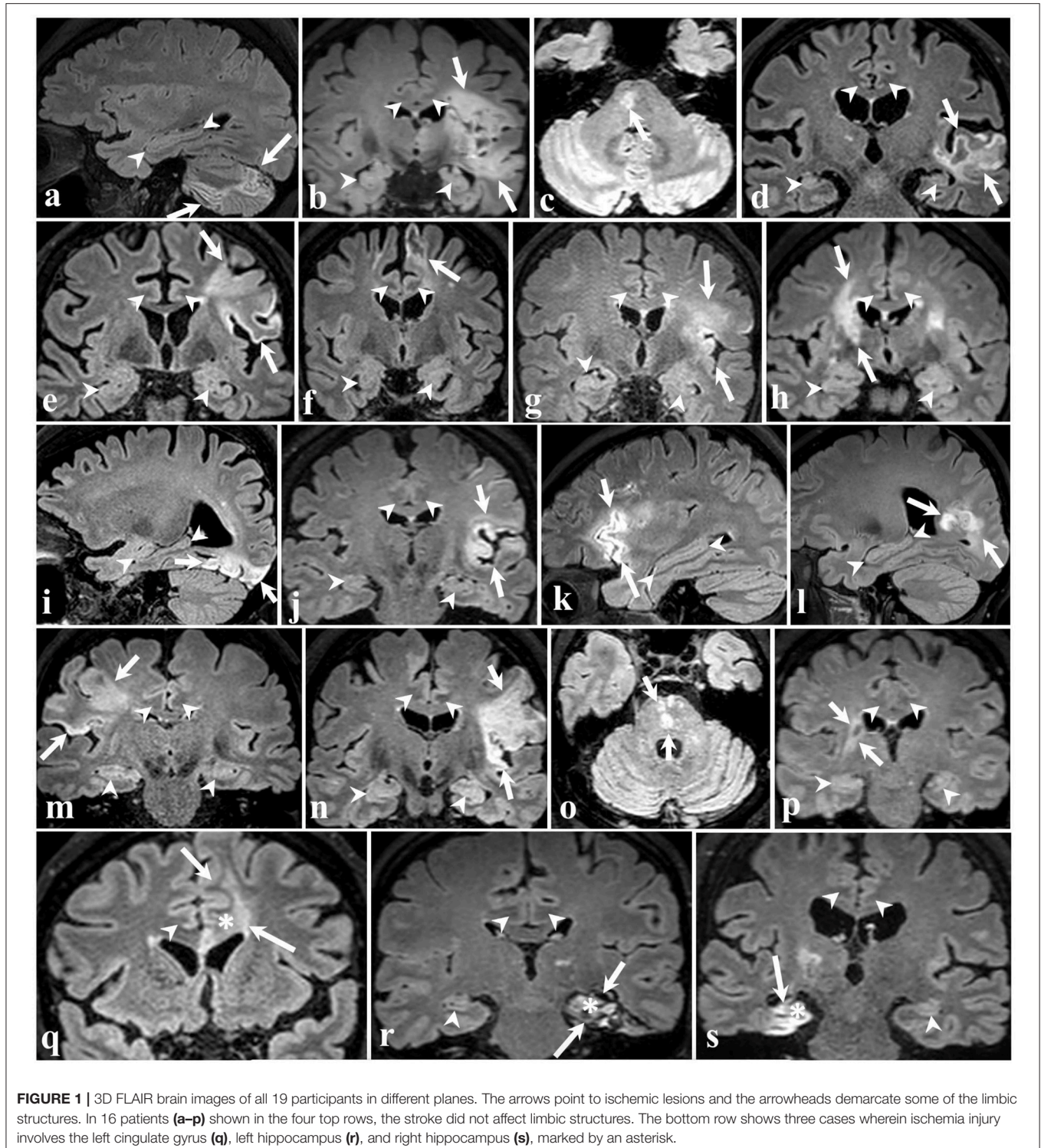
PID	Laterality	Lesion location	NIHSS				Lesion volume (mL)			Figure 1
			Acute	01M	03M	12M	01M	03M	12M	
P01	Left	Genu and body of CC, SFG,CR, PreM	5	0	0	0	15.06	14.82	10.72	1f
P02	Left	STG, SMG, FG, OC, wernicke	4	4	4	2	61.47	78.80	79.89	1d
P03	Right	LPC, SMG, insula, TL, PRF	13	NA	5	3	55.22	35.46	39.73	1m
P04	Left	Insula, broca, RF, LN	20	NA	0	0	22.16	22.06	25.50	1g
P05	Left	FG, OL, VP	5	3	1	1	15.37	11.75	12.33	1i
P06	Right	Caudate and basal ganglia, parahippocampal	8	2	4	1	13.47	11.14	7.72	1h
P07	Left	Frontal lobe, precentral gyrus, operculum	2	1	0	–	18.14	8.31	–	1q
P08	Left	LN, caudate, putamen	7	2	0	–	16.56	19.13	–	1n
P09	Left	Cerebellum	1	0	–	0	32.57	NA	22.13	1a
P10	Right	Thalamic, TL, hippo, parahippocampal	6	3	2	2	24.75	16.90	13.52	1s
P11	Left	Thalamic, TL, hippo, parahippocampal	7	3	2	1	43.60	26.77	28.18	1r
P12	Left	FG, TL, BG, broca	25	2	2	3	34.75	32.27	43.68	1j
P13	Right	Pontine	6	2	1	0	0.37	0.34	0.37	1c
P14	Right	Occipital lobe	2	1	1	0	6.64	2.65	1.50	1l
P15	Right	Basal ganglia and motor cortex	14	2	0	–	30.06	15.39	–	1k
P16	Left	Insula, basal ganglia	20	15	9	8	77.60	60.80	55.57	1b
P17	Left	Operculum and caudate	20	5	4	4	87.19	61.95	66.83	1e
P18	Center*	Pontine	1	1	2	1	0.92	1.61	1.54	1o
P19	Right	Internal capsule	5	1	0	1	3.38	4.82	5.12	1p

CC, corpus callosum; SFG, superior frontal gyrus; CR, corona radiata; PreM, pre motor area; STG, superior temporal gyrus; SMG, superior marginal gyrus; FG, fusiform gyrus; OL, occipital lobe; LPC, lateral prefrontal cortex; TL, temporal lobe; PRE, peri-rolandic fissure; RF, Rolandic fissure; LN, lentiform nucleus; VP, visual pathway; BG, basal ganglia; NIHSS, National Institute of Health Stroke Scale. NA, no data obtained; –, missed follow-up visit; \*, lesion center to right (right hemisphere as ipsilesional).



(ipsilesional and contralateral), serial visits, and interactions between laterality, visits, and lesion volume. The random effects included the patient and interactions between patients and laterality. The specified random effects led to a nested covariance matrix and accounted for correlation of measurements of the

same patient and different level of correlation for measurements on the same side of brain. Tests were two tailed and  $p$ -values  $<0.05$  were considered significant. Statistical analyses were performed using the SAS software (version 9.4, the SAS Institute, Cary, NC).



## RESULTS

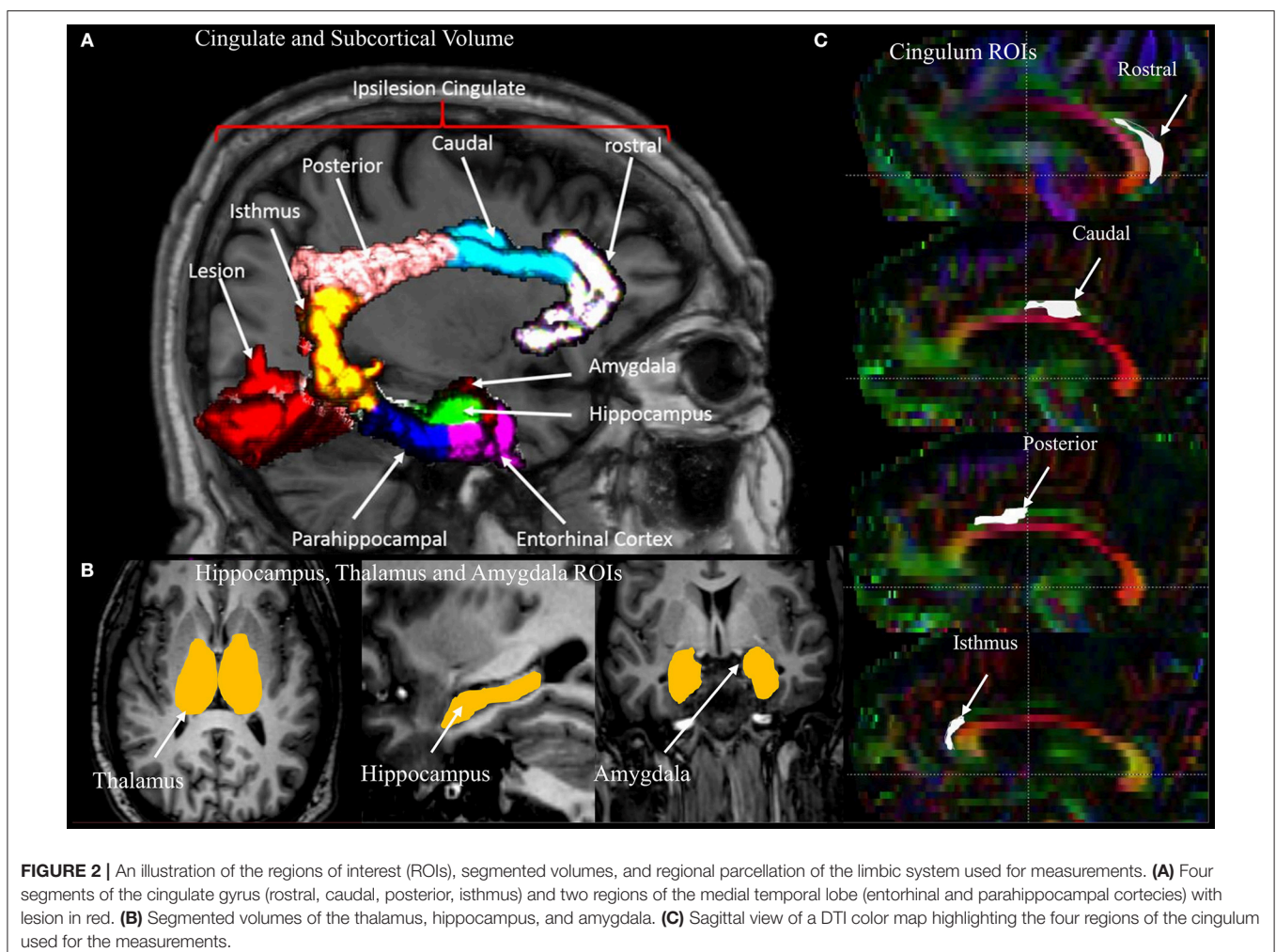
The patients had a median NIHSS of 6 (range 1–25). All patients completed the 1 month MRI. One patient missed the 3 month MRI and three patients missed 12 month MRI. The lesions were identified as middle cerebral artery infarct ( $n = 10$ ), posterior cerebral artery infarcts ( $n = 5$ ), anterior cerebral artery infarct ( $n = 2$ ), and pontine infarct ( $n = 2$ ). The average lesion size was  $29.44 \pm 25.4 \text{ mm}^3$  with inter-rater reliability of 97% at 1 month. Eleven patients had left hemispheric stroke and eight had right hemispheric stroke. The serial lesion volume, location, and severity of individual patients are listed in **Table 1**.

Representative MRI images depicting the approximate size and location of the lesions are shown in **Figure 1**. Most of the patients had lesions remote from the limbic system. **Figure 2** is a graphic illustration of the segmented limbic system structures on T1w MRI and the ROI locations for the DTI measurement in one patient.

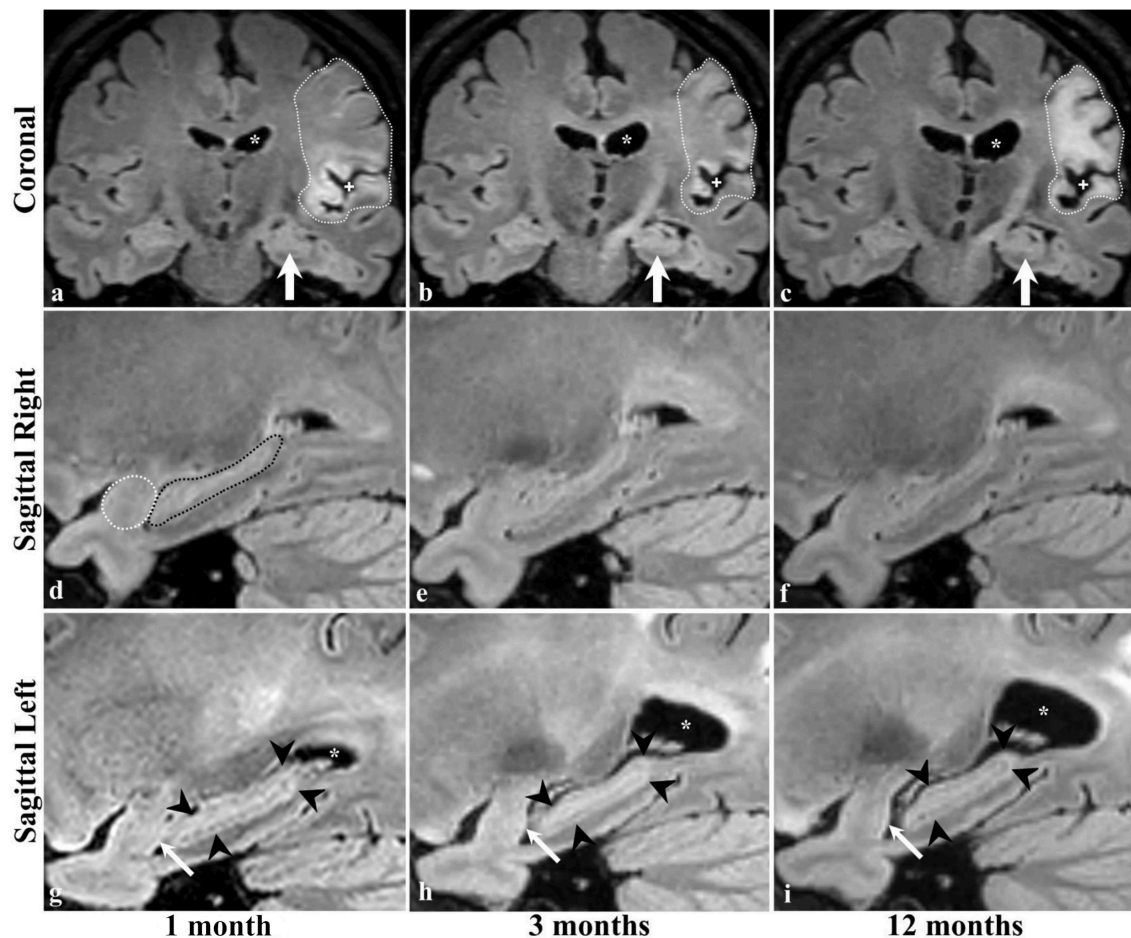
### Subcortical Volumes

Serial radiological assessment revealed remote structural atrophy in the ipsilesional hippocampus and amygdala, as shown

for a representative patient in **Figure 3**. This was validated by quantitative volumetric analysis which demonstrated a significant reduction in the ipsilesional amygdala and hippocampus volume at 12 months ( $p < 0.05$ ). The temporal ipsilesional thalamus volume was also significantly decreased ( $p < 0.05$ ). The hemispheric comparison between ipsi and contralesional sides revealed significant reduction in the hippocampal and amygdala volumes but not the thalamic volume. The ipsilesional cortical thickness of the two sub-regions of the medial temporal lobe, entorhinal, and perirhinal cortices, was significantly reduced at 12 months compared to the contralesional side ( $p < 0.05$ ), with the exception of the parahippocampal region ( $p = 0.10$ ). There was no temporal decrease in the three ipsilesional medial temporal regions. The serial changes in the ipsilesional and contralesional volumes and cortical thickness are illustrated in **Figure 4**. The cortical thickness of the ipsilesional cingulate cortices was progressively decreased in the caudal anterior and isthmus regions ( $p < 0.05$ ). The cortical thickness of the posteriorcingulate and rostral anterior regions showed longitudinal change, but no hemispheric differences were observed. The serial changes in the ipsi- and contralesional cingulate are shown in **Figure 5**.







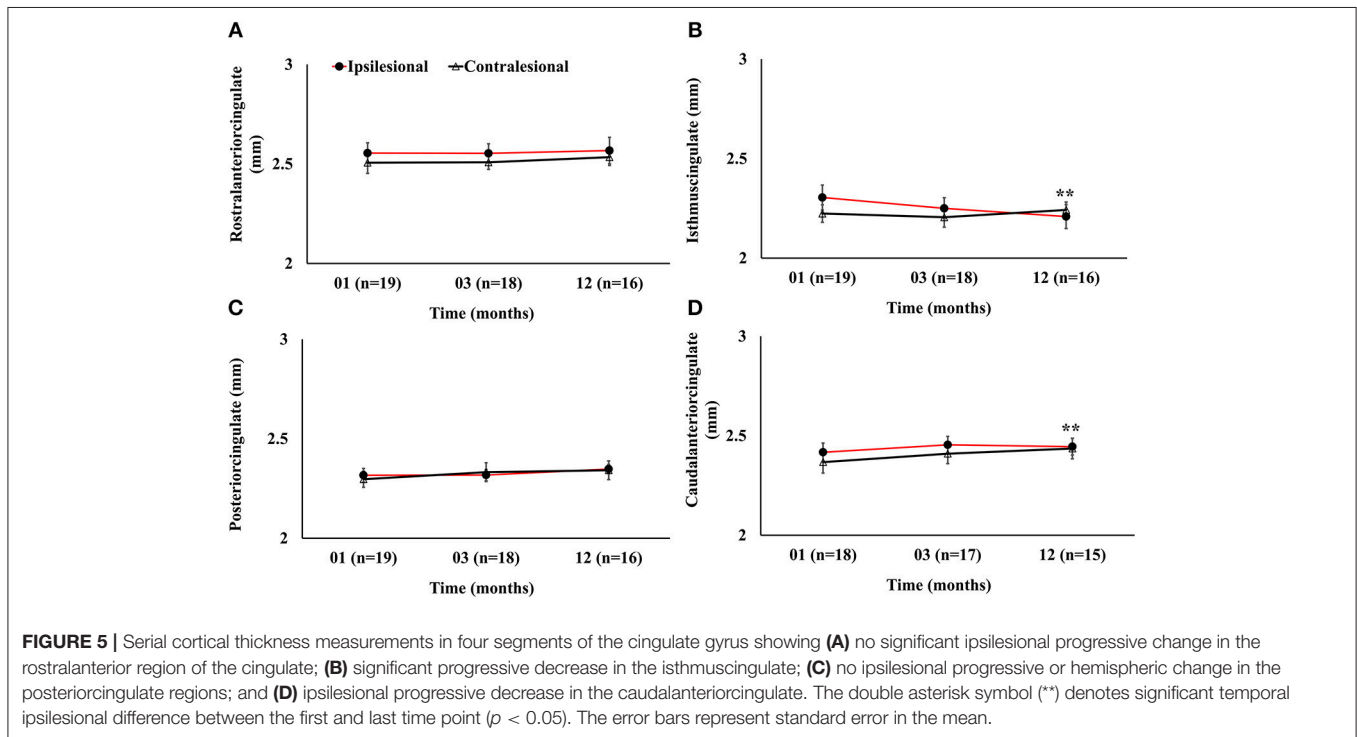
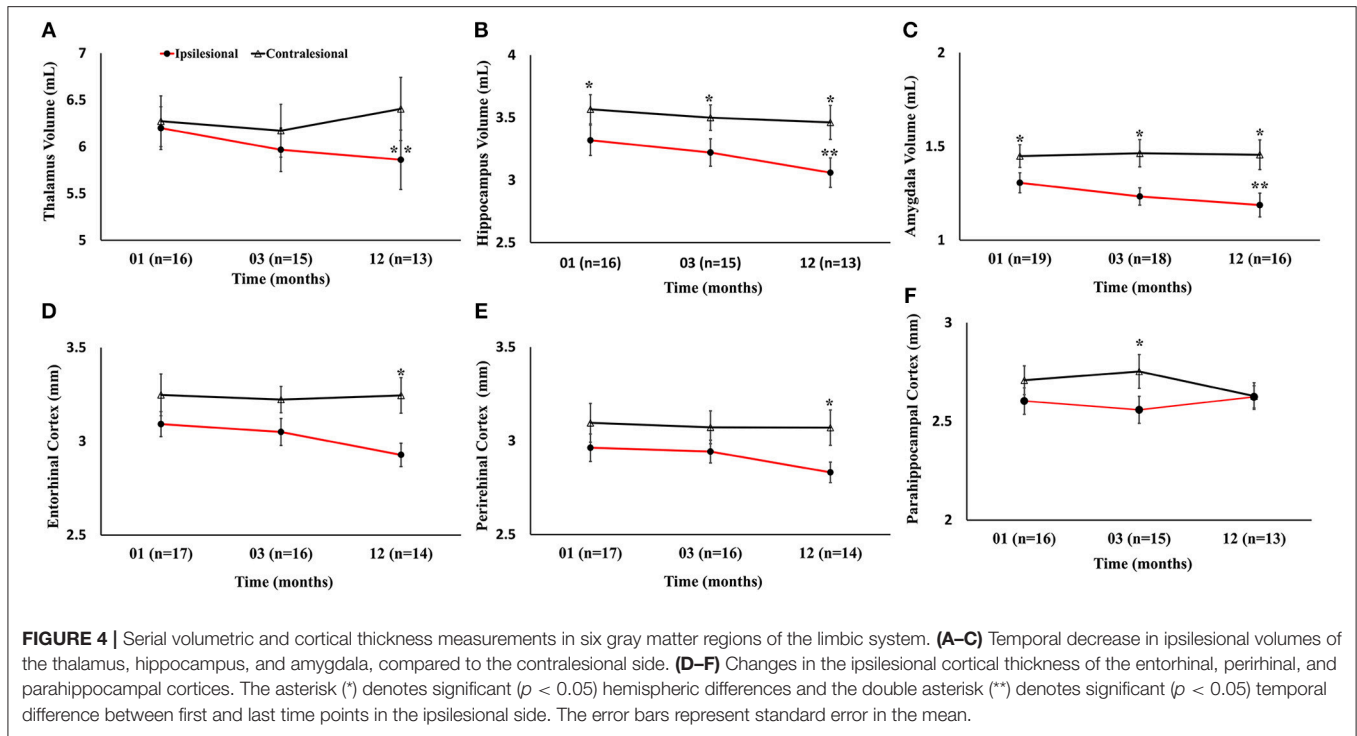
**FIGURE 3 |** Brain MRI of a patient with ischemic stroke in the left MCA territory. Coronal images are at the level of the interthalamic adhesion at 1, 3 and 12 months of stroke (**a–c**, respectively), with concurrent sagittal images centered at the right (**d–f**) and left (**g–i**) hippocampi. Dotted lines (**a–c**) delineate the infarcted brain tissue and its expected evolution in time, including the increase in the parenchymal signal intensity (gliosis), the *ex-vivo* dilatation of the corresponding lateral ventricle (\* in the top and bottom rows), and the widening of the adjacent subarachnoid space (+ in top row) indicative of parenchymal volume loss and scarring. The left hippocampus was not affected by the ischemic event (arrows in **a–c**). Dotted lines in the image of the right temporal lobe (**d**) delineate the normal appearing amygdala (white) and hippocampus (black) and no changes were identified at 3 (**e**) or 12 (**f**) months. The left amygdala and hippocampus are normal in size and morphology at baseline examination (**g**); however, there is progressive hippocampal atrophy in the follow-up scans (arrowheads in **g–i**). Interval volume loss of the left temporal amygdala can be identified by gradual straightening of its posterior contour (arrows in **g–i**).

## Diffusion Tensor Analysis Gray Matter Fractional Anisotropy and Mean Diffusivity

There were no progressive changes in ipsilesional FA and no differences between the ipsi- and contralesional thalamus, hippocampus, and amygdala. A significant increase in the MD of the ipsilesional thalamus, and amygdala was found at 12 months compared to 1 month ( $p < 0.05$ ). The comparison between ipsi- and contralesional MD of these regions showed significantly higher values in the ipsilesional hemisphere ( $p < 0.05$ ) at 12 months. The DTI findings in subcortical gray matter are summarized in **Figure 6**.

## White Matter Fractional Anisotropy and Mean Diffusivity

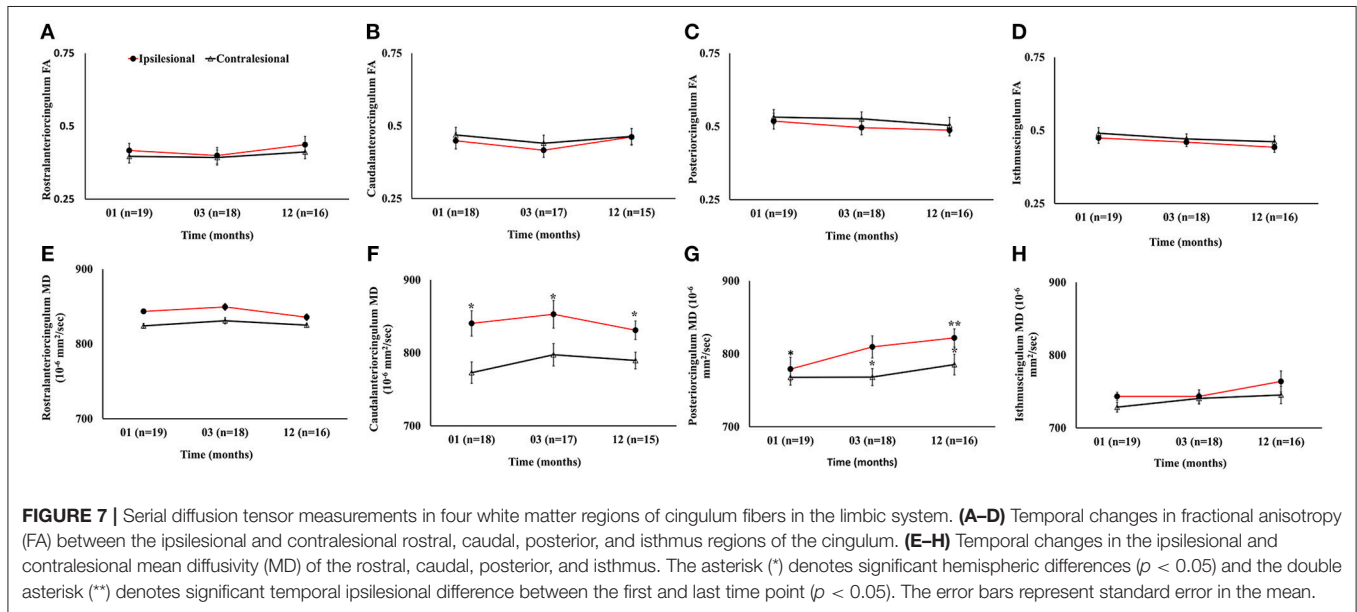
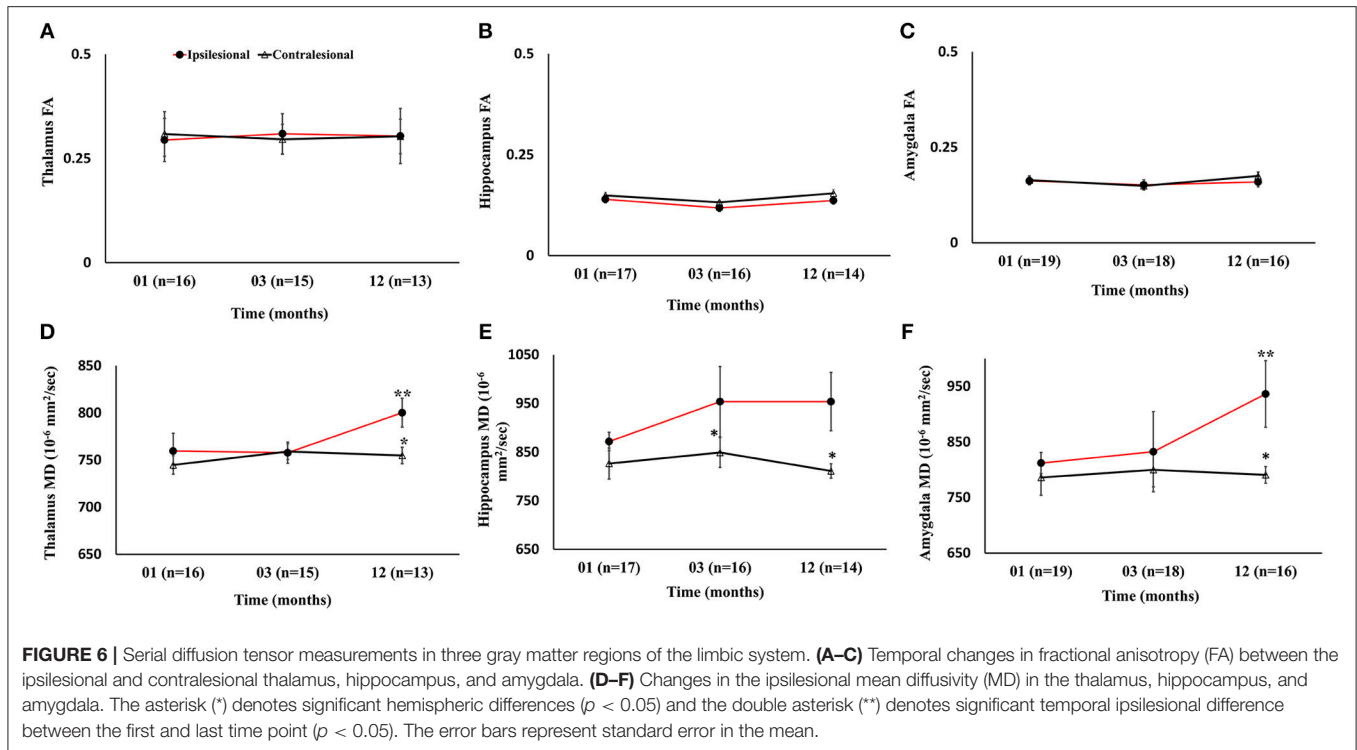
The ipsilesional caudal and posterior cingulum fibers showed significant increase in MD as compared to the contralesional side ( $p < 0.05$ ). However, only the ipsilesional posteriorcingulum fiber demonstrated elevated MD between 1 and 12 months ( $p < 0.05$ ). There were no progressive or hemispheric differences in MD of the rostral and isthmus regions of the cingulum. Unlike MD, there was no statistically significant difference between ipsi- and contralesional FA in any segment. The overall changes in FA and MD (**Figure 7**) and volumetric, cortical thickness, and microstructural changes are summarized in **Table 2**.



The correlation between lesion volume and changes in subcortical volumes, cortical thickness, FA, and MD showed no effect except for MD of the ipsilesional amygdala, which correlated significantly with lesion volume ( $p < 0.05$ ).

## DISCUSSION

To the best of our knowledge, this is the first longitudinal study to combine volumetry, cortical thickness, and DTI measurements to track post-stroke regional macro- and microstructural changes in



the limbic system. We found the rate of progressive degeneration, when compared to the contralesional side, to be heterogeneous. While none of the patients in this study had a subcortical infarct, alterations in the subcortical structures of the limbic system were observed. We identified ipsilesional thalamus, hippocampus, amygdala, entorhinal, and perirhinal cortices to be most vulnerable to degeneration. Unlike the thalamus, the hippocampal and amygdala volumes were significantly reduced within 1 month of onset, suggesting degeneration onset within

the acute to subacute period. This was further supported by increased MD of the ipsilesional hippocampus and amygdala at the sub-chronic phase, which occurred later in the thalamus during the chronic phase. Our data show no correlation between lesion volume and neuronal degeneration, with the exception of increased MD in the amygdala.

In animal models, positron emission tomography studies have shown secondary degeneration as a result of neuronal loss in stroke (10). Our data suggest that neuronal loss in the



**TABLE 2** | A summary of results comparing progressive changes in the ipsilesional measurement between 1 and 12 months (progressive), and between the ipsilesional and the contralesional hemispheres (Hemispheric).

Structures	Measurements	Progressive <sup>†</sup>	Hemispheric <sup>††</sup>
Amygdala	Volume	↓	↓
	MD	↑	↑
	FA	—	—
Hippocampus	Volume	↓	↓
	MD	—	↑
	FA	—	—
Thalamus	Volume	↓	—
	MD	↑	↑
	FA	—	—
Entorhinal cortex	Cortical thickness	—	↓
Perirhinal cortex	Cortical thickness	—	↓
Parahippocampal	Cortical thickness	—	↓
Caudalanteriorcingulate	Cortical thickness	↓	—
Isthmusingulate	Cortical thickness	↓	—
Posteriorcingulate	Cortical thickness	—	—
Rostralanteriorcingulate	Cortical thickness	—	—
Caudalanterior cingulum	MD	—	↑
	FA	—	—
Isthmus cingulum	MD	—	—
	FA	—	—
Posterior cingulum	MD	↑	↑
	FA	—	—
Rostralanterior cingulum	MD	—	—
	FA	—	—

<sup>†</sup>Ipsilesional increase (↑) or decrease (↓) in measurements between 1 and 12M.

<sup>††</sup>Comparisons between ipsi- and contralesional measurement side at any visit. ↑ and ↓ are statistically significant  $P < 0.05$ . — No statistically significant change.

ipsilesional amygdala and hippocampus precedes that in the thalamus. Hippocampal volume loss has been recognized as a hallmark of cognitive impairment in Alzheimer's disease, and was recently suggested to play a role in stroke as well (21, 26). Post-stroke hippocampal atrophy is thought to be a predictor for post-stroke cognitive impairment (35). In a recent study, entorhinal atrophy and hippocampal structural deformation, but not hippocampal volume, were strongly associated with post-stroke cognitive impairment (36). We also found ipsilesional perirhinal atrophy, in addition to the entorhinal atrophy.

In our study, the observed tissues volume observed was accompanied by microstructural changes on DTI-derived scalar measures. The significantly increased MD in the ipsilesional thalamus, hippocampus, and amygdala suggests changes in water mobility due to early cellular breakdown. These findings are in agreement with previous post stroke imaging studies (37–40) and supports its potential role as a surrogate marker of poststroke unilateral neuronal loss. These microstructural changes could be secondary to Wallerian degeneration of the white matter pathways of the limbic system, which are connected to the brain and cerebellum. The other mechanism could be cytotoxic, vasogenic edema following the primary injury to fiber tracts.

Global neuroinflammation suggested in animal stroke models could be a contributing factor to remote neuronal loss. Furthermore, absence of temporal changes in structural and

microstructural measures in the contralesional hemisphere suggests that structural disintegration is confined to the ipsilesional hemisphere. Future studies with a larger number of participants are needed to determine the existence and clinical significance of unilateral progressive volume loss, cortical thinning, and increased diffusivity, and to determine their contributions to cognitive impairment.

Our results show significant, progressive ipsilesional cortical thinning in the caudalanterior and isthmus regions of the cingulate cortex, compared to previously reported significant thinning in the posterior regions of cingulate (20). This may be due to differences in measurement methods. We used FreeSurfer analysis for average thickness, while the previous study used voxel-based morphometry (20).

Furthermore, our results show significant elevated ipsilesional MD in the caudal and posterior segments of the cingulum but not in the rostral and isthmus regions, suggesting selective white matter vulnerability. Unexpectedly, we did not observe changes in ipsilesional FA in the caudal and posterior regions, suggesting that changes in MD may precede those in FA.

The limitations of our study include the small sample size and heterogeneity in lesion volume and location between patients. The study duration of 12 months may be too short to track long-term tissue loss. Lack of serial neurophysiological measurements prevents ascribing causality of these structural changes to post-stroke neuropsychological impairment. Cognitive assessment and neurological functional will be collected in future studies to determine the clinical relevance of our imaging findings.

In conclusion, this study uncovered ongoing micro- and macrostructural changes in the limbic system long after injury, even with clinical improvement assessed with NIHSS scores. These findings provide insight into the mechanisms leading to cognitive and psychiatric impairments often observed after stroke. Further, we showed that many of these effects exist even when the injury site is remote from the limbic structures. Nonetheless, these ongoing and non-localized complications of stroke demand further studies to better understand their mechanisms and develop appropriate interventions.

## HUMAN SUBJECT PARTICIPANTS

This Study was approved by the McGovern Medical School, University of Texas Health Sciences Center at Houston Committee for the Protection of Human Subjects and by the Memorial Hermann Office of Research.

## AUTHOR CONTRIBUTIONS

MH designed the study, carried MRI quantitative analysis, and drafted the manuscript. RG contributed to data analysis, assisted in optimizing MRI acquisition. KH provided qualitative and quantitative quality assurance of images and data analysis. SG and SA recruited, consented, and obtained patient's serial neurological assessment. OA and AZ performed region of interest analysis. JJ provided free surfer analysis. MM assisted in patient's recruitment from his facility. NS assisted in

creating plots and figures. XZ performed statistical analysis. EF and CS provided radiology reports and assisted in lesion locations. SS supervised the study and provided necessary resources. All authors contributed to and approved the final manuscript.

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