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Original Research

Whole brain and corpus callosum diffusion tensor metrics: How do they correlate with visual and verbal memory performance in chronic traumatic brain injury

Charalambos Yiannakkaras[1,*], Nikos Konstantinou[2], Fofi Constantinidou[3,4], Eva Pettemeridou[3,4], Eleni Eracleous[5], Savvas S. Papacostas[6] and Ioannis Seimenis[1]

[1]*Medical Physics Laboratory, Department of Medicine, School of Health Sciences, Democritus University of Thrace, Alexandroupolis-68100, Greece*

[2]*Department of Rehabilitation Sciences, Faculty of Health Services, Cyprus University of Technology, Limassol-3036, Cyprus.*

[3]*Center for Applied Neuroscience, University of Cyprus, Nicosia-1678, Cyprus*

[4]*Department of Psychology, University of Cyprus, Nicosia-1678, Cyprus*

[5]*Medical Diagnostic Center "Ayios Therissos", Nicosia-2033, Cyprus*

[6]*Neurology Clinic B', The Cyprus Institute of Neurology and Genetics and The Cyprus School of Molecular Medicine, Nicosia-1683, Cyprus.*

*Correspondence: CAN@ucy.ac.cy (Charalambos Yiannakkaras)

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This research investigates the chronic effect of moderate to severe traumatic brain injury on brain white matter integrity, as reflected by diffusion tensor imaging metrics, and the assessment of their correlation to neuropsychological response. Thirteen male participants with traumatic brain injury (8.4 years average post-injury time) were compared to a matched group of neurologically healthy controls. None of the traumatic brain injury subjects had received post-acute neurocognitive and/or neuropsychological rehabilitation. Between-group comparison of fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity was performed for the whole brain and corpus callosum. An extensive battery of visual and verbal memory tasks was employed for the comparative assessment of neurocognitive performance. Between-group and within-group performance differences were correlated with fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity of corpus callosum. Significant changes in global fractional anisotropy, mean diffusivity, and radial diffusivity were associated with traumatic brain injury. Visual memory capacity was reduced in traumatic brain injury, and this deficit was correlated to white matter integrity loss at the corpus callosum. Participants with traumatic brain injury underperformed controls in verbal memory as well, but no correlation with corpus callosum diffusion tensor imaging properties was established. Between-group performance difference was correlated with corpus callosum diffusion metrics in several tasks. Significant correlations were found between corpus callosum diffusion tensor imaging metrics and neuropsychological response within the traumatic brain injury group. Changes in whole brain and corpus callosum diffusion tensor metrics inflicted by moderate to severe traumatic brain injury are still evident several years post-injury and relate to neurocognitive impairment, while loss of white matter integrity seems to correlate with episodic and working memory impairment.

Keywords

Traumatic brain injury; chronic effects; diffusion tensor imaging; white matter; corpus callosum; visual memory; verbal memory

List of Abbreviations

AD	Axial Diffusivity
AVLT	Auditory Verbal Learning Test
CC	Corpus Callosum
CL	Confidence Level
DTI	Diffusion Tensor Imaging
EPI	Echo Planar Imaging
FA	Fractional Anisotropy
FMRIB	Functional Magnetic Resonance Imaging of the Brain
HC	Healthy Controls
MD	Mean Diffusivity
MRI	Magnetic Resonance Imaging
RD	Radial Diffusivity
ROI	Region of Interest
TBI	Traumatic Brain Injury
TBSS	Track Based Spatial Statistics
WM	White Matter
VSTM	Visual Short Term Memory

1. Introduction

Traumatic brain injury (TBI) is a public health challenge of vast proportion (Maas and Menon, 2017). Moderate to severe TBI is increasingly regarded as a condition with neuropsychological effects that extend through acute and chronic phases for several years post-injury, resulting in a neurodegenerative cascade (Konstantinou et al., 2016; Laglois et al., 2006). Diffusion tensor imaging (DTI), can assist in injury characterization and monitoring of TBI evolution (Kwon and Jang, 2012) and observation of neural regeneration (Jiang et al., 2013). Specifically, DTI-derived metrics assess injury-induced changes in oriented tissue such as brain white matter (WM) (Hutchison et al., 2016; Levin et al., 2008; Moseley et al., 2008). The characterization and quantification of changes inflicted by TBI on brain microstructure, the assessment of their effect on neuropsychological response, as well as the tracking of their time evolution, may provide guidance for patient management in terms of rehabilitation, participation in community life, and ability to work (Lorenz and Katz, 2015). Brain microstructure assessment by DTI during the TBI sub-acute phase may constitute a tool for predicting long-term cognitive outcome (Endlow et al., 2016; Messè et al., 2011; Wang et al., 2011; Wilde et al., 2008). Although further standardization of DTI implementation and reporting may be needed (Asken et al., 2017; Fortin et al., 2017), residual microstructural abnormalities and functional loss are generally reported as chronic consequences of TBI (Faber et al., 2016; Hashim et al., 2017; Moen et al., 2016).

Memory functions require uni- and bi-lateral transfer of information through the corpus callosum (CC), a WM structure vulnerable to TBI (Arenth et al., 2014; Mathias et al., 2004; Pollmann et al., 2004; Schulte et al., 2005; Treble et al., 2013). Damaged microstructural integrity of CC due to TBI may disrupt this connectivity, thus impair memory. A longitudinal study in participants with severe TBI reported that corpus callosal DTI measures at the acute phase can predict long-term outcomes in learning and memory (Wang et al., 2011). Other TBI-related studies have documented declarative memory correlates with fractional anisotropy (FA), a DTI-derived metric, at the splenium, while working memory correlates with FA at the genu (Schulte et al., 2005; Treble et al., 2013). Although FA has been reported to predict outcome of memory rehabilitation interventions (Strangman et al., 2012), inconsistent findings have been reported as to whether corpus callosal FA declines at the chronic phase of moderate to severe TBI (Voelbel et al., 2012). Thus, CC qualifies as a structure of particular interest in the study of traumatic changes and in the attempt to unravel structural predictors of memory performance after TBI.

This study aims to characterize and assess brain WM integrity loss as a chronic consequence of moderate to severe TBI. Therefore, DTI metrics were assessed in the whole brain and at the CC for participants with TBI and healthy controls. The relationship of these metrics with the neuropsychological response to brain injury was also investigated. It was hypothesized that loss of CC WM integrity would be correlated with working and episodic memory impairment.

2. Materials and Methods

Twenty males, native speakers of the Greek language, with a primary diagnosis of significant moderate to severe (moderate-severe) closed head injury at least 12 months prior to study recruitment were initially included as TBI subjects and underwent a magnetic resonance imaging (MRI) exam. All subjects with TBI had sustained diffuse axonal injury with contusions as evidenced by their initial MRI scans performed soon after injury. Detailed inclusion and exclusion criteria are provided by Konstantinou et al. (2016). Thirteen subjects with TBI remained in the study after excluding MRI scans that presented difficulties in non-linear alignment with the tools described in the following image processing and statistical analysis section. The age of the remaining subjects (TBI group) ranged from 23 to 60 years at the time of testing and their education ranged from 8 to 18 years. Subjects with TBI were recruited on average 8.4 years post-injury. Table 1 provides demographic information as well as information from the Galveston Outcome Scale Expanded (GOSE), a functional outcome scale obtained at the time of the study. GOSE scores indicate the presence of moderate-severe disability several years post-injury for the majority of the subjects with the following distribution of recovery: three subjects had achieved good recovery (scores 7 or 8), six subjects demonstrated moderate upper or lower disability, required some assistance with daily activities, and were unable to return to work (scores 5 or 6), and three subjects exhibited severe upper or lower disability and could not live independently or perform daily activities without considerable assistance (scores 3 or 4). All subjects demonstrated focal neurological signs at the time of injury and were hospitalized for several days during the acute phase of injury management. Glasgow Coma Score (GCS) information was not consistently available and therefore is not included.

Table 1. Demographic data of participants with TBI, includes Galveston Outcome Scale Extended (GOSE) score at the time of study admission.

TBI subject	Age (Years)	Education (Years)	TSI (Months)	GOSE
1	41	8	36	4
2	23	13	63	6
3	24	15	60	4
4	24	13	24	6
5	29	12	166	8
6	29	14	76	5
7	60	11	156	5
8	24	14	84	3
9	47	12	274	6
10	30	18	179	-
11	35	17	27	7
12	29	16	24	8
13	37	16	139	6
	M = 33.2; SD = 10.8	M = 13.8; SD = 2.71	M = 101; SD = 76.5	M = 5.7; SD = 1.6

TBI: traumatic brain injury, TSI: time since injury, M: mean, SD: standard deviation

Seventeen non-injured, Greek speaking males were carefully recruited to the group of healthy controls (HC). One participant was unable to complete the MRI examination due to claustrophobia and was excluded from subsequent analysis. Two participants were also excluded due to image artefacts identified at the

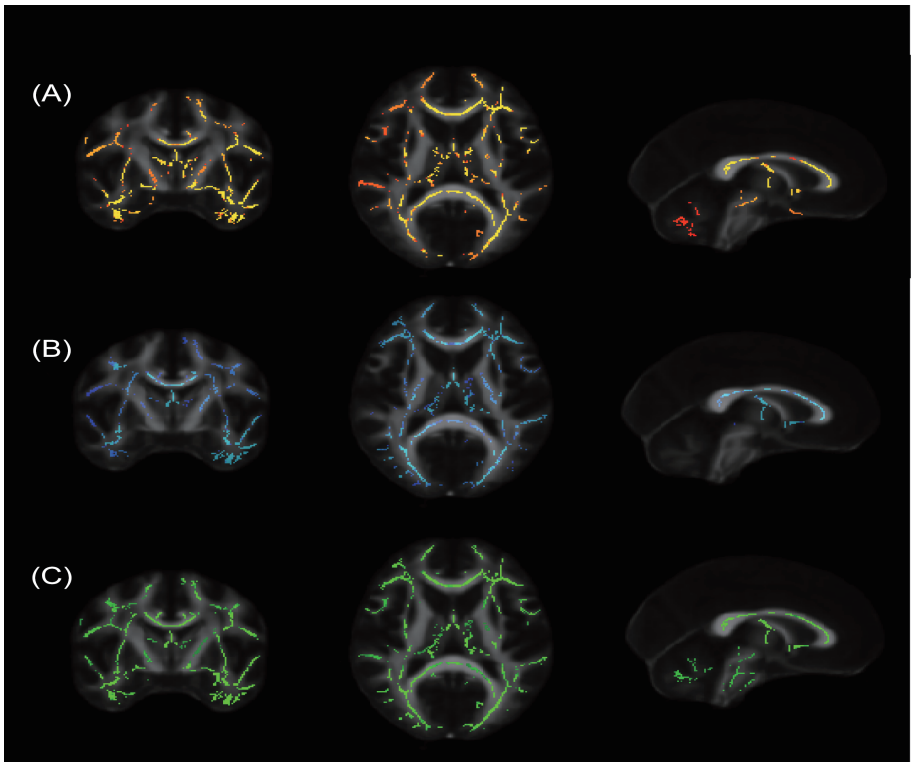

Figure 1. Areas of observed statistically significant difference (FWE-corrected $p < 0.05$) between control and TBI groups, super-imposed on FMRIB-58 template. Within these areas TBI group presented (A) lower FA, (B) higher Mean Diffusivity and (C) higher Radial Diffusivity, compared to the control group.

stage of image pre-processing. The two groups (TBI and HC) were closely matched on age, education, and socioeconomic status (with the exception of two participants, all subjects with TBI were pair matched with controls). While the sample sizes are modest, this study was unique as none of the TBI group had received any post-acute neurocognitive and/or neuropsychological rehabilitation and data analysis used extensive neuropsychological measures along with imaging indices. Approval was granted by the National Bioethics Committee for all procedures followed and written consent was obtained from each participant.

A spin-echo, echo-planar-imaging, parallel imaging enhanced (SENSE factor 2) three dimensional acquisition scheme (TE = 43 msec, TR = 18477 msec, EPI factor = 61, acquisition voxel size 2 $\times$ 2 $\times$ 2 mm^3) was employed on a three Tesla MRI system (Achieva, Philips Medical Systems, the Netherlands), to acquire reference (b = 0 sec/mm^2) and diffusion-weighted (b = 1000 sec/mm^2) volumes for all participants. Thirty-two gradient directions were used for diffusion sensitization. Following each acquisition, 68 axial slices of the brain were reconstructed with a voxel size of 1.8 $\times$ 1.8 $\times$ 2.0 mm^3. The scanning session also included other pulse sequences to exclude significant brain pathology of different etiology (e.g., T1-weighted rapid acquisition gradient-echo, T2-weighted turbo spin echo, fluid-attenuated inversion recovery). Average scanning time per participant was 29.5 minutes.

Reconstructed diffusion-weighted images were corrected for patient motion (between the two acquisitions with different b values) and for eddy current distortions. Voxel wise statistical analysis for the calculation of FA, mean diffusivity (MD), radial diffusivity (RD) and longitudinal or axial diffusivity (AD) maps was carried out using Tract Based Spatial Statistics (TBSS) (Smith et al., 2006). TBSS is part of the FMRIB software library, abbreviated as FSL. Multiple comparisons correction was applied by controlling Family-Wise Error (FWE) rate at the level of 0.05.

Four working and two episodic memory tasks were employed to assess visual memory performance, while verbal memory capacity was evaluated by means of five working and three episodic memory tests. Individual visual and verbal memory test scores were combined to give two composite scores corresponding to visual and verbal memory constructs, respectively, using a method advocated by Cahn et al. (1998), and previously used in TBI research (Cojen et al., 2003; Constantinidou et al., 2008; Konstantinou et al., 2016). Tests employed are shown in Table 2.

The Visual Memory tests included the Rey Complex Figure Test (immediate, delayed recall and recognition total score, Meyers and Meyers (1995), the Visual Span Forward and Backwards (adapted from WMS-R, Wechsler (1997), and two experimental memory tasks enabling the separate assessment of two distinct mechanisms of spatial and object VSTM: 1. the spatial visual short-term memory (VSTM) experimental task threshold, and 2. the object VSTM experimental task threshold (described in Konstantinou et al. (2016). The Verbal Memory test battery included the Greek adaptation of the Auditory Verbal Learning Test (AVLT), total score of trials 1–5, difference score between trial 5 and trial 1, short delay free recall (Trial 6), long delay free recall (Trial 7), and the list A true positive recognition score (Constantinidou and Evripidou, 2012), the Digit Span Forward and

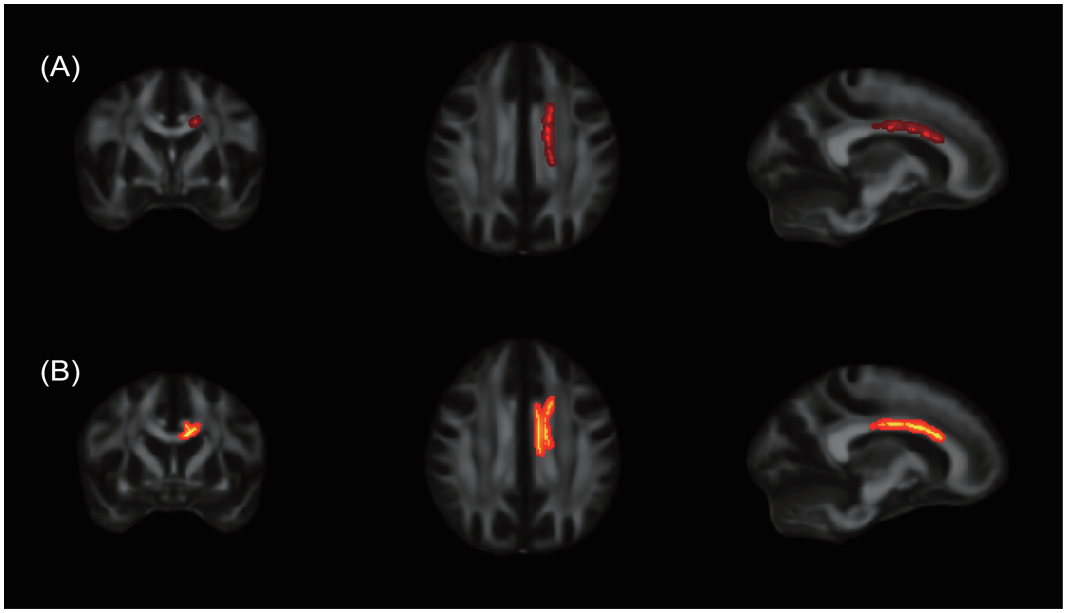

Figure 2. (A) Object VSTM Threshold Score vs. Axial Diffusivity. Statistically significant slope difference between control and TBI groups on the left side of the body of Corpus Callosum (FWE Corrected $p \leq 0.05$), and (B) positive linear correlation (FWE Corrected $p \leq 0.05$) between Object VSTM Threshold Score and Fractional Anisotropy within TBI group on the left side of the body of Corpus Callosum.

Backwards total score (Greek adaptation of the Wechsler Memory Scale-Revised, WMS-R), and the adapted paragraphs from the WMS-R Story Recall subtests (sum of the immediate recall and the sum of the delayed recall scores). The combined scores (composites) of these tasks were also evaluated to perform a comprehensive assessment of visual and verbal memory.

Participants performed a delayed match-to-sample VSTM task maintaining a set of shapes in VSTM throughout a one second retention interval by visually projecting them onto a screen while not verbalizing them. During the response period of the task, participants pressed a button to respond whether the memory probe item appeared at the same location as any of the memory-set items for the spatial VSTM task or whether the memory probe item appearing at fixation was identical to any of the previously viewed memory set items. This design enabled separate assessment of two distinct mechanisms of spatial and object VSTM. Further details concerning the following VSTM task procedures can be found in Konstantinou et al. (2016).

A one-way ANCOVA (26 degrees of freedom) was conducted to determine whether a statistically significant difference in cognitive performance existed between the participants with TBI and the matched HC group, when controlled for age and education. The Levene's test was utilized to check equality of variances (homogeneity of groups) prior to the ANCOVA test, and homogeneity of regression slopes was assessed to verify the reliability of ANCOVA results. Differences between groups are quoted with Bonferroni corrected p-values (Table 1).

Between-group comparison of whole brain WM diffusion properties was conducted by TBSS implementation. The four explanatory variables (FA, MD, AD, and RD) were fitted to a General Linear Model, whilst age and education of the participants were included as confounding parameters. Two contrasts were used for statistical testing. One to designate controls higher than participants with TBI and one for the opposite relation.

Between-group comparisons using ROI-based TBSS analyses were conducted to determine areas of CC with statistically significant slope differences regarding correlations between individual cognitive performance scores and diffusion tensor metrics (FWE corrected $p \leq 0.05$). To this end, a CC structure extracted from the MNI structural atlas was used. Following thresholding at 10%, the CC structure was binarized and used to mask the skeletonized data employed for the subsequent process of permutation testing.

Within-group ROI-based TBSS analyses were also conducted separately for each group to determine CC areas with statistically significant correlations between cognitive performance and diffusion metrics. The aim was to identify diffusion tensor metrics that could potentially function as within-group predictors of cognitive performance.

To determine the direction (positive or negative) of the correlations mentioned above, multiple regression models were employed. The input parameters included the cognitive performance score as the dependent variable, and the diffusion metric of interest (mean value within the area delineated via TBSS), age, and education level as the independent variables. Before running the multiple regression models, a Grubb's outlier test ($\alpha = 0.01$) was used to identify possible outliers of DTI metrics.

3. Results

Controls outperformed TBI subjects in several visual and verbal working memory tasks ("immediate recall of Rey complex figure", "Object VSTM threshold", "AVLT total score in trials 1–5", "digit span sum", and "immediate story recall") and some of the episodic memory tasks ("delayed recall of Rey complex figure", "long delay free recall", and "story delayed recall"). Additionally, there was a statistically significant group difference in visual and verbal composites, demonstrating the adverse long-term effects of

Table 2. Visual and verbal memory performance comparison between control and TBI groups.

	Neuropsychological Assessment	Group Difference (significant at $\alpha = 0.05$)
Visual Memory Tasks	Working Memory Tasks	
	Rey Figure Immediate Recall	Yes, (F(1, 25) = 12.45, $p = 0.002$)
	Visual Span Total	No, (F(1, 25) = 1.30, $p = 0.27$)
	Spatial VSTM Threshold	No, (F(1, 25) = 2.09, $p = 0.16$)
	Object VSTM Threshold	Yes, (F(1, 25) = 8.72, $p = 0.007$)
	Episodic Memory Tasks	
	Rey Figure Delayed Recall	Yes, (F(1, 25) = 7.50, $p = 0.011$)
	Rey Figure Recognition	No, (F(1, 25) = 1.38, $p = 0.25$)
	Visual memory Composite*	Yes, (F(1, 25) = 7.14, $p = 0.014$)
Verbal Memory Tasks	Working Memory Tasks	
	AVLT** Total Score in trials 1-5	Yes, (F(1, 25) = 5.02, $p = 0.035$)
	Learning Curve (AVLT 5-1)	No, (F(1, 25) = 0.004, $p = 0.95$)
	Short Delay Free Recall	No, (F(1, 25) = 2.18, $p = 0.15$)
	Digit Span Total	Yes, (F(1, 25) = 4.5, $p = 0.044$)
	Story Immediate Recall	Yes, (F(1, 25 = 7.6, $p = 0.011$)
	Episodic Memory Tasks	
	Long Delay Free Recall	Yes, (F(1, 25) = 4.2, $p = 0.052$)
	AVLT Recognition List A	No, (F(1, 25) = 2.1, $p = 0.16$)
	Story Delayed Recall	Yes, (F(1, 25 = 14.87, $p = 0.001$)
	Verbal memory composite	Yes, (F(1, 25 = 7.92, $p = 0.01$)

∗ Visual and verbal memory composites are the combined scores from all relevant cognitive tasks administered.

∗∗ Auditory verbal learning test.

TBI in memory capacity. Table 2 gives group comparison results for each test.

Whole brain TBSS analysis helped unravel the chronic effect of TBI on WM integrity by detecting statistically significant differences in DTI properties between HC and TBI groups. The latter exhibited global reduction in FA and increase in MD and RD compared to HC (FWE-corrected $p < 0.05$, Fig. 1). The two groups did not present statistically significant differences in AD. However, such difference was found at a lower threshold (CL 90%) in a large part of the WM network.

Table 3 presents FA, MD, and RD numerical data from the TBSS-detected areas (Fig. 1) along with statistically significant differences between the HC and TBI groups. The AD values correspond to the whole brain as no areas with statistically significant intergroup differences were identified by TBSS.

The measured neuropsychological group differences in visual memory performance were correlated with CC diffusion metrics in one working (Object VSTM Threshold) and one episodic (delayed recall of Rey complex figure) memory related task. For the "Object VSTM Threshold" task, between-group cognitive performance difference was found to correlate with AD on the left side of the CC body (Fig. 2A). In the same region, AD was found to correlate negatively ($p = 0.007$) with the "Object VSTM Threshold" score among participants with TBI. No relevant correlation was established for the HC group. Within-group analysis also yielded a positive ($p = 0.002$) linear correlation between the "Object VSTM Threshold" score and FA on the left side of the body of CC for the participants with TBI (Fig. 2B), similarly with no corresponding correations for the HC group.

For the delayed recall of the Rey complex figure, TBSS analysis showed a group difference for the correlation slope between the cognitive score and FA in part of the CC body (Fig. 3A). Between-group slope difference was also found for the correlation between the score for the delayed recall of the Rey complex figure and MD on the left side of the body and the anterior CC (Fig. 3B). Further regression analysis showed a positive linear relationship of the Rey complex figure delayed recall performance score with FA ($p = 0.002$) and a negative linear relationship with MD ($p = 0.007$) for TBI subjects, in the corresponding anatomical areas just mentioned.

In both the cases just quoted, it is assumed that the observed within-group correlations could account for the observed group differences. In the case of immediate recall of the Rey complex figure, the detected group difference in neuropsychological performance was not accompanied by any observations from comparative TBSS analysis at the CC.

Statistically significant group differences in correlations between "Object VSTM Threshold" score and axial, mean, and radial diffusivity were also found by TBSS group comparison at the splenium of CC. However, they were rejected by the Grubb's test ($\alpha = 0.01$) as outlier-driven correlations.

Within-group TBSS and regression analysis for the subjects with TBI presented a statistically significant positive linear correlation ($p = 0.0002$) between the combined cognitive performance score of all visual memory tasks and FA at the body of CC, extending from the isthmus anteriorly to the limits of the genu (Fig. 4). The same regression model for the HC group indicated a statistically significant positive correlation of the composite score with education level ($p = 0.045$) and a marginally statistically significant negative correlation with age ($p = 0.057$), but no significant

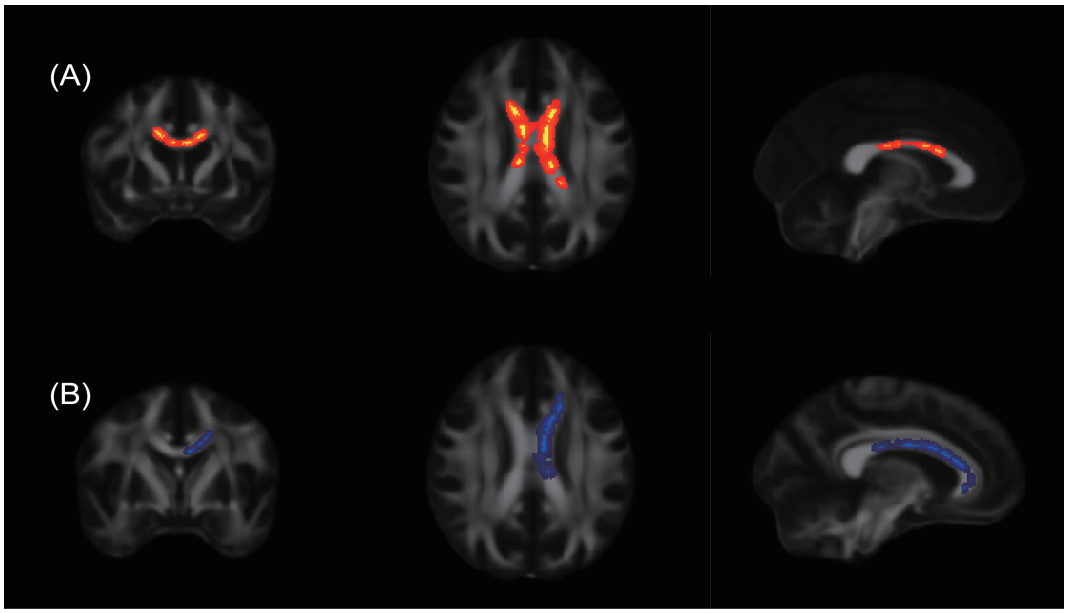

Figure 3. (A) Statistically significant slope difference between control and TBI groups for the correlation of Rey Delayed Recall Score and Fractional Anisotropy at the body of Corpus Callosum (FWE-corrected $p \leq 0.05$), and (B) Statistically significant slope difference between control and TBI groups for the correlation of Rey Delayed Recall Score and Mean Diffusivity on the left side of the body and anterior Corpus Callosum (FWE-corrected $p \leq 0.05$).

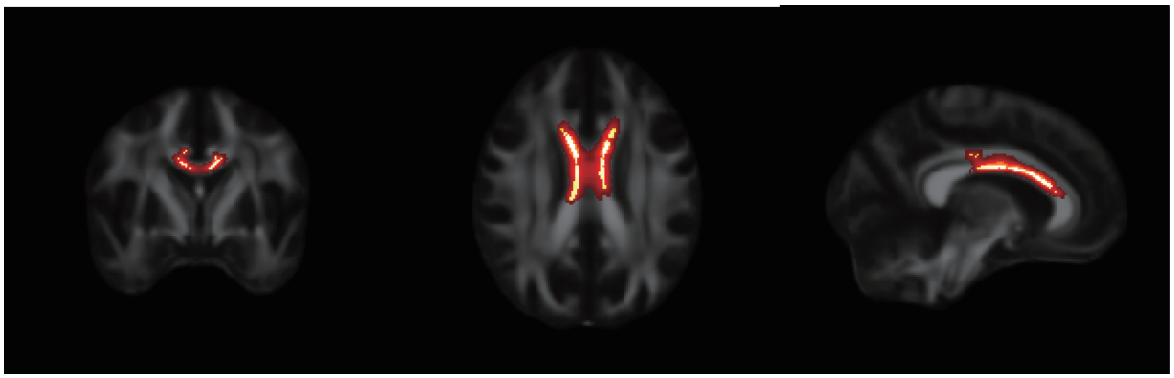

Figure 4. Positive linear correlation (FWE Corrected $p \leq 0.05$) between Visual Memory Composite and Fractional Anisotropy within the TBI group at the body and anterior Corpus Callosum.

correlation with FA.

The measured neuropsychological group differences in verbal working and episodic memory performance (i.e. AVLT total score in trials 1–5, digit span sum, story immediate recall, long delay free recall, and story delayed recall) were not found by statistical testing to be correlated with CC diffusion metrics.

For the delayed free recall task, however, an area extending through the whole length of CC was found to exhibit statistically significant negative linear correlation ($p = 0.007$) between performance score and MD (Fig. 5A) within the TBI group. A smaller CC area of negative correlation ($p = 0.005$) was also revealed for RD (Fig. 5B).

Although no significant group difference was found in neuropsychological performance for the List A true positive recognition task, TBSS analysis indicated a statistically significant between-group difference in the correlation slope between performance score and MD through the whole CC length (Fig. 6A). Such difference was also shown for the correlation between performance score and RD, extending from the CC body to the posterior left part through the junction with the splenium (Fig. 6B). Subsequent multiple regression analysis among TBI subjects yielded a statistically significant negative linear correlation of List A true positive recognition score with MD ($p = 0.00005$) and age ($p = 0.0005$). A similar correlation was found between performance score and RD ($p = 0.00008$) and age ($p = 0.0008$). It is assumed that the observed correlations within the TBI group account for the corresponding group differences, since no such correlations were found within the HC group.

The subjects with TBI also presented a statistically significant negative linear correlation ($p = 0.0001$) between List A true positive recognition performance score and AD at the anterior left part of the CC body (Fig. 7). Regression analysis in this area also yielded a statistically significant negative correlation of performance score with age ($p = 0.001$).

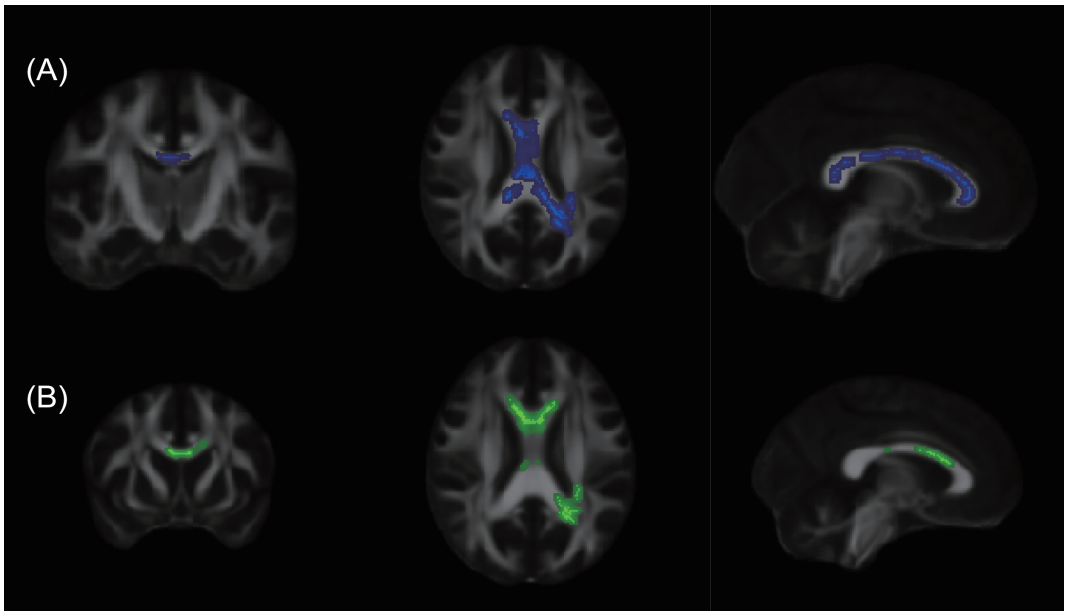

Figure 5. (A) Statistically significant negative linear correlation (FWE-corrected $p \leq 0.05$) between the delayed free recall performance score and Mean Diffusivity at the Corpus Callosum determined by TBSS for the TBI group, and (B) Statistically significant negative linear correlation (FWE-corrected $p \leq 0.05$) between the delayed free recall performance score and Radial Diffusivity at the anterior and posterior part of corpus callosum determined by TBSS for the TBI group.

Although a statistically significant difference between the two groups was measured for the composite verbal memory score, no significant correlations were established with CC diffusion metrics.

4. Discussion

It has previously been suggested that moderate-severe brain injuries inflict both axonal and myelinic damage, as opposed to mild injuries in which damage is primarily axonal (Kraus et al., 2007). Widespread brain network decrease in FA and increase in MD have been reported in both the sub-acute and chronic phases of TBI. This probably reflects demyelination and swelling of axons (Benson et al., 2007; Hashim et al., 2017; Endlow et al., 2016; Kennedy et al., 2009; Kinnunen et al., 2011; Kraus et al., 2007). Some studies have also reported increase in AD (Hashim et al., 2017; Kinnunen et al., 2011). In this study, four diffusion tensor measures were employed to assess the global WM and CC changes present in the chronic phase of moderate-severe TBI. Global decrease in FA, as well as increase in MD and RD, were evident in these subjects. Nevertheless, no difference was found in global AD between HC and TBI subjects at the 95% confidence level. It has been suggested that RD in WM is modulated by myelin, whereas AD primarily relates to axonal integrity (Alexander et al., 2007). Hence, demyelination is associated with an increase in RD and may not have a significant influence on AD (Harsan et al., 2006; Song et al., 2002, 2005; Tyszka et al., 2006). Therefore, current whole-brain results suggest myelin-related damage, as no group difference in global AD was noted. Nevertheless, CC results revealed that although most of the between-group differences in the correlation slope involved RD, some correlations within the TBI subject group, as well as between-group differences, involve AD. This indicates that although myelin-related damage affects global WM integrity several years post-injury, residual microstructural damage relating to axonal loss may be also present at the CC, a suggestion that adds to previous reports of apparent irreversible myelin damage observed in moderate-severe TBI (Kraus et al., 2007).

Current neuropsychological results demonstrated that HC outperformed TBI subjects in several tasks, as well as in the overall assessment of visual and verbal memory. The employment of composites for the overall memory assessment helped minimize specificity issues related to individual tasks and multiple statistical comparisons, thus improving reliability. In a recent study, composite memory scores were found to correlate with FA and RD in various tracts, including the corpus callosal fiber pathway, indicating persistent functional loss in chronic TBI (Hashim et al., 2017).

Using comparative TBSS analysis, an attempt was made to detect CC regions with group differences regarding the correlation between individual task performance scores and diffusion metrics. This scenario was confirmed for two visual memory tasks (one working and one episodic) in which HC outperformed TBI subjects, and for one verbal episodic memory task in which the two groups performed similarly. No statistically significant group difference was detected for the correlation slope between verbal working memory performance and CC diffusion metrics. These findings indicate that the extent of the involved CC area depends on the selected diffusion metric.

Analysis within the control group of the visual memory composite showed that visual memory performance is driven by a positive correlation with education level and a negative correlation with age. Within the group of TBI subjects however, a positive correlation with FA at the CC body, extending anteriorly from the isthmus, was revealed (Fig. 4). Despite the observed correlations between overall visual memory performance and FA within TBI

Table 3. Diffusion metric measurements from TBSS-detected areas with statistically significant difference between the HC and TBI groups (Fig. 1) for each metric (AD values correspond to the whole brain as TBSS analysis indicated no areas with statistically significant intergroup differences).

Metric		Mean	SD	SEM	95% Confidence Interval for group difference	Statistical Significance
FA	HC	0.51	0.03	0.01	(0.0063 , 0.064)	$t = 2.6, p = 0.0015$
	TBI	0.47	0.04	0.01		
MD(x10^{-4} mm^2/s^{-1})	HC	6.06	0.21	0.06	(-1.43 , -0.03)	$t = -2.23, p = 0.034$
	TBI	6.79	1.26	0.34		
RD(x10^{-4} mm^2/s^{-1})	HC	8.34	0.57	0.15	(-3.42 , -0.18)	$t = -2.34, p = 0.025$
	TBI	10.14	2.78	0.77		
AD(x10^{-4} mm^2/s^{-1})	HC	10.15	0.22	0.06	(-0,95 , 0,06)	$t = -1.98, p = 0.06$
	TBI	10.60	0.81	0.23		

HC: Healthy Control, TBI: Traumatic Brain Injury subjects, TBSS: track-based spatial statistics, FA: fractional anisotropy, MD: mean diffusivity, RD: radial diffusivity, AD: axial diffusivity, SD: standard deviation, SEM: standard error of the mean.

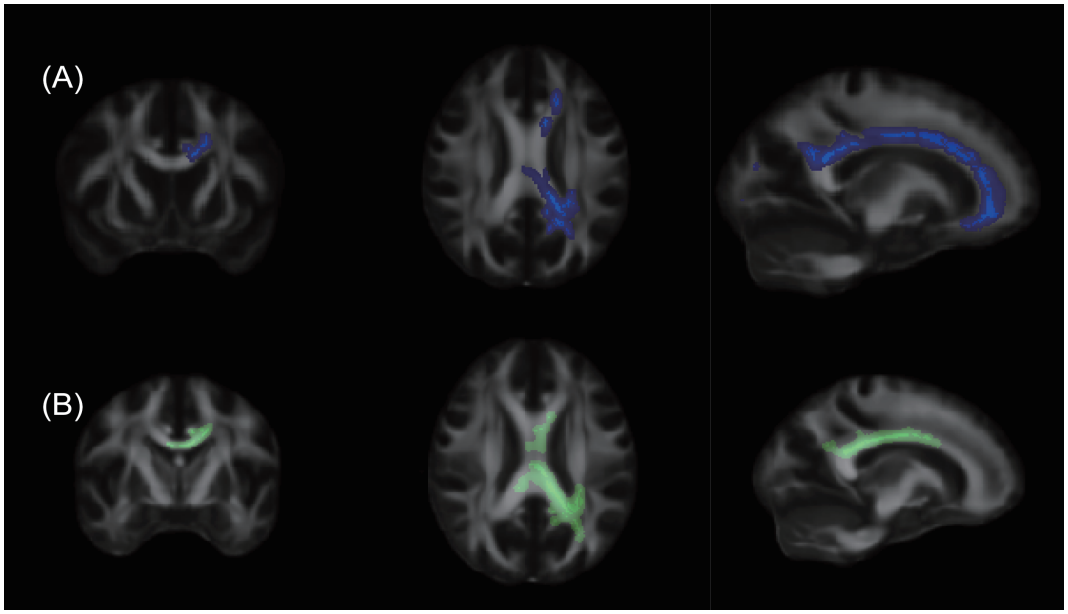

Figure 6. Statistically significant slope difference between control and TBI groups (FWE-corrected $p \leq 0.05$) for the correlation between list A true positive recognition score and (A) Mean Diffusivity extending over the whole length of Corpus Callosum and being prominent on the left, and (B) Radial Diffusivity extending posteriorly and leftwards from the body of Corpus Callosum.

subjects, no corresponding between-group differences were observed at the chosen confidence level (95%). Yet, such differences were evident at a lower threshold (CL 90%). This suggests that a firm conclusion on the correlation of visual memory loss with CC diffusion metrics could possibly be documented with a larger sample size.

Salmond et al. (2006) reported correlation of reduced FA at CC with learning and memory measures in chronic TBI. In the current study, within-group CC analysis of learning and memory individual tasks showed correlation between memory performance and various diffusion metrics only for the TBI group. FA correlated positively with performance in one visual working memory task, whilst AD and MD correlated negatively with performance in two verbal episodic memory tasks. Arenth et al. (2014) reported that, in TBI, reduced FA at the splenium relates to declarative memory and at the genu relates to working memory. Here, CC diffusion was found to correlate with working and episodic visual memory, as well as with episodic but not working verbal memory. Consideration of the current and previous findings suggest that inferences in this type of correlation analysis depend on the DTI metric employed and the specific anatomical sub-region involved and can therefore be complicated. Nevertheless, the employed approach of correlating DTI changes to neuropsychological response seems to enable insight even when microstructural changes in WM are not followed by neurological or neuropsychological changes.

Inherent methodological limitations mainly relate to the spatial resolution of MRI, which is of the order of millimetres while diffusion occurs at a scale of micrometres. Minor quantities of cerebrospinal fluid that may be measured as water diffusion, image noise, and artefacts, as well as correction, pre-processing and registration algorithm limitations, add to the overall analytic uncertainty (Alexander et al., 2007). Apart from the approximations imposed by partial volume effects, the multi-compartmental nature of water diffusion in nervous tissue is underestimated by the

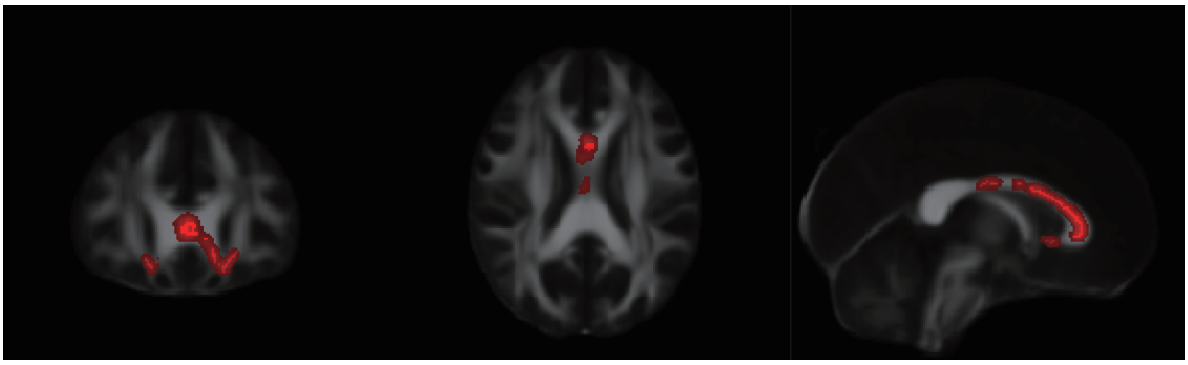

Figure 7. Statistically significant negative linear correlation (FWE-corrected $p \leq 0.05$) between list A true positive recognition score and axial diffusivity at the anterior left Corpus Calossum as determined by TBSS for the TBI group.

mono-exponential fitting of a diffusion-weighted signal intensity. Thus, employment of other models, such as the continuous time random walk model (Gatto et al., 2019), could be considered in the future to mitigate shortcomings of the DTI model. Gender differences have also been identified as a factor to consider in study of the corpus callosum (Luders et al., 2010). The pooling of uniquely male subjects, leaves a question as to whether inferences are valid for females. On the other hand, findings are not obscured by potential sex differences. The presence or absence of rehabilitation and training-induced brain plasticity, which has been reported to continue through chronic phases of TBI is another possible confounding factor (Han et al., 2017). A systematic review has indicated that community-based interventions improve the cognitive outcome of adolescents following TBI (Clasby et al., 2018). In this study, none of the TBI group had attended any post-acute rehabilitation programs, therefore, performance improvement post-injury, if any, was restricted to the limited capacity of endogenous repair mechanisms (Guan and Kong, 2015). A considerable number of exclusion and inclusion criteria were adopted to secure sample uniformity and specificity that inevitably restricted subject group size. The specificity issues of neuropsychological testing also constitute an additional factor that limits the reliability and accuracy of the conclusions made here. Nevertheless, this project was enhanced by the use of extensive neuropsychological measures, imaging indices, and also by between-group CC comparison that was based on correlation differences, not individual values.

5. Conclusions

Results reported here indicate that changes in whole brain WM diffusion properties inflicted by moderate-severe TBI are still evident several years post-injury in a group of survivors who have not received systematic rehabilitation. Overall visual memory capacity was reduced in the TBI group and this decline was correlated to WM integrity loss at the CC body, while group performance differences in visual memory were correlated with DTI metrics at the body and anterior left part of CC. Overall verbal memory was also reduced in TBI subjects, but no correlation between this decline and CC diffusion properties was established. Nevertheless, group performance difference in one task relating to verbal episodic memory was correlated with MD and RD at various CC locations. Within the TBI group, significant correlations between neuropsychological performance and CC diffusion metrics were found for one visual working memory and two verbal episodic memory tasks, although corresponding group differences were not found to be significant.

Future work is warranted for similar investigation of the cingulum, fornix, and cerebral cortex. Accounting for the fact that current results are based on a cross-sectional study and that recent work has revealed lifelong evolving consequences of TBI-induced structural lesions and functional outcomes (Wilson et al., 2017), it is imperative that this investigation is extended into a longitudinal study.

Authors' Contributions

NK, FC and IS established the concept of the study. CY, NK, FC and IS designed the study. CY conducted the literature search. NK, FC, EP, EE and SP contributed in the clinical studies. NK conducted the experimental studies. EP and EE contributed to the data acquisition CY analysed the data, prepared and edited the manuscript. NK, FC, SP and IS reviewed the manuscript.

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Ethics Approval and consent to participate

The National Bioethics Committee of the Republic of Cyprus approved this project. The approval number is EEBK/EII/2011/10.

Conflict of interest

The authors declare no competing interests.

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