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Progressive Neurodegeneration Across Chronic Stages of Severe Traumatic Brain Injury

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Objective: To examine the trajectory of structural gray matter changes across 2 chronic periods of recovery in individuals who have sustained severe traumatic brain injury (TBI), adding to the growing literature indicating that neurodegenerative processes occur in the months to years postinjury. **Participants:** Patients who experienced posttraumatic amnesia of 1 hour or more, and/or scored 12 or less on the Glasgow Coma Scale at the emergency department or the scene of the accident, and/or had positive brain imaging findings were recruited while receiving inpatient care, resulting in 51 patients with severe TBI. **Methods:** Secondary analyses of gray matter changes across approximately 5 months, 1 year, and 2.5 years postinjury were undertaken, using an automated segmentation protocol with improved accuracy in populations with morphological anomalies. We compared patients and matched controls on regions implicated in poorer long-term clinical outcome (accumbens, amygdala, brainstem, hippocampus, thalamus). To model brain-wide patterns of change, we then conducted an exploratory principal component analysis (PCA) on the linear slopes of all regional volumes across the 3 time points. Finally, we assessed nonlinear trends across earlier (5 months-1 year) versus later (1-2.5 years) time-windows with PCA to compare degeneration rates across time. Chronic degeneration was predicted cortically and subcortically brain-wide, and within specific regions of interest. **Results:** (1) From 5 months to 1 year, patients showed significant degeneration in the accumbens, and marginal degeneration in the amygdala, brainstem, thalamus, and the left hippocampus when examined unilaterally, compared with controls. (2) PCA components representing subcortical and temporal regions, and regions from the basal ganglia, significantly differed from controls in the first time-window. (3) Progression occurred at the same rate across both time-windows, suggesting neither escalation nor attenuation of degeneration across time. **Conclusion:** Localized yet progressive decline emphasizes the necessity of developing interventions to offset degeneration and improve long-term functioning. **Key words:** *chronic decline*, *longitudinal*, *neurodegeneration*, *traumatic brain injury*

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RESEARCH INTO the neurological damage following traumatic brain injury (TBI) typically focuses on the immediate, postimpact primary injuries and early secondary neural damage.[1] However, a growing number of longitudinal studies have revealed progressive losses to brain volume (including whole brain and substructures)[2–8] and cognitive function (notably memory and executive functioning)[9–11] in the months and years following injury. Such deterioration stands in contrast to prevailing assumptions about brain recovery after TBI and may contribute to poor long-term functional outcomes.[12]

Several studies examining the brain across 2 time points within the chronic stages of injury have shown global deterioration. Whole-brain volume loss has been observed across a range of windows postinjury, including 2 to 12 months,[7] 3 to 14 months,[8] 5 to 30 months,[6] and 5 to 12-56 months (with an average of 20).[5] Bilateral volumes of frontal, temporal, and occipital cortices were found to be significantly lower in patients at

approximately 1 year postinjury than in controls, with continued decline at a greater rate than nondamaged areas approximately 1 year later.[4] Visible lesion expansion from approximately 5 to 30 months postinjury has also been observed.[6]

Several studies have also examined regional volume loss. Our group found significant decreases in volume of the hippocampus (HPC) between 2 scan times compared with a normative sample.[6] On the basis of monthly percent volume changes to the whole brain, corpus callosum, and bilateral HPC, we also found that the majority of patients showed significant volume loss in at least one region compared with healthy controls (≥2 SDs below controls).[5] Another longitudinal study found significant volume reductions in subcortical regions in patients compared with controls, including the thalamus, putamen, caudate, and amygdala.[4]

To date, few studies have examined gray matter volume loss in humans commencing in the chronic stages of TBI,[3] at multiple time points, and comparing multiple brain regions. Understanding the trajectories of decline, and how they might differ across individuals, is important for prognostication and treatment. Therefore, the goal of the present study was to examine changes to gray matter volume across 3 time points encompassing early and later time-windows in the chronic stages of severe TBI, approximately 5 months to 1 year, and 1 to 2.5 years postinjury. To examine changes across multiple brain regions, we employed a recently developed automated segmentation method (MALP-EM[13,14]). Manual segmentation is generally accepted as more accurate for individual regions than automatic protocols, with higher measured atrophy rates,[15,16] and more sensitivity for detecting volume changes. We chose MALP-EM, however, as it has been validated with both healthy controls and patients with TBI of varying severity (in the HPC, thalamus, putamen, occipital pole[13]), showing significantly improved accuracy for this and other clinical populations[17] compared with other automated methods. Although automated tools may lead to inconsistencies with manual segmentation for certain injury and lesion characteristics, they show high reliability across time points and are thus particulary beneficial for longitudinal studies.

Objective 1 focused on specific brain regions (accumbens, amygdala, thalamus, HPC, brainstem), as their reduced acute volumes have been shown to predict poorer clinical outcomes at chronic follow-up.[14] Specifically, smaller volumes of the thalamus in the chronic stages of moderate-to-severe TBI significantly correlated with deficits in memory, attention, and executive functioning.[18] As well, the accumbens, an important part of the dopaminergic pathway, is implicated in chronic TBI deficits, including poorer executive functioning.[19] Furthermore, neural circuitry implicated in mood disorders[20] (which are prevalent post-TBI[21,22]) encompasses these regions. For Objective 1a, we therefore examined rates of volume loss bilaterally in these regions, from early to later chronic time points, predicting they would show significant rates of degeneration. For Objective 1b, we incorporated matched healthy controls in the first time-window, as the delay between scan times was similar between controls and patients in this time-window. Bilateral volume changes were compared between groups in this time-window, and we predicted accelerated degeneration, meaning a greater rate of volume loss than would be expected from normal, time-related changes in controls.

In Objective 2, we employed a brain-wide approach to explore patterns of degeneration across all 62 segmented gray matter regions. In Objective 2a, we examined which groups of regions showed similar linear rates of volume reduction across the 2 chronic time-windows. On the basis of previous longitudinal findings,[4,5] we expected widespread degeneration in both cortical and subcortical regions. For Objective 2b, we again incorporated the controls to determine which sets of regions showing significant rates of degeneration in patients from Objective 2a also showed accelerated, pathological degeneration in the first time-window. Finally, for Objective 2c, we examined nonlinear trends between the 2 time-windows in the patients to ascertain whether rates of degeneration across the 62 regions changed across the 2 chronic time-windows.

METHODS

Participants

Magnetic resonance imaging (MRI) files were drawn from a database of patients who had participated in a longitudinal study, originally recruited from the inpatient Acquired Brain Injury service at the University Health Network, Toronto Rehabilitation Institute (UHN-TRI). Neuroimaging was acquired at approximately 5 months, 12 months, and 1 to 3 years postinjury. Inclusion criteria were as follows: (1) clinically diagnosed TBI with injuries serious enough for inpatient rehabilitation; (2) posttraumatic amnesia (PTA)[23] of 1 hour or more, and/or Glasgow Coma Scale (GCS)[24] score of 12 or less either at the emergency department or at the scene of the accident, and/or positive neuroimaging findings; (3) resolution of PTA by 3 months postinjury; (4) ability to use at least one upper extremity; (5) older than 17 years; (6) sufficient English fluency to complete assessments; (7) competency to provide informed consent or availability of a legal decision maker; and (8) completed all 3 MRI scans. Exclusion criteria were as follows: (1) history of prior TBI; (2) diagnosis of neurological disorders primarily affecting the central

nervous system; (3) history of a psychotic disorder; (4) failure on a Test of Memory Malingering[25] at any session; and (5) additional TBI or neurological events between scans. For the present study, anyone older than 60 years at the first scan was also excluded to control for age-related volume loss.[26]

The resultant sample of 51 patients (33 males) were on average 38.4 years old at the first scan (SD = 14.0; range, 18-60), with a mean of 15.1 years of education (SD = 2.7; range, 9-20). Injury severity was based on the GCS[24] score and/or PTA,[23] obtained through medical records or interviews with patients and/or caregivers. Where there were discrepancies, PTA scores were used (see Table 1). Injury severity was moderate or greater for all patients, with the majority being severe. Functional independence for motor-related tasks at discharge is also noted in Table 1, based on scores on the Functional Independence Measure–Motor subscale.[27,28] Additional characterization variables at approximately 5 months postinjury are also summarized in Table 1, including scores and categorization on the Beck Depression Inventory (BDI-II),[29] Beck Anxiety Inventory (BAI),[30] scores and categorization of likely posttraumatic stress disorder (PTSD) as indicated by the Trauma subscale of the Anxiety-Related Disorders Scale of the Personality Assessment Inventory (PAI),[31,32] and indicators of alcohol and drug misuse based on the associated subscales of the PAI.[31] Average scores on these measures were overall low in our patient sample, with only one patient requiring assistance associated with motor impairments, fewer than 6 patients exhibiting moderate-severe symptoms on the BDI and the BAI, only one patient with likely PTSD, 2 patients showing likely alcohol abuse, and no patients showing evidence of drug abuse or dependency.

Healthy control participants were scanned at 2 time points, approximately matching the delay between patients' first 2 scans. Inclusion criteria for the parent study were as follows: (1) older than 17 years; and (2) commitment to complete both scans. Exclusion criteria were as follows: (1) history of TBI or concussion requiring hospitalization; and (2) any disorder affecting the central nervous system. Additional exclusion criteria for the current study were more than 60 years of age, and a delay between scans of more than 2 years. The resultant sample ($N = 20$; 8 males) was on average 36.0 years old (SD = 12.2; range, 18-60) at the first scan, with a mean education of 16.0 years (SD = 2.4; range, 12-19). There were no significant differences between patients and controls for age ($t_{39} = 0.59$, $P = .55$, $d = 0.15$) or education ($t_{38} = 1.52$, $P = .14$, $d = 0.39$).

Procedure

This study was approved by the UHN Research Ethics Board and conducted in accordance with the Declaration of Helsinki. Following recruitment, informed consent, and discharge from UHN-TRI (patients only), all MRI sessions were conducted at UHN-Toronto General Hospital. The first scan for patients was completed at a mean of 5.3 months postinjury (SD = 1.1; range, 4.0-8.9); the second scan at a mean of 12.9 months postinjury (SD = 1.7; range, 10.8-17.2), with a mean delay of 7.7 months (SD = 1.9; range, 4.2-12.7); and the third scan at a mean of 32.8 months postinjury (SD = 10.8; range, 21.8-80.2), with a mean delay of 20.8 months (SD = 12.6; range, 7.6-69.4). The mean delay between scans for healthy controls was 11.16 months (SD = 3.7; range, 7.6-20.1), significantly longer than patients ($t_{23} = 3.98$, $P < .001$, $d = 1.17$). This difference would have likely resulted in overestimation of the volume decline rate in controls and introduced a conservative bias in the detection of significant decline in patients, against the direction of our hypotheses.

Image acquisition

Magnetic resonance images were acquired using a General Electric (GE) Signa-Echospeed 1.5-Tesla HD scanner (SIGNA EXCITE; GE Healthcare, Milwaukee, Wisconsin), with an 8-channel head coil. The acquisition protocol for the T1-weighted images used in the present analyses was as follows: high-resolution isotropic T1-weighted 3-dimensional IR-prepped radiofrequency-spoiled-gradient recalled-echo (3D IRSPGR) acquired on an axial plane (TR = 300 ms; TE = 5 ms, TI [inversion time] = 12 ms), flip angle (FA) = 20°, slice thickness = 1 mm no gap, matrix = 256 × 256, field of view (FOV) = 25 cm.

Image processing

The images were transferred onto a workstation, received in Digital Imaging and Communications in Medicine file format and converted into Neuroimaging Informatics Technology Initiative file format using the mri_convert utility in FreeSurfer.[33] Following a postinstallation test run with the sample data provided,[13] the images were submitted to the MALP-EM protocol. The outlined protocol is readily available online (https://github.com/ledigchr/MALPEM) and is briefly summarized in Appendix 1.

Analyses

Analyses of volumetric change in patients incorporated linear slopes across the 2 time points for all gray matter regions (58 bilateral and 4 unpaired; see Appendix 2). Analyses of volumetric change between patients and controls incorporated monthly percent volume change based on the patients' first time-window

TABLE 1 *Demographics and injury variables for patient sample*

	M	SD	*n*	%
Age at injury, years	37.92	14.06		
Education, years	15.06	2.66		
Male			33	64.71
Type of injury				
Motor vehicle accident			32	62.74
Fall			16	31.37
Assault			1	1.96
Sports injury			2	3.92
Severity of injury variables				
Acute care length of stay, days	38.63	22.34	48	
GCS (lowest recorded score)	5.98	3.39	45	
Mild (13-15)			4	7.84
Moderate (9-12)			3	5.88
Severe (<9)			38	74.51
Missing data			6	11.76
Length of posttraumatic amnesia				
<60 min, mild			0	0.00
1-24 h, moderate			1	1.96
>24 h, severe			47	92.16
Missing data			3	5.88
Injury severity rating				
Mild			0	0.00
Moderate			1	1.96
Severe			49	96.08
Missing data			1	1.96
Functional independence (motor)				
Score	87.78	5.35		
Full independence			15	29.41
Full or with help of a device			28	54.90
With supervision			0	0
With supervision or minimal assistance			1	1.96
Missing data			7	13.72
Depression scores (BDI)				
Total score	9.07	7.85		
Normal			33	64.70
Minimal			8	15.69
Mild-moderate			1	1.96
Moderate-severe			4	7.84
Severe			1	1.96
Missing data			4	7.84
Anxiety scores (BAI)				
Total score	6.04	6.75		
Minimal			35	68.63
Mild			8	15.69
Moderate			5	9.80
Severe			1	1.96
Missing data			2	3.92
Anxiety-related disorders—Trauma (PAI)				
T-score	49.36	8.42		
Not likely to have PTSD			43	84.31
Exceeds cutoff of likely PTSD			1	1.96
Missing data			7	13.72
Alcohol use (PAI)				
T-score	50.64	9.65		
No use			39	76.47
Normal use			3	5.88
Likely abuse			2	3.92
Likely severe abuse			0	0
Missing data			7	13.72
Drug use (PAI)				
T-score	51.09	7.79		
No use			33	64.70
Some use			11	21.57
Likely drug abuse			0	0
Likely drug dependency			0	0
Missing data			7	13.72

Abbreviations: BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; GCS, Glasgow Coma Scale; PAI, Personality Assessment Inventory; PTSD, posttraumatic stress disorder.

(5 months-1 year postinjury), and both scans for controls, using the following formula:

$$100 \times [(\text{volume at scan 2}) - (\text{volume at scan 1})/(\text{volume at scan 1})/\text{delay (months)}]$$

For a full overview of data processing, see Appendix 2.

Before the formal analyses, correspondence between the segmentation methods was examined. HPC volumes from the same data processed using manual segmentation from a previous study[5] were compared with the volumes extracted from MALP-EM. Correlations between volumes derived from each method were calculated for each hemisphere (right, left) and time point (scan 1, scan 2, scan 3) using R software.[34]

For Objective 1a, we examined changes in patients across the 3 time points by conducting one-sample *t* tests of the linear slopes of the 5 regions of interest (ROIs; accumbens, amygdala, brainstem, HPC, and thalamus) using R,[34] corrected for multiple comparisons (Holm-Bonferroni). For Objective 1b, we examined accelerated degeneration when accounting for normal decline in the first time-window by conducting separate analyses of covariance (ANCOVAs) for the monthly percent volume change for each ROI using R,[34] with group as the independent variable (patients vs controls), controlling for intracranial volume change across scans, and corrected for multiple comparisons (Holm-Bonferroni).

For Objective 2a, we examined patterns of changes across earlier to later time-windows in patients by submitting the linear slope of each region into a principal component analysis (PCA) with varimax rotations using jamovi software,[35] using the scree plot to select the number of meaningful components. Component scores for each region were then computed by calculating the product of the component loading and individual linear slope of each region within a component. One-sample *t* tests were used to compare the average component scores for each component to a mean of zero, where a score significantly below zero would represent significant degeneration of the component's respective set of regions.

For Objective 2b, we examined whether the sets of regions showing significant degeneration across the 3 time points in patients earlier (represented by the PCA components with component scores significantly below zero) would also show evidence of accelerated degeneration when compared with controls in the first time-window. Multivariate analyses of covariance (MANCOVAs) were conducted for each interpreted component, with group as the independent variable (patients vs controls), and controlling for intracranial volume change across scans. The dependent variables for each MANCOVA were the monthly percent volume changes of the regions in each of the components, weighted by the PCA loading of each region. Follow-up Pillai's trace analyses were conducted for MANCOVAs reaching significance after correcting for multiple comparisons (Holm-Bonferroni). For Objective 2c, we examined degeneration rates in patients across the earlier and later time-windows by conducting an additional PCA on the quadratic betas extracted from nonlinear regression models of the volume of each region.

RESULTS

Comparison of segmentation methods

Manual and automated segmentation using MALP-EM showed an overall moderately strong, significant correlation ($M = 0.52$, $SD = 0.06$; individual correlations are listed in Appendix 3).

Objective 1: Regions of interest

Before addressing Objective 1, volumes of the unilateral ROIs were compared between patients and controls to categorize absolute group differences at both time points (see Table 2). Independent sample *t* tests revealed significantly smaller patient volumes at scan 1 (5 months postinjury for patients) for the brainstem, left HPC, and right and left thalamus than controls and marginally for the right HPC.

At scan 2 (1 year postinjury for patients), patient volumes of most regions were significantly smaller than controls, with only the right accumbens and right amygdala not exhibiting a significant group difference (see Table 2).

Objective 1a was to determine whether ROIs whose acute volumes postinjury predict clinical outcome chronically[14] also lose volume over time (across both time-windows in patients). Four of the regions had a slope significantly below zero, whereas the HPC (bilaterally) showed marginal decline (see Table 3). As past studies showing significant HPC volume loss examined change unilaterally,[5] follow-up one-sample *t* tests were conducted for each HPC hemisphere. These analyses indicated that the left HPC drives most of the region's bilateral volume change.

Objective 1b was to determine whether the same ROIs would exhibit accelerated degeneration in the patients' first time-window compared with changes in controls. First, by conducting a one-way analysis of variance with group as a factor (patient, control), no differences in intracranial volume change were found ($F_{1,69} = 0.04$, $P = .85$, partial $\eta^2 = 0.0005$), suggesting minimal intrascanner variability between groups. On the basis of the individual ANCOVAs for each of the ROIs, the accumbens showed significantly more decline in patients than in controls; the thalamus, amygdala, and brainstem showed marginally more decline in patients than controls; and decline in the HPC was not significantly

TABLE 2 *Comparison of average absolute volumes for unilateral regions of interest between the patient and control groups*

Scan time	Region	Patient volume	Control volume	*t*	*P*	Adjusted α	*d*
1	Brainstem	17.63	19.16	3.33	<.01[a]	.008	0.84
	Left accumbens	0.47	0.52	1.81	.08	.0125	0.48
	Right accumbens	0.47	0.48	0.35	.73	.025	0.09
	Left amygdala	1.05	1.10	0.97	.34	.017	0.22
	Right amygdala	1.05	1.06	0.32	.75	.05	0.05
	Left hippocampus	2.86	3.02	3.73	<.001[a]	.007	0.88
	Right hippocampus	2.60	3.14	2.63	.012[b]	.01	0.65
	Left thalamus	7.17	8.10	5.08	<.0001[a]	.005	1.05
	Right thalamus	7.04	7.83	4.53	<.0001[a]	.006	1.05
2	Brainstem	17.20	19.30	4.39	<.0001[a]	.007	1.10
	Left accumbens	0.45	0.55	3.51	<.01[a]	.0125	0.99
	Right accumbens	0.44	0.50	2.10	.04	.025	0.58
	Left amygdala	1.00	1.15	2.91	<.01[a]	.017	0.70
	Right amygdala	1.00	1.09	1.72	.09	.05	0.40
	Left hippocampus	2.53	3.01	4.09	<.001[a]	.008	0.95
	Right hippocampus	2.80	3.16	3.48	<.01[a]	.01	0.82
	Left thalamus	6.98	8.05	6.08	<.0001[a]	.005	1.44
	Right thalamus	6.91	7.81	5.02	<.0001[a]	.006	1.17

[a] $P < .01$.
[b] $P < .05$.

TABLE 3 *Summary of Objective 1 results*[a]

Objective 1a					
Region	**Decline (patients only)**	***t***	***P***	**Adjusted α**	***d***
Brainstem	−0.04	7.51	<.01[b]	.0125	1.05
Accumbens	−0.004	5.30	<.01[b]	.017	0.74
Amygdala	−0.003	2.70	<.01[b]	.025	0.37
Hippocampus	−0.003	1.87	.07	.05	0.26
Left	−0.001	2.04	.047[c]	.05	0.28
Right	−0.002	1.11	.27	.05	0.16
Thalamus	−0.03	9.85	<.01[b]	.01	1.38

Objective 1b						
Region	**Patient change**	**Control change**	***F***	***P***	**Adjusted α**	**Partial η^2**
Brainstem	−0.32	0.06	5.08	.03[c]	.025	0.07
Accumbens	−0.48	0.65	10.43	<.01[b]	.01	0.13
Amygdala	−0.53	0.41	5.47	.02[c]	.017	0.07
Hippocampus	−0.26	0.10	2.13	.15	.05	0.03
Left	−0.60	0.05	3.37	.07	.05	0.05
Right	−0.41	0.16	1.74	.19	.05	0.03
Thalamus	−0.28	0.04	5.88	.02[c]	.0125	0.08

[a] Decline in Objective 1a was categorized as the linear slope across the 3 scan times in patients; decline in Objective 1b was based on the average monthly percent volume change.
[b] $P < .01$.
[c] $P < .05$.

TABLE 4 *Summary of Objective 2 results*[a]

Objective 2a						
Component	**Eigenvalues**	**% Variance**	**Total variance**	**Component scores**	***t***	***P***
1	14.58	23.51	23.5	−0.008	−2.19	.033
2	6.60	10.64	34.2	−0.001	−0.98	.33
3	4.98	8.03	42.2	−0.005	−2.29	.026
4	3.36	5.42	47.6	−0.001	−0.81	.42
5	3.26	5.26	52.9	−0.006	−2.41	.019
6	2.60	4.19	57.1	−0.0003	−0.46	.65
7	2.35	3.80	60.9	−0.006	−5.40	<.0001

Objective 2b				
MANCOVAs		***F***	***P***	**Adjusted α**
Component 1		0.70	.81	.05
Component 3		2.89	<.01[b]	.017
Component 5		1.43	.18	.025
Component 7		6.74	<.01[b]	.013
Follow-up	**Region**	***F***	***P***	**Partial η^2**
Component 3	Thalamus	5.88	.02[c]	0.08
	Parahippocampal gyrus	6.67	.01[c]	0.09
	Amygdala	5.47	.02[c]	0.07
Component 7	Putamen	17.58	<.01[b]	0.20
	Accumbens	10.43	<.01[b]	0.13
	Parahippocampal gyrus	6.67	.01[c]	0.09

Objective 2c						
Component	**Eigenvalues**	**% Variance**	**Total variance**	**Component scores**	***t***	***P***
1	17.12	27.61	27.6	0.0002	0.45	.65
2	5.96	9.61	37.2	−0.00001	−0.09	.93
3	4.94	7.97	45.2	−0.001	−0.84	.40
4	4.08	6.59	51.8	0.0001	0.37	.71
5	3.87	6.25	58.0	0.00004	0.14	.88
6	3.11	5.02	63.0	−0.0001	−1.41	.16
7	2.28	3.67	66.7	0.00005	0.45	.65

Abbreviation: MANCOVA, multivariate analysis of covariance.
[a]Objective 2a: summary of eigenvalues and component scores analysis for meaningful components. Objective 2b: results of follow-up MANCOVAs of components with scores significantly below zero (1, 3, 5, and 7), and follow-up Pillai's trace analyses. Objective 2c: summary of eigenvalues and composite scores analysis for meaningful components.
[b]$P < .01$.
[c]$P < .05$.

different between groups (see Table 3 and Appendix 4). As shown previously, follow-up ANCOVAs were conducted for each hemisphere of the HPC, revealing a marginal difference between groups in the left HPC but no significant difference in the right HPC.

Objective 2: All gray matter regions

Objective 2 was to examine patterns of decline across all gray matter regions. To address Objective 2a, a PCA based on the linear slopes of the regions across the 3 time points in patients revealed 7 meaningful components accounting for 60.9% of the variance (see Table 4, 2a). Four of these components (1, 3, 5, and 7) had scores significantly below zero (representing significant degeneration) and were selected for further interpretation (see Table 4, 2a).

Component 1, with 21 regions loading onto it and accounting for the most variance, represents cortical atrophy mostly involving dorsal regions, most notably the superior parietal lobule, middle frontal gyrus, precentral gyrus, angular gyrus, postcentral gyrus, precuneus, middle occipital gyrus, and superior occipital gyrus, which had the greatest negative individual component scores (see Figure 1). Component 3 included 9 regions representing subcortical atrophy, mostly

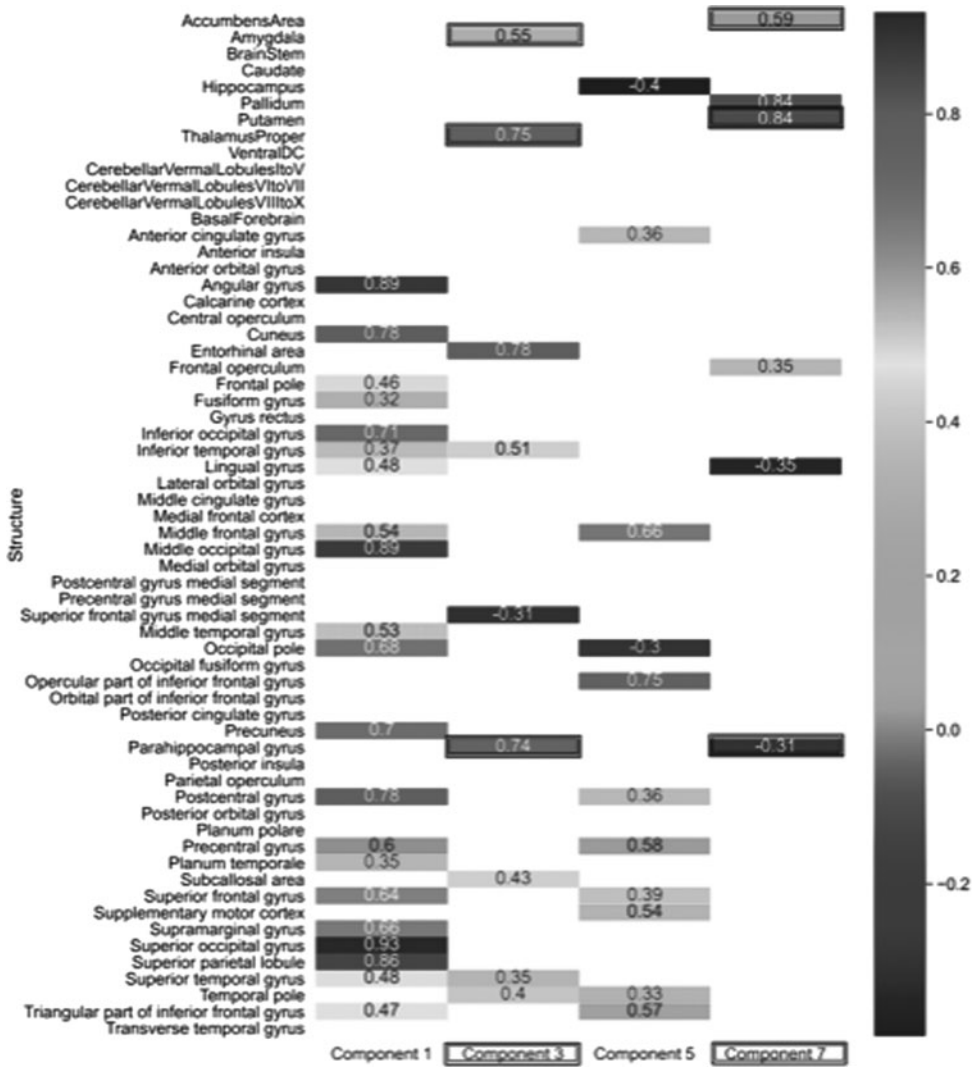

Figure 1. Objectives 2a and 2b. Components from principal component analysis results examining linear slopes in patients between the 3 chronic time points postinjury, including regions loading onto each component and their respective loadings, alphabetically ordered by subcortical regions and then by cortical regions. Components and regions highlighted in boxes are those that remained significant in follow-up multivariate analyses of covariance controlling for age-related changes in the first time-window.

ventral temporal, driven by the thalamus, temporal pole, parahippocampal gyrus, superior temporal gyrus, and amygdala, which had the greatest negative individual component scores. Component 5 included 12 regions representing frontal-central atrophy, with the middle frontal gyrus, precentral gyrus, postcentral gyrus, temporal pole, and supplementary motor cortex notably having the greatest negative individual component scores. Finally, the 6 regions loading onto Component 7 represent atrophy to the basal ganglia, with the putamen, pallidum, and accumbens contributing the most through the greatest negative individual component scores.

Objective 2b was to determine whether the patterns of degeneration resulting from the original PCA were based on injury-related (not normal time-related) volume changes, which would reflect accelerated degeneration. On the basis of the 4 separate MANCOVAs, significant group differences emerged for Components 3 and 7 but not for Components 1 or 5 (see Table 4, 2a, and highlighted in Figure 1). Follow-up analyses revealed that within Component 3, the thalamus, parahippocampal gyrus, and amygdala showed significant differences between groups. Within Component 7, significant group differences emerged for the putamen, accumbens, and parahippocampal gyrus.

Finally, Objective 2c was to examine whether the same rates of degeneration persisted across the chronic time-windows postinjury. On the basis of an additional PCA with quadratic betas, 7 meaningful components emerged, accounting for 66.7% of the variance (see

Table 4, 2c). Submitting the calculated average component scores for each of the components to individual *t* tests, however, did not reveal any significant patterns, suggesting no non-linear degeneration patterns.

DISCUSSION

In the present study, we first showed that key subcortical and temporal ROIs–the accumbens, amygdala, brainstem, thalamus, and HPC (marginally)–exhibit degeneration in the chronic stages postinjury. This confirms that these regions, whose smaller volumes at the acute stage are associated with poor clinical outcome in the chronic stage,[14] continue to lose volume over time. Damage to these specific regions could help explain observed deficits in emotional and sensorimotor functioning in TBI,[36,37] and further degeneration in the chronic stages of injury may affect long-term outcomes of patients. In the first time-window, only the accumbens showed significantly reduced volume compared with controls, suggesting that although these 4 regions may contribute to patients' later clinical outcomes, accelerated degeneration specifically in the accumbens may be the strongest predictor. Nonetheless, further investigation is warranted to determine whether the relationship between these regions and clinical outcome is driven by (1) their absolute volume either premorbidly or acutely postinjury, or (2) the extent to which their volumes decline between acute and chronic stages.

Corroborating patterns from previous studies highlighting the HPC's vulnerability to injury-related damage,[4,5,14] patients presented smaller HPC volumes than controls at 5 months postinjury. The results of the chronic degeneration patterns in the HPC were not as strong, however, likely driven by differences in the adopted segmentation method, as the previous study with this patient cohort employed manual segmentation.[5] Despite the lack of full convergence, both the extracted HPC volumes and the directionality of the results were consistent between segmentation methods in the present study. Therefore, the accumulating evidence points to the HPC's vulnerability to chronic degeneration.

The aforementioned findings notwithstanding, the central focus and unique contribution of this study were the exploration of brain-wide patterns of degeneration. In line with other longitudinal findings,[4] the brain-wide analyses suggest that both cortical (frontal, temporal) and subcortical regions are vulnerable to chronic degeneration up to at least 1 to 2.5 years postinjury. Only the subcortical and temporal regions showed evidence of accelerated degeneration in the first time-window, corresponding with the limbic system, and the basal ganglia. As the sets of regions showing accelerated degeneration overlap with the ROIs in the first set of analyses summarized earlier, this pattern may also help explain injury-related deficits in emotional, sensorimotor, as well as cognitive functioning.[9–11,36,37] Interestingly, the parahippocampal gyrus was positively associated with the component representing the limbic system but negatively associated with the component representing the basal ganglia, raising the question whether postinjury neural compensation[38] may drive competition between declarative[39] (parahippocampal gyrus) and more habitual cognitive processes[40] (basal ganglia). Overall, although chronic degeneration is widespread cortically and subcortically across chronic stages, accelerated degeneration seems more exclusive to subcortical regions, at least up to 1 year postinjury. Alternatively, cortical regions may show proportionally more progressive atrophy in the later time-window.

An important clinical question is the impact of chronic degeneration. In combination with aging-related processes and injury-related reduction in brain and cognitive reserve,[41] TBI leads to an increased vulnerability to neurodegenerative diseases,[42,43] which are associated with a reduction in cognitive capacities.[44] There is also more direct evidence of cognitive declines in the chronic stages of TBI.[9–11,36,37] For example, in a previous study of moderate-to-severe TBI, 27% of patients showed significant declines on at least 2 neuropsychological tests (2 SDs below healthy controls), mainly in verbal fluency and delayed verbal recall memory.[9] A more recent study replicating and extending these findings showed that left HPC loss between 5 and 12 months postinjury was significantly associated with cognitive decline between 12 and 30 months postinjury.[45] Interestingly, one recent study found that although significant reductions to cortical thickness were observed postinjury, they were only found in patients who self-reported chronic cognitive symptoms.[46] This further supports a relationship between chronic neurodegeneration and cognitive decline. However, more research is needed to gain a clearer understanding of the cognitive impact of neurodegeneration.

Whether premorbid and/or injury-related comorbidities (eg, mood disorders, alcohol and drug misuse, chronic pain, PTSD) impact chronic neurodegeneration should also be examined. In the present sample, there was an overall low rate of these types of comorbidities at 5 months postinjury. This may be due to an unawareness of deficits, where an overestimation of abilities and the tendency to use denial as a defense mechanism has been associated with lower scores on the BDI.[47] Nonetheless, how these variables may interact with injury-related declines should be systematically examined in samples with greater variability across each relevant measure.

Unlike other longitudinal studies that either did not have a control group[3] or where controls were not age-matched,[4] here we incorporated a matched control

group for the first time-window. As we did not have control scans mapping onto the second time-window (ie, approximately 23 months between scans), a limitation was that we were unable to empirically compare the rate of atrophy between the early and later time-windows when accounting for normative decline in healthy controls. A notable aspect of the present study was the availability of multiple scan times, with the present findings converging with one other study comparing multiple time points.[3] Specifically, they found subcortical decline in their second time-window between 3 and 12 months; in the present study, we observed a comparable pattern. We further examined the trajectory of volume change across early to later chronic time-windows post-TBI. These analyses did not reveal any reliable changes, suggesting neither abatement nor acceleration of earlier rates of degeneration. Future studies with multiple time points should examine changes past the initial 2 or 3 years postinjury to further establish the trajectory of postinjury changes.

With regard to causes of chronic neurodegeneration, diffuse axonal injury, chronic inflammation, hypoxia, blood-brain barrier disruptions, and delayed apoptosis[48–53] are candidate mechanisms. Specific to subcortical regions, their vulnerability to chronic volume loss may stem from activation of microglia underlying chronic inflammatory processes,[54] metabolic dysregulation,[55] or diaschisis independent of axonal injury.[56] Environmental factors may also play a role. Moderate-to-severe TBI typically results in a reduction of activities and cognitive stimulation, leading to neurological disuse, which may, in turn, lead to atrophy in the chronic stages of injury.[57,58] Consistent with classic rodent studies on the detrimental effects of impoverished environments,[59,60] reductions in environmental enrichment have been associated with decreases in bilateral HPC volume in chronic TBI.[61,62]

CONCLUSION

Despite not being widespread, the present study highlights the persistence of chronic degeneration in severe TBI, particularly in subcortical regions. These results, together with known mechanisms of chronic neurodegeneration, can be used to inform the development of methods for improving the outcomes and quality of life of individuals with TBI. Clinical implications emphasize the necessity for rehabilitation to continue past typical acute and subacute schedules to offset the continuing chronic consequences, potentially by engaging patients in activities or training that would increase their environmental enrichment.

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APPENDIX 1

Summary of MALP-EM Protocol

The central steps in the MALP-EM protocol include (1) N4 bias correction for intensity normalization; (2) brain extraction using label propagation and group agreement (using pincram[63]), an iterative atlas-based method meant to increase robustness by using 35 manually annotated brain images from 30 subjects, provided by Neuromorphometrics, Inc (http://Neuromorphometrics.com); (3) registration with relaxed probabilistic priors based on the probabilistic fusion estimates and the image intensities to allow for greater segmentation accuracy for brains with atrophy; and (4) label refinement and segmentation using a combination of spatial priors taken from joint label fusion and posteriors taken from intensity-based expectation-maximization optimization. The protocol has been validated against manual segmentation using healthy control brain images, resulting in approximately 77% overlap across all regions, as measured by their similarity indices. It has been further validated in individuals with a TBI of varying degrees of severity by expert raters on 4 representative regions (HPC, thalamus, putamen, and occipital pole[13]). The completed report provides volumetric output for 138 atlas-labeled regions based on the Neuromorphometrics atlases, including 65 symmetric regions and 8 unpaired regions (third ventricle, fourth ventricle, brainstem, cerebrospinal fluid, optic chiasm, cerebellar vermal lobules I-V, cerebellar vermal lobules VI-VII, and cerebellar vermal lobules VIII-X).

A sample of the visual segmentation masks derived from MALP-EM from 2 of the patients in the present study is shown in Supplemental Digital Content Figure 1 (available at: http://links.lww.com/JHTR/A452). The masks in this figure were derived using MALP-EM at scan 1, with one example of a patient providing a sample from sagittal view (left) and another example of a patient providing a sample from coronal view (right).

APPENDIX 2

Data Processing

As gray matter volume changes were the focus of the present study, only gray matter volumes were examined from the volumetric output. ROI analyses in the patients were conducted on the bilateral volume changes in the accumbens, amygdala, brainstem, HPC, and thalamus, as modeled by a linear slope across the 3 time points postinjury for each bilateral region. To calculate the slopes, each scan time was mean-centered by the group grand mean of all scan times (17.3 months) to account for temporal differences between scan times. On the basis of the mean-centered scan times, volume change was linearly modeled by calculating a single slope across the 3 time points for each region, with the middle time point providing a more representative slope of the pattern of change across time. To control for normal time-related volumetric changes to the ROIs, monthly percent volume change was calculated on the basis of the patient's first time-window (5 months-1 year postinjury) and scans 1 to 2 for matched controls. Monthly percent volume change was calculated using the following formula:

$$100 \times [(\text{volume at scan 2}) - (\text{volume at scan 1})/(\text{volume at scan 1})/\text{delay (months)}]$$

To explore brain-wide patterns of degeneration across all gray matter regions and maximize dimension reduction, volumes for each symmetric region were summed at each time point, resulting in 58 bilateral and 4 unpaired gray

matter volumes, including: accumbens area, amygdala, brainstem, caudate, HPC, pallidum, putamen, thalamus, ventral diencephalon, cerebellar vermal lobules I-V, VI-VII, and VIII-X, basal forebrain, anterior cingulate gyrus, anterior insula, anterior orbital gyrus, angular gyrus, calcarine cortex, central operculum, cuneus, entorhinal area, frontal operculum, frontal pole, fusiform gyrus, gyrus rectus, inferior occipital gyrus, inferior temporal gyrus, lingual gyrus, lateral orbital gyrus, middle cingulate gyrus, medial frontal cortex, middle frontal gyrus, middle occipital gyrus, medial orbital gyrus, postcentral gyrus (medial segment), precentral gyrus (medial segment), superior frontal gyrus (medial segment), middle temporal gyrus, occipital pole, occipital fusiform gyrus, opercular part of inferior frontal gyrus, orbital part of inferior frontal gyrus, posterior cingulate gyrus, precuneus, parahippocampal gyrus, posterior insula, parietal operculum, postcentral gyrus, posterior orbital gyrus, planum polare, precentral gyrus, planum temporale, subcallosal area, superior frontal gyrus, supplementary motor cortex, supramarginal gyrus, superior occipital gyrus, superior parietal lobule, superior temporal gyrus, temporal pole, triangular part of inferior frontal gyrus, and transverse temporal gyrus.

We conducted a PCA on the mean-centered linear slope of changes to all bilateral volumes and unpaired regions in patients from across the 3 postinjury time points. Follow-up MANCOVAs were conducted for each interpreted component, involving monthly percent volume change and accounting for normal time-related change, based on the patients' first time-window and the control participants' 2 scans. The dependent variables for each MANCOVA were the monthly percent volume changes of the regions in each of the components, weighted by the PCA loading of each region. A follow-up PCA examining nonlinear rates of degeneration across the first and second time-windows in patients utilized quadratic betas obtained from the nonlinear regression models of the volume of each region.

APPENDIX 3

See Supplemental Digital Content Table 1 (available at: http://links.lww.com/JHTR/A453).

APPENDIX 4

See Supplemental Digital Content Figure 2 (available at: http://links.lww.com/JHTR/A454).

This figure represents the results from Objective 1b, representing monthly percent change of each ROI in each group (patient, control).